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Sex-Specific Genetic and Non-Genetic Effects on Life History Traits in the Bean Beetle (*Callosobruchus maculatus*)

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
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Abstract

Sex-Specific Genetic and Non-Genetic Effects on Life History Traits in the Bean Beetle

(*Callosobruchus maculatus*)

By Christopher Minjae Kim

Genetic and non-genetic effects play a large role in determining life history traits. Previous studies have looked to understand the effects of these genetic and non-genetic effects on life history traits. However, these effects have often been observed in isolation. Therefore, we were interested in how these effects may interact and influence offspring life history traits in bean beetles, *Callosobrunchus maculatus*. Virgin females and males from different combinations of bean host backgrounds were mated while comparing offspring on different combinations of bean hosts. For both mated adults and offspring, black-eyed peas (*Vigna unguiculata*) or adzuki beans (*Vigna angularis*) were used as the host types. Then, the life history traits of egg size, size at emergence, time to emergence, and adult lifespan were measured. Overall, we found a mixed outcome with the presence of both genetic and non-genetic effects. We then observed the relationships between the parental and offspring traits to understand if certain traits affect the outcome of other traits which could help conclude if certain genetic or non-genetic effects were present. There was greater evidence for a predictive SEM model when observing male offspring compared to female offspring. For future directions, we discussed the implementation of observing traits such as clutch size and emergence success. Also, observing the parental and offspring microbiome to understand a microbial effect that may also be based genetically or non-genetically.

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Introduction:

Phenotypic variation among individuals of the same species can be due to genetic and non-genetic effects. Genetic effects are inheritable traits passed down from parental generations (Fox 1995). These inheritable traits may have been selected for generations due to their beneficial role in increasing an organism's reproductive success. On the other hand, non-genetic effects also play a key role in the evolution of different organisms. Also denoted as environmental effects, non-genetic factors such as the maternal phenotype or environment may affect the offspring's fitness. For example, the parental generation's environment may result in phenotypic variation in traits such as preference for a particular environment to rear their offspring. Now, offspring will be adapted to this environment without having to sacrifice any energetic output.

Previous studies have examined both genetic and non-genetic parental effects in organisms, particularly insects. For example, seed beetles, *Stator limbatus*, display different genetic and non-genetic parental effects depending on the fitness trait observed (Fox 1995). Overall, no evidence that the beetles were acclimated to perform better through non-genetic parental effect was observed in survivorship, development time, and body size of the offspring (Fox 1995). However, they observed a non-genetic maternal effect on the development times and size of the offspring based on the host the mother was reared on (Fox 1995). They further observed both non-genetic maternal and paternal effects on offspring survivorship (Fox 1995).

However, the extent of simultaneous expression of genetic and non-genetic effects was not accounted for.

Egg size is a notable life history trait that can be influenced by genetic or non-genetic effects. For example, in the frog *Bombina orientalis*, it was found that a greater non-genetic effect is present where egg size was positively associated with a shift in female body mass due to the environment (Kaplan 1997). In another study, *Nezara viridul*a (Southern green stink bug) displayed a decrease in egg mass under highly stressful conditions (McLain & Mallard 1991). Non-genetic effects that influenced the egg mass included the location of the egg relative to the cluster of eggs laid at once (McLain & Mallard 1991). Furthermore, larger females produced larger eggs (McLain & Mallard 1991).

The time to emergence and size at emergence is other life history traits that can be influenced by genetic or non-genetic effects. Time to emergence has been linked to egg mass in prior studies. *Callosobruchus chinesis* displayed a decreasing development time as the egg size increased (Yanagi 2010). This paved the way for a potential interpretation that a non-genetic maternal effect was present where larger females may have simply laid larger eggs, which would have taken a shorter time to develop (Yanagi 2010). Interestingly, size at emergence varies greatly where insects such as *Stator limbatus* grow to a constant size independent of the egg mass (Fox 1997). This is usually at the cost of development where the time to emergence is elongated to help account for the small egg mass and develop an adult with a constant size (Fox

1997). Therefore, tradeoffs between time to emergence and size to emergence have been observed.

The life history trait of lifespan has displayed both a genetic and non-genetic effect. *Caenorhabditis elegans* have displayed varying lifespan data when their diet has been restricted (Stastna 2015), suggesting a non-genetic effect on lifespan. Furthermore, in Latvian brown cows, the non-genetic factor of farm size has affected the cow's lifespan; larger farms were found to decrease the length of productive life of these cows (Cielava 2014). Within *Callosobruchus maculatus*, the maternal age also appeared to affect the lifespan of offspring (Fox, Bush & Wallin 2003). The older the maternal age was, the longer the lifespan was for the offspring (Fox, Bush & Wallin 2003). This trend may be due to the mother's ability to package different mRNAs and proteins into the eggs that are produced later in the mother's lifespan (Fox, Bush & Wallin 2003).

To explore the extent of genetic and non-genetic effects, bean beetles were used as the organism of interest. Bean beetles, *Callosobrunchus maculatus*, are agricultural pest insects most commonly found in Africa and Asia (Beck & Blumer, 2014). They lay their eggs on the surface of beans, which the larvae burrow into to obtain nutrients. The beetles exclusively lay their seeds on legumes, notably dry beans with the genus *Vigna* (Beck & Blumer, 2014). The life cycle of a bean beetle starts as a fertilized egg on the surface of its host beans. Soon after burrowing into the bean as larvae, the bean beetle will feed on the insides of the bean to obtain nutrients that will last them their entire lifespan. This is because bean beetles do not feed as adults. The emergence time from the beans takes about three to four weeks under ideal conditions. Previous studies have found that temperature and humidity greatly determine the time of emergence (Howe and Currie 1964, Schoof 1941). Therefore, a temperature of 30˚C and relative humidity of 50% was determined to provide the most success in the emergence rate from the beetles (Beck & Blumer, 2014). Once the beetles emerge, they will be fully developed adults in 24 to 36 hours (Beck & Blumer, 2014). The lifespan of adults is usually between 10-14 days (Beck & Blumer, 2014).

Previous studies have tried to observe maternal genetic influences on egg size and larval performance (development time and adult size) on *Vigna angularis*, azuki beans. (Fox 1993b). Initially predicting a maternal genetic effect, it was found that *Callosobruchus maculatus* body size and egg size were heritable characters with no non-genetic maternal effect (Fox 1993b). It was determined that body and egg size exhibit additive genetic variation that has been passed down through generations (Fox 1993b). However, when it comes to early larval life history traits such as egg size, there was a maternal size effect where larger mothers produced larger eggs (Fox 1993b). This in turn lengthened the development time of the offspring (Fox 1993b). However, this trend did not continue into adulthood where egg size had no significant lasting effect on adult size (Fox 1993b).

However, in these studies, genetic and non-genetic maternal and paternal effects and offspring host effects have been studied in isolation. Therefore, we were interested in how these effects may interact and influence offspring life history traits.

Therefore, within our study, we isolated virgin females and males from different combinations of bean host backgrounds that were mated while comparing offspring on different combinations of bean hosts. For both mated adults and offspring, black-eyed peas (*Vigna unguiculata*) or adzuki beans (*Vigna angularis*) were used as the host types.

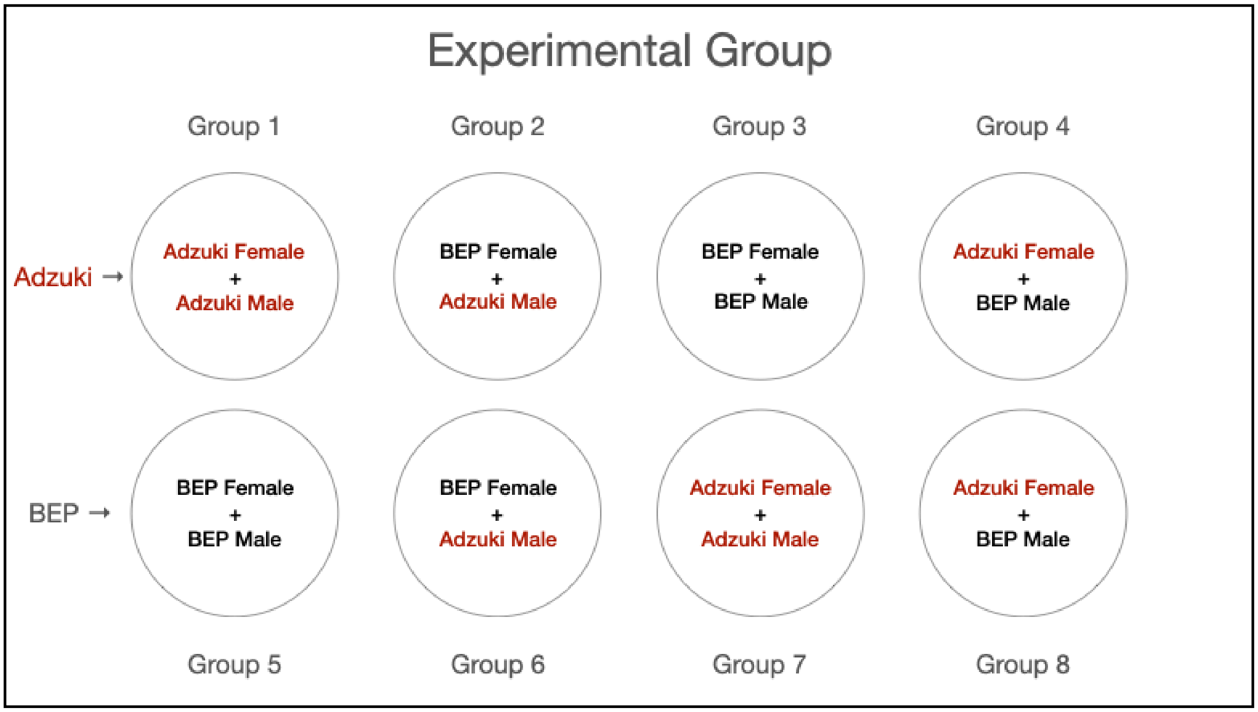


Figure 0: Eight experimental groups. Groups 1-4 used adzuki beans as the host type. Groups 5-6 used BEP as the host type.

This was to allow us to isolate maternal and paternal genetic and non-genetic effects, as well as offspring host effects, on four life history traits (egg size, development time, emergence size, and adult lifespan).

It was predicted that overall non-genetic effects would have the greatest impact on the life history traits of bean beetles. For example, it has been previously observed that black-eyed peas are considered higher-quality environments that support the normal development of bean beetles compared to adzuki beans, which are considered a lower-quality environment (Beck & Blumer, 2014). Therefore, it is reasonable to assume that regardless of the offspring's genetics, if the host type of the offspring is black-eyed peas, then the offspring may naturally become larger adults or have a longer lifespan. There may also be the possibility that larger females may produce larger offspring regardless of genetics. Of course, there is the possibility that the mothers may hold a gene that codes for larger beetles, which may in turn allow their offspring to become relatively larger. The same can be said for time to emergence and adult lifespan.

Methods:

The sex-specific genetic and non-genetic effects and offspring host effects on four life-history traits were observed in the bean beetle, *Callosobruchus maculatus*. The four life-history traits included egg size, time to emergence, size at emergence, and adult lifespan.

Therefore, within our study, we decided to isolate virgin females and males while comparing offspring on different combinations of host groups black-eyed peas (*Vigna unguiculata*) or adzuki beans (*Vigna angularis*). The experimental groups were as depicted in Figure 0. This was to allow us to isolate maternal and paternal genetic and non-genetic effects, as well as offspring host effects, on four life history traits (egg size, development time, emergence size, and adult lifespan).

The source population of the bean beetles used in the study was originally collected from a culture of beetles that infested beans in a grocery store in Columbus, OH. Large replicate stock cultures have been maintained on organic black-eyed peas (*Vigna unguiculata*) and organic adzuki beans (*Vigna angularis*) at 30˚C for over 20 years.

To isolate virgin adult beetles from different bean hosts for experimental crosses, beetles from multiple stock populations were isolated from black-eyed peas or adzuki beans. Initially, 50 plates with 18 adzuki beans each were set up with a single female bean beetle on each plate from an adzuki stock culture. One week later, 50 plates with 30 black-eyed peas each were set up with single female bean beetles on each plate from a black-eyed pea stock culture. The delayed setup for black-eyed pea cultures was because adult emergence from adzuki beans takes 2-3 weeks longer for beetles reared on adzuki beans as compared to those reared on black-eyed peas (Beck & Blumer, 2014). All the bean beetles were incubated within an incubator set at 30˚C. After setting up the cultures, they were constantly observed for beans with eggs on them. Whenever a bean with at least one egg was observed, the bean was removed from the plate and placed in a 12-well tissue culture plate to prevent further eggs from being laid on the bean and maximize the probability of maintaining an emerged adult's virginity. Virgins were assigned to eight different groups (Figure 0). Virgins were randomly assigned to each group. However, for experimental groups in which males and females were from the same host type, we made sure to select males and females from different parents to prevent inbreeding. Once all the virgin adults were added to their respective groups, the pairs were allowed to mate and freely lay eggs on their separate bean types for three days. Each treatment group was replicated 10 times.

Initially, with the groups that had adzuki beans as the offspring host type (Groups 2, 4, 6, 8), the parental generations were allowed to mate for 4 days, which resulted in at least 10 eggs on average per group. However, for the groups that have BEP as the offspring host type (Groups 1, 3, 5, 7), the parental generation was allowed to mate for 7 days (3 days longer than the adzuki host) because, after 4 days, there were less than 10 eggs on average per group. After the respective times passed, the parental generation was removed and the beans with eggs were incubated at 30˚C. As the offspring emerged, the date on which the beetle emerged was noted to determine the time to emergence. It is important to note that there may be some variance in the time at which the beetles emerged from the eggs because eggs may have been laid at any point during the mating period which was not recorded. The egg from which the beetle emerged was also marked and measured in width and length to obtain the egg size. The beetle's emergence size (the size of the beetle that emerges from the egg) was also measured using a microscope, defined by the wing length of the beetle.

Four male and four female offspring from each replicate of each treatment group were used to measure adult lifespan. The objective was to obtain at least four male and four female offspring from each replicate to measure the lifespan while the remaining offspring were used to measure the emergence time and beetle size. This was to ensure a high sample size. The individuals that were measured for lifespan were individually placed in a tissue culture plate and left alone until they were dead. The day on which the beetle was non-responsive was referred to as the "death date" and adult lifespan was defined as the number of days between the emergence date and death date. Bean beetles are known to feign death and it was important to determine that the beetle was dead and not feigning death (Beck & Blumer, 2014).

To determine the effects of the maternal host, paternal host, and offspring host on offspring life history traits, we used a series of linear mixed-effects models with main effects, all two-way interactions, and the three-way interaction as the fixed effects and replicate as the random effect to control for the non-independence of offspring from the same mating. The size at emergence, time to emergence, and lifespan models were run separately for males and females due to sexual size dimorphism in bean beetles. The models were estimated with the *lme4* package in R (Bates, Mächler, Bolker, Walker 2015). The significance of fixed effects was estimated with the *car* package (Fox, Weisberg 2019), and estimated marginal means were estimated with the *emmeans* package (Russell 2023).

To examine potential relationships among life history traits that might explain the effects of host types, we constructed a series of path models based on potential biological linkages between life history traits (Figure 12, 13).

The significance of each effect on the life history traits was estimated with the *sem* function (Jorgensen, Pornprasertmanit, Schoemann, & Rosseel 2022) in the *lavaan* package. Path models were visualized with the *lavaanPlot* package (Rosseel 2012).

Results:

Part 1: Overall Effect of Host Type on Life History Traits

Egg Size

Maternal, paternal, and offspring host, as well as all two-way interactions, and the three-way interaction, had a statistically significant impact on egg size (P<0.001, Table 1). When both the male and female in a mating were reared on adzuki beans and the eggs were laid on BEP, the egg size was the smallest (Figure 1). However, no other host treatment group combinations were statistically different.

Table 1: ANOVA for a linear mixed-effects model examining maternal, paternal, and offspring host effects on egg size.

Source	**Chisq**	**df**	**P**
Maternal Host	92.79	1	< 0.001
Paternal Host	27.78	1	< 0.001
Offspring Host	55.58	1	< 0.001
Maternal Host : Paternal Host	69.48	1	< 0.001
Maternal Host : Offspring Host	35.67	1	< 0.001
Paternal Host : Offspring Host	56.09	1	< 0.001
Maternal Host : Paternal Host : Offspring Host	41.05	1	< 0.001

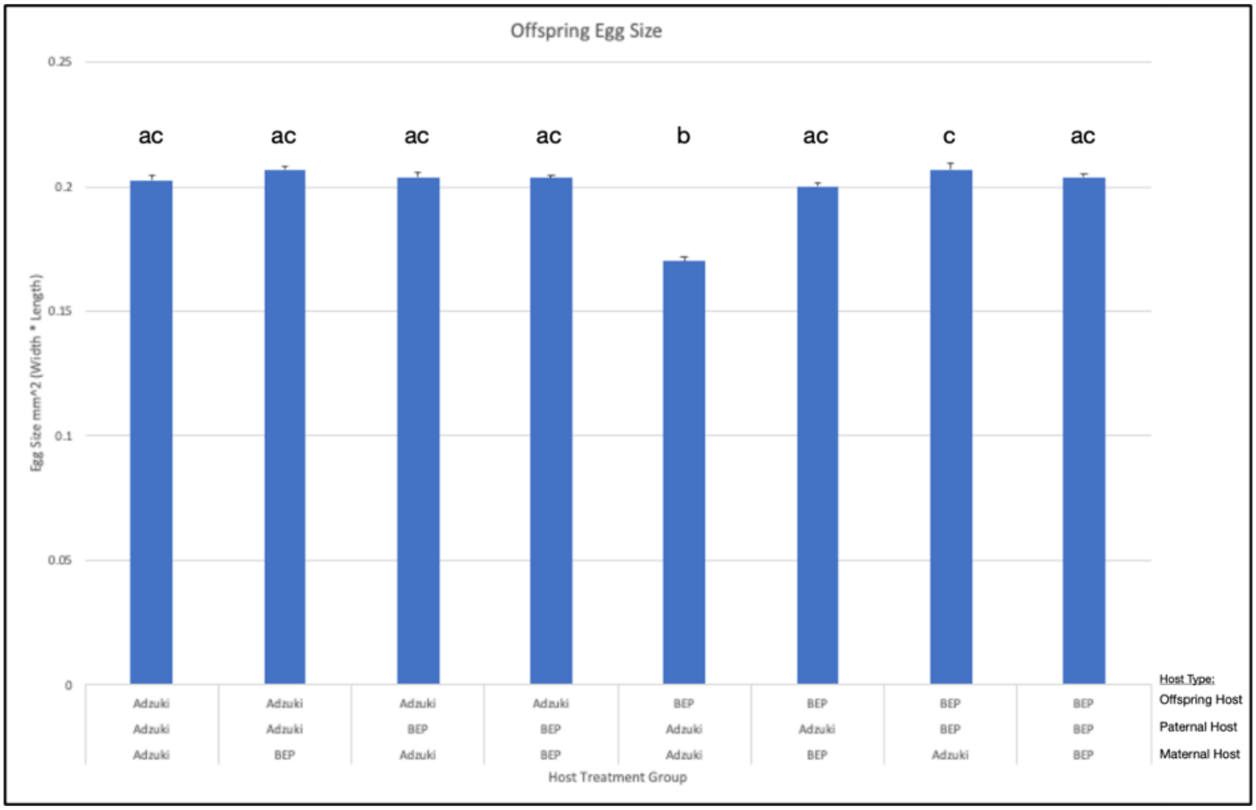


Figure 1: Effect of different host treatment combinations on egg size. The graph is based on the estimated marginal means (±1 SE). The letters above the bar graph depict the relative significance of the individual host treatment group compared to the other groups. If the same letter is shared, the groups have no statistical significance.

Time to Emergence

For female offspring, time to emergence was significantly affected by the maternal host and all two-way interactions ($P<0.05$, Table 2). When the maternal and offspring hosts were the same while the paternal host differed, the emergence time of females was significantly shorter (Figure 2). For example, when the maternal and offspring hosts were adzuki beans and the paternal host was BEP time to emergence was shorter. A similar result was found when the paternal host was adzuki beans and the maternal and offspring hosts were BEP (Figure 2).

Table 2: ANOVA for a linear mixed-effects model examining maternal, paternal, and offspring host effects on female offspring emergence time.

Source	**Chisq**	**df**	**P**
Maternal Host	6.84	1	0.0089
Paternal Host	0.46	1	0.497
Offspring Host	0.39	1	0.533
Maternal Host : Paternal Host	12.06	1	< 0.001
Maternal Host : Offspring Host	8.57	1	0.0034
Paternal Host : Offspring Host	7.30	1	0.0069
Maternal Host : Paternal Host : Offspring Host	3.47	1	0.063

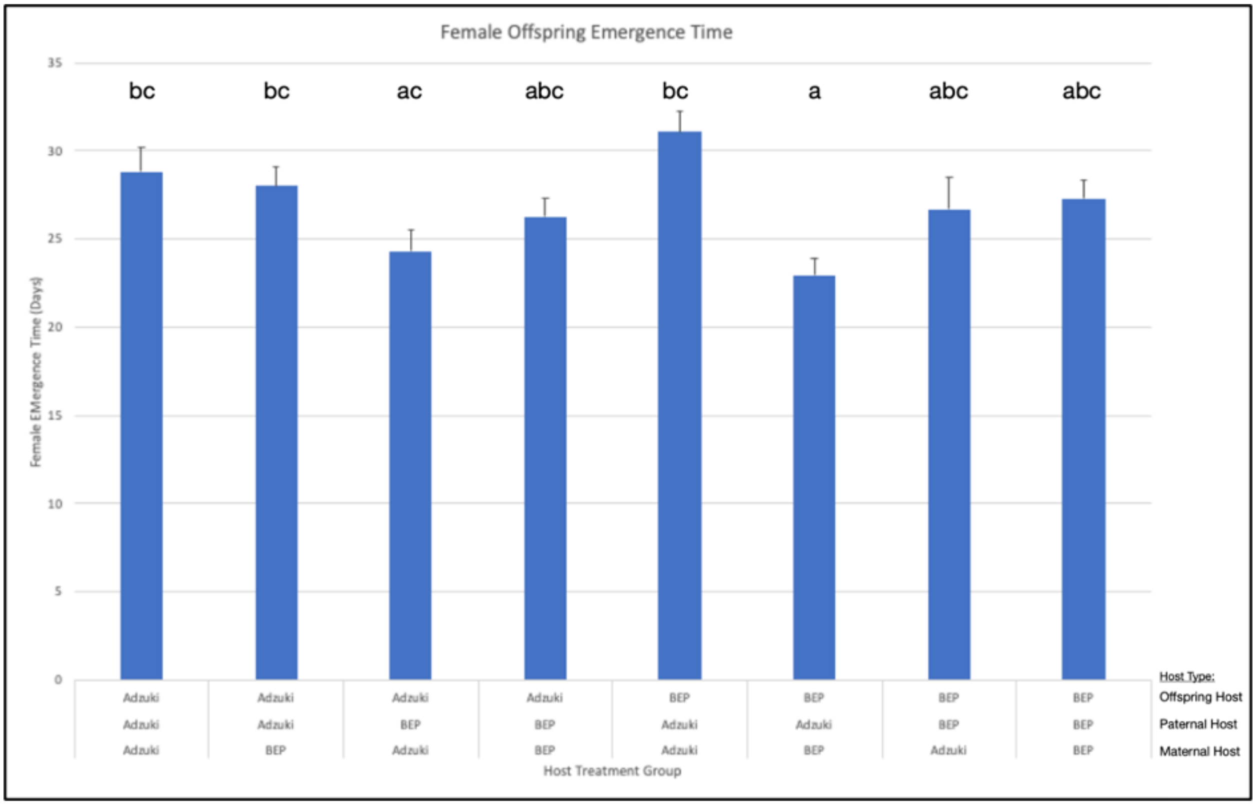


Figure 2: Effect of different host treatment combinations on female offspring emergence time. The graph is based on the estimated marginal means (±1 SE). The letters above the bar graph depict the relative significance of the individual host treatment group compared to the other groups. If the same letter is shared, the groups have no statistical significance.

In contrast to female offspring, time to the emergence of male offspring showed a significant effect of paternal and offspring hosts and all two-way interactions ($P<0.05$, Table 3). When the maternal and offspring host was the same while the paternal host differed, the emergence time of males was significantly shorter as compared to when the maternal and paternal host was the same but differed from the offspring host (Figure 3). For example, the offspring, paternal, and maternal host combinations of adzuki, BEP, and adzuki, respectively, had a significantly shorter time to emergence (Figure 3). Furthermore, the time to emergence appeared to be slightly longer (Figure 3).

Table 3: ANOVA for a linear mixed-effects model examining maternal, paternal, and offspring host effects on male offspring emergence time.

Source	**Chisq**	**df**	**P**
Maternal Host	3.73	1	0.0533
Paternal Host	5.81	1	0.0159
Offspring Host	5.71	1	0.0168
Maternal Host : Paternal Host	25.37	1	< 0.001
Maternal Host : Offspring Host	13.90	1	< 0.001
Paternal Host : Offspring Host	8.23	1	0.0041
Maternal Host : Paternal Host : Offspring Host	1.63	1	0.202

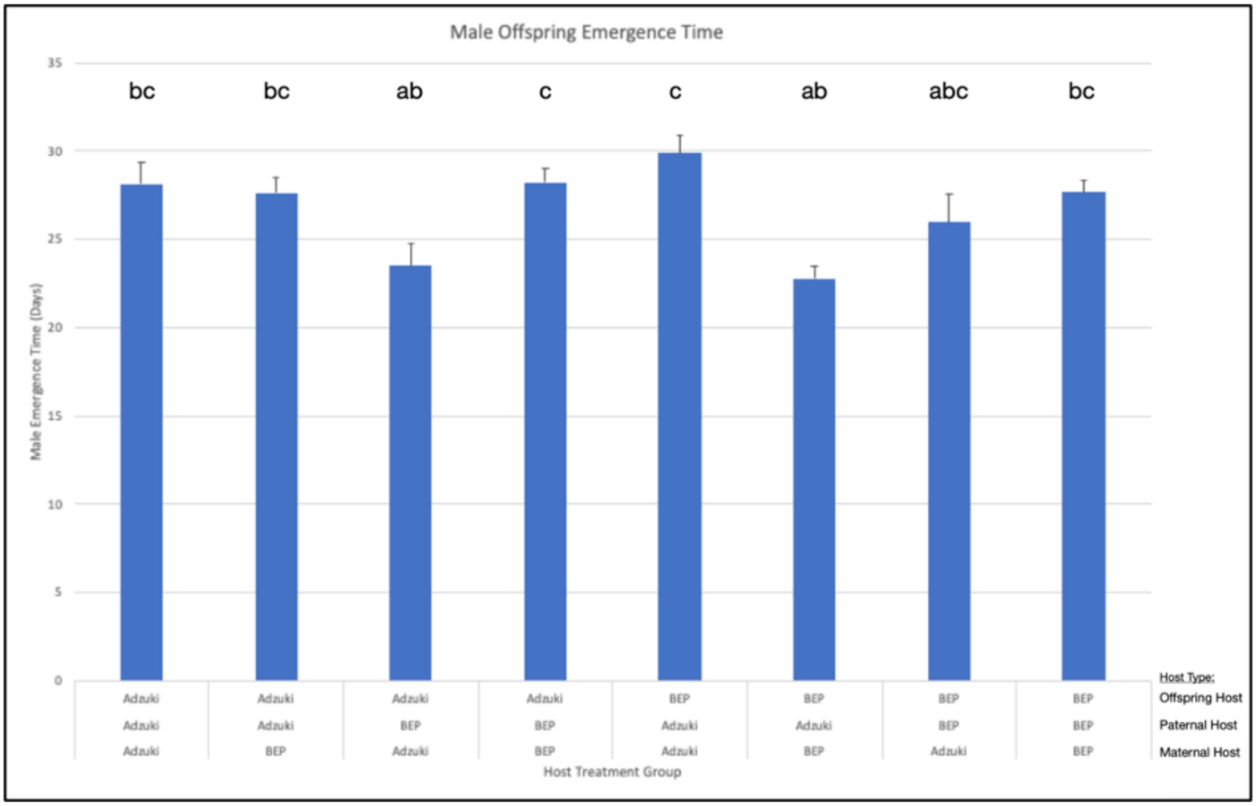


Figure 3: Effect of different host treatment combinations on male offspring emergence time. The graph is based on the estimated marginal means (±1 SE). The letters above the bar graph depict the relative significance of the individual host treatment group compared to the other groups. If the same letter is shared, the groups have no statistical significance.

Size at Emergence

For female offspring, size at emergence was significantly affected by all individual effects, two-way interactions, and the three-way interaction (P<0.001, Table 4). The female size was significantly smaller when the offspring host was adzuki and the maternal host was BEP (Figure 4). Furthermore, when the maternal and paternal hosts were both adzuki and the offspring host was BEP, the female size was larger (Figure 4).

Table 4: ANOVA for a linear mixed-effects model examining maternal, paternal, and offspring host effects on female offspring size.

Source	**Chisq**	**df**	**P**
Maternal Host	543.73	1	< 0.001
Paternal Host	61.43	1	< 0.001
Offspring Host	866.18	1	< 0.001
Maternal Host : Paternal Host	90.53	1	< 0.001
Maternal Host : Offspring Host	80.12	1	< 0.001
Paternal Host : Offspring Host	34.16	1	< 0.001
Maternal Host : Paternal Host : Offspring Host	22.68	1	< 0.001

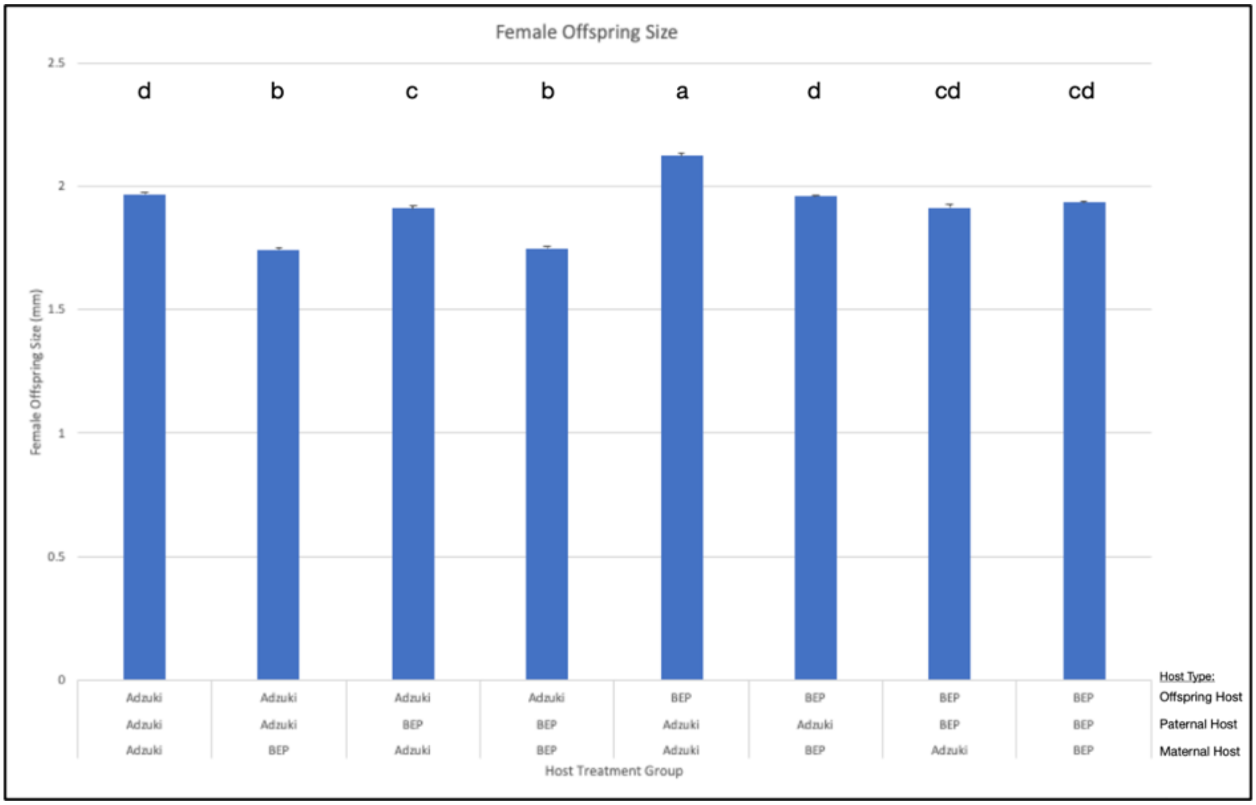


Figure 4: Effect of different host treatment combinations on female offspring size. The graph is based on the estimated marginal means (±1 SE). The letters above the bar graph depict the relative significance of the individual host treatment group compared to the other groups. If the same letter is shared, the groups have no statistical significance.

For male offspring, the size at emergence showed a significant effect of all the individual hosts, two-way interactions, and three-way interactions (P<0.001, Table 5). When the offspring host and maternal host were the same while the paternal host was adzuki, the offspring size was smaller (Figure 5). Furthermore, when the paternal and maternal hosts were the same and the offspring host was BEP, the offspring size was larger (Figure 5).

Table 5: ANOVA for a linear mixed-effects model examining maternal, paternal, and offspring host effects on male offspring size.

Source	**Chisq**	**df**	**P**
Maternal Host	73.20	1	< 0.001
Paternal Host	186.78	1	< 0.001
Offspring Host	9.88	1	0.00167
Maternal Host : Paternal Host	13.22	1	< 0.001
Maternal Host : Offspring Host	31.45	1	< 0.001
Paternal Host : Offspring Host	41.56	1	< 0.001
Maternal Host : Paternal Host : Offspring Host	134.20	1	< 0.001

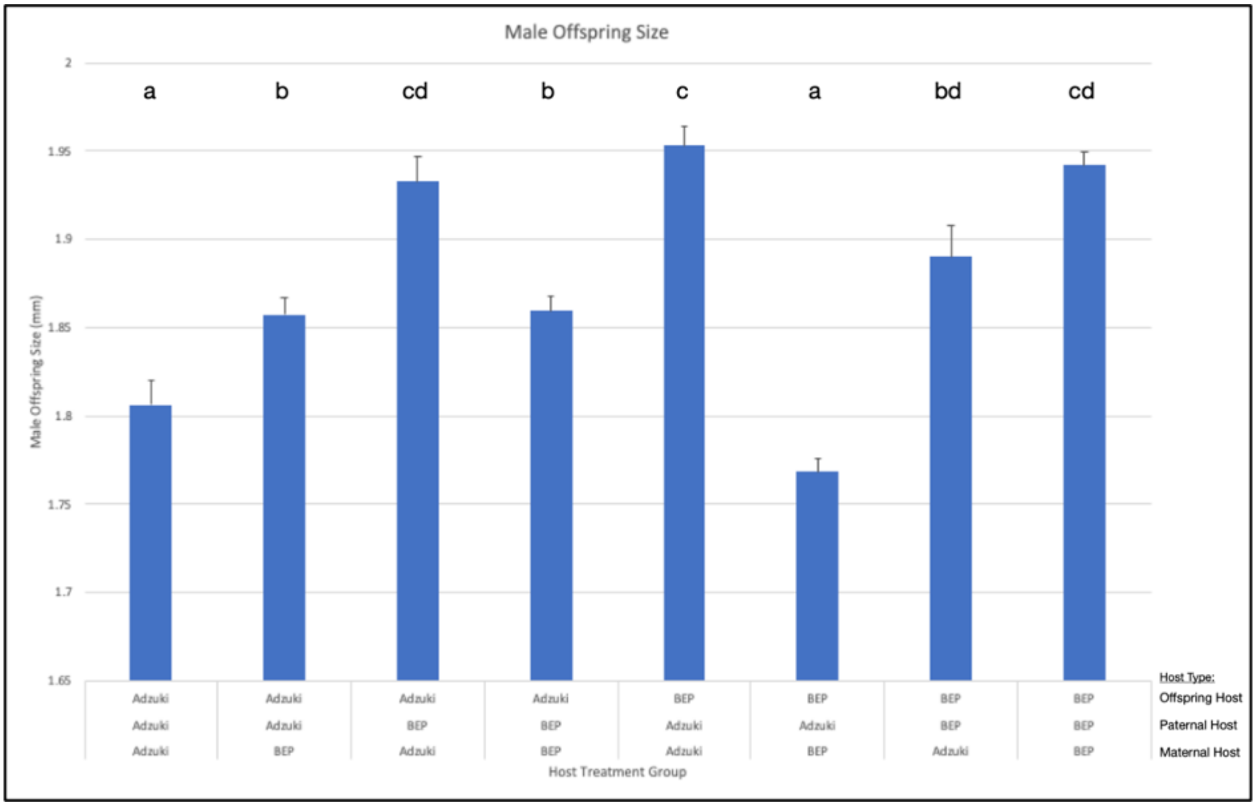


Figure 5: Effect of different host treatment combinations on male offspring size. The graph is based on the estimated marginal means (±1 SE). The letters above the bar graph depict the relative significance of the individual host treatment group compared to the other groups. If the same letter is shared, the groups have no statistical significance.

Adult Lifespan

For female offspring, the adult lifespan was significantly affected by all individual host types, and the two-way interaction between maternal and offspring hosts ($P<0.05$, Table 6). When the offspring host was adzuki and the maternal host was BEP, the female lifespan was significantly shorter (Figure 6). In contrast, all other combinations of host types did not show statistically significant differences in lifespan.

Table 6: ANOVA for a linear mixed-effects model examining maternal, paternal, and offspring host effects on female offspring lifespan.

Source	**Chisq**	**df**	**P**
Maternal Host	28.64	1	< 0.001
Paternal Host	7.33	1	0.0068
Offspring Host	25.45	1	< 0.001
Maternal Host : Paternal Host	0.037	1	0.857
Maternal Host : Offspring Host	21.91	1	< 0.001
Paternal Host : Offspring Host	0.101	1	0.750
Maternal Host : Paternal Host : Offspring Host	2.60	1	0.107

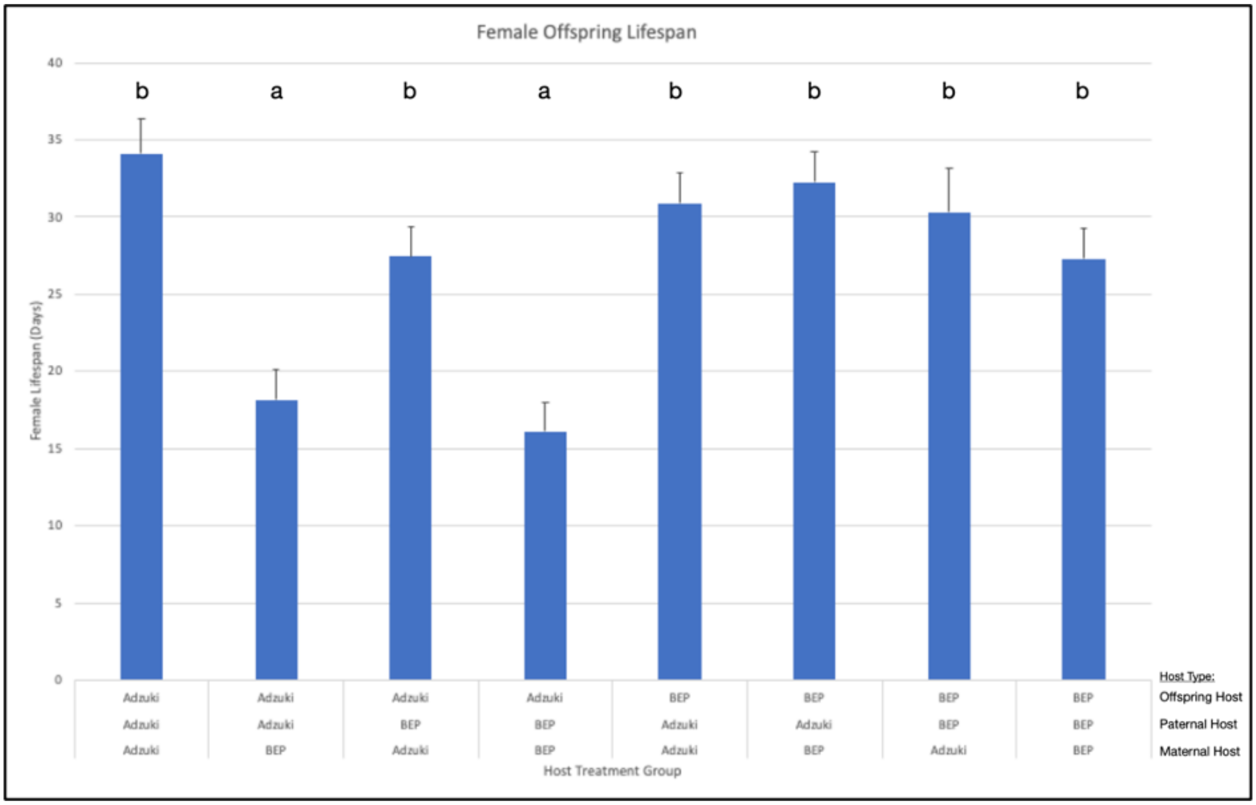


Figure 6: Effect of different host treatment combinations on female offspring lifespan. The graph is based on the estimated marginal means (±1 SE). The letters above the bar graph depict the relative significance of the individual host treatment group compared to the other groups. If the same letter is shared, the groups have no statistical significance.

For male offspring, the adult lifespan showed a significant effect from maternal and offspring hosts and the two-way interactions that included the maternal host ($P<0.05$, Table 7). When the offspring host was adzuki and the maternal host was BEP, the male lifespan was significantly shorter (Figure 7). Furthermore, when the maternal and paternal hosts differed, the male lifespan was slightly shorter; however, not all groups displayed this trend.

Table 7: ANOVA for a linear mixed-effects model examining maternal, paternal, and offspring host effects on male offspring lifespan.

Source	**Chisq**	**df**	**P**
Maternal Host	11.60	1	< 0.001
Paternal Host	1.54	1	0.215
Offspring Host	10.20	1	0.001
Maternal Host : Paternal Host	5.88	1	0.015
Maternal Host : Offspring Host	18.96	1	< 0.001
Paternal Host : Offspring Host	0.123	1	0.726
Maternal Host : Paternal Host : Offspring Host	0.0166	1	0.897

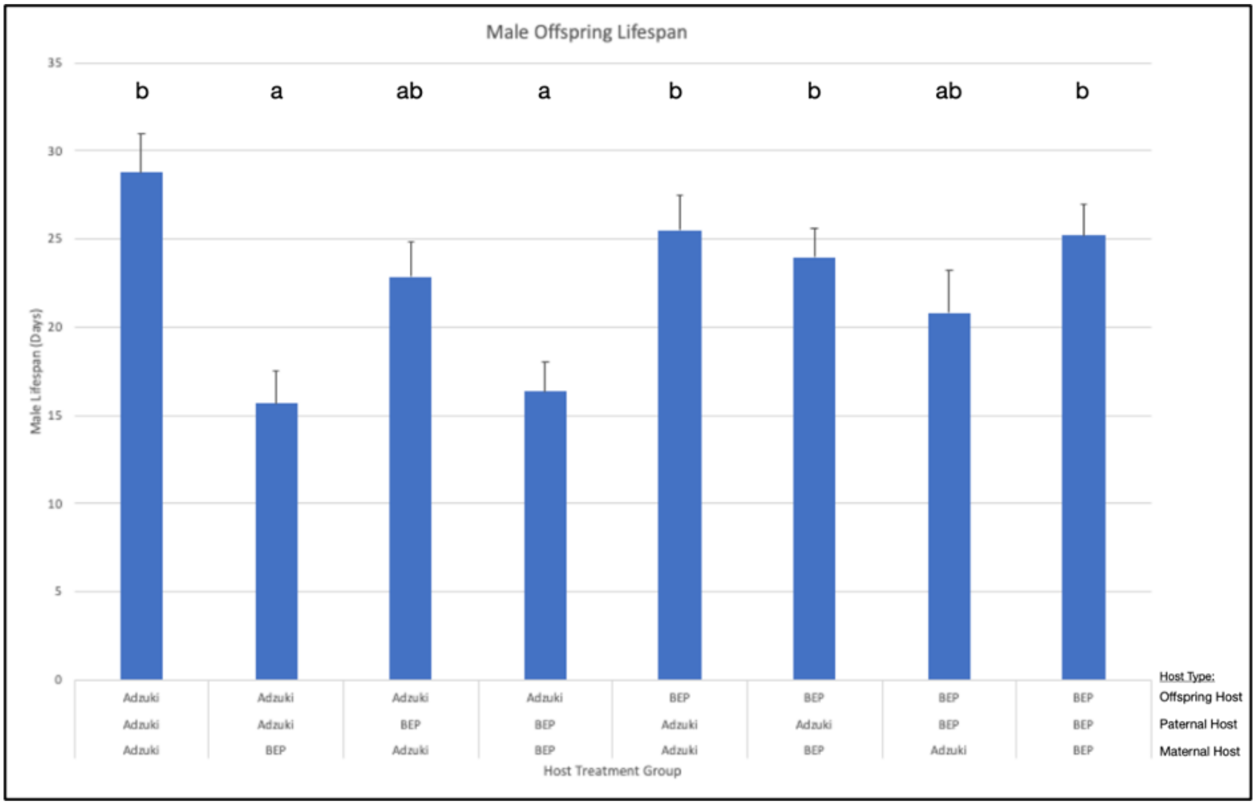


Figure 7: Effect of different host treatment combinations on male offspring lifespan. The graph is based on the estimated marginal means (±1 SE). The letters above the bar graph depict the relative significance of the individual host treatment group compared to the other groups. If the same letter is shared, the groups have no statistical significance.

Part 2: Mechanisms of how Life History Traits Interact

Before using structural equation models (SEMs) to understand the relationships between the offspring's life history traits and parental traits, the effect of the maternal and paternal host on the maternal and paternal size, respectively, was observed. For both paternal and maternal size, the host from which the mother or father emerged did not affect the size of the parental generation we used in mating (Maternal: F=1.406, df=78, P=0.239, Paternal: F=0.3924, df=78, P=0.533) (Figure 8).

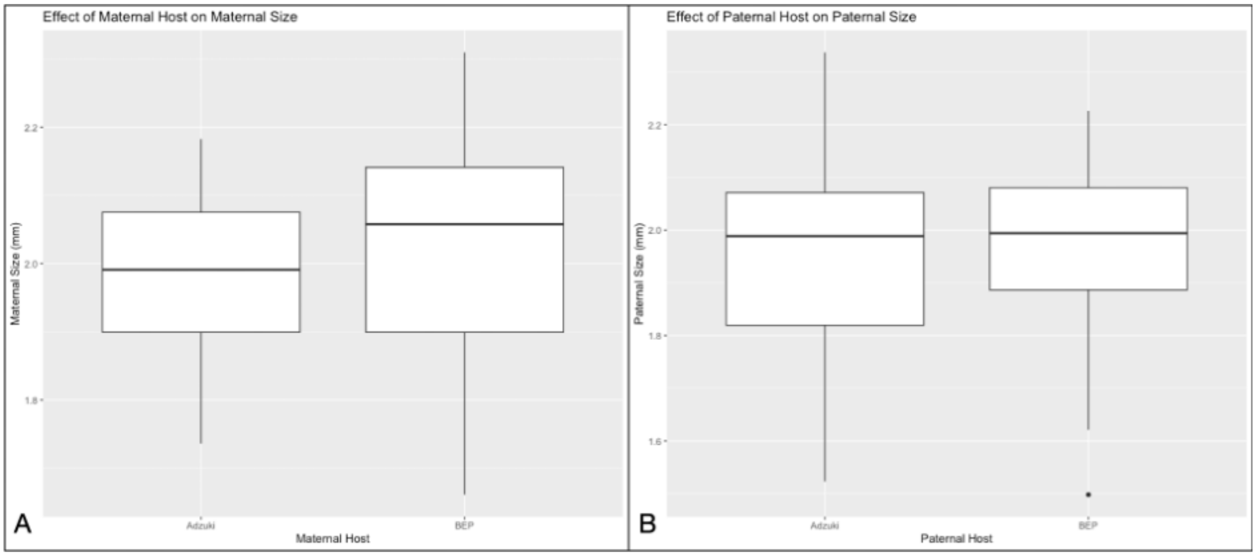
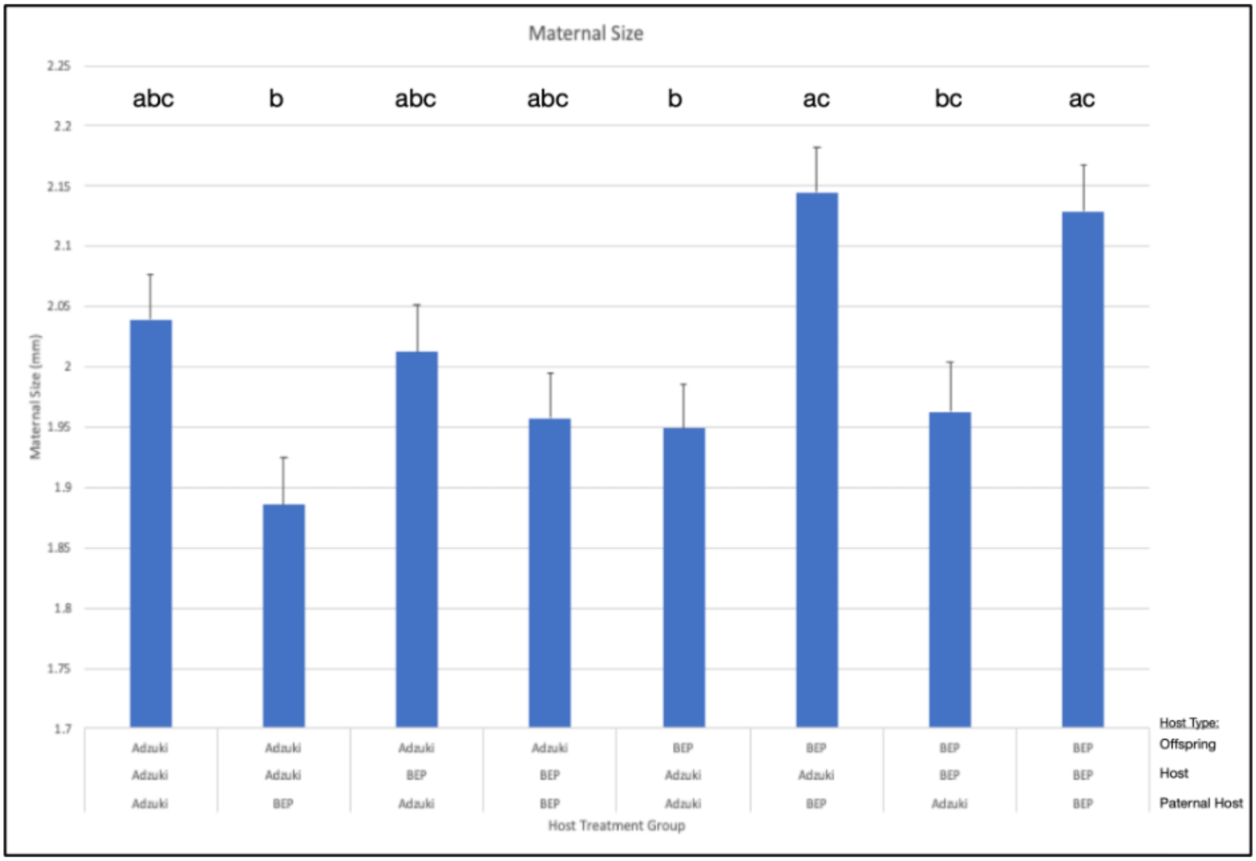


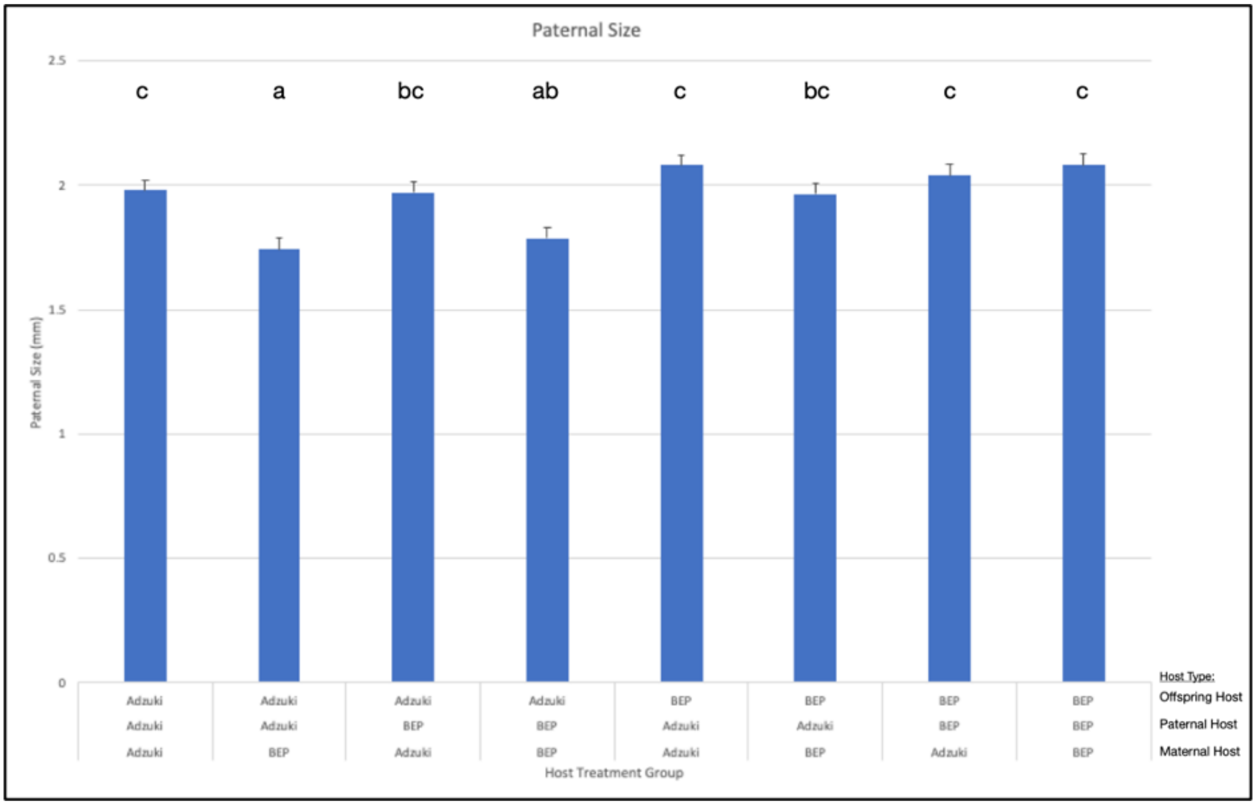
Figure 8: Box plot displaying the effect of maternal (A) and paternal (B) host treatments on maternal and paternal size. No statistical significance is observed in the relation between parental host type and parental size.

However, there were significant differences in maternal and paternal size across mating combinations due to chance that could have resulted in host effects of offspring life history traits (Figure 9, 10).



Source	**Sum Sq**	**df**	**P**
Maternal Host	0.02582	1	0.1737
Paternal Host	0.00213	1	0.6939
Offspring Host	0.10149	1	0.00818
Maternal Host : Paternal Host	0.00587	1	0.5145
Maternal Host : Offspring Host	0.38694	1	< 0.001
Paternal Host : Offspring Host	0.00283	1	0.6506
Maternal Host : Paternal Host : Offspring Host	0.01929	1	0.2389

Figure 9: Effect of different host treatment combinations on maternal size. (A) The graph is based on the estimated marginal means (±1 SE). The letters above the bar graph depict the relative significance of the individual host treatment group compared to the other groups. If the same letter is shared, the groups have no statistical significance. (B) The table depicts ANOVA for a linear mixed-effects model examining maternal, paternal, and offspring host effects on maternal size.



Source	**Sum Sq**	**df**	**P**
Maternal Host	0.30970	1	< 0.001
Paternal Host	0.01471	1	0.3693
Offspring Host	0.55428	1	< 0.001
Maternal Host : Paternal Host	0.05099	1	0.0971
Maternal Host : Offspring Host	0.13581	1	0.0077
Paternal Host : Offspring Host	0.00164	1	0.7634
Maternal Host : Paternal Host : Offspring Host	0.01325	1	0.3940

Figure 10: Effect of different host treatment combinations on paternal size. (A) The graph is based on the estimated marginal means (±1 SE). The letters above the bar graph depict the relative significance of the individual host treatment group compared to the other groups. If the same letter is shared, the groups have no statistical significance. (B) The table depicts ANOVA for a linear mixed-effects model examining maternal, paternal, and offspring host effects on paternal size.

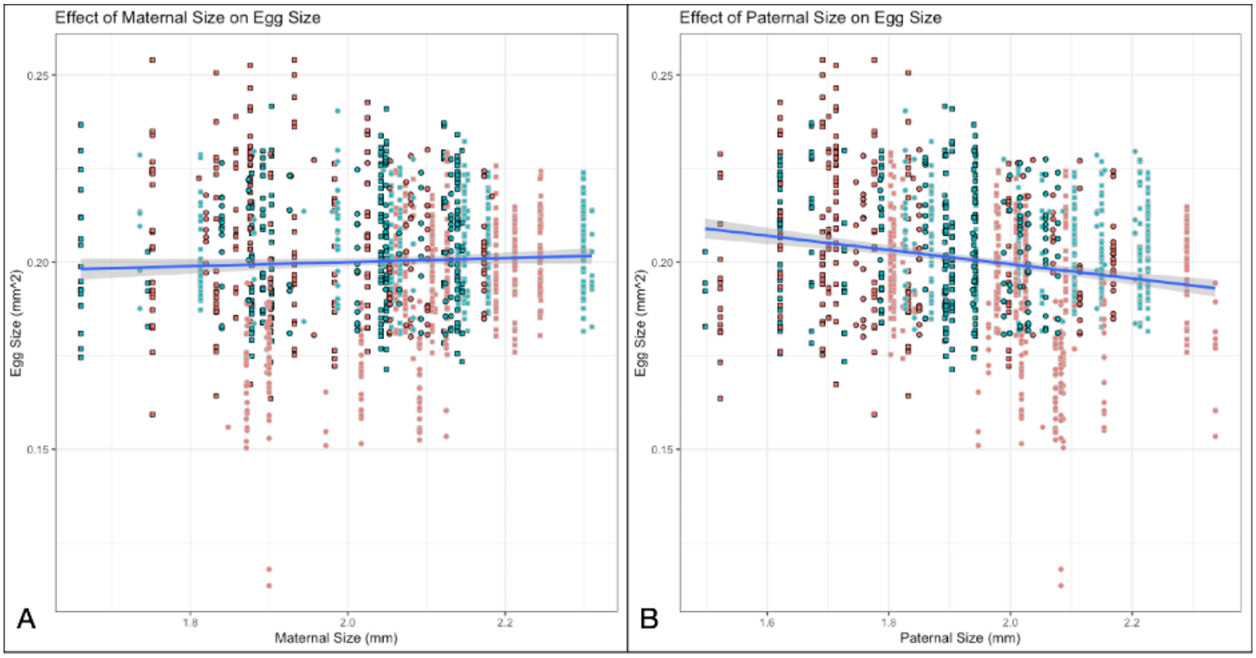


Figure 11: Scatter plot displaying the effects of maternal (A) and parental (B) size on egg size. No statistical significance is observed in the relation between maternal size and egg size. A negative trend is observed between the paternal size and egg size.

Then, the effects of the maternal and paternal size on egg size were plotted. The maternal size showed no effect on the egg size produced (Chisq=1.9534, df=1, P=0.1622) (Figure 11A). However, the egg size showed a decreasing trend as the paternal size increased (Chisq=8.1786, df=1, P=0.004) (Figure 11B). However, no interaction between the maternal and paternal size along with the host types was observed (Table 8).

Table 8: ANOVA for a linear mixed-effects model examining maternal, paternal, and offspring host & size effects on egg size.

Source	**Chisq**	**df**	**P**

Maternal Size	0.689	1	0.4067
Paternal Size	1.085	1	0.2977
Maternal Host	60.428	1	< 0.001
Paternal Host	35.918	1	< 0.001
Offspring Host	51.473	1	< 0.001
Maternal Host : Paternal Host	65.542	1	< 0.001
Maternal Host : Offspring Host	22.950	1	< 0.001
Paternal Host : Offspring Host	60.197	1	< 0.001
Maternal Host : Paternal Host : Offspring Host	38.717	1	< 0.001

SEM Model for Emergence Time and Offspring Size

When observing the SEM model fit for female offspring and their emergence time and size at emergence, the model was a poor fit for the data (P=0.007), with a significant p-value indicating that the relationships explained by the SEM were significantly different from the relationships in the data. On the contrary, when observing the SEM model fit for male offspring, their emergence time and size at emergence fit the model (P=0.217). The specific treatment groups that displayed significant relationships in the SEM model included the offspring, paternal, and maternal host combinations of “adzuki, adzuki, BEP”, “adzuki, BEP, BEP”, “BEP, adzuki, adzuki”, “BEP, adzuki, BEP”, “BEP, BEP, adzuki”, and “BEP, BEP, BEP”, respectively. The offspring, paternal, and maternal host combinations of “adzuki, adzuki, BEP” showed a strong trend negative trend between maternal size and offspring size. (Figure 12A) Furthermore, a weak negative trend was present between paternal size and offspring size. The offspring, paternal, and maternal host combinations of “adzuki, BEP, BEP” displayed a weak positive trend between paternal size and offspring size (Figure 12B). The offspring, paternal, and maternal host combinations of “BEP, adzuki, adzuki” displayed a strong positive trend between egg size and offspring size. In the offspring, paternal, and maternal host combinations of “BEP, adzuki, BEP”, egg size strongly negatively affected offspring size (Figure 12E). Paternal size also had a weak negative effect on offspring size. However, the maternal size had a strong positive effect on offspring size. The offspring, paternal, and maternal host combinations of “BEP, BEP, adzuki” displayed a strong positive effect between egg size and emergence time (Figure 12C). Finally, the offspring, paternal, and maternal host combinations of “BEP, BEP, BEP” showed a strong positive effect between paternal and egg sizes (Figure 12F).

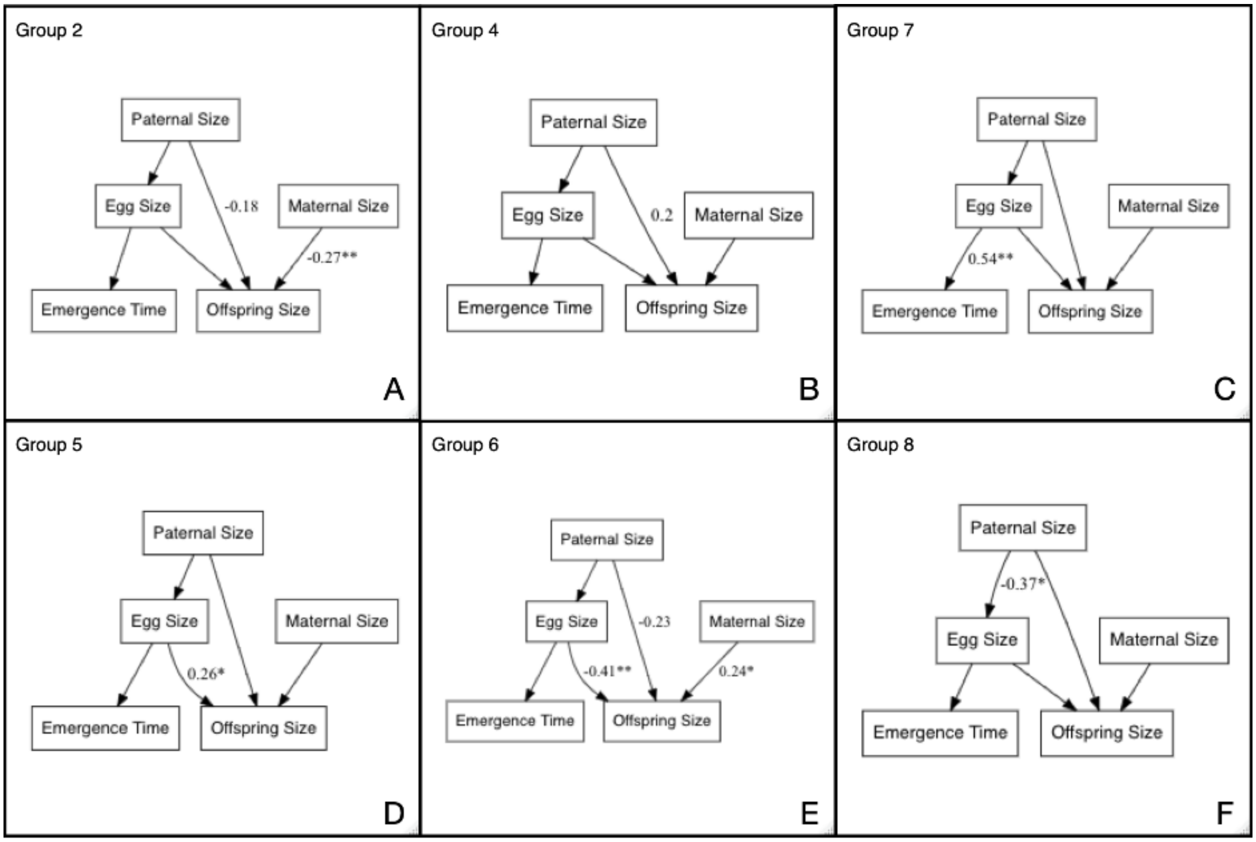


Figure 12: Structural equation model (SEM) of paternal, maternal, and egg size on the emergence time and size at emergence (offspring size). Each letter depicts a different group (host treatment combination) that displayed some sort of significance on the offspring's life history traits. Group 2 (adzuki, adzuki, BEP), Group 4 (adzuki, BEP, BEP), Group 5 (BEP, adzuki, adzuki), Group 6 (BEP, adzuki, BEP), Group 7 (BEP, BEP, adzuki), Group 8 (BEP, BEP, BEP).

SEM Model for Lifespan

When observing the SEM model fit for female offspring and their lifespan, the model was again a poor fit for the data (P<0.001). On the contrary, when observing the SEM model fit for male offspring, the model did fit their lifespan data (P=0.316). Males from the offspring, paternal, and maternal host combinations of “BEP, adzuki, adzuki” and “BEP adzuki, BEP” displayed a weak positive effect between offspring size and lifespan (Figure 13A, B). The offspring, paternal, and maternal host combinations of “BEP, BEP, adzuki” displayed a strong negative trend between offspring size and lifespan (Figure 13C).

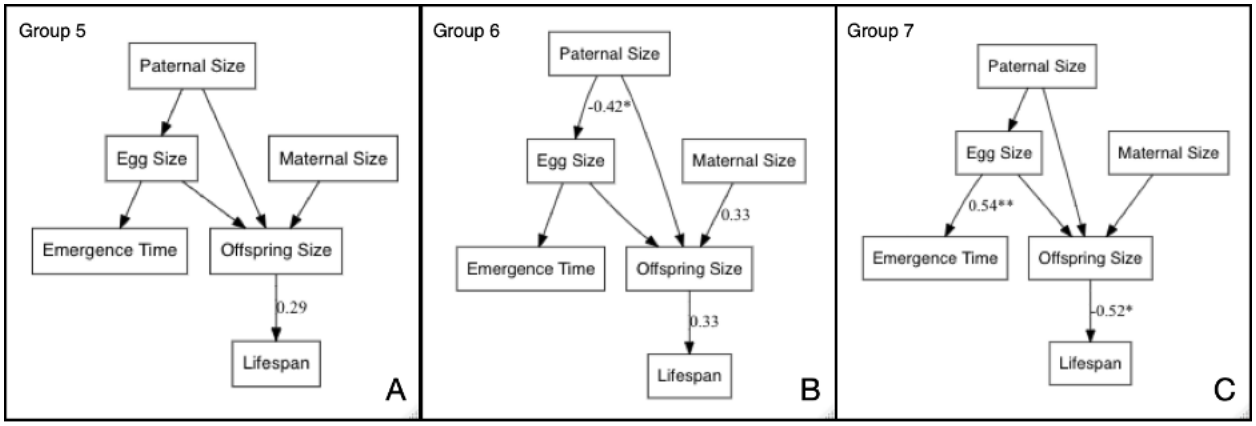


Figure 13: Structural equation model (SEM) of paternal, maternal, and egg size on the offspring’s lifespan. Each letter depicts a different group (host treatment combination) that displayed some sort of significance on the offspring's life history trait. Group 5 (BEP, adzuki, adzuki), Group 6 (BEP, adzuki, BEP), Group 7 (BEP, BEP, adzuki).

Discussion:

Previous studies have studied genetic and non-genetic maternal and paternal effects and offspring host effects in isolation. Therefore, we were interested in how these effects may interact and influence offspring's life history traits in *Callosobruchus maculatus*, bean beetles, on host groups of black-eyed peas (*Vigna unguiculata*) or adzuki beans (*Vigna angularis*).

We initially predicted that overall non-genetic effects would have the greatest impact on offspring life history traits of bean beetles. However, the results do not show a clear conclusion that non-genetic effects have the greatest impact. Instead, complex interactions of sex-specific genetic and non-genetic effects were present in the data. For all offspring life history traits (egg size, time to emergence, size at emergence, and adult lifespan) at least one two-way, if not three-way, interactions between the host type combinations were significant, which can be attributed to both genetic and non-genetic effects.

We also found varying results in our study compared to previous studies. In one previous study on bean beetles, the non-genetic maternal effects on the development times and size of the offspring based on the maternal host type were observed with no paternal effect (Fox 1995). However, we did not observe this clear trend where potential paternal genetic and non-genetic effects were present to give us mixed results.

Previously, larger females were found to produce larger eggs (Yanagi, 2010). However, in our data, we found no statistically significant trend where larger females produced larger eggs (Figure 11A). Time to emergence was also linked to egg size whereas shorter development time was linked to larger egg size (Yanagi, 2010). However, this trend was insignificant within our paper, except for the offspring, paternal, and maternal host type combination of adzuki, adzuki, and BEP, respectively, that produced a positive correlation between egg size and development time (Figure 12C).

Regarding the relationships between the offspring’s life history traits (time of emergence, size at emergence, and lifespan) and parental traits, a mix of genetic and non-genetic effects was present for the male offspring. However, it is difficult to conclusively state that a certain parental trait directly affects a particular offspring's life history trait all the time. It is difficult to state a clear effect attributed to the offspring’s life history trait because of the high significance in interactions between host types and variability in time to emergence, offspring size, and lifespan traits. An explanation for this may be that bean beetles can express both genetic and non-genetic effects but, are adapted to display certain effects under specific conditions that benefit them the most at a certain period. For example, with the offspring, paternal, and maternal host type combination of adzuki, adzuki, and BEP, respectively, the larger the parents are, the smaller the offspring may be to adjust for the possibility that more eggs were laid, and with larger males having larger spermatophore that allows for a greater clutch size.

The proposed mechanistic models (Figure 12, 13) were not a good estimate for the relationships between parental traits and female offspring life history traits. This may indicate that the variables affecting their life history traits were not observed for female offspring, and possible other traits such as clutch size may need to have been observed. Clutch size could play a role in determining if an observed parental trait was a genetic or non-genetic effect. Previous research found that insects such as bean weevil, *Acanthoscelides obtectus*, laid a larger clutch size on larger seeds (Szentesi, 2003). Overall, with clutch size, we can connect the other offspring life history traits and determine if trade-offs such as laying smaller eggs to adjust for a larger clutch size was present.

In future iterations of this experiment, one could implement the observation of traits such as clutch size and emergence rate to increase the number of relationships that can be observed between offspring life history traits and parental traits. An introduction of other high and low-quality beans can be introduced such as mung, *Vigna radiata*, and pigeon pea, *Cajanus cajan*, respectively. The introduction of greater diversity in bean qualities could give us a greater range of nutrients that could affect the life history trait variations present in the bean beetle.

Future directions could also include a continuation of this procedure and further observing the microbiomes of the parents and offspring. By observing the microbiome, we could observe the composition of the beetle's microbiome and understand if there is a differentiating microbe that is present within a beetle that is specific to a certain host type, or if the combination of different parental host types, can affect the microbiome of the offspring. It has been previously discussed how conserved, heritable, or variable the microbiome community of bean beetles is, where bean beetles were found to harbor a heritable bacterial community (Berasategui, Moller, Weiss, Beck, et al., 2021). With this data, we can try to observe if a particular microbe is present to better break down nutrients found within adzuki compared to BEP and if this microbe is carried on from the parental generation or is generated based on the offspring host type. Therefore, the possibility of a vertical transfer of genes can be observed for genetic, or non-genetic parental effects by observing variability in a similar mating set-up (Figure 0) as our experiment.

Bibliography:

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*(1), 1–48.

Beck, C. W., Blumer L. S. (2014). A Handbook on Bean Beetles, *Callosobruchus maculatus*. National Science Foundation

Berasategui, Aileen et al. (2021). "Symbiont Genomic Features and Localization in the Bean Beetle *Callosobruchus maculatus*." *Applied and environmental microbiology* vol. 87,12.

Cielava, Lāsma & Jonkus, Daina & Paura, Liga. (2014). Effect of farm size on the productivity and longevity of Latvian brown cows. Research for Rural Development. 1. 95-99.

Fox, C. (1994).Maternal and genetic influences on egg size and larval performance in a seed beetle (*Callosobruchus maculatus*): multigenerational transmission of a maternal effect?. *Heredity* **73**, 509–517.

Fox, C. W. (1993a). The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus (Coleoptera, Bruchidae).* Oecologia 96:139-146.

Fox, C. W. (1993b). Maternal and genetic influences on egg size and larval performance in a seed beetle (*Callosobruchus maculatus*) – multigenerational transmission of a maternal effect. Heredity 73:509-517.

Fox, Charles W. (2000). "Natural Selection on Seed-Beetle Egg Size in Nature and the Laboratory: Variation among Environments." *Ecology*, vol. 81, no. 11, pp. 3029–35. *JSTOR*

Fox, C.W., Bush, M.L. and Wallin, W.G. (2003), Maternal age affects offspring lifespan of the seed beetle, *Callosobruchus maculatus*. Functional Ecology, 17: 811-820.

Fox, C. W., Waddell, K. J., & Mousseau, T. A. (1995). Parental Host Plant Affects Offspring Life Histories in a Seed Beetle. Ecology, 76(2), 402–411.

Fox J, Weisberg S (2019). *An R Companion to Applied Regression*, Third edition. Sage, Thousand Oaks CA

Harney, E., Paterson, S. and Plaistow, S.J. (2017), Offspring development and life-history variation in a water flea depends upon clone-specific integration of genetic, non-genetic and environmental cues. Funct Ecol, 31: 1996-2007.

Jorgensen, T. D., Pornprasertmanit, S., Schoemann, A. M., & Rosseel, Y. (2022). semTools: Useful tools for structural equation modeling. R package version 0.5-6

Kaplan, Robert H., and Elizabeth G. King. (1997) “Egg Size Is a Developmentally Plastic Trait: Evidence from Long Term Studies in the Frog *Bombina Orientalis*.” *Herpetologica*, vol. 53, no. 2, pp. 149–65. *JSTOR*

Kawecki, T. J. (1995). Adaptive plasticity of egg size in response to competition in the cowpea weevil, *Callosobruchus maculatus (Coleoptera, Bruchidae).* Oecologia 102:81-85.

McGinley, MA, DH Temme, and MA Geber. (1987). Parental investment in offspring in variable environments: theoretical and empirical considerations. The American Naturalist 130: 370-398.

McLain, Denson & Mallard, Stephen. (1991). Sources and Adaptive Consequences of Egg Size Variation in *Nezara viridula* (Hemiptera: Pentatomidae). Psyche: A Journal of Entomology. 98. 10.1155/1991/10123.

Rosseel Y (2012). “lavaan: An R Package for Structural Equation Modeling.” *Journal of Statistical Software*, **48**(2), 1–36

Sorci, G., Clobert, J. (1997). Environmental maternal effects on locomotor performance in the common lizard (*Lacerta vivipara*). *Evol Ecol* **11**, 531–541.

S. R. Searle, F. M. Speed & G. A. Milliken. (1980). Population Marginal Means in the Linear Model: An Alternative to Least Squares Means, The American Statistician, 34:4, 216-221

Stastna, J., Snoek, L., Kammenga, J. *et al.* (2015). Genotype-dependent lifespan effects in peptone-deprived *Caenorhabditis elegans*. *Sci Rep* **5**, 16259

Stillwell, R.C. and Fox, C.W. (2005), Complex patterns of phenotypic plasticity: interactive effects of temperature during rearing and oviposition. Ecology, 86: 924-934.

Szentesi, Á. (2003), Resource assessment and clutch size in the bean weevil, *Acanthoscelides obtectus*.Pest. Manag. Sci., 59: 431-436

Vasudeva, R. (2023). Experimental evidence for stronger impacts of larval but not adult rearing temperature on female fertility and lifespan in a seed beetle. *Evol Ecol.*