Title : A proximate mechanism for evolutionary trade-offs in the bean beetle *Callosobruchus maculatus*

Running head: A proximate mechanism for trade-offs

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Conflict of interests

The authors have no conflict of interest to disclose.

Author's Contributions

SJLG contributed to generating the concept of the study as well as manuscript revision. FB contributed to generating the concept of the study, designing study methods, treatment preparations and applications, data analysis, manuscript drafting and revision and supervision of the study. DIUN helped design the study, carried out the outlined experimental methods, performed male behavioral data analysis, and helped draft the manuscript. BD carried out dissections, imaging, morphological data collection and helped revise the manuscript.

Abstract

The mechanisms underlying resource allocation and covariances in life history traits are at the core of evolutionary biology. Whilst previous studies have detailed multiple mechanisms for the differential allocation of resources toward life history traits, few studies have tested the contributions of these mechanisms toward both resource allocation and life history trait covariance. To address this, we explored the role of Juvenile Hormone (JH) in modulating resource allocation and trait correlations in the bean beetle *Callosobruchus maculatus*. We hypothesized that JH plays a critical role in determining the differential resource allocation to dispersal traits and gonads. To test our hypotheses, we manipulated JH levels in developing larvae using methoprene (a synthetic JH analog) and precocene (a JH synthesis inhibitor). We found support for our hypothesis as decreased JH generated individuals with larger wings and smaller gonads relative to the control group. We found consistent negative covariances between reproductive and dispersal traits across treatments, with JH shifting individuals along this trade-off axis without altering the relationship. Notably, these effects were sex-specific: in males, wing size was shaped by both testes size and JH treatment, whereas in females, wing size was related to ovariole size but unaffected by JH manipulation. Interestingly, we found no significant effect of increased JH on wing and gonad size. These findings provide direct evidence of JH's involvement in resource allocation and trait covariances in bean beetles.

Introduction

Life history trade-offs are fundamental to understanding the mechanisms behind ecological and evolutionary processes. Trade-offs are often quantified as negative covariances between costly traits (*e.g.*, body size, ornament length, pre-copulatory behaviors) in response to varying environmental conditions. In the face of limited resources, costly traits will compete, leading to the emergence of environment-specific strategies that selection can act upon, *i.e.* trade-offs (Roff, 2002; Stearns, 1989). Varying resource availability allows investigators to study the roles of resource allocation and covariance between an organism's costly traits (Messina & Fry, 2003). However, simply observing phenotypic correlations may lead to inaccurate accounts of negative correlations since spurious correlations may be observed between unrelated traits (de Jong & van Noordwijk, 1992; van Noordwijk & de Jong, 1986). Thus, it is also important to study the proximate causes of trade-offs since natural selection directly acts upon these physiological mechanisms (Sinervo & Svensson, 1998; Zera & Harshman, 2001).

Multiple ecological and physiological mechanisms have been associated with moderating the induction of trade-offs, which in turn can contribute to the development and maintenance of polymorphisms in many insect species (Fry, 2006; Gascoigne et al., 2022; Zera & Mole, 1994). Ultimate causes, like ecological perturbations, can shape populations through trade-offs. For example, the generation of dispersal morphs in some species results from intra-specific competition with increased larval density and low resource availability (Garland et al., 2022; Gascoigne et al., 2022). At the proximate level, hormones often act as underlying mechanisms behind life history trade-offs. In insects, one of the primary hormones associated with life history trade-offs is juvenile hormone (JH) (Sinervo & Svensson, 1998; Stearns, 1989; Zera & Harshman, 2001). In addition to its conserved role in larval development across insects (Emlen

& Nijhout, 2001; Fry, 2006; Gotoh et al., 2011; Nijhout, 1998), JH has also been implicated as a developmental switch determining the generation of dispersal and non-dispersal morphs in wing-polymorphic insects . JH acts by concurrently impacting wing size and other costly morphological and behavioral traits associated with reproduction, *e.g.* gonad size, courtship, fecundity, and timing of egg production in females and mate acquisition in males (Gascoigne et al., 2022; Guerra, 2011). Studies across numerous insect species have demonstrated that high larval density conditions typically lead to reduced juvenile hormone titers, promoting the development of dispersal morphs with enhanced flight capability, while low-density environments maintain higher JH levels that favor reproductive investment (Hartfelder & Emlen, 2012; Zera & Tiebel, 1988). This endocrine response to crowding provides a mechanistic link between ecological conditions and the adaptive resource allocation trade-offs observed in wing-polymorphic insects.

The bean beetle (*Callosobruchus maculatus*) serves as an excellent model organism for studying trade-offs due to its well-documented wing dimorphism (Caswell, 1960; Messina, 1991; Roff, 1986; Sanghvi et al., 2022; Southgate et al., 1957; Utida, 1956). Increased competition due to high larval density in the environment during early development results in individuals with long wings relative to body size thereby improving flight capability (Messina & Renwick, 1985; Taylor, 1974; Utida, 1972). Additionally, recent studies have demonstrated correlations between specific morphological traits and behavioral patterns in *C. maculatus* (Dick & Credland, 1984; Gascoigne et al., 2022; Katsuki & Lewis, 2015). In Gascoigne et al. (2022), we induced differential resource allocation to dispersal traits by allowing beetles to develop under different larval density treatments. We found that increased larval density generated individuals with larger wings and smaller gonads in both males and females. Overall, Gascoigne et al. (2022)

demonstrated that density during development affects trade-offs between dispersal and reproduction in *C. maculatus*. However, the proximate mechanisms governing the differential allocation of resources to traits that cause these trade-offs are not fully understood.

To test this mechanism, we exposed *C. maculatus* larvae to either a juvenile hormone (JH) analog, a JH antagonist, or a control. We predicted that if JH mediates trade-offs between dispersal and reproductive traits in *C. maculatus*, results from JH manipulations would mirror the effects of larval density on dispersal and reproductive traits observed in Gascoigne et al. (2022) in comparison to the control group. Specifically, (1) treatment with the JH analog (methoprene) will shift resource allocation toward reproduction, leading to individuals with relatively shorter wings and larger gonads, as seen in low density environments, while the JH antagonist (precocene) would produce the opposite effect, relatively longer wings and smaller gonads as observed in high larval density environments; and (2) we further predicted that altering JH levels may influence the trade-off (*i.e.*, the covariance) between dispersal and reproductive traits. Specifically, if JH regulates the trade-off directly, we expect to see systematic changes in trait covariation across treatments. However, if trade-offs are developmentally constrained, we expect JH to shift individuals along an unchanging trade-off slope.

Methods

Laboratory Population

The individuals in our study were sourced from a controlled laboratory population bred from a strain of *C. maculatus* at Lake Forest College in Illinois, USA. This cowpea-adapted strain is housed at 25°C under a 12hr:12hr light:dark cycle in 4-liter plastic containers containing cowpeas (*Vigna unguiculata*) and sealed with mesh plastic covers to ensure adequate ventilation.

Juvenile Hormone (JH) Treatments

To test the effect of JH in changing resource allocation towards wing and gonad size in *C. maculatus*, we treated the cowpeas used for larval development. Specifically, the cowpeas used for the generation of experimental individuals were treated with either precocene (a synthetic JH inhibitor), methoprene (a synthetic JH analog) and acetone as a control. We soaked 50 grams of cowpeas in a solution of either precocene diluted in acetone to a concentration of 10 mg/ml, methoprene diluted in acetone to a concentration of 10 mg/ml or pure acetone for 48 hours. The concentrations for all treatments were based on previous work in *C. maculatus* using juvenile hormone analogs applied to cowpea seeds (Abo El-Ghar, 1992) and supported by studies demonstrating strong developmental effects of methoprene in Coleoptera at lower concentrations when applied topically (Parthasarathy & Palli, 2009). This combination of prior research and our own pilot testing supports the biological relevance of our chosen treatment level and larval exposure method. Subsequently, the cowpeas were added to 48-well cell culture plates with a single bean in each well. To minimize potential plate effects, all three treatments were evenly distributed across every plate. Females selected haphazardly from the laboratory population were allowed 48 hours to oviposit, after which they were removed from all plates. Each female was confined to a single cowpea placed in an individual well of the 48-well plate, with the wells closed off to prevent access to other cowpeas. After oviposition, we manipulated larval densities to achieve medium density (5-7 eggs per cowpea) to minimize the confounding effects of larval density on resource allocation for the treatment groups. The plates were kept in an incubator (Powers Scientific environmental chamber DS27SD) at 27°C under a 12hr:12hr light:dark photoperiod for three to four weeks, the generation time of an adult *C. maculatus*. Towards the end of the third week, we checked the plates daily for the emergence of adults.

Once adults emerged, they were sexed and isolated. We only collected a single adult from each cowpea to standardize individuals by age and not use individuals who were siblings.

Dissections and morphological trait measurements

We dissected experimental individuals under a microscope (Leica MZ60-series stereo microscope) using FST fine forceps and Pyrex nine-well dissection well-plates. For all experimental individuals, we obtained one hind wing and gonad (a testis for males and an ovariole for females) as primary traits of interest. All specimens were mounted in DMHF (2,5-dimethyl-4-hydroxy-3(2H)-furanone) medium. Imaging was done using a Leica MZ16A stereoscope with a Leica DFC429 digital camera and Leica Application Suite imaging software. Trait sizes were quantified using ImageJ (Rasband, 1997).

Statistics and analysis

All gonad and wing size measurements were natural log-scaled to correct for increases in variance as absolute measures increase. Furthermore, we measured relative trait size as a measure of selective resource allocation by dividing the natural log of an individual's trait size by the natural log of the same individual’s body mass (Karp et al., 2012).

Rather than categorizing individuals into discrete groups, we treated dispersal and reproductive relative trait sizes as continuous variables since we found them to vary continuously (Messina & Renwick, 1985). This approach allowed us to detect treatment-induced shifts in trait allocation without imposing artificial categorical boundaries. To test our first prediction that increased juvenile hormone (JH) will alter resource allocation leading to the development of non-dispersal morphs, one-way ANOVAs were used to test for sex-specific main effects of the treatments on relative gonad and wing size and relative dispersal traits. Subsequently, Tukey-HSD post-hoc tests were used to show differences between groups.

To test our second prediction that JH may influence the covariation between reproductive and dispersal traits, we conducted sex-specific analyses of covariance (ANCOVAs) to identify the effects of JH treatment, gonad size, and gonad size × treatment interaction on relative wing length. Whilst the one-way ANOVA testing the first prediction demonstrates the main effect of JH treatment on reproductive and dispersal trait size, these effects are not independent. In many ectotherms, increases in reproductive trait size often lead to reductions in dispersal trait size and *vice versa* (Gascoigne et al., 2022; Miller et al., 2019; Roff & Fairbairn, 2007; Zera & Denno, 1997). Importantly, such trade-offs may either remain developmentally constrained, resulting in a consistent negative covariation across treatments, or they may be plastic. Manipulations to the underlying mechanisms from which trade-offs arise can alter the slope of allometric scaling between traits (Shingleton et al., 2007; Shingleton & Frankino, 2013). To distinguish between these possibilities, we included the gonad size × treatment interaction in the ANCOVA model. A significant interaction would indicate that JH modulates the slope of the trade-off, whereas a non-significant interaction would suggest that JH shifts individuals along a fixed trade-off axis.

All statistical analyses were performed in JMP® 13 Software (SAS Institute Inc. 2016).

Results

Resource allocation: Reduced juvenile hormone leads to allocation to dispersal

To test our first prediction that increased juvenile hormone (JH) will alter resource allocation by increasing investment in gonads, we measured gonad and wing sizes in beetles exposed to varying levels of JH during larval development.

JH treatment had a significant effect on testis size and wing size in male and female *Callosobruchus maculatus*. In males, treatment with the JH inhibitor precocene led to a significant decrease in testis size ($F_{(2,57)} = 4.25$, $p = 0.019$, Figure 1A) and increased wing length relative to body mass ($F_{(2,57)} = 9.93$, $p = 0.001$, Figure 1B) compared to the acetone control. Similarly, treatment with the JH inhibitor precocene in females led to a decrease in ovariole size ($F_{(2,57)} = 3.61$, $p = 0.03$, Figure 1C) and an increase in wing length relative to body mass ($F_{(2,57)} = 9.08$, $p<0.001$, Figure 1D) compared to the acetone control. In turn, treatment with a JH inhibitor led to an increased investment in dispersal-related morphological traits and a decreased investment in reproductive-related morphological traits in both male and female *C. maculatus*. However, the JH analog methoprene did not show the opposite effect of the JH inhibitor precocene. Contrary to our prediction, the JH analog methoprene showed no effect on gonad size (Figure 1A,C) or wing size (Figure 1B,D) in male and female *C. maculatus* relative to the acetone control ($p > 0.05$).

Trait covariances: Reproductive and dispersal traits covary negatively across treatments

To test our second prediction that JH levels may influence the trade-off (*i.e.*, the covariance) between dispersal and reproductive traits, we regressed relative wing length against relative gonad size controlling for JH treatment and relative gonad size × treatment interaction.

We found negative covariances between reproductive and dispersal traits in both sexes. In males, there was a significant effect of relative testis area ($F_{1,63} = 29.6$, $p <0.0001$) and JH treatment ($F_{1,63} = 3.72$, $p = 0.03$) on wing size, but no testis area × treatment interaction ($F_{1,63} = 0.04$, $p = 0.19$, Figure 2A). In females, we found an effect of relative ovariole area on wing size ($F_{1,63} = 31.85$, $p<0.0001$), but no effect of treatment ($F_{1,63} = 0.26$, $p = 0.77$) or ovariole area × treatment interaction ($F_{1,63} = 2.25$, $p = 0.11$, Figure 2B). These results, along with the average trait shifts shown in Figure 1, suggest that while JH affects mean trait values, it does not change the underlying allometric scaling (the trade-off slope) between these traits. Instead, JH treatment shifts individuals along the trade-off slope without altering the slope itself, indicating that the relationship between reproductive and dispersal traits remains stable despite the JH treatment (Frankino et al., 2019).

Discussion

Our results support the hypothesis that juvenile hormone (JH) mediates the physiological relationship governing trade-offs between dispersal and reproductive traits in response to environmental conditions. In *Callosobruchus maculatus*, these conditions, specifically larval crowding, appear to generate shifts in trait investment. Using the juvenile hormone analog (methoprene) and antagonist (precocene), we investigated the physiological effect of varying environmental conditions during development, *i.e.*, larval density, observed at a phenotypic level in Gascoigne et al. (2022). We found significant effects of the JH treatment on the reproductive and dispersal traits of both males and females. Treatment with the JH antagonist precocene mirrored the effects of high larval density during development, resulting in trade-offs between gonad size and wing length, with individuals developing smaller gonads and longer wings, indicative of a shift toward dispersal. However, treatment with the JH analog methoprene did not significantly show the reverse effects, with larger gonads and shorter wing lengths as expected.

This asymmetry may be explained by threshold effects in hormone responses. According to the classical JH-wing-morph hypothesis, trait expression is determined by whether JH titers fall above or below certain thresholds, with elevated levels typically reducing wing development (Zera, 2016). If beetles reared at medium density (5-7 eggs per cowpea) already maintain endogenous JH levels favoring reproduction, further methoprene exposure may not have been sufficient to induce further shifts and thus show significant differences between the control and methoprene group. Additionally, acetone itself has been shown to have an effect on morph determination in some insects, potentially through stress-mediated responses regulated by neurohormones (Zera & Tanaka, 1996). If acetone produced a mild brachypterizing (wing-reducing) effect in our control beetles, this could have blurred the observable differences between control and methoprene treatments.

Importantly, our results suggest that JH modulates where individuals fall along a fixed trade-off axis by enhancing investment in one trait at the expense of another, rather than altering the shape or slope of the trade-off itself. This further suggests that the underlying resource-allocation “architecture” is developmentally constrained, and hormonal cues serve to toggle trait prioritization within these limits. From an evolutionary perspective, such constraints may channel phenotypic variation along predictable axes, shaping life history strategies in response to environmental cues without requiring shifts in the trade-off structure itself. Such findings are consistent with other insect systems, such as the wing-polymorphic cricket where JH application shifts individuals along a trade-off slope betweeen flight muscle and ovarian development (Zera & Bottsford, 2001). Similarly, in planthoppers, JH analogs influence wing length without disrupting the negative correlation between wing and reproductive development (Ayoade et al., 1999). It has also been observed in caste differentiation in social insects, where endocrine regulation preserves trade-off integrity while enabling plastic responses (Hardie & Lees, 1985). Our work extends these findings to Coleoptera, suggesting the evolutionary conservation of these physiological mechanisms across diverse insect orders and thus supports the view of endocrine mechanisms acting as modulators rather than designers of phenotypes. This developmental constraint perspective helps explain why life history trade-offs are often maintained across environments despite selection potentially favoring their breakdown (Frankino et al., 2019).

Our results also reveal some sex-specific influences of JH. While many studies in *Callosobruchus maculatus* have focused on males due to clear trade-offs between testes and wing size, females also allocate resources between reproduction and dispersal traits (Canal et al.

2021). We included both males and females in our study to test whether juvenile hormone (JH) similarly regulates reproductive and dispersal trait allocation in both sexes. In males, wing size was shaped by both testes size and JH treatment, whereas in females, wing size was tightly coupled to ovariole size but unaffected by JH manipulation. This aligns with our previous findings in Gascoigne et al. 2022, where larval density influenced wing and gonad size more strongly in males than females. Together, these findings suggest that the dispersal–reproduction trade-off in response to larval crowding is more hormonally plastic in males, likely reflecting sex differences in life-history strategies (Gascoigne et al. 2022). Male fitness may benefit from flexible dispersal strategies under variable mating opportunities, whereas female fitness is more strongly tied to maximizing reproductive output, favoring tighter integration of reproductive and dispersal traits and reduced hormonal modulation. Although we observed some treatment effects on female wing and gonad size, significant trade-offs mediated by juvenile hormones may involve other unmeasured traits, such as immunity or lifetime fecundity, which could be more relevant to female fitness and mediate their resource allocation patterns (Rolff & Siva-Jothy, 2002; Zuk & McKean, 1996).

The trade-offs we observed likely stem from selective resource application shaped by a a physiological hierarchy regulated by JH (Flatt & Kawecki, 2007; Zera & Zhao, 2006). While we frame our results in terms of environmental “pressure,” it is more accurate to describe the context as developmental conditions simulating resource limitation. These conditions likely trigger stress pathways that modulate hormone sensitivity or production (Noriega, 2014). For example, precocene, which suppresses JH activity, led to greater investment in dispersal traits, while methoprene was expected to promote reproductive investment. These results are consistent with the idea that organisms must balance growth, survivial and reproduction under developmental constraints, and that developmental hormone cues help manage this balance, resulting in phenotypic dimorphism (Clifton & Noriega, 2012; Zera et al., 2007).

Mechanistically, JH interacts with nutrient-sensitive signaling pathways such as the insulin/insulin-like growth factor (IIS)/target of rapamycin (TOR) pathway, where reduced insulin signaling has been linked to lower JH levels and decreased fecundity (Pan et al., 2022). High JH titers promote vitellogenin synthesis, a hormone critical for ovarian development, through binding to the transcription factor methoprene-tolerant (Parthasarathy et al., 2009). These dynamics may explain our expectations of enhanced gonad development in methoprene-treated, low-density individuals (Gascoigne et al., 2022), though the lack of observed effect could stem from suboptimal dosing or limited trait variance in our laboratory population (Zera, 2016). Conversely, JH downregulation has been associated with increased lipid storage, particularly under reduced reproductive investment (Li et al., 2022). In insects, energy resources often fuel both flight and reproduction, and investment in dispersal traits like wing development may trade off with fecundity, particularly in crowded conditions where dispersal offers a fitness advantage (Katsuki & Lewis, 2015).

Despite these insights, our study has several limitations. First, we did not directly quantify *in vivo* concentrations of methoprene or precocene, and the efficiency of larval uptake remains uncertain. While previous studies have shown that surface application to cowpea seeds can influence *C. maculatus* development (Abo El-Ghar, 1992), direct measurement of hormone uptake would improve the precision of causal inference. Future work using tracer compounds or analytical chemistry approaches could help clarify dose-response dynamics. Second, we did not measure endogenous JH levels under different rearing densities. Although the hypothesis that density modulates hormone production is well supported in other insect systems (Ishikawa et al., 2013; Iwanaga & Tojo, 1986) empirical confirmation in *C. maculatus* would strengthen the link between environment, endocrine response, and phenotype. Finally, while topical JH analog application is widely used and effective, genetic tools such as RNA interference or CRISPR-based modulation of JH pathway genes would offer more targeted tests of causality. Such methods remain challenging in *C. maculatus* due to larval concealment within seeds and limited genomic resources, but adapting these tools could open new avenues for dissecting endocrine regulation of life history trade-offs.

In conclusion, our study demonstrates that juvenile hormone is a key proximate mechanism mediating the trade-off between dispersal and reproduction in *Callosobruchus maculatus*. By experimentally altering JH signaling, we demonstrate that hormonal modulation can shift resource allocation between competing traits without altering the underlying covariance structure. These findings underscore the importance of physiological pathways in translating environmental variation into adaptive phenotypic outcomes and offer a mechanistic foundation for understanding how life history strategies evolve in response to ecological constraints.

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