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E PLURIBUS UNUM, EX UNO PLURA[1]: Quantitative and Single-Gene Perspectives on the Study of Behavior

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■ **Abstract** Genetic studies of behavior have traditionally come in two flavors: quantitative genetic studies of natural variants and single-gene studies of induced mutants. Each employed different techniques and methods of analysis toward the common, ultimate goal of understanding how genes influence behavior. With the advent of new genomic technologies, and also the realization that mechanisms underlying behavior involve a considerable degree of complex gene interaction, the traditionally separate strands of behavior genetics are merging into a single, synthetic strategy.

INTRODUCTION

The contrast between quantitative genetic and single-gene perspectives on heredity is as old as the science of genetics itself (Provine 1971). Francis Galton, the original quantitative geneticist, published his foundational paper in the same year as did Gregor Mendel, originator of the concept of single, stable hereditary factors (later called genes). Galton maintained that heredity is controlled by a multitude of factors, each of small effect, that sum to give the overall trait. His first efforts were directed at demonstrating the hereditary nature of "genius" in men (Galton 1865). The Mendelian perspective, although originating in the study of garden peas, was applied to human behavior as soon as it became well known to the general scientific community at the beginning of the twentieth century, largely through the efforts of Charles B. Davenport (1911), founder of the Eugenics Records Office at the Cold Spring Harbor Laboratory in 1910. During those years, an academic battle raged between biometricians and Mendelians over the issue of whether Darwinian evolution occurred by selection on small continuous variation or by discontinuous leaps. Although the proximate conflict was resolved by the development of theoretical population genetics (Provine 1971), the ultimate conflict has persisted in the form

[1]Out of many, one; out of one, many.

of two contrasting approaches: quantitative versus single-gene. These perspectives have influenced studies of the genetic contributions to behavior as much as any other area of genetic research, and they represent the classic distinction between a systems approach and a reductionist approach.

Quantitative genetics has generally taken for its raw material the naturally occurring genetic variation that affects behavior, whereas single-gene mutant analysis often creates its own raw material by inducing and isolating mutations affecting behavior and neural function. The two approaches began from different premises, and despite the fact that they had the same ultimate goal—an understanding of the genetic underpinnings of behavior—they necessarily had different proximate goals that depended on what was feasible. Quantitative genetics concerned itself with naturally occurring variation, either in the wild or in laboratory selection experiments. The focus was on the composite genotype, variance due to genetics, and evolutionary concerns; much of it is based on the theoretical work of R.A. Fisher (1930). Throughout most of this field's history, the genetic factors responsible for such naturally occurring, continuously varying traits were unknown, and the analysis consisted mainly of general statistical inferences based on phentoypic comparisons—e.g., rough esimates of the number of contributing loci and of the independence (additivity) or nonindependence (epistasis) of their interactions. Often, these results followed from artificial selection experiments in the laboratory (Ehrman & Parsons 1981, Greenspan 2004).

In its contemporary incarnations, the most commonly practiced form of quantitative genetics is quantitative trait locus (QTL) analysis. A QTL is a polymorphic locus that contains alleles with differential effects on the expression of a continuously distributed phenotypic trait. Usually it is detected by means of a DNA polymorphism, often not actually part of the gene in question, that shows association with quantitative variation in a particular phenotypic trait. The full extent of variation for the phenotype is assumed to be determined by the cumulative action of alleles at many loci, as well as by nongenetic factors. In QTL analysis, a phenotypic difference between two strains is mapped against an extensive set of distributed, gratuitous genetic markers that also differ between them, chromosomal regions mediating significant effects are mapped, and candidate genes in those segments are identified with the ulitmate goal of identifying DNA polymorphisms (QTNs or quantitative trait nucleotides) that mediate the phenotypic effects (e.g., Belknap et al. 2001, Bucan & Abel 2002, Mackay 2002). Many of the applications of this approach have been carried out in the mouse because of the existence of many well-characterized inbred strains whose inbreeding has minimized any intrastrain genetic variation (Nguyen & Gerlai 2002, Williams 2000).

In addition to inbred strains, mouse geneticists also have at their disposable a series of recombinant inbred (RI) lines. These are sets of inbred lines made from F1 hybrids between two strains, such that random mixtures of their genomes are fixed in nearly homozygous condition after repeated generations of brother-sister mating (Bailey 1971). Known polymorphisms between the two starting strains are then scored in each RI line, and a rough map is made of the chromosomal segments originating from each parental strain. The characterization of these RI lines over the

decades has allowed accumulation of a detailed set of markers, whose resolution has been vastly improved with the advent of sequence length polymorphisms (Dietrich et al. 1992) and now single-nucleotide polymorphisms (Lindblad-Toh et al. 2000). The upshot of this work is a standard set of well-characterized lines for QTL analysis of any phenotype.

Single-gene mutant analysis as a means of defining mechanisms traces its origins to microbial genetics, which concentrated on cellular mechanisms to the virtual exclusion of all evolutionary or environmental questions. It consisted of the induction of mutants to identify relevant genes and direct demonstration of biochemically definable roles and interactions among them. A particular strength of the approach is the theoretical ability to perform saturation mutagenesis for a particular trait and thereby presumably identify all of the genes that are mutable to produce alterations in the selected phenotype. Initially worked out on such prokaryotic phenomena as phage assembly (Wood et al. 1968), saturation-scale mutagenesis was later applied to more complex multicellular phenotypes such as embryonic pattern formation (Nusslein-Volhard & Wieschaus 1980). [Recent, genome-level analysis suggests that saturation mutagenesis is actually quite difficult to achieve in practice (cf. Giaever et al. 2002).]

As first applied to behavior by Seymour Benzer (1967), this approach represented a distinct departure from traditional "behavior genetics," a largely quantitative genetic discipline focused on the behavioral influences of natural genetic variation, most of which seemed to result from relatively mild effects of multiple genes, or on spontaneous mutations that occasionally appeared in laboratory strains. The single-gene, induced-mutation school was not interested in whether a gene would exhibit natural variation. Instead, the interest lay in which genes contributed to the behavior and which neural or biochemical components were selectively altered.

The majority of mutations uncovered in these searches caused disruptions more severe than allelic variants found in the wild. The single-gene practitioners' concern is with whether a mutant is "normal," not with subtle variations from the mean. The severity of a mutant phenotype is a complex function of many factors. It depends, among other things, on the nature and extent of inactivation or alteration of the gene in question, on the gene's role in development and behavior, and on the genetic background in which it is expressed (deBelle & Heisenberg 1996, Hall 1994, Gerlai 1996). Many of the recent knock-out mutations in the mouse have been notable for their lack of an obvious abnormal phenotype, sometimes referred to inappropriately as having no phenotype (cf. Hall 1994 for discussion). (Knock-out refers to the engineering of a mutation that completely eliminates a gene's product, thus producing a complete loss of function. Traditionally, these are known in the genetics literature as null mutations. Mutations producing partial loss of function are called hypomorphs and generally result from lower levels of expression, or from a change in amino acid sequence that reduces the activity, broadly defined, of the gene product.)

In flies and nematodes, most of the mutations were obtained from screening procedures deliberately designed to reveal distinctive changes in phenotype

("forward" genetics), as opposed to the mouse where most knockouts have been generated from cloned genes in an effort to ask if mutations in the particular gene will produce a distinctive phenotype ("reverse" genetics). Forward genetics is now possible in the mouse as well (Bucan & Abel 2002).

Despite the nature of Benzer's mutant hunting strategy in flies, aggressive relative to the collection of natural variants, some of the resulting mutations were considerably milder in phenotype, and often more informative, than null mutations of the same genes. The milder phenotypes associated with these mutations are still more drastic than those commonly found in nature, but they begin to approach the subtler influence of those occurring naturally. At the same time, recent molecular studies of natural variation in single genes influencing behavior have begun to close the gap between the two schools.

The purpose of this article is not to review this extensive literature, which has been done repeatedly, and more focally (e.g., Bucan & Abel 2002, Greenspan & Ferveur 2000, Hall 2003, Mackay 2002, Sokolowski, 2002, Waddell & Quinn 2001). Nor is the purpose to decide which is "better," a task analogous to asking if eating with a fork is better than eating with a spoon. They are independent ways of getting to a common goal: understanding the genetic foundations and mechanisms of behavior. Environmental effects, another critical set of parameters in behavioral genetic studies (Sokolowski & Wahlsten 2001), and interspecies differences are also not considered here. Instead, the goal is to consider the characteristics of the two major genetic approaches and the synthesis between them that is now emerging (cf. Tully 1996).

Sources of Genetic Variation

Studies of the genetic aspects of behavior have drawn on naturally occurring variants and on induced mutations. Natural variants are defined simply as spontaneously occurring, heritable variations of a gene. As such, they can be found in the wild and also in laboratory populations, the main difference residing in which kinds of new mutations will survive and be tolerated in the different environments. Artifical selection experiments represent a special case of variation within a species, in which the experimenter applies external constraints on the population by selecting for a particular phenotype. This procedure biases the polymorphisms and combinations of variants passed on to the next generation. A corollary concern of this approach is genetic background—the naturally occurring variation in laboratory strains—and its ability to influence phenotype.

Induced mutations are the mainstay of single-gene analysis. Originating in Muller's X-ray-induced mutations in the fruit fly in the 1920s, generated in an effort to ask what the gene itself is, they began to be used as an analytical tool in microbial genetics for elaborating metabolic pathways in the 1940s and 1950s, and contributed to the blossoming of eukaryotic developmental genetics in the 1980s. In contrast to quantitative genetics, less attention has been paid to genetic background.

Although genetic studies of behavior have drawn on both sources of genetic variation, each has contributed a dimension that could not have come from the other.

THE CLASSICAL APPROACHES

Genetic Architecture: Geotaxis in *Drosophila*

Artificial selection in the laboratory has been the conventional method for detecting the presence of naturally occurring genetic variation in behavior (Ehrman & Parsons 1981). Such variation has been clearly demonstrable in virtually every behavioral selection attempted (Greenspan 2004), as had already been shown for nonbehavioral phenotypes (Lewontin 1974). Behavioral differences are generally due to the contribution of many genes, usually of rather small effect, and thus refractory to conventional mapping techniques. Only their approximate number and putative interactions could be estimated from statistical analyses of selected lines and of F1, F2, and backcross progeny.

Bidirectional selection for positive versus negative geotaxis constitutes one of the classic experiments in behavioral genetics. Inaugurated by Hirsch in the 1950s, it was inspired by the selectional experiments on maze learning in rats carried out by his mentor, Robert Tryon (Hirsch & Tryon 1956). Hirsch chose *Drosophila* as his experimental material because of its genetic prowess and performed his selection on flies collected from various spots in the New York area. (One of these was a farm in Syosset, Long Island, which shows just how long ago these experiments were undertaken.) Using a choice maze that required the flies to choose 16 times between going down (positively geotaxic) or up (negatively geotaxic), he began a multigenerational selection.

Over the course of the next thirty years, he and his students selected, reselected, reverse selected, performed F1, F2, and backcrosses, tested for the relative contributions of each chromosome, and tested for correlated effects on other behaviors (reviewed by Greenspan 2004). Chromosomal analysis studies, in which strains are constructed containing various combinations of chromosomes from each of the original selected strains, were undertaken in large measure to demonstrate that the behavior had a genetic component. The strategy was originally developed for morphological studies in *Drosophila* (Robertson 1954) and has recently been rediscovered in the mouse (Nadeau et al. 2000). Because these studies compared scores with and without particular chromosomes from each selected line, they provided a very rough map of relevant loci. Moreover, since they could be put back together in various combinations after being tested individually, the presence of interactions between genes on separate chromosomes could also be inferred. The analyses revealed contributions from all chromosomes (Hirsch & Erlenmayer-Kimling 1962, Hirsch & Ksander 1969, Ricker & Hirsch 1988). A statistical (biometrical) analysis of these data subsequently revealed extensive interactions among all three chromsomes (McGuire 1992).

Hirsch's experiments demonstrated clearly that behavioral phenotypes respond to selection and have heritable components. They also hint at the complexity of the genetics, both in the number of loci likely to be involved and in the intricacy of their interactions. The studies could not say anything, however, about the identities of any of the genes.

Genetic Dissection: Circadian Rhythms in *Drosophila*

The clearest contribution single-gene mutants have made is in the realm of identifying individual genes that are central to behavioral mechanisms, pointing the way to the unraveling of the cellular mechanisms. Perhaps the best example of this strategy can be seen in studies of the circadian clock. These discoveries began with the isolation of long-day, short-day, and arrhythmic mutants in *Drosophila*, all of which proved to be alleles of the same gene, dubbed *period* (Konopka & Benzer 1971). The subsequent isolation of additional mutants in flies, fungi, and mice and the cloning of these genes revealed the cellular mechanism of the circadian clock to be nearly universal in the biological world (Hall 1995, 2003; Harmer et al. 2001; Takahashi 2004). Many of the actual genes involved, such as *period*, are conserved between flies and mammals.

The screen for these mutants was begun by Benzer's student Ron Konopka, who placed flies in the lab's spectrophotometer and left them there for days to track their daily activity rhythms. From measuring eclosion (emergence of the adult after metamorphosis) and locomotor activity came the first rhythm mutants of the *period* gene (Konopka & Benzer 1971). Konopka's *period* mutants exhibit normal activity cycles as a function of the light:dark cycle, but they cannot maintain that rhythm in constant darkness. The arrhythmic mutant (*pero*) has no discernible cycle, the short-day mutant (*pers*) has a 19-h cycle, and the long-day mutant (*perl*) has a 29-h cycle in constant darkness.

Following the discovery of the *period* gene, the quest progressed (after a hiatus of ~15 years, see Greenspan 2003) with the isolation and cloning of additional mutants: *timeless*, *doubletime*, *Clock* (neé *Jerk*), *cycle*, and *cryptochrome* (Hall 2003). What has emerged is a picture of the circadian clock as a transcriptional regulatory sequence in which each cell counts out its own 24-h period by means of an oscillating cycle of transcription and translation. A key element is that transcription of *period* and *timeless* (in *Drosophila*) is negatively regulated by their own protein products, with the consequence that as the proteins accumulate, they begin to dampen their own synthesis. Interjection of a time delay between transcription, translation, and import back into the nucleus sets the period of the events to the circadian cycle. This serves as the central time-keeping mechanism for all circadian rhythmic activities.

The first few clock genes isolated in *Drosophila* came from forward genetic screens, testing behaviorally for alterations either in adult eclosion (*per*) or in locomotor activity (*timeless*, Sehgal et al. 1994; *doubletime*, Price et al. 1998; *Clock*, Allada et al. 1998; *cycle*, Rutila et al. 1998). These make up much of the

basic cycling mechanism, *per* and *tim* repressing their own transcription, which requires activation by the transcription factors *Clock* and *cycle*, with the *doubletime* kinase regulating the degradation of *period* and *timeless* proteins.

Subsequent mutant screens played variations on the original paradigm. s*haggy*, a kinase well known for its role in embryonic development, showed up as a clock gene in a screen for dominant effects of overexpressed genes (Martinek et al. 2001), and it phosphorylates the product of *timeless*. Another kinase, casein kinase 2α, was isolated as a dominant mutation (*Timekeeper*) that suppresses the short-day phenotype of per^s (Lin et al. 2002), and it phosphorylates the products of both *period* and *timeless*. A screen based on a molecular phenotype, monitoring alterations in the circadian cycling of a reporter gene (luciferase) fused to the *per* promoter, identified *cryptochrome*, a flavin-containing protein involved in extraocular light-reception for entrainment (Stanewsky et al. 1998). The discovery of the neuropeptide gene that mediates the implementation of circadian locomotor rhythms, *Pdf* (*Pigment-dispersing-factor*), was discovered accidentally while staining immunocytochemically with antisera to the PDF neuropeptide as part of a control experiment (Renn et al. 1999). These represent only the starting members of an ever-expanding set of genes, many of which were isolated by forward genetic screens and isolated in mutant screens for totally different behaviors or for developmental defects, and subsequently shown to affect rhythms (Hall 2003).

The picture that has emerged of the cellular clock is a tour de force of the Benzerian, single-gene mutant approach. In many respects, the analysis of circadian rhythms at the level of the cellular clock resembles the pathway dissections of microbial genetics. By themselves, however, these studies do not address evolutionary issues of natural variation in these genes (see below).

Reverse Genetic Dissection: Pheromone Response in Mice

Reverse genetics has become the predominant mode of analysis in the mouse world, where the mutants are called knockouts in reference to the technology of gene replacement used to create null alleles. It falls squarely into the single-gene camp, but unlike the foregoing examples of random mutagenesis or natural variant analysis, reverse genetics starts from knowledge of a particular gene and then works backward to create a knockout (or some other specific and deliberate alteration) of that gene. It has the virtue (when it works properly, cf. Maldonado et al. 1996) of being well defined from the outset and of providing a clear test of the requirement for that gene. It suffers from the drawback that it relies on our preexisting knowledge of the relevant universe of genes, and our imagination as to which genes will play important roles. Often, the results confirm our expectations based on previous, nongenetic experiments (e.g., the effect of a serotonin receptor on aggression, Sadou et al. 1994, or of an NMDA receptor on learning, Tang et al. 1999). There are occasional surprises in these experiments, but usually the surprise is in the lack of an expected effect of the mutation on a particular phenotype.

A case illustrating all of these features concerns the vomeronasal organ (VNO) in the mouse, the part of the rodent olfactory system involved in pheromone perception. A phospholipase-activated ion channel, TRP2, that shows highly restricted expression to the VNO (Liman et al. 1999) was knocked out as a test of its requirement in pheromone discrimination. VNO neurons in the resulting mice did indeed show a failure to respond to urine-derived pheromones, as expected, and they displayed decreased aggression, also as expected. But the mutant male mice did not live up to expectations in failing to initiate sexual advances toward females. Instead, these males indiscriminately tried to mount both sexes (Leypold et al. 2002, Stowers et al. 2002). On the other hand, knockouts of a specific set of VNO receptor genes (of the *V1ra* and *V1rb* clusters) did reduce the likelihood that a male will mount a female (Del Punta et al. 2002). The failure to achieve this result in the TRP2 knockout may be due to incomplete elimination of VNO function in this mutation.

By making cloned genes accessible to targeted mutagenesis, reverse genetics has opened up a new major avenue of genetic analysis, especially in the mouse where forward genetics is more difficult. It does not, however, offer the same range of alleles that emerge from forward genetic screens, such as the long-day and short-day alleles of the *period* gene, or from natural variants (see below). This is because our imaginations pale in comparison to the myriad variations that emerge from the generation of random variants and selection for specific phenotypes, as occurs in both forward genetics and in natural selection.

MUTUAL CRITICISMS

A measure of the separation between single-gene and quantitative geneticists can be seen in their criticisms of each other. The genetic architecture camp criticizes its single-gene counterpart for the lack of relevance of the extreme phenotypes of its mutants to anything that could contribute to species evolution, as well as for its failure to take into account the contributions of genetic background to phenotype. On the other hand, the mono-geneticists disparaged the architecturists for their failure to say anything about which genes might be involved, as well as for the lack of relevance of small-effect genes to underlying mechanism. As it turns out, induced mutants do have natural counterparts, small-effect genes are relevant to mechanism, and now the genes for complex natural variation can be identified.

Natural Variants of a Single-Gene Mutant: *period*

In contrast to the severe consequences of the mutant alleles used to define the circadian clock, the *per* gene also exhibits milder, natural, polymorphic variation. This, in itself, is not surprising because virtually all genes show some degree of DNA sequence variation. The key question is whether there are detectable, functional consequences to the polymorphisms.

Two alleles of the *per* gene predominate in the wild: one containing 17 repeats of the pair of amino acids threonine and glycine $(Thr\text{-}Gly)_{17}$ in the middle of the coding reigion, and one containing 20 repeats $(Thr\text{-}Gly)_{20}$ (Costa et al. 1992). These two alleles are nonrandomly distributed in the wild, following a north-south gradient (known as a latitudinal cline) from northern Europe down to North Africa, with $(Thr\text{-}Gly)_{20}$ predominating in the north and $(Thr\text{-}Gly)_{17}$ predominating in the south. Distributions of this sort are traditionally explained as an adaptive response to climatic variation. In this case, the strongest correlation is with temperature range.

If these variants are undergoing selection, as the existence of a latitudinal cline implies, then the functional differences between them become relevant. A clue to such a functional difference was seen in tests of temperature compensation—one of the distinctive features of circadian rhythms that allow cold-blooded organisms to maintain their 24-h cycle at different temperatures (Pittendrigh 1954). Flies carrying the northerly $(Thr\text{-}Gly)_{20}$ allele exhibit more robust temperature compensation than those with the more southerly $(Thr\text{-}Gly)_{17}$ allele. The temperature range is much greater in the north and presumably imposes greater demands on the temperature-compensating ability of the clock (Sawyer et al. 1997). The size of the effect is small, perhaps reflecting a trade-off between temperature compensation and conservation of the overall 24-h rhythm.

By showing that naturally occurring variation with selectional and functional implications could be found for one of the canonical rhythm genes, Kyriacou and colleagues effectively answered the objection that induced mutations do not contribute to our understanding of natural processes. On the other hand, the *per* effects could not account for all of the detailed aspects of the variation between natural isolates, underlining the (inevitable) genetic complexity of the strains isolated from northern versus southern locales.

Reverse Genetic Natural Variation: The 5-HT Transporter in Humans

Human genetic studies must necessarily rely on analysis of the existing natural variation in the population, as no one (not even the Raelians) is proposing that we deliberately induce new mutations in ourselves. In one incarnation, the analysis is no different from QTL analysis in laboratory animals, with the exception that the available materials for study (identified subjects and recombination events for mapping purposes) are finite. The challenges to the identification and verification of individual genes from such studies, as compared to the analogous studies in laboratory animals, are correspondingly greater. Yet, these studies constitute much of the contemporary work on mapping disease susceptibility and pharmacogenomics, the genetic influences of drug response in disease treatment. In a variation on this theme, the ability to take a known gene, look for polymorphisms in it, and test for the association of a given allele with some phenotype is entirely feasible and has yielded a series of interesting insights.

The serotonin transporter (5-HTT) occupies a key position in the physiology of mood and anxiety, most notably because of its role as a target for the most popular and effective antidepressants and antianxiolytics. A polymorphism in the length of the transcriptional control region of this gene affects its transcriptional efficiency (Lesch et al. 1996). In a survey of some 500 individuals, an association was found between the short form of the polymorphism (low efficiency of transcription) and various personality measures of anxiety and depression. The short allele behaved dominantly in that there was no difference between homozygotes and heterozygotes. The effect, though statistically significant, was nonetheless small because the allelic association accounted for only 7%–9% of the variance for those measures. This kind of result—statistically significant but small in effect—is typical of such human association studies. It also exemplifies the difficulties and multigenic nature of genetic analysis in humans.

Subsequent attempts to find an overall association between 5-HTT and depression have been inconsistent, a common problem in human association studies (Merikangas et al. 2002). When the subjects' life histories are also taken into account, however, the strength of the association goes up considerably. In a study of nearly 1000 young adults, the correlation of the 5-HTT short allele with depression increased if the individual had also experienced either childhood maltreatment or several major stressful life events, according to a set of standardized measures (Caspi et al. 2003). In this study, there was a graded effect when homozygotes and heterozygotes were compared: Homozygotes for the short allele were more likely to experience depression after stressful life events or childhood maltreatment than heterozygotes, who were more likely to experience depression than those homozygous for the long allele. The subjects in this study had been followed since birth, and their traumatic life events were documented long before the diagnosis of depression, thus strengthening the association between cause and effect.

Thus, nongenetic (i.e., environmental) factors must be added to genetic factors as components of the inherent complexity of these phenotypes (Kendler et al. 2003). It is possible, nonetheless, to assign functional significance to identified, natural variants even if their contribution is only as one among a plethora of others. Moreover, it is easy to imagine that if fruit flies were capable of depression, a severe 5-HTT mutation might produce a strain of consistently and totally "bummed out" flies.

Exceptions to Natural Multi-Genicity

Not all naturally occurring variation is necessarily complex and polygenic. A few notable exceptions illustrate that a single-gene mode of inheritance can occasionally account for the majority of natural variation for a trait.

Natural and laboratory populations of *D. melanogaster* harbor two behavioral types with respect to food foraging behavior: Rovers, which search widely, and sitters, which do not (Sokolowski 1980). The effect is not merely a difference in locomotor activity because it is expressed only in the presence of food. Analysis

of F1 and F2 generation progeny showed that the vast majority of the behavioral variance could be accounted for by a single gene for which the sitter phenotype is recessive to Rover (Sokolowski 1980). Utilizing some of the more sophisticated tools of *Drosophila* chromosome manipulation, the locus was mapped and X-ray mutagenesis used to produce new alleles of the locus that was dubbed *foraging* (de Belle et al. 1989). Molecular analysis showed it to be one of two structural loci for cGMP-dependent protein kinase (*dg2*) and that natural Rovers have ~12% more activity of the enzyme than sitters, with correspondingly higher levels of *dg2* mRNA and protein (Osborne et al. 1997). Severe mutations of the *foraging* gene are lethal (de Belle et al. 1993), emphasizing once again the mild character of natural variants as compared to null alleles (Greenspan 1997).

An analogous set of findings has identified a locus in the nematode *Caenorhabditis elegans* similar to vertebrate neuropeptide receptors that accounts for natural variation in aggregation, feeding, and foraging behavior (de Bono & Bargmann 1998, de Bono 2003). These nematodes exist as "solitary" or "social" strains that feed either individually or in clumps. In all natural isolates examined, a single amino acid substitution in the *npr-1* (neuropeptide receptor resemblance) gene distinguishes the 5 solitary cases from the 12 social cases. Solitariness is dominant, null mutants are social, and social animals can be made solitary by expression of the solitary allele or by overexpression of the naturally occurring social allele. The *npr-1* mutants are not affected exclusively in aggregation behavior but also differ with respect to hyperactivity on food, burrowing into agar, and accumulating on the border of a bacterial lawn. Sequence comparisons among natural isolates of three other *Caenorhabditis* species as well as *C. elegans* indicate that the solitary allele is only found in *C. elegans*, which suggests that the social allele is ancestral (Rogers et al. 2003).

An emerging story in the human genetics literature attributes handedness to a single gene difference, with the unusual feature that the recessive phenotype is stochastic. Handedness has generally been assumed to exhibit a complex mode of inheritance (e.g., McManus 1985), if it is heritable at all (e.g., Bishop 2001). By postulating that individuals homozygous for the Non-Right-Handed (*NRH*) allele have a 50:50 chance of being non-right-handed (where non-right-handed is defined as anyone who favors their left hand for any task), the population data fit very well (Klar 1996, 1999, 2003). Heterozygosity or homozygosity for the Right-Handed (*RH*) allele ensures consistent right-handedness. This model accounts well for the population frequency of nonright-handedness if one assumes a 40% frequency of the *NRH* allele. It also accounts very well for the observation of discordance in handedness among indentical (MZ) twins, as well as for the frequency of such discordant twins and for the 50:50 proportion of non-right-handed:right-handed children of the discordant twins. A stochastic, recessive phenotype of this sort finds a precedent in another developmental asymmetry: the *situs inversus* mutation of the mouse, in which half of the homozygous mutant individuals have reversed asymmetry of their internal organs (Layton 1976). One salient feature of this mode of inheritance is the challenge it presents to mapping the pertinent locus, especially

if one is not consciously looking for it. This may account for lack of progress in identifying the putative *NRH* locus in humans. If the hypothesis is confirmed, it may eventually break the long-standing logjam in the identification of genetic factors in schizophrenia (Harrison & Owen 2003), if non-right-handedness (*NRH*/*NRH*) proves to be a predisposing factor in the disease (Klar 1999).

The foregoing examples, however, are single-gene exceptions to the quantitative complexity of most polymorphic traits.

Genetic Background: Bane of the Single-Gene Approach

Behavioral mutants are notoriously sensitive to variations in genetic background, the natural, genetic heterogeneity in laboratory stocks (also known in the classical genetics literature as modifiers). Whereas quantitative geneticists have long been aware of this ever-present variable, single-gene practitioners have not, in general, sometimes to their embarassment. A case in point is the erroneous identification of the temperature-sensitive paralytic mutation *shibire* as the voltage-sensitive sodium channel (Kelly 1974), based on the greater resistance to tetrodotoxin of the mutant relative to a control strain. The control, however, was not of the same genetic background as the mutant. As it turned out, the "control" strain was exceptionally sensitive to tetrodotoxin (Gitschier et al. 1980), whereas the *shibire* mutant was as resistant to it as most other fly strains (mutant or normal). The genetic basis of resistance in this control strain has never been properly determined.

Not only do experiments need to be conducted such that mutants and controls are on the same background (Dubnau & Tully 1998), but also mutant phenotypes will often fade over time as the result of unintended selection for such modifiers. The dependence of mutant phenotype on strain background has been well documented for learning mutants in the mouse (Gerlai 1996). The spontaneous disappearance of mutant phenotypes in the laboratory over time, well known at the level of folklore, has been documented for mutants affecting learning and brain development in the fly (de Belle & Heisenberg 1996). The range of phenotypic effects of several anatomical brain mutants is narrowed or widened in different genetic backgrounds (de Belle & Heisenberg 1996).

The ubiquity of the problem has bedeviled mutant studies all along. In a legendary incident at a *Drosophila* meeting in the early 1970s, one investigator began his talk by saying, "I would like to announce that *Hyperkinetic* is now a recessive" (J.C. Hall, personal communication, but not the speaker). That is, the strain no longer showed a dominant mode of inheritance for the mutation, as reported originally. Such phenomena are presumed to be the result of spontaneous selection for modifying alleles that are present in the population—a distinctly quantitative genetic problem! Moreover, the potency with which a given background can mask or exacerbate the phenotype of a mutation underlines its relevance to the issue of genetic mechanism. A graphic example of the range of these effects was shown in a study of modifiers of the *sevenless* mutation in *Drosophila*, a mutant originally isolated as part of a genetic dissection of phototaxis behavior (Harris et al.

1976) and subsequently studied in great depth for its role in cell fate determination in photoreceptors (e.g., Brennan & Moses 2000). When a moderate allele of *sevenless* (roughly midway between the most severe and wild-type) was placed on a range of different genetic backgrounds, phenotypes were found that ranged from fully wild-type to more severe than the most effective enhancer mutations previously isolated (Polaczyk et al. 1998). Clearly, genetic mechanisms cannot be properly understood without paying attention to such background effects. That is, single-gene effects fade into quantitative genetics at the margins.

MUTUAL BENEFITS

At this point in the discussion, it would probably seem to the single-gene school that quantitative genetics would have a lot to gain from the single-gene studies, in the form of information on the function of individual genes and on cellular mechanisms underlying behavior. Similarly, it would probably seem to the quantitative school that single-gene studies would have a lot to gain from quantitative genetics, in the form of a wider net for capturing relevant genes, and a conceptual framework for confronting the problems of gene interaction at the systems level. Both schools, on the other hand, would certainly expect everyone to gain from the new genomic technologies.

Gene Interactions: A Common Currency

Gene interactions have been an explicit part of the quantitative and single-gene programs from the outset. For the quantitative approach, the issue of interaction arises as soon as a trait is seen to be polygenic. If many genes are involved, their interactions are either additive (independent) or nonadditive (epistatic). For the single-gene approach, interactions at the molecular level are inseparable from the issue of mechanism. They constitute one of the main strategies for identifying, through screens for enhancer and suppressor mutations, components that interact molecularly. These sometimes lead to demonstration of direct, physical interactions between gene products. It is fitting, therefore, that gene interactions should emerge as one of the crossover points at which the two genetic approaches meet.

The first major proponent of gene interaction as an important element in evolutionary genetics was Sewall Wright, one of the architects of the modern synthesis of Darwinian theory with Mendelian genetics. His view contrasted with that of his coarchitects, J.B.S. Haldane (1932), who believed that alterations in a few, major individual genes were critical, or R.A. Fisher (1930), who emphasized the importance of small contributions from multiple genes acting in an additive manner. Beginning with his detailed studies of gene interaction in the formation of coat color in guinea pigs (Wright 1916) and continuing through his work on evolution by alterations in gene frequencies (Wright 1932), Wright saw a vast universe of potential gene interaction (1963). With the development of more sophisticated analyses and statistical models for quantitative trait analysis, epistasis could be

inferred from the phenotypic scores of the various classes of progeny from test crosses of wild strains or of selected lines (e.g., McGuire 1992, Zeng et al. 1999).

The problem of epistasis emerged in some of the earliest studies by Mendelians (Bateson 1909) but became of major conceptual importance when metabolic pathways began to be analyzed by means of mutants. In this context, epistasis took on a biochemical meaning when a mutation in an upstream enzyme masked the presence of a mutation in one that was downstream (Beadle & Tatum 1941). The analogy would later be applied to developmental pathways and become the paradigm for demonstrating gene interactions in molecular genetic analyses (Avery & Wasserman 1992, Greenspan 2001).

One immediately obvious difference between the use of the term epistasis by the two schools (cf. Phillips 1998) is the insistence in single-gene analysis on a strong interfering effect—e.g., blockage of a pathway's output by a knockout mutation and rescue of that blockage by an activating mutation downstream. That is, an epistatic relationship represents either a direct physical interaction between gene products, or else an interaction only one or two steps away from being directly physical, as in a metabolic or signal transduction pathway. Directness, in turn, is valued as evidence of specificity, and specificity is the shibboleth of modern molecular biology (Greenspan 2001). No such requirement is imposed by the quantitative geneticists. For that school, epistasis occurs whenever two genes interact nonadditively, regardless of how far removed physically or temporally their sphere of activity may be. The corresponding shibboleth for this view is that anything that affects the final phenotype is fair game for natural selection, and is thus relevant.

Are these two views of epistasis reconcilable? More specifically, because the quantitative version of epistasis encompasses the single-gene version, the question is whether less direct varieties of interaction are relevant for understanding core, functional mechanisms. One step in favor of satisfying this demand is the increasing evidence that genes influencing behavior are pleiotropic (Greenspan 1997, Hall 1994, Pflugfelder 1998, Sokolowski 2002). That is, the genes that have mutated to produce behavioral variants are, almost universally, genes that also play other, often vital, roles in the organism's biology. This imposes a stringent selection on behavioral mutants. They must pass through the eye of a needle in order to retain viability and relative normality in most respects, while exhibiting defects in the behavior in question.

A case in point is the *optomotor-blind* mutation in a T-box domain transcription factor, which affects the optomotor response to horizontal motion in *Drosophila* (Heisenberg et al. 1978). It is an unusual allele of *bifid*, a vital gene required for many aspects of fly development, selectively altering transcription in a restricted part of the fly's optic lobes (Heisenberg 1997). As a consequence, these mutants are missing critical, motion-detecting neurons. Similarly, the learning mutant *Volado* is a special allele of the vital *scab* locus, which encodes an α-integrin required in many aspects of fly development (Grotwiel et al. 1998). That the same can be said of the *foraging* gene, i.e., null alleles are lethal (de Belle et al. 1989; and see above), illustrates a fundamental similarity between quantitative and single-gene

traits: Behavioral variants are special or mild mutations of genes that have much broader roles.

If pleiotropy is widespread (perhaps even ubiquitous) among genes that affect behavior, then the potential for interactions between genetic variants is vastly expanded. The wider the network of contacts a gene product makes, the more chances there are for an alteration in another gene to influence it. This suggests the basis for a rapprochment between single-gene and quantitative analyses. If the traits studied in single-gene mutant studies were measured more sensitively (i.e., quantitatively) so that less extreme phenotypes were also examined, then mutant screens would yield a wider range of genes. As a result, many more of these wide-ranging interactions would be revealed, many more elements of the core mechanisms would likely emerge, and our concept of a core set of genes would be correspondingly enlarged. Such an approach has already shown its value in the application of a large set of mild, quantitative variants in bristle number to the study of peripheral nervous system development in *Drosophila* (Norga et al. 2003).

Identifying "All" of the Genes: A Second Crossover Point

A prominent feature of our current genomic era is the drive for encyclopedic coverage, to identify "all" of the genes subserving a given process. There were precedents in the saturation mutant screens of the past, carried out in microorganisms (e.g., Wood et al. 1968, for phage morphogenesis), and in a few instances in *Drosophila* (e.g., Nusslein-Volhard & Wieschaus 1980, for embryonic cuticle patterning) and *C. elegans* (Ferguson et al. 1987, for vulva development). For the quantitative geneticists, this has been the quest all along: to know all of the players and not to assume that there are only a few major ones. For the single-gene practitioners, it has motivated the extensive mutant hunts.

Both approaches are currently benefiting from the capabilities offered by genomic technologies. The availability of whole-genome sequences has vastly accelerated the process of identifying a new mutation, high-resolution DNA polymorphism maps have given a major boost to QTL studies, and genome-wide expression analysis with DNA microarrays has provided a new avenue into the range of gene activities that underlie a phenotype. These technologies have enabled a new synthesis between the quantitative and single-gene strategies, not owing to any conceptual breakthrough but simply to the newfound ability to identify individual genetic factors.

CLOSING THE GAP

Geotaxis in *Drosophila* Revisited

The ability to monitor the expression level of every gene in the genome under different conditions and in different genotypes is a technique whose promise has been touted widely (e.g., Lander 1999, White 2001). Use of the technology has already complemented and extended quantitative genetic analysis. One of the first

applications of the new technology to quantitative genetic problems utilized microarrays to identify gene expression differences between Hirsch's bidirectionally selected geotaxis lines (described above). Although not all such mRNA expression differences would be due to actual genetic polymorphisms in the affected genes, some would, and others could be indicative of downstream effects of polymorphic genes. With these caveats in mind, RNA from adult heads of the positively geotaxic (Lo) and negatively geotaxic (Hi5) lines were analyzed on an array representing roughly two thirds of the fly genome (Toma et al. 2002). Many genes were differentially expressed (~250), representing a wide range of functions (e.g., transcription, signal transduction, cytoskeleton, and metabolism).

To test for the functional significance of the differences, mutants in some of the affected genes were tested in the geotaxis maze, after first being standardized with respect to genetic background (Toma et al. 2002). Several of the mutants were found to deflect the geotaxis response in the predicted direction from the geoneutral response of the background strain. The predicted direction for a mutant was based on the expression level of that gene in the selected lines; a null or hypomorphic mutant should behave like the line with the lower expression level. Thus, for several of the loci, a severe, single-gene lesion could mimic a selected phenotype. In most cases, however, no single-gene effect was as strong as that of the aggregate effect in a selected line.

The genes found to affect geotaxis are pleiotropic. (Actually, this had to be true, simply because they were existing mutants that had been isolated on the basis of some other phenotype.) One is relatively restricted in the nervous system: the neuropeptide gene *Pigment-dispersing-factor* (*Pdf*), which is involved in mediating the locomotor output of the fly's circadian rhythms (Park et al. 2000, Renn et al. 1999). The others are rather widely distributed, including *Pendulin*, encoding the nuclear import protein importin-α (Torok et al. 1995), and *cryptochrome*, encoding a flavin-binding protein that serves as an extraocular photoreceptor in circadian rhythms (Stanewsky et al. 1998).

The foregoing example bridges quantitative and single-gene studies in two ways: first, the use of selected strains to identify the many contributions of individual genes, and second, the use of preexisting single-gene mutants to test and validate the functional relevance of genes differing between the selected strains (cf. Long et al. 1996). The idea of using laboratory selection as an avenue toward identifying genetic mechanisms, unthinkable in the traditional single-gene world, has now become the method of choice in certain instances (e.g., Dierick & Greenspan 2003).

A QTL for Sleep EEG in Mice

Many aspects of sleep behavior and physiology vary among inbred strains of the mouse (Tafti & Franken 2002). EEG measurements revealed a particularly prominent difference in theta oscillations (4–12 Hz) during both paradoxical (REM) and slow-wave sleep among mouse strains (Franken et al. 1998). Theta

oscillations are thought to modulate REM sleep insofar as treatments that suppress theta also suppress REM sleep (M. Tafti, personal communication). The theta-peak frequency (TPF) was found to vary from 6.12 to 7.61 Hz in the lowest (A/J) and highest (C58BR/cdJ) strains.

To apply genetic analysis to theta oscillations during paradoxical sleep, Tafti et al. (2003) first determined that slow TPF was recessive by making F1 hybrids between a slow TPF strain (BALB/cByJ) and a fast TPF strain (C57BL/6J). By producing F2 progeny from these two strains and scoring TPF phenotype and chromosomal markers, they found a major QTL on chromosome 5. Further crosses were performed, designed to subdivide the region further, and a 2.4 cM chromosomal segment was identified—small but likely to contain upwards of 20 genes.

At this point, the analysis would have stalled had it not been for the existence of relatively severe mutations in two of the promising candidate genes in that region: a knockout in *Nos1* (neuronal nitric oxide synthase), and a naturally occurring mutation of *Acads* (short-chain acyl-coenzyme A dehydrogenase), the first enzymatic step in fatty acid beta-oxidation. *Nos1* was a likely candidate because nitric oxide is known to affect theta oscillations (Datta et al. 1997), but the mutant's TPF score was similar to its own background C57BL/6J score. The other well-defined variant in that chromosomal segment is the *Acads* mutation, a spontaneously arising deletion of several hundred base pairs in the gene in Balb/cByJ that produces a null phenotype (Reue & Cohen 1996).

The *Acads* mutation in Balb/cByJ arose spontaneously after it had been separated in the 1980s from its parental Balb/cBy strain. Thus, these two strains differ exclusively (or nearly so) in the *Acads* gene, and when TPF was compared between Balb/cByJ and Balb/cBy, it differed. Further tests of recombinant inbred lines between the original C57BL/6J and Balb/cBy strains, and of additional recombinant progeny generated from them, confirmed that there were no other loci influencing TPF segregating in these strains. Additional evidence supporting *Acads* involvement came from DNA microarray studies and metabolite administration, strengthening the correlation between TPF and fatty acid beta-oxidation in mitochondria. As an enzyme involved in energy metabolism, the *Acads* gene has potential significance for sleep insofar as one of the active hypotheses for understanding the underlying function of sleep postulates a central role for energy metabolism (Benington & Heller 1995, Kong et al. 2002).

This study draws on nearly all aspects of the genetic arsenal available in the mouse: strain differences, DNA markers, genome sequence information, spontaneous and induced mutants, recombinant inbred strains, and microarrays. In making a successful gene identification, all were needed.

Long-Term Memory in *Drosophila*—Combining Forward and Reverse Genetics

In a forward genetic, single-gene mutagenesis attempt to get at all of the genes subserving a behavior, Dubnau et al. (2003) carried out a large-scale screen for new

mutants ($N = 60$) defective in long-term memory, and in parallel, they performed a DNA microarray analysis to identify genes ($N = 42$) expressed in the brains of flies under conditions that produce long-term memory. These genes (both sets) run the gamut of biological functions: transcription, translation, signal transduction, cytoskeleton, and metabolism. The investigators then determined the overlap between the two sets of genes and tested mutants identified on the arrays that fell into the overlap. The results suggested a requirement for the machinery of mRNA localization and translational regulation in the consolidation of long-term memory. The approach relies on training protocols that had previously produced 3-h-versus-1-d memory of an odor made aversive when coupled to electric shock in the fly (Tully et al. 1994). The distinction in memory duration depends on whether these trials are administered all at once (massed) for short-term memory or with 15-min intervals (spaced) for long-term memory. This distinction provided an internal control for judging mutants, e.g., are they normal for immediate memory but abnormal for long-term memory? The same criterion—massed versus spaced training—was the differential applied to the gene profiling results.

The microarrays pointed to the mRNA localization genes *staufen* and *moesin* and the translational regulation genes *pumilio*, *orb*, and *eIF2G* as upregulated selectively after spaced training. The mutant screen isolated new alleles of the related genes ($oskar^{norka}$, $pumilio^{milord}$, and $eIF5C^{krasavietz}$) as showing defective long-term memory. Extant mutants in *staufen*, identified on the arrays, were tested and also found to have abnormal long-term memory. Further confirmation came from a temperature-sensitive genotype of *staufen*, which permitted a demonstration that the gene product is needed in the period soon after training to be effective. The issue of a critical period for these effects is especially relevant, given that all of these genes are capable of affecting development and viability: *staufen*, *oskar*, and *pumilio* were isolated originally as maternal effect genes, certain alleles of which produce grossly abnormal embryos when the mother is mutant (Palacios & St. Johnston 2001), and the one preexisting allele of *eIF5C* has severely reduced viability (Spradling et al. 1999).

The foregoing is an example of a single-gene study that begins to look quantitative based on having cast such a wide net for "all" (or at least as many as possible) of the genes mutable to that phenotype. The spectrum of biological functions revealed in the mutant screen alone should dispel any notions of the unimportance of pleiotropic genes and their far-ranging activities for a core mechanism.

Odor-Guided Behavior in *Drosophila*

A study of odor-guided behavior in the fruit fly bridges quantitative with single-gene analysis in a different way. A series of insertional mutations was identified in which flies fail to jump in response to benzaldehyde (Anholt et al. 1996). Fourteen *smell impaired* (*smi*) mutant lines were recovered from several hundred strains in which a *P* element, a transposable DNA sequence routinely used for insertional mutagenesis in *Drosophila*, had been inserted at random on a chromosome. Any

lines that were not viable and healthy as homozygotes were discarded, as were those that showed any locomotor defects. Because they are insertional mutants, the resulting 14 variants could be easily mapped to their exact chromosomal location using genomic information, and the genes cloned and sequenced (e.g., Ganguly et al. 2003, Kulkarni et al. 2002).

A classical quantitative genetic analysis was then performed: interactions among the genes were assessed by constructing pairwise combinations of the mutants and testing the resulting double heterozygotes (i.e., *mutant1*/+, *mutant2*/+) for their olfactory responsiveness. Many of the pairwise combinations showed epistatic interactions: a more potent effect of the combination than predicted from the average effect of each mutant by itself (Federowicz et al. 1998).

Had the study stopped there, the conclusion would have been that mutants isolated for a common phenotype can show interactions—this is not particularly surprising or informative. Fortunately, it did not, and the investigators went on to measure the genome-wide transcriptional profiles of five of these *smell impaired* (*smi*) mutants with DNA microarrays (Anholt et al. 2003). The results showed, once again, a wide range of genes whose transcription levels are altered in each mutant. More importantly, there was an overlapping set of gene expression effects among the interacting *smi* mutants. Behavioral tests of extant mutants in several of the genes identified on the microarrays (e.g., the ion channels *Shaker* and *trp1*, the component of synaptic release *Syntaxin1A*, *calmodulin*, and the GABA receptor *Rdl*) further demonstrated their functional relevance to the *smi* phenotype.

BEHAVIOR-GENETIC ANALYSIS: THE NEW SYNTHESIS

> Almost never can a complex system of any kind be understood as a simple extrapolation from the properties of its elementary components.
>
> D. Marr (1982)

Reductionist schools of thought usually define themselves in opposition to systems schools. This certainly describes the relationship between the single-gene analysts of behavior and the behavior-genetic architecturalists. The disagreement usually revolves around each side's view that the other is missing some important point. The behavior geneticists felt that the single-gene approach ignored the complexity of interactions of genes and the inherent variability of genes in each population. For their part, the single-gene analysts objected to the lack of identification and mechanistic explanation of the entities being described by the behavior geneticists. Neither was wrong because neither had the whole answer.

The passage of time and the elaboration of findings in opposing schools sometimes allow a reconciliation. As a result, there is now a basis for synthesizing the viewpoints and principles from each school.

Single Genes in Genetic Architecture Terms

From its inception, the concept of genetic architecture implicitly acknowledged the existence of single genes influencing behavior and of the interactions of several or many such genes determining a particular phenotype. The expansion in the modes of analysis described above enlarges our view of the varieties of gene action and sensitizes us to the network aspects of the system.

The recognition of the ubiquity of pleiotropy in gene action (Greenspan 1997, Hall 1994, Pflugfelder 1998, Sokolowski 2002) means that each gene has, in effect, its own architecture—a distributed pattern of action through the various stages and tissues of the organism. In this sense, the summated action of the genes is not so much a jigsaw puzzle in which each piece fits together with its immediate neighbors in one spot, but is rather a flexible, multilayered network (cf. Greenspan 2001)—a viewpoint that was implicit in quantitative genetics and that single-gene genetics has been slowly approaching.

Genetic Architecture in Single-Gene Terms

In populations, the pleiotropic, network attributes of genes have consequences for how genetic variation can produce behavioral variation. Each allele of a gene can potentially contribute in several ways to phenotype. These contributions, in turn, depend on the partners with which a gene interacts. Variation can thus occur in a restricted portion of a gene's range of activities if its interacting partners are more sensitive to perturbation in one place than in another. If its interacting partners also come in allelic variants, a further dimension is added.

Phenotypic variation in a population, which is what one measures, is thus not a monotonic function of allelic variation. Instead, it may well represent a more complex fabric than the distribution of alleles alone might suggest. This may seem to present an even-more-bewildering picture than the traditional view. Its saving grace, however, is knowing that the network nature of a gene's interactions ultimately makes its contributions to phenotype more comprehensible. Further study of the interacting nature of one gene's variation with that of another, in turn, brings its population genetic architecture within the realm of comprehensibility.

The two perspectives can be distilled into one: many genes for each behavior (e pluribus unum), many behaviors from each gene (ex uno plura).

The Relative Contributions of Genes Differ

The concept of genetic architecture has always assumed that where there are multiple genes, they do not necessarily contribute equally to the behavioral phenotype. Some are strong effect, some weak, and every stripe in between, but all are subject to changes in the strength of their effect in different genetic backgrounds. This view finds support in both classical and molecular studies.

In contrast, the idea that the various contributing genes sum to produce the phenotype, R.A. Fisher's concept of additive, independent factors in quantitative

genetics (Fisher 1930), has found less support (e.g., Weber et al. 1999, 2001) than its converse, nonadditive interactions (e.g., Clark & Wang 1997, Federowicz et al. 1998, Mackay 2002). The recent findings described above provide further support for nonadditivity by suggesting a molecular basis for it in the wide-ranging effects seen on expression across the genome.

Synergism and network flexibility make it easier to conceive of how new properties in behavior can emerge: Tune an allele up here, tune another one down there, combine them with some other preexisting variants, allow it all to ripple through the networks, and boom! you have a new behavior. Although no one is yet at the point of demonstrating this in the lab, the threshold effects frequently seen in selection experiments, in which the phenotype does not move at all for many generations and then diverges rapidly (e.g., Erlenmeyer-Kimling et al. 1962), or in which the phenotype fluctuates dramatically before diverging consistently (e.g., Manning 1961), suggest that such effects can occur in the laboratory, where they can be studied in the ways exemplified above.

At the same time, it is also easy to imagine that the number of ways for genes to influence behavior will be manifold. It will depend on the context of other alleles present (i.e., genetic background), as well as on the actual role(s) a given gene plays in that behavior. The impact of one level, the individual gene, on the other, the gene system, is reciprocal: individual genes influence the network, and the network properties, in turn, influence the action of individual genes.

At the beginning of the single-gene era of behavioral studies, Sydney Brenner (1973) remarked, "Understanding the genetic foundations of behavior may well require solving all of the outstanding questions of biology." The thirty years that have passed since then suggest that this may not be quite true. But to the extent that we must understand the nature and principles of how gene networks influence complex phenotypes, the synthesis of quantitative and single-gene approaches currently underway would seem to be a prerequisite.

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CONTENTS