

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

how frequently different *Drosophila* species encounter wasps, but it is possible that if mechanosensation is adaptive then divergent fly populations that experience differential parasite loads will have differential mechanosensation.

To get an initial picture of phenotypic variation in broadly defined mechanosensory response across *Drosophila*, we measured the touch response of eight *Drosophila* species and 26 of the 50 *D. melanogaster* lines from the *Drosophila* Population Genomics Project (DPGP), potentially capturing both inter- and intra-specific variation. Our results show small but significant genotypic variation within *D. melanogaster* and no significant variation between tested species. Our data are weakened by low replicate sizes within genotypes/species and by a significant effect of who performed the experiment.

Materials and Methods

We measured mechanosensory response in eight *Drosophila* species (*D. ananassae*, *D. erecta*, *D. parabiopectinata*, *D. melanogaster*, *D. sechellia*, *D. simulans*, *D. santomea*, *D. yakuba*), ranging in divergence from ~0.5 to 15 million years. Multiple lines of *D. melanogaster* were used to uncover any intraspecific variation (*w¹¹¹⁸*, *yw*, and 26 Raleigh lines collected from Raleigh, NC by T. MacKay). Flies were reared on cornmeal-yeast-agar medium at 20°C with 12-h light-dark cycle. Tactile response was measured after Caldwell *et al.* (2003). Late 2nd and early 3rd instar larvae were placed in the center of a 1% agar/distilled water 55mm petri dish after gentle washing with distilled water at room temperature. Once individuals began unidirectional motion they were gently stroked along their 2nd or 3rd thoracic segment with an eyebrow hair. Responses were scored: 0, showing no response; 1, showing brief hesitation and continued motion in the same direction; 2, anterior recoil and continued motion in the same direction; 3, turning between 0 and 90 degrees; 4, turning greater than 90 degrees. Individuals were tested 4 times with ~5 seconds between stroking. Larvae that did not move were discarded. Overall scores for individuals were summed, and responses ranged from 0 to 16. Measurements within genotype/species were taken over multiple days at various times by both EE and BW. Mean and standard error were measured for each genotype or species.

Our results did not fit a normal distribution and are heteroscedastic. Thus we used non-parametric analyses to see if genotypes/species had significantly different touch responses. We used Kruskal-Wallis rank sum test (KW) and a general linear model (GLM). In our model, our total response by test (*y*) could be influenced by variance in genotype/species (*G*), experimenter (*E*), and their interaction(*G*×*E*).

$$y = b_0 + Gx + Ex + GxEx + \varepsilon$$

We analyzed *D. melanogaster* (*D. mel*) and *D. simulans* (*D. sim*) independently, sorting by genotype. For cross-species comparisons, all *D. mel* and *D. sim* genotypes were taken to calculate their species' respective values. All statistical analyses were performed with R (2005).

Results/Discussion

We found variation in touch response within both genotype (*D. mel* and *D. sim*) and species (Figure 1a-c). *D. melanogaster*, in particular, had a broad range of responses, with some genotypes

contributing heavily to the species range (307, 315, and 357). Within *D. simulans* and within other species responses were much less varied.

Within *D. melanogaster*, genotype contributed significantly to total response (KW: $\chi^2 = 51.5$, $p = 0.003$) with genotypes 774 ($p = 0.032$), 786 ($p = 0.034$), w^{1118} ($p = 0.003$), and yw ($p = 0.011$) showing significantly different contributions to overall variance in touch response. Overall, the experimenter did not have a significant influence on touch response ($p = 0.556$), but the interaction of genotype with experimenter did on genotypes 315 ($p = 0.002$), 357 ($p = 7.2e-06$), and 399 ($p = 0.032$). *D. simulans* did not show any difference between genotypes (KW: $\chi^2 = 1.88$, $p = 0.596$), and no genotype, experimenter, or interaction contributed to overall variance. Across all species, we found no significant differences in response due to species alone (KW: $\chi^2 = 9.42$, $p = 0.224$). We did find a significant effect of experimenter; however, (KW: $\chi^2 = 29.82$, $p = 4.74e-08$). No genotype, experimenter, or G×E interaction contributed significantly to overall response variance.

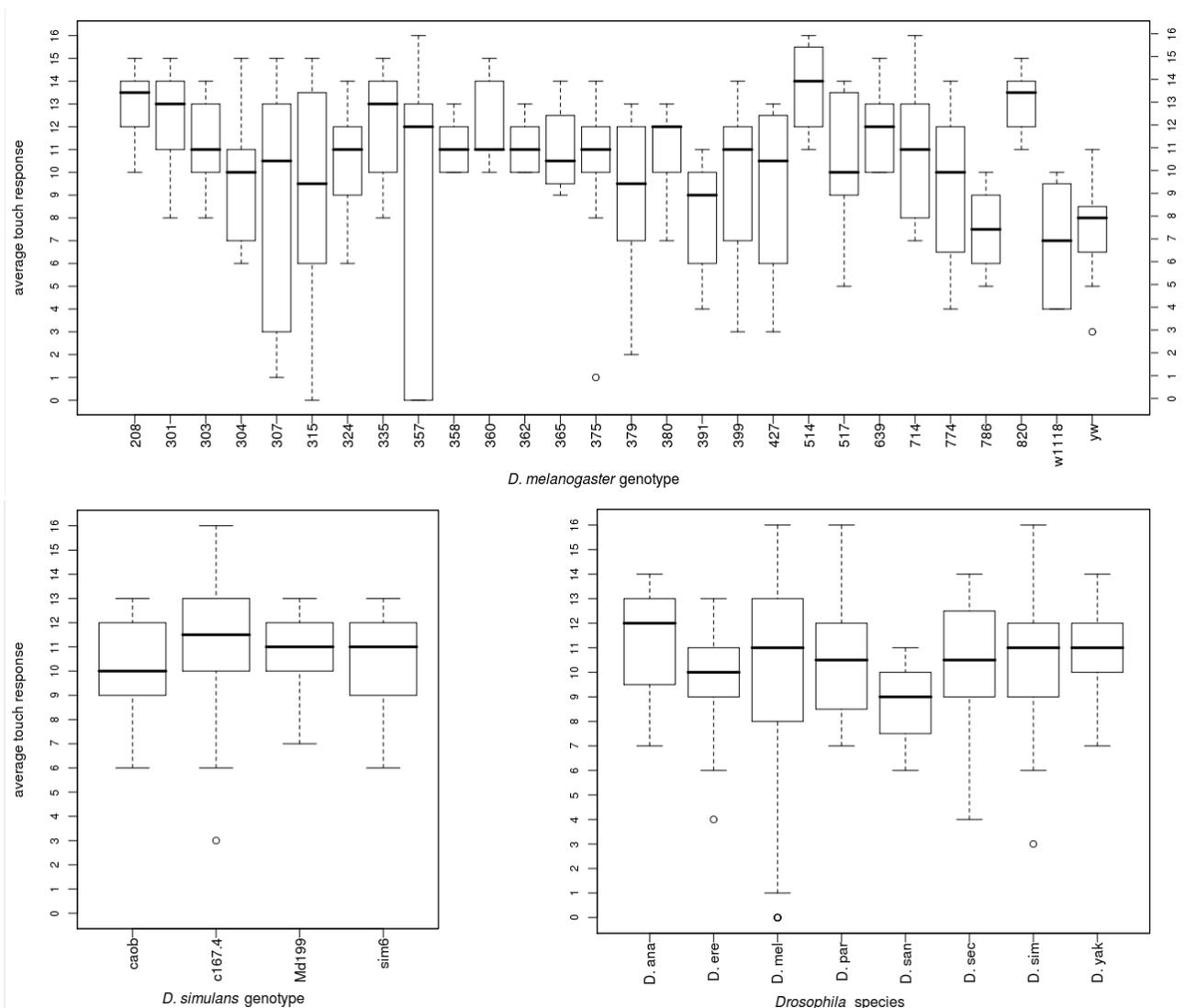


Figure 1. Box-and-whisker plot surveying mechanosensation across, a) *D. melanogaster* Raleigh inbred lines, b) *D. simulans* genotypes, and c) multiple *Drosophila* species. Aggregate results from *D. melanogaster* and *D. simulans* are contained within their species' averages in c.

Our replicate sizes were not equal across tests. For example, we tested 196 *D. melanogaster* larvae but only 8 *D. sechellia*. When standard deviation for response mean was regressed against sample size for each species, we found a positive correlation ($R^2 = 0.311$), and we found an even stronger positive correlation between inter-quartile range for response means and species sample size ($R^2 = 0.575$). The correlation coefficients, however, are somewhat low, and when *D. melanogaster* is removed this correlation disappears.

We found weak but significant differences in genotype touch response within *D. melanogaster*. Interestingly, we found strong genotype by experimenter effects in certain genotypes. Two factors may account for this. First, we attempted to elicit larval response as uniformly as possible, but subtle differences in technique could have driven dynamic responses in some genotypes. Thus, the interaction could be real, and certain genotypes are more sensitive to the quality of touch than others (for example, accidentally poking instead of stroking). Second, this interaction could also be an artifact of varied replicate sizes for each experimenter within genotype. The large influence of experimenter on species-wide response variance could also be due to this asymmetry.

We chose the method of Caldwell *et al.* (2003), because it appeared to elicit consistent responses in our pilot experiments with *D. simulans* even with multiple experimenters. Expanding our tests to other species uncovered unexpected variation, and this could easily have been due to technical error (for example, elicitation differences and unequal sample sizes). However, when using this test or others like it in the future, the unconscious influence of experimenter should be tested and controlled.

Mechanosensation could contribute to larval fitness. Parasitoid wasp encounters are common in many *Drosophila* populations, and rolling behavior is a typical defense behavior in response to ovipositor touch. In biomedical research, *Drosophila* offers great opportunity to dissect the genetic and molecular mechanisms of mechanosensation. Understanding the genetic variation existing across *Drosophila* species would thus inform a variety of fields. While we found some evidence of genetic variation in mechanosensation within *D. melanogaster*, some confirmation is required. Future tests may take "high" and "low" genotypes, having responses on the extreme ends, and cross them to see if touch response is heritable.

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