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A survey of the trans-regulatory landscape for *Drosophila melanogaster* abdominal pigmentation

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ABSTRACT

Trait development results from the collaboration of genes interconnected in hierarchical networks that control which genes are activated during the progression of development. While networks are understood to change over developmental time, the alterations that occur over evolutionary times are much less clear. A multitude of transcription factors and a far greater number of linkages between transcription factors and *cis*-regulatory elements (CREs) have been found to structure well-characterized networks, but the best understood networks control traits that are deeply conserved. Fruit fly abdominal pigmentation may represent an optimal setting to study network evolution, as this trait diversified over short evolutionary time spans. However, the current understanding of the underlying network includes a small set of transcription factor genes. Here, we greatly expand this network through an RNAi-screen of 558 transcription factors. We identified 28 genes, including previously implicated *abd-A*, *Abd-B*, *bab1*, *bab2*, *dsx*, *exd*, *hth*, and *jing*, as well as 20 novel factors with uncharacterized roles in pigmentation development. These include genes which promote pigmentation, suppress pigmentation, and some that have either male- or female-limited effects. We show that many of these transcription factors control the reciprocal expression of two key pigmentation enzymes, whereas a subset controls the expression of key factors in a female-specific circuit. We found the pupal Abd-A expression pattern was conserved between species with divergent pigmentation, indicating diversity resulted from changes to other loci. Collectively, these results reveal a greater complexity of the pigmentation network, presenting numerous opportunities to map transcription factor-CRE interactions that structure trait development and numerous candidate loci to investigate as potential targets of evolution.

Introduction

A major undertaking in evolutionary developmental biology is to understand how gene regulatory networks that control trait development change during evolution. Most physical traits develop through coordinated programs of gene expression, orchestrated by a network of linkages between transcription factors and downstream target genes. Linkages between transcription factors and target genes are encoded within *cis*-regulatory element (CRE) sequences that determine when, where, and at what level a gene is expressed during development (Arnone and Davidson, 1997). The structure of regulatory networks, including the genes, CREs and their transcription factor linkages are of great interest to the field of developmental biology as they embody the programs for cell, tissue, and organ development (Davidson, 2006). Gene networks in extant organisms represent the product of a complex, genome-wide evolutionary process. Although several individual networks in a single species have been well-characterized (Bonn and Furlong, 2008; Imai et al., 2009; Levine and Davidson, 2005; Ochoa-Espinosa et al., 2005; Oliveri et al., 2008; Peter and Davidson, 2011; Sandmann et al., 2007; Zeitlinger et al., 2007), and several examples of macro-evolutionary changes to networks have been explored (Hinman et al., 2007; Weatherbee et al., 1999; Zinzen et al., 2006), a mechanistic understanding of the incipient events of network evolution can be considered to be in its infancy. To better understand these early events in network evolution, well characterized gene networks are needed for traits that evolved between closely-related species.

One well-suited model to study the incipient stages of regulatory network evolution is the cascade of transcription factors and enzymes that generate abdominal pigmentation patterns among fruit fly species from the genus *Drosophila* (*D.*). These species evolved extensive diversity in pigmentation since diverging from a

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common ancestor ~50 million years ago (Wittkopp et al., 2003). This diversity includes sexual dimorphism such as that exhibited by *D. melanogaster*, where the dorsal cuticle tergites on the posterior two (A5 and A6) abdominal segments are fully pigmented in males. Female tergite pigmentation, though, is typically limited to a posterior stripe (Fig. 1A). Among related species, male pigmentation can be limited to the A6 tergite (e.g. *D. baimaii*) or spans the A4–A6 tergites (e.g. *D. prostipennis*). This range of dimorphic patterns is thought to have evolved from a monomorphic ancestor that gave rise to extant species such as *D. willistoni* (Fig. 1A) (Jeong et al., 2006). In distantly-related lineages, male-specific pigmentation has seemingly evolved convergently (e.g. *D. funebris*). As the genes encoding pigmentation enzymes and most transcription factors are conserved between fruit fly species with sequenced genomes (Clark et al., 2007; Richards et al., 2005), it seems that abdominal pigmentation diversity evolved largely by changes in the structure of the pigmentation network, causing differences in pigmentation enzyme expression.

In *D. melanogaster*, many of the genes encoding pigmentation enzymes have been extensively characterized (True et al., 2005; Wittkopp et al., 2003). In particular, *yellow* and *tan* are required for the production of black pigments, and are expressed specifically in the abdominal epidermal cells that underlie black cuticle, such as male A5 and A6 segments (Jeong et al., 2008, 2006). In a pattern reciprocal to *yellow* and *tan*, *ebony* is expressed in more anterior A2–A4 segments in males and throughout the abdomen of females to promote a yellow cuticle color (Fig. S1) (Rebeiz et al., 2009a; Richardt et al., 2003). Some of the patterning mechanisms that sculpt these reciprocal patterns of pigmentation are known.

A network of four transcription factors has been shown to regulate the pigmentation gene battery directly or indirectly (Fig. 1B). Activation of *yellow*, and presumably *tan*, in the A5 and A6 segments requires direct regulation by the Hox protein Abdominal-B (Abd-B) which interacts with CRE binding sites (Jeong et al., 2008, 2006). The absence of comparable *yellow* and *tan* expression in females is due to the Bric-à-brac 1 and 2

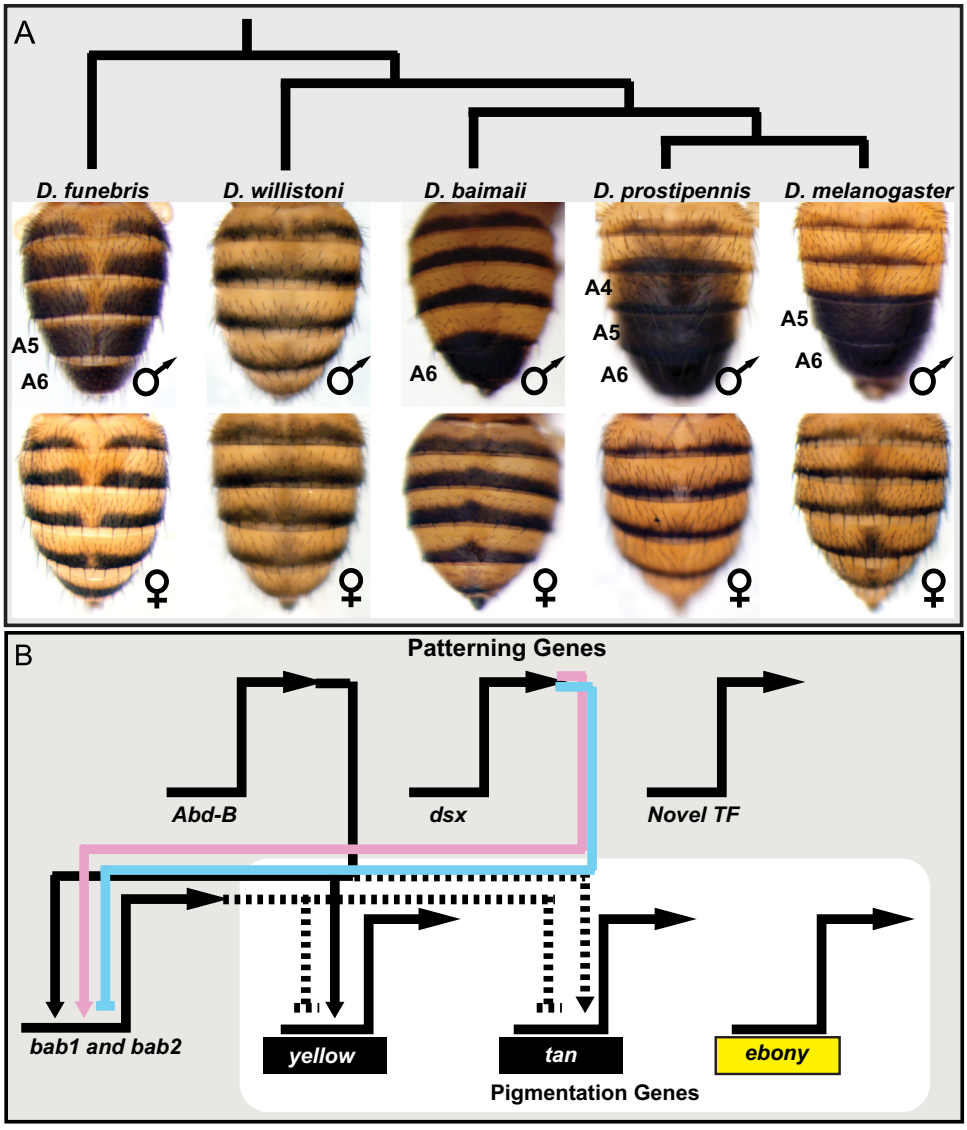

Fig. 1. Abdominal pigmentation pattern and gene network. (A) The dorsal abdomens of fruit flies are covered by cuticle plates, called tergites that exhibit diverse patterns of pigmentation. This includes the sexually monomorphic pattern of *D. willistoni*, and dimorphic patterns that evolved convergently between *D. funebris* and *D. melanogaster*. The number of pigmented tergites differs for *D. baimaii* and *D. prostipennis* males, two species more closely-related to *D. melanogaster*. (B) Contemporary understanding of the *D. melanogaster* abdominal pigmentation network. Direct regulatory interactions between transcription factors and target gene *cis*-regulatory elements are represented as solid lines. Dashed lines indicate a regulatory relationship that has not been demonstrated to be direct. Regulatory interactions terminating with arrowheads and nail heads respectively indicate activating and repressing regulatory inputs.