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—Review—
Review Series: Animal Bioresource in Japan

Drosophila Genetic Resource and Stock Center; The National BioResource Project

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Abstract: The fruit fly, *Drosophila melanogaster*, is not categorized as a laboratory animal, but it is recognised as one of the most important model organisms for basic biology, life science, and biomedical research. This tiny fly continues to occupy a core place in genetics and genomic approaches to studies of biology and medicine. The basic principles of genetics, including the variations of phenotypes, mutations, genetic linkage, meiotic chromosome segregation, chromosome aberrations, recombination, and precise mapping of genes by genetic as well as cytological means, were all derived from studies of *Drosophila*. Recombinant DNA technology was developed in the 1970s and *Drosophila* DNA was the first among multicellular organisms to be cloned. It provided a detailed characterization of genes in combination of classical cytogenetic data. *Drosophila* thus became the pioneering model organism for various fields of life science research into multicellular organisms. Here, I briefly describe the history of *Drosophila* research and provide a few examples of the application of the abundant genetic resources of *Drosophila* to basic biology and medical investigations. A Japanese national project, the National BioResource Project (NBRP) for collection, maintainance, and provision of *Drosophila* resources, that is well known and admired by researchers in other countries as an important project, is also briefly described.
Key words: genetic resource, genomics, human diseases model animal, proteomics, transgenesis

Why *Drosophila* Is an Important Model Organism for Genetic Research

The fruit fly, *Drosophila melanogaster*, is a small two-winged insect with characteristic red eyes, whose body size is about 2–3 mm in total length and weighs about 1 mg. They are cultured very easily and do not require any special equipment. A glass or transparent plastic vial (H100 mm × 25 mm in diameter) with about 7.5 ml of fly media, which can be made in the kitchen (ingredients: 1,400 g corn flour, 700 g corn grit, 1,200 g dry yeast, 3,000 g glucose, 216 g powdered agar, and 27.4 l water; after 30 min cooking, 150 ml propionic acid and 150 ml 10% methyl parahydroxybenzoate in 70% ethanol are added), is enough to grow about 100 flies. A larger bottle can grow 300–500 flies and even a population cage can be used to collect hundreds of thousands of eggs within 30 min to one hour, if they need to be

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collected in a short time interval. When fruit flies are reared at 24°C about 10 days are required from egg to imago. In two to three days the adult females mature, mate and start laying eggs. Hatchability from the egg to the first instar larva of the wild-type strain is about 95%, and 10 to 20 eggs a day are laid by a single female for more than 20–30 days, or an even longer period under laboratory conditions. An individual fly can be examined under the microscope while anesthetized. A live adult fly can also be observed with a low vacuum scanning electron microscope and the examined fly can be recovered alive (unpublished). The fruit fly is easy to breed and many individuals (100,000–200,000) can be used for studies on mutations and in screening experiments.

Genomic studies using *Drosophila melanogaster* have been most comprehensively performed and it is recognized as a model reference organism [1]. The genome size is about 1.6×10^8 bp, about one twentieth the size of the human and mouse genomes, but the genetic map is so complete that the numerous mutations found to date have been mapped genetically, cytologically as well as physically on the DNA sequences. The *Drosophila* gene number is estimated as roughly 14,000, approximately half that of humans [11]. The genome project revealed explicitly gene homology across different species, indicating that biological functions are regulated by the same systems encoded in the homologous genes. Although we do not look like fruit flies, some of the proteins and genes which are involved in the development and morphogenesis of legs in *Drosophila* are similar to those in humans that are necessary in bone formation. Since the discovery of four-winged fruit flies and abnormal segmentation mutations by E. Lewis [21–23] and T. Kaufman [20], research has demonstrated that humans and fruit flies are similar at both the cellular and molecular levels. This basic understanding has prompted not only *Drosophila* researchers but also medical researchers to work on fruit flies to advance the understanding of human body formation, behavior, and diseases.

The reason why *Drosophila melanogaster* has become such an important genetic material and contributed so many fundamental findings to genetics in general is largely attributed to the polytene chromosomes in the salivary glands that are known as salivary gland chromosomes. Before molecular studies on the genome spread to a variety of organisms, cytological analyses of chromosome complements were performed [28, 43]. Animals and plants like locusts and lilies which possess large genome sizes were preferred as research materials because they tend to have larger chromosome complements [17]. Up to around 1985, cytological observations were preferably carried out using the organisms having high C-values [18, 19] simply because they are easier to examine under the light microscope. Beautiful meiotic configurations of chromosomes at chiasma formation and segregation provided us with the basic knowledge of genetics. However, *Drosophila* was an exception. The extremely large salivary gland chromosomes (Fig. 1) were first studied in detail by Bridges [8]. He sketched and counted the number of bands on the chromosomes, and allocated genes to the bands. The band numbers Bridges counted were challenged later by electron microscopy but the number was the same [36]. It should be noted here that the banding pattern is consistent among individuals and through the age of larvae. Sturtevant [40] soon demonstrated that the genes are arranged along the chromosomes in order and the gene arrangement was shown to be the same as that revealed by genetic recombination. The salivary gland chromosomes provided us the cytological genome map which is about 1,000 times larger than that of the mitotic chromosomes. Since *Drosophila* genetics began with collecting and mapping mutations onto the salivary chromosome bands, we still refer to the classical *Drosophila* genetic system. Molecular information is now integrated into the genetic system and the entire genome can be read by browsing several excellent *Drosophila* databases.

Drosophila Stocks

After the historical finding of the white-eyed mutant by Morgan [26], genetic studies using *Drosophila* expanded explosively. Many young scholars gathered in the "fly room" of Morgan's laboratory at Princeton University, who did not know fatigue, promoted careful observations with full of curiosity and discovered many mutants, chromosome aberrations, and even aneuploids. The fruits of their labors were published as *The mutants of Drosophila melanogaster* [9], which is the first *Dros-*

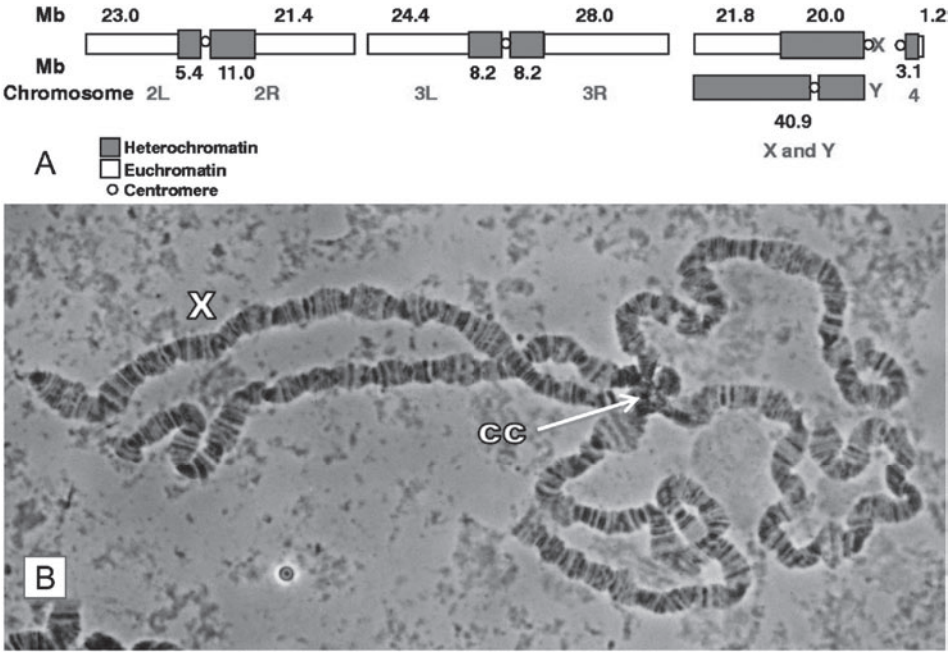

Fig. 1. Chromosome of *Drosophila melanogaster*. A: Mitotic chromosomes showing euchromatin, heterochromatin, and centromeres. Arms of the autosomes are designated 2L, 2R, 3L, 3R, and 4. Euchromatic lengths shown above the arms were estimated from the sequence analysis. The heterochromatic lengths were estimated from direct measurements of mitotic chromosomes of the larval blastoderm cells [50]. B: Polytene chromosomes in a salivary gland cell. All chromosome arms are attached together at the chromocenter (cc) which corresponds to the heterochromatic region of mitotic chromosomes. Only the euchromatic region of all arms are polytenized. The band pattern provides specific landmarks to identify each chromosome and to make cytological maps.

ophila mutation data book and became the basis of the current most comprehensive *Drosophila* research database, FlyBase (http://flybase.org/). *Drosophila* strains established as results of genetic research or collections from the wild populations are given to researchers free when strains are requested. This has long been a tradition of the *Drosophila* research community. The importance of *Drosophila melanogaster* as a research resource is well recognized internationally [24].

Stock Maintenance

There are many advanced *Drosophila* specific techniques to generate mutants: mutagenesis screen, transgenesis, construction of chromosome rearrangements, mosaic analysis, etc. *Drosophila* resources are all maintained alive. At present neither techniques of freezing embryos nor sperm injection have been developed. All fly stocks are taken care of by transferring flies from old culture vials to new culture vials. It is very laborious to look after and maintain large collections of stocks, but since the flies are maintained alive they can be delivered immediately and the recipients can use the flies right away. It is important to describe how *Drosophila* stocks can be maintained on a large scale.

Castle [10] reported the effects of inbreeding on fertility and concluded that *Drosophila* can be maintained by constant inbreeding (brother with sister) with no effects on the variability of the number of teeth on the sex-comb and productiveness of offspring. It is convenient for us to maintain stocks by inbreeding because there is no need to select flies carrying phenotype(s) specific to the stock every generation. Here, I briefly describe the most important chromosome aberration which is absolutely necessary for maintaining mutant stocks by inbreeding.

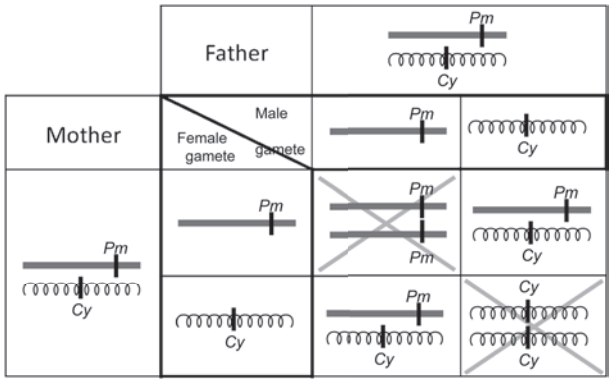

Fig. 2. Balancer chromosome and examples of its utility in crossing experiments. The parental males and females both carry chromosomes marked with the dominant eye color mutation *Plum* (*Pm*) which is associated with a recessive lethal effect and a balancer, in this case bearing the dominant wing mutation *Curly wings* (*Cy*). Zygotes from the cross of *Pm*/*Cy* males and females are expected in four classes as shown here, but the homozygotes for *Pm*, and *Cy* do not survive because of the recessive lethal effects intrinsic to dominant mutations (shown by the cross). The surviving progeny are all *Pm*/*Cy* heterozygotes which have the same genotype as their parents. The stock can be maintained by inbreeding with this chromosome combination. If the chromosome in question carries a deficiency instead, which lacks at least one vital gene, the deficiency can be maintained for generations as illustrated here.

An inversion is known to prevent the recovery of recombinants. Although exchanges occur in the inverted region at meiosis between the homologs heterozygous for an inversion, the recombinants fail to be recovered as gametes [4]. Only gametes bearing the parental nonrecombinant chromosomes are produced. This suppression should be perfect if an inversion has additional inversions. These types of chromosomes are called "balancer chromosomes" or "balancers" and carry at least one dominant visible mutation and a recessive lethal mutation which might be intrinsic to the inversion or the dominant mutation (Fig. 2). The new version of balancers bear transgenic markers of fluorescent fusion proteins. The purpose of balancers is to maintain flies carrying the same constitution of chromosomes for generations, and to make the chromosome of interest homozygous, so that the culture population can be maintained by inbreeding without selection. We call such an inbreeding line a stock. This technique is only applicable to *D. melanogaster*.

Drosophila Stock Centers

Drosophila genetic resources were created as the results of research and maintained as laboratory stocks. The *Drosophila* stock center began with the first collection of mutants established at the California Institute of Technology. This collection was brought from Princeton University by Thomas Hunt Morgan with his students, Calvin Bridges and Alfred Sturtevant, in 1928. The collection remained there with Sturtevant and came under the direction of E. B. Lewis until 1987. The California Institute of Technology did not want to keep the collection without Lewis as a director. The collection then moved to Indiana University under the directorship of Thomas Kaufman. At that time there were 1,500 mutant lines in the collection. Bowling Green State University also maintained a large number of mutant lines, but all stocks were combined into the Bloomington stock center at Indiana University. There was the European *Drosophila* stock center at Umeå University in Sweden, but it was closed in 2000. In Japan, the *Drosophila* Genetic Resource Center (DGRC) at Kyoto Institute of Technology was founded in 1999 as a governmental research facility and the building was completed in 2000. DGRC began with my collection of about 2,400 mutants and chromosomal rearrangements, which was the largest collection in Japan at the time. Soon after the establishment, important lines from the collection at the Umeå stock center were transferred to Kyoto. DGRC is now functioning as an international *Drosophila* stock center in collaboration with the Bloomington stock center. There are several universities and institutions at which a large collection of *Drosophila* stocks are maintained as a laboratory task. Harvard University (Exelixis deficiency lines), and Wien University (RNAi lines) are functioning but Szeged University (*P-element* insertion lines) was closed in 2009.

National BioResource Project

The Japanese government commenced the National BioResource Project (NBRP) in 2002. DGRC was nominated as the core institute for collection, maintenance, and provision of *Drosophila* resources which are live stocks of *D. melanogaster* and other *Drosophila*

Table 1. Subsets of stock collection

NP lines	GAL4 enhancer trap lines
GAL4 / GAL80	Stage- and/or tissue-specific, or non-specific expression of GAL4 / GAL80 blocks GAL4 activity
GS lines	Gene Search System: Forced expression induced by GAL4
LA lines	Mae-UAS.6.11 Insertions: Misexpression induced by GAL4
UAS	UAS responders
Protein Trap	GFP/YFP fusion protein expression lines
GFP, YFP, RFP etc.	Fluorescent protein insertion lines
MARCM	Mosaic Analysis with a Repressible Cell Marker system
FRT-lethal (BruinFly)	Lethal lines with FRT and ey-FLP
FRT	FRT (FLP recombination target) strains
FLP	Flipase producing lines
phiC31 (ϕC31)	phiC31 Integration System Components
P-element mutagenesis	*P-element* mutagenesis starter kits and controls
DrosDel lines	Deletion lines generated by FRT-mediated recombination
Deficiency Kits	Selected deficiency strains
Deficiencies	Genome wide deficiencies
Mapping	Marker strains useful for recombination (classical) mapping
Balancers	Stocks carrying balancer(s)
Duplication Kit 1	Selected duplication strains for the *X* chromosome
T(1;Y) & *T(Y;A)*	Translocations between *X* and *Y*, or between *Y* and autosomes
Wild type stocks	Wild type stocks of *D. melanogaster*
Other species	Species other than *D. melanogaster*

species, and molecular resources like cDNAs, BAC clones, and others. The aim of NBRP is to collect, maintain, and provide the bioresources that are the basic materials of life science research, and to upgrade the bioresources responding to demands as well as adding to their quality through developing preservation technologies, genome analysis, and others. Provision of bioresource information is another important mission.

In our stock collection under NBRP, besides the classical stocks essential for basic manipulation of *Drosophila* stocks, transposon insertions, transgenic lines, RNAi strains, and wild-type strains of other species, are held. At this time we have about 39,000 stocks. DGRC at the Kyoto Institute of Technology is the core institute for the NBRP mission in collaboration with three subinstitutes, the National Institute of Genetics (NIG), Ehime University, and Kyorin University. Approximately 25,000 stocks are maintained at DGRC, 12,000 at NIG, and 1,000 each at Ehime and Kyorin Universities. NIG has a collection of RNAi strains. These RNAi strains are called "inducible RNAi strains" since the RNAi flies produce double stranded RNA *in vivo* at specific tissues or organs at specific developmental stages by using the UAS-GAL4 driver system. Because the *Drosophila* genome project provides DNA sequence information, a couple of RNAi constructs to a specific gene had been designed to make RNAi strains which produce inducible RNA causing loss-of-function phenotypes in the inducible condition. NIG is now providing about 12,000 stocks of this type. Ehime University specializes in wild-type strains of Japanese and Asian *Drosophila* species, and the genomic DNA of the species identified. Kyorin University specializes in mutant lines of *Drosophila ananassae*, *Drosophila hydei*, *Drosophila virilis*, and *Drosophila auraria*. The standard 12 species strains used for genomic sequencing are maintained at both Ehime and Kyorin Universities. At DGRC, a variety of important stocks are maintained as shown in Table 1. At present about 24,000 lines are listed for distribution.

The strains which are absolutely necessary for *Drosophila* genetics are classified as "basic strains". They comprise about 4,000 lines consisting of visible mutants, chromosome deficiencies and duplications, balancers associated with multiple inversions with dominant mutation(s), and drivers. Deficiencies and duplications are useful for mapping genes on the chromosomes by simple breeding experiments, screening for recessive loss-of-function type mutations, and changing gene dosage. At present, the whole *Drosophila* genome is almost

covered by a series of deficiencies, but new deficiencies are constantly being synthesized by techniques recently developed for transgenesis and homologous recombination (see below).

Although it is of great genetic importance and is useful, one of the major problems with *Drosophila* stock maintenance is that it has no inert period in its life cycle. Each line is maintained in three culture vials. Maintaining the stocks thus requires a considerable labor force. The maintenance problem is compounded by the fact that we keep a duplicate set of the whole complete collection in separate incubators in order to avoid unexpected problems with maintenance, and tragic damage or loss due to disaster. This means that right now, in the whole collection, there are at least 150,000 viable cultures in progress. In order to maintain a high quality, 80 lines are randomly selected every week from those unchecked and each line is examined for phenotypes and infection problems. All results are, of course, recorded on our database. The labor intensive maintenance problems as described above have led us to attempt sperm injection to eggs and long term storage of frozen sperm, both of which are currently under investigation.

New mutation and transgenic lines of *Drosophila melanogaster* developed recently are collected from laboratories all over the world. The lines are then subjected to extensive quarantine before being added to the collection. All flies are examined for their phenotypes, any infections by mites, bacteria, or fungi, and recorded on the database. The database (http://kyotofly.kit.jp/stocks/ or http://www.dgrc.jp/flystock/) is open to public search and order of stocks through the web page. Stocks are sent out every week to the users who make requests in the previous weekends.

Contents of Collection at DGRC

The current *Drosophila* stock collection at DGRC is shown in Table 1. All subsets are important, but here I will explain two major techniques, homologous recombination-based genome-wide chromosomal deficiencies, and a variety of transgenesis methods and site-specific transgenesis to overcome the problems associated with *P-element*-mediated integration. I should stress here that transposon-mediated insertion stocks have recently increased very rapidly and modern technologies are accelerating the production of more sophisticated transgenic lines on a genome-wide scale.

1. Deficiency chromosomes

Among the accumulated collection of numerous chromosomal rearrangements, deficiencies have become more important, particularly because transgenetic techniques have progressed and can make any kind of insertional duplication. In *Drosophila*, targeted mutagenesis is not an easy technique, so a chromosomal region or a single gene deletion is useful for studying the effects of introduced genomic DNA, like a BAC clone carrying the corresponding region or gene.

Golic and Golic [13] introduced an innovative method to construct chromosome rearrangements. They used the FLP site specific recombinase of the yeast 2 μ plasmid. When flies bearing FLP recombination targets (*FRTs*), which are FRT lines, are crossed to a fly carrying *hsFLP* (heat inducible FLP gene), which are FLP lines, FLP synthesis is induced and promotes recombination between *FRTs*. *FRTs* are short asymmetric sequences that FLP recognizes and recombines with directionally (Fig. 3). The recombination frequency is very high, nearly 100% when *FRTs* lie a few kilobases apart on the same chromosome. *FRTs* on homologous chromosomes are also efficiently recombined. Ryder *et al*. [33–35] made a pair of *P-elements*, p{RS3} and p{RS5}, collected 3,243 lines and mapped them to the *D. melanogaster* genome sequence. By using these p{RS} insertion lines, they produced 665 lines by design to obtain large deficiencies covering >77% of the euchromatic genome sequence. Each deletion uncovers an average 368 kb. Above all they made available a core collection of 209 validated and healthy stocks which cover more than 60% of the genome. In addition to the Ryder's DrosDel collection, Parks *et al*. [30] generated deletions by *FRTs* recombination in flies with the *piggyBac* elements made Exelixis group. The average deletion size of Exelixis deletion is about 140 kb and coverage is about 56% of the genome.

2. Integration of transgenes

In *D. melanogaster* the transposon-mediated integration of transgenes has been of great utility in numerous

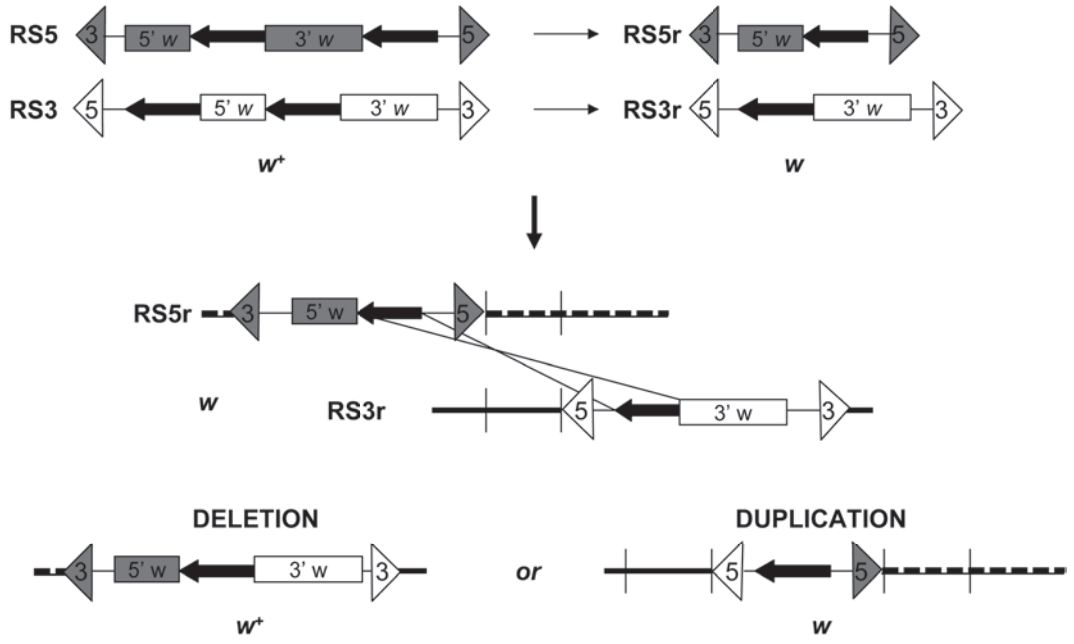

Fig. 3. Creating genomic deletions and duplications with RS elements. The P{*RS3*} and P{*RS5*} elements contain a functional *white* gene (shown split into two parts) and two FRT sites (shown as thick arrows in the *P-element*). The 5' and 3' ends of the *P-element* are marked with triangles. The elements differ in the position of the second FRT sites in the element and the orientation of the construct within the *P-element* ends. Internal recombination between the FRT sites mediated by FLP recombinase produces the remnant form of the elements, P{*RS3r*} and P{*RS5r*}; each now has a non-functional half of the *white* gene and a single FRT site. If a P{*RS3r*} and a P{*RS5r*} are arranged *in trans* at different locations on homologous chromosomes and they are in the orientation shown, a FLP-mediated recombination between them produces a reconstituted *P-element* with a functional *white* gene. The intervening genomic DNA is deleted. The reciprocal recombinants create a tandem duplication of the deleted segment, but the eye color is white and there is no way to identify whether it carries the duplication by phenotype.

experiments. Our collection listed in Table 1 shows it so clearly that subsets of mapping, balancers, deficiency kits, duplication kits, and *T(X;Y)* & *T(A;Y)*s are not in this category, but the others are all produced by transgene integration into the genome and further mobilization. They can be broadly divided into two major groups, gene disruptions and transgenic insertions. Many transgene technologies utilize *P-elements*. The Gene Disruption Project was established to make *P-element* insertion mutagenesis [39]. The *P-element*-mediated enhancer trapping [29, 44], the GAL4-UAS system developed by Brand and Perrimon [7], the FLP/*FRT* system for creating mutant clones by inducing mitotic recombination [48] and generating chromosomal rearrangements for manipulating the genome (as described above), gene trapping using fluorescent fusion proteins [27], gene targeting [32], and the genome-wide library of RNAi transgenic insertions (as noted above), are all transgenesis technologies. The *P-element* tends to be inserted into the 5' regulatory region of genes, causing major problems through disruption of other genes and position effect variegation. To overcome the problems, Groth *et al*. [14] invented site-specific integration of transgenes using bacteriophage ΦC31 integrase. This method allows large DNA fragments as BAC clones to be integrated into the fly genome [5, 42].

How to Obtain *Drosophila* Strains

There are two major *Drosophila* stock centers in the world.

1. The Drosophila Genetic Resource Center (DGRC) at the Kyoto Institute of Technology:

DGRC has the largest collection of *Drosophila* stocks and also has three sub-centers as described above. The entry sites to DGRC and its sub-centers of NBRP are listed below:

a. http://www.DGRC.kit.ac.jp/
   DGRC home page
b. http://kyotofly.kit.jp/stocks/
   Stock search and order
c. http://www.dgrc.jp/flystock/
   Integrated page for NBRP "*Drosophila*"
   DGRC stock finder & Genome viewer
d. http://www.shigen.nig.ac.jp/fly/nigfly/
   RNAi at The National Institute of Genetics
e. http://www.dgrc.jp/ehime/
   Wild-type strains of *Drosophila* species
f. http://www.dgrc.jp/kyorin/
   Mutant strains of *Drosophila* species

2. The Bloomington Drosophila Stock Center at Indiana University:

This is the stock center that many Drosophila researchers have relied on to search stocks and make orders. The web site is as below:

a. http://flystocks.bio.indiana.edu/bloomhome.htm

3. Other stock centers:

a. The Vienna *Drosophila* RNAi Center
   http://stockcenter.vdrc.at/control/main
b. The Exelixis Collection at the Harvard Medical School
   https://drosophila.med.harvard.edu/
   Exelixis deficiencies
c. BDGP Gene Disruption Project of the Bellen/Hoskins/Spradling Laboratories
   http://flypush.imgen.bcm.tmc.edu/pscreen/
d. The *Drosophila* Species Stock Center at University of California, San Diego
   https://stockcenter.ucsd.edu/info/welcome.php
e. The Australian *Drosophila* Biomedical Research Support Facility
   http://www.ozdros.com/

All these sites allow open access and search for stocks through web databases. Guides are presented on each page and e-mail contact addresses are provided for queries.

Human Disease Model Organism

The genomic sequence of *Drosophila* has estimated that as many as 75% of the genes associated with genetic diseases in humans have sequence homologs in flies. As the number of such human genes is steadily increasing, *Drosophila* can therefore advance human disease research in various categories as summarized in Table 2. In October 2006, the conference entitled "*Drosophila* as a Model for Human Diseases" was organized in Barcelona and a group of scientists gathered to discuss the ways in which *Drosophila melanogaster* could contribute to the field of human diseases [6]. At the meeting, diseases such as cancer, neurodegenerative disorders, muscular dystrophy, cardiac failure, diabetes, and drug addiction were discussed together with specific topics of basic biology that are directly relevant to the diseases, including signaling pathways, genomic regulatory networks, stem cell proliferation, planar cell polarity, aging, oxidative stress, and innate immunity. The Genetics Society of America (GSA) also organized a 2006 annual meeting entitled "Genetic Analysis: Model Organisms to Human Biology". In 2010, the GSA is setting up a meeting covering substantial contributions and further potential of research. In 2008, at the 49th Annual *Drosophila* Research Conference in San Diego, a number of laboratories presented exciting findings on *Drosophila* models of human diseases [31]. Research on neurodegenerative disorders, cancer, diabetes, cardiac disease, etc. are always popular, but new fields such as obesity, infectious diseases, alcoholism, drug addiction, and prion diseases were also presented. Although the numbers of research articles of this kind are too numerous to cite here, I briefly introduce two examples of research on human diseases using *Drosophila* as a model organism.

The first example is a model system for genetics of cardiomyopathy. The adult fly has an open circulatory system with the cardiac chamber located directly beneath the dorsal cuticle between the thorax and the abdomen as shown in Fig. 4. For examination of the *Drosophila*

Table 2. Number of *Drosophila* homologues to human genetic diseases*

Category	Number of genes	
Immunological		31
Complement mediated	11	
Other	20	
Endocrine		47
Diabetes	9	
Other	38	
Deafness		12
Syndromic	7	
Nonsyndromic	5	
Pleitropic developmental		31
Growth, immune, cancer	7	
Apoptosis	1	
Other	23	
Hematologic		37
Erythrocyte, general	24	
Porphyrias	7	
Platelets	6	
Skeletal development		23
Craniosynostosis	5	
Skeletal dysplasia	13	
Other	5	
Storage		42
Glycogen storage	10	
Lipid storage	20	
Mucopolysaccaridosis	9	
Other	3	
Cardiovascular		25
Cardiomyopathy	10	
Conduction defects	4	
Hypertension	6	
Atherosclerosis	3	
Vascular malformations	2	
Neurological		67
Neuromuscular	18	
Neuropsychiatric	8	
CNS/Developmental	7	
CNS/Ataxia	8	
Mental Retardation	6	
Other	20	

Category	Number of genes	
Metabolic/mitochondrial		103
Dermatologic	30	
Coagulation abnormalities	28	
Connective tissue	15	
Gastrointestinal	13	
Renal	12	
Pharmacologic	11	
Peroxisomal	9	
Complex other	6	
Pulmonary	4	
Soft tissue	2	
Malignancies		73
Brain	3	
Breast	4	
Colon	11	
Other GI	3	
Genitourinary	4	
Gynocologic	3	
Endocrine	3	
Dermatologic	2	
Xeroderma	6	
Other/sarcomas	11	
Hematologic	25	
Ophthalmologic		38
Aniridia	1	
Rieger syndrome	1	
Mesenchymal dysgenisis	2	
Iridogoniodysgenisis	2	
Corneal dystrophy	2	
Cataract	3	
Glaucoma	2	
Retinal dystrophy	1	
Choroiderimea	1	
Color vision defects	2	
Cone dystrophy	2	
Cone rod dystrophy	2	
Night blindness	7	
Leber amaurosis	1	
Macular dystrophy	2	
Retinitis pigmentosa	7	
Total		659

*Kaufman (2006) personal communication.

heart, a few imaging techniques have been developed [46]. Optical coherence tomography (OCT) is one of them, and has been successfully applied to imaging of live adult flies. Because OCT is a non-destructive, non-invasive imaging technology, serial measurements on the same fly and direct visualization of the cardiac chamber throughout the cardiac cycle provides direct measurements of end-diastolic and end-systolic dimensions as well as heartbeat rate (Fig. 5). This sophisticated imaging technology enabled the identification of the genes that influence dilated cardiomyopathy and heart failure through large scale screening for mutations in *Drosophila* [2, 45, 46]. Wolf *et al*. [45] reported that a flightless mutation at the *wings up A* (*wupA*) gene, namely the

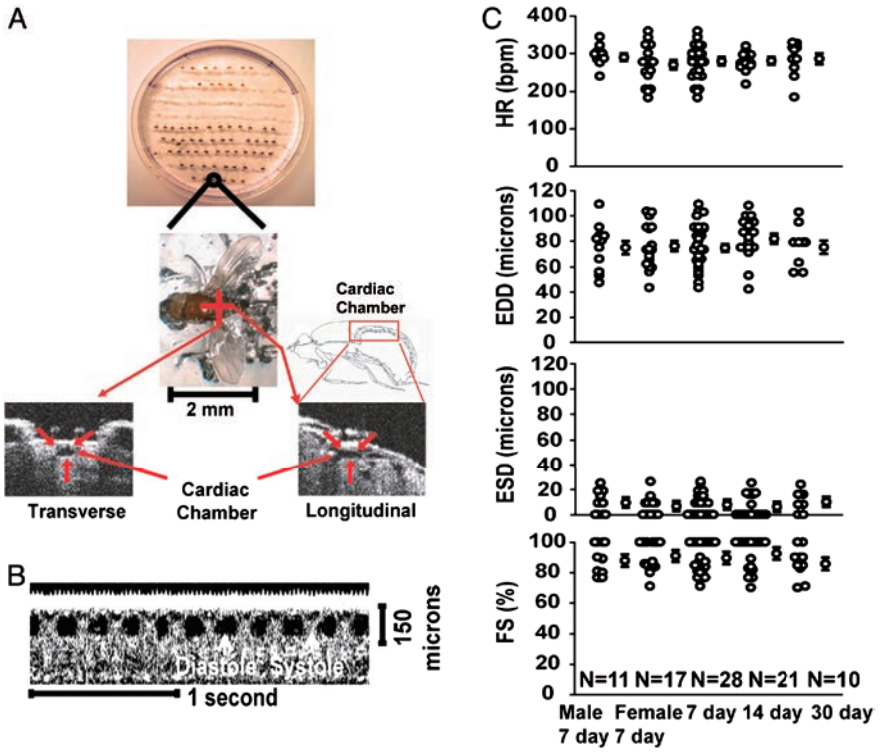

Fig. 4. Optical coherence tomography (OCT) imaging of cardiac function in live, adult *Drosophila* [46]. (A) A plate of immobilized *Drosophila* on transparent gel support and acquired OCT images in longitudinal and transverse orientations. The location of the cardiac chamber is also indicated on the lateral view of the adult fly. (B) Continuous OCT transverse image data of a 7-day-old fly. Depth scale, 150 μm; time scale, 1 s. (C) Cardiac chamber parameters of flies at 7, 14, and 30 days of age maintained at 22°C showing heart rate (HR) in beats per minute (bpm), end-diastolic dimension (EDD) in μm, end-systolic dimension (ESD) in μm, and fractional shortening (FS). All data are expressed as individual data points (left) and mean ± SE (right); no statistically significant differences were found in HR, EDD, ESD, or FS.

Troponin I (TnI) mutation, showed an abnormal cardiac phenotype of dilated cardiomyopathy. Similarly, another flight muscle protein, tropomyosin 2, when mutated, also showed significant impairment of systolic function. Furthermore, Wolf *et al*. examined the effects of a mutation of human δ-Sarcoglycan gene, δsg^{S115A}, that had been speculated to be associated with dilated cardiomyopathy. They proved the association by exhibiting the dilated cardiomyopathy phenotype in transgenic *Drosophila* carrying the human δsg^{S151A} mutant DNA[45].

Another interesting approach to human biology is the study of drug addiction and the decoding of the complex genetic and neuro-pharmacological reinforcement of behaviors induced by drug of abuse. Cocaine's most characteristic properties, motor activity and stereotypic behaviors, are observed in the fly as well as behavioral sensitization, a certain aspect of addiction. Several assay systems have been developed to administer cocaine and quantify its effect. One of them is a video tracking system as illustrated in Fig. 6. McClung and Hirsh [25] first demonstrated that upon exposure to free-base cocaine, volatilized off a heated nichrome filament (Fig. 6A), fruit flies perform a range of unusual behaviors: low doses induce continuous grooming, intermediate doses lead to circling (Fig. 6C) and other abnormal walking behavior, while higher doses cause fast and uncontrolled movements (Fig. 6D), and even akinesia or death. By using several behavioral assays, fly behaviors can be quantified, and a few novel genes regulating cocaine-induced behavior have been identified. They are the *moody* gene coding the G-protein-coupled receptor, the *loco* gene,

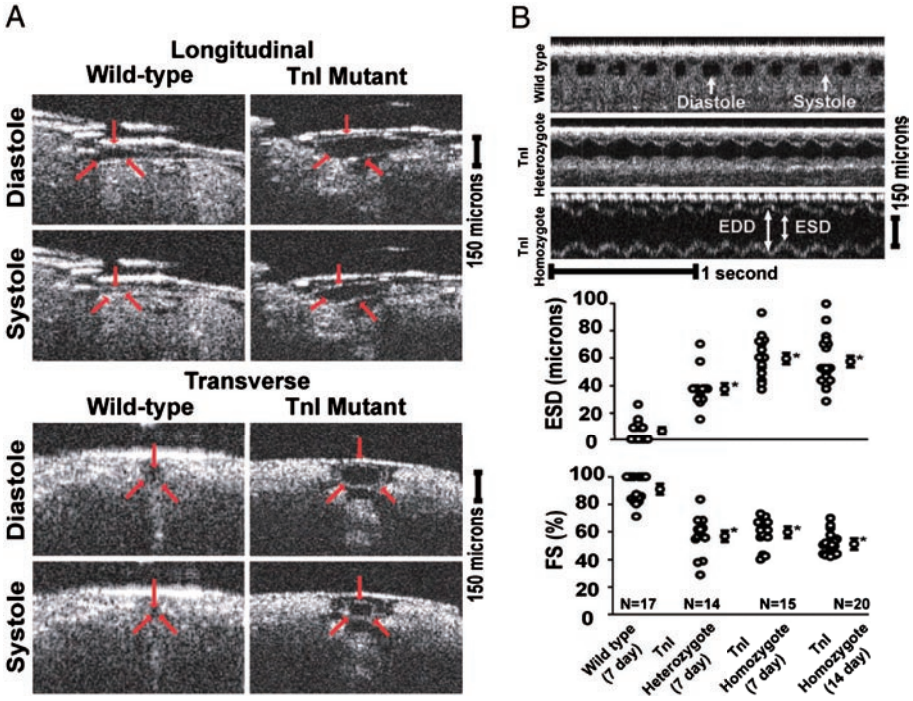

Fig. 5. A point mutation in Troponin I causes dilated cardiomyopathy in adult *Drosophila* [46]. (A) Representative longitudinal and transverse two-dimensional OCT images of the cardiac chamber during diastole and systole in wild-type and homozygotes for the TnI mutation of *Drosophila* at 7 days of age. Arrows outline the edges of the cardiac chamber in both phases of the cardiac cycle. (Scale, 150 μm.) (B) Representative continuous OCT images and summary data for ESD and FS from 7-day-old fly, heterozygote and homozygote for the TnI mutation. Time scale, 1 s; dimension scale, 150 μm. *, $P<0.005$.

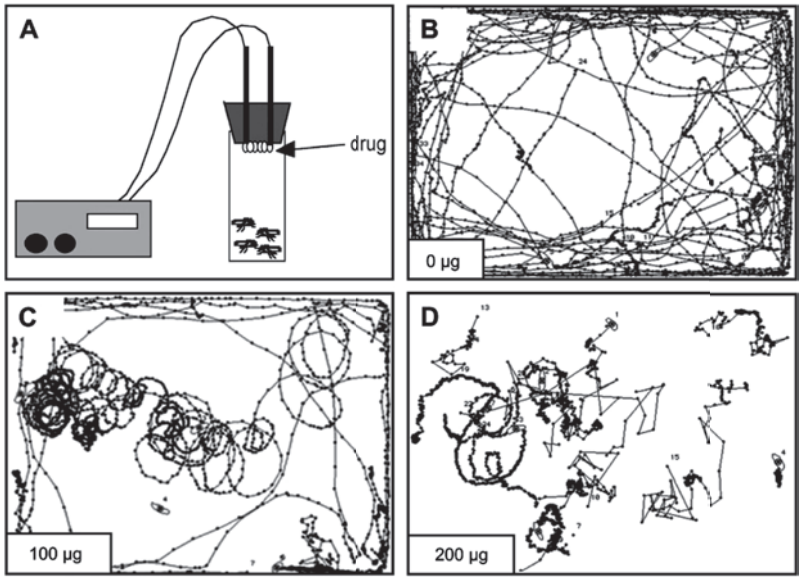

Fig. 6. Cocaine delivery and cocaine-induced locomotor behaviors in *Drosophila* [15]. (A) Illustration of the system used to volatilize free-base cocaine [25]. Cocaine (dissolved in ethanol) is deposited on the nichrome coil and volatilized by heating the coil. Flies "inhale" the volatilized cocaine and are then transferred to an observation chamber in which their behavior is filmed and analyzed using software. (B–D) Computer-generated traces of the locomotor behavior of a group of five flies exposed to volatilized free-base cocaine. Each panel corresponds to a 1 min period starting 2 min after the end of the cocaine exposure. (B) Mock exposure, (C) 100 mg cocaine, and (D) 200 mg cocaine [3, 15].

the fly homolog of RGS4 encoding a regulator/inhibitor of G-protein signaling, and the *white rabbit* gene encoding a Rho-family GTPase activating protein.

Many *Drosophila* homologs of genes of vital importance to humans, mice, and insects should be targeted for extensive analysis, to achieve better understanding of the general genetic mechanisms. I should point out that a significant proportion of the human genome is constitutive heterochromatin in which highly repetitive DNA sequences are enriched, though its function is not yet well understood. The sequence information of heterochromatin is far more advanced in *Drosophila* than in any other eukaryotes. Heterochromatin has been a mystery since its first discovery, but its secrets are now steadily being revealed and heterochromatic genes are being identified [12, 16, 37, 38, 47, 49].

Compared with experiments in vertebrates, large screens are expedited in *Drosophila* at low cost, short generation time, with capacity for experiments using large numbers of animals, sophisticated techniques to manipulate genes and integration of the transgenes, and above all the availability of numerous live resources maintained in stock centers. It is difficult to pick an example to describe how important and how interesting use of *Drosophila* as a research tool is, because there are so many different researches in progress. I would like to stress here that upon completion and updating of the sequencing of the *Drosophila* genome, the next omics studies like proteomics, metabolomics, and peptidomics will be actively pursued. *Drosophila* resources have been maintained in the laboratory for a very long time by inbreeding, and by using balancers and other techniques for producing homozygous populations on any scale. This homozygous constant genetic background is very valuable in proteomic analyses [41, 50]. A high frequency of SNPs is convenient for mapping genes, but it causes serious problems in comprehensive proteomic profiling of specific tissues and organs. *Drosophila* will surely continue to fly on.

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