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AMAZING PAPERS IN NEUROSCIENCE
Exploring the Genetic Underpinnings of Aggression in *Drosophila melanogaster*

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Aggression is a multidimensional social behavior observed in a wide range of animal species. Displays of aggression serve as an integral component of intraspecies competition for access to resources, territory, and mates. Despite being seen across nearly every facet of the animal kingdom, our understanding of how genes mediate aggression remains limited. A growing body of contemporary research has chosen to adopt the vinegar fly *Drosophila melanogaster* as an important tool in the modelling and study of aggression. Although references to aggression in *Drosophila* appear in the early 1900s (Sturtevant, 1915), the study of aggression in *Drosophila* received limited attention from researchers until the early 2000s. In 2006, Dierick and Greenspan were the first to investigate the genetic underpinnings of *Drosophila* aggression in an unbiased fashion. They described in detail how candidate genes for *Drosophila* aggression were identified by genetically screening fly strains that were selectively bred for heightened aggression. They identified that the *Cyp6a20* gene mediated aggression at the phenotypic level. Dierick and Greenspan (2006) is an excellent demonstration of how the application of behavioral genetic concepts to genetics research can inform our understanding of how genes mediate behavior. This paper constitutes an excellent teaching resource for any behavioral neuroscience course and is a fine example of how comparatively simple model organisms like *Drosophila* can be used to dissect the genetic underpinnings of complex behavior.

Key words: Behavioral genetics; Aggression; Drosophila melanogaster

The origin of the field of behavioral genetics is largely attributed to the work of Francis Galton. This early work consists of an article on the heritability of “talent” and “character,” and a book titled *Heritability Genius* which asserted that social success (and the talents and character pertinent for this success) was heritable and could be passed down to one’s offspring (Galton, 1865; Galton, 1869). This research was heavily influenced by the publication of T*he Origin of Species* by Galton’s half cousin Charles Darwin, (Fancher, 2009). Much of the early history of behavioral genetics is marred with controversy, with many of Galton’s core ideas being later used as justification for the unsavory practices of the 20th century eugenics movements across Europe and North America. In fact, the term “eugenics” was originally coined by Galton himself in a book titled: *Inquiries into human faculty and its development*, with the term itself equating to “well-born” when translated from its Greek roots (Galton, 1883).

The frequent association of behavioral genetics with eugenics has led many to criticize the entire field of behavioral genetics, with these critiques persisting long after the death of the eugenics movement (McGue, 2008). In defense of behavioral genetics, McGue (2008) notes that although the field is not undeserving of criticism, many contemporary critiques unfairly ignore the many positive contributions behavioral genetics has made to our understanding of the genetic underpinnings of behavioral differences between individuals. Quantitative behavioral genetics research has repeatedly demonstrated that traits such as intelligence, propensity for substance abuse, and even personality are strongly influenced by genetic factors (Kendler et al., 2000; Haworth et al., 2009; Vinkhuyzen et al., 2012). Notably, these breakthroughs in our understanding of how genes contribute to individual differences in psychological traits has provided many promising avenues for improving our approaches to education and the treatment of substance abuse and depression (Sturgess et al., 2011; Haworth and Plomin, 2012; Jukić et al., 2018).

Although our understanding of genetics has advanced tremendously since Galton’s time, our understanding of how specific genes contribute to specific behaviors and the relative importance of genes in comparison to environmental factors remains underdeveloped. The human genome contains a vast amount of genetic information and making sense of how each gene contributes to a specific behavioral phenotype is an extremely complex task. This complexity problem has led many researchers to utilize organisms with comparatively simple genomes such as the vinegar fly *Drosophila melanogaster* as model systems when investigating the genetic underpinnings of neurological disorders and basic neural processes. Notably, *Drosophila* have served as a powerful tool in the modelling of Alzheimer’s Disease (Shulman et al., 2013), Parkinson’s disease (Feany and Bender, 2000), insomnia (Seugnet et al., 2009), and have provided valuable insights that have informed our understanding of circadian rhythms (Allada et al., 1998), learning (Yu et al., 2004), and memory (Walkinshaw et al., 2015).

The roots of *Drosophila* behavioral genetics can be traced to seminal work by the laboratories of Jerry Hirsch and Seymour Benzer (Tully, 1996). Hirsch and Benzer adopted contrasting approaches to *Drosophila* behavioral genetics, with Hirsch and colleagues selectively breeding flies for specific behavioral phenotypes, while Benzer and colleagues used mutagenesis to disrupt single genes in order to screen for behavioral phenotypes (Tully, 1996). A large body of contemporary research in the field of *Drosophila* behavioral genetics has followed in the footsteps of Hirsch and colleagues and Benzer and co-workers by integrating many of the experimental methods employed by both labs.

References to *Drosophila* aggression first appear in scientific literature from the early 1900s; a notable example can be found in an account by Sturtevant (1915) of two males competing for the same female by "spreading their wings and rushing towards each other." Although the scientific community has known about *Drosophila* aggression since then, the study of *Drosophila* aggression would receive only limited research attention until the early 2000s. In 2002, a paper published by Baier, Wittek, and Brembs was the first to suggest that *Drosophila* might be used as a novel neurobiological model of aggression. Around the same time, the Kravitz laboratory published a comprehensive analysis of *Drosophila* aggression, in the form of an ethogram (a record of behaviors) detailing an assortment of agonistic interactions (Chen et al., 2002). This major contribution (along with many others) by the Kravitz laboratory and others laid the foundation for future studies on *Drosophila* aggression. Soon thereafter, Dierick and Greenspan (2006) conducted the first unbiased (not looking for a specific gene) and comprehensive genetic screen for candidate genes linked to aggression in *Drosophila*, using an ingenious forward genetic approach. The use of both artificial selection and mutant analysis by Dierick and Greenspan (2006), represents an interesting integration of elements of both Hirschian and Benzerian behavioral genetics.

ARTICLE SUMMARY

The authors begin by introducing aggression as a poorly understood and complex social behavior, influenced by genetic and environmental factors alike, and seen throughout the animal kingdom. They provide a brief overview of past research on aggression in *Drosophila*, noting that although several genes linked to aggression had been previously reported in mice, these genes were primarily "serendipitous" findings, and that a comprehensive analysis of aggression at the molecular level had yet to be attempted in any lab species. Following this, the authors detail how a forward genetic approach, involving the selective breeding of flies for enhanced aggression, was used to perform an unbiased genetic screen of the brains of extremely aggressive flies, to identify potential candidate genes responsible for mediating aggression in *Drosophila*.

In order to form the starting population, the authors first crossed a roughly equal number of males and females taken from two independent Canton-S lines. They note that this was repeated for two generations, and that selection for aggression began on the 3rd generation (Gen3). In order to create a fly line that expressed heightened aggression in an unbiased manner, the authors placed a large group of flies into a cage with several small food-filled containers affixed to its base. The aggression selection protocol for Gen1 – Gen7 involved extracting 15-30 aggressive males (i.e., males that won territorial fights against other males) per generation and then mating these males with an equal number of random virgin females from the same generation. The authors note that the aggression selection protocols were changed for the 8th generation (Gen8) onward, with only males that showed fight-escalating behaviors (i.e., standing on their hind legs and wrestling other males) being extracted for future breeding. In order to establish a neutrally aggressive control group the authors first placed another similarly sized group of flies into a separate but identical cage. According to the authors, the protocol used for creating the control group consisted of first extracting and discarding 15-30 aggressive males from the control cage per generation. Followed by randomly selecting 30 males from the remaining control population and mating them with an equal number of virgin females from the same generation. Both of these selection processes were replicated, producing two aggressive (i.e., AggrI and AggrII) and two neutrally aggressive fly lines (i.e., NeurI and NeurII).

The authors developed several behavioral assays to measure the resultant changes in aggression across generations. The first behavioral assay placed pairs of male flies inside of a small plexiglass arena containing food and a female. Male pairs from both Gen8 Aggr lines demonstrated a higher number of fights during the one-hour observation time. This difference was only significant between the NeurII and AgrrI lines.

The second behavioral assay placed several pairs of males into multiple separate arenas. These were contained within a larger chamber, which contained neither food nor females. The male pairs placed within these arenas were then observed and rated for four distinct fighting behaviors: "fight frequency", "fight latency", "fighting index" (i.e., time spent fighting), and "fight intensity" (i.e., number of escalating behaviors). The authors reported that Gen11 males from both Aggr lines had a significantly higher fight frequency than those from either Neutr line, while noting that the other three fighting behaviors' categories only significantly differed when non-fighting pairs were also included. Additionally, Gen21 males from both Aggr lines showed significantly higher levels of aggression across all four fighting behaviors when compared to both Neutr lines. Dierick and Greenspan (2006) concluded that aggressive behaviors increase in intensity in later generations.

The third behavioral assay recorded the total number of "escalating encounters" observed within each "population cage" (the cage used during aggression selection). Males from both Aggr lines showed significantly more escalating behaviors than those from either Neutr line. This trend was even more extreme within cages containing a mix of 50% aggressive and 50% neutral males, with aggressive males being responsible for virtually all instances of escalation.

To determine if selection for heightened aggression led to any other phenotypic changes, the authors evaluated the mean intergroup differences for males from Gen22-23, in three key areas: activity level, mating success, and body mass. Surprisingly, they found that NeutrII males had significantly more mating success, and were significantly heavier than AggrII males, however no other traits significantly differed between any other combination of lines.

To assess how genetic expression within the brains of Aggr flies differed from that found in Neutr flies, the authors conducted microarray expression analysis on the heads of Gen21 flies. They reported that the overall differences in genetic expression between the Aggr and Neutr lines were very small. In order to remedy this problem, they narrowed their focus to only genes with expression levels that differed in the same direction in both Aggr and Neutr lines (i.e., if gene A is upregulated in AggrI, then gene A is also upregulated in AggrII). They then confirmed the expression differences for candidate genes with available mutant strains using qPCR. Five mutant strains were evaluated using a behavioral assay which indicated that only the *Cyp6a20* gene (downregulated in Aggr flies) significantly mediated aggression. The authors noted that the *Cyp6a20* gene encodes a cytochrome P450, an enzyme with a variety of functions, but most notably is involved in pheromone detection. They suggested that mutations to the *Cyp6a20* gene may result in a hypersensitivity to male pheromones that leads to heightened aggression.

VALUE

Genetic analysis techniques have advanced considerably since the early 2000s, with the microarray technique utilized by Dierick and Greenspan (2006) now largely replaced by newer genetic analysis tools such as RNA sequencing. However, despite its use of older genetic analysis techniques, this paper is of great teaching value for several reasons. Firstly, although the paper does not claim to be on the topic of behavioral genetics, it serves as a superb working example of how the application of behavioral genetic concepts in the lab can help to inform our understanding of how genetic differences contribute to behavioral differences. Secondly, the field of behavioral genetics continues to generate controversy to this day with many contemporary works in the field (see: Richardson and Norgate, 2006) drawing the ire of other researchers (see: Lerner, 2006). If this paper were presented alongside another behavioral genetics paper which has attracted harsh criticism, it could be used to introduce the concept of controversy within the scientific community. Thirdly, this paper serves as an excellent resource for demonstrating how a comparatively simple model organism such as *Drosophila* can be used in the lab to investigate complex behaviors. The experimental techniques presented within this paper may be of value to any student working with *Drosophila* as part of a university research project. Finally, the forward-genetic approach presented within this paper presents an excellent example of the power of non-hypothesis driven research.

AUDIENCE

The content within Dierick and Greenspan (2006) would be useful lecture material for any behavioral neuroscience course. The key concepts within this paper could be easily presented in a journal club style presentation, where a summary of the paper's key findings is presented alongside a description of its experimental methods. Educators could follow-up this presentation by providing students with a brief overview (e.g., a paper handout or short lecture) of how genetic analysis techniques have changed in the years since this paper was published (see: Ansorge, 2009; Ozsolak and Milos, 2010). This could easily springboard into a stimulating classroom activity where students are asked to evaluate the limitations of the genetic techniques presented by Dierick and Greenspan, explaining how (or if) they would make use of these previously described novel genetic tools if given the opportunity to replicate this study. This would be a great way to get students to think critically about previous research, and to more carefully assess the implications of the methods of a study.

An educator might also use this paper to acquaint 1st year undergraduate students with university research databases. It has been over a decade since the publication of Dierick and Greenspan (2006), and a multitude of other papers on *Drosophila* aggression have since been published, with many of these newer papers citing this earlier work (e.g., Seugnet et al., 2009; Zwarts et al., 2011). In this activity, an educator would take their class to a university computer lab or the university library, and then provide students with a list of scientific search engines (i.e., Google scholar, PubMed, Web of Science). The educator could then give a quick introductory lecture describing the key findings and experimental methods of Dierick and Greenspan (2006), noting how it fits in with other literature. Students could then be asked to track (using a scientific search engine of their choice) how later papers have cited and made use of the findings presented by Dierick and Greenspan. This activity would culminate with students writing a brief essay explaining how the Dierick and Greenspan paper has contributed to later neuroscience research. This activity could help new students who may be unfamiliar with scientific search engines familiarize themselves with these tools, while also allowing students to see first-hand the impact that foundational research can have on a field.

Educators may also consider teaching this topic in the form of a lab based practical. In this activity students would be shown videos of fruit fly aggression (Certel and Kravitz, 2012) and asked to identify aggressive behaviors and form an ethogram documenting the occurrence of these behaviors (Chen et al., 2002). Before completing this task, students would be provided with a brief overview (paper handout or short lecture) explaining what ethograms are, why they are important, and how they are used in the field of ethology. At the end of this activity, students would be given the opportunity to compare their ethogram to the ethogram presented in Chen et al. (2002). This activity would provide students with hands-on experience in the field of behavioral genetics and would provide excellent context for the Dierick and Greenspan paper.

Although the validity of behavioral genetics has been questioned by some members of the scientific community, the field of behavioral genetics has evolved considerably since Galton's time and has made many valuable contributions to modern neuroscience. The findings of modern behavioral genetic studies like Dierick and Greenspan (2006) highlight the crucial role genes play in the development of psychological traits. Developing a better understanding of how genes mediate behavior may improve the quality of pharmacological therapeutics and psychological interventions by allowing these to be tailored to the specific needs of an individual. In summary, behavioral genetics is a historically misunderstood field that has undergone a significant evolution and offers many promising prospects for teaching neuroscience.

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