Keltner, L. University of Oregon, Eugene, Oregon. Low temperature enhancement of fluorescence as an aid in chromosome banding.

A technique developed by the author for investigation of inorganic crystalline solids is being used to enhance resolution of fluorochrome stained chromosome preparations.

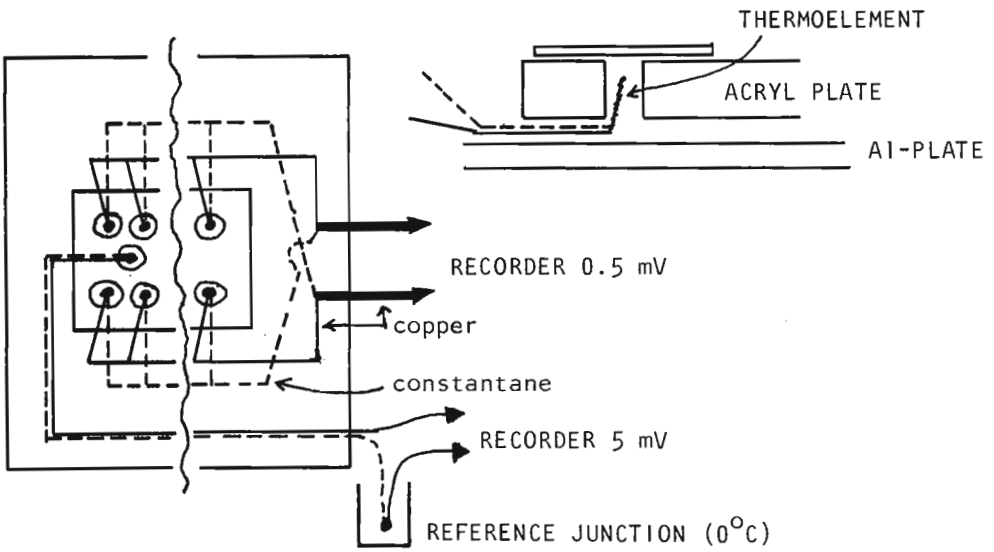
As the relative temperature drops, the probability of non-radiative decay of excited atoms to the ground state decreases, resulting in proportionally greater photon emission. Excitation of fluorogenic substances with radiation in the ultraviolet range thus elicits enhanced fluorescence when the temperature is lowered. Photometric studies of two variations of the acridine molecule, quinacrine mustard dihydrochloride and acridine orange, demonstrate significant increases in fluorescent behavior over the lowering temperature range of 0 to -190°C, with the dyes both in aqueous solutions and in solutions containing calf thymus DNA.

Preliminary results show enhanced resolution of fluorescent banding in chromosomes treated with quite low concentrations of quinacrine mustard dihydrochloride and examined at temperatures in the -100 to -190° region. The stability of the fluorochrome may also be accentuated. Dr. David Wagner of the University of Oregon has also found striking increases in fluorescence at low temperatures of chromatographic preparations of lichen extracts, assisting in identification of difficult materials.

Further studies are underway to determine precise fluorochrome concentrations and optimum temperatures for maximum resolution, and to overcome difficulties encountered with apparatus at the required temperatures.

Lumme, J. University of Oulu, Finland. An efficient instrument to measure freezing points of insects.

A widely used technique to study one aspect of cold resistance (which may play a role in winter resistance) is to determine the freezing point (also called supercooling point) of a biological specimen. Conventionally, the freezing point of one individual is measured by one channel of a recorder in one run. Gradual cooling of an object is followed by a small thermoelement, and the freezing is seen as a sudden increase in the temperature. Here I present a modification which significantly increases the working capacity of a recorder, an expensive essential part of most methods. My purpose is to point out that our knowledge on the winter resistance of Drosophila species is really poor, and it can be improved significantly with easy and cheap methods.



Thermoelements with very small tips are soldered from 2 x 0.1 mm copper-constantane double wire. Ten (or even more) of them are connected in parallel, and two such groups are connected oppositely parallel via a thicker copper cable to a 0.5 mV recording channel. The voltage of this circuit approximates zero in all temperatures, until a fly (or pupa) in contact with one of the thermoelement tips freezes. At this moment, a short peak is seen in a slowly running (0.5 mm/min) recording paper. The peak is either negative or positive depending on the group, which the frozen fly belongs to. The individuals within groups cannot be identified. This circuit does not measure the temperature, but a separate sensor must be built into the device. This will occupy one channel of a recor-

der, if the temperature is not under independent control. Anyway, the freezing points in two experimental groups, both of them containing 10 or more individuals, can be measured simultaneously by one channel of the recorder.

Some technical solutions in the instrument constructed for Drosophila are presented in the figure. I drilled 2 x 10 holes (Ø 3 mm) in a 5 mm acryl plate. The small thermoelement tips were inserted into the holes from the bottom, and this system was mounted tightly on an aluminum plate, which is to equalize temperatures in the holes. A separate thermoelement was inserted into a similar hole to measure the actual temperature of the system. The temperature was recorded by the second channel (5 mV) of Kipp & Zonen BD9 two-channel recorder. The whole measuring unit was placed into an insulating styrofoam box, and regular, constantly cold (-35°C) freezer was used to cool this package. Using this primitive temperature regulator the cooling rate is rather repeatable, and it can be varied by modifying the insulation. This instrument has been used for measuring the freezing points of adults, pupae, and 3rd instar larvae of Drosophila species. The contact of individuals with the thermoelements is usually good enough without any cementing.

References: Asahina, E. 1969, Adv. Insect Physiol. 6:1-49; Crumpacker, D.W., J. Pyati and L. Ehrman 1977, Evol. Biol. 10:437-469; Hudson, J.E. 1978, Can. J. Zool. 56:1697-1709.

Mahowald, A.P. Indiana University, Bloomington, Indiana. Improved method for dissecting late ovarian stages.

A number of procedures have been devised for isolating sufficient quantities of larval and adult tissues for molecular studies (Zweidler and Cohen 1971; Petri, Wyman and Kafatos 1976). Unfortunately, both adult fat body and ovarian follicles undergo the "heat shock" response following isolation and culture (Petri, Wyman and Hessikoff 1977; Spradling and Mahowald 1979). In order to avoid this response, it is necessary to carry out labeling in vivo. We have recently developed a rapid method for accumulating large numbers of individual ovarian follicles from flies which have received injections of radio-labeled precursors. For the most part this has obviated the need for mass isolation techniques.

The flies are lightly anesthesized and submerged individually in buffered ringer's solution. While holding the fly on his back with one forceps, a small hole is made in the cuticle at the level of the 5th to 6th sternite. The tip of the watchmaker's forceps is pushed into the posterior tip of the exposed ovary. The object is to open the ovarian sheath at a level where the individual ovarioles enter a common chamber.

Immediately, follicles are extruded from individual ovarioles, and these emerge through the opening in the abdominal wall. With the aid of gentle teasing of the original hole in the ovary, it is possible to obtain extrusion of all stage 10 to stage 14 egg chambers. As they are extruded from the ovariole sheath, each egg chamber breaks free of the more anterior chamber so that little dissection is needed.

After the ovarian follicles of one ovary have been exhausted, the same procedure can be carried out on the second ovary. After both ovaries have been exhausted of late ovarian stages, the ovaries can now be removed and any final dissection carried out. With practice, most of the flies can be induced to extrude their eggs. In addition to the greater speed, the follicles are undamaged and clean of any adhering debris.

We have little information concerning the mechanism of this release. Since the ovary must remain in situ for success, a reasonable assumption is some neural response is involved. In some insects neurosecretory cells appear to be involved in ovulation and oviposition (Maddrell 1974). We have recently found neurosecretory neuron endings in both the oviduct and the surrounding muscular sheaths. It is possible that these nerve endings are involved in ovulation in Drosophila.

References: Maddrell, S.H.P. 1974, in: Insect Neurobiology (ed. J.E. Treherne), Amer. Elsevier, NY, pp. 307-558; Petri, W.H., A.R. Wyman and S. Hessikoff 1977, DIS 52:80; Petri, W.H., A.R. Wyman and F.C. Kafatos 1976, Develop. Biol. 49:185-199; Spradling, A.C. and A.P. Mahowald 1979, Cell 16:589-598; Zweidler, A. and L.H. Cohen 1971, J. Cell Biol. 51:240-248.