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Vitamin A deprivation does not decrease fluorescence of ARF72-RFP, a label for Golgi apparatus, in *Drosophila* visual receptors.

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Each ommatidium of the *Drosophila* compound eye has 8 photoreceptors (retinula cells); the rhabdomere of each is the specialized organelle that houses rhodopsin and the visual transduction molecules. One of the primary functions of the retinula cell is maintenance of the rhabdomere, including turnover of membrane and protein (Lee *et al.,* 1996). Vitamin A deprivation reduces or eliminates rhodopsin in *Drosophila* rhabdomeres (Harris *et al.,* 1977). Vitamin A replacement synchronizes *de novo* synthesis and export of rhodopsin (Sapp *et al.,* 1991).

Although the quality of fixation of *Drosophila* photoreceptors has always been variable, electron micrographs from our lab archives showed that vitamin A deprived flies are more likely to be plagued by what we refer to as "ghosty cytoplasm" (marked with asterisks [*], Figure 1, Top left) than vitamin A replete controls (Figure 1, Middle left). (Electron micrographs are labeled thus: R is rhabdomere, G is Golgi, > is desmosome, N is nucleus, and PG is pigment granule.) We hypothesized that cytoplasmic organelles dedicated to biosynthesis, rough endoplasmic reticulum and Golgi apparatus, might be reduced by vitamin A deprivation. We tested our hypothesis using a fly stock we had been using to visualize Golgi apparatus, ARF72-RFP (ADP ribosylation factor tagged with red fluorescent protein).

Flies were lightly etherized and fixed to a glass slide for visualization of the deep pseudopupil. A typical fluorescence micrograph is presented (Figure 1, Top right); the blurry appearance compared with rhabdomere fluorescence (Stark and Thomas, 2004) is explained since Golgi apparatus is distributed throughout retinula cells. Fluorescence was quantified using a fluorescence microscope with a photometer system (Stark *et al.,* 1985). The pseudopupil image was delimited by the photometer and fed to the photomultiplier tube. Rhabdomeres were excited with a calibrated amount of 488 nm light, and a voltage response proportional to the level of fluorescence being emitted was recorded by a computer. Flies were raised at room temperature either on our yellow cornmeal food (supplemented with beta-carotene, vitamin A replete) or on Sang's medium lacking vitamin A. A strong correlation between age and level of fluorescence was noted (Figure 1, Middle right). Thus, for control, measurements were performed on flies within 12 hr of eclosion.

RFP fluorescence of vitamin A deprived flies (n = 28) was the same as that of flies reared on vitamin A replete medium (n = 27), as witnessed, since the error bars (95% confidence intervals)

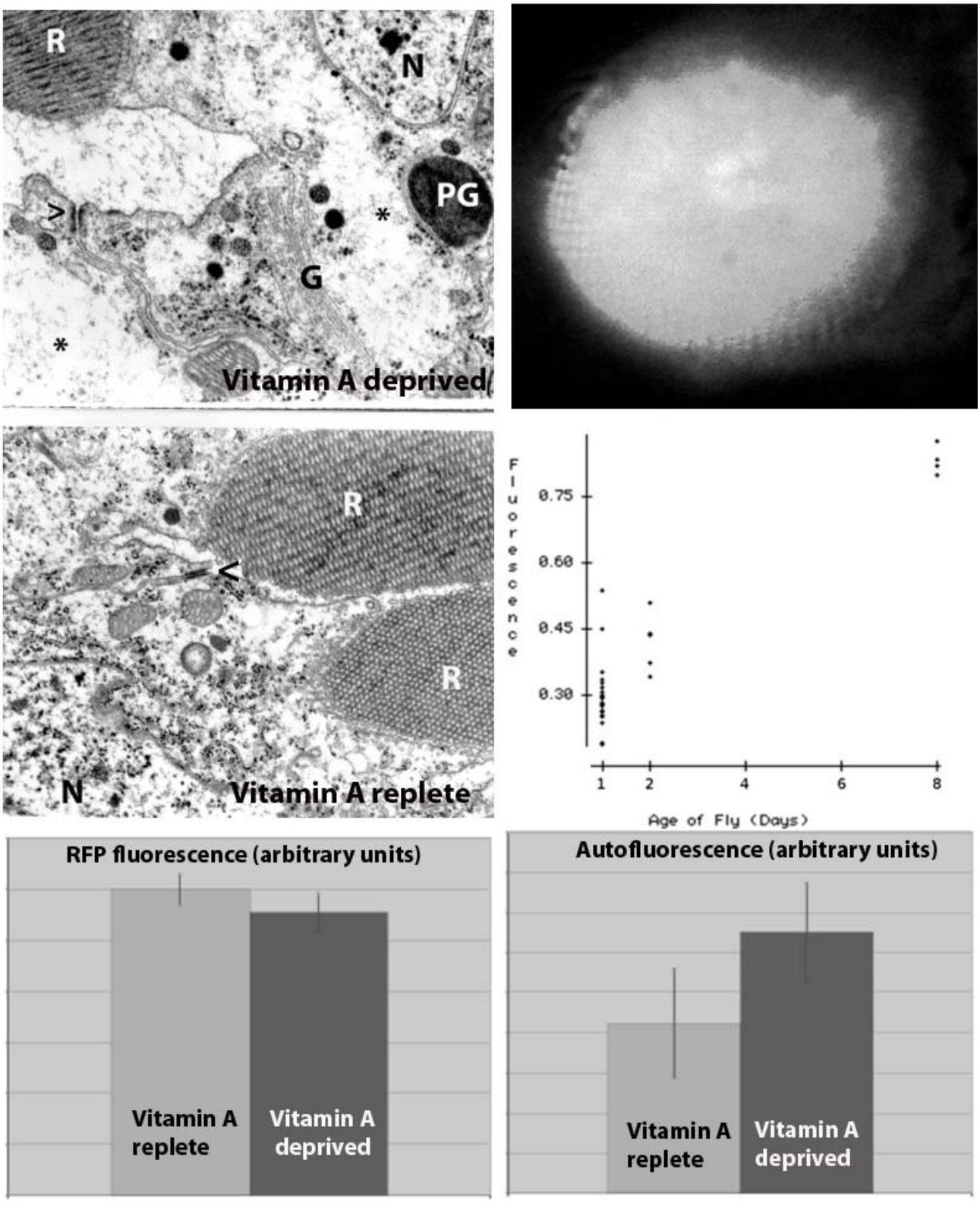
R
N
PG
G
Vitamin A deprived
R
R
N
Vitamin A replete
Fluorescence
0.75
0.60
0.45
0.30
1
2
4
6
8
Age of Fly (Days)
RFP fluorescence (arbitrary units)
Vitamin A replete
Vitamin A deprived
Autofluorescence (arbitrary units)
Vitamin A replete
Vitamin A deprived

Figure 1.

overlap (Figure 1, bottom left). The stock of flies was not genetically pure; only 28.7% of the ARF72-RFP flies raised on the replete medium and 18.3% of the flies raised on the deficient food showed RFP fluorescence. Furthermore, we noted a variation in eye color – ranging from slightly pink to pure white (in the white-eyed stock). However, there was no correlation found between eye color and whether a fly showed RFP fluorescence. Measuring at a higher sensitivity to quantify background autofluorescence among flies that were negative for RFP, the level was higher in vitamin A deprived flies (n = 125) than in those raised on replete food (n = 67) (Figure 1, Bottom right); however, again, this difference was not statistically significant as witnessed by the overlapping 95% confidence intervals. The purpose of this last control was to verify the expectation that background fluorescence did not predominate in our measurements of RFP fluorescence.

In conclusion, we reject our hypothesis that Golgi apparatus, as quantified by ARF72-RFP fluorescence, is higher in vitamin A replete *Drosophila* than in vitamin A deprived flies; further, we conclude that any difference in quality of fixation between replete *vs* deprived *Drosophila*, if real, cannot be attributed to different amounts of Golgi complexes in retinula cells.

Acknowledgments**:** We thank Prof. J.E. O'Tousa at University of Notre Dame for the white-eyed ARF72-RFP stock.

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Mechanosensation diversity across and within *Drosophila* species.

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Introduction

Mechanosensation remains a largely ignored area of organismal perception. Smell, taste, vision, and hearing are well characterized in *Drosophila*, yet we know little about mechanosensation and the somatosensory system in general. Knowledge of the genetics, morphology, and molecular biology of olfaction (smell) and gustation (taste) in *Drosophila* have in particular fueled an incredible diversity of important work in many fields. In evolutionary biology, we know that olfactory and gustatory receptors have evolved as adaptations within *Drosophila* species (McBride, 2007; Kopp *et al.*, 2009), and many other non-*Drosophila* species (for example, Hayden *et al.*, 2009; Steiger *et al.,* 2009). We have little information, however, on the role of mechanosensation in evolution.

Detecting the attack of a parasitoid may be an important role for mechanosensation. In wild *Drosophila* populations, depending on the season, as many as 35-85% of individuals can be parasitized by a variety of wasp species, and it is thought that wasps could play a major role in controlling fly population size (Carton *et al.,* 1986). Hwang *et al.* (2007) showed that nociceptive neurons, a type of mechanosensory perception, are important for larval rolling behavior, a defensive response to parasitoid wasp attack. Apart from cellular immunological response, larval rolling behavior may be *Drosophila'*s main defense against parasites. Critical to this response is immediate and accurate mechanosensation.

If mechanosensory response contributes largely to parasite avoidance, and if parasitic wasps exert strong selective pressure, then mechanosensation may serve as an adaptation. We do not know