Expression of Genes Involved in *Drosophila* Wing Morphogenesis and Vein Patterning Are Altered by Spaceflight

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**INTRODUCTION**

Imaginal wing discs of *Drosophila melanogaster* (fruit fly) defined during embryogenesis ultimately result in mature wings of stereotyped (specific) venation patterning. Major regulators of wing disc development are the epidermal growth factor receptor (EGF), Notch, Hedgehog (Hh), Wingless (Wg), and Dpp signaling pathways. Highly stereotyped vascular patterning is also characteristic of tissues in other organisms flown in space such as the mouse retina and leaves of Arabidopsis thaliana. Genetic and other adaptations of vascular patterning to space environmental factors have not yet been systematically quantified, despite widespread recognition of their critical importance for terrestrial and microgravity applications. Here we report changes in gene expression with space flight related to *Drosophila* wing morphogenesis and vein patterning. In addition, genetically modified phenotypes of increasingly abnormal ectopic vein venation in the *Drosophila* wing were analyzed by NASA’s VESGEN Genetration Analysis (VESGEN) software. Our goal is to further develop insightful vascular mappings associated with bioinformatic dimensions of genetic or other molecular phenotypes for correlation with genetic and other molecular profiling relevant to NASA’s GeneLab and other Space Biology exploration initiatives.

**METHODS**

**Gene Expression Analyses from Drosophila.** Spaceflight-reared larvae and adult samples were collected, processed and analyzed as described previously by Marcu et al. Briefly, the Gal4-UAS transgenic line of *Drosophila melanogaster* that expresses two copies of eGFP under the control of the hemolymph promoter was used in all experiments. RNA samples were processed and hybridized to Drosophila 2.0 Affymetrix® arrays. Six sets of larval arrays and 3 sets of adult arrays were used to provide repeats for statistical validation. The False Discovery Rate (FDR) criterion by Benjamini and Hochberg was applied to p-values.

**VESGEN Mapping and Quantification.** Binary vascular patterns extracted from grayscale images published by Johannes and Preiss (Figure 1) were analyzed by automated, user-interactive VESGEN software to generate parameters that include vessel diameter ($d$), fractal dimension ($D$) and densities of vessel area ($A_d$), length ($L_d$), number ($N_d$), and branch point ($B_d$) as described previously (Figure 2).

**RESULTS**

Microarray data from larval (Table 1) and adult flies (Table 2) returned from space measured significant changes in genes important for wing development and vein patterning compared to ground controls. For instance, the hedgehog pathway regulates the positioning of longitudinal veins such as L3 and L4. Expression of the gene *Smoothened* with hedgehog receptor activity was significantly down regulated (-0.8 fold; p-value=0.00) in space-retained adult flies. Similarly, expression of *Rhomboid 7* (-0.7 fold; ecopic veins increased in number by $N_d$ from 1 in the wildtype to 18 in Class 5; for the ecopic vesicles, $L_d$ increased from 0.0004 to 0.0095 px²; $A_d$, $L_d$, and $N_d$ for ecopic vesicles are 24, 42× and 18× greater compared to wildtype.

**CONCLUSIONS AND DISCUSSION**

Major regulators of wing disc development include genes important for the epidermal growth factor receptor (EGF), Notch, Hedgehog (Hh), Wingless (Wg), and Dpp signaling pathways. Most of these genes also play a vital role in wing vein morphogenesis. We measured significant changes in expression for a number of such genes including *Smoothened*, *Rhomboid 7*, and *Ash2*. Alternative venation of *Drosophila* resulting from a series of increasingly perturbed gene expression was successfully mapped by NASA’s VESGEN software to reveal that normal stereotyped vascular patterning was not significantly changed, despite the presence of increasingly abnormal ectopic vasculization. In the future, space-dependent changes in vascular patterning may be mapped by VESGEN to offer useful phenotypic read-outs of changes in genetic and other molecular signaling during *Drosophila* development and vascular adaptations of other important experimental model tissues such as Arabidopsis leaves and the rodent GI and retina (Figure 2).

**REFERENCES**


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