Examining Genetic Background and Synaptic Morphology with Heterozygotes

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***Short Abstract* — Most genetic studies in *Caenorhabditis elegans* are done in the N2 background, but it is unclear how this laboratory background affects these genetic studies. To examine these subtle effects, particularly on synapse development, we examine subtle synaptic mutants, using high-throughput microfluidics and computer vision to obtain the requisite sample size. With heterozygotes between these and wildtype strains, we examine different backgrounds rapidly without extensive breeding, identifying an unknown interaction between a true wildtype and the genes *jkk-1* and *unc-104*. This provides a methodology for studying multigenic and background interactions, particular their interaction with genetic studies in *C. elegans*.**

***Keywords* — Microfluidics, *C. elegans*, Quantitative Phenotyping, Computer Vision, Epistasis, Synapses, Synaptic Trafficking, Synaptic Morphology, High-throughput**

I. Background

THE model organism *Caenorhabditis elegans* is prized for ease of handling and genetic manipulation. This encourages use for genetic studies, an endeavor that has yielded ground-breaking results. However, the vast majority of genetic studies in *C. elegans* have been done on the strain N2, in which decades of cultivation has resulted in behavioral, physiological, and genetic divergence from wild populations [1]. It is probable that this genetic background modifies the results obtained in genetic studies, but the exact significance of these effects is unknown.

Evaluation of this has been bottlenecked by difficulties in experimental procedure, as well as the subtlety of background effects. Large-scale phenotypic effects drown out genetic background effects, while subtler effects require much larger sampler sizes, fluorescent markers introduced into every background under study, and detailed observation, rendering examination of more than a handful of genetic backgrounds impracticable.

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II. Methodology and Results

To address these difficulties, we introduce a methodology tying together several innovations made by this lab. These include the use of **microfluidics for high-throughput imaging**, **computer vision** for rapid and accurate quantitative phenotyping, and the use of **heterozygotes for comparison of genetic backgrounds** without burdensome, repeated outcrossing. The former two reflect innovations demonstrated previously by this lab [2], while the latter is a novel approach to a challenging experimental problem.

We choose to use a combination of the two recently introduced **dominant** synaptic mutants *jkk-1 (km2)* and *unc-104 (wy673)*, along with the synaptic marker *Pmig-13:snb-1::yfp*, all in the N2 background. These were chosen because of the relative subtlety of the phenotypes involved, as well as the relatively unexplored nature of synaptic morphology.

By crossing these strains with wildtype strains of *C. elegans*, producing heterozygous F1 progeny, we show that **background effects on a subtle feature like synaptic morphology can be discerned**, using the N2-cross as a control. In particular, we show that the genetic background of the Hawaiian strain CB4856 exerts an effect on synaptic morphology similar to the *km2* and *wy673* alleles, without reinforcing these mutations when present. Since CB4856 does not carry mutations known to affect synaptic morphology, the CB4856 wildtype background exerts a novel effect on these phenotypes. The choice of genetic background used for a given study can thus have a strong effect, and it is important to understand the interactions of genetic background with phenotype.

We thus demonstrate that our method is **capable of detecting subtle genetic background effects**, and also that **these effects are an important confound to genetic discovery**.

References

[1] McGrath, PT. (2011) Parallel evolution of domesticated *Caenorhabditis* species targets pheromone receptor genes. Nature 477, 321-325

[2] Chung, K., Crane, M. M. & Lu, H. (2008) Automated on-chip rapid microscopy, phenotyping and sorting of C. elegans. Nat. Methods 5, 637–643.