

**At the Crossroads of Developmental Plasticity: Epigenetic and Endocrine Regulators
Mediate Environmental Influence in Fire Ant Development**

Navid Bahramifarid

Thesis submitted to the University of Ottawa
in partial Fulfillment of the requirements for the
Master of Science in Biology

Department of Biology
Faculty of Science
University of Ottawa

© Navid Bahramifarid, Ottawa, Canada, 2026

Abstract

From plants to animals, our genomes are responsive to environmental variation, the interaction of which has profound consequences on our life-history and evolution. Environmental variation can influence developmental processes, and gene-by-environment interactions (GxE) have been shown to be mediated by the action of hormones and epigenetic mechanisms including histone modifications. Ants are an emerging model organism to study how these regulatory mechanisms facilitate development in response to environmental variation. Ant development is plastic: environmental changes can generate diverse phenotypic variation, generating individuals of different castes that vary in several traits including size, behaviour, and reproduction. We are beginning to understand how hormonal and epigenetic mechanisms can play a role in generating caste-specific trait variation. However, the interplay of these mechanisms and the process by which they mediate environmental variation to produce trait variation in ants remains enigmatic. Here, we use the highly invasive fire ant *Solenopsis invicta* known for both its extreme size variation in the worker caste and its adaptability to highly variable and stressful environments, to investigate the interplay of GxE mechanisms in response to environmental variation. We found that size variation and developmental timing are both thermally and nutritionally plastic. Next, we show that sizing and developmental timing are influenced by the key insect hormones juvenile hormone and ecdysone, as well as by histone modifiers, and that these regulators are both thermally and nutritionally plastic. Finally, we show the interaction of hormones and histone modifier activity. Collectively, this data suggests that size plasticity in ants is influenced by multiple environmental cues and that hormones and epigenetic processes together orchestrate this response. More generally, understanding the influence of the environment on regulatory mediators of

developmental plasticity may provide insight into the mechanistic crosstalk underlying conserved developmental pathways of insect growth.

Acknowledgements

First and foremost, I would like to dearly thank my supervisor Dr. Rajendhran Rajakumar for his endless support and guidance in completing my Master's degree. I am greatly appreciative of the time, knowledge, and passion for science that he has shared with me over these past years as his student. Next, I would like to thank my thesis advisory committee members, Dr. Heath MacMillan and Dr. Gilda Stefanelli for their continuous support and feedback, providing new outlooks and approaches over the course of this project. Furthermore, I am deeply grateful for all members of the Rajakumar lab for providing a collaborative environment to learn from each other and to provide unconditional aid to one another throughout our academic and research endeavours. In particular, I would like to thank Vie Tran, Kami Fabien, and Akash Dahiya for their contributions to this work, and Erica Vong for her endless support. Finally, I am grateful to Ehab Abouheif and his lab for providing the Rajakumar lab with several *Solenopsis invicta* colonies, which allowed me the opportunity to work with this incredible ant species.

Table of Contents

<i>Abstract</i>	<i>i</i>
<i>Acknowledgements</i>	<i>iv</i>
<i>List of Figures</i>	<i>vii</i>
Chapter 1: Introduction	1
1.1 Insects & Developmental Plasticity	1
1.2 Developmental Plasticity & Temperature	5
1.3 Developmental Plasticity & Nutrition	7
1.4 Developmental Plasticity & Endocrine Signaling	10
1.5 Developmental Plasticity & Epigenetics	14
1.6 Connecting the Environment to Epigenetics & Hormonal Modulation of Phenotype	17
1.6.1 Temperature & Endocrine Modulation	18
1.6.2 Temperature & Histone Modifications	18
1.6.3 Nutrition & Endocrine Modulation	19
1.6.4 Nutrition & Histone Modifications	20
1.6.5 Endocrine Modulation & Histone Modifications	21
1.7 Objectives and Hypotheses	25
Chapter 2: Methods	28
2.1 Ant husbandry	28
2.2 Experimental replicate setup	28
2.3 Morphological data collection	29
2.4 RT-qPCR primer design	30
2.5 RNA extraction and cDNA synthesis	31
2.6 RT-qPCR	32
2.7 Temperature treatments	32
2.8 Nutrition treatments	33
2.9 Endocrine manipulations	34
2.10 Pharmacological histone modification inhibitors	35
2.11 Statistical analyses	35
Chapter 3: Results	37
3.1 Natural Plasticity in <i>S. invicta</i>	37
3.2 Thermal Plasticity in <i>S. invicta</i>	40
3.3 Nutritional Plasticity in <i>S. invicta</i>	43
3.4 Influence of Hormones on Developmental Plasticity in <i>S. invicta</i>	46

3.4.1 <i>Effects of Juvenile Hormone Analog on Fire Ant Development</i>	46
3.4.2 <i>Effects of 20-hydroxyecdysone on Fire Ant Development</i>	48
3.5 Influence of Pharmacological Inhibitors of Histone Modifiers on Developmental Plasticity in <i>S. invicta</i>	50
Chapter 4: Discussion	53
4.1 Environmental Variation & Worker Polyphenism in Fire ants	54
4.1.1 <i>Thermal Plasticity of Size and Developmental Timing</i>	54
4.1.2 <i>Nutritional Plasticity of Size and Developmental Timing</i>	55
4.2 Mediators of GxE and Plasticity in fire ants	55
4.2.1 <i>JH Signalling: Environmental response and influence on sizing</i>	56
4.2.2 <i>Ecdysone Signalling: Environmental Response and influence on Sizing</i>	57
4.2.3 <i>Histone (de)acetylation: Environmental Response and Influence on Sizing</i>	57
4.2.4 <i>Histone (de)methylation: Environmental Response and Influence on Sizing</i>	59
4.2.5 <i>Epigenetic-Endocrine Crosstalk Underlying Size Variation in Fire Ants</i>	60
Supplemental Material	62
References	65

List of Figures

Figure 1.	Proposed connections between the environmental, endocrine, and epigenetic facets of developmental plasticity in <i>Solenopsis invicta</i> .	24
Figure 2.	<i>Solenopsis invicta</i> natural comparison of targeted endocrine and histone genes in developing larvae.	37
Figure 3.	The effects of temperature variation on developmental plasticity in <i>Solenopsis invicta</i> .	40
Figure 4.	The effects of nutritional variation on developmental plasticity in <i>Solenopsis invicta</i> .	43
Figure 5.	The effects of the juvenile hormone analogue methoprene on developmental plasticity in <i>Solenopsis invicta</i> .	46
Figure 6.	The effects of 20-hydroxyecdysone (20E) on developmental plasticity in <i>Solenopsis invicta</i> .	48
Figure 7.	The effects of pharmacological inhibitors of histone modifications on developmental plasticity in <i>Solenopsis invicta</i> .	50
Figure 8.	Connecting the environmental, endocrine, and epigenetic facets of developmental plasticity in <i>Solenopsis invicta</i> .	53

Chapter 1: Introduction

1.1 Insects & Developmental Plasticity

The versatility of a genotype to exhibit alternative phenotypes in diverse environmental conditions is referred to as phenotypic plasticity (Beldade et al., 2011; Schlichting, 1986; West-Eberhard, 1989; Whitman & Agrawal, 2009). The concept of plasticity was notably demonstrated in plants through multiple experiments on various species of plants by Clausen et al. (1940). Different populations were reared in contrasting environments of sunlight and humidity, which led to variations in the morphology of specific characters, such as stem number and height (Clausen et al. 1940). In the context of plants, Bradshaw (1965) further discussed the concept of plasticity and distinguished between morphological and physiological plasticity; morphological plasticity has a developmental origin, whereas physiological plasticity typically refers to adult physiological responses to environmental variation.

Following this pioneering plasticity research and theory in plants, the concept of plasticity was further investigated in other organisms, including insects. Various insects have been shown to be developmentally sensitive to environmental factors (Simpson et al., 2011), ranging from nutrient-dependent horned morphs in beetles and seasonal-dependent eyespot morphs in butterflies (Emlen, 1994; Prudic et al., 2011). Furthermore, there are extensive examples of behavioural plasticity across insects, including solitary versus gregarious forms of locusts and the caste-specific behaviours found in social insects (Simola et al., 2016). A classic insect model to explore developmental plasticity is the fruit fly, *Drosophila melanogaster*. The developmental trajectory of *D. melanogaster* can be altered through various environmental factors, such as temperature. Differing temperatures can affect fruit fly development, such that lower temperatures can result in

the development of larger individuals (Nunney & Cheung, 1997). Another classic system, *Tribolium castaneum*, showed sensitivity to altering nutritional states, where higher quality diets led to faster developing individuals (Ming & Cheng, 2012). Both *D. melanogaster* and *T. castaneum* are solitary insects that demonstrate developmental sensitivities to their environments, showcased through changes in their body sizing and developmental timing. Both of these traits are important proxies that can be used to quantify developmental plasticity.

Variations in body sizing and developmental timing is particularly dramatic in relation to eusocial insects including many bees and ants (Wilson, 1953; Hölldobler & Wilson, 1990; Chole et al., 2019). Eusocial insects form groups called colonies and have the following features: overlap of multiple generations of individuals; cooperative care of developing brood; a division of labour that distinguishes individuals into various castes (Hölldobler & Wilson, 1990). Generally, these castes categorize individuals by their behavioural, physiological, and morphological features, comprising the reproductive queens and males and the non-reproductive workers (Hölldobler & Wilson, 1990; Robinson, 2009). For the vast majority of eusocial insects, the developmental differentiation of castes is not determined genetically, but rather it is plastic and altered through the surrounding environment in a hormone-dependent manner (Nijhout & Wheeler, 1982). Therefore, the ability to be developmentally plastic is crucial for eusocial insects to form their complex societies. While numerous environmental factors can influence various critical windows of development in social insects, we will emphasize the effects of temperature and nutrition based on their known potential in affecting insect growth.

Ants are an emerging model organism for studying developmental plasticity. Ant colonies are primarily made up of a female caste system (Hölldobler & Wilson, 1990). Individuals of each caste carry out specific reproductive (queen) and non-reproductive (minor workers and major

soldiers) tasks (Hölldobler & Wilson, 1990). Depending on the species of ant, minors are often smaller ants that temporally progress from having nursing tasks to foraging tasks, while the larger majors have roles in colony defense and processing prey (Wilson, 2003). During development, minors and majors begin from the same plastic, undifferentiated larvae, yet can be subjected to differing external stimuli leading to the alteration of the physiology, morphology, and behaviour of the adult ant (Chittka et al., 2012). For example, the ant genera *Pheidole* and *Camponotus* are known for having a clear morphological distinction between their minor worker and major soldier castes (Hölldobler & Wilson, 1990; Wilson, 2003). Furthermore, there are behavioural differences between the minor worker and soldier castes that are associated with the differences in sizing of various morphological traits, including soldiers exhibiting a disproportionately larger head-to-body ratio (Rajakumar et al., 2018).

Recently, endocrinological mechanisms and epigenetic modifications enabling the plastic development of ant castes have been characterized. In particular, these two developmental processes have been shown to mediate growth in various ant species (Passera & Suzzoni, 1979; Wheeler & Nijhout, 1981; Brian, 1974; Alvarado et al., 2015). However, how environmental variation influences the activity of these processes and how these processes interact with each other remains poorly understood. To properly establish an ant model linking gene-by-environment interactions and developmental plasticity, a focus is needed on a species for which there is extensive morphological variation, and an understanding of their developmental timing, developmental response to environmental factors, and some aspects of developmental endocrinology and/or epigenetics have been explored. Based on that, currently the best candidate species is the fire ant *Solenopsis invicta*.

S. invicta is a thoroughly investigated ant species, both in a curiosity-driven context and in a pest-eradication context. *S. invicta* is a highly invasive species native to South America that is estimated to have first began its invasion of North America in 1933, outperforming other invasive fire ants (Tschinkel, 2006). As the geographic distribution of *S. invicta* has expanded, they have devastated ecological niches and have displaced native wildlife (Allen et al., 2004). Alternatively, it is thought that this devastation is due to the anthropomorphic disruption of wildlife rather than fire ants, and that fire ants are simply capable of withstanding human-caused ecological disruptions (Tschinkel & Wilson, 2014). *S. invicta* are within the top 10 list of most economically damaging species recorded, at a total cost of \$16.7 billion USD (Cuthbert et al., 2022). Unlike most ants, *S. invicta* workers vary dramatically in their sizing from very small workers to massive-sized workers in the form of a size continuum (Mirenda & Vinson, 1981; Tschinkel, 2006). However, unlike *Pheidole* and *Camponotus*, which have a distinct large soldier caste, the largest workers of *S. invicta* are not considered soldiers as they do not have a disproportionately large head-to-body (Tschinkel, 2006). Across fire ant development, an individual progresses from an embryo to four separate larval instars until they metamorphose into a prepupa, then pupa, until eclosion into an adult ant. At key points during these stages, there are environmentally sensitive windows where environmental cues and responding hormones are known to influence which caste the developing fire ant becomes. During embryogenesis, optimal temperature and photoperiod induce embryos to become queens, while later during larval development, individuals are thought to undergo varying duration/timing of their last instar to enable the generation of differently sized workers, although this is poorly understood (Wheeler, 1990; Tschinkel, 2006). To better understand the mechanisms that underlie developmental plasticity in *S. invicta*, that have facilitated their ecological and invasive success, we will explore how variations in environmental factors and their underlying

endocrine and epigenetic mediators affect and modulate their development. The following sections highlight insect literature, ranging from solitary to eusocial species (with an emphasis on *S. invicta*), related to key environmental (temperature and nutrition) variation and both endocrinological and epigenetic mechanisms related to modulating this variation during development.

1.2 Developmental Plasticity & Temperature

Temperature has been shown to be a key driver of alternative phenotypes across a wide array of insects. For solitary insects, development of the fruit fly *Drosophila melanogaster* is known to be thermally sensitive, specifically affecting their growth rate (Powsner, 1935). Powsner investigated the effects of rearing *D. melanogaster* at temperatures ranging from 15-32°C. Each developmental stage (embryo, larva, pupa) demonstrated a decrease in the number of hours required to complete their respective developmental periods as their rearing temperatures increased (Powsner, 1935). Body sizing in *D. melanogaster* is another trait that is susceptible to temperature variation (De Moed et al., 1997; Ghosh et al., 2013; Karan et al., 1998; Nijhout et al., 2014). These studies found that sizes of various body traits decrease as temperature increases. In contrast, lower temperatures lead to the development of larger individuals (Nunney & Cheung, 1997). The sensitivities and relationship of body sizing and developmental timing of fruit flies to varying temperatures is a common trend observed in many solitary insect species ranging from hornworms (Nijhout et al., 2006) to crickets (Rohde et al., 2015) to beetles (Tseng et al., 2018). The generality of this temperature sensitivity seen in these solitary insects can be further expanded when considering the nature of ectotherms and the temperature-size rule (TSR). This concept denotes that individuals reared at warmer temperatures terminate development sooner, and form a decreased body size, while those reared at colder temperatures have the opposite effects (Arendt, 2011). Aspects of the TSR appear to hold true for some eusocial insects as well: temperature

increases has been shown to decrease forewing size in honeybees (Medina et al., 2018), while regional thermal decreases are associated with larger body sizes in termites (Lin et al., 2024). However, in ants, the TSR may be put into question. Passera (1974) demonstrated a clear relationship between the presence of the larger major soldier *Pheidole* ants and rearing temperatures above 24°C. When thermal conditions were increased by 2°C, to 26°C, this led to a 6-fold increase in the number of soldiers produced, furthermore, a decrease of 2°C to 22°C (and below) repressed soldier development entirely (Passera, 1974). Furthermore, in *Camponotus floridanus*, a species that has small workers and large soldier, soldier development was enhanced by increases in temperature (MacMillan et al., 2025). In contrast, developing individuals of *Aphaenogaster* responded to higher temperatures by accelerating their developmental timing, while decreasing the overall sizing of adults (Oms et al., 2017). Overall, temperature as an environmental factor has a significant influence on the developmental plasticity of several solitary and eusocial insects, including ants. However, how does the fire ant *Solenopsis invicta* respond to changes in thermal conditions across development?

Given their South American origin, *S. invicta* populations flourish in hotter environments and perform less efficiently at colder temperatures (Tschinkel, 2006). Several studies have investigated how variations in rearing temperatures can affect developmental trajectories of *S. invicta* individuals. In terms of sizing, a study was done to test the effects of three incubation temperatures (25°C, 30°C, and 35°C) on larval development and subsequent adult worker body sizes (Cassill & Tschinkel, 2000). As an extension to TSR, the Von Bertalanffy (1960) rule states that higher developmental temperatures lead to faster growth and smaller body size. Testing the Von Bertalanffy rule, Cassill and Tschinkel investigated varying incubation temperatures on fire ant development and they found conflicting results. For individuals who developed into workers,

no changes in body size were found in relation to varying incubation temperatures. Interestingly, large reproductive (queen) larvae were generated at higher temperatures, which is a conflict to TSR. In addition to sizing, developmental timing for workers was investigated in *S. invicta* and it was found to be sensitive to changes in rearing temperatures (Porter, 1988). This study tested seven different incubation temperatures (°C): 17, 21, 24.5, 28.5, 30, 32, and 35. While larvae were unable to grow at 17°C and 21 °C, the duration was measured for each developmental stage across the life history of *S. invicta* individuals, where the timing of the larval stage decreased from 28 days at the minimum surviving temperature (24.5°C) to 11 days at the maximum temperature (35°C). However, overall colony growth in terms of worker production displayed a temperature performance curve, where peak performance was reached at 32°C (Porter, 1988). Although this study had thoroughly investigated the influence of temperature variation during *S. invicta* growth, the effects of each thermal condition on individual sizing was not examined. To build on both Cassill & Tschinkel (2010) and Porter (1988), further work needs to be done to test the effect of varying temperatures on body sizing of developing *S. invicta* individuals to better understand how this species grows during development in the context of TSR and to further characterize the degree of developmental plasticity this species possesses.

1.3 Developmental Plasticity & Nutrition

In parallel to temperature, nutrition is one of the most extensively investigated environmental cues in the context of developmental plasticity in insects. Multiple insect phenotypes are affected by an individual's diet, including their reproductive success (Wigglesworth, 1960). Females have certain dietary requirements before they can begin laying their eggs. For example, the *Lucilia* blowfly needs to be fed a source of protein (Evans, 1935; Mackerras, 1933, as cited in Wigglesworth, 1960); the *Musca* housefly requires the three

components of sugar, protein, and water (Glaser, 1923; Roubaud, 1922, as cited in Wigglesworth, 1960); the *Dermestes* beetle only requires drinking water (Dick, 1937, as cited in Wigglesworth, 1960). Despite the varying levels of nutrients required for egg laying, insects do require a minimum distribution of the major nutrient classes of carbohydrates, proteins, lipids, vitamins, and minerals, for optimal nutritional supply and growth (House 1965, as cited in House, 1969). In *D. melanogaster*, studies have investigated the effects of alternative diets in fly growth, quantified through changes in body size and developmental time. As described by Robertson (1960), observing alterations in body size exclusively is insufficient to adequately describe the environmental effects of nutrition on developing flies. A deficient diet can prolong developmental timing of larval growth without changing the final size of the individual adults (Edgar, 2006; Robertson, 1960). This can be explained through the concept of critical weight (CW), where a minimum weight is required of the developing individual prior to progression to the next developmental stage (De Moed et al., 1999). Once CW is attained, larval feeding is stopped, metamorphosis is initiated, and no further increases in size occur (De Moed et al., 1999; Nijhout et al., 2014). Changes in diets of developing larvae can therefore manipulate the interactions between body sizes and developmental time to produce variations in growth and adult phenotypes (De Moed et al., 1999). For example, restrictions in both protein and dietary RNA led to smaller adults to be produced (Edgar, 2006; Robertson, 1960), with the RNA-deficient treatments taking more time to development (Robertson, 1960), while protein-rich diets led to larger adults to form (Kristensen et al., 2011). Moreover, sugar-deficient diets formed larger individuals, while simultaneously shortening their developmental time (Robertson, 1960).

Beyond solitary insects, the importance of diets among the female castes of social insects is notably seen in the honeybee *Apis mellifera*. Within a colony of social honeybees, developing

individuals with identical genomes can take a trajectory to either become a reproductive queen caste or a mostly non-reproductive worker caste (Barchuk et al., 2007). The initial source for this difference is through the diets supplied to the developing larvae. There is a distinction between royal jelly (RJ) diet, which is abundantly fed to queen-destined larvae, and worker jelly (WJ) diet, which is fed to worker-destined larvae (Wang et al., 2016). Notably, RJ diets contain higher contents of protein, sugars (fructose and glucose), and certain minerals and trace elements, such as iron and zinc levels. Through these dietary differences, queen-destined larvae become much bigger in size despite a faster development, as compared to worker-destined larvae (Weaver, 1957). Both solitary and social insects are thus developmentally plastic and exhibit alternative phenotypes in response to variations in nutrient intake. However, a key distinction is that solitary insects have known requirements of specific types and amounts of different nutrients for optimal growth (Csata & Dussutour, 2019). For social insects, including honeybees and ants, the type of nutrients required differs based on an individual's stage across development within a colony, where larvae and reproductives require higher levels of protein content (Holldobler & Wilson, 2009; Howard & Tschinkel, 1981; Cassill & Tschinkel, 1999; Markin, 1970; Sorensen & Vinson, 1981), while adult workers require more carbohydrate-rich diets (Markin, 1970; Wilson & Eisner, 1957). Furthermore, variations in diet contents can affect caste-specific development and differentiation in ants. In the ant *Pheidole pallidula*, developmental trajectories were investigated between a group fed with a honey and insect carcass diet as compared with another group fed exclusively with a honey diet (Passera, 1974). With a lack of a protein source, the honey diet group was unable to produce soldier ants and produced minor worker ants exclusively. Another study looked at the effects of a sugar diet on caste differentiation in the ant *Myrmica rubra*, demonstrating that when

this protein-deficient diet is used to rear undifferentiated larvae, it favoured formation of the smaller worker caste rather than the larger reproductive queens (Brian, 1956).

In *S. invicta*, investigations in nutritional plasticity have been primarily focused on social interactions (Cassill & Tschinkel, 1995, 2000; Lee Cassill & Tschinkel, 1999). Notable findings include the role of the 4th instar larvae in acting as a metabolic caste or communal gut (Tschinkel, 1988; M. L. Allen et al., 2018), which utilizes its specialized hairs and mouthparts to digest solid foods to then store and distribute digested protein contents for colony usage (Csata & Dussutour, 2019; Dussutour & Simpson, 2009; Petralia & Vinson, 1978, 1979). The effects of altering diet on the developmental plasticity of size and developmental time have yet to be described for *S. invicta*. More recently, several studies have found both the insulin and Target of Rapamycin (TOR) signaling pathways act as key regulators of nutrient-dependent development (Koyama & Mirth, 2018; Mirth & Riddiford, 2007; Nijhout et al., 2014). These signaling pathways have been shown to play key roles in modulating developmental plasticity and producing variation in growth within and between species across insects (Edgar, 2006; Lavine et al., 2015). Altogether, investigating the influence of nutrition and the developmental mechanisms that connect nutritional variation with developmental differences is pivotal towards gaining a better understanding of how fire ant colonies respond to changing nutritional environments, producing individuals that differ in their size and the time they take to develop.

1.4 Developmental Plasticity & Endocrine Signaling

Hormones, specifically juvenile hormone (JH) and ecdysteroids play necessary roles in insect metamorphosis (Truman & Riddiford, 1999). Flies, bees, and ants are holometabolous insects, meaning that they undergo complete metamorphosis and development progresses through the life stages including egg, larva, pupa, and is completed following metamorphosis into the adult

stage (Truman & Riddiford, 1999). The hormones JH and ecdysteroids, specifically ecdysone and its active form 20-hydroxyecdysone (20E), interact by alternating between surging and decreasing concentrations to allow holometabolous insects to molt between larval instars and progress through each developmental stage (Edgar, 2006; Hölldobler & Wilson, 1990; Truman & Riddiford, 1999). JH prevents morphogenesis into adulthood, enabling prolonged growth across development (Belles, 2020). Ecdysone promotes individuals to molt, enabling the shedding of the preceding larval instar cuticle for resorption and secretion of a new cuticle for the next instar (Williams, 1968; Edgar, 2006). The two key downstream transcriptional effectors of the JH and 20E pathways are Krüppel homolog 1 (*Kr-h1*), preventing metamorphosis, and *E93*, promoting metamorphosis, respectively (Belles, 2020). Biosynthesis of JH occurs in the corpora allata (CA) gland (Nijhout et al., 2014), and experiments in *Drosophila* demonstrate that removal of the CA reduces JH concentrations, thereby forming pupae at a reduced size that complete development prematurely (Riddiford et al., 2010). Furthermore, biosynthesis of ecdysone occurs in the prothoracic gland (PG) and ecdysone feeding experiments in the last instar of *D. melanogaster* larvae leads to the development of individuals with smaller body size and shortened developmental time (Ono, 2014). Generally, ecdysone is understood to negatively regulate insect growth through its regulation of developmental timing (shortening development), however in lepidopterans and fruit flies, ecdysone was also found to amplify tissue-specific growth (Nijhout et al., 2014).

Both JH and ecdysone are necessary for proper insect development, and they often act antagonistically to ensure sufficient growth occurs until a critical weight is attained and metamorphosis to the next developmental stage can begin (Liu et al., 2018). In social insects, and honeybees specifically, work has been done to characterize the relationship between JH and ecdysone in the context of caste differentiation and their division of labour (Hartfelder, 2000). In

addition to the nutritional effects of RJ and WJ, an increase in production of JH levels are observed in queen larvae, whereas worker larvae have a significant decrease in JH synthesis (Hartfelder, 2000). The additional JH concentration not only leads to more growth and larger sizes in queen-destined larvae but also triggers the prothoracic gland to increase ecdysone levels, resulting in a faster growth rate (Hartfelder, 2000). Termites are another social insect displaying caste differentiation in response to JH exposure (Cornette et al., 2008). The soldier caste in various termite species was induced using CA gland transfers (Lüscher, 1958), and through treatments of JH analogs (JHA) (Cornette et al., 2006; R. W. Howard & Haverty, 1979; Hrdý & Křeček, 1972; Koshikawa et al., 2005; Lelis & Everaerts, 1993; Ogino et al., 1993). Finally, the effects of JH on development and caste differentiation have also been investigated in ants. The influence of JH on ant development can induce caste-specific developmental differences, including distinct variations in sizing, developmental timing, and allometry. In *Pheidole*, the role of JH has been shown to trigger two developmental switch points in caste differentiation (Passera & Suzzoni, 1979; Wheeler & Nijhout, 1981, 1983). The first occurs between the reproductive queen and the sterile worker castes, where high JH exposure during embryonic development exclusively led to queen ants (Passera & Suzzoni, 1979). The second switch point occurs in the bipotential last larval stage, where treatment with high levels of JH-analogue induced soldier development, where soldiers develop longer and grow larger (Wheeler & Nijhout, 1981, 1983). Rajakumar et al. (2012) demonstrated that an additional JH switch point controlling soldier-supersoldier development exists in a handful of species that have supersoldier castes that exhibit a massive disproportionate head compared to their body. Furthermore, this additional threshold is retained in a dormant/inactive form in species that do not exhibit supersoldiers, yet it can be activated artificially using a JH-analogue, demonstrating that the developmental potential to develop supersoldiers is

retained throughout the genus and its activation has facilitated the independent evolution of supersoldiers across the genus.

In the context of ecdysone, very little work has been done in the context of caste-specific development. Brian (1974) injected the third/final larval instar of the ant *Myrmica rubra* with ecdysone to observe the effects on queen/worker caste differentiation. Ecdysone treatments to queen-potential larvae were shown to promote an even distribution of worker : queen proportions, while ethanol control groups predominantly produced queens (Brian, 1974). Increased ecdysone levels promote a quicker larval molt, preventing the developmental time required for adequate queen growth.

In *S. invicta*, several studies have investigated the effects of JH and its analogs on fire ant behaviour and reproduction (Ren et al., 2023; Starkey et al., 2023; Starkey, 2023). Mature queens contained high concentrations of JH which promoted their own reproductive capabilities while the reproductive potential of winged female virgins (alates) was suppressed through Corpora allata inhibition, thereby preventing JH formation (Brent & Vargo, 2003). In terms of development in *S. invicta*, high levels of JHA have been found to prevent larval growth and metamorphosis (Troisi and Riddiford, 1974). Furthermore, Wheeler (1990) investigated the effects of JHA on developing *S. invicta* larvae and showed that it led to increases in pupal sizing and longer developmental periods. Regarding the effects of ecdysone in *S. invicta*, very limited work has been done thus far. One study tested injections of ecdysone in mature alate virgin females to measure its influence on flight muscle apoptosis, a phenomenon observed in several insects, however no significant findings were found (Azizi et al., 2009). No other studies have investigated the effects of ecdysone on *S. invicta* development. To complement the work done regarding the effects of JH in *S. invicta*, it is imperative to understand the functional roles of ecdysone in fire ant development. Furthermore,

this will provide a more holistic view of how underlying endocrinological signaling can mediate the plastic development of fire ants.

1.5 Developmental Plasticity & Epigenetics

The term *epigenetics* was coined in the 1940s by Conrad Waddington (Waddington, 1942, 1957; Jablonka & Lamb, 2002), and it describes reversible, heritable, and environmentally sensitive alterations in gene activity without influencing the original DNA sequences (Kiefer, 2007). Two of the major epigenetic mechanisms include DNA methylation and histone modifications (Gibney & Nolan, 2010). These mechanisms have been conserved across a vast array of organisms, and they have each been demonstrated as key players in differential gene expression (Allis et al., 2007; Yung & Elsässer, 2017; de Mendoza et al., 2020). In bees, differential DNA methylation controls queen versus worker development such that high levels of DNA methylation leads to the production of workers (Kucharski et al., 2008). In ants, DNA methylation has been shown to regulate sizing among the complex worker caste system in *Camponotus floridanus* (Alvarado et al., 2015). Specifically, DNA methylation can control continuous size variation across workers and developmental timing through global methylation levels, and particularly through methylation of the gene *Egfr* (Alvarado et al., 2015). While progress has been made in understanding the role of DNA methylation and caste-specific growth in social insects, the role of histone modifications in this process remains unknown.

Histone modifications

Histone modifications alter chromatin structure through post-translational modifications (PTMs) that are carried out by enzymes that can either read, add (write), or remove (erase) specific chemical modifications, including acetyl and methyl marks, to the N-terminal tails of the histone proteins (Jenuwein & Allis, 2001; Berger, 2007; Nicholson et al., 2015). Chromatin is composed

of clusters of nucleosomes, which are formed from long DNA strands wrapped around histone protein octamers (Jenuwein & Allis, 2001). An octamer contains two dimers of H2A-H2B proteins and a tetramer of H3-H4 proteins, linked together with the core H1 protein (Jenuwein & Allis, 2001). The histone tails contain amino acid residues, namely lysine and arginine, which are the targets for histone modifications (Nicholson et al., 2015). Writers add chemical modifications to histone tails and include histone methyltransferases (HMTs) and histone acetyltransferases (HATs), which add methyl and acetyl groups, respectively (Nicholson et al., 2015). In contrast, erasers remove these marks and include histone demethylases (HDMTs) and histone deacetylases (HDACs), that remove methyl and acetyl groups, respectively (Nicholson et al., 2015). From these enzymes, HDACs, as well as HMTs typically, have a common role in altering chromatin function and structure into a heterochromatic state, condensing the chromatin, and repressing transcription (Jenuwein & Allis, 2001; Tamaru, 2010; Nicholson et al., 2015). HATs and HDMs (typically) both promote the formation of a euchromatic state, opening the chromatin, promoting active transcription. In solitary insects, an example of histone modifications controlling trait sizing is seen in the broadhorned flour beetle *Gnatocerus cornutus* (Ozawa et al., 2016). RNA interference (RNAi) was used to knockdown (KD) two histone deacetylases during larval development. HDAC1 KD led to a size reduction in mandibles and increased the size of hind wings in adults. In contrast, HDAC3 KD increased mandible size while reducing hind wing size in adults. Modifications to HDACs has also been shown to affect *D. melanogaster* development, where treatment with the HDAC inhibitor (HDACi) trichostatin A (TSA) led to developmental delays (Pile et al., 2001).

Recently, histone modifications have been shown to play key developmental roles in eusocial insects, specifically in caste differentiation (Okwaro & Korb, 2023). In honeybee larval

diets, RJ has been described to contain approximately 5% HDACi contents, suggesting its potential role in RJ-led caste differentiation between queen and worker honeybees (Spannhoff et al., 2011). In the termite *Zootermopsis nevadensis*, soldier-destined larvae showed increased expressions of two HMTs, *Ash1* and *Hmt4-20*, and one HDM, *Utx* (Suzuki et al., 2019). RNAi of these genes led to increased developmental time during the presoldier molting periods. In ants, behavioural and lifespan studies further demonstrate the effects of histone modifications on caste-specific differences. For behaviour, histone writers and erasers were investigated in the ant *C. floridanus*, where pharmacological treatments of the HDACi TSA were shown to promote majors to express more foraging activity that is typical of minor workers (Simola et al., 2016). Conversely, treatments using the histone acetyltransferase inhibitor (HATi) C646 demonstrated less foraging activity in minors, mimicking major-like behaviours (Simola et al., 2016). For lifespan, histone (de)acetylation has been investigated in the ant *Temnothorax rugatulus*, where it was shown to play distinct roles in worker ant lifespan (Choppin et al., 2021). Furthermore, workers from queen-less colonies were able to reproduce and had increased lifespans compared to colonies containing queens, and this increased lifespan was blocked when queen-less workers were fed histone acetylation inhibitors (Choppin et al., 2021). Overall, while we have an increased understanding of the role of histone epigenetics during adulthood in ants, namely in behaviour (Simola et al., 2016) and lifespan (Choppin et al., 2021), the roles of this process in a developmental context remain unknown.

In *S. invicta*, data regarding the expression of genes encoding histone modifiers has been obtained through transcriptomic studies while protein level data for some has also been obtained through proteomics (Allen et al., 2018; Ye & Li, 2020). Allen et al. (2018) found that among 17 differentially expressed histone transcripts, 16 demonstrated increased activity in larvae. In the

scope of proteomics, lysine acetylation (Kac) was measured across hundreds of proteins in worker adults and it was found that diverse forms of lysine acetylation of protein targets was conserved and therefore this histone modification may play a role in regulating cellular energy metabolism and stress responses as it does across animals (Ye & Li, 2020). These -omic scale studies alongside the sequencing of the *S. invicta* genome (Wurm et al., 2011) altogether confirm the evolutionary conservation of all the major histone modifiers, but further work needs to be done to understand their role in generating caste-specific development and phenotypic variation. Specifically, understanding the effects of histone modifications on influencing fire ant sizing and developmental timing is critical to further characterize the developmental mechanisms underlying size plasticity in fire ants.

1.6 Connecting the Environment to Epigenetics & Hormonal Modulation of Phenotype

Thus far, we have reviewed developmental plasticity in four facets, two of which are the environmental factors of temperature and nutrition, while the two others are underlying molecular mediators of plasticity, being endocrine modulators and histone modifications. We progressed from solitary to eusocial insects with an emphasis on ants and a further focus on our species of interest *S. invicta*. Our proposed model (Fig. 1) to investigate developmental plasticity in *S. invicta* illustrates potential connections among the four facets based on the current state of the literature, with an additional link addressing changes in morphological phenotypes. Links (arrows) between environment (temperature/nutrition), developmental plasticity modulators (hormones and histone modifications), and phenotypic variation in our proposed model are based on current research done in other insects, providing hypotheses and predictions of their links in the context of growth, developmental timing, and overall development in fire ants. Below, I briefly review how each connecting arrow has been established in insect developmental plasticity literature.

1.6.1 Temperature & Endocrine Modulation

In the solitary beetle *Monochamus alternatus*, a study found that temperature-dependent termination of larval diapause is mediated by both the JH and 20E pathways (Yang et al., 2023). For this beetle species, 25°C is considered a high temperature that promoted early diapause termination in larvae. Transcriptomic analyses from the temperature treated groups revealed differentially expressed genes (DEGs) related to the regulation of biosynthesis and degradation of JH and 20E (Yang et al., 2023). Knocking down the expression of several of these genes with RNAi affected diapause duration, demonstrating a shared role between temperature and JH & 20E activity in a developmental context (Yang et al., 2023). In the eusocial termite *Coptotermes formosanus*, JH titers are known to induce development of soldiers (Henderson, 2019; Mao et al., 2005; Park & Raina, 2005). Furthermore, JH-dependent soldier caste development in these termites has been shown to be sensitive to temperature (Y. Liu et al., 2005). Specifically, several temperatures were tested in workers, which can postnatally become presoldiers and soldiers (as they are hemimetabolous) and both extremes of 20°C and 36°C were unable to produce presoldiers and soldiers (Liu et al., 2005). JH titers of the workers were also measured for each temperature at various developmental days, demonstrating no changes in JH concentrations at both extreme temperatures (Liu et al., 2005). However, at the rearing temperatures of 24, 28, and 32°C, there were steady increases of JH levels until soldier formation at the 60-day developmental mark. Therefore, there is an association between higher temperatures (maximum at 32°C) and higher JH levels, corresponding to soldier development in this eusocial termite species.

1.6.2 Temperature & Histone Modifications

There are distinct connections found between temperature variations and histone modifier activity in several non-insect species, ranging from the plant *Arabidopsis thaliana* (Pajoro et al.,

2017) to the oyster *Crassostrea gigas* (Fellous et al., 2015). However, findings within insect literature are still emerging. Thus far, important results have been found in the fly *Eurosta solidaginis* and in the moth *Epiblema scudderiana*, demonstrating links between cold-stress and histone modifications, enabling entry into diapause for these insects (Yu et al., 2024). Conserved H3 tail lysine targets for both acetylation and methylation levels were examined following cold-stress treatments of developing larvae. Interestingly, *E. solidaginis* larvae showed increased levels of H3 lysine acetylation and methylation, while *E. scudderiana* showed decreased levels in response to colder rearing temperatures (Yu et al., 2024). The study demonstrates the role that histone modifications can play in mediating temperature-dependent entry into diapause, providing a connection between temperature and histone epigenetics. Studies focusing on this connection in the context of eusocial insects are limited. However, transcriptomic analysis following heat stress experiments with six species of the thermophilic desert ant *Cataglyphis*, revealed that several genes encoding histone modifiers of methylation (*Utx*, *SET7*) and acetylation (*Kat6b*) were upregulated with increased temperature (Perez et al., 2021). This work suggests that there may be an association between heat stress response of *Cataglyphis* ants to the expression levels and potential activity of key epigenetic histone modifiers.

1.6.3 Nutrition & Endocrine Modulation

Variations in nutritional states have been linked with both JH and ecdysone. In the solitary mosquito, *Aedes aegypti*, starvation experiments led to a reduction in JH biosynthesis from the corpora allata (Perez-Hedo et al., 2014). In parallel, lack of food supply in *D. melanogaster* leads to inhibition of oogenesis and induces apoptosis within the egg chambers through the increase of ecdysteroid levels (Terashima et al., 2005). Together, these examples show a link between nutritional plasticity and downstream endocrinological activity of JH and ecdysone in insects. In

eusocial insects, *Coptotermes formosanus*, the same eusocial termite discussed earlier in the context of thermal and endocrine associations, soldier caste differentiation is also nutritionally dependent and correlated with JH titers (Y. Liu et al., 2005). Higher quality diets led to a higher proportion of soldiers being formed, which corresponded with high JH titers in the developing soldiers. Given that JH titers are known to induce soldier caste development in termites (Henderson, 2019; Mao et al., 2005; Park & Raina, 2005), this work connects nutritional state with hormone activity and the plasticity of caste development.

1.6.4 Nutrition & Histone Modifications

Extensive research has investigated histone modifications as an underlying mechanism of nutritional plasticity in *D. melanogaster*. In flies, the histone deacetylase *Rpd3* (ortholog is *Hdac1* in ants), which is known to deacetylate several H3 tail marks, is critical in tolerating starvation stress (Nakajima et al., 2016). When developing flies were starved from all nutritional resources, *Rpd3/Hdac1* activity was increased, leading to downstream effector proteins to be expressed and enabling resistance to starvation stress. In a complementary study where the nutrition was restricted rather than removed (starvation), *Rpd3/Hdac1* activity was decreased instead (Cridge et al., 2017). Furthermore, *Hdac1* regulates lifespan plasticity in flies (Rogina et al., 2002). Another histone modifier, *E(z)*, which is a histone H3 lysine 27 (H3K27) methyltransferase, has been shown to be nutritionally sensitive in flies (Cridge et al., 2017). Flies that were fed a low-protein (LP) diet showed increased expression of the *E(z)* protein, which resulted in reduced fly longevity (Xia et al., 2016). When a LP diet was supplemented with a pharmacological inhibitor of *E(z)*, longevity reduction effects were alleviated, indicating the mediatory effects of histone methyltransferase activity in the nutrition-dependent plastic response of aging (Xia et al., 2016). In eusocial insects, nutritional state has been suggested to influence queen vs worker caste determination through

histone modifications in *A. mellifera* (Alhosin, 2023). Specifically, an active ingredient of royal jelly is 10-Hydroxy-2-decenoic acid (10HDA), which is a fatty acid that inhibits activity of the histone deacetylase HDAC3 (Alhosin, 2023; Polsinelli & Yu, 2018; Spannhoff et al., 2011). Suppression of HDAC3 with this molecule in mammalian cells permits histone acetylation activity to remain active. This suggests that queen development may nutrient-sensitive through the action of 10HDA in suppressing HDAC3 activity.

1.6.5 Endocrine Modulation & Histone Modifications

To better understand the intricacies of the molecular mechanisms of developmental plasticity in solitary and social insects, recent work has been done to understand how hormones influence histone (de)acetylation and (de)methylation modifier activity, and vice versa. For histone acetylation, *Tribolium castaneum* cells were treated with RNAi to knockdown the transcripts of a gene encoding a key histone acetyltransferase effector protein (*CBP*) and found subsequent decreases in *Kr-h1* activity (Roy & Palli, 2018). In *D. melanogaster* correlations were found with histone H3K23 acetylation and ecdysone-led activation of the ecdysone-inducible genes *Eip74EF* and *Eip75B*, which are downstream transcriptional effectors of the ecdysone pathway (Bodai et al., 2012). In silkworm embryo cell lines, histone acetylation has been shown to mediate ecdysone-responsive genes (Cheng et al., 2018, 2022). Specifically, histone H3K27 acetylation in downstream ecdysone effector genes was increased after 20E treatments (Cheng et al., 2022). Although the link between histone acetylation and metamorphosis hormones is present, their shared effects in a developmental context, namely in social insects, have yet to be explored.

For histone deacetylation, a reciprocal suppressive regulation of JH and histone deacetylation has been found in *T. castaneum*, where larval treatments with JH analog suppressed *HDAC1* expression, while *HDAC1* suppresses JH signalling based on *HDAC1* KD causing an

increase in the expression levels of the JH pathway effector *Kr-h1* (George et al., 2019). In parallel, several *HDACs*, including *HDAC1* was found to suppress *Kr-h1* expression in the mosquito *Aedes aegypti* (Gaddelapati & Palli, 2025). In both beetles and mosquitoes, *HDAC1* KD led to important development effects, ranging from delayed pupation (Gaddelapati & Palli, 2025) to death of larvae (George et al., 2019). Interestingly, RNAi KD of another histone deacetylase, *HDAC3*, was shown in the eusocial termite *Cryptotermes secundus* to downregulate *Kr-h1* expression, which is a queen-specific hallmark for this insect (Okwaro & Korb, 2024). Furthermore, *HDAC3* was shown to dysregulate 20E related genes in the solitary moth *Helicoverpa armigera* (Chang et al., 2022). In ants, ecdysone-histone deacetylase developmental studies have yet to emerge. However, a relation has been shown between the *HDAC1*-scaffolding partner CoREST and degraders of JH (Glastad et al., 2020). Treatments with the histone deacetylase inhibitor TSA led to an overall increase in histone acetylation, leading to a reprogramming of foraging behaviours in *C. floridanus* majors via repression of JH-degraders. No clear roles for ecdysone were found in this treatment, but it is worth noting that majors were shown to exhibit higher ecdysone activity in wildtype individuals. Therefore, histone deacetylase activity, primarily the *HDAC* genes, demonstrate shared activity with metamorphosis-related hormones affecting insect development and behaviour.

Histone methylation has been linked with both JH and ecdysone activity in *Bombyx* and *Drosophila* (Yang et al., 2021). A significant fraction of the methylation of histone H3K27 is completed by a complex of genes that encode for the polycomb repressive complex 2 (PRC2) enabling the trimethylation of histone H3K27 (Ringrose & Paro, 2004). The PRC2 is critical for gene transcription silencing and its gene encoding the component proteins include the aforementioned *E(z)* gene and the Suppressor of zeste 12 (*Su(z)12*) gene (Ringrose & Paro, 2004). Inhibition of the PRC2 and *Su(z)12* in *Bombyx* and *Drosophila*, respectively, led to decreases in

biosynthesis of ecdysone and disrupting developmental transition between the larval and pupal stages (Yang et al., 2021). Furthermore, both *Su(z)12* expression and H3K27me3 levels were downregulated after JHA application, indicating that JH negatively regulates *Su(z)12* and repressive methylation marks of H3K27 (Yang et al., 2021). Altogether demonstrating a multidirectional relationship between *Su(z)12* histone H3K27 methylation with JH and ecdysone (Yang et al., 2021). In the eusocial termite *Z. nevadensis*, key histone modifying genes were inhibited using RNAi and JH activity was measured (Suzuki et al., 2019). Knockdown of two histone methyltransferase, *Hmt4-20* and *Ash1*, led to decreased expression of JH synthetic genes and JH receptor genes, respectively, and also extended presoldier molting periods. The relationship between histone methylation and ecdysone have yet to be investigated in social insects.

Demethylation of histone H3K27me3 through the demethylase activity of *Utx* has been linked to ecdysone-mediated cell death of salivary glands in *Drosophila* (Denton et al., 2013). During the prepupal developmental period, mutants of *dUtx* showed reduced transcription of ecdysone-induced apoptosis and autophagy encoding genes, causing delays in salivary gland degradation (Denton et al., 2013). In social insects, *Z. nevadensis* JH activity was also measured after knockdown treatments of histone demethylases (Suzuki et al., 2019). RNAi treatments of the histone demethylase *Utx* in developing termites led to a decreased expression of the JH receptor gene (*Met*) and *Kr-h1*, and also led to an extension of the presoldier molting period. Overall, histone demethylation, notably *Utx* activity, demonstrates an important connection between both JH and ecdysone across various insects.

Altogether, this review of insect literature provides a foundation to understand the effects of thermal and nutritional variation as well as endocrine and epigenetic modulation underlying developmental plasticity. It emphasizes their interactions, producing diverse phenotypic responses.

Social insects have demonstrated sensitivities to both temperature and nutrition, affecting their size and developmental timing. Likewise for hormone and histone activity, both mechanisms are necessary for insect growth. However, there remain gaps and lack of clarity in several connections between our four targeted facets in the context of insect developmental plasticity. To provide a holistic view aimed at connecting these various facets of developmental plasticity (Fig. 1), we use *S. invicta* fire ants as our model to explore each facet and how they interact with one another.

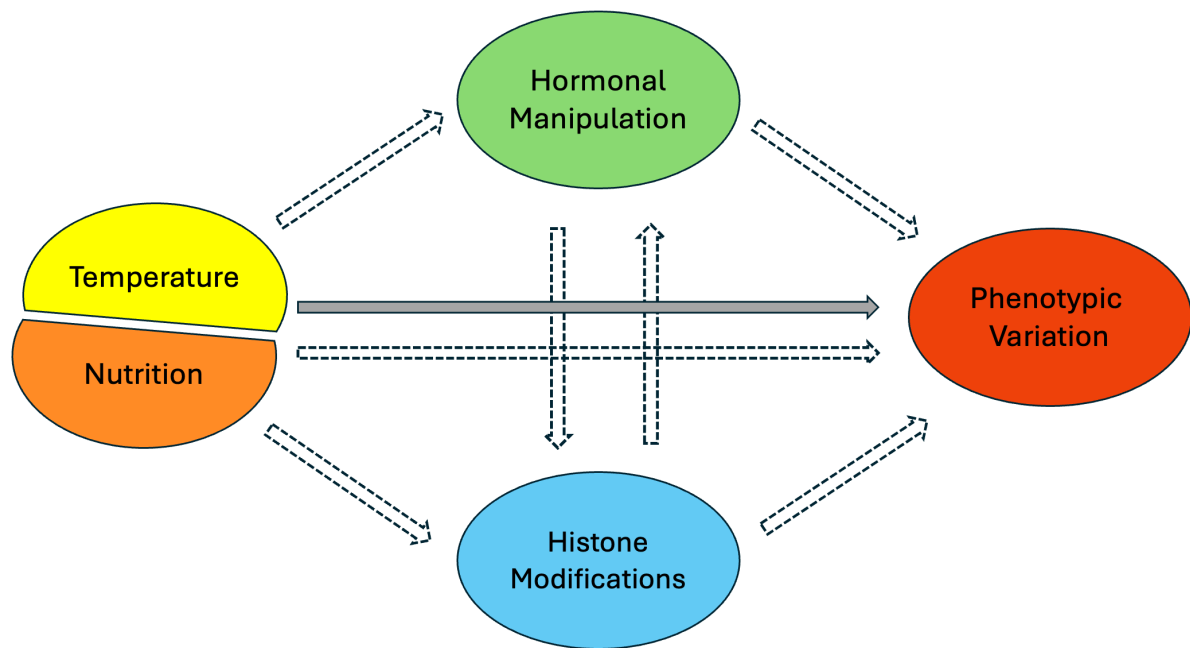


Fig. 1. Proposed connections between the environmental, endocrine, and epigenetic facets of developmental plasticity in *Solenopsis invicta*. Each facet is represented by their respective colours and are linked by arrows to indicate their potential interactions, as explored in various insects. Dotted empty arrows indicate connections that have yet to be established in *S. invicta*, while full lines demonstrate relationships previously characterized in fire ant literature.

1.7 Objectives and Hypotheses

Objective 1

Our overall aim is to investigate the activities of temperature, nutrition, hormones, and histones in the context of developmental plasticity. However, we will first describe the expressional differences of our genes of interest in natural *S. invicta* individuals across three larval stages of the third instar, early fourth instar (E4) and late fourth instar (L4). For metamorphosis-related hormones, we will measure the activities of two downstream transcriptional end-products of the JH and ecdysone pathways, *Kr-h1* and *E93*, respectively. Furthermore, the expressions of key histone toolkit genes in ants, *Kat6b*, *Hdac1a* and its paralog *Hdac1b*, *Su(z)12*, and *Utx*, will be investigated to represent each of the four facets of histone acetylation, deacetylation, methylation, and demethylation, respectively.

Hypothesis 1

JH and Ecdysone signaling alongside histone modifiers are differentially expressed across larval stages in *S. invicta*.

Objective 2

We aim to understand how environmental factors alter the developmental trajectories and to describe their effects on the activity of underlying mediators in *S. invicta* individuals. Therefore, we will characterize the effects of thermal and nutritional variations on developing *S. invicta* larvae to discover morphological changes in phenotype. We will then describe the effects of both environmental factors on our JH and ecdysone pathway targets, *Kr-h1* and *E93*, respectively. Furthermore, the expressions levels of the histone toolkit genes, *Kat6b*, *Hdac1a* and its paralog *Hdac1b*, *Su(z)12*, and *Utx*, will be investigated.

Hypothesis 2.1

Based on the temperature-size rule that applies broadly across holometabolous insects, temperature increase will lead to a size reduction and a shortening of developmental timing of developing *S. invicta* ants.

Hypothesis 2.2

Changes in temperature will be associated with differential expression of histone modifiers and JH/ecdysones signalling in developing *S. invicta* ants.

Hypothesis 2.3

Nutritional variation influences size and developmental timing of developing *S. invicta* ants, with concurrent differential expression of histone modifiers and JH/ecdysones signalling.

Objective 3

Our next objective aims to address the effects of hormone modulation and histone modifications and their interplay on morphological phenotypes, using functional approaches. First, we will manipulate the hormonal levels JH and ecdysone by using JHA and 20E treatments, respectively, to characterize their influence on the size and developmental timing of developing *S. invicta* larvae, followed by characterizing the expressional differences in our histone target genes. Next, we will implement pharmacological inhibitors of histone modifications to understand their effects on the morphological changes in size and developmental timing.

Hypothesis 3.1

Juvenile hormone treatment will lead to an increase in size and an extension of developmental timing and ecdysone treatment will lead to a decrease in size and a shortening of developmental timing in developing *S. invicta* ants.

Hypothesis 3.2

JH and ecdysone levels influence expression levels of histone acetylation and methylation modifiers in developing *S. invicta* ants.

Hypothesis 3.3

Inhibition of histone modifiers will affect sizing and developmental timing of developing *S. invicta* ants.

Chapter 2: Methods

2.1 Ant husbandry

Monogynous *Solenopsis invicta* colonies originally collected in Florida, USA, were donated by the Abouheif lab. All colonies were maintained in incubators set to 25°C, 60% humidity and a 12:12 h light:dark cycle. Colonies were kept in plastic boxes with mesh-covered holes to allow for airflow, and fluon-coated walls to prevent insect climbing. The boxes were lined with test tubes filled with either water or sugar water and plugged with cotton to allow for enclosed living quarters for the ants. A layer of acetate paper covered the tubes to mimic a subterranean environment. Colonies were fed 3x/week with a diet of mealworms, fruit flies, and Bhatkar-Whitcomb diet (Bhatkar & Whitcomb, 1970). All experiments except for ecdysone and nutritional treatments were performed on the same colony to allow for more consistent data production.

2.2 Experimental replicate setup

From the *S. invicta* colonies, all experimental treatments were composed of worker ants and early 4th (E4) instar larvae, isolated in a 4:1 ratio. E4 larvae were targeted because the last larval instar of *S. invicta* is the 4th instar, which is a developmentally plastic period that allows for the highest growth potential to occur (Alvarado et al., 2015). The E4 larvae have yet to experience these high levels of growth, enabling this developmental stage to respond to various experimental manipulations and observe their effects on growth and gene activity. Furthermore, the smallest observable workers were selected for each replicate. The smallest fire ant workers are known to be primarily dedicated to brood care (Wilson, 1978), thus ensuring sufficient care for developing larvae within replicates. Each replicate was separated into small plastic containers containing 1-2 small cotton-bound water tubes, mimicking the environment of their source colony. Larvae were

measured using the Zeiss Axio Zoom. V16 microscope. Early 4th (E4) instar larvae were categorized based on the outlined measurements of head width (0.26-0.33 mm) and body length (0.910-1.200 mm), and with the emergence partially sclerotized mouthparts, as described by Petralia & Vinson (1979). Furthermore, 3rd instar larval head width ranged from 0.20-0.25 mm and body length from 0.60-0.90 mm, with the absence of mouthparts (Petralia & Vinson, 1979). Late 4th (L4) instar larval head width had the same range as the E4 individuals, but with a body length ranging from 1.50-1.82 mm (Petralia & Vinson, 1979). Head width is the widest head capsule segment, while body length measures the top of the larval head to the base of its body (larvae were placed ventral side up and dorsal side down). These measurements were specific to the ‘minor worker’ destined larvae for *S. invicta*, meaning that these larval size ranges led to the smaller workers to be produced (Petralia & Vinson, 1979). All of our experimental manipulations focused on the ‘minor worker’ destined larval size ranges. Furthermore, collected E4 instar larvae were separated into smaller (0.910-1.100 mm) and larger (1.101-1.200) groups and split evenly among each set of trials to allow for an equal distribution of ‘early’ and ‘late’ E4 larvae per treatment. With the exception of the nutritional experiments, all treatments were fed 3x/week with a diet 2 mealworms cut into 3-4 pieces and a cube of Bhatkar-Whitcomb diet, until final pupation.

2.3 Morphological data collection

Treatments were checked every 2-4 days to record the progression of pupal distribution by dumping out glass tube contents and counting the pupae present, providing our developmental timing data. Counts could not be made daily without disturbing larval growth and promoting worker stress. Dumped pupae were then collected and the Zeiss Axio Zoom. V16 microscope was used for pupal measurements. The anterior of the pupae faces forward, with their dorsal segments facing upwards, as demonstrated by Rajakumar et al. (2018). From this, head width is measured

using the widest head segment. Body length is measured from the top of the head to the base of the thorax, at a consistent physical landmark found among pupae, found in between the first and second petioles.

2.4 RT-qPCR primer design

Our overall RT-qPCR primer design largely followed the MIQE guidelines (Bustin et al., 2009; Bustin & Huggett, 2017; Rodríguez et al., 2015; Taylor et al., 2010; Thornton & Basu, 2015). Using the National Center for Biotechnology (NCBI) database, we designed 1-3 pairs of forward and reverse primers for each of our genes of interest (Taylor et al., 2010). The protein sequence of each gene was first found within the *D. melanogaster* database, given the high level of gene conservation in the robust fly genome. The protein sequence was then run through BLAST against the *S. invicta* database, in search for the corresponding sequence in fire ants (Ye et al., 2012). Nucleotide sequences were then acquired and imported into the Geneious software, allowing for sequence annotations. Exon-exon junctions were located and placed along each sequence. Primers were then designed along the identified exon-exon junctions to avoid intron amplification (Rodríguez et al., 2015). Primer sizes ranged from 18-22 base pairs, with melting temperatures (T_m) ranging from 55-65°C, with each pair being within 2 degrees of each other (Taylor et al., 2010; Thornton & Basu, 2015). Their GC base pair content required a range of 50-60% (Taylor et al., 2010). Primers could not include four of the same bases in a row and we aimed for our total amplicon lengths to span 80-115 base pairs (bp), but could range from a minimum of 75 bp to a maximum of 150 bp (Rodríguez et al., 2015; Taylor et al., 2010; Thornton & Basu, 2015). Using Oligo Calc, self-complementarity and self-annealing of primers were verified and adjusted for. Each primer and amplicon were then NCBI blasted against *S. invicta*, to check for the specificity of the amplicon relative to the gene of interest (Rodríguez et al., 2015; Taylor et al., 2010). A small

E-value was required (~ 0), which would indicate a decreased probability that the similarity between the sequences is due to chance (Rodríguez et al., 2015). Additionally, query coverage needed to be $>90\%$ for our genes of interest, while undesirable genes could not surpass 70% (Rodríguez et al., 2015). Finalized primer sequences were then ordered from ThermoFisher Scientific and tested using PCR/gel-electrophoresis from *S. invicta* cDNA, to select for the optimal primer pair for our targeted genes. Exceptionally, to ensure primers targeting paralogs, in this case *Hdac1a* and *Hdac1b*, were paralog-specific, their nucleotide sequences were aligned, and we designed primers in regions that were variable and lacked conservation between the two.

2.5 RNA extraction and cDNA synthesis

For each experimental manipulation with the intention for RT-qPCR analysis, 6 biological replicates were produced, each containing 5 larvae, for a total of 30 larvae per treatment group. Larvae were frozen within 1.5 mL centrifuge tubes at -80°C for a minimum of 24 hours prior to extraction and purification of RNA. This allowed larvae to be easily crushed and hand homogenized in 100 μL of Trizol. Tubes were filled up to 1 mL of Trizol and were left for 5 minutes to incubate at room temperature (RT). To separate the aqueous phase, 200 μL of chloroform was added with a 3-minute RT incubation, followed by a centrifugation at $12,000 \times g$ at 4°C for 15 minutes. 500 μL of the aqueous phase was separated and combined with 500 μL of 99% ethanol. The Direct-zol RNA MicroPrep kit (Zymo Research) was then used to complete RNA extraction. To purify the RNA of any DNA contaminants, a Turbo DNase treatment (Invitrogen) was implemented. The purified RNA was then verified for its concentration and purify level using a nanodrop (ThermoFischer). From this, 500 ng (within 21 μL of solution) of cDNA was produced by first normalizing samples based on RNA concentrations, followed by using the Super Script IV kit (Invitrogen). To match the concentration of cDNA produced, RNA dilution of

500 ng in 21 uL of DEPC water was prepared, followed by a collective 20-fold dilution of both RNA and cDNA samples for RT-qPCR application.

2.6 RT-qPCR

With the established primers for our genes of interest, we then used the extracted RNA and synthesized cDNA for RT-qPCR using relative quantification analysis. All RT-qPCR plates were run on a C1000 CFX96 RT-qPCR device, using SSO advanced SYBR green (BioRad). To understand how our genes of interest change in expression across treatments groups, we require a conserved gene that is highly developmentally and experimentally stable in its expression levels. The housekeeping gene *Rpl32* is a robust normalization gene, conserved in both solitary (Ponton et al., 2011) and social insects (Freitas et al., 2019). With our housekeeping gene, relative fold changes were calculated using the DDCT method (Livak & Schmittgen, 2001), to compare changes in gene expression between groups. First, CT values for the gene of interest and housekeeping gene were calculated, followed by the DCT value, which is the difference between the CT values of both genes. This normalized our sample, which was then subtracted from the DCT value of our control group, providing the DDCT value. To provide biological relevance, 2^{-DDCT} was calculated demonstrating relative fold changes of both treatment and control groups, for further statistical analysis.

2.7 Temperature treatments

The three thermal conditions of low (25°C), medium (28.5°C), and high (32°C) were used to investigate effects of temperature variation on morphology. 25°C is the temperature at which colonies are normally reared, 32°C is the temperature for which *S. invicta* colonies are known to undergo maximum growth (Porter, 1988), and 28.5°C provides an intermediate temperature. Three incubators were used to set each temperature, while humidity (60%) and photoperiod (12:12 h LD)

were kept constant. Larvae were reared until pupation for morphological data collection. Three modified temperatures of a wider range including low (18°C), medium (25°C), and high (30.4°C) conditions, reflective of the range examined by Porter (1988), were used to rear larvae for 48 hours. This was followed by flash freezing at -80°C for RT-qPCR analysis. In both sets of temperatures, the 25°C groups were the controls, given that the source *S. invicta* colony is kept in a constant 25°C environment. All temperatures chosen were based on Porter's (1988) study, describing the ranges of temperatures that affect colony growth and developmental timing.

2.8 Nutrition treatments

To investigate the effects of nutrition on developmental plasticity in *S. invicta*, two replicates were prepared by first starving workers prior to E4 larvae being added to the experimental groups. Cassill & Tschinkel (1995) implemented a 48-hour worker starvation period, presumably to deplete workers of any residual nutrients. However, our trials suffered mass deaths from this method. Therefore, we starved workers for 24 hours prior to being added to experimental boxes for both groups. Both replicates were fed the same diet, consisting of 2 mealworms and a cube of Bhatkar-Whitcomb diet (Bhatkar & Whitcomb, 1970), but differed in the frequency of feeding, where the control group was fed daily, while the group in a caloric deficit was intermittently fasted, being fed every 4 days. Larvae were reared until pupation for morphological data collection. Furthermore, to determine the effects of nutrient deprivation on underlying endocrine and epigenetic activities, another set of replicates were prepared, where the control group was fed daily, while the treatment group was starved of any nutrients for 4 days (96 hours). Based on our findings from the nutrient deprivation setup, where there is a high mortality of adult workers caring for the experimental larvae, our 4-day starvation setup for quantifying the influence of nutritional variation on endocrine and epigenetic modifier expression levels, adult workers were

not pre-starved to ensure adult workers could survive during the 96hr experimental period to care for the larvae being investigated. Larvae were then collected and flash frozen at -80°C for RNA extraction and RT-qPCR analysis. Finally, another set of replicates were arranged to investigate the effects of a high sugar diet, while being protein (among other nutrients) deficient. The control group was supplied with a saturated solution of sucrose and fed regularly every 2 days with two mealworms and a Bhatkar-Whitcomb diet, while the treatment only received the sucrose solution. Larvae were reared until pupation for morphological data collection.

2.9 Endocrine manipulations

Hormonal treatments to target the JH pathway implemented the JH analogue methoprene (CAT: 33375; Millipore Sigma). 5 mg/mL aliquots were made using acetone as the solvent and were further diluted to 2.5 mg/mL (Fig. 5). Treatments were carried out on E4 instar larvae, as previously described. Larvae were placed on their dorsal side on a wet Kimtech tissue. 1 ul of acetone (control) or methoprene (treatment) was applied topically on larval abdomens, with the high volatility of acetone allowing for rapid cuticular absorption (Wheeler, 1990). Larvae were reared until pupation for morphological data collection or were collected after 24 hours post treatment and flash frozen at -80°C for RNA extraction and RT-qPCR analysis.

For ecdysone treatments, 20-hydroxyecdysone (20E) (CAT: H5142; Millipore Sigma) was dissolved in a 99% ethanol solvent, and 1 mg/mL aliquots were used for microinjection experimental manipulations, using techniques described by Bear et al., (2017) as guidelines. Both the 99% ethanol control and 20E treatment were dissolved in a 1x phosphate-buffered saline (PBS) solution, at a 1:9 ratio, to allow for optimal hormonal delivery. E4 instar larvae were microinjected with either 99% ethanol or with 1 mg/mL 20E, using a combination of a Zeiss SteREO Discovery V8 microscope with an Eppendorf CellTram 4r Oil microinjector and a Narishige

micromanipulator. A Stutter Instrument P-97 needle puller was used to prepare needles for larval injection. Larval bodies were placed ventral-up, and their cuticles were pierced at the lateral midline, which is a fatty body section, and in between larval segments, to minimize fatal wounds. Larvae were reared until pupation for morphological data collection or were collected after 24 hours post treatment and flash frozen at -80°C for RNA extraction and RT-qPCR analysis.

2.10 Pharmacological histone modification inhibitors

The pan-histone deacetylase inhibitor trichostatin-A (TSA) (CAT: T1952; Millipore Sigma) was acquired at 5 mM, dissolved in a dimethyl sulfoxide (DMSO) solvent for experimental manipulation (Simola et al., 2016). E4 instar larvae were microinjected using the same methodology as in the 20E treatments. However, given the high mortality rate of DMSO dissolved treatments, the proportion of the PBS vehicle present was increased to mitigate the lethality of DMSO. Therefore, DMSO controls and TSA treatments were dissolved in 1x PBS at a 0.5:9.5 ratio. The histone methyltransferase inhibitor GSK343 (CAT: SML0766; Millipore Sigma), which targets a component of the PRC2 silencing complex, was dissolved in DMSO at a concentration of 5 mM (Werner et al., 2023). Methodology for PBS dilution and microinjection followed the same protocol as the TSA treatments. Larvae were reared until pupation for morphological data collection or were collected after 48 hours post treatment (Simola et al., 2016) and flash frozen at -80°C for RNA extraction and RT-qPCR analysis.

2.11 Statistical analyses

All datasets were tested for normality using the Shapiro-Wilk test, while the developmental timing results were tested using a combination of the Shapiro-Wilk and D'Agostino & Pearson tests, given the several identical values found within the developmental timing datasets. If following normal distribution, the means of head width, body length, developmental timing, and

RT-qPCR relative fold changes were respectively compared using one-way ANOVA analyses (followed by Tukey's multiple comparison test) for treatments that were experimenting with three parameters (natural expression and temperature treatments), while unpaired two-tailed Student's *t*-tests were used for treatments with two parameters (nutrition, endocrine, and epigenetic). If the dataset is not normally distributed, a Kruskal-Wallis test (followed by Dunn's multiple comparison test) was used rather than an ANOVA, while a Mann-Whitney test was used instead of a Student's *t*-tests. A one-tailed *t*-test was used to assess the effect of JHA on *Su(z)12* (Fig. 5I), because it has been established that *Su(z)12* is negatively regulated by JH signalling and specifically by JHA treatments in *Bombyx* and *Drosophila* (Yang et al., 2021). Furthermore, a Welch's *t*-test was used in the analysis of TSA treatments on head and body sizes (Fig. 7A, B), given their significance in F test variance. Scatterplots were log transformed and both slopes and (when possible) y-intercepts were compared between groups. All analyses were done using GraphPad Prism.

Chapter 3: Results

3.1 Natural Plasticity in *S. invicta*

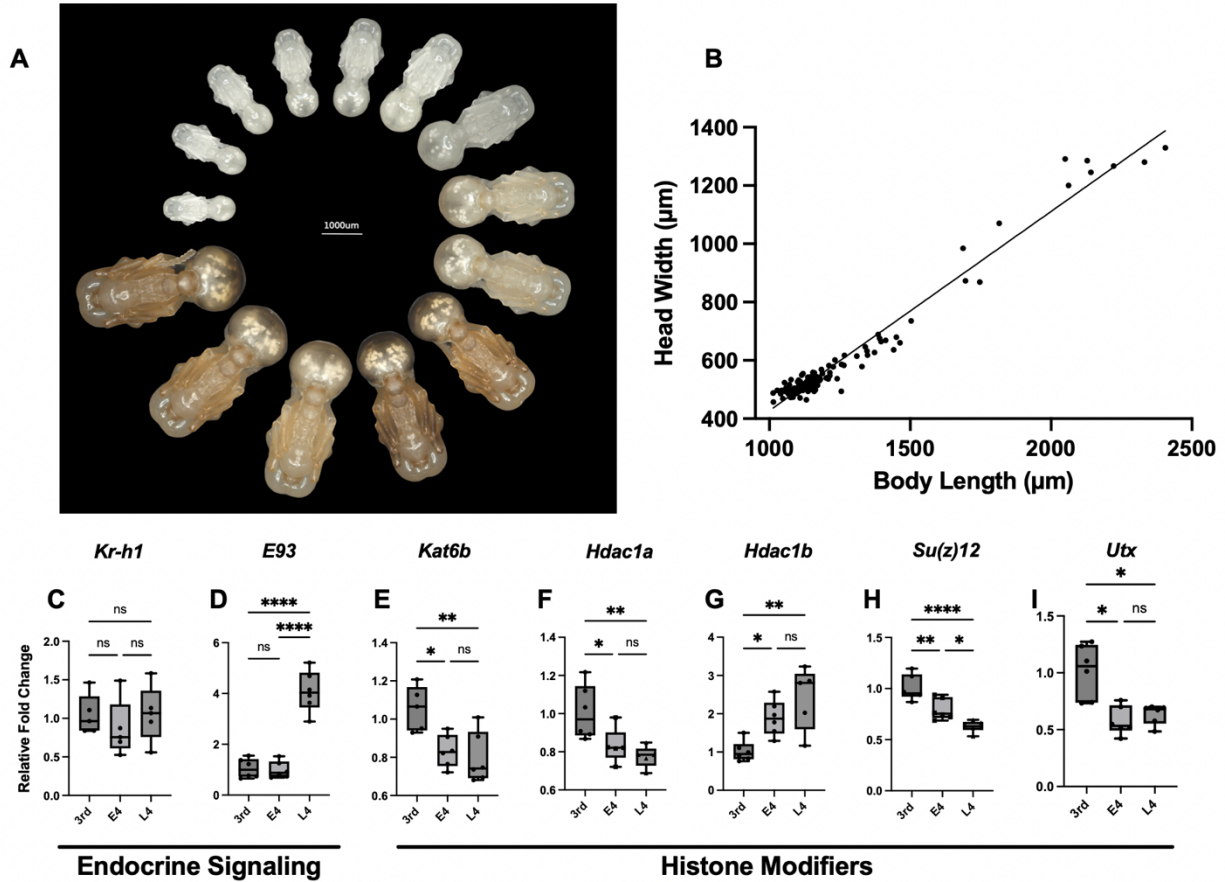


Fig. 2. *Solenopsis invicta* natural comparison of targeted endocrine and histone genes in developing larvae. (A) Pupal size pinwheel representing the general size continuum of differently sized individuals at their pupal stage within the *S. invicta* worker caste, separated by intervals of ~100 μm within a fire ant colony. (B) An XY scatterplot to compare the natural head width-to-body length distribution of individuals qPCR analyses were used to compare the relative expression levels of target genes in developing 3rd, early 4th (E4) and late 4th (L4) instar larvae. One-way ANOVA analyses were used to compare expression differences in (C) *Kr-h1*, (D) *E93*, (E) *Kat6b*, (F) *Hdac1a*, (G) *Hdac1b*, (H) *Su(z)12*, and (I) *Utx*. Bars represent means, with standard deviation (SD) indicated with error bars. Values for statistical significance of ANOVA and Kruskal-Wallis tests include * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. Sample size for each group ranges from 5-6 biological replicates, each containing 5 individual larvae.

Size variation within the worker caste system of *Solenopsis invicta* is extensive and has been well characterized (Tschinkel, 2006). Size variation in holometabolous insects is generated during larval stages that precede metamorphosis (Hanna et al., 2023). Body size variation and head-to-body allometry can be visualized and quantified at the pupal stage following metamorphosis when individuals have obtained their final size (Fig1A, B). Before investigating the role of both critical factors of the histone epigenetic toolkit and major hormonal pathways, in regulating growth and size variation in fire ants, we first wanted to assess their expression dynamic during the key growth stages of larval development (Fig.1C-I). Growth variation underlying adult size variation in *S. invicta*, emerges towards the end of larval development (Wheeler, 1990). To understand how the expression levels of our target genes progress in natural *S. invicta* development, we sampled larvae from the 3rd, early 4th and late 4th instars and measured expression levels of key hormonal and histone epigenetic toolkit genes. Taking an RT-qPCR approach, we measure the expression of *Kr-h1*, the key effector of JH signalling pathway, and *E93*, a critical effector of the ecdysone signalling pathway across instar 3 (3rd), early 4th (E4) and late 4th (L4). While *Kr-h1* activity remained stable across the developmental stages (Fig. 2C; $p=0.6178$), *E93* had an increase in activity in the L4 stage (Fig. 2D; $p<0.0001$). We next measured the expression of the histone acetyltransferase *Kat6b* (also known as *enok*) and the histone deacetylase paralogs *Hdac1a* and *Hdac1b*, essential writers/erasers of histone acetylation as well as histone methyltransferase *Su(z)12* and histone demethylase *Utx*, essential writers/erasers of histone methylation (Nicholson et al., 2015; Marmorstein & Zhou, 2014; Seto & Yoshida, 2014; Ringrose & Paro, 2004). These genes have been implicated both in the ant comparative genomic literature as candidate genes facilitating adaptive radiations, size variation, and caste diversity (Romiguier et al., 2022; Vizueta et al., 2025) as well as the ant transcriptomic literature as candidate mediators of environmental

response and caste plasticity (Gospocic et al., 2021; Perez et al., 2021; Simola et al., 2016). For acetylation modifiers, we found that *Kat6b* decreased in expression from the 3rd to E4 (Fig. 2E; p=0.0147) and from the 3rd to L4 (Fig. 2E; p=0.0052), while the developmental expression of histone deacetylase paralogs *Hdac1a* and *Hdac1b* progressed in opposite directions, where *Hdac1a* decreased both between 3rd to E4 (Fig. 2F; p=0.0456), and between E4 to L4 (Fig. 2F; p=0.0086), and *Hdac1b* increased both between 3rd to E4 (Fig. 2G; p=0.0365) and between 3rd and L4 (Fig. 2G; p=0.0022). For methylation modifiers, we found that *Su(z)12*, decreased in expression across stages (Fig. 2H; 3rd vs E4, p=0.0062; 3rd vs L4, p<0.0001; E4 vs L4, p=0.0287) while the expression of the histone demethylase *Utx* decreased between 3rd and E4 as well as 3rd and L4 (Fig. 2I; 3rd vs E4, p=0.0105; 3rd vs L4, p=0.0283). Overall, expression levels of our targeted genes are dynamic across stages and provide a natural context to better understand the activity of these genes following environmental, endocrine, and epigenetic perturbations.

3.2 Thermal Plasticity in *S. invicta*

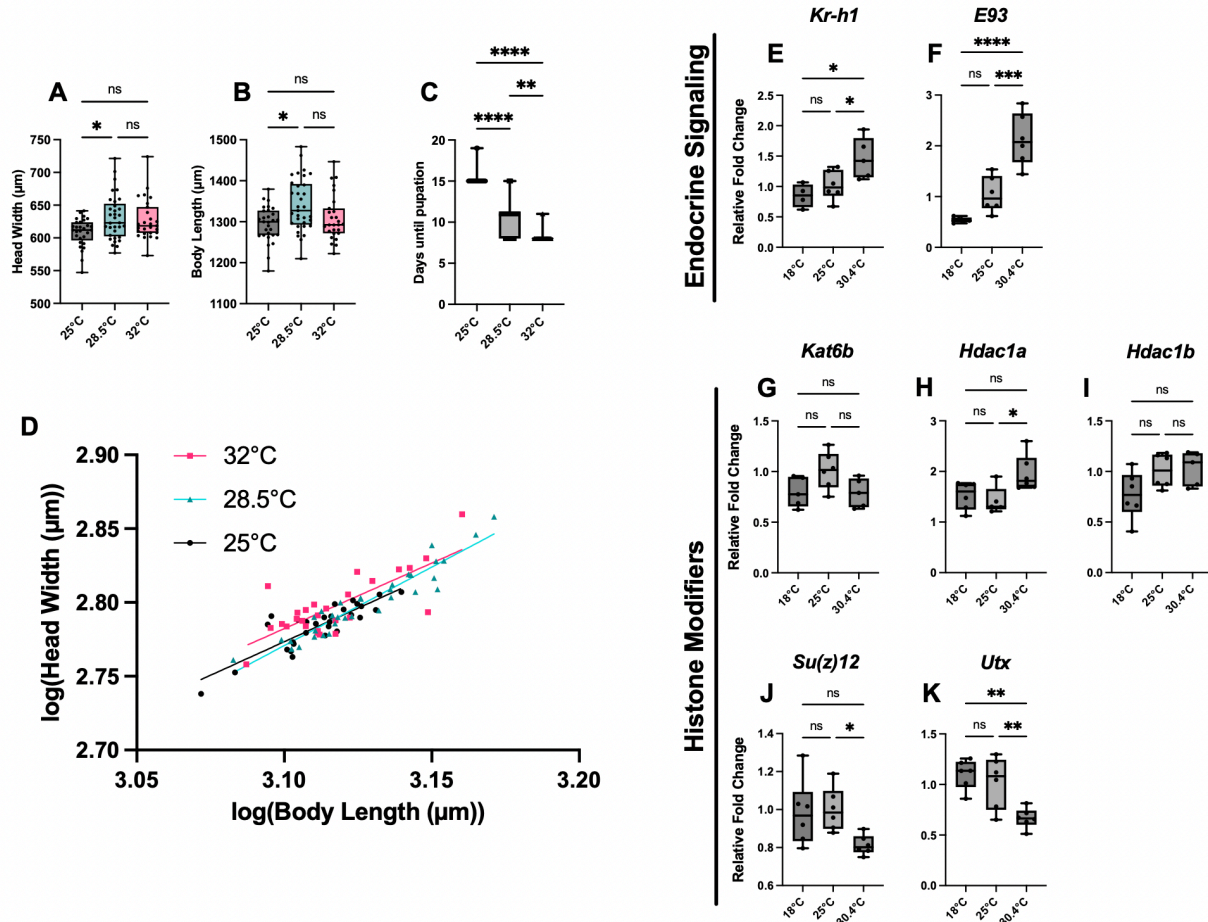


Fig. 3. The effects of temperature variation on developmental plasticity in *Solenopsis invicta*. Comparison between measurements of (A) head width, (B) body length, (C) developmental timing, and (D) head-to-body allometry of developing *S. invicta* larvae in 25 $^{\circ}\text{C}$ (n=29), 28.5 $^{\circ}\text{C}$ (n=33), and 32 $^{\circ}\text{C}$ (n=27) treatment groups. qPCR analyses of 48-hour treatments comparing (E) *Kr-h1*, (F) *E93*, (G) *Kat6b*, (H) *Hdac1a*, (I) *Hdac1b*, (J) *Su(z)12*, and (K) *Utx* between 18, 25, and 30.4 $^{\circ}\text{C}$ groups. Bars represent means, with standard deviation (SD) indicated with error bars. Values for statistical significance of ANOVA and Kruskal-Wallis tests include * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. Sample size for qPCR treatment groups range from 4-6 biological replicates, each containing 5 individual larvae.

We next wanted to investigate the influence of environmental perturbations on developmental growth and developmental timing in fire ants. Variation in thermal conditions is known to affect developmental timing and worker production in *S. invicta* colonies (Porter, 1988). Higher temperatures led to exponentially more rapid growth of developing brood (embryos, larvae, pupae), while colony sizes peaked at 32°C, followed by a rapid decline thereafter. Based on this, we characterized the effects of temperature variation on size and developmental timing, as well as potential endocrine and epigenetic modifiers underlying fire ant thermal plasticity. To test this, we reared *S. invicta* E4 larvae in at low (25°C), medium (28.5°C), and high (32°C) temperatures and characterized head width and body size of pupae. Relative to the 25°C group, we found increases for 28.5°C reared individuals for both head width (Fig. 3A; $p=0.0411$) and body length (Fig. 3B; $p=0.0190$). Next, we compared the differences in developmental timing between larvae reared among the three temperatures and found that increasing temperatures shortens the total developmental period (Fig. 3C; 25 vs 28.5°C, $p<0.0001$; 25 vs 32°C, $p<0.0001$; 28.5 vs 32°C, $p=0.0043$). Furthermore, we plotted log transformed measurements for head width against body length to analyze the allometric effects of varying temperatures. We performed a simple linear regression and found a decrease between the intercepts of the 28.5°C treatment, relative to the 25°C and 32°C groups (Fig. 3D; $p=0.0038$). Based on our findings of thermal plasticity of size and developmental timing, we reared E4 larvae at low (18°C), medium (25°C), and high (30.4°C) temperatures, and then assessed the expression of the endocrine (*Kr-h1*, *E93*) and histone modifier (*Kat6b*, *Hdac1a*, *Hdac1b*, *Su(z)12*, and *Utx*) toolkit genes after a 48hr exposure. For endocrine signalling, increasing temperatures resulted in expressional increases of both *Kr-h1* (Fig. 3E; 18 vs 30.4°C, $p=0.0132$; 25 vs 30.4°C, $p=0.0457$) and *E93* (Fig. 3F; 18 vs 30.4°C, $p<0.0001$; 25 vs 30.4°C, $p=0.0005$). For histone acetylation modifiers, while *Kat6b* (Fig. 3G) did not respond to

temperature variation, expression levels of *Hdac1a* increased with temperature (Fig. 3H; 25 vs 30.4°C, $p=0.0298$), yet *Hdac1b* was unaffected (Fig. 3I). For histone methylation modifiers, *Su(z)12* (Fig. 3J; 25 vs 30.4°C, $p=0.0477$), and *Utx* (Fig. 3K; 18 vs 30.4°C, $p=0.0020$; 25 vs 30.4°C, $p=0.0098$) decreased with temperature. Collectively, at the phenotypic level, higher temperatures can increase sizing of individuals and shorten developmental timing, while at the molecular level, ecdysone signalling as well as histone acetylation modifiers increase with temperature while histone methylation modifiers decrease.

3.3 Nutritional Plasticity in *S. invicta*

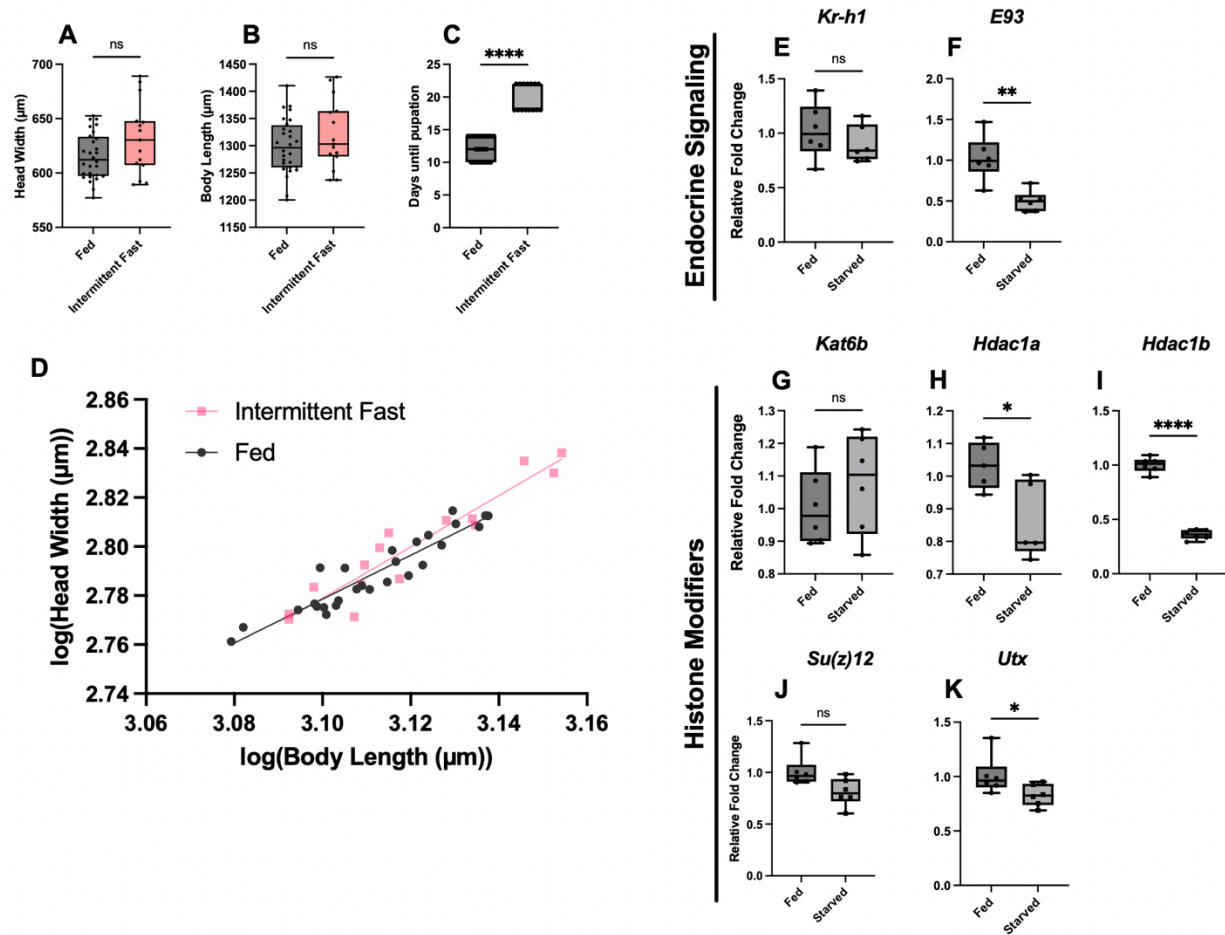


Fig. 4. The effects of nutritional variation on developmental plasticity in *Solenopsis invicta*. Comparison between measurements of (A) head width, (B) body length, (C) developmental timing, and (D) head-to-body allometry of developing *S. invicta* larvae in sated ($n=28$) and intermittently fasted ($n=15$) treatment groups. qPCR analyses of 96-hour treatments comparing (E) *Kr-h1*, (F) *E93*, (G) *Kat6b*, (H) *Hdac1a*, (I) *Hdac1b*, (J) *Su(z)12*, and (K) *Utx* between sated and starved groups. Bars represent means, with standard deviation (SD) indicated with error bars. Values for statistical significance of Student's *t*-tests and Mann-Whitney tests include * $p < 0.05$, ** $p < 0.01$, and **** $p < 0.0001$. Sample size for qPCR treatment groups range from 5-6 biological replicates, each containing 5 individual larvae.

Expanding on our thermal plasticity findings regarding the influence of environmental variation on growth and developmental timing, we next wanted to investigate the influence of nutritional plasticity. We first reared *S. invicta* E4 larvae in either an intermittently fasted group with or a fed group and characterized head width and body size, developmental timing, and head-to-body allometry of pupae. Head, body size, and allometry remained unchanged in the fasting group as compared to the sated group (Fig. 4A; $p=0.0536$; Fig. 4B; $p=0.2398$; Fig. 4D). Despite the lack of change in sizing, intermittent fasting individuals prolonged development compared to the fed group (Fig. 4C; $p<0.0001$). This developmental timing extension likely is the cause for intermittently fasted individuals catching up in size to the fed group. To further investigate nutrition-dependent size variation, we tried an alternative nutritional regime of nutrient deprivation, where individuals were fed only sugar water and compared to fed individuals. While this nutritional regime led to a high rate of mortality, four individuals survived, and both their head and body sizes were dramatically smaller than the fed group, such that they were smaller than any individuals found in the natural size distribution (Fig. 1A, B; Fig. S1). Furthermore, this change in size was mirrored by a dramatic increase in developmental timing, prolonging development of nutrient-deprived individuals by an extra 2-5 weeks as compared to fed controls (2-to-3-fold extension). Based on our findings of nutritional plasticity of size and developmental timing, we reared E4 larvae in either a fed or starvation condition and then assessed the expression of the endocrine and histone modifier toolkit genes after a 96hr treatment. For endocrine signalling, while starvation did not lead to a change in *Kr-hl* expression relative to fed controls (Fig. 4E), *E93* decreased in expression levels (Fig. 4F; $p=0.0016$). For histone acetylation modifiers, while *Kat6b* did not change following starvation (Fig. 4G), both *Hdac1a* (Fig. 4H; $p=0.0249$) and *Hdac1b* (Fig. 4I; $p<0.0001$) decreased in expression levels compared to fed controls. For histone methylation

modifiers, *Utx* decreased in expression levels following starvation, as compared to fed controls (Fig. 4K; $p=0.0411$), while *Su(z)12* did not change (Fig. 4J). Altogether, at the phenotypic level, nutritional deficiency prolongs developmental timing and can negatively affect growth, while at the molecular level, ecdysone signalling, as well as both histone acetylation and histone demethylation modifiers all decrease.

3.4 Influence of Hormones on Developmental Plasticity in *S. invicta*

3.4.1 Effects of Juvenile Hormone Analog on Fire Ant Development

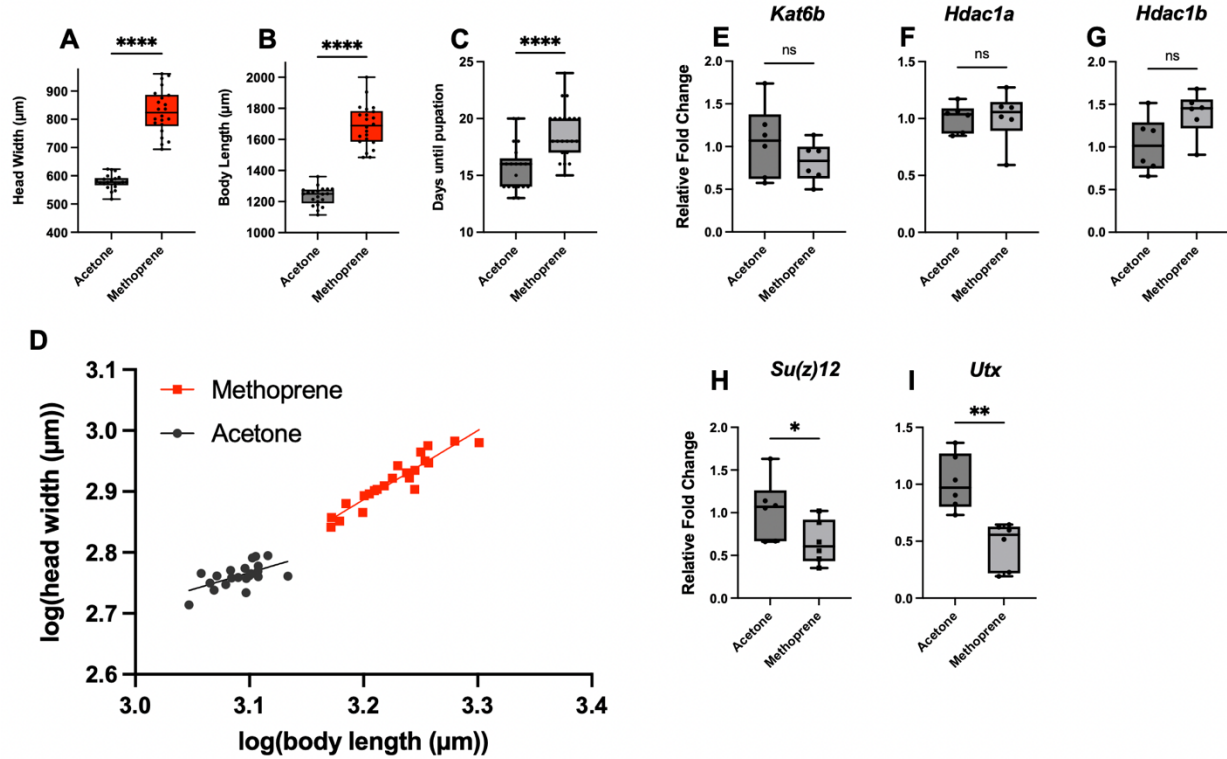


Fig. 5. The effects of the juvenile hormone analogue methoprene on developmental plasticity in *Solenopsis invicta*. Comparison between measurements of (A) head width, (B) body length, (C) developmental timing, and (D) head-to-body allometry of developing *S. invicta* larvae in acetone controls (n=21) and methoprene (n=23) treatment groups. qPCR analyses of 24-hour treatments comparing (E) *Kat6b*, (F) *Hdac1a*, (G) *Hdac1b*, (H) *Su(z)12*, and (I) *Utx* between acetone and methoprene groups. Bars represent means, with standard deviation (SD) indicated with error bars. Values for statistical significance of Student's *t*-tests include ** $p < 0.01$ and **** $p < 0.0001$. Sample size for qPCR treatment groups include 6 biological replicates, each containing 5 individual larvae.

Juvenile hormone is known to influence size and developmental timing in *S. invicta* in a dose-dependent manner. Wheeler (1990) demonstrated that treatment of JH to developing *S. invicta* larvae led to an increase in body size and an extension of larval development. How JH influences epigenetic processes, and specifically histone modifiers is unknown. Therefore, we

treated developing *S. invicta* individuals with JH analog (JHA) and assayed the influence of JH on sizing, head-to-body allometry, and histone modifier expression levels. We injected E4 larvae with JHA and as expected, we found that compared to the control, JHA increased head width (Fig. 5A; $p < 0.0001$) and body size (Fig. 5B; $p < 0.0001$) as well as extended developmental timing (Fig. 5C; $p < 0.0001$). As the influence of JH on head-to-body allometry has yet to be described in fire ants, we therefore analyzed the allometric effects of JHA and found that treatments led to an increase in slope as compared to controls (Fig. 5D; $p = 0.0041$). Based on our findings of the effects of JH signalling on sizing, allometry, and developmental timing, we next treated E4 larvae with JHA and characterized the influence of JH signalling on the expression levels of histone modifiers. For histone acetylation modifiers, individuals treated with JHA for 24 hours had no effect on expression levels as compared to controls (Fig. E-G), while JHA treatment led to decreased expression of both histone methylation modifiers *Su(z)12* (Fig. 5H; $p = 0.0292$) and *Utx* (Fig. 5I; $p = 0.0018$). Altogether, at the phenotypic level, JH signalling prolongs developmental timing, positively affects sizing and allometry, while at the molecular level, histone acetylation modifiers are unresponsive to JH, yet histone methylation modifiers decrease.

3.4.2 Effects of 20-hydroxyecdysone on Fire Ant Development

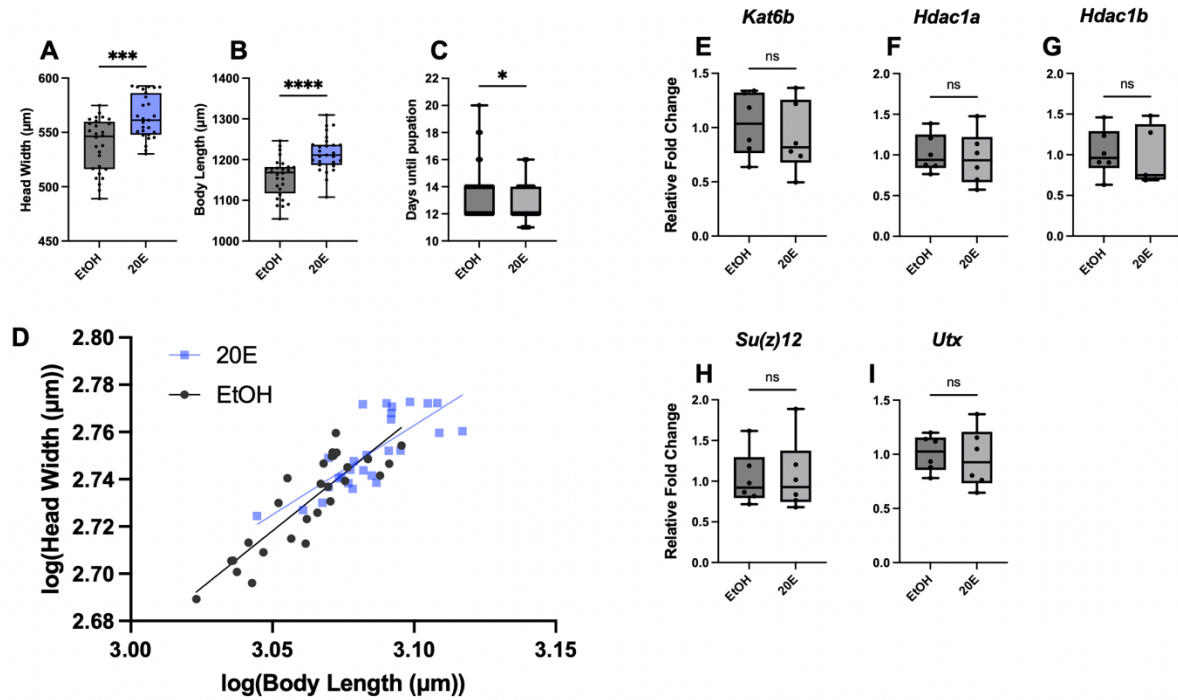


Fig. 6. The effects of 20-hydroxyecdysone (20E) on developmental plasticity in *Solenopsis invicta*. Comparison between measurements of (A) head width, (B) body length, (C) developmental timing, and (D) head-to-body allometry of developing *S. invicta* larvae in ethanol controls (n=28) and 20E (n=27) treatment groups. qPCR analyses of 24-hour treatments comparing (E) *Kat6b*, (F) *Hdac1a*, (G) *Hdac1b*, (H) *Su(z)12*, and (I) *Utx* between ethanol and 20E groups. Bars represent means, with standard deviation (SD) indicated with error bars. Values for statistical significance of Student's *t*-tests and Mann-Whitney test include * $p < 0.05$, *** $p < 0.001$, and **** $p < 0.0001$. Sample size for qPCR treatment groups range from 5-6 biological replicates, each containing 5 individual larvae.

The steroid hormone ecdysone is another insect growth regulator that works complementarily to JH during insect growth and metamorphosis (Mirth & Riddiford, 2007). In ants, while very little has been done to assess the role of ecdysone during development, it has been shown in *Myrmica rubra* to cause a decrease in both size and developmental timing (Brian, 1974), consistent with that shown in other insects (Colombani et al., 2005; Edgar, 2006; McBrayer et al., 2007). The effects of ecdysone treatments in *S. invicta*, specifically on developmental sizing,

timing, allometry, and on histone modifier expression levels have yet to be explored. We therefore injected E4 instar larvae with 20-hydroxyecdysone (20E), an active form of ecdysone. Consistent with that found in other insects, 20E shortened developmental timing (Fig. 6C; $p=0.0349$). Surprisingly, rather than decreasing size, we found that, compared to controls, 20E increased both head (Fig. 6A; $p=0.0007$) and body size (Fig. 6B; $p<0.0001$) proportionally, as there were no changes in head-to-body allometry (Fig. 6D). Based on our findings of the effects of ecdysone signalling on sizing, allometry, and developmental timing, we next treated 20E to E4 larvae and described the influence of ecdysone on histone modifier expression levels. After a 24 hours treatment, larvae treated with 20E did not change in the activity of histone acetylation nor histone methylation modifier levels, as compared to controls (Fig. 6E-I). Therefore, the action of ecdysone signalling on sizing and developmental timing is independent of histone modifier activity. Altogether, we found that 20E treatments led to an unexpected increase in head and body sizes, and further, this increase in sizing occurred despite a shortening of developmental timing.

3.5 Influence of Pharmacological Inhibitors of Histone Modifiers on Developmental Plasticity in *S. invicta*

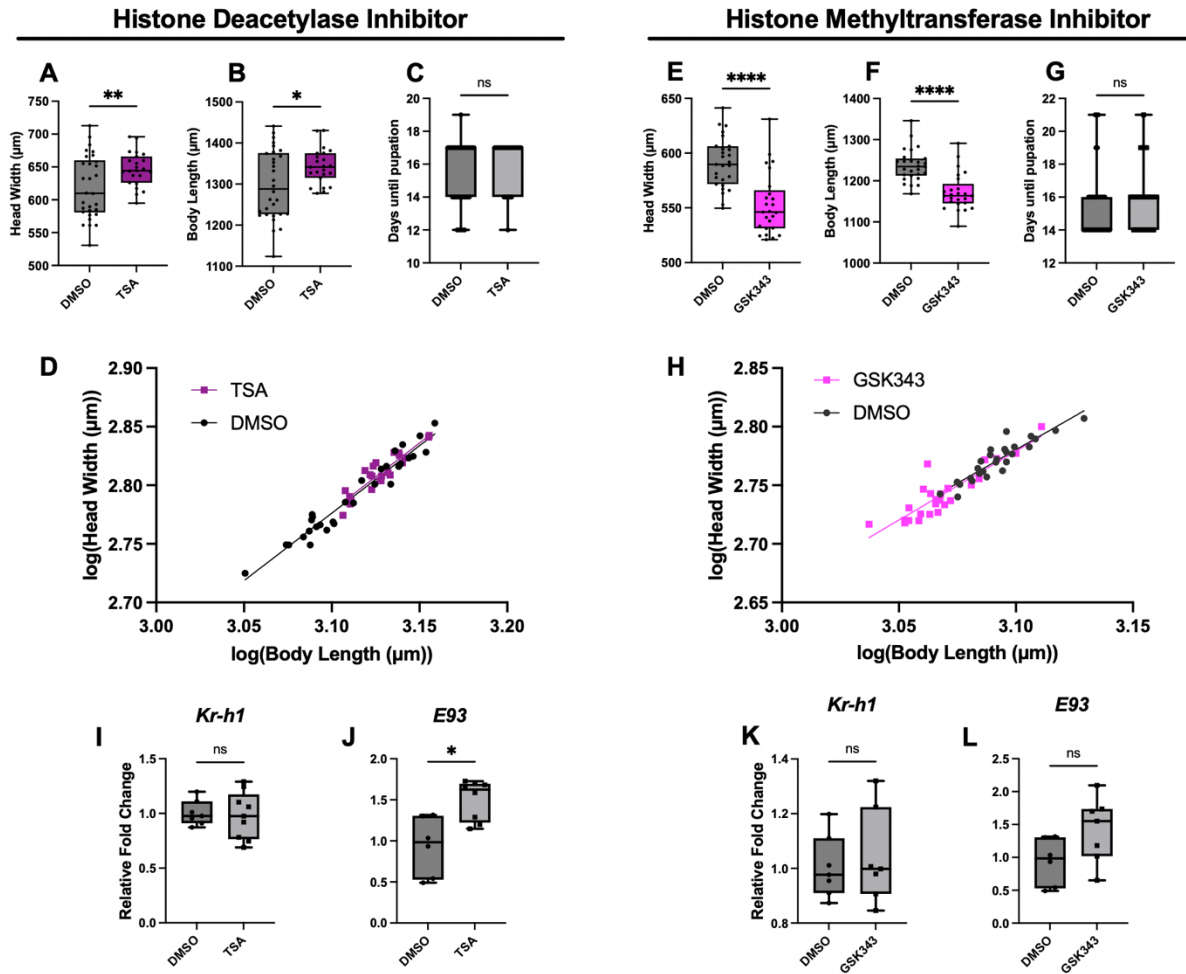


Fig. 7. The effects of pharmacological inhibitors of histone modifications on developmental plasticity in *Solenopsis invicta*. Comparison between measurements of (A) head width, (B) body length, (C) developmental timing, and (D) head-to-body allometry of developing *S. invicta* larvae in DMSO controls (n=16) and histone deacetylase inhibitor TSA (n=13) treatment groups. Comparison between measurements of (E) head width, (F) body length, (G) developmental timing, and (H) head-to-body allometry of developing *S. invicta* larvae in DMSO controls (n=27) and histone methyltransferase inhibitor GSK343 (n=25) treatment groups. qPCR analyses of 48-hour treatments comparing (I) *Kr-h1* and (J) *E93* between DMSO and TSA groups, as well as (K) *Kr-h1* and (L) *E93* between DMSO and GSK343 groups. Bars represent means, with standard deviation (SD) indicated with error bars. Values for statistical significance of Student's *t*-tests, Mann-Whitney tests, and Welch's *t* tests include * $p < 0.05$, ** $p < 0.01$ and **** $p < 0.0001$. Sample size for qPCR treatment groups range from 6-9 biological replicates, each containing 3 individual larvae.

Based on our findings, histone (de)acetylation and histone (de)methylation are responsive in varying degrees to environmental and hormonal variation and are dynamic in expression during caste-specific development. We therefore wanted to functionally manipulate this modification to investigate their role in sizing, allometry, and developmental timing. Histone deacetylases mediating developmental plasticity, as shown through inhibitor experiments (histone deacetylase inhibitor trichostatin A; HDACi, TSA) in both beetles and flies (Werner et al., 2023). In ants, TSA treatments have demonstrated that histone deacetylases regulate caste-specific behavioural plasticity (Simola et al., 2016). In parallel to the erasure activity of histone deacetylation, the writer activity of histone methylation coordinate with histone deacetylation, and together they promote heterochromatin formation and transcriptional repression (Jenuwein & Allis, 2001). Therefore, to characterize the role of these heterochromatin-forming histone modifications, we used a pharmacological approach to inhibit them. Developing E4 larvae that were injected with the HDACi TSA had increases in head width (Fig. 7A; $p=0.0099$) and body size (Fig. 7B; $p=0.0158$), without altering developmental timing nor allometry (Fig. 7C, D), as compared to controls. We next injected E4 larvae with the histone methyltransferase inhibitor (HMTi) GSK343, and despite the expected coordination of these two modifications, this resulted in the opposite effect of HDACi. Surprisingly, HMTi of E4 larvae led to a decrease in head width (Fig. 7E; $p<0.0001$) and body sizes (Fig. 7F; $p<0.0001$), without changing developmental timing (Fig. 7G) nor allometry (Fig. 7H), relative to controls. While our earlier findings suggest that developmental hormones can, to varying degrees, influence histone modifiers, very little is known of the capacity for histone modifiers to influence hormonal signalling in developing ants. Furthermore, given our findings of the effects of inhibitors of histone deacetylation and methylation modifiers on sizing, we next treated E4 larvae to determine the influence of HDACi and HMTi on the expression levels of

hormonal signaling. After a 48-hour treatment, TSA treatments led to an increase in expression of *E93* (Fig. 7J; $p=0.0200$), without influencing *Kr-h1* levels (Fig. 7I). Treatments with GSK343 did not affect expression levels of *Kr-h1* nor *E93* (Fig. 7K, L). Altogether, histone (de)acetylation and (de)methylation influence head and body size in a bidirectional manner, and histone deacetylation is negatively associated with an ecdysone activity.

Chapter 4: Discussion

Our findings have illuminated how environmental variation can influence size variation, how endocrine and epigenetic mechanisms respond to environmental variation, and how these mechanisms can influence size variation. The following sections attempt to synthesize these facets that altogether have generated fundamental insight into how a developing fire ant responds to its environment and generates a wide array of sizes.

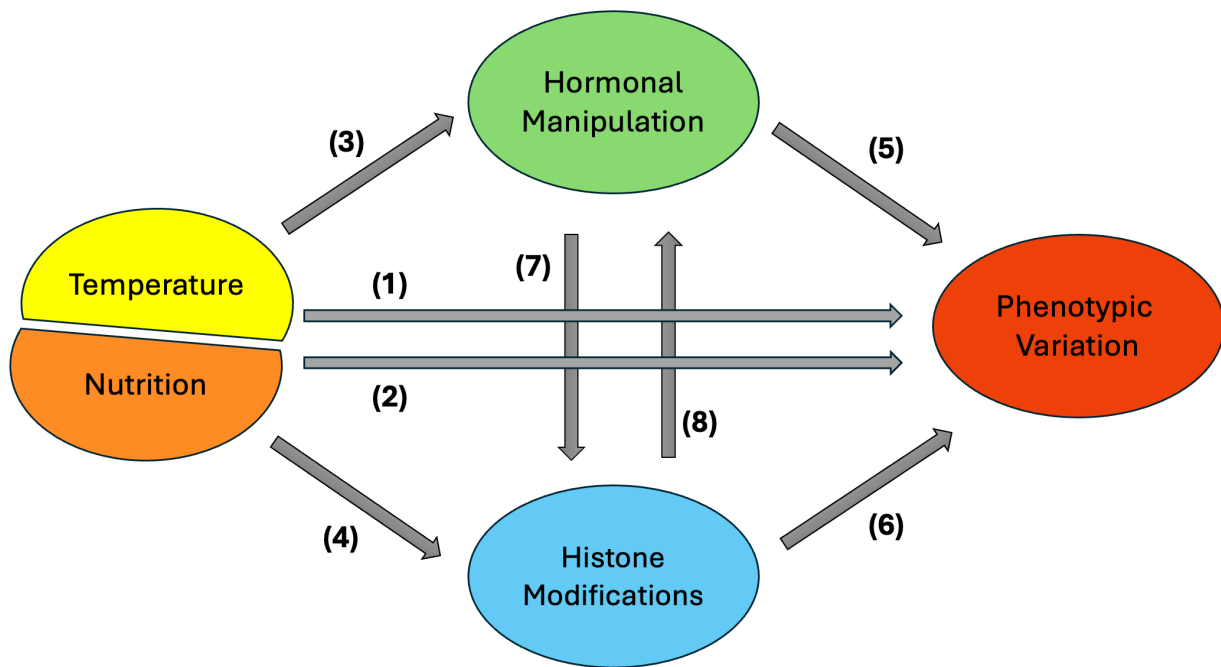


Fig. 8. Connecting the environmental, endocrine, and epigenetic facets of developmental plasticity in *Solenopsis invicta*. Each facet is represented by their respective colours and are linked by arrows to indicate their interactions within fire ants. Full lines demonstrate relationships that have been characterized across *S. invicta* development. Each number represents the interplay between each respective facet. (1) Effects of changes in temperature on phenotypic variation. (2) Effects of changes in nutrition on phenotypic variation. (3) Effects of changes in temperature and nutrition on hormonal signalling. (4) Effects of changes in temperature and nutrition on histone modifier activity. (5) Effects of hormonal manipulation on phenotypic variation. (6) Effects of histone modifications on phenotypic variation. (7) Effects of hormonal manipulations on histone modifier activity. (8) Effects of histone modifications on hormonal signalling.

4.1 Environmental Variation & Worker Polyphenism in Fire ants

Environmental variation is known to influence growth and caste in several species of ants (Wheeler, 1986; Hölldobler & Wilson, 1990). Here we found that for *S. invicta* developing workers, developmental timing and sizing are influenced by both thermal and nutritional plasticity.

4.1.1 Thermal Plasticity of Size and Developmental Timing

Temperature increase led to an increase in size and a decrease in total duration of development (Fig. 3; Fig. 8, arrow 1). This finding is consistent with what has previously been demonstrated for both ant species *Pheidole pallidula* and *Camponotus floridanus*, where increasing temperature leads to an increase in growth and induction of the large soldier caste (Passera, 1974; MacMillan et al., 2025). Together, these findings indicate that ants violate the Temperature Size Rule (TSR). Across insects, TSR predicts that an increase in temperature would lead to a shorter duration of development, producing smaller individuals. Conversely, TSR also predicts that lower temperatures would extend duration of development, producing larger individuals. While fire ants (and the other ants described above) are consistent with TSR when predicting temperature increase leading to a shorter period of development, they exhibit an unexpected increase in size. This raises the question of how ants are capable of obtaining a larger final size with increasing temperatures. We propose that ants have co-opted aspects of hormonal signalling in such a way that increasing temperatures retain their influence on developmental timing relative to TSR, yet ants have decoupled sizing from developmental timing in the context of temperature, and instead, temperature can increase the rate of growth.

4.1.2 Nutritional Plasticity of Size and Developmental Timing

In parallel, we found nutritional deficiency ranging from intermittent fasting to nutrient deprivation, influenced sizing and developmental timing to varying degrees (Fig. 4; Fig. 8, arrow 2). In the context of intermittent fasting, we found that individuals delayed metamorphosis and substantially extended their last instar, but they obtained the same final body size as optimally fed individuals (Fig. 4A, B, C). In the context of nutrient deprivation, where individuals obtained minimal nutrients, individuals extended their developmental duration and were substantially smaller than optimally fed individuals (Fig. S1). Our findings that nutritional variation can influence both developmental timing and sizing is consistent to what is known in other ant species. For example, in *Pheidole pallidula*, increasing levels of protein has been shown to increase growth and induce the production of large soldier and even supersoldiers (Goetsch, 1937; Passera, 1974). The induction of soldiers in *Pheidole* when optimal nutrients are provided suggests that nutritional variation may influence developmental hormones, including JH signalling because that pathway is upregulated during the development of soldiers (Rajakumar et al., 2012). While we do not find that nutritional deficiency influences JH signalling in *S. invicta*, we do see a change in ecdysone signalling (Fig. 4F). Future work, characterizing the supplement of typical rearing diet with high levels of macronutrients may uncover a role for JH signalling in nutrition-dependent size variation.

4.2 Mediators of GxE and Plasticity in fire ants

Endocrinological and epigenetic mechanisms are critical in mediating a developing organism's response to a changing environment, generating a diverse array of phenotypic variation (West-Eberhard, 2003; Jablonka & Lamb, 2014; Sultan, 2015). Here we found that for *S. invicta* developing workers, developmental timing and sizing are influenced to varying degrees by both JH and ecdysone signalling as well as both histone (de)acetylation and (de)methylation.

Furthermore, we found that histone modifier expression is regulated by JH signalling and that both histone (de)acetylation and (de)methylation modifiers function to regulate sizing independent of developmental timing.

4.2.1 JH Signalling: Environmental response and influence on sizing

For hormone signalling, we found that JH influences the sizing of head and body, and developmental timing (Fig. 5A, B, C; Fig. 8, arrow 5). Based on extensive work done in fire ants, and ants of *Pheidole*, and *Camponotus*, our work reinforces the role of JH in regulating sizing and developmental timing. Furthermore, we found that JH can change the allometry of developing *S. invicta* workers (Fig. 5D). The increase in the head-to-body slope of JHA-treated individuals, as compared to controls, phenocopies the slope differences exhibited by *Solenopsis geminata* (Tschinkel, 2006), a species of fire ant that has a soldier caste, as compared to the slope of *S. invicta*. In fact, the change in slope in combination with profound changes in both head and body size generates individuals that, when compared to their control, reflect a morphospace similar to that of the minor worker and soldier of *Solenopsis geminata*, a closely related fire ant species with a distinct soldier caste (Tschinkel, 2006). We also found that temperature increase led to an increase in JH signalling, through *Kr-h1* activity (Fig. 3E; Fig. 8, arrow 3), yet nutritional variation (fed versus starved) did not influence JH signalling (Fig. 4E). It is possible that nutritional variation can affect JH signalling in either other developmental stages or is responsive to nutritional variation at other timepoints than we characterized. Future work should characterize the role of JH outside of the E4 larval time period and further characterize, at shorter and longer timepoints, the influence of starvation on JH signalling.

4.2.2 Ecdysone Signalling: Environmental Response and influence on Sizing

For ecdysone signalling, we found that ecdysone influences the sizing of head and body and developmental timing (Fig. 6A, B, C; Fig. 8, arrow 5). Surprisingly, ecdysone generates larger individuals, and these larger individuals reach their final size in a shorter time period, as compared to controls. This would indicate that ecdysone stimulates a faster growth rate to positively regulate size in fire ants as opposed to the extension of the duration of development that is regulated by JH. Ecdysone promoting faster growth of large individuals is reminiscent to the development of queens in *Apis mellifera*. Kamakura (2011) demonstrated that ecdysone, regulated by EGFR/MAPK signalling, controls developmental timing such that queens have a shorter duration of development compared to workers. Furthermore, we found that increases in temperature led to an increase in head and body size, yet this was achieved through a shorter developmental duration (Fig. 3A, B, C). This suggests that ecdysone controlling growth rate is potentially thermally plastic. Based on our JH and ecdysone experiments, and both of these pathways being positively influenced by temperature increase (Fig. 3E, F; Fig. 8, arrow 3), we therefore suggest that social insects may have independently co-opted JH and ecdysone signalling, and their synergy, to coordinate growth rates with developmental timing, facilitating the evolution of size variation within and between castes.

4.2.3 Histone (de)acetylation: Environmental Response and Influence on Sizing

For histone (de)acetylation, we found that histone deacetylation modifiers (*Hdac1a* and *Hdac1b*) are associated with temperature and nutritional states that would enable larger sized individuals (Fig. 8, arrow 4). An increase in temperature as well as an increase in nutrition when comparing fed versus starvation both lead to increases in size and *Hdac1* activity (Fig. 3A, B, H; Fig. 4H; Fig. S1). In contrast, when inhibiting histone deacetylation, rather than obtaining smaller

individuals, we obtained individuals with both larger head and body sizes (Fig. 7A, B; Fig. 8, arrow 6). Furthermore, HDACi treatments led to a shift in the distribution of individual sizes formed that span on the upper size range, relative to DMSO controls (Fig. 7A, B). This distribution differs from the shift in morphospace seen in the JHA treatments, where individuals with non-overlapping head and body sizes were formed. Changes in sizing through hormonal and histone epigenetic mechanisms in fire ants is consistent with that found in context of castes in both the honeybee *A. mellifera* and the Florida Carpenter ant *Camponotus floridanus* (Alvarado et al., 2015; Spannhoff et al., 2011). In *A. mellifera*, royal jelly is supplied to developing larvae to induce the development of queen larvae as opposed to worker diet supplied to induce the development of smaller workers. This royal jelly contains a natural HDACi (10-Hydroxy-2-decenoic acid) suggesting that the inhibition of deacetylation is associated with the generation of the large queen found in a colony (Spannhoff et al., 2011). In *C. floridanus*, the large soldier has lower levels of *Hdac1* expression than the smaller minor workers suggesting that histone deacetylation promotes the growth of smaller individuals (Alvarado et al., 2015). Therefore, histone acetylation in ants and bees is thought to be associated with a decrease in size and this matches our finding that by supplying E4 fire ant larvae with an HDACi, you generate larger individuals likely due to an increase in growth rate. Furthermore, our HDACi treatment generated larger individuals that obtained their increase in size independent of developmental timing (Fig. 7C), suggesting that their larger size was through an increase in the rate of growth rather than the duration of growth. In the context of behavioural plasticity and regulating rate of foraging, Simola et al., (2016), found that by supplying adult *C. floridanus* soldier individuals with HDACi, it promoted individuals to increase their foraging rate, comparable to our increase in developmental growth rate. In the context of both thermal and nutritional plasticity, histone deacetylation may be facilitating an environmental

response that elicits changes in developmental timing. Altogether, histone (de)acetylation may be an important integrator of plasticity and growth to coordinate developmental timing, growth rates, and size variation as a whole.

4.2.4 Histone (de)methylation: Environmental Response and Influence on Sizing

For histone (de)methylation, we found that the histone demethylation modifier *Utx* is positively associated with nutrition-mediated increase in size (Fig. 4K; Fig. S1), and both *Utx* and the histone methylation modifier *Su(z)12* are negatively associated with temperature-mediated increase in size (Fig. 3K, J; Fig. 8, arrow 4). We found that by inhibiting histone methylation using an HMTi (GSK343), this causes a decrease in size (Fig. 7E, F; Fig. 8, arrow 6). Given that *Su(z)12* and *Utx* both target the Histone H3 lysine 27 residue, it could be suggested that histone demethylation (*Utx*) is analogous to HMTi activity (GSK343). Therefore, a decrease in size induced by HMTi (GSK343) treatment mirrors the lower temperature-induced increase in *Utx* expression and decrease in size (Fig. 3K). Lower temperatures leading to an upregulation of *Utx* in fire ants underlying size plasticity is reminiscent of higher temperatures leading to a downregulation of its ortholog *Kdm6b* in turtles, underlying the plasticity of sex. Finally, based on our HDACi experiments, both HDACi and HMTi generate size variation independent of developmental timing, suggesting that histone modifiers regulate growth rate and size plasticity independent of environmental variation and molecular factors, that regulate the duration of development. GSK343 has been previously shown to both suppress reproduction (Pereira et al., 2018) and developmental transitions (Roquis et al., 2018) in trematode worms, proliferation in neuroblastoma cells (Bownes et al., 2021), and suppress cell proliferation and cell cycle progression as well as downregulate the nutrient-sensing growth regulating TOR pathway in pancreatic cancer cells (Xu et al., 2019). Future work should characterize the role of GSK343 and

the inhibition of histone methylation in size regulation and growth in other organisms to explore the generalities of this histone modification associated with sizing beyond ants.

4.2.5 Epigenetic-Endocrine Crosstalk Underlying Size Variation in Fire Ants

In the context of endocrine-epigenetic crosstalk, we found that juvenile hormone has a dramatic influence on sizing, causing a change in the sizing of head, body, and allometry, as well as developmental timing (Fig. 5A, B, D, C). These phenotypic consequences are associated with our findings that JH signaling downregulates both histone methylation (*Su(z)12*) and demethylation (*Utx*) yet did not influence the activity of histone acetylation modifiers (Fig. 5H, I; Fig. 8, arrow 7). Similar to our findings, it has been shown in both flies and moths that *Su(z)12* is negatively regulated by JH signaling (Yang et al., 2021), and therefore the influence of JH on histone methylation may be universal across holometabolous insects. In contrast, ecdysone had no effect on histone modifiers (Fig. 6E-I), suggesting that its regulatory role in growth rate and sizing may be downstream of histone epigenetic mechanisms of size. Furthermore, investigating the influence of histone modifiers on hormonal signaling has demonstrated a negative relationship between histone deacetylase activity and ecdysone signaling (Fig. 7J; Fig. 8, arrow 8). Consistent with our results, histone acetylation has been shown to be crucial in regulating ecdysone response genes in a positive manner (Bodai et al., 2012; Cheng et al., 2022; Kirilly et al., 2011; Roy & Palli, 2018). The CREB-binding protein (CBP) is a highly conserved gene encodes a protein that has histone acetyltransferase activity (Bordoli et al., 2001). In both developing flies and silkworm embryonic cell lines, ecdysone response genes have been shown to be positively regulated by the HAT activity of CBP at H3K23 and H3K27 residues (Bodai et al., 2012; Cheng et al., 2022; Kirilly et al., 2011; Roy & Palli, 2018). More specifically, in *Drosophila*, *sox14* is an ecdysone response gene that is positively regulated by CBP activity, while overexpression of *Rpd3* (*Hdac1* ortholog)

led to negative regulation of *sox14* (Kirilly et al., 2011). The directionality of the regulatory activities of both histone acetylation and deacetylation on ecdysone response genes is consistent with the responsiveness of the ecdysone pathway demonstrated in our results. Throughout this study, an increase in ecdysone activity has been correlated with an increase in size in the contexts of thermal, nutritional, and histone modifier manipulations (Fig. 2, 3, 7) and has been demonstrated to increase size and shorten developmental timing (Fig. 5). Thus far, we know that HDAC activity has a negative influence on ecdysone activity in *Drosophila* (Kirilly et al., 2011) and that both HDAC activity and ecdysone activity demonstrate respective negative and positive relationships with honeybee queen development (Alhosin, 2023; Kamakura, 2011; Polsinelli & Yu, 2018; Spannhoff et al., 2011). Therefore, we suggest that histone (de)acetylation may regulate ecdysone activity to determine appropriate sizing across insects. Future work in social insects may better illuminate the complex dynamics between the regulation of both developmental timing and growth underlying sizing and size evolution across castes. Collectively, the evolution of extreme size variation within the worker caste system in fire ants specifically, and across plants and animals more generally, may be the product of thermal and nutritional plasticity that is dynamically mediated by an endocrine-epigenetic axis.

Supplemental Material

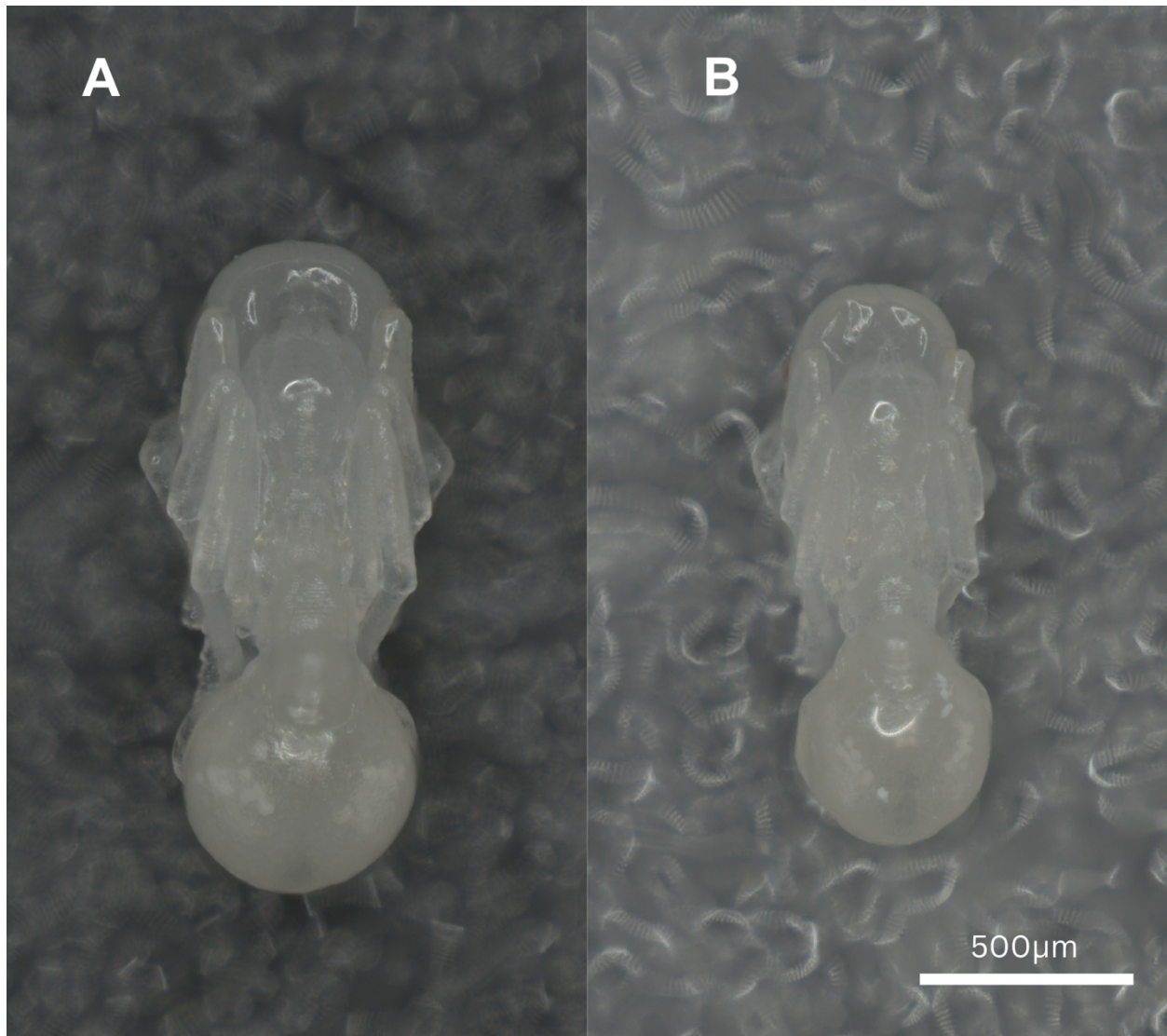


Fig. S1. Comparison between the average sized fed and sugar-diet individual *S. invicta* pupae. (A) The average sized individual from 47 acquired pupae in a fed experiment. **(B)** The average sized individual from 4 acquired pupae in the sugar-diet (starved) experiment.

Table S1: Summary chart outlining the effects of each experimental manipulation on sizing, developmental timing, and allometry. Arrows indicate direction of change and number of arrows are representative of the significance level, based on statistical analyses: ↑/ ↓ represents $*p<0.05$; ↑↑/ ↓↓ represents $**p<0.01$; ↑↑↑/ ↓↓↓ represents $***p<0.001$; ↑↑↑↑/ ↓↓↓↓ represents $****p<0.0001$. Equal symbols demonstrate stability in gene expression.

	Head Width	Body Length	Dev Timing	XY changes
Increasing temperatures	↑ at 28.5°C	↑ at 28.5°C	↓↓↓↓ at 28.5°C and 32°C	↓↓ in y-intercept at 28.5°C
Intermittent fasting	No change	No change	↑↑↑↑	No change
Methoprene	↑↑↑↑	↑↑↑↑	↑↑↑↑	↑↑ in slope
20E	↑↑↑	↑↑↑↑	↓	No change
TSA	↑↑	↑	No change	No change
GSK343	↓↓↓↓	↓↓↓↓	No change	No change

Table S2: Summary chart outlining the responsiveness of JH/ecdysone signalling and of histone modifiers after various experimental manipulations. Diagonal arrows for Natural Development indicate the direction of change, leading to an overall increase or decrease in gene expression. Arrows indicate direction of change and number of arrows are representative of the significance level, based on statistical analyses: ↑/ ↓ represents $*p<0.05$; ↑↑/ ↓↓ represents $**p<0.01$; ↑↑↑/ ↓↓↓ represents $***p<0.001$; ↑↑↑↑/ ↓↓↓↓ represents $****p<0.0001$. Equal symbols demonstrate stability in gene expression.

	Natural Development	Temperature (Increased)	Nutrition (Starved)	JHA	20E	TSA	GSK343
<i>Kr-h1</i>	=	↑	=	/	/	=	=
<i>E93</i>	↗	↑↑↑	↓↓	/	/	↑	=
<i>Kat6b</i>	↘	=	=	=	=	/	/
<i>Hdac1a</i>	↘	↑	↓	=	=	/	/
<i>Hdac1b</i>	↗	=	↓↓↓↓	=	=	/	/
<i>Su(z)12</i>	↘	↓	=	↓	=	/	/
<i>Utx</i>	↘	↓↓	↓	↓↓	=	/	/

Table S3: List of both forward (for) and reverse (rev) primers designed for RT-qPCR genes.

Endocrine Signalling Primers	
<i>Kr-h1</i> for	5' CACAGCGTACAGTTTCCTCA 3'
<i>Kr-h1</i> rev	5' TGGAGTCTCTAAACCGGTTG 3'
<i>E93</i> for	5' TGGACGAGGTGGACGACAAA 3'
<i>E93</i> rev	5' CCTTACGAGATCCTCCAACGAG 3'
Histone Modifier Primers	
<i>Kat6b</i> for	5' GCCCAAGGCTCCATTACCGA 3'
<i>Kat6b</i> rev	5' GCTTCTCGTTGGCATTCCGC 3'
<i>Hdac1a</i> for	5' GATGGTGTGGAGGAAGCTT 3'
<i>Hdac1a</i> rev	5' ATACTTTCCTTTTCCTGCTCCTAC 3'
<i>Hdac1b</i> for	5' CATATAAAGGATCTAGGAAGCGG 3'
<i>Hdac1b</i> rev	5' AGCATCTGTCTTCATATCCTCTTC 3'
<i>Su(z)12</i> for	5' AGCAACGGTCTGGTAGATAGAC 3'
<i>Su(z)12</i> rev	5' GTTCGAAACCAGCGTTGGC 3'
<i>Utx</i> for	5' GAGCAAGGACGTAGAAGTGC 3'
<i>Utx</i> rev	5' GAACACCTCGATCTCGCATT 3'
<i>Rpl32</i> for	5' AACTCAAGCGCAACTGGC 3'
<i>Rpl32</i> rev	5' GCTACCGTAACCAATGCTAGG 3'

References

- Alhosin, M. (2023). Epigenetics Mechanisms of Honeybees: Secrets of Royal Jelly. *Epigenetics Insights*, 16, 25168657231213717. <https://doi.org/10.1177/25168657231213717>
- Allen, C. R., Epperson, D., & Garmestani, A. (2004). Red imported fire ant impacts on wildlife: A decade of research. *The American Midland Naturalist*, 152(1), 88–103.
- Allen, M. L., Rhoades, J. H., Sparks, M. E., & Grodowitz, M. J. (2018). Differential Gene Expression in Red Imported Fire Ant (*Solenopsis invicta*) (Hymenoptera: Formicidae) Larval and Pupal Stages. *Insects*, 9(4), 185. <https://doi.org/10.3390/insects9040185>
- Allis, D., Jenuwein, T., Reinberg, D., & Caparros, M.-L. (2007). *Epigenetics*.
- Alvarado, S., Rajakumar, R., Abouheif, E., & Szyf, M. (2015). Epigenetic variation in the Egfr gene generates quantitative variation in a complex trait in ants. *Nature Communications*, 6(1), 6513.
- Azizi, T., Johnston, J. S., & Vinson, S. B. (2009). Initiation of flight muscle apoptosis and wing casting in the red imported fire ant *Solenopsis invicta*. *Physiological Entomology*, 34(1), 79–85. <https://doi.org/10.1111/j.1365-3032.2008.00655.x>
- Bear, A., Prudic, K. L., & Monteiro, A. (2017). Steroid hormone signaling during development has a latent effect on adult male sexual behavior in the butterfly *Bicyclus anynana*. *PLoS One*, 12(3), e0174403.
- Becher, M. A., Scharpenberg, H., & Moritz, R. F. A. (2009). Pupal developmental temperature and behavioral specialization of honeybee workers (*Apis mellifera* L.). *Journal of Comparative Physiology A*, 195(7), 673–679. <https://doi.org/10.1007/s00359-009-0442-7>
- Beldade, P., Mateus, A. R. A., & Keller, R. A. (2011). Evolution and molecular mechanisms of adaptive developmental plasticity. *Molecular Ecology*, 20(7), 1347–1363.

- Berger, S. L. (2007). The complex language of chromatin regulation during transcription. *Nature*, 447(7143), 407–412.
- Bhatkar, A., & Whitcomb, W. (1970). Artificial diet for rearing various species of ants. *Florida Entomologist*, 229–232.
- Bodai, L., Zsindely, N., Gáspár, R., Kristó, I., Komonyi, O., & Boros, I. M. (2012). Ecdysone Induced Gene Expression Is Associated with Acetylation of Histone H3 Lysine 23 in *Drosophila melanogaster*. *PLoS ONE*, 7(7), e40565. <https://doi.org/10.1371/journal.pone.0040565>
- Bordoli, L., Netsch, M., Lüthi, U., Lutz, W., & Eckner, R. (2001). Plant orthologs of p300/CBP: conservation of a core domain in metazoan p300/CBP acetyltransferase-related proteins. *Nucleic Acids Research*, 29(3), 589–597.
- Bownes, L. V., Williams, A. P., Marayati, R., Stafman, L. L., Markert, H., Quinn, C. H., Wadhwani, N., Aye, J. M., Stewart, J. E., & Yoon, K. J. (2021). EZH2 inhibition decreases neuroblastoma proliferation and in vivo tumor growth. *PLoS One*, 16(3), e0246244.
- Bradshaw, A. D. (1965). Evolutionary Significance of Phenotypic Plasticity in Plants. In *Advances in Genetics* (Vol. 13, pp. 115–155). Elsevier. [https://doi.org/10.1016/S0065-2660\(08\)60048-6](https://doi.org/10.1016/S0065-2660(08)60048-6)
- Brent, C. S., & Vargo, E. L. (2003). Changes in juvenile hormone biosynthetic rate and whole body content in maturing virgin queens of *Solenopsis invicta*. *Journal of Insect Physiology*, 49(10), 967–974. [https://doi.org/10.1016/S0022-1910\(03\)00166-5](https://doi.org/10.1016/S0022-1910(03)00166-5)
- Brian, M. V. (1956). Studies of caste differentiation in *Myrmica rubra* L. 4. Controlled larval nutrition. *Insectes Sociaux*, 3(3), 369–394. <https://doi.org/10.1007/BF02225758>
- Brian, M. V. (1974). Caste differentiation in *Myrmica rubra*: The rôle of hormones. *Journal of Insect Physiology*, 20(7), 1351–1365. [https://doi.org/10.1016/0022-1910\(74\)90238-8](https://doi.org/10.1016/0022-1910(74)90238-8)

- Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., & Shipley, G. L. (2009). *The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments*.
- Bustin, S., & Huggett, J. (2017). qPCR primer design revisited. *Biomolecular Detection and Quantification*, 14, 19–28.
- Cassill, D. L., & Tschinkel, W. R. (1995). Allocation of liquid food to larvae via trophallaxis in colonies of the fire ant, *Solenopsis invicta*. *Animal Behaviour*, 50(3), 801–813.
[https://doi.org/10.1016/0003-3472\(95\)80140-5](https://doi.org/10.1016/0003-3472(95)80140-5)
- Cassill, D. L., & Tschinkel, W. R. (2000). Behavioral and developmental homeostasis in the fire ant, *Solenopsis invicta*. *Journal of Insect Physiology*, 46(6), 933–939. [https://doi.org/10.1016/S0022-1910\(99\)00200-0](https://doi.org/10.1016/S0022-1910(99)00200-0)
- Chang, H., Xu, Z., Li, W., Cai, C., Wang, W., Ge, P., Jia, X., Li, Y., Ding, T., Ma, W., Banaei-Moghaddam, A. M., Mo, H., & Ren, M. (2022). HDAC3 Knockdown Dysregulates Juvenile Hormone and Apoptosis-Related Genes in *Helicoverpa armigera*. *International Journal of Molecular Sciences*, 23(23), 14820. <https://doi.org/10.3390/ijms232314820>
- Cheng, D., Cheng, T., Yang, X., Zhang, Q., Fu, J., Feng, T., Gong, J., & Xia, Q. (2018). The genome-wide transcriptional regulatory landscape of ecdysone in the silkworm. *Epigenetics & Chromatin*, 11(1), 48. <https://doi.org/10.1186/s13072-018-0216-y>
- Cheng, D., Dong, Z., Lin, P., Shen, G., & Xia, Q. (2022). Transcriptional Activation of Ecdysone-Responsive Genes Requires H3K27 Acetylation at Enhancers. *International Journal of Molecular Sciences*, 23(18), 10791. <https://doi.org/10.3390/ijms231810791>
- Chittka, A., Wurm, Y., & Chittka, L. (2012). Epigenetics: The Making of Ant Castes. *Current Biology*, 22(19), R835–R838. <https://doi.org/10.1016/j.cub.2012.07.045>

- Chole, H., Woodard, S. H., & Bloch, G. (2019). Body size variation in bees: Regulation, mechanisms, and relationship to social organization. *Current Opinion in Insect Science*, 35, 77–87.
- Choppin, M., Feldmeyer, B., & Foitzik, S. (2021). Histone acetylation regulates the expression of genes involved in worker reproduction in the ant *Temnothorax rugatulus*. *BMC Genomics*, 22(1), 871.
- Clausen, J., Keck, D., & Hiesey, W. (1940). *Experimental studies on the nature of species. I. Effect of varied environments on western North American plants*. (Issue 520). Washington D.C. CABI Databases.
- Colombani, J., Bianchini, L., Layalle, S., Pondeville, E., Dauphin-Villemant, C., Antoniewski, C., Carré, C., Noselli, S., & Léopold, P. (2005). Antagonistic actions of ecdysone and insulins determine final size in *Drosophila*. *Science*, 310(5748), 667–670.
- Cornette, R., Gotoh, H., Koshikawa, S., & Miura, T. (2008). Juvenile hormone titers and caste differentiation in the damp-wood termite *Hodotermopsis sjostedti* (Isoptera, Termopsidae). *Journal of Insect Physiology*, 54(6), 922–930. <https://doi.org/10.1016/j.jinsphys.2008.04.017>
- Cornette, R., Koshikawa, S., Hojo, M., Matsumoto, T., & Miura, T. (2006). Caste-specific cytochrome P450 in the damp-wood termite *Hodotermopsis sjostedti* (Isoptera, Termopsidae). *Insect Molecular Biology*, 15(2), 235–244.
- Cridge, A., Harrop, T., Lovegrove, M., Remnant, E., & Dearden, P. (2017). Nutrition and epigenetic change in insects: Evidence and implications. In *Advances in insect physiology* (Vol. 53, pp. 31–54). Elsevier.
- Csata, E., & Dussutour, A. (2019). *Nutrient regulation in ants (Hymenoptera: Formicidae): a review*. https://doi.org/10.25849/MYRMECOL.NEWS_029:111

- de Mendoza, A., Lister, R., & Bogdanovic, O. (2020). Evolution of DNA methylome diversity in eukaryotes. *Journal of Molecular Biology*, 432(6), 1687–1705.
- De Moed, G., De Jong, G., & Scharloo, W. (1997). Environmental effects on body size variation in *Drosophila melanogaster* and its cellular basis. *Genetics Research*, 70(1), 35–43.
- De Moed, G. H., Kruitwagen, C. L. J. J., De Jong, G., & Scharloo, W. (1999). Critical weight for the induction of pupariation in *Drosophila melanogaster*: Genetic and environmental variation. *Journal of Evolutionary Biology*, 12(5), 852–858. <https://doi.org/10.1046/j.1420-9101.1999.00103.x>
- Denton, D., Aung-Htut, M. T., Lorensuhewa, N., Nicolson, S., Zhu, W., Mills, K., Cakouros, D., Bergmann, A., & Kumar, S. (2013). UTX coordinates steroid hormone-mediated autophagy and cell death. *Nature Communications*, 4(1), 2916.
- Dussutour, A., & Simpson, S. J. (2009). Communal Nutrition in Ants. *Current Biology*, 19(9), 740–744. <https://doi.org/10.1016/j.cub.2009.03.015>
- Edgar, B. A. (2006). How flies get their size: Genetics meets physiology. *Nature Reviews Genetics*, 7(12), 907–916. <https://doi.org/10.1038/nrg1989>
- Emlen, D. J. (1994). Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 256(1346), 131–136.
- Fellous, A., Favrel, P., & Riviere, G. (2015). Temperature influences histone methylation and mRNA expression of the Jmj-C histone-demethylase orthologues during the early development of the oyster *Crassostrea gigas*. *Marine Genomics*, 19, 23–30. <https://doi.org/10.1016/j.margen.2014.09.002>

- Freitas, F. C., Depintor, T. S., Agostini, L. T., Luna-Lucena, D., Nunes, F. M., Bitondi, M. M., Simões, Z. L., & Lourenço, A. P. (2019). Evaluation of reference genes for gene expression analysis by real-time quantitative PCR (qPCR) in three stingless bee species (Hymenoptera: Apidae: Meliponini). *Scientific Reports*, 9(1), 17692.
- Gaddelapati, S. C., & Palli, S. R. (2025). Histone deacetylases synergistically regulate juvenile hormone signaling in the yellow fever mosquito, *Aedes aegypti*. *Insect Biochemistry and Molecular Biology*, 177, 104256. <https://doi.org/10.1016/j.ibmb.2024.104256>
- George, S., Gaddelapati, S. C., & Palli, S. R. (2019). Histone deacetylase 1 suppresses Krüppel homolog 1 gene expression and influences juvenile hormone action in *Tribolium castaneum*. *Proceedings of the National Academy of Sciences*, 116(36), 17759–17764. <https://doi.org/10.1073/pnas.1909554116>
- Ghosh, S. M., Testa, N. D., & Shingleton, A. W. (2013). Temperature-size rule is mediated by thermal plasticity of critical size in *Drosophila melanogaster*. *Proceedings of the Royal Society B: Biological Sciences*, 280(1760), 20130174.
- Gibney, E., & Nolan, C. (2010). Epigenetics and gene expression. *Heredity*, 105(1), 4–13.
- Glastad, K. M., Graham, R. J., Ju, L., Roessler, J., Brady, C. M., & Berger, S. L. (2020). Epigenetic regulator CoREST controls social behavior in ants. *Molecular Cell*, 77(2), 338–351.
- Goetsch, W. (1937). Die Entstehung der „Soldaten“ im Ameisenstaat. *Naturwissenschaften*, 25(50), 803–808.
- Gospocic, J., Glastad, K. M., Sheng, L., Shields, E. J., Berger, S. L., & Bonasio, R. (2021). Kr-h1 maintains distinct caste-specific neurotranscriptomes in response to socially regulated hormones. *Cell*, 184(23), 5807–5823.

- Hanna, L., Lamouret, T., Poças, G. M., Mirth, C. K., Moczek, A. P., Nijhout, F. H., & Abouheif, E. (2023). Evaluating old truths: Final adult size in holometabolous insects is set by the end of larval development. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 340(3), 270–276.
- Hartfelder, K. (2000). Insect juvenile hormone: From “status quo” to high society. *Brazilian Journal of Medical and Biological Research*, 33(2), 157–177. <https://doi.org/10.1590/S0100-879X2000000200003>
- Henderson, G. (2019). Primer pheromones and possible soldier caste influence on the evolution of sociality in lower termites. In *Pheromone communication in social insects* (pp. 314–330). CRC Press.
- Hölldobler, B., & Wilson, E. O. (1990). *The ants*. Belknap Press of Harvard University Press.
- Hölldobler, B., & Wilson, E. O. (2009). *The superorganism: The beauty elegance and strangeness of insect societies*. WW Norton & Company.
- House, H. L. (1969). EFFECTS OF DIFFERENT PROPORTIONS OF NUTRIENTS ON INSECTS. *Entomologia Experimentalis et Applicata*, 12(5), 651–669. <https://doi.org/10.1111/j.1570-7458.1969.tb02560.x>
- Howard, D. F., & Tschinkel, W. R. (1981). The flow of food in colonies of the fire ant, *Solenopsis invicta*: a multifactorial study. *Physiological Entomology*, 6(3), 297–306. <https://doi.org/10.1111/j.1365-3032.1981.tb00274.x>
- Howard, R. W., & Haverty, M. I. (1979). Termites and juvenile hormone analogues. *A Review of Methodology and Observed Effects. Sociobiology* 4: 277-285.

- Hrdý, I., & Křeček, J. (1972). Development of superfluous soldiers induced by juvenile hormone analogues in the termite, *Reticulitermes lucifugus santonensis*. *Insectes Sociaux*, 19(2), 105–109.
<https://doi.org/10.1007/BF02224728>
- Jablonka, E., & Lamb, M. J. (2002). The changing concept of epigenetics. *Annals of the New York Academy of Sciences*, 981(1), 82–96.
- Jablonka, E., & Lamb, M. J. (2014). *Evolution in four dimensions, revised edition: Genetic, epigenetic, behavioral, and symbolic variation in the history of life*. MIT press.
- Jenuwein, T., & Allis, C. D. (2001). Translating the histone code. *Science*, 293(5532), 1074–1080.
- Jones, J. C., Helliwell, P., Beekman, M., Maleszka, R., & Oldroyd, B. P. (2005). The effects of rearing temperature on developmental stability and learning and memory in the honey bee, *Apis mellifera*. *Journal of Comparative Physiology A*, 191(12), 1121–1129.
<https://doi.org/10.1007/s00359-005-0035-z>
- Kamakura, M. (2011). Royalactin induces queen differentiation in honeybees. *Nature*, 473(7348), 478–483.
- Karan, D., Morin, J., Moreteau, B., & David, J. (1998). Body size and developmental temperature in *Drosophila melanogaster*: Analysis of body weight reaction norm. *Journal of Thermal Biology*, 23(5), 301–309.
- Kirilly, D., Wong, J. J. L., Lim, E. K. H., Wang, Y., Zhang, H., Wang, C., Liao, Q., Wang, H., Liou, Y.-C., & Wang, H. (2011). Intrinsic epigenetic factors cooperate with the steroid hormone ecdysone to govern dendrite pruning in *Drosophila*. *Neuron*, 72(1), 86–100.
- Koshikawa, S., Cornette, R., Hojo, M., Maekawa, K., Matsumoto, T., & Miura, T. (2005). Screening of genes expressed in developing mandibles during soldier differentiation in the termite *Hodotermopsis sjostedti*. *Febs Letters*, 579(6), 1365–1370.

- Koyama, T., & Mirth, C. K. (2018). Unravelling the diversity of mechanisms through which nutrition regulates body size in insects. *Current Opinion in Insect Science*, 25, 1–8.
<https://doi.org/10.1016/j.cois.2017.11.002>
- Kristensen, T. N., Overgaard, J., Loeschcke, V., & Mayntz, D. (2011). Dietary protein content affects evolution for body size, body fat and viability in *Drosophila melanogaster*. *Biology Letters*, 7(2), 269–272. <https://doi.org/10.1098/rsbl.2010.0872>
- Kucharski, R., Maleszka, J., Foret, S., & Maleszka, R. (2008). Nutritional Control of Reproductive Status in Honeybees via DNA Methylation. *Science*, 319(5871), 1827–1830.
<https://doi.org/10.1126/science.1153069>
- Lavine, L., Gotoh, H., Brent, C. S., Dworkin, I., & Emlen, D. J. (2015). Exaggerated trait growth in insects. *Annual Review of Entomology*, 60(1), 453–472.
- Lee Cassill, D., & Tschinkel, W. R. (1999). Regulation of Diet in the Fire Ant, *Solenopsis invicta*. *Journal of Insect Behavior*, 12(3), 307–328. <https://doi.org/10.1023/A:1020835304713>
- Lelis, A., & Everaerts, C. (1993). Effects of juvenile hormone analogues upon soldier differentiation in the termite *Reticulitermes santonensis* (Rhinotermitidae, Heterotermitinae). *Journal of Morphology*, 217(2), 239–261.
- Lin, W.-J., Chiu, C.-I., & Li, H.-F. (2024). Divergent effects of climatic factors on termite body size: Alate versus worker castes. *Environmental Entomology*, 53(6), 1157–1168.
- Liu, S., Li, K., Gao, Y., Liu, X., Chen, W., Ge, W., Feng, Q., Palli, S. R., & Li, S. (2018). Antagonistic actions of juvenile hormone and 20-hydroxyecdysone within the ring gland determine developmental transitions in *Drosophila*. *Proceedings of the National Academy of Sciences*, 115(1), 139–144.

- Liu, Y., Henderson, G., Mao, L., & Laine, R. A. (2005). Effects of Temperature and Nutrition on Juvenile Hormone Titers of *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *Annals of the Entomological Society of America*, 98(5), 732–737. [https://doi.org/10.1603/0013-8746\(2005\)098%255B0732:EOTANO%255D2.0.CO;2](https://doi.org/10.1603/0013-8746(2005)098%255B0732:EOTANO%255D2.0.CO;2)
- Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻ ΔΔCT method. *Methods*, 25(4), 402–408.
- Lüscher, M. (1958). Experimentelle Erzeugung von Soldaten bei der Termite *Kaloterme flavicollis* (Fabr.). *Die Naturwissenschaften*, 45(3), 69–70. <https://doi.org/10.1007/BF00638631>
- MacMillan, O., Singer, J., Perrakis, S., Craig, A., Ntanga, D., Qiu, D., & Rajakumar, R. (2025). Juvenile Hormone Signalling Underlies the Switchpoint and Differentiation of Soldiers in *Camponotus floridanus*. <https://doi.org/10.1101/2025.08.25.672237>
- Maleszka, J., Barron, A. B., Helliwell, P. G., & Maleszka, R. (2009). Effect of age, behaviour and social environment on honey bee brain plasticity. *Journal of Comparative Physiology A*, 195(8), 733–740. <https://doi.org/10.1007/s00359-009-0449-0>
- Mao, L., Henderson, G., Liu, Y., & Laine, R. A. (2005). Formosan Subterranean Termite (Isoptera: Rhinotermitidae) Soldiers Regulate Juvenile Hormone Levels and Caste Differentiation in Workers. *Annals of the Entomological Society of America*, 98(3), 340–345. [https://doi.org/10.1603/0013-8746\(2005\)098%255B0340:FSTIRS%255D2.0.CO;2](https://doi.org/10.1603/0013-8746(2005)098%255B0340:FSTIRS%255D2.0.CO;2)
- Markin, G. P. (1970). Food distribution within laboratory colonies of the argentine ant, *Tridomyrmex humilis* (Mayr). *Insectes Sociaux*, 17(2), 127–157. <https://doi.org/10.1007/BF02223074>
- Marmorstein, R., & Zhou, M.-M. (2014). Writers and readers of histone acetylation: Structure, mechanism, and inhibition. *Cold Spring Harbor Perspectives in Biology*, 6(7), a018762.

- McBrayer, Z., Ono, H., Shimell, M., Parvy, J.-P., Beckstead, R. B., Warren, J. T., Thummel, C. S., Dauphin-Villemant, C., Gilbert, L. I., & O'Connor, M. B. (2007). Prothoracicotropic hormone regulates developmental timing and body size in *Drosophila*. *Developmental Cell*, 13(6), 857–871.
- Medina, R. G., Paxton, R. J., De Luna, E., Fleites-Ayil, F. A., Medina Medina, L. A., & Quezada-Euán, J. J. G. (2018). Developmental stability, age at onset of foraging and longevity of Africanized honey bees (*Apis mellifera* L.) under heat stress (Hymenoptera: Apidae). *Journal of Thermal Biology*, 74, 214–225. <https://doi.org/10.1016/j.jtherbio.2018.04.003>
- Ming, Q.-L., & Cheng, C. (2012). Influence of Nutrition on Male Development and Reproduction in *Tribolium castaneum*. *Journal of Economic Entomology*, 105(4), 1471–1476. <https://doi.org/10.1603/EC12057>
- Mirenda, J. T., & Vinson, S. B. (1981). Division of labour and specification of castes in the red imported fire ant *Solenopsis invicta* Buren. *Animal Behaviour*, 29(2), 410–420.
- Mirth, C. K., & Riddiford, L. M. (2007). Size assessment and growth control: How adult size is determined in insects. *BioEssays*, 29(4), 344–355. <https://doi.org/10.1002/bies.20552>
- Münch, D., & Amdam, G. V. (2010). The curious case of aging plasticity in honey bees. *FEBS Letters*, 584(12), 2496–2503. <https://doi.org/10.1016/j.febslet.2010.04.007>
- Nakajima, E., Shimaji, K., Umegawachi, T., Tomida, S., Yoshida, H., Yoshimoto, N., Izawa, S., Kimura, H., & Yamaguchi, M. (2016). The Histone Deacetylase Gene Rpd3 Is Required for Starvation Stress Resistance. *PLOS ONE*, 11(12), e0167554. <https://doi.org/10.1371/journal.pone.0167554>

- Nicholson, T. B., Veland, N., & Chen, T. (2015). Writers, Readers, and Erasers of Epigenetic Marks. In *Epigenetic Cancer Therapy* (pp. 31–66). Elsevier. <https://doi.org/10.1016/B978-0-12-800206-3.00003-3>
- Nijhout, H., Davidowitz, G., & Roff, D. (2006). A quantitative analysis of the mechanism that controls body size in *Manduca sexta*. *Journal of Biology*, 5(5), 16.
- Nijhout, H. F., Riddiford, L. M., Mirth, C., Shingleton, A. W., Suzuki, Y., & Callier, V. (2014). The developmental control of size in insects. *WIREs Developmental Biology*, 3(1), 113–134. <https://doi.org/10.1002/wdev.124>
- Nijhout, H. F., & Wheeler, D. E. (1982). Juvenile hormone and the physiological basis of insect polymorphisms. *The Quarterly Review of Biology*, 57(2), 109–133.
- Nunney, L., & Cheung, W. (1997). THE EFFECT OF TEMPERATURE ON BODY SIZE AND FECUNDITY IN FEMALE *DROSOPHILA MELANOGASTER*: EVIDENCE FOR ADAPTIVE PLASTICITY. *Evolution*, 51(5), 1529–1535. <https://doi.org/10.1111/j.1558-5646.1997.tb01476.x>
- Ogino, K., Hirono, Y., Matsumoto, T., & Ishikawa, H. (1993). *Juvenile hormone analogue, S-31183, causes a high level induction of presoldier differentiation in the Japanese damp-wood termite.*
- Okwaro, L. A., & Korb, J. (2023). Epigenetic regulation and division of labor in social insects. *Current Opinion in Insect Science*, 58, 101051. <https://doi.org/10.1016/j.cois.2023.101051>
- Okwaro, L. A., & Korb, J. (2024). Histone Deacetylase 3 Is Involved in Maintaining Queen Hallmarks of a Termite. *Molecular Ecology*, 33(22), e17541. <https://doi.org/10.1111/mec.17541>
- Oms, C. S., Cerdá, X., & Boulay, R. (2017). Is phenotypic plasticity a key mechanism for responding to thermal stress in ants? *The Science of Nature*, 104(5–6), 42. <https://doi.org/10.1007/s00114-017-1464-6>

- Ono, H. (2014). Ecdysone differentially regulates metamorphic timing relative to 20-hydroxyecdysone by antagonizing juvenile hormone in *Drosophila melanogaster*. *Developmental Biology*, 391(1), 32–42. <https://doi.org/10.1016/j.ydbio.2014.04.004>
- Ozawa, T., Mizuhara, T., Arata, M., Shimada, M., Niimi, T., Okada, K., Okada, Y., & Ohta, K. (2016). Histone deacetylases control module-specific phenotypic plasticity in beetle weapons. *Proceedings of the National Academy of Sciences*, 113(52), 15042–15047. <https://doi.org/10.1073/pnas.1615688114>
- Pajoro, A., Severing, E., Angenent, G. C., & Immink, R. G. H. (2017). Histone H3 lysine 36 methylation affects temperature-induced alternative splicing and flowering in plants. *Genome Biology*, 18(1), 102. <https://doi.org/10.1186/s13059-017-1235-x>
- Park, Y. I., & Raina, A. K. (2005). Regulation of juvenile hormone titers by soldiers in the Formosan subterranean termite, *Coptotermes formosanus*. *Journal of Insect Physiology*, 51(4), 385–391. <https://doi.org/10.1016/j.jinsphys.2005.02.001>
- Passera, L. (1974). Différenciation des soldats chez la Fourmi *Pheidole pallidula* Nyl. (Formicidae Myrmicinae). *Insectes Sociaux*, 21(1), 71–86. <https://doi.org/10.1007/BF02222980>
- Passera, L., & Suzzoni, J. (1979). Le rôle de la reine de *Pheidole pallidula* (Nyl.) (Hymenoptera, Formicidae) dans la sexualisation du couvain après traitement par l'hormone juvénile. *Insectes Sociaux*, 26(4), 343–353.
- Pereira, A. S., Amaral, M. S., Vasconcelos, E. J., Pires, D. S., Asif, H., daSilva, L. F., Morales-Vicente, D. A., Carneiro, V. C., Angeli, C. B., & Palmisano, G. (2018). Inhibition of histone methyltransferase EZH2 in *Schistosoma mansoni* in vitro by GSK343 reduces egg laying and decreases the expression of genes implicated in DNA replication and noncoding RNA metabolism. *PLoS Neglected Tropical Diseases*, 12(10), e0006873.

- Perez, R., De Souza Araujo, N., Defrance, M., & Aron, S. (2021). Molecular adaptations to heat stress in the thermophilic ant genus *Cataglyphis*. *Molecular Ecology*, 30(21), 5503–5516.
<https://doi.org/10.1111/mec.16134>
- Perez-Hedo, M., Rivera-Perez, C., & Noriega, F. G. (2014). Starvation Increases Insulin Sensitivity and Reduces Juvenile Hormone Synthesis in Mosquitoes. *PLoS ONE*, 9(1), e86183.
<https://doi.org/10.1371/journal.pone.0086183>
- Petralia, R. S., & Vinson, S. B. (1978). Feeding in the Larvae of the Imported Fire Ant, *Solenopsis invicta*: Behavior and Morphological Adaptations. *Annals of the Entomological Society of America*, 71(4), 643–648. <https://doi.org/10.1093/aesa/71.4.643>
- Petralia, R. S., & Vinson, S. B. (1979). Developmental Morphology of Larvae and Eggs of the Imported Fire Ant, *Solenopsis invicta*. *Annals of the Entomological Society of America*, 72(4), 472–484. <https://doi.org/10.1093/aesa/72.4.472>
- Pile, L. A., Lee, F. W.-H., & Wassarman, D. A. (2001). The histone deacetylase inhibitor trichostatin A influences the development of *Drosophila melanogaster*: *Cellular and Molecular Life Sciences*, 58(11), 1715–1718. <https://doi.org/10.1007/PL00000809>
- Polsinelli, G. A., & Yu, H. D. (2018). Regulation of histone deacetylase 3 by metal cations and 10-hydroxy-2E-decenoic acid: Possible epigenetic mechanisms of queen-worker bee differentiation. *PLOS ONE*, 13(12), e0204538. <https://doi.org/10.1371/journal.pone.0204538>
- Ponton, F., Chapuis, M.-P., Pernice, M., Sword, G. A., & Simpson, S. J. (2011). Evaluation of potential reference genes for reverse transcription-qPCR studies of physiological responses in *Drosophila melanogaster*. *Journal of Insect Physiology*, 57(6), 840–850.
<https://doi.org/10.1016/j.jinsphys.2011.03.014>

- Porter, S. D. (1988). Impact of temperature on colony growth and developmental rates of the ant, *Solenopsis invicta*. *Journal of Insect Physiology*, 34(12), 1127–1133.
[https://doi.org/10.1016/0022-1910\(88\)90215-6](https://doi.org/10.1016/0022-1910(88)90215-6)
- Prudic, K. L., Jeon, C., Cao, H., & Monteiro, A. (2011). Developmental plasticity in sexual roles of butterfly species drives mutual sexual ornamentation. *Science*, 331(6013), 73–75.
- Rajakumar, R., Koch, S., Couture, M., Favé, M.-J., Lillico-Ouachour, A., Chen, T., De Blasis, G., Rajakumar, A., Ouellette, D., & Abouheif, E. (2018). Social regulation of a rudimentary organ generates complex worker-caste systems in ants. *Nature*, 562(7728), 574–577.
- Rajakumar, R., San Mauro, D., Dijkstra, M. B., Huang, M. H., Wheeler, D. E., Hiou-Tim, F., Khila, A., Cournoyea, M., & Abouheif, E. (2012). Ancestral developmental potential facilitates parallel evolution in ants. *Science*, 335(6064), 79–82.
- Ren, Q., Ma, L., Zhang, X., Chen, L., Mao, Z., Li, D., Zhang, L., & Jiang, X. (2023). Effect of Juvenile Hormone on Worker Behavioral Transition in the Red Imported Fire Ant, *Solenopsis invicta* (Hymenoptera: Formicidae). *Insects*, 14(12), 934.
- Riddiford, L. M., Truman, J. W., Mirth, C. K., & Shen, Y. (2010). A role for juvenile hormone in the prepupal development of *Drosophila melanogaster*. *Development*, 137(7), 1117–1126.
<https://doi.org/10.1242/dev.037218>
- Ringrose, L., & Paro, R. (2004). Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. *Annu. Rev. Genet.*, 38(1), 413–443.
- Robertson, F. W. (1960). The ecological genetics of growth in *Drosophila* 1. Body size and developmental time on different diets. *Genetical Research*, 1(2), 288–304.
<https://doi.org/10.1017/S0016672300000264>
- Robinson, E. (2009). Physiology as a caste-defining feature. *Insectes Sociaux*, 56(1), 1–6.

- Rodríguez, A., Rodríguez, M., Córdoba, J. J., & Andrade, M. J. (2015). Design of primers and probes for quantitative real-time PCR methods. In *PCR primer design* (pp. 31–56). Springer.
- Rogina, B., Helfand, S. L., & Frankel, S. (2002). Longevity Regulation by *Drosophila* Rpd3 Deacetylase and Caloric Restriction. *Science*, 298(5599), 1745–1745.
<https://doi.org/10.1126/science.1078986>
- Rohde, K., Dreher, E., & Hochkirch, A. (2015). Sex-specific phenotypic plasticity in response to the trade-off between developmental time and body size supports the dimorphic niche hypothesis: Sex-specific density effects. *Biological Journal of the Linnean Society*, 115(1), 48–57.
<https://doi.org/10.1111/bij.12460>
- Romiguier, J., Borowiec, M. L., Weyna, A., Helleu, Q., Loire, E., La Mendola, C., Rabeling, C., Fisher, B. L., Ward, P. S., & Keller, L. (2022). Ant phylogenomics reveals a natural selection hotspot preceding the origin of complex eusociality. *Current Biology*, 32(13), 2942-2947.e4.
<https://doi.org/10.1016/j.cub.2022.05.001>
- Roquis, D., Taudt, A., Geyer, K. K., Padalino, G., Hoffmann, K. F., Holroyd, N., Berriman, M., Aliaga, B., Chaparro, C., & Grunau, C. (2018). Histone methylation changes are required for life cycle progression in the human parasite *Schistosoma mansoni*. *PLoS Pathogens*, 14(5), e1007066.
- Roy, A., & Palli, S. R. (2018). Epigenetic modifications acetylation and deacetylation play important roles in juvenile hormone action. *BMC Genomics*, 19(1), 934. <https://doi.org/10.1186/s12864-018-5323-4>
- Schlichting, C. D. (1986). The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics*, 667–693.

- Seto, E., & Yoshida, M. (2014). Erasers of histone acetylation: The histone deacetylase enzymes. *Cold Spring Harbor Perspectives in Biology*, 6(4), a018713.
- Simola, D. F., Graham, R. J., Brady, C. M., Enzmann, B. L., Desplan, C., Ray, A., Zwiebel, L. J., Bonasio, R., Reinberg, D., Liebig, J., & Berger, S. L. (2016). Epigenetic (re)programming of caste-specific behavior in the ant *Camponotus floridanus*. *Science*, 351(6268), aac6633.
<https://doi.org/10.1126/science.aac6633>
- Simpson, S. J., Sword, G. A., & Lo, N. (2011). Polyphenism in insects. *Current Biology*, 21(18), R738–R749.
- Sorensen, A. A., & Vinson, S. B. (1981). Quantitative food distribution studies within Laboratory colonies of the imported fire ant, *Solenopsis invicta* Buren. *Insectes Sociaux*, 28(2), 129–160.
<https://doi.org/10.1007/BF02223701>
- Spannhoff, A., Kim, Y. K., Raynal, N. J. -M, Gharibyan, V., Su, M., Zhou, Y., Li, J., Castellano, S., Sbardella, G., Issa, J. J., & Bedford, M. T. (2011). Histone deacetylase inhibitor activity in royal jelly might facilitate caste switching in bees. *EMBO Reports*, 12(3), 238–243.
<https://doi.org/10.1038/embor.2011.9>
- Starkey, J. A. (2023). *The Influence of Brood and Juvenile Hormone on Worker Behavior and Associated Gene Expression in the Red Imported Fire Ant, Solenopsis invicta*.
- Starkey, J., Hawkings, C., & Tamborindeguy, C. (2023). Influence of juvenile hormone analog on behavior in the red imported fire ant, *Solenopsis invicta*. *Scientific Reports*, 13(1), 14726.
- Sultan, S. E. (2015). *Organism and environment: Ecological development, niche construction, and adaptation*. Oxford University Press.

- Suzuki, R., Yaguchi, H., & Maekawa, K. (2019). Histone modifying genes are involved in the molting period during soldier differentiation in *Zootermopsis nevadensis*. *Journal of Insect Physiology*, 117, 103892. <https://doi.org/10.1016/j.jinsphys.2019.103892>
- Tamaru, H. (2010). Confining euchromatin/heterochromatin territory: Jumonji crosses the line. *Genes & Development*, 24(14), 1465–1478. <https://doi.org/10.1101/gad.1941010>
- Taylor, S., Wakem, M., Dijkman, G., Alsarraj, M., & Nguyen, M. (2010). A practical approach to RT-qPCR—publishing data that conform to the MIQE guidelines. *Methods*, 50(4), S1–S5.
- Terashima, J., Takaki, K., Sakurai, S., & Bownes, M. (2005). Nutritional status affects 20-hydroxyecdysone concentration and progression of oogenesis in *Drosophila melanogaster*. *Journal of Endocrinology*, 187(1), 69–79. <https://doi.org/10.1677/joe.1.06220>
- Thornton, B., & Basu, C. (2015). Rapid and simple method of qPCR primer design. In *PCR primer design* (pp. 173–179). Springer.
- Troisi, S. J., & Riddiford, L. M. (1974). Juvenile Hormone Effects on Metamorphosis and Reproduction of the Fire Ant, *Solenopsis invicta*. *Environmental Entomology*, 3(1), 112–116. <https://doi.org/10.1093/ee/3.1.112>
- Truman, J. W., & Riddiford, L. M. (1999). The origins of insect metamorphosis. *Nature*, 401(6752), 447–452. <https://doi.org/10.1038/46737>
- Tschinkel, W. R. (1988). Social control of egg-laying rate in queens of the fire ant, *Solenopsis invicta**. *Physiological Entomology*, 13(3), 327–350. <https://doi.org/10.1111/j.1365-3032.1988.tb00484.x>
- Tschinkel, W. R. (2006). *The Fire Ants*. Harvard University Press.
- Tschinkel, W. R., & Wilson, E. O. (2014). Scientific natural history: Telling the epics of nature. *BioScience*, 64(5), 438–443.

- Tseng, M., Kaur, K. M., Soleimani Pari, S., Sarai, K., Chan, D., Yao, C. H., Porto, P., Toor, A., Toor, H. S., & Fograscher, K. (2018). Decreases in beetle body size linked to climate change and warming temperatures. *Journal of Animal Ecology*, 87(3), 647–659.
- Vizueta, J., Xiong, Z., Ding, G., Larsen, R. S., Ran, H., Gao, Q., Stiller, J., Dai, W., Jiang, W., & Zhao, J. (2025). Adaptive radiation and social evolution of the ants. *Cell*.
- Von Bertalanffy, L. (1960). Principles and theory of growth. *Fundamental Aspects of Normal and Malignant Growth*, 493, 137–259.
- Waddington, C. H. (1942). Canalization of development and the inheritance of acquired characters. *Nature*, 150(3811), 563–565.
- Waddington, C. H. (1957). *The Strategy of the Genes*. George Allen & Unwin.
- Wang, Y., Ma, L., Zhang, W., Cui, X., Wang, H., & Xu, B. (2016). Comparison of the nutrient composition of royal jelly and worker jelly of honey bees (*Apis mellifera*). *Apidologie*, 47(1), 48–56. <https://doi.org/10.1007/s13592-015-0374-x>
- Weaver, N. (1957). Effects of Larval Age on Dimorphic Differentiation of the Female Honey Bee. *Annals of the Entomological Society of America*, 50(3), 283–294. <https://doi.org/10.1093/aesa/50.3.283>
- Werner, M. S., Loschko, T., King, T., Reich, S., Theska, T., Franz-Wachtel, M., Macek, B., & Sommer, R. J. (2023). Histone 4 lysine 5/12 acetylation enables developmental plasticity of *Pristionchus* mouth form. *Nature Communications*, 14(1), 2095.
- West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics*, 249–278.
- West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press.

- Wheeler, D. E. (1986). Developmental and physiological determinants of caste in social Hymenoptera: Evolutionary implications. *The American Naturalist*, 128(1), 13–34.
- Wheeler, D. E. (1990). The developmental basis of worker polymorphism in fire ants. *Journal of Insect Physiology*, 36(5), 315–322. [https://doi.org/10.1016/0022-1910\(90\)90012-5](https://doi.org/10.1016/0022-1910(90)90012-5)
- Wheeler, D. E., & Nijhout, H. F. (1981). Soldier determination in ants: New role for juvenile hormone. *Science*, 213(4505), 361–363.
- Wheeler, D. E., & Nijhout, H. F. (1983). Soldier determination in *Pheidole bicarinata*: Effect of methoprene on caste and size within castes. *Journal of Insect Physiology*, 29(11), 847–854.
- Whitman, D., & Agrawal, A. (2009). What is Phenotypic Plasticity and Why is it Important? In D. Whitman & T. Ananthakrishnan (Eds.), *Phenotypic Plasticity of Insects*. Science Publishers. <https://doi.org/10.1201/b10201-2>
- Whitman, D. W., & Agrawal, A. A. (2009). What is phenotypic plasticity and why is it important. *Phenotypic Plasticity of Insects: Mechanisms and Consequences*, 1–63.
- Williams, C. M. (1968). Ecdysone and ecdysone-analogues: Their assay and action on diapausing pupae of the cynthia silkworm. *The Biological Bulletin*, 134(2), 344–355.
- Wilson, E. O. (1953). The origin and evolution of polymorphism in ants. *The Quarterly Review of Biology*, 28(2), 136–156.
- Wilson, E. O. (1978). Division of labor in fire ants based on physical castes (Hymenoptera: Formicidae: Solenopsis). *Journal of the Kansas Entomological Society*, 615–636.
- Wilson, E. O. (2003). *Pheidole in the New World: A dominant, hyperdiverse ant genus*. Harvard University Press.
- Wilson, E. O., & Eisner, T. (1957). Quantitative studies of liquid food transmission in ants. *Insectes Sociaux*, 4(2), 157–166. <https://doi.org/10.1007/BF02224149>

- Wurm, Y., Wang, J., Riba-Grognuz, O., Corona, M., Nygaard, S., Hunt, B. G., Ingram, K. K., Falquet, L., Nipitwattanaphon, M., & Gotzek, D. (2011). The genome of the fire ant *Solenopsis invicta*. *Proceedings of the National Academy of Sciences*, *108*(14), 5679–5684.
- Xia, B., Gerstin, E., Schones, D. E., Huang, W., & Steven de Belle, J. (2016). Transgenerational programming of longevity through E(z)-mediated histone H3K27 trimethylation in *Drosophila*. *Aging*, *8*(11), 2988–3008. <https://doi.org/10.18632/aging.101107>
- Xu, H., Zhang, L., Qian, X., Zhou, X., Yan, Y., Zhou, J., Ge, W., Albahde, M., & Wang, W. (2019). GSK343 induces autophagy and downregulates the AKT/mTOR signaling pathway in pancreatic cancer cells. *Experimental and Therapeutic Medicine*, *18*(4), 2608–2616.
- Yang, M., Li, G., Yu, L., Du, S., Jiang, D., Chu, X., Wang, K., Wu, S., Wang, R., Zhang, F., & Hu, X. (2023). Temperature and metal ions regulate larval diapause termination via the 20-hydroxyecdysone and juvenile hormone pathways in *Monochamus alternatus*. *Pest Management Science*, *79*(1), 437–446. <https://doi.org/10.1002/ps.7212>
- Yang, Y., Zhao, T., Li, Z., Qian, W., Peng, J., Wei, L., Yuan, D., Li, Y., Xia, Q., & Cheng, D. (2021). Histone H3K27 methylation-mediated repression of *Hairy* regulates insect developmental transition by modulating ecdysone biosynthesis. *Proceedings of the National Academy of Sciences*, *118*(35), e2101442118.
- Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics*, *13*(1), 134.
- Ye, J., & Li, J. (2020). First proteomic analysis of the role of lysine acetylation in extensive functions in *Solenopsis invicta*. *PLOS ONE*, *15*(12), e0243787. <https://doi.org/10.1371/journal.pone.0243787>

- Yu, Z., Pei, T., Wang, H., Wang, C., Liu, J., & Storey, K. B. (2024). Lysine Methylation and Histone Modifications during Cold Stress of Insects: Freeze-Tolerant *Eurosta solidaginis* and Freeze-Avoiding *Epiblema scudderiana*. *Insects*, *15*(7), 498. <https://doi.org/10.3390/insects15070498>
- Yung, P. Y. K., & Elsässer, S. J. (2017). Evolution of epigenetic chromatin states. *Current Opinion in Chemical Biology*, *41*, 36–42.