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April 12, 2022

Effect of Maternal Bean Host and Carry-Over Effect on  
Life-History Traits of Offspring after Diet Switch in *Callosobruchus maculatus*

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## Abstract

### Effect of Maternal Bean Host and Carry-Over Effect on Life-History Traits of Offspring after Diet Switch in *Callosobruchus maculatus*

By Tianyi Xu

The maternal rearing environment can not only directly influence mothers' fecundity and fitness but can also indirectly affect phenotypes, fitness, and behaviors of the offspring generation. Herbivorous insects can potentially respond to environmental cues and produce offspring that are acclimated to specific host conditions with improved overall performance. In addition, offspring larval rearing environment can also influence adult life-history traits, a type of offspring carry-over effect. In this study, we are interested in examining the transgenerational maternal effects and within-generation carry-over effects on offspring's life-history traits by rearing *Callosobruchus maculatus* offspring on three different bean hosts that were either the same or different from maternal hosts. We hypothesized that if maternal effects were significant, offspring with the highest quality maternal bean host would have the best performance, whereas if larval carry-over effects had a more significant impact, offspring would have the best performance when reared on the highest quality bean host regardless of the maternal bean host. Our results suggested that both maternal host and offspring host significantly influenced offspring performance, in which the type of maternal host via maternal effects had some influence over offspring body size and body mass, but not survival, fecundity, or egg size, and the type of offspring host significantly impacted all measured offspring life-history traits possibly due to carry-over effects. However, the presumed highest quality maternal and offspring host did not necessarily result in the highest offspring fitness, and the complex interaction effects require further interpretation and investigation.

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## Introduction

Maternal effects can influence offspring through a variety of ways. In many cases, the maternal rearing environment can not only directly influence mothers' fecundity and fitness but can also indirectly affect phenotypes, fitness, and behaviors of offspring. Factors of abiotic elements, resource abundance and quality, predators and preys in the maternal rearing environment can all influence offspring life-history traits (Slater *et al.*, 2019). In addition, environmental conditions under which organisms are reared can lead to preferences for a particular habitat or host type for development. This natal habitat preference induction (Davis and Stamps, 2004) can give rise to potentially increased fitness and better adaptation to maternal hosts in offspring (Mousseau and Dingle, 1998). Such maternal effects could potentially lead to speciation and other evolutionary consequences (Slater *et al.*, 2019).

Maternal nutrition also has cascading effects on offspring reproductive function and other life-history traits in many organisms (Dupont *et al.*, 2012). These non-genetic components of maternal effects can have significant influences on offspring phenotypes, as females can have acclimated behavioral changes and provide resources to larval progeny (Fox *et al.*, 1995). Some examples of adjustments include parental care, oviposition, and changes in reproductive behavior in response to environmental cues to predict future environmental conditions (Mousseau and Fox, 1998). For example, females of *Pieris rapae* (cabbage butterfly) have been shown to be able to alter egg sizes according to protein levels in diets (Rotem and Agrawal, 2003). In addition, offspring had better adaptation to environments with extreme protein levels if their mothers were reared in these conditions, which could possibly indicate the existence of adaptative maternal effects.



Studies have shown that herbivorous insects responding to environmental cues could potentially produce offspring that are acclimated to specific types of host condition with improved overall performance (Fox *et al.*, 1995; Spitzer, 2004; McLean *et al.*, 2009; Cahenzli and Erhardt, 2013). For instance, these insects may respond to the physical, nutritional, and defense qualities of plant hosts to adjust their behaviors and result in distinctive offspring phenotypes. Previous research showed variation in offspring fitness and survivorship of *Stator limbatus* (seed beetle) reared on different plant hosts. Specifically, mothers reared on *C. floridum* produced offspring with larger body mass and faster developmental rates than mothers reared on *A. greggii*, which may be contributed by non-genetic maternal effects and rapid adaptation (Fox *et al.*, 1995). However, very few studies provided evidence for maternal effects that could lead to better performance of offspring when reared under maternal rearing environments, or transgenerational acclimatization in herbivore-host plant systems. One exception is a study on *Coenonympha pamphilus* (small heath) by Cahenzli and Erhardt (2013), where mothers experiencing predictive environmental cues were able to acclimatize their progeny to specific plant host qualities. Though evidence showed that maternal effects could enhance offspring performances, most attempts to demonstrate such offspring acclimatization to the specific maternal rearing environments or food resource via maternal adjustment of progeny phenotypes have not been successful, including cases of *Ophraella notulata* (leaf beetle) (Futuyma *et al.*, 1993), *Stator limbatus* (Fox *et al.*, 1995; Amarillo-Suarez and Fox, 2006), *Saissetia coffeae* (soft scale insect) (Spitzer, 2004), and *Acyrtosiphon pisum* (pea aphid) (McLean *et al.*, 2009). Other research on plant-aphid-parasitoid interactions even indicated that maternal effects of *A. pisum* played a relatively insignificant role in offspring fitness and outcome of its interactions with parasitoid wasps (Slater *et al.*, 2019). Overall, studies in offspring acclimatization to maternal

rearing environments and maternal effects in herbivorous insects remain a heated debate and require further investigation.

Offspring larval rearing environmental conditions and life-history traits can also affect adult traits, a carry-over effect defined as a type of developmental plasticity (Moore and Martin, 2019). Originally a terminology extensively used in biomedical fields, carry-over effect has now been defined as “to occur in any situation in which an individual’s previous history and experience explains their current performance in a given situation” by O’Connor *et al.* (2014) in an ecological context. Therefore, carry-over effects could be identified as the influences of factors occurring across life-history stages and phases within a single generation. Many organisms encounter and inhabit distinctive environments during their development. Consequently, environmental settings during early life stage could potentially affect adult phenotypes, fitness, survivorship, and reproductive behaviors (De Block and Stoks, 2005). Carry-over effects across different life-history stages are thus influenced by forces of selection and could have implications on evolution of developmental plasticity. Factors including stress from lack of food resources and nutrition, or low-quality environment could all potentially influence fitness of organisms in later life stages (Pechenik, 2006; Stoks and Cordoba-Aguilar, 2012). For instance, under nutritional constraints, larval *Lestes viridis* (damselfly) takes longer to emerge and has smaller sizes, lower mating success, and reduced survival patterns as adults (De Block and Stoks, 2005).

The system of interest in this study, *Callosobruchus maculatus* (bean beetle or cowpea weevil), is a serious insect pest specifically feeding on stored bean that has resulted in worldwide economic loss and deterioration of food quality (Beck and Blumer, 2014). This *Bruchidae* species has been widely used for many empirical studies of evolutionary and ecological theories

as they are easy to handle (Fox and Tatar, 1994) and have a relatively short life cycle. Previous studies on *C. maculatus* have examined cost of reproduction and age-specific mortality (Tatar *et al.*, 1993; Tartar and Carey, 1995), oviposition substrate's effect on adult mortality (Fox and Tatar, 1994), sexual selection (Martinossi-Allibert *et al.*, 2018) and inbreeding-stress interactions (Springer *et al.*, 2019) but left a gap in studies of potential effects of the maternal rearing environment and carry-over effects across life stages on offspring fitness and survivorship.

*C. maculatus* is especially suitable for testing such maternal and carry-over effects since its development involves both larval and adult stages and it can successfully complete its life cycle on a variety of legume hosts. The species experiences a complete metamorphosis inside individual beans, and adults no longer require water or food resources after emergence, which may potentially lead to stronger maternal effects on offspring fitness as there are limited factors influencing offspring performance. Therefore, we were interested in testing if transgenerational maternal effects could leave a “legacy” on fitness and survivorship of offspring after mothers' oviposition on different bean types, or if larval carry-over effects could play a more significant role in offspring development. Studies on maternal and carry-over effects on offspring life-history traits and fitness in *C. maculatus* can help us better understand insect evolutionary and behavioral ecology and explore potential mechanisms of maternal effects such as preference-performance hypothesis (Gripenberg *et al.*, 2010) or vertical transmission of maternal microbes (Funkhouser and Bordenstein, 2013). This information can also improve our understanding on qualities of rearing environments in the agricultural pest-control industry.

To study maternal and carry-over effects on offspring's performance in *Callosobruchus maculatus*, we reared offspring on three different bean hosts that were either the same or different from the ones experienced by mothers. We hypothesized that offspring with mothers

reared on the highest quality bean host would have the best performance via maternal effects. Predictions included larger body size and mass, higher fecundity, and increased life span compared to the offspring with mothers reared on other bean hosts. Factors of the nutritional components and toxicity levels of the three bean hosts were considered since legume species differ in nutrient quality (USDA Agricultural Research Service) and secondary compounds (Bisby *et al.*, 1994) that may potentially influence offspring performance and give rise to within-generation carry-over effects that are independent of maternal effects. Black-eyed peas are considered a high-quality host for its nutritional values, and adzuki beans are a relatively low-quality host that usually leads to a longer beetle emergence time (Beck *et al.*, 2013), controlling other factors. Therefore, if larval carry-over effects have a more significant impact on the adult life-history traits, we would expect to see the best performance in offspring reared on black-eyed peas, regardless of maternal bean host.

## **Methods**

### **(a) Study System**

*Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae), also known as bean beetle or southern cowpea weevil, is an agricultural pest insect found throughout Africa and Asia (Beck and Blumer, 2014). The larvae feed exclusively on legumes (Fabaceae) and can feed on various legume species, though *Vigna unguiculata* (black-eyed pea, or cowpea) is preferred. Adult bean beetles do not require food or water and spend their limited lifespan mating and laying eggs on the beans (Beck and Blumer, 2014). The species' life cycle involves a complete metamorphosis inside the bean hosts. After mating, female adults will lay fertilized eggs on the bean surface. The larvae then hatch from the eggs and burrow down into the inside of the bean

and start to feed on the bean endosperm and embryo. The larvae will undergo molting through four larval instars and eventually burrow right below the bean surface to start pupation (Beck and Blumer, 2014). After metamorphosis, adults will chew through the seed coat and emerge. This entire process usually takes from 3 to 7 weeks depending on bean host quality. The beetles become fully mature and start mating one or two days after emergence, and usually live for one to two weeks (Beck and Blumer, 2014).

### (b) Legume Hosts

Populations of *C. maculatus* that have been living in three different stored bean environments for generations were used as mothers (F0 generation). Three legume species used to culture *C. maculatus* were *Vigna unguiculata* (black-eyed pea), *Vigna radiata* (mung bean), and *Vigna angularis* (adzuki bean). They differ in nutrient qualities, and components and concentrations of secondary compounds, which may lead to carry-over effects from larval to adult stages. For instance, black-eyed peas are considered a bean host with higher nutritional values compared to mung beans and adzuki beans (Beck *et al.*, 2013).

### (c) Experimental Design

*C. maculatus* stocks were cultured on beans in plastic snap-lid containers at 30°C. Mated females were selected from each bean host environments and allowed to oviposit on fresh bean hosts in single 35 mm petri dishes, with one beetle in each dish. With three bean types and three lines of beetle cultures, a 3 by 3 factorial design was used, resulting in nine unique treatments each with ten replicates (Figure 1). Maternal effects would be suggested by differences in maternal host independent of offspring host independent of offspring host (e.g., ADZ-\*, BEP-\*,

Mung-\*), whereas offspring carry-over effects would be suggested by the effect of offspring host independent of maternal host (e.g., \*-ADZ, \*-BEP, \*-Mung). Three out of the nine treatments include the same maternal hosts and offspring hosts, whereas offspring beetles in the other six treatments experience novel hosts different from their maternal hosts. If treatments with the same maternal and offspring hosts have higher performance than the novel combinations, this would suggest genetic adaptation to specific host types. F1 eggs in the petri dishes were then incubated at 30°C.

#### (d) Data Collection

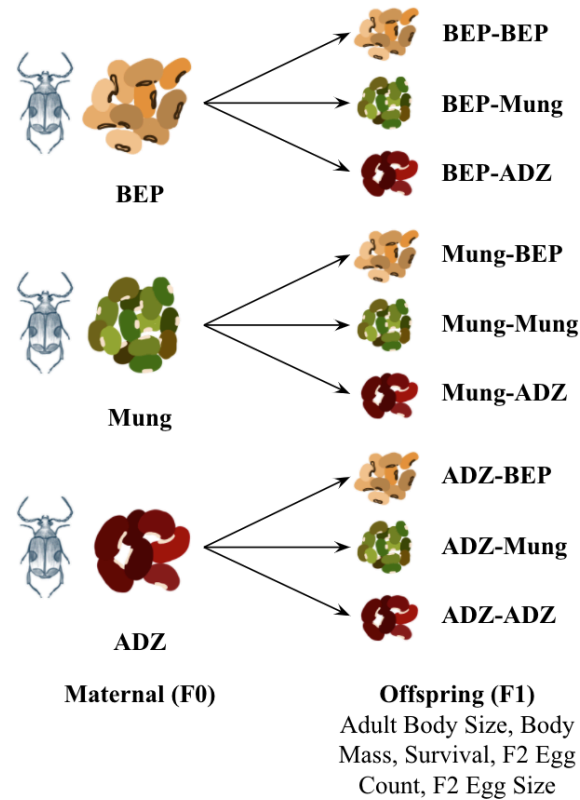
After the first offspring beetle (F1 generation) emerged, the data collection phase began. Newly emerged beetles were relocated to 90 mm petri dishes corresponding to each replicate.

##### *Adult Body Size and Mass*

Three males and females were removed from each replicate within 24 hours after their emergence and their body mass were measured to 0.1 mg using an Ohaus Analytical Balance Model PA84. The beetles were placed back in the 90 mm petri dishes after being measured. Elytra length of three females and males were measured from each replicate after they died using NIH Image J (version 2.1.0) as the data for adult body size. Large body size and body mass usually indicate better nutritional intake and higher fitness.

##### *Adult Survival*

Offspring populations were monitored daily for data collection on sexes and numbers of beetles emerged and died on that specific day. Dead beetles were instantly removed to an empty 35 mm petri dish corresponding to the replicate ID and were stored in a freezer (below 0°C).



**Figure 1. Experimental design.** Mated *C. maculatus* females selected from all three host environments were cultured in different host environments and allowed to lay eggs. They were the F0 mother generation shown on the left side of the graph. Their offspring, or the F1 generation, were shown on the right. Thus, a total of nine treatments were included, each with ten replicates. Offspring treatment was identified by “maternal host – offspring host”, as indicated above. For F1 offspring generation, their body mass and body size were measured, and their survival were monitored daily. Selected F1 offspring were allowed to lay eggs on the same bean host as what they were reared on. These F2 eggs laid by the F1 offspring were then counted as the fecundity data, and their sizes were also measured.

### *Egg Count and Size*

From each replicate, one F1 virgin female was relocated to a 90 mm petri dish with beans of the same type that she was reared on and mated with two healthy males from a black-eyed pea

stock environment. After the F1 females finished laying eggs and died, numbers of F2 eggs on the bean surface were counted as the F1 fecundity data. In addition, images of five randomly sampled eggs from each replicate were taken under a dissection microscope on a calibration slide. Egg sizes were then measured using the same linear measurement method as elytra length.

#### (e) Statistical Analysis

All statistical analyses were performed through R (version 3.6.2) and R Studio (version 1.2.5033). Linear mixed effect models were performed on life-history data including body mass at emergence and elytra length for both sexes, F2 egg count, and F2 egg size with the lme4 package (Bates *et al.*, 2015). Maternal host, offspring host, and their interaction were included as the fixed effects, and the replicate ID was included as a random effect because F1 individuals within the same replicate were all originated from the same F0 mother, and such dependence needs to be controlled. If the interaction effect was not significant, then it was removed and the models with only maternal and offspring carry-over effects were run. Significance of maternal effects and carry-over effects, determined by the maternal host and the offspring host fixed effects, and their interactions, was calculated using the car package (Fox and Weisberg, 2019) through the Type III ANOVA test. Tukey pairwise comparisons between nine treatments were conducted using the emmeans package (Lenth, 2022), and results were indicated as compact letter displays on boxplots generated through the ggplot2 package (Wickham, 2016).

The survival data were fitted through Cox proportional-hazards models. Specifically, the survival curves of all nine treatments were plotted with functions in the survival (Therneau and Grambsch, 2000) and survminer (Kassambara, 2021) packages, and pairwise comparisons of



survival curves were conducted using the coxme package (Therneau, 2020) with log-rank test, taking the potential random effects into account.

## Results

### (a) Adult Body Size and Mass

The longest and the shortest adult male elytra length were 2.469 cm and 1.544 cm, and the largest and the smallest adult male body mass were 0.0068 g and 0.0022 g. The average elytra length and body mass were 2.015 cm (n = 262, sd = 0.134) and 0.004562 g (n = 198, sd = 0.0007), respectively. The association between interaction effects and both male elytra length and body mass were insignificant, but both maternal effects and offspring carry-over effects were significant (Table 1).

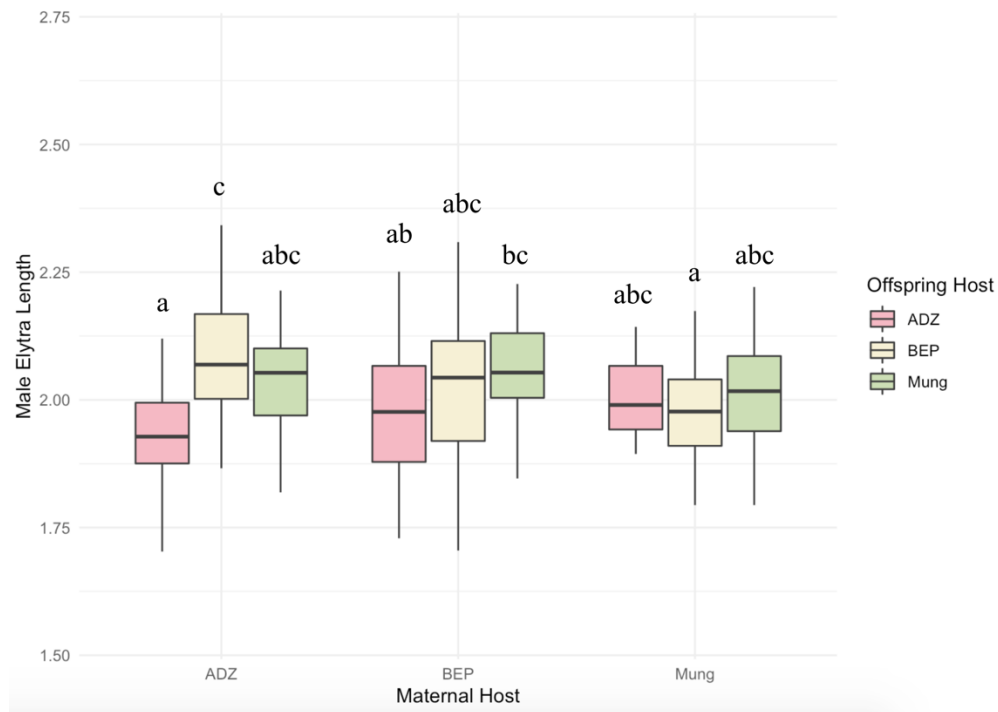
**Table 1. Results of linear mixed models investigating F1 male offspring body size and mass.** Fixed effects of maternal host and offspring host and their interaction were included in the models to investigate their association with adult male elytra length and body mass. Replicate ID was included as a random effect in all models. Since the interaction effects were insignificant, they were removed and the models with only maternal and offspring effects were re-run.

Response Variable	Fixed Effect	Chi-square	Df	P-value
a. Male Elytra Length	Maternal Host	10.43	2	0.0054
	Offspring Host	19.42	2	<0.001
b. Male Body Mass	Maternal Host	13.38	2	0.0012
	Offspring Host	32.39	2	<0.001

When comparing male offspring reared on the same offspring host to examine maternal effects, offspring with mothers reared on mung beans were smaller in size than offspring with mothers reared on adzuki beans ( $p = 0.009$ ) and black-eyed peas ( $p = 0.0153$ ) on average. For instance, ADZ-BEP had a larger body size than Mung-BEP (Figure 2;  $p = 0.0235$ ). In addition, similar patterns occurred in male body mass, in which offspring with mothers reared on mung

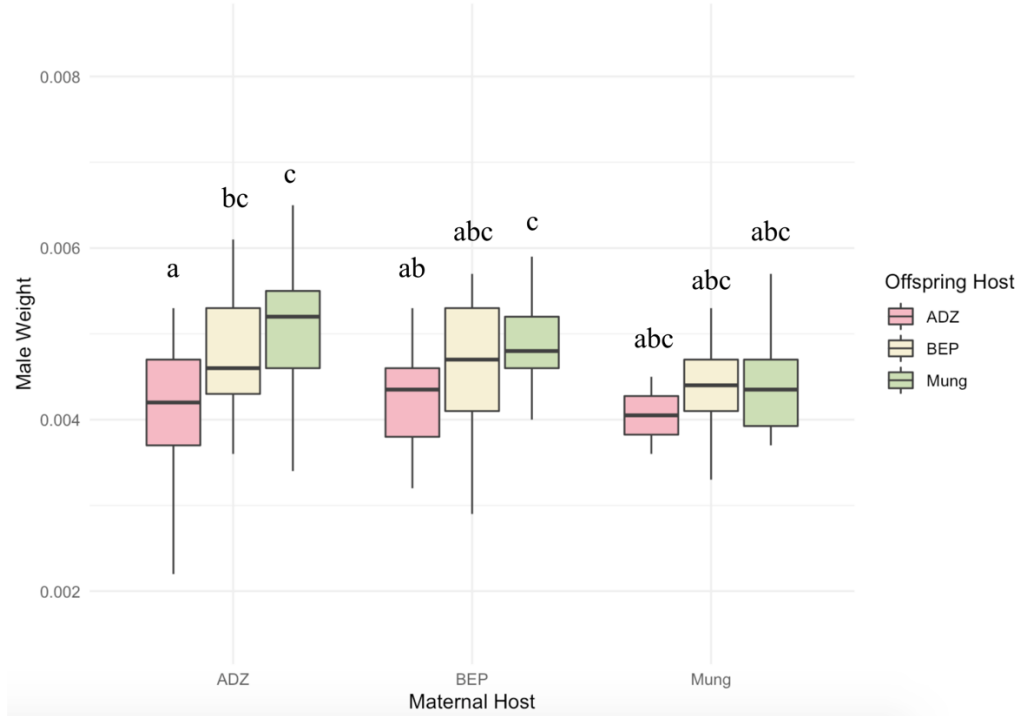
beans were smaller in size than offspring with mothers reared on adzuki beans ( $p = 0.0024$ ) and black-eyed peas ( $p = 0.0046$ ) on average.

For males across the three offspring host types with the same maternal rearing environments (i.e., offspring carry-over effects), offspring reared on adzuki beans were significantly smaller than offspring reared on black-eyed peas and mung beans (both  $p < 0.001$ ). Specifically, replicates reared on adzuki beans and black-eyed peas with adzuki bean as the maternal host had a significant difference in body size (Figure 2;  $p = 0.002$ ), and ADZ-BEP had a larger body size than ADZ-ADZ on average. Again, similar patterns were observed in male body mass controlling the maternal host, in which males had a significantly smaller body mass when reared on adzuki beans versus on black-eyed peas and mung beans ( $p < 0.001$ ) (Figure 3; e.g., [ADZ-ADZ] – [ADZ-BEP]  $p = 0.021$ , [ADZ-ADZ] – [ADZ-Mung]  $p = 0.0011$ , [BEP-ADZ] – [BEP-Mung]  $p = 0.0053$ ).



**Figure 2. F1 male elytra length across treatments.** Treatments were assigned compact letter displays (CLD) using Tukey pairwise comparison method. Groups with the same letter are not detectably different. Groups with different letters are significantly different. Groups can have more than one letter (“ab”) to reflect overlap between different

sets. (ADZ-ADZ: n = 23, ADZ-BEP: n = 29, ADZ-Mung: n = 34, BEP-ADZ: n = 40, BEP-BEP: n = 28, BEP-Mung: n = 46, Mung-ADZ: n = 3, Mung-BEP: n = 33, Mung-Mung: n = 26)



**Figure 3. F1 male body mass across treatments.** Treatments were assigned CLDs. (ADZ-ADZ: n = 21, ADZ-BEP: n = 21, ADZ-Mung: n = 25, BEP-ADZ: n = 28, BEP-BEP: n = 29, BEP-Mung: n = 29, Mung-ADZ: n = 2, Mung-BEP: n = 25, Mung-Mung: n = 18)

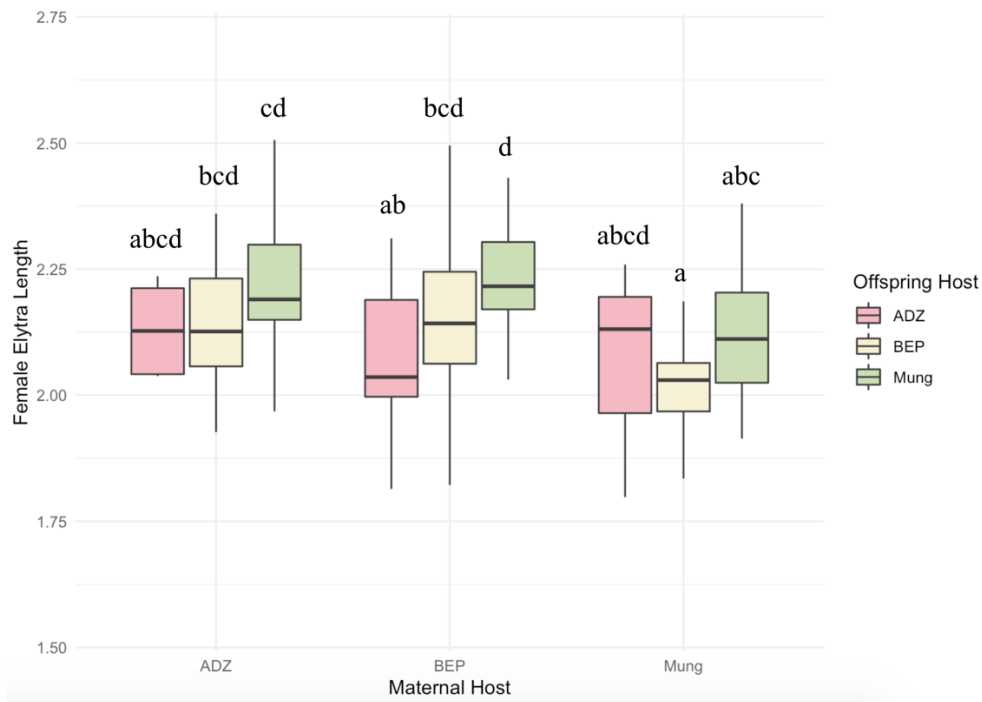
For females, the longest and the shortest adult elytra length were 2.639 cm and 1.786 cm, and the largest and the smallest body mass were 0.0081 g and 0.0017 g. The average elytra length and body mass were 2.136 cm (n = 234, sd = 0.145) and 0.005775 g (n = 198, sd = 0.001), respectively. The association between interaction effects and elytra length was insignificant, but both maternal effects and offspring carry-over effects were significant (Table 2a). However, for body mass, the effects of offspring host and the interaction were significant, whereas maternal host had no direct effect (Table 2b).

**Table 2. Results of linear mixed models investigating F1 female offspring body size and mass.** Fixed effects of maternal host and offspring host and their interaction were included in the models to investigate their association with adult female elytra length and body mass. Replicate ID was included as a random effect in all models. Since the interaction effect for elytra length was insignificant, it was removed and the model with only maternal and offspring effects was re-run.

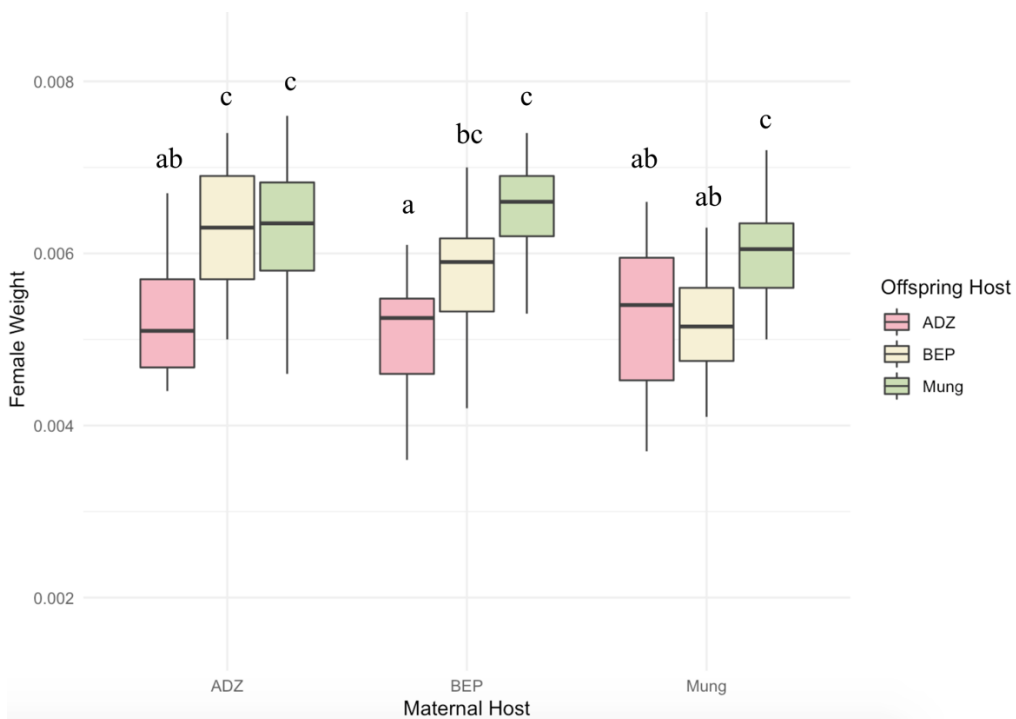
Response Variable	Fixed Effect	Chi-square	Df	P-value
a. Female Elytra Length	Maternal Host	27.49	2	<0.001
	Offspring Host	39.28	2	<0.001
b. Female Body Mass	Maternal Host	1.46	2	0.48
	Offspring Host	20.34	2	<0.001
	Maternal Host : Offspring Host	15.14	4	0.0044

When comparing female offspring reared on the same environments to study maternal effects, offspring with mothers reared on mung beans were smaller in size than offspring with mothers reared on adzuki beans and black-eyed peas (both  $p < 0.001$ ) on average (Figure 4; e.g., [Mung-BEP] – [ADZ-BEP]  $p = 0.015$ , [Mung-BEP] – [BEP-BEP]  $p = 0.0077$ , [Mung-Mung] – [BEP-Mung]  $p = 0.0318$ ).

When comparing female offspring with the same maternal host to study carry-over effects, offspring reared on mung beans were significantly larger than offspring reared on adzuki beans and black-eyed peas (both  $p < 0.001$ ). Specifically, the within-treatment-group variance of female elytra length occurred between treatments reared on adzuki beans and mung beans with black-eye peas as the maternal host (Figure 4;  $p < 0.001$ ), in which offspring reared on mung beans had a larger body size than offspring reared on adzuki beans on average. Female offspring had the smallest body mass when reared on adzuki beans than the other two hosts when their mothers were reared on adzuki beans and black-eyed peas (Figure 5; [ADZ-Mung] – [ADZ-ADZ]  $p = 0.0058$ , [BEP-Mung] – [BEP-ADZ]  $p = 0.0423$ , ([ADZ-BEP] – [ADZ-ADZ]  $p = 0.0015$ , [BEP-BEP] – [BEP-ADZ]  $p = 0.001$ ). In addition, when maternal host was mung bean, offspring reared on adzuki beans and black-eyed peas had smaller body mass than offspring reared on mung beans (Figure 5; [Mung-Mung] – [Mung-ADZ]  $p = 0.0257$ , [Mung-BEP] – [Mung-Mung]  $p = 0.0062$ ).



**Figure 4. F1 female elytra length across treatments.** Treatments were assigned CLDs. (ADZ-ADZ: n = 8, ADZ-BEP: n = 32, ADZ-Mung: n = 31, BEP-ADZ: n = 33, BEP-BEP: n = 26, BEP-Mung: n = 42, Mung-ADZ: n = 3, Mung-BEP: n = 29, Mung-Mung: n = 30)



**Figure 5. F1 female body mass across treatments.** Treatments were assigned CLDs. (ADZ-ADZ: n = 16, ADZ-BEP: n = 24, ADZ-Mung: n = 24, BEP-ADZ: n = 26, BEP-BEP: n = 26, BEP-Mung: n = 30, Mung-ADZ: n = 10, Mung-BEP: n = 24, Mung-Mung: n = 18)

(b) Survival

The longest life spans were 25 days for males and 20 days for females, and the shortest life spans were 0 day for males (emerged and died on the same day) and 1 day for females. The average life spans were 8.69 days for males (n = 617, sd = 2.481) and 8.06 days for females (n = 637, sd = 2.558). For both male and female beetles, survival was significantly related to offspring host and the interaction between maternal host and offspring host (Table 3). In addition, maternal host was also significantly associated with female survival time (Table 3b).

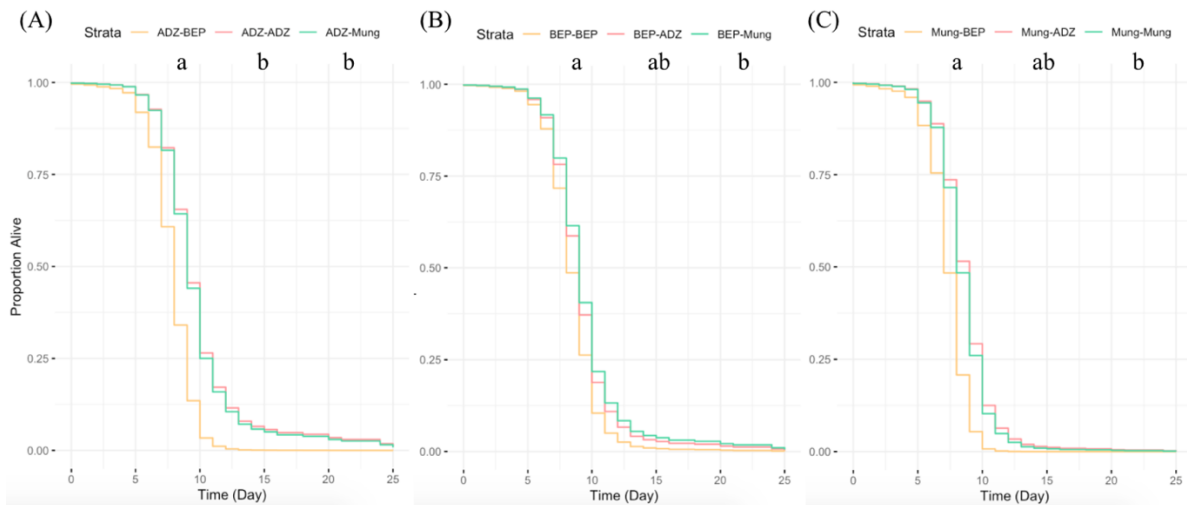
**Table 3. Results of Cox proportional-hazards model with mixed effects.** Fixed effects of maternal host and offspring host and their interaction were included in the models to investigate their association with F1 adult survival time after emergence. Replicate ID was included as a random effect in the models.

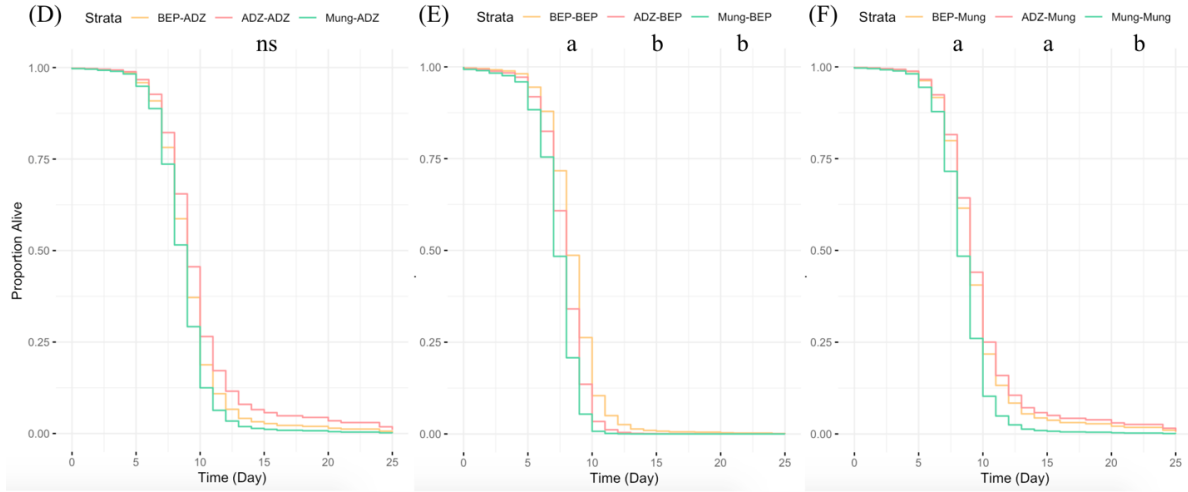
Response Variable	Fixed Effect	Chi-square	Df	P-value
a. Male Survival Time	Maternal Host	3.54	2	0.17
	Offspring Host	43.72	2	<0.001
	Maternal Host : Offspring Host	14.12	4	0.0069
b. Female Survival Time	Maternal Host	6.59	2	0.037
	Offspring Host	16.46	2	<0.001
	Maternal Host : Offspring Host	31.16	4	<0.001

Figures 6D-F show male survival curves grouped by the same offspring hosts to examine maternal effects. For offspring reared on adzuki beans, no significant difference was detected in the pairwise comparison (Figure 6D). For offspring reared on black-eyed peas, the treatment with black-eyed pea as the maternal host had a significantly higher survival probability (Figure 6E; [BEP-BEP] – [ADZ-BEP] p = 0.00309, [BEP-BEP] – [Mung-BEP] p < 0.001). Surprisingly, the offspring with mung bean maternal host had a significantly lower survival probability when

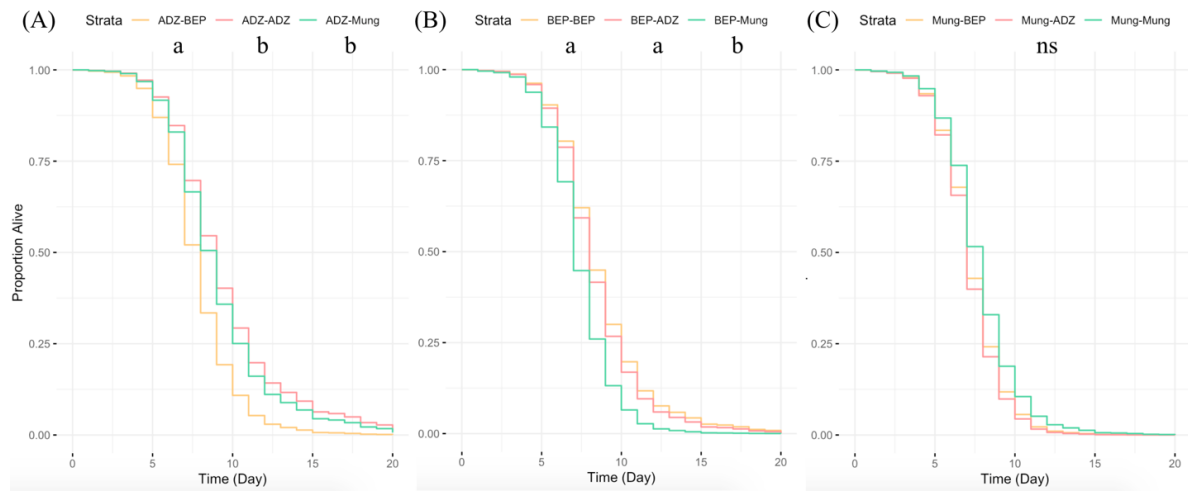
reared on mung beans than offspring with black-eyed pea or adzuki beans as the maternal host (Figure 8F; [Mung-Mung] – [ADZ-Mung]  $p = 0.0302$ , [Mung-Mung] – [BEP-Mung]  $p = 0.01698$ ).

Figures 6A-C show male survival curves grouped by the same maternal hosts to examine offspring carry-over effects. Offspring reared on black-eyed peas had significantly a lower survival probability than offspring reared on mung beans regardless of their maternal hosts (Figure 6; [ADZ-BEP] – [ADZ-Mung]  $p < 0.001$ , [BEP-BEP] – [BEP-Mung]  $p < 0.001$ , [Mung-BEP] – [Mung-Mung]  $p = 0.00309$  using log-rank pairwise comparison). Offspring reared on adzuki beans had no significant difference compared with offspring reared on the other two beans within the black-eyed pea and mung maternal host treatment groups but had a significantly higher survival probability than offspring reared on black-eyed pea when their maternal host environments were adzuki beans (Figure 6A;  $p = 0.0068$ ).

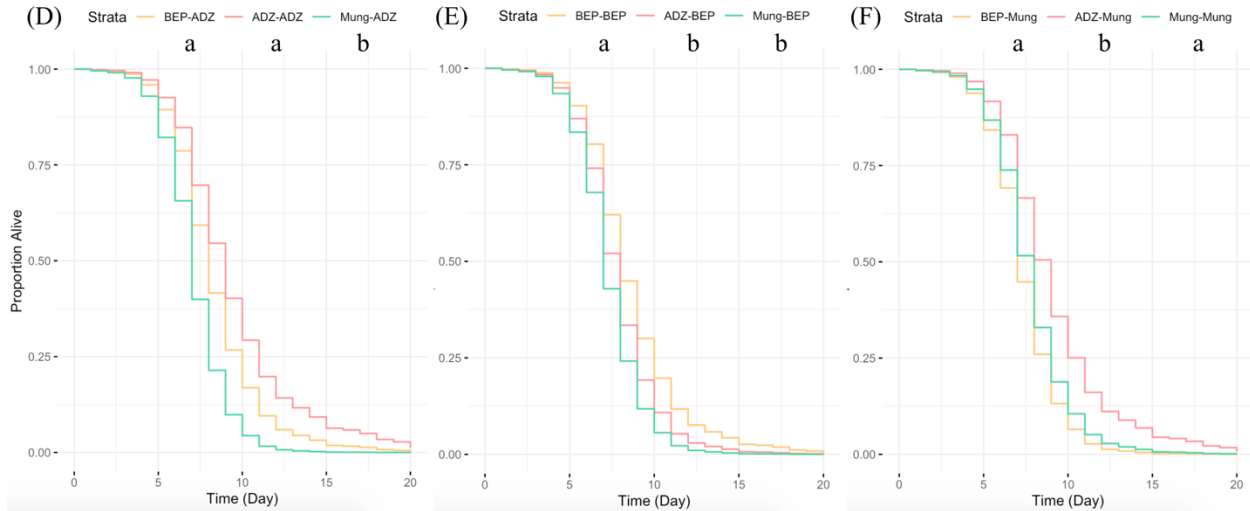




**Figure 6. F1 male survival curves across treatments.** Treatments were assigned CLDs within each group. For better visualization of the fixed effects, survival curves from treatments were grouped by (A) adzuki maternal host, (B) black-eyed pea maternal host, and (C) mung maternal host to study offspring carry-over effects, and by (D) adzuki offspring host, (E) black-eyed pea offspring host, and (F) mung offspring host to study maternal effects. Note that survival curves in A-C and D-F are the same nine curves but are grouped differently. (ADZ-ADZ: n = 31, ADZ-BEP: n = 95, ADZ-Mung: n = 46, BEP-ADZ: n = 79, BEP-BEP: n = 155, BEP-Mung: n = 125, Mung-ADZ: n = 3, Mung-BEP: n = 53, Mung-Mung: n = 30)







**Figure 7. F1 female survival curves across treatments.** Treatments were assigned CLDs within each group. For better visualization of the fixed effects, survival curves from treatments were grouped by (A) adzuki maternal host, (B) black-eyed pea maternal host, and (C) mung maternal host to study offspring carry-over effects, and by (D) adzuki offspring host, (E) black-eyed pea offspring host, and (F) mung offspring host to study maternal effects. Note that survival curves in A-C and D-F are the same nine curves but are grouped differently. (ADZ-ADZ:  $n = 18$ , ADZ-BEP:  $n = 99$ , ADZ-Mung:  $n = 65$ , BEP-ADZ:  $n = 72$ , BEP-BEP:  $n = 122$ , BEP-Mung:  $n = 125$ , Mung-ADZ:  $n = 11$ , Mung-BEP:  $n = 65$ , Mung-Mung:  $n = 60$ )

Figures 7D-F show female survival curves grouped by the same offspring hosts to examine maternal effects. For offspring reared on adzuki beans, the treatment with mung bean as the maternal host had a significantly lower survival probability than treatments with black-eyed pea or adzuki bean as the maternal host (Figure 7D; [Mung-ADZ] – [ADZ-ADZ]  $p = 0.03484$ , [Mung-ADZ] – [BEP-ADZ]  $p = 0.03853$ ). For offspring reared on black-eyed peas, the treatment with black-eyed pea as the maternal host had a significantly higher survival probability (Figure 7E; [BEP-BEP] – [ADZ-BEP]  $p = 0.04667$ , [BEP-BEP] – [Mung-BEP]  $p = 0.00203$ ). However, such trend did not exist in the offspring treatment group reared on mung beans (Figure 7F).

Figures 7A-C show female survival curves grouped by the same maternal hosts to study offspring carry-over effects. When comparing survival curves between treatments with the adzuki maternal host, black-eyed pea offspring host was associated with a significantly lower survival probability (Figure 7A; [ADZ-BEP] – [ADZ-ADZ]  $p = 0.02692$ , [ADZ-BEP] – [ADZ-

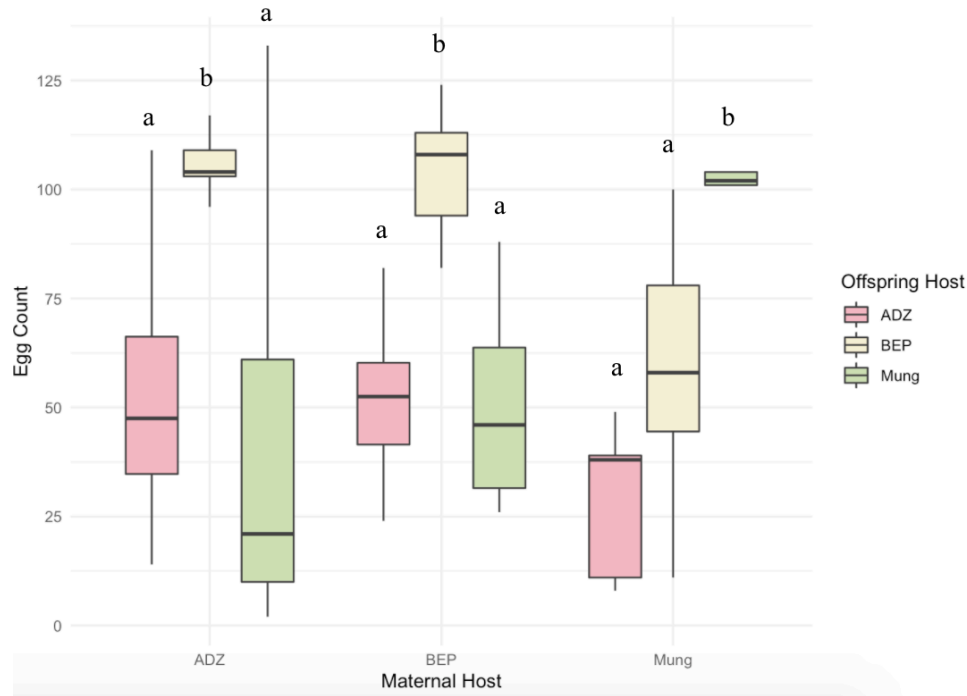
Mung]  $p = 0.00203$ ). However, offspring reared on mung beans had a significantly lower survival probability within the black-eyed pea maternal host treatment group (Figure 7B; [BEP-Mung] – [BEP-ADZ]  $p = 0.01127$ , [BEP-Mung] – [BEP-BEP]  $p < 0.001$ ). Offspring reared on adzuki beans had no significant difference in survival even compared to treatments with the same maternal and offspring host environments. No significant difference was observed between the offspring host treatments with mung bean as the maternal host.

### (c) Egg Count

Not every F1 female was able to lay eggs. Within the replicates that successfully oviposited, the highest number of eggs laid by F1 females was 133, and the lowest was 2, with an average of 65.9 ( $n = 72$ ,  $sd = 35.96$ ). Both offspring host and the interaction between maternal host and offspring host significantly influenced the numbers of eggs laid by F1 females, whereas maternal host did not contribute significantly to F1 fecundity (Table 4).

**Table 4. Results of linear mixed models investigating F1 offspring fecundity.** Fixed effects of maternal host and offspring host and their interaction were included in the models to investigate their association with F2 egg count. Replicate ID was included as a random effect in all models.

Response Variable	Fixed Effect	Chi-square	Df	P-value
F2 Egg Count	Maternal Host	3.12	2	0.21
	Offspring Host	31.76	2	<0.001
	Maternal Host : Offspring Host	43.29	4	<0.001



**Figure 8. F2 egg counts across treatments.** Treatments were assigned CLDs. (ADZ-ADZ: n = 8, ADZ-BEP: n = 9, ADZ-Mung: n = 9, BEP-ADZ: n = 8, BEP-BEP: n = 9, BEP-Mung: n = 8, Mung-ADZ: n = 5, Mung-BEP: n = 11, Mung-Mung: n = 5)

Although maternal host was not significantly related to F1 fecundity, there were detectable differences in the number of eggs laid between offspring host groups with the same maternal host. On average, offspring reared on black-eyed peas laid higher numbers of eggs than offspring reared on adzuki beans ( $p < 0.001$ ) and on mung beans ( $p = 0.0046$ ). For example, when the maternal host was either black-eyed peas or adzuki beans, F1 females reared on black-eyed peas laid significantly higher numbers of eggs compared to other offspring host treatments (Figure 8; [ADZ-BEP] – [ADZ-ADZ]  $p = 0.0044$ , [ADZ-BEP] – [ADZ-Mung]  $p < 0.001$ ; [BEP-BEP] – [BEP-ADZ]  $p = 0.0028$ , [BEP-BEP] – [BEP-Mung]  $p = 0.0016$ ). However, in contrast, when the maternal host was mung beans, offspring reared on mung beans, the same environment as their mothers, laid the highest numbers of eggs on average ([Mung-Mung] – [Mung-ADZ]  $p < 0.001$ , [Mung-Mung] – [Mung-BEP]  $p = 0.0463$ ).

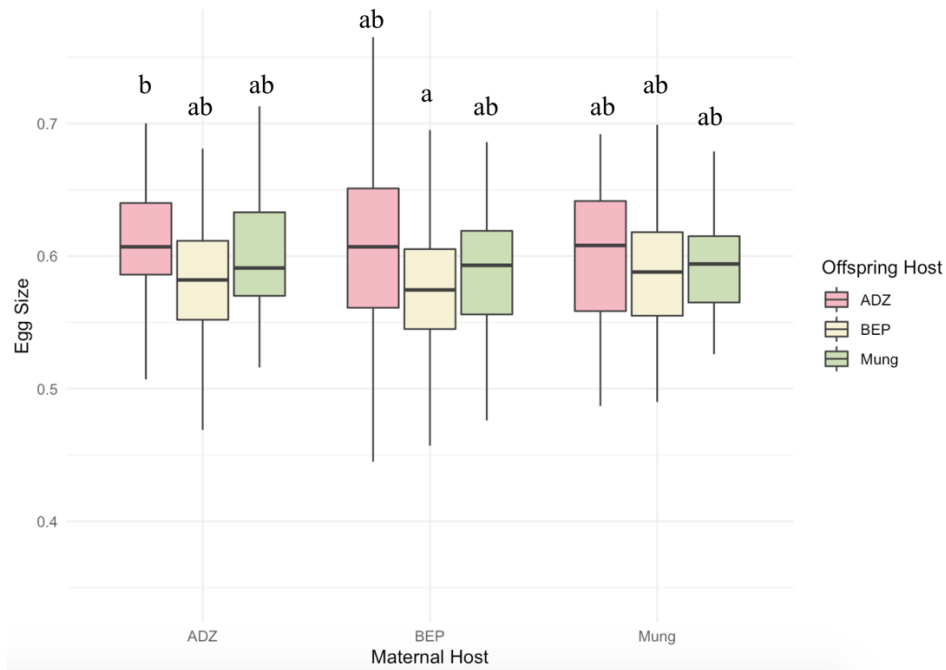
#### (d) Egg Size

Across all nine treatments, the largest egg laid by F1 females was 0.765 cm in length, and the smallest egg laid was 0.345 cm, with an average of 0.59 cm ( $n = 530$ ,  $sd = 0.054$ ). When F2 egg size was fitted against the three fixed effects, the only significant effect was offspring host (Table 5).

**Table 5. Results of linear mixed models investigating size of eggs laid by F1 offspring.** Fixed effects of maternal host and offspring host and their interaction were included in the models to investigate their association with F2 egg count. Replicate ID was included as a random effect in all models. Since the interaction effects were insignificant, they were removed and the models with only maternal and offspring effects were re-run.

Response Variable	Fixed Effect	Chi-square	Df	P-value
F2 Egg Size	Maternal Host	1.51	2	0.47
	Offspring Host	13.96	2	<0.001

Controlling the factor of maternal host, offspring reared on adzuki beans laid significantly larger eggs than offspring reared on black-eyed peas ( $p < 0.001$ ), and eggs laid by offspring reared on mung beans had no significant difference with the other two treatment groups. Specifically, offspring reared on black-eyed peas whose mothers were reared on the same type of bean had smaller eggs on average compared to offspring reared on adzuki beans whose mothers were also reared on adzuki beans (Figure 9;  $p = 0.0063$ ).



**Figure 9. F2 egg sizes across treatments.** Treatments were assigned CLDs. (ADZ-ADZ: n = 41, ADZ-BEP: n = 111, ADZ-Mung: n = 41, BEP-ADZ: n = 45, BEP-BEP: n = 116, BEP-Mung: n = 49, Mung-ADZ: n = 27, Mung-BEP: n = 67, Mung-Mung: n = 33)

## Discussion

Here we report experiments that study the maternal and carry-over effects on offspring's life-history traits by rearing *Callosobruchus maculatus* offspring on three different bean hosts that were either the same or different from maternal hosts. Our hypothesis was that offspring with mothers reared on the highest quality bean host would have the best performance via transgenerational maternal effects. However, if within-generation larval carry-over effects have a more significant impact on the life-history traits, offspring would have the best performance when reared on the highest quality bean host regardless of maternal bean host. Larger body size, body mass and egg size, higher fecundity, and increased life span could all be indications of higher fitness in beetles. In addition, black-eyed peas are considered a high-quality host for its nutritional values, and adzuki beans with less nutrient and higher concentrations of plant toxins

are a relatively low-quality host that usually leads to longer beetle emergence time (Beck *et al.*, 2013), controlling other factors.

According to the results, the effects of maternal host, offspring host (carry-over effect), and their interactions contributed differently and inconsistently to each measured life-history trait (Table 6), which was different from the hypothesis that maternal effect would be the most significant factor that associated with all life-history traits of *C. maculatus*. The hypothesis that if larval carry-over effect was significant, then offspring reared on the bean host with the highest quality would have the best performance regardless of the maternal host was also not supported due to the complex observations and results.

**Table 6. Summary table of fixed effects significance in the models.** This table shows whether each fixed effect was significant to explain the response traits. For response variables without significant interaction effects, orders of fitness suggested by the pair-wise comparisons were included.

Response Variable	Sex	Maternal Host	Offspring Host	Maternal Host : Offspring Host
Body Size	Male	Mung < ADZ = BEP	ADZ < BEP = Mung	Not Significant
	Female	Mung < ADZ = BEP	ADZ = BEP < Mung	Not Significant
Body Mass	Male	Mung < ADZ = BEP	ADZ < BEP = Mung	Not Significant
	Female	Not Significant	Significant	Significant
Survival Time	Male	Not Significant	Significant	Significant
	Female	Significant	Significant	Significant
Egg Count	/	Not Significant	Significant	Significant
Egg Size	/	Not Significant	BEP = Mung < ADZ	Not Significant

### *Maternal Effects*

The associations between the fixed effects of maternal host environment and adult body size of both males and females, body mass of males, and survival time of females were significant, which could potentially indicate that transgenerational maternal effects could contribute to body size, body mass and survival time of adult offspring. However, maternal

effects were not completely independent of the offspring carry-over effects in the case of female survival since there were interaction effects. Offspring fecundity and size of eggs that offspring laid were not related to maternal host type.

Offspring with mung beans as the maternal host environment had the smallest body sizes in both males and females and the smallest body mass in males, regardless of the offspring host environments that F0 generation laid eggs on (i.e., the within-generation carry-over effects were controlled). There was no detectable difference between the body size and mass of offspring reared on adzuki beans and black-eyed peas. However, mechanisms of such potential transgenerational maternal effects remain unclear and require further investigation.

According to the female survival curves (Figures 7D, E), treatments with beetles reared on adzuki beans and black-eyed peas and their mothers reared on the same environments (ADZ-ADZ and BEP-BEP) had a relatively high survival probability, which may suggest that the offspring were genetically adapted to the maternal hosts. However, this trend was not universal across all treatment groups, as ADZ-ADZ did not differ significantly with BEP-ADZ in the survival curve (Figure 7D), and the Mung-Mung treatment did not have a significantly higher survival probability than other groups (Figure 7F). In addition, interaction effects were observed in this model, which indicates that the maternal host effects were not independent of the offspring host effects (see *Interaction Effects* below).

In summary, we can conclude that maternal effect existed in *C. maculatus* and had some influence over offspring body size and body mass. However, it did not match our previous prediction as there was no evidence showing its effects on offspring survival, fecundity, and egg size. This is different from the finding by Cahenzli and Erhardt (2013) that mothers were able to adjust offspring phenotypes based on the environmental cues they received, an indication of

strong maternal effects, but somewhat similar to the result of a study conducted by McLean *et al.* (2009) that maternal environment did not influence offspring fecundity or host type preference. Another study found that maternal host had no effect on overall offspring performance (Spitzer, 2004). Such differences across studies might be due to the inherent differences of insect systems used, but they also indicate that further research is necessary to better understand effects of maternal rearing environment on offspring fitness in insects.

### *Offspring Carry-over Effects*

The fixed effects of offspring host were significant in determining all measured traits, which indicated that larval carry-over effect was probably the most important factor in determining life-history traits in bean beetles. Specifically, offspring reared on adzuki beans were the smallest in body size of males and body mass of both males and females. The females were also smaller than offspring reared on mung beans but not significantly different from offspring reared on black-eyed peas. As previously mentioned, adzuki is a lower-quality host and not a natural host for *C. maculatus* (Beck *et al.*, 2013), and it is very likely that the high concentrations of secondary compounds such as saponins and flavonoids (Liu *et al.*, 2017) could reduce fitness of the beetles and affect their acquisition of nutrition during the larval stage, independent of their maternal rearing environments.

In addition, offspring reared on black-eyed peas had the highest egg counts, and offspring reared on adzuki beans had the lowest egg counts. Therefore, larval carry-over effect may potentially lead to high offspring egg counts, as black-eyed pea is considered as the bean host with the highest quality (Beck *et al.*, 2013). Moreover, offspring reared on adzuki beans were able to lay eggs with the largest size among all three treatment groups, independent of the



maternal effects. It is possible that females could increase its numbers of eggs laid when encountering a high-quality host, instead of increasing the quality of their offspring through larger eggs (Spitzer, 2004). Other than supporting offspring carry-over effects, this could also be an indication of a trade-off between the number and provisioning of eggs (Smith and Fretwell, 1974; Rotem and Agrawal, 2003) by offspring reared on adzuki beans. Offspring reared on adzuki beans laid the fewest eggs on average, and larger eggs (better egg provisioning) usually provide a larger amount of nutrition to offspring (Fox and Czesak, 2020), resulting in higher fitness in offspring (Smith and Fretwell, 1974).

Despite our assumption that black-eyed peas are the bean host with the highest quality, male offspring reared on black-eyed peas had the lowest survival probability (Figures 6A-C), and offspring reared on adzuki beans had no significant difference from offspring reared on mung beans. Although a study by Fox and Tartar (1994) found that mothers reared on black-eyed peas had a lower survival probability than mothers reared on adzuki beans, no offspring survival data were presented. Since offspring reared on black-eyed peas had the highest abundance, mating competition could occur in the population and thus resulted in shorter survival time in offspring reared on black-eyed peas, which could be a potential confounding variable in the experiment that needs to be controlled in future studies. In addition, a reason for male and female survival curves to be inconsistent and hard to interpret could be the harmful copulation process that could result in a cost to female survival but no direct cost to male survival (Tartar et al., 1993; Yanagi and Miyatake, 2003; Edvardsson and Tregenza, 2005).

### *Interaction Effects*

Interaction effects are generally hard to interpret. In this study, they were present in association with female body mass, survival time of both females and males, and egg count. A previous study (Beck *et al.*, 2013) found that the overall offspring performance on a high-quality host when their mothers were reared on a low-quality host was higher than when their mothers were reared on another high-quality host, indicating that maternal adaptation to a low-quality host may pre-adapt offspring to high-quality hosts and result in higher fitness in offspring. However, the pre-adaptation was not observed in this study in both females and males (Figures 5, 6A-C, 7A-C), as female offspring with mothers reared on adzuki beans almost always had the lowest body mass regardless of their rearing environments, and both male and female survival patterns of ADZ-BEP treatments were lower than other groups with adzuki beans as maternal host.

One interesting finding was that offspring reared on black-eyed peas laid the highest numbers of eggs when their mothers were reared on adzuki beans and black-eyed peas, but not mung beans (Figure 8). Females from the Mung-Mung offspring treatment were able to lay the highest number of eggs, possibly due to the interaction effects of maternal host and offspring host. However, this result might be an indication of genetic adaptation of the offspring to specific hosts (Cahenzli and Erhardt, 2013) instead of temporary acclimatization moderated by the maternal generation.

To improve this study, numbers and sizes of eggs laid by the maternal generation should be measured as the oviposition substrate may have direct influence over mother reproductive behaviors (Fox and Tartar, 1994; Tartar and Carey, 1995), and sample sizes for the fecundity data should be increased. This project can be followed by a future study that measures offspring

life-history traits in the larval stage, as adaptative maternal effects are often more significant in early stages of offspring development (Rotem and Agrawal, 2003). Some insects are able to alter egg provisioning based on their experiences and provide offspring with more resources during their larval development processes (Awmack and Leather, 2002). In addition, non-genetic transgenerational acclimatization of offspring to maternal hosts can be studied if effects of genetic inheritance are controlled in the experimental design. Another research direction is to study latent effects, a type of carry-over effect that originates in early stages but only exhibits in later development (Pechenik, 2006), by analyzing adult life-history traits of larvae with the same body size but have emerged from different offspring bean hosts to focus on long-term effects of offspring rearing environment.

Though our data provided no evidence for transgenerational acclimatization of offspring to maternal host, we found that Possible mechanisms of transgenerational maternal effects such as vertical transmission of maternal microbes (Funkhouser and Bordenstein, 2013) and epigenetic responses (McCaw *et al.*, 2021) and within-generation carry-over effects should be further investigated in both *C. maculatus* and other insect systems, as these studies could provide valuable insights to understand insect-plant association and the process of insect evolution. This information can also help propose effective ways of insect pest control since we would have a better understanding of factors influencing their life-histories and fitness.

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