Genome size and phenotypic plasticity in the seed beetle, *Callosobruchus maculatus*

Jesper Boman

Degree project in biology, Bachelor of science, 2017
Examensarbete i biologi 15 hp till kandidatexamen, 2017
Biology Education Centre and the Animal Ecology Program at the Department of Ecology and Genetics, Uppsala University
Supervisors: Göran Arnqvist and Johanna Liljestrand Rönn
Abstract

It has long been evident that genome size is not an accurate measure of organismal complexity. This paradox was “solved” with the discovery of nonfunctional and selfish DNA in the 1970s. However, emerging from this explanation was an enigma of complexity. Neither neutral nor adaptive models can account for all genome size variation across the tree of life. An organism with intraspecific variation is needed to investigate the functional role of genome size differences. Here I use different populations of the seed beetle, Callosobruchus maculatus, with a known intraspecific genome size variation of ~4%. It has previously been shown that a larger genome is associated with higher scores in fitness-related traits for this species. In this study, genome size is regressed with phenotypic plasticity along three different environmental gradients. Genome size did not correlate with plasticity in mass and development time along environmental gradients of temperature and host types. However, the results show that larger genomes are consistent with higher canalization of fitness under different food regimes. This further supports the idea that natural selection acts on genome size variation in this species.
# Table of contents

1. Introduction ........................................................................................................... 4.

   2.1 Populations ........................................................................................................ 5.
   2.2 Fitness assay .................................................................................................... 5.
   2.3 Larval development ......................................................................................... 6.
   2.4 Statistical analysis .......................................................................................... 6.

3. Results .................................................................................................................... 7.
   3.1 Fitness assay .................................................................................................... 7.
   3.2 Larval development: effects of temperature .................................................. 9.
   3.3 Larval development: effects of host type ....................................................... 12.
   3.4 Trends in plasticity among populations ......................................................... 14.

4. Discussion .............................................................................................................. 15.

5. Acknowledgements .............................................................................................. 17.

6. References ............................................................................................................ 17.

1. Introduction

The realization that genome size was inconclusive in explaining organismal complexity was summarized by Thomas (1971) as ‘The C-value paradox’, with C-value being the haploid DNA content of an organism. This suggested that the genome contained more than the blueprint needed for the function and structure of an organism. Shortly after its origination the paradox was solved with the discovery of noncoding DNA. Ever since, the nature of noncoding DNA has been a major focus of genome biology and the original question has been rewritten as ‘The C-value enigma’, suggesting the possibility of wide set of answers (Gregory 2001, 2005a). A plethora of hypotheses pertaining to explain the C-value enigma has been formulated. They can roughly be summarized by the example of two classical theories: ‘The Selfish DNA hypothesis’ emphasize the role of transposable elements (TE) that may self-proliferate until the arising genomic obesity is stalled by selection, due to snowballing costs of replication (Doolittle & Sapienza 1980, Orgel & Crick 1980). 'The Bulk DNA hypothesis' emphasizes the spatiotemporal properties of the genome. If for example the size of a cell is the primary target of selection then a greater genome may be needed to expand the nuclear membrane to supply a larger cell with transcripts (Cavalier-Smith 1978). The theories are not mutually exclusive in that the former is the mechanism of the latter. They differ however in their answer to what is causing genome size evolution: selection for bulk or neutral proliferation of TEs. Ultimately the relative rate of insertions and deletions is what determines the size of the neutral and thus nonfunctional part of the genome (Lynch 2007). Furthermore if genomic obesity becomes a target of selection then it is the effective population size and the magnitude of the negative effect that determines the fate of a certain genome.

It has been suggested that a substantial part of many genomes is entirely nonfunctional and represents ‘junk’ DNA (Ohno 1972). However, the ENCODE project assigned a biochemical “function” to 80 % of the human genome (The ENCODE Project Consortium 2012). This was met with a hard resistance from population geneticists and genome biologists alike and resulted in a discussion on the biological definition of function (Graur et al. 2013, Palazzo & Gregory 2014). Irrespective of our definition of functionality, genome size does correlate with physiological and morphological factors (Lynch 2007) (Hessen et al. 2013). This central tendency is most apparent across wide evolutionary scales. For example cell volume scale with haploid genome size in prokaryotes and unicellular eukaryotes (Lynch 2007). Furthermore the picture of the neutralism of TEs has been altered and the adaptive significance of gene duplication has been highlighted (Kidwell & Lisch 2001, Gregory 2005b). With progress in the field of intraspecific genome size it has become apparent that genome size produces conspecific differences in phenotype, enabling natural selection to act on genome size (Ellis et al. 2014, Arnqvist et al. 2015). Variation of functional DNA most likely amount to differences in gene duplication number and/or presence of non-coding DNA with a regulatory role, but could also include some undiscovered genomic aspect. Furthermore Ellis et al. (2014) showed that plasticity was affected by genome size in inbred Drosophila lines. This study aims to assess this phenomenon in populations of cowpea seed beetle, Callosobruchus maculatus, by investigating the role of genome size for variation in phenotypic plasticity.
Arnqvist et al. (2015) measured the size of the genomes for both sexes of 18 different populations of *C. maculatus*. They found approximately 4% variation in genome size among populations and significant correlations between genome size and important traits, such as female fecundity and male competitive fertilization success.

*C. maculatus* (Coleoptera, Bruchidae) is a West African species that have spread across the world’s arid, semi-arid, and tropical regions by trade in beans from legumes (Southgate 1978, 1979). Easy maintenance and intraspecific genome size variation among populations make *C. maculatus* a promising model for genome biology research. Here I continue the foundation lain by Arnqvist et al. (2015) by expanding from trait phenotypes to trait plasticity. Using the same populations as the previous study, three experiments are performed: (i) fitness assay in which beetles are paired and offspring production is measured under different adult food regimes, (ii) keeping larvae in different temperatures and (iii) on three different host beans. In the latter two assays, body mass and development time are the measured traits. The overarching question addressed is: is genome size associated with phenotypic plasticity across populations?

2. Material & methods

2.1 Populations

In this study 18 populations of *Callosobruchus maculatus* with known genome sizes were used (Arnqvist et al. 2015). As adults they are facultatively aphagous meaning that they don’t need water or food to reproduce (Fox 1993). The beetles have been collected from natural populations during different occasions between 1975-2010. The genome size variation is not related to time since collection (Arnqvist et al. 2015). The beetles have been reared aphagously in laboratory climate cabinets at 29°C and 60-75% with a 12:12 hour dark and light cycle.

A couple of hundred individuals from each population were transferred to 2 L glass containers with open lids covered by filter paper and 2 dL of their main natural host: black-eyed peas, *Vigna unguiculata*. After 15 days beans with eggs were transferred to virgin chambers. The individuals hatching from these beans were later used for all experiments in the current study. All beans used in this study were sterilized through freezing for at least 48h in -18°C.

2.2 Fitness assay

Virgin males and females were collected from the prepared populations. They were placed in 60 mm diameter petri dishes filled with 55 *V. unguiculata* beans (~12-12.5 grams). Three different treatment groups were used: “NF” (no food or water), “W” (only water) and “F” (food and water). The water treatment consisted of an Eppendorf tube filled with water plugged with cotton. For the food treatment, the Eppendorf was filled with 20% sucrose solved in water. The F treatment group were also served ~4 pieces of *Manuka health* bee pollen per petridish. The virgin beetles were paired and placed in the cabinets at 29°C. After 12 days the adults were removed.
and the dishes were kept for another 26 days at 29°C. Subsequently the dishes were frozen for a couple days and then offspring number (as an estimate of fitness) was counted. Offspring were counted in \( N=5\) to 10 dishes per population per treatment.

2.3 Larval development

Two environment gradients were used to test larval development: temperature and bean preference.

Recently emerged beetles from each population were collected and sorted by sex. For the temperature treatment 30 males and 30 females from each population were placed in glass containers filled with 200 black eyed peas at 29°C for 4 hours. The adults were carefully removed and 96 of the beans were placed in virgin chambers (1:1:2, for treatment temperatures: 22°C, 29°C, 35°C). The virgin chambers were stored at their treatment temperatures awaiting emergence of adult beetles.

The beans used for the larval development host preference experiment are the previously mentioned black-eyed pea *Vigna unguiculata*, chickpea, *Cicer arietinum* and adzuki, *Vigna angularis*. The same method was employed for bean type as for temperature with the exception that only 15 males and 15 females were collected per population for chickpea and adzuki, and 80 beans was used instead of 200. This resulted in one virgin chamber (24 beans with a few eggs on each) of adzuki beans per population and 1-2 for chickpeas. These virgin chambers were stored at 29°C. The larval development data from 29°C black-eyed pea was used both for the temperature- and bean gradient.

Emerged F1 beetles were sorted after sex and weighed at one or two occasions per day, depending on the rate of emergence. The body mass were measured using a Sartorius Genius scale. All emerged beetles of the same sex per observation were weighed simultaneously to increase measurement precision and alleviate workload. The number of beetles constituting one measurement of weight was also noted. The development time was calculated as the sum of the time from when the end of the 4 hour window when eggs could be lain up until the last observation before emergence plus half of the time until the time of emergence.

2.4 Statistical analysis

The aim of the study was to explore if there is a connection between genome size and phenotypic plasticity in seed beetles. Consequently, the first step in the statistical analysis was to test the hypothesis of significant differences in reaction norms among populations. A two-way ANOVA was employed for all assays, and the focal term here was the population x environment interaction. This and all analyses for this project were performed using the statistical software R v. 3.3.2 (R Core Team 2016). One-way ANOVAs were then used to retrieve an estimate, the \( F \)-statistic of the magnitude of phenotypic plasticity for each population. Put simply, the \( F \)-statistic compares the variance in offspring number within each environment (NF, W, F, see above) to the variance between different environments. These estimates, and their counterparts from the larval development data, were then used to correlate the degree of phenotypic plasticity with genome size.
The larval development data consisted of two continuous variables: body mass and development time. ANCOVA was used to analyse the differences in reaction norms in two separate models, one for each treatment (host type and temperature). All models for larval development data were weighted by the amount of beetles constituting one measurement of average body mass. Three different F-statistics were produced for each population and treatment: (i) with mass as response variable and development time as covariate, in other words: the growth rate, (ii) with mass as response variable and no covariate and (iii) development time as response variable and no covariate. The latter two were used to detect plasticity masked by robustness in the other variable.

3. Results

3.1 Fitness assay

The pronounced difference in reaction norms among the 18 populations used in this study in all of three experiments illustrates the large genetic variation across C. maculatus. For the fitness assay, a conservative model for the two-way ANOVA is significant (p << 0.001) for the interaction population x environment, i.e. the reaction norm. Several trends were observed where some populations appeared less plastic with flatter reaction norms, for example Brazil Leicester. In other words their response to the different environments was more robust (Figure 1). A group of populations did not seem to react much to the presence of water but had a significant fitness increase in the food environment. This trend was most apparent in Zaire. Others like Oyo had a more linear relationship along the environmental gradient and Brazil London had a low offspring count overall.

When correlating the F-statistic for each population with genome size, a significant negative trend is found ($r^2 \approx 0.41 \ p < 0.01$). Larger genome size is consistent with more robust phenotypes (Figure 2.). To investigate the composition of the reaction norms, the $F$-statistic for each population was correlated with the mean offspring count in total and in each environment (Figure 3.). The only significant correlation was that a larger offspring count in the food environment was consistent with higher value of $F$ ($r^2 \approx 0.37 \ p < 0.01$). This indicated a trade-off between fitness in “NF” and “F” but that correlation was insignificant ($p > 0.78$). Furthermore genome size does not correlate significantly with total mean fitness ($p > 0.16$), which means that the genome size is correlating not with the trait itself but its plasticity under different food regimes.
Figure 1. Fitness assay showing that the populations vary significantly in their reaction norms across the environmental gradient \( p \ll 0.001 \). The figure shows the mean offspring count (fitness) in the three environments “NF” (no food or water), “W” (only water) and “F” (sugar water and bee pollen). The general trend is an environmental gradient from “NF” to “F” but the deviations from this trend is apparent in this graph illustrating the mean fitness across the environments.

Figure 2. Significant negative correlation between genome size and phenotypic plasticity as measured by the \( F \)-statistic in the fitness assay \( r^2 \approx 0.41 \ p < 0.01 \).
Figure 3. Scatter plots showing the relationships between the $F$-statistics of each population in total and in each environment with respect to their mean fitness. A positive correlation is found in the food environment ($r^2 \approx 0.37$ $p < 0.01$).

3.2 Larval development: effects of temperature

The development time data from the temperature gradient revealed three distinct clusters along the development time axis (Figure 4). A higher temperature resulted in a shorter development time and lower body mass. The sexes were clearly separated with females weighing more than males, though that distinction is somewhat less clear at 22°C (Supplementary figure 1). The populations had a uniform environmental response in development time but not body mass (Figure 5 & 6). This indicates that body mass is a trait with more genetic variation in plasticity than development time across a temperature gradient for *C. maculatus*. Variation among populations in plasticity for both traits combined (growth rate) was confirmed using an ANCOVA approach ($p \ll 0.001$) (Figure 7). There was no significant correlation with genome size for neither growth rate (mass as response variable, development time as covariate: $p \approx 0.75$) nor the decomposed parts: body mass ($p \approx 0.12$) or development time ($p \approx 0.64$).
**Figure 4.** Effect of temperature on development time for the larva and body mass at emergence. Higher temperature during development is consistent with shorter development time and lower body mass.

**Figure 5:** Temperature is an excellent predictor of mean development time for *C. maculatus*. The variation in plasticity between populations in development time along the temperature gradient is minor.
Figure 6: In contrast to development time, there is much variation in reaction norms in mean body mass between populations. However, this variation cannot be attributed to differences in genome size ($p \approx 0.12$). Temperature does not uniformly predict body mass.

Figure 7: The populations follow two general patterns in growth rate for the temperature treatments: i) no difference in mass over time/temperature or ii) increased mass at lower temperature/longer development time, especially at 22°C. The variation among populations is significant ($p << 0.001$).
3.2 Larval development: effects of host type

The development time data from the larval development host type experiment constitute more of a gradient (Figure 8) compared with the triclustered data of the temperature experiment (Figure 4). In general the development time of the beetles is greatly dependent on host type (Figure 9). A majority of populations have their shortest mean development time on black-eyed peas and longest on adzuki beans but some like the Mali beetles emerged later on chickpeas and in very few numbers. The reaction norms for mean body mass show a great variation in plasticity among the populations (Figure 10). A model with body mass as response and time as covariate confirms variation in plasticity ($p < 0.001$) (Figure 11). There is no significant correlation between plasticity and genome size for growth rate (mass as response variable, development time as covariate: $p \approx 0.7$), body mass ($p \approx 0.85$) or development time ($p \approx 0.35$).

![Graph showing development time vs mass for different host types](image)

**Figure 8:** Effect of host type on development time for the larva and body mass at emergence. The different environments form a gradient in development time, from black-eyed pea (*V. unguiculata*) to chickpea (*C. arietinum*) and adzuki (*V. angularis*). There is also trend in low body mass of beetles emerging late on chickpea and adzuki, most of them male (Supplementary figure 2).
**Figure 9:** There are plastic variation in development time among populations on different beans and most populations follow a uniform pattern with longest mean development time (y-axis) on adzuki and shortest on black-eyed peas. A notable exception is Mali in which only three individuals emerged on the chickpeas and considerably later than on adzuki.

**Figure 10:** The populations vary considerably in reaction norm with respect to mean body mass on different beans.
Figure 11: There is a significant variation in growth rate among populations (p<< 0.001). Noteworthy in this figure is the deviating positive Oman trend line, which did not translate into a relative high $F$-statistic in growth rate (standardized to -0.41, Supplementary table 1). It can be compared with e.g. the relative flat Benin trend line with a much greater $F$-statistic (standardized to 3.24).

3.3 Trends in plasticity among populations

Though some populations seem to be inherently more plastic (Supplementary table 1), there are no overall significant trends when fitting regression models between plasticity in the fitness assay and the larval development experiments (Table 1). If anything, populations with more canalized fitness tended to show more plasticity for other types of traits.

Table 1. Linear regressions of $F$-statistics between the fitness and the body or development time in the larval development (=LD) experiments. $\beta$ is the coefficient of the slope of the fitted regression line.

<table>
<thead>
<tr>
<th>$F$-statistic regressions</th>
<th>P-values</th>
<th>$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD temperature growth rate vs. Fitness Assay</td>
<td>$p &gt; 0.99$</td>
<td>0.0006</td>
</tr>
<tr>
<td>LD temperature body mass vs. Fitness Assay</td>
<td>$p \approx 0.62$</td>
<td>-0.09</td>
</tr>
<tr>
<td>LD temperature development time vs. Fitness Assay</td>
<td>$p \approx 0.22$</td>
<td>0.002</td>
</tr>
<tr>
<td>LD host type growth rate vs. Fitness Assay</td>
<td>$p &gt; 0.16$</td>
<td>-0.34</td>
</tr>
<tr>
<td>LD host type body mass vs. Fitness Assay</td>
<td>$p &gt; 0.68$</td>
<td>-0.10</td>
</tr>
<tr>
<td>LD host type development time vs. Fitness Assay</td>
<td>$p \approx 0.07$</td>
<td>-0.13</td>
</tr>
</tbody>
</table>
4. Discussion

The seed beetle populations in this study showed not only a remarkable variation in phenotypic plasticity, they also exhibited variation in the degree of plasticity of different traits (Table 1 & Supplementary table 1). The main hypothesis tested in this contribution was that larger genomes allow individual beetles to modulate their expression of functional traits to prevail under different environmental conditions. This means that larger genomes should be associated with a higher degree of phenotypic plasticity. As a result of this underlying flexibility, we predicted that large genomes should be associated with more canalized fitness (Waddington 1942, Flatt 2005). While I failed to find direct support for the former prediction, I did found support for the latter. This study shows a negative correlation between genome size and plasticity in the amount of offspring due to resource availability (Figure 2). Plasticity in fitness did correlate with higher offspring count in the food environment, which indicates that the more plastic populations were the ones able to utilize this resource (Figure 3D). There are indications of a trade-off between the sparse “NF” environment and the resource rich “F” environment when comparing some populations, but not all. These findings might suggest that a larger genome simply is a fitness disadvantage in this experimental setting with monogamous pairs as opposed to the competitive fertilization success tested previously (Arnqvist et al. 2015). That would, however, not explain the non-significant correlation between mean total fitness and genome size ($p > 0.16$). For the fitness assay there are two reaction norm trends on each end of a continuum: (i) either canalized fitness consistent with a larger genome or (ii) smaller genome that somehow allow for a more plastic fitness response to food availability but perhaps at the cost of fitness in aphagous conditions. Using a more sophisticated statistical approach such as polynomial fitting of the reaction norm could elucidate some the intricacies of these results (e.g. DeWitt & Scheiner 2004). It is also reasonable to believe that male and female body mass is correlated with offspring count and controlling for weight in the seed beetle pairs could give a better measure of relative fitness (Bilde et al. 2008). Due to time limitations this was not done in this study.

The main conclusion to be drawn from the larval development experiments is that genome size is not a main contributor to plasticity in body mass or development time variation in *C. maculatus*. For severalfold variations in genome size there seems to exist a relationship with body mass for some arthropods such as fruit flies and ostracods (Gregory & Johnston 2008, Jeffery et al. 2017). Arnqvist et al (2015) failed to find such a link among a sample of bruchines and the correlation was weak at best for Gregory & Johnston’s (2008) dataset, which included a 2.5x variation in genome size among *Drosophilidae* species. For these population 0.04x variation in genome size of these *C. maculatus* populations, it is perhaps not surprising that genome size did not correlate with body mass. However, the lack of correlation between genome size and a certain trait does not exclude the possibility of genome size related effects acting on plasticity in that trait. The best example of this is the results from the fitness assay of this study.

If genome size would affect body mass and development time, then it most likely would have inflated the cell volume and/or cell division rate of the populations with the larger genomes (Gregory 2001). This seems to be the case in for example vertebrate erythrocytes across taxa (Olmo 1983). This however may not be the case
for insects at the intraspecific level (Tsutsui et al. 2008, Arnqvist et al. 2015). Larger cell size has been documented as a plastic response to low temperature in Drosophila (French et al. 1998). However the evolutionary adaptation to cold climates in this clade is most likely an increase in cell number (Partridge et al. 1994). If C. maculatus follows the same pattern then a significant positive correlation with GS and plasticity in mass and/or development time for the larval development temperature experiment would be predicted. If the plastic body mass increase in low temperature is mainly in cell number for C. maculatus then a slower cell division and therefore perhaps a longer development time would have been consistent with larger genome size. The data however showed no indications of genome size correlations with plasticity for the temperature experiment. Two possible explanations are: (i) genome size does not scale with cell size, cell division rate or other components affecting development time and/or body mass. (ii) The intraspecific variation in genome size is too minute to give a pattern through the noise of variation attributable to other factors. One such factor could be increased ribosome copy number as a response to reduced enzymatic efficiency in low temperature (Woods et al. 2003). This could lead to larger body mass, through an increase in cytoplasm to regulate ribosomal concentration and thus an increase in cell size (Hessen et al. 2013).

The host type experiment showed considerable among-population variation in plasticity in body mass and development time none of which could be significantly attributed to genome size (Figure 9-11). We predicted that populations with larger genomes would be more plastic in their response to legume toxins (e.g. Gupta 1987). Translating this prediction into reaction norms was not straightforward since longer development time could have meant a canalized reaction or a bigger body mass as in the temperature experiment. The trend was opposite in the host type experiment in part due to very small late-emerging beetles on adzuki beans (Figure 8.) The genetic basis of host type adaptation may reside in regions unrelated to genome size variation in C. maculatus or the environmental gradient might be too narrow to elucidate a genome size effect. A future study could use a wider range of beans or different strains of chickpea to increase the environmental gradient (e.g. Erler et al. 2009, Savković et al. 2016).

There are no indications that the intraspecific variation in genome size of C. maculatus is due to supernumerary chromosomes or polyploidy (Angus et al. 2011). The size difference among population may reside mainly in heterochromatic regions as Arnqvist et al (2015) recognized it did among a sample of bruchine species. However, some kind of molecular effect must invariably be present to explain the genome size correlations with different traits (as shown previously) and plasticity in fitness. A bulk effect via cell volume is unlikely considering that genome size does not affect neither body mass itself nor its plasticity in C. maculatus. The linear relationships of genome size with traits and their plasticity is consistent with a dosage effect, which in a future experiment could be measured using transcriptomic data (Mathers et al. 2017). This can be done by identifying differences among populations in the expression of plasticity related genes and investigate if these are related to the still unknown functional content of genome size variation in C. maculatus. Having numerous duplicated copies of such a gene could help to moderate the environmental stimulus and produce a more robust fitness response (Proulx & Phillips 2005). Furthermore, the variation could also reflect differences in the amount of noncoding DNA elements such as DNA binding sites and/or ncRNAs or transcription factors
Both of these mechanisms could, either separately or in tandem, explain the phenomenon observed here that larger genomes are consistent with more canalized fitness.

5. Acknowledgments
I would sincerely like to thank Göran Arnqvist for giving me the opportunity to explore this fascinating topic and for inviting me wholeheartedly to the wonderful Arnqvist lab group. Without your enthusiasm, advice and belief in me this undertaking would have been impossible. A very warm thanks to Johanna Liljestrand Rönn who taught me everything I needed to know for the lab work (and much more) and also assisted me during many hours when the beetles was emerging en masse. Again, this project would not have been what it is without your excellent advice and encouragements. Thank you Karl Grieshop for helping me navigate in the jungle of variance. Furthermore thank you Ivain Martinossi, David Berger, Elina Immonen, Zorana Kurbalija Novičić, Helen Bayram and Ahmed Sayadi for your excellent and warm collegiality. And last but not least, much thanks to Rafael Augusto and Emma Thilliez for great company during long days and weekends in the lab.

6. References


size Variation in D. melanogaster Reflects Life History Variation and Plasticity. PLOS Genetics 10: e1004522.


## Appendix

**Supplementary table 1:** Standardized values for plasticity ($F$-statistic) and genome size. LD = larval development.

<table>
<thead>
<tr>
<th>Population</th>
<th>Genome size</th>
<th>Fitness Assay</th>
<th>LD TEMP (Growth rate)</th>
<th>LD TEMP (Mass)</th>
<th>LD TEMP (Time)</th>
<th>LD HOST (Growth rate)</th>
<th>LD HOST (Mass)</th>
<th>LD HOST (Time)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benin</td>
<td>0.44</td>
<td>-0.78</td>
<td>-0.83</td>
<td>0.92</td>
<td>0.17</td>
<td>3.24</td>
<td>3.18</td>
<td>1.62</td>
</tr>
<tr>
<td>Brazil Leicester</td>
<td>1.55</td>
<td>-1.17</td>
<td>-0.69</td>
<td>0.57</td>
<td>-0.60</td>
<td>0.77</td>
<td>-0.53</td>
<td>-0.64</td>
</tr>
<tr>
<td>Brazil London</td>
<td>1.04</td>
<td>-1.02</td>
<td>-0.84</td>
<td>-1.36</td>
<td>-1.08</td>
<td>-0.60</td>
<td>-0.52</td>
<td>0.68</td>
</tr>
<tr>
<td>Brazil USA</td>
<td>1.02</td>
<td>-0.45</td>
<td>0.97</td>
<td>-0.63</td>
<td>0.06</td>
<td>-0.55</td>
<td>-0.02</td>
<td>1.82</td>
</tr>
<tr>
<td>California</td>
<td>-1.08</td>
<td>1.14</td>
<td>-0.74</td>
<td>-1.36</td>
<td>2.03</td>
<td>-0.64</td>
<td>-0.54</td>
<td>-0.60</td>
</tr>
<tr>
<td>IITA</td>
<td>-0.45</td>
<td>-0.86</td>
<td>-0.27</td>
<td>-0.52</td>
<td>-0.92</td>
<td>-0.63</td>
<td>-0.34</td>
<td>1.23</td>
</tr>
<tr>
<td>Lome</td>
<td>0.00</td>
<td>-0.57</td>
<td>-0.60</td>
<td>-1.31</td>
<td>-0.92</td>
<td>-0.07</td>
<td>0.30</td>
<td>-0.56</td>
</tr>
<tr>
<td>Lossa</td>
<td>0.42</td>
<td>0.11</td>
<td>0.00</td>
<td>0.90</td>
<td>1.33</td>
<td>-0.58</td>
<td>-0.40</td>
<td>-0.73</td>
</tr>
<tr>
<td>Mali</td>
<td>0.03</td>
<td>-0.15</td>
<td>-0.11</td>
<td>0.35</td>
<td>0.85</td>
<td>-0.50</td>
<td>-0.12</td>
<td>1.72</td>
</tr>
<tr>
<td>Nigeria Mix</td>
<td>-0.40</td>
<td>1.31</td>
<td>0.21</td>
<td>1.30</td>
<td>-0.42</td>
<td>-0.65</td>
<td>-0.42</td>
<td>-1.45</td>
</tr>
<tr>
<td>Ofuya</td>
<td>-0.65</td>
<td>-0.56</td>
<td>3.05</td>
<td>-0.10</td>
<td>-1.39</td>
<td>1.17</td>
<td>-0.66</td>
<td>-0.93</td>
</tr>
<tr>
<td>Oman</td>
<td>-2.42</td>
<td>2.31</td>
<td>-0.42</td>
<td>-0.88</td>
<td>0.65</td>
<td>-0.41</td>
<td>-0.55</td>
<td>-0.23</td>
</tr>
<tr>
<td>Oyo</td>
<td>0.83</td>
<td>-0.74</td>
<td>-0.49</td>
<td>1.21</td>
<td>1.91</td>
<td>-0.68</td>
<td>-0.70</td>
<td>0.34</td>
</tr>
<tr>
<td>South India</td>
<td>-1.50</td>
<td>0.45</td>
<td>-0.58</td>
<td>-0.20</td>
<td>-0.54</td>
<td>-0.32</td>
<td>-0.14</td>
<td>-0.44</td>
</tr>
<tr>
<td>Uganda</td>
<td>0.21</td>
<td>-0.24</td>
<td>-0.85</td>
<td>0.83</td>
<td>0.04</td>
<td>0.10</td>
<td>0.02</td>
<td>-0.49</td>
</tr>
<tr>
<td>Upper Volta</td>
<td>0.64</td>
<td>-0.71</td>
<td>0.50</td>
<td>1.31</td>
<td>-0.72</td>
<td>0.19</td>
<td>-0.57</td>
<td>-0.13</td>
</tr>
<tr>
<td>Yemen</td>
<td>0.82</td>
<td>1.61</td>
<td>0.24</td>
<td>0.47</td>
<td>-0.43</td>
<td>-0.72</td>
<td>0.04</td>
<td>-0.94</td>
</tr>
<tr>
<td>Zaire</td>
<td>-0.50</td>
<td>0.32</td>
<td>1.44</td>
<td>-1.49</td>
<td>-0.04</td>
<td>0.88</td>
<td>1.98</td>
<td>-0.26</td>
</tr>
</tbody>
</table>
Supplementary figure 1: The females (red) are consistently heavier than males (blue) in the larval development temperature experiment.

Supplementary figure 2: Host type does not diminish the sexual size dimorphism in *C. maculatus.*