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 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 wing venation in the Drosophila wing were analyzed by NASA’s VESGEN platform software.

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 hemelotrin promoter was used in all experiments. RNA samples were processed and hybridized to Drosophila 2.0 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 ($D_v$), fractal dimension ($D_f$) and densities of vessel area ($A_v$), length ($L_v$), number ($N_v$), and branch point ($B_v$) as described previously (Figure 2).

RESULTS
Microarray data from larvae (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 Smoothed with hedgehog receptor activity was significantly down regulated (-0.8 fold; p-value = 0.00) in space-retuned adult flies. Similarly, expression of Rhomboid 7 (-0.7 fold; p-value = 0.00) and Aveugle (-0.8 fold; p-value = 0.00) were significantly down regulated in space-retuned adult flies compared to ground controls. Expression of Rhomboid and Aveugle is critical in EGF-regulated stereotyped patterning of veins. In the case of space-retuned larvae, expression of ash2 was significantly up-regulated (+0.6 fold; p-value = 0.00), suggesting possible changes in interven cell fate that determines interven patterning.

By comparing vascular parameters generated with VESGEN (Table 3, Figure 1), the eight stereotyped wing veins remained quite constant in genetically perturbed phenotypes compared to wildtype, including the most perturbed phenotype, Class 5. For example, $A_v$ and $L_v$ for stereotyped Class 5 vessels are 1.03x and 1.13x that of the wildtype. In Class 5, only the stereotyped PCV is incomplete. However, ectopic veins increased in number by $N_v$ from 1 in the wildtype to 18 in Class 5; for the ectopic vessels, $L_v$ increased from 0.0004 to 0.0095 px². $A_v$, $L_v$, and $N_v$ for ectopic vessels are 24x, 42x and 18x 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 that include Smoothed, Rhomboid, 7, Aveugle, and ash2. Altered wing venation of Drosophila resulting from a series of increasingly perturbed gene expression was successfully mapped by NASA’s VESGEN software to reveal that normal stereotypical vascular patterning was not significantly changed, despite the presence of increasingly abnormal ectopic vasculatization. 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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