ments on several smaller, long-lived satellites, each carrying a detector tailored for optimum observations in a portion of the broad x- and gamma-ray energy range. Without doubt the lunar surface will be the ultimate platform for gamma-ray telescopes, but many modest programs in the next few years could make significant gains in knowledge and maintain the interest of younger scientists in solving some of the intriguing mysteries before us. For now, the astrophysics community can rejoice in the continuous flow of the exciting insights about the gamma-ray sky provided by Compton and Granat.

REFERENCES AND NOTES

1. With few exceptions gamma rays are photons with energies above 100 keV [extending to $>10^{15}$ eV (1 PeV)], which are produced by the interactions of high-energy charged particles with matter and fields. Since they have no charge, they are undeflected by electric and magnetic fields and therefore they directly probe high-energy phenomena in the gamma-ray source.
2. C. D. Dermer and R. Schlickeiser, *Science* **257**, 1642 (1992).
3. P. Sreekumar *et al.*, *Astrophys. J.* **400**, L67 (1992).
4. G. Kanbach *et al.*, *Astron. Astrophys. Suppl. Ser.*, in press.
5. *Proceedings of the Conference on Recent Advances in High Energy Astronomy*, Toulouse, *Astronomy and Astrophysics Supplement Series*, (Springer Verlag, Heidelberg, in press).
6. P. Durouchoux and N. Prantzos, Eds., *Gamma-Ray Line Astrophysics, AIP Conference Proceedings No. 232* (American Institute of Physics, New York, 1991).
7. Central Bureau for Astronomical Telegrams, International Astronomical Union, Smithsonian Astrophysical Observatory, Cambridge, MA 02138.
8. W. N. Johnson, Ed., *Proceedings of Gamma Ray Observatory (GRO) Science Workshop* (Goddard Space Flight Center, Greenbelt, MD, 1989); C. R. Schrader, N. Gehrels, B. Dennis, Eds., *Proceedings of Compton Observatory Science Workshop (NASA Conf. Pub. 3137*, NASA, Greenbelt, MD, 1992).

9. The author gratefully acknowledges discussions with C. Fichtel, N. Gehrels, G. Kanbach, and R. Ramaty, and support from M. M. Chupp.

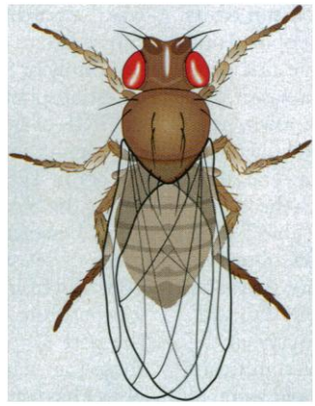
Frosted Flies

Michael Ashburner

Drosophila—the geneticist's fruit fly—are easy to freeze, 'tis the waking 'em up that's the hard part. But no longer. Peter Mazur and colleagues show in a paper in this week's issue of *Science* (p. 1932) that FlyCryo Inc. is now a reality. *Drosophila* embryos can be frozen in liquid nitrogen and then thawed to develop into viable and fertile adult flies. This is the breakthrough that the *Drosophila* community has been waiting for.

Why is the cryogenic preservation of *Drosophila* so important? First, a little history and a moral tale. The genetic analysis of *Drosophila melanogaster* began in Thomas Hunt Morgan's laboratory at Columbia University just over 80 years ago. There are many factors that have contributed to the success of this fly as an experimental organism. Some of them are intrinsic—its short (10-day) life cycle, its relative ease of laboratory culture, its small chromosome number (and its relatively small genome size), its giant chromosomes at the larval stage—to mention but a few. These factors would have been of little importance had Morgan, and his brilliant students, C. B. Bridges, H. J. Muller, and A. H. Sturtevant, not proselytized, actively encouraging other biologists to work with their favorite fruit fly. As the Columbia school discovered more and more mutations and constructed more and more genetically marked strains, they freely gave these to colleagues for both research and teaching. As more mutations and genetically characterized strains accumulated, so the use of *Drosophila* as a research organism grew. There is a limit to the degree of sophistication in genetic analysis of a species with only one or a few mutations and no special chromosomes. However, with several thousand genes marked by mutant alleles and with the construction of thousands of strains with special chromosomes that allow, for example, the trivial maintenance of recessive lethal mutations, our ability to ask increasingly difficult questions of *Drosophila* increases.

The generosity of Morgan's group in sending their precious strains to colleagues began a tradition in research that continues to this day. It is this tradition that has fueled the recent explosion in our knowledge of many fundamental biological problems that has come from *Drosophila* research. There is, however, a heavy price to pay for this. The price is the maintenance of thousands of different stocks of *Drosophila* by the expensive, time-consuming, and precarious process of culture. A reasonably large *Drosophila* research laboratory might keep 2000 different stocks. At any one time only a fraction of these, perhaps 20%, will be in active use. The others will be kept either because they represent an essential living archive of research "completed" or because they may be of use for future work, either by the laboratory itself or by others. Since the 1930s, the *Drosophila* community has been in the habit of publishing its stock lists in its house journal, *Drosophila Information Service*. But even in the 1930s the need for centralized stock centers was realized. First at the California Institute of Technology (Caltech) (to which the Morgan group migrated in the late 1920s), then at Cold Spring Harbor, and then in Bloomington, Indiana (under Herman Muller), *Drosophila* stock centers were established to maintain in perpetuity stocks for researchers. Today there are three such stock centers, two in the United States (supported by the Natural Science Research Council) and one in Europe (supported by the Swedish Natural Science Research Council). The Caltech collection is now in Bloomington and carries well over 5000 stocks; the original Bloomington collection of Muller is now at Bowling Green, Ohio, and is only slightly smaller; the European Stock Center (about 1600 stocks) is in Umeå. Each of these centers publishes stock lists and all of their stocks are freely available to anyone. In 1985, Dan Lindsley estimated that there were at least 15,000 different stocks of *D. melanogaster* being maintained. Since then there has been a dramatic growth, particularly in stocks carrying marked P-elements and transformed DNA sequences. In addition, there is a collection of stocks of other species of *Drosophila*, originally at Austin, Texas, but now at Bowling Green; this carries about 1300 stocks.



For many years *Drosophila* geneticists have been jealous of their colleagues who work with mice or even *Caenorhabditis elegans*, for they can maintain their stocks by the "simple" expedient of freezing in liquid nitrogen. The *C. elegans* stock center in Missouri maintains all of its stocks by slow freezing of larvae. Major centers for mouse stocks, such as that at the Jackson Laboratory in Bar Harbor, Maine, are in the process of freezing eight-cell embryos of their stocks. Once frozen, of course, the stocks require relatively little care. Just as important, the genotypes of these stocks will not change with time, which will inevitably occur if they are kept from generation to generation by live culture. This is an important point. Natural selection does not stop

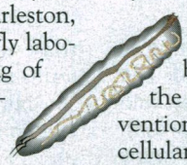
The author is in the Department of Genetics, University of Cambridge, Cambridge CB2 3EH, United Kingdom.

while an organism is grown in the laboratory. A stock labeled *y w v f* in 1927 will have a different genotype today, at least with respect to its genetic background.

Jealousy can be a destructive force; in this case it was not. In 1985, Dan Lindsley was motivated to persuade the National Science Foundation to convene a meeting of fly researchers and cryobiologists in Charleston, South Carolina. Before 1985 many fly laboratories had dabbled in the freezing of *Drosophila*. With the notable exception of larval ovaries, successfully frozen in 1973 by Brüschweiler and Gehring (*1*), nothing had worked. Given that mouse embryos had been successfully frozen in 1972, this was embarrassing. The Charleston workshop was perhaps the first time *Drosophila* biologists had admitted their problems to the professionals in the cryobiology field. As a direct consequence of this meeting two groups of drosophilists and cryobiologists were funded to solve the problem of cryogenically preserving flies and to do so in a way that would serve the purposes of stock maintenance. These groups were those of Peter Mazur at Oak Ridge, with Tony Mahowald (now in Chicago) as the fly expert, and Peter Steponkus at Cornell, with Ross MacIntyre for fly expertise.

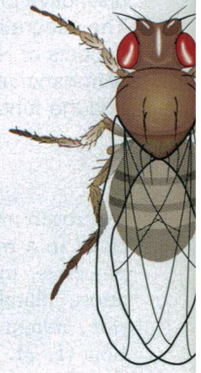
We learned in 1985 that the freezing of *Drosophila* would not be easy, but I am glad we did not know just how difficult it would be. The first problem is that the *Drosophila* embryo is impermeable to water. In conventional freezing, water is withdrawn osmotically from cells and freezes extracellularly. Since 1973 we have known that embryos can be made permeable by dissolving their waxy vitelline membrane in alkanes (such as hexane or octane). The simple method developed by Limbourg and Zalokar (*2*) for doing this is not satisfactory for freezing, because the viability of permeabilized embryos is too poor. The Cornell and Oak Ridge groups both solved this problem, developing techniques to fully permeabilize embryos (*3*, *4*). The Oak Ridge group discovered the critical importance of using very precise alcohol and alkane treatments (*4*). Making embryos permeable, allowing the efflux of water and the influx of a cryoprotectant, was the first success.

The next barrier was the discovery that *Drosophila* embryos are very sensitive to cold (*5*). If cooled at a slow rate, for example at less than 1°C per minute, embryos, especially those at younger stages (up to 6 hours after fertilization), die well before any ice is formed. That result suggested that conventional freezing, used to such good effect with mammalian embryos and *C. elegans* larvae, would fail for *Drosophila*. The alternative strategy was to "outrace" the lethal consequences of chilling by cooling (and later by warming) the embryos very rapidly—at about 100,000°C per minute. Were that to be done with the cryoprotectant at the concentration usually used for conventional freezing (1 to 1.5 M), then intracellular ice formation would simply kill the embryos. Both research groups realized that the only way ahead was to avoid ice crystal formation altogether, to vitrify the water during cooling and effect a direct glass-to-water transition during warming (*6*). Vitrification, rather than conventional freezing, has already been seen as an alternative for the preservation of mammalian embryos by Rall and Fahy (*7*). It requires very high concentrations of cryoprotectant to dehydrate the cells (8.5 M ethylene glycol is used for fly embryos).



At this stage some success in cryopreservation was announced by the Cornell group (*6*)—they found about 18% hatching of frozen embryos, but the frequency of viable adults was disappointingly low (about 0.5% of frozen embryos). Even worse, there were large variations in survival from experiment to experiment. The solution to the problem seems to be twofold. On the one hand, the precise conditions used to permeabilize the embryos are critical. On the other hand, so is the developmental stage of the embryos that are frozen. The former problem can be solved by permeabilizing a "monolayer" of embryos held between polycarbonate filters and by using a simple apparatus that allows flow rates and exposure to solvents to be precisely controlled (*4*). As for the importance of the developmental stage, Mazur and co-workers find that embryos frozen 14.5 hours after egg-laying survive far better (68% hatch to larvae) than those even 45 minutes older or younger. This is a mixed blessing. It means that a population of embryos to be frozen must be staged rather accurately to achieve maximum success (this is not difficult, but it could be tedious). However, 14.5-hour embryos have completed many of the critical steps of embryogenesis. By this time the embryo is running on its zygotic, rather than maternal, genome, most cell division has ceased, and the larval cuticle is just being synthesized. The significance of this is that variation among stocks in their ability to withstand cryopreservation may be low.

The combined efforts of the Oak Ridge and Cornell groups have given us a method to preserve cryogenically wild-type *Drosophila* embryos with a success rate of about 25% (that is, of frozen embryos that develop to fertile adult flies). This stands up well by comparison with the success of cryopreservation of *C. elegans* larvae or eight-cell mouse embryos. There is no reason to think that the process has been optimized, and the method has not yet been tested with mutant stocks. There was always a risk that the cryobiologists would develop a method to preserve fly embryos that was technically elegant but very demanding. The beauty of the new method is that it is technically facile—indeed, it has already been repeated in one other laboratory. Fly laboratories the world over should repeat this work with a wide range of mutant stocks. For this they will need a detailed protocol and a way of rapidly sharing their experiences [for example, through the electronic *Drosophila Information Newsletter* (*8*)].



The vitrification of *Drosophila* embryos will not only have a great impact on the *Drosophila* community, but also on those working with other experimentally important insects, such as mosquitoes. Although it may be some years before *Drosophila* geneticists are confident enough of the method to dispense with conventional stock keeping, the day when we can satisfy the wish of the recent inquirer to the Bloomington stock center, who asked for his stocks to be shipped frozen, may not be far away.

REFERENCES AND NOTES

1. W. Brüschweiler and W. Gehring, *Experientia* **29**, 135 (1973).
2. B. Limbourg and M. Zalokar, *Dev. Biol.* **35**, 382 (1978).
3. D. V. Lynch *et al.*, *Cryobiology* **26**, 445 (1989).
4. P. Mazur *et al.*, *ibid.* **29,** 210 (1992).
5. P. Mazur *et al.*, *ibid.*, p. 39.
6. P. L. Steponkus *et al.*, *Nature* **345**, 170 (1990).
7. W. F. Rall and G. M. Fahy, *ibid.* **313**, 573 (1985).
8. *Drosophila Information Newsletter* is available by sending the electronic mail message SUB DIS-L followed by your name to LISTSERV@IUBVM.UCS.INDIANA.EDU.