

Of Flies and Man: *Drosophila* as a Model for Human Complex Traits

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Annu. Rev. Genomics Hum. Genet. 2006.7:339–67

First published online as a Review in Advance on June 6, 2006

The *Annual Review of Genomics and Human Genetics* is online at genom.annualreviews.org

This article's doi: 10.1146/annurev.genom.7.080505.115758

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1527-8204/06/0922-0339\$20.00

Key Words

quantitative trait loci, pleiotropy, epistasis, genotype by environment interaction, transgenic models of human disease

Abstract

Understanding the genetic and environmental factors affecting human complex genetic traits and diseases is a major challenge because of many interacting genes with individually small effects, whose expression is sensitive to the environment. Dissection of complex traits using the powerful genetic approaches available with *Drosophila melanogaster* has provided important lessons that should be considered when studying human complex traits. In *Drosophila*, large numbers of pleiotropic genes affect complex traits; quantitative trait locus alleles often have sex-, environment-, and genetic background-specific effects, and variants associated with different phenotypic are in noncoding as well as coding regions of candidate genes. Such insights, in conjunction with the strong evolutionary conservation of key genes and pathways between flies and humans, make *Drosophila* an excellent model system for elucidating the genetic mechanisms that affect clinically relevant human complex traits, such as alcohol dependence, sleep, and neurodegenerative diseases.

Quantitative trait locus (QTL): a region of the genome affecting a complex trait bounded by molecular markers; these regions can be large in an initial genome scan, or as small as a single gene in a high-resolution mapping study

GENETIC DISSECTION OF HUMAN COMPLEX TRAITS: CHALLENGES AND PROSPECTS

Human populations harbor a rich diversity of phenotypic variation for aspects of morphology, physiology, behavior, and susceptibility to common diseases. A spectrum of genetic architectures underlies this spectrum of phenotypic diversity. At one end of this spectrum are alleles with large phenotypic effects that segregate in a Mendelian or nearly Mendelian fashion, giving rise to qualitative differences in phenotype. The genes at which these alleles segregate can be identified with relative ease by linkage mapping in large pedigrees because the genotype can be unambiguously inferred by observing the phenotype, and typically the large effects are attributable to an obvious genetic lesion. At the other end of the spectrum are quantitative traits, so called because they give rise to quantitative differences in trait phenotypes between individuals, such that the distribution of phenotypes in the population approximates a statistical normal distribution (42). This continuous quantitative variation is attributable to the simultaneous segregation of multiple interacting quantitative trait loci (QTLs) with individually small effects, whose expression is sensitive to the environment. Identifying the genes at which these alleles segregate is much more problematic because the phenotypes are often difficult to quantify accurately and the relationship between genotype and phenotype is not simple. Because the expression of alleles affecting quantitative traits is sensitive to environmental variation, one genotype gives rise to multiple phenotypes. Conversely, many genotypes can give rise to the same phenotype, because alleles at multiple loci with similar effects segregate in the population. Furthermore, the genetic variants affecting complex phenotypes may be in noncoding regions of the genome, and not easily discerned by examination of the primary DNA sequence. Unfortunately, there is an inverse correlation between the population frequency of allelic variants af-

fecting complex phenotypes and their effect on the trait. Alleles with large phenotypic effects are often deleterious and are consequently rarely encountered. Alleles affecting quantitative traits are more common, accounting for the bulk of observable phenotypic variation.

A comprehensive understanding of the genetic architecture of human complex traits requires that we answer the following questions. How many loci affect variation in the trait, and what is the distribution of allelic effects? The questions are related: For a fixed amount of genetic variation, the magnitude of allelic effects per locus decreases as the number of loci increases. Thus, “complex” could run the gamut from one extreme of a few (e.g., 10) loci with rather large effects to the other of many (e.g., 100) loci with very small effects, to an intermediate situation in which the distribution of effects is exponential, where a few loci with large effects account for most of the variation, and increasingly larger numbers of loci with increasingly smaller effects make up the residual variation (144). Clearly these scenarios have contrasting implications regarding the likelihood of identifying all of the genetic variants contributing to variation in complex traits. What genes causally contribute to the observable variation? Do the genes interact additively, or epistatically? If the latter, expression of variants at one locus will be suppressed or enhanced by variants at another; this context dependence increases the difficulty of identifying the individual players. What are the pleiotropic effects of alleles affecting variation in one trait on variation in others? Pleiotropic effects on reproductive fitness determine what balance of evolutionary forces is responsible for maintaining the segregating variation. How does the expression of alleles differ between males and females, and in different physical and social environments? Again, any context dependence is vitally important in terms of personalized medicine, but increases the difficulty of genetic dissection. What are the molecular polymorphisms

causing the difference in expression of QTL alleles, and what are the molecular mechanisms causing the variation in expression in different genetic backgrounds, sexes, and environments? What is the relationship between natural variation in transcript abundance and variation in trait phenotypes?

Twin studies clearly implicate moderate to strong heritabilities of human complex traits, including aspects of morphology, behavior, longevity, and disease susceptibility (42, 103). Nevertheless, it has proven difficult to unambiguously identify the causal genes. Because QTLs have small and environmentally sensitive effects, they must be mapped by linkage disequilibrium (LD) with molecular markers that do exhibit Mendelian segregation. The methods are computationally complicated (103) and will not be reviewed here, but the principle is simple and was recognized more than 80 years ago (151). If a QTL is linked to a marker locus, there will be a difference in mean values of the quantitative trait among individuals with different genotypes at the marker locus. Alternatively, for threshold traits [which are scored on a binary scale of “affected” or “not affected,” but which have an underlying continuous liability (41)], there will be a difference in marker allele frequency between affected and unaffected individuals.

To date, two common designs have been used to map QTLs for human complex traits: whole genome scans utilizing LD in pedigrees, and association mapping of candidate genes or candidate gene regions in unrelated individuals that capitalizes on historical LD. Although thousands of such studies have been reported, repeatability is generally poor. Both designs suffer from the problem of accurate phenotypic definition of the traits (47, 129, 163); different clinical phenotypes can yield discordant results due to underlying genetic heterogeneity. Small sample sizes limit the statistical power to reliably detect QTLs unless they have large effects (42, 103), and the effects of those that are detected will be overestimated (14), leading to underpowered replication studies. Small sample sizes

also preclude attempts to tease out context-dependent effects, such as sex, environment, and background genotype, which, if pervasive, will also contribute to failure to replicate findings across studies. Issues of bias and precision also plague efforts to understand the genetic architecture of complex traits. Whole genome linkage scans are unbiased, at least within the context of the study population, but localization of QTLs tends to be in the 20-cM range—fine-scale mapping requires informative recombinants within this region, and hence much larger samples and a high density of informative polymorphic markers within the pedigree. Association studies targeting a candidate gene (or gene region implicated by QTL mapping) are precise but potentially biased by population admixture (42, 103) or missing true causal variants by selective genotyping of markers (93).

In the near future, many of these issues can be addressed using large-scale whole genome association analyses (143). This design is predicated on the discovery of the block-like LD structure of the human genome, with blocks of variable length with low haplotype diversity, separated by regions of high recombination (175); thus, each block need only be “tagged” by a small number of markers to recover the majority of haplotypes. However, it is important to consider the likely genetic architecture of human complex traits in designing these large and expensive studies. Furthermore, the very block-like structure of LD in the human genome is an impediment to identifying the genes and molecular variants corresponding to the QTLs. Few to many genes may be imbedded in the haplotype block associated with the trait, and most are likely computationally predicted genes with unknown function. Finally, this is about as far as one can go with human genetics, which is of necessity a descriptive endeavor. One solution to this impasse is to functionally test hypotheses regarding candidate genes using the mouse as a genetically tractable mammalian model. However, even mice have drawbacks *vis à vis* generation interval and the expense of rearing

Linkage disequilibrium (LD): the nonrandom association of alleles at two or more polymorphic loci in a population

SNP: single nucleotide polymorphism

GAL4/UAS: a binary expression system whereby a gene tagged by a *P*-element containing the yeast transcription factor *GAL4* is expressed in the presence of a second *P*-element containing the yeast upstream activating sequence (*UAS*), which can be fused to *lacZ* or *GFP* for analysis of tissue-specific expression, or to a promoter or introduced human gene for targeted overexpression

the large numbers of animals required for quantitative genetic analyses. In this review, we present the case for using *Drosophila* as a model system for understanding the genetic basis of human complex traits, both in terms of general principles and discovery of orthologous genes and pathways. As it is not possible to summarize this vast literature in a few pages, we highlight a few examples, and apologize in advance to authors whose work is not cited due to space constraints.

WHY FLIES? *DROSOPHILA* AS A MODEL FOR THE STUDY OF COMPLEX TRAITS

Two general and somewhat surprising themes to emerge from the plethora of whole genome sequence data are (*a*) a large fraction of these genomes is uncharted phenotypic territory, and (*b*) there is great evolutionary conservation of genes affecting common biological processes and molecular functions across a diverse array of taxa. In *Drosophila*, less than 20% of the 13,600 genes and predicted genes have been characterized by classic genetic and molecular methods (1). Furthermore, there is direct homology between *Drosophila* genes and genes that affect human disease. Of all the genes known to affect human disease, more than 60% have *Drosophila* orthologs, and more than half of all *Drosophila* protein sequences are similar to those of mammals (149). Thus, lessons learned from studies of *Drosophila* complex traits will provide guidance for experimental design of human studies. Determining the effects of mutations and natural variants affecting evolutionarily conserved complex traits in *Drosophila* will suggest positional candidate genes to include in human association study designs. Further, *Drosophila* models of human diseases directly implicate cellular mechanisms that underlie the etiology of these disorders, and are potentially powerful systems for identifying genetic modifiers, therapeutic targets, and drug testing.

The *Drosophila* genome has been sequenced (1) and well annotated (39). Pub-

licly available resources include collections of mutations at single loci [the goal of the Berkeley *Drosophila* Genome Gene Disruption Project is to obtain mutations in each of the genes and computationally predicted genes (15), many of which have been generated in a defined isogenic background (176)], and deficiencies that cover nearly 80% of the genome that are useful for high-resolution mapping, many of which have molecularly defined breakpoints in an isogenic background (133). A battery of common single nucleotide polymorphism (SNP) and insertion/deletion variants is available for high-resolution recombination mapping (16). The *P* transposable element has been harnessed as an efficient vector for transformation and insertional mutagenesis, and the binary *GAL4/UAS* system (19) can be used to analyze tissue-specific expression patterns, general overexpression of candidate genes, and targeted expression in space and time (182). There are now efficient techniques for targeted gene knockouts and allelic replacement as well as RNAi (54), and several platforms are available for whole genome analysis of transcript abundance.

Drosophila exhibit a rich repertoire of complex traits, some of which have clear human homologs; e.g., circadian rhythm, sleep, drug responses, locomotion, aggressive behavior, and longevity. Furthermore, natural populations of *Drosophila* harbor substantial genetic variation for practically any trait that can be defined and measured (42), and thus exploited to map QTLs. The large numbers of individuals required for analysis of quantitative trait phenotypes can be reared economically under controlled environmental conditions, and the short generation interval facilitates construction of replicated “designer” genotypes. Segregating variation for any trait of interest can be frozen in a panel of lines derived from nature by inbreeding, or by essentially cloning wild derived chromosomes using balancer stocks and placing them in a common background to create chromosome substitution lines (36, 84, 102, 190, 191). Extreme lines are useful for QTL mapping, and the

entire panel can be used for LD mapping studies. Repeatedly selecting the extreme scoring individuals from the population and mating them to construct divergent artificial selection lines is an efficient method for rapidly concentrating together all alleles that affect increasing or decreasing values of the trait, providing excellent starting material for QTL mapping (42, 61, 97). In addition to the usual backcross and F2 designs for QTL mapping in organisms that can be inbred and crossed (42, 103), one can construct whole genome recombinant inbred lines by inbreeding the F2 generation to homozygosity, as well as isogenic recombinant chromosomes in a common background (61, 97, 190, 191) and interval-specific congenic lines (95, 100, 102).

The ability to replicate genotypes is important because heritabilities of most quantitative traits rarely exceed 0.5, and can be much less for components of behavior and reproductive fitness. Low heritabilities mean that an individual's phenotype is a poor guide to its genotype because of variation attributable to the environment (no matter how strictly controlled). Thus, the ability to measure any number of individuals of the same genotype effectively cancels out the environmental variation, and gives an accurate quantification of the genotypic effect (42). This is essential if one is to assess QTL alleles with subtle as well as large effects.

GENETIC ARCHITECTURE OF COMPLEX TRAITS: LESSONS FROM *DROSOPHILA*

How Many Loci?

There are two distinct contexts in which we seek to understand the number of loci that affect a quantitative trait: the number of loci required to produce the trait, and the subset of these loci that harbor naturally occurring allelic variation for the trait. The first endeavor is best carried out by mutational analysis, and the answer to the second is the province of QTL mapping.

Analysis of subtle, quantitative effects of adult viable and fertile mutations generated by single *P*-element insertions in an isogenic background is an effective and direct approach to identifying genes that affect complex traits (104). Conducting such screens in an isogenic background is critical for detecting subtle mutational effects, because effects of segregating QTLs in an outbred strain will be of the same magnitude as the effects we wish to detect. Further, it is necessary to evaluate multiple individuals bearing the same mutation for the trait phenotype, because mutations with quantitative effects are sensitive to environmental variation. To date, these screens have been conducted for activities of enzymes involved in intermediary metabolism (31), sensory bristle number (101, 123), olfactory behavior (6), and resistance to starvation stress (62).

Two major conclusions emerge from this work: (a) Screening for quantitative effects of induced mutations is a highly efficient method both for discovering new loci affecting complex traits and determining pleiotropic effects of known loci on these traits. (b) In each case a substantial fraction of the *P*-elements tested (at least 4%) affected each trait; therefore, the number of genes potentially affecting any one trait is large, and most genes must have pleiotropic effects on multiple complex traits. The conclusion that large numbers of loci potentially affect complex traits is backed up by the observation of extensive transcriptional coregulation in response to coisogenic *P*-element-induced mutations; mutations at coregulated loci in turn interact epistatically with the focal mutations (5). Examples of pleiotropy include *P*-element insertions in transcribed regions of the neurodevelopmental loci *extra macrochaetae*, *roundabout*, *tram-track*, and *kekkon-1*, which affect both bristle number (123) and starvation tolerance (62). A *P*-element insertion in *scribble* (*smi97B*), which is essential for establishing polarity in epithelial cells during embryonic development (17), affects bristle number (101, 123) and olfactory behavior (6, 50). *P*-element insertions in

***P*-elements:** genetically engineered *Drosophila* transposable elements that are used for insertional mutagenesis, enhancer trap studies, targeted overexpression, RNAi, and homologous recombination

Pleiotropy: the phenomenon in which a single gene affects more than one phenotype

scribbler affect adult bristle number (123) and larval turning behavior (169).

Although large numbers of loci are potentially mutable to affect complex traits, it is possible that the situation for segregating variation is simpler; that is, relatively few loci contribute to naturally occurring variation. Initial QTL mapping experiments in *Drosophila* indicated that natural variation might be of manageable complexity, with 7–11 QTLs affecting abdominal bristle number (60, 97) and 8–9 QTLs affecting sternopleural bristle number (60, 61) in different mapping populations. Similar studies revealed small numbers of QTLs affecting longevity (5), ovariole number (2), olfactory behavior (1), courtship signal (3), flight (2), and measures of metabolism (6), resistance to starvation stress (5), and male mating behavior (4) (43, 51, 62, 115, 117, 126, 188). On the other hand, 10 and 11 QTLs affecting wing shape were detected on the second and third chromosomes alone, respectively (190, 191).

Numbers of QTLs detected in genome scans are always minimum numbers: Larger mapping populations have the potential to separate closely linked QTLs by recombination, and have the power to detect more QTLs with smaller effects. Furthermore, one mapping population represents a restricted sample of the total segregating variation; therefore, different QTLs may be identified in different mapping populations (42). High-resolution mapping is required to determine whether QTLs detected in initial genome scans correspond to single genes or multiple closely linked loci, and such studies suggest that the latter scenario is more common for *Drosophila* QTLs. For example, recombination mapping of QTLs affecting abdominal and sternopleural bristle numbers indicated that at least 53 QTLs affect one or both traits (37). Deficiency complementation mapping is a powerful method for mapping *Drosophila* QTLs to subcM intervals (135). Deficiency complementation mapping revealed that the 4 QTLs affecting variation in longevity between two strains fractionated into at least 15 QTLs

(106). Similarly, the 5 QTLs affecting variation in resistance to starvation stress were resolved to 14 QTLs (62). If the splitting of single QTLs into multiple closely linked QTLs on detailed examination is a general hallmark of the genetic architecture of complex traits, the level of difficulty for genetic dissection of QTLs in humans will increase considerably.

Candidate Genes

The regions to which QTLs map typically contain several positional candidate genes, many of which are computationally predicted. In *Drosophila*, one can use quantitative complementation tests to mutations at positional candidate genes and LD mapping to identify which of the genes correspond to the QTL. The logic of quantitative complementation tests to mutations is identical to that of complementation tests to deficiencies (96). This method has been used to identify candidate genes corresponding to QTLs affecting sensory bristle number (61, 96), olfactory (43) and mating (115) behavior, longevity (36, 106, 134), and resistance to starvation stress (62). These studies have shown that many of the QTLs that affect natural variation in bristle numbers mapped to the same location as candidate genes that affect the development of sensory bristles (61, 96), as implicated by mutation screens. More commonly, though, novel genes that were not previously implicated to affect the trait have been identified by these tests, highlighting both the importance of quantitative genetic analysis as a tool for functional genome annotation, and our ignorance of the underlying genetic architecture of most complex traits. Examples of novel genes affecting longevity include *shuttle craft* (134), *tailup*, and *Lim3* (106), all of which affect motor neuron development, and three genes [*Dopa decarboxylase* (*Ddc*), *Catecholamines up*, and *Dox-A2*] in the catecholamine biosynthesis pathway (36, 106). These genes are thus excellent candidates for inclusion in human studies seeking to identify genes associated with variation in life span.

The goal of obtaining a living library of mutations for all *Drosophila* genes has not yet been achieved (15); therefore, complementation tests are not possible for all positional candidate genes. In these cases (and also in cases where complementation tests positively identify a candidate gene), LD mapping can be used to determine whether molecular polymorphisms in the candidate gene are associated with phenotypic variation in the trait. In *Drosophila*, LD decays rapidly with physical distance in regions of normal recombination (94), which is a favorable scenario for identifying the actual polymorphisms (quantitative trait nucleotides, or QTNs) that cause the differences in phenotype between QTL alleles. Theoretical considerations indicate that LD mapping requires large samples—at least 500 individuals are necessary to detect a QTN contributing 5% of the total phenotypic variance with 80% power (93). The ability to construct chromosome substitution lines in *Drosophila* greatly increases the power of LD mapping: genetic variance attributable to chromosomally unlinked loci is eliminated, measuring multiple individuals per line increases the accuracy of the estimate of genotypic value, and all markers are homozygous, which circumvents the problem of inferring haplotypes. Further increases in power can be achieved by introgressing the candidate gene alleles into a common isogenic chromosome background (94, 100, 145). Inferences from LD mapping studies in *Drosophila* that are relevant to similar studies of human complex traits are that phenotypic variation is associated with both common and rare alleles; all kinds of molecular variants (single nucleotide polymorphisms, insertions/deletions, transposable elements), and, most importantly, variants in noncoding regions (including introns) as well as coding regions (36, 85, 104).

Effects

Although large numbers of loci potentially affect complex traits, *Drosophila* studies reveal

that the distributions of homozygous effects of *P*-element insertions (101, 123) and QTLs (37, 159) are exponential, with a few genes (QTLs) with major effects and increasingly more with smaller effects, down to the limit of detection imposed by the scale of the experiments. This is of practical importance because it implies that most of the variation in natural populations could be accounted for by relatively few QTLs with large effects, even though large numbers of QTLs contribute to the total variation. On the other hand, alleles affecting complex traits are highly context dependent, with effects that vary according to sex, environment, and genetic background.

One surprising result to emerge from quantitative genetic analyses of *Drosophila* sensory bristle numbers was that *P*-element insertions (101, 123), spontaneous mutations (105), QTLs (37, 60, 61, 97, 125), and SNPs in candidate genes (94, 95) often had large sex-specific effects. That is, they showed genetic variation in the magnitude of sex dimorphism, such that some QTLs had greater effects in males than females, or vice versa. Subsequent studies revealed sex-specific effects to be a near-ubiquitous feature of the genetic architecture of complex traits in *Drosophila*, and have been documented for QTLs affecting longevity (88, 89, 106, 126, 135, 183) and for *P*-element insertions and QTLs affecting olfactory behavior (6, 43) and resistance to starvation stress (62).

Although the effects of QTL alleles vary with changes in the environment, they will not necessarily exhibit environment-specific effects [known as genotype by environment interaction (GEI)]. GEI occurs when there is variation among genotypes in the rank order or relative magnitude of effects in different environments (42). There have only been a few *Drosophila* studies evaluating the extent to which QTLs exhibit GEI, because detecting GEI requires that the same genotypes are reared in multiple environments. These studies indicate that GEI is pervasive. For example, when QTL mapping populations were reared in three temperature

QTN: quantitative trait nucleotide

GEI: genotype by environment interaction

Epistasis: the phenomenon in which the effect of a genotype at one locus is modulated (suppressed or enhanced) by the genotype of another locus

environments, genotype by temperature interaction accounted for approximately 14% of the total genetic variance for sensory bristle number (37, 60). In one study (37), 33.3% of the QTLs that affected sternopleural bristle number and 55.3% of the QTLs that affected abdominal bristle number had significant QTL by temperature interactions. The situation for life span is even more dramatic. The life span of recombinant inbred lines was evaluated under standard culture conditions and four stressful environments (183). Remarkably, GEI accounted for 79% of the total genetic variance.

A final source of context dependence is genetic background; i.e., epistasis. In quantitative genetics, the term epistasis indicates any nonadditive interaction between segregating alleles at two or more loci (42, 103). Epistasis occurs when the effect of variation at one locus is suppressed or enhanced by the genotype at another locus. Traditional analyses of quantitative traits using correlations among relatives and observations of inbreeding depression and heterosis were consistent with largely additive genetic variation for most traits, with possible epistatic interactions for components of reproductive fitness (42, 103). However, *Drosophila* studies using introgression (20, 158) and chromosome substitution lines (21, 78) documented strong epistasis for the archetypical additive traits, numbers of sensory bristles, hinting that epistasis is more common than previously thought.

Recent studies have used three methods to evaluate the presence of epistasis. The first is to construct all nine two-locus genotypes between a pair of biallelic loci, or all $n(n - 1)/2$ double heterozygotes between n biallelic loci. These methods have been applied to *P*-element insertional mutations in a common coisogenic background affecting metabolic activity (30) and olfactory behavior (46). Strong epistasis was observed in both cases. The latter method provides a way to identify genetic networks affecting complex traits. Of the 12 *P*-element insertional mutations affecting olfactory behavior, 8 could

be placed in a single interaction network, and two additional mutations interacted but could not be joined with the main network (46). Thus, interactions were observed for 83% of mutations affecting olfactory behavior, indicating that epistasis is an essential feature of the genetic architecture of complex traits in *Drosophila*, at least for induced mutations.

The second method of evaluating the presence of epistasis is to assess interactions in a genome scan for QTLs. This method only has power to detect large epistatic interactions because of the statistical penalty paid for evaluating $n(n-1)/2$ interactions between significant QTLs, or between all markers. Nevertheless, epistatic interactions between QTLs affecting sensory bristle numbers were not only common, but the effects were of the same magnitude as the main effects, and often sex-specific (37, 61, 97). Epistasis was also detected between QTLs affecting longevity (88, 89) and wing shape (190, 191). In the latter case, the epistatic interactions contributed negligibly to the total phenotypic variance because the interactions were balanced between positive and negative effects. In addition, genome scans for pairwise epistasis affecting longevity revealed more interactions than expected by chance; most interactions were between markers that did not have significant main effects (106).

The third possible method for fine-scale dissection of molecular polymorphism-trait associations is to combine *in vitro* mutagenesis with *P*-element mediated germ line transformation to test the functional effects of each polymorphic site associated with the trait, together and in combination. Remarkably, this method revealed epistatic interactions between three polymorphic sites in a 2.3-kb intronic region affecting natural variation in alcohol dehydrogenase protein concentration (164).

If the sex-, environment-, and genetic background-specific QTL effects of *Drosophila* QTLs are general features of the genetic architecture of complex traits, the implication is that one should expect similar

complexity for alleles affecting complex traits in humans. Indeed, sex-specific effects have been reported for markers at candidate genes affecting longevity (35) and serum triglyceride levels (173); alleles affecting asthma susceptibility, violence, and depression exhibit GEI (22, 23, 127); and there is evidence of epistasis between two loci affecting plasma apolipoprotein E levels (80). Therefore, studies of human complex traits need to explicitly test for effects of sex, GEI, and epistasis, which requires incorporating epidemiologic and genetic data, and ensuring sample sizes that are large enough for statistically partitioning the data into the relevant main effects and interactions. Failure to consider this complexity will lead to incorrect inferences, rejecting important associations in a particular context if they do not have a significant effect averaged across sexes, environments, or genotypes at other loci.

Below, we provide recent examples highlighting the utility of *Drosophila* studies to provide insights about clinically relevant human complex diseases. The expression of human disease proteins in transgenic flies combined with classical mutagenesis and enhancer/suppressor screens have proven to be particularly powerful approaches.

DROSOPHILA AS A MODEL FOR ALCOHOLISM AND SLEEP

It might seem hard to imagine that *Drosophila* could be useful for studies of such quintessential human physiological traits as alcoholism, drug abuse, or sleep. Nonetheless, experiments during the last five years have shown that flies can present a surprisingly informative and experimentally amenable model system for those traits.

Alcoholism presents widespread social and health problems throughout the industrialized world. The National Institute on Alcohol Abuse and Alcoholism has estimated that approximately 14 million people in the United States suffer from alcoholism. Alcohol and nicotine addiction are mediated via

similar neural mechanisms that involve modulation of the dopaminergic mesolimbic system in the ventral tegmental area (86). Alcohol sensitivity, the development of tolerance to alcohol, and susceptibility to addiction vary in the population. Whereas the neural mechanisms in the mammalian brain that mediate alcohol addiction have been studied extensively, there is no functional correlate of addiction in *Drosophila*. However, alcohol sensitivity and the development of alcohol tolerance in flies show remarkable similarities to alcohol intoxication in vertebrates, suggesting that at least some aspects of these processes may be similar across species. There is evidence for overlapping pathways that mediate responses to cocaine administration and ethanol exposure and these pathways may interact with the neural circuit that regulates circadian activity (10, 131, 148). Although fruit flies have also been proposed as a model for cocaine sensitivity (111), we focus here on sensitivity to alcohol, because the genetic basis and neural circuitry for ethanol sensitivity and tolerance have been more extensively documented.

The prevalence of sleep disorders is widely appreciated, yet the mechanisms that regulate sleep, including its genetic underpinnings, remain largely unknown. In the past five years *Drosophila* has emerged as a suitable experimental model to investigate the genetic basis that underlies the transition from and duration of activity to rest periods. Such periods of inactivity are distinct from the well studied circadian system in flies and show features that are reminiscent of sleep (66, 157). Here we survey the progress to date on sleep in *Drosophila* and its broader implications.

Alcohol Sensitivity

Flies are naturally exposed to ethanol, as they feed on fermented food. Exposing flies to low concentrations of ethanol stimulates locomotor activity, whereas high concentrations induce an intoxicated phenotype that shows marked similarities to human alcohol intoxication, characterized by locomotor

impairments, loss of postural control, sedation, and immobility (161, 195). Alcohol sensitivity in *Drosophila* has been measured by tracking locomotor activity and by measuring knockdown time in an “inebriometer.” The inebriometer is a 4-ft long vertical glass column, which contains a series of slanted mesh partitions to which flies can attach. Flies are introduced in the top of the column and exposed to ethanol vapors. As they lose postural control they fall through the column. The elution time from the column is used as a measure of sensitivity to alcohol intoxication (32, 189).

Whereas the role of alcohol dehydrogenase as a critical factor in ethanol metabolism has been well established in flies (57, 65, 130), mice (192, 203), and humans (120, 141), mutant screens to date have implicated only a limited number of additional gene products in ethanol sensitivity in *Drosophila*. One of the best documented genes is *cheapdate*, an allele of *ammesiac* (118), which encodes a neuropeptide that has also been implicated in olfactory memory (140). The *ammesiac* gene product is produced in two dorsal paired medial cells, from where it is released onto the mushroom bodies, the major integrative center of the *Drosophila* brain (184). The *ammesiac* neuropeptide is thought to activate the cyclic adenosine 3,5-monophosphate (cyclic AMP) signaling pathway (44). The calcium/calmodulin-dependent adenylate cyclase encoded by the *rutabaga* gene (118) and the axonal migration and cell adhesion receptor, fasciilin II (25), both of which are expressed in mushroom bodies, have also been implicated in ethanol sensitivity. Flies with reduced cyclic AMP-dependent protein kinase activity show decreased sensitivity to ethanol exposure (131). Dopamine has also been implicated in the acute response of *Drosophila* to alcohol exposure (10) and could be linked to the adenylyl cyclase pathway. Targeted expression of an inhibitor of cyclic AMP-dependent protein kinase to specific brain regions using the *GAL4-UAS* binary expression system identified a small group of cells that decrease sensitivity for ethanol-

induced locomotor impairments (146). However, hydroxyurea-mediated ablation of the mushroom bodies did not affect ethanol sensitivity (146). These studies indicate that alcohol sensitivity, as assessed by loss of motor coordination, is mediated, at least in part, by a cyclic AMP signaling pathway in specific neural circuits, but the precise connectivity of these circuits remains to be further elucidated.

Repeated exposure to ethanol induces ethanol tolerance in flies, not unlike habituation observed in humans (155). A single exposure to ethanol induces lowered sensitivity to a subsequent ethanol exposure. This induction of tolerance is maximal 2 h after the first exposure in the inebriometer and dissipates over a period of 24–36 h (155). Neurons of the central complex that mediate locomotor activity (109) have been implicated in induction of ethanol tolerance (155), and octopamine, the neurotransmitter homolog of noradrenaline in vertebrates (116, 147), has been implicated as a mediator of ethanol tolerance in flies (56, 148, 155). Exposure to heat stress induces tolerance to subsequent exposure to ethanol, indicating that both of these environmental stressors converge on a similar cellular stress response mechanism (156). A nucleic acid-binding zinc-finger protein encoded by the *hangover* (*hang*) gene has been identified as a component of this pathway. Mutant flies in which *hang* expression is abolished show reduced tolerance following ethanol exposure. Tolerance is virtually completely abolished in flies carrying mutations both in *hang* and in the gene encoding tyramine beta-hydroxylase; this suggests that octopamine mediates a separate pathway involved in the induction of ethanol tolerance (154, 156). The bioamines dopamine (10) and tyramine (112) have also been implicated in the responses of flies to cocaine.

Although these studies have identified important signposts in the genetic and neural networks that mediate ethanol sensitivity and development of ethanol tolerance in *Drosophila*, the information gained, thus far, is rudimentary. A comprehensive understanding

of the genetic architecture of alcohol sensitivity and tolerance in *Drosophila* will require whole genome approaches that can identify interactive ensembles of genes that shape these quantitative traits.

Sleep

Periods of inactivity in *Drosophila* that occur during the circadian day and are independent of circadian activity per se were first characterized as rest episodes that resemble sleep. Characteristic features of such periods of inactivity included increased arousal threshold and sleep homeostasis, i.e., increased resting following sleep deprivation (66, 157). A detailed analysis of sleep homeostasis in *Drosophila* showed that flies exhibit all the major characteristics of sleep rebound after sleep deprivation usually observed in people (70). Furthermore, periods of inactivity were age dependent, with young flies sleeping more than old flies, similar to human sleep (157). Early studies implicated monoamine catabolism (157) and adenosine receptors (66) in the regulation of sleep in *Drosophila*. Subsequently, cyclic AMP and its interactions with the cyclic AMP response element binding (CREB) protein were implicated as one regulatory mechanism that controls rest-activity transitions in flies (67). In addition, local field potentials recorded from the medial brain near the mushroom bodies of flies correlated with locomotor activity and were proposed to represent a characteristic electrophysiological feature of sleep and arousal in flies (122, 180).

Although sleep can operate independently from the circadian clock in *Drosophila*, circadian rhythms and rest-activity homeostasis in *Drosophila* appear to be interrelated. Many genes that show altered transcriptional regulation in spontaneously awake, sleep-deprived, or sleeping flies, also cycle according to the fly's circadian rhythm (29). When loss-of-function mutants in circadian clock genes, including *period*, *timeless*, *clock*, and *cycle*, were sleep deprived, they showed an abnormally large homeostatic rebound response

and died after 10 h of sleep deprivation. Expression of heat-shock proteins could protect against the effects of sleep deprivation in the *cycle* mutant (157). This is perhaps not surprising as heat-shock proteins appear to mediate protective responses to diverse stressors. What sets the phenomenon of sleep apart from the well understood circadian clock mechanism, however, is its enticing relationship to arousal (180) and by extension the complex concept of consciousness, forever the holy grail of neurobiological research (181).

Characterization of the sleep phenotype in *Drosophila* has laid the foundation for two scientific endeavors. The first is to identify genes that are associated with sleep and, ultimately, to understand how ensembles of genes orchestrate the expression of sleep and how this genetic architecture is influenced by environmental effects (27, 29). The second is to identify compounds that can be used for pharmacological intervention in treating human sleep disorders. A mutant screen of 9000 fly lines found a line that exhibited three times shorter sleep periods than normal flies. This *minisleep* (*mms*) mutant shows normal sleep rebound and is not impaired in performance in behavioral assays following sleep deprivation, as are wild-type flies, including geotaxis responses and escape responses to heat and combined noise/vibration stimuli. Their life span, however, was shortened. The *minisleep* phenotype results from a point mutation in the *Shaker* gene, which encodes a voltage-gated potassium channel (28).

Another mutant, *fumin* (*fmm*), also shows abnormally high levels of activity and reduced periods of sleep. In contrast to the *mms* mutant, *fmm* flies are deficient in their sleep rebound response following sleep deprivation and have a normal life span. The *fmm* mutation was mapped to the dopamine transporter gene, implicating a function for dopamine in the regulation of sleep and arousal (83). Inhibitors of dopamine synthesis promote sleep in *Drosophila*, whereas administration of metamphetamine suppresses sleep and promotes arousal (4). Sleep was also suppressed

AD: Alzheimer's disease

APP: amyloid precursor protein

by the drug modafinil (68). It is already clear from these early studies that *Drosophila* has an enormous potential to contribute significant new insights into the regulation of sleep, and may provide a powerful bioassay system for the development of pharmaceuticals that may promote either sleep or wakefulness.

DROSOPHILA AS A MODEL FOR NEURODEGENERATIVE DISEASES

A shared feature of the most common neurodegenerative diseases—Alzheimer's disease, Parkinson's disease, and Huntington's disease, as well as other polyglutamine disorders—is the aberrant processing, degradation, and progressive accumulation of specific misfolded proteins or their fragments in areas of the brain (β -amyloid, α -synuclein, and huntingtin, respectively). The causes for the accumulation of these specific polypeptides and the mechanisms of their neurotoxicity remain poorly understood. Especially intriguing is the paradox that these proteins are widely expressed in the central nervous system, yet only certain classes of cells, characteristic for each disease, undergo neurodegeneration. Thus, it is clear that their neurotoxic effects depend on a specific cellular context, which implies interactions with specialized cellular components and pathways. Defining these cellular contexts is necessary to understand the pathogenic mechanisms that underlie these neurodegenerative diseases and is an endeavor that will benefit greatly from powerful genetic models that allow resolution of epistatic modifiers or quantitative genomic approaches. For example, in a *Drosophila* model of spinocerebellar ataxia, type 1 cell-specific neurodegeneration may be mediated by association of a conserved interaction domain in the protein ataxin with the *Drosophila* zinc-finger transcription factor Senseless or its mammalian homolog Gfi-1 (178).

Overexpression of neurodegenerative proteins in the *Drosophila* brain gives rise to neurodegenerative phenotypes with behav-

ioral and physiological correlates that show remarkable similarities to the human disease syndromes. Consequently, *Drosophila* has materialized as a better in vivo model system than could have been a priori predicted for unraveling the cellular mechanisms that underlie the etiology of these disorders. Furthermore, transgenic fly models are potentially powerful systems for the identification of genetic modifiers, therapeutic targets, and drug testing. Here we review advances that have been made in understanding the most prominent neurodegenerative diseases, Alzheimer's disease, Parkinson's disease, and Huntington's disease, using *Drosophila melanogaster*. Note that the utility of *Drosophila* is by no means limited to these disorders, and that other neurological diseases, e.g., fragile X-syndrome (200) and susceptibility to seizures, a correlate of epilepsy (186), as well as ocular hypertension, a common prelude to glaucoma (18), may also benefit from the genetic power and experimental convenience of this model system.

Alzheimer's Disease

Alzheimer's disease (AD) is the most common form of senile dementia and affects about four million Americans, with a substantial increase on the horizon as the population ages. The disease was first described in 1906 by Dr. Alois Alzheimer, a German neuropathologist and psychiatrist (3), and is a progressive neurodegenerative disease that affects men and women equally, usually after the age of 65, although the disease can also be diagnosed earlier in the 40s or 50s. The diagnosis of AD can only be ascertained postmortem by observing characteristic neuritic plaques and neurofibrillary tangles in the cerebral cortex. The plaques contain β -amyloid protein, a 42-amino acid proteolytic fragment of a larger amyloid precursor protein (APP), whereas the tangles are formed by twisted fragments of the tau protein. Cholinergic neurons appear most susceptible during the progression of AD. The mechanisms of neurotoxicity of β -amyloid and tau, and the

interrelationships between these proteins, are not yet understood.

Two transmembrane proteolytic enzymes, presenilin 1 and presenilin 2, contribute to the proteolysis of APP, and missense mutations in presenilin 1 have been implicated in early-onset familial AD. Presenilins not only cleave APP, but also mediate proteolytic cleavage of the integral membrane domain of Notch, a critical receptor that triggers developmental processes that result from cell-cell interactions (114). The *D. melanogaster* genome contains a gene that encodes an APP-like protein (APPL), which is expressed in the nervous system (98, 110), as well as a single presenilin gene, which may generate alternatively spliced messages (107). Loss-of-function mutants in *Drosophila* presenilin phenocopy lethal Notch mutants with loss of lateral inhibition within proneural cell clusters and absence of wing margin formation (124, 168, 199). The proteolytic activity of presenilin requires additional proteins, including nicastrin (26), APH-1 (48, 81), and PEN-2 (48), all of which have *Drosophila* homologs (170), which are essential for trafficking and localization of presenilin and form a complex that confers the characteristic γ -secretase activity, which leads to the formation of the 42-amino acid β -amyloid fragment (171).

Overexpression of wild-type human APP in *Drosophila* resulted in cuticular defects and a blistered wing phenotype (197). Deletion of the endogenous *appl* gene or overexpression of mutant forms of APP or APPL resulted in defects in axonal transport, similar to phenotypes observed with kinesin and dynein mutants (58). Moreover, expression of the 42-amino acid β -amyloid fragment in the *Drosophila* brain resulted in amyloid deposits, age-dependent learning defects assessed by a conditioned avoidance learning paradigm in which exposure to an odorant was paired with an electric shock, and extensive neurodegeneration (73). Cleavage of APP in vivo in transgenic flies by targeted coexpression of specific proteolytic enzymes, including presenilins, also led to β -amyloid plaques, and

age-dependent neurodegeneration, as well as to a shortened life span, and defects in wing vein development (55). When transgenic expression of APP and proteases was targeted to the retina, age-dependent neurodegeneration of photoreceptor cells was evident (55).

The precise function of APP under normal conditions is not known. Flies in which APPL has been deleted show minor behavioral defects, but are otherwise viable, fertile, and morphologically normal (99). Overexpression of APP gives rise to phenotypes that suggest interference with the Notch signaling pathway during neural development (113), consistent with the dual action of presenilin on proteolytic cleavage of both Notch and APP (114, 124, 168, 199). Overexpression of APPL in adult flies results in disruption of axonal transport (58, 177) and induces axonal arborizations (90). This process of APP-induced neuritic differentiation may be mediated via a tyrosine kinase pathway that modulates the JNK kinase signaling cascade (90, 172). These findings suggest a role for APP in developing the nervous system or stabilizing and maintaining axonal connectivity.

Studies on the effects of APP on neurodegeneration in *Drosophila* were paralleled by studies on the mechanism by which overexpression of tau results in neurotoxicity in AD. When wild-type and mutant forms of the human tau protein were overexpressed in *Drosophila* under a pan-neuronal driver, cholinergic neurons underwent progressive degeneration, which became evident only with age and was more pronounced with mutant than with wild-type tau protein (194). This neurodegenerative process resulted in accelerated death. However, despite the accumulation of the tau protein, no neurofibrillary tangles were evident, suggesting that the formation of such tangles is not a prerequisite for neurotoxicity (194). A subsequent study, however, which combined overexpression of tau with misexpression of the kinase Shaggy, the *Drosophila* homolog of glycogen synthase kinase 3 (GSK3 β), previously implicated in tau phosphorylation, showed that

tau-induced neurodegeneration in *Drosophila* is exacerbated when transgenic tau is hyperphosphorylated and implicated the Wnt signaling pathway as a mediator of tau-induced neurodegeneration (75). A genetic screen for modifiers of tau-induced neurodegeneration independently confirmed the importance of the state of phosphorylation of tau by identifying three kinases and four phosphatases as the major class of enhancers-suppressors of the tau-induced neurodegenerative phenotype (160). One of these kinases, PAR-1, initiates the phosphorylation of tau on two serine residues and this event enables further phosphorylation by other kinases, such as GSK3 β (121). Furthermore, coexpression of constitutively active GSK3 β exacerbates tau-induced inhibition of axonal transport and locomotor defects, whereas inhibitors of phosphorylation by GSK3 β reverse the effects of tau overexpression (119).

The mechanisms that give rise to progressive neurodegeneration during the etiology of AD are clearly complex, but *Drosophila* *in vivo* models are now well poised to unravel the mechanisms that lead from the overproduction of proteolytic fragments of APP and tau to the cellular programs that result in neuronal cell death. Thus, the *Drosophila* model will be useful in identifying therapeutic targets and intervention strategies for treating AD (34).

Parkinson's Disease

In his essay on the "shaking palsy," James Parkinson (132) described the symptoms of the disease that would later bear his name as an "involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported, with a propensity to bend the trunk forward, and to pass from walking to a running pace, the essence of intellect being unaffected." Parkinson's disease (PD) is now recognized as the second most common neurodegenerative disorder characterized by resting tremor, slowness of movement, rigidity of the extremities and neck,

and stooped posture. In some patients these motor disorders are accompanied by dementia. The neuropathology of PD results from loss of dopaminergic neurons in the substantia nigra pars compacta. Neuronal loss is associated with the accumulation of the protein α -synuclein, which forms filamentous aggregates, known as Lewy bodies, and mutations in the gene encoding this protein have been linked to congenital PD (82, 139, 162, 201).

A *Drosophila* model for PD was developed by generating transgenic fly lines that express normal human α -synuclein or two mutant proteins linked to familial Parkinson's disease, A30P and A53T α -synuclein (45). Flies in which these proteins were expressed either under the control of a pan-neuronal promoter or the promoter of DOPA decarboxylase showed progressive age-dependent loss of dopaminergic neurons with the formation of aggregates similar to Lewy bodies. The same phenotypes were observed with wild-type and mutant α -synuclein transgenes and were accompanied by locomotor dysfunction. Thus, the *Drosophila* model recapitulates the essential features of PD. Furthermore, expression of α -synuclein in the *Drosophila* eye under the control of the *gmr* promoter resulted in neurodegeneration (45). Because motor dysfunction in PD can be treated effectively by administering the dopamine precursor, L-DOPA, the effects of L-DOPA and other dopamine agonists were tested in the transgenic fly model of PD. Inclusion of L-DOPA and the dopamine agonists pergolide, bromocriptine, and 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine (SK&F 38,393) in the food of α -synuclein expressing transgenic flies restored their impaired locomotor activity (136).

A carefully executed study that used high-density oligonucleotide microarrays to analyze transcriptional changes in heads from transgenic flies that express α -synuclein found 36 genes that showed altered expression levels in one-day-old flies, a time well before neurodegeneration and locomotor

abnormalities become evident (153). Whereas about half of these genes encoded predicted transcripts of unknown function, significant differential expression was evident for three genes involved in catecholamine biosynthesis: *Henna*, which encodes phenylalanine hydroxylase; *Punch*, which encodes GTP cyclohydrolase; and *purple*, which encodes 6-pyruvoyl tetrahydrobiopterin synthase. Other transcripts with altered regulation encoded products associated with lipid binding and metabolism, and with mitochondrial function, including *walrus*, which encodes an electron transfer flavoprotein, and the ATPase gamma-subunit, which is a component of complex V of the respiratory electron transfer chain (153). Mitochondrial dysfunction had been implicated previously in some forms of PD (152), observations later confirmed in the *Drosophila* model (53, 138). Thus, altered transcriptional regulation of key genes that underlie the pathogenesis of PD occurs long before symptoms of the disease manifest. Detection of presymptomatic transcriptional changes diagnostic of imminent PD could conceivably lead to early intervention of disease progression in PD patients.

The *Drosophila* model for PD has provided insights into the relationship between α -synuclein and the formation of Lewy bodies. Motivated by the observation that α -synuclein in Lewy bodies is phosphorylated on serine 129 (49, 128, 150), the effects of phosphorylated and nonphosphorylated forms of α -synuclein expressed in transgenic flies were evaluated (24). Phosphorylation could be suppressed either by mutating serine 126 to an alanine or mimicked by mutating this serine to a negatively charged aspartate. Blocking phosphorylation of transgenic α -synuclein suppressed the loss of dopaminergic neurons, whereas phosphorylation aggravated neurotoxicity. In the absence of phosphorylation α -synuclein was more effectively sequestered into Lewy bodies, suggesting that the formation of Lewy bodies may protect neurons from α -synuclein toxicity (24).

Significant attention has focused on the role of chaperones in PD under the hypothesis that misfolding of α -synuclein may contribute to its neurotoxicity. Coexpression of the molecular chaperone Hsp70 with α -synuclein in transgenic flies prevented loss of dopaminergic neurons, whereas interference with the expression of Hsp70 aggravated α -synuclein-induced neurodegeneration (8). Furthermore, the drug geldanamycin, which elicits stress responses and consequently activation of Hsp70, promoted neuronal survival in flies expressing α -synuclein, despite the continued formation of Lewy bodies (7, 9).

A second focus for the pathogenesis of PD has centered on parkin, an ubiquitin-protein ligase implicated in an autosomal recessive juvenile form of PD (79). A *Drosophila* homolog of parkin was identified and null mutants for this gene show reduced life span, locomotor defects, and male sterility. The locomotor defects appeared to result from apoptotic muscle degeneration due to mitochondrial dysfunction, suggesting that mitochondrial impairment and increased sensitivity to oxidative stress may play a role in the neurodegeneration observed in PD (53, 138). An important role for parkin in the etiology of PD is further corroborated by the observation that in fly brains expression of Pael-R, the parkin substrate, phenocopies the age-dependent selective degradation of dopaminergic neurons observed with the α -synuclein transgene. Furthermore, coexpression of parkin with Pael-R rescues the phenotype (198) and coexpression of parkin with α -synuclein suppresses the latter's neurotoxic effects (64, 198).

It has been reported both that dopaminergic neurons remain intact in parkin null mutants (136) and that a subset of dopaminergic neurons degenerate in such mutants (193). Genetic screens for modifiers of the parkin phenotype showed that double mutants of parkin and glutathione-S-transferase S1 exhibited enhanced neurodegeneration. Conversely, overexpression of glutathione-S-transferase S1 in dopaminergic neurons suppressed parkin-induced neurodegeneration

(193). As glutathione transferases may protect against oxidative stress, it is reasonable to propose (193) that this enzyme counterbalances the increased sensitivity to oxygen radicals described previously (138).

Huntington's Disease

First described in 1872 by George Huntington (71), Huntington's disease (HD) is an autosomal dominant heritable neurodegenerative disorder, which primarily affects the caudate nucleus and putamen and to a lesser extent the frontal and temporal cortices. The disease is caused by the accumulation of CAG repeats in the first exon of the gene that encodes the huntingtin (Htt) protein leading to the formation of long glutamine (polyQ) tracts (11, 72, 204). It is a late-onset progressive disease with increasing cognitive deficits and motor impairments (chorea) that results in death within 10–20 years of onset. In healthy people there are 37 or fewer glutamines in the polyQ tract of Htt, but in HD patients their number can exceed 150. The age of onset correlates with the length of the polyQ repeats (137, 167). Fragments of polyQ-Htt form intracellular aggregates that are toxic to neurons by inducing apoptosis (40) and disrupting axonal transport (59, 87). The polyQ expansion interferes with nuclear export of Htt and causes accumulation of Htt in the nucleus and the formation of nuclear aggregates, which may hinder transcription (33). Mutant Htt proteins interact with molecular chaperones and interfere with protein degradation, thereby causing the accumulation of misfolded polypeptides (38, 63). In addition, the N terminus of Htt binds to regions of the plasma membrane that are enriched in phosphatidylinositol (4, 5) biphosphate (77), and expanded glutamine repeats may compromise this interaction. However, the precise function of Htt in the human brain remains enigmatic, and the pathogenic mechanisms by which aggregates of polyQ-Htt peptides lead to neurodegeneration are not fully understood.

Although a putative *Drosophila* homolog of Htt has been reported, the gene encoding this homolog shows a vastly different intron/exon structure than its human counterpart and does not contain the CAG repeats that encode the polyQ tracts (91). Despite the absence of both a close Htt homolog in the *Drosophila* genome and a naturally occurring phenotype that would resemble Huntington's chorea in flies, *D. melanogaster* has served as a remarkably powerful in vivo experimental model for studies on Htt-induced neurodegeneration. Jackson and colleagues (74) generated transgenic flies in which fragments of human Htt containing tracts of 2, 75, or 120 glutamines were expressed in photoreceptor cells. The resulting phenotypes were dramatic. The long polyQ repeats resulted in degeneration of the photoreceptor cells and its onset occurred sooner and was more severe with the 120 glutamine repeats than with the 75 glutamine expansion. As in the case of human HD, neurodegeneration developed progressively. This pioneering study established *Drosophila* as a convenient model in which the effects of modifiers on Htt-induced neurodegeneration could be readily assessed and therapeutic strategies could be tested.

Lievens et al. (92) used the binary *GAL4-UAS* expression system in *Drosophila* to target expression of polyQ-Htt peptides to a subset of glia under the control of the promoter of the glutamate transporter *EAAT1* gene. Transgenic flies showed nuclear inclusions with a decrease in EAAT1 expression and a shortened life span. Glial cells, however, did not undergo apoptosis. Based on the observation that maintenance of EAAT1 expression depends on epidermal growth factor (EGF) signaling, Lievens et al. (92) showed that polyQ peptides may interfere with EGF signaling and hence compromise glial function.

The inclusion bodies that are formed by polyQ-containing fragments of Htt include transcription factors, chaperones, proteasome subunits, and ubiquitin (12, 52, 185).

Coexpression of the molecular chaperone Hsp70 with polyQ-Htt suppresses neurodegeneration (187). Ubiquitination, which tags proteins for degradation, may also play a role in inducing neurodegeneration by polyQ-containing fragments of Htt. Modification of such fragments by the small ubiquitin-like modifier (SUMO) or by ubiquitin on lysine residues of mutant Htt expressed in the *Drosophila* eye exacerbates the neurodegenerative loss of rhabdomeres, whereas reduced levels of SUMO and increased ubiquitination provide protection against neurodegeneration (166). However, mutations of the lysine residues that are modified by SUMO also ameliorate the neurodegenerative effects of polyQ-Htt in transgenic flies (166). It is not surprising that impairments of the cell's capability to remove neurodegenerative proteins would result in pathology. Whereas correlative evidence based on SUMO colocalization with neurodegenerative proteins or areas of brain lesions has been reported previously (174, 179), to date only the transgenic fly model has enabled the establishment of a direct causal link between SUMO levels and severity of disease.

As chaperones and ubiquitination have been implicated in both HD and PD, it is difficult to escape the notion that parallel processes may contribute to the etiologies of these two distinct neurodegenerative disorders. The *Drosophila* model will prove useful in delineating the mechanistic similarities and differences between these two important neurological diseases.

The *Drosophila* model for HD has been used extensively to identify therapeutic targets and to develop compounds that might prevent or slow the progression of HD. Inhibitors of histone deacetylase, including sodium butyrate and suberoylanilide hydroxamic acid administered as fly food supplements, substantially reduced the neurodegeneration of rhabdomeres in transgenic flies that express polyQ-Htt (165). Subsequent studies showed that suberoylanilide hydroxamic

acid also ameliorates motor deficits in a mouse model of Huntington's disease (69).

Another strategy aimed at preventing the formation of aggregates by Htt fragments through their polyQ repeats employed a bivalent peptide with two polyQ(25)-containing arms (76). The hypothesis underlying this strategy was that blocking the binding sites of polyQ-Htt for aggregation would prevent the formation of large inclusion bodies and rescue neurodegeneration. Indeed, survival increased dramatically in transgenic flies, which coexpressed this bivalent peptide with polyQ(108)-Htt under the pan-neuronal *elav* promoter from 1% to about 50% (76). A variation of this approach was the intracellular expression of an engineered single-chain antibody that recognizes a unique Htt epitope and interferes with Htt aggregation when coexpressed in transgenic flies with polyQ-Htt. Such flies showed a substantial reduction in the progression of neurodegeneration and an extended life span compared to untreated transgenic polyQ-Htt-expressing controls (196).

Whereas these studies underscore the critical importance of aggregate formation for the manifestation of the disease, delivery of peptides or driving intracellular expression of engineered antibodies in the brains of HD patients would be highly invasive. Consequently, a search for small organic molecules with therapeutic potential was conducted using a high-throughput screening system in yeast to identify inhibitors of polyQ-Htt aggregation. Four potential lead compounds were identified and tested in the *Drosophila* HD model. The most effective compound showed nearly 25% rescue of the neurodegenerative phenotype when included in fly food at the highest dose (300 μ M) (202). Rapamycin, a compound that readily crosses the blood brain barrier, also slows the progression of HD in both mouse and fly models. Rapamycin interacts with a kinase, mTOR (mammalian Target of Rapamycin), which is a regulator of autophagy, a possible pathway for clearance of

Htt polypeptides. Inhibition of mTOR by rapamycin promotes autophagy and attenuates cell death (142).

Based on the hypothesis that diverse cellular mechanisms contribute to HD and that drugs that target separate aspects of cellular pathology might have synergistic or additive beneficial effects, combinations of drugs were tested at nontoxic threshold concentrations for their effects on polyQ-Htt-induced neurodegeneration in the brains and retinas of flies (2). These studies showed that suberoylanilide hydroxamic acid in combination with either cystamine or Congo Red, or in combination with either geldanamycin or Y-27,632 (an inhibitor of the Rho-associated kinase p160ROCK), significantly enhanced suppression of the HD phenotype in flies, even though expression of the polyQ-Htt transgene was unaffected (2).

Although a magic bullet for preventing and treating HD in at-risk individuals is still far in the future, the *Drosophila* model thus far has proved to be an advantageous system for advancing our understanding of the pathogenesis of this disease as well as for developing and testing therapeutic approaches (13, 108).

CONCLUSION

Drosophila melanogaster provides an excellent model system for gaining insights in human complex traits, because genetically identical individuals can be reared in large numbers under controlled environmental conditions, genetic parameters such as pleiotropy and epistasis can be readily dissected, and evolutionary conservation of cellular mechanisms more often than not enables extrapolation of observations from flies to humans. Substantial evidence validates the use of this powerful genetic model system for studies of such quintessential human disorders like alcoholism, sleep disorders, and the most prevalent neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and Huntington's disease. In each instance, genomic approaches using mutant or transgenic flies can be used to elucidate disease mechanisms at the genetic level. Furthermore, flies also provide an *in vivo* screening system for drug development. Comparative genomic approaches that include *Drosophila* versus human comparisons are likely to gain increasing popularity in the future.

SUMMARY POINTS

1. Most phenotypic variation for aspects of morphology, physiology, behavior, and susceptibility to common diseases in human populations is attributable to multiple, interacting genes with small effects whose expression is sensitive to the environment.
2. Understanding the genetic basis of variation for human complex traits is complicated by uncontrolled environmental and genetic background effects, the need for large sample sizes and accurate definitions and measurements of trait phenotypes, and the existence of haplotype blocks in the human genome, which hinders fine-scale mapping of causal genes within blocks of high linkage disequilibrium.
3. *Drosophila* provides a powerful genetic model for the study of complex traits, because unlimited numbers of genetically identical individuals can be reared rapidly under controlled environmental conditions and subjected to sophisticated genetic approaches.
4. There is considerable evolutionary conservation of genes and pathways affecting key biological processes between flies and humans, including human disease genes.

5. Quantitative genetic studies of *Drosophila* complex traits have identified novel pleiotropic genes that had not been previously implicated to affect the traits, and which can be incorporated in human studies of orthologous traits.
6. Alleles affecting *Drosophila* complex traits exhibit sex-, environment-, and genetic background-specific effects, with variation that is attributable to molecular polymorphisms in noncoding as well as coding regions.
7. Expression of human disease proteins in transgenic flies combined with classical mutagenesis and enhancer/suppressor screens can provide mechanistic insights into clinically relevant human complex traits, including alcohol dependence, sleep, and even neurodegenerative diseases unique to humans, such as Alzheimer's and Parkinson's disease, and glutamate expansion disorders, such as Huntington's disease.
8. Future comparative genomic approaches between the *Drosophila* model and human populations can provide powerful strategies for the genetic dissection of complex physiological and behavioral traits, including many human diseases, the identification of disease susceptibility genes, and the establishment of in vivo screening systems for developing new therapeutic agents.

ACKNOWLEDGMENTS

The authors acknowledge support from grants from the National Institutes of Health (GM59469 and EY015873 to R.R.H.A.; GM45146 and AA015348 to T.F.C.M.). This is a publication of the W. M. Keck Center for Behavioral Biology at North Carolina State University.

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45. The first convincing demonstration that *Drosophila* can be used as a model for human neurodegenerative diseases, showing that overexpression of α -synuclein in the fly brain results in progressive age-dependent loss of dopaminergic neurons by forming aggregates and locomotor dysfunction, symptoms that are strikingly reminiscent to Parkinson's disease.

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Contents

A 60-Year Tale of Spots, Maps, and Genes <i>Victor A. McKusick</i>	1
Transcriptional Regulatory Elements in the Human Genome <i>Glenn A. Maston, Sara K. Evans, and Michael R. Green</i>	29
Predicting the Effects of Amino Acid Substitutions on Protein Function <i>Pauline C. Ng and Steven Henikoff</i>	61
Genome-Wide Analysis of Protein-DNA Interactions <i>Tae Hoon Kim and Bing Ren</i>	81
Protein Misfolding and Human Disease <i>Niels Gregersen, Peter Bross, Søren Vang, and Jane H. Christensen</i>	103
The Ciliopathies: An Emerging Class of Human Genetic Disorders <i>Jose L. Badano, Norimasa Mitsuma, Phil L. Beales, and Nicholas Katsanis</i>	125
The Evolutionary Dynamics of Human Endogenous Retroviral Families <i>Norbert Bannert and Reinhard Kurth</i>	149
Genetic Disorders of Adipose Tissue Development, Differentiation, and Death <i>Anil K. Agarwal and Abhimanyu Garg</i>	175
Preimplantation Genetic Diagnosis: An Overview of Socio-Ethical and Legal Considerations <i>Bartha M. Knoppers, Sylvie Bordet, and Rosario M. Isasi</i>	201
Pharmacogenetics and Pharmacogenomics: Development, Science, and Translation <i>Richard M. Weinshilboum and Liewei Wang</i>	223
Mouse Chromosome Engineering for Modeling Human Disease <i>Louise van der Weyden and Allan Bradley</i>	247

The Killer Immunoglobulin-Like Receptor Gene Cluster: Tuning the Genome for Defense <i>Arman A. Bashirova, Maureen P. Martin, Daniel W. McVicar, and Mary Carrington</i>	277
Structural and Functional Dynamics of Human Centromeric Chromatin <i>Mary G. Schueler and Beth A. Sullivan</i>	301
Prediction of Genomic Functional Elements <i>Steven J.M. Jones</i>	315
Of Flies and Man: <i>Drosophila</i> as a Model for Human Complex Traits <i>Trudy F.C. Mackay and Robert R.H. Anbolt</i>	339
The Laminopathies: The Functional Architecture of the Nucleus and Its Contribution to Disease <i>Brian Burke and Colin L. Stewart</i>	369
Structural Variation of the Human Genome <i>Andrew J. Sharp, Ze Cheng, and Evan E. Eichler</i>	407
Resources for Genetic Variation Studies <i>David Serre and Thomas J. Hudson</i>	443

Indexes

Subject Index	459
Cumulative Index of Contributing Authors, Volumes 1–7	477
Cumulative Index of Chapter Titles, Volumes 1–7	480

Errata

An online log of corrections to *Annual Review of Genomics and Human Genetics* chapters may be found at <http://genom.annualreviews.org/>