Excerpt from "Genetic Resources at Risk: Scientific Issues, Technologies, and Funding Policies". 1990. P.E. McGuire and C.O. Qualset, eds. Report No. 5, University of California Genetic Resources Conservation Program, Davis CA. 42

THE DROSOPHILA STOCK AND RESOURCE CENTER: ITS ROLE IN BIOLOGICAL RESEARCH

Thomas C. Kaufman, Kathleen Matthews, and David Cribbs

Abstract. Drosophila *has an extensive history as a tool in genetic research. This long-standing interest has resulted in* Drosophila *being perhaps the best genetically characterized eukaryote. Unfortunately, flies, unlike many other research organisms, cannot yet be maintained by cryogenic storage techniques. This fact has necessitated establishing stock centers for the safe keeping of mutation-bearing strains. The minimal function of these centers is to stock the most valuable germ line variants and to make these freely available. As an aid in this function, we have developed computerized stock lists which allow access to the collection. In addition to this minimal function the stock centers also 1) establish new mutant lines; 2) consult with investigators; and 3) are attentive to new technologies reported in the literature so that these may be incorporated into the collection. In light of this last goal, we have established a collection of P-element transposon stocks. These insertion elements serve as unique DNA markers and allow the ready cloning of adjacent genomic sequences. The* Drosophila *stock centers will continue to perform an essential function for the research community. However, this will only be so if an adequate funding level is maintained.*

Thomas C. Kaufman, *who presented this paper, received his B.A. from California State University at Northridge in 1967 and his Ph.D. from the University of Texas, Austin in 1970. He served as a postdoctoral fellow with support from the National Research Council of Canada at the University of British Columbia, Vancouver in 1971. He joined the faculty of Indiana University as Assistant Professor in 1976 where he is now Professor of Genetics. He has been a Senior Fellow in the Institute for Molecular and Cellular Biology at Indiana University since 1984. His research interests are the genetic, molecular, and developmental characterization of the member loci of the* Antennapedia *gene complex in* Drosophila.

In the past few years, *Drosophila* has gone through a major resurgence and has now become a darling of the molecular biology community. Presently our service as a stock center is to the members of that research community. Since many of the users are new to the field, often the presence of the Center and its workings are accepted as the norm. However, there is a good deal going on behind the scenes. My comments today should shed some light on the way the Center works. I would like to divide my remarks into four basic sections, the history of the Stock Center, its current functions, a prospectus for the near future, and the nature of the funding we currently have.

There is a bit of numerology to keep in mind through all of this. One of the reasons *Drosophila* is prominent as a genetic research tool is that it has a small genome. Current genetic estimates are that there are somewhere between five- and ten-thousand mutable loci in that genome. Put another way, there are 5 to 10 $\times$ 10^3 loci that will mutate and produce a phenotype, either lethality or a visible phenotype such as eye color or wing shape. This number being relatively small, one stands a chance of actually mutationally saturating the genome and understanding the complete genetic repertoire of this higher eukaryote. Please keep that five- to ten-thousand number in mind.

History of the Center

The Stock Center began with the first collection of mutants established at the California Institute of Technology. This collection was brought by Thomas Hunt Morgan when he moved there from Columbia University with his students, Calvin Bridges and Alfred Sturtevant, in 1928. The collection remained at Cal Tech with Sturtevant and came under the direction of Professor E.B. Lewis, remaining there until 1987. The collection had to find a new home because Professor Lewis was approaching retirement and Cal Tech did not want to maintain it without him as director. At a *Drosophila* research conference a few years ago, I agreed to take on the directorship, and moved the stocks to Indiana University. Most of you are more familiar with Indiana University in the context of plant genetics; Marcus Rhodes, Ralph Cleland, and several other well known plant geneticists worked at IU. However, we should remember that H.J. Muller and Fernandus Payne established a tradition of *Drosophila* research there as well. Currently we have six fly labs and all the facilities for maintaining the collection. When Dr. Lewis transferred the fly stocks to IU in May of 1987, there were 1,500 mutant lines in the collection. Since that time we have expanded the number of mutant lines to roughly 3,500. We have also begun a collection of stocks containing P elements. At this time we have about 1,000 stocks of this type. Our long-term goal is to obtain a saturating set of these insertion elements, and I will elaborate on this point later. At present, the total number of mutant lines is about 4,500.

Despite its great genetic utility, one of the major problems with *Drosophila* is that there is no quiescent period in its life cycle. You

cannot freeze them. They do not make the equivalent of seeds. What this means, of course, is you have to keep the stocks reproducing continually. Maintaining the stock collection is therefore very labor intensive. this maintenance problem is compounded by the fact that we keep a duplicate set of the complete collection as a hedge against incubator failure. Each member mutant line is represented by three rotating vial cultures. This means that right now in the mutant collection, not counting the P-element collection, there are minimally 18,000 viable cultures going. Since these need to be changed every 12 to 15 days, we need to have several employees just to maintain the stocks in good condition. Based on current funding levels, the major function of the Stock Center has been simply to maintain this set of mutant lines.

Contents and functions

At this point, I will present some information about content of the stock collection. The largest number of stocks contain point mutations that produce interesting phenotypes. The most familiar are the white eye mutations, curly wings, forked bristles, the kinds of things that bedevil undergraduate students in their first biology lab. We also have some rather esoteric things such as a mutant called *shibire*. These flies are perfectly normal at 22°C, but if you shift them to 29°C, they become paralyzed. Another example are the homoeotic mutations, like *Antennapedia* which causes legs rather than antennae to grow out of the head of the fly. We also have a large number of chromosomal rearrangements, balancer chromosomes, and interesting marker combinations for mapping and stock construction, very much like the Tomato Genetics Stock Center.

In addition to the genetic stocks of *Drosophila melanogaster*, we do keep some germplasm stocks. These exist in the form of wild flies caught from sundry locations around the world. For example, we have Swedish, Swiss, and Japanese fruit flies. However, there is a difficulty with putting too much importance on these. They are all *Drosophila melanogaster* which population geneticists have referred to as a "garbage pail" species. *Drosophila melanogaster* is so closely tied to human activity, it is hard to know whether the variation in these collections is due to the geographical origin of an individual population or whether the collection was brought in on somebody's garbage truck from another site.

With this large and growing collection we have found it necessary to utilize a commercially available database management system called Paradox. This system has greatly aided our ability to keep records and to disseminate information. We also are setting up a bulletin board on BITNET for *Drosophila* workers.

The other thing we do, of course, is send out stocks. In the first one-and-one-half years that the Stock Center has been at IU, we had 956 requests for stocks. Those requests resulted in our sending out 3,627 individual mutant lines to 24 different countries including the US.

We also are in the process of collecting and putting together valuable mutants and further expanding the stock collection. Current

research in *Drosophila* makes it important to increase the number of mutants that have interesting developmental defects. We have put together a set of chromosome deficiencies which will allow people to map readily mutations in the *Drosophila* genome and which saturates essentially 50% of that genome. We have also put together a set of stocks that we have obtained from Dr. William Engels at the University of Wisconsin which facilitates the ability to mobilize transposable elements within the genome of the fly. This mobilizer stock has resulted in the induction and recovery of a large number of new mutations that have very interesting effects on the anatomy and the development of the organism. Moreover, the presence of a transposon in the mutant locus makes the molecular cloning of these loci quite easy.

Finally, we are putting together what I referred to earlier as the P-element collection. These P elements are transposable entities within the *Drosophila* genome very much like the Ac-Ds system in maize. The molecular biology of P elements has proceeded to the point where they can actually be inserted into the genome mechanically by injecting P-element DNA into the developing embryo. Subsequently, one can recover individual insert lines in which there is transposable element DNA flanking a cotransformed genetic marker that allows you to detect the presence of the transposon in the genome. Using the Engel mobilization stocks, these individual insert lines can be jumped around the genome at will and moved to new positions. Our goal at the Stock Center is to assemble a set of P-element stocks such that there is a P-element insert every 10 to 20 kilobases in the genome. The reason for assembling this collection is that P-element DNA in these lines is unique in the genome and allows the molecular biologist access to every position in the genome. What that means is that the potential exists that the entire genome of this higher eukaryote could be cloned and characterized. This perhaps seems to be a rather lofty goal, but I think the important aspect of our function in the community is to put together this collection and have it ready.

A further important function of the Center lies in the construction of new stocks by juxtaposing novel mutant combinations and by combining multiple balancer chromosomes that are useful. One construction in particular we have just finished is the insertion of marker genes in which specific promoter elements drive the structural gene for β-galactosidase into balancer chromosomes. What these allow one to do is to distinguish histochemically different embryonic genotypes before any phenotype is revealed by a mutant lesion. Again, the ability to do this has come out of the recent advances in molecular biology combined with the genetic utility of *Drosophila*.

We also exist as a clearinghouse for information for the *Drosophila* research community. It turns out that a lot of the molecular biologists who are foraying into *Drosophila* for the first time need help with *Drosophila* husbandry and genetics. We can recommend the usefulness of certain stocks and the best culture conditions.

Prospectus

Our prospective for growth is limited by our carrying capacity in terms of the number of people we can accommodate in the laboratory and the number of incubator square feet available. With our current resources, we could handle approximately 5,000 mutant lines. As stated earlier, we now have 3,500. Also mentioned previously is the potential of identifying every locus in the genome of this organism (5,000 to 10,000); yet we do not have the potential to keep mutant alleles for every gene. We could, perhaps, keep one mutant allele of every locus, but we could not keep multiple alleles. However, if we kept point mutations at each locus, we could not maintain any aberration sets, any inversions, any multiple balancers, or any of the other interesting, useful stocks. This, of course, is a problem.

If the P-element Stock Center reaches its ultimate goal, we would like to have somewhere between 20 to 50 insertion points in every numbered segment of the polytene chromosomes. The optimum would be 50. There are 104 of those numbered polytene chromosome segments, so simple arithmetic tells you that this stock collection is going to be somewhere between two- and five-thousand stocks. If we assume the larger of the two numbers, that means that we would need a laboratory that would house 10,000 separate mutant lines. That would require much more of a facility than we presently have.

Drosophila is burgeoning as a research tool. The number of people who call and the number of stocks we send out is enormous. We had no idea when we agreed to take on this Stock Center that the activity level would be like it is. We have had to install a telephone answering machine in the lab, due to the the number of inquiries and the fact that sometimes people in Europe do not pay attention to what time it is in Indiana.

Funding

The National Science Foundation (NSF) pays for the mutant stock collection inherited from Lewis. The funded amount is $100,000 a year. The University takes 47% of that for overhead, so actually we have only $53,000 annually. The Howard Hughes Institute is funding the P-element Stock Center. They have given us a sufficient amount of money to establish the Center, but there was a string attached to it. That is, they said they would buy the equipment, set up the P-element Stock Center, and fund it for two years only. There is no more money for longer maintenance. For long-term support for what I feel is an extremely valuable stock collection for this research organism, we are going to have to look to other sources for funding. We are at present negotiating with the NSF. If nothing comes from them, perhaps the National Institutes of Health (NIH), although I have been told the NIH is not in the habit of funding this sort of facility.

DISCUSSION

Question: ***Do you have any difficulty with USDA in shipping?***

Kaufman: First of all, we do not write "fruit fly" on the packages because that immediately raises attention. We write, "*Drosophila* for research only. No cash value. Open immediately." Our only problem in terms of shipping has been to the Indian subcontinent where apparently overzealous customs officials like to open things. Usually people making a request, for example, from Australia or Canada, will send us special customs forms to use.

Question: ***How many people are involved in this; how much space is required?***

Kaufman: Right now there are two curators, Dr. Kathleen Matthews and Dr. David Cribbs. Cribbs runs the P-element collection. Matthews runs the mutant collection which employs three half-time changers and one full-time media prep person. Cribbs runs the P-element collection which employs two technicians, one full-time and one half-time. The reason we can get away with so few (we do not have bottle washers, for example) is because there are six *Drosophila* research labs at Indiana University. Essentially the Stock Centers are being supplemented by that group of people. We are doing it on a shoestring.

Question: ***What would you consider to be an adequate level of funding in order to do everything you want to do the way it should be done?***

Kaufman: Probably double the current figure; $200,000 per annum. There are two grants extant right now to develop cryogenic methods of keeping fly stocks. I do not know what progress has been made on those. I do not know that such a capability will necessarily be a panacea. The Jackson Lab's freezing facility for mouse embryos works well for some stocks but not so well for others, so cryopreservation is variable in its efficacy.

Question: ***Since* Drosophila *is not self-pollinated, a population produces new genotypes. How do you handle that for clientele that want to have the same thing they had before?***

Kaufman: The lines that we keep are inbred, they are reproduced by sibling matings, so that works well. When we create new stocks, that is a problem. It has become a very big problem now with all of the interest from molecular biologists. The background in which a mutation has been recovered is extremely important because there is tremendous RFLP variability within the background of even this sort of ubiquitous species.

Question: ***You must have to handle orders very personally because you need to know what a person got before, if it is wanted again. You may not be able to supply it because of something critical some place down the line.***

Kaufman: Correct. What happens is we get inbreeding depression. There is no way we can get around that. When we go through those bottlenecks, then we have to outcross to get the vigor back. When we do that we have to warn the recipients. The great thing about our database system is that we can keep not only all the genetic information, but also we have all the records of who got shipped what and when. We can keep very nice records of when outcrosses have taken place.

Question: ***Do you have any backup in other labs at other locations?***

Kaufman: There are two other *Drosophila melanogaster* mutant stock centers. One of them is in Bowling Green, Ohio run by Ron Woodruff, also supported by the NSF. There is a European stock center in Umea, Sweden. Their collection is not as large as the two centers in the States, and they are having a big problem with funding. They are trying to get EMBO money, and I do not know that they are going to be able to get it. There is a species stock center also at Bowling Green which houses wonderful, strange, and esoteric different species of *Drosophila*.

Question: ***How do you decide what to put into a collection?***

Kaufman: Right now we have not had to make any really critical decisions. What Kathy, Dave, and I do is go through the literature and look at the kinds of new things that are coming out. Recently we have just added the Tübingen collection of Nusslein-Volhard and stocks from Eric Weischaus. These are very large collections of important developmental mutations. Whenever we see new break points we try to get them, because break points are invaluable. It is terrible to see these things get lost. We also inherited the collection of Larry Sandler after his untimely death. There were some things in there that no one else had. We keep an eye on what is happening in the literature and the scientific obituary column which is the grimmer of the two.