Interaction between inbreeding and assortative mating in the Cowpea Weevil *Callosobruchus maculatus*

Yin Yuan

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Biology Education Centre and Department of Animal Ecology, Uppsala University, Uppsala University
Supervisors: Mats Bjorklund and Emma Rova
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Summary

The models of assortative mating are many, and the theory and empirical data on inbreeding is rich, however studies of the combination of the two are basically absent. This project therefore aims at studying the interaction between assortative mating and inbreeding by using the population Cowpea weevil, *Callosobruchus maculatus* (*Fabr*). After six generations of inbreeding, the results show that female fecundity was indeed affected by inbreeding depression; females laid fewer eggs after mating with males that were closely related. Both the number of eggs laid and individuals hatched became fewer and fewer over generations, which indicate that inbreeding decrease female fecundity and reproductive rate. My study also shows food resource affected the female fecundity significantly, and offspring from black-eyed beans always had larger body size than those from mung beans. This project conclude that the effect of mating patterns on female fecundity during lifetime was significant. The extent of inbreeding depression is larger at smaller population sizes, which means inbreeding happened more frequently in smaller population size. The results show deviations from Hardy-Weinberg equilibrium, which means assortative mating was avoided mostly. One of the most conceivable reasons could be inbreeding depression. This suggest that inbreeding is a factor that disturb the effects of assortative mating on sympatric speciation. The populations that can mate assortatively and at the same time avoid inbreeding are the ones that survive and may evolve into new species. This study is a benificial understanding in research of interaction between ecological patterns and inbreeding as well as assortative mating in both theoretical and laboratorial aspect, in the end, speciation.
Introduction

Theories of speciation

Speciation is the evolutionary process where new biological species evolves. Whether speciation is achieved normally via genetic drift or natural selection is the subject of much ongoing discussion in biology. In nature there are four geographic modes of speciation, based on the extent to which speciating populations are geographically isolated from one another: allopatric, peripatric, parapatric, and sympatric (Mayr 1963, Coyne 1992, Doebeli et al. 2005, Bolnick and Fitzpatrick 2007). The study of speciation is an elementary biological problem. An increasing number of studies found that speciation played an important role in evolution (Crockford 2004, Sadedin 2005). However, many open questions remain and need further study.

It has been discussed whether speciation can occur at different geographic areas (allopatry), or in the same geographic area (sympatry). It is widely believed that many species have originated through allopatric divergence. This means that new species arise from geographically isolated populations of the same ancestral species (Mayr 1963, Coyne 1992). According to Gavrilets (2003), sympatric speciation is the emergence of new species from a population where mating is random with respect to the birthplace of the mating partners (Gavrilets 2003). Unfortunately, the origin of species by sympatric speciation has not gained much support.

One of the key factors in sympatric speciation is assortative mating. Assortative mating mechanisms reduce the break-up of the ancestral population into diverging and reproductively isolated descendent species (Doebeli 2005). It appears when individuals select to mate with individuals that are more phenotypically similar to themselves than expected under random mating. Assortative mating carries cost not only in terms of time and effort spent searching for the right mates, but there is also the risk of inbreeding to be considered. In small population, on an island for instance,
assortative mating could happen on the basis of an ecological trait (Kirkpatrick and Servedio 1999). In a small-population, individuals most similar to one another which are often close kin. In the recent classic models of sympatric speciation (Dieckmann and Doebeli 1999), the evolution of assortative mating depends either on an ecological character affecting resource use or on a selectively neutral marker trait.

Inbreeding has been shown to cause decrease of fecundity and reduced survival in natural populations. To avoid inbreeding depression in small populations is a major concern in conservation biology. Genetic factors such as the pattern of expression of deleterious alleles (Lacy 1996), or the effect of past history including purging of deleterious alleles (Lacy and Ballou 1998) is important to understand the possible risk of inbreeding depression (Björklund 2003).

Inbreeding depression caused by mating with close relatives is a commonly reported phenomenon in natural populations. Even if it is not clearly shown what factor causes the reduction in fitness caused by inbreeding, it is interesting to design a project to test how inbreeding affect the population size or female fecundity. Inbreeding increases the frequency of homozygotes in the population and decreases the frequency of heterozygotes. There is year-to-year variation in population size when inbreeding happens. (Li 1963, Heschel and Paige 1995, Kokko and Ots 2006). An increase of inbreeding coefficient, or level of homozygosity can manifest itself as a loss in individual fitness (Franklin 1980).

**Aims and hypotheses**

The models of assortative mating are many (Dieckmann and Doebeli 1999, Doebeli 2005) (Kirkpatrick and Servedio 1999), and the theory and empirical data on inbreeding is rich (Li 1963, Heschel and Paige 1995, Kokko and Ots 2006), however studies of the combination of the two are basically absent. This project therefore aims at studying the interaction between assortative mating and inbreeding.

The effects of inbreeding are difficult to predict since it ultimately depends on the amount of deleterious mutations that accumulate in a population. In addition, in a
small population it also depends on the stochastic effects of drift, whether the deleterious alleles will be purged or simply drive the population to extinction. This predicts that we can make experiments for local adaptation under local resources, as well as the evolution of assortative mating. But the chameleonic effects of inbreeding will affect the outcomes.

In some studies, inbreeding is thought to be a factor which decrease the population fitness (Li 1963). Within small populations, there will always be selection for inbreeding avoidance, most easily through an absence of assortative mating. Alternatively, some research indicated in some circumstances inbreeding can be beneficial (Kokko and Ots 2006). Can assortative mating be beneficial too in different populations? Unfortunately, this point has not been studied at any depth. Thus, we face a conflict of selection on different levels. The populations that can mate assortatively and at the same time avoid inbreeding are the ones that survive and may evolve into new species.

We designed the study using the seed beetle *Callosobruchus maculatus* (Fabr.) as the study species because it is easy to raise in large populations. This experiment was divided into two parts: inbreeding and assortative mating.

The hypotheses are as follow: (1) The number of eggs laid and individuals hatched are expected to decrease over generations, which indicate that inbreeding depression significantly affect female fecundity. (2) Inbreeding depression is hypothesized to impact assortative mating significantly. This project allows us to understand better what is the importance of inbreeding and assortative mating for the process of speciation.
Material and Methods

Model organism

The Model Organism in this experiment is the Cowpea weevil, *Callosobruchus maculatus* (Fabr.), which originate from Brazil. This species is a pest on stored legumes (*Fabaceae*), particularly beans of the genus *Vigna* (Rankin and Arnqvist 2008). The females attach their eggs to the surface of beans, and the larvae develop inside (Edvardsson and Tregenza 2005). This species has non-overlapping generations, and the time of a generation is approximately 28 days. Females generally mate several times during their lifetime, although only one, or occasionally two, matings are required to fertilize all of their eggs (Fox 1993). After mating, if seeds are available, females start to lay eggs on the surface of the host seeds within minutes (Messina 1991). Larvae typically develop by utilising the nutrition from dried peas, they chew near the surface and leave a thin covering uneaten which appears as a window from which the adults later emerge.

These insect populations have been kept in the Department of Animal Ecology of Uppsala University, Sweden for 8 years as a model system for various research purposes.

Equipment and experimental design

Part I- Inbreeding

Two naturally occurring colour morphs of the bean weevil *Callosobruchus maculatus* was raised on two different food resources. One was mung beans (M), *Vigna radiat* (*L.*), and the other was black-eyed beans (BE), *Vigna unguiculata* (*L.*). To cause and test for the effects of inbreeding, we induced different levels of inbreeding by creating three treatment groups of different population sizes. The population sizes consisted of 2, 8 and 20 pairs respectively. One control treatment, used for comparison and consisting of a couple of hundred pairs, were also put on the two resources M (mung
beans) and BE (black-eyed beans). Each treatment contained ten replicates and female fecundity was measured on a sub-sample of four females replicate for the 4 pair and the 8 pair groups. For apparent reasons, female fecundity could only be measured on two females in the 2 pair group.

At the start of the experiment, mung beans and black-eyed beans with eggs were taken from an uninbred population of several thousands of individuals and put into virgin chambers. The larvae were maintained to develop within their seeds to the adult stage in a climate chamber at 26° C, 50 % RH, and 24 H light. After approximate 15 days, the seed beetles started to hatch. Virgin males and females were singled out and put into separate 92×16mm Petri dishes. All virgins were maintained solitarily before mating. When enough virgin males and females had been extracted, 2 males+2 females, 8 males+8 females, 20 males+20 females, for the three levels respectively, males and females were placed together within their treatment group and allowed to mate for 24 hours. After mating, I picked out 2 mated females from the 2 males+2 females treatment, (2×2 treatment in short), 4 mated females from the 8 males+8 females treatment, (8×8 treatment in short), 4 mated females from the 20 males+20 females treatment, (20×20 treatment in short) from each resource, and put into Petri dishes with 20±0.05 g mung beans or black-eyed beans for eggs laying. Except for the two males of the 2×2 treatment that were discarded, all the rest of the mated individuals were treatment organized put into plastic jars with 40±0.05 g mung beans or black-eyed beans, stored to contribute to the next generation. The same procedure was done for all six generations. Then I counted all of the eggs laid by the two mated females and individuals hatched from treatment 2×2. The number of eggs laid and individuals hatched were counted for a subsample of 4 females from the treatment 8×8 and 20×20. The same procedure was also done for all six generations. Female fecundity and larval survival was calculated from this data. Afterwards I measured the nature and strength of assortative mating in the three treatments. To count eggs and individuals from all females in the experiment would have been far to time consuming for a 30 weeks project.
Part II- Assortative mating

In order to understand how inbreeding may affect or create assortative mating in the Cowpea weevil, after three generations of induced inbreeding, twenty pairs from each level of inbreeding and food resource were put together to mate freely within each level of inbreeding. The nature and intensity of assortative mating was measured in the different treatments by comparing the amount of heterozygotes in the succeeding generation to that of Hardy-Weinberg equilibrium in case of random mating.

The first generation of the second part experiment (assortative mating) was from the third generation of the first part experiment (inbreeding), to make the result clearer, I kept the generation number the same. That means there were G1 (generation 1), G2, G3, G4, G5 and G6 in Part I, but G3, G4, G5 and G6 appeared in Part II. From G3 of Part I, I picked out 20 virgin males and 20 virgin females from each food resource, i.e. 80 individuals in total, and mated them within each level of inbreeding in glass jars with 40 ± 0.05 g mung beans and 40 ± 0.05 g black-eyed beans mixed. This was done for 4 generations and for all ten replicates. In each generation, the individuals hatched were analysed for colour morphs. I distinguished the colour morphs by assigning them one of three colours: black, intermediate and brown, and counted the total number of each colour morphs in every treatment or inbreeding level.

Statistical analyses

I chose an analysis of variance (ANOVA) to compare the total number of eggs laid, between different resources, treatments and generations. ANOVA was also used to test the number of eggs laid between the three treatments in generation 6 and the control treatment. An analysis of covariance (ANCOVA) was used to compare the amount of individuals hatched between the three treatments (2 × 2, 8 × 8 and 20 × 20 treatment). To analyse the amount of larvae hatched over generations within the three treatments, ANCOVA was generally used. The data of the colour morphs was analysed by a non-parametric analysis of variance, the Kruskal–Wallis test. The analysis of non-parametric approach was used because of heteroscedasticity and departures from
normality. Kruskal–Wallis test is a non-parametric method for testing equality of population medians among groups. It is identical to a one-way analysis of variance with the data replaced by their ranks (Adams and Anthony 1996). To compare observed number of intermediate colours with expected number of intermediate colours, \( \chi^2 \) value was calculated by \( \frac{(\text{observed}-\text{expected})^2}{\text{expected}} \). If the \( \chi^2 \) value is 3.84 or more, the p-value is 0.05 or less (Urbanek et al. 1999).

**Hardy-Weinberg equilibrium**

The Hardy-Weinberg equilibrium law states that both allele and genotype frequencies in a population remain constant over time. In other words, they are in equilibrium from generation to generation unless specific disturbing influences are introduced. Those disturbing influences include non-random mating, mutations, selection, limited population size, random genetic drift and gene flow (Stern 1943, Finney 1952, Crow 1999).

It has been shown that in a completely randomly mating population, in which two alleles, A and a, occur in the frequencies p and q, the summation of p and q equals one. With no mutation, selection or immigration, the three genotypes AA, Aa and aa are expected to remain in equilibrium from generation to generation at frequencies of \( p^2 \), \( 2pq \) and \( q^2 \) (\( p^2 + 2pq + q^2 = 1 \)) (Stern 1943). This means that if there is more of one genotype there must be non-random mating, which is what we tested for.

In this experiment system there were three morphs, brown homozygotes, black homozygotes and intermediates (heterozygotes). After 40 pairs (20 pairs from each resource type) mated on a mixture of the two resource types (mung beans and blace-eyed beans), the F\(_1\) generation was expected to have 25% of black, 25% of brown and 50% of intermediate colours if the populations were in Hardy-Weinberg equilibrium. But if either the percentage of black or brown raised, positive assortative mating happened. Therefore, the key factor for testing assortative mating is to see if the percentage of intermediates is larger or smaller than 50%. If there are fewer intermediates than expected, we can conclude that the population is not in
Hardy-Weinberg equilibrium, which is an indication of positive assortative mating. On the other hand, if there are more intermediates than expected, we can draw the conclusion of negative assortative mating, i.e. inbreeding avoidance.
Results

Part I- Inbreeding

Number of eggs laid

The result showed that the effect of generation and treatment was significant. The interaction of generation, treatment and resource was not significant and the effect of resource was not significant (Table 1).

From the left graph of Fig. 1, we can see that the number of eggs changed between generation 2 to generation 6. For black-eyed beans, as seen in the right graph of Fig. 2, treatment $2 \times 2$ and $20 \times 20$ varied in the same trend as in mung beans. Even though the impact of resource was not significant, the detailed changes from these two graphs may give us better understanding of how inbreeding affects the female fecundity.

The last generation (G6) was compared with the treatment control (Fig. 2). The impact of treatment was significant, but the interaction between treatment and resource was not significant (Table 2). The total number of eggs was always less than treatment control, especially treatment $2 \times 2$.

Number of individuals hatched

The results showed that both generation and treatment affected the number of individuals significantly. The impact of resource was not significant, and the interaction between generation and treatment had no significant effects either (Table 3). Number of eggs was set as covariate (Fig. 3).

The control treatment was also compared with G6 in this part (Fig. 4). The interaction between treatment and resource was significant. Both treatment and resource affected the number of individuals significantly (Table 4). The mean values of individuals among four treatments ($2 \times 2$, $8 \times 8$, $20 \times 20$, control treatment) were almost the same in mung beans. The mean values of individuals varied a lot in black-eyed beans. The order of mean value of individuals from large to small was
control, 8 × 8, 20 × 20 and 2 × 2 treatment. The number of individuals on black-eyed beans was always more than that on mung beans except 2 × 2 treatment.

Part II- Assortative mating

*Percentage of intermediates*

The results showed that generation affected percentage of intermediate colour significantly. The multiple comparisons between generation 3 and 5 were significant, the interaction between generation 3 and 6 was significant (Table 5).

All of the percentages of intermediate colour over generations were larger than 50%. The trend line of intermediate colours’ percentage increased significantly with a steady rate from generation 3 to 6 (Fig. 5). In control treatment, the percentage was 57%.

The results showed that treatment was not significant when affected percentage of intermediate colours. The multiple comparisons between each two treatment were not significant (Table 6).

All of the percentages of intermediate colour over generations did not significantly change within three treatments (Fig. 6).

As mentioned in Material and Methods, to compare observed number of intermediate colours with expected number of intermediate colours, $\chi^2$ value is calculated. The number of $\chi^2$ value ($\chi^2 \geq 3.84$) increased over generations. From generation 3 to 6, the possibility of significant from number of intermediate colours increased (Table 7).
Discussion

Effects of inbreeding on female fecundity

As many studies of animal and plant species show, inbreeding results in the decline of female fecundity, which is the expression of inbreeding depression; females laid fewer eggs after mating with males that were closely related. Both the number of eggs laid and individuals hatched became fewer and fewer over generations, which indicate that inbreeding decrease female fecundity and reproductive rate.

Effects of food resource on female fecundity

In this experiment, food resource was not significant in the analysis of combination of generation, treatment and lifetime fecundity. However, food resource affected the female fecundity significantly when resource and treatment was compared to the number of eggs laid and individual hatched. Females of *Callosobruchus maculatus* feed on black-eyed beans (BE), *Vigna unguiculata (L.*) may enhance reproductive success since females appear to produce more offspring than those feed on mung beans (M), *Vigna radiat (L.*)*. Even though this experiment did not test the weight and size of eggs as well as individuals, according to six month observation, the offspring from black-eyed beans always had larger body size than those from mung beans. Since the individuals hatched from BE was generally more than M, we can predict that larvaees from BE has stronger survival ability. Since it was a 30 weeks project, I did not test the differences between the two resources.

Effects of mating patterns on female fecundity

Mate selection is a main biological event and it leads to a substantial determinant of an individual’s return on its reproductive investment, which was suggested by Darwin in 1859 (Darwin 1859). It is therefore hardly surprising that considerable interest has been devoted to the study of mate choice and in particular the effects of random and
non-random mating on the fecundity of females. In this study, inbreeding was brother-sister mating, and part of the estimated population were random mating, part were non-random mating. Wilson (1999) pointed that random mating can enhance female fecundity. Within a population, if relatives tend to mate with each other more often than expected under random mating, there will be stronger inbreeding than that attributable solely to population size. Even though I did not directly test which mating pattern impacted the female fecundity, or whether it appeared positive or negative effect, the effect of mating patterns on female fecundity during lifetime was obvious.

As results show in $2 \times 2$ treatment, less eggs and offsprings could be found over generations, which indicates female fecundity is affected strongly. The mating system was non-random in $2 \times 2$ treatment. The parents in the $8 \times 8$ and $20 \times 20$ treatments were partly from random mating, partly from non-random mating. Relatives are more closely related by ancestry than are randomly chosen members of the population. Inbreeding results from non-random mating produced leads to relatives mate more often within a population (Bateson 1983). Thus, the fecundity of females and survival probability of offsprings are not stronger than that with rare inbreeding caused by random mating.

**Effects of population size on female fecundity**

Species that exhibit large variations in population size due to demographic and environmental stochasticity and catastrophes are probably to be particularly sensitive to inbreeding. In contrast, populations that have had quite small effective population sizes in a long period, in other words, those that have recovered from population bottlenecks, should be less sensitive to inbreeding depression because of the purging of deleterious recessive alleles (Brook et al. 2002). The result of this project showed that female fecundity had less decrease in fecundity in larger population sizes, which means inbreeding happened more frequently in smaller population size. Some studies have shown the similar outcomes: in small population models, larger population sizes have more possibility to avoid the effects of inbreeding depression (Heschel and Paige
Furthermore, the relative impact of all stochastic effects on extinction risk decreases with increasing population size (Menges 1992). These theories predict that larger population sizes have stronger fecundity than smaller ones because they are less affected by inbreeding depression. Another evidence to support this hypothesis is that the offsprings from $8 \times 8$ and $20 \times 20$ treatment always started to hatch 1-2 days earlier than those from treatment $2 \times 2$ during the six generations.

**Effects of inbreeding avoidance and assortative mating**

Since there were more intermediates than expected, we can conclude that the population is not in Hardy-Weinberg equilibrium. Furthermore, more intermediates than predicted were observed, which is an indication of assortative mating avoidance. The reason could be inbreeding depression. The results show that percentage of intermediate colours increased with a steady rate over generations, which predict that assortative mating was avoided more from generation 3 to 6. If the conclusion of inbreeding depression results assortative mating avoidance is established, inbreeding depression occurred increasingly over generations is concluded. This conclusion was proved by many studies (Husband and Schemske 1996, Wang et al. 1999, Pemberton 2004). This suggest that inbreeding is a factor that disturb the effects of assortative mating on sympatric speciation. Inbreeding increases the frequency of homozygotes in the population and decreases the frequency of heterozygotes. Genetic factors such as the pattern of expression of deleterious alleles (Lacy 1996), or the effect of past history including purging of deleterious alleles (Lacy and Ballou 1998) is thought to be the possible results of inbreeding depression. The populations that can mate assortatively and at the same time avoid inbreeding are the ones that survive and may evolve into new species. This means the possibility that new species arise from populations in the same area of the same ancestral species. That makes better understanding of whether speciation can occur in the same geographic area, which is sympatric speciation.

Another key factor in sympatric speciation is assortative mating. Assortative
mating mechanisms reduce the break-up of the ancestral population into diverging and reproductively isolated descendent species (Doebeli 2005). It appears when individuals select to mate with individuals that are often close kin of themselves. The risk of inbreeding affects assortative mating cost was indicated in this project. Within different levels of inbreeding, assortative mating could happen on the basis of an ecological trait. In the recent classic models of sympatric speciation, the evolution of assortative mating depends either on an ecological character affecting resource use or on a selectively neutral marker trait. This study is a benificial understanding in research of interaction between ecological patterns and inbreeding as well as assortative mating in both theoretical and laboratorial aspect, in the end, speciation.
Acknowledgements

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Lastly, I would like to dedicate this thesis to my parents Hongqi Yuan and Aixue Yin. Without their support and love I couldn’t stand and live in Sweden to finish my master degree.
Table 1. Univariate Tests of Significance for No. of Eggs (categorical predictors: generation, resource and treatment).

<table>
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<th>Effect</th>
<th>SS</th>
<th>Degree of Freedom</th>
<th>MS</th>
<th>F</th>
<th>p</th>
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Table 2. Univariate Tests of Significance for No. of Eggs (categorical predictors: generation and treatment).

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Table 3. Univariate Tests of Significance for No. of Individuals (categorical predictors: generation, resource and treatment).

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<td>2</td>
<td>1055.6</td>
<td>19.679</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Generation*Resource</td>
<td>200.6</td>
<td>4</td>
<td>50.2</td>
<td>0.935</td>
<td>0.443</td>
</tr>
<tr>
<td>Generation*Treatment</td>
<td>117.6</td>
<td>8</td>
<td>14.7</td>
<td>0.274</td>
<td>0.974</td>
</tr>
<tr>
<td>Resource*Treatment</td>
<td>285.0</td>
<td>2</td>
<td>142.5</td>
<td>2.657</td>
<td>0.071</td>
</tr>
<tr>
<td>Generation<em>Resource</em>Treatment</td>
<td>563.6</td>
<td>8</td>
<td>70.4</td>
<td>1.313</td>
<td>0.233</td>
</tr>
<tr>
<td>Error</td>
<td>51978.1</td>
<td>969</td>
<td>53.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Univariate Tests of Significance for No. of Individuals (categorical predictors: resource and treatment).

<table>
<thead>
<tr>
<th>Effect</th>
<th>SS</th>
<th>Degree of Freedom</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>87.13</td>
<td>1</td>
<td>87.13</td>
<td>3.302</td>
<td>0.070</td>
</tr>
<tr>
<td>No. of Eggs</td>
<td>92185.37</td>
<td>1</td>
<td>92185.37</td>
<td>3494.273</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Resource</td>
<td>663.27</td>
<td>1</td>
<td>663.27</td>
<td>25.141</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment</td>
<td>559.97</td>
<td>3</td>
<td>186.66</td>
<td>7.075</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Resource*Treatment</td>
<td>269.52</td>
<td>3</td>
<td>89.84</td>
<td>3.405</td>
<td>0.018</td>
</tr>
<tr>
<td>Error</td>
<td>6094.21</td>
<td>231</td>
<td>26.38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Multiple Comparisons p values (2-tailed) of percentage of intermediate colours.

Independent (grouping) variable: generation. Kruskal-Wallis test: H (3, N = 109) = 18.100, p = 0.0004.

<table>
<thead>
<tr>
<th>Generation 3 - R:36.321 4 - R:52.056 5 - R:61.375 6 - R:71.308</th>
<th>3</th>
<th></th>
<th></th>
<th>&lt;0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>0.390</td>
<td>0.018</td>
<td>0.160</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.018</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>&lt;0.001</td>
<td>0.160</td>
<td>1.000</td>
</tr>
</tbody>
</table>
Table 6. Multiple Comparisons p values (2-tailed) of percentage of intermediate colours.


<table>
<thead>
<tr>
<th>Generation</th>
<th>2*2 - R:51.414</th>
<th>20*20 - R:51.512</th>
<th>8*8 - R:61.333</th>
</tr>
</thead>
<tbody>
<tr>
<td>2*2</td>
<td>-</td>
<td>1.000</td>
<td>0.602</td>
</tr>
<tr>
<td>20*20</td>
<td>1.000</td>
<td>-</td>
<td>0.494</td>
</tr>
<tr>
<td>8*8</td>
<td>0.602</td>
<td>0.494</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 7. Number of $\chi^2$ values ($\chi^2 \geq 3.84$) from generation 3 to 6 within treatment $2 \times 2$, $8 \times 8$ and $20 \times 20$.

<table>
<thead>
<tr>
<th>Generation</th>
<th>Treatment</th>
<th>No. of $\chi^2$ values ($\chi^2 \geq 3.84$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2*2</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>8*8</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>20*20</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>2*2</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>8*8</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>20*20</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>2*2</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>8*8</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>20*20</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>2*2</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>8*8</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>20*20</td>
<td>10</td>
</tr>
</tbody>
</table>
Fig. 1 Analysis of variance of generation, resource and treatment. The Y axis represents lifetime fecundities (total number of eggs laid) of *C. maculatus* females. The categorical predictors are generation (from 2 to 6, generation 1 is not inbred), resource (M: mung beans, BE: black-eyed beans) and treatment (2×2, 8×8 and 20×20). Vertical bars denote 0.95 confidence intervals.
Fig. 2 Analysis of variance of resource and treatment. The Y axis represents total number of eggs laid of *C. maculates* females from generation 6 and treatment control. The categorical predictors are resource (M: mung beans, BE: black-eyed beans) and treatment (2×2, 8×8, 20×20 and control). Vertical bars denote 0.95 confidence intervals.
Fig. 3 Analysis of covariance generation and treatment. The Y axis represents lifetime fecundities (total number of individuals hatched) of *C. maculates* females. The categorical predictors are generation (from 2 to 6, generation 1 is not inbred) and treatment (2×2, 8×8 and 20×20). Vertical bars denote 0.95 confidence intervals.
Fig. 4 Analysis of covariance at their means of resource and treatment. The Y axis represents lifetime fecundities (total number of individuals hatched) of *C. maculatus* females of generation 6 and treatment control. The categorical predictors resource (M: mung beans, BE: black-eyed beans) and treatment (2×2, 8×8, 20×20 and control).
Fig. 5 Non-parametric analysis of variance (Kruskal–Wallis test). The Y axis represents percentage of intermediate colours of *C. maculates* females. The X axis represents independent grouping variable generation (from 3 to 6, generation 1 and 2 are not inbred).
Fig. 6 Non-parametric analysis of variance (Kruskal–Wallis test). The Y axis represents percentage of intermediate colours of *C. maculates* females. The X axis represents independent grouping variable treatment (2 × 2, 8 × 8, 20 × 20).
References


Fox, C. W. 1993. The Influence of Maternal Age and Mating Frequency on Egg Size and Offspring Performance in Callosobruchus-Maculatus (Coleoptera,
Bruchidae). Oecologia 96:139-146.


