

EXAMINING THE GENETIC BASIS FOR A PHENOTYPIC CHANGE IN THE RED
SHOULDERED SOAPBERRY BUG, *JADERA HAEMATOLOMA*

By

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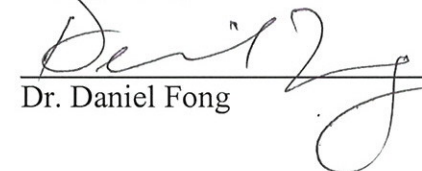
In

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ABSTRACT

The red shouldered soapberry bug *Jadera haematoloma* (Heteroptera: Rhopalidae) has provided an unique opportunity to study the genetic basis for phenotypic differences between populations. Among *J. haematoloma* in Southern Florida, individuals are found feeding on the native balloon vine (*Cardiospermum* sp.). Recently derived (~60 years) populations also feed on goldenrain tree (*Koeleruteria* sp.). As a result of this host shift, rostrum length in derived race has declined from almost 70% of body length to roughly 50%. This study looks into the development of the mouthparts, focusing on three genes- *Distal-less*, *dachshund*, and *homothorax* known to play a role in the mouthpart development. RNA interference was used to characterize the roles of these genes in mouthpart development. Treatment groups for all the genes resulted in phenotypic differences from the control group. Maternal RNAi for *Dll* resulted in hatchlings without proper appendage development. Juvenile RNAi showed that *Dll* significantly reduced labrum length and *dac* showed a significant reduction in labium segment 3 and 4. It has been concluded that *Distal-less*, *dachshund*, and *homothorax* play a developmental role in *J. haematoloma* mouthparts.

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CHAPTER 1

EXAMINING THE GENETIC BASIS FOR A PHENOTYPIC CHANGE IN THE RED-
SHOULDERED SOAPBERRY BUG, *JADERA HAEMATOLOMA*

In the natural world one can observe phenotypic variation among individuals of the same species due to environmental influences or genetic changes such as mutation. It is assumed that individuals of the same species would utilize identical genetic processes to develop the same structures. With this knowledge, observation of significant variation of an anatomical feature between populations of the species is interesting. Questioning how this is possible leads us to looking at the genetic control of developmental pathways. Although relative few examples of the genetic basis for phenotypic evolution are known (e.g.(Morrissey and Ferguson, 2011; Paez et al., 2010; Shapiro et al., 2004; Wittkopp et al., 2003), a unique opportunity is available with a heteropteran, *Jadera haematoloma*, the red-shouldered soapberry bug. This insect has undergone a reduction in mouthpart length after a host shift in the past 60 years (Carroll & Boyd, 1992). This thesis presents the first molecular genetic investigation of development processes in *J. haematoloma*. Based upon research conducted with *Oncopeltus fasciatus*, the large milkweed bug (Heteroptera:Lygaeidae) genes which were found to have a function in mouthpart development were evaluated in this study (Angelini & Kaufman, 2004). There have been no molecular, genetic or developmental studies of *J. haematoloma* to date so this project will establish a developmental framework for future studies of phenotypic variation in this species.

Jadera haematoloma (Heteroptera: Rhopalidae) is commonly referred to as the red-shouldered soapberry bug, based on its coloration and its preference for feeding on plants of the soapberry family (*Sapindaceae*). These “true bugs” have modified mouthparts, used for piercing and extracting nutrients from seeds. Among *J. haematoloma* in Southern Florida, individuals are found feeding and reproducing on the native balloon vine (*Cardiospermum corindum*), a member of the soapberry family. This vine is a woody perennial with seeds contained in a hollow pod, approximately 2.5 cm in diameter. Although the exact timing of the preference change is unknown, approximately 60 years ago a population of *J. haematoloma* diverged to begin feeding on the goldenrain tree (*Koelreuteria sp.*). This tree was introduced by landscapers as an ornamental plant to the American Southeast from Taiwan (Carroll and Boyd, 1992). This is a small to medium sized deciduous tree with hollow pods approximately 2 cm in diameter. Subsequent to the tree’s introduction and the bugs’ host shift, Carroll and colleagues (2003) described a number of morphological and life history traits from *J. haematoloma* populations living on the ancestral food source and those living on the introduced host plant. These investigators reported that the mouthparts of the population living on the goldenrain tree are 30% shorter relative to body size than those living on the ancestral host, the balloon vine. This shows a change in relative allometry (Stern and Emlen, 1999; Thompson, 1917); or the scaling relationship among soapberry bug individuals between total body size and one organ, in this case the length of the mouthparts. This rapid evolution has piqued the interest of biologists as it is typical for phenotypic evolution to take significantly more time than can be seen in one human lifetime.

All insect mouthparts consist of modifications of three appendage pairs: the mandibles, maxillae and labium, which are used in the collection and processing of food. Heteroptera, such as *J. haematoloma*, have modified mouthparts used for piercing seeds and extracting nutrients (Figure 1). These mouthparts are modified from what is considered to be the ancestral form found in the majority of insect orders such as Orthoptera (grasshoppers), Blatteria (roaches), and Coleoptera (beetles). In these orders the anatomy is termed “mandibulate” due to presence of unjointed chewing mandibles. The mandibles are utilized as powerful cutting jaws, the maxillae are used in the manipulation of food, and the labium is a lower cover of the mouthparts. In mandibulate mouthparts, the maxillae and labial appendages have similarities in their anatomy with the exception of the labial appendages being fused mid-ventrally into the labium. Both have large proximal podomeres bearing two pairs of medial endites, or articulated outgrowths. The maxillary and labial palps may have up to seven segments depending on the insect group. In the Heteroptera, the labium is a segmented structure lacking palps, which develops from the fusion of embryonic appendages. Two pairs of slender bristle-like stylets run down a groove in the labium. Narrow spaces between the stylets form channels for the secretion of saliva and the up-take of liquid food. The outer, anterior pair of stylets are derived from the mandibles and the inner, posterior pair correspond to the maxillae. The labrum is present at the anterior base of the labium and appears as a flap covering it ventrally (Snodgrass, 1935). The entire mouthpart structure is generally referred to as the “beak” or “rostrum”. Although the anatomy of *J. haematoloma* has not been studied in detail, studies of another heteropteran, the large milkweed bug,

Oncopeltus fasciatus (Lygaeidae), have described mouthpart morphology and development (Angelini and Kaufman, 2004; Butt, 1960; Newcomer, 1948).

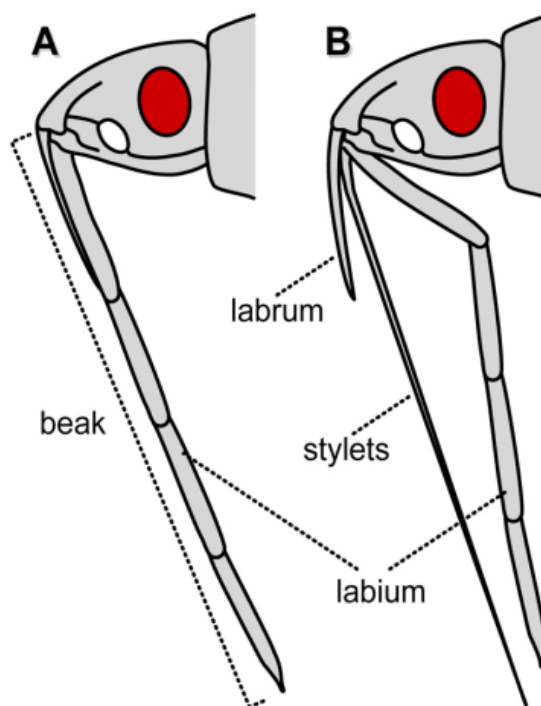


Figure 1: Anatomical overview of the Heteropteran mouthparts. (A) The beak is the complete feeding structure of the soapberry bug. It consists of several individual parts. (B) Mouthparts separated to show specific anatomical features including the slender four segmented labium, two pairs of stylets and labrum.

To date, studies have approached the phenotypic changes in *J. haematoloma* from the perspective of natural history. Little is known about the ecology of this species. Cross rearing and hybridization experiments have discovered that genetic differences between the two races largely determine the phenotypic differences. In the cross rearing experiments, hatchlings from both hosts were reared on seeds from the native and introduced host plants. These experiments determined that host plant influences on beak

length were minor therefore demonstrating that environment only plays a part in determining length of the labium. Thus there is a significant genetic divergence between the populations (Carroll et al., 1997).

In the cross rearing experiments, investigators raised individuals from both populations on the reciprocal host plant as well as on their natal host plant. In the reciprocal cross each population performed better on its original host. It was observed that the derived population was less fecund on the balloon vine and displayed enhanced fecundity on the goldenrain tree. The ancestral population produced eggs at the same rate regardless of host. The results of these cross rearing experiments showed that increased performance on the introduced host has evolved with surprising speed and magnitude, as have reductions in the performance on the native host (Carroll et al., 1998). Compared to the balloon vine, the diameter of the seeds of the goldenrain tree are smaller and their nutritional composition is 50% higher in lipids and 50% lower in protein. Also, there is a difference between the species, in that the balloon vine has a smaller seed crop for longer periods of the year and the goldenrain tree has a larger seed crop for a shorter period of the year (Carroll et al 1998). Seeds can become removed from their protective hollow pod, allowing any individual to have access to the food source. However many seeds remain within the pod, creating a situation in which possessing a beak of the correct length is advantageous so they may access seeds despite the physical barrier. As the pods of the derived host are smaller in diameter, it potential would be energetically favorable for *J. haematoloma* to produce shorter beaks and allocate that energy elsewhere, such as reproduction. As predicted, the beak length has declined from almost 70% of body length to slightly greater than 50% in the derived populations. Other elements of body size have

not been found to have significant changes, and analysis of covariance has shown that beak length evolution is independent of body size (Carroll and Boyd, 1992). Results have also shown that the change in beak length is genetically based. The leading hypothesis is that this change is evolutionary, due to adaptation to host pod size (Carroll and Boyd, 1992; Carroll et al., 1997). Further studies have found a significant interaction between body size, development time and growth rate. With size and development rate being complex traits, these probably result from the interaction of many genes (Carroll et al., 2001; Carroll et al., 1997).

Carroll and colleagues' study shows that the reduction in mouthpart length is a genetic effect, reaching beyond phenotypic plasticity and environmental influences (Carroll et al. 2001). In host-race hybridization experiments, it was found that mouthpart length variation between the two populations is a result of a combination of additive and non-additive genetic variance, including genes-of-major effect. Additive variance is the phenotypic variation resulting from frequency differences in alleles with incremental, additive influences on phenotype. Non-additive variance is the portion of the phenotypic changes due to epistatic interactions among genes. Epistasis is where a single phenotypic trait is influenced by multiple, interacting genes. This is important to note, as when one gene is knocked down, the phenotypic change that results may not give a complete understanding of that gene's function due to possible interactions with other genes. However, valuable insight on genetic networks can still be obtained by looking at these indirect genetic interactions.

To determine the genetic architecture of *J. haematoloma*, purebred, hybrid, and back-cross lines were compared in a two-generation study. For mouthpart length, additive

genetic variance was large, and it was also seen that the interaction of dominance, maternal effects, and epistasis were important for other traits, including body size and development time (Carroll et al 2001).

Due to the known genetic influence on mouthpart length in *J. haematoloma* there has been interest in using techniques from developmental genetics to help understand phenotypic variation in this species. Three target genes have been selected for this study based on their known function in appendage development in arthropods, and strong sequence conservation among animal species. The first gene, *Distal-less (Dll)*, is known to encode a homeodomain transcription factor, and it is expressed in distal structures of appendages during development (Cohen et al., 1993). This gene's expression and function is widely conserved and seen in the appendages of all invertebrates investigated to date. Orthologs of *Dll* are also expressed in the jaws and placode-derived structures of chordates (Panganiban et al., 1997). In *Oncopeltus fasciatus*, another heteropteran, *Dll* is required in the labium for development of the most distal portion, and knockdown by RNA interference (RNAi) during juvenile stages results in a reduction of labium length (Angelini and Kaufman, 2005). *Oncopeltus fasciatus Dll* is not expressed in the mandibular appendages, which give rise to the mandibular stylets, but this gene is expressed in the maxillary appendages. Regardless of maxillary expression *Dll* RNAi had no effect on the maxillary stylets, which indicates that this gene is not necessary for proper development of the stylets (Angelini and Kaufman, 2004).

The second gene, *dachshund (dac)*, is a transcription factor expressed throughout the length of the embryonic mandibular and maxillary limb buds of *O. fasciatus* (Angelini & Kaufman, 2004). This gene plays a role in the proper differentiation of a

subset of segments in the developing leg. Further, *Drosophila dac* has a function in sensory structures, including the eyes (Mardon et al., 1994) which is also seen in *Tribolium castaneum* (Yang et al., 2009). Expression of *dac* also appears in a small proximal domain in the labium of *O. fasciatus*. Knockdown of *dac* in *O. fasciatus* shows that it is required for maturation of the stylets; however no mouthpart phenotypes in the labrum or labium were reported (Angelini & Kaufman, 2004).

The third gene of interest in this study is *homothorax (hth)*, which encodes a homeobox transcription factor. Expression of *hth* appears in the most proximal portion of the labium and throughout the length of the mandibular and maxillary limb buds of *O. fasciatus*. It is required for proper elongation of the heteropteran stylets. In *O. fasciatus hth* RNAi depletions, the labium was transformed distally to legs and the labrum was reduced or absent (Angelini and Kaufman, 2004).

This thesis looks at the developmental function of these genes, which are known from mouthpart development in *O. fasciatus* embryos (Angelini & Kaufman, 2004), in the soapberry bug. These previous studies have focused on the embryos of *O. fasciatus* and results may not be immediately comparable to this study, which is focused on juvenile gene function due to the interest in adult allometry phenotypes. However gene function conservation can still be compared between species. Relatively few examples of the genetic basis for phenotypic change are known, but such investigations are an active part of the field of evolutionary developmental biology (e.g. Wittkopp et al 2003, Shapiro et al 2004 (Morrissey and Ferguson, 2011; Paez et al., 2010). There have been no molecular genetic or developmental studies of *J. haematoloma* to date. This thesis project establishes a developmental genetic framework in which to examine recent phenotypic

evolution in the soapberry bug. The first objective of this study was to utilize RNA interference to look at the developmental function of these genes. By knocking down the target gene, the resulting phenotypic abnormalities show the function of the gene. Measurements of the mouthparts allowed for quantitative comparisons between treatment groups. The second objective of this study was to utilize quantitative real time PCR to validate gene knockdown and elucidate gene interactions in the mouthparts of this species. These objectives will be accomplished utilizing individuals collected from populations feeding on the goldenrain tree and representing the derived phenotype. This is due to a wider range of this population and ease of collection due to location. As no molecular work had been performed before, we wished to establish that these techniques would yield results before attempting to work with the ancestral population which is more difficult to obtain and maintain in the lab.

Significance of Work

This study incorporates many different disciplines of biology, including genetics, development and evolution. The ultimate goal is to establish an understanding of the genetic basis for rapid evolutionary change in *J. haematoloma*. The determination that this rapid change is genetically influenced and not environmental is a key piece to this puzzle. While it is possible that many developmental genes may influence mouthpart length, including genes with no known orthologs in model species, a candidate gene approach is a simple and fast first means to approach these goals. Evolution is not commonly in the span of a human lifetime, and *J. haematoloma* offers an opportunity to explore the developmental genetic effects of evolution on an unusually small time scale.

It may be too big a leap for us to say that genes examined in this study are the direct selective targets for phenotypic evolution, but this work will be a solid first step in understanding the developmental genetic landscape on which selection can operate. Once this landscape is known, it can be used as a starting point to look at the generality, parallelism, repeatability and predictability of evolution within the soapberry bug clades (Stern and Orgogozo, 2009). Recent theory predicts that early in adaptation, alleles with large effects and high pleiotropy are most likely the main targets for selection. (Orr, 2005; Stern, 2011).

As the field of evolutionary developmental biology grows, we are expanding our knowledge from genetic model organisms, such as *Drosophila* and *C. elegans*, reaching to *Oncopeltus* and now *Jadera*. These new research organisms pose many interesting evolutionary questions.

Materials and Methods

Animal Husbandry

Soapberry bugs were kept in 12.7-cm by 13.9-cm by 21.6-cm plastic containers. Tissue paper was placed between the cover and container to prevent the bugs from escaping and allowing air flow. Spring water was continuously available in 50 mL Pyrex glass flasks with a paper towel wick and a cotton plug to prevent individuals from falling into the water. Bugs were fed *Koelreuteria paniculata* seeds collected in the Washington, DC area in summer 2010 and were replaced every 3-4 days. Containers for the 2 populations utilized in this experiment (Davis CA and Washington DC) were changed weekly. Davis CA individuals represented the derived race and were obtained from collaborators from UC Davis. Collection and shipments were made throughout the year at our request. The Washington DC population were collected from July 2010-August 2010 from a goldenrain tree location on American University campus. These individuals also represent the derived race. All containers were kept in an incubator set at 30° C with a 12:12 light cycle. Each cage housed 5- 40 individuals at a time of various instars and number of containers per a population fluctuated. Due to the prevalence of cannibalism, eggs were removed and kept in Petri dishes until hatching, when juveniles were removed to a separate container.

Isolation of candidate gene sequences

Candidate genes from *J. haematoloma*, as well as several house-keeping genes (*rps18*, *actb*, *syx1*, and *18S*- Table 1), were cloned through degenerate PCR. Total RNA was extracted from *J. haematoloma* juveniles of mixed instars using the PureLink RNA Mini Kit (Invitrogen/Life Technologies). The first-strand synthesis of cDNA was done by reverse transcription, using AMV reverse transcriptase (Promega) and a poly-T primer to enrich for protein-coding transcripts. Primers were designed by Dr. Dave Angelini based on published ortholog protein sequences, aligned to find areas of conservation. Once gene fragments were isolated by degenerate PCR, they were ligated into a plasmid vector (Invitrogen Topo4) for transformation into competent *E. coli*. Purified clone plasmids with insert DNAs were sequenced off-campus to confirm their identity (Beckman Coulter Genomics, Danvers, MA).

Table 1

Jadera haematoloma Gene Sequences

| Gene | Symbol | Fragment size (bp) |
|-------------------------------|--------------|--------------------|
| <i>Distal-less</i> | <i>Dll</i> | 173 |
| <i>dachshund</i> | <i>dac</i> | 923 |
| <i>homothorax</i> | <i>hth</i> | 701 |
| <i>sarcomere length short</i> | <i>sals</i> | 404 |
| <i>ribosomal protein</i> | <i>rps18</i> | 408 |
| <i>β-actin</i> | <i>actb</i> | 1002 |
| <i>syntaxin-1</i> | <i>syx1</i> | 585 |
| <i>ribosomal RNA</i> | <i>18S</i> | 225 |

RNA interference

Gene function can be efficiently and quickly determined in many insect species with RNA interference. This technique has been successful in *O. fasciatus* and based on its relationship to *Jadera*, it is likely that these insects share mechanisms of RNA interference (Hannon, 2002). Exact custom primers were ordered (Sigma Custom Oligos) with the T7 viral promoter sequences at their 5' ends and used for the in vitro synthesis of double-stranded RNAs (dsRNAs). This process begins with the plasmid vector containing a cloned gene fragment. Linear DNA was synthesized using PCR with these T7-tagged primers. The result is the target gene fragment with T7 promoters on each end, and once T7 polymerase is added, it synthesizes complimentary RNAs in both directions. Three genes are a target for RNAi: *Distal-less (Dll)*, *dachshund (dac)*, *homothorax (hth)*, as well as Green Fluorescent Protein (GFP), which is used as a non-specific control dsRNA.

After dsRNA is synthesized, it is injected into individual soapberry bugs, either an adult female or fifth instar. Each dsRNA corresponds to the nucleotide sequence for the targeted gene, allowing it to be specifically knocked down, causing a loss of function phenotype (Belles, 2010). Once the dsRNA enters the hemolymph it is transported into cells through a specific dsRNA transporter protein, Sid-1 (Feinberg and Hunter, 2003). Inside cells, the dsRNA is cleaved into ~23-base pair short interfering RNAs (siRNAs) by the Dicer enzyme. The RNA Induced Silencing Complex (RISC) then utilizes the antisense strand of the siRNA to bind to the complementary mRNA and proceed to degrade the mRNA. This degradation of the mRNA causes a loss or knockdown of the gene's biochemical and developmental function (Terenius et al., 2011). For maternal

RNAi, approximately 5 μ l of 1 μ g/ μ l dsRNA was injected into adult females under a metathoracic leg. The females were then cohabitated with males and their offspring were scored for specific gene knock-down phenotypes (modified from Hughes and Kaufman 2000).

An alternative method was to inject fifth instars (the final stage before molting into an adult), in order to observe knock down phenotypes in the resulting adults. Juveniles were anaesthetized with CO₂ exposure and injected with a pulled-glass capillary needle with 3-4 μ l of 1 μ g/ μ l dsRNA under a metathoracic leg (Angelini & Kaufman, 2005). To control for possible nonspecific effects of injection or dsRNA toxicity, dsRNA encoding GFP, which does not naturally occur in insect genomes, was also introduced.

Specimens were preserved in 75% ethanol, examined under a dissection microscope and compared to the control specimens for phenotypic abnormalities, particularly in the mouthparts. The length of each labial segment, the labrum, antennae and legs as well as pronotum width and distance across the eyes were measured on a VistaVision dissecting microscope (VWR) using an ocular micrometer (Figure 2) Comparisons of labium (beak) length relative to the size of the body and other appendages were based upon these measurements and were necessary to examine the effects of RNAi on relative beak allometry. Pearson's product moment correlation was calculated to determine the significance of the correlation between anatomical characters (defined as $p < 0.05$).

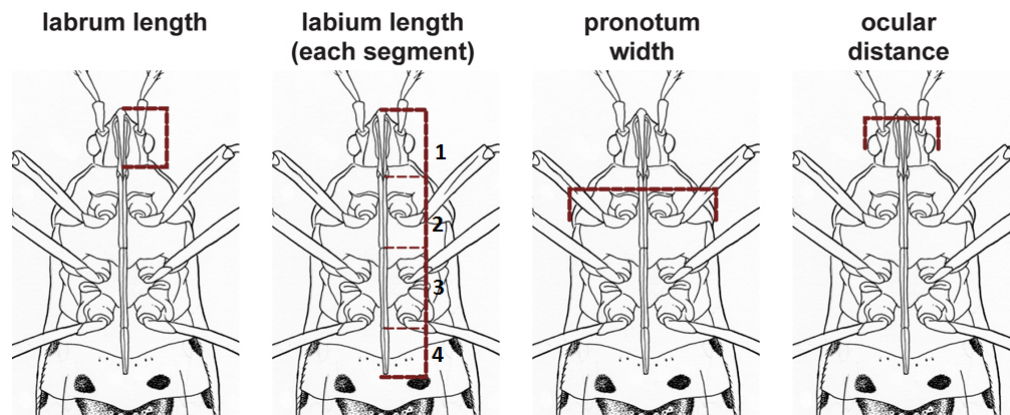


Figure 2: Measurements of anatomical features taken.

To test the effects of RNAi on quantitative phenotypes, ANOVA was used to determine whether dsRNA treatments differed significantly. Tukey's HSD post hoc test was used to determine the significance of pairwise differences between the control (GFP) and the target gene (*Dll*, *dac* and *hth*) dsRNA treatments for uncorrected measurements, as well as measurements normalized for pronotum width and ocular distance (measures of body size).

Validation of RNAi

Real-time PCR (rt-PCR) can be used to find quantitative differences in mRNA expression by using sequence specific primers to determine the number of transcript copies in a sample. The procedure is similar to standard PCR, however the products of the reaction are detected in real-time based on the fluorescence of SYBR Green bound to double stranded DNA products. If a standard series of known template RNA concentrations are assayed on the same plate, it is possible to calculate the starting number of mRNA templates in samples. For validation three biological replicates were

used for each gene and RNA was extracted from the head, without antennae. Each gene knockdown was compared to the other 3 gene knockdowns for analysis of expression level differences. To determine the effectiveness of RNAi, quantitative real-time PCR was used to verify that the target gene was knocked down relative to control RNAi specimens for *Dll* and *hth* (Bustin, 2004). For *dac* relative real-time PCR was used due to failure of the standards and analyzed as described in (Pfaffl, 2001). For this purpose, primers (Appendix) have been designed that bind to the transcript sequences outside the dsRNA region. Primers overlapping the dsRNA sequence will also amplify from the reverse-transcription products of dsRNA molecules introduced by injection or those subsequently amplified in vivo. Product dissociation curves were examined to verify that primer pairs yielded only a single product.

Results

***Jadera haematoloma* Gene Sequences**

Three candidate genes: *Distal-less*, *dachshund*, and *homothorax* were successfully cloned and sequenced from *J. haematoloma*. In addition, a transcription factor involved in muscle development, *short sarcomere length (sals)* and four house-keeping genes (*β -actin*, *rps18*, *syntaxin-1*, and *18S rRNA*) have also been cloned and sequenced (Table 1). Double-stranded RNA has been synthesized for *Dll*, *dac*, *hth* and GFP. RNA interference with GFP, *Dll*, *dac*, and *hth* dsRNA has been performed on juveniles with an average survival rate of 88%.

Qualitative Results of RNA interference

Table 2 shows the genes, number of individuals injected and the number which survived to molt and were measured successfully (scored). Table 3 shows the measurements taken in millimeters using a microscope. Treatment refers to when the bugs were injected.

Table 2

RNAi Treatments

| dsRNA | Population | Individuals injected | Individuals scored |
|------------|------------|----------------------|--------------------|
| GFP | Davis | 21 | 17 |
| <i>Dll</i> | Davis | 33 | 31 |
| <i>dac</i> | Davis | 34 | 29 |
| <i>hth</i> | Davis | 21 | 21 |
| <i>hth</i> | DC | 15 | 11 |

Table 3

Measurement of individuals in millimeters. Abbreviations are as follows: lr=labrum, lb=labium, l1=labial segment 1, l2=labial segment 2, l3= labial segment 3, l4=labial segment 4, od=ocular distance, pw=pronotum width

| Treatment | dsRNA | lr | lb | l1 | l2 | l3 | l4 | od | pw |
|-----------|------------|------|------|------|------|------|------|------|------|
| 2 | GFP | 1.66 | 6.03 | 1.14 | 1.51 | 1.66 | 1.72 | 2.60 | 2.34 |
| 2 | GFP | 2.03 | 6.14 | 1.46 | 1.51 | 1.56 | 1.61 | 2.24 | 2.29 |
| 2 | GFP | 1.87 | 5.04 | 1.09 | 1.30 | 1.20 | 1.46 | 2.34 | 2.08 |
| 2 | GFP | 2.03 | 6.34 | 1.35 | 1.56 | 1.66 | 1.77 | 2.24 | 1.77 |
| 2 | GFP | 1.98 | 5.30 | 1.14 | 1.30 | 1.40 | 1.46 | 2.08 | 2.34 |

| Treatment | dsRNA | lr | lb | l1 | l2 | l3 | l4 | od | pw |
|-----------|------------|------|------|------|------|------|------|------|------|
| 2 | GFP | 2.08 | 5.77 | 1.30 | 1.46 | 1.40 | 1.61 | 2.29 | 2.24 |
| 2 | GFP | 2.13 | 5.62 | 1.30 | 1.30 | 1.40 | 1.61 | 2.03 | 2.03 |
| 10 | GFP | 1.82 | 6.14 | 1.35 | 1.40 | 1.77 | 1.61 | 2.18 | 2.18 |
| 10 | GFP | 1.98 | 5.82 | 1.14 | 1.51 | 1.56 | 1.61 | 2.08 | 1.98 |
| 10 | GFP | 1.25 | 4.63 | 0.73 | 1.25 | 1.35 | 1.30 | 1.87 | 1.61 |
| 10 | GFP | 1.72 | 5.36 | 1.14 | 1.35 | 1.40 | 1.46 | 2.03 | 1.82 |
| 10 | GFP | 1.87 | 5.82 | 1.20 | 1.40 | 1.66 | 1.56 | 2.18 | 2.03 |
| 10 | GFP | 2.18 | 6.14 | 0.78 | 1.82 | 1.77 | 1.77 | 2.29 | 2.18 |
| 10 | GFP | 1.98 | 6.76 | 1.35 | 1.56 | 1.92 | 1.92 | 2.29 | 2.24 |
| 10 | GFP | 1.87 | 5.82 | 1.30 | 1.35 | 1.56 | 1.61 | 2.24 | 2.08 |
| 10 | GFP | 1.61 | 5.41 | 1.20 | 1.14 | 1.46 | 1.61 | 2.03 | 1.82 |
| 3 | <i>Dll</i> | 1.61 | 5.51 | 1.40 | 1.30 | 1.30 | 1.51 | 2.03 | 2.03 |
| 3 | <i>Dll</i> | 1.20 | 4.00 | 0.83 | 0.99 | 0.99 | 1.20 | 1.87 | 2.08 |
| 3 | <i>Dll</i> | 1.30 | 5.46 | 1.35 | 1.30 | 1.30 | 1.51 | 2.08 | 2.03 |
| 3 | <i>Dll</i> | 1.30 | 5.93 | 1.30 | 1.56 | 1.51 | 1.56 | 2.55 | 2.55 |
| 4 | <i>Dll</i> | 1.72 | 5.62 | 1.25 | 1.40 | 1.46 | 1.51 | 2.18 | 1.92 |
| 4 | <i>Dll</i> | 1.51 | | 1.30 | 1.98 | | | 2.50 | 2.44 |
| 4 | <i>Dll</i> | 1.51 | 5.41 | 0.94 | 1.46 | 1.51 | 1.51 | 2.13 | 1.82 |
| 1 | <i>Dll</i> | 1.51 | 5.82 | 1.25 | 1.46 | 1.56 | 1.56 | 2.44 | 2.08 |
| 1 | <i>Dll</i> | 1.46 | 5.36 | 1.25 | 1.30 | 1.35 | 1.46 | 2.08 | 1.92 |
| 1 | <i>Dll</i> | 1.46 | 5.15 | 1.14 | 1.25 | 1.35 | 1.40 | 2.03 | 1.82 |
| 1 | <i>Dll</i> | 1.56 | 6.29 | 1.40 | 1.46 | 1.72 | 1.72 | 2.18 | 2.08 |
| 1 | <i>Dll</i> | 1.35 | 4.89 | 1.14 | 1.20 | 1.20 | 1.35 | 1.92 | 1.98 |
| 1 | <i>Dll</i> | 1.25 | 4.42 | 0.73 | 1.30 | 1.30 | 1.09 | 1.98 | 1.98 |
| 1 | <i>Dll</i> | 1.30 | 5.51 | 1.30 | 1.30 | 1.40 | 1.51 | 2.13 | 2.03 |
| 1 | <i>Dll</i> | 1.25 | 5.36 | 1.20 | 1.30 | 1.30 | 1.56 | 2.29 | 2.18 |
| 1 | <i>Dll</i> | 1.56 | 4.42 | 1.09 | 1.09 | 1.14 | 1.09 | 1.87 | 1.82 |
| 1 | <i>Dll</i> | 1.25 | 4.21 | 0.57 | 1.09 | 1.20 | 1.35 | 1.87 | 1.66 |
| 1 | <i>Dll</i> | 1.77 | 4.47 | 0.73 | 1.20 | 1.20 | 1.35 | 1.92 | 1.87 |
| 1 | <i>Dll</i> | 0.99 | 5.25 | 1.04 | 1.30 | 1.40 | 1.51 | 2.24 | 2.13 |
| 9 | <i>Dll</i> | 1.77 | 4.94 | 1.14 | 1.30 | 1.14 | 1.35 | 1.98 | 2.03 |
| 9 | <i>Dll</i> | 1.61 | 4.94 | 1.09 | 1.14 | 1.30 | 1.40 | 2.08 | 1.87 |
| 9 | <i>Dll</i> | 1.66 | 4.89 | 0.94 | 1.40 | 1.46 | 1.09 | 2.44 | 2.34 |
| 9 | <i>Dll</i> | 1.87 | 6.19 | 1.46 | 1.51 | 1.61 | 1.61 | 2.44 | 2.44 |
| 9 | <i>Dll</i> | 2.13 | 6.66 | 1.20 | 1.72 | 1.87 | 1.87 | 2.34 | 2.13 |
| 9 | <i>Dll</i> | 1.66 | 6.03 | 1.30 | 1.40 | 1.66 | 1.66 | 2.13 | 1.98 |
| 9 | <i>Dll</i> | 1.77 | 6.24 | 1.30 | 1.40 | 1.77 | 1.77 | 2.18 | 2.55 |
| 9 | <i>Dll</i> | 1.56 | 4.78 | 0.99 | 1.20 | 1.30 | 1.30 | 2.13 | 2.18 |
| 9 | <i>Dll</i> | 1.35 | 4.94 | 1.04 | 1.30 | 1.30 | 1.30 | 1.98 | 1.98 |
| 7 | <i>dac</i> | 1.87 | 5.93 | 1.25 | 1.51 | 1.56 | 1.61 | 2.18 | 2.08 |
| 7 | <i>dac</i> | 1.87 | 4.94 | 1.20 | 1.25 | 1.30 | 1.20 | 1.92 | 1.82 |
| 7 | <i>dac</i> | 1.25 | 4.47 | 1.09 | 1.14 | 1.09 | 1.14 | 1.87 | 1.66 |
| 7 | <i>dac</i> | 1.92 | 5.51 | 0.88 | 1.51 | 1.51 | 1.61 | 2.29 | 2.24 |
| 7 | <i>dac</i> | 1.66 | 6.40 | 1.46 | 1.35 | 1.82 | 1.77 | 2.18 | 1.98 |

| Treatment | dsRNA | lr | lb | l1 | l2 | l3 | l4 | od | pw |
|-----------|------------|------|------|------|------|------|------|------|------|
| 7 | <i>dac</i> | 1.09 | 6.34 | 1.46 | | | 1.25 | 2.29 | 2.13 |
| 7 | <i>dac</i> | 1.46 | 5.36 | 1.04 | | | 1.56 | 2.18 | 2.08 |
| 7 | <i>dac</i> | 1.77 | 5.72 | 1.25 | 1.30 | 1.56 | 1.61 | 2.60 | 2.18 |
| 7 | <i>dac</i> | 1.61 | 4.68 | 1.09 | 1.09 | 1.14 | 1.35 | 2.18 | 1.61 |
| 7 | <i>dac</i> | 2.08 | 5.67 | 1.30 | 1.25 | 1.46 | 1.66 | 2.24 | 1.72 |
| 7 | <i>dac</i> | 1.56 | 4.68 | 1.04 | 1.20 | 1.35 | 1.09 | 1.92 | 1.77 |
| 7 | <i>dac</i> | 2.08 | 5.10 | 1.09 | 1.30 | 1.30 | 1.40 | 2.29 | 2.18 |
| 7 | <i>dac</i> | | 3.48 | | 1.09 | 1.09 | 1.30 | 1.92 | 1.87 |
| 7 | <i>dac</i> | 1.82 | 4.21 | 1.04 | 1.04 | 0.99 | 1.14 | 2.29 | 2.34 |
| 7 | <i>dac</i> | 1.82 | 5.98 | 1.25 | 1.51 | 1.56 | 1.66 | 2.03 | 1.82 |
| 7 | <i>dac</i> | 1.35 | 4.58 | 0.57 | 1.30 | 1.25 | 1.46 | 2.13 | 2.03 |
| 7 | <i>dac</i> | 2.70 | 5.82 | 1.25 | 1.30 | 1.66 | 1.61 | 2.44 | 2.50 |
| 7 | <i>dac</i> | 1.30 | 5.41 | 1.25 | 1.30 | 1.35 | 1.51 | 2.13 | 2.08 |
| 8 | <i>dac</i> | 1.35 | 5.15 | 1.04 | 1.30 | 1.30 | 1.51 | 1.98 | 1.77 |
| 8 | <i>dac</i> | 1.61 | 5.82 | 1.14 | 1.56 | 1.56 | 1.56 | 2.13 | 1.87 |
| 8 | <i>dac</i> | 1.82 | 4.99 | 1.04 | 1.30 | 1.30 | 1.35 | 1.87 | 1.72 |
| 8 | <i>dac</i> | | 5.67 | 0.99 | 1.56 | 1.56 | 1.56 | 2.13 | 1.98 |
| 8 | <i>dac</i> | 1.92 | 5.04 | 1.04 | 1.30 | 1.30 | 1.40 | 1.87 | 1.56 |
| 8 | <i>dac</i> | 2.13 | 5.67 | 1.20 | 1.46 | 1.46 | 1.56 | 2.13 | 2.18 |
| 8 | <i>dac</i> | 1.66 | 4.63 | 1.04 | 1.20 | 1.20 | 1.20 | 1.82 | 1.56 |
| 23 | <i>dac</i> | 1.72 | 4.63 | 1.04 | 1.25 | 1.25 | 1.09 | 1.98 | 1.77 |
| 23 | <i>dac</i> | 1.66 | 5.20 | 1.20 | 1.30 | 1.35 | 1.35 | 2.03 | 1.82 |
| 23 | <i>dac</i> | 1.72 | 4.99 | 1.20 | 1.20 | 1.30 | 1.30 | 1.77 | 1.82 |
| 23 | <i>dac</i> | 1.56 | 4.63 | 1.04 | 1.14 | 1.25 | 1.20 | 2.03 | 1.77 |
| 14 | <i>hth</i> | 2.50 | 7.02 | 1.40 | 1.82 | 1.87 | 1.92 | 2.55 | 2.50 |
| 14 | <i>hth</i> | 1.87 | 5.77 | 1.35 | 1.40 | 1.46 | 1.56 | 2.24 | 2.08 |
| 14 | <i>hth</i> | 1.92 | 6.60 | 1.61 | 1.56 | 1.72 | 1.72 | 2.34 | 2.18 |
| 14 | <i>hth</i> | 2.24 | 7.75 | 1.61 | 1.98 | 2.18 | 1.98 | 2.44 | 2.55 |
| 14 | <i>hth</i> | 2.34 | 6.97 | 1.46 | 1.66 | 2.08 | 1.77 | 2.29 | 1.98 |
| 14 | <i>hth</i> | 1.40 | 4.99 | 1.04 | 1.30 | 1.30 | 1.35 | 2.03 | 1.82 |
| 14 | <i>hth</i> | 2.03 | 5.72 | 1.30 | 1.35 | 1.46 | 1.61 | 2.08 | 2.24 |
| 14 | <i>hth</i> | 1.40 | 5.62 | 1.09 | 1.35 | 1.61 | 1.56 | 2.13 | 1.92 |
| 14 | <i>hth</i> | 1.87 | 5.98 | 1.20 | 1.40 | 1.61 | 1.77 | 2.18 | 2.08 |
| 14 | <i>hth</i> | 1.20 | 4.68 | 0.99 | 1.09 | 1.35 | 1.25 | 1.92 | 1.82 |
| 14 | <i>hth</i> | 1.87 | 6.34 | 1.46 | 1.56 | 1.66 | 1.66 | 2.29 | 2.24 |
| 15 | <i>hth</i> | 1.92 | 5.25 | 1.20 | 1.30 | 1.30 | 1.46 | 2.08 | 1.92 |
| 15 | <i>hth</i> | 1.92 | 4.84 | 1.09 | 1.25 | 1.25 | 1.25 | 2.08 | 1.77 |
| 15 | <i>hth</i> | 1.92 | 4.68 | 0.99 | 1.20 | 1.25 | 1.25 | 2.03 | 1.82 |
| 15 | <i>hth</i> | 1.98 | 5.30 | 1.14 | 1.30 | 1.35 | 1.51 | 2.13 | 1.98 |
| 15 | <i>hth</i> | 2.18 | 5.41 | 1.14 | 1.30 | 1.40 | 1.56 | 2.44 | 2.18 |
| 15 | <i>hth</i> | 1.87 | 5.04 | 1.14 | 1.25 | 1.30 | 1.35 | 1.98 | 1.82 |
| 15 | <i>hth</i> | 1.61 | 4.84 | 1.04 | 1.09 | 1.30 | 1.40 | 1.92 | 1.66 |
| 15 | <i>hth</i> | 2.03 | 5.77 | 1.30 | 1.46 | 1.46 | 1.56 | 2.13 | 1.98 |
| 19 | <i>hth</i> | 1.61 | 5.10 | 1.09 | 1.20 | 1.30 | 1.51 | 2.13 | 1.82 |

| Treatment | dsRNA | lr | lb | l1 | l2 | l3 | l4 | od | pw |
|------------------|--------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| 22 | <i>hth</i> | 1.92 | 5.20 | 1.09 | 1.25 | 1.35 | 1.51 | 2.24 | 2.13 |
| 22 | <i>hth</i> | 1.92 | 5.30 | 1.09 | 1.30 | 1.35 | 1.56 | 2.50 | 2.29 |
| 22 | <i>hth</i> | 1.61 | 5.41 | 1.14 | 1.25 | 1.40 | 1.61 | 2.13 | 1.82 |
| 22 | <i>hth</i> | 1.66 | 5.04 | 1.04 | 1.25 | 1.35 | 1.40 | 2.03 | 1.72 |
| 22 | <i>hth</i> | 1.72 | 5.15 | 1.30 | 1.25 | 1.30 | 1.30 | 2.08 | 1.82 |
| 22 | <i>hth</i> | 1.66 | 5.25 | 1.20 | 1.25 | 1.35 | 1.46 | 2.08 | 1.82 |
| 22 | <i>hth</i> | 1.30 | 4.42 | 1.04 | 1.30 | 1.04 | 1.04 | 1.98 | 1.56 |
| 22 | <i>hth</i> | 1.66 | 4.84 | 1.25 | 0.94 | 1.40 | 1.25 | 2.08 | 1.56 |
| 22 | <i>hth</i> | 1.40 | 6.08 | 1.40 | 1.56 | 1.56 | 1.56 | 2.18 | 1.56 |
| 22 | <i>hth</i> | 1.04 | 4.68 | 1.14 | 1.46 | 0.88 | 1.20 | 1.82 | 1.30 |
| 22 | <i>hth</i> | 1.20 | 5.20 | 1.30 | 1.30 | 1.30 | 1.30 | 1.92 | 1.72 |
| 22 | <i>hth</i> | 1.04 | 5.20 | 1.40 | 1.30 | 1.46 | 1.04 | 2.18 | 1.66 |
| 22 | <i>hth</i> | 0.88 | 3.95 | 0.78 | | | 1.30 | 2.24 | |

Individuals injected with *Dll*, *dac*, and *hth* showed visible phenotypic abnormalities in comparison to the control GFP dsRNA specimens. The control individuals appeared normal in comparison to unmanipulated specimens, with no obvious qualitative or quantitative changes to the mouthparts, antennae or legs (Fig. 3A). The labium remained straight, with all four segments present while the labrum and stylets lay flat on top.

In comparison to those individuals injected with GFP, 14 out of 31 individuals injected with *Dll* dsRNA had stylets present, though visibly abnormal, curving away in various degrees from the beak instead of lying flat along the labium. The labrum was also deformed, either shortened by 33% or curved, not lying flat on the labium (Fig. 3B). Eight of the 29 injected with *dac* dsRNA also had deformed stylets and labrum. Two individuals of the 29 scored also had a fusion of the second and third segment of the labium (Fig. 3C). This fusion could represent that *dac* is located upstream of genes which control segmentation. Further this could be showing the need for this gene for both allometry and patterning, even at this late stage of development. Individuals injected

with *hth* dsRNA have also been observed with curved stylets (9 out of 32) (Fig. 3D). No other drastic phenotypes were observed.

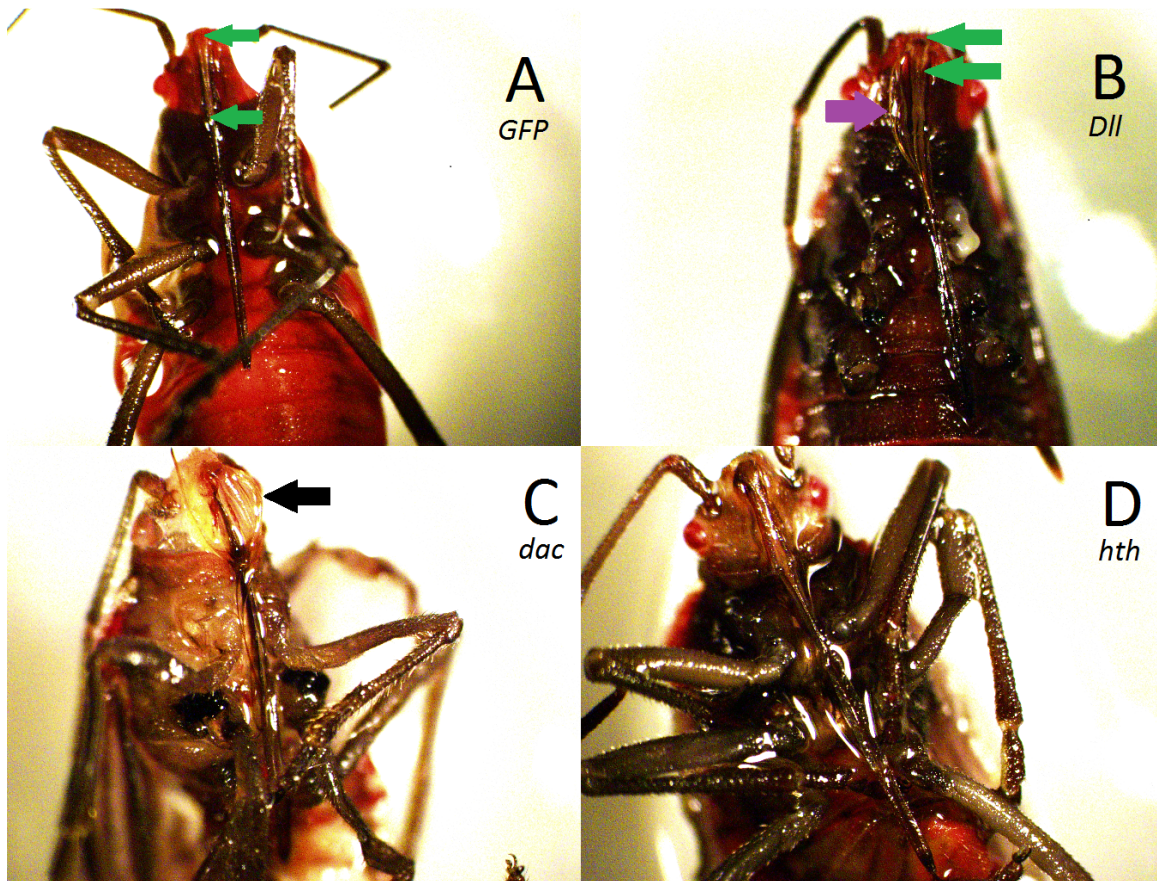


Figure 3: *J. haematoloma* adult after injection at instar L5 of GFP (A), injection of *Dll* (B), injection of *dac* (C), and injection of *hth* (D). Green arrows show decrease in labrum length between control and *Dll*. Purple/Black arrows show deformed stylets.

Parental RNAi was also performed on females from the Washington, DC population. For each gene, 2 females were injected and placed in a cage with a male from the same population. Only the *Dll* dsRNA injected females produced eggs, which were collected daily. Four eggs hatched, with all hatchlings lacking the distal regions of

appendages when compared to a wild type hatchling (Figure 4A,B). Significantly, this effect included a truncated labium (Figure 4C,D).

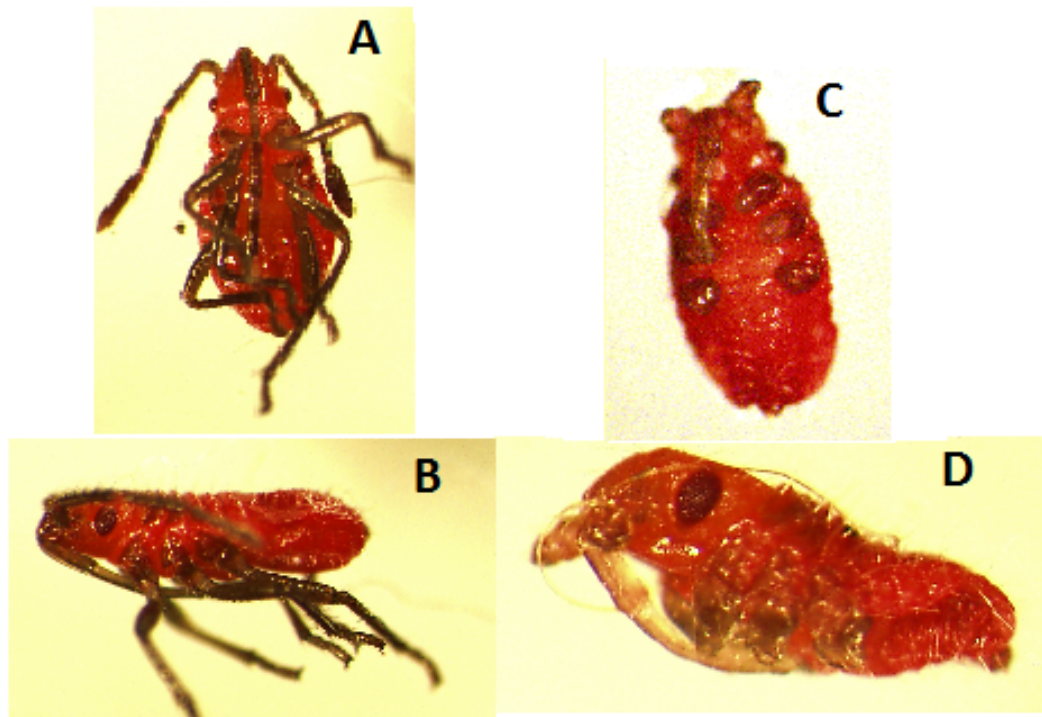


Figure 4: *J. haematoloma* hatchling from a *Dll* dsRNA injected female (C,D) in comparison to wild type hatchling (A,B). Note the distinct lack of distal appendage structures and the labium consists of only 3 segments.

Allometric Results of RNA interference

Measurements of specimens taken with an ocular micrometer were analyzed with the statistical program R (Ihaka and Gentleman). Significance indicates a difference between the measurements of the gene treatment were different from one another for a reason other than chance alone. ANOVA was used to determine overall treatment effect and was followed up with a Tukey's HSD test to look at specific gene pair comparisons

between treatment groups (GFP, *Dll*, *dac*, and *hth*) for differences in labrum length, labium segment 1, 2, 3 and 4, total labium length, ocular distance, and pronotum width. Values were also normalized for the two indicators of body size, pronotum width and ocular distance and analyzed with ANOVA. The labrum was significantly reduced as a result of *Dll* RNAi (ANOVA $p=0.0023$; Tukey's HSD for *Dll*-GFP $p=0.0017$). Labium segment 4 (most distal from the head) was significantly shortened as a result of *dac* RNAi (ANOVA $p=0.0231$; Tukey's HSD for *dac*-GFP $p=0.0143$). Labium segment 3 did not appear to be significantly affected by leg patterning gene knock down (ANOVA $p=0.0572$) however there was a significant difference in labium segment 3 length (Tukey's HSD *dac*-GFP $p=0.0143$). Figures 5-12 show the ANOVA boxplots for the uncorrected measurements in millimeters, significant Tukey's HSD are shown in cornsilk. Figures 13-18 show the ANOVA results with measurements (mm) normalized to pronotum width. Figures 19-24 show the ANOVA results for measurements (mm) normalized to ocular distance.

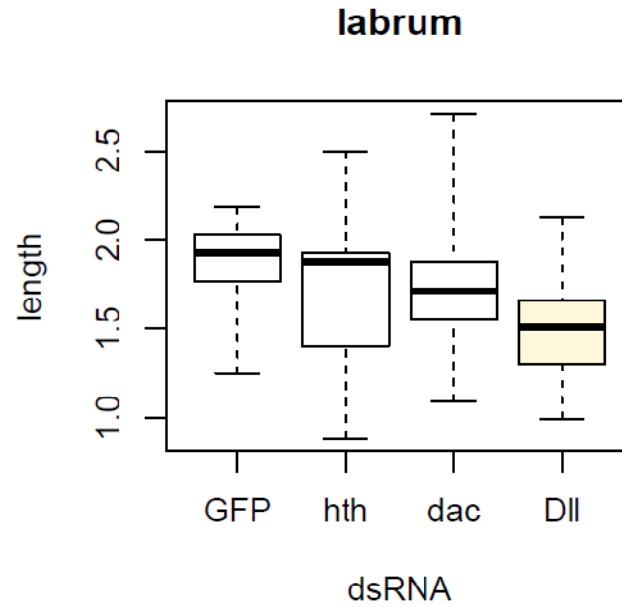


Figure 5: ANOVA results for uncorrected labrum length between treatment groups. *Dll* was found by Tukey's HSD to be significantly different from the control (GFP) and is represented by the color cornsilk.

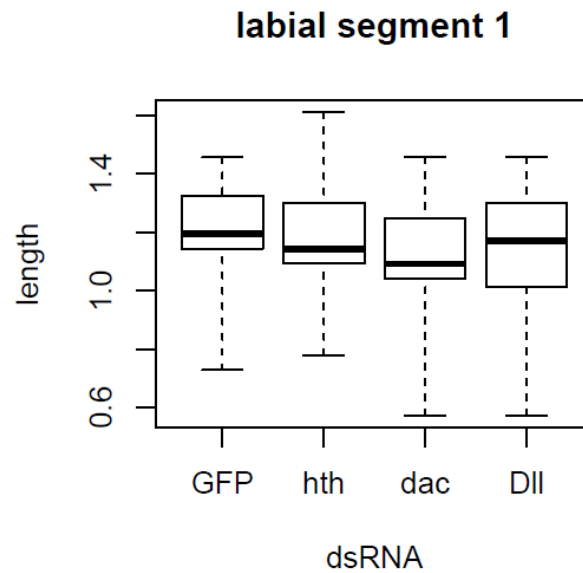


Figure 6: ANOVA results for uncorrected labial segment 1 length between treatment groups.

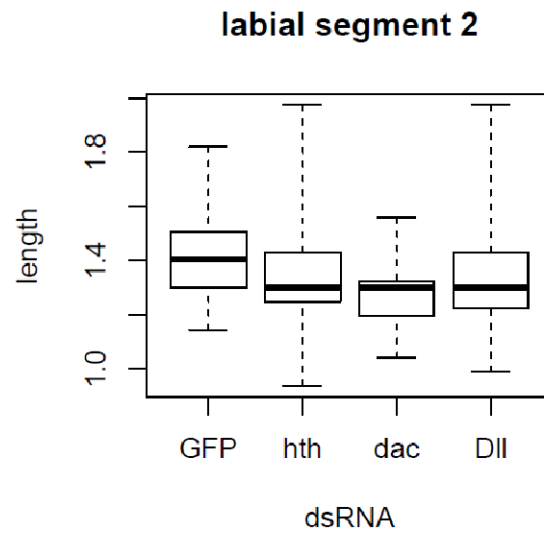


Figure 7: ANOVA results for uncorrected labial segment 2 length between treatment groups.

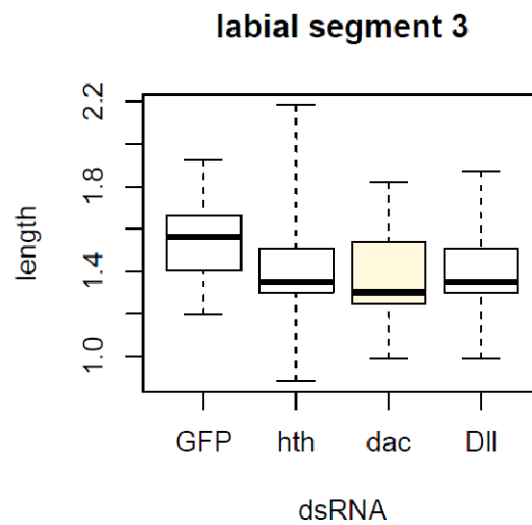


Figure 8: ANOVA results for uncorrected labial segment 3 length between treatment groups. *dac* was found by Tukey's HSD to be significantly different from the control (GFP) and is represented by the color cornsilk.

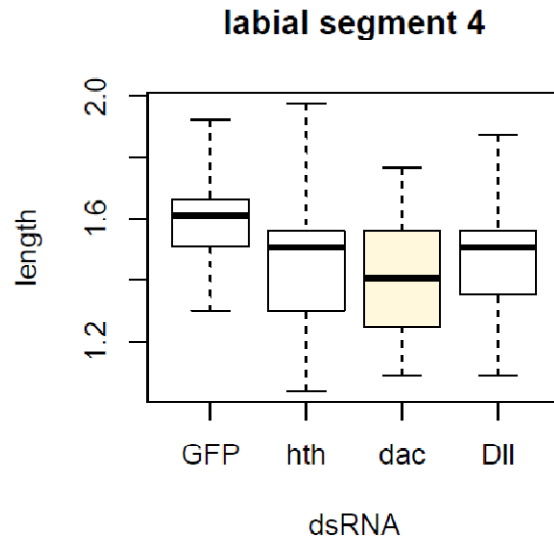


Figure 9: ANOVA results for uncorrected labial segment 4 length between treatment groups. *dac* was found by Tukey's HSD to be significantly different from the control (GFP) and is represented by the color cornsilk.

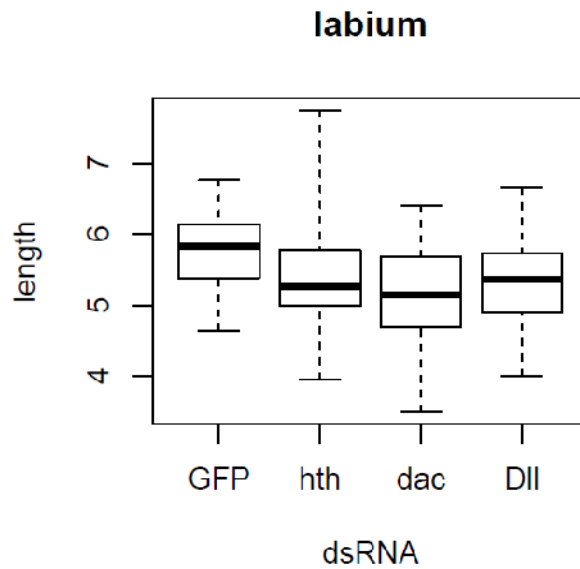


Figure 10: ANOVA results for uncorrected total labium length between treatment groups.

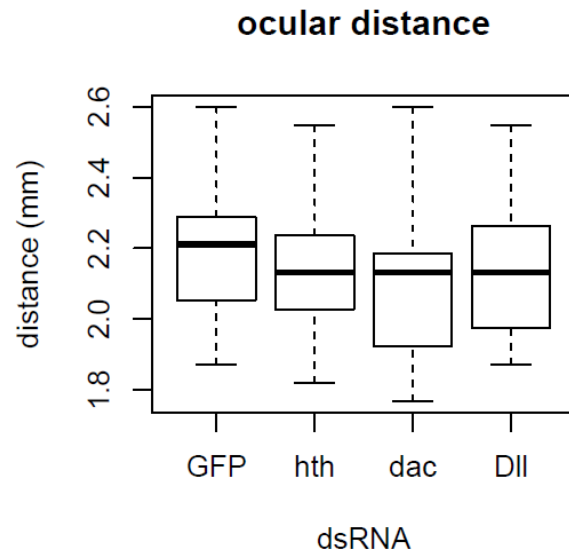


Figure 11: ANOVA results for uncorrected ocular distance between treatment groups.

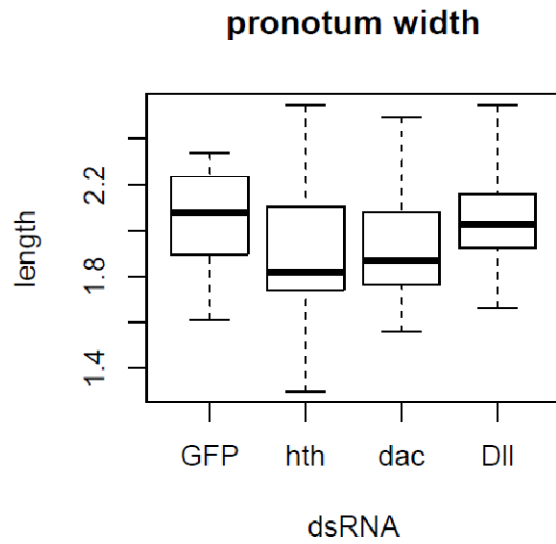


Figure 12: ANOVA results for uncorrected pronotum width between treatment groups.

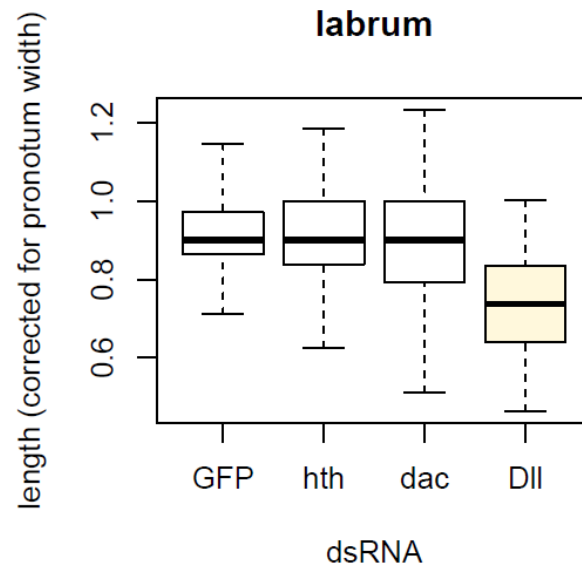


Figure 13: ANOVA results for labrum normalized to pronotum width between treatment groups. *Dll* was found by Tukey's HSD to be significantly different from the control (GFP) and is represented by the color cornsilk.

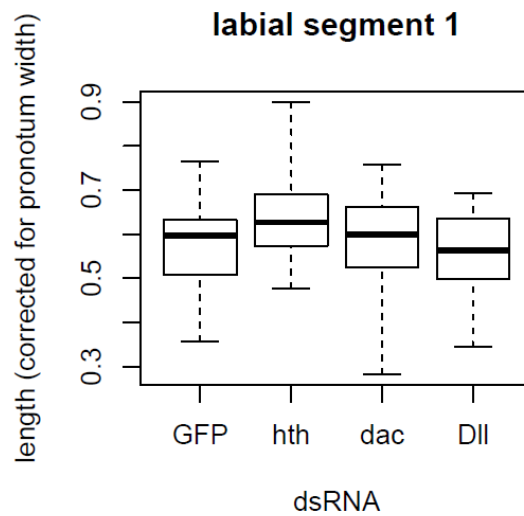


Figure 14: ANOVA results for labial segment 1 normalized to pronotum width between treatment groups.

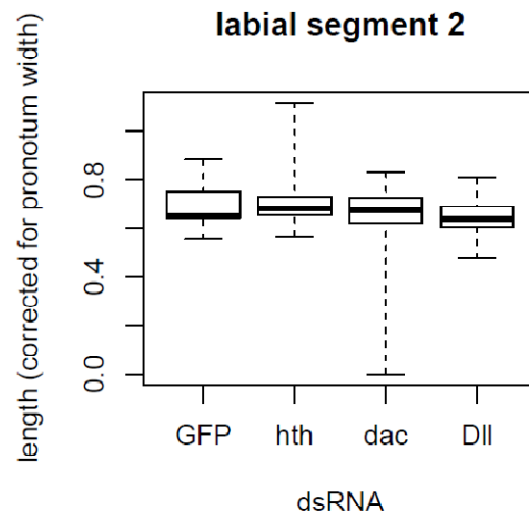


Figure 15: ANOVA results for labial segment 2 normalized to pronotum width between treatment groups.

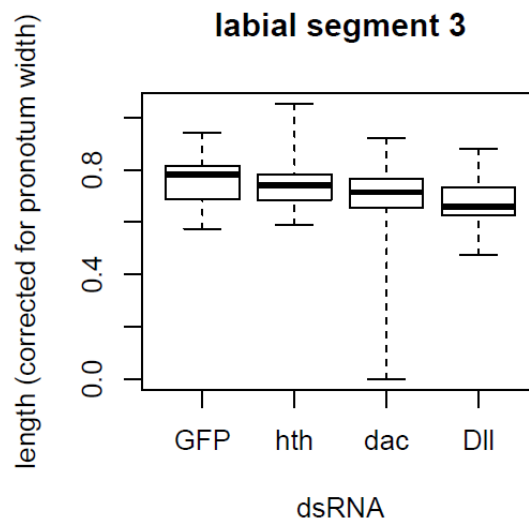


Figure 16: ANOVA results for labial segment 3 normalized to pronotum width between treatment groups.

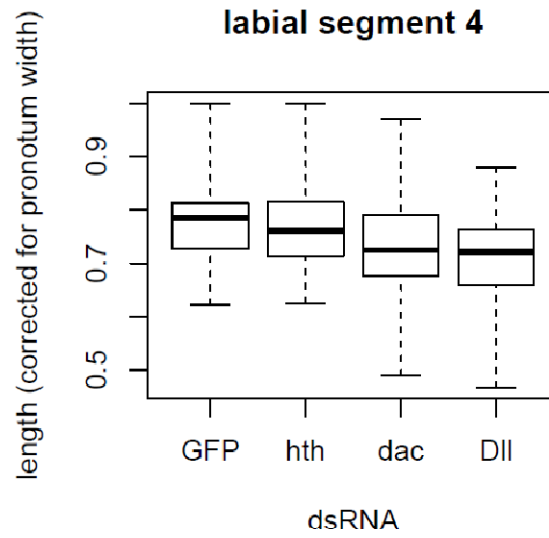


Figure 17: ANOVA results for labial segment 4 normalized to pronotum width between treatment groups.

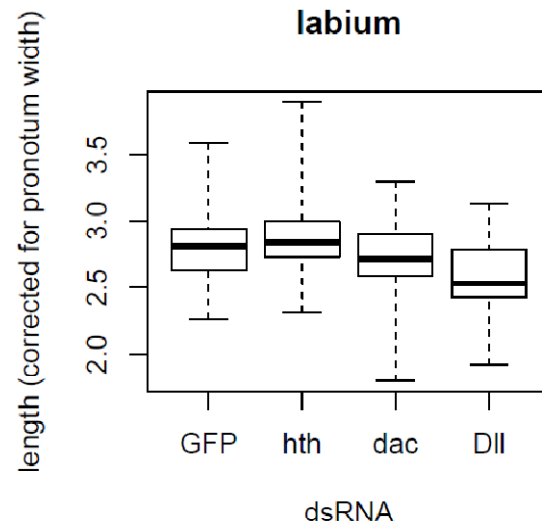


Figure 18: ANOVA results for total labium length normalized to pronotum width between treatment groups.

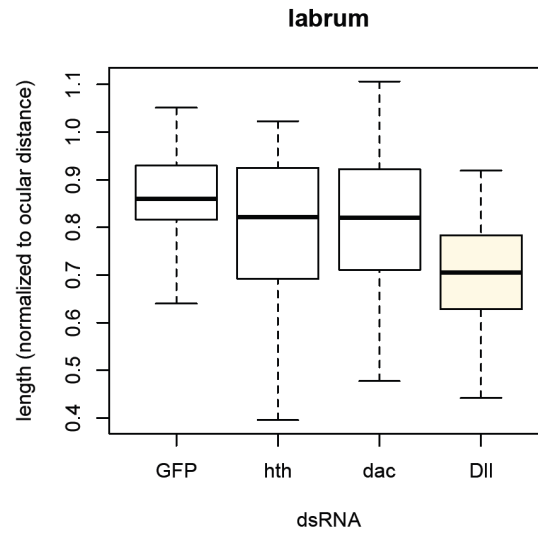


Figure 19: ANOVA results for labrum normalized to ocular distance between treatment groups. *Dll* was found by Tukey's HSD to be significantly different from the control (GFP) and is represented by the color cornsilk.

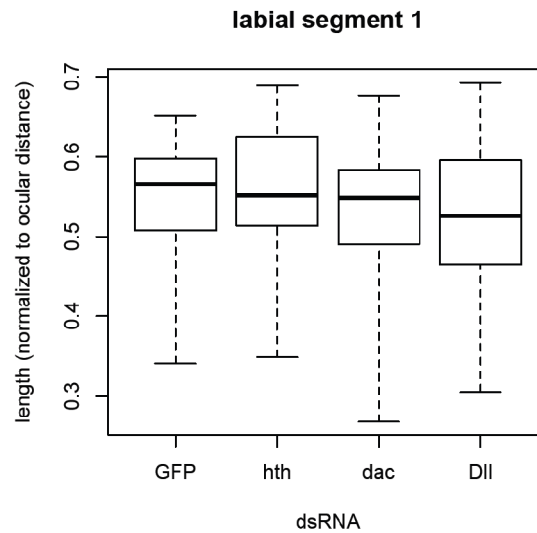


Figure 20: ANOVA results for labial segment 1 normalized to ocular distance between treatment groups.

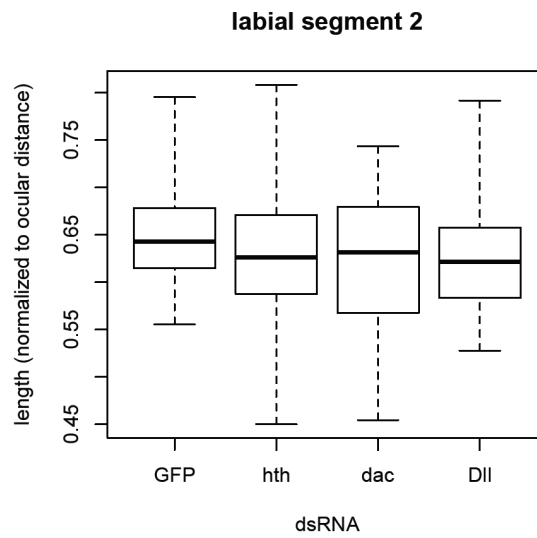


Figure 21: ANOVA results for labial segment 2 normalized to ocular distance between treatment groups.

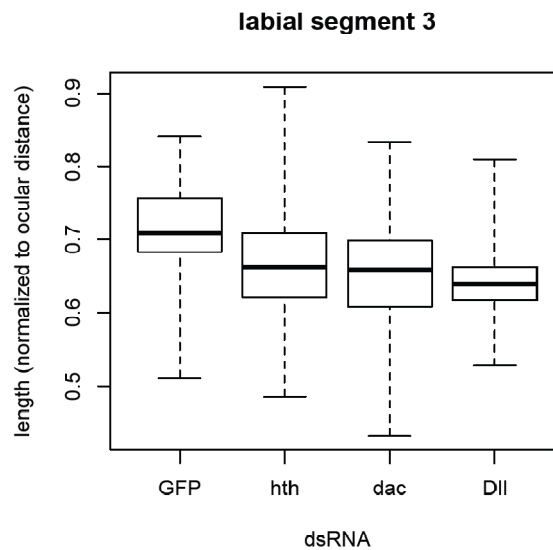


Figure 22: ANOVA results for labial segment 3 normalized to ocular distance between treatment groups.

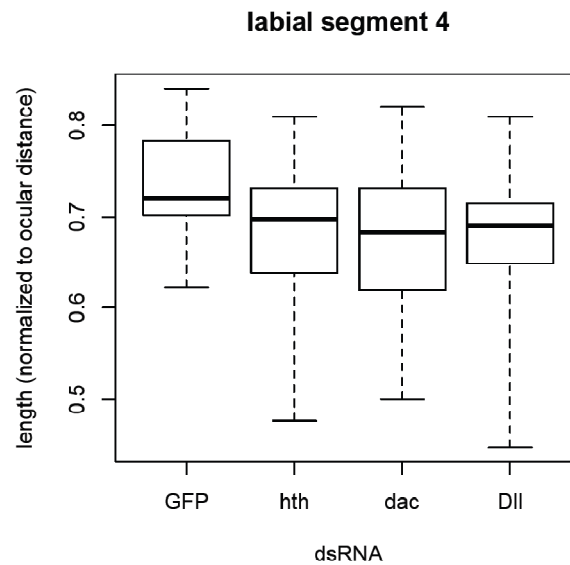


Figure 23: ANOVA results for labial segment 4 normalized to ocular distance between treatment groups.

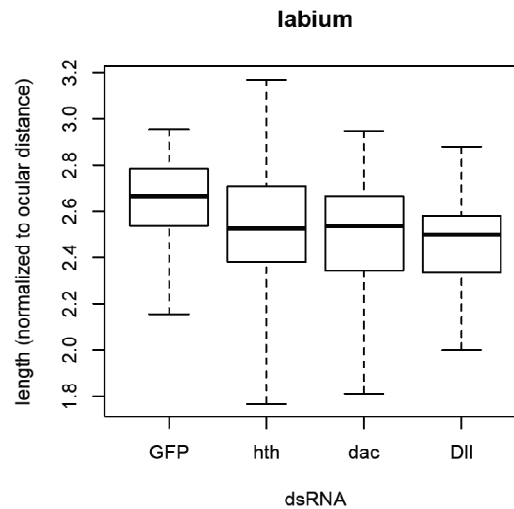


Figure 24: ANOVA results for total labium length (mm) normalized to ocular distance between treatment groups.

Utilizing a Pearson's product-moment correlation I tested for correlation between the anatomical portions of the mouthparts. This test allows us to look for an allometric

relationship between the individual parts. It was found that all comparisons were significantly correlation ($p < 0.05$), in particular the labrum and labium ($p = 2.10 \times 10^{-8}$). ANOVA was also used to determine if there were any significant differences between treatments groups for the same gene. As injections were performed on different dates, each group of injections received a treatment number. Significant differences were found between groups of the same gene and are reported in bold in Table 4.

Table 4

ANOVA Between Treatment Groups of each Gene

| Gene | Number of treatments | lr | l1 | l2 | l3 | l4 | lb | od | pw |
|------------|----------------------|---------------|--------|---------------|---------------|---------------|---------------|--------|---------------|
| GFP | 2 | 0.1572 | 0.2010 | 0.9752 | 0.1552 | 0.9824 | 0.9514 | 0.1647 | 0.1505 |
| <i>Dll</i> | 4 | 0.0056 | 0.6434 | 0.0392 | 0.2323 | 0.8464 | 0.5609 | 0.4202 | 0.1556 |
| <i>dac</i> | 3 | 0.7397 | 0.8868 | 0.3294 | 0.5105 | 0.0472 | 0.2919 | 0.0700 | 0.1829 |
| <i>hth</i> | 4 | 0.0010 | 0.1873 | 0.0621 | 0.0100 | 0.0044 | 0.0079 | 0.2586 | 0.0019 |

RNAi Validation

Validation of RNAi was done with rt-PCR measurement of target gene expression and differences were analyzed in R. Percent expression in comparison to the GFP control specimens are reported in Table 5. The diagonal of the table shows the percent knockdown for the targeted gene (*hth*: 54.4%, *dac*: 71.3%, *Dll*: 78.5%) when compared to the control (GFP). The rest of Table 3 shows each gene knockdown compared to the other 3 gene knockdowns for analysis of expression level differences.

Comparing expression levels in different RNAi backgrounds also allowed the analysis of indirect genetic interactions. Three of these interactions were found to be significant (Table 5). In *Dll* knockdowns *dac* expression increased (178.2%)

demonstrating that *Dll* inhibits *dac*. Also when *dac* was knockdown *hth* expression increased (134.4%) as well as *Dll* (167%) showed that *dac* inhibits these two genes. Figure 25 shows these gene interactions and also those found in the *Drosophila* leg (from (Abu-Shaar and Mann, 1998)).

Table 5

RNAi Validation of Gene Knockdown and Interactions Between Genes

| | <i>hth</i> expression | | | <i>dac</i> expression | | | <i>Dll</i> expression | | |
|------------|-----------------------|--------|---------------|-----------------------|--------|---------------|-----------------------|--------|---------------|
| RNAi | mean (%GFP) | RDSE | p | mean (%GFP) | RDSE | p | mean (%GFP) | RDSE | p |
| <i>hth</i> | 53.5% | ±25.0% | 0.0014 | 150.9% | ±44.4% | 0.3431 | 117.8% | ±39.6% | 0.2181 |
| <i>dac</i> | 134.4% | ±31.4% | 0.0085 | 71.3% | ±21.3% | 0.0161 | 167.0% | ±46.9% | 0.0012 |
| <i>Dll</i> | 100.9% | ±22.8% | 0.9250 | 178.2% | ±47.0% | 0.0240 | 78.5% | ±15.2% | 0.0707 |

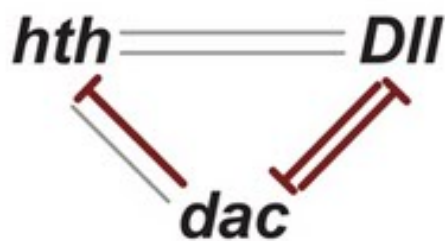
J. haematoloma beak*D. melanogaster* leg

Figure 25: Gene interactions for the *Jadara* beak and the *Drosophila* leg. Significant negative interactions are drawn in red, insignificant or inconclusive in grey. No positive interactions were detected.

Discussion

Despite *Jadera haematoloma* not being studied at a molecular level previously, this study successfully cloned several genes and utilized RNAi to determine developmental functions. Use of the candidate gene approach allowed us to test whether several candidate genes contribute to patterning of the mouthparts in *J. haematoloma*. The three genes examined at in this study, *Distal-less*, *dachshund*, and *homothorax*, offered valuable insight into the patterning of the beak in this particular species. Phenotypic differences observed between the control and knock down treatments (Figure 3:A-D) demonstrate that these genes are necessary for the proper development of the mouthparts. Due to their allometric influence during late juvenile development, these genes are potential targets for selection to act upon during the evolution of beak length.

Patterning of the Mouthparts

Despite all three knockdown treatments showing phenotypic deformities, ANOVA and Tukey HSD identified only a few significant allometric differences between the control (GFP) and treatment groups. Also ANOVA showed a significant difference between treatment groups, particularly for *hth* indicating that there may be population level differences of these genes, which should be explored further in the future.

A significant difference was determined between the groups for the length of the labrum, specifically between GFP and *Dll*. This illustrates *Dll*'s importance to the development of length in this portion of the mouthparts and is expected due to its known role for distal appendage development. In *Oncopeltus fasciatus* *Dll* knockdown in embryos also had a reduced labrum. Further the labium was reduced overall, which was not seen in *Jadera*. A discrepancy however is that this study focused upon juvenile RNAi, whereas Angelini

and Kaufman (2004) based their results on maternal RNAi and could contribute to differences seen based on variation in gene expression during different stages of development. In *O. fasciatus* juvenile RNAi resulted in a reduced labium in the instar following injection, with the reduction of length appearing to be uniform and not restricted to the distal segment. This suggests that *Dll* has a role in regulating adult beak length (Angelini and Kaufman, 2005). The *J. haematoloma* hatchlings from a *Dll* knockdown female were observed missing their appendages and with only 3 segments to the labium. This is consistent with the truncation observed in *O. fasciatus* *Dll* depleted hatchlings (Angelini and Kaufman, 2004) showing conservation in gene function between these species. Further studies may explore the differences between knockdowns occurring at the various stages, including a more extensive study using maternal RNAi so better comparisons can be made.

Despite major anatomical differences in the mouthparts between heteropterans and other arthropods, comparisons can still be made to look at gene function and patterning. A study by Simonnet & Moczek (2011) on *Onthophagus* beetles represent a look into the role of the three major leg gap genes in what is considered to be the ancestral form of mouthparts for arthropods. This study found that *Dll* RNAi greatly reduced overall labrum size. A study in *Tribolium castaneum*, the red flour beetle, represents another mandibulate insect with robust mandibles. It was found that *Dll* truncated the palps, although these structures are not found in the modified true bug mouthparts it shows conservation in function (Angelini et al., in review). *Drosophila melanogaster* mouthparts are modified from the ancestral form, having reduced mandibular and maxillary appendages, with the labium's function as a sponging

proboscis (i.e. labium). It has been seen that *Dll* mutants lack portions of the proboscis and maxillary structures (Abzhanov et al., 2001).

Labium segments were measured individually and each was analyzed separately. Significance was found for the fourth or most distal segment from the head, which can be attributed to *dac*, which was also significant in the 3rd segment. This provides insight as to where in the labium *dac* could be expressed, as in-situ hybridization in *Oncopeltus* embryos showed expression as being restricted to the more proximal segment of the labium (Angelini and Kaufman, 2005). Although these two species have similar mouthpart morphology, it is possible there are differences in expression locations of these three genes. This could also show a difference in timing of the gene between the different juvenile stages. It was found that *O. fasciatus dac* RNAi had no effect on the labrum or labium (Angelini and Kaufman, 2004) which is in contrast with the current *Jadera* findings. In *Onthophagus* beetles they found that *dac* RNAi resulted in loss of the elongation of the mandible. The authors hypothesize that this gene may have played a role in the evolution from a short mandible utilized for chewing into the elongated flat mandible of modern filter feeding beetles (Simonnet and Moczek, 2011). This could represent another role of *dac* in allometry of the mouthparts. In the red flour beetle knockdown of *dac* resulted in reduced length of palps and fusion of segments, indicating its requirement for the proper development of the intermediate portion of the palps (Angelini et al, in revision). *D. melanogaster* does not express *dac* in the maxillary or labial primordia (Abzhanov et al., 2001). Future studies should look at the specific location of expression of these genes at each stage in various species for comparison.

Despite *O. fasciatus hth* knockdowns having drastic results including the labium being transformed distally to legs and the labrum being reduced or absent, this was not found in *Jadera*. None of the measurements were found to be significant and phenotypic abnormalities observed were of the stylets not lying flat along the labium. The fruit fly also showed a more drastic result of a partial proboscis to leg transformation (Inbal et al., 2001). In the cricket *Gryllus bimaculatus hth* RNAi resulted in the proximal portion of the mouthparts transforming to a more antennal identity and the distal portion transforming into leg identity (Ronco et al., 2008). This shows the diversity across mouthpart morphology and each gene's role particularly between embryos and juveniles.

Modularity of Mouthparts

The Pearsons product moment correlation result shows the high correlation between the specific portions of the beak, particularly between the labrum and labium. This high correlation demonstrates that as the length of the labium fluctuates, so does the length of the labrum. These results could potentially start the basis for a case for modularity. Further it makes sense as it is suspected that the soapberry bug is under selection on the functional morphology of the mouthparts. This is also supported as the labrum and labium were effected (or not) by the same genes. As genes are interacting to develop the mouthparts, a study looking specifically at the larger gene network responsible for developing the mouthparts could provide insight as to what selection is acting upon.

Interactions

Validation of the RNAi with qPCR was successful and showed a significant knockdown in the expression of the target gene. The interactions showed *Dll* having an

inhibitory effect on *dac* and *dac* having an inhibitory effect on *Dll* and *hth* in the mouthparts. This is consistent with gene interactions found in *Drosophila* legs. Despite this comparison being made between two different anatomical features, they are still appendages and most likely utilizing the same genes for patterning and allometry. However, further studies should tease apart each of the appendages, including the antennae, legs, and genitalia to look at variations of these gene interactions.

Evolutionary Implications

These results are convincing for the role of these specific genes in patterning the mouthparts in *J. haematoloma*, however further analysis is needed to understand the extent of that role. This study does prove that techniques utilized on other systems can also be used on this species and therefore further candidate gene studies can be performed. This is particularly exciting as no previous studies have worked with this organism at a molecular level. The ability to knockdown specific genes utilizing RNA interference is a huge advantage in continuing to look at the genetic basis for the evolution of the mouthparts. Despite the disadvantage of not having the genome sequence for *J. haematoloma* it is possible to design primers for PCR as shown in this study. Analysis of these three leg gap genes has given us the beginning of an answer and is a point where other studies can springboard from. Interactions of the genes can play a role and can be easily explored with qPCR. Other genes known to be involved in appendage development such as *Deformed (Dfd)*, *proboscipedia (pb)* and *Sex combs reduced (Scr)* may be of interest to determine function (Hughes and Kaufman, 2000). A broader analysis of candidate genes can provide a more complete look at the development of *J.*

haematoloma mouthparts. As there are potentially thousands of genes in the genome, three genes only represents a small percentage of what could be selected upon to result in such rapid evolution. It is demonstrated that these three genes contribute to controlling the mouthpart phenotype and could provide the variation between individuals in the wild on which selection is acting.

APPENDIX A: PRIMER SEQUENCES

Primers for degenerate PCR

| gene | primer | aminoacid sequence | DnAoligosequence | length |
|---------------------------------|-------------|-----------------------|---------------------------|--------|
| <i>Distal-less</i> | Jh'DII-f1 | YPFRPMHQ | TAyCCnTTymGGCCnATGCAYCA | 23 |
| | Jh'DII-r1 | KKMMKAAQ | TGrGCrGCyTTCATCATyTTyTT | 23 |
| | Jh'DII-f2 | GKGKKMRK | GGnAArGGnAArAArATGmGGAA | 23 |
| | Jh'DII-r2 | KIWFQNR | CkCCkrTTyTGrAACCAAdATyTT | 23 |
| <i>dachshund</i> | Jh'dac-f1 | CLPQAFEL | TGCCTbCCnCArGCyTTCGAnCT | 23 |
| | Jh'dac-r1 | EKAELKMD | TCCATyTTnAGyTCrGCyTTyTC | 23 |
| | Jh'dac-f2 | LVCNVEQV | CTnGTyTGCAAyGTYGArCArGT | 23 |
| | Jh'dac-r2 | AADNARQQ | TGyTGCCkyGCrTTrTCnGCnGC | 23 |
| <i>homothorax</i> | Jh'htb-f11 | FNEDIA (M/V) | TTyAAyGArGAYAThGCnrT | 20 |
| | Jh'htb-r11 | QVNNWF IN | TTdATrAACCArTTrTTnACyTG | 23 |
| | Jh'htb-f12 | QAIQVLR | CArGCnATmCArGTnCTbmGGTT | 23 |
| | Jh'htb-r12a | QKKQLAQ | TGnGCnAryTGyTTyTTyT | 19 |
| | Jh'htb-r12b | KGKMPIDL | ArrTCdATnGGCATyTTnCCyTT | 23 |
| | Jh'da-f1 | LDDAINV | TNGAYGAYGCNATHAAYGT | 19 |
| <i>daughterless</i> | Jh'da-r1 | EEEKAED | TCYTCNGCYTTYTCYTCYTC | 20 |
| | Jh'da-f2 | KKRKEPPD | AARAARMGNAARGARCCNCCNGA | 23 |
| | Jh'da-r2 | NPKAACL | ARRCANGCNGCYTTNGGR | 20 |
| | Jh'da-f2 | NPKAACL | ARRCANGCNGCYTTNGGR | 20 |
| <i>rps18</i> | d-rps18-f | IPEKFQHI | AThCCnGArAArTTyCArCAyAT | 23 |
| | d-rps18-r | GQHTKT | CCnGTnGTyTTnGTrTGyTGnCC | 23 |
| <i>syntaxin1</i> | d-syx1-f1 | MIDKIQAN | ATGAThGAYAArAThCArGCnAA | 23 |
| | d-syx1-r1 | KKALKYQS | GAYTGrTAYTTnArnGCyTTyTT | 23 |
| | d-syx1-f2 | VEEVKKKH | GTnGArGArGTnAArAArAArCA | 23 |
| | d-syx1-r2 | EHAVDYVQ | TGnACrTArTCnACnGCrTGyTC | 23 |
| <i>β-actin</i> | d-actb-f1 | MCD (D/E) EVAA | ATGTGyGAYGAnGArGTnGCnGC | 23 |
| | d-actb-r1 | KIKIIAPP | GGnGGnGCdATdATyTTdATyTT | 23 |
| | d-actb-f2 | GMCKAGFA | GGnATGTGyAArGCnGGnTTyGC | 23 |
| | d-actb-r2 | MQKEITAL | ArnGCnGTdATyTCyTTyTGCAT | 23 |
| <i>18S rRNA</i> | Of'18S-f1 | | ATGTCCTGTCGGTGGCGGATAG | 22 |
| | Of'18S-r1 | | AACCAACAAAATAGAACCAAGGTCC | 25 |
| | Of'18S-f2 | | ATAAACGATGCCAGCCAGCGAT | 22 |
| | Of'18S-r2 | | CTGTCAATCCTTCCAATGTCCG | 22 |

Realtime PCR Primers

| gene | primer name | DNA oligo sequence | dir | length |
|---------------------------------|--------------|-------------------------|-----|--------|
| <i>Dll</i> | Jh'Dll-qf1 | AGGTTCCAGAGGACGCAGTA | F | 20 |
| | Jh'Dll-qr1 | TGTCTGAGTGAGTCCAAGGG | R | 20 |
| | Jh'Dll-qf2 | CCTCGCACCATTCTACTCAAG | F | 20 |
| | Jh'Dll-qr2 | AAGGTACTGCGTCCTCTGGA | R | 20 |
| <i>dac</i> | Jh'dac-qf1 | GCCAATGGTTACAATCACCC | F | 20 |
| | Jh'dac-qr1 | CGGATTAAGGATGGCTGTGT | R | 20 |
| | Jh'dac-qf2 | AGTCTAACTGCGAAGCGAGC | F | 20 |
| | Jh'dac-qr2 | TTCTTGGTCAGATTCGGGAC | R | 20 |
| <i>hth</i> | Jh'hth-qf1 | AGGAAAGTGGTGTGGACGAC | F | 20 |
| | Jh'hth-qr1 | CCCAGGAACACGGAAGAGTA | R | 20 |
| | Jh'hth-qf2 | TAGCTGCCTGAAAGGGAAGA | F | 20 |
| | Jh'hth-qr2 | GCGTTTGATCTTCCATCGTT | R | 20 |
| <i>sals</i> | Jh'sals-qf1 | CAGCATCTGTAGCAGACGTAGTG | F | 23 |
| | Jh'sals-qr1 | ATCATCACGGACCTTGTCTATGT | R | 23 |
| <i>rps18</i> | Jh'rps18-qf1 | CAAAGGTGTTGGTAGGAGGTATG | F | 23 |
| | Jh'rps18-qr1 | CTCTTCTTCAGAGCATTCACCAG | R | 23 |
| <i>β-actin</i> | Jh'actb-qf1 | CTAACTGAGCGTGGTTACAGCTT | F | 23 |
| | Jh'actb-qr1 | AAGTTCATAGGACTTCTCGAGGG | R | 23 |
| <i>syx1</i> | Jh'syx1-qf1 | ACTACCGAGAAAGGTGTAAAGGG | F | 23 |
| | Jh'syx1-qr1 | GACAGCTGGATTTCCCTTGTCTA | R | 23 |
| <i>18S</i> | Jh'18S-qf1 | CGATAACGAACGAGACTCTAACC | F | 23 |
| | Jh'18S-qr1 | AGACCTGTTATTGCTCAATCTCG | R | 23 |

T7-appended primers for dsRNA synthesis

| gene | primer name | DNA oligo sequence | dir | length |
|------------|-------------|--|-----|--------|
| | T7 | taatacgactcactataggg | - | 20 |
| | T3 | ATTAACCCTCACTAAAGGGA | - | 20 |
| | M13F-40 | GTTTTCCAGTCACGAC | | 17 |
| | M13F-21 | TGTAAAACGACGGCCAG | | 17 |
| | M13R | CAGGAAACAGCTATGAC | | 17 |
| <i>GFP</i> | T7-GFP-f1 | taatacgactcactataggg GCTGTTACCGGGGTGGTGC | F | 40 |

| | | | | | |
|------------|--------------|----------------------|-----------------------|---|----|
| | T7-GFP-r1 | taatacgactcactataggg | GCGGACTGGGTGCTCAGGTA | R | 40 |
| <i>Dll</i> | T7-Jh'Dll-f1 | taatacgactcactataggg | ACCTCGCACCATTTACTCAA | F | 40 |
| | T7-Jh'Dll-r1 | taatacgactcactataggg | CACCTGTGTCTGAGTGAGTC | R | 40 |
| <i>dac</i> | T7-Jh'dac-f1 | taatacgactcactataggg | CAGCTGAACCACCCTGGCTC | F | 40 |
| | T7-Jh'dac-r1 | taatacgactcactataggg | AGCGCCAACGCTCGCTCACT | R | 40 |
| | T7-Jh'dac-f2 | taatacgactcactataggg | CTGACCAAGAAGACACTTCA | F | 40 |
| | T7-Jh'dac-r2 | taatacgactcactataggg | TACTTTTCAGGAGACCCTGGA | R | 40 |
| <i>hth</i> | T7-Jh'hth-f1 | taatacgactcactataggg | ACTTCTGCCACCGCTACATT | F | 40 |
| | T7-Jh'hth-r1 | taatacgactcactataggg | CACATCGGGGGTACTAGCCC | R | 40 |
| | T7-Jh'hth-f2 | taatacgactcactataggg | GCAGGAAAAGTGGTGTGGACG | F | 40 |
| | T7-Jh'hth-r2 | taatacgactcactataggg | GATTCCACGTTTTTTCTGGT | R | 40 |

APPENDIX B: JADERA HAEMATOLOMA DNA SEQUENCES

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LOCUS      TBA                               173 bp ss-DNA      linear      SYN
04-Jun-2010
DEFINITION Jadera haematoloma Distal-less partial CDS
ACCESSION  -
KEYWORDS   -
SOURCE     Jadera haematoloma (red-shouldered soapberry bug)
           ORGANISM Jadera haematoloma (Davis GRT population)
               Eukaryota; Metazoa; Arthropoda; Hexapoda; Insecta;
Pterygota;
           Neoptera; Paraneoptera; Hemiptera; Euhemiptera;
Heteroptera;
           Panheteroptera; Pentatomomorpha; Coreoidea; Rhopalidae;
           Jadera.
REFERENCE  TBA
AUTHORS    Stacey L. Baker, David R. Angelini
COMMENT     Sequence of clone Jh'D11-1A
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ACTGCAGCAG
     61 CTCAACAGGA GGTTCAGAG GACGCAGTAC CTTGCGCTTC CCGAGCGGGC
AGAGCTTGCC
    121 GCCTCCCTTG GACTCACTCA GACACAGGTG AAAATCTGGT TCCAAAACCG GCG
//LOCUS      TBA                               923 bp ss-DNA      linear
SYN 04-Jun-2010
DEFINITION Jadera haematoloma dachshund partial CDS
ACCESSION  -
KEYWORDS   -
SOURCE     Jadera haematoloma (red-shouldered soapberry bug)

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ORGANISM  Jadera haematoloma (Davis GRT population)
          Eukaryota; Metazoa; Arthropoda; Hexapoda; Insecta;
Pterygota;
          Neoptera; Paraneoptera; Hemiptera; Euhemiptera;
Heteroptera;
          Panheteroptera; Pentatomomorpha; Coreoidea; Rhopalidae;
          Jadera.
REFERENCE TBA
AUTHORS   Stacey L. Baker, David R. Angelini
COMMENT   Consensus sequence of clones Jh'dac-1A and 1F
FEATURES  Location/Qualifiers
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TCTAGCCAAT
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GAACCACCTT
361 GGCTCCGGCC ACACAGCCAT CCTTAATCCG CAACTTCAAC ATCACCTAAT
CAAACCACCT
421 CCACCCATGG ACGCRCTCTC AAGATCTGGC ATTTGGGAAA ATTGCAGAGC
TGCCTATGAG
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GCGAGCGTTG
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GAACCAGARC
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AGGTGGTACC
661 GGCCCTGAAG ATGAGGACGA GGAAGACGAA GGTCCCGAAT CTGACCAAGA
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CGCAGTCTCC
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841 GACCCTACAA TCTCATCCAC TGAGACTCTC CTCAGGAACA TCCAGGGTCT
CCTGAAAGTA
901 GCRGCCGACA ACGCAMGGCA ACA
//

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LOCUS TBA 701 bp ss-DNA linear SYN
 04-Jun-2010
 DEFINITION Jadera haematoloma homothorax partial CDS
 ACCESSION -
 KEYWORDS -
 SOURCE Jadera haematoloma (red-shouldered soapberry bug)
 ORGANISM Jadera haematoloma (Davis GRT population)
 Eukaryota; Metazoa; Arthropoda; Hexapoda; Insecta;
 Pterygota;
 Neoptera; Paraneoptera; Hemiptera; Euhemiptera;
 Heteroptera;
 Panheteroptera; Pentatomomorpha; Coreoidea; Rhopalidae;
 Jadera.
 REFERENCE TBA
 AUTHORS Stacey L. Baker, David R. Angelini
 COMMENT Consensus sequence of clones Jh'hth-1A, 1B and 1H
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                  /note=polymorphism (clone B&H form shown
                  /note=in clone A this reads: ATGTTGCAG)
BASE COUNT      192 A          191 C          186 G          120 T          11
OTHER
ORIGIN
      1 CAGGCRATAC AGGTRCTGKG GTTTCACCTT CTTGAACTTG AAAAGGTTCA
CGAGCTTTGC
     61 GATRACTTCT GCCACCGCTA CATTAGCTGC CTGAAAGGGA AGATGCCCAT
CGACCTAGTG
    121 ATAGACGAGA GAGAAAGCTC CAAACCTCTT GGTGAACTGG GGACACCGGC
GAACGGCAAC
    181 GATGGAAGAT CAAACGCTGA TTCGACCTCG CACACAGACG GGGCTAGTAC
CCCCGATGTG
    241 GCATTTTCAA GCAACTCAAA TGGATACAGG CCTCCCTCCA GCTCACTCTC
ATACCCTGGC
    301 CATGGGAGTG AAGACGTGAG GTCACCAGGA TCTGGTGGAA CCCCTGGTCC
TCTCYCTCAG
    361 GCGCCCCAGC TTGACCACTC TGATGCAGGA AAGTGGTGTG GACGRCGGGA
ATGGCCCTCA
    421 CCRGCAGAGG CACGAGCAGC GYCTGACGCT GCGCGGCGCG GAGTCCTCTA
CTCTTCCGTG
    481 TTCCTGGGCA GCCCCGGAGA ATACAACTCA TGTGATGCGA GCAATGCAAG
CATCGGAAGC
    541 GGGGAAGGCA CAGGAGAAGA AGACGACGAT ACAAACGGAA AGAAAAACCA
GAAAAAACGT
    601 GGAATCYTCC CGAAAGTAGC GACGAATATA CTGAGAGCCT GGTTATTCCA
ACACCTAACG
    661 CACCCGTATC CGTCGGAAGA CCAGAAAAAA CARCTCGCMC A
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LOCUS TBA 404 bp ss-DNA linear SYN
 20-Jul-2010
 DEFINITION Jadera haematoloma sarcomere length short partial CDS
 ACCESSION -
 KEYWORDS -
 SOURCE Jadera haematoloma (red-shouldered soapberry bug)
 ORGANISM Jadera haematoloma (Davis GRT population)
 Eukaryota; Metazoa; Arthropoda; Hexapoda; Insecta;
 Pterygota;
 Neoptera; Paraneoptera; Hemiptera; Euhemiptera;
 Heteroptera;
 Panheteroptera; Pentatomomorpha; Coreoidea; Rhopalidae;
 Jadera.
 REFERENCE TBA
 AUTHORS Stacey L. Baker, David R. Angelini
 COMMENT Consensus sequence of clones Jh'sals-1A and 1B
 FEATURES Location/Qualifiers
 source 1..404
 /organism="Jadera haematoloma"
 /mol_type="cDNA"
 gene <1..>404
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 mRNA <1..>404
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 /product="sarcomere length short"
 CDS <1..>404
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 /codon_start=3
 /product="sarcomere length short"

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 EEIITKSASVADVAVASQPDELDDIDKVRDDLQPQSRLP"
 primer_bind 2..22
 /note=Jh'da-f2 (degenerate
 /note=not exact in 5' region)
 misc_feature 71..71
 /note=C/T polymorphism
 misc_feature 158..158
 /note=A/G polymorphism
 primer_bind 304..326
 /note=Jh'sals-qf1
 primer_bind complement(358..380)
 /note=Jh'sals-qr1
 primer_bind complement(385..404)
 /note=Jh'da-r2 (degenerate
 /note=not exact)
 BASE COUNT 155 A 70 C 91 G 85 T 2
 OTHER
 ORIGIN
 1 TAGAGAGGAA GGAGCCGCCG GACTGGACTG ATATGATGAA GGAAGTAGAA
 CAAGGAGTAA

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        61 AACTAAATCA YGTAAAGTGT AATGACAGGA GTGCCCCAGT TATTCCAAAA
GCAAAAGCTA
       121 AGGGTCAATT TGTATATGAG TCAGAAAAAG AAAATTCRCA TAATCCTCAC
AATCAGCTGT
       181 TGAAAGAAAT CCAGTCAGGT GTACATTTAA AGAAAACGAA AACAAATGAC
AGAAGTAAAC
       241 CAATGTTAGA AGGTTTAAGA AAGTTTAGGC GGCAAATGAC CATTGAAGAA
ATTATTACGA
       301 AATCAGCATC TGTAGCAGAC GTAGTGGCTG TTGCTTCACA ACCTGACGAA
CTTGATGACA
       361 TAGACAAGGT CCGTGATGAT TTACAACCCC AAAGCCGCCT GCCT
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LOCUS TBA 1002 bp ss-DNA linear SYN
 24-Jul-2010
 DEFINITION Jadera haematoloma beta-actin partial CDS
 ACCESSION -
 KEYWORDS -
 SOURCE Jadera haematoloma (red-shouldered soapberry bug)
 ORGANISM Jadera haematoloma (Davis GRT population)
 Eukaryota; Metazoa; Arthropoda; Hexapoda; Insecta;
 Pterygota;
 Neoptera; Paraneoptera; Hemiptera; Euhemiptera;
 Heteroptera;
 Panheteroptera; Pentatomomorpha; Coreoidea; Rhopalidae;
 Jadera.
 REFERENCE TBA
 AUTHORS Stacey L. Baker, David R. Angelini
 COMMENT Sequence of clone Jh'actb-1F
 FEATURES Location/Qualifiers
 source 1..1002
 /organism="Jadera haematoloma"
 /mol_type="cDNA"
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 mRNA <1..>1002
 /gene="Jh'actb"
 /product="beta-actin"
 CDS <1..>1002
 /gene="Jh'actb"
 /codon_start=2
 /product="beta-actin"

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 LRVAPEEHPILLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGR
 TTGIVLDSGDGVSHTVPIYEGYALPHAILRLDLAGRDLTDYLMKILTERGYSFT
 TTAEREIVRDIKEKLCYVALDFEQEMATAAAASTSLEKSYELPDGQVITIGNERF
 RCPEALFQPSFLGMESCGIHETVYNSIMKCDVDIRKDLYANTVLSGGTTMYPGI
 ADRMQKEITALAPSTIKIKIIAP"
 primer_bind 2..24
 /note=d-actb-f1 (degenerate)
 primer_bind 980..1002
 /note=d-actb-r1 (degenerate)
 BASE COUNT 248 A 294 C 238 G 221 T 0
 OTHER
 ORIGIN
 1 TATGTGCGAT GAGGAGGTGG CGGCTCTTGT TGTTGACAAT GGTTCGGGA
 TGTGCAAGC
 61 CGGCTTCGCC GGAGATGACG CCCCAGGGC CGTCTTCCCC TCCATCGTCG
 GTAGACCTAG

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121 GCACCAGGGT GTCATGGTCG GTATGGGTCA AAAGGACAGC TATGTAGGTG
ATGAGGCCCA
181 GAGCAAGAGA GGTATTCTCA CCCTGAAATA CCCAATTGAA CACGGTATCA
TCACCAACTG
241 GGACGACATG GAGAAAATCT GGCACCACAC CTTCTACAAC GAGCTGCGAG
TCGCCCCAGA
301 GGAACACCCA ATCCTCTTGA CTGAGGCCCC ACTCAACCCT AAGGCCAACA
GGGAAAAGAT
361 GACCCAAATC ATGTTTGAAA CCTTCAACAC ACCCGCCATG TATGTTGCCA
TCCAGGCTGT
421 CCTTTCCTTG TACGCCTCCG GTCGTACCAC CGGTATTGTA CTTGACTCCG
GTGATGGTGT
481 CTCCCACACT GTCCCAATCT ATGAAGGTTA TGCCCTCCCC CACGCCATCC
TCCGTCTGGA
541 CTTGGCTGGA CGAGACTTGA CTGATTACCT CATGAAGATC CTAAGTACAG
GTGGTTACAG
601 CTTACCACC ACCGCTGAAA GGGAAATTGT CAGGGACATC AAGGAAAAAC
TTTGCTATGT
661 CGCCCTCGAC TTCGAGCAGG AAATGGCTAC CGCCGCTGCC TCCACCTCCC
TCGAGAAGTC
721 CTATGAACTT CCCGACGGTC AGGTCATCAC CATTGGTAAC GAAAGGTTCC
GTTGCCCAGA
781 GGCTCTCTTC CAGCCTTCCT TCTTGGGTAT GGAATCTTGC GGTATCCATG
AGACTGTATA
841 CAACTCCATC ATGAAGTGCG ATGTTGACAT CAGGAAAGAC TTGTACGCCA
ACACCGTCCT
901 CTCAGGAGGT ACTACCATGT ACCCAGGTAT TGCTGACAGG ATGCAGAAGG
AAATCACAGC
961 CCTCGCACCC TCAACAATTA AGATCAAGAT CATCGCACCC CC
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LOCUS TBA 408 bp ss-DNA linear SYN
 16-Jul-2010
 DEFINITION Jadera haematoloma ribosomal protein S18 partial CDS
 ACCESSION -
 KEYWORDS -
 SOURCE Jadera haematoloma (red-shouldered soapberry bug)
 ORGANISM Jadera haematoloma (Davis GRT population)
 Eukaryota; Metazoa; Arthropoda; Hexapoda; Insecta;
 Pterygota;
 Neoptera; Paraneoptera; Hemiptera; Euhemiptera;
 Heteroptera;
 Panheteroptera; Pentatomomorpha; Coreoidea; Rhopalidae;
 Jadera.
 REFERENCE TBA
 AUTHORS Stacey L. Baker, David R. Angelini
 COMMENT Sequence of clone Jh'rps18-1A
 FEATURES Location/Qualifiers
 source 1..408
 /organism="Jadera haematoloma"
 /mol_type="cDNA"
 gene <1..>408
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 mRNA <1..>408
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 /product="ribosomal protein S18"
 CDS <1..>408
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 /codon_start=2
 /product="ribosomal protein S18"

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 primer_bind 2..24
 /note=d-rps18-f (degenerate)
 primer_bind complement(386..408)
 /note=d-rps18-r (degenerate)
 BASE COUNT 143 A 66 C 96 G 102 T 0
 OTHER
 ORIGIN
 1 TATTCCTGAG AAGTTTCAGC ATATCCTTCG TATCATGGGT ACTAATATCG
 ATGGTAAAAG
 61 GAAAGTTATG TTCGCTATGA CAGCTATCAA AGGTGTTGGT AGGAGGTATG
 CCAATATTGT
 121 TCTTAAAAAA GCCGATGTTG ATTTAGATAA GAGAGCTGGT GAATGCTCTG
 AAGAAGAGGT
 181 AGACAAAATT TTCACAATTA TGCAATATCC TAGACAATAT AAAATTCCGG
 ACTGGTTCTT
 241 GAATAGACAA AAAGATATTG TTGATGGAAA ATACAACCAG TTGACCTCCG
 CAAATCTTGA
 301 CAGCAAACCTT CGAGAAGATT TGGAAAGGCT CAAGAAAATC AGGGCCCACA
 GAGGAATGAG
 361 GCACTATTGG GGTTTGAGGG TGAGAGGACA ACACACCAAA ACCACCGG

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LOCUS TBA 585 bp ss-DNA linear SYN
 16-Jul-2010
 DEFINITION Jadera haematoloma syntaxin-1 partial CDS
 ACCESSION -
 KEYWORDS -
 SOURCE Jadera haematoloma (red-shouldered soapberry bug)
 ORGANISM Jadera haematoloma (Davis GRT population)
 Eukaryota; Metazoa; Arthropoda; Hexapoda; Insecta;
 Pterygota;
 Neoptera; Paraneoptera; Hemiptera; Euhemiptera;
 Heteroptera;
 Panheteroptera; Pentatomomorpha; Coreoidea; Rhopalidae;
 Jadera.
 REFERENCE TBA
 AUTHORS Stacey L. Baker, David R. Angelini
 COMMENT Sequence of clone Jh'syx1-1A
 FEATURES Location/Qualifiers
 source 1..585
 /organism="Jadera haematoloma"
 /mol_type="cDNA"
 gene <1..>585
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 mRNA <1..>585
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 CDS <1..>585
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 AKLKVIEQNIQEEHTNKSSAGLRIRKTQHSTLSRK FVEVMTVYNRTQTDYRER
 CKGRIQRQLGITGR TTTNEELEEMLEQGNPAVFTQGIIMETQQAKRTLADIEAR
 primer_bind HADI IKLENSIRELHDMFMDMAMLVESQGEMIDRIEYHVEHAVDYV"
 2..24
 primer_bind /note=d-syx1-f2 (degenerate)
 complement(563..585)
 /note=d-syx1-r2 (degenerate)
 BASE COUNT 219 A 104 C 143 G 118 T 0
 OTHER
 ORIGIN
 1 TGTGGAGGAG GTGAAGAAGA AGCATAGTGC CATCCTCAGT GCTCCACAAA
 CAGATGAAAA
 61 GGTCAAACAA GAATTGGAAG ACCTTATGGC TGACATTAAA AAAGCAGCCA
 ACAAAGTCCG
 121 TGCCAAACTT AAAGTTATCG AACAAAACAT AGAGCAGGAA GAACATACAA
 ATAAATCGTC
 181 TGCCGGCTTA AGGATACGAA AAACCCAACA CTCAACTTTA TCTAGGAAGT
 TTGTAGAGGT
 241 AATGACAGTA TACAATCGGA CACAGACTGA CTACCGAGAA AGGTGTAAAG
 GGAGGATACA


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      301 ACGGCAACTG GGAATTACTG GTAGGACAAC AACGAATGAG GAATTAGAAG
AAATGTTAGA
      361 ACAAGGAAAT CCAGCTGTCT TCACTCAGGG GATCATAATG GAGACCCAAC
AGGCAAAGCG
      421 GACATTGGCT GATATAGAGG CAAGGCATGC TGATATAATC AAATTAGAAA
ATTCCATTAG
      481 GGAACTCCAT GATATGTTCA TGGACATGGC TATGCTCGTT GAGAGCCAGG
GAGAAATGAT
      541 CGACCGTATA GAGTACCATG TTGAGCATGC GGTTGATTAT GTACA
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LOCUS          TBA                      225 bp ss-DNA      linear      SYN
16-Jul-2010
DEFINITION    Jadera haematoloma 18S ribosomal RNA partial sequence
ACCESSION     -
KEYWORDS      -
SOURCE        Jadera haematoloma (red-shouldered soapberry bug)
ORGANISM      Jadera haematoloma (Davis GRT population)
               Eukaryota; Metazoa; Arthropoda; Hexapoda; Insecta;
Pterygota;   Neoptera; Paraneoptera; Hemiptera; Euhemiptera;
Heteroptera; Panheteroptera; Pentatomomorpha; Coreoidea; Rhopalidae;
               Jadera.
REFERENCE     TBA
AUTHORS       Stacey L. Baker, David R. Angelini
COMMENT       Sequence of clone Jh'18S-1A
FEATURES      Location/Qualifiers
               source          1..225
                               /organism="Jadera haematoloma"
                               /mol_type="cDNA"
               gene            <1..>225
                               /gene="Jh'18S"
BASE COUNT    51 A              53 C              58 G              62 T              0
OTHER
ORIGIN
      1 GCCGTTCTAG TTGGTGGACT GATTTGTCTG GTTAATTCCG ATAACGAACG
AGACTCTAAC
      61 CTATTAACTA GGCGTTTCCG GTATACAAAT CTACCGGCGA GATTTTTTCT
TCTTAAGGGG
      121 ACAGGCGGCT CTTAGCCGCA CGAGATTGAG CAATAACAGG TCTGTGATGC
CCTTAGATGT
      181 TCTGGGCCGC ACGCGCGCTA CACTGAAGGA ATCAGCGTGT GCTCC
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- morph and life history differences during host race radiation in the soapberry bug, *Jadera haematoloma* Herrich-Schaeffer (Hemiptera : Rhopalidae). *Ann Entomol Soc Am* 96, 135-143.
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Oostra, V., Ozaki, K., Papakonstantinou, M., Popadic, A., Rajam, M.V., Saenko, S., Simpson, R.M., Soberon, M., Strand, M.R., Tomita, S., Toprak, U., Wang, P., Wee, C.W., Whyard, S., Zhang, W.Q., Nagaraju, J., Ffrench-Constant, R.H., Herrero, S., Gordon, K., Swelters, L., Smagghe, G., 2011. RNA interference in Lepidoptera: An overview of successful and unsuccessful studies and implications for experimental design. *J. Insect Physiol.* 57, 231-245.

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