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Is the genetic architecture of behavior exceptionally complex?

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Are traits with high levels of plasticity more complex in their genetic architecture, as they can be modulated by numerous different environmental inputs? Many authors have assumed that behavioral traits, in part because they are highly plastic, have an exceptionally complex genetic basis. We quantitatively summarized data from 31 genome-wide association studies (GWAS) and 87 traits in *Drosophila melanogaster* and found no evidence that behavioral traits have fundamental differences in the number of single-nucleotide polymorphisms or the significance or effect size of those associations, compared with nonbehavioral (morphological or physiological) traits. We suggest the assertion that behavioral traits are inherently more complex on a genetic basis compared with other types of traits should not be assumed true, and merits further investigation.

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Genetic architecture as a window into biological complexity

A central goal in biology is to elucidate the genotype-to-phenotype map, that is, the genetic architecture of traits. Genetic architecture is generally thought to exist on a gradient between simple, Mendelian traits controlled by single genes and complex, polygenic traits underlain by potentially thousands of interacting genetic loci [1]. There has been intense interest in understanding the genetic basis of complex traits [2]. Complex traits can have several defining characteristics, such as the involvement of multiple cell populations or tissues, and the occurrence of phenotypic subcomponents, or endophenotypes [3]. For example, courtship behavior consists of multisensory signaling and receiving (olfactory, visual, and mechanosensory) and also involes multiple body parts (brain, reproductive organs, muscle systems, and endocrine and exocrine glands), and thus variation in traits such as courtship are expected to involve a large swath of genes that influence various components of this complex trait [4,5].

Plasticity, the ability of an organism to produce different phenotypes from the same genotype in response to environmental cues, is another important aspect of biological systems that should be considered in understanding phenotypic and genetic complexity. Many recent studies have aimed to identify specific genes and pathways associated with organisms' plastic responses to different environmental stimuli [6]. Genetic variants such as single-nucleotide polymorphisms (SNPs) can be significantly associated with organismal responses to environmental variation [6]. For example, behavioral plasticity for docility in beef cattle has a small but significant genetic component [7], and genome-wide association studies (GWAS) have identified specific candidate genes related to reproductive plasticity in cattle raised in different rearing environments [8]. In addition, olfactory responsiveness in *Drosophila melanogaster* depends on the nutritional rearing environment, and up 50% of the variation in adult behavior is attributable to gene-by-environment interactions [9]. Thus, studies to date suggest traits with high levels of phenotypic plasticity are likely to be polygenic, related to relatively large networks of interacting genes [6], and a general prediction is that traits with high levels of phenotypic plasticity should manifest complex genetic architectures.

Behavior as a complex and plastic trait

In particular, traits related to animal behavior have long been assumed to be among the most complex phenotypically, leading to the widely held belief that behavioral traits have an especially complex genetic architecture [10]. The assumption that behavior has a complex genetic basis compared with other types of traits, such as morphology or physiology, has intuitive appeal. This thinking may in part stem from early statements such as E.O. Wilson's assertion in the widely read classic book *Sociobiology* that behavior is the trait 'furthest away from DNA' [11]. Authorities in the study of behavioral genetics have stated "behaviors are exceptionally complex quantitative traits" [12], and "behaviors are complex traits, with variation attributable to multiple interacting loci with individually small effects, whose expression depends on the environment" [5]. This thinking has led to statements such as, "When compared to many other types of traits (e.g. morphological or physiological traits), all behavioral traits may have added genetic complexity in terms of the numbers of genes and molecular pathways that influence them" [13]. But is this actually true?

There are several logical reasons for these assertions. First, behavioral traits by definition are responses to the environment, requiring integration of information from multiple organismal systems (sensory, metabolic, endocrine, neurobiological, etc.) and are thus expected to be affected by a large number of genes, potentially each with small, modulating effects. Even within the brain itself, multiple neurons, networks, and regions of the brain are required to elicit even a relatively simple behavioral program [14]. In addition, the brain is also thought to be the most transcriptionally diverse tissue in the body, with numerous region- and neuron-specific patterns of gene expression [15]. This suggests many target sequences throughout the genome could result in changed gene expression in different brain regions or neural populations, and thus modulate behavior. Furthermore, behavior can change and develop over an organism's lifetime, and different genes may be involved in the organization of behavior during early life compared with the maintenance or modulation of behavioral states during later stages, such as adulthood. For example, behavioral maturation in honey bees involves several shifts in behavioral state (e.g. care of offspring, guarding of the nest, and foraging for food) in an age-associated way; each stage of life is associated with unique changes in brain structure, physiology, and gene expression [16]. Thus, behavioral traits can be characterized by plasticity on multiple timescales, from developmental to physiological [10,17]. Finally, behavioral traits are often 'noisier' to measure than physical traits due to the subjectivity of human observation. This could result in underpowered statistical tests for allelic associations with behavior, and in lower levels of significance for behavior-associated genetic loci.

Furthermore, behavior, by definition, can have a large amount of plasticity in its phenotypic expression [17]. Genes 'for' phenotypic plasticity are expected to be part of complex interacting gene networks that may modulate suites of behavioral and physiological traits [18,19], suggesting the underlying genetic architecture of plastic traits should generally be complex [6]. While many nonbehavioral traits are known to have high levels of plasticity (e.g. neck spine formation in *Daphnia* water fleas in response to predation risk [20]), it has been argued that plasticity is especially high for behavioral traits, as behaviors are often more rapid and reversible than developmental, morphological, or physiological traits. For example, honey bee workers show high levels of plasticity during behavioral maturation, with the ability to accelerate, delay, or even reverse the order of which behavioral tasks they perform, depending on colony needs [21]. Thus, although any trait may in principle be plastic, there may be some difference in degree between trait types, with behavioral traits thought to have more extreme plasticity than other types of traits [22].

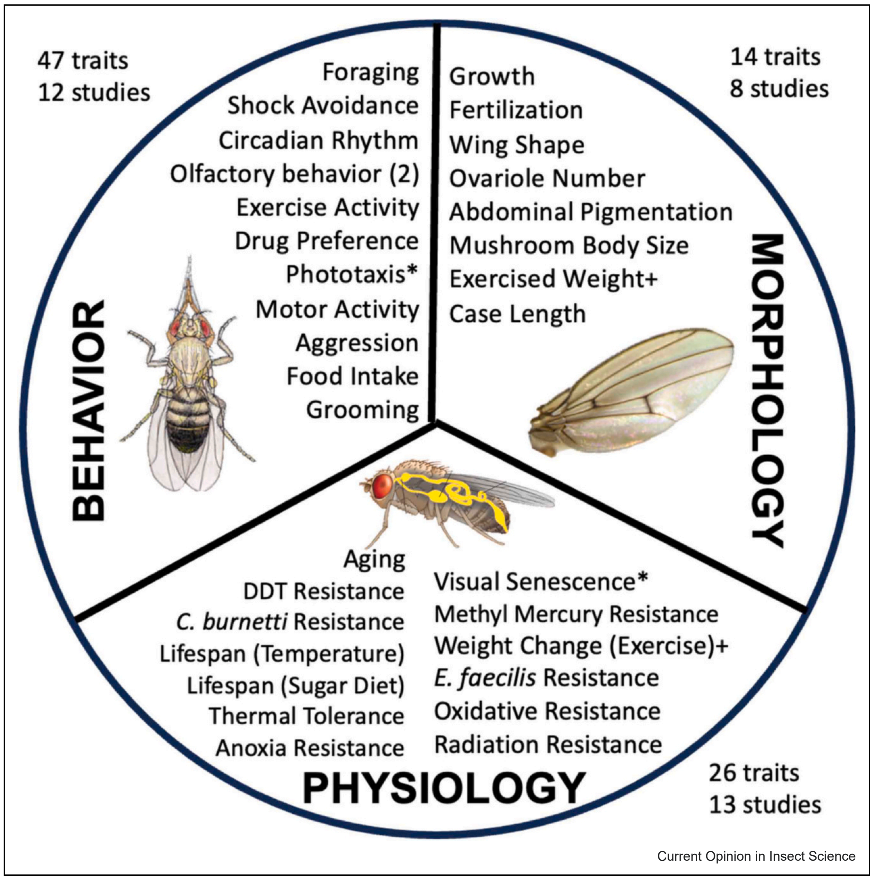
However, while behavior appears to be intuitively more plastic and complex than physical traits due to the complexity of the brain, the changes to behavior over the lifetime of an individual, and sensitivity to environmental influences, this may not necessarily be the case. There are reasonable doubts about whether behavior really has more plasticity than nonbehavioral traits [22], and the measurement of behavioral traits can be further complicated by subjectivity in how behavior is measured by researchers, and the presence of endophenotypes (behavioral subtraits) within many complex behavioral traits. Despite the many reasons to support the idea that behavioral traits should be highly complex both on a phenotypic and genetic level to our knowledge, there have been few attempts to directly assess the genetic architecture of behavior alongside nonbehavioral phenotypes (e.g. developmental, morphological, or physiological organismal-level traits).

Do behavioral traits differ quantitatively in their genetic architecture from nonbehavioral traits?

A more objective way to address this question is with a controlled quantitative comparison across many traits. To begin to investigate this question in an empirical way, we summarized the results from published GWAS from the model organism *Drosophila melanogaster*. We sought a collection of studies, all on the same organism with a standardized set of genetic backgrounds, analyzing many different behavioral and nonbehavioral traits, and using consistent methodology and study design. We capitalized on the highly useful resource of the Drosophila Genetic Reference Panel (DGRP), a set of inbred genetic lines derived from natural populations, and with sequenced and characterized genomes [23]. A previous study demonstrated the utility of a meta-analytic approach with GWAS and the DGRP, finding evidence of a larger genetic component to feeding and courtship behaviors than other types of behavioral traits, which may be more strongly environmentally controlled [24].

With genetic background thus controlled, we gathered data from 31 GWA studies using the DGRP, and divided the traits studied into morphological, physiological, and behavioral trait categories (resulting in 87 total traits,

Figure 1

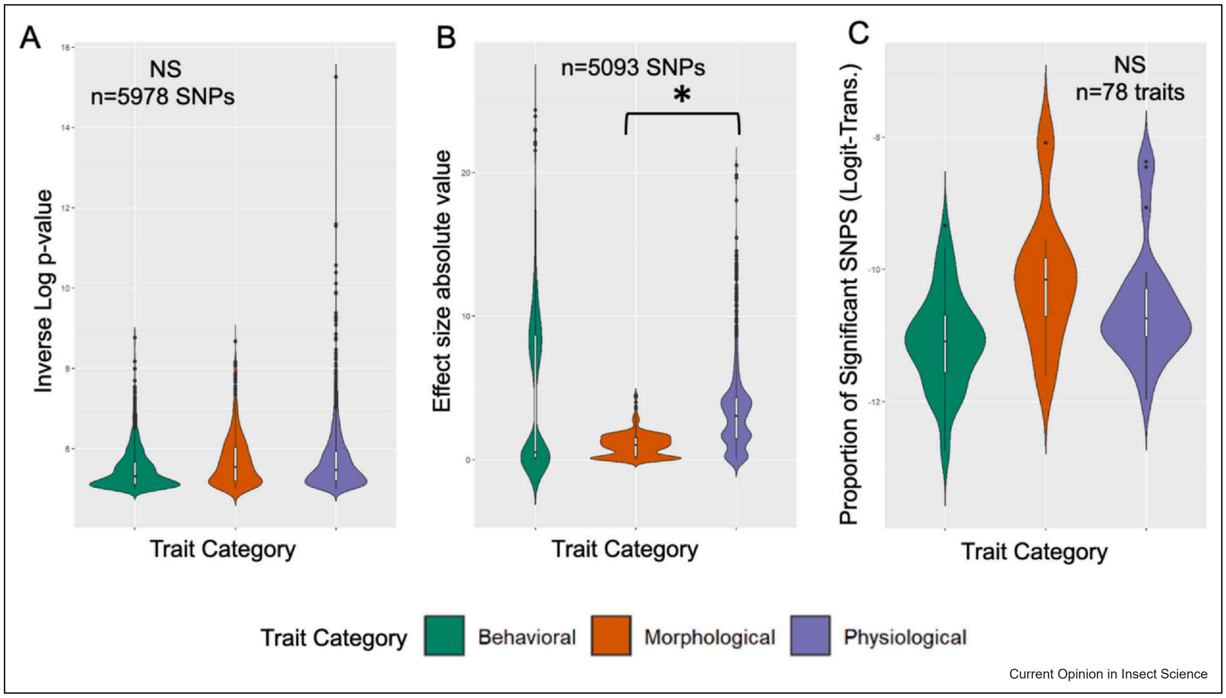


List of studies included per trait category. Note that many studies included multiple traits, thus, the total number of traits (n = 87) is larger than the total number of studies (n = 31). Two studies (indicated with * and +) included traits from multiple trait categories. Also, note that not every study was included in every analysis due to unavailable data. For more information, see Cameron R Fay, MS thesis, Iowa State University, 2022. Grooming image from [33], wing image from Wikimedia, internal anatomy image from [34].

Figure 1, data fully accessible at https://github.com/cfay75/Behavioral_Architecture_MetaAnalysis). From each study, we collected published data on effect sizes per SNP, significance of each SNP for the given trait, and proportion of SNPs found to be significant for the trait in question. Studies were excluded if fewer than 30 lines were examined, and SNPs were trimmed by significance ($p < 10^{-5}$, which is a common threshold in GWAS). We also investigated a more stringent SNP significance threshold of $p < 10^{-6}$, but this did not affect our overall results (data not shown). General linear models were then created for each metric of interest, and combined datasets from up to 31 studies were used to compare behavioral, morphological, and physiological traits to each other using pairwise Tukey's t-tests. Each model included trait category, data type, and number of included DGRP lines as fixed effects, and study as a random effect to account for individual publications that assessed multiple phenotypes in different trait categories. Additional analyses excluded the potential effects of differences in data distributions, type of data (discrete vs. continuous). A detailed description of these methods and associated results is available in Cameron R Fay, MS thesis, Iowa State University, 2022.

What do the data from across 87 traits and 31 studies, in examining these three GWAS metrics suggest? First, there were no significant differences between behavioral and nonbehavioral traits in terms of the level of significance of SNPs (Tukey's t-tests of estimated marginal means [Behavioral–Morphological: z-ratio = 1.390, p = 0.3459; Behavioral–Physiological: z-ratio = 0.503, p = 0.8699; Morphological–Physiological: z-ratio = −1.116, p = 0.5041; p = 0.4551, Figure 2a]). Second, for effect sizes, the absolute value of the effect size was significantly smaller for morphological than physiological traits, but all other comparisons were not significantly different from one another (Tukey's t-tests of estimated marginal means; Behavioral–Morphological: z-ratio = 0.794, p = 0.7068; Behavioral–Physiological: z-ratio =

Figure 2



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(a) Violin plots showing significance (inverse log of the p-value) for the three trait categories. Increased values on the y-axis correspond to an increase in significance, and within each violin plot is a boxplot showing median and quartiles, n = 5978 SNP (single-nucleotide polymorphisms), NS= no significant differences between trait categories. **(b)** Violin plots showing absolute values of the effect sizes for the three trait categories. Increased values on the y-axis correspond to larger effect sizes, and within each violin plot is a boxplot showing median and quartiles, n = 5093 SNPs, *= significant difference based on Tukey's t-test, p = 0.0026. **(c)** Violin plots showing the proportion of significant SNPs (logit-transformed) for the three trait categories with boxplot of median and quartiles. Higher values on the y-axis indicate more SNPs found to be significant, n = 78 studies.

−1.486, p = 0.2978; Morphological–Physiological: z-ratio = −3.319, p = 0.0026, Figure 2b). As there were no significant differences for effect size in comparison to behavioral traits, this also does not support the assertion that behavioral traits are more complex. Third, there were no differences between trait categories (behavioral, physiological, and morphological) in the proportion of SNPs that were significantly associated with a given trait (Tukey's t-tests of estimated marginal means, Behavioral–Morphological: z-ratio = −2.099, p = 0.0900; Behavioral–Physiological: z-ratio = −0.923, p = 0.6257; Morphological–Physiological: z-ratio = 1.409, p = 0.5041; p = 0.3363, Figure 2c). While there was a visible (but nonsignificant) trend for behavioral traits to have a lower proportion of significant SNPs than morphological traits, this is in the opposite direction of the prediction if behavioral traits have a more complex genetic basis.

Overall, these data from across 31 *Drosophila* GWAS provide no evidence that behavioral traits have a more complex genetic architecture. If anything, the data show weak support for the idea that morphological traits may have a more complex genetic basis than other traits. However, the apparent difference between morphological and physiological traits was not significant using a more stringent SNP significance threshold (data not shown). Thus, the most parsimonious interpretation from all these results combined is that there are no global differences in GWAS-based estimates of genetic architecture between types of traits, and no indication of behavioral traits having a more complex genetic basis.

Prospects for future research

More studies are needed to quantitatively and systematically address the question of how genetic architecture varies by trait type, and if this is related to trait complexity or plasticity. While preliminary, the analysis we provide above challenges the common assumption that highly plastic traits, such as behavior, have a more complex genetic architecture than other trait categories.

Although the analysis presented here rejects the hypothesis that behavior has a more complex genetic architecture than other traits, this comparison is limited in several ways. These limitations are delineated here in detail as they can help guide future research aimed at directly addressing this important question. First, the analysis presented here looked only at *Drosophila* inbred lines — a highly useful genetic resource, but representing just a handful of genetic lines from a single species.

Further analysis in other types of animals — especially species with numerous different complex forms of behavior such as sociality, learning and memory, and other highly plastic traits — is necessary to better understand genetic and behavioral connections. In addition, this meta-analysis is also limited by the informational constraints of the GWA studies that comprise it [25]. Although some of the traits we use in this study can be used as proxies to estimate genetic architecture, GWAS is not a direct metric of genetic architecture [26]. In addition, SNP-based analysis ignores other types of genetic polymorphisms. For example, variation caused by inversionsare known to influence morph differences linked to behavior and morphology in male ruffs [27]. Similarly, epigenetic variation has been found to influence traits such as human personality and psychological traits [28], and is also thought to be important in the regulation of phenotypic plasticity [29]. Other limitations include the influence of environmental differences between laboratories on GWAS results [30], as well as the fact that effect size estimates are most reliable for SNPs with moderate-to-large effect sizes. Future studies should also account for colocalized SNPs and use this information to get a more accurate count of the true number of independent genetic variants associated with each trait. We recommend a comprehensive future study involving coordinated GWAS of a large set of behavioral and nonbehavioral traits, using a controlled panel of genetic backgrounds, conducted simultaneously across laboratories, and with high levels of replication that can allow for detection of SNPs with smaller effect sizes.

Although some disease traits with major fitness consequences may stem from relatively few, rare allelic variants, evidence from modern GWAS studies suggests an exceptionally complex genetic architecture for many common traits. Such studies have led to the proposal of an ‘omnigenic’ model, which suggests a few ‘core’ genes with direct effects modulate many complex traits, but a large amount of heritability is actually attributable to a huge number of variants with miniscule effect sizes and barely detectable levels of significance [31]. These genes most likely act through indirect modulation of core genes and as part of vast interconnected gene networks. The analysis presented in this article focuses on SNPs using significance thresholds, thus and thus likely misses many subtle modulating alleles in favor of those with more easily detectable statistical associations. Nonetheless, while not directly tested, the thesis of this article is not inconsistent with the omnigenic model of inheritance for complex traits; rather, we suggest that such a view should apply equally well to all types of complex traits, whether physiological, morphological, or behavioral.

This opinion piece is not meant to provide a definitive answer to the question of whether behavior has a more complex genetic basis than other types of behavior. Rather, we invite researchers to use caution in making blanket statements about behavior being unusual in its genetic architecture. Our analysis suggests a rigorous re-examination of the assumption that behavioral traits have an exceptionally complex genetic basis is warranted. In addition, not all behavioral traits are equally plastic, thus, it would be insightful for future studies to quantify plasticity, such as with a plasticity index as demonstrated by Kapheim [32], and assess whether this is directly related to aspects of genetic architecture, both within and across different types of traits.

Data Availability

Data and code are provided on a public repository in GitHub, that link is given in the paper.

Declaration of Competing Interest

None.

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