

Gene Regulatory Network Homoplasy Underlies Recurrent Sexually Dimorphic Fruit Fly Pigmentation

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ABSTRACT

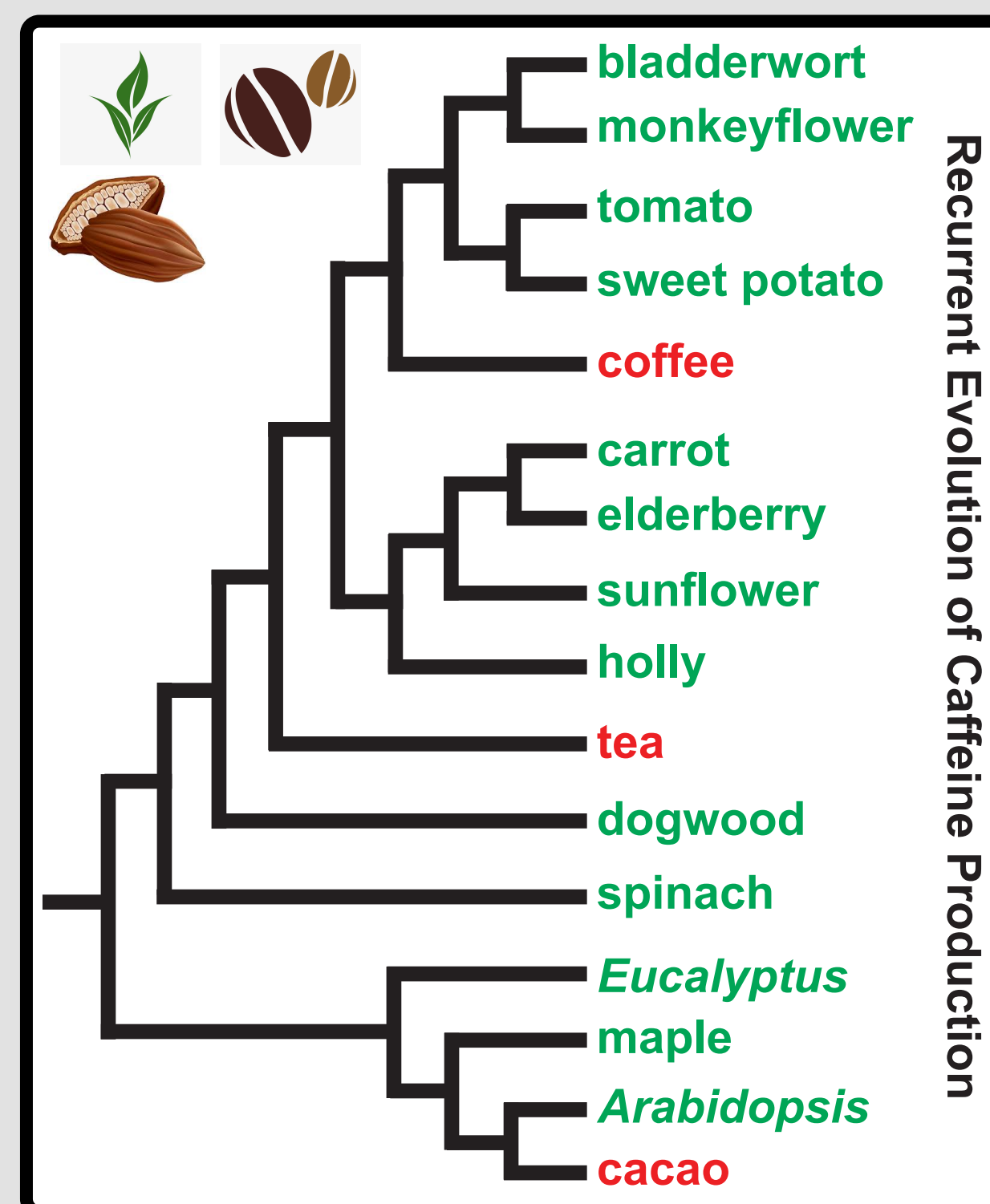
Traits that appear discontinuously along phylogenies may be explained by independent origins (homoplasy) or repeated loss (homology). While discriminating between these models is difficult, the dissection of gene regulatory networks (GRNs) which drive the development of such repeatedly occurring traits can offer a mechanistic window on this fundamental problem. The GRN responsible for the male-specific pattern of *Drosophila* (*D.*) melanogaster melanic tergite pigmentation has received considerable attention. In this system, a metabolic pathway of pigmentation enzyme genes is expressed in spatial and sex-specific (i.e., dimorphic) patterns. The dimorphic expression of several genes is regulated by the Bab transcription factors, which suppress pigmentation enzyme expression in females, by virtue of their high expression in this sex. Here, we analyzed the phylogenetic distribution of species with male-specific pigmentation and show that this dimorphism is phylogenetically widespread among fruit flies. The analysis of pigmentation enzyme gene expression in distantly related dimorphic and monomorphic species shows that dimorphism is driven by the similar deployment of a conserved metabolic pathway. However, sexually dimorphic Bab expression was found only in *D. melanogaster* and its close relatives. These results suggest that dimorphism evolved by parallel deployment of differentiation genes, but was derived through distinct architectures at the level of regulatory genes. This work demonstrates the interplay of constraint and flexibility within evolving GRNs, findings that may foretell the mechanisms of homoplasy more broadly.

Widespread Recurring Traits in the Natural World

Recurring traits are widespread in nature, suggesting that evolution has predictable solutions to certain ecological challenges.

One such example being the evolution of caffeine production in multiple plants as a form of protection from insects and competition against other plants.

This raises a few questions. (1) How does this situation play out at the molecular level? (2) In cases where we have species that have converged upon the same phenotype, will we see the recruitment of the same sets of genes into the network regulating this characteristic or will nature find multiple ways to the same outcome? (3) What insights could this give us into the evolutionary history of a trait?



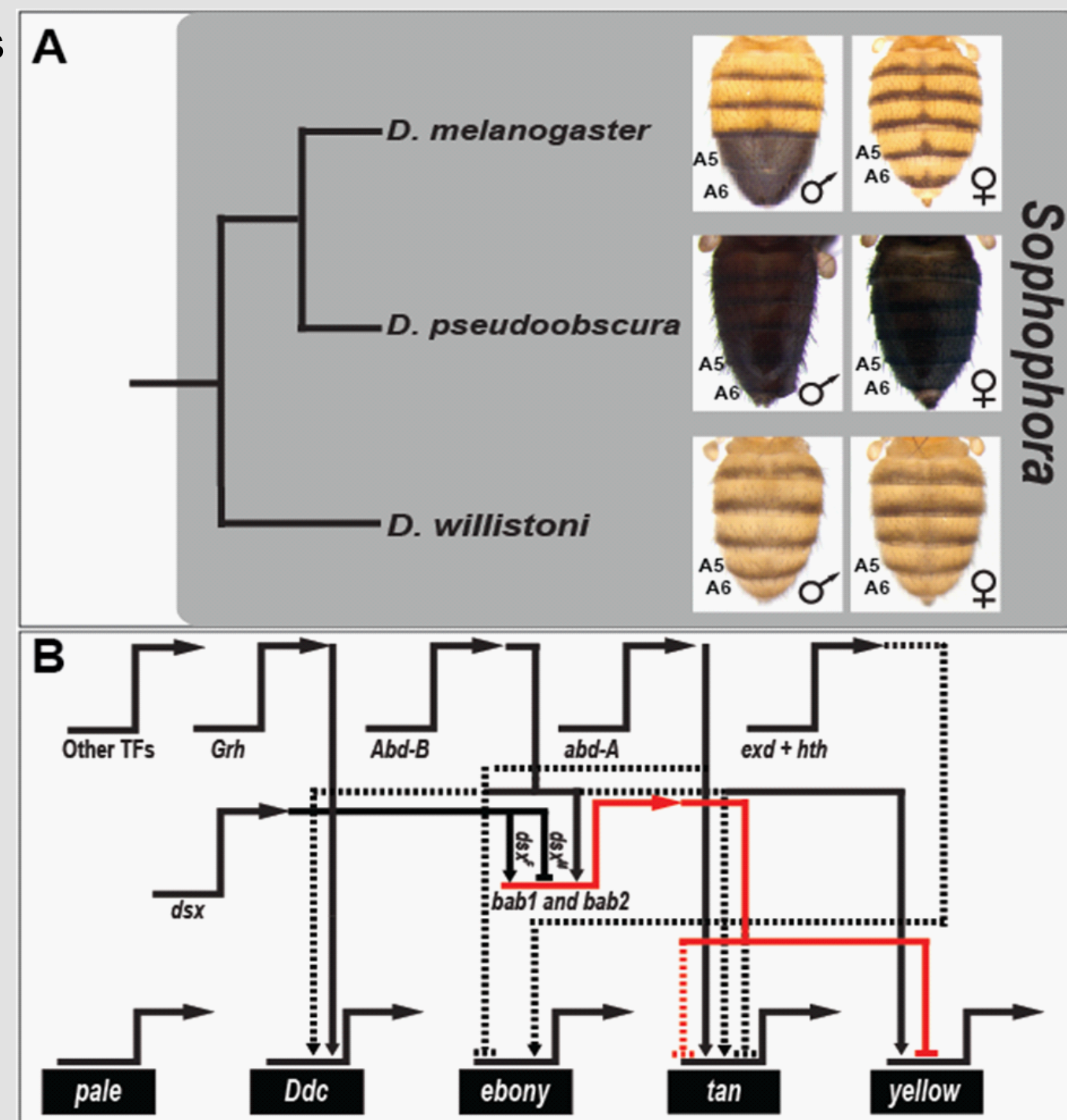
We aim to answer these questions using the sexually dimorphic pigmentation of multiple fruit fly species.

Model for Sexual Dimorphism and Underlying Network

(A) Phylogenetic relationship of model species of the *Sophophora* subgenus. Here, monomorphism is presumed to be ancestral, and dimorphism derived.

(B) The current understanding of the gene regulatory network for the *D. melanogaster* phenotype has key transcription factor genes (regulatory genes) driving the expression of genes that encode proteins that participate in a pigment metabolic pathway (differentiation genes). The horizontal-pointing arrows represent expression occurring from the named loci. Key nodes within this network drive the male-limited expression of the *tan* and *yellow* genes, whose expression in females is repressed by the activity of the *bab1* and *bab2* transcription factor genes.

Lines ending in arrows indicate activation while nail-heads indicate repression.



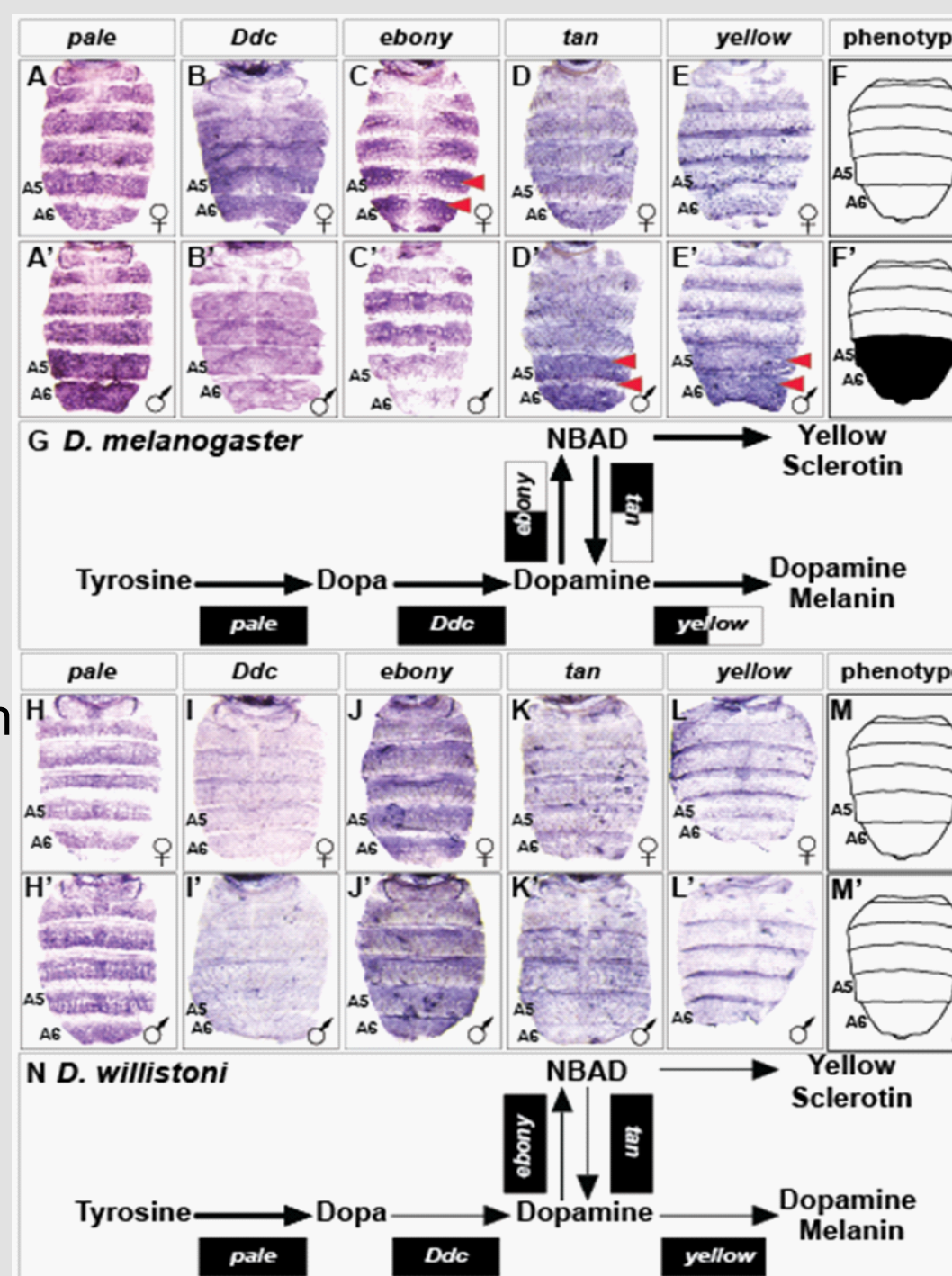
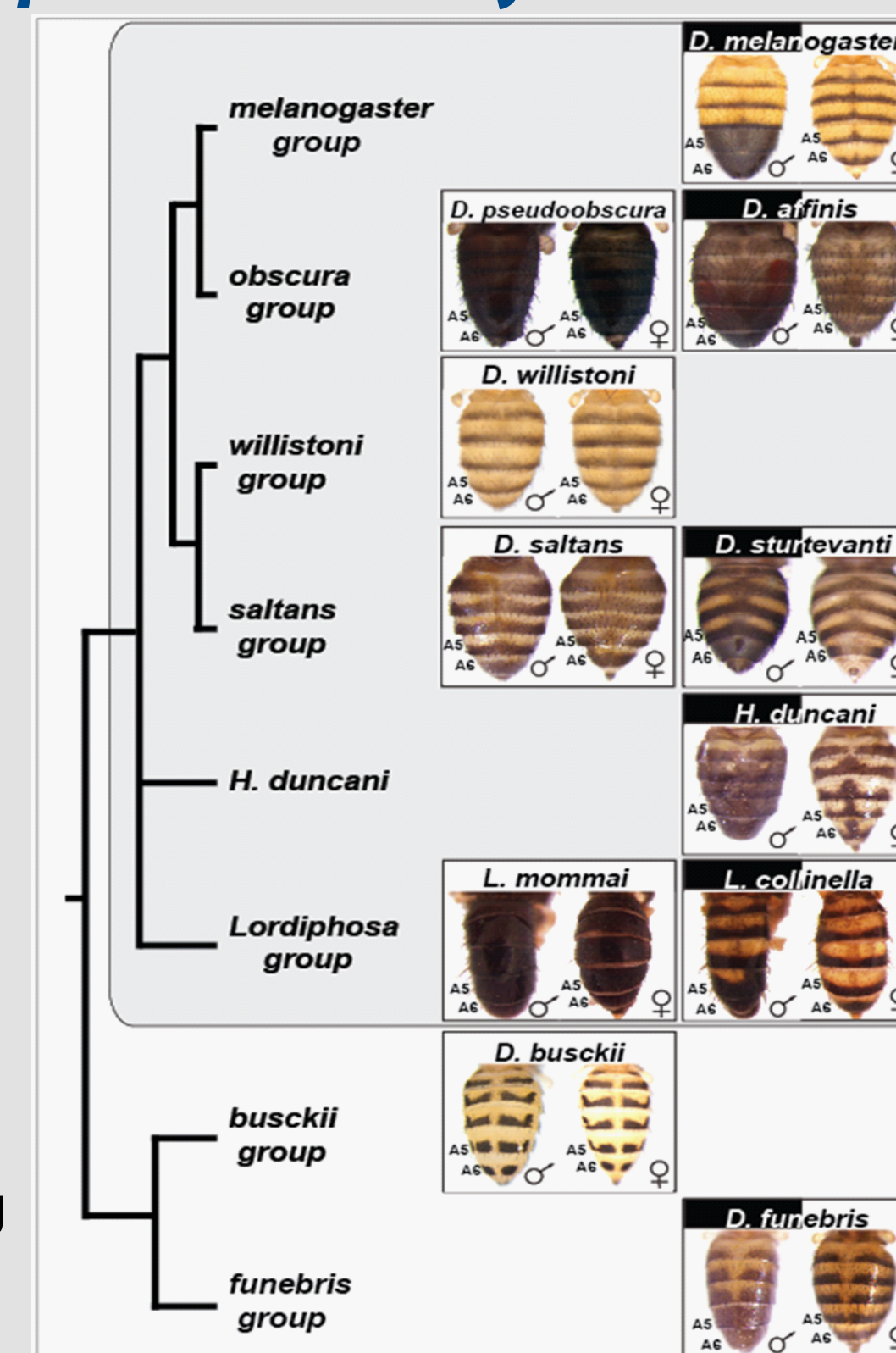
Widespread Dimorphism in *Sophophora* and Beyond

Species with dimorphic tergite pigmentation are widespread throughout the *Drosophila* genus. *Sophophora* subgenus species groups and species are indicated by the gray background. *D. busckii* and *D. funebris* are included as non-*Sophophora* species from the *Drosophila* genus that respectively exhibit monomorphic and dimorphic patterns of tergite pigmentation. The homologous A5 and A6 segment tergites are indicated for each species, the segments bearing the dimorphic pigmentation in *D. melanogaster*. While the *obscura*, *saltans*, and *Lordiphosa* groups are predominately populated by monomorphic species (e.g. *D. pseudoobscura*, *D. saltans*, and *L. mommai*), they possess a few dimorphic species, including *D. affinis*, *D. sturtevantii*, and *L. collinella*.

Dimorphic fruit fly pigmentation is another example of a recurrent trait. We first sought to know what is taking place at the level of the differentiation genes that are responsible for making this trait.

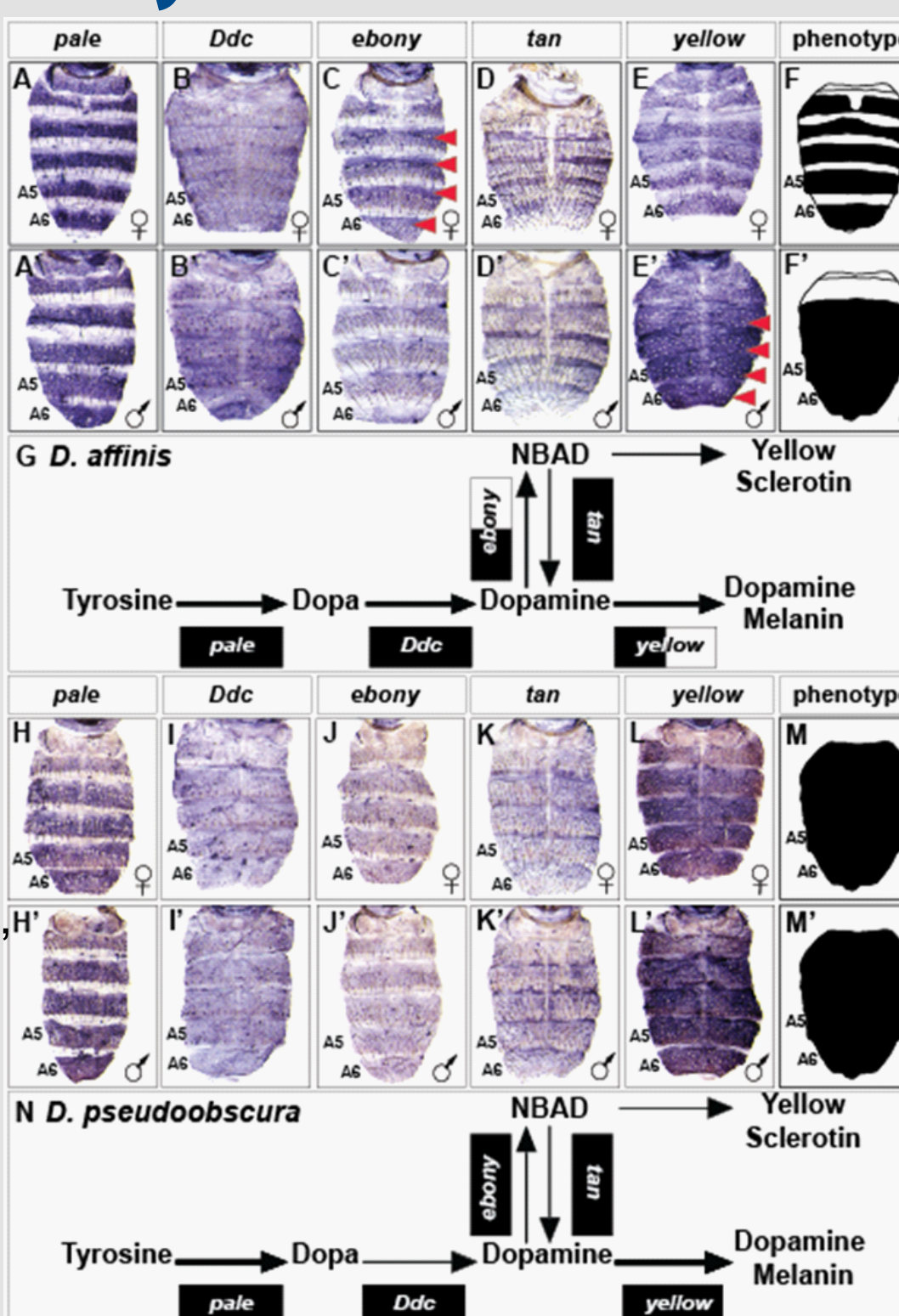
Contrast in Pigment Metabolic Pathway: *D. melanogaster* and *D. willistoni*

(A-E) Female and (A'-E') male expressions of *D. melanogaster* pigment metabolic pathway genes, and (F and F') cartoon representation of the pigmentation phenotype. (G) Summary of the *D. melanogaster* pathway use includes robust expression of all genes, with dimorphic expressions of *ebony*, *tan*, and *yellow*. (H-L) Female and (H'-L') male expressions of *D. willistoni* pigment metabolic pathway genes, and (M and M') cartoon of the pigmentation phenotype. (N) Summary of the *D. willistoni* pathway use includes modest and monomorphic expression of most genes. (A, A', H, and H') *pale*, (B, B', I, and I') *Ddc*, (C, C', J, and J') *ebony*, (D, D', K, and K') *tan*, and (E, E', L, and L') *yellow*. Red arrowheads indicate robust patterns of dimorphic expression in the A5 and A6 segment epidermis.



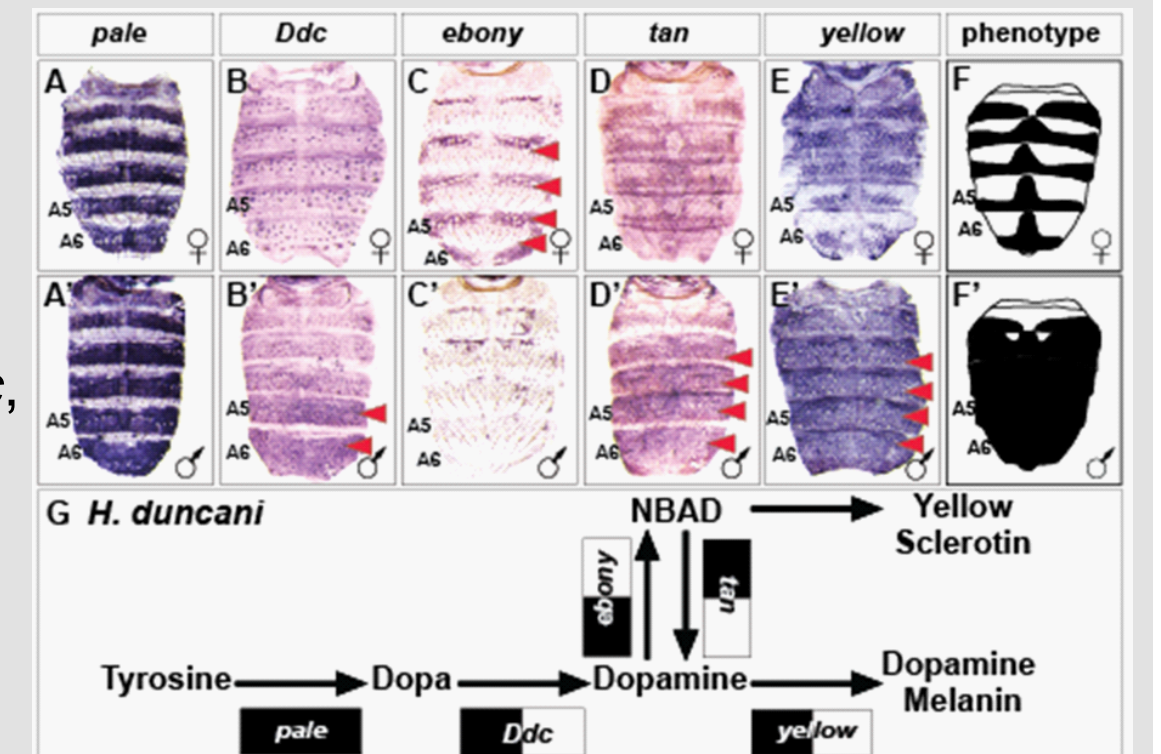
Contrast in Pigment Metabolic Pathway: *D. affinis* and *D. pseudoobscura*

(A-E) Female and (A'-E') male expressions of *D. affinis* pigment metabolic pathway genes, and (F and F') cartoon representation of the pigmentation phenotype. (G) Summary of the *D. affinis* pathway use includes robust expression of all genes, with dimorphic expressions of *ebony*, and *yellow*. (H-L) Female and (H'-L') male expressions of *D. pseudoobscura* pigment metabolic pathway genes, and (M and M') cartoon representation of the pigmentation phenotype. (N) Summary of the *D. pseudoobscura* pathway use includes monomorphic expression of most genes. (A, A', H, and H') *pale*, (B, B', I, and I') *Ddc*, (C, C', J, and J') *ebony*, (D, D', K, and K') *tan*, and (E, E', L, and L') *yellow*. Red arrowheads indicate robust patterns of dimorphic expression in the A3-A6 segment epidermis.



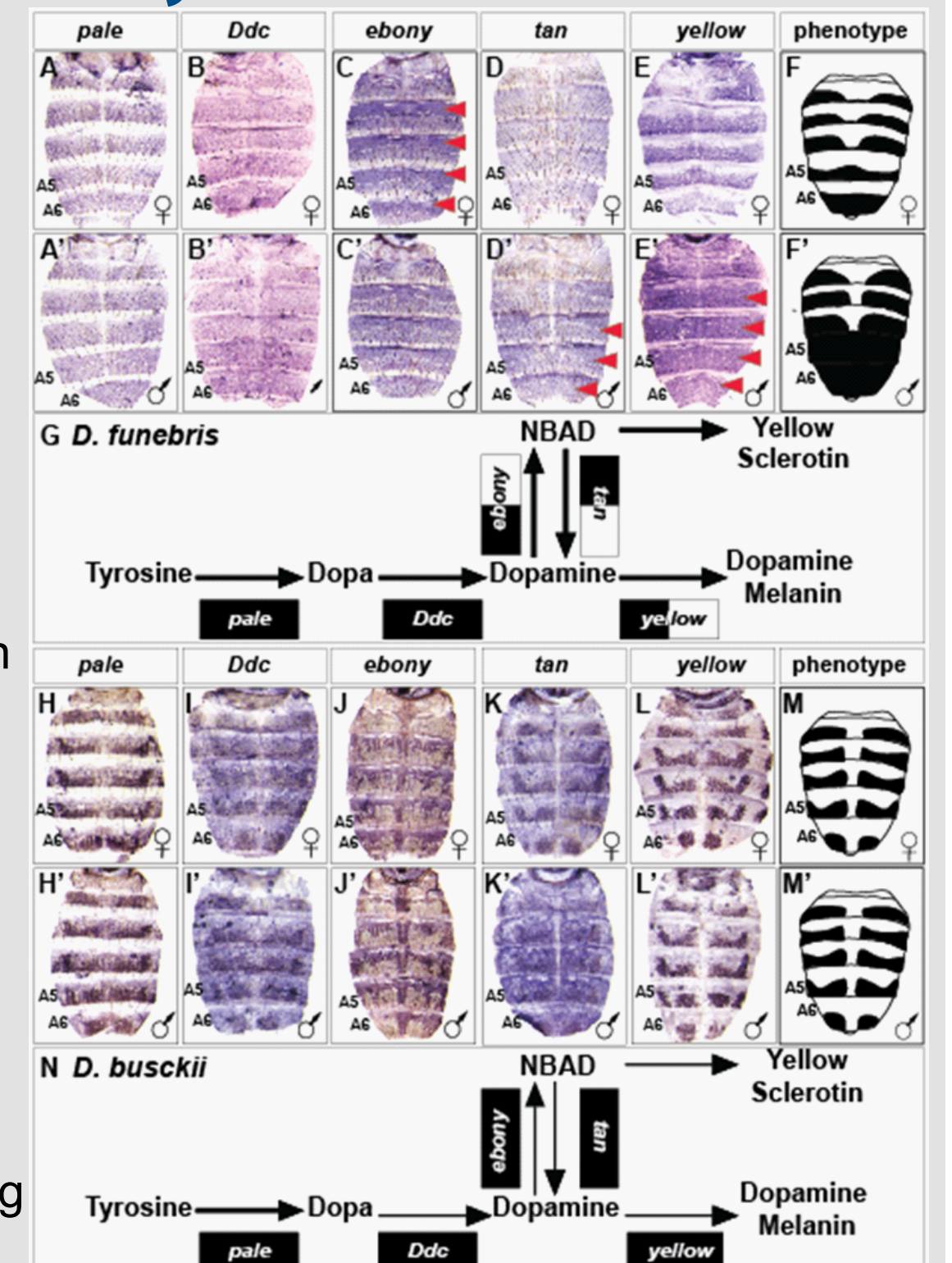
Pigment Metabolic Pathway Utilization in *H. duncani*

(A-E) Female and (A'-E') male expressions of *H. duncani* pigment metabolic pathway genes, and (F and F') cartoon representation of the pigmentation phenotype. (G) Summary of the *H. duncani* pathway use includes robust expression of all genes, with dimorphic expressions of *Ddc*, *ebony*, *tan*, and *yellow*. (A, A') *pale*, (B, B') *Ddc*, (C, C') *ebony*, (D, D') *tan*, and (E, E') *yellow*. Red arrowheads indicate robust patterns of dimorphic expression in the dorsal abdominal epidermis.



Contrast in Pigment Metabolic Pathway: *D. funebris* and *D. busckii*

(A-E) Female and (A'-E') male expressions of *D. funebris* pigment metabolic pathway genes, and (F and F') cartoon representation of the pigmentation phenotype. (G) Summary of the *D. funebris* pathway use includes robust expression of all genes, with dimorphic expressions of *ebony*, *tan*, and *yellow*. (H-L) Female and (H'-L') male expressions of *D. busckii* pigment metabolic pathway genes, and (M and M') cartoon representation of the pigmentation phenotype. (N) Summary of the *D. busckii* pathway use includes robust and monomorphic expression of most genes. (A, A', H, and H') *pale*, (B, B', I, and I') *Ddc*, (C, C', J, and J') *ebony*, (D, D', K, and K') *tan*, and (E, E', L, and L') *yellow*. Red arrowheads indicate robust patterns of dimorphic expression in the dorsal abdominal epidermis.



After observing that there are predictable gene expression patterns at the level of differentiation genes in the creation of dimorphic pigmentation, we next wanted to see if this predictability also exists at the regulatory level by observing Bab1 expression.

Bab1 Expression Hints to an Independent Origin of Dimorphism

(A-P) Pigmentation phenotype from a representative region of the adult A5 segment.

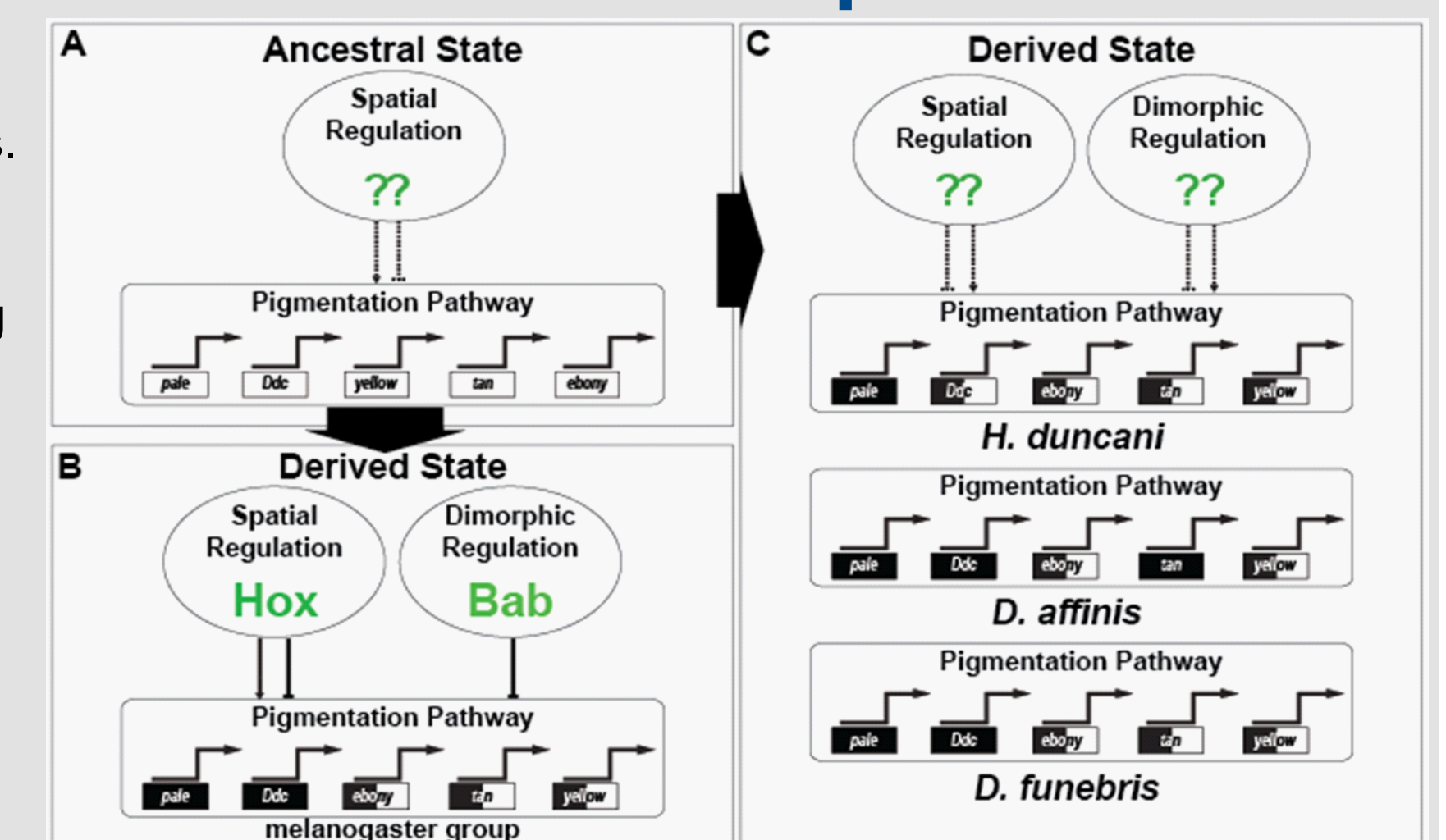
(A'-P') Bab1 expression during late pupal development in the region underlying our representative A5 pigmentation.

Bab1 expression is only dimorphic in *D. melanogaster* (A' and B'), while all other species show monomorphic Bab expression. This result indicates that the Bab1 transcription factor is a unique regulator of dimorphism in *D. melanogaster*.

Convergent Networks and Dimorphism

(1) There is predictability at the differentiation level of the network but not at the level of the regulators. (2) There appears to be flexibility for the genes that may be used to regulate the evolution of a recurring trait.

(3) When a trait appears to have evolved multiple times among different species, understanding the molecular mechanisms by which the traits are built allows us to determine what is ancestral and what is derived. Here, the lack of a consistent regulatory network regulating dimorphism suggests that this trait has independent origins.



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