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Dissecting the Genetic Architecture of Behavior in *Drosophila melanogaster*

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Abstract

Variation in behaviors in natural populations arises from complex networks of multiple segregating polymorphic alleles whose expression can be modulated by the environment. Since behaviors reflect dynamic interactions between organisms and their environments, they are central targets for adaptive evolution. *Drosophila melanogaster* presents a powerful system for dissecting the genetic basis of behavioral phenotypes, since both the genetic background and environmental conditions can be controlled and behaviors accurately quantified. Single gene mutational analyses can identify the roles of individual genes within cellular pathways, whereas systems genetic approaches that exploit natural variation can construct genetic networks that underlie phenotypic variation. Combining these approaches with emerging technologies, such as genome editing, is likely to yield a comprehensive understanding of the neurogenetic underpinnings that orchestrate the manifestation of behaviors.

Introduction

Behaviors are the ultimate expression of the nervous system and the quintessential manifestations of living organisms. From a genetics perspective, behaviors are quantitative phenotypes, *i.e.* traits that vary among individuals and arise from multiple interacting and segregating genes that are modulated by the organism's developmental history and its environment [1]. The fraction of the variance of a behavioral phenotype within a population that is due to genetic variation is its broad sense heritability (H^2). The heritability represents the sum of genetic variances contributed by all genes and their interactions, and a substantial H^2 is a prerequisite for gene mapping studies as well as artificial selection [2]. Because both the genetic background and the environmental rearing conditions can be controlled precisely, *Drosophila melanogaster* presents an attractive model system for investigating the genetic architecture of behavior. Flies display a wide repertoire of behaviors, many of which occur across the animal kingdom (*e.g.* aggression [3,4], courtship and mating [5–7], sleep [8,9], learning and memory [10]). Evolutionary conservation of fundamental molecular mechanisms and cellular pathways allows us to uncover general principles that apply across behavioral phenotypes and across phyla.

Studies aimed at the genetic dissection of behaviors in *Drosophila* have utilized both mutational analyses of individual genes and quantitative genetic approaches. The former approach relies on a change in the behavior as a consequence of disruption of a specific gene, whereas the latter correlates variation in the behavioral phenotype among individuals with genotypic differences to identify simultaneously multiple genes that contribute to the behavioral phenotype. Furthermore, systems genetics approaches in which DNA sequence variants are correlated with variation in transcript abundance levels and variation in organismal phenotype have demonstrated that behaviors are dynamic phenotypic manifestations that emerge from transcriptional networks of pleiotropic genes [11,12]. Both environmental effects and epistatic interactions [13–18] modulate emergent behavioral phenotypes. In addition, epigenetic regulation may contribute to long-term behavioral modifications [19]. This entire genetic system is further influenced by the development of the organism and is a culmination of its evolutionary history while at the same time providing targets for future evolutionary adaptation (Figure 1). Both single gene studies and systems genetics approaches, and a combination of these strategies, have contributed to our understanding of the genetic underpinnings of behaviors.

Single gene approaches – mechanistic snippets of a polygenic puzzle

Classically, identification of genes that contribute to *Drosophila* behaviors has relied on chemical or *P*-element insertional mutagenesis. Unlike studies on development, which focus on events that happen pre-eclosion, studies on behavior generally require the survival of viable and functional adults. Thus, hypomorphic rather than null mutants are typically used for the study of behaviors. Early mutagenesis screens identified genes that, when disrupted, give rise to large behavioral effects. For example, *period* mutations have dramatic effects on circadian rhythm [20] and the *paralytic* mutation results in unambiguous locomotion deficits [21].

Statistical analyses of the effects of transposon insertions in carefully controlled co-isogenic backgrounds, which considered more than two standard deviations from the control mean phenotypic value a criterion for aberrant behavior, showed that behavioral phenotypes present large mutagenic targets, ranging from ~4–6% of the genome for olfactory behavior [15] to ~37% of the genome for startle behavior [22]. This observation implies a highly polygenic and pleiotropic architecture for behaviors and is consistent with their modularity, *i.e.* complex behaviors can be dissected into constituent components, with overlapping genetic networks underlying each component (*e.g.* locomotion would be a constituent component of aggression as well as mating behavior; Figure 1).

Large phenotypic effects that arise from disruption of a single gene can lead to the identification of cellular pathways or mechanisms that are instrumental in enabling the behavior. A classic example is the elucidation of the regulatory feedback loops that control the circadian clock which largely came from studies on single gene mutations [23,24]. However, effects on downstream gene products and genes that regulate these feedback loops and their interactions that lead to the ultimate expression of circadian behavior remain to be fully understood.

Similarly, disruption of single genes, including *tailless* [25] and *Tachykinin* (*Tk*) and its receptor, *Takr86C* [26], and inactivation of the neurons in which they are expressed have identified a neural circuit that contributes to aggression and originates in the pars intercerebralis, a brain structure also implicated in the control of sleep [27]. However, other brain regions, including the mushroom bodies [28], dopaminergic projections to the central complex [29], octopaminergic neurons in the suboesophageal ganglion [30] and input via the olfactory projection [31,32] are also implicated in aggression. Furthermore, transposon insertions in as many as 57 genes from among 170 genes surveyed resulted either in reduced aggression or hyper-aggression [33]. Thus, the mechanisms that regulate aggression depend on integrated neural circuits and a complex and extensive underlying genetic architecture, in which disruption of any major single gene component can result in an abnormal behavioral phenotype.

These examples illustrate how mutagenesis studies can provide significant insights in some of the underlying cellular mechanisms that drive behaviors. For any given behavioral phenotype, however, such studies have also led to a pantheon of genes with diverse annotated functions, each of which affects the phenotype, but without indication of how these genes interact together as a functional ensemble that gives rise to the phenotype. Putting these independent snippets of information in a common framework is the goal of systems genetics approaches (Figure 2).

Systems genetics approaches – harnessing the power of natural variation

Systems genetics approaches have become feasible in *Drosophila* with the generation of reference mapping panels, such as the *Drosophila melanogaster* Genetic Reference Panel (DGRP) [34] and the *Drosophila* Synthetic Population Resource (DSPR) [35]. The DGRP consists of 205 inbred lines derived from isofemale lines from a wild North Carolina population with fully sequenced genomes. The most recent release of the DGRP documents 4,853,802 single nucleotide polymorphisms (SNPs) and 1,296,080 non-SNP variants (insertions, deletions and copy number variants) as well as 16 polymorphic inversions [36]. Sequence variation in this population can be correlated with phenotypic variation. The *Drosophila* genome is highly polymorphic and an extensive history of recombination has led to little local linkage disequilibrium, except within chromosomal inversions [36]. Linkage disequilibrium decays within a few hundred base pairs [34]. The absence of local linkage disequilibrium, as is found in the human genome [37], prevents the use of tagging SNPs for association studies and instead requires comprehensive analyses of whole genome DNA sequences. The advantage is that causality can be more readily assigned to a gene or even a polymorphism within a gene. Thus, naturally occurring variants that survived the sieve of natural selection are a treasure trove for the analysis of complex traits, including behaviors.

All traits that have been measured on the DGRP to date show extensive phenotypic variation, including behavioral traits, such as sleep parameters [38], startle behavior [17] and olfactory response to the odorant benzaldehyde [18]. Genome wide association (GWA) studies employ a relatively small number of lines compared to the numbers of polymorphic markers that are tested and, thus, polymorphic markers that are associated with variation in

behavior rarely reach genome-wide statistical significance based on Bonferroni correction for multiple testing or permutation thresholds. This issue is, however, mitigated by several factors. First, since there is minimal genetic variation among individuals within a line, phenotypic values can be determined with great precision, since essentially the same genotype can be measured repeatedly. Second, since all polymorphisms in the population are known those with the highest *P*-values for association can be selected as candidate genes for downstream analyses (Figure 3). Third, mutational analyses using the vast public resources available for the *Drosophila* community can verify that mutations in candidate genes identified in the GWA study indeed affect the behavioral phenotype. The fraction of such validation tests that confirm association of the gene with the behavior provides an estimate for an empirical false discovery rate. Finally, lines from each extreme of the phenotypic distribution can be intercrossed to form an advanced intercross population. Individuals from this population can be assayed and DNA from individuals with extreme low and high phenotypic values can be pooled and subjected to bulk DNA sequencing and extreme QTL mapping to identify alleles that contrast the high and low responders (Figure 3) [17,18]. One advantage of this approach is that alleles that were present at low frequency in the DGRP and could not be detected by GWA can be represented at intermediate frequencies in the base population used to generate the advanced intercross. In addition, extensive recombination generates a vast number of outbred individuals so that sample size in the advanced intercross population is no longer limiting. Finally, changes in allele frequencies that occur during many (>25) generations of intercrossing can result in changes in additive effects of single variants that participate in gene-gene interactions, enabling significant associations to be uncovered in the extreme QTL mapping population that were not identified in the original GWA study in the DGRP [17,18]. Combining the results from GWA analyses and extreme QTL mapping studies can reveal comprehensive genetic networks that underlie variation in the behavioral phenotype (Figure 3).

A number of generally applicable insights have emerged from these studies: (1) Most behavioral phenotypes are sexually dimorphic, implying distinct genetic architectures for males and females. (2) Epistasis dominates the genetic architecture of complex traits, including behaviors [17,18,39,40], and suppressing epistasis buffers the genome against the effects of newly arising mutations [39,40]. (3) Common alleles have small to moderate effects on phenotypic variation, whereas rare alleles, that have perhaps appeared in more recent evolution, tend to have large effects [41,42]. (4) The genes that contribute to variation in behaviors are pleiotropic and span a wide range of gene ontology categories; however, developmental genes and genes associated with neural connectivity and neuronal function are prominently represented among diverse behavioral phenotypes [17,28]. This is perhaps not surprising as the expression of behaviors is itself a property of the nervous system.

Genes, genomes and the environment

Since behaviors encompass interactions between organisms and their environments, the relationship between the genome and organismal phenotype is not static, but the genetic networks that orchestrate the behavioral phenotype are expected to be dynamic and plastic. Examination of whole genome transcriptional profiles of an DGRP-derived advanced intercross population using Affymetrix expression microarrays under 20 different

environments showed that only ~15% of the transcriptome is environmentally plastic to macro-environmental changes, encompassing among others proteases and rapidly evolving multigene families [13]. The remainder of the transcriptome is remarkably buffered (canalized) against environmental perturbations. Different genotypes can respond differently to environmental changes, which is the definition of “genotype-by-environment interactions”. For example, food deprivation during the larval stage differentially affects food search behaviors in adult flies that have different alleles of the *foraging* gene [43,44]. Similarly, isofemale lines of *D. simulans* that were reared on different diets and at different temperatures showed differences in cuticular hydrocarbon profiles, which could affect variation in mating behavior, since some cuticular hydrocarbons function as pheromones [45]. The topology of genetic networks is altered by environmental interactions and these effects are dependent on epistatic modifiers [46]. Phenotypic plasticity and genotype-by-environment interactions enable organisms to rapidly adapt to changing environmental conditions and thus affect fitness. Variation in adaptability among individuals to changing environments provides a framework for natural selection, in which the balance for homeostasis and plasticity is optimized.

Conclusions

The combination of single gene mutational analyses with quantitative genetic and genomic approaches has led to fundamental widely applicable insights into the genetic underpinnings of behaviors. Behaviors are emergent properties of complex genetic networks, characterized by pleiotropy and widespread epistasis. These networks are sexually dimorphic and sensitive to environmental modulation. They provide at the same time stability and flexibility to the genotype-phenotype relationship. The studies reported to date provide a foundation for more comprehensive mapping of gene-gene interactions and investigations of the robustness of genetic networks for behaviors during genetic or environmental perturbations. Furthermore, it will be important to incorporate studies on epigenetic mechanisms in systems level analyses of behaviors.

Behavioral genetic studies have benefitted from a range of new emerging technologies, such as next generation sequencing and optogenetics. New technologies, such as CRISPR-mediated genome editing [47–49], will enable a more precise dissection of the context-dependent action of individual alleles on the behavioral phenotype and associated transcriptional networks. Single cell transcriptional analysis [50,51] may in the future provide insights in how transcriptomes in individual neurons within neuronal circuits interact to enable the expression of behaviors. Linking the dynamics of complex neural circuits to the dynamics of complex genetic networks that drive behaviors is the next frontier in neurogenetics research.

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Figure 1. Genetic underpinnings of behavioral phenotypes

The diagram illustrates how complex behavioral phenotypes arise as emergent properties of transcriptional networks of pleiotropic genes. The connectivity of these networks is influenced by sequence variation at the DNA level, which in turn is subject to gene-gene interactions. Gene expression is affected by the environment and network interactions create non-linearity in the relationship between genotype and phenotype. These systems genetics relationships are further shaped by the developmental history of the individual and are subject to evolutionary forces.

Figure 2. Contributions of single gene mutational analysis and analysis of natural variants to the dissection of the genetic architecture of behavioral phenotypes

GWA analyses and whole genome transcriptional analyses can identify networks of candidate genes associated with the behavioral phenotype. Single gene mutants can serve to validate candidate genes within the network and identify genes that contribute to the phenotype, but show little or no allelic variation within the population. This approach, however, generally falls short of identifying connectivity among genes that contribute to the phenotype. Combining both approaches, as illustrated in the diagram, leads to a comprehensive description of the genetic basis that underlies the behavioral phenotype.

Figure 3. Complementary approaches for the analysis of the genetic basis of natural variation of behaviors

The diagram shows how variation in DNA sequence in the DGRP can be associated with variation in phenotypic values. The probability for association of each polymorphic marker with phenotypic variation across the chromosomes identifies a limited set of markers with P -values that clearly stand out at a nominal $P < 10^{-5}$. DGRP lines with extreme low and high phenotypic values can be intercrossed and alleles can be identified that differentially segregate among individuals with extreme behavioral phenotypes in an advanced intercross population. The results from both the GWA study and the extreme QTL mapping analysis can be combined to identify a genetic network associated with phenotypic variation. Subsequent mutational analysis can be employed to verify that mutations in genes within the network indeed affect the phenotype, and in doing so establish an empirical false discovery rate.