Effects of *nlg3* expression level on fecundity

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GOAL: Identifying the fecundity in *Drosophila melanogaster* with diverse genetic crossings.

INTRODUCTION:

Neuroligin 3

In humans, *neuroligin* genes have been linked to several neurodevelopmental and mental disorders, including autism spectrum disorder and schizophrenia. Neuroligins belong to the family of cell adhesion proteins that are important for synaptic maturation and functioning (Xing et al., 2014). These molecules are essential for the formation and maintenance of chemical synapses, which are the fundamental units of neural circuitry (Xing et al., 2014). In *Drosophila melanogaster* (a key model species), the autism-related *nlg3* protein is crucial for synapse development and regulation, influencing social spacing behaviour (Robinson et al., 2025).

Why *Drosophila*?

Drosophila serves as an advantageous model organism because it breeds efficiently, is easy to handle, and female flies produce large number of progeny, up to 100 eggs per day and up to 2,000 over their lifetime (Giansanti et al., 2025, Simon lab personal communication). *Drosophila* provides a powerful system due to the high conservation of human-associated genes within its genome. This allows researchers to generate mutants across multiple genes to investigate protein interactions and mimic pleiotropic characteristics observed in various human diseases, such as cancer, sleep disorders, aging, and neurological or neuromuscular disorders such as Parkinson's disease (Giansanti et al., 2025).

Fecundity

In *Drosophila*, reproduction and lifespan are closely connected through a fecundity-longevity trade-off, where investing energy into reproduction can shorten lifespan. Because both reproduction and somatic maintenance rely on shared metabolic resources, higher reproductive output often reduces longevity, while reduced fecundity can extend it. This balance is also shaped by factors such as social environment, stress, and genetic background, which influences how flies allocate energy between survival and reproduction (Brenman-Suttner et al., 2020).

Preliminary data in Simon's lab show that *nlg3* deficient flies have an increased number of progeny, and a decreased life-span. Flies missing only one copy of *nlg3* live longer, but their fertility has not been assessed. This project will assess fecundity of flies with diverse alteration of *nlg3* in various experiments.

GAL4/UAS System

The GAL4-upstream activating sequence (UAS) is a binary gene expression tool adapted from yeast that allows tissue-specific or temporal control of transgene expression in *Drosophila melanogaster* (Brand & Perrimon, 1993). It consists of two separate transgenic lines including a

driver line that expresses the yeast transcription factor GAL4 under the control of a specific promoter (for neurons *elav-GAL4*), and a responder line that carries the upstream activating sequence (UAS) placed before a target gene. When the two lines are crossed, GAL4 binds to the UAS sites in the progeny, activating transcription of the downstream gene specifically in tissues where the driver promoter is active. This allows researchers to either overexpress or silence genes.

EXPERIMENT 1:
Effects of *Nlg3* Gene Deletion on Fecundity in *Drosophila melanogaster*

HYPOTHESIS:
Homozygote deletion of the *nlg3* gene in *Drosophila melanogaster* will lead to reduced fecundity due to the loss of *nlg3* expression affecting reproductive processes in the flies, while the heterozygote deletion will lead to reduced fecundity because they live longer.

HUSBANDRY:
In this experiment investigating the effects of *nlg3* gene deletion on fecundity in *Drosophila melanogaster*, three fly genotypes will be maintained: Def/Def representing the deletion treatment group, Cs/Cs as the wild-type control group, and Def/Cs resulting from the cross between deletion and wild-type flies, also considered a treatment group.
In the wild, fruit flies feed on yeast, bacteria, and plant matter found within ripe or rotting fruit. In the laboratory, flies will be cultured on a cornmeal, yeast, and agar-based medium supplemented with carbohydrates and preservatives. The firmness of the food will be adjusted by varying agar concentration depending on strain health and larval activity. Ingredients will be combined with water, boiled, and poured hot into vials to cool and form a solid food plug at the bottom.
Adult flies will be transferred into these vials capped with cotton or foam plugs, where they will lay eggs on the surface of the food. Once hatched, larvae will burrow into the medium and progress through three instar stages before wandering third instar larvae leave the food to pupate along the vial walls. Because freezing flies or larvae is not currently effective, stocks will be maintained by transferring adults to fresh food every two to three weeks to prevent contamination from bacteria, mold, or mites. In some labs, strains not in active use are stored at 18 degrees Celsius to slow development and reduce maintenance frequency, in this case, at room temperature. These husbandry practices are based on those described in *Genetics on the Fly: A Primer on the Drosophila Model System* and will ensure healthy flies for consistent fecundity testing across all genotypes (Hales et al., 2015).

DELETION:
Deletion refers to the removal of the *nlg3* gene from the genome of *Drosophila melanogaster*. Flies that are homozygous for this deletion (Def/Def) lack functional copies of *nlg3*, which is expected to negatively affect biological processes regulated by this gene. Since *nlg3* is believed to play a role in reproductive functions, its absence leads to reduced fecundity in these flies. Heterozygous flies (Def/Cs) carry one deleted and one normal copy of the gene and show intermediate levels of fecundity. By comparing both deletion groups to wild-type flies (Cs/Cs) with intact *nlg3* genes, the experiment aims to determine how loss of *nlg3* impacts reproductive capacity, with the hypothesis that fecundity will decrease due to the gene deletion.

CROSSES:
Def/Def x Cs/Cs → Def/Cs (heterozygous deletion treatment group)

In this experiment, a single genetic cross will be performed between homozygous *nlg3* deletion flies (Def/Def) and wild-type flies (Cs/Cs) to generate heterozygous offspring (Def/Cs). The Def/Def flies will be maintained as the deletion treatment, and the Cs/Cs flies will function as the wild-type control. This cross allows comparison between homozygous deletion, heterozygous deletion, and wild-type flies to assess the impact of *nlg3* deletion on fecundity.

EXPERIMENT 2:
Knockdown of n*lg3* using RNA Interference

GAL4/UAS System and RNAi
RNA interference (RNAi) is a method used to reduce gene expression. It works by producing double-stranded RNA which triggers degradation of complementary mRNA, reducing gene expression (Dietzl et al., 2007; Ni et al, 2011). This is controlled using the GAL4/UAS system which allows for activation of the RNAi.

HYPOTHESIS:
Reducing *nlg3* expression through RNAi will decrease fecundity because of its role in lifespan regulation.

CROSS:
RNAi/cs X nlg3GAL4/attp40 = nlg3-Gal4>UAS-RNAi = decreased fecundity

EXPERIMENT 3:
Upregulation of n*lg3* using cDNA

HYPOTHESIS:
Upregulating nlg3 expression (via UAS-nlg3-cDNA driven by nlg3-Gal4) will increase fecundity of the Drosophila, because its longevity is reduced.

Gal4/UAS System and cDNA
Complementary DNA (cDNA) is synthesized from messenger RNA (mRNA) using the enzyme reverse transcriptase. Because cDNA lacks introns removed during RNA processing, it is particularly useful for gene cloning and gene expression studies (Pelley, 2007).

As mentioned above, the UAS-Gal4 system is a genetic tool in which the GAL4 transcription factor binds to upstream activating sequences (UAS) to drive the expression of genes located downstream of the UAS promoter. In this experiment, *nlg3-Gal4* flies will be crossed with flies carrying *nlg3* cDNA to test the following hypothesis: if the Gal4/UAS system activates the cDNA construct, it will lead to an increase in *nlg3 levels.*

CROSSES:

1. nlg3-Gal4/nlg3-Gal4 X UAS-nlg3-cDNA/UAS-nlg3-cDNA = nlg3-Gal4/UAS-nlg3-cDNA = increased nlg3 activation = increased fecundity

2. UAS-nlg3-cDNA/UAS-nlg3-cDNA X Cs/Cs = UAS-nlg3-cDNA/CS = normal nlg3 activation= normal fecundity

3. nlg3-Gal4/nlg-Gal4 X Cs/Cs = nlg3-Gal4/Cs = normal nlg3 activation = normal fecundity

AMPLIFICATION METHODOLOGY

Flies will be transferred from one bottle to another by gently tapping the original bottle down on a soft surface. The plug will be removed carefully ensuring no contamination, and the bottle will be inverted over a fresh food bottle. The flies will be gently tapped again to transfer, after which the top bottle will be removed quickly, and the new bottle will be sealed with a clean plug.

Protocol for Fertility Assay (Simon Social Lab provided by Brenda)

TIMELINE:

Week 1	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week 2	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week 3	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday

(sample calendar timeline, can be adjusted as needed)

AMPLIFICATION STEPS:

1. Dump flies out *(Sunday week 1)*
2. Collect virgin flies between 4 to 8 hours of dumping *(Monday week 2)*
3. Leave for 3 to 4 days to grow and then separate 5 males and 5 females into vials (3 replicates) *(Thursday week 2)*
4. After another 3 to 4 days, dump the parents out to allow for the eggs to start the reproduction cycle *(Tuesday week 3)*
5. When 3rd instar pupae are visible, start counting the number of enclosed flies daily, this marks day 10. You will be counting everyday on carbon dioxide until day 20. (Sunday week 3)

NOTES:

- Count carefully between days 10-15
- Expect numbers to start decreasing after day 17-18 to almost none on days 19-20

STEPS TO ACHIEVE:

- ✓ Read articles & reviews
- ✓ Amplify
- × Cross the flies
- × Collect virgins
- ✓ Use calendar to plan
- ✓ Learn protocol

References

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