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Research Idea

Identifying genetic factors that increase cognitive reserve: A theoretical approach

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Abstract

Studies have demonstrated that some individuals display pathological hallmarks of Alzheimer's disease (AD) but are not afflicted with cognitive decline. The ability to maintain cognitive function despite the presence of pathology is referred to as cognitive reserve. This project aims to identify the molecular pathways involved in cognitive reserve using *Drosophila melanogaster* (*Drosophila*) models of AD. Specifically, a theoretical approach using experimental evolution to drive a population of AD-like *Drosophila* carrying a tau mutation to develop cognitive reserve is proposed. To accomplish this, a population of AD-like *Drosophila* will be placed in a single population cage along with wild-type flies and forced to compete for food and water. The first generation of AD-like *Drosophila* will be generated using random mutagenesis of the initially isogenic AD-like fly. The selected tau mutant displays a rough eye condition which allows for easy distinction between tau mutant and wild-type flies. It is hypothesised that AD-like flies with cognitive decline will be unable to survive because their limited cognitive abilities will prevent them from effectively competing for food and water. In contrast, AD-like flies with mutations that promote cognitive reserve will be better capable of survival. After 90-99% of mutant flies have died, the surviving mutant flies will be back-crossed to the P1 mutant to maintain tau mutation stability. It is expected that artificial selection will result in the creation of a generation of tau mutant flies that demonstrate cognitive abilities comparable to those of wild-type flies despite maintaining an AD-like tau mutation. This approach will monitor the successful

trajectory of the evolution of increased cognitive reserve through survival curve analysis and measures of cognition. A limitation of the method is that only a dominant mutation or series of dominant mutations would be identified using this approach.

Keywords

Alzheimer's disease, tauopathy, experimental evolution, *Drosophila melanogaster*, animal model, cognition, dementia

Overview and background

Although there has been success in extending human lifespan, less success has been obtained related to "mindspan" or maintaining mental abilities over time. A major contributor to a declining quality of life associated with mindspan is Alzheimer's disease (AD). AD is a progressive neurodegenerative disorder affecting nearly 20 million people worldwide with no treatments or cures available. While drugs have been effective in clearing the neuropathology, the positive effects on cognition have been minimal. The observation that clearance of plaques and tangles is distinct from a parallel improvement in cognition, along with substantial evidence that certain individuals with high neuropathology display negligible defects in cognition, suggests that distinct molecular pathways are involved in cognitive decline and neuropathology. Amongst the theories put forward to explain the maintenance of cognition in some individuals with neuropathology, the concept of cognitive reserve has emerged. We hypothesise there is a divergent molecular pathway and that altered gene expression within a cognitive reserve pathway contributes to resilience of cognition to neuropathological burden. Such a hypothesis is testable using modern experimental methods that can drive an organism to develop cognitive reserve in the presence of high AD pathology.

Cognitive reserve is the observation that an individual shows unusually strong cognition despite aging, disease or other factors (McQuail et al. 2021). Evidence of cognitive reserve has been demonstrated. For example, postmortem examinations (Katzman et al. 1988) revealed that neuropathology of AD did not necessarily correspond to cognition. It has been well established that individuals with higher educational attainment are less at risk of AD-mediated cognitive decline than individuals with less education (Stern 2012). The results of a 1995 study indicated that educated individuals did not live as long after being diagnosed with AD as uneducated individuals (Stern et al. 1995). A possible explanation is that education is linked with greater levels of cognitive reserve. Such reserve allowed certain individuals to display an extended mindspan which delayed the manifestation of symptoms. It was also determined that engagement in leisure activities is associated with a decreased risk of dementia (Scarmeas et al. 2001). Cognitive reserve has also been examined in relation to head injury. Kesler and colleagues determined that individuals with higher educational attainment maintained better cognition after severe head injuries than individuals with lower educational attainment (Kesler et al. 2003). Cognitive reserve is different from brain maintenance, in which strong cognition is kept due to slow brain aging (

Katzman et al. 1988, McQuail et al. 2021). Research on cognitive reserve in humans examines three domains: cognitive performance, brain aging (determined through examination of brain anatomy) and cognitive reserve itself (McQuail et al. 2021).

It is proposed that an experimental model using AD-mutant *Drosophila* can be used to select for increased cognition, despite the presence of high AD pathology. To accomplish this goal, a homogeneous population of *Drosophila* expressing mutant tau protein, such as those used by others (Wittmann et al. 2001, Cowan et al. 2011), will be irradiated to generate random mutations within this population. These particular tau mutants have the benefit of both a measurable decline in cognitive performance and the presence of the rough eye phenotype that can be used to track the persistence of the tau mutation. This initial "library" of mutated, mutant tau *Drosophila* will then be used in an experimental evolution-based experimental design as described below (see Methods). Alternatively, with the results shown by Levy et al. (Levy et al. 2022), a direct measurement of continued tau aggregations is also possible to verify that flies have not reverted to normal tau expression. Additional screening will include a battery of cognitive tests to validate that cognition is improved. Next-generation sequencing methods can then be used to compare the mutants that developed cognitive reserve (improved cognition, despite the presence of disease) and have memory comparable to wild-type to the original P1 mutant flies.

Tau in AD

Tau is a microtubule-associated protein involved in the maintenance of axonal stability. During AD progression, tau becomes post-translationally modified in several ways, with the most research interest directed toward changes in the phosphorylation state. Indeed, recent work has focused on excessive tau phosphorylation at specific sites as a biomarker of AD (Itoh et al. 2001, Aguillon et al. 2022, Johansson et al. 2023). Such an important role of tau in AD is noted in animal models that carry various tau mutations, such as P301L (Bukhari and Feany 2022) or R406W (Wittmann et al. 2001), amongst others. Abnormal accumulation and aggregation of tau leads to phenotypic changes in animals similar to those observed in humans. Therefore, the use of tau mutant *Drosophila* in the present study should be well-suited to identifying relevant molecular targets of cognitive reserve corresponding to those in AD (Bagyinszky et al. 2014, de Rojas et al. 2021).

Phenotypes of AD

AD is one of many causes of dementia, which is widely accepted as a progressive decline in two or more areas of cognition. Thus, individuals with AD are characterised by a range of phenotypes. Individuals suffering from very mild AD show variable deterioration of functional, cognitive and behavioural abilities (Atri 2019) that begin years before a clinical diagnosis. During the later stages, dysphoria, psychosis, aggression, wandering and improper sexual behaviour may also be observed (Mega et al. 1996). Notably, a typical phenotype is the sleep cycle disruption (Wang and Holtzman 2020), along with shifts in mood, increased anxiety, depression, withdrawal and apathy (Atri 2019), as well as locomotive dysfunction. Unlike late-onset AD, in which the medial lobe is most susceptible

to atrophy, studies have suggested that the parietal and occipital cortex are most susceptible to atrophy in early-onset AD patients (Adriaanse et al. 2014). The observation of such phenotypes supplies an opportunity to use behavioural assessments (e.g. sleep patterns, motor function) to monitor *Drosophila* progression or degeneration.

Genetics of cognitive reserve

The expression of specific genes has been suggested to be linked to cognitive reserve. Yegla and Foster (Yegla and Foster 2022) studied cognitive reserve-associated genes in the CA1 region of the hippocampus of young and old rats. Genes that have been linked to cognitive reserve include *Fos*, *Hmgcr* and *Rgn.* Usually, cognitive reserve genes are found to function as negative regulators of the aging process, affecting factors such as cognitive stress (Yegla and Foster 2022). Heuer and colleagues (Heuer et al. 2020) used a systems genetics approach to examine how gene expression relates to cognition in the aging process and AD. An examination of the molecular processes that affect cognitive reserve in mice found that molecular pathways related to microglia and astrocytes function as biomarkers for AD (Heuer et al. 2020). Specifically, a negative relationship between the expression of Fibroblast growth factor-2 (*Fgf2)* and short-term memory outcomes was observed. In contrast, Kiyota and colleagues (Kiyota et al. 2011) determined that *Fgf2* may help prevent the development of AD pathology. Although studies disagree on the exact effects of the expression of *Fgf2*, it is clear that this gene plays a role in cognitive function during AD. In addition to *Fgf2*, many other genes have been associated with cognitive reserve. For example, Ramos-Miguel et al. examined components of the SNARE complex and found that *STX1A, SYT12, SNAP25* and GABAergic *STXBP1* were linked with stronger cognition despite AD pathology (Ramos-Miguel et al. 2021).

Cognitive reserve has also been studied with respect to other neurological conditions or pathologies in addition to AD (Afimova et al. 2013). They found that a higher frequency of the G allele of *SNAP-25* was correlated with greater cognitive fitness and that a G allele might increase the likelihood of greater cognitive reserve (Afimova et al. 2013). It was also determined that the expression of *CNTLN* and *PROK1* in the coronary arteries and the expression of *PRSS50* in the atrial appendage interacted with deposition on episodic memory performance (Hohman et al. 2017). In addition, predicted gene expression levels interacted with amyloid deposition on executive functioning performance (Hohman et al. 2017). Overall, this suggests that subsets of genes impact resilience and demonstrate that cell-cycle regulation, angiogenesis and heme biosynthesis affect the progression of AD.

Drosophila as a model for neurodegeneration and cognitive reserve

Many advantages exist for modelling neurodegenerative disease using *Drosophila*. *Drosophila* is simple to maintain, has a short life span and reproduces rapidly. Although *Drosophila* is a relatively simple organism, it engages in various complex types of behavior, such as courtship behaviour, learning, memory, social interaction, aggression, grooming and addiction (Cowan et al. 2011). *Drosophila* is an ideal model for studying AD because its genetics are tractable, a variety of gene disruption methods exist for *Drosophila*, rapid

production of novel transgenic flies is possible and its genome has been sequenced and annotated. In addition, the cellular and molecular bases that drive *Drosophila's* biological processes are essentially the same as those of mammals, with over 75% of human disease-related genes having *Drosophila* orthologues (Cowan et al. 2011).

Assessment of cognitive reserve

As cognitive reserve has been defined as the maintenance of good cognition despite neurodegenerative pathologies, the success of this experiment can be determined by the extent to which flies demonstrate strong cognition while continuing to carry a tau mutation. Currently, tests such as the Mini-Mental State Exam are used to assess cognitive health in humans. This particular test determines cognitive health by examining how participants perform on assessments measuring factors such as orientation, working memory, memory, visuospatial skills and language (Farias et al. 2008). To accurately gauge the cognitive health of the flies in this experiment, it would be ideal to find and assess the equivalent of each of these six factors. In this study, a T-maze for the assessment of short-term memory (Dissel et al. 2017), courtship conditioning to assess long-term memory (Broughton et al. 2003) and a heat-maze (Foucaud et al. 2010) (analogue of the Morris water maze) to assess spatial memory would be used. A review by Gistelinck and colleagues (Gistelinck et al. 2012) describes the impact of tau overexpression on olfactory learning and memory as measured by aversive phototaxis suppression that can be used to monitor tau toxicity in *Drosophila* that overexpress human tau. It will be important to monitor the original tau mutant using the appropriate measure of cognition that most closely aligns with the model to confirm that experimental evolution is taking place with the particular human tau isoform overexpression system being used.

What is experimental evolution?

Experimental evolution is a method of studying the evolutionary process by examining the genetic changes that occur when natural selection is applied to a population of organisms within a lab environment (Izutsu et al. 2016). Numerous advantages exist when using *Drosophila* as a model organism for experimental evolution. They have a short generation time, are simple to maintain and abundant public genomic resources exist (Burke and Rose 2009). Selection can produce physiological changes quickly because it is simple to maintain *Drosophila* populations with many variations (Burke and Rose 2009). Several models exist for guiding *Drosophila* evolution that are available for the present study. A 2016 paper by Fuse and colleagues records how researchers in Japan kept a *Drosophila* population under constant darkness for 1,500 generations (Izutsu et al. 2016). Flies in this population adapted to their environment and displayed phototactic behaviour and increased fecundity (Izutsu et al. 2016). Although it is possible to allow mutations to occur naturally, the process can be speeded up by inducing mutagenesis. Currently, two methods exist for subjecting *Drosophila* to mutagenesis. The first method uses an alkylating agent, such as ethyl methanesulphonate, or EMS, to substitute a nucleotide base (Lin et al. 2014). It is cost-efficient and helpful in producing mutations of different natures in a single gene (Lin et al. 2014). The second method is irradiation, proposed here.

Objectives

The objective of this study is to identify genes associated with cognitive reserve using *Drososphila* as a model organism.

Impact

Amongst people with AD, some individuals display little evidence of significant cognitive decline despite elevated levels of neuropathology. It is probable that such resistance to decline is due to underlying differences in these individuals' gene expression. Therefore, it is likely that resistance to AD-driven cognitive decline is a heritable trait.

The hypothesis that gene expression within a cognitive reserve pathway exists can be tested using experimental evolution to drive the expression of cognitive reserve in animal models of AD. Although the present proposal is theoretical, prior research has shown that age-related cognition genetics in *Drosophila* can be evaluated using experimental evolution methods (Zwoinska et al. 2017). This prior work demonstrates the feasibility of the approach proposed here. In the proposed project, experimental evolution designed to drive *Drosophila* to overcome the cognitive effects of AD pathology will parallel human evolution that has resulted in genetic variants that include the ability of some individuals to defeat pathology-driven cognitive decline. The expected cognitive improvements resulting from the evolutionary process are understood to be an indication of increased cognitive reserve.

Analysis of the genome of *Drosophila* (see Ready to Validate in Figure 3) that has undergone multiple generations of experimental evolution will likely result in many genetic changes to be evaluated. While such a task appears daunting, the results of this study are not intended to be “simple" to analyse, but possible. An initial examination would be to group the identified genes into functional categories as has been done in prior studies with a primary focus on the genes associated with synapse formation. By determining the genetic sequences resulting from this experimental evolution method, it is hypothesised that genes associated with clinically meaningful outcomes relevant to the human condition would be identified. Future drug development could then target these genes and/or their molecular pathways with the intention of improving cognitive reserve in human patients. Such treatments would not focus on reducing pathologies, but on allowing individuals to perform and carry out activities of daily living longer than disease progression would normally allow. Furthermore, these findings would add to the body of knowledge in a meaningful way by using a robust and established animal model to interrogate a molecular pathway that is otherwise difficult to analyse in a human population.

As part of this theoretical method, confirmation of the influence of the mutations on cognition rather than physical performance or some other phenotype that could allow mutant flies to compete with wild-type is proposed. Memory testing is an important aspect of this study as it validates cognitive improvement. If it is observed that mutant flies are improving in their ability to seek food and water without measurable improvement in cognition, it is expected that sensory or motor skills were also being impacted. These

findings could be confirmed using appropriate assays to analyse such functions. If improvements in sensory or motor skills were confirmed, the findings would be equally valuable, albeit regarding unintended targets of the evolutionary pressure such as olfaction, muscle coordination, strength, endurance or other relevant variables to competing for resources.

Identifying a molecular pathway involved in cognitive reserve would profoundly impact cognitive research beyond AD. For example, it would be expected that the pathways found using this method would open the door to new drug interventions to improve cognition. Such a development would profoundly impact the array of diseases and conditions that result in cognitive decline. Additionally, with current trends in "brain-hacking," pharmacological or other interventions in the identified pathway(s) would be helpful to enhance cognition and have applications for emergency personnel or the warfighter who must make critical decisions under stressful conditions. Therefore, if this study proves successful, the identification of molecular targets to enhance normal cognition or prevent/reverse memory deficits is closer than ever before.

Implementation

Drosophila wild-type and mutants

Mutant *Drosophila* flies that express human tau protein will be used (Wittmann et al. 2001). Several mutant flies have been previously characterised to have cognitive decline. This mutation is easily monitored by the continued presence of the rough eye phenotype. The tau mutant used is associated with early-onset familial dementia and significantly shortened the lifespan of afflicted flies as compared to wild-type tau (Wittmann et al. 2001). A mutation such as this would allow the wild-type flies to rapidly outcompete the mutant flies resulting in a shorter generation time. However, any of several well-characterised tau mutations may be used if impaired cognition and rough eye phenotype are displayed (Ambegaokar and Jackson 2010).

Population maintenance and breeding plan

A stock of P1 mutants to provide a supply of females is to be maintained at a temperature of 25°C, at 60% humidity, at 12-hour light/dark cycles in order to be bred to males from each generation of experimental flies created. This is to maintain the presence of dominant mutations of tau between generations. These flies will be kept on a yeast, dark corn syrup and agar diet. Given that this study will be longitudinal over many generations, an awareness and strategy for managing potentially confounding variables is warranted with excellent work available from Piper and Partridge that informed the present design (Piper and Partridge 2016). In addition, in order to account for generational effects, early emergence and to maintain age-matched adult flies, we will use the methods employed by Linford and colleagues (Linford et al. 2013).

Mutation strategy

Initially, the tau mutant male *Drosophila* flies will be exposed to irradiation to initiate a non-selective mutation pattern. The goal is to expand the initial pool of mutations that may be further refined by the selective pressure of competing with wild-type flies for limited food/water. As a result of irradiation, it is possible that the tau mutation is lost. This is monitored by removing any flies that lose the rough eye phenotype from inclusion in the study.

Experimental evolution

Population cages will be used to each house 5,000 total flies. Population cages may be constructed on-site or purchased from Carolina Biological Supply Company. Two primary cages will be used in this experiment. Each cage will consist of a combination of 2,500 AD mutant male flies carrying a tau mutation and 2,500 sterile male wild-type *Drosophila*. Flies will be introduced into the cage environment as adults. The cage environment will be made as natural as possible, with limited food and water. Each cage is monitored twice per day to remove and count dead flies.

The control cage will contain Population 1 (the original, non-irradiated tau mutant fly). Mutant flies that survive to the 90-99% threshold are removed from the cage and bred with females from the initial stock of tau mutants. The resulting generation will repopulate the population cage and compete with a new generation of wild-type male flies. Population 1 will represent natural evolutionary pressure and provide a control for changes in lifespan that may be caused by variables like breeding effects or other confounds (Jafari 2010, Promislow et al. 2022).

The experimental cage will contain Population 2 (the irradiated mutant flies). Population 2 will also consist of 2500 mutant flies that compete against 2500 wild-type flies for limited food and water. When between 90 and 99% of the mutants have died, the surviving mutants will be removed and bred with females from the original stock of tau mutants. Half of the resulting generation of mutants will be irradiated to introduce new mutations. No mutagenesis will be induced in the remaining half of the generation. Both the irradiated and non-irradiated flies will be placed together in the population cage to compete against a new generation of wild-type males. As a result, the experimental population cage will consist of a mix (1:1) of the offspring of surviving male mutant flies that are not irradiated and the offspring of surviving male mutant flies that are irradiated (introducing additional mutations). This process of repeated irradiation and mixing 1:1 of the irradiated and non-irradiated flies will occur with every generation. Thus, new mutations will regularly be introduced to compete with the prior group of successful flies. Observation of the rough eye phenotype will be used to assess mutation maintenance in AD mutant flies. See Fig. 1 for a graphical representation of the method to be employed to drive experimental evolution over generations of directed evolution.

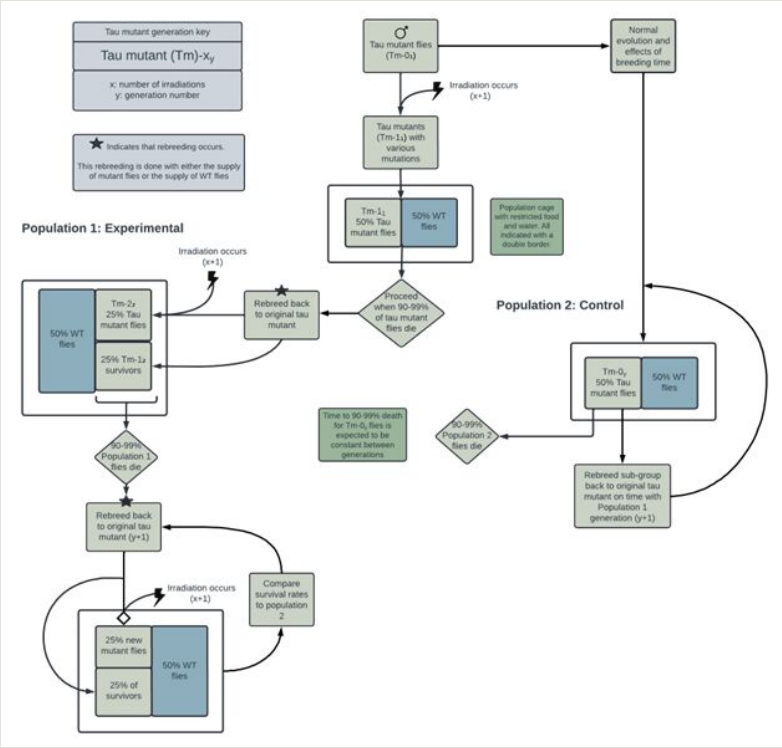

Figure 1. doi

Flow chart representation of the evolutionary process proposed. The initial population of tau mutant flies is divided into two groups, Population 1 and Population 2. Population 2 functions as a control, representing evolution in the absence of additional mutations, but under selective pressure. Population 2 is placed into a population cage that includes 2500 tau mutant flies that will compete with an equal number of wild-type flies for limited food and water. When between 90 and 99% of the mutants have died, the surviving mutants are bred with female flies from the initial stock to produce a new generation. The males of this new generation are placed back into the population cage to compete against a new group of wild-type male flies.

Population 1 functions as the experimental population. Two thousand five hundred tau mutants from Population 1 will be irradiated to induce random mutagenesis on the tau mutant background. These 2500 flies will then be placed into a population cage to compete against an equal number of wild-type flies. When between 90 and 99% of these mutants have died, the surviving mutants are bred with the female flies from the initial stock. Two thousand five hundred flies will be removed from the resulting generation and divided into two equal groups. One group will receive treatment with irradiation before being placed into the population cage and the second group will receive no additional mutagenic treatment before being placed into the population cage. Together, these two groups will compete against 2500 new wild-type male flies for limited resources. Such a strategy ensures that new mutations are introduced while increasing the likelihood that beneficial mutations that have already occurred are maintained in the population. This process is then repeated until increased longevity is seen based on statistical comparison.

Techniques for assessment of cognitive reserve

Multiple techniques are available for assessing cognition. Aversive phototoxic suppression (T-maze) has been used by others (Seugnet et al. 2008,Dissel et al. 2017) to determine short-term memory. This method is based on the flies' ability to remember which chamber in a T-maze holds an aversive stimulus (e.g. an illuminated vs. a darkened chamber). Cognition may be assessed by examining long-term memory using courtship conditioning in flies (Broughton et al. 2003). Spatial organisation may be used to assess its level of reserve. An example of this using a mouse model would be the Morris water maze. A 2015 study (Granger et al. 2016) examined sex-based differences in cognitive reserve in a mouse model using the Morris water maze. The researchers associated reserve with using effective navigational strategies to complete the maze successfully. Male mice more efficiently switched to spatial learning strategies instead of systematic learning strategies, allowing them to better compensate for amyloid pathology (Granger et al. 2016). Analogues for the Morris water maze exist that use a heat maze to assess spatial learning in *Drosophila*. For example, it was shown that *Drosophila* use visual cues to increase their navigational efficiency (Foucaud et al. 2010). See Fig. 2 for a graphical representation of the generalised scheme to assess successful generational improvements in cognition using the assessments described.

Short-term memory testing

Short-term memory will be assessed through Aversive Phototaxic Suppression (Dissel et al. 2017). First, flies will be placed in a T-maze and choose between light and dark rooms. Then, an adverse stimulus (quinine/humidity) is paired with the light room. Over a series of trials, the phototaxis of the fly is observed. Ideally, experimental evolution will drive mutant flies to have corrected wild-type like memory.

Long term memory

The long-term memory of the flies will also be assessed through analysis of courtship conditioning. This method was employed by Dissel and colleagues (Dissel et al. 2017) and it is based on exposing a male fly to a non-receptive female. This same fly is later exposed to a receptive female to examine his associative memories.

Statistical analysis

To determine the extent to which the experimental system drives evolution towards improved cognitive reserve, survival curves will be used as an end-point measure. Fig. 3 shows a theoretical set of expected survival curves as experimental evolution drives revertants to become more like wild-type. Survival curves in aging studies across animal models, including *Drosophila*, are well-established as appropriate indicators of change in longevity. In addition, the method of data collection proposed includes the possibility of other measures of evolutionary success, including median lifespan, mean lifespan and maximum lifespan, amongst others (Blumenstiel et al. 2009, Haelterman et al. 2014, Ziehm

et al. 2015). Progress towards increased survival will be assessed using the Kaplan-Meier estimation method. A Kaplan-Meier curve will be constructed for each generation of mutant flies and compared to the control group using a log-rank test with significance set to p = 0.05. The control comparison is the generational matching group of the original isogenic tau mutant fly that is maintained with the same generational frequency. By selecting and propagating only those mutant flies that survive in a population of wild-type, it is expected that the genetic variables that allow cognitive reserve will be enriched with each generation.

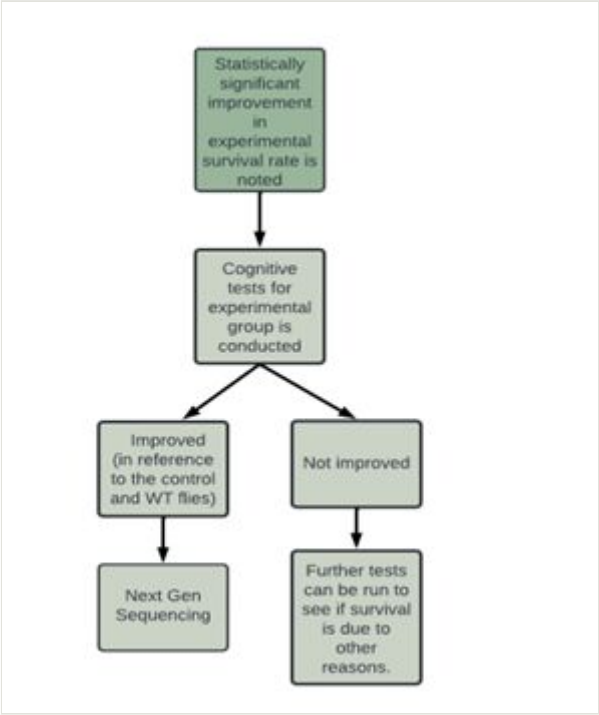

Figure 2. doi

Flow chart representing the assessment of improved longevity. Increased longevity may be a result of mutations that have induced cognitive reserve or it may be a result of mutations linked to genes impacting longevity itself. The extent to which increased lifespan may be attributed to improved cognition may be assessed using a T-maze or courtship rituals. If improved cognition is the driving factor behind increased longevity, then next-generation sequencing is used to determine which mutations have impacted cognition. If longevity is increased, but cognition is not improved, then further testing and sequencing are required to determine which genes have been affected by the process of mutagenesis.

Using next-generation sequencing methods, the genomes of these surviving *Drosophilas* can be compared to ascertain the mutations responsible for cognitive reserve development. In this study, the flies resulting from experimental evolution would be pooled and their genome sequenced and compared to the original tau mutant to identify the genes altered by experimental evolution. Sequencing technologies, such as those by Oxford

Nanopore, Pacific Biosciences and others, when combined with various algorithms to assemble such data, have the ability to provide complete genome information (Ludwig et al. 2022). Examples in which *Drosophila* genomes have been compared include work by Blumenstiel and colleagues (Blumenstiel et al. 2009), who used Illumina next-generation sequencing to identify EMS-induced mutations affecting eggshell morphology in *Drosophila* . Similarly, Lee and colleagues (Lee et al. 2016) used whole genome sequencing to identify EMS-induced mutations affecting competition between wild-type and RpS3 heterozygous cells. Feng and colleagues have also successfully explored the genomic differences amongst various *Drosophila* species using NGS methods (Feng et al. 2023). While the approach is focused on whole genome comparisons, with the prior knowledge of putative cognitive reserve genes, more specific comparisons could also be completed. The successful completion of this approach would support more traditional experimental approaches to determine the mechanistic basis and potential pharmacological strategies to regulate the predicted molecular pathways.

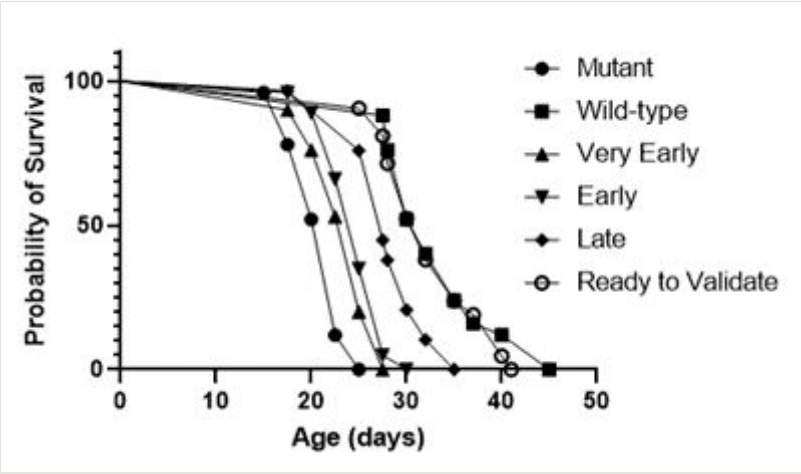

Figure 3. doi

Simulated data for the change in survival rates over time. As the generations increase from very early to late, we expect that the survival of the irradiated flies will increase and look more like the curve for wild-type flies. Observation of a trend towards statistical insignificance compared to the initial mutant fly would strongly indicate that the population was evolving (the curve is shifting to the right). The determination that the population has evolved to be like wild-type will be at the point in which there is no statistical difference ($p > 0.05$) between the wild-type and mutant flies as illustrated as Ready to Validate.

Conclusion

We have suggested a novel approach to understanding the genetic pathways that underlie cognitive reserve. Although we have focused on AD, this approach could be applied to other neurodegenerative disorders with an accurate animal model. In particular, with evidence for the existence of cognitive reserve in Dementia with Lewy Bodies and

Frontotemporal Dementia (Perneczky et al. 2007, Borroni et al. 2009, Carli et al. 2021), researchers could apply the same method. Results from such studies would provide insights into the specific molecular pathways that regulate the different phenotypic expressions of these diseases as they relate to cognitive decline or reserve. The power of experimental evolution could similarly be used by selection of animals that demonstrate increased resistance to the cognitive decline accompanying the aging process. Such a finding would profoundly impact our understanding and ultimately reveal cellular processes that underlie age-related cognition decline.

Hosting institution

University of West Florida

Conflicts of interest

The authors have declared that no competing interests exist.

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