Genetics on the Fly: A Primer on the *Drosophila* Model System

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ABSTRACT Fruit flies of the genus *Drosophila* have been an attractive and effective genetic model organism since Thomas Hunt Morgan and colleagues made seminal discoveries with them a century ago. Work with *Drosophila* has enabled dramatic advances in cell and developmental biology, neurobiology and behavior, molecular biology, evolutionary and population genetics, and other fields. With more tissue types and observable behaviors than in other short-generation model organisms, and with vast genome data available for many species within the genus, the fly's tractable complexity will continue to enable exciting opportunities to explore mechanisms of complex developmental programs, behaviors, and broader evolutionary questions. This primer describes the organism's natural history, the features of sequenced genomes within the genus, the wide range of available genetic tools and online resources, the types of biological questions *Drosophila* can help address, and historical milestones.

KEYWORDS *Drosophila*; development; comparative genomics; model organism;

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Natural History

Drosophila are small flies in the order Diptera and family Drosophilidae. Commonly known as fruit or vinegar flies, they are often found on rotting fruit or other decaying matter (Powell 1997). *Drosophila melanogaster*, in the subgenus *Sophophora*, was first made famous at the beginning of the 20th century when the Morgan lab at Columbia University confirmed the chromosome theory of inheritance (Morgan 1910; Bridges 1916). Now studied by >1800 labs around the world, *D. melanogaster* is a powerful model organism. Flies are easily cultured in the lab and have many offspring and short generation times; in addition, they have a compact genome, are easy to manipulate genetically, and have many orthologous genes associated with human disease. In phylogenetic terms, the next-closest common invertebrate model, with an evolutionary divergence time of at least 600 million years (Adoutte *et al.* 2000), is the nematode *Caenorhabditis elegans*.

D. melanogaster is a human commensal species that inhabits all of the earth's continents except Antarctica. Around 15,000 years ago, *D. melanogaster* migrated from its ancestral range in southern Sub-Saharan Africa to Europe and subsequently colonized much of the rest of the world, reaching the Americas in just the last few hundred years (David and Capy 1988; Lachaise *et al.* 1988). Humans are largely responsible for *D. melanogaster* migration in recent history, probably through the trade of fruit (David and Capy 1988; Markow and O'Grady 2007). Because the recent dispersion from its native home in tropical Sub-Saharan Africa required surviving in habitats with temperate climates, *D. melanogaster* are used to study adaptation to new environments (*e.g.*, Schmidt *et al.* 2005).

There are >2000 described *Drosophila* species (Powell 1997; Markow and O'Grady 2006); however, the phylogenetic relationships between most of these species are not resolved and are debated among taxonomists (Dalton 2009; O'Grady and Markow 2009). Researchers study species across the genus *Drosophila* for everything from the size of their sperm (Pitnick *et al.* 1995) to their speciation history (Coyne and Orr 1998; Orr *et al.* 2007). Nearly two dozen *Drosophila* species have available genome sequences (more on this below), of which *D. melanogaster* was the first to be completed (Adams *et al.* 2000). Together, these species span a wide range of ecological habitats, life history characteristics, and evolutionary divergence times (Singh *et al.* 2009). This tractable complexity makes *Drosophila* a powerful model for comparative genomics studies on topics such as gene family evolution (Hahn *et al.* 2007), gene regulation (Stark *et al.* 2007), and ecological adaptation (Markow and O'Grady 2007).

Life Cycle

A major advantage of using *D. melanogaster* and related species as model systems is their particularly short life cycle, which allows for the rapid generation of large numbers of progeny to use in genetic crosses (Ashburner 1989). In *D. melanogaster*, the process of developing from a fertilized egg to adult (Figure 1) requires on average only 9–10 days at 25°; however, temperature can greatly influence the speed of this process, with flies cultured at 18° requiring ~19 days from egg to adult. Upon fertilization, embryogenesis is completed in ~24 hr, followed by three larval stages (termed first, second, and third instar) with a molting event at each stage transition. The first two instars each last on average 1 day, whereas the third instar typically requires 2 days. Thus, 5 days after fertilization, larval development is complete and the animals metamorphose within a hard, protective chitin-based pupal case (or puparium) that forms from the outer larval cuticle. The steroid hormone ecdysone is a central player in *Drosophila* metamorphosis, mediating gene expression shifts from the larval to adult fly pattern (Yamanaka *et al.* 2013). The animal remains in the pupal case for 4–5 days, during which most larval tissues break down and many adult structures develop from 19 imaginal discs present in the larvae. Imaginal discs are a collection of tissue-specific progenitor
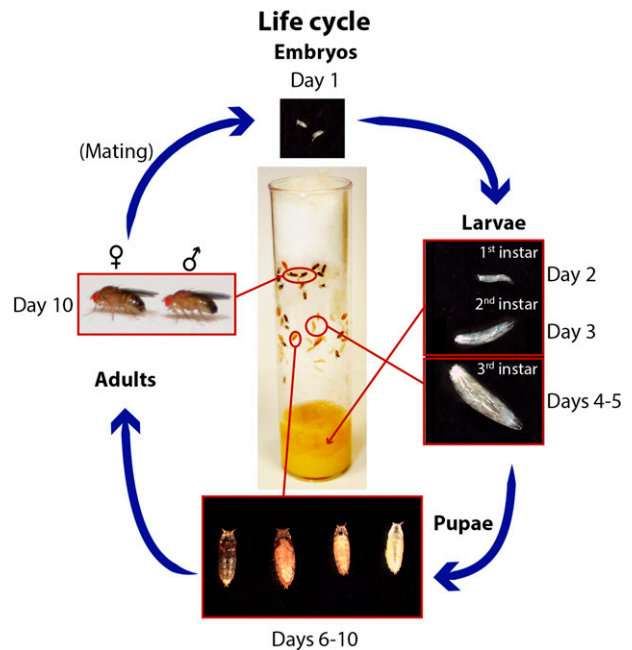
Figure 1 Life cycle of *D. melanogaster*. *D. melanogaster* are cultured in vials with food in the bottom and a cotton, rayon, or foam plug at the top. The pictured vial shows each major stage of the life cycle, which is completed in 9–10 days when flies are maintained at 25°. Embryos hatch from the egg after ~1 day and spend ~4 days as larvae in the food. Around day 5, third instar larvae crawl out of the food to pupate on the side of the vial. During days 5–9, metamorphosis occurs, and the darkening wings within the pupal case indicate that maturation is nearly complete. Adult flies eclose from pupal cases around days 9–10.

cells that develop during embryonic and larval stages and later give rise to most adult structures (such as eyes, legs, and wings) during pupal stages. Adult flies emerge from the pupal case in a process termed eclosion and become sexually mature in ~8–12 hr, allowing the life cycle to repeat itself.

Food and Husbandry

In the wild, fruit flies feed on yeast, bacteria, and plant matter within ripe or rotting fruit. In the lab, *Drosophila* media generally consists of a cornmeal/yeast/agar base supplemented with various carbohydrates and preservatives (see http://flystocks.bio.indiana.edu/Fly_Work/media-recipes/media-recipes.htm for recipe variants). The firmness of the food can be adjusted with varying amounts of agar depending on the health of the strain and level of larval activity. Ingredients are typically mixed with water, boiled, poured hot into vials or bottles, and then allowed to cool to create a solid plug of food at the bottom of the container. Some commercially available instant fly foods (with a potato-flake base) are simply mixed with water directly in the culture vial. Adult flies are transferred into the container (which is capped with a cotton or foam plug), where they lay eggs on the surface of the food. When eggs hatch into larvae, the larvae burrow into the food and progress through the instar stages until ready to undergo metamorphosis. As "wandering" third instar larvae,

Higher X chromosome dosage in XX flies
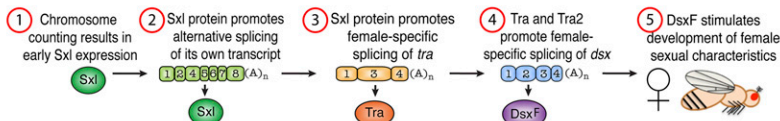
Lower X chromosome dosage in XY flies
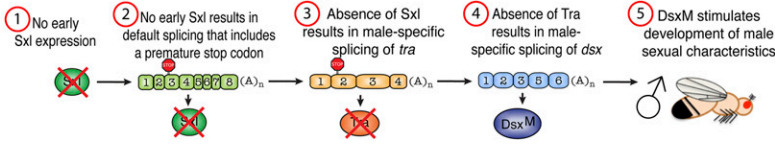
Figure 2 Sex determination. The number of X chromosomes in *D. melanogaster* is determined by an X chromosome counting mechanism. In XX females, early expression of the RNA-processing gene *Sex lethal* (*Sxl*) later results in female-specific processing of its own transcript. Sxl then begins a cascade of alternative splicing events that ultimately result in generation of the female-specific isoform of Dsx (DsxF). Note that Fru is not shown here for clarity. In males, the absence of early Sxl expression results in default processing of *Sxl* and *tra* transcripts that contain an early stop codon. Dsx pre-mRNA is then processed for a male-specific isoform of Dsx (DsxM). These Dsx isoforms then promote expression of downstream genes that govern sex-specific decisions related to morphology and behavior.

they crawl out of the food and pupate along the side of the container. Currently there are no efficient methods to freeze and recover adult flies or larvae, so the typical approach to maintain fly lines is continual transferring of adult flies to fresh media. It is recommended that flies are transferred to new food frequently (once every 2–3 weeks) to avoid bacterial/mold contamination and mite accumulation; however, this timing is commonly extended for strains that are not being actively used by maintaining them at 18°.

Sex Determination

While the presence of a Y chromosome is sufficient for male determination in mammals, it does not directly participate in sex determination in fruit flies. Instead sex determination in *D. melanogaster* is regulated by an X chromosome counting mechanism that "senses" the dosage of X chromosomes (Erickson and Quintero 2007). Thus, normal female flies are XX and males XY, but XO flies are males due to the decreased X dosage. In addition, each cell in the animal undergoes its own sex determination, with some cells continually monitoring X dosage. It is therefore possible to find gynandromorphs, in which part of the fly body has male characteristics and other parts female (for example, due to loss of one X chromosome in some cells within an otherwise XX female).

At the molecular level, sex determination in *Drosophila* is controlled by activation of the RNA processing gene S*ex-lethal* (*Sxl*) in females but not males (Bell *et al.* 1988; Salz and Erickson 2010; Verhulst *et al.* 2010). XX dosage in females results in early expression of Sxl, which then initiates a cascade of alternative splicing events that ultimately regulate differential splicing of the transcription factors *doublesex* (*dsx*) and *fruitless* (*fru*) (Figure 2). Sex-specific isoforms of Dsx and Fru then mediate expression of downstream effectors that govern sexual morphology and behavior (Baker *et al.* 1987; Demir and Dickson 2005). For example, female-specific isoforms promote expression of yolk proteins in the fat body of females but not males. Work is underway to identify specific genes regulated by sex-specific isoforms of Fru (Neville *et al.* 2014; Nojima *et al.* 2014; Vernes 2014) and Dsx (Clough *et al.* 2014; Luo and Baker 2015). For a review on sex determination in *Drosophila*, see Salz and Erickson (2010).

Drosophila Genome Features

With its extraordinarily well-curated genome and large genetic toolkit (see below), *D. melanogaster* is a powerful genetic system. The entire *D. melanogaster* genome size is estimated at ~180 Mb (Bosco *et al.* 2007), ~120 Mb of which is euchromatin (Adams *et al.* 2000; Celniker *et al.* 2002). Of the four chromosome pairs (Figure 3A), the first is the sex chromosomes, including an acrocentric X chromosome and the submetacentric Y chromosome that is gene poor and almost entirely composed of heterochromatin (highly compacted, transcriptionally silent DNA, which is dense in repeats). The remaining three pairs are autosomes. Chromosomes 2 and 3 are large metacentric autosomes, whose left and right arms are sometimes referred to separately as 2L, 2R, 3L, and 3R. Chromosome 4 (the dot chromosome) is a very small autosome. The first physical maps of these chromosome arms were created by Calvin Bridges in Thomas Hunt Morgan's group (Bridges 1935) based on polytene chromosomes, which are giant chromosomes (commonly isolated from salivary glands) usually consisting of >1000 endoreplicated DNA copies adhering in register. Adding a chemical dye to polytene chromosomes gives rise to unique banding patterns for each chromosomal region; these banded regions were numbered by Bridges and standardized as map coordinates that are still in use today. Before the *D. melanogaster* genome was sequenced, mapping of a DNA fragment to a genomic region was typically performed by *in situ* hybridization to polytene chromosomes.

The small genome size and longtime use as a model organism made *D. melanogaster* an appealing candidate genome to use as a proof of principle for whole genome shotgun sequencing and assembling of larger, more complex genomes (Adams *et al.* 2000). As a result, *D. melanogaster* was the second multicellular genome to be sequenced, after *C. elegans* (C. elegans Sequencing Consortium 1998). The first assembly was published in 2000 (Adams *et al.* 2000), and several iterations of assembly "finishing" were published subsequently (Celniker *et al.* 2002; Hoskins *et al.* 2007).

Initial genome annotation efforts identified ~13,600 genes, ~2500 of which had already been characterized (Adams *et al.* 2000; Misra *et al.* 2002). The current number of protein-coding genes predicted based on the latest genome assembly is 13,920 (R6.05; http://flybase.org/static_pages/docs/release_notes.html).
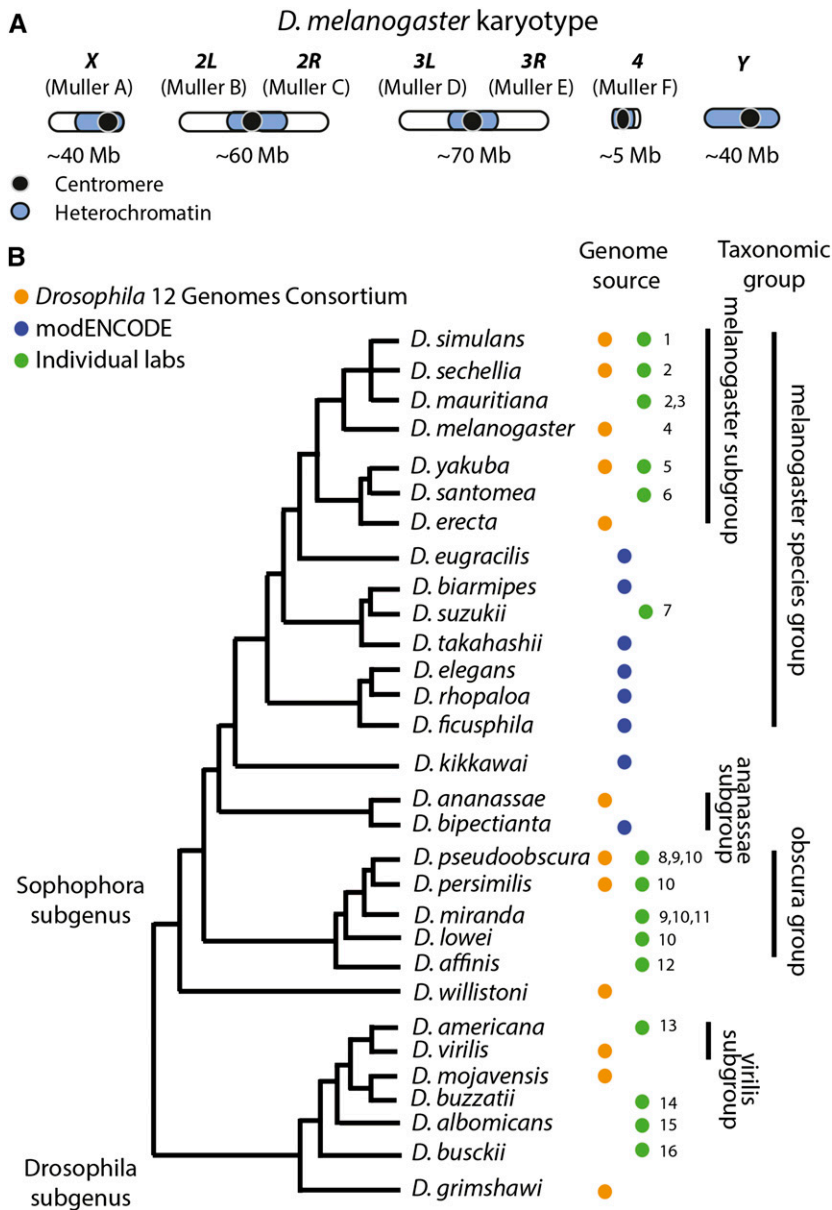
A

D. melanogaster karyotype

X (Muller A)	2L (Muller B)	2R (Muller C)	3L (Muller D)	3R (Muller E)	4 (Muller F)	Y
~40 Mb	~60 Mb		~70 Mb		~5 Mb	~40 Mb

● Centromere
● Heterochromatin

B

● *Drosophila* 12 Genomes Consortium
● modENCODE
● Individual labs

Genome source | Taxonomic group

- D. simulans — 1
- D. sechellia — 2
- D. mauritiana — 2,3
- D. melanogaster — 4
- D. yakuba — 5
- D. santomea — 6
- D. erecta
- D. eugracilis
- D. biarmipes
- D. suzukii — 7
- D. takahashii
- D. elegans
- D. rhopaloa
- D. ficusphila
- D. kikkawai
- D. ananassae
- D. bipectianta
- D. pseudoobscura — 8,9,10
- D. persimilis — 10
- D. miranda — 9,10,11
- D. lowei — 10
- D. affinis — 12
- D. willistoni
- D. americana — 13
- D. virilis
- D. mojavensis
- D. buzzatii — 14
- D. albomicans — 15
- D. busckii — 16
- D. grimshawi

melanogaster subgroup
melanogaster species group
ananassae subgroup
obscura group
virilis subgroup

Sophophora subgenus
Drosophila subgenus

Figure 3 Genome organization and phylogeny. (A) Organization of the *Drosophila melanogaster* genome. *D. melanogaster* has two metacentric autosome arms (chromosomes 2 and 3; Muller elements B and C and D and E), a small autosome (chromosome 4; Muller element F) and a pair of sex chromosomes (chromosome X—Muller element A—and chromosome Y). The approximate sizes and division of heterochromatin/euchromatin are shown. (B) Comparative genomics resources. Phylogeny of *Drosophila* species whose genomes were sequenced either by a large consortium (*i.e.,* Drosophila 12 Genomes Consortium *et al.* 2007 or modENCODE https://www.hgsc.bcm.edu/drosophila-modencode-project) or other community or individual lab sequencing project. While likely not an exhaustive list, it highlights the power to do comparative genomics in *Drosophila*. The tree topology is derived from several sources (*Drosophila* 12 Genomes Consortium *et al.* 2007; Gao *et al.* 2007; Seetharam and Stuart 2013), as the phylogenetic relationships between some of these species are not well resolved. The references for each genome are as follows: (1) Hu *et al.* (2013); (2) Garrigan *et al.* (2012); (3) Nolte *et al.* (2013); (4) Adams *et al.* (2000); (5) Rogers *et al.* (2014); (6) http://genomics.princeton.edu/AndolfattoLab/Dsantomea_genome.html; (7) Chiu *et al.* (2013); (8) Richards *et al.* (2005); (9) Kulathinal *et al.* (2009); (10) McGaugh *et al.* (2012); (11) Zhou and Bachtrog (2012); (12) Palmieri *et al.* (2014); (13) Fonseca *et al.* (2013); (14) Guillen *et al.* (2014); (15) Zhou *et al.* (2012); and (16) Zhou and Bachtrog (2015).

Computationally predicted gene models are supported by evidence from sequenced transcripts (*i.e.*, ESTs, cDNAs, and RNAseq data) and homology to known genes in GenBank (Misra *et al.* 2002). Each gene is assigned an annotation ID that begins with two letters—in *D. melanogaster,* "CG" for protein-coding genes and "CR" for nonprotein-coding genes—and followed by a series of numbers. Annotation IDs in other *Drosophila* species are identified by a different prefix (*e.g.,* "GA" for *D. pseudoobscura* IDs and "GD" for *D. simulans* IDs). Each genomic feature in the *D. melanogaster* genome is assigned a unique FlyBase identifier: genes begin with "FBgn" and transcripts begin with "FBtr." Genes typically are not assigned a descriptive name until they have been studied—those not yet studied are referred to by their "CG" number. *Drosophila* biologists have a tradition of assigning gene names based on their mutant phenotypes rather than their wild-type functions. For example, *dunce* mutants have a

learning defect (Dudai *et al.* 1976), *shaven baby* mutants are missing the small hairs (trichomes) of the larval cuticle (Wieschaus *et al.* 1984), and *tinman* mutants lack a heart (Evans *et al.* 1995). Gene names and abbreviations are italicized but usually not capitalized, unless the allele for which they are named is dominant to wild type. Protein names and abbreviations are usually capitalized but not italicized.

Defining functional elements in the D. melanogaster genome

In 2007, the modENCODE project was launched with the goal of defining and describing all functional elements in the genomes of *D. melanogaster* and *C. elegans* (Celniker *et al.* 2009; modEncode Consortium *et al.* 2010). As a result of this large-scale collaboration, the fly community now has access to large datasets documenting many facets of *Drosophila* genetics, including transcription across the genome at different

stages, transcription start and stop sites, alternative splicing patterns, promoter and other regulatory elements (and proteins that bind them), histone variants, and chromatin structure. Guidance in understanding and navigating available modENCODE data can be found in Boley *et al.* (2014).

modENCODE data indicate that the *D. melanogaster* transcriptome is much more complex than previously thought, suggesting an increased number of predicted protein-coding genes (14,692) along with 2872 noncoding genes, collectively covering 72% of the genome (Brown *et al.* 2014). More than half of *D. melanogaster* genes show alternative splicing, sometimes with differential promoter-driven use of alternative first exons, and 45% of genes encode more than one protein isoform. Many paralogous pairs of genes exist as a result of past gene duplication events, and in most cases the newer copy shows enriched expression in the testis at first, with gradually broadening expression over evolutionary time (Assis and Bachtrog 2013). Nearly 2000 long noncoding RNAs (lncRNAs) were identified in the modENCODE project, of which many overlap with protein-coding genes in an antisense orientation and likely are involved in regulation of the overlapping genes.

The broader view of genome-wide gene regulation obtained through modENCODE and other complementary efforts (de Graaf and van Steensel 2013) suggests (consistent with work in other organisms) that the three-dimensional folding of DNA in the nucleus brings together disparate regions of the genome into coregulated domains of genes showing similar expression patterns (modEncode Consortium *et al.* 2010). Binding of 84 transcription factors has been mapped at different developmental stages (Slattery *et al.* 2014). The map of transcription across the *D. melanogaster* genome was validated via comparison with transcriptional patterns in other *Drosophila* species (Chen *et al.* 2014).

Transposable elements

Transposable elements (TEs) are mobile genetic elements, first discovered in maize (McClintock 1950), that are common in *Drosophila* genomes. The two main classes of TEs are retrotransposons and DNA transposons (Craig *et al.* 2002). Retrotransposons move in the genome through an RNA intermediate using a "copy and paste" mechanism—DNA is transcribed to RNA, reverse transcribed, and inserted in new genomic location. DNA transposons move in the genome through a DNA intermediate using a "cut and paste" mechanism—transposon DNA is excised and moved to a new genomic location. In total there are ~150 different TE families in *D. melanogaster* (Mackay *et al.* 2012), most of which are retrotransposons (Kaminker *et al.* 2002). These families, such as *gypsy*, *copia*, and *minos*, vary in their abundance and activity across *Drosophila* genomes (*Drosophila* 12 Genomes Consortium *et al.* 2007). One type of DNA transposon, the *P* element, was recently introduced to *D. melanogaster* from a distantly related species, *D. willistoni* (Daniels *et al.* 1990) and spread through *D. melanogaster* populations in a staggering <75 years (Bingham *et al.* 1982; Engels 1983; Kidwell

1983)! TEs are often deleterious, as they can insert into genes or cause chromosomal rearrangements. RNA interference mechanisms control the activity of TEs by silencing endogenous elements (Brennecke *et al.* 2007; Czech *et al.* 2008). These pathways involve 24–31 nucleotide Piwi-interacting RNAs (piRNAs) that associate with Piwi proteins (Brennecke *et al.* 2007) mainly in the germline, and ~21 nucleotide endogenous siRNAs that associate with Dicer2 (Czech *et al.* 2008) in both the soma and germline. Not all TEs cause problems in the genome; rather, some TEs now serve an important biological role. For example, the activity of some retrotransposon families (*Het-A*, *TART*, and *Tahre*) maintains telomere length (George *et al.* 2006), a feature unique to *Drosophila* species. In the lab, researchers have developed ways to use TEs as tools for mutagenesis and transformation (see *A Drosophila Genetic Toolkit* below).

Conserved chromosome arms across Drosophila species

The gene content of chromosome arms tends to be conserved across *Drosophila* species, but the order and orientation of these arms are shuffled (Muller 1940). These conserved chromosome arms, lettered from A to F, are called Muller elements and correspond to the X, 2L, 2R, 3L, 3R, and 4 chromosome regions of *D. melanogaster*, respectively (Figure 3A). In general, the karyotype of each *Drosophila* species can be achieved by reorganizing these Muller elements. In *Drosophila*, chromosomal translocations involving the centromere and paracentric inversions (those not involving the centromere) are more common and fixed more often than pericentric inversions (those involving the centromere) (Sturtevant and Novitski 1941). The conservation of some Muller elements extends beyond *Drosophila* into other Dipterans such as botflies, house flies (Boyes and Vanbrink 1965), and even mosquitos (Bolshakov *et al.* 2002).

Drosophila as a model for comparative genomics

Since the publication of the *D. melanogaster* genome sequence (Adams *et al.* 2000), researchers have sequenced the genomes of over two dozen *Drosophila* species to varying levels of completion. The second sequenced *Drosophila* genome was *D. pseudoobscura*, a species with historical importance in evolutionary genetics (Richards *et al.* 2005). *D. pseudoobscura* is well studied for its abundance of chromosomal inversions (Sturtevant and Dobzhansky 1936) and the possibility that these genomic arrangements contribute to adaptation and the formation of new species (Noor *et al.* 2001; Navarro and Barton 2003). One motivating factor for sequencing *D. pseudoobscura* was to discover *cis*-regulatory elements; however, it turned out that there is little conservation between the noncoding regions of *D. pseudoobscura* and *D. melanogaster* (Richards *et al.* 2005). The *Drosophila* 12 Genomes Consortium involved a community-wide effort to sequence 10 additional species with a wide range of divergence times from *D. melanogaster* (*Drosophila* 12 Genomes Consortium *et al.* 2007). Of these sequenced species, the closest relatives

to *D. melanogaster* are members of the *simulans* clade—*D. sechellia* and *D. simulans*—species that differ in ecology and life history characteristics despite diverging just 240,000 years ago (Garrigan *et al.* 2012). *D. sechellia* is endemic to the Seychelles Archipelago and specializes on a fruit toxic to other *Drosophila* species (*Morinda citrifolia*), whereas *D. simulans* is a geographically widespread and cosmopolitan species (Lachaise *et al.* 1988). The most distant relative to *D. melanogaster* is the Hawaiian "picture wing" species *D. grimshawi*. Hawaiian *Drosophila* are the most diverse group of drosophilids (Kaneshiro 1997) and are studied for their interesting morphological characteristics and speciation history (Carson 1975, 1982, 1997; Templeton 1981). Species with intermediate divergence times from *D. melanogaster* allowed for more efficient identification of conserved *cis*-regulatory elements and noncoding RNAs (Stark *et al.* 2007). One goal of the modENCODE project has been to sequence the genomes of eight additional species with next-generation technology (Celniker *et al.* 2009). Several more *Drosophila* species genomes are available through the efforts of individual labs (*e.g.*, Garrigan *et al.* 2012; Zhou and Bachtrog 2012; Zhou *et al.* 2012; Nolte *et al.* 2013; Guillen *et al.* 2014) (Figure 3B). The large number of sequenced *Drosophila* genomes provides an important resource for comparative genomics and offers a powerful approach to the discovery of functional elements in genomes and their evolution (Singh *et al.* 2009), and ecological genomics (Markow 2015).

A *Drosophila* Genetic Toolkit

A strong attribute of the *Drosophila* experimental system is the ability to ask a wide variety of questions about the role of genes in the development and function of the organism, such as the following: Which genes are involved in the development of particular organs and tissues, or in a behavior, and what are the molecular pathways? Where is a certain gene expressed in the fly during development and/or later in the adult? What genes mediate basic cell biological events within specific cell types? Can controlled expression of a particular gene (or variants thereof) in developmental time and cellular location help elucidate that gene's function? Ingenious genetic tools to address these questions in *D. melanogaster* have enabled dramatic advances in our understanding of basic cell biology and development. In addition, since most human genes associated with genetic disease have a *Drosophila* counterpart, genetic analysis in flies has led to important applications toward human health.

How can I identify the genes involved in my favorite developmental or behavioral process?

Finding genes associated with a developmental process or behavior can be accomplished in a variety of ways but most often is associated with a forward genetic screen in which mutagenized fly lines are examined for alterations in the phenotype of interest. Before we discuss how genetic screens are carried out, we need a brief overview of the basics of doing a genetic cross and some of the tricks of the trade.

Genetic crosses and balancer chromosomes: Hallmarks of a model genetic organism are the ability to create stable inbred stocks carrying mutations or other experimental genome manipulations and the ability subsequently to cross these stocks to other stocks to generate desired genotypes. In *D. melanogaster*, multigenerational crossing schemes can be easily designed and carried out by isolating unmated or "virgin" females <8 hr old (since females who previously mated with siblings can store sperm for later use) and mixing them with males of defined genotypes. Males and females are distinguished primarily by abdominal pigmentation patterns (Figure 1) as well as genital structures and presence of "sex combs" on the front legs of males. A typical component of a *Drosophila* researcher's day is the collecting of virgins upon arriving in the lab in the morning and leaving the lab at night. Accurate genetic crosses are enabled by visible "marker" mutations that allow selection of offspring that inherited one version or the other of a chromosome. Classic examples of these markers are *yellow* and *white* on the X chromosome (affecting body and eye color, respectively), *Curly* (*Cy*) on the second chromosome (affecting wing shape), and *Stubble* (*Sb*) on the third chromosome (affecting bristle length) (Greenspan 1997). For example, a geneticist may collect non-curly winged flies to obtain those that inherited the parent's other copy of the second chromosome with a mutation of interest. Observant genetics students might wonder whether meiotic crossing over could be a big problem here—what if the mutation of interest had recombined onto the *Curly* chromosome during meiosis in the parent? To prevent such movement, the chromosomes with visible markers also contain multiple inversions to prevent viable recombinant offspring; thus the only viable offspring are those with one or the other of the parent's two intact chromosomes (either with the mutation of interest or with the dominant marker). These engineered chromosomes with visible markers and multiple inversions are called "balancer chromosomes." Balancer chromosomes have a third feature as well—recessive lethal mutations—to prevent mutations of interest from being selected out of an inbred population. For example, if you are studying mutation *f* that is homozygous lethal, you must propagate *f* via heterozygotes that have one wild-type and one mutant copy. Over time in a population, the *f* allele would gradually decrease in frequency since *f/f* homozygotes die while heterozygotes and wild-type homozygotes reproduce. If the *f* mutation is instead maintained with a balancer chromosome, the *f* allele persists since balancer homozygotes die, and heterozygotes are the only viable genotype. See Greenspan (1997) for a deeper examination of the logistics for setting up crosses and using balancer chromosomes.

Inducing mutations: The first step in designing a genetic screen is choosing how you will disrupt the genome in hopes
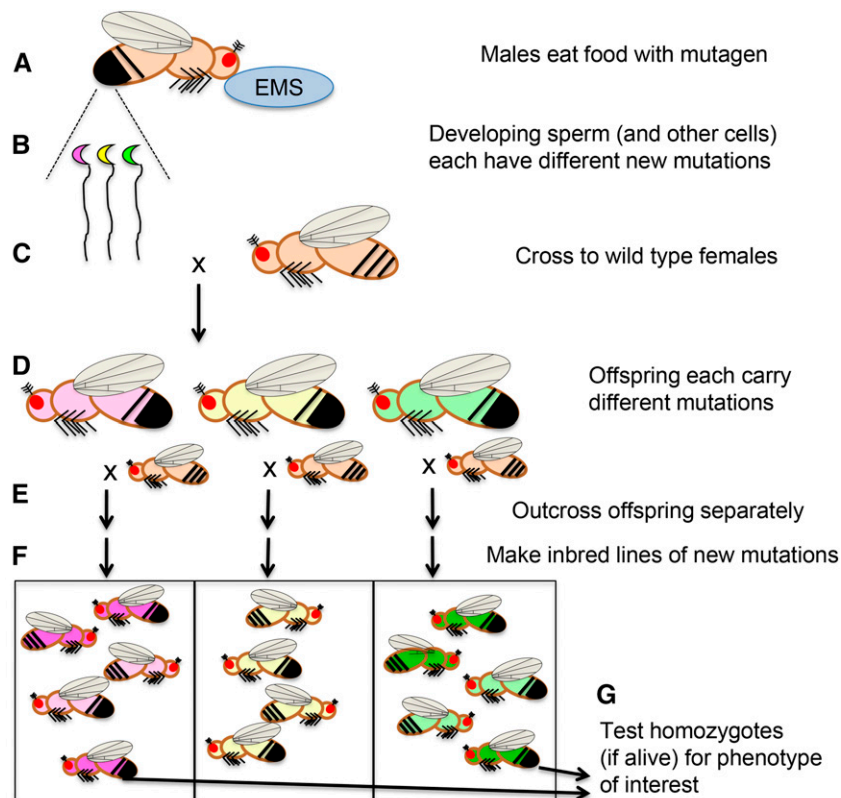
Figure 4 Generalized scheme for a forward genetic screen using chemical mutagenesis. (A) Male flies eat food laced with ethane methyl sulfonate (EMS), an alkylating agent which typically causes point mutations. (B) Different mutations occur in each cell of the feeding flies, including sperm (indicated by pink, yellow, and green sperm cells). (C) Outcrossing the mutagenized flies to untreated females yields (D) offspring that each potentially have a different new mutations throughout their bodies, indicated schematically by body colors corresponding to the sperm cells above. (E) Outcrossing these flies individually and (F) inbreeding each set of offspring gives a population of flies for each new mutation. (G) Researchers then test homozygous flies (darker pink and green) for the phenotype of interest. In some cases, adult homozygotes are not viable (as in the yellow population) and so researchers interested in earlier developmental steps may examine embryos and larvae within these populations to find dying homozygotes.

that particular genes affecting your process of interest are among the many genes randomly mutated. As with other model systems, the fly community has used chemical mutagens such as ethyl methane sulfonate (EMS) added to fly food or ionizing radiation such as X-rays to trigger everything from single base pair mutations to larger chromosomal changes (Greenspan 1997). As described earlier, the identification of transposable elements provided an additional tool to alter genetic sequences through the mobilization of genetic elements throughout the genome. The Gene Disruption Project has used different transposable elements to achieve broad coverage of insertions and to generate a variety of extremely useful types of insertions, although this has not completely saturated the genome due to the particular idiosyncrasies of the elements (Bellen *et al.* 2004, 2011; Venken *et al.* 2011). To date, the project has created >12,000 insertion lines that are available for public use in the stock collections. Current estimates suggest that only 30% of the coding sequences in the genome have been targeted using these traditional methods (Kondo 2014). These insertions have also been used in creative ways to create small deletions through imprecise transposon excision (Voelker *et al.* 1984), mutations in nearby genes through "local hopping" of the element (Tower *et al.* 1993), and defined sets of isogenic deletions that cover the genome through the excision of DNA between elements at defined insertion points (Parks *et al.* 2004; Ryder *et al.* 2004; Thibault *et al.* 2004). Most often researchers will use a combination of transposable element insertion libraries and chemical mutagenesis to provide

broad mutational coverage of all the chromosomes in hopes of "saturating" or creating mutations in all the possible genes that may play a role in their favorite developmental or behavioral process.

A basic forward genetic screen: The goal of a forward genetic screen is to identify genes involved in a particular developmental process, biochemical activity, or behavior, with no *a priori* model of which genes might be important. Success is often dependent on how efficiently a particular phenotype can be observed. A variety of innovative screening approaches have identified genes associated with many aspects of *D. melanogaster* biology (St Johnston 2002), with an early example being the Nobel Prize-winning embryonic lethal zygotic screen to dissect the basis of early development (Nusslein-Volhard and Wieschaus 1980). Their hypothesis was that mutations causing embryonic lethality likely affected genes that normally played a role in early development. Indeed, when they visualized embryonic morphology of the many mutants they isolated, the observed phenotypes suggested different levels of genetic and spatial organization in the developing embryo. Most mutagenesis screens start with mutagenizing males via chemicals or radiation and crossing them to females carrying balancer chromosomes (Figure 4). Single male offspring from this cross are outcrossed again, and male and female siblings within these individual lines are then crossed to start inbred lines that propagate different founding mutations. Within these lines, heterozygotes (with a balancer) propagate the stock, while

homozygotes can be examined for recessive phenotypes of interest. Independent inbred lines with similar phenotypes can be crossed together in complementation tests to see if mutations happen to affect the same gene.

An interesting recent application of genetic screening is the elucidation of the fly as a model for alcoholism and related behaviors (Singh and Heberlein 2000). The Heberlein lab invented an inebriometer—a tube containing ethanol vapor and multiple platforms from which flies can fall from level to level—to screen mutagenized flies and identify genes associated with alcohol sensitivity (Singh and Heberlein 2000). The *barfly* mutant showed decreased alcohol sensitivity, and the *tipsy* mutant showed increased sensitivity compared to wild type. These are further examples of the creative naming of *D. melanogaster* gene mutations based on the phenotypes they express. Another mutant identified by their lab, *cheapdate*, turned out to be an allele of the *amnesiac* gene, which encodes a PACAP-like neuropeptide and thus connects the cAMP pathway with ethanol sensitivity (Moore *et al.* 1998). The implications of this work for understanding effects of alcohol on the nervous system go beyond just the biology of the fly. Although the structures and networks associated with the fly and human brains are different, the molecular mechanisms uncovered in the fly associated with the neural response to alcohol and addictive behaviors are, at a basic level, shared with humans (Robinson and Atkinson 2013).

Enhancer and suppressor screens: A forward genetic screen to find additional genes of interest can be performed starting from an already-mutant genetic background—the goal is to find new mutations that make worse ("enhance") the preexisting mutant phenotype or that alleviate ("suppress") that original phenotype. Often the new genes found in an enhancer or suppressor screen are directly involved in the biological phenomenon of interest but would not have had a significant enough effect to be found in the original forward genetic screen. In other cases, enhancers and suppressors that function through reduced dosage are genes that would cause lethality alone as homozygotes and thus perhaps would not be found in an original screen.

Mapping mutations identified in mutagenesis screens: The next step is mapping a mutation to a chromosomal region to enable identification of candidate genes. Researchers commonly map genes using parallel complementation tests against a collection of known chromosomal deficiencies that collectively span the chromosome in question. A "deficiency kit" for a given chromosome can be obtained from the Bloomington Stock Center (Cook *et al.* 2010). An older approach is meiotic or recombination mapping with visible markers (Greenspan 1997). Many multiply marked chromosomes with recessive mutations are available at the Bloomington *Drosophila* Stock Center for this purpose, and a more recent approach using dominant markers is available (Sapiro *et al.* 2013). As sequencing technologies increase in speed and decrease in price, brute force sequencing will become a more common way to identify new mutations in inbred fly strains as well as in individual flies within a population.

I have identified a candidate gene possibly altered in an inbred line from a mutant screen—How can I make sure that that gene is truly associated with the effect I am observing? How can I test the effects of different versions of that gene?

An important next step in characterizing a new mutation found in a screen is testing whether a candidate gene you identified is truly associated with the phenotype you are observing, instead of being a random secondary occurrence. In the fly community, this is called doing a "rescue" experiment. The question you are asking is whether adding back a wild-type copy of the gene will reverse, or rescue, the mutant phenotype. (Once you have confirmed that the gene you found is the correct one, you can also add back different, manipulated versions of the gene to do structure–function studies.) The key innovations that allow us to answer these questions are *P*-element-mediated transformation as well as the Gal4/UAS bipartite expression system.

Transformation: Much of *D. melanogaster* genetics was literally "transformed" with the identification and development of the *P*-element as a germ-line transformation vector. The *P*-element is a classic transposable element (Bingham *et al.* 1982; Rubin *et al.* 1982) that originally contained a gene encoding the transposase enzyme, which together with the inverted repeats found on the terminus of the DNA element permitted movement within the genome. Rubin and Spradling (1982) hypothesized that replacing the internal transposase gene with a gene of interest would produce an ideal system for inserting DNA into the fly genome. This *P*-element construct could then be co-injected into early (still syncytial) embryos along with an independent source of the transposase enzyme to insert the transposable element into the developing germline. The proof-of-principle experiments demonstrated heritable and stable transformation of the *D. melanogaster* germline using the wild-type *rosy* gene as a marker (Rubin and Spradling 1982). Later work developed the now classic wild-type *white* (w^+) eye color marker gene that is used to identify transformants in a *white* mutant background (Klemenz *et al.* 1987). This discovery and the development of a variety of other elements was the genesis of much of the genetic technology that makes the fruit fly such a powerful model system. The entire spectrum of their uses is beyond the scope of this article (Venken and Bellen 2005, 2007) but their use as a mutagenesis tool and the design of the binary Gal4/UAS expression system are two of the most important stages in the development of the fly as a modern genetic system.

GAL4/UAS expression system: Brand and Perrimon (1993) took the *P*-element transformation vector and used it to create a gene expression system that would eventually allow for the expression of any gene of interest in any particular
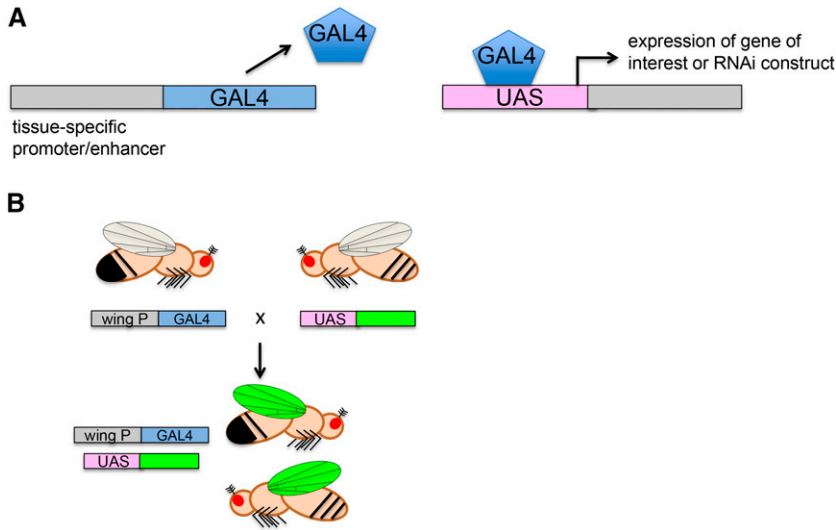
Figure 5 GAL4/UAS system for modular expression of transgenes in specific tissues. To express a transgene or RNAi construct in a particular tissue, one needs flies carrying (A) a "driver" with a tissue-specific promoter/enhancer placed 5' of the gene encoding the yeast GAL4 transcription factor (left) and (right) the gene of interest placed 3' of the upstream activating sequence (UAS), which is activated by GAL4. (B) Transgenic flies carrying either of the two constructs alone (top) do not express the gene of interest, but when crossed into the same fly, the tissue-specific promoter (a wing promoter in this example) drives expression of GAL4, which turns on the gene of interest (here indicated by green) in the specified tissue. The system can also be used to express a hairpin RNA to knock down a gene in the target tissue.

tissue within the fly. They cloned the yeast transcription factor GAL4 into a *P*-element vector and showed that one could place a defined promoter upstream of GAL4 or integrate the GAL4 element into the genome to use endogenous transcriptional enhancers to express GAL4 (an enhancer trap). To accompany this "driver" of gene expression, they created a corresponding *P*-element vector, pUAST, containing the upstream activating sequences (UAS) to which GAL4 protein can bind. These UAS sequences were connected to a general promoter and a cloning site to allow for the insertion of any gene of interest. This bipartite expression system could be used to drive the expression of a gene in a defined way (Figure 5), allowing researchers to perform rescue experiments and other manipulations (see below).

Gene expression libraries that enabled the GAL4-UAS system: The advent of transposable-element-based genetics in *D. melanogaster* spurred the development of reagents to identify promoter sequences driving cell-type-specific gene expression throughout development. Early screens aimed to find genes important for particular processes based on their expression patterns. Random genome-wide insertion of *P*-elements containing a cellular marker such as *Beta-Galactosidase* (*LacZ*) or *GFP* would be expressed if inserted just after an endogenous promoter (Bellen *et al.* 1989; Grossniklaus *et al.* 1989; Wilson *et al.* 1989). Later modifications inserted a transposable element with the GAL4 gene to create cell-type-specific GAL4 expression lines, which then allowed the manipulation of specific cell types using the GAL4/UAS system. These "enhancer-trap" lines were the first generation of expression tools that would eventually lead to the unprecedented specificity in the expression of particular transgenes. A second approach is to clone small enhancer regions from genes of interest to create GAL4 expression lines that replicate the expression pattern of that gene. Systematic approaches to the generation of enhancer specific expression of GAL4 has led to the creation of the Janelia Farm GAL4

collection that uses small defined regions of noncoding or intronic DNA to create expression patterns that are more restricted than previous generations—in some cases only one or two cells (Pfeiffer *et al.* 2008).

The GAL4-UAS system has been modified in various ways to improve utility (reviewed in Duffy 2002). One example of a challenge addressed is lack of control over final resting place of a transposable element as a transformation vector within the genome. An insertional event may disrupt an endogenous gene's function, or the local transcriptional/chromatin environment may reduce expression of either the GAL4 transcription factor or the genes associated with the UAS elements. Unpredictable expression levels can add uncertainty regarding whether a rescue experiment, or other manipulations, are working as designed. To remedy this unpredictability, a new transformation system was developed using the serine recombinase protein from the PhiC31 bacteriophage, which recombines sequences using the attB and attP site-specific DNA sequences (Groth *et al.* 2004). A series of attP landing sites have been created at distinct points through out the genome providing molecularly defined positions for the high-efficiency creation of transgenic flies using transformation vectors with an incorporated attB sequence (Venken *et al.* 2006). These sites have also been characterized for the levels of expression of an inserted transgene, thus allowing for more finely tuned expression of a transgene as well as the comparison of different transgenes at the same chromosomal location (Ni *et al.* 2008).

I have identified many different genes using a mutant screen, but I suspect some specific additional genes may be involved in my biological process of interest, too. How do I test whether they are involved in this process?

We have already described the forward genetics approach of the mutant screen, in which you go from biological phenomenon to gene(s). In contrast, a reverse genetics approach is one in which you start with a gene and try to determine its function

via direct genetic manipulation and assessment of phenotypes. A gene's sequence or expression pattern may flag it as possibly interesting to people studying a particular process. How do researchers alter a gene if no chemically induced mutations exist or no transposable elements are inserted? Candidate gene experiments have led to new approaches to altering the genome of the fly. Initially researchers turned to homologous recombination processes similar to familiar mouse knock-out technology (Rong and Golic 2000). While this system achieved the desired outcomes, it was inefficient and not ideal for large-scale implementation throughout the genome. The two most promising approaches have been genome editing technology and RNA interference.

Genome editing: The recent excitement about purposefully altering genomes has centered on genome-editing techniques that take advantage of particular types of nuclease enzymes. Genome-editing technologies have enabled significant progress toward the goal of a complete collection of mutant strains for every gene. Several different technologies have been successfully used in *D. melanogaster*, including zinc finger directed nucleases, and transcription activator like effector nucleases (TALENs) (Beumer and Carroll 2014). The most promising editing technology is the modified bacterial clustered regularly interspaced short palindromic repeat (CRISPR/Cas9) system. In its original form, the targeted endonuclease is used by bacteria as a defense against viruses and plasmids by combining an endonuclease and a targeting guide RNA. Modification of this guide RNA allows the targeting of particular genes to induce the endonuclease to produce a double strand break. This double strand break efficiently results in the creation of short insertions/deletions and large deletions in a gene through nonhomologous end joining repair (Gratz *et al.* 2013; Bassett and Liu 2014). Development of this technology in the fly has created an efficient mechanism by which the community can generate complete loss-of-function (or "null") mutations in all of the ∼14,000 genes in the genome (Gratz *et al.* 2013; Bassett and Liu 2014). This technology also has the potential to "knock-in"/create specific mutations within a coding sequence to model the effects of particular genetic mutations, to probe the function of a particular protein domain, characterize splice sites, or fuse specific reporters (*e.g.*, GFP, YFP, etc.) at precise locations within a gene.

RNA interference: A second approach to creating genome-wide libraries to examine gene function has been the development of RNA interference in both transgenic animals and cell lines. Transgenic approaches use the GAL4/UAS system described earlier to express short inverted repeat RNA hairpins that target specific genes to reduce or "knock-down" the expression of the gene's messenger RNA (mRNA) (Kennerdell and Carthew 2000). These are not permanent alterations of a gene's coding sequence, but rather eliminate a gene's function by reducing mRNA levels from that gene. The original

versions of these systems used random insertions of transgenes carrying the UAS–RNA interference (RNAi) constructs specifically designed to target a particular candidate gene. These were inefficient in their ability to knock-down RNA expression of a particular gene to null levels and could produce lethality or sterility depending on the insertion site (Dietzl *et al.* 2007; Ni *et al.* 2008). In addition, work in cell culture suggested that the inverted repeat could produce off-target effects where multiple genes' expression was altered by the system (Perrimon and Mathey-Prevot 2007). Some of these issues have been addressed with new libraries that use the phiC31a/AttP site-specific integration system (described above) to insert the UAS–RNAi transgene in a well-characterized location on the chromosome that ensures high levels of expression and an insertion in a position that does not affect other genes (Ni *et al.* 2008). These libraries at the *Drosophila* RNAi Screening Center and Vienna *Drosophila* Research Center can be used to screen almost 90% of the protein coding genes for phenotypes of interest. With the use of well-characterized GAL4 driver lines that are activated at specific stages or in specific cell types, researchers can elucidate the function of particular genes in cellular and developmental processes from embryo to adult by expressing the RNAi hairpin constructs (and thus knocking down the gene) at those developmental stages or in those cell types (Boutros *et al.* 2004; Armknecht *et al.* 2005; Mathey-Prevot and Perrimon 2006; Dietzl *et al.* 2007).

If a mutation produces lethality at some point in development, how do I examine a gene's role in other stages of the life cycle? How do I determine in which cells the gene is exerting its effect?

Assessing the effects of a mutation on a particular developmental/cellular/behavioral process can be difficult for genes that play multiple roles at different stages of the life cycle. If a mutation prevents the completion of embryonic development (lethality) then analysis of the gene's role in adult tissues can be preempted. In response to this challenge, geneticists have created tools that allow more fine-tuned analysis of gene function at any stage of development. For example, the previously discussed RNAi system allows researchers to knock down gene function at any stage of development or in specific cell types using the GAL4/UAS system; stage- or cell-specific GAL4 drivers can knock down a candidate gene or can drive a whole library of RNAi lines to do a genome-wide screen. If instead of RNAi knock-down, a fly biologist would like to analyze how a lethal mutation or other allele of a gene affects a tissue (or determine in which cell type a mutation has to to exert an effect), they can use clonal analysis.

Clonal analysis: To characterize later roles of a gene essential for early development, one can produce mosaics that have homozygous mutant patches of cells (clones) in an otherwise heterozygous animal via mitotic recombination (Figure 6). While triggering mitotic recombination can be triggered by radiation, a more efficient approach hinges on genetic

manipulation of flies to incorporate on their chromosomes the site-specific recombination components associated with the *Saccharomyces cerevisiae* 2-μm plasmid, the FLP recombinase and its site-specific recombination sites (FRTs) (Golic and Lindquist 1989). The original versions of this system placed FRT recombination sites on all of the arms of the *D. melanogaster* chromosomes to enable mitotic recombination between homologous chromosomes in the presence of the FLP recombinase. A researcher would recombine a mutation of interest onto a FRT chromosome, distal of the FRT site. In flies heterozygous for that mutation but with FRTs on both homologous chromosomes, FLP-triggered mitotic recombination would lead to patches of homozygous mutant cells, typically identified by a linked recessive marker or loss of a linked fluorescent gene product. The first FLP transgenic flies had the gene under the control of the *hsp70* promoter to permit the induction of the FLP and thus recombination at specific time points during development with an increase of temperature (Golic and Lindquist 1989). In *D. melanogaster* this technique was originally developed to examine maternal contributions to development through the creation of germline clones in females, and then it was adapted for somatic clones to observe the behavior of mutant cells in a variety of tissues throughout development and in the adult (St Johnston 2002).

Early in development of the embryo, the maternal contribution of RNA and protein from a heterozygous mother to a homozygous mutant embryo may mask an early developmental role for a gene. Germline clones in which homozygous mutant germ cells are created within a heterozygous female permits the characterization of earlier functional roles for such a gene. This technique takes advantage of the dominant ovo^D mutation, which in females leads to atrophic ovaries that produce no eggs (Perrimon 1984). In females transheterozygous for ovo^D and the mutation of interest on FRT-containing chromosomes, heat shock induces FLP-mediated mitotic recombination, allowing production of homozygous mutant clones free of the ovo^D allele—these are the only cells that can continue through oogenesis to produce eggs. The phenotype of these cells, if any, can reveal a role for the gene of interest in germline and early development.

A variation of this process can produce somatic clones in any cell type at any stage of development. Rather than ovo^D, the nonmutant chromosome contains a marker gene such as eye color (*white*⁺), body color (*yellow*⁺), or a fluorescent cell maker (GFP) to identify the nonlabeled clonal mutant cells in the labeled wild-type background (Perrimon 1998). As the FLP-FRT system has developed, a variety of promoters have been fused to the FLP recombinase gene to allow for more precise control of mitotic recombination during developmental time or in particular tissues. In addition, a more sophisticated system has been built, mosaic analysis with a repressible cell marker (MARCM), to allow for the analysis of individually marked mutant cells in an unlabeled heterozygous background (Lee and Luo 2001). This innovation has created
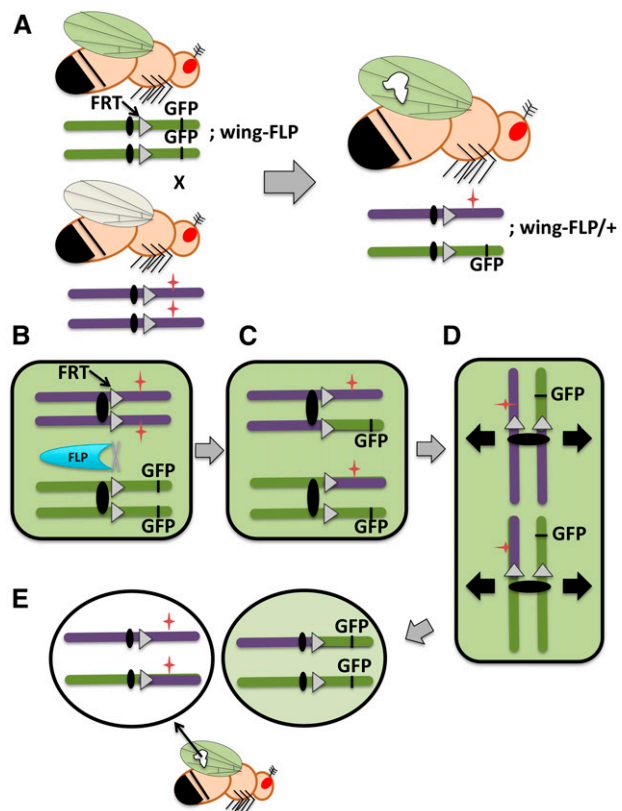

Figure 6 Clonal analysis in somatic tissue. (A) Schematic diagram of a genetic cross used to create homozygous somatic clones of a mutation of interest within a heterozygous background. In this example, the clone is a patch of mutant cells within a wild-type wing. One parent is homozygous for a chromosome that carries the FLP recombinase recognition site, FRT (triangles) and a distal GFP marker that is being expressed under the control of a wing promoter. All of the cells of this fly's wings will express GFP and appear green under a fluorescent microscope. This fly is also homozygous on another chromosome (not drawn) for the FLP recombinase gene, which is being expressed under the control of a wing promoter. The other parental fly is homozygous for a mutation of interest (red star) on the same FRT-carrying chromosome. Progeny from this cross will be transheterozygous for the GFP-marked chromosome and the mutant chromosome, and they will be heterozygous for FLP. During development, FLP-mediated mitotic recombination in the developing wing will produce patches of unmarked homozygous mutant cells (white patch). Panels B–E show the mechanics of clone production through mitotic recombination in the progeny. (B) Cells in mitotic G2 have replicated chromosomes with sister chromatids. In some wing cells, the FLP recombinase triggers recombination between FRT sites on nonsister chromatids, and (C) one copy each of the GFP marker and the mutation of interest will switch between homologous chromosomes. (D) One of two possible chromatid alignments at mitotic metaphase for the cell pictured in C. (The other alignment, not shown, leads to two heterozygous daughter cells.) The black arrows show the direction of sister chromatid separation during the completion of mitotic division. (E) Daughter cells produced upon completion of cell division for the cell pictured in D. One daughter cell is homozygous for the mutation of interest and will subsequently divide to give rise to a homozygous patch of cells, which can be identified for phenotypic analysis based on loss of the GFP marker. The other daughter cell is homozygous for the GFP marker and will blend into the surrounding heterozygous cells that also fluoresce.

a significant step forward in resolution, allowing for the examination and genetic manipulation of single mutant neurons within the adult brain to determine which neurons and which genes affect certain behaviors.

***Drosophila* cell lines:** While *Drosophila* is touted primarily as a fast genetic system for studying biological processes in a live animal, culturing of fruit fly cells can also be extremely advantageous for certain applications such as testing effects of gene knock-down at the subcellular level. Nearly 100 different *D. melanogaster* cell lines exist, with the S2 and Kc lines being the most commonly used (Echalier and Ohanessian 1969; Schneider 1972). These lines, originally derived by mechanical dissociation of embryos, became immortalized spontaneously and are likely of hematopoietic lineage. Other protocols established cell lines from protease-digested imaginal discs (Ui *et al.* 1987); however, a *Drosophila* cell line of true epithelial origin has yet to be derived. In addition, no method of forced immortalization exists (like retroviral infection in mammalian cells), and so establishing a new *Drosophila* cell line is dependent on spontaneous immortalization and can be somewhat variable (Baum and Cherbas 2008). As in cultured cells from other organisms, chromosome loss, duplication, and rearrangement can lead to altered karyotypes in some lines. *D. melanogaster* cell lines are commonly used to express and purify recombinant proteins when problems in protein activity or solubility are found in prokaryotic systems (Davis *et al.* 1993; Ikonomou *et al.* 2003). Transfection frequency in S2 cells is fairly high, making transgene analysis relatively straightforward (*e.g.*, for immunoprecipitation or subcellular localization or proteins). Cell culture enables scaled-up biochemical approaches that can be difficult to perform on isolated tissues. Perhaps the most powerful approach using *D. melanogaster* cells is RNAi (Hannon 2002). While RNAi in mammalian cell culture typically involves transfection of a pool of short double-stranded RNAs (dsRNAs) matching the target gene, *D. melanogaster* S2 cells readily absorb dsRNA from the culture media, increasing the percentage of cells with knock-down (Echeverri and Perrimon 2006). Also, this response is not length dependent in flies, as dsRNA of 500–1000 bp can be added to cultured cells, with Dicer then chopping this into shorter pieces of dsRNA. Including such a large dsRNA increases the likelihood of effective knock-down. These properties make *D. melanogaster* S2 cells particularly amenable to high-throughput RNAi approaches (Armknecht *et al.* 2005; Perrimon and Mathey-Prevot 2007), and several RNAi libraries have been developed for this purpose.

Using the Power of *Drosophila* Genetics to Study Developmental and Cell Biology

Genetic approaches in *D. melanogaster* have proven highly successful in elucidating the mechanisms that regulate a wide variety of biological processes ranging from developmental pathways to cytoskeletal regulation to organelle trafficking.

In this section, we describe key developmental steps and cell biological processes whose molecular mechanisms have been explored largely through genetic analysis. A resource for more information on these processes is Campos-Ortega and Hartenstein (1997).

Fertilization

Embryogenesis begins with fertilization of the oocyte. Upon mating, female flies store sperm for up to 2 weeks in specialized organs called seminal receptacles and spermathecae (Lefevre and Jonsson 1962). Sperm storage is thought to allow for the coordination of ovulation with sperm release and to reduce ecological costs associated with multiple matings (Wolfner 2003). After mating, females tend not to mate again for several days; however, sperm from more than one male can be stored at a given time. Sperm competition and sperm preference have thus been observed based on the genetics of both the male and female. Fertilization itself does not occur until the egg is ready to be laid. Mature eggs leave the ovaries and travel through the oviduct, during which time some of the stored sperm are released. As the egg passes through the oviduct, one or a few sperm enter the egg through a small, anterior opening in the chorion called the micropyle. Interestingly, fertilization in flies does not involve membrane fusion, but rather the sperm completely enters into the egg, with subsequent sperm plasma membrane breakdown occurring in the cytosol of the egg. Also, fertilization can only occur in what will become the anterior pole of the developing embryo.

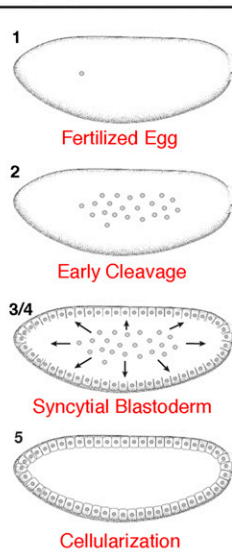### Superficial cleavage and cellularization

As in most insects, *D. melanogaster* development begins with nuclear divisions in a common cytoplasm with no new cellular membranes (thus it is a syncytium). The first nuclear divisions occur in the center of the egg and are coordinated such that all nuclei divide simultaneously in a cycle. After 10 division cycles, most of the nuclei migrate to the periphery where they become partially encapsulated by cytoskeletal proteins that create furrow canals. Bulk zygotic transcription initiates shortly thereafter and occurs prior to true cellularization. Cellularization marks the beginning of asynchronous cell divisions and occurs through invagination of the oocyte's plasma membrane along the furrow canals. The cellular blastoderm, formed 3 hr postfertilization, now consists of a layer of ~6000 cells lining the periphery of the zygote with yolk on the interior (Figure 7).

Gastrulation/morphogenesis

Next, gastrulation serves to specify the three germ layers of the animal (mesoderm, endoderm, and ectoderm), and is characterized by cell fate decisions and shape changes that drive cells to move in sheets to different regions of the embryo. The mesoderm is specified along the ventral surface as cells fold inward along the ventral midline and pinch off to form a hollow tube immediately beneath the ventral ectoderm (Leptin 1995). A video illustrating these movements is at

Cleavage Divisions
(Longitudinal section)

1 Fertilized Egg
2 Early Cleavage
3/4 Syncytial Blastoderm
5 Cellularization

Cellularization to the end of Embryogenesis
(Surface view)

5 Cellularization
8 Gastrulation
9 Germband Elongation
11 Parasegmental Furrows
12 Germband Retraction
13 Dorsal Closure
15 Head Involution
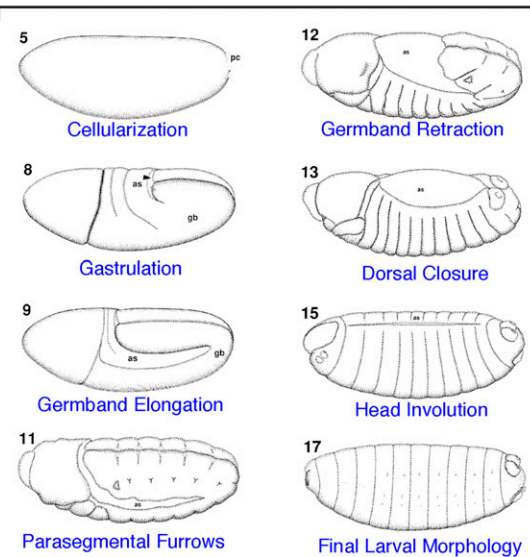17 Final Larval Morphology

Figure 7 Stages of embryonic development. *D. melanogaster* development begins in a syncytium characterized by nuclear divisions without cytokinesis (stage 2). After 10 synchronized rounds of division, nuclei migrate to the periphery where they become partially encapsulated by actin-based furrow canals (stage 3/4). True cellularization occurs in stage 5, followed by gastrulation (stage 8), which determines the three germ layers. Dramatic morphogenetic movements then reshape the body plan as cells from the posterior migrate toward the anterior in germband extension (stage 9) followed by later retraction to the posterior (germband retraction; stage 12). Epithelial cells then migrate toward the dorsal midline in dorsal closure (stage 13), and head structures begin to mature (head involution; stage 15). Finally the larva reaches its mature state (stage 17) and hatches from the eggshell. Images adapted from the Atlas of *Drosophila* Development (Hartenstein 1993) and used with permission. In each panel, anterior is to the right and dorsal is up.

https://www.youtube.com/watch?v=ymRYxFYLsZ4. Similar cell-shape changes drive anterior and posterior midgut invaginations of cells fated to become the endoderm.

Following specification of the germ layers, dramatic movements then reshape the body plan. Convergent extension (a process whereby cells narrow along one axis and extend along the perpendicular axis) causes cells of the prospective trunk region (germband) to first extend toward the anterior of the animal and later retract to the posterior. Videos showing these movements are at https://www.youtube.com/watch?v=FChS4KU5jDM and https://www.youtube.com/watch?v=MefTPoeVQ3w. Several important processes begin while the germband is in the extended position including organogenesis, segmentation, and neurogenesis. As the germband retracts, epithelial tissue from the cellular blastoderm (amnioserosal cells) spreads to cover the dorsal surface. The lateral ectoderm from both sides of the embryo then migrates over the top of these amnioserosal cells in a process termed "dorsal closure." Amnioserosal cells then degenerate and cuticle deposition begins. Now embryogenesis is nearly complete; the trachea fills with air, and the first muscle twitches can be observed before the first instar larva hatches from the eggshell (Figure 7). A useful resource illustrating these events and the development of particular organ systems is Volker Hartenstein's *Atlas of Drosophila Development* (Hartenstein 1993), available online at http://www.sdbonline.org/sites/fly/atlas/00atlas.htm.

Anterior/posterior patterning

While the sperm entry site determines anterior/posterior polarity in some organisms such as *C. elegans*, this polarity is already specified in fly eggs before fertilization. In *D. melanogaster*, maternally contributed mRNAs are differentially localized within the embryo through anchoring to the cytoskeleton (van Eeden and St Johnston 1999; Lasko 2012). Translation of these mRNAs after fertilization results in protein gradients, with the highest concentration of protein being adjacent to its mRNA pool. For example, *bicoid* mRNA is localized to the anterior, and analysis of *bicoid* mutants revealed that it is critical for head and thorax formation (Berleth *et al.* 1988; Driever and Nusslein-Volhard 1988). In contrast, *nanos* localizes to the posterior and regulates abdominal segment formation (Gavis and Lehmann 1992). These protein gradients are then used to specify expression of a series of zygotic genes involved in segmentation and cell fate determination. There are three groups of segmentation genes (gap genes, pair-rule genes, and segment polarity genes), each of which is sequentially expressed and serves to further specify positioning within the embryo (St Johnston and Nusslein-Volhard 1992). Finally, after establishing identity within each segment, the homeotic selector genes (also called HOX genes) control the specification of tissues or organs in particular segments (Lewis 1978). The HOX genes in flies are all related transcription factors found in two complexes: the Antennapedia complex, whose five genes control the identity of the segments anterior to the midthorax and the Bithorax complex, whose three genes control the identity of the segments posterior to the midthorax. When HOX genes are mutant, a "homeotic" transformation occurs in which segmental identities are misspecified, thus resulting in replacement of one structure with another that is normal in form, but inappropriately positioned. For example, legs may develop in place of antennae in an Antennapedia mutant, or a second pair of wings may replace small dorsal appendages called halteres because of Ultrabithorax abnormalities. This work in *Drosophila* set the stage for our current understanding that HOX genes help determine segmental patterns in a way broadly conserved across widely diverged species; many

human developmental disorders stem from aberrations in these genes.

Dorsal/ventral patterning

Like anterior/posterior patterning, the dorsal/ventral axis in *D. melanogaster* is established through protein gradients, but using a very different mechanism (Morisato and Anderson 1995). Genetic studies revealed that the ventral embryonic region is specified by the transcription factor Dorsal and its cytoplasmic anchor Cactus (thus embryos mutant for *dorsal* show only dorsalized structures). Dorsal mRNA is maternally provided to the oocyte, yet translation of the transcript does not occur until cellularization and occurs uniformly throughout the embryo. Complex signaling interactions then occur between the oocyte and follicle cells of the egg chamber to regulate nuclear localization of Dorsal protein only in cells fated to become the ventral surface (Neuman-Silberberg and Schupbach 1993). These signaling events involve a cascade of proteases that ultimately activate Pelle kinase, which phosphorylates Cactus, and stimulates Cactus destruction. Without its cytoplasmic anchor, Dorsal is now free to move into the nucleus and stimulate expression of genes specifying ventral fate. More information about embryonic patterning and axis determination in *D. melanogaster* can be found at http://www.ibiology.org/ibioseminars/development-stem-cells/eric-wieschaus-part-1.html and http://www.ibiology.org/ibioseminars/development-stem-cells/trudi-schupbach-part-1.html.

Imaginal discs, with eye development as an example:
Following embryogenesis, flies undergo three larval stages prior to pupation. These larvae feed, thereby building energy reserves, and they make (and specify) new cells in preparation for metamorphosis. As in most insects, adult structures in fruit flies arise from imaginal cells that are fated during embryogenesis. These imaginal cells form "disc"-shaped tissues, and each disc will ultimately become a unique structure in the adult animal, such as adult mouthparts, eyes/antennae, legs, wings, and genitalia. Each disc originates from a small cluster of cells in the embryo that invaginate to form a flattened sac of epithelium. Continued cell divisions expand the discs during embryonic and larval stages, until they ultimately develop during metamorphosis into adult structures. Imaginal discs can serve as useful systems to elucidate molecular events, and here we discuss eye development as an example of the process.

The adult *D. melanogaster* compound eye serves as a useful model system to elucidate a number of biological processes not only related to visual systems, but also general signaling pathways like the Ras pathway. The fruit fly eye is a highly ordered array of nearly 800 ommatidia, each functioning as an individual visual receptor. The ommatidium is composed of eight photoreceptor cells, with photoreceptors 1–6 positioned radially and photoreceptors 7 and 8 in the middle (with R7 positioned above R8). The photoreceptor cells are surrounded by two primary pigment cells, themselves surrounded by secondary pigment cells shared by adjacent ommatidia. Cone cells overlay the photoreceptors, and a bundle of eight neurons runs to the preoptic stalk.

Cells of the prospective eye are first fated in the embryo through expression of regulatory transcription factor families like *eyeless*. Initially ~20 cells are fated to become eye cells; however, the disc grows through cell division to 10,000 cells by the third larval stage. Cell proliferation then ceases and differentiation begins, with BMP, Hedgehog, and EGFR signaling controlling differentiation of the photoreceptor cells and formation of the ommatidia. In the pupal stage, the disc tissue undergoes dramatic morphological changes to acquire its final shape, and it develops its light-sensing capabilities. Differentiation events sweep across the eye tissue in a seeming wave, at the crest of which is the so-called "morphogenetic furrow." Photoreceptor cells acquire their rhabdomeres (organelles specialized for phototransduction), and differential expression of rhodopsins allows for some photoreceptors to be involved in motion detection and image construction, while others detect color differences.

The compound eye has served as a useful model system to elucidate a number of biological processes not only related to visual systems, but also general signaling pathways like the Ras pathway. In 1976, mutant flies specifically lacking photoreceptor 7 were reported (Harris *et al.* 1976), and cloning of the gene (named *sevenless*) revealed that it was a receptor tyrosine kinase (RTK) with homology to the EGF receptor. A subsequent enhancer screen using temperature-sensitive alleles of *sevenless* identified other components that cooperate with *sevenless* in this process, including *bride of sevenless* (*boss*), *son of sevenless* (*sos*), and surprisingly the oncogene Ras. Bride of Sevenless turned out to be the ligand for the pathway, and Son of Sevenless a Ras guanine exchange factor (GEF); thus these genetic studies in the fly eye helped elucidate the major players in Ras signaling.

Gametogenesis: The development of egg cells in females
(oogenesis) and sperm cells in males (spermatogenesis) involves not only the reductional chromosomal divisions of meiosis but also dramatic cellular reorganization and shape changes. In both the ovary and testis, germline stem cells at one end of the organ replenish their own populations while generating cells that undergo four mitotic divisions to make a cluster ("cyst") of 16 still-diploid cells, each encompassed in a layer of somatic cells. In the ovary, one of the 16 diploid germ cells becomes the oocyte, eventually undergoing meiosis. The remaining 15 cells, nurse cells, are directly connected to the oocyte through actin-based tubes called ring canals (King 1970; Bastock and St Johnston 2008). These nurse cells are essentially RNA and protein factories, and late in oogenesis their actin cytoskeletons contract to squeeze their cytosolic contents into the oocyte. This "dumping" will provide the fertilized oocyte with the mRNA and proteins necessary to begin embryogenesis. (Consequently, a homozygous mutant zygote for a gene required early can develop up to a point as long as wild-type RNA and protein from the heterozygous mother

lasts; on the other hand, a homozygous mutant mother's embryos will be defective regardless of their own genotypes—this is called a maternal effect mutation.) The nurse cells then undergo apoptosis and are eliminated, while the follicle cells secrete both a vitelline membrane and outer chorion shell to protect the maturing egg. In the testis, all 16 diploid germline cells in a cyst undergo meiosis to form a cyst of 64 haploid round spermatids. These cells undergo dramatic reshaping of the cytoskeleton, mitochondria, membranes (Fabian and Brill 2012) to give rise to mature sperm with a condensed nucleus and a long tail containing a microtubule-based axoneme.

Gametogenesis in flies has served as a useful system to study stem cell specification and maintenance (Matunis *et al.* 2012), cell shape changes, and other events. Spermatocytes are a particularly advantageous system in which to study cytokinesis since the cells are large and easy to image and have a weak spindle assembly checkpoint (Cabernard 2012). Aspects of cytokinesis can therefore be studied in mutant cells with a spindle defect that would otherwise activate a checkpoint that precludes analysis of effects on cytokinesis. Genetic studies in *Drosophila* spermatocytes have revealed important mechanisms regulating cleavage furrow formation, aspects of contractile ring formation and function, as well as the role of membrane trafficking from internal compartment in cytokinesis (Giansanti and Fuller 2012).

Drosophila as a Genetic Model for Studying Neurobiology and Behavior

Genetic analysis of the fruit fly has helped the neuroscience community understand all levels of neural function from the development of a nervous system, neuronal function and plasticity at the molecular level, adult neural networks, and the neurobiology of complex behaviors (Bellen *et al.* 2010). Maybe most surprising to non-Drosophilists is the variety of complex behaviors exhibited by the fly that can be dissected to identify their underlying genetic and biological components. This approach to behavior, defined as neurogenetics, was pioneered in the laboratory of Seymour Benzer in the 1960s and has led to a bounty of discoveries that touch on the control of biological rhythms, sensory biology, learning and memory, sleep, aggressiveness, sexual behavior, and more (Vosshall 2007). Simple but powerful genetic screens underlie many of these findings. The field's initial forays into biological circadian rhythms was through the identification of a series of *period* mutants that had altered daily cycles—too long, too short, or none at all (Konopka and Benzer 1971). The subsequent molecular cloning of the *D. melanogaster period* gene in 1984 led to identification of the transcriptional feedback loop of *period*/*timeless* and a continually growing set of interconnected pathways that play a role in behavioral rhythms (Bargiello *et al.* 1984; Zehring *et al.* 1984). The cloning of the *period* locus also represents the first time the gene responsible for a particular phenotype was identified using the newly developed *P*-element transformation technology (Bargiello *et al.* 1984; Zehring *et al.* 1984). Jonathan Weiner's

book *Time, Love, Memory* covers Benzer's important contributions in detail and in a compelling way (Weiner 1999).

In fact, many of our genetic footholds in the field of neurobiology and behavior are the result of a simple behavioral assay paired with a genetic screen. The findings within the fly system have relevance to understanding of all of these processes through to higher vertebrate systems. Evolutionary conservation of gene functions have shown that much of what we have learned about behaviors such as circadian rhythms and memory is applicable to the more complex versions of these processes in mice and humans (Bellen *et al.* 2010). Furthermore, the conservation of these genetic processes has also provided significant insight into the underlying mechanisms of the human diseases and disorders that result from the alteration of typical neuronal function (Fortini and Bonini 2000; Muqit and Feany 2002). This fundamental connection between fly research and human biology is highlighted in the many examples of similar gene mutations giving rise to similar functional phenotypes. For example, shortened rhythm mutations in a *per* homolog in humans have been identified in individuals with a sleep syndrome (Toh *et al.* 2001).

The fly system has developed in parallel with significant technological advances in our ability to probe and manipulate neuronal systems, making it one of the best systems to move from gene discovery to the study of the development, function, and plasticity of neural networks. While the connectome of all 302 *C. elegans* adult neurons was determined by TEM many years ago (White *et al.* 1986), the efforts to map connectomes of more complicated adult nervous systems is being aided by the genetic and cell biological reagents available in the fly. Thus, the more complex nervous system of the brain has become more tractable with each passing decade. The *Drosophila* brain has ~100,000 neurons, and the recent publication of single-cell resolution maps of the projections of 16,000 of them has pushed this field in new directions (Chiang *et al.* 2011). The map, paired with the ability to manipulate, ablate, and activate/repress individual neurons within a network, will allow researchers to ask increasingly sophisticated questions about the neural control of behavior and the higher cognitive calculations being made within networks (Kazama 2015). One important new technology is optogenetics, which allows the stimulation or repression of individual neurons using light of a specified wavelength (Fenno *et al.* 2011). Some early optogenetic work was done in the fly to address a variety of behavioral circuits including odorant learning and responses (Bellmann *et al.* 2010), proboscis extension (Gordon and Scott 2009), nocioception (Hwang *et al.* 2007), and the escape response (Zimmermann *et al.* 2009). The small size of the *Drosophila* brain has also been exploited to do single-cell recordings within defined networks either through technically challenging electrophysiological recordings or with calcium imaging on intact animals who are often performing a particular behavior task (Kazama 2015; Owald *et al.* 2015). Work over the past decade on the olfactory system in the fly has mapped neural connections associated with response to individual odorants. Electrophysiology and calcium imaging of animals in behavioral assays has indicated

how the fly computes the response to directionally applied odors (Gaudry *et al.* 2013). The single-cell resolution in defined networks of neurons makes *Drosophila* one of the key systems for future dissection of the complex interplay between neural network computation and behavioral output.

Drosophila in Studies of Population and Evolutionary Genetics

The *Drosophila* community has been instrumental in combining evolutionary biology with other disciplines to understand the differences within and between species from molecular to phenotypic levels, yet another example of the tractable complexity of *Drosophila*. Below we highlight how work with *Drosophila* has enabled advances at the intersections of evolutionary biology with genetics and molecular biology, genome biology, and developmental biology.

Evolutionary genetics and molecular evolution

By the mid-20th century, the previously disparate fields of genetics and evolutionary biology (Fisher 1930; Haldane 1932; Dobzhansky 1937) came to be integrated into what is now known as the Modern Synthesis (Huxley 1942). Out of these ideas came much of what we understand about evolution today, including that populations evolve by natural selection acting on genetic variation. Early Drosophilists like Muller and Dobzhansky had an important influence on the Modern Synthesis and the fields of evolutionary and population genetics. The first studies of population variation were at the level of chromosomal inversion polymorphisms (Dobzhansky and Queal 1938) and the frequency of lethal mutations (Dubinin 1946; Greenberg and Crow 1960). Drosophilists also pioneered surveys of molecular genetic variation, first using protein electrophoresis to study allozyme variation (Lewontin and Hubby 1966) and then DNA sequence polymorphism (Kreitman 1983). DNA sequence variation holds information on the frequency, mode, and strength of natural selection acting in the genome. To make inferences from DNA sequence variation data, *Drosophila* population geneticists spearheaded statistical methods to detect the effects of deleterious mutations (Charlesworth *et al.* 1993), infer population demographic history [*e.g.*, Glinka *et al.* 2003; Haddrill *et al.* 2005) and adaptive evolution (*e.g.*, the McDonald–Kreitman test (McDonald and Kreitman 1991), and the Hudson–Kreitman–Aguade test (Hudson *et al.* 1987)]. Several software packages exist to run tests using these and other methods on user-supplied data, such as DNAsp (Rozas *et al.* 2003) and libsequence (Thornton 2003). Studies in a wide variety of species have employed these tests and their derivatives to study adaptive evolution throughout the genome (reviewed in Eyre-Walker 2006).

Population genomics, quantitative genetics, and "evo- devo"

Recent high throughput sequencing technologies (reviewed in van Dijk *et al.* 2014) have made it feasible to sequence and compare entire genomes of many individuals—the basis of a field now known as population genomics. The *Drosophila* community boasts powerful resources for population genomics and quantitative genetics, with genome sequences available for hundreds of *D. melanogaster* individuals from different geographic populations. The *Drosophila* Genetic Reference Panel (DGRP) is a panel of >200 inbred, mostly homozygous lines of *D. melanogaster* from Raleigh, NC, whose genome sequences (Mackay *et al.* 2012; Huang *et al.* 2014) combined with phenotypes assayed from the same lines are an unprecedented resource for genome-wide association studies (GWAS) (Ober *et al.* 2012). To map a trait of interest, a researcher can order the DGRP flies from the Bloomington Stock Center (http://flystocks.bio.indiana.edu/), measure phenotypes in these lines, and access the genome data to perform analyses (http://dgrp2.gnets.ncsu.edu/). GWAS analyses using these lines have identified significant genomic associations with sleep traits (Harbison *et al.* 2013), life span and fecundity (Durham *et al.* 2014), olfactory behavior (Arya *et al.* 2015), abdominal pigmentation (Dembeck *et al.* 2015), starvation resistance, and startle response (Mackay *et al.* 2012; Ober *et al.* 2012). In several cases, the genes identified in these studies have orthologs implicated in similar phenotypes in humans (*e.g.*, Harbison *et al.* 2013).

Complementary to the DGRP is the *Drosophila* Population Genomics Project (DPGP)—a large-scale resequencing project focused on the genomes of *D. melanogaster* from populations in Sub-Saharan Africa and France (Pool *et al.* 2012; Lack *et al.* 2015). The latest version of DPGP (DPGP3; http://www.dpgp.org/dpgp3/DPGP3.html) involves the sequencing of >300 individuals from a single population in Zambia, in what is presumed to be the ancestral range of the species (Pool *et al.* 2012; Lack *et al.* 2015). The *Drosophila* Genome Nexus is a recent compilation of each of these population genomic sequences assembled against a single common reference genome assembly, which facilitates direct comparisons among datasets (Lack *et al.* 2015). In total, the Nexus contains 623 genomes with representation from populations that span much of *D. melanogaster*'s current geographic range. These population data allow for the study of *D. melanogaster* demographic and migratory history (Pool *et al.* 2012), natural selection and the genetic basis of local adaptation (Langley *et al.* 2012), chromosomal inversion polymorphism (Corbett-Detig and Hartl 2012), and copy number variation (Langley *et al.* 2012). For example, *D. melanogaster* has recently evolved resistance to some common pesticides. The molecular basis of resistance is well studied. Single nucleotide changes in *acetyl-cholinesterase* (*Ace*) cause resistance to organophosphates and carbamates (Mutero *et al.* 1994; Menozzi *et al.* 2004), whereas TE insertions and copy number variants of the cytochrome P450 gene *Cyp6g1* causes DDT resistance (Schmidt *et al.* 2010). Studies using the DGRP identified these and other regions of the genome as targets of recent positive selection (Garud *et al.* 2015).

Prior to the early work of Dobzhansky (1937) and Muller (1942), little was known of the genetic basis of species differences.

What is now known as the Dobzhansky–Muller model of speciation is the basis of many contemporary studies of speciation genetics (*e.g.*, Coyne and Orr 1989, 1997). Owing to the abundance of new genetic and molecular tools, the field of speciation genetics is growing (reviewed in Presgraves 2010). Population genomic resources now exist for several species closely related to *D. melanogaster*, including *D. simulans* (Rogers *et al.* 2014), *D. mauritiana* (Nolte *et al.* 2013; Garrigan *et al.* 2015), and *D. yakuba* (Rogers *et al.* 2014) with several additional datasets on the way (http://www.dpgp.org/1K_300genomes.html). Population-level data on transcriptomes (RNA sequence datasets) are also available for studying gene regulatory evolution and *de novo* genes (Zhao *et al.* 2014). Because *Drosophila* species vary widely in many phenotypic aspects (Markow and O'Grady 2007), these resources enable *Drosophila* researchers to study the function and evolution of species genomes in a comparative framework (Figure 3B).

Comparative population genomic resources also empower the field of evolutionary developmental biology (evo-devo), which arose from an effort to understand species morphological differences by studying the evolution of developmental processes. The pathways involved in building organism body plans are remarkably conserved between distantly related species (Carroll 2008). The homeobox—a conserved motif found in genes involved in patterning during development across metazoans, including the HOX genes described above—was first identified in *D. melanogaster* (McGinnis *et al.* 1984; Scott and Weiner 1984). Through the genetic manipulation of *cis*-regulatory elements (regulatory sequences that govern the spatial and temporal patterns of gene expression) (Arnone and Davidson 1997) Drosophilists led the evo-devo studies of traits like pigmentation (Gompel *et al.* 2005; Jeong *et al.* 2008; Williams *et al.* 2008) and trichome patterns (Sucena *et al.* 2003; Frankel *et al.* 2011).

Drosophila Databases and Online Resources

As the fly community expanded in the mid-20th century, the journal *Drosophila Information Service* catalogued the growing list of known mutants. Eventually the "Red Book" collected descriptions of mutant strains and genes associated with phenotypes (Lindsley and Grell 1968; Lindsley and Zimm 1992). Now, in the internet and postgenomic age, many online resources are available to help researchers find strains, molecular reagents, and data on genomes, genes, proteins, and molecular interactions.

FlyBase, http://flybase.org, (St Pierre *et al.* 2014) is the typical starting point when a researcher wants to find information on a particular gene or a genomic region. Its user-friendly interface provides access to genome data and annotations thereof from multiple *Drosophila* species. FlyBase also enables searching for batches of genes based on expression pattern or other criteria. A gene entry includes information on gene structure, genomic neighborhood, protein sequence, homologs, known alleles and phenotypes, and references in the literature; it also provides links to the relevant sections of other online databases. Genome data and annotations available via FlyBase continue to be refined and updated by the Berkeley *Drosophila* Genome Project (BDGP), http://www.fruitfly.org/ (*e.g.*, Hoskins *et al.* 2007). Another useful starting point is FlyMine, http://www.flymine.org/, which integrates genome and protein data from multiple sources to allow for complex queries (Lyne *et al.* 2007). Data from the modENCODE project (described above) can be accessed at http://www.modencode.org/ and through various linked interfaces such as modMine, http://intermine.modencode.org/ (Contrino *et al.* 2012). For beginning researchers, the Interactive Fly, http://www.sdbonline.org/sites/fly/aimain/1aahome.htm, is a good starting point for exploring the roles of genes in developmental processes.

Beyond those broadly applicable sites, published reviews of *Drosophila* resources (Matthews *et al.* 2005; Cook *et al.* 2010; Mohr *et al.* 2014) are good starting points to find specialized resources; however, since these reviews go out of date quickly, FlyBase maintains updated lists of links to databases and sources for stocks, reagents, and services (including both nonprofit and for-profit operations) at http://flybase.org/static_pages/allied-data/external_resources5.html. A few examples of online resources with more narrow foci are described below, listed according to the questions they help address.

How can I find flies with mutations in my gene of interest?

The Gene Disruption Project, http://flypush.imgen.bcm.tmc.edu/pscreen/about.html (Bellen *et al.* 2011), has yielded >12,000 insertion mutants, with the majority of genes represented. This project represents an additional aspect of the many efforts of the BDGP.

Where and when is a gene expressed?

FlyAtlas2, http://flyatlas.gla.ac.uk/flyatlas/index.html, catalogs gene expression at the level of mRNA enrichment across multiple tissues for most genes, based on microarray data (Chintapalli *et al.* 2007; Robinson *et al.* 2013). The BDGP embryo *in situ* database, http://insitu.fruitfly.org/cgi-bin/ex/insitu.pl, documents specific gene expression patterns within embryos (Tomancak *et al.* 2007).

Where in the genome is a certain transcription factor predicted to bind?

The *Drosophila* Transcription Factor Database, http://www.flytf.org/, allows exploration of genome and protein data for transcription factors with DNA sequence specificity (Adryan and Teichmann 2006).

With what genes and proteins does a gene/protein interact?

DroID, the *Drosophila* Interactions Database, http://www.droidb.org/ (Murali *et al.* 2011), includes protein interaction data from the *Drosophila* Protein Interaction Map, https://interfly.med.harvard.edu/ (Guruharsha *et al.* 2011), and other sources in a searchable format.

In the fly brain, what genes are active in what cells, and how do neurons connect?

Virtual FlyBrain, http://www.virtualflybrain.org/site/vfb_site/home.htm (Milyaev *et al.* 2012), integrates images and other data on brain anatomy at the cellular level.

Where can I find data on genetic variants comprising a population?

The *Drosophila* Population Genomics Project (DPGP), http://dpgp.org/, has made available sequence data from multiple genomes within several different populations of flies.

Where can I obtain fly stocks?

Tens of thousands of genetically distinct fly stocks of *D. melanogaster*, from mutants to those with fluorescently tagged genes to those carrying RNAi-inducing transgenes, are available from various sources including the following three large stock centers:

Bloomington *Drosophila* Stock Center (Indiana University), http://flystocks.bio.indiana.edu/.
Kyoto *Drosophila* Genetic Resource Center, https://kyotofly.kit.jp/cgi-bin/stocks/index.cgi.
Vienna *Drosophila* RNAi Center, http://stockcenter.vdrc.at/control/main.

In addition, stocks from related *Drosophila* species can be obtained from the *Drosophila* Species Stock Center, https://stockcenter.ucsd.edu/info/welcome.php.

Where can I obtain cell lines, vectors, reporter constructs, cloned fly cDNAs, and other molecular reagents?

The *Drosophila* Genomics Resource Center (DGRC), https://dgrc.cgb.indiana.edu/Home, collects and distributes cellular and molecular reagents generated by many individuals and organizations, including the BDGP (*e.g.*, Stapleton *et al.* 2002).

Drosophila Milestones and Timeline to the Present

The first scientist to adopt *D. melanogaster* as a research organism was William Castle in 1900, and by a decade later a few others had followed his lead. Thomas Hunt Morgan's group at Columbia University (and later at the California Institute of Technology), including students Alfred Sturtevant, Calvin Bridges, and Hermann Muller, made groundbreaking discoveries that established *D. melanogaster* as a valuable genetic model organism. Morgan ultimately became known as the founder of *Drosophila* genetics—all current *Drosophila* researchers can trace their intellectual pedigree back to Morgan.

Morgan's group provided important evidence for the chromosome theory of heredity through correlating the inheritance of red- and white-eyed variants with the segregation of the X chromosome, especially in rare offspring resulting from sex chromosome nondisjunction (Morgan 1910; Bridges 1916). They were the first to deduce linkage of genes in a linear order on a chromosome (Sturtevant 1913) and to dissect chromosomal rearrangements, starting with deficiencies (Bridges 1917). Morgan was later awarded the 1933 Nobel Prize in Physiology or Medicine. Stories of the work in Morgan's "Fly Room" at Columbia became legendary and are recounted in various books such as *Lords of the Fly* (Kohler 1994), *Fly* (Brookes 2001), and *Mendel's Legacy* (Carlson 2004). The 2014 film *The Fly Room*, produced by Alexis Gambis, is told from the perspective of Calvin Bridges's daughter; the production included construction of a detailed replica of the laboratory space that was temporarily on display in a New York gallery in 2013, and which can still be seen online.

Another Fly Room accomplishment involved polytene chromosomes, which consist of many replicated but not separated DNA molecules adhering in register; these are easily stained and visualized, with each chromosome showing a unique banding pattern. After their discovery in the 1930s in other insects, polytene chromosomes were described in *D. melanogaster* salivary gland cells (Painter 1933) and established by Bridges (still in Morgan's group after their move to CalTech) as a primary tool for chromosome mapping (Bridges 1935). His meticulous drawings of polytene chromosomes are still in use 80 years later.

The Soviet-born geneticist Theodosious Dobzhansky spent time in Morgan's Fly Room and through his studies connected ideas of genetic variability in a population with how populations shift over time to become reproductively isolated new species. His book *Genetics and the Origin of Species* (Dobzhansky 1937) was crucial in the formulation of the Modern Synthesis, which connected genetics with other areas to create a broad understanding of the mechanism of evolution.

After leaving Morgan's group in 1915, Herman Muller discovered through examining offspring of irradiated flies that gene mutations are inducible by X-rays in a dose-dependent way (Muller 1927). This seminal work on the nature and origin of mutations earned Muller a Nobel Prize in 1946 and set the stage for scientists to generate (using radiation, and, later, chemicals) vast collections of mutant strains for dissecting the biology of many processes.

Ed Lewis was a driving force in the *Drosophila* community from the 1940s until his death in 2004; his early work, among many other things, helped develop models for the nature of the gene based on intragenic recombination. His later work using radiation-induced and spontaneous mutants focused on development of the body plan in *Drosophila*, setting the stage for *Drosophila* as an important model organism in developmental biology (Lewis 1978). His work on patterning earned him a share of the 1995 Nobel Prize.

Christiane Nusslein-Volhard and Eric Wieschaus were the other corecipients of the 1995 Nobel Prize for their mutagenesis screens to identify and describe mutations affecting the early patterning steps of embryonic development (Nusslein-Volhard and Wieschaus 1980); this later allowed in-depth molecular characterization of conserved genes that govern the basic body plan of most metazoans.

David Hogness, along with Ed Lewis and colleagues performed the first cloning and genomic analysis of a gene, *Ubx*, within the Bithorax complex (Bender *et al.* 1983), setting the stage for the broad use of molecular biology in *Drosophila* genetics.

Mutational analysis also led to important advancements in behavioral genetics, as Seymour Benzer and colleagues identified the first known genes associated with circadian rhythms and learning and memory by analysis of flies with relevant defects (Konopka and Benzer 1971; Dudai *et al.* 1976; Weiner 1999).

The 1980s saw Allan Spradling and Gerry Rubin's discovery of methods for making transgenic flies revolutionize the experimental capabilities of the fly community (Rubin and Spradling 1982; Spradling and Rubin 1982). For example, researchers could test whether an added wild-type copy of a gene rescued a mutant phenotype, and they could add tagged versions of genes to the genome to trace protein localization and isolate interactors.

Molecular characterization of *D. melanogaster* genes exploded in the 1980s and 1990s, making clear how conserved many developmental pathways were between flies and mammals. For example, Walter Gehring's work on the Pax6 gene as a master regulator of eye development showed that this pathway was shared between flies and humans (reviewed in Gehring 2002).

While gene knock-out technology was more difficult to achieve in flies than in other model systems, Kent Golic's group developed such techniques around the turn of the 21st century (Rong and Golic 2000, 2001). The sequencing of the fly genome in 2000 by a collaboration between the company Celera and the Berkeley *Drosophila* Genome Project was a major milestone (Adams *et al.* 2000) and served as a trial run in preparation for Celera's contributions to the Human Genome Project. The 21st century thus far has seen development of new targeted gene knock-down and knock-out approaches using RNA interference and the CRISPR/Cas9 system, the latter of which is quickly and dramatically expanding in popularity and use (Gratz *et al.* 2013; Bassett and Liu 2014).

The most recent Nobel Prize for work using *D. melanogaster* was awarded to Jules Hoffman in 2011 for his 1996 work on the innate immune response. With a wide range of genetic manipulations available, and with close conservation between a large number of fly and human genes, the fly continues to be used for molecular characterization of basic cell biological and other processes, many of which directly relate to medically relevant human conditions such as neurodegeneration, cancer, and aging.

Conclusion

As we begin the second century of using *Drosophila* as an experimental tool in the fields of genetics, cell biology, developmental biology, neurobiology, and evolutionary biology, the groundwork laid during the first century will enable the tractable complexity of the fly to continue offering valuable insights into basic science as well as applied, translational research toward human health. Students entering a research career will continue to find exciting and groundbreaking opportunities to contribute to scientific knowledge in the many *Drosophila* labs around the world.

Glossary

Balancer chromosomes

Chromosomes engineered with multiple inversions, dominant markers, and recessive lethal mutations that, respectively, prevent viable recombination products from being produced during meiosis, allow easy discernment of which progeny inherit which chromosome, and keep mutations on the homologous chromosome from being selected out of the population.

Cellularization

The stage when nuclei in the early syncytial embryo become surrounded by their own individual plasma membranes.

CRISPR/Cas9

A powerful gene editing system, used in many organisms and contexts, that enables the generation of mutations in specific genes through targeting the Cas9 endonuclease to a desired DNA region with a complementary "guide RNA."

Eclosion

The process at the end of metamorphosis during which a new adult fly emerges from the pupal case.

Enhancer trap

An approach that allows for the identification of promoter/enhancers that express in unique cell types or during specific developmental stages. Typically this approach involves random integration of a reporter gene into the genome, followed by screening for expression of the reporter gene in the desired cell type/temporal pattern.

Enhancer/suppressor screen

A type of genetic screen used to identify additional genes that interact with an already identified gene in a biological process. Additional mutations need only be heterozygous in some cases. The approach starts with an allele of a gene with marginal function such that any additional increase or decrease in function in the pathway results in a change in the phenotypic severity. Heterozygous mutations in the additional genes may be sufficient to have a detectable phenotypic difference in combination with the initial allele. Not requiring homozygosity at loci greatly simplifies the scheme for the screen.

FLP/FRT clonal analysis

Site-directed mitotic recombination approach frequently used to generate patches of cells homozygous for a particular mutant allele in an otherwise heterozygous background. Clonal analysis is especially useful to study the role of a gene in a cell population when animals fully mutant for the gene are lethal, and it involves expression of the flippase recombinase (FLP) to mediate mitotic recombination at the flippase recombination target (FRT) sequences, downstream of which the original cell is heterozygous for a mutation. In a subset of mitotic recombination instances, chromosome segregation to daughter cells results in a homozygous mutant cell that subsequently divides to create a patch (or "clone") of homozygous cells whose phenotype can be assessed.

Forward genetic screen

A screening approach used to identify genes involved in a particular biological process, typically involving random mutagenesis (by chemicals or radiation) and searching among animals in subsequent generations for mutant animals displaying a desired phenotype.

GAL4/UAS system

A modular experimental method that allows for controlled spatial and temporal expression of a gene or RNAi knockdown construct. Transgenic flies expressing the yeast transcription factor GAL4 under the control of a desired promoter/enhancer are selected and these are crossed to flies with a second transgene carrying the upstream activating sequence (UAS), which responds to GAL4, upstream of the gene of interest. Offspring with both transgenes will express the gene of interest in the pattern dictated by the chosen promoter.

Germline clones

Specific type of clonal analysis used to assess mutant phenotypes in the absence of both maternal contribution and zygotic expression. Typically accomplished using a modified FLP/FRT protocol that incorporates the dominant female sterile ovo^D (ensures that only females where recombination occurred in the germline can lay eggs).

Imaginal discs

Tissues within a larva that, during metamorphosis, will undergo dramatic changes to become a particular adult structure like an eye, wing, or leg.

MARCM

Mosaic analysis with a repressible cell marker (MARCM) is a specific type of clonal analysis in which the cells to be analyzed are recognized by expression of a reporter gene. This utilizes the Gal80 repressor, which prevents expression of UAS/Gal4 constructs. The genetics are designed such that the desired recombination also removes the Gal80 repressor, allowing reporter gene expression to be activated only in cells where recombination has occurred.

modENCODE

A large scale collaborative project to define functional elements in the genomes of *Drosophila* and the nematode *C. elegans*.

Muller elements

Conserved chromosome arms where, across species, the gene content of an arm is conserved but may differ in order or orientation. *Drosophila* species have five Muller elements labeled A through F.

Ommatidium

Unit of the *Drosophila* compound eye consisting of eight photoreceptor cells and surrounded by pigment and support cells.

PhiC31 system

An approach to generate transgenic lines in which the genomic integration site is not random, but rather can be selected from a number of available landing sites. Avoids the difficulties of position affect variation associated with random insertions as integration sites can be selected that are known to express well. Transgenes can also be inserted at the same location, allowing direct phenotypic comparison of different transgenes at the same site.

Polytene chromosomes

Chromosomes that form after multiple rounds of endoreplication (DNA replication without mitosis), which results in multiple sister chromatids that remain synapsed. The banding pattern of polytene chromosomes can be used to visualize genomic reorganizations and deletions.

Puparium

Hard, exoskeleton of the last larval stage. Serves to protect the fly as it undergoes metamorphosis inside the pupal case.

Reverse genetics

An experimental approach to determine the function of a gene in which the phenotypes associated with mutation or knock-down of that gene are studied. It is considered "reverse" genetics only because the forward approach was practiced first, and the reverse approach became much more common only after the *D. melanogaster* genome was sequenced, with many uncharacterized genes revealed.

Syncytium

Developmental state in which multiple nuclei share a common cytoplasm as a result of mitosis without cytokinesis, as in the early *Drosophila* embryo.

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