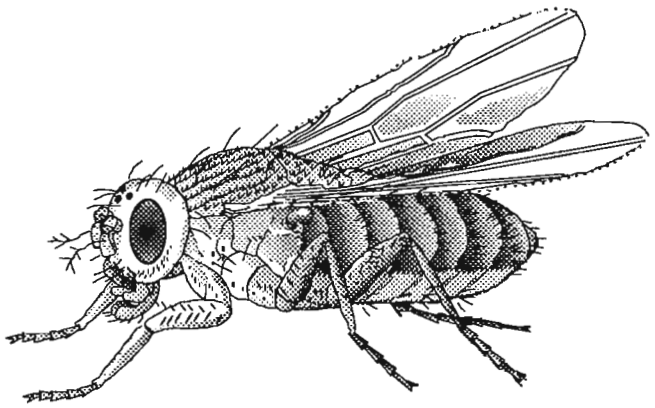
Drosophila Information Service



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Preface

The production of DIS 71 could not have been completed without the generous efforts of many people. Jean Ware, Caroline Tawes, April Sholl, Coral McCallister, Christine LaFon, Stanton Gray, Barrie Ryan and Mingull Jeung in the Department of Zoology have assisted in the preparation and correction of typescripts, maintenance of computer records, shipping, and correspondence. Melva Christian at the University of Oklahoma Printing Services oversaw the publication. The Bowling Green Stock List was prepared by Ron Woodruff, and the Bloomington Stock List and excerpts from DIN were prepared by Kathy Matthews. We hope that you find a lot of useful information here, and we invite you to let us know what can be done to improve DIS as an informal source of communication among *Drosophila* researchers. The publication of Drosophila Information Service is supported in part by a grant from the National Science Foundation to R.C. Woodruff for the Mid-America Drosophila Stock Center, Bowling Green, Ohio.

Drosophila Information Service

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Technique Notes

Hughes, K.D., G. Lagos and M.B. Sokolowski. York University, Ontario, Canada. A new method for testing digging behavior in *Drosophila*.

Previous studies of larval digging behavior in *Drosophila* species have used a technique in which medium containing charcoal has been examined to measure categorically digging behavior (Godoy-Herrera, 1977, 1978; Sokolowski, 1982). Eggs were placed in vials containing medium divided into two layers: a bottom layer with charcoal and a top layer without charcoal. Larvae were scored as "diggers" by counting the number of larvae with charcoal in their digestive tracts at subsequent larval stages of development. This assay has a number of limitations. Firstly, the behavior of individual larvae can not be observed during the test period. Secondly, larval digging done prior to the time of sampling might not be detected if the charcoal has already passed through the digestive tract. Thirdly, there may be interactions between larvae tested in groups.

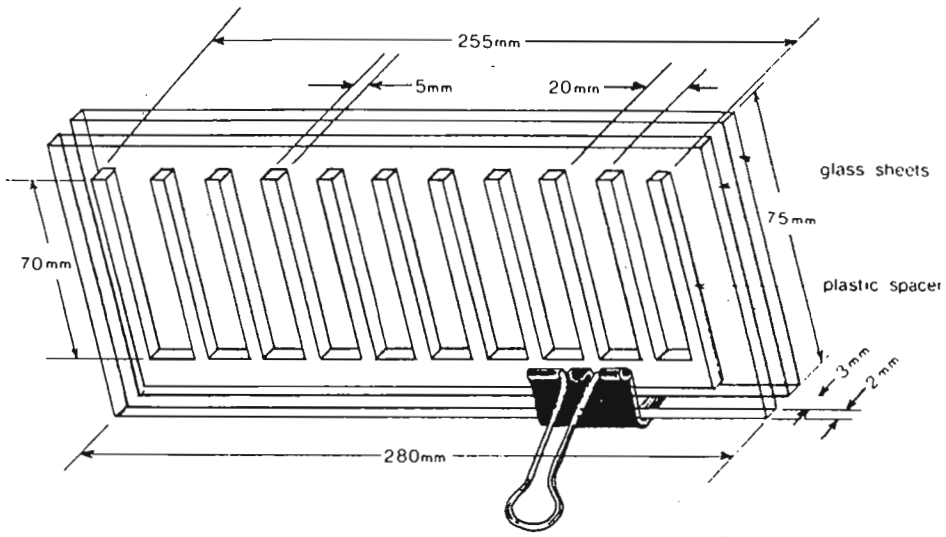
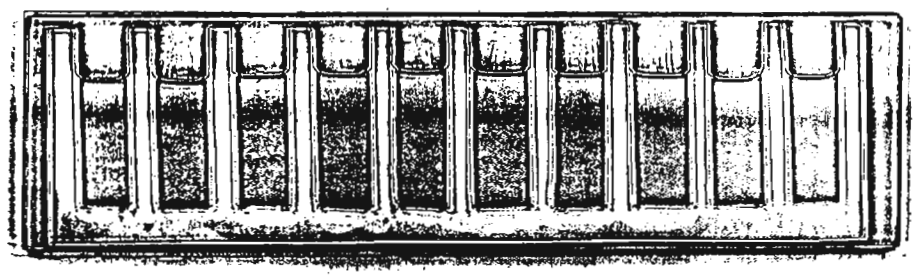


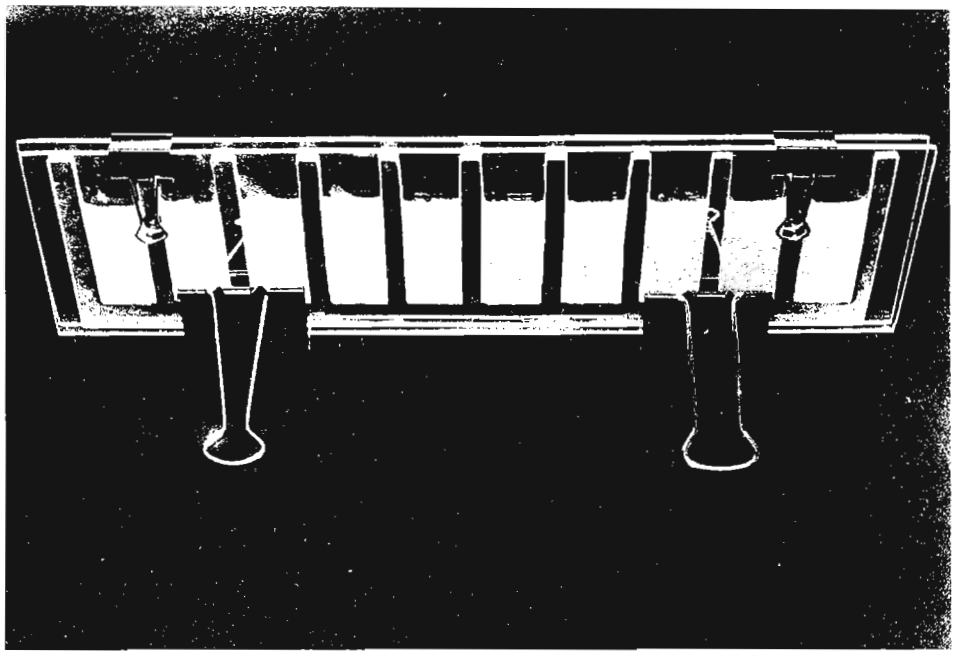
Figure 1. Perspective view of the digging apparatus. Glass (or plastic) sheets are separated by a plastic spacer. Only one out of four clips is shown.

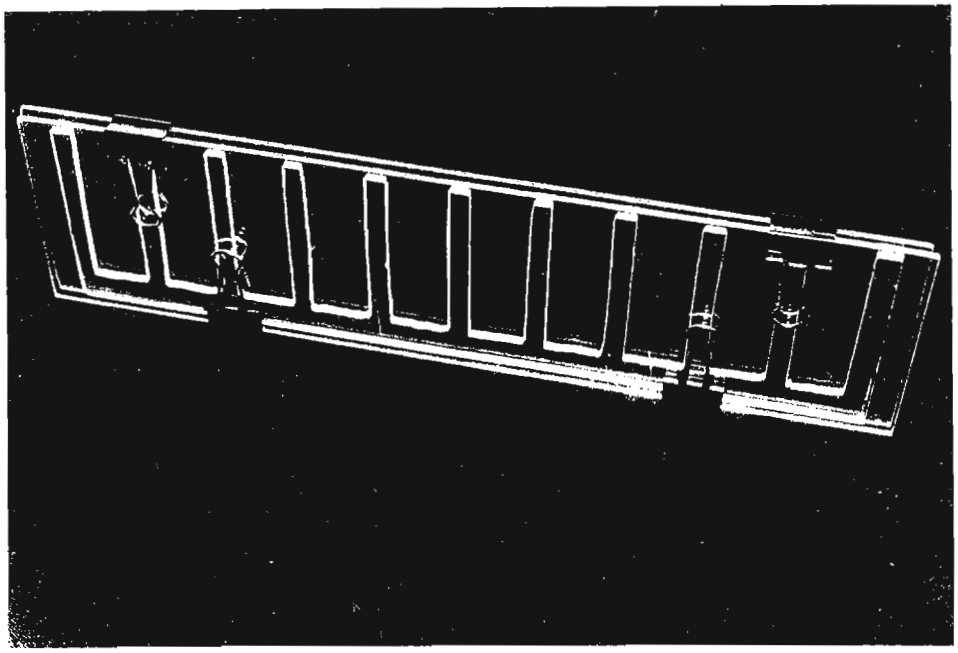
Figure 2 (see next page). Photographs of the digging apparatus with medium added (A) and without medium added (B).

Figure 3. Photocopy of the apparatus without the clips. Notice the surface of the medium shown as a definite line. Clips must be carefully removed prior to photocopying.



We have devised a new assay for digging behavior which enables us to study larval digging while testing large numbers of individual larvae. Based on an "ant-farm design", two sheets of glass are separated by a plastic spacer and held together with large clips which also serve to hold the apparatus upright (Figures 1 and 2). A dead yeast-agar-sucrose medium is poured into the "wells" and given a few minutes to harden after which individual larvae are added (one larva/well). Depending on the thickness of the spacer, all three larval instars can be tested and observed from either side of the glass. Marks can be made on the glass to record the depth that an individual has dug after a number of minutes, hours or even days. A hard-copy of the results can be obtained by photocopying the entire apparatus without the clips (Figure 3). This step is useful for two reasons. First, it allows for a





large number of larvae to be tested consecutively, thereby minimizing differences in ages of larvae. Second, it allows the experimenter to visualize clearly the surface of the medium which ordinarily might be hard to discern due to meniscus formation.

This technique for testing larval digging behavior can also be used for long-term studies. Providing that the medium used can support larval growth and development, larvae can remain in this apparatus until eclosion. Therefore, questions with regard to digging and other types of behavior, such as larval activity and pupation site preference, can be investigated under a number of different conditions. More specifically, these conditions might include use of light, temperature, salt and alcohol gradients to examine larval digging behavior. Overall, our method for testing digging behavior allows the experimenter both to observe individual larval responses and quantitatively measure digging behavior.

References: Godoy-Herrera, R. 1977, Behav. Genet. 7:433-439; Godoy-Herrera, R. 1978, Behav. Genet. 8:475-479; Sokolowski, M.B. 1982, Animal Behav. 30:1252-1253.

Fryxell, K.J., C.P. Wood and J.P. Kumar. Department of Biology, University of California, Riverside CA 92521.
Improved procedures for P element-mediated transformation of *D. melanogaster*.

P element-mediated germline transformation was developed by Spradling and Rubin (1982) and has proven to be one of the most powerful techniques in *Drosophila* molecular genetics. Although some aspects of this technique have been refined (Spradling, 1986), most of the protocol remains unchanged from its original description. We report here modifications that have proven to be useful in our laboratory. These include several strategies for increasing egg yields, and for increasing the survival rate after DNA injection.

In most cases, we have been able to obtain adequate egg yields by maintaining and handling our stocks and egg-laying bottles under constant dim light (an orange night light), and by controlling the age (5-12 days after eclosion at 22°C) and number of flies. Egg yields were linearly proportional to the number of flies within the range from 0 to 1000 flies / 10 cm petri. If the flies were collected from bottles that are not overcrowded and are maintained in split bottles for only one day, then as many as 1000 flies / 10 cm petri could be used without any increase in mortality or egg retention. If the flies were maintained in split bottles for 2-3 days, then 500 flies / petri gave better results. The number of flies was quickly estimated by referring to volume marks on our anesthetizer (200 adult *D. melanogaster* occupy a volume of about 1.0 ml when loosely packed under CO_2 anesthesia).

In cases where egg-laying yields were still inadequate, we found that brief CO_2 anesthesia at the beginning of the egg-laying period significantly increases the number of eggs laid. Pure CO_2 gas was introduced into the split bottle by inserting a plastic pipette past the foam stopper. A gentle flow rate was maintained just long enough to cause the majority of the flies to fall off the sides of the bottle (about 10 sec), and the flies were immediately returned to a 22°C incubator under constant dim light. Our split bottles are not airtight, and the flies recover rapidly. In the first such experiment, CO_2-treated flies laid 100 eggs / 2 hr, while a control bottle laid 40 eggs / 2 hr. This result was replicated with several different batches of flies, all of which gave 2-3 fold stimulation of egg production in the period from 0-2 hr after CO_2 anesthesia. Egg-laying rates were also increased at 2-4 hr, but decreased to below control levels at 4-6 hr and 6-8 hr. In order to rule out the possibility that this method stimulates the release of immature, fragile, or unfertilized eggs, we compared the hatching rates of CO_2-induced and control eggs. After dechorionation, mounting on double-stick tape, and immersion in mineral oil, 6/10 CO_2-induced eggs hatched into first-instar larvae, compared with 4/10 of the control eggs. Thus, CO_2-induced eggs appear to have normal viability.

Most protocols call for injected embryos to be immersed in the smallest possible amount of oil, in order to avoid hindering the supply of oxygen to the embryos (Santamaria 1986). In our experience, however, thicker layers of mineral oil (i.e., a depth of about 10 embryo diameters) give higher hatching rates than thin layers (about 2 embryo diameters), as if limited gas exchange were less important than other potential problems such as dessication, oil leakage, or distortion by surface tension. Thick layers of mineral oil must be confined inside some sort of border, typically produced by extruding Vaseline from a syringe. Vaseline is semisolid and partially soluble in mineral oil, so that Vaseline borders sometimes leak and larvae sometimes crawl through them. These problems can be eliminated by using a mixture of 50% Vaseline and 50% paraffin wax (w/w). The mixture is more solid than Vaseline, less brittle than wax, and melts at a lower temperature than wax. The mixture is prepared by placing the ingredients in a covered pyrex petri dish, melting them on a hot plate set to "low", and mixing with a spatula. The melted mixture is applied to a glass slide with a small paint brush until the border is about 4 mm high.

After optimal dessication and injection, embryos do not appear to be wrinkled (Spradling 1986). Nevertheless, many injected embryos appear to become overdessicated (wrinkled) after 24 hr in a humidified chamber, as if the mineral oil were absorbing a small amount of water from the embryos. In order to test this possibility, we prepared "hydrated mineral oil" by combining equal volumes of dH_2O and conventional commercial mineral oil and repeatedly shaking it vigorously over a period of hours. "Hydrated mineral oil" appears to have distinctly reduced hydrophobicity, based on its surface behavior at paraffin borders, and presumably has adsorbed a significant amount of water. In any case, we obtained a hatching rate of 57% (28/49) in our first DNA injection experiment that used hydrated mineral oil. Our previous results with the same batch of commercial mineral oil (not hydrated) and the same chemical dechorionation procedure (Spradling 1986) had varied from 0 to 40% hatching and averaged about 20%. Our subsequent results with hydrated mineral oil have varied from 40% to 60% hatching and averaged about 50%. Thus, the use of hydrated mineral oil apparently produced a 2-3 fold increase in hatching rates.

If droplets of pure water were accidently pipetted along with the hydrated mineral oil, then dechorionated embryos might, in principle, suffer some osmotic damage. In practice, the embryos are apparently resistant to this kind of osmotic stress. We tried purposely adding various volumes of water to the hydrated mineral oil layer on the coverslips, and none caused any obvious decrease in the hatching rate after DNA injection into embryos. In fact, embryos frequently hatched inside the water droplets. We do not recommend adding water in this way, because large water droplets may come into direct contact with the double-stick tape, causing the tape to gradually become opaque, and making the larvae more difficult to locate.

Acknowledgments: We thank M. Kim, B. Kuo, and J. Chang for their help with this work.

References: Santamaria, P. 1986, in: *Drosophila*: A Practical Approach, IRL Press, Washington DC, pp 159-173; Spradling, A.C. and G.M. Rubin 1982, Science 218:341-347; Spradling, A.C. 1986, in: *Drosophila*: A Practical Approach, IRL Press, Washington DC, pp 175-197.

Spencer, R. Mark. TriKinetics, Inc., 256 Charles Street, PO Box 1055, Waltham, MA 02154 USA. Multi-channel *Drosophila* Activity Monitor.

Osgood, Powell, and Wagner (1991) describe a simple apparatus for counting passes of a single *Drosophila* through an infrared beam. A computerized system for performing a similar function for 32 *Drosophila* simultaneously is shown in the accompanying photograph (Figure 1). 5mm glass tubes containing individual *Drosophila* are snapped into clips built into the plastic case. A separate infrared beam bisects each tube, allowing an onboard microprocessor to concurrently count *Drosophila* passes for each tube individually. To insure that passes through a beam are counted accurately, the microprocessor uses an adaptive detection algorithm which compensates for changes in ambient light as well as emitter/detector sensitivity.

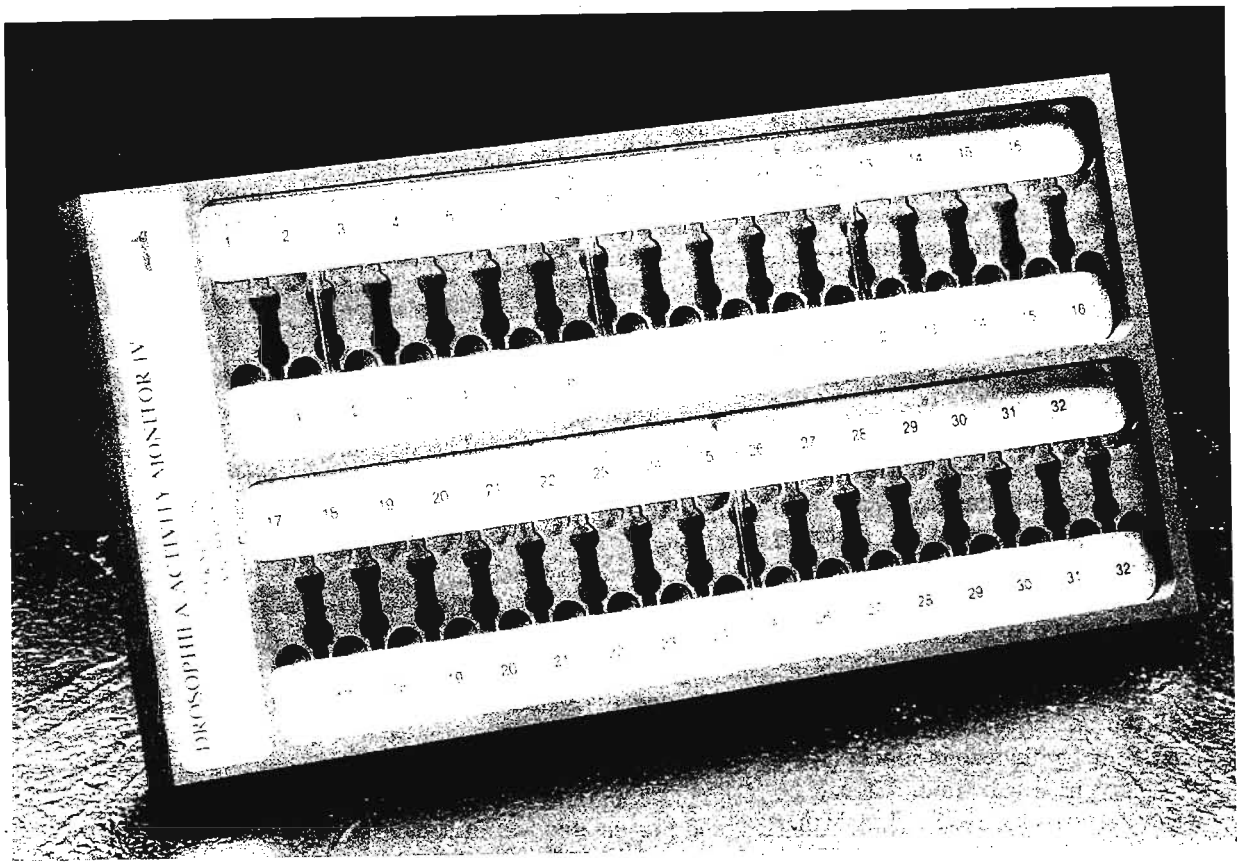


Figure 1. *Drosophila* Activity Monitor IV.

A Macintosh computer is connected to one or more of these monitoring units over a simple multi-drop cable, allowing the count totals for as many as 2048 channels to be periodically uploaded and stored on disk. Use of this system allows the activity data to be automatically logged over an extended period of time, providing for the analysis of circadian rhythms or other long-term patterns of activity.

Reference: Osgood,C., B. Powell and J. Wagner 1991, DIS 70:256-257.

Korochkin, L. Department of Biology, Yale University, New Haven, USA. Simple histochemical method for the acetyl cholinesterase staining in whole *Drosophila* embryos.

The histochemical study of the nervous system in *Drosophila* is used in neurobiology and neurogenetics. These methods are useful for the analysis of genetic control in brain development and to compare morphological and molecular cell maturation. The morphological differentiation of *Drosophila* nerve cells and their specific metabolism have been studied and compared (rev. Hall, 1982). Histochemical staining of acetyl

cholinesterase has been shown to be wide-spread in such investigations (Chase and Kankel, 1988).

Brown and Schubiger (1981) modified the classic histochemical Gerebtzoff technique (1953) for this purpose and have adapted it to *Drosophila* embryos. They demonstrated the ability to detect acetyl cholinesterase activity in a central nervous system (CNS) of a whole *Drosophila* embryo without histological sectioning. It is of great importance because one can analyze the enzyme distribution in the nerve tissue more adequately with this method.

The Brown-Schubiger recipe is generally accepted and recommended in the "Laboratory Manual" by M. Ashburner (1989). To use this method we need to remove not only chorion but the vitelline membrane too. Some inconvenient procedures are used in this technique (saturated, 40% hot Na_2SO_4, and a part of Na_2SO_4 is crystallized during cooling).

It was for these reasons that I tried to simplify this method on the basis of Karnovsky's technique (Karnovsky a. Roots, 1964) which is less complicated than Gerebtzoff's. Some changes were also included in the pretreatment of embryos before staining: 1. Continuous vigorous shaking of the n-heptan-fixative vial containing embryos. 2. The time of postfixation in formaldehyde was reduced. 3. The vitelline membrane was removed by shaking with the use of fine forceps. 4. The use of acetyl-B-methylthiocholin iodide (ICN, Cleveland, Ohio) as a substrate. It can be rather effective (Burstone, 1962). 5. A higher substrate concentration was used. 6. The incubation in the histochemical reagent was protracted.

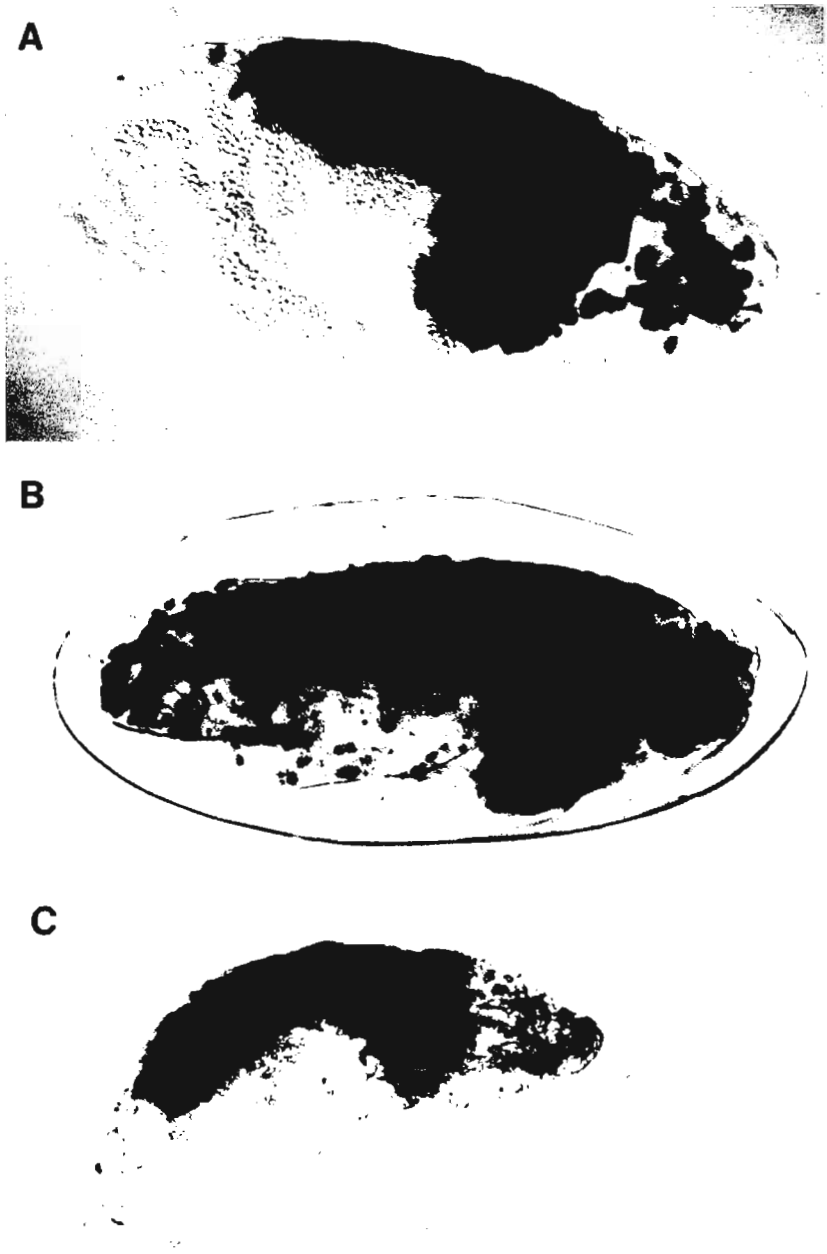


Figure 1. Results of histochemical reaction for acetyl cholinesterase. A, The late *Drosophila* embryo; B, The late *Drosophila* embryo inside vitelline membrane; C, The earliest first instar larva. Magnification 160x.

The following protocol gave consistently reproducible results.

1. Wash embryos, dechorionation with 50% clorox.
2. Wash embryos in distilled water.
3. Transfer embryos in 2 ml screw-capped vial with 1 ml n-heptan and 1 ml 3.7% formaldehyde fixative (3.7% formaldehyde in 133 mM cacodylate buffer with 1mM $CaCl_2$, pH 7.2). Shake vigorously 30-40 min.
4. Fix in 3.7% formaldehyde - 10 min.
5. Line the bottom of a 35 mm Petri dish with double Scotch tape and fill the dish with enough fixative to cover bottom.
6. Transfer embryos from vial to Petri dish. Allow embryos to adhere to tape. Remove vitelline membrane with tungsten needle or by shaking with fine forceps (I prefer last one). Embryos will pop out of their vitelline membrane.

7. Fix for an additional 15-20 min.
8. Rinse in distilled water.
9. Stain in histochemical reagent for acetyl cholinesterase for 5-6 hours (it can be overnight, I had best results in this case). I propose the composition of this reagent:
   acetic buffer (preparation: 0.1 M sodium acetate 39.0 ml, 0.1 N acetic acid 1.0 ml, pH 6.2) - 1.25 ml
   distilled water - 0.950 ml
   3.75% glycine - 0.050 ml
   0.1 M $CuSO_4$ (or 2.49% $CuSO_4 \cdot 5H_2O$) - 0.050 ml
   substrate mixture - 0.200 ml
   Preparation of substrate mixture: dissolve 40 mg of substrate in 0.78 ml of distilled water, add 0.26 ml of 0.1 $CuSO_4$ and centrifuge. Supernatant is used as incubation solution.
10. Rinse briefly in distilled water.
11. Treat with dilute ammonium sulfide (5-10% yellow ammonium sulfide) - 1.0 - 3.0 min.
12. Wash in distilled water and mount in glycerol.

Acetyl cholinesterase appears as a dense brown or black precipitate (Figure 1A).

It was possible to stain CNS of the earliest 1st instar larvae (after the same treatment) and embryos without removing the vitelline membrane in some cases (Figure 1B,C). It is possible to find conditions to stain acetyl cholinesterase in the brain of *Drosophila* larvae and other insects at different ages (after perforation by tungsten needle).

Using this technique I compared the dynamics of the acetyl cholinesterase reaction in Canton S and Oregon R embryos. The results were identical in both cases. I did not find any differences. At first, traces of acetyl cholinesterase staining can be seen at 10-11 hours after egg laying. The staining dramatically increases between 13-15th hours after egg laying. A whole larval CNS was successfully stained after 3.7% formaldehyde fixation by this method.

The method is rather simple and reproducible. It can be recommended for rapid histochemical staining of acetyl cholinesterase in whole *Drosophila* embryos and in earlier larvae at different stages of development.

Acknowledgments: The author expresses profound thanks to Prof. D. Kankel, G. Fitzgerald and Mark Kankel for their help and advice.

References: Ashburner, M. 1989, *Drosophila*. A Laboratory Manual. Cold Spring Harbor Laboratory Press, CSH, 434 p.; Brown, E. and G. Schubiger 1981, Segmentation of the central nervous system in ligated embryos of *Drosophila melanogaster*. Wilhelm Roux's Arch. Dev. Biol. 190:62-64; Chase, B. and D. Kankel 1985, On the role of normal acetylcholine metabolism in the formation and maintenance of the *Drosophila* nervous system, Dev. Biol. 125:361-380; Gerebtzoff, M. 1953, Recherches histochimiques sur les acetylcholine et choline esterase. 1. Introduction et technique. Acta Anat. 19:219-230; Karnovsky, M. and L. Roots 1964, "A direct-coloring" thiocholine method for cholinesterases. J. Histochem. Cytochem. 12:219-221.

Bojic-Stamenkovic, G., M. Milanovic and M. Andjelkovic. University of Belgrade, Yugoslavia. Maintaining *Drosophila busckii* by modifying *Drosophila* standard substrate.

D. busckii is the only known species of subgenus *Dorsilopha* (Throckmorton, 1975). Individuals of this species can most often be found on various decomposing materials which promote bacterial growth, like decaying vegetables (potatoes, cauliflower, squash, mushrooms), rotten fish, chicken coops and, very rarely, fermenting fruit (only bananas and melons; Atkinson and Shorrocks, 1977). It is a characteristic of these environments that they are rich in proteins and starch and higher order alcohols like soluble oils (Daggard, 1981).

There is a relative difficulty of culturing *D. busckii* under laboratory conditions. Namely, the feature of this species is that it cannot complete its development on the standard cornmeal diet which is otherwise successfully used for rearing all known species of *Drosophila*.

Establishing of *D. busckii* on the standard *Drosophila* substrate is possible only with considerably larger quantities of dead brewers yeast (Krivishenco, 1963; Chein *et al.*, 1977; Slavicek and Krider, 1987). However, *D. busckii* is normally reared in laboratories on different potato media (Frankham, 1977; Rockwell and Leving, 1986).

Experimenting with the above mentioned media in our laboratory, we managed to establish the *D. busckii* culture on a standard *Drosophila* substrate which had been prepared without saccharose and living yeast. The ingredients of this modified standard substrate are as follows: corn meal 8%, dead yeast 2%, agar 0.8% and Nipagin 0.1%. *D. busckii* survives on this medium, and the development lasts 2 or 3 weeks in both cases.

It is difficult to give a definite explanation of how saccharose can prevent the development of *D. busckii* on the standard substrate in view of the fact that this disaccharide in itself has no toxic effects on living organisms. This

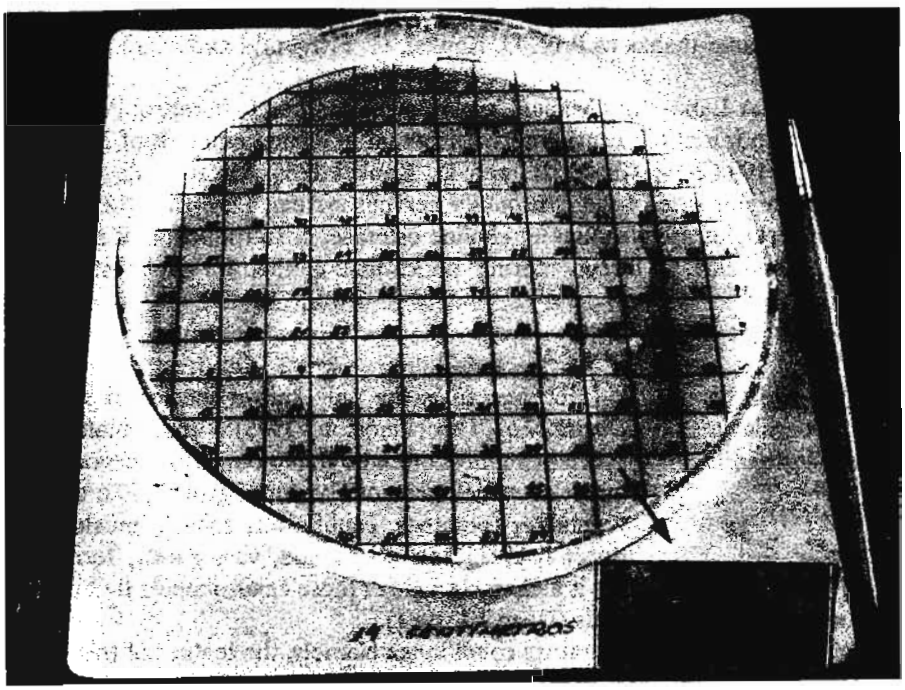
phenomenon could be related to the release of ethanol from saccharose during the yeast fermentation in the standard substrate. Namely, alcohol dehydrogenase (E.C.1.1.1.1. alcohol:NAD+ oxidoreductase), the enzyme that detoxicates alcohol out of *Drosophila* food, has low activity in *D. busckii*. Daggard (1981) found that this enzyme's activity is several times lower in adult *D. busckii* compared with *Drosophila melanogaster* which is successfully reared on the standard substrate.

Since the preparation of modified standard (saccharose - free) substrate was even simpler than the preparation of potato substrates, we recommend this substrate for culturing *D. busckii* under laboratory conditions.

References: Atkinson, W. and B. Shorrocks 1977, Oecologia (Berl.) 29:223-232; Chen, P.S., H.K. Mitchell and M. Neuweg 1977, Insect Biochem. 8:279-286; Daggard, G.E. 1981, In: Genetic Studies of *Drosophila* Population (Gibson, J.B., ed.), Australian National University Press, Canberra, pp: 59-75; Frankham, R. 1973, D.I.S. 50:199; Krivshenko, J.D. 1963, Genetics 48:1239-1258; Rockwell, R.F. and L. Levine 1986, Beh. Gen. 16:543-551; Slavicek, J.M. and H.M. Krider 1987, Genet. Res. Camb. 50: 173-180; Throckmorton, L. 1975, In: Handbook of Genetics, Vol. 3 (King, R.C., ed.), Plenum Press, New York.

Jung, I.B.C. and A.K. Oliveira. Departamento de Genêtica, Instituto de Biociências, UFRGS, Porto Alegre, Brasil. A technique for rapid collection and individual developmental analysis of large pupae numbers.

The analysis of abnormalities in *Drosophila* development during the imaginal disc morphogenetic process is limited by the small number of individuals that can be obtained. Here is described an easy method to accomplish higher individual amounts of these developmental stages. This method is important when we need to treat *Drosophila* populations with hormones and other substances that probably modify the normal development.



During the interval time from zero hour until after eight hours, and by using a wet brush, pupae were collected and transferred to a Petri dish placed above a paper circle with the dish diameter divided in 1 cm x 1 cm squares and numbered. A 14 cm diameter dish can contain up to 450 pupae (Figure 1). To maintain the humidity the Petri dish border is padded with wet absorbent paper, and for gaseous changes the Petri dish is covered with a thin plastic perforated with a histological needle. The development can be individually accomplished until the eclosion of adult flies, when they are transferred to a useful bottle. The results are recorded in special tables using appropriate symbols for abnormalities or normal pupal development.

This method was used in a recent experiment with *Drosophila melanogaster* Oregon-R populations selected for developmental rate and treated with steroids and analogues, being observed a viability of 98% in 3486 control pupae studied.

Lofdahl, K. L. and J. Hirsch. University of Illinois, Urbana-Champaign, Illinois USNA. Shock-avoidance (via learned orientation) of electric current of measured intensity by *Drosophila melanogaster.*

We are developing a new method for the instrumental conditioning of *individual D. melanogaster* that produces rapid learning of a shock-avoidance response in nearly 100% of unselected (wild-type) flies. This learning paradigm permits characterization of single flies for both rate of initial shock-avoidance learning and retention rate (memory decay rate).

A key and clear advantage of the present method over standard and/or popular methods in the literature (e.g., Murphey, 1967, 1973; Spatz, Emanns, and Reichert, 1974; Tully and Quinn, 1985; Wallace and Sperlich, 1988) is that it measures the stimuli as received by the subjects.

Future applications of this method of operant conditioning will include: (1) a test for generality of increased learning ability in lines of flies selectively bred for an increased rate of excitatory classical conditioning (Lofdahl et al., 1992) and (2) a test of the "learning and memory" mutants (such as *dunce* and *amnesiac*, previously isolated in a *group-learning* paradigm, see Tully and Quinn, 1985) for defects in instrumental conditioning. This method can therefore provide an independent test of whether such mutations affect specifically learning or memory.

Methods:

Apparatus: The test apparatus (learning chamber) is a plastic cylinder lined with conducting wires with odd wires connected among themselves (positive) and even wires also connected (negative). These wires are connected to a voltage stimulator that can deliver either dc voltage or square voltage pulses.

There is only one path for ohmic current flow in the chamber, and that is when a fly, placed in the chamber, simultaneously touches two adjacent wires (which have opposite polarities). Then the fly ohmically completes the electrical circuit and the stimulus (electric current shock) is received by the fly.

Electrical Measurements: The intensity of this stimulus, i.e. the intensity of the electrical current flowing through the fly, is measured with the aid of an electrometer and a monitor resistor. This is necessary because of the extremely small electrical currents flowing through the fly when the latter is being shocked. Since the fly has a very high electrical resistance (approximately 1-10 gigaohms, due to the minute area of contact between the fly's tarsi and the wire as well as the minuscule pressure of that contact), even a large voltage (e.g., 50 volts) delivered from the stimulator will cause only very small currents (10-50 nanoamperes) to pass through the fly (calculated from Ohm's Law: Current (I) = Voltage/Resistance). Such small currents do not damage the fly during training.

Behavioral Observations: Nevertheless, the fly finds even such weak currents to be aversive. The fly will jump to avoid such a shock. The usual movement pattern of a "naive" fly causes the fly to be constantly touching adjacent wires (and thus receiving shocks, see Figure 1a). Within a few minutes, however, the fly learns to adopt a "safe" position by orienting itself longitudinally along one wire and freezing there (Figure 1b). A fly meets our learning criterion if it remains in this safe position (i.e., touching only one wire) for 3 minutes without being shocked.

In contrast, when the magnitude of the current is below the threshold of shock, a fly receiving it fails to find it aversive and remains resting on two wires (Figure 1c). Nevertheless, we can measure such a low rate of electric current flow through the fly on the oscilloscope (see Figure 1c).

Results:

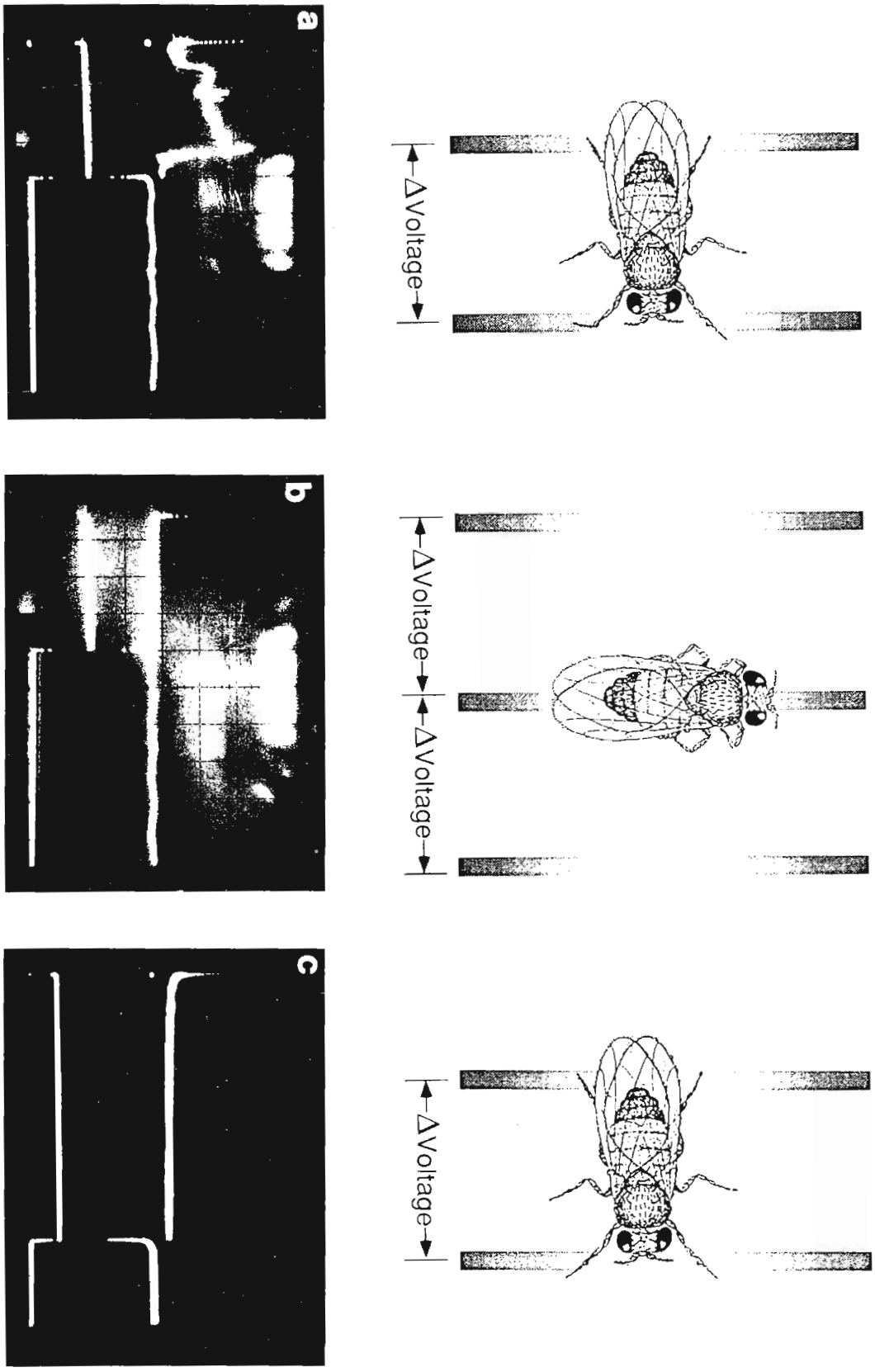
Unanesthetized males of the Canton-S wild-type stock were repeatedly tested to see whether learning rate improved with practice. Each fly was placed in the chamber until it met the learning criterion. Then it was removed to a rest vial for 5 minutes. After this rest, the fly was returned to the test chamber and its learning rate was measured on a second run. Each fly was given 5 runs with 5-minute rest periods between runs.

Most individuals (84%) improved their learning rate by Run 5 (Sign-Test, $p < 0.001$) and the majority (79%) of flies had already improved by Run 3 (Sign-Test, $p < 0.001$). Some flies needed no shocks on Runs 2-5 to adopt the safe position after being returned to the test chamber (Chi-square = 35.6, d.f. = 4, $p < 0.001$).

The average learning times of the population sample also decrease monotonically with practice (Figure 2). This pattern is seen more clearly when each fly's learning rate is standardized for that fly's initial learning rate on Run 1 before averaging over the population. This improvement of learning time with practice is not due to habituation of the

Figure 1 (next page). Fly position in chamber and corresponding recordings of shock intensity*. a) Fly receives an aversive shock and jumps to avoid the shock. b) Fly has adopted the "safe" position (touching only 1 wire) so it receives no shock. c) Fly receives a non-aversive electrical stimulus and does not jump to avoid shock.

*Note: The lower oscilloscope trace shows a square-wave voltage pulse delivered by the stimulator. The upper trace permits measurement of the electrical current (stimulus) passing through the fly.


a
ΔVoltage
b
ΔVoltage
ΔVoltage
c
ΔVoltage

fly to the shocks, since no fly ever rests on two wires (as predicted by the habituation hypothesis) when electrical current intensities equal to those used for training flies in the present experiment are applied (produced here using a stimulator voltage of 50 volts).

Summary: This new operant conditioning paradigm measures the learning rates of identified individuals when electrical stimuli of small but known intensity are delivered by the voltage stimulator and received by the fly. The method has a fundamental advantage because all other methods developed for operant conditioning of *Drosophila* (and also, to the best of our knowledge, of rats) fail to assure the reception of shock by the subject during training (e.g., not even oscilloscope traces of both: 1) the output of the stimulator and 2) a second trace of a voltage drop enabling calculation of the electrical current passing through the fly are presented). Nearly 100% of all flies in an unselected population have learned. Furthermore, a majority (84%) improve their learning rates with practice. This method of training individual flies with aversive stimuli of known intensity should be suitable for artificial selection experiments to increase rates of learning or retention of memory in a shock-avoidance paradigm. This new method of operant conditioning is simple to apply and non-invasive, using small (non-perturbing) currents to rapidly train individual flies.

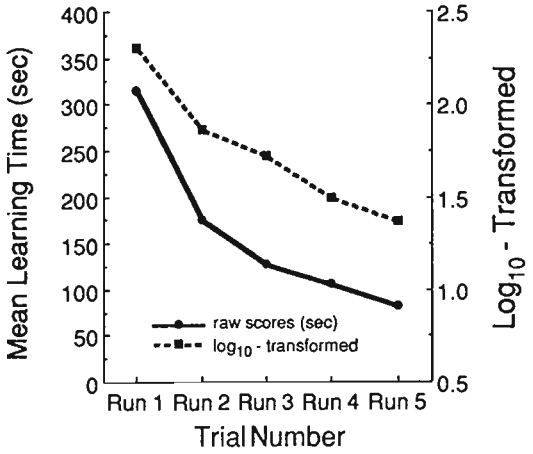


Figure 2. Mean learning time vs. run number for male flies of Canton-S wild-type stock.

References: Lofdahl, K.L., M. Holliday and J. Hirsch 1992, in press, J. Comp. Psych.; Murphey, R.M. 1967, Anim. Behav. 15:153-161; Murphey, R.M. 1973, Anim. Behav. 21:687-690; Spatz, H.Ch., A. Emanns and H. Reichert 1974, Nature 248: 359-361; Tully, T. and W.G. Quinn 1985, J. Comp. Physiol. 157:263-277; Wallace, B. and D. Sperlich 1988, Proc. Nat. Acad. Sci. USA 85:2869-2872.

Aspi, J.[1], H. Laajalahti[2] and T. Leppänen[2]. [1]Department of Genetics, [2]Department of Electronic Engineering, University of Oulu, Finland. A *Drosophila* courtship song simulator constructed as a PC expansion card.

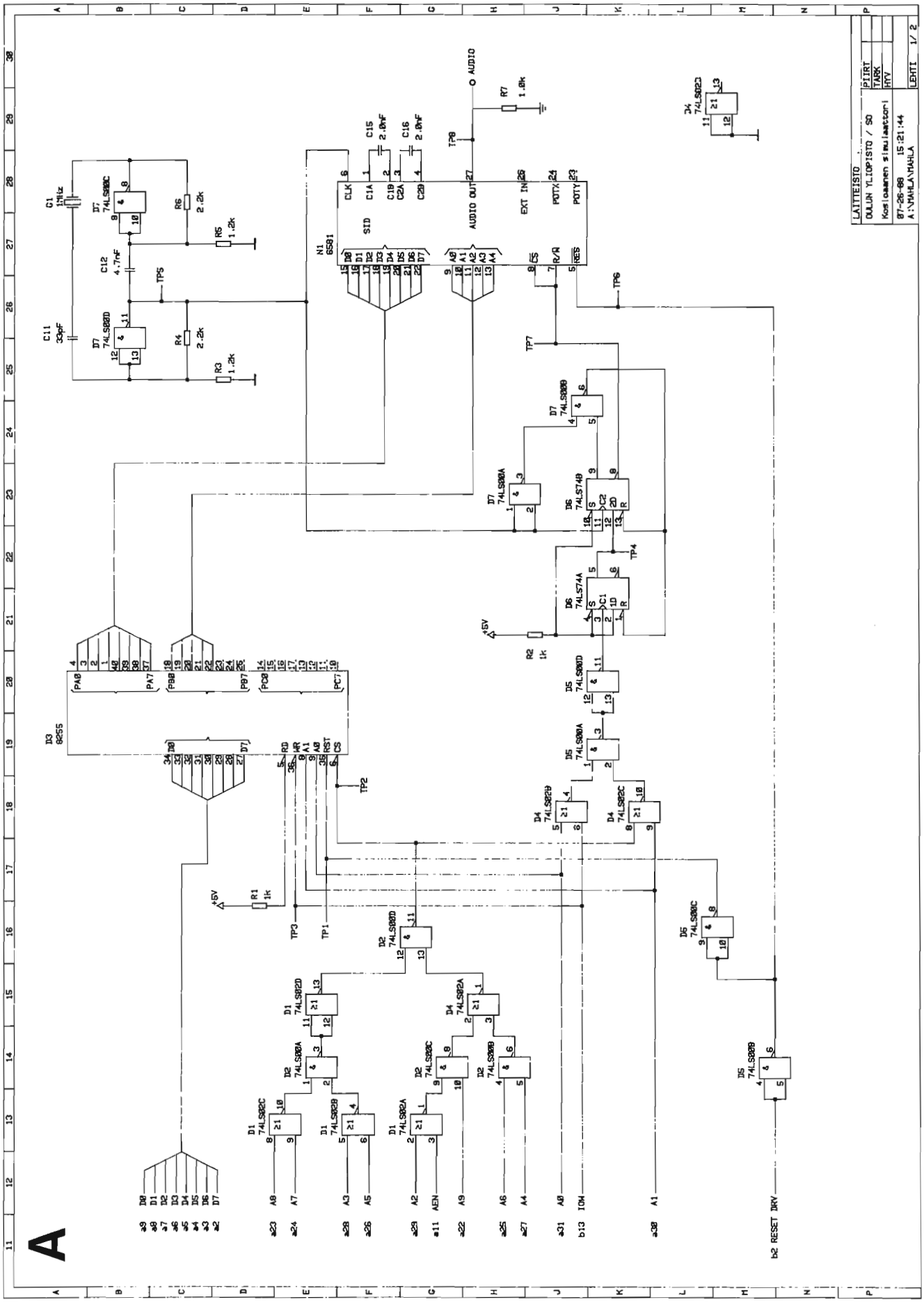
Drosophila experiments with wing-amputated males or wingless mutants have shown that the absence of a courtship song significantly reduces mating success in most species studied (e.g., Bennet-Clark and Ewing 1967; Hoikkala 1988). The mating success of wingless males can be restored by subjecting females to songs with some species-specific characters during the courtship (Bennet-Clark and Ewing 1969; von Schilcher 1976; Kyriacou and Hall 1982).

Tape-recorded natural songs could be played back when examining the species recognition function of songs, but these are tedious to use when studying the importance of interspecies variation in song characters, since it is difficult to find male songs which are different with respect to one feature but similar with respect to all others. The background noise (white noise) recorded together with natural songs for unavoidable technical reasons can also become a serious problem, since it can inhibit copulation in *Drosophila* (Kyriacou and Hall 1982; van den Berg 1987).

These difficulties can be avoided with the aid of song simulators. Different song characters can be easily varied separately without altering the others (Robinson and Ewing 1978), and the problem of white noise can be largely avoided. Song simulators also allow one to go beyond the limits of 'normal' natural variation.

The first song simulators to be used were simple transistor-based oscillators (Bennet-Clark and Ewing 1971). Although their output lacked some of the elements of a normal song, even these allowed the importance of certain song characteristics for female choice to be demonstrated. With the aim of imitating natural songs more closely, however, more complicated simulators based on integrated circuits have been constructed (Robinson and Ewing 1978; Johnson and Cowling 1980; Beukema et al. 1986). These normally contain many interconnected pulse generators and timers, control of the song characters taking place through ordinary potentiometers. Adding more parameters to the songs make the simulators more complicated, and in some simulators there have been difficulties in locking the many time-

Figure 1 (next two pages). Circuit diagrams of the song simulator (A) and the amplifier (B).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C16 2.0nF
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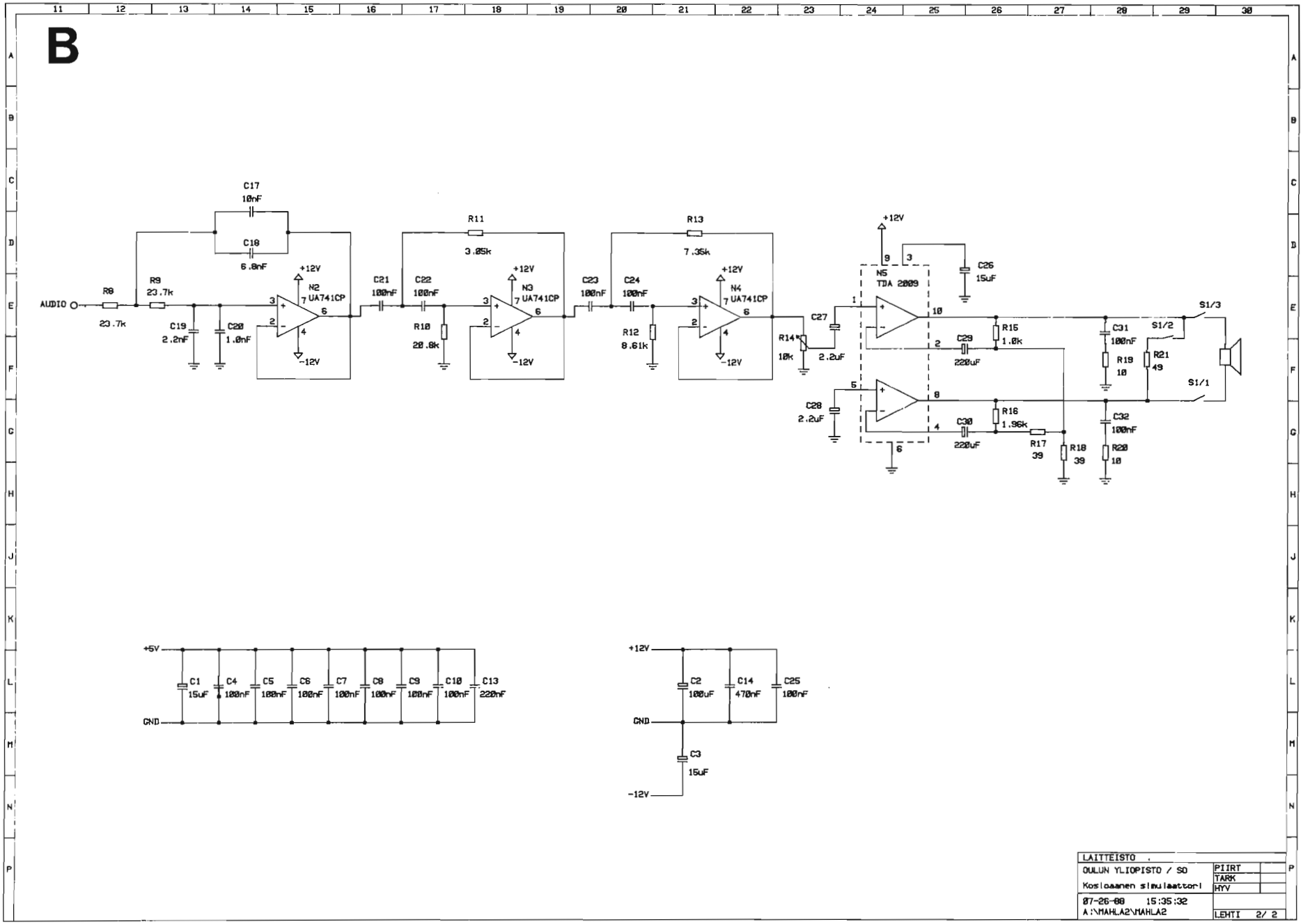
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N5
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C29
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C32
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R21
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controlling elements in phase (see Beukema et al. 1986). Because of the increasing complexity of the simulators, many features of the songs (e.g., harmonics) have often been ignored in their construction. Usually simulators can be used to mimic the songs of only one or just a few species.

Our aim was to construct a more flexible and simpler sound simulator based on a programmable sound generator connected to the I/O space of a personal computer, the songs being controlled by software. Within the limits of the sound generator circuit, the simulator can be adjusted to mimic the songs of quite different groups of animals simply by altering the software. The variables can be defined directly and exactly from the user's interface without measuring the output of the simulator. No extra power supply or enclosure is needed for the equipment. Physically, our song simulator is a normal half-length PC/AT (IBM and compatibles) expansion card, which can be adjusted to the I/O slot. The Intel programmable I/O device 8255A was used to interface the expansion card with the slot in the PC (Figure 1a). The selection signal of the device chip is coded from the lines A2..A9 and AEN (ADDRESS ENABLE). A "low" in the CS input pin of the interface device enables communication between the device and the PC. The data bus of the interface device is connected directly to the system (PC) data bus. Output data is sent to port A and the address data for the sound generator to port B. Port selection is coded from the output pins A0 and A1 of the system bus.

The sound generator chip is Intel 6581 (Sound Interface Device, henceforth SID). It is fully programmable and contains tree sound generators which can be used in conjunction. Each generator consists of a tone oscillator (4 waveforms per oscillator), an envelope generator and an amplitude modulator. The frequency range of the tone generators is between 0 and 4000 Hz. The master clock for the SID is a crystal oscillator with a operating frequency of 1 MHz. Because the microcomputer is running at different speed (normal clock speed is 4.7 MHz in a PC and 8.0 in an AT), external logic is necessary to phase the clock speeds when writing data in the registers of SID. At the same time as data is written into the 8255, the chip selection signal is also coded in the SID. This signal, which is also connected to the read/write pin of the SID, is phased with the aid of D-flip-flops allowing data entry only when it is in the right phase with respect to the clock signal of the SID. Both 8255 and SID are reset by the control signal sent via the RESET DRV-line of the I/O slot of the PC.

Table 1. Definable variables of the sound simulator output

Variable description	Control range
Length of one complete pulse train (song sequence)	0 - 30,000 ms
Length of pause between trains	0 - 30,000 ms
Length of pulse	0 - 1,000 ms
Interpulse interval (IPI)	0 - 30,000 ms
Basic frequency - frequency of first harmonic	0 - 4,000 Hz
Frequency increase - increase in frequency during a song pulse	0 - 4,000 Hz
Intensity increase - increase in sound intensity during a pulse train (O = O dB, 10 = 8 dB)	0 - 10 units
Difference in harmonics (only two harmonics) - difference in intensity between first and second harmonic frequency (O = same amplitude,5 = 2nd harmonic is about 3 dB weaker than 1st harmonic)	0 - 5 units
Output volume	0 - 9 units

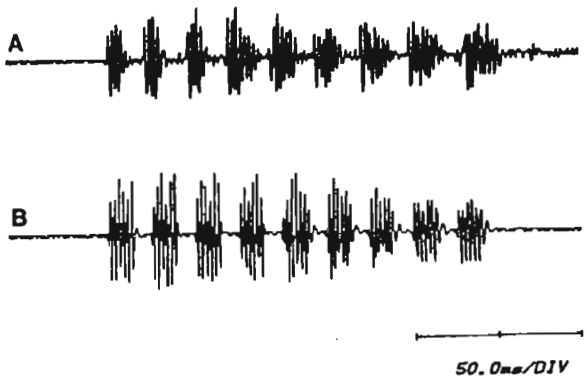


Figure 2. Oscillogram of the tape-recorded song of a male of *Drosophila montana* caught in the wild (A) and a simulator produced song (B).

The video output of the SID is fed first to high-pass and low-pass filters and then to the amplifier (Figure 1b). The limiting frequency to the high-pass filter is 200 Hz and that to low-pass filter 1200 Hz. If different limiting frequencies are needed, the values of resistors R10-R13 or capacitors C17-C20 should be changed. The bridge-connected amplifier is designed around a stereo audio amplifier circuit TDA 2009 (SGS). The expansion card has an audio socket for a loudspeaker. Parallel with the socket is a 48 ohms resistor, which is connected when the plug of speaker cord is not on. The output power is 5.8 W and output impedance 8 ohms. The rate of output signal can be varied by the trimmer R14.

We have also designed software which is able to produce the courtship songs of all the *Drosophila virilis* group species using the expansion card. A detailed description of courtship songs of this group has been presented by Hoikkala et al. (1982). Each song consist of a series of pulses (pulse trains), as in many other *Drosophila* species. Songs of this

group contain some characters which are not unique but which have often been ignored when constructing hardware-based sound simulators. The basic frequency (1st harmonic) of these pulses is about 300 Hz, and there are also two or three other harmonics with decreasing intensities. The basic frequency of the sound increases during the production of a song pulse. In some species, (but not all) the intensity of the songs also increases during the production of a pulse train. User menu-driven interface of our software is very straightforward, all the 'special' characters can be varied (Table 1). The software also offers three levels of accuracy in respect of the maximum error in timing: 0.5, 1 (default) and 2 milliseconds. The more accurate timings are selected, the slower the execution of the program becomes. Although the software is designed for this particular purpose, the application can be used to produce a very wide range of songs.

The software is written mainly in the C programming language, and the modules were compiled and linked with Microsoft C (ver. 5). Some parts were easier to implement with assembly language than with C. The program uses the Timer Tick interrupt of DOS, which activates the sound-producing subroutine, the Timer Tick interrupt vector being altered to point to this function. This new address is set by an assembly language subroutine. The display of help screens also makes use of assembler subroutines. The compiled version and the source codes of the program (with fully documentation on the functions of the modules) are available on request (Send floppy disc mailer with formatted 5 1/4' or 3.5' disk enclosed in envelope to J.A).

The simulator seems to be well equipped to produce the songs of the *virilis*-group species. Oscillograms of the song of a male of *D. montana* caught in the wild and the corresponding simulator-produced song are given as an example in Figure 2. The songs were first recorded on a Sony TC-FX 33 cassette recorder and then fed into a Gold digital 1425 oscilloscope. The oscillograms were drawn by a Hitachi 672 Graph Plotter.

References: Bennet-Clark, H.C. and A.W. Ewing 1969, Anim. Behav. 17:755-759; Beukema, W.J., T.W. Nyboer, and M.J. van der Berg 1986, DIS 63: 138-139; --- 1987, DIS 66:147-148; Johnson, P. and D.E. Cowling 1980, DIS 55:152-154; Hoikkala, A., S. Lakovaara, and E. Romppainen 1982, In: Lakovaara, S. Advances in Genetics; Hoikkala, A. 1988, Ann. Zool. Fenn. 25:257-263; Robinson, D.J. and A.W. Ewing 1978, Behav. Res. Meth. Instrum. 10: 848-851.

Frankham, R., K.E. Weber[1], S. Rousseau, T. Davidson, P. Fanning and B. Clisby. Macquarie University, Sydney, NSW, Australia. [1]Univ. Southern Maine, Portland, Maine, USA. An apparatus for measurement and selection on larval-pupal development time.

Reproductive fitness traits represent the most difficult and least understood traits in quantitative genetics, yet they are among the most important in plant and animal production. The major limitation of laboratory animal research in quantitative genetics is the labour involved in recording and scoring individuals for quantitative characters. While a range of automatically scoreable characters have been developed in recent years (e.g. Weber 1988a-g), few convenient and reliable systems exist for reproductive fitness characters. Ideally such a fitness character should be easily measurable in both sexes with minimal labour, have a high repeatability and as high a heritability as possible. We have developed a convenient model system for automated sorting on developmental time (DT) from larva to pupa that is highly repeatable, reasonably heritable and measurable in both sexes. "Developmental time" is recorded by lowering a vial containing a fixed number of known aged larvae down a perspex tube at a rate of 25mm per hour, such that the position of pupae in the tube reflects their developmental time, the most rapidly developing individuals pupating nearer the top of the tube and the slowest developing individuals pupating nearer the bottom of the tube. The apparatus is shown in Figure 1. It consists of a frame on castors, with clips to attach twelve 1 m long perspex tubes. To the left of the power plug is a slow electric motor attached to gears. These drive the light coloured aluminium spindle that runs across the apparatus near the top. Twelve lengths of fishing line are attached at points across the spindle. These lengths are carefully wound around the spindle in a single layer and have weighted hooks on their other end. The hooks are used to attach fishing line extending up from the vials. The fishing line from the spindle passes over a grooved rod before dropping down to attach a vial. A handle can be attached to the right hand end of the spindle to wind the fishing line up at the conclusion of a run. A black cloth is placed over the black frame at the top of the apparatus to achieve near-darkness during runs.

Vials are made by inserting rubber stoppers into one end of 25mm diameter x 50mm long polycarbonate tubes. A length of fishing line is threaded through the stopper and tied and the other end passed up through the medium and ended in a loop. Fifty larvae of known age are placed in a vial filled to the top with PS medium (Frankham et al. 1988) and yeasted with a pinch of freeze-dried yeast. The vial is placed in a 50 ml centrifuge tube (with four small holes bored in it for aeration) and kept at 25°C to complete most of the larval development period. Vials are loaded into the DT machine shortly before larvae are due to pupate. Each vial is placed in a 1 m long x 26 mm internal diameter perspex tube (marked in 25mm divisions) and the loop coming from the vial attached to a weighted hook on the end of the

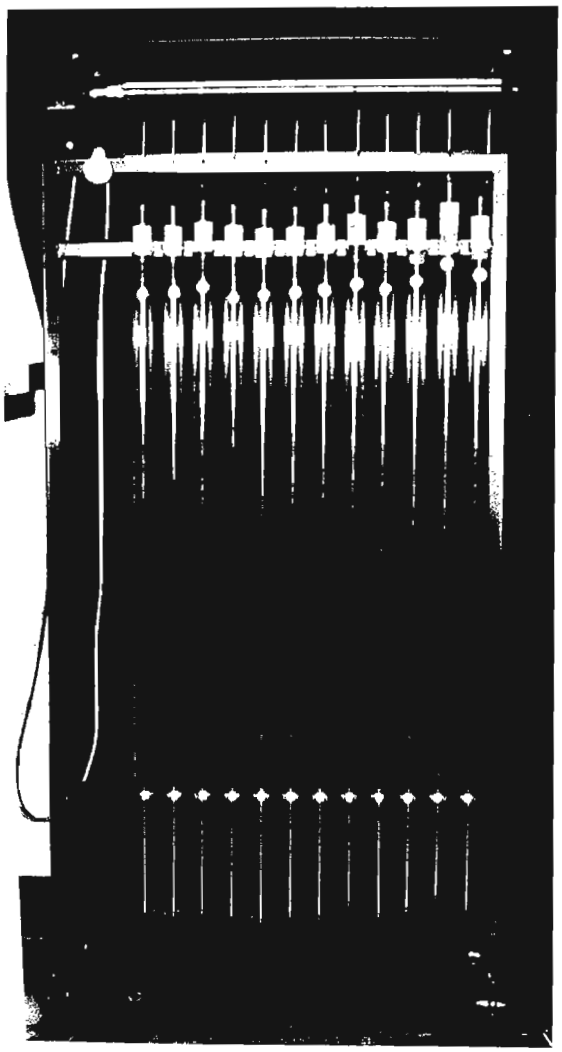


Figure 1. The developmental time apparatus with a vial loaded into each of the 12 perspex tubes and a sinker hung beneath each vial. Vials are attached to the apparatus via a looped length of fishing line (secured through the rubber stopper in the bottom of the vial) that loops over a weighted hook attached to the end of fishing line carefully wound around the spindle at the top of the apparatus.

fishing line coming down from the spindle of the DT machine. A 50g sinker is attached to the bottom of each vial to ensure that it runs smoothly down the perspex tube. The electric motor is turned on and run for 39 hours. As the larvae crawl out of the medium to pupate, they attach to the dry wall of the perspex tube in the vicinity of the top of the vial. The numbers of pupae in each graduation of the tube are recorded and converted into "developmental times". Twelve vials containing a total of 600 larvae can be run simultaneously on the DT machine. Runs are done at 25°C in near darkness. At the end of a run, vials are detached, a clutch between the gears and the spindle disengaged, a removable handle attached to the far end of the spindle, and the spindle wound so that the fishing line rewinds tightly around the spindle in a single layer. Detailed manufacturing drawings are available on request.

Selection of fast, slow or control pupae is carried out by wiping the required pupae from top, bottom or throughout the tube, until sufficient numbers are obtained. To facilitate wiping of the pupae from the tube, cold water is first run through the tubes. A stick with dampened foam rubber attached to one end is used to wipe the pupae free. After selection, pupae are placed in a papered bottle containing yeasted PS medium and allowed to eclose.

Response to selection has been demonstrated for this character, with response being evident within 1-2 generations. Full details will be presented elsewhere.

Acknowledgment: We thank R. Davis for technical assistance. This work was supported by an Australian Research Council grant.

References: Frankham, R., B.H. Yoo and B.L. Sheldon 1988, Theor. Appl. Genet. 76:909-914; Weber, K.E. 1988a, DIS 67:91-93; 1988b, DIS 67:93-94; 1988c, DIS 67:96; 1988d, DIS 67:96-97; 1988e, DIS 67:97-102; 1988f, DIS 67:102-104.

Rand, David M., Graduate Program in Ecology and Evolution, Brown University, Providence, RI 02912. A dot blot hybridization method for estimating the frequencies of mitochondrial DNA haplotypes in experimental populations of *Drosophila*.

A number of recent studies have used *Drosophila* population cage or bottle experiments in an attempt to detect the presence or absence of selection on mtDNA (MacRae and Anderson, 1988; Fos *et al.*, 1990; Nigro and Prout, 1990). As the statistical power of such experiments is largely a function of the number of replicate cages used and the number of individual flies sampled per cage, it is desirable to conduct experiments that require the scoring of a large number of individual flies.

The procedure described below is aimed at increasing the feasibility of large scale population cage experiments. The method uses specific oligonucleotides to screen dot blots prepared in a microtiter tray format. By designing oligos that differ at a single nucleotide in the middle of the sequence, differential hybridization is achieved and can distinguish haplotypes that might be used in a typical population experiment. By using pairs of differentially hybridizing oligos in reciprocal hybridization experiments, one can also monitor the population experiment for paternal leakage of mtDNAs between strains.

DNA Preparation

Two protocols for preparing the DNA are listed. The first is the "careful" method which produces cleaner blots but makes use of a centrifuge which can spin microtiter plates. The second is a "quick and dirty" method which can be done rapidly without a centrifuge but has a slightly higher signal to noise ratio.

Careful Method

1. Using an eight-tipped repeating pipettor (e.g. Oxford 8800, Fisher # 21-243-1) dispense 25 ul of grinding buffer (0.2 M sucrose, 50 mM EDTA, 0.1 M Tris, pH 9.0 and 0.5% Sodium Dodecyl Sulfate) to each well of a 96-well flat-bottomed microtiter plate (Fisher Scientific, # 08-758-15).

2. Using a Rainin electronic repeating pipettor, dispense 0.5 ul of Diethylpyrocarbonate (Sigma Chemical Co.) into each 25 aliquot of grinding buffer.

3. Place a single female Drosophila in each well of the grinding plate and homogenize the flies by several circular strokes with a 96-pronged grinder which fits into the corresponding wells of the microtiter plate (see Figure 1). Frozen flies are easiest to manage as one does not have to worry about keeping the flies anesthetized. Check to make sure that the flies are well homogenized.

4. Place a lid on the the microtiter plate containing the homogenized flies and put the plate at 68°C for 15 minutes. The use of a covered water bath containing a platform submerged by about 2 mm of water on which the grinding plate can be placed is ideal. The high humidity limits evaporation from the wells so the homogenate molarity does not change significantly.

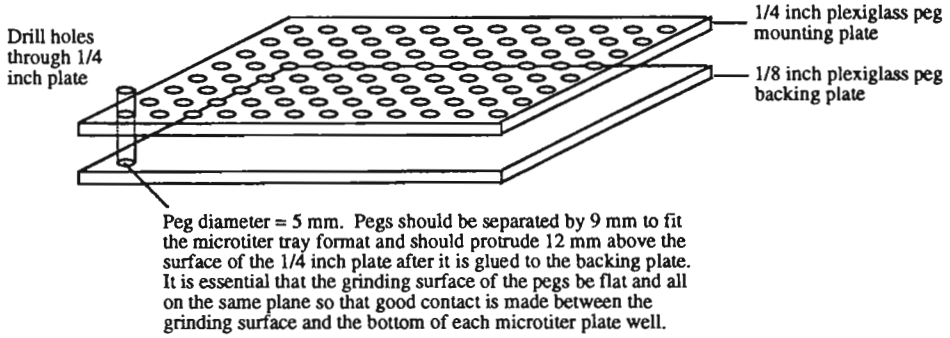


Figure 1. Pestel plate for single fly homogenization in a microtiter tray format.

5. Using the repeating multitipped pipettor add 5 ul of 8M potassium acetate to each well. If care is taken in rinsing the tips after any contact with homogenate, it is not necessary to use clean pipette tips each time. Deliver the potassium acetate to the sides of each well first and then, using the pipettor as a multitipped stirrer, mix the potassium acetate into the homogenate. Rinse the tips in distilled H_2O before stirring the next set of wells.

6. Place the plate on a slurry of ice/slush for 15 minutes to precipitate proteins and SDS.

7. Spin the plate in a centrifuge at 4000 rpm for five minutes to pellet the proteins and debris.

8. While the grinding plate is spinning, add 50 ul of EtOH to each well of a second microtiter plate (Falcon round-bottom #3911, Fisher # 08-772-8, or equivalent). The supernatant from the grinding plate will be added to the second plate for DNA precipitation.

9. Pull up the supernatant (about 20 ul) from the homogenized flies using a multitipped pipettor and add it to the corresponding wells of the round-bottomed plate holding the EtOH. Mix the supernatant with the EtOH to ensure proper precipitation. As before, the same tips can be used for each set of wells if care is taken in rinsing the tips.

10. Place the plate containing the DNA at -80°C for 10 minutes.

11. Spin the plate in a centrifuge at 4000 rpm for 10 minutes to pellet the DNA.

12. Carefully pull off the EtOH supernatant without removing the pellet and place the plate in an oven (37 - 80°C) to evaporate residual EtOH. The amount of residual EtOH is not crucial; there should not be standing liquid, but avoid overdrying as this may hinder the resuspension of the DNA pellet.

13. Add 25 ul of TE (10 mM Tris, pH 7.5, 1 mM EDTA) to resuspend the pellets and then heat the plate to 98-100°C for 1-2 minutes to denature the DNA strands.

14. Place the plate on a slurry of ice/slush to chill the samples. Add 25 ul of 20X SSC to each well before adding the homogenate to the dot blot apparatus.

Quick and Dirty Method

1. Using an eight-tipped repeating pipettor dispense 25 ul of squishing buffer (10 mM Tris, pH 8.2, 1 mM EDTA, 25 mM NaCl, 200 ug/ml Proteinase K [Gloor and Engels, 1991]) to each well of a 96-well flat-bottomed microtiter plate (Fisher Scientific, # 08-758-15).
2. Place a single female Drosophila in each well of the grinding plate and homogenize the flies by several circular strokes with a 96-pronged grinder which fits into the corresponding wells of the microtiter plate (see Figure 1). Check to make sure that the flies are well homogenized.
3. Place the plate at 37°C for 20 min; then place the plate at 98-100°C for 2 minutes to denature the proteinase K and denature the DNA strands.
4. Place the plate on a slurry of ice/slush and add 25 ul of 20X SSC. The samples are ready to be added to the dot blot apparatus.

Dot-Blotting

1. Prepare the hybridization membrane (e.g., GeneScreen) by first wetting it in deionized H_2O and then soaking in 10X SSC for five minutes (different membranes may require different wetting procedures).
2. Using the multi-tipped pipettor, pull up as much of the denatured homogenate as possible (about 40-50 ul) and dispense the liquid to the corresponding wells of a dot-blot apparatus (e.g., Bio Rad) hooked to a vacuum pump. The dot-blot apparatus should be set up as a sandwich of a sheet of hybridization membrane and Whatman 3 MM paper, with the membrane on top so that the homogenate is applied to the surface of the membrane. The same eight tips can be used for all samples if the tips are rinsed sufficiently by up and down pipetting in a large beaker of deionized water.
3. Apply the vacuum for five minutes (longer if there is still fluid in the wells of the dot-blotter).
4. Remove the filter and before it dries lay it, DNA side up, on a sheet of 3MM paper saturated with denaturation buffer (1.5 M NaCl, 0.5 M NaOH). Denature for five minutes to insure that the DNA strands are separated.
5. Move the filter to a sheet of 3 MM paper saturated in neutralization buffer (1.5 M NaCl, 0.5 M Tris, pH 7.5). Neutralize for five minutes.
6. Move the filter to a sheet of 3 MM paper saturated with 10X SSC for five minutes and then blot it dry on sheet of 3 MM paper.
7. Bake the filter for 30 minutes in a 80°C vacuum oven or cross link the DNA using an appropriate UV light source (e.g., Stratalinker from Stratagene). Baking or crosslinking should follow the recommendations of the supplier depending on the membrane used.

End Label the Oligonucleotide

The following standard end labelling reaction (pg 1131, Sambrook *et al.*, 1989) has provided good probes for the dot-blots prepared as described above. I have found that there is no need to separate the unincorporated [g-^{32}P]-ATP from the labelled oligonucleotide. The entire reaction is simply added to the hybridization solution containing the filter. Moreover, reactions using half the recommended amount of polynucleotide kinase have produced effective probes.

1ul Oligonucleotide (10 pmoles/ul)
2ul 10X T4 polynucleotide kinase buffer
10ul sterile deionized H_2O
2ul polynucleotide kinase (4 units; Sambrook *et al.*, 1989 calls for 8 units)
5ul [g-^{32}P]-ATP (New England Nuclear, #NEG-002A, 3000 Ci/mmol)
Incubate at 37°C for 45 minutes

Hybridization

The conditions for hybridization depend on the length and base composition of the oligonucleotides used as probes. Refer to pages 6.4.1 - 6.4.2 in Current Protocols, Ausubel *et al.*, 1988 for suggested temperatures. The experiments reported here involved a 15 mer and a 19 mer at the temperatures indicated below.

Pre-Hyb buffer: 6X SSC, 5X Denhardt's Solution, 0.05% sodium pyrophosphate (F.W.=446.06), 100ug/ml boiled herring sperm DNA (yeast tRNA or sheared salmon sperm DNA will work), 0.5% SDS.

SSC Hybridization Buffer: 6X SSC, 1X Denhardt's solution, 100ug/ml herring sperm DNA (or yeast tRNA or sheared salmon sperm DNA), 0.05% sodium pyrophosphate.

1. Prehybridize for one hour at 37°C in Pre-Hyb buffer.
2. Remove Pre-Hyb buffer and add enough Hybridization buffer to cover the membrane(s) (5 - 10 ml). Add the entire end-label reaction and hybridize for 14 to 48 hours at:

14 mer room temperature
15 mer room temperature (about 23°C)
17 mer 37°C
19 mer 40°C
20 mer 42°C
23 mer 48°C

As with any hybridization there should be no opportunity for evaporative loss from the hybridization chamber which would increase the salt concentration and cause the oligo to melt off the target.

Wash the Filters

1. Wash the filters three times at room temperature for 10-15 minutes each in 6X SSC, 0.05% Sodium Pyrophosphate.

2. The second washes should be at higher temperatures in the same wash buffer. The following conditions are from Ausubel *et al.*, 1988 or from the experiments reported here (15 mer, 19 mer):

Oligomer	First Wash	Second Wash
14 mer	37°C	41°C
15 mer	39°C	43°C
17 mer	48°C	53°C
19 mer	53°C	56°C
20 mer	55°C	63°C
23 mer	60°C	70°C

The washing temperatures are critical so it is wise to check the temperature with an accurate thermometer and be certain that the desired temperatures are not exceeded. Washes at temperatures below those suggested have produced unambiguous dot-blot hybridization patterns (e.g., 15 mer: 35°C then 40°C)

Exposure

The filters are wrapped in plastic food wrap and exposed to X-ray film as with a normal autoradiograph.

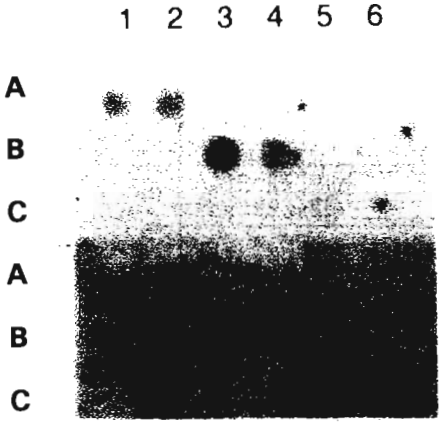
Rehybridization

The filters can be stripped of the probe and rehybridized with a different oligo. The stripping should be done according to the recommendations supplied with the hybridization membrane.

Differential oligonucleotide hybridization to dot-blots.

D. melanogaster homogenate was loaded into wells A1, A2, B3, B4 and that of *D. simulans* loaded into wells C5 and C6. Top half of the figure: positive hybridization of the oligonucleotide AGGAATTTTATTCTT to *D. melanogaster* mtDNA and no hybridization to the two *D. simulans* flies whose mtDNA carries a C in the position of the underlined T in the oligo above. Bottom half of figure: the dot-blot was stripped and rehybridized with the oligo AATTCGAGCCGAATTAGGA which bound to *D. simulans* and did not hybridize to the four *D. melanogaster* flies which carry a T in the position of the underlined C in the oligo (rows A and B).

The primers were based on published sequences (Satta *et al.*, 1987) and were designed intentionally with different lengths (15 and 19 bases) and GC contents (3/15=20%; 8/19=42%)to test the robustness of the technique. A sharp threshold of hybridization will be best achieved if relatively short oligos are used (15 to 20 bp) and the nucleotide that differs between strains lies in the middle of the sequence.



General Comments

It is highly recommended that female *Drosophila* be used for the dot blots since the eggs they carry hold many copies of mtDNA molecules. Depending on one's sampling regime, it is further recommended that adults not be allowed to age significantly before freezing. Old and tired flies, and especially old males, do not produce good results.

As with any method, it is recommended that one try slightly different conditions (e. g., oligos of different length and GC content) to establish the best combination of variables for the strains or species under study. Since the rehybridization of filters can be done with relative ease, it is also recommended that oligos be designed that give positive hybridization to each of the two (or more) mtDNA haplotypes in the population experiment. Filters should be rehybridized with the alternative oligo to insure that the reciprocal dot-blot pattern is produced.

In light of the recent evidence for paternal leakage of mtDNAs (Kondo *et al.*, 1990; Hoeh *et al.*, 1991; Gyllensten *et al.*, 1991), reciprocal hybridizations become all the more important. Any dot in a pair of reciprocal autoradiographs from the same dot-blot that shows positive hybridization to both oligos could be a heteroplasmic fly carrying mtDNA from both strains. Indeed, the use of population cages and dot-blot hybridization could be an effective means of studying the frequency of such paternal leakage effects in *Drosophila*.

Acknowledgements: This work was supported by a grant to R. C. Lewontin from NIH and Funds from the Division of Biology and Medicine, Brown University.

References: Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith and K. Struhl 1988, J. Wiley and Sons, New York; Fos, M. M. A. Dominguez, A. Latorre and A. Moya 1990, PNAS USA 87: 4198-4201; Gloor, G. and W. Engels 1991, *Drosophila* Information Newsletter, Vol 1; Gyllensten, U., D. Wharton, A. Josefsson and A.C. Wilson 1991, Nature 352: 255-257; Hoeh, W.R., K.H. Blakley and W.M. Brown 1991, Science 251: 1488-1490; Kondo, R., Y. Satta, E.T. Matsuura, H. Ishiwa, N. Takahata and S.I. Chigusa 1990, Genetics 126: 657-663; MacRae, A. F. and W.W. Anderson 1988, Genetics 120: 485-494; Nigro, L. and T. Prout 1990, Genetics 125: 551-555; Sambrook, J., E.F. Fritch and T. Maniatis 1989, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Satta, Y., H. Ishiwa and S.I. Chigusa 1987, Mol. Biol. Evol. 4(6):638-650.

Fukui, H.H., A.A. Keso, C. Belinco, and J.W. Curtsinger. University of Minnesota, Minneapolis, MN USA. Thanatometer: A chamber designed for longevity measurement for *Drosophila melanogaster*.

Longevity of *D. melanogaster* has often been measured by using vials or standard population cages. Some of the deficiencies of these methods are: 1) Vials require frequent transfer and are labor intensive when large number of flies are tested; 2) It is difficult to control microenvironment. To overcome these deficiencies, we have developed and tested a new type of chamber for longevity studies.

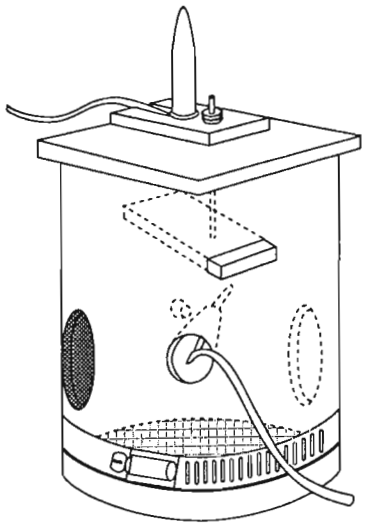
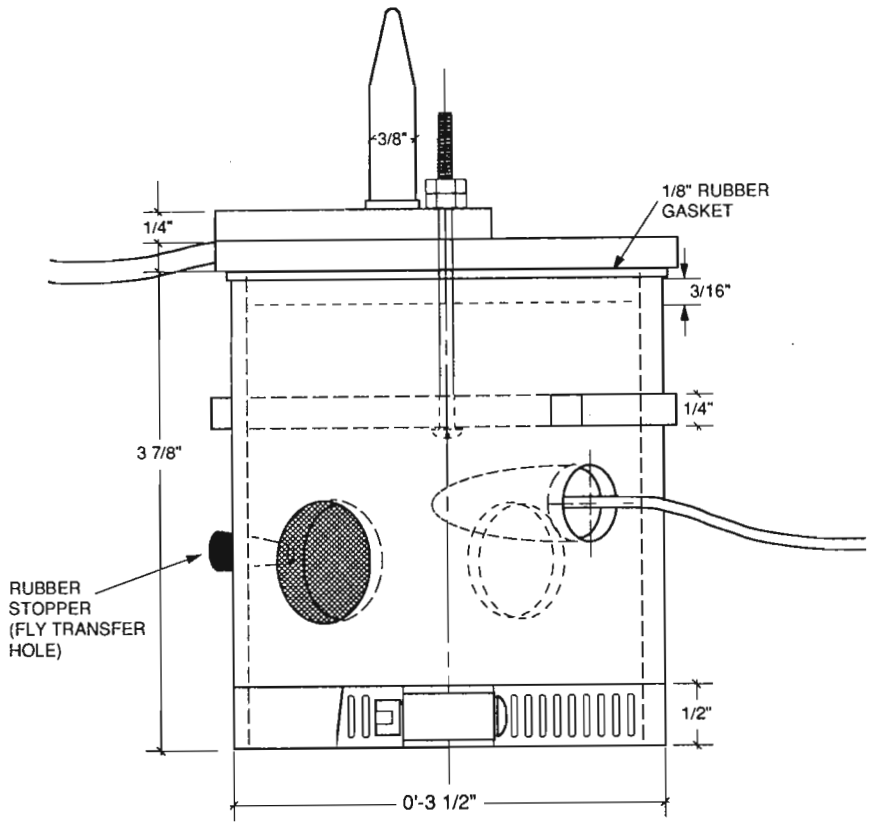


Figure 1. Assembled thanatometer.

Figures 2 to 4. Dimensions and parts of thanatometer.



Chamber: An assembled chamber is described in Figure 1, and the dimensions and parts are described in Figures 2-4. Clear plexiglass is used to build the top and cylinder units. The two units are connected by a 1 1/4" bolt.

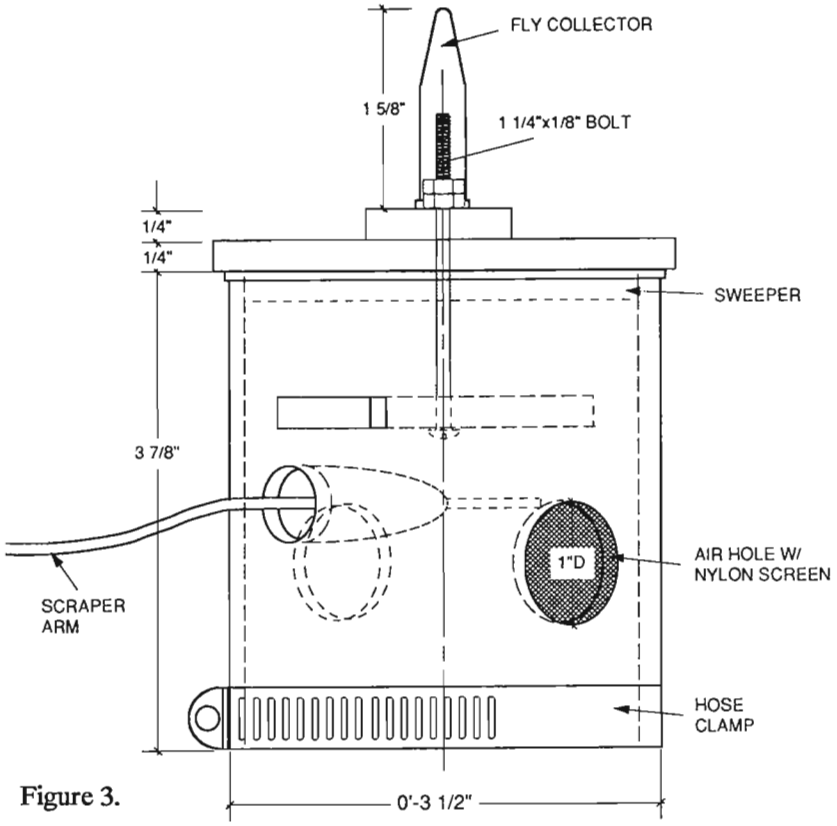


Figure 3.

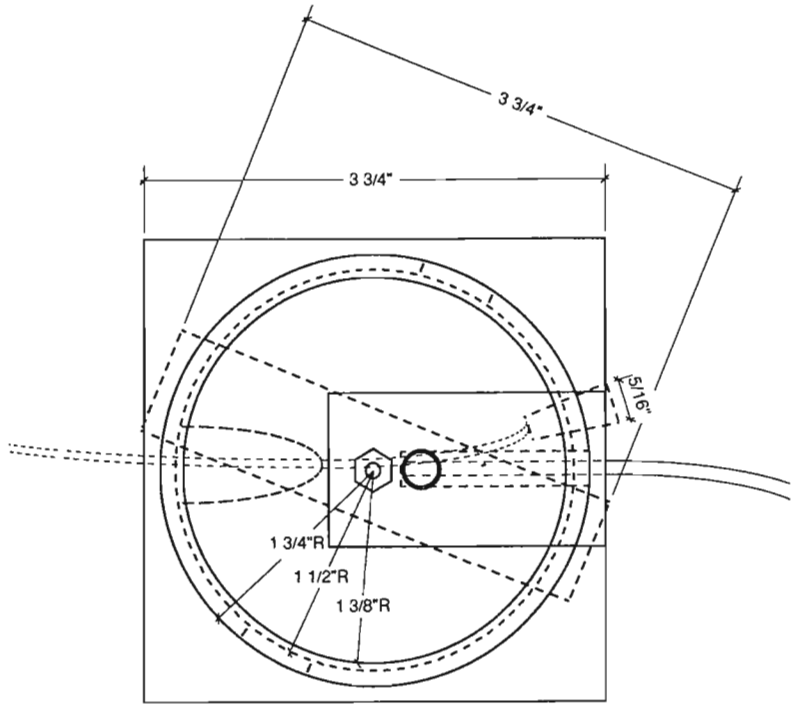


Figure 4.

Widemouth jar gaskets are used to seal the connection between the two units. The fly collector unit is a 1.5 ml eppendorf tube. A 15 cm long vinyl coated wire (#7004231106, Bulldog Jordan Co.) is used as a scraper arm. It is inserted to the cylinder unit through a rubber finger tip (size # 11 1/2) and is used to remove dead flies from the wall. Nylon screen is used to seal the two ventilation holes and the bottom of the cylinder unit. A hose clamp is used to secure the nylon screen on the bottom. A standard yeasted-molasses-cornmeal-agar medium is poured into disposable polystyrene petri dishes (100 x 15 mm) and attached to the bottom of the cylinder unit. Thus, flies can get the media through the screen. To control moisture, the surface of the culture media is covered with two to four layers of cheese cloth.

To remove dead flies, the medium is removed from the chamber and the chamber is set on the stand upside-down. The groove stopper is pulled to the mouth of the groove. The dead flies are swept into the groove by turning the cylinder unit. Dead flies are then pushed toward the fly collector by pushing the groove stopper until the mouth of the collector is completely covered with the stopper. Finally, the fly collector is detached from the chamber and dead flies are removed from the collector onto an index card where they can be counted.

Experiment: Virgin males from three inbred strains of *D. melanogaster* were tested for their longevity by using the thanatometer. The base population from which these strains were derived was maintained as a large random-mating laboratory population ("LF350", Weber and Diggins, 1990). Mated females were drawn from the base population to initiate lines which were inbred for 50 generations by half-sib mating. The flies from the inbred lines were subjected to two generations of density control (100

Table 1. Sample size and mean virgin male longevity with standard error (S.E.) for the three inbred lines. The data from the vial method were included to compare the two methods. The chambers and/or vials sharing a letter designation within the inbred line do not differ significantly at P = 0.05 in the Tukey multiple comparison test.

Inbred line	N	Mean longevity (Day)	S.E.	Tukey
2 (Cham. 1)	55	27.2	1.2	A
2 (Cham. 2)	52	35.6	1.2	B
2 (vial)	155	33.9	1.1	B
3 (Cham. 3)	63	55.6	1.9	A
3 (Cham. 4)	108	60.5	1.2	B
3 (vial)	248	35.3	0.8	C
4(Cham. 5)	88	50.5	1.5	A
4 (vial)	236	52.5	1.4	A

eggs / 8-dram vial) before testing. This treatment was carried out to control the environmental factors attributable to larval density (Clare and Luckinbill, 1985; Luckinbill and Clare, 1985). The flies were collected when they were 0- to 5-hr post-emergence. They were sexed when they were 2- to 7-hr post-emergence under light CO_2 anesthesia to reduce the age-specific toxic effects of CO_2 (Perron, et al., 1972). The culture media were changed three times a week until the last fly died.

The mean longevity measured by using thanatometers are summarized in Table 1. About eight percent of flies were lost during the experiment, which is comparable to that of the vial method. The means were compared with the ones previously measured by using 4-dram vials (5 to 6 flies/vial). The one-way ANOVA with the Tukey multiple comparison was used for this analysis (SAS Inc, 1985). The most notable difference between the two methods is that the mean longevity is increased by 20 to 25 days by using the thanatometers in the inbred stock #3. This may be due to improved ventilation in the thanatometer (there are two 1" ventilation holes on the cylinder unit), and the stock #3 may be more susceptible to ventilation. Pearl and Parker (1922) showed that the mean longevity of the flies maintained in well ventilated vials increased the mean longevity by about 4 days compared to the control. For the other stocks, the means measured by thanatometer are not significantly different from that measured by vials in most of the cases. An exception is a significantly shorter mean longevity than the vial mean for one of the thanatometer in the inbred stock #2.

The survival curve of the three inbred stocks are summarized in Figure 5 to Figure 7. Again the

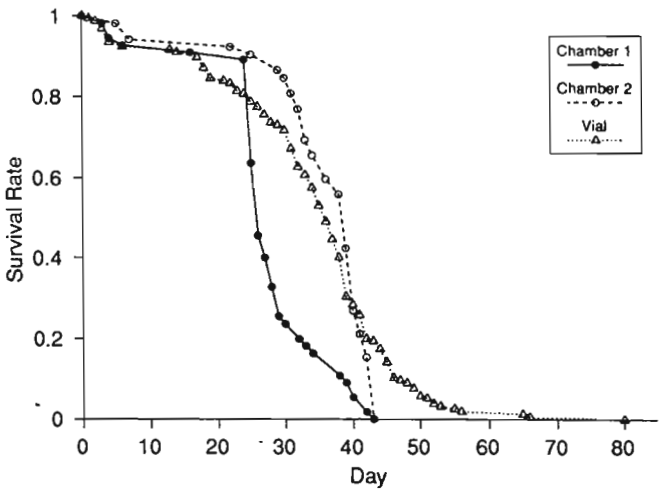


Figure 5. Survival curve of the inbred line #2.

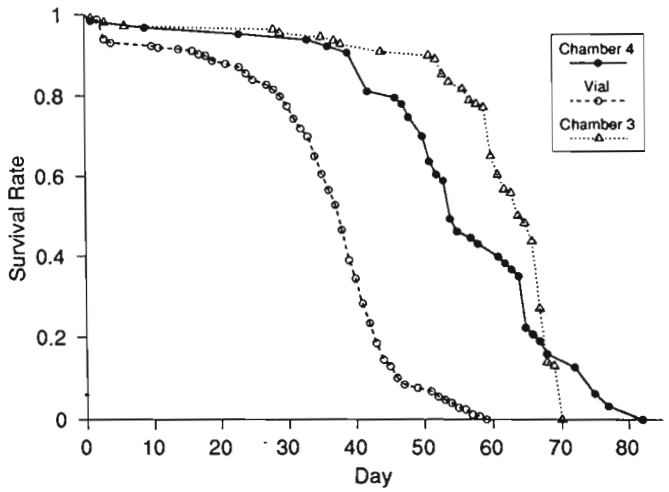


Figure 6. Survival curve of the inbred line #3.

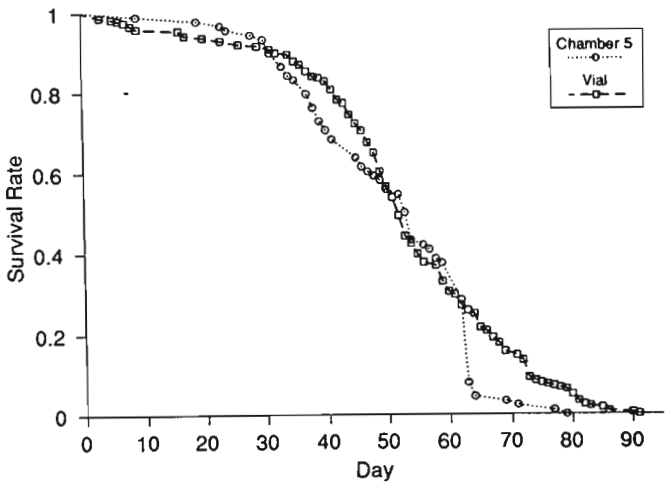


Figure 7. Survival curve of the inbred line #4.

data from the thanatometers tests were compared with the vial tests data. It is notable from the inbred stocks #2 and #4 data that the maximum age at death is shorter in the thanatometer tests than that of the vial tests (Figures 5 and 7). It may be due to aged individual having difficulty getting food from the culture through the screen. However, preliminary data indicated the maximum age at death in the thanatometer method could be as long as the one measured by the vial method when the thanatometers were in a temperature controlled room. If this is the case, environmental differences between the room and the incubator have significant effects on the maximum age of death.

The thanatometer simplifies the measurement of longevity considerably, because we transfer culture media than flies, and also because each fly is counted only once at death. This method allows a large number of flies to be subjected to similar microenvironments. The mean longevity for the tested inbred lines were longer or similar to that of the results of vial tests in most of the cases. The maximum age at death, however, may be shorter than that of the vial tests in some of the inbred lines tested in the incubator.

Acknowledgment: Ken Webber gave much technical advice during the development. This work was supported by NIH grants on "Oldest-old Mortality" to J. W. Curtsinger and J. Vaupel (PO1 AG08761).

References: Clare, M.J. and L.S. Luckinbill 1985, Heredity 55:19-29; Luckinbill, L.S. and M.J. Clare 1985, Heredity 55:9-18; Parl, R. and S.L. Parker 1922, Am. Nat. 56:385-405; Perron, J.M., L. Huot, G.-W. Corrivault and S.S. Chawla 1972, J. Insect Physiol. 18:1869-1874; SAS Institute, Inc., 1985, SAS Inst., Cary; Weber, K.E. and L.T. Diggins 1990, Genetics 125:585-597.

Dapkus, D. and M. Murray[1]. Winona State University, Winona, Minnesota and [1]Mayo Clinic, Rochester, Minnesota. A new apparatus for testing resistance to volatile anesthetics.

Several workers have published methods for measuring anesthetic resistance including Rasmusen (1955), Gamo (1981), and Krishnan and Nash (1990). We have developed a new apparatus which allows the unobstructed viewing and repeated scoring of several samples of flies exposed to the same concentration of inhalation anesthetic.

The basic apparatus (see Figure 1) consists of an eight inch culture dish (Carolina Biological Supply, Burlington, North Carolina, #74-1006) closed with a top made of a 9 inch octagon of 1/4 inch plate glass. The top of the culture dish was flattened by grinding on a glass plate covered with a slurry of 220 grit grinding compound. The plate glass top was pierced by a centrally located 1/4 inch hole drilled with a diamond-coated drill. The opening was fitted with a plastic washer attached to the glass with epoxy glue; the area of attachment on the glass was roughened with coarse sand paper. Delrin plastic was used to manufacture the plastic piece. It was machined to be 0.375 inch thick and 1 inch in diameter; one side of the washer was flattened by removing 0.188 inch (to allow closing of the stopcock) and a 0.125 inch hole was drilled and reamed to fit a luer lock needle taper. This opening was fitted with a three-way anesthetic stopcock (Terumo Corp., Tokyo, Japan).

The inside of the chamber was fitted with six fly-containing containers made from four ounce clear, straight-sided, round specimen jars (Fisher, Chicago, Illinois, #03-320-3E). The threaded end of each glass jar was cut off to a height of 5/8 inch using a diamond saw and smoothed by grinding with abrasive compound. Six of the resulting glass rings were attached to the plate glass in a 7.25 inch circle with Dow Corning (Midland, Michigan) silicone rubber sealant.

The phenolic caps of the jars had a 1.75 inch hole bored through the top with a hole saw. A 2 inch disk of 1/2 mm nylon mesh (Wildco Wildlife Supply Co., Saginaw, Michigan) was fitted inside the jar lid and cemented into place with silicone sealant.

In use, the plate glass top is removed

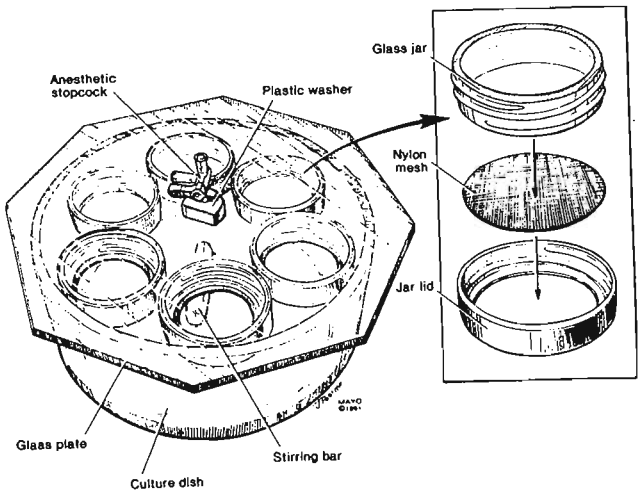


Figure 1. Anesthetic test apparatus.

from the culture dish and placed upside down. Groups of 25 or fewer flies of one sex are counted out under CO_2 anesthesia and placed in the glass jar. We have found it important, in producing uniform results, that the flies to be compared are the same age and are conditioned on fresh medium for at least 24 hours before testing. The lid is screwed on to restrain the fly samples. We wait 20-30 minutes to allow complete recovery from CO_2 anesthesia. A 3 inch x 1/2 inch teflon magnetic stirring bar with a twist of copper wire around its center (to improve its spinning) is placed in the culture dish and the plate glass is sealed to the culture bowl with a bead of silicone high vacuum grease (Dow Corning, Midland, Michigan). To begin the test a sample of liquid anesthetic is injected through the opened stopcock with a gas-tight syringe. The stopcock is closed and the contents are stirred at a high speed for one to two minutes on a magnetic stirrer.

Readings of the number of flies knocked down are made through the plate glass top using a five inch illuminated magnifier (Hosfelt Electronics Inc., Steubenville, Ohio). The criterion for a "knock down" for those flies not obviously "out" and on their backs is that they are tipped far enough over to the side so that a wing touches the screen. We have found that taking the mean of two or three readings decreases the variability in the scoring. Also, since flies in the early stages of anesthesia fall down and get up, it is best to observe and score each fly just once, rather than to try to count all the flies down. As the observer scans the flies, one at a time, those that are struggling are observed for 2 seconds and scored according to whether they are up or down more than half of the time.

Using this apparatus, two different experimental designs are possible. In one method, several bowls are set up with various concentrations of anesthetic and readings are made at a preordained time such as 1 or 2 hours. The results can then be analyzed using probit analysis (Finney, 1971). Alternatively, flies to be compared can be tested at a single concentration with readings made at various time intervals, such as every ten minutes. The results can then be analyzed using survival analysis (Lee, 1980).

Studies of anesthetic concentrations in the bowls were conducted using a Perkin Elmer MGA 1100 mass spectrometer. The suction line of the machine was attached to the stopcock and allowed to withdraw and analyze a small sample (1 ml per second, approximately 5-10 seconds) of the gas in the bowl. We studied five bowls set up with 40 microliters of liquid halothane. Each bowl was tested three times, with a 5-15 minute "airing" in between tests. The average concentrations in the three sets of replicates were 0.42. 0.45 and 0.45 percent halothane when read after 2 minutes of stirring. Repeated readings were made on the first series of bowls at 2, 10, 30, 60 and 120 minutes. Over the first 30 minutes the mean concentration decreased by about 7% from 0.42 to 0.39 and remained stable at that level for the next 1.5 hours. From these studies we conclude that the bowls leak little if any halothane over a two hour time period. Rather, the decrease in halothane concentration in the bowls is most likely due to absorption into the silicone sealant and silicone vacuum grease inside the apparatus. We base this conclusion on the stability of the concentrations from 30 minutes to 2 hours and the higher 2 minute readings in the second and third uses of the bowls, both of which are consistent with a saturation of the silicone sealant and grease with halothane. Ideally, if a mass spectrometer, or other means of measuring the concentration of anesthetic agent, were available, the concentration of anesthetic in each bowl could be checked after each test. However, the tests made with the apparatus are still useful even if the concentration connot be measured since tests can be set up so that comparisons are made between up to six groups of flies all exposed to the same concentration within a bowl.

We have used this apparatus with halothane, enflurane, isoflurane, chloroform and ether with good results. The advantages of this method are that it: 1) allows repeated scoring through a flat viewing surface. 2) exposes up to six groups of flies to the same concentration of anesthetic, under the same conditions. 3) is able to reliably detect the effects of subtle genetic or environmental alterations on anesthetic resistance.

References: Finney, D. J., 1971. Probit Analysis. Cambridge U. Press, London; Gamo, S., M. Ogaki and E. Nakashima-Tanaka, 1981. Anesthesiology 54: 289-293; Krishnan, K. and H. Nash, 1990. PNAS 87: 8632-8636; Lee, E., 1980. Statistical Methods for Survival Data Analysis. Wadsworth, Belmont, California; Rasmuson, B., 1955. Hereditas 41: 147-208.

Research Notes

Stoltenberg, S. F., J. Hirsch and S. H. Berlocher. University of Illinois, Urbana-Champaign, Illinois USNA. Allozyme monomorphism and allozyme correlates of geotaxis in evolved populations.

Lines of *D. melanogaster* that have been intermittently, divergently selected for geotactic performance for approximately half of the 700+ generations since their isolation have evolved phenotypically stable, extreme expressions of geotaxis (Ricker and Hirsch, 1985a). Earlier, relaxed selection had resulted in loss of selection gains. Such regression was described by Dobzhansky and Spassky (1969) as natural selection reasserting itself following the cessation of artificial selection. Despite stability, each line retained a substantial amount of additive genetic variation (i.e. was not homozygous) at the loci influencing geotaxis, evidenced by a response by both lines to reverse selection (Ricker and Hirsch, 1985b, 1988a). Chromosomal analyses had indicated that each of the three major chromosomes influences geotactic behavior (Hirsch and Erlenmeyer-Kimling, 1962; Hirsch and Ksander, 1969; Ricker and Hirsch, 1988 a and b).

We report now the results of an allozyme survey of the positively (Low) and negatively (High) selected geotaxis lines that was an effort to refine the resolution of our understanding of the genetic systems influencing geotaxis from the chromosomal level to the level of gene products (i.e., allozymes). Nineteen gene-enzyme systems known to be polymorphic to varying degrees in the species (see Kojima et al., 1970; Band, 1975) were surveyed. Loci on each of the three major chromosomes were included (see Table 1).

Table 1. The nineteen gene-enzyme systems assayed and their map positions, as well as the number of individuals assayed for each system from the Hight and Low lines. Those gene-enzyme systems fixed for alternative alleles in the selected lines are marked with an asterisk, all others are fixed for the same allele in both lines

Chromosome	Map position	Number of individuals assayed	
		High	Low
X			
*PGD	0.5	40	40
II			
GOT- 2	3.0	30	30
GPDH	17.8	30	30
MDH-1 (c)	37.2	30	30
*ADH	50.1	75	75
GOT-1	75.0	30	30
*AMY	77.7	66	66
III			
EST-6	35.9	54	54
PGM	43.6	30	30
EST-C	47.7	54	54
OCT-DH	49.2	24	24
MEN	51.7	30	30
XDH	52.0	40	40
ALDOX	57.2	24	24
MDH-2 (m)	62.6	24	24
LAP-A	98.3	24	24
LAP-D	98.3	24	24
ACPH	101.1	60	60
Unmapped			
ACON	???	30	30

Abbreviations and symbols are from Ashburner and Gelbart (1991).

Individuals were frozen, stored, homogenized and electrophoresed in Berlocher's laboratory. All gene-enzyme systems were assayed using 12% (w/v) starch gels, except amylase which was assayed using 7% acrylamide gels (see Stoltenberg, in preparation, for procedures). A minimum of 24 individuals from each line was assayed for each gene-enzyme system. A total of 214 individuals were assayed.

The nineteen gene-enzyme systems and their abbreviations are: acid phosphatase (ACPH), aconitase (ACON), alcohol dehydrogenase (ADH), aldehyde oxidase (ALDOX), amylase (AMY), esterase (EST-C, EST-6), glutamate oxaloacetic transaminase (GOT-1, GOT-2), glycerophosphate dehydrogenase (GPDH), leucine amino peptidase (LAP-A, LAP-D) malate dehydrogenase (MDH-1 [cytoplasmic], MDH-2 [mitochondrial]), malic enzyme (MEN), octanol dehydrogenase (ODH), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucomutase (PGM), and xanthine dehydrogenase (XDH).

No electrophoretic variation between the lines was detected at 16 of the 19 loci surveyed. That is, both the High and Low lines are isogenically fixed for the same homozygous allele (listed above) at 16 of the 19 allozymes assayed. Whereas the remaining three allozymes (ADH, AMY and 6PGD) have segregated between the lines with the lines heterogenically fixed for alternative homozygous alleles, i.e. the High line is homozygous for ADH-S, AMY2,3, and 6PGD-A and the Low line is homozygous for ADH-F, AMY1, and 6PGD-B.

Earlier, Rollin Richmond and Steve McKechnie independently had found that the lines were differentiated with respect to the two ADH alleles (both unpublished). This survey confirms their finding and now we report the first evidence for a difference with respect to the AMY and 6PGD loci.

ADH, AMY and 6PGD or closely linked loci may be involved in the genetic systems influencing geotaxis in the selected lines. These allozyme correlates of geotaxis may be acting as genetic markers for geotaxis or the result of a chance fixation due to the population bottlenecks through which the lines have passed (Ricker and Hirsch, 1988a).

References: Ashburner, M. and W. Gelbart 1991, DIS 69; Band, H.T. 1975, Genetics 80:761-771; Dobzhansky, T. and B. Spassky 1969, Proc. Nat. Acad. Sci. USNA 62:75-80; Hirsch, J. and L. Erlenmeyer-Kimling 1962, J. Comp. Physiol. Psych. 55:732-739; Hirsch, J. and G. Ksander 1969, J. Comp. Physiol. Psych. 67:118-122; Kojima, K., J. Gillespie, and Y.N. Tobari 1970, Bioch. Gen. 4:627-637; Ricker, J.P. and J. Hirsch 1985a, DIS 61:141-142; Ricker, J.P. and J.

Hirsch 1985b, J. Comp. Psych. 99:380-390; Ricker, J.P. and J. Hirsch 1988a, J. Comp. Psych. 102:203-214; Ricker, J.P. and J. Hirsch 1988b, Behav. Gen. 18:13-25; Stoltenberg, S.F., in prep. Master's Thesis, Univ. Illinois.

Stoltenberg, S.F., J. Hirsch and S.H. Berlocher. University of Illinois, Urbana-Champaign, Illinois USNA. Alcohol dehydrogenase as a possible genetic marker for geotaxis in evolved populations.

Lines of *D. melanogaster* that have evolved stable extreme expressions of negative and positive geotaxis (Ricker and Hirsch, 1985) are homozygous for different alleles of alcohol dehydrogenase (ADH), amylase (AMY) and 6-phosphogluconate dehydrogenase (6PGD) (Stoltenberg et al. 1992). These allozyme correlates of geotaxis may themselves be involved in the genetic system(s) that influence the behavior (or closely linked to loci that do) or be due to chance fixation as a result of the population bottlenecks through which these lines have passed (Ricker and Hirsch, 1988a). Crossley and Hirsch (1991) report that the ADH genotype is positively associated with geotactic performance in individuals resulting from one reciprocal cross (Low female x High male) and that the association is reversed in the HL category.

We report two experiments designed to clarify and extend the findings of Crossley and Hirsch (1991) by increasing the statistical power of the experiment (by increasing sample size) and by including AMY in the analysis. The first experiment was performed to estimate the recombination rate between ADH and AMY. The structural genes for ADH and AMY are located on the second chromosome at map positions 50.1 and 77.7, respectively (Ashburner and Gelbart, 1991). The second experiment was performed to estimate the effects of ADH and AMY on geotactic performance in F_2 generation individuals in a manner similar to that used by Crossley and Hirsch (1991).

To estimate the recombination rates between ADH and AMY, males and females from the negatively (High) and positively (Low) selected lines were reciprocally crossed (H female x L male, L female x H male). Female F_1 progeny were mated to males from each selected line resulting in four classes of backcross progeny (LH female x H male, LH female x L male, HL female x H male, HL female x L male).

Backcross individuals were then electrophoresed using polyacrylamide gels (7% w/v) and stained for both ADH and AMY in Berlocher's laboratory (see Stoltenberg in preparation for details). A total of 264 individuals were genotyped (see Table 1).

The observed recombination rate between ADH and AMY (0.227) did not differ significantly from the expected rate (0.212) when arcsine transformed proportions were compared ($p = .01$, Cohen, 1988). Thus no deviation from free recombination between ADH and AMY was indicated.

To assess the effects of ADH and AMY on geotactic performance, i.e. their utility as genetic markers for geotaxis, we tested F_2 individuals in Hirsch type geotaxis mazes, then genotyped them for ADH and AMY.

Reciprocal crosses of the High and Low lines were used to produce F_1 generation offspring (HL, LH) that were then mated within each reciprocal cross to produce the F_2 generation. Individuals resulting from reciprocal crosses (HL, LH) were kept separate throughout the experiment.

Samples of approximately 220 F_2 individuals for each sex resulting from each reciprocal cross were tested for geotactic performance. Individuals were then frozen and stored at -70°C in their respective geotaxis categories until they were electrophoresed. Polyacrylamide gel electrophoresis and staining for ADH and AMY were carried out as above.

Results of ANOVA indicate that Sex and ADH genotype each accounted for a significant amount of variance in geotaxis score ($p = .0001$, see Table 2). The effects of Mating type (HL, LH) and AMY genotype on geotaxis score were not statistically significant ($p = .06$ and .56 respectively). The Mating x Sex and Mating x AMY interactions are interesting, but do not reach statistical significance when the total number of comparisons is considered due to the fact that when many comparisons are made the likelihood of finding differences due to chance increases. Therefore the significance level should account for the number of comparisons made (i.e. alpha = .05/15 comparisons = 0.003).

X-chromosome effects on geotaxis have been documented (Erlenmeyer-Kimling et al. 1962, Hirsch and Ksander 1969, Ricker and Hirsch 1988 a and b). They are consistent with our finding of an effect of Sex. It is interesting that the High and Low lines are homozygous for alternative alleles of 6PGD (Stoltenberg et al. 1992) as the structural gene coding for this enzyme is located on the X-chromosome. The association of 6PGD and geotaxis has yet to be studied.

That ADH genotype has a significant effect on geotactic performance in F_2 generation individuals indicates the utility of ADH as a genetic marker for geotaxis in these lines, whereas AMY does not appear to be a genetic marker. Table 3 shows a significant performance bias in F_2 generation individuals with parental line ADH genotypes in the expected direction (i.e. ADH S/S-high, ADH F/F-low). This association is consistent for each reciprocal cross. These results extend the finding of Crossley and Hirsch (1991) with a substantially increased sample size (i.e., from n = 87 to

n = 773).

Thus, it appears that the structural gene for ADH or (a) closely linked loci (locus) play(s) some role in the genetic system(s) that influence geotaxis in the High and Low lines. The ADH region on chromosome II would be a reasonable starting point in the search for gene correlates of geotaxis in the selected lines.

Table 1 Genotypic distributions of the progeny from each backcross are presented. Recombinant types are marked with an asterisk (*)

Genotype (ADH-AMY)	Backcross Type HL♀ x L♂	LH♀ x L♂	HL♀ x H♂	LH♀ x H♂
S--2,3 / F--1	20	27		
S--1 / F--1*	8	7		
F--2,3 / F--1*	4	10		
F--1 / F--1	23	26		
S--2,3 / S--2,3			21	30
S--1 / S--2,3*			4	5
F--2,3 / S--2,3 *			9	13
F--1 / S--2,3			26	31

Table 2. ANOVA partitioning of variance in geotaxis score into mating (LH or HL), Sex, AMY, ADH, and associated interactions. Degrees of freedom as well as F and p values are given

Source	ANOVA Df	F	p
Model	27	8.63	0.0001
Mating	1	2.90	0.09
Sex	1	37.91	0.0001
AMY	2	0.57	0.56
ADH	2	12.89	0.0001
Mating x Sex	1	6.07	0.01
Mating x AMY	2	2.89	0.06
Mating x ADH	2	0.46	0.63
Sex x AMY	2	1.78	0.17
Sex x ADH	2	0.34	0.71
AMY x ADH	2	1.25	0.29
Mating x Sex x AMY	2	0.32	0.73
Mating x Sex x ADH	2	1.00	0.37
Mating x AMY x ADH	2	1.12	0.33
Sex x AMY x ADH	2	1.89	0.15
Mating x Sex x AMY x ADH	2	4.63	0.01

Table 3. The percentage of individuals from parental line (i.e. High and Low) and F_2 generation geotaxis distributions that were found in "low" (0 - 3) and "high" (12 - 15) geotaxis categories. Only individuals that were ADH homozygotes (F/F and S/S) are included (i.e. F_2 generation ADH heterozygotes are not shown).

	Percent Geotaxis Categories 0 - 3	12 - 15	N
Low ADH F/F	78.4	0.0	445
High ADH S/S	0.0	92.8	431
F_2 ADH F/F	34.6	23.2	211
F_2 ADH S/S	12.0	54.3	184

References: Ashburner, M. and W. Gelbart 1991, DIS 69; Cohen, J. 1988, Statistical Power Analysis For The Behavioral Sciences, Lawrence Erlbaum Associates, Inc., 179-213; Crossley, S. and J. Hirsch 1991, DIS 70:54-57; Dobzhansky, T. and B. Spassky 1969, Proc. Nat. Acad. Sci. USNA 62:75-80; Erlenmeyer-Kimling, L., J. Hirsch, and J. Weiss 1962, J. Comp. Physiol. Psych. 55:722-731; Hirsch, J. and G. Ksander 1969, J. Comp. Physiol. Psych. 67: 118-122; Ricker, J.P. and J. Hirsch 1985, DIS 61: 141-142; Ricker, J.P. and J. Hirsch 1988a, J. Comp. Psych. 102:203-214; Ricker, J.P. and J. Hirsch 1988b, Behav. Gen. 18:13-25; Stoltenberg, S.F., in prep. Master's Thesis, Univ. Illinois; Stoltenberg, S.F., J. Hirsch, and S.H. Berlocher 1992, DIS 71:185-186.

Cusick, S.M. and J.G. Pelliccia. Department of Biology, Bates College, Lewiston, Maine. Chlorate inhibits fertility in *Drosophila*.

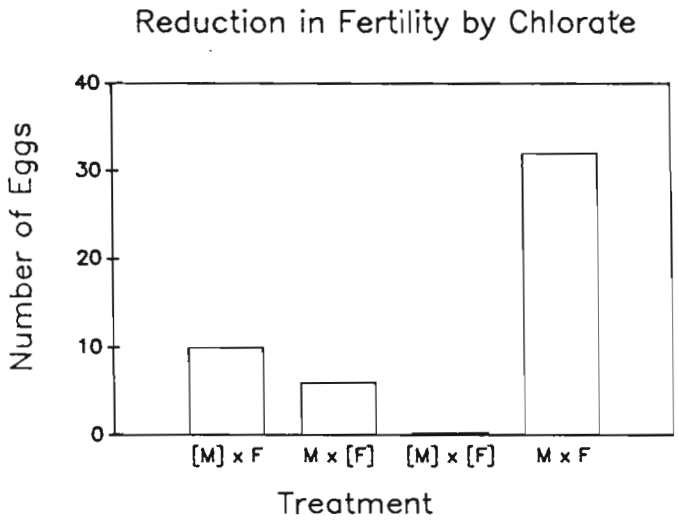
Chlorate is a sulfate analogue that can inhibit the sulfation of proteins in intact cells (Baeuerle and Huttner, 1986). Chlorate works by inhibiting the action of ATP-sulfurylase which is an enzyme required for the production of PAPS (3'-phosphoadenosine 5'-phosphosulfate), the sulfate donor in eukaryotic sulfate conjugations. One enzyme whose function is thus affected is tyrosyl protein sulfotransferase (TPST).

We have found that adult flies exposed to sodium chlorate at concentrations up to 100 mM show no obvious loss in viability. However, these flies produce no progeny. We looked more closely at this phenomenon by separately treating previously mated adult males and females with chlorate by culturing them for 24 hours on Carolina Instant Drosophila Medium 4-24 reconstituted with 100 mM sodium chlorate. Males and 5-7 females were then mixed and eggs were collected for 5 hours on agar laying plates. Results are shown in Figure 1. Bracketed symbols indicate that flies were treated with chlorate prior to egg collection. Unbracketed symbols indicate control flies.

The data show that when females are treated with chlorate, egg production decreases roughly five fold. Surprisingly, treatment of males alone resulted in a three fold decrease in egg laying while treating both males and females caused no eggs to be laid. This inhibition of egg laying was rapidly reversible when chlorate treated adults were

transferred to fresh, control media.

We tested the effect of chlorate on embryonic and larval development in two ways. Eggs collected on laying plates from control females were placed on filter paper soaked in a chlorate, sucrose, yeast extract solution. These eggs hatched and development proceeded normally. However, if we cultured untreated adult males and females in a vial containing media reconstituted with chlorate, no larval development was observed. We assume, in this experiment, that some of the first eggs laid would not be affected by chlorate if the primary route of chlorate exposure requires ingestion by the female. Yet, even these eggs could not develop. Thus, there must be a functional difference between an egg's exposure to chlorate on filter paper and exposure made upon reconstituted media.



Chlorate inhibits PAPS production and thus all of a fly's sulfotransferases should be affected. Baeuerle and Huttner (1985) showed that, although the spectrum of sulfated proteins in male and female flies was similar if protein bound oligosaccharide sulfation was followed, the patterns of tyrosine sulfation were quite different due to the presence of sulfated yolk proteins in the female. Preliminary results in our lab show that TPST function is affected by a reduction in PAPS and that the sulfation of yolk proteins is reduced by chlorate. This fact may account for the observed decrease in female fertility. The explanation for the decrease in male fertility is unclear but sulfation of at least one abundant protein in males is also reduced by chlorate. We are interested in looking more closely at the affects of chlorate on protein sulfation and on fertility.

References: Baeuerle, P.A. and W.B. Huttner 1985, J. Biol. Chem. 260: 6434-6439; Baeuerle, P.A., and W.B. Huttner 1986, Biochem. Biophys. Res. Comm. 141: 870-877.

Eisses, K. Th. and P. Bets. Department of Plant Ecology and Evolutionary Biology, Rijksuniversiteit Utrecht, The Netherlands. Attraction of *Drosophila melanogaster* toward acetic acid and ethanol dependent on alcohol dehydrogenase alleles.

Effects of ethanol on the *Drosophila* alcohol dehydrogenase (*Adh*) polymorphism in natural and in laboratory conditions and the role of ADH activity in adults and larvae have been studied extensively. The product of ethanol oxidation by ADH and micro organisms is acetic acid, which is a major compound in the *Drosophila* niche besides ethanol. However, its role in the interaction with different *Adh* gene-enzyme systems has been neglected. Whether acetic acid poses any selective forces, and in which direction, on different *Adh* genotypes or on different stages of the life cycle of *D. melanogaster* has not yet been studied.

The adaptation process is determined by the interaction between species and their environment. One of the most important components of this interaction in insects is the oviposition-site, because of the restricted area that larvae can explore. The oviposition-sites for a certain species are characterized by a specific smell, caused by one or several chemical components, that can be detected somehow by the olfactory sense of the organism involved. Olfactory response toward volatile chemical determinants of a certain bait is a main factor in habitat selection. Fermenting fruit is very attractive to *D. melanogaster,* as are some of the natural compounds of fermenting and degrading fruit in olfactometers, e.g. acetic acid (Barrows, 1907; Fuyama, 1976). Acetic acid acts as an attractant in olfactory measurements in very low doses; down to 1/1000 of that of ethanol to provoke a response (Fuyama, 1976; Parsons and Spence, 1981). Acetic acid not only attracts flies to the bait or oviposition habitat, but it has a profound influence on the oviposition rhythm in *D. melanogaster*. Pulses of acetic acid fumes induce oviposition on a neutral medium (Fluegel, 1981). Laudien and Iken (1977) posed that the choice of special substrates for egg-deposition is according to the smell experienced as larvae and that it is a form of ecological imprinting. They measured a high preference for oviposition on acetic acid from adults developed in an initially 8 % ethanol medium. Because of microbial ethanol oxidation the third stage larvae, started as eggs on ethanol medium, certainly encounter appreciable levels of acetic acid (Hageman et al., 1990). McKenzie and

McKechnie (1979) measured ethanol and acetic acid concentrations in a transect of a degrading grape pile at different stages of degradation. At stage 2 they measured at the surface 4.7 % (v/v) acetic acid and below the surface as far as a depth of 10 cm the larvae were concentrated in a zone with 7.1 % ethanol and 3.1 % acetic acid. Laying eggs at this surface enable the *D. melanogaster* larvae to dig into a zone without interspecific competition with *D. simulans*. The presence of the olfactory stimulus acetic acid possibly implies simultaneously the presence of certain levels of ethanol, acting in this way as a resource recognition compound (Ehrman and Parsons, 1981).

Olfactory responses not only differ between species but also between geographic strains of one species (Parsons and Spence, 1981; Parsons 1982), so genetic variation exists for this trait. We have tried to measure the involvement of ADH activity variation in choice behavior as proposed by Gelfand and McDonald (1980), but extended the experiment into a multiple choice including acetic acid. Four *Adh* genotypes of *D. melanogaster*, Adh^{F}, Adh^{S}, $bAdh^{n4}$, and Adh^{71k}

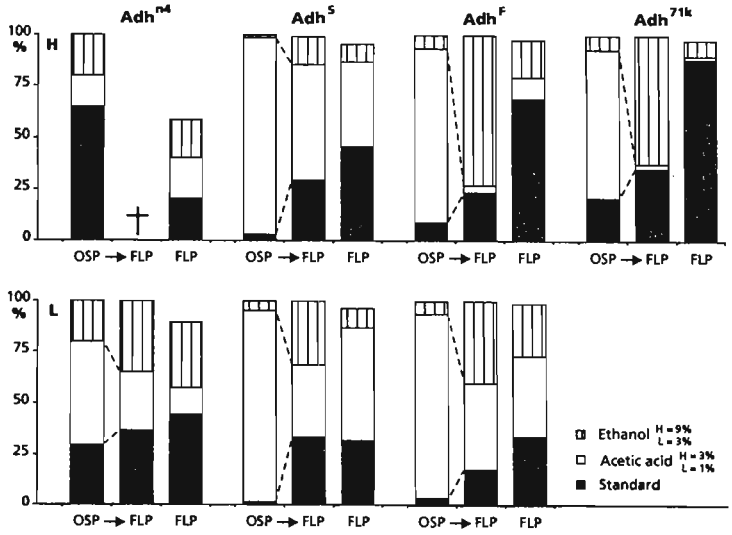


Figure 1. The stacked bars are grouped per genotype. The left bars show the percentages of eggs laid during 24 h on the three medium types. The middle ones show the medium preference distribution of the first instar larvae, starting from the oviposition sites that are distributed according to the OSP bars. The right bars show the medium preference distribution of first instar larvae, starting from the disc center. In the latter case eggs were laid on plain agar, collected, counted and transferred to the choice disc. L = low concentrations of acetic acid (1%) and ethanol (3%), H = higher concentrations of acetic acid (3%) and ethanol (9%).

were tested for Oviposition Site Preference (OSP) and First instar Larval food Preference (FLP) using a Multiple Choice Device (Eisses, 1991). FLP has been measured independently and combined with an OSP experiment. Three medium types were offered simultaneously in two concentration ranges: standard sugar-yeast medium, and ethanol and acetic acid supplemented medium (Figure 1).

* The strains showed significantly different OSP and FLP distribution patterns (Figure 1). Acetic acid supplemented medium was predominantly chosen as oviposition site, whereas larvae avoided this substrate and even crawled away when hatching on this medium. Adh^{S} larvae did not show much aversion toward acetic acid.

* The $bAdh^{n4}$ flies proved to be less fastidious with respect to OSP; even ethanol patches were chosen. The absence of ADH activity has likely to do with this phenomenon (Gelfand and McDonald, 1980). Even when choices could be made, these choices did not prevent larvae from an early death in the chosen medium, especially when $bAdh^{n4}$ is concerned.

* Much more larvae preferred ethanol supplemented patches when laid as egg on acetic acid, in contrast with larvae hatching from eggs laid on a plain agar medium. Acetic acid seemed to trigger ethanol attraction.

References: Barrows, W. M., 1907, J. Exp. Zool. 4:511-535; Ehrman, L. and P. A Parsons, 1981, Behavior Genetics and Evolution. McGraw-Hill, New York; Eisses, K. Th., 1991, DIS 70:241-242; Fluegel, W., 1981, J. Insect Physiol. 27:705-710; Fuyama, Y., 1976, Behav. Genet. 6:407-420; Gelfand, L. J. and J. F. McDonald, 1980, Behav. Genet. 10:237-249; Hageman, J., K. Th. Eisses, P. J. M. Jacobs and W. Scharloo, 1990, Evolution 44:447-454; Laudien, H. and H. H. Iken, 1977, Z. Tierpsychol. 44:113-129; McKenzie, J. A. and S. W. McKechnie, 1979, Oecologia 40:299-309; Parsons, P. A. and G. E. Spence, 1981, Austral. J. Zool. 29:671-678.

Eisses, K. Th. Department of Plant Ecology and Evolutionary Biology, Rijksuniversiteit Utrecht, The Netherlands. Expression of heterozygous *radius-incompletus* dependent on different Octanol Dehydrogenase and Alcohol Dehydrogenase alleles.

A *Cy/Pm;TM3/Pr* strain was used to construct strains homozygous for certain combinations of Alcohol Dehydrogenase (*Adh*) and Octanol Dehydrogenase (*Odh*) alleles. The *TM3* balancer chromosome has dominant markers *Serrate* and *Stubble*, and a recessive marker *radius-incompletus* (*ri*). At a certain stage of the crosses I noticed *TM3* flies with an interruption of vein L2 at the wing edge (Figure 1b). Later on *TM3* flies with almost completely deleted L2 veins were noticed (Figure 1a) as in homozygous *ri* (Lindsley and Grell, 1968), or flies with L2 veins interrupted in the middle (Figure 1c).

Then I observed large differences in the penetrance of heterozygous *ri* between *TM3* flies combined with different *Odh* alleles (Table 1). The effect must be closely linked to the *Odh* allele, because $TM3/Odh^S$ flies were crossed with Odh^F homozygotes, and $TM3/Odh^F$ flies with Odh^S homozygotes. Parental flies with low penetrance produced progeny with high penetrance, and vice versa (Table 2). All homozygous *Odh* flies were wild type with respect to wing phenotype, without

Table 1. *ri* pentrance in heterozygotes

	mean over all *Adh* strains (range)
$TM3/Odh^F$	81.1 (53- 99%)
$TM3/Odh^S$	23.0 (2- 40%)
$TM3/Odh^{NC1}$	3.6* (0- 42%)
$TM3/Odh^{NC1}$	1.1** (0- 4%)

* included b Adh^{nLA248} *cn bw* strain
** without b Adh^{nLA248} *cn bw* strain

Table 2. *ri* penetrance in heterozygotes

	parents	cross	progeny
b Adh^{nLA248} *cn bw*; $TM3/Odh^F$	94.6	Odh^S/Odh^S	3
b Adh^{nLA248} *cn bw*; $TM3/Odh^S$	39.3	Odh^F/Odh^F	100

Table 3. Apparent involvement of Alcohol dehydrogenase alleles or second chromosome interactions in *ri* pentrance

Adh^F; $TM3/Odh^S$	36.4	Adh^F; $TM3/Odh^F$	98.9
Adh^S; $TM3/Odh^S$	17.2	Adh^S; $TM3/Odh^F$	83.0
Adh^{n4}; $TM3/Odh^S$	2.9	Adh^{n4}; $TM3/Odh^F$	58.9
Adh^{71k}; $TM3/Odh^S$	1.8	Adh^{71k}; $TM3/Odh^F$	53.3

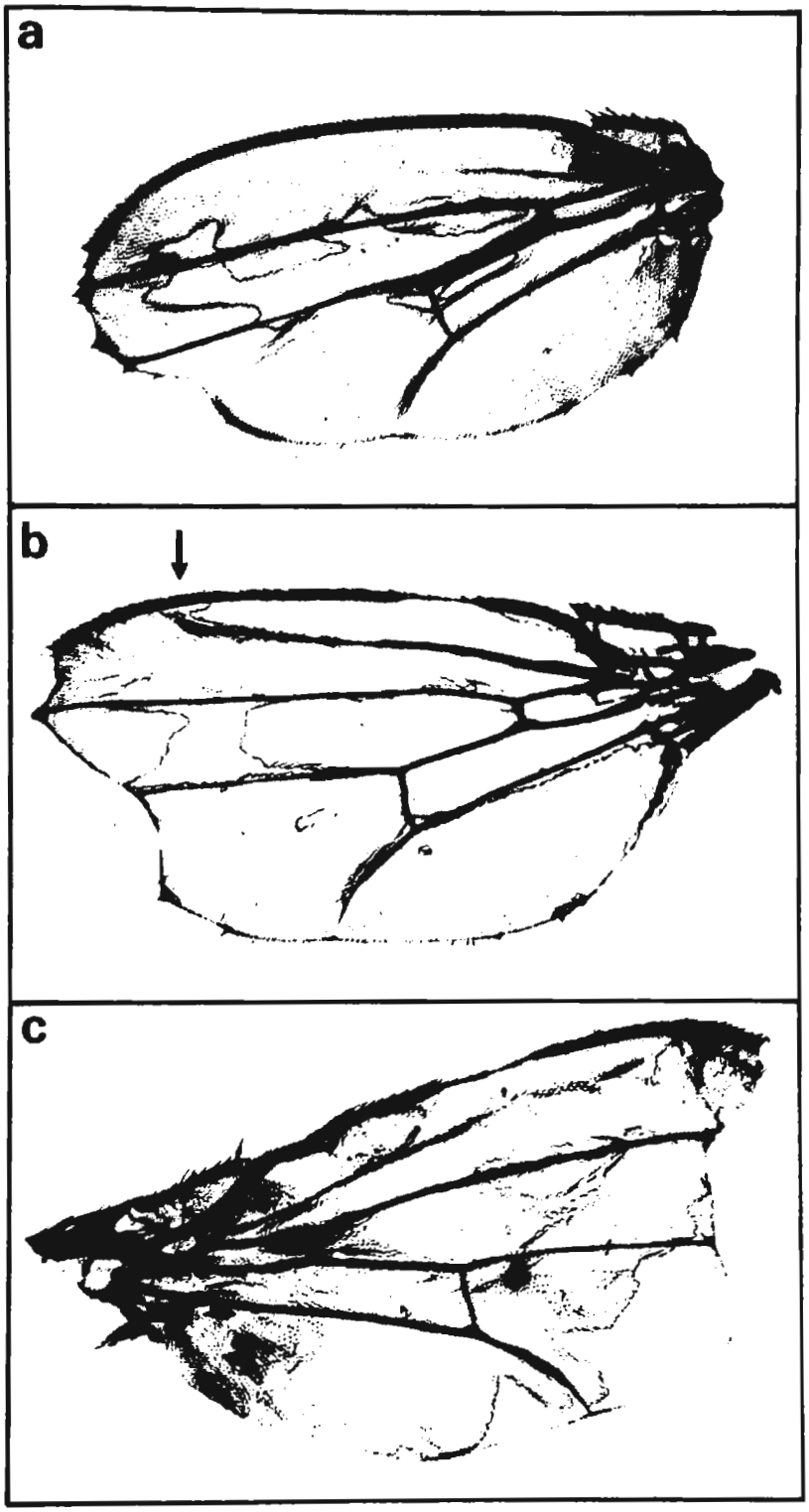


Figure 1. Wing phenotypes of TM3 (*Ser ri*) flies with various expressions of *radius-incompletus* penetrance. a: large deletion of L2 vein as in homozygous *ri* flies; b: small deletion of L2 vein at the wing edge; c: interruptions in L2 vein.

without any *ri* expression. Although a difference between the mean penetrances in *TM3/Odh*^{F}, *TM3/Odh*^{S} and *TM3/Odh*^{NC1} strains is noticeable, the wide range of values is possibly due to interactions with second chromosomes, defined by different *Adh* alleles. A consistent pattern of values can be noticed (Table 3). The *b Adh*^{nLA248} *cn bw* strain is an exceptional one because it was the only homozygous *Adh* strain among twenty that showed L2 vein deletions at the wing edge in combination with *TM3/Pr*, as in Figure 1b. Moreover it was the only strain with a rather high *ri* penetrance, in combination with *TM3/Odh*^{NC1} (Table 1). The *Adh*^{71k} strain with high ADH activity showed a very low penetrance, so it is probably not the *Adh* locus itself that is involved. The genes for *Odh* (3:49.2) and *ri* (3:46) are in close vicinity, but the nature of this penetrance phenomenon remains unclear. One could think of *ri* modifiers linked to the *Odh* locus.

Emans, H. J. B. and K. Th. Eisses. Department of Plant Ecology and Evolutionary Biology, Rijksuniversiteit Utrecht, The Netherlands. Acetic acid: a neglected selective force on *Adh* polymorphism in *Drosophila melanogaster*.

For many years studies on the *alcohol dehydrogenase* gene-enzyme polymorphism have been concentrated on the role of environmental ethanol in selection processes. The presence of active ADH allows larvae and flies to tolerate considerable amounts of ethanol, whereas *Adh-null* mutants die with rather low concentrations. However, in non-sterile conditions ethanol is rapidly converted by microorganisms into acetic acid (Hageman *et al.*, 1990). In natural environments both ethanol and acetic acid are present (Barrows, 1907; McKenzie and McKechnie, 1979). See Eisses and Bets (1992) for other implications of acetic acid on *D. melanogaster*. The question can be posed whether or not acetic acid as a product of ethanol oxidation acts differently on flies and larvae with different *Adh* alleles.

Four *Adh* genotypes of *D. melanogaster*, *Adh*^{71k}, *Adh*^{F}, *Adh*^{S}, and *bAdh*^{n4} with decreasing ADH activity in this order, were tested in experiments on adult longevity. Glass vials with 400 mg cotton wool and 4 ml of 1% sucrose

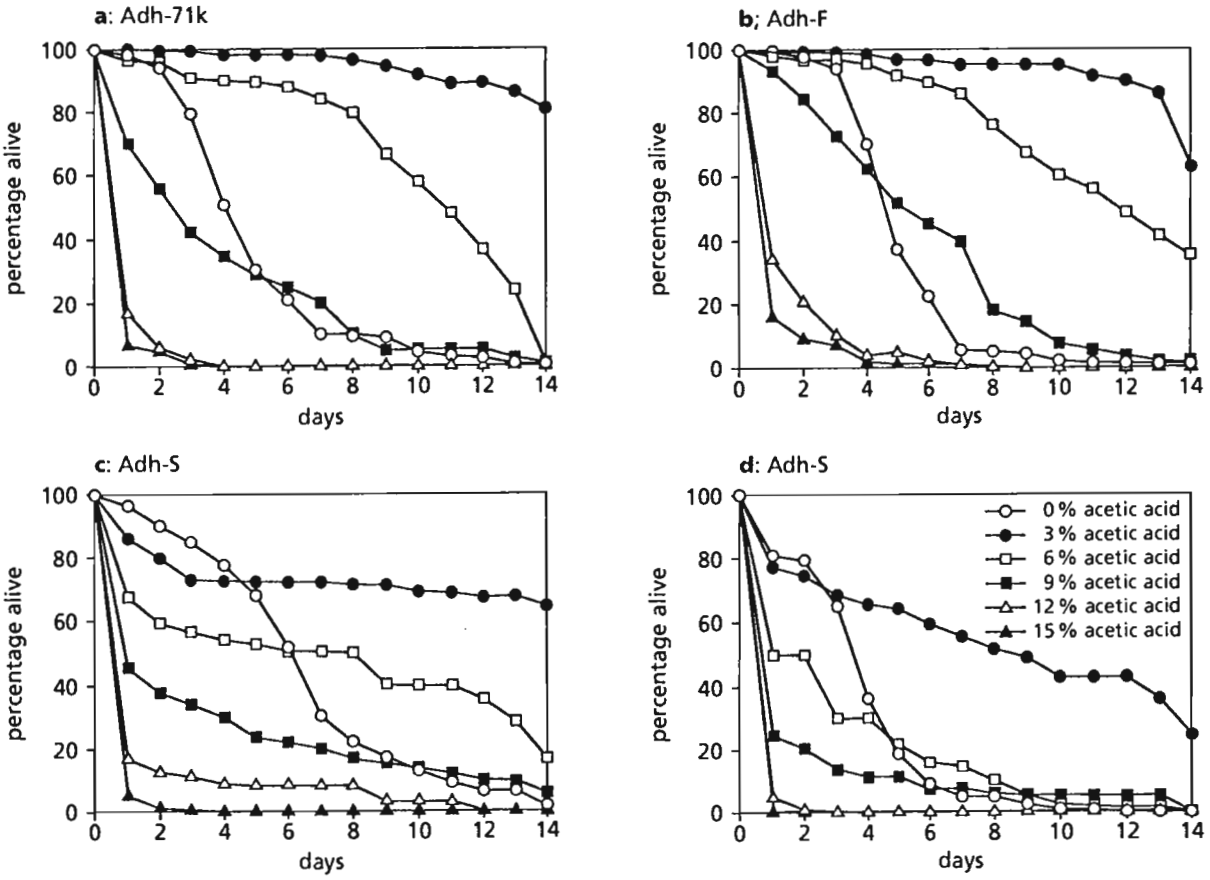


Figure 1. Percentage of surviving flies during 14 days. Four replicates of 20 flies each per strain per concentration. Four *Drosophila melanogaster* strains, homozygous for alcohol dehydrogenase alleles *Adh*^{71k}, *Adh*^{F}, *Adh*^{S}, and *bAdh*^{n4} were tested.

solution supplemented with various concentrations of acetic acid were used. Per concentration four replicates of each strain with 10 males and 10 females (aged 1-2 days) per vial were placed in a climate room at 25°C and 60% Relative Humidity. Surviving flies were scored during 14 days. The LT50-values, the time at which 50% of the flies had died, were calculated by linear interpolation, using the rankit method.

Figure 1 shows the beneficial effect of 3% acetic acid on the longevity of the flies from all strains, whereas 12 and 15% acetic acid is disadvantageous. Because of different LT50(control) values the relative LT50-values of each strain were calculated as the ratio of LT50/LT50(control) (Parsons, 1982). Figure 2 shows the positive effect of low concentrations of acetic acid on the adult longevity and the negative effect of high concentrations. Differences can be noticed between the strains in the extent of the positive effect of 3% acetic acid. The ranking order is the same as with ADH activity. The strains differ also in the concentrations of acetic acid at which the curves cross the line LT50/LT50(control) = 1 (threshold concentration). *Adh-null* cross the line at the lowest concentration, whereas Adh^{71k} and Adh^{F} are the least sensitive strains. The experiments have demonstrated that similar curves occurred with acetic acid as occurred with ethanol when using strains differing in ADH activity (Parsons and Spence, 1981; Parsons, 1982). The effects of acetic acid on longevity point in the same direction as the effect of ethanol with similar ADH activity rankings. An explanation for this phenomenon could be a correlated expression of enzymes involved with the detoxification and metabolism of ethanol, including an intermediate substrate as acetic acid.

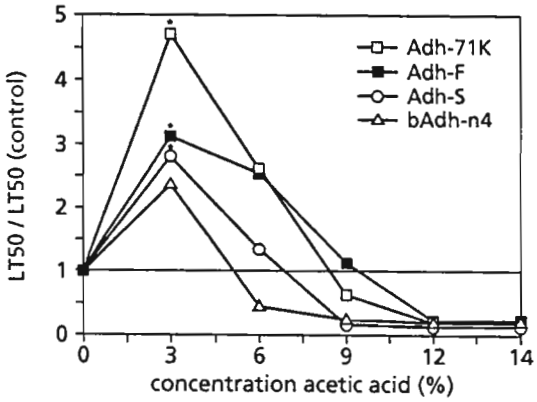


Figure 2. Relative longevity of four *Adh* strains of *D. melanogaster* expressed as the ratio of LT50/LT50(control). * means that the LT50-value has been extrapolated, because more than 50% of the flies were still alive.

References: Barrows, W. M., 1907, J. Exp. Zool. 4:511-535; Eisses, K. Th., 1991, DIS 70:241-242; Hageman, J., K. Th. Eisses, P. J. M. Jacobs and W. Scharloo, 1990, Evolution 44:447-454; McKenzie, J. A. and S. W. McKechnie, 1979, Oecologia 40:299-309; Parsons, P. A., 1982, Aust. J. Zool. 30:427-433; Parsons, P. A. and G. E. Spence, 1981, Austral. J. Zool. 29:671-678.

Bruins, B.G., W. Scharloo and G.E.W. Thörig. Department of Plant Ecology and Evolutionary Biology, Rijksuniversiteit Utrecht, The Netherlands. Dietary vitamin C protects *Drosophila simulans* against light.

Light is an important environmental factor, which influences survival and developmental time of *Drosophila melanogaster* larvae (Northrop, 1925; Kyriacou, 1990; Bruins et al., 1991a and b). Light increases mortality and retards developmental time from egg to adulthood. We showed that the effect of light is diet dependent (Bruins et al., 1991a). High yeast concentrations in the food protect larvae and pupae against the injurious effect of light. At low yeast concentrations larvae and pupae become extremely light sensitive. Survival under high light intensities improves strongly on media supplemented with vitamin C (Bruins et al., 1991b).

Table 1. Survival from egg to adulthood of *Drosophila simulans* and *Drosophila melanogaster* (expressed as % flies) at two different light intensities on 16G media (16 g yeast/l) supplemented with vitamin C (11 mM). Four vials were tested (each with 100 eggs) at each condition. The light sources were Philips 58W/33 fluorescent tubes. Both species do have the same geographic origin (Ormos Panagias, Greece).

	60 lx	400 lx
Sim. 16G	33.5 ± 4.4	0.25 ± 0.5
Sim. 16G + Vit C	33.5 ± 7.7	28.8 ± 04.9
Mel. 16G	71.5 ± 4.1	10.5 ± 11.7
Mel. 16G + Vit C	69.3 ± 5.1	68.0 ± 01.6

The present study shows that *Drosophila simulans* reacts in a similar way (Table 1). *Drosophila simulans* is light sensitive, light induces high mortality during the development from egg to adulthood and retards larval developmental time. Moreover survival under high light intensities is improved on media supplemented with vitamin C. Besides there are differences between the two sibling species. Survival under low light intensities is lower in *simulans* than in *melanogaster*. However, *simulans* is more sensitive to light than *melanogaster*. Because light induces high mortality we expect that this light sensitivity finds expression in phototactic behavior. Measurements of the slow phototactic response show 'dark reactions' (Jacob

et al., 1977). The initially positive response at low light intensities changes into a negative one at high light intensities. Kekić and Marinković (1979) showed that flies avoid in their experimental design the compartments with the highest light intensities. Light induced mortality could be an important factor underlying photonegative behavior of *Drosophila*. However, the possibility that light induced mortality and phototactic behavior are correlated in *Drosophila* needs further investigation.

References: Bruins, B.G., W. Scharloo and G.E.W. Thörig 1991a, Insect Biochem. 21: 535-539; Bruins, B.G., W. Scharloo and G.E.W. Thörig 1991b, Insect Biochem. 21: 541-544; Jacob, K.G., R. Willmund, E. Folkers, K.F. Fischbach and H. Ch. Spatz 1977, J. comp. Physiol. 116: 209-225; Kekić, V. and D. Marinković 1979, Aquilo Ser. Zool. 20: 118-128; Kyriacou, C.P., M. Oldroyd, J. Wood, M. Sharp and M. Hill 1990, Heredity 64: 395-401; Northrop, J.H. 1925, J. gen. Physiol. 9: 81-86.

Band, H. T. Michigan State University, E. Lansing, MI 48824 and UVA's Mt. Lake Biological Station, Pembroke, VA 24136. An update on *Drosophila* work at chilling and subzero temperatures.

The following list of references on drosophilid chilling and cold survival has been compiled from Cryobiology News Notes and other sources. Papers and books published since 1988 are included and any earlier works not in Band (1991) DIS 70. Note that the successful cryopreservation of *D. melanogaster* embryos, adult emergence and fertility have been achieved (Steponkus et al. 1990). Of more general interest is the finding now that heat shock protects against cold shock. Such diverse species as the flesh fly (Joplin et al. 1990) and spinach (Neven et al. 1990) produce proteins related to hsp70 of *Drosophila melanogaster*.

Burton, V., H. K. Mitchell, D. Young and N. S. Peterson. 1989. Heat shock protection against cold stress of *Drosophila melanogaster*. Mol. Cell Biol. 8: 3550-3552.

Czajka, M. L. and R. E. Lee, Jr. 1990. A rapid cold-hardening response protecting against cold shock injury in *Drosophila melanogaster*. J. exp. Biol. 148: 245-254.

Denlinger, D. L., K. H. Joplin, C-P. Chen, and R. E. Lee, Jr. 1990. Cold shock and heat shock, pp. 131-148. In R. E. Lee and D. L. Denlinger (eds.). Insects at Low Temperature. Chapman and Hall, New York.

Hoffman, A. A. and P. A. Parsons. 1991. Evolutionary genetics and environmental stress. Oxford University Press, Oxford.

Izquierd, J. I. 1991. How does *Drosophila melanogaster* overwinter? Ent. exp. appl. 59 (1): 51-58.

Joplin, K. H., G. D. Yocum, and D. L. Denlinger. 1990. Heat shock proteins are induced by cold shock in the brain and integument of *Sarcophaga crassipalpis*. Cryobiology 27: 661.

Kimura, M. T. 1980. Bionomics of Drosophilidae (Diptera) in Hokkaido. IV *Drosophila sexuittata, D. trivittata*, and *D. alboralis*. Zool. Mag. (Tokyo) 89: 206-209.

_____. 1988a. Adaptations to temperate climates and evolution of overwintering strategies in the *Drosophila melanogaster* species group. Evolution 42: 1288-1297.

_____. 1988b. Interspecific and geographical variation of diapause intensity and seasonal adaptation in the *Drosophila auraria* species complex (Diptera: Drosophilidae). Functional Ecology 2: 177-183.

Lee, R. E., Jr. and D. L. Denlinger (eds.). 1991. Insects at Low Temperature. Chapman and Hall, New York.

_____, C-F. Chen and D. L. Denlinger. 1987. A rapid cold-hardening process in insects. Science 238: 1415-1417.

Leibo, S. P., S. P. Myers, R. E. Pitt and P. L. Steponkus. 1988. Survival of *Drosophila melanogaster* embryos cooled to subzero temperature. Cryobiology 25:545-546.

Lin, T. T., R. E. Pitt, and P. L. Steponkus. 1988. Permeability of *Drosophila melanogaster* embryos to ethylene glycol and glycerol. Cryobiology 25:527-528.

_____, _____, and _____. 1989. Osometric behavior of *Drosophila melanogaster* embryos. Cryobiology 26: 453-471.

Lynch, D. V., S. P. Myers, S. P. Leibo, R. J. MacIntyre, R. E. Pitt, and P. L. Steponkus. 1988. Permeabilization of *Drosophila* eggs using isopropanol and hexane. DIS 67: 89-90.

_____, T. T. Lin, S. P. Myers, S. P. Leibo, R. J. MacIntyre, R. E. Pitt and P. L. Steponkus. 1989. A two-step method for permeabilization of *Drosophila* eggs. Cryobiology 26: 445-452.

Mazur, P. 1990. Kinetics of killing of unfrozen intact *Drosophila* embryos at low temperaures. Cryobiology 27: 650.

_____, K. W. Cole, P. D. Schreuders, and A. P. Mahowald. 1991. Confirmation of the ability of permeabilized 12 hr *Drosophila* embryos to survive cooling to -200°C. Cryobiology 28:524-525.

_____, U. Schneider, K. E. Jackson and A. P. Mahowald. 1988. Chilling injury in intact *Drosophila* eggs at various stages of embryonic development between 0° and -25°C in the absence of ice formation. Cryobiology 25: 544.

_____, _____, and A. P. Mahowald. 1992. Characteristics and kinetics of subzero chilling injury to *Drosophila* embryos. Cryobiology 29: 39-68.

Myers, S. P., D. V. Lynch, R. E. Pitt and P. L. Steponkus. 1987. Cryobiology of *Drosophila* embryos. Cryobiology 24: 549.

_____, _____, D. C. Kipple, S. P. Leibo and P. L. Steponkus. 1988. Tolerance of *Drosophila melanogaster* embryos to ethylene glycol. Cryobiology 25: 545.

_____, R. E. Pitt, D. V. Lynch and P. L. Steponkus. 1989. Characterization of intracellular ice formation in *Drosophila melanogaster* embryos. Cryobiology 26: 472-484.

_____, T. T. Lin, R. E. Pitt and P. L. Steponkus. 1989. Tolerance of *Drosophila melanogaster* embryos to permeating cryoprotectants. Cryobiology 26: 550.

Myers, S. P. and P. L. Steponkus. 1990. Subzero chilling sensitivity of *Drosophila melanogaster* embryos. Cryobiology 27: 651-652.

Neven, L. G., D. W. Haskell, and C. L. Guy. 1990. A heat shock cognate comes out in the cold. Cryobiology 27: 661.

Pitt, R. E., T. T. Lin, S. P. Myers, and P. L. Steponkus. 1989. Intracellular ice formation in *Drosophila melanogaster* embryos: implications for conventional cryopreservation. Cryobiology 26: 550.

Rancourt, D. L. 1990. Holfish antifreeze protein from transgenic *Drosophila*. Bio-Technol. 8 (5): 453-457.

Saunders, D. S., V. C. Henrich, and L. I. Gibert. 1989. Induction of diapause in *Drosophila melanogaster* photoperiodic regulation and the impact of arrhythmic clock mutations on time measurement. Proc. Nat. Acad. Sci., U.S.A. 86: 3748-3752.

Shimada, K., and A. Riihimaa. 1990. Cold-induced freezing tolerance in diapausing and non-diapausing larvae of *Chymomyza costata* (Diptera:Drosophilidae) with accumulation of trehalose and proline. Cryo-Letters 11: 243-250.

Steponkus, P. L. 1990. Cryopreservation of *Drosophila melanogaster* embryos. Nature 345 (6271): 170-172.

_____. 1991a. Cryobiology of *Drosophila melanogaster* embryos, pp. 408-423. In R. E. Lee, Jr. and D. L. Denlinger (eds.). Insects at Low Temperature. Chapman and Hall, New York.

_____, S. P. Myers, and L. C. Gardner. 1990. Cryopreservation of *Drosophila melanogaster* embryos: recovery of adults. Cryobiology 27: 651.

Band, H. T. Michigan State University, E. Lansing, MI 48824 and UVA's Mt. Lake Biological Station, Pembroke, VA 24136. Lack of evidence for clinal speciation in Virginia *Chymomyza amoena*.

The Hawaiian *Drosophila* provide a laboratory for the study of speciation. *Drosophila silvestris* and *D. heteroneura* are sibling species diverging in head shape and behavior (Templeton, 1977; Spieth, 1981) but F_1 and F_2 are fully fertile. Asymmetric isolation has been reviewed by Kaneshiro (1989). Typically, females of an ancestral population accept males from both ancestral and descendant populations in contrast to females of a descendant population who mate only with descendant males. Female oviposition and larval feeding behavior divide *Drosophila grimshawi* into generalist and specialist groups. Ecologically similar populations remain interfertile. Some degree of hybrid sterility exists between ecologically dissimilar populations (review: Ohta, 1989). A fourth type, called clinal speciation, occurs when populations at either end of a geographic distribution are reproductively incompatible (Murray, 1972). Laboratory incompatibility of *D. melanogaster* males and females grown at different temperatures (Cohet and David, 1974) foreshadowed the discovery of geographical races of this species (Cohet and David, 1980), also an example that weedy species do not speciate (Carson, 1975).

Chymomyza amoena in Virginia gave tentative evidence of clinal speciation. No progeny resulted from crosses of mountain (Rt. 700) and Pamplin populations, the easternmost locality from which *C. amoena* populations have been obtained (Band 1988a). It was also a time of severe drought in the South.

Determination that *C. amoena* was breeding in acorns on the Mt. Lake Biological Station grounds made it advisable to repeat the crosses. Acorn emergees readily oviposited and larvae developed in apples (Band, 1991), demonstrating the same generalist nut/fruit breeding behavior of mid-Michigan *C. amoena* (Band, 1988b).

Duration of mating was measured in four populations, ML-acorn, Rt. 700, Blacksburg, and Pamplin populations to assess similarities in mating behavior. Newly emerging flies in the population bottles were lightly etherized, sexes separated and typically aged at least one week prior to undertaking mating duration studies for each population. Matings typically occur within a half-hour when flies are aged. Population bottles enable up to three matings to be satisfactorially timed at any one observation period. Females not mated within an hour were considered unmated, especially since these ultra small populations were also used to compare fertility of ML-acorn versus Pamplin populations. Three to five males were introduced into cultures of one to three females from the same population without

Table 1. Mating duration in minutes for Virginia *Chymomyza amoena* populations

Location	Western Virginia			Piedmont
Population	ML-acorn	Rt. 700	Blacksburg	Pamplin
Duration	17.9 ± 2.8	17.1 ± 2.4	17.7 ± 1.2	19.1 ± 1.5
n	10	8	10	14

Table 2. Reciprocal crosses among Virginia *Chymomyza amoena*

Cross			
Females	Males	F_1	F_2
ML-acorn	Pamplin	yes	no
Pamplin	ML-acorn	yes	yes
Rt. 700	Pamplin	yes	yes
Pamplin	Rt. 700	yes	yes
Blacksburg	Pamplin	yes	?
Pamplin	Blacksburg	yes	no (mites)
ML-acorn	Rt. 700	yes	yes
Rt. 700	ML-acorn	yes	yes
ML-acorn	Blacksburg	yes	yes
Blacksburg	ML-acorn	yes	yes

etherization and duration of matings timed.

Table 1 shows the mating duration for the four Virginia populations. Mating duration is similar and the grand mean is 18.1 $\pm$ 1.0 minutes. For the 42 observations, the 95% confidence limits are: L_1 = 16.2 and L_2 = 20.1 minutes. All except the ML-acorn population derive from adults reared from apples. However for this larval overwintering, cold hardy species (Band and Band, 1984, 1987) only the Blacksburg population may be an exclusively apple breeding population, as reported in Band (1988a).

Comparison of intrapopulation sterility yielded similar results for the ML-acorn and Pamplin populations. Of the 10 ML-acorn females mating in 6 cultures, an additional 4 females did not mate and only 3 of the cultures yielded progeny. Among an equal number of mated Pamplin females, 5 did not mate and only 2 of 5 cultures produced progeny. Therefore whether females unmated within an hour mated later appears not to have improved overall culture success.

To reassess interpopulation fertility, no choice reciprocal small mass matings were employed: females of one population were crossed with males of another population. Table 2 shows the crosses among the western Virginia (ML-acorn, Rt. 700, Blacksburg) and Pamplin populations, and whether or not the F_1 hybrids produced an F_2. Loss of the Pamplin population made it impossible to repeat crosses where no information was obtained on fertility of the F_1s. However previous crosses between Pamplin and Blacksburg populations showed gene exchange could take place. Previous work also showed that the potentially specialist Blacksburg population remained interfertile with mountain populations (Band, 1988a). Hence interfertility of Virginia populations is compatible with past findings of interfertility of Michigan and Virginia populations.

References: Band, H. T., 1988a, Va. J. Sci. 39: 378-392; _____, 1988b, Amer. Midl. Nat. 120, 163-182; _____, 1991, Great Lakes Entomol. 24: 45-50; Band, H. T. and R. N. Band, 1984, Experientia 40: 889-891; _____ and _____, 1987, Experientia 43: 1027-1029; Carson, H. L., 1975, Amer. Nat. 109: 83-92; Cohet, Y. and J. David, 1974, Experientia 30: 1266-1268; _____ and _____, 1980, Genetica 54: 161-165; Kaneshiro, K. Y., 1989, pp. 279-296 in L. V. Giddings, K. Y. Kaneshiro and W. W. Anderson (eds.). Genetics, Speciation and the Founder Principle, Oxford University Press, N. Y.; Murray, J. J., 1972, Genetic Diversity and Natural Selection, Oliver and Boyd, Edinburgh; Ohta, A. T., 1989, pp. 315-329 in L. V. Gidding, K. Y. Kaneshiro, and W. W. Anderson (eds.). Genetics, Speciation and the Founder Principle, Oxford University Press, N. Y.; Spieth, H. T., 1981, Evolution 35: 921-930; Templeton, A. R., 1977, Evolution 32: 630-641.

Band, H. T. Michigan State University, E. Lansing, MI 48824 and Mt. Lake Biological Station, Pembroke, VA 24136. On the ability of *Chymomyza amoena* to breed in *Endothia parasitica*.

Chymomyza amoena has been found to breed in chestnuts, acorns, apples, and cherries in Switzerland (Burla and Bachli, 1992). In this country it has been found in a variety of nuts and fruits also (Band, 1988, 1991). Dorsey and Carson (1956) found that this species was among the drosophilids which would come to oak wilt fungus bait. Griswold (1958) bred it and other drosophilids from oak wilt fungus traps and found some drosophilids were also found capable of transmitting the fungus to damaged oak stems.

The question arises, if *C. amoena* can breed in chestnuts, can it also breed in chestnut blight fungus? *Endothia parasitica*, the fungus causing chestnut blight, will grow in *C. amoena* medium when propionic acid is omitted. A Swiss *C. amoena* mated female was allowed to oviposit in *Endothia parasitica* cultured on *C. amoena* medium for 24 hours. At least 3 eggs were counted; one adult emerged 36 days later, but died before it could be transferred to new medium to see if it would also transfer the fungus. The experiment was repeated with Virginia (ML-acorn) *C. amoena*. Three mated females oviposited longer in *Endothia/C. amoena* medium. Ten adults emerged between days 26 and 32 after eggs were first seen in the culture. Adults were transferred to new propionic acid free medium to see if they would also transfer over *Endothia parasitica*. Although moulds grew, as may be found in lab cultures of *C. amoena* from natural

substrates, it was not possible to isolate *E. parasitica* on potato-dextrose agar medium. Adults emerging from the chestnut blight fungus-coated medium were fertile.

Roane et al. (1986) note that the American chestnut *Castanea dentata* had been the most abundant tree in the eastern deciduous forest, but was destroyed by chestnut blight over a period of about 40 years after the fungus was detected on an American chestnut in the Bronx Zoological Park in New York City in 1904. The fungus was found on European chestnut *Castanea sativa* in Northern Italy in 1938. A hypovirulent *E. parasitica* strain was detected in 1950 and 1951 whose spread arrested the course of the disease and saved the European chestnut. The hypovirulent strain has been found in the United States. So far vectors for its spread in the United States have proved elusive. Agents responsible for the spread of the hypovirulent strain in Europe remain unknown.

I thank Martha Roane for the *Endothia parasitica* culture.

References: Band, H. T., 1988, Amer. Midl. Nat. 120:163-182; ____, 1991, Great Lakes Entomol. 24: 45-50; Burla, H. and G. Bachli, 1992, Mitt. Schweiz. Ent. Ges. in press; Dorsey, C. K. and H. L. Carson, 1956, Ann. Entomol. Soc. Amer. 49: 177-181; Griswold, C. L., 1958, J. Econ, Entomol. 51: 733-735; Roane, M. K., G. J. Griffin and J. R. Elkins, 1986, Chestnut Blight, Other *Endothia* Diseases and the Genus *Endothia*, APS Press.

Lunz, R., W. Johnson, M. Ghosh, and D.K. Hoshizaki. University of Illinois at Chicago. Identification of fatbody enhancer activity in *Drosophila melanogaster*.

The *Drosophila* fatbody is analogous to mammalian adipose tissue and liver. It is the principal tissue involved in storage and intermediary metabolism and is the primary site for triglyceride and phospholipid synthesis. The fatbody is also the main source for hemolymph proteins, lipids and carbohydrates that serve as metabolic precursors in other tissues.

One of our research interest is the tissue specific regulation of triglyceride and phospholipid biosynthesis in the fat body. We have screened "enhancer trap" lines (Bellen *et al.* 1989; Wilson *et al.* 1989; Bier *et al.* 1989) for specific expression of the reporter gene *LacZ* in either the adult or larval fat cell. The isolation of such enhancers and their associated genes should lead to the identification of genes involved in fat cell development or fat cell function.

We have identified a total of 13 lines in which *LacZ* expression was detected in a limited number of tissues including the fat cells of the adult and the larva (Table 1). Three of these lines were from a collection of 537 "enhancer trap" lines obtained through A. Spradling (Carnegie Insitute of Washington); 7 from a collection of 69 "enhancer trap" lines previously identified by R. Davis (Baylor College of Medicine) to have *LacZ* expression in the fat cells of the adult head; 3 from a collection of 12 lines previously identified by L.Y. Jan and Y. N. Jan (Bier *et al.* 1989 and personal communication) to have expression in the embryonic fat; and a single line from G. Rubin (University of California at Berkeley).

To determine if any of these "enhancer trap" lines identify known genes which are expressed in the fat cell, *in situ* hybridization to salivary gland polytene chromosomes was carried out.

Table 1. Fat body enhancer trap lines

Strain Designation	Fat body		Other Tissues		cytological position	Source
	Adult	larva	Adult	larva		
RD1937	+	+	none	none	2F	1
X8-157A	+	+	none	none	5CD	3
J3-76a	+	+	follicle cells	midintestine	18D	3
RD721	+	+	none	imaginal disc	24A	1
rP445	+	+	none	anterior midintestine	25A	2
AS0330	+	+	Follicle cells	gastric caeca; hindintestine	25AB	2
S3358	+	+	oenocytes	none	25F	3
RD61	+	-	none	gastric caeca	54BC	1
RD1272	+	+	nurse cells; follicle cells	none	64B	2
AS1649	+	+	follicle cells; nurse cells; malpighian tubes; accessory gland; anterior ejaculatory duct	midintestine	65F	4
AS1708	+	+	nurse cells; follicle cells; accessory gland; posterior ventriculus	none	65F	4
S3360	+	+	oenocytes; posterior ventriculus	midintestine; gastric caeca	70D	1
S3259	+	+	oenocytes; posterior ventriculus	midintestine; gastric caeca	85B	4

[1]R. Davis; [2]G. Rubin; [3]L.Y. Jan and Y. N. Jan; [4]A. Spradling

The cytological site of the *LacZ* insertion in each "enhancer trap line" was determined (Table 1). In only one case was there a correlation to a known "fat" gene. S3360 cytologically maps to same region as the larval serum protein P1 gene; however the beta-galactosidase activity of S3360 is not restricted to the larval fat cells.

We have detected several fat cell enhancers that are active in the adult and or larva. Each of these enhancers appears to be unique and therefore should identify novel fat cell genes.

References: Bellen, H.J., C.J. O'Kane, C. Wilson, U. Grossniklaus, R.K. Pearson, and W.J. Gehring 1989, Genes Dev. 3:1288-1300; Bier, E., H. Vaessin, S. Shepherd, K. Lee, K. McCall, S. Barbel, L. Ackerman, R. Carretto, T. Uemura, E. Grell, L.Y. Jan, and Y.N. Jan 1989, Genes Dev. 3:1273-1287; Wilson, C., R.K. Pearson, H.J. Bellen, C.J. O'Kane, U. Grossniklaus, and W.J. Gehring 1989, Genes Dev. 3:1301-1313.

Collett, J.I. and S. Seymour-Jones. School of Biological Sciences, University of Sussex, Brighton, Sussex, BN1 9QG, U.K. Leucine amino peptidase (LAP) is coded for by Jonah family genes in the chromosomal region of 99 in *D. melanogaster.*

Carlson *et al.*s' (1985) isolation of RNA from larval salivary glands included an 'unexpected' (contaminant) RNA of a species present copiously in the gut, which, hence, Carlson named 'Jonah'. Its cDNA hybridized to seven chromosomal sites, and thus described a family of Jonah genes. But the most prominent hybridization was at sites in 99C and 99F at the tip of 3R. *In situ* hybridization of the cDNA to gut tissue demonstrated the exclusive presence of transcripts in the posterior mid-gut of larvae (Akam and Carlson, 1985). Translation of extracted RNA complementary to the cDNA generated a protein of 28 kDa. No protein of equivalent size was found in a search of the soluble components of the posterior mid-gut. The less soluble proteins of the sedimenting component of the posterior mid-gut were not examined. Restriction and heteroduplex maps of the Jonah genomic regions of 99C and F revealed that each region contains a pair of sites of homology with the cDNA.

Characterization of the LAP genes from their proteins shows striking resemblances to the description of Jonah genes from their transcripts. For instance, electrophoretic variants of LAP (Beckman and Johnson, 1964) and discrimination of its components (Hall, 1986; Seymour-Jones and Collett, 1992) have implicated two genes, LAP A and D, in the generation of active LAP proteins. Electrophoretic variants of LAP D have been mapped by recombination to a site at 98.3 $\pm$ 0.5 (Falke and MacIntyre, 1966). Beckman and Johnson (1964) found no recombination between LAP A and D variants among 178 progeny. Ising *et al.*s' (1984) recombinaton mapping of TEs bearing w^+ alleles at the chromosomal sites of 94A and 100(D)C places a recombinaiton map site of 98.3 in the region of 99C. Measurement of LAP activity in dissected parts of guts also indicates that the exclusive tissue site of copious LAP activity is also the exclusive tissue site of transcription of the 99C Jonah gene. Further, silver-staining of SDS-associated and electrophoretically separated proteins released from the insoluble component of gut with Triton X-100 indicated that the most prominent of a limited number of proteins was of a 28-29 kDa size. Moreover, Northern analysis of mid-gut RNA from adults of both sexes and various ages and probed with Jonah cDNA demonstrated the presence of amounts of Jonah RNA proportional to the characteristic activities of LAP of each preparation.

With these striking coincidences of map sites, protein and transcript sizes, tissue sites and dynamics of activities, it would be remarkable if the LAP and at least one Jonah gene site in the 99 region are not one and the same.

References: Akam, M.E. and J.R. Carlson 1985, EMBO 4:155; Carlson, J.R. and D.S. Hogness 1985, Dev. Biol. 108:355; Hall, N.A. 1986, Biochem. Gen. 24:775; Ising, G. and K. Block 1984, Mol. Gen. Genet.196:6; Seymour-Jones, S. and J.I. Collett 1992, Dros. Inform. Serv. 71.

Manolakou, E. and C.B. Krimbas. Agricultural University of Athens, Greece. Changes in recombination values in *D. subobscura* due to the presence of inversions.

The percentage of recombination between two markers differs from cross to cross in *Drosophila subobscura*. Three different causes might be responsible for these differences: the presence of a gene that reduces the total length of the genetic map (Koske and Maynard Smith 1954), the heterozygosity for an inversion on the same chromosome on which these markers are located in spite of the fact that these markers are outside the inversion, and finally the heterozygosity for inversions on other chromosomes (the Schultz-Redfield effect). The difficulty encountered in constructing genetic maps is illustrated from the results of the following crosses, showing the effect of inversions on recombination.

The values of recombination between *cherry* eyes and *curled* wings, two recessive markers located on the O

chromosome (homologous to 3R of *D. melanogaster*) were recorded in three different crosses. In the first the mother was homozygous for the gene arrangement O_{3+4} without any heterozygote inversion in its remaining chromosomes. The percentage of recombination increased by 6 units when the same mother was heterozygous for two to four inversions on its remaining chromosomes (for A_2, U_{1+2}, E_8 or E_{1+2+9}, J_1) This is apparently due to the Schultz-Redfield effect, the increase of recombination due to the presence of heterozygous inversions on the other chromosomes; this effect was also documented for markers of the J chromosome by Pinsker and Sperlich (1984). The third cross reported the mothers were heterozygotes for inversion O_7, which, however, is outside the region comprising the markers *ch* and *cu*: inversion 7 extends from 77B/C to 85/86, while *cu* and *ch* are in the region between the sections 87 and 91. In spite of this their recombination percentage is drastically reduced.

Table 1. Percentage of recombination between the markers *ch* and *cu*, located on the O chromosome, in three different crosses. N is the number of flies counted.

	Mother's karyotype	number of inversions on other chromosomes	recombination	N
1.	O_{3+4} / O_{3+4}	0	35.9 ± 0.8	3682
2.	O_{3+4} / O_{3+4}	2 - 4	42.6 ± 0.8	3853
3	O_{3+4+7} / O_{3+4}	0	3.0 ± 0.7	535

The inversion polymorphism in natural populations of *D. subobscura* is extremely rich and therefore it is common to get different recombination values in different crosses; a careful control of the female karyotype is needed if we are to compare recombination percentages from different crosses.

References: Koske, Th., and J. Maynard Smith, 1954, J.of Genetics 52:521-541. Pinsker, W., and D. Sperlich, 1984, Genetics 108:913-926.

Thörig, George E.W. University of Utrecht, Department of Plant Ecology and Evolutionary Biology. Section of Evolutionary Genetics, Padualaan 8, 3584 CH Utrecht, The Netherlands. When the hybrid sterility barrier between *Drosophila melanogaster* and *Drosophila simulans* breaks down.

Hybrid sterility between *Drosophila melanogaster* and *Drosophila simulans* is a fact based on many investigations during the past seventy years. Regardless of this statement, my crosses showed that fertile hybrids can be found at a low frequency. Based on the male offspring I found an abnormal class of hybrids. Their phenotypes phenocopy that of either *D. sim* or *D. mel* males, although the ADH electromorphs showed their origin. This phenotypic instability is coupled to certain strains and the success depends on temperature and season. The *simulans* strains carry a new gene system *Lhr*. It rescues females in the cross *simulans* females x *melanogaster* males.

Thörig, George E.W. University of Utrecht, Plant Ecology and Evolutionary Biology, Utrecht, The Netherlands. Consequences of *in vivo* transformations.

Crosses between *Drosophila simulans* females X *Drosophila mauritiana* males produce fertile females as offspring. I used this effect to transport wildtype genes from *Drosophila mauritiana* into *Drosophila simulans* by backcrossing. The transformation of the *mau* wildtype gene st^+ into *sim* was subjected to selection. The selection pressure expelled the wildtype gene after six generations of breeding with seven replicates. Selection by myself on st^+ *mau* in the backcrossed *sim* stocks produced stocks with st^+ *mau* homozygotes. These results put questions on the efficiency of transformations during generations on the population level. Because the mutant *st* always has a selective disadvantage against the st^+ wildtype *simulans* allele, in my case it gains advantage over the st^+ *mau* allele after *in vivo* transformation. In the reverse crosses fertile females F_1, (*sim* x *mau*) x *mau* males, I never succeeded in transporting the mutant *st sim* in the *mau* background. Both species are closely related (Lemeunier et al., 1986).

References: F. Lemeunier, J.R David, L. Tsacas and M. Ashburner (1986). The *melanogaster* Species Group. The Genetics and Biology of *Drosophila*. eds. M. Ashburner, H.L. Carson and J.N. Thompson, Jr. vol 3e pp 218-221.

Kumar, Ajai, K.K. Gupta, and J.P. Gupta. Genetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi, India. A report of Drosophilidae of Sikkim, India.

Sikkim is a small and mountainous Indian state in the eastern Himalayas, covering an area of about 7096 sq. Km. One third of its land is covered with dense forests. It also harbors a large number of different kinds of orchids. Despite its remarkably varied physiographic conditions, the state remained unexplored for its drosophilid fauna until recently. During November, 1988 and March, 1989 intensive field collections were undertaken for the first time at and around two different localities, Gangtok and Ranipool in Sikkim, using different methods of collection.

Altogether 2595 flies representing 45 species under 7 genera of Drosophilidae were collected. Out of them, 14 species are detected as new, while 3 other species are newly recorded from India. The taxonomic account of these species has already been published (Gupta and Gupta, 1990, 1991; Kumar and Gupta, 1990, 1991, 1992a, b, in press). The details of these species are given in Table 1.

Acknowledgment: The authors are indebted to Prof. T. Okada, Japan, for his help in confirming the identifications. Financial assistance in the form of a Teacher Fellowship and Senior Research Fellowship to AK and KKG respectively from the U.G.C., New Delhi is gratefully acknowledged.

References: Gupta, K.K. and J.P. Gupta, 1990, Senckenberg. biol. 74(1/3):20-23; Gupta, K.K. and J.P. Gupta, 1991, Proc. Zool. Soc. 44(2):119-126; Kumar, Ajai and J.P. Gupta, 1990, Proc. Zool. Soc. 43(1):25-30; Kumar, Ajai and J.P. Gupta, 1991, Senckenberg. biol. (in press); Kumar, Ajai and J.P. Gupta, 1992a, Orient Insects 26:207-212; Kumar, Ajai and J.P. Gupta, 1992b, Entomon (in press).

Table 1. Distribution of drosophilid species at two different localities in Sikkim, India.

Species collected	No. of specimens at: Gangtok	Ranipool	Total
Genus *Drosophila*			
Subgenus *Lordiphosa*			
D. acutissima	03	—	03
*D. himalayana**	22	—	22
*D. parantillaria**	—	04	04
*D. peniglobosa**	—	03	03
Subgenus *Sophophora*			
D. ananassae	133	115	248
D. biarmipes	—	11	11
D. bipectinata	121	74	195
D. eugracilis	272	128	400
D. kikkawai	28	22	50
D. kurseongensis	17	—	17
D. malerkotliana	91	168	259
D. nepalensis	145	59	204
D. prolongata	—	15	15
D. pulchrella	117	74	191
D. punjabiensis	97	—	97
*D. suborosa**	—	03	03
D. takahashii	82	48	130
D. trapezifrons	68	23	91
D. trilutea	65	—	65
D. tristipennis	—	23	23
Subgenus *Scaptodrosophila*			
D. paratriangulata	17	—	17
*D. vazrae**	—	07	07
*D. zingiphila**	—	11	11
Subgenus *Drosophila*			
D. annulipes	10	—	10
D. fuscicostata	—	24	24
D. immigrans	140	57	197
*D. ovilongata**	10	—	10
D. paralongifera	12	—	12
D. setitarsa	—	11	11
*D. sikkimensis**	22	—	22
Subgenus *Dorsilopha*			
D. busckii	38	23	61
Genus *Scaptomyza*			
Subgenus *Scaptomyza*			
*S. tistai**	—	53	53
*S. clavata***	02	—	02
*S. parasplendens***	18	—	18
Subgenus *Parascaptomyza*			
S. elmoi	33	—	33
Genus *Liodrosophila*			
L. quadrimaculata	10	—	10
L. penispinosa	17	—	17
L. angulata	03	—	03
Genus *Microdrosophila*			
*M. bilineata**	—	09	09
*M. sikkimensis**	—	02	02
*M. gangtokensis**	04	—	04
Genus *Mulgravea*			
*M. ranipoolensis**	—	06	06
Genus *Zaprionus*			
*Z. cercociliaris**	11	—	11
Z. pyinoolwinensis	03	—	03
Genus *Hypselothyrea*			
H. gutata	—	11	11
Total No. of flies	1611	984	2595
Total no. of species	30	26	46

* New species ** Newly recorded species

Ribaudo R.M., G. Di Lemma and A. Di Pasquale. University of Palermo, Italy. Possible "chromosomal contamination" in *Drosophila melanogaster.*

Previous cytological observations have evidenced peculiar configurations recurring preferably at specific sites along the 2nd and 3rd salivary gland polytene chromosomes of the *tu-pb* melanotic tumor mutant strain. The kind of irregularities (rings, hairpins, extra inserted bands or band deletions, unusual ectopic pairing, asynapsis, an inversion 2R 52A-56E/F) and their inter- and intra- individual variability raise the possibility that these features may be related to the mobility of transposable elements (Di Pasquale et al., 1988). On the other hand, Oregon-R salivary chromosomes do not appear to be affected by these irregularities; while features very similar to that of the *tu-pb* strain emerged from preliminary observations on polytene chromosomes of heterozygous Oregon-R/*tu-pb* individuals. A cytological analysis has been undertaken to gain information on the possibility of "contamination" of the Oregon-R chromosomes by the *tu-pb* ones. Crosses were made between Oregon-R and *tu-pb* flies, F1 Oregon-R/*tu-pb* heterozygous males were backrossed to Oregon-R females. The offspring from these crosses were examined for salivary chromosome patterns. According to each type of irregularity, the sites and the frequency of occurrence among individuals or nuclei of the same individual were scored. In order to allow a direct comparison between F_1 and backross progeny the results of these observations are summarized in the histograms presented in Figure 1.

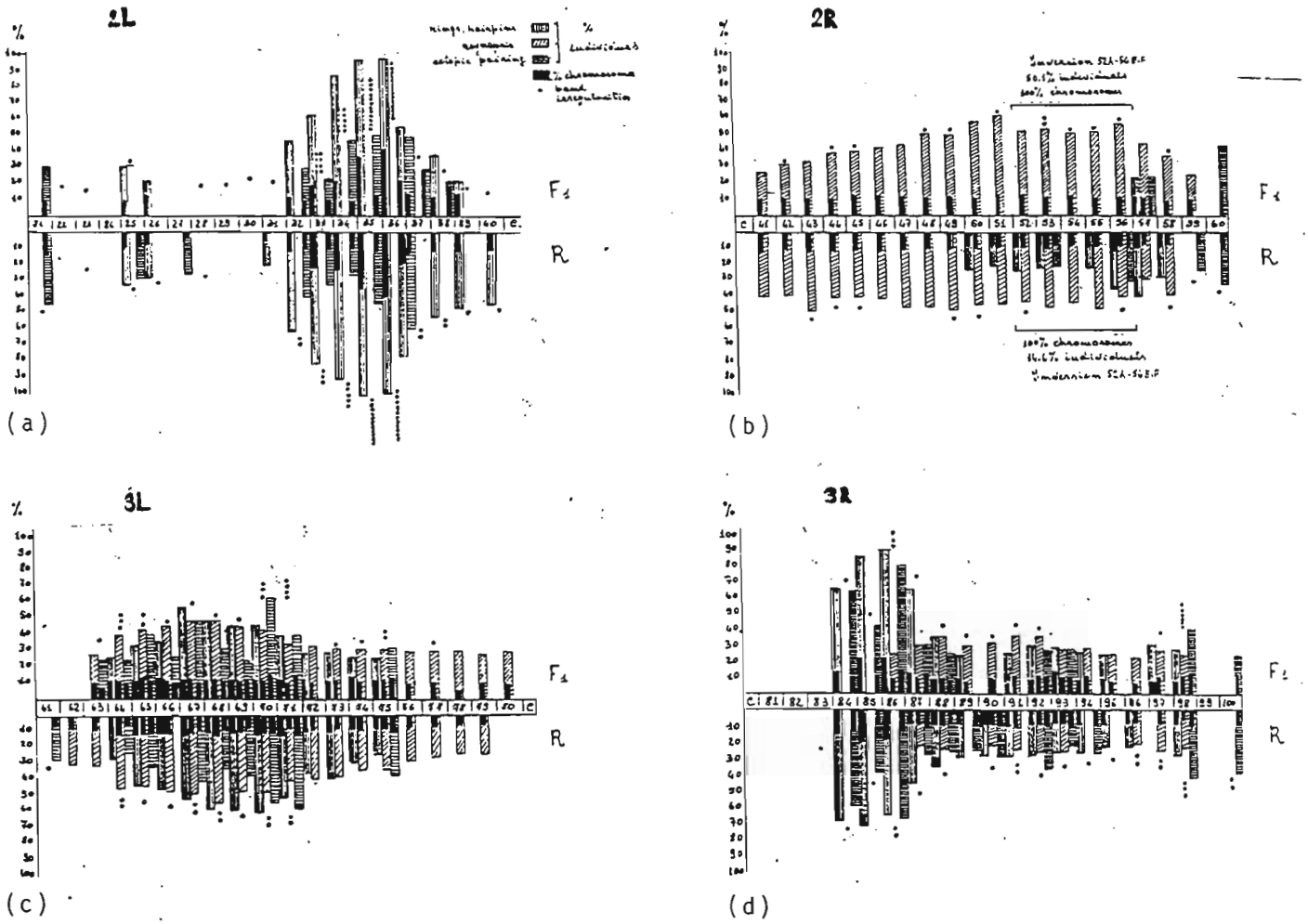


Figure 1. Distribution of irregularities in chromosome arms: (a) 2L, (b) 2R, (c) 3L, (d) 3R.

What emerges from these comparisons is that distribution, frequency and types of irregularity are almost identical in the F_1 and backross individuals, moreover they resemble those found in the *tu-pb* strain. It can be assumed that in the F_1 heterozygous individuals, Oregon-R chromosomes are involved in peculiar configurations owing to the somatic pairing with the *tu-pb* ones. If this is the case, the frequency of individuals exibiting the unusual configurations must be halved in the backross offspring since this includes only one half Oregon-R/*tu-pb* heterozygotes. Owing to the considerable similarity between F1 and backross individuals we think it possible that the Oregon-R chromosomes have in some way acquired the peculiar characteristics of the *tu-pb* ones through a sort of "contamination". These observations strengthen the hypothesis of an involvement of transposable elements in the *tu-pb* peculiar features. Further investigations will provide more information on the nature of this phenomenon.

References: Di Pasquale Paladino, A., P. Cavolina, G. Romano and R. Ribaudo 1988, DIS 67:32.

Ribaudo, R.M., G. Di Lemma and A. Di Pasquale. Universita di Palermo, Italy. Somatically unstable transposable elements in a strain of *Drosophila melanogaster*.

During the examination of the salivary gland polytene chromosomes of the *tu-pb* melanotic tumor strain of *Drosophila melanogaster*, we observed peculiar configurations in which were involved some 2nd and 3rd chromosome regions. Since the distribution of the irregularities in the chromosomes was far from random

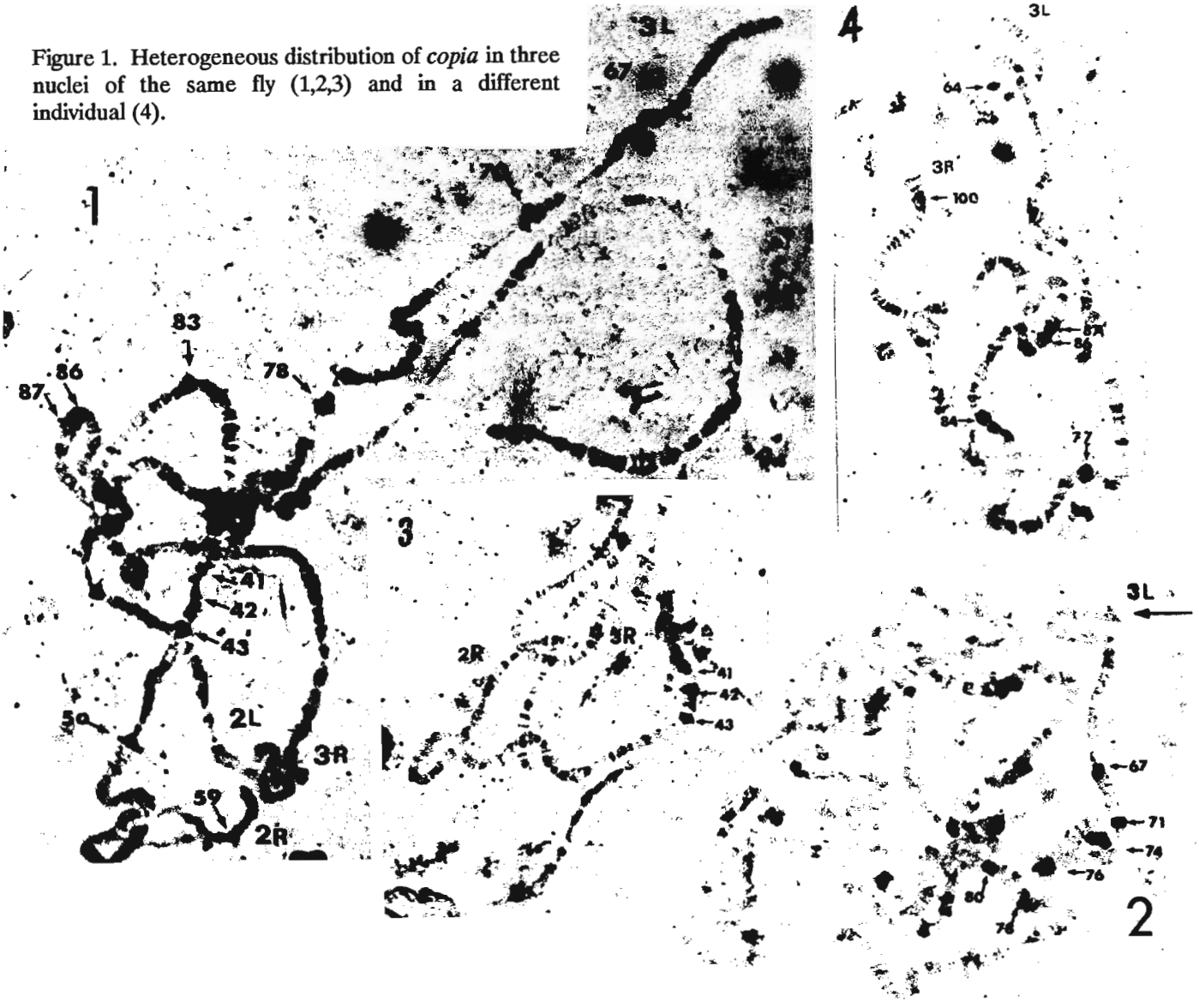


Figure 1. Heterogeneous distribution of *copia* in three nuclei of the same fly (1,2,3) and in a different individual (4).

and at the same time the individuals were heterogeneous as regarding the occurrence of these configurations in their nuclei, we hypothesized that the features observed might be related to the mobility of transposable elements (Di Pasquale et al., 1988). To verify this hypothesis, we have extended our studies by analyzing distribution pattern of some *D. m.* transposons along the salivary chromosomes of the *tu-pb* strain. By *in situ* hybridization, we tested the presence of *copia, mdg1, mdg4, 1731* and *hobo* in the 2nd and 3rd autosomes looking for heterogeneous insertion site distribution among nuclei of the same individual. The results of this analysis revealed homogeneous distribution of the sites occupied by *mdg1* and *mdg4* in all nuclei of the individuals examined; moreover, *copia, 1731* and *hobo* exibited stable insertion sites which were present in all nuclei of the individuals analyzed, and unstable sites which were heterogeneous among individuals as well as among nuclei of the same gland. It is worth noting that this analysis evidenced a strong correspondence between the sites occupied by the transposons and the regions involved in the chromosome configurations.

These results seem to account for transposition events in somatic cells and also to support the hypothesis of a relation between mobility of transposable elements and the chromosomal irregularities observed. Heterogeneous distribution of the *copia* element is presented in Figure 1.

References: Di Pasquale Paladino, A., P. Cavolina, G. Romano, and R. Ribaudo 1988, DIS 67:32.

Chatterjee, R.N., Department of Zoology, University of Calcutta, India. Influence of X chromosomal aneuploidy on X chromosomal organization in *Drosophila melanogaster.*

In *Drosophila*, studies of aneuploids indicate that, survival is inversely related to the extent of the duplications and deficiencies of genetic material. Lethality is mainly due to cumulative disruption of genic balance. Works of Lucchesi and his co-workers (Lucchesi et al., 1977; Maroni and Lucchesi, 1980) and Chatterjee (1985, 1990) have shown that X chromosome aneuploidy could alter the expression of the X-linked genes in *Drosophila*. In this report, I have analyzed the consequences of extensive X chromosomal aneuploidy of *Drosophila melanogaster* on the puffing activity pattern of the salivary gland X chromosomes.

The results seem to indicate that when duplication for the segments 9A-11A, 15EF-20F and 8C-20F of the X chromosome are combined in males, a locus specific X chromatin transformation develops in both duplicated and non-duplicated segments of the X chromosome. A critical analysis of data further reveals that formation of large puffs at 1A-2B, 10BCD, 11BC, 15BC and 18BCD sites of aneuploid X chromosome is correlated with an elevated level of X activity (including abnormal longitudinal differentiation) in the aneuploids. In case of Dp 3E-20F, the X chromosome are expressed at a female level and the puffing activity pattern of the X chromosome is almost the same as normal female. It may also be noted that in metamales (1X;3A) and triploid intersexes (2X;3A) the puffing pattern in the X chromosome matches that of the diploid male aneuploid larvae, in general. However, in hyperploid females (2X+Dp;2A); metafemales (3X;2A) and triploid females (3X;3A), no change in puffing activity pattern was observed as compared to the normal Oregon R^+ female X chromosome. The X chromosome aneuploidy has also little effect on the autosomal puffing pattern. These results suggest that large X chromosomal aneuploidies cause an abnormal puffing pattern of the X chromosome in male *Drosophila*.

Acknowledgments: The work is supported by a research grant from University Grants Commission to RNC.

References: Chatterjee, R.N. 1985, Chromosoma 91:259-266; Chatterjee, R.N. 1990, Ind. J. Exptl. Biol. 28:101-105; Lucchesi, J.C., J.M. Belote, and G. Maroni 1977, Chromosoma 65:1-7; Maroni, G. and J.C. Lucchesi 1980, Chromosoma 77:253-261.

Chatterjee, R.N. and R. Mukherjee. Department of Zoology, University of Calcutta, India. RNA synthesis in *Drosophila melanogaster* polytene chromosomes after *in vitro* treatment of cAMP.

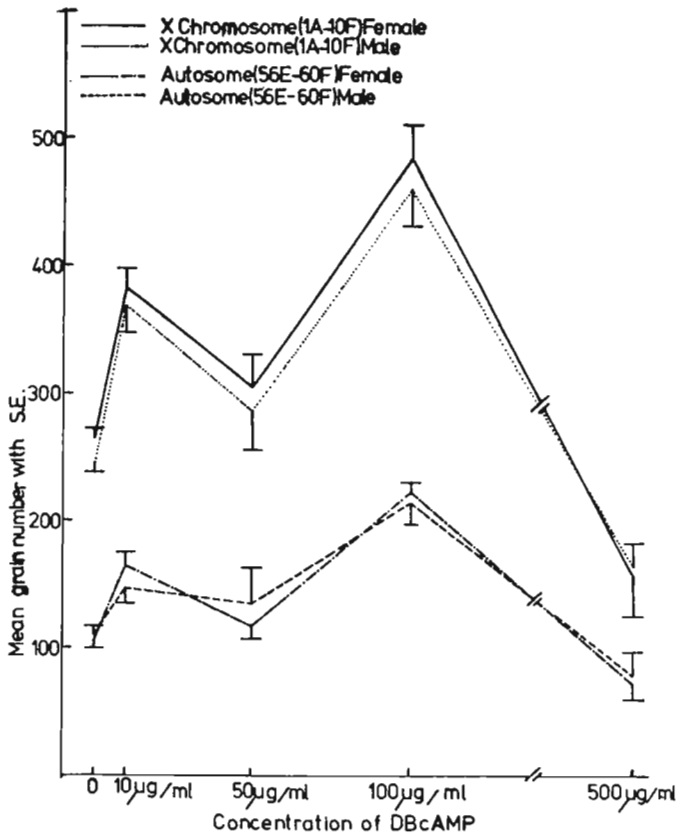
Rensing and Hardeland (1972) reported that pretreatment of DBcAMP caused temporal variations of puff response in *Drosophila melanogaster.* The present communication deals with the effects of DNcAMP on the ^{3}H-Uridine (500 microliter Ci/ml; Sp. activity 13,600 mCi/mM, obtained from BARC, Bombay, India) containing DBcAMP. The concentrations of DNcAMP were 10, 50, 100, 150 microgram/ml. Cytological preparations of chromosomes were then made and processed for autoradiography.

Data on the ^{3}H-uridine incorporation pattern in an X chromosomal and autosomal segments of *D. melanogaster* following *in vitro* treatment with different concentrations of cAMP are presented in Figure 1. As it appears from the data, the induction effect of cAMP was not linearly proportional to the dose of cAMP for both X and autosomal

Table 1. Puffing activity indices and ^{3}H-uridine incorporation pattern in the different puff site of X and autosomes of female salivary glands of *D. melanogaster* after *in vitro* treatment of DBcAMP at a concentration 100 µg/ ml.

Puff site	No. of nuclei	Mean puffing activity indices		Mean grain number ± S.E.	
		Control Female	DBcAMP treated Female	Control Female	DBcAmp treated Female
2AB	20	2.04	1.81	42.90 ± 4.35	29.15 ± 2.38*
3DE	22	1.50	1.71	11.18 ± 2.96	24.05 ± 1.75*
9EF	20	1.52	1.74	19.35 ± 1.99	35.95 ± 2.12
32CD	20	1.27	1.68	23.40 ± 2.38	44.00 ± 2.76*
34A	23	1.09	1.35	10.39 ± 1.55	14.04 ± 1.99
47A	20	2.11	2.39	25.25 ± 2.46	36.45 ± 4.85*
50CD	20	1.78	1.96	21.90 ± 2.87	46.85 ± 5.05*
61CD	21	1.02	1.35	9.43 ± 1.23	18.05 ± 2.23*
62EF	20	1.63	1.74	16.55 ± 2.25	21.55 ± 2.36
63E	20	1.53	1.83	16.55 ± 1.30	23.15 ± 2.94
67F	20	1.31	1.42	12.40 ± 1.29	15.50 ± 2.21
88EF	18	1.32	1.61	19.28 ± 2.14	29.33 ± 3.32*

*Significantly different from respective control.



segments. The synthetic activity pattern of the polytene of cAMP. On the other hand, at 100 microgram/ml concentration of cAMP, this chemical induced greater incorporation of ^{3}H-uridine into all chromosomes. However, at 500 micrograms/ml concentration of cAMP, the overall incorporation of ^{3}H-Uridine appears significantly depressed.

In order to have precise evaluation of the effect of cAMP, 12 puff sites of *D. melanogaster* were selected (Table 1). As the synthetic activity pattern of the polytene chromosomes of *Drosophila* is concentration dependent and as 100 microgram/ml concentration of cAMP caused optimum induction of synthetic activity pattern in the polytene chromosomes of *Drosophila*, we have used 100 microgram/ml concentration of cAMP for analyzing the cAMP responsive sites of *D. melanogaster* polytene chromosomes. Data in Table 1 reveal that treatment of DBcAMP seems to cause selective induction and/or repression of puffs at many sites of the X and autosomes of *Drosophila*. The puffs at 2AB, 3DE, 61CD, 62EF and 63EF were found to react differently (Table 1). It may also be noted here that cAMP caused higher incorporation of ^{3}H-uridine at 3D site of the X chromosome (Figure 2). This is expected, since one of the cAMP phosphodiesterase gene (Form-I) which helps to maintain the intracellular level of cAMP, is present in 3D site of the X chromosome (Kiger and Salz, 1985). In this context, it may be noted here that the cellular level of cAMP is regulated by balanced synthesis and degradation. Data in Table 1 further indicate that most of the cAMP responsive puff sites incorporate ^{3}H-uridine in accordance with the mean puffing activity indices.

Acknowledgments: This work is supported by "Rameshwar Das Smarak Kosh Endowment Fund" to RM.

References: Kiger, J.A. Jr. and H.K. Salz 1985, Adv. Insect Physiol. 18:141-179; Rensing, L. and R. Hardeland 1972, Exptl. Cell Res. 73:311-318.

Figure 1. Data show the ^{3}H-uridine incorporation pattern over X chromosome segment (1A-10F) and an autosome (56E-60F) of the salivary gland chromosomes of *D. melanogaster* after *in vitro* treatment with different concentrations of DBcAMP.

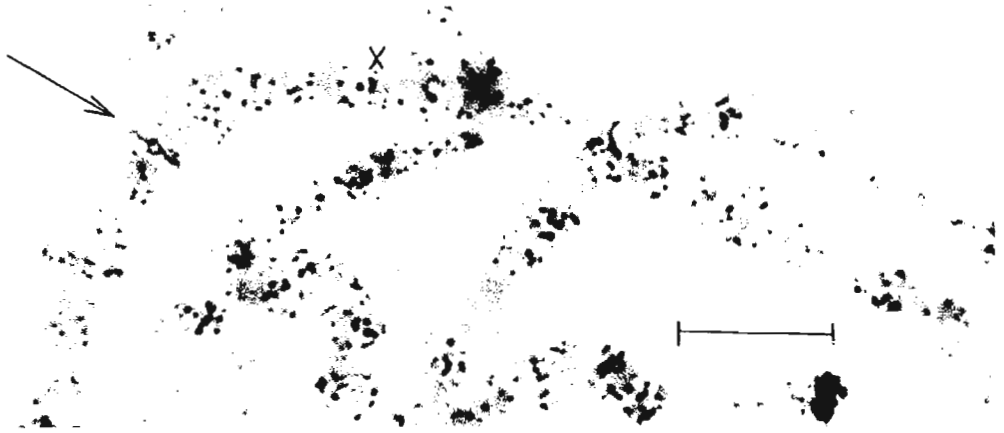


Figure 2. Autoradiogram showing RNA synthesizing activity of the polytene chromosomes of *D. melanogaster* treated with 100 ug/ml DBcAMP. Note the ^{3}H-uridine incorporation pattern at 3D (arrow) site of the X chromosome. X=X chromosome, Bar represents 10 um.

Held, Lewis I., Jr. Texas Tech University, Lubbock, Texas, USA. Pupal development outside the pupal case.

As in other cyclorrhaphous dipterans, the last instar *Drosophila* larva hardens its skin to form a puparium wherein it metamorphoses into an adult fly. This "pupal case" shields the fragile pupa but otherwise serves no apparent function. Because its opacity hinders *in situ* observations of the underlying epidermal cells, I tried removing it at different stages. Complete removal at 12 h after puparium formation (just after head eversion) does not interfere with the remaining 3.5 days of pupal development, provided that sufficient humidity is maintained. (Earlier removals failed because the epidermis rips too easily, and operations at 24 h yielded low frequencies of survival for the same reason.) The procedure is as follows. Individuals are collected as white prepupae and aged for 12 h at 25°C in a humidified atmosphere. The pupae are then cleaned, dried, and placed ventral-side-down on a piece of double-stick tape on a microscope slide. Under a dissecting microscope (fiber-optic illumination prevents overheating), the opercular flap is pried open and torn off using fine forceps (Dumont #5 microdissecting tweezers, Roboz Surg. Instr. Co.). One tong of the forceps is inserted parallel to the body between the pupal case and the thorax to a distance of one or two segments, and the forceps are closed and tilted to pinch, lift, and tear the cuticle along the dorsal midline. (A second pair of forceps can be used to help in tearing.) The cuticle is then peeled laterally (the case splits naturally into annular strips) and removed, and the process is repeated until the front half of the pupa is exposed. The forceps are then inserted along the abdomen (one tong on each side), which allows the pupa to be extracted. In one experiment 20 wild-type (Oregon R) pupae were subjected to this protocol. All developed full eye color and body pigmentation: 17 became healthy adults and walked normally, two could only twitch their legs, and one died. The only notable abnormality was misshapen wings: 15 of the flies had two deformed wings, four had one deformed wing, and one fly was normal.

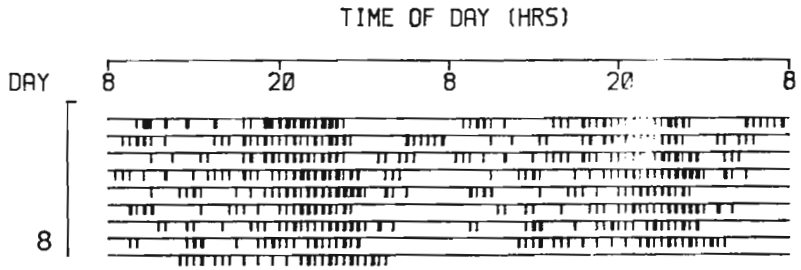
Because the legs and wings of young pupae can be lifted off the chest and bent to the side, they can be observed under a compound microscope without any light refraction from thoracic fat globules. This technique should therefore facilitate time-lapse studies of imaginal development at the tissue and cell level.

Acknowledgments: The author's research is supported by Grant 003644-044 from the Texas Advanced Research Program.

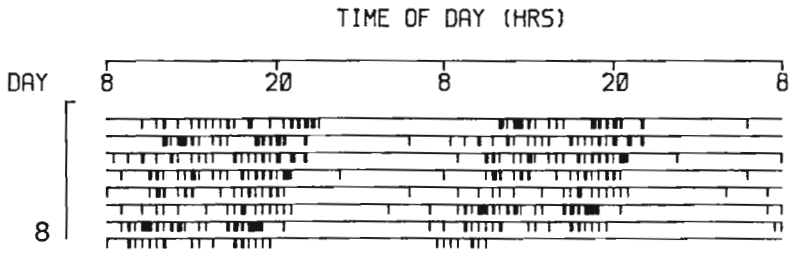
Hall J.C., M.J. Hamblen-Coyle, L. Moroz, J.E. Rutila, Q. Yu, M. Rosbash, and D.A. Wheeler, Brandeis University, Waltham, MA. Circadian rhythms of *D. melanogaster* transformed with DNA from the period gene of *D. simulans*.

Several *per* transgenic strains have been generated (Wheeler *et al.*, 1991). The "hosts" for these carried a non-functional allele of the X-chromosomal period gene (per^{01}) and a rosy marker (ry^{506}); the incoming DNA included (in addition to ry^{+} and P-element sequences)

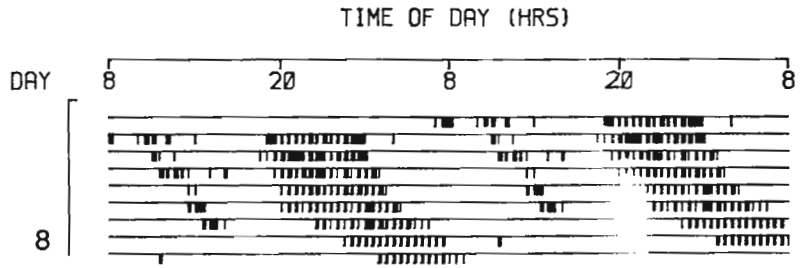
a *D. melanogaster* τ = 24.0 h



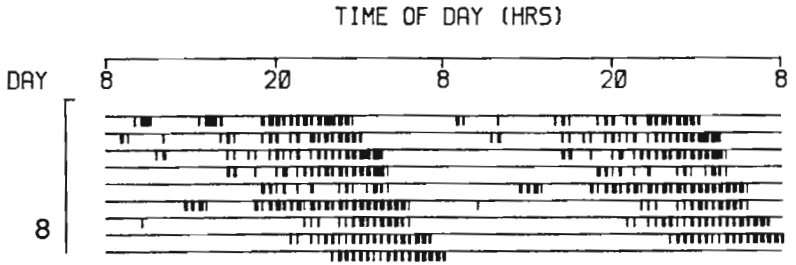
b *D. simulans* τ = 23.7 h



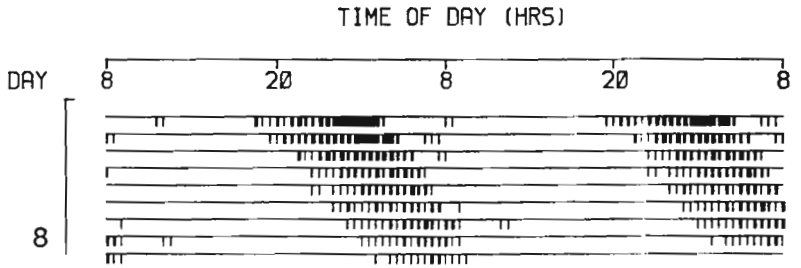
c 13.2m-TGm τ = 25.0 h



d 13.2s-TGs τ = 24.6 h



e 13.2m-TGs τ = 24.5 h



f 13.2s-TGm τ = 24.5 h

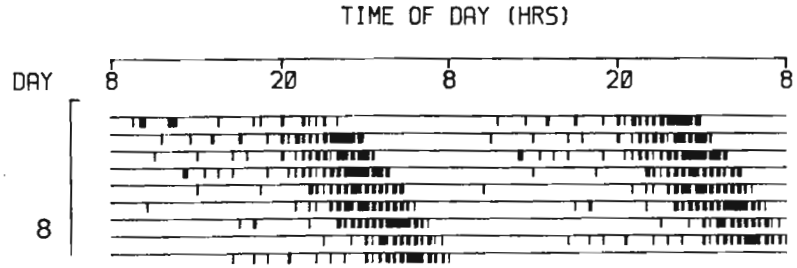


Figure 1 (Previous page).
Locomotor activity rhythms of wild-type and of transformed flies involving *per* DNA. Normal rhythmicities, as exhibited by wild-type *D. melanogaster* and *D. simulans* adults in constant darkness (DD), are exemplified in **a** and **b** (males from respectively, the Oregon-R and Kenscoff strains). The flies had been reared in light:dark (LD) cycling conditions, then exposed as adults to 3-4 days of 12h:12h LD (light-on at none), before DD monitoring. The latter was performed as described in Hamblen et al. (1986) and involved placing the flies in narrow, cylindrical glass tubes, flanked by infrared emitter/detector pairs; a fly unusually breaks the infrared light beam (to which its visual, including circadian, systems are insensitive) when it moves. Each beam breakage is recorded digitally (using software and hardware described in Hamblen et al., 1986) as an "event," and these records can be converted (by computer) to the analog "actograms" shown here, in which each vertical marking represents an activity event. The actograms are double plotted: days 1-2 of DD behavior on the top line, days 2-3 on the next, etc. The digital versions of each time series were analyzed by Chi-square periodogram, as in Hamblen et al. (1986), but using newer software, written for a VAX computer by one of us (D.A.W.). These analyses, from which significant rhythmicity was obtained in every case (alpha = 0.05), led to the best estimates of the circadian periodicities (τ) that accompany each of the six panels. The longer-than-normal τ values for the four transformant types, whose behaviors are exemplified in **c** through **f** (see text and Table 2), are reflected in the fact that the active portions of these activity cycles occur later and later on succeeding days.

Table 1. Circadian rhythms of locomotor activity exhibited by wildtype males of *D. melanogaster* (m) and *D. simulans* (s).

Strain	No. rhythmic	Mean period (h ± SEM)	No. arrhythmic
m Oregon-R	61	24.0 ± 0.1	1
m Canton-S	56	24.0 ± 0.2	0
s Kenscoff	27	23.9 ± 0.1	2
s Australia	9	24.2 ± 0.2	0
s Georgetown	11	23.8 ± 0.2	0

The sources of the *D. simulans* strains are given in Wheeler et al. (1991). The method by which these circadian rhythms are monitored is described in the caption to Figure 1. The "significance"of a given rhythm (i.e., one that is not "arrhythmic"), and the determination its period, was by Chi-square periodogram analysis (cf. Hamblen et al., 1986).

Table 2. Circadian rhythms of locomotor activity exhibited by transformed males. The intraspecific (13.2m-TGm) and partially hybrid (13.2s-TGs) transformants involved per DNA cloned from *D. melanogaster* wild-type (Oregon-R) and *D. simulans* wild-type (Kenscoff), respectively. The "per-chimeric" transformants involved transduced pieces of DNA, most of which were from *D. melanogaster* ("13.2m") or from *D. simulans* ("13.2s"), with a central portion of the gene (including the "TG" repeat, see text) removed and replaced by that of the other species ("TGm for *D. melanogaster's* per, and "TGs" for *D. simulans's*).

Stain type/ Insert location	No. rhythmic	Mean period (h ± SEM)	No. arrhythmic
13.2m-TGm: 89A	40	25.0 ± 0.1	0
13.2s - TGs: 18F	5	24.7 ± 0.2	1
13.2s - TGs: 44F	13	25.2 ± 0.1	0
13.2s - TGs: 55C	8	24.3 ± 0.2	1
13.2s - TGs: 62C	4	25.4 ± 0.1	1
13.2m - TGs: 4C	10	24.1 ± 0.3	4
13.2m - TGs: 16B	9	24.1 ± 0.1	0
13.2m - TGs: 30B	13	24.7 ± 0.1	0
13.2m - TGs: 79D	12	24.3 ± 0.2	0
13.2s-TGm: 8E	23	24.6 ± 0.1	0
13.2s-TGm: 12F	17	24.7 ± 0.1	0
13.2s-TGm: 55D	13	25.1 ± 0.2	0

Each male whose locomotor activity was monitored and analyzed (as described in Table 1 and Figure 1) carried one copy of the transduced per DNA. These insert locations were determined by in situ hybridization, using biotinylated probes and acid phospatase histochemical detection. For the 13.2s-TGs strains 44F and SSC, and for 13.2m-TGs 30B, the insert is a homozygous lethal; the other autosomal inserts are homozygous viable. The 13.2s-TGs strains whose male had their song rhythms analyzed in Wheeler et al. (1991) were 18F, 44F, and 62C; song-analyzed 13.2m-TGs strains were 16B and 79D; and for 13.2sTGm, 12F and SSD.

the *D. simulans* homolog of a ca. 13.2 kb genomic fragment, cloned originally from the *per* locus of *D. melanogaster*. The latter had been previously shown -- when transduced into per^{01} flies -- to rescue the arrhythmicity which is associated with adult locomotor activity or courtship singing behavior of males expressing only that "null" allele (Citri *et al.*, 1987; Yu *et al.*, 1987).

The main reason for producing these partial-hybrid transformants was to analyze the periodicities associated with their courtship song rhythms (see Wheeler *et al.*, 1991). But locomotor activity rhythms were analyzed as well -- against a background of what appeared to be essentially identical circadian rhythmicities, as recorded from wild-type flies of these two species (Table 1, Figure 1 a,b). The transformed flies' activity rhythms are described in Table 2, which reveals that the *melanogaster* form of the per^{+} allele mediates essentially the same rhythmicities as does the closely related form of this gene (c.f. Wheeler *et al.*, 1991) from *D. simulans* (also see Figure 1 c,d). In contrast, *D. melanogaster* males whose only functional *per* gene is that from *D. simulans* sang with relatively short-period -- i.e., *simulans*-like -- courtship rhythms (Wheeler *et al.*, 1991); these periodic-

ities were at least 35% shorter than those associated with normal *D. melanogaster* males or transformed ones carrying the conspecific (wild-type) form of this clock gene (Yu *et al.*, 1987; Wheeler *et al.*, 1991). The "intra-locus" etiology of this *per*-controlled behavioral difference was mapped to a central portion of the gene, by constructing chimeric forms of the period gene and generating transformants with them (Wheeler *et al.*, 1991). The chimeras contained "flanking" (coding plus non-coding) regions from one species and a ca. 700 bp central region from the other; the latter includes the "Thr-Gly repeat" that is encoded within either species's form of this locus. The bioassays of these chimeric *per* genes with regard to the circadian phenotype are also listed in Table 2 (with examples shown in Figure 1e,f).

In all of these transformants -- the one intraspecific type tested here, and the various kinds of partially hybrid strains -- the circadian periodicities (Table 2) tended to be longer than those exhibited by the wild-type of either species (cf. Table 1). This observation -- which is consistent with previous results from the "89A" all-*melanogaster* strain (Table 2), and from another intraspecific *per* transformant as well (Citri *et al.*, 1987; Yu *et al*, 1987) -- suggests that the 13.2 kb fragments of *per*-locus DNA are slightly inadequate (e.g., missing some positive-regulatory sequences); or that the transduced pieces of DNA do not function quite optimally in ectopic genomic locations (which are given in the left-hand column of Table 2); or both.

References: Citri, Y., H.V. Colot, A.C. Jacquier, Q. Yu, J.C. Hall, D. Baltimore, and M. Rosbash 1987, Nature 326:42-47; Hamblen, M., W.A. Zehring, C.P. Kyriacou, P. Reddy, Q. Yu, D.A. Wheeler, L.J. Zwiebel, R.J. Konopka, M. Rosbash and J.C. Hall 1986, J. Neurogenet. 3:249-291; Wheeler, D.A., C.P. Kyriacou, M.L. Greenacre, Q. Yu, J.E. Rutila, M. Rosbash and J.C. Hall 1991, Science, in press; Yu, Q., H.V. Colot, C.P. Kyriacou, J.C. Hall and M. Rosbash 1987, Nature 326:765-769.

Chakrabarti, C. S. Deparment of Zoology, Centre for Life Sciences, University of North Bengal, Siligure - 734 430, Darjeeling, India. SEM obervation on some sensory hairs of the third instar larva of *Drosophila ananassae*.

Hertweck (1931) at first described the larval cuticular sense organs of *Drosophila*. Kankel *et al.* (1980) reported six different types of cuticular sensory hairs in *Drosophila melanogaster* and classified them as type A, B, C, D and E.

In the case of *Drosophila ananassae* I have noticed five different types of cuticular sensory hairs, which are morphologically different from the types reported earlier for *D. melanogaster*. For the convenience of study I have classified them as type 1, 2, 3, 4 and 5.

Type-1: This hair is 20 micrometers long and originates from a cuticular depression. Basal part of the hair is broad but it narrows gradually. The tip of the hair is almost pointed (Figure 1a).

Type-2: This hair has an undivided broad basal part with two minute grooves on one side and a bifurcated apical part. Two hairs that originate after bifurcation are arranged in the form of a forcep. Total length of this hair is 15 micrometers (Figure 1b).

Type-3: It is a complex structure, composed of two kinds of hair, which have originated side by side. One kind of hair is provided with a broad base and a needle like straight spike. Other kind of hair is narrow, elongated and curved. The length of each hair is about 10 micrometers (Figure 1c).

Type-4: This sensory hair has a common basal part and two slender arms originating from the base. The length of each arm is about 10 micrometers (Figure 1d). This type of hair distinctly varies from type 2.

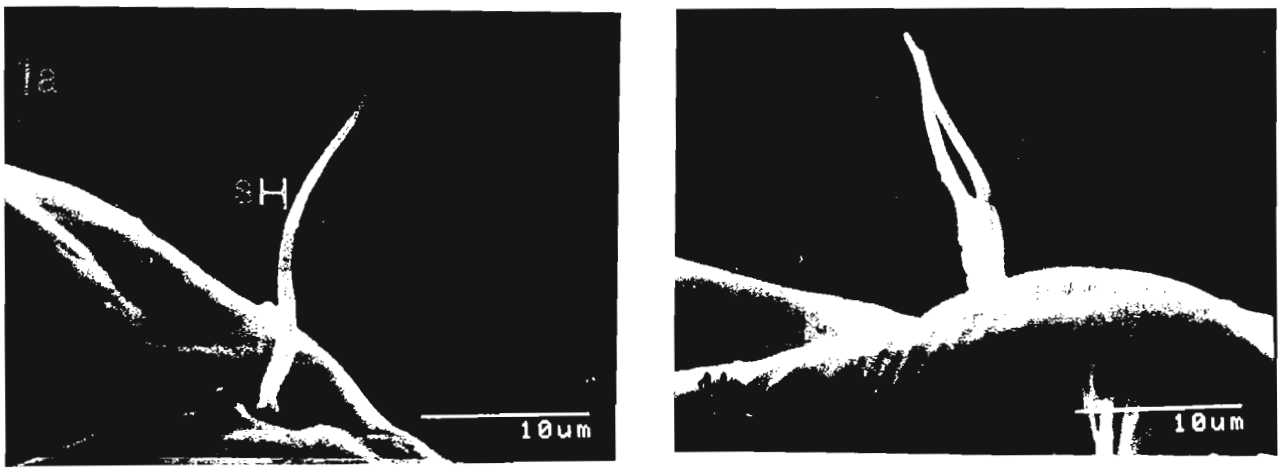


Figure 1. Sensory hairs of the third instar larva: a, Type 1 sensory hair; b, Type 2 sensory hair.

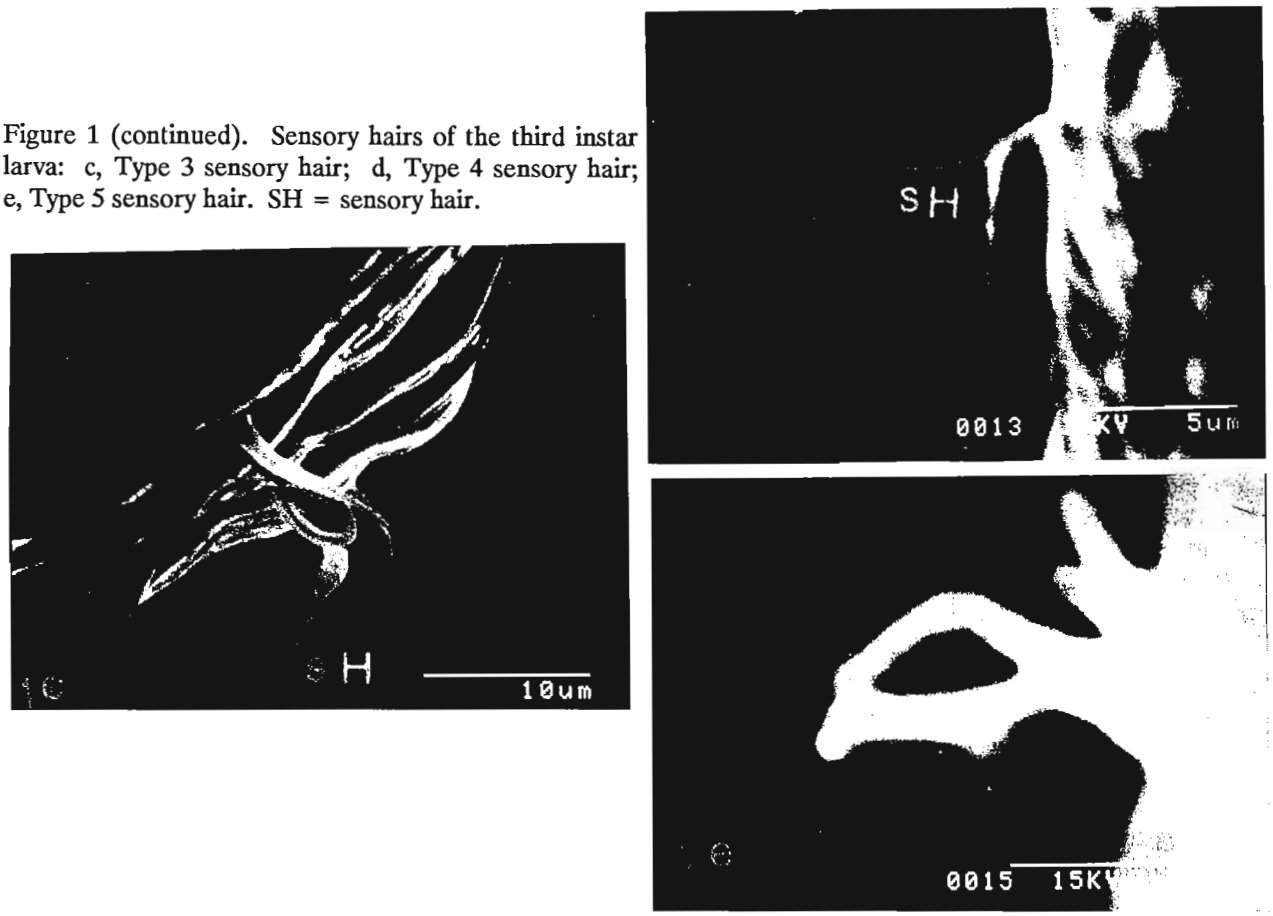


Figure 1 (continued). Sensory hairs of the third instar larva: c, Type 3 sensory hair; d, Type 4 sensory hair; e, Type 5 sensory hair. SH = sensory hair.

Type-5: This complex sensory structure is short and stumpy and is formed by two distinct types of hair. One type of hair is with a broad base, from which two non-identical fingerlike projections originate. Of these two fingers one is virtually straight, with a hammer headed tip and a bud like protuberance at the middle of the arm, whereas the other finger is slender and curved, its tip is united with the tip of the former finger. At the base of this complex structure here is located another form of sensory hair, having a common base and two unequal spike like projections (Figure 1e).

References: Hertweck, H. 1931, Meigen. Z. Wiss. Zool. 139:559-663; Kankel, D. R., A. Rerrus, S.H. Garen, P.J. Harte and P.E. Lewis 1980, The Genetics and Biology of *Drosophila*, Vol. 2d, pp. 295-368; Chakrabarti, C.S. 1990, Ultrastructural and genetical studies on the preadult and adult of *Drosophila ananassae*, Ph.D. thesis, University of Burdwan (unpublished).

Chakrabarti, C.S. Deparment of Zoology, Centre for Life Sciences, University of North Bengal, Siligure 734 430 Darjeeling, India. SEM observation on the filaments of the egg surface of *Drosophila ananassae*.

The egg of *Drosophila* is bilaterally symmetrical, elongated structure with narrower anterior end and broader posterior end. Usually a pair of delicate filaments as extension of the chorion are found on the dorsal surface of the egg at its anterior end. These are variable in number (Sturtevant, 1921). Wieschaus (1980) considered the filament as respiratory appendages.

The present scanning electron microscopic studies on the eggs of *Drosophila ananassae* have clearly expressed the structural detail of the paired filaments. Average length of the filament is 230 micrometers. They originate from a common platform but after their full growth they assume the shape of a forcep (Figure 1a). The filaments are not identical in shape. One of the two filaments is straight with a club shaped apical end. This is much broader than the other and is provided with distinct cellular patterns (Figure 1b). The other filament is narrow, little bigger than the former and with distinct curvature at the mid position. Tip of this filament is slightly swollen and is provided with two prominent hook-like projections (Figure 1c). Presence of these hook-like structures suggests that these appendages not only perform respiratory function as suggested by Wieschaus (1980) but also act as attachment and sensory organ.

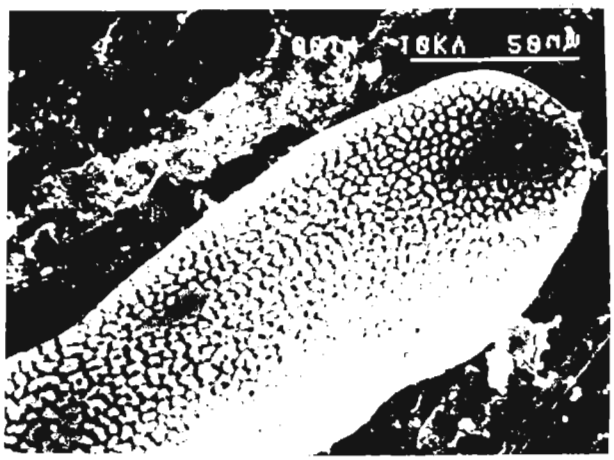
References: Sturtevant, A. H. 1921, The North American species of *Drosophila*; Weischaus, E. 1980, Development and Neurobiology of *Drosophila* pp. 85; Chakrabarti, C. S. 1990, Ultrastructural and genetical studies on the preadult and adult of *Drosophila ananassae*. Ph.D. thesis, University of Burdwan (unpublished).

Figure 1a. Egg of *Drosophila melanogaster* with extended filaments.

Figure 1b. Broad tip of one filament.

Figure 1c. Tip of the narrow filament with hooks.

FL = filament; HK = hook.



Chakrabarti, C.S. Department of Zoology, Centre for Life Sciences, University of North Bengal, Siligure - 734 430 Darjeeling, India. SEM observation on the oral sensory structures of the third instar larva of *Drosophila ananassae*.

A number of larval cephalic sensory structures were reported for *D. melanogaster* by different authors. For example, the ventral organ and antenno-maxillary complex was reported by Hertweck (1931); the labial organ was reported by Strasberger (1932). The scanning electron microscopy of some of these organs had been reviewed by Kankel *et al.* (1980).

In the present scanning electron microscopic study I have noticed most of these structures for *D. ananassae* also. In addition, I have observed different types of sensory spinules and papillae in the oral part of the 3rd instar larvae. Some of these structures are morphologically different from that of *D. melanogaster*.

The larval mouth aperture is bordered by two dorsal lips and a ventral lip. Each dorsal lip is provided with a distinct ovoid structure called the antennal element (Figure 1a).

By the dorso-lateral side of the antennary complex, the maxillary element is located. This structure morphologically differs from the same structure of *D. melanogaster* (Figure 1b).

On the ventral side of the lower lip, rostral spinules are arranged in a number of rows. Average length of the spinule is 4 micrometers, a spinule is provided with a broad base and pointed tip (Figure 1c). In addition, a

Figure 1a. Antennal element of the third instar larva.

Figure 1b. Maxillary element of the third instar larva.

Figure 1c. Rostral spinules of the third instar larva.

Figure 1d. Oral papillae of the third instar larva.

AE = antennal element; ME = maxillary element; RS = rostral spinules; OP = oral papillae.

number of delicate, bushy oral papillae are found around the mouth aperture and mandibular hooks. These papillae are arranged in a number of rows. Each papilla is slender and slightly curved with pointed tip (Figure 1d).

References: Hertweck, H. 1931, Meigen, Z. Wiss. Zool. 139:559-663; Strasberger, M. 1932, Meigen, Z. Wiss. Zool., 140:539-649; Kankel, D.R., A. Rerrus, S.H. Garen, P.J. Harte, and P.E. Lewis 1980, The Genetics and Biology of *Drosophila*, Vol. 2d Edited by M. Ashburner and T. R. F. Wright; Chakrabarti, C.S. 1990, Ph.D. thesis, University of Burdwan (unpublished).

Chakrabarti, C.S. Department of Zoology, Centre for Life Sciences University of North Bengal, Siliguri - 734 430, Darjeeling, India. SEM study on the sensory cones of the third instar larva of *Drosophila ananassae*.

Herweck (1931) described seven large sensory cones on either side of the larval abdominal segment. He classified them into three types. Ferris (1950) reported the presence of six sensory cones for *D. melanogaster* and classified them into three types.

During scanning electron microscopic study of the third instar larvae of *D. ananassae* I have noticed five types of sensory cones, which differ from each other very slightly but their difference from that of *D. melanogaster* is significant. I have classified them into five categories. Which are as follows:

Category-A: This cone is provided with rows of backwardly projected spinules. Each spinule is approximately 8-9 micrometers long. The terminal end of the cone is blunt but is provided with a number of slender soft sensory hairs (Figure 1a).

Category-B: This type of cone is covered by a number of spinules; which are arranged in different directions. These spinules are much more delicate than the category A spinules (Figure 1b).

Category-C: In this cone the spinules of its basal part are backwardly directed and are arranged in definite rows but the spinules of the distal part of the cone being longer and slender, form an entangled mass at the apex (Figure 1c).

Category-D: This cone is provided with curved sensory spinules, which are arranged irregularly at the base of the

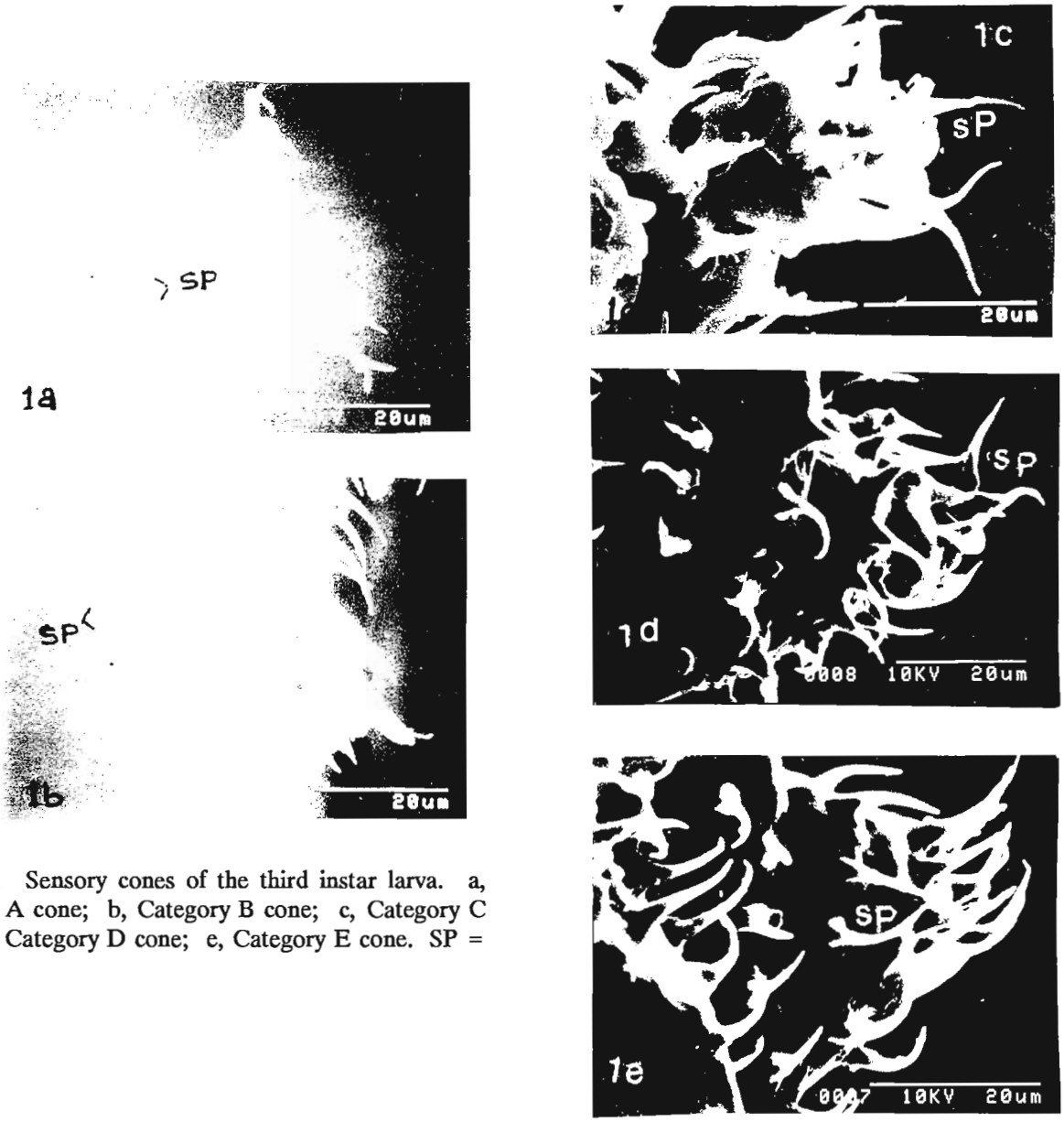


Figure 1. Sensory cones of the third instar larva. a, Category A cone; b, Category B cone; c, Category C cone; d, Category D cone; e, Category E cone. SP = spinule.

cone but the spinules of the distal part overlap each other (Figure 1d).

Category-E: Spinules of this cone are also curved but are arranged in a regular manner at the basal part of the cone. At the apex many spinules are found very close to each other (Figure 1e).

References: Hertweck, H. 1931, Anatomic und variabilitat des nerven system und der sinnesorganne von *Drosophila melanogaster* Meigen. Z. Wiss. Zool. 139:559-663; Ferris, G.F. 1950, Biology of Drosophila, pp. 368-418; Chakrabarti, C.S. 1990, Ultrastructural and genetical studies on the preadult and adult of *Drosophila ananassae*, Ph.D. thesis, University of Burdwan (unpublished).

Ziemba, S.E. Biological Sciences, Wayne State University, Detroit, MI, USA. Speciation of aerobic and facultative anaerobic microorganisms associated with *Drosophila melanogaster.*

A variety of extracellular microorganisms are normally associated with *Drosophila melanogaster*, the majority found as apparent commensal flora of the gut and in fecal materials. These microorganisms exist in a symbiotic relationship with healthy *D. melanogaster*, are present in both larva and adult flies, and are passed from adults to newly emerging larvae primarily by fecal contamination of the eggs by the adults (Ashburner,1989). Brooks

(1963) and Bakula (1969) have noted the commensal flora of *Drosophila* as Bacillus and Brevibacteria, but a detailed list of the specific species associated with *Drosophila* proved hard to find. Using *Drosophilia* wild type and mutant strains (almost all derived from the Oregon-R strain of *D. melanogaster*) maintained by Dr. Robert Stephenson, Wayne State University, I have compiled such a list.

Drosophila stock bottles were chosen so that dates of fly transfer, age of the adult flies, and the internal conditions of the bottles and their media were as similar as possible. The *Drosophila* selected for isolation were of healthy flies maintained two to four weeks on standard cornmeal media, supplemented with yeast. Adult flies were ether-anesthetized, surface sterilized (2 minute serial immersions in 5% bleach, 95% ethanol, sterile distilled water), and aseptically ground in sterile water to provide material used for microbial culture. Five to ten flies were sacrificed per *Drosophila* stock culture; twenty-two different stock cultures used in all.

Bacterial isolation was achieved using standard microbial isolation techniques with enriched (sheep blood agar) and selective (MacConkey, CNA, BCG agars) media (BBL, Remel products). Every different colony type observed after 48 hours incubation at 32°C was isolated, Gram stained for cell morphology and identified using standard biochemical tests (BBL, Remel, API, Flowlabs products). Twenty to thirty different biochemical characteristics were assessed on each isolate; these results then were correlated with cell and colonial morphologies and compared to established microbial species characteristics to speciate each separate isolate.

The following list encompasses those microorganisms I have identified as commensal flora of *D. melanogaster*. Amounts given correspond to the relative amount of microbial growth seen over the surface of a sheep blood agar plate obtained from one or two crushed *Drosophila* spread evenly over the agar surface. Amounts and presence of each species will vary with culture age, media type, and bottle conditions of the fly stock cultures examined.

1. *Bacillus* species - moderate to heavy growth:
   a. *B. panthothenticus*
   b. *B. sphaerius*
   c. *B. babius*
   d. *B. circulans*
   e. *B. stearothermophillis*
2. *Brevibacterium* species - moderate to heavy growth:
   a. *Br. linens*
   b. *Br. epidermidis*
   c. *Br. oxidans*
   d. *Brevibacterium* sp. (unable to speciate by biochemical parameters)
3. *Curtobacterium pusillemas* - moderate growth
4. *Candida krusei* - none to moderate growth (There is an inverse relationship of the presence of *Candida* as commensal flora when compared to the presence of bacterial commensals; the common microbiota is heavy bacterial growth and none to rare yeast present.)
5. Gram negative bacilli - normally absent as microflora of *Drosophila* (Presence of Pseudomonas or Enterobacteriacae species is usually due to a transient contamination of the *Drosophila* culture.)

Bakula (1969) commented on the selective (genetic?) preference for the Gram positive bacilli listed, as compared to the more common mix of Gram negative bacilli plus relatively fewer Gram positive bacteria present as intestinal microflora of other organisms. But *Enterococcus faecalis*, a Gram positive coccus found normally in the human gut, did appear consistently in some for the *Drosophila* strains. This suggests the possibility that some enteric organisms common to other species are able to inhabit a stable niche in the *Drosophila* microbiota. Further studies of these symbiotic relationships are indicated.

References: Ashburner, M. 1989, Drosophila: A Laboratory Handbook, p.1195; Bakula, M. 1969, The persistence of a microbial flora during postembryogensis of *Drosophila melanogaster*. J. Invert. Path. 14:365-374; Brooks, M. 1963, The microorganisms of healthy insects. Insect Pathology 1:215-250, Academic Press, New York; Claus, D. and R. Berkeley 1984, Bacillus, In: Bergey's Manual of Systematic Bacteriology 1:1105-1139; Dasch, G., *et al.* 1984, Endosymbionts of Insects, In: Bergey's Manual of Systematic Bacteriology 1:811-833; Jones, D. and R. Keddie 1984, Brevibacterium, In: Bergey's Manual of Systematic Bacteriology 2:1301-1313; Komagata, K. and K. Suzuki 1984, Curtobacterium, In: Bergey's Manual of Systematic Bacteriology 2:1313-1317; Richards, A. and M. Brooks 1958, Internal Symbioses in Insects. Annual Review of Entomology 3:37-56; Silva-Hutner, M. and B. Cooper 1980, Yeasts of Medical Importance, In: Manual of Clinical Microbiology, Third Edition, pp.562-576, American Society for Microbiology, Washington, D.C.

Alexandrov, I.D. Joint Inst. for Nuclear Research, P.O. Box 79, Moscow, Russian Federation (RF). Different patterns of spontaneous mutability in two wild-type sib stocks of *D. melanogaster* with long-term laboratory history.

Out of the wild-type base population founded by Toropanova (1960) from one pair flies collected at the northern edge of area of the species (Domodedovo, Moscow Region) two inbred lines (D-18 and D-32) have been simultaneously established by one sib pair mating during ten generations (Glembotski et al., 1962). At the time of derivation and shortly after that, the lines were remarkable for apparently different rates of the spontaneous sex-linked recessive lethals (SLRL) in male germ cells (Tables 1 and 2). Bearing in mind these features, both lines have been introduced in our researches on radiation genetics since 1966 (Alexandrov, 1969). As a result extensive data on the spontaneous rates of SLRL as well as visible mutations in germ cells of males from the lines named have been collected over the last three decades. Thereto, it is important to note that all the time the base lines were maintained by 2-vials mass cultures.

Analysis of data concerning D-18 (Table 1) shows that, firstly, the spontaneous rate of SLRL in male germ cells has been reduced by 1966 (the beginning of our researches) to that generally observed in long-term wild-type laboratory stocks of *D. melanogaster* (Schalet, 1986) and secondly, mutation rates were practically the same over period under consideration.

In contrast, the mutability pattern in D-32 male germ cells over the same time was not such as uniform as that for D-18 males. In particular, at least two periods of highly increased mutation rate may be apparently noted. The first of them (Table 2) has fallen on 1980-1981 and was characterized by the high level of SLRL only whereas the second started in the end of 1986 is steady continuing up until today displaying high specific locus mutability as well. Thereby, the average recessive visible mutation rate for 5 loci used in D-32 line turned out to be almost one order higher than that usually observed in "normal" wild-type laboratory stocks (Shukla et al., 1979). The further genetic and cytological analysis of visibles scored showed that a bulk of them are fertile, transmitted, not mosaically expressed, stable, and point, i.e., unassociated with any detectable cytological changes, mutants.

At the same time, it was found that flies from D-32 stock display some traits of the reduced fitness. In particular, the fertility of females broadly varies so that the average fecundity of fertile females from D-32 line is one sixth as high as of that for females from "usual" wild-type laboratory stock (Table 3). Thereby, the females with low

Table 1. Spontaneous mutation rates in D-18 male germ cells over three decades of observation

Time of analysis	Mutations scored*					
	SLRL			Specific locus mutations		
	No. of chromosomes tested	No. of lethals	% of lethals	No. of alleles tested	No. of visibles	Mutation rate (x 10^{-5} /locus)
Mar., 1959 - Feb., 1961**	10363	36	0.347	—	—	—
Jan., 1966 - Mar., 1967	1764	2	0.113	493979	1^{s} (vg)	0.20
Jan., 1970	1478	3	0.203	—	—	—
Mar., 1973	1215	1	0.082	87935	0	0
Apr., 1974	962	1	0.103	—	—	—
Mar., 1986	1076	0	0	40714	0	0
Feb., 1987	5494	6	0.109	—	—	—
Total for 1966-1987	11989	13	0.108	622628	1	0.16

* In all experiments individual (or 2) males were daily mated to 5 (or 10) fresh virgin females of KL stock, Ins (l) sc^{SI}, sc^{8} + dl-49, y w^{a} (M5), b cn vg throughout 5 days. Visibles of 5 loc i (y, w, b, cn, vg) were scored among F_1 whereas SLRL were detected by standard M5 method in F_2 and re-tested in F_3; ** Data from Glembotski et al., 1962; ^{s} Sterile mutant.

Table 2. Spontaneous mutation rtes in D-32 male germ cells over three decades of observation

Time of analysis	Mutations scored*					
	SLRL			Specific locus mutations		
	No. of chromosomes tested	No. of lethals	% of lethals	No. of alleles tested	No. of visibles	Mutation rate (x 10^{-5} /locus)
Mar., 1959 - Feb., 1961**	9891	5	0.051	—	—	—
Jan., 1966 - Mar., 1967	3736	2	0.053	364393	3^{s}(w^{a}, b,vg)	0.82
Apr.,1973	1974	1	0.051	166258	0	0
Nov., 1977	1051	1	0.095	69277	0	0
Dec., 1980	633	1	0.157	17606	0	0
Dec., 1981	794	3	0.378	40618	0	0
Mar., 1986	1135	1	0.088	53891	0	0
Mean for 1966-1986	9323	9	0.096	712043	3	0.42
Dec., 1986	2059	25	1.214	—	—	—
Apr.-Aug., 1987	4768	15	0.314	199356	3^{t}(w, 2b)	1.50
Feb., 1988	2497	6	0.24	322057	7^{t}(3y, 2w, b, vg)	2.17
Apr., 1988	2201	12	0.545	333305	4^{t}(b, 3vg)	1.20
July, 1988	2304	5	0.217	423351	7^{t}(2y,2b, 3vg)	1.65
Nov.-Dec., 1991	—	—	—	314735	9^{t}(y,w,2b, cn,4vg)	2.85
Mean for 1986-1991	13829	63	0.455	1592804	30	1.88

*, **, s- see table 1; t = transmitted and not associated with cytologically detectable chromosomal changes mutants.

fertility show the bilateral atrophy of ovaries degree of which proved to vary from female to female. In addition, the average life-time of D-32 flies was found to be half as short again as that of the flies of Seychelles ("usual") wild-type inbred stock (50 and 80 days, respectively).

Thus, in the case of D-32 line we deal with somewhat unusual system of genetic instability displaying the dysgenic (high mutability, reduced fertility, atrophy of gonadal tissues) as well as an atypical for P-M, I-R or hobo-mediated dysgenesis traits (intrinsic to the same stock genetic instability, the lack of chromosomal changes and reversions in mutant alleles). To study what kind of mobile element may be responsible for genome instability described, the number and distribution patterns of ten different elements in D-32 line and spontaneous visibles isolated were studied by *in situ* hybridization and the results obtained are reported in this issue elsewhere.

Table 3. The mean of progeny from inter-or intrastrainpair crosses* for 12 days

♂ \ ♀	D-32	S+(**)
D-32	61.5 ± 14.0	295.0 ± 81.8
S+	34.8 ± 12.3	310.3 ± 39.2

* = In each crossing not less than 15 pairs were examined;** = Seychelles wild-type inbred (F_{40}) stock.

References: Alexandrov, I.D. 1969, DIS 44:78, 114; Glembotski, J.L., E.A. Abeleva and Yu.A. Lapkin 1962, In "Radiation Genetics", Engel'gardt, V.A. (Ed.), Publisher's house AS USSR, Moscow, pp. 300-311; Schalet, A.P. 1986, Mutation Res. 163:115-144; Shukla, P.T., K. Sankaranarayanan and F.H. Sobels 1979, Mutation Res., 61:229-248; Toropanova, T.A. 1960, C.R. (Doklady) Acad. Sci. USSR 132:460-463.

Alexandrova, M.V. and I.D. Alexandrov, Joint Inst. for Nuclear Research, P.O. Box 79, Moscow, Russian Federation (RF). Frequency spectra of mobile elements in two wild-type inbred stocks of *D. melanogaster* with different spontaneous mutability.

To define a likely DNA rearrangement in D-32 genome as a putative cause of its continual and over-all high spontaneous mutability (Alexandrov, this issue) the analysis of number and distribution patterns of ten different mobile elements in the salivary gland chromosomes of line named was performed. Simultaneously, the same work was done with sib D-18 line displaying a "usual" low spontaneous mutability (Alexandrov, ibid.). For experiments started in January of 1987, samples of D-32 and D-18 populations were allowed to develop at lower temperature (18-19°C) to obtain the larvae with chromosomes suitable for *in situ* hybridization. The purified probe DNAs were labeled with ^{3}H-TTP to a specific activity of about 2×10^7 dpm/ug of DNA (Rigby et al., 1977) and *in situ* hybridization was done as described in Pardue and Gall (1975) with a minor modification (Belyaeva et al., 1984). 3-6 larvae were used for each probe from each line. As a rule, either half of the gland of individual larva was hybridized with two different denatured DNA probes on the same slide. After staining by Giemsa, the consistent labeling sites of full intensity were scored to bands within the lettered subdivisions of polytene chromosomes.

The quantitative data available in terms of the average number of insertion sites of particular element per genome and of unstable sites (i.e., observed in some, but not all, larvae examined) among them per genome are shown in Table 1. As seen, both lines have in a single copy of P and gypsy elements and appear the frequency patterns of mdg-1, mdg-2, mdg-3, I, and jockey elements that usually show in "typical" wild-type laboratory strains (Bingham and Zachar, 1989). It is striking that the distribution patterns of mdg-1, mdg-2 and mdg-3 along chromosomes was found to be practically the same for all larvae and lines analyzed. For example, there were 21 insertion sites of mdg-1 common to both D-32 and D-18 lines (in threes larvae examined), from a total of 24 and 25 labeling sites for those lines, respectively.

In contrast, a copy number of B104 and, especially, copia as well as their distribution patterns throughout the genome proved to be not so remarkably constant even among the larvae from some line under study. As a result, the average per genome polymorphism values (the ratio of mean of unstable sites to mean of all detected sites of a particular element) are essentially higher than those found for mdg-elements (Table 1). Moreover, these values for high mutable D-32 line are twice as high as those observed in the mutationally stable D-18 line. Therefore, D-32 genome proved to display at least two interconnected features of genetic instability (high rates of spontaneous mutations and transpositions).

To test further as to whether either unstable B104 and copia (or both simultaneously) are responsible for high mutation rate in D-32 male germ cells the existence of these retrotransposons at the specific loci was assessed by *in situ* hybridization of polytene chromosomes in spontaneous *y, w, b* and *vg* mutants. Previous to this study, it was ascertained that a genetically silent insertions of B104 and copia in non-mutant D-32 larvae were constantly observed around *y, w* (sites 1B1-4 and 3C6-8 for copia) and *vg* (site 49D1,2 for B104) loci.

At present, the analysis of 4 *y*, 3 *w*, 3 *b*, and 7 *vg* spontaneous and cytologically point mutants have been performed

and the results obtained are following: 3 of 4 *y* mutants have an intact copia insertion site at the 1B1-4, but the fourth (y^{88c41}) has fully lost a copia from this site indicating that y^{88c41} seems to result from an excision of copia and neighboring unique genetic loci as well since this mutation genetically proved to be a multilocus deletion (*arth*$^-$*y*$^-$*ac*$^-$). Further, all of 3 *w* mutations examined show an extra copia insertion at the 3C2,3 band where the *w* locus is known to be located. The same picture, i.e., the appearance *de novo* copia at the regions containing our target genes (34D4-6 for *b* and 49D3-6 for *vg* loci), is observed for all *black* and 5 out of 7 *vg* mutations studied.

Table 1. Frequency spectra of different mobile elements in high-(D-32) and low- (D-18) mutable stocks of *D. melanogaster*

	D-32			D-18		
Mobile elements	Mean of sites/genome	Mean of unstable site/genome	Vp*	Mean of sites/genome	Mean of unstable site/genome	Vp*
P	1 (17C)	0	0	1 (17C)	0	0
gypsy	1 (96F)	0	0	1 (89C)	0	0
jockey	21	0	0			
I	33	0	0	20.3	2.67	0.13
mdg-1	23.6	0.33	0.01	23.7	0.67	0.03
mdg-2	20.7	2.75	0.13	22.7	0.50	0.02
mdg-3	23.7	0.25	0.01	27.0	0	0
Dm 297	53.0	6.00	0.11	48.0	7.00	0.15
B104 (roo)	80.0	36.00	0.45	85.0	20.00	0.23
copia	105.3	64.33	0.61	70.4	20.00	0.28

* - The average per genome polymorphism value

Thus, the data obtained show that (a) active transposition of mobile elements and (b) insertion mutations are not limited to hybrid dysgenesis only but can also be observed in long-term inbred lines maintained in the laboratory by small mass cultures.

The causes as well as the mechanisms underlying the continual molecular instability of D-32 genome remain to be clarified. Nevertheless, the striking phenomenology ascertained (the dysgenic traits in the intrastrain crosses, an increase in transposition and excision rates for two copia-like retrotransposons only, the stability of insertion gene mutations, and the lack of chromosomal changes in visibles) shows that we seem to deal with a new type of unstable genetic system that is characterized first of all by the overproduction of two (copia and B104) retrotransposons at the same time. It is interesting that both elements are known to have the same (in size) duplications of five bases of host sequence at the insertion sites. A further analysis of unstable genetic system in question may give a unique opportunity to assess the copy number control mechanisms and some general questions of the genetics and biology of retrotransposons as well.

Acknowledgments: We thank Prof. V.A. Gvozdev for DNA probes employed in the present study. We are also thankful to Drs. E.Sp. Belyaeva and E.G. Pasyukova for research assistance and useful discussion.

References: Belyaeva, E.Sp., E.V. Ananiev and V.A. Gvozdev 1984, Genetica (USSR) 20:1255-1263; Bingham, P.M. and Z. Achar 1989, In "Mobile DNA" (Eds. Berg, D.E., M.M. Howe), Washington, D.C., Amer. Soc. Microbiol., 485-502; Pardue, M.L. and J.G. Gall 1975, Methods Cell Biol. 10:1-17; Rigby, P.W.J., M. Dieckmann, C. Rhoades and P. Berg 1977, J. Mol. Biol. 113:237-251.

Alexandrov, I.D. and M.V. Alexandrova. Joint Inst. for Nuclear Research, P.O. Box 79, Moscow, Russian Federation (RF). The distribution patterns of roo and copia retrotransposons under reduction of fitness.

As it was shown (Alexandrov, this issue), the flies from D-32 wild-type inbred stock are displaying as high spontaneous mutation rate as a certain traits of the reduced fitness, in particular, a low female fertility. A high insertion rate of copia and roo retrotransposons in D-32 lines (Alexandrova and Alexandrov, this issue) suggested that the flies with the most reduced fertility may have the greatest copy number and distribution polymorphisms of the elements named. To check this supposition, the number and distribution patterns of copia, roo, mdg-1, and mdg-3 elements in one of the semisterile D-32 subculture were analyzed by *in situ* hybridization of ^{3}H-labeled DNA probes on giant salivary gland chromosomes. 3-4 larvae were used and 608 nuclei per larva were analyzed for each of elements examined.

To data, the results obtained appear as follows. Firstly, no polymorphism was found for mdg-1 and mdg-3 retrotransposons. Their copy number and location on the chromosomes of semisterile subline were the same as that observed for an initial population. Secondly, the picture for copia and roo retrotransposons proved to be surprisingly different from that supposed. As seen (Table 1), there is striking stability, but not the increase as proposed, of copy number of both elements if these data to compare with those for the initial population (Table 1, in Alexandrova and Alexandrov, this issue). However, the location of some roo and many of the copia insertion sites on the chromosomes of larvae from semisterile subline were found to be quite different from distribution patterns of these elements observed

in the initial population. For example, the stable sites 35A, 53C, 64D 73C for roo and 2B3.5, 7A, 12E, 15F, 18A, 22E, 26E, 39B, 44A, 51B, 54D, 57A,C, 59E, 66A, 67B, 68C, 73D,F, 85F, 93D and 99A for copia which were noted in all larvae from semisterile subline proved to be never found in larvae from the initial population. Thereby, any cytologically detected chromosomal changes associated or unassociated with the insertion sites of copia or roo in semisterile D-32 subline were not observed.

Table 1. Copy number of roo and copia retrotransposons on the chromosome arms of individual larvae from semisterile subline out of D-32 unstable stock of *D. melanogaster*

Chromosome arms	roo						copia				
	Larvae No.					No.of unstable	Larvae No.				No.of unstable
	1	2	3	4	mean	sites	1	2	3	Mean	sites
X	13	14	15	13	13.7	2	23	22	23	22.6	3
2L	14	14	14	14	14.0	0	18	20	19	19.0	5
2R	9	9	9	9	9.0	0	21	21	22	21.3	7
3L	15	17	17	16	16.2	4	22	26	25	24.3	21
3R+4	14	14	14	14	14.0	0	15	20	26	20.3	13
Total	65	68	69	66	67.0	6	99	109	115	107.6	49
Mean of unstable sites /genome						1.5					16.3
V_p*					0.02					0.15	

* V_p = the average per genome polymorphism value (see text in Alexandrova and Alexandrov, this issue).

Thus, the reduction of fitness (via sterility) seems to be accompanied (or brought forth) by a broad molecular reshifting of the facultative components of the genome resulting to direct and cooperative mutagenic effects of retrotransposons themselves rather than by secondary deleterious effects of putative chromosomal changes usually attendant, as in a typical hybrid dysgenesis, the process of activation of transposable elements. A high frequency of flies with reduced fitness in D-32 lines shows that the formation of deleterious sets of mobile elements by random genetic drift and selection against chromosomes with such sets to decrease abundance of retrotransposons in D-32 line to "optimal" level is continuing so far. How long will these genetic processes continue, and what is the mechanism(s) generating the abundance of copia and roo in D-32 unstable stock? The answers to these questions remain still obscure.

Seymour-Jones, S. and J.I. Collett, School of Biological Sciences, University of Sussex, Brighton, Sussex, BN1 9QG, U.K. Two genes, two polypeptides and eight (or more) leucine amino peptidases.

The activity of Leucine amino peptidase in *D. melanogaster* is readily detectable in electrophoretic separations and measurable spectrophotometrically. Electrophoresis of preparations by the standard methods of protein separation in starch and acrylamide gels however do not provide satisfactory clarity of activity, but Hall (1986) by introducing the nonionic detergent Triton X-100 into homogenate preparations and into gel media was able to distinguish two Leucine amino peptidase activities in both electrophoretic and chromatographic separations. Their elution from Sephadex and Sepharose columns indicated that the size of one is substantially greater than the size of the other. The larger is associated exclusively with larval and adult guts (LAP G) and the smaller appears more prominently in pupal hemolymph (LAP P), but is also present in ovaries and in guts.

A search for conditions of electrophoresis that would provide greater clarity of separation of the two types of LAP's proved successful in revealing that LAP G and LAP P each consist of further distinguishable activities. Acrylamide gels (39:1 acrylamide to bis-acrylamide) were prepared following the method of Studier (1973) for separation in a discontinuous buffer system consisting of gels made in 50 mM Tris-citrate, pH 7.0 and 10 mM lithium-borate, pH 8.2, containing 0.1% Triton X-100 and separation in 93 mM lithium-borate buffer, pH 8.2 containing 0.025% Triton X-100. LAP activity was identified by the method of Beckman and Johnson (1964). The percentages of gels used here were varied from 4.0 to 7.5%, in 0.5% increments. The patterns of separation were markedly different at each gel concentration. In gels of 4-5% acrylamide the LAP from dissected guts appeared consistently in three to five closely spaced bands of activity of varying intensities close to the cathode. The spacing and intensities of the bands were consistent with five molecular structures of which the three less mobile were more active (in these conditions). These activities correspond to Hall's (1986) LAP G. At higher acrylamide concentrations, similarly prepared whole pupae of stage 8 (Bainbridge and Bownes, 1981) generated a thick but considerably weaker band of activity at the cathodal position of LAP G, but in addition, three more mobile and distinctive activities. These activities correspond to Hall's (1986) LAP P. Other still more mobile bands of indistinct band morphology and less activity appear in some gels and in different patterns in preparations of flies carrying the LAP A^{null} and LAP A^{fast} alleles (Beckman and Johnson, 1964).

An explanation that encompasses these greatly refined electrophoretic distinctions among the LAPs, their differences in size (Hall, 1986) and the variation of electrophoretic patterns found in preparations of flies with LAP variants is that the two genes, LAP A and D, identified by Beckman and Johnson (1964) and mapped by Falke and MacIntyre (1966), generate both tetramers and dimers of polypeptide subunits coded for by the LAP A and D genes. The large LAP G thus appears as a tetramer and its five forms are the five possible constructs of two different polypeptides coming from two different genes, LAP A and D. It is a highly "sticky" molecule and therefore without release from the gut with detergent remains largely insoluble or at best as a smear of activity in electrophoretic separations. Similarly, the smaller, more mobile and less "sticky" LAP P appears to be a dimer of the same polypeptide subunits, LAP A and D, constructed in all three possible ways. The other (highly mobile) activities seen less consistently in electrophoretic separations of these preparations may be monomeric forms of LAP A and D. Are these eight or more forms functionally distinctive?

References: Bainbridge, S.P. and M. Bownes 1981, J. Embryol. Exp. Morph 66:57; Beckman, L. and F.M. Johnson (1964) Hereditas 51:221; Falke, E.V. and R.J. MacIntyre 1966, Dro. Inform. Serv. 41:169; Hall, N.A. 1986, Biochem. Genetics 24:775; Studier, F.W. 1973, J. Mol. Biol. 79:237.

J.P. Kundu and A.S. Mukherjee. Genetics Research Unit, Dept. of Zoology, Calcutta Univ., 35 Ballygunge Cir. Road, Calcutta 700 019, India. Evidence for the existence of HMG-like proteins in *Drosophila melanogaster* and functional implications through *in situ* transcription assay.

The high mobility group (HMG) proteins have been isolated and purified from mammals, chicken and other vertebrate systems (see Goodwin and Bustin, 1988). This group of proteins are extractable in 0.35 to 0.4M NaCl or 5% PCA, and precipitated in 18 to 20% TCA. When these proteins are fractionated through acetone-HCl and acetone precipitation and run on AUT-PAGE or SDS-PAGE, they migrate faster and show four distinct bands, two at 27-29 kd region and two (or three) at 12-15 kd region. Attempts have been made earlier to identify the HMG proteins from invertebrates and specially from insects. However, their existence in insects including *Drosophila* has not been definitely substantiated. Bassuk and Mayfield (1982) have isolated two proteins of 63 kd and 13 kd size from *Drosophila*, which they have claimed to qualify the properties of HMG-like proteins, with respect to mobility profile in AUT-PAGE and SDS-PAGE and also in respect of the amino acid composition at the N- and C-terminal ends.

Table 1. Mean grain number [^{3}H-UMP incorporation] + S.D. into the X chromosomes and 2R segments and X/A ratios following *in situ* transciption assay

	MALES		FEMALES	
	CONTROL [HMG-]	TREATED [HMG+]	CONTROL [HMG-]	TREATED [HMG+]
X-Chromosome [1A-10F]	133.3 ± 36.8	274.7 ± 66.3	124.7 ± 46.6	244.7 ± 67.0
2R [56F-60F]	59.2 ± 30.4	105.0 ± 31.6	66.5 ± 30.4	107.1 ± 34.9
X/A	2.2 ± 0.4	2.7 ± 0.3	1.8 ± 0.3	2.3 ± 0.3
	SIGNIFICANT AT 5% LEVEL			

However, since the proteins isolated by Bassuk and Mayfield (1982) have different molecular size, and no sequence homology has been done, the true nature of the HMG-like proteins remains controversial.

Schroeter et al. (1985) have shown that HMG 14, 17 can be exclusively extracted from the chromatin of chicken erythrocytes upon intercalation with ethidium bromide (5 to 10 mM) and subsequent precipitation in 18% TCA followed by acetone-HCl and acetone fractionation. This technique has also been successfully used for mammalian cells (Mukherjee et al., 1990). We have carried out an investigation for isolating and fractionating the putative HMG proteins from *Drosophila* using the ethidium bromide intercalation and subsequent extraction in low ionic strength buffer, and compared with the profiles of the proteins isolated by using the conventional 0.4M NaCl and 5% PCA extraction procedures. The results are presented below.

For this purpose, late third instar larvae of *Drosophila* were homogenized in a minimum volume of NE buffer, pH 7.5 (75 mM NaCl, 25 mM EDTA), containing 100 mM PMSF, and spun at 6000 rpm for 10 minutes. The pellet was collected and homogenized in buffer E, (10 mM Tris HCl, pH 7.0, 2mM $CaCl_2$, 0.2mM PMSF, 10 mM Beta-mercapto ethanol, pH 7.5) containing 5% Triton X-100 and incubated at 0°C for 4-5 hours. The homogenate was spun at 6000 rpm for 10 minutes and the pellet was collected. To the pellet, buffer E + 0.35 M sucrose + 7.5% DMSO were added,

and centrifuged at 6000 rpm for 10 minutes. The pellet was washed with the buffer E, and dissolved in low ionic strength TEA buffer, pH 7.5 (1 mM Triethanol-amine and 0.2 mM EDTA) containing 7.5 mM EtBr and incubated for 30 minutes on ice. 20% TCA was added and centrifuged at 20000 rpm for 20 minutes, and the pellet was dispersed in acidified acetone (acetone:HCl, 7:1), centrifuged at 10000 rpm and to the supernatant 3.5 vols of acetone was added, kept on ice for 2 to 3 hours and centrifuged at the same speed.

The proteins obtained from acetone-HCl and acetone precipitated pellets were dissolved in tris (10 mM) - EDTA (1 mM) buffer, pH 7.5, and run on SDS-PAGE (after Laemmli 1970). The acetone precipitated protein profiles are presented in Figure 1. It is clear that two bands and only two bands appear at the 10-14 kd molecular region. This profile, therefore, suggests that the proteins in the two bands might represent the two HMG 14,17 protein bands.

We used *in situ* transcription assay on the polytene chromosomes of *D. melanogaster*, in presence of the proteins thus isolated by EtBr intercalation-extraction procedure, using *E. coli* RNA polymerase holoenzyme (obtained from Sigma) following the technique of Umiel and Plaut (1973; see Chatterjee et al., 1981). The results revealed that transcription is augmented by a factor of two, in presence of the putative HMG proteins isolated from *Drosophila*. The results on the SDS-PAGE profile and *in situ* transcription assay with those proteins, taken together thus suggest that the HMG proteins, and at least HMG 14, 17, exist in *Drosophila*, and further that they show functional homology with those of mammals (Weisbrod and Weintraub, 1979). The amino acid analysis and other assays are in progress.

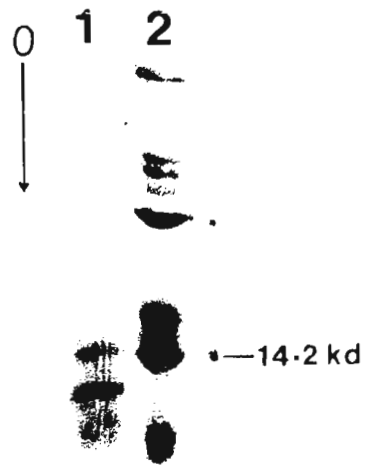


Figure 1. 13% SDS-PAGE profile of EtBr extracted putative HMG 14, 17 from third instar larvae of *Drosophila melanogaster*. Lane 1 = Protein bands of HMG 14, 17; Lane 2 = Marker protein (Dalton VII).

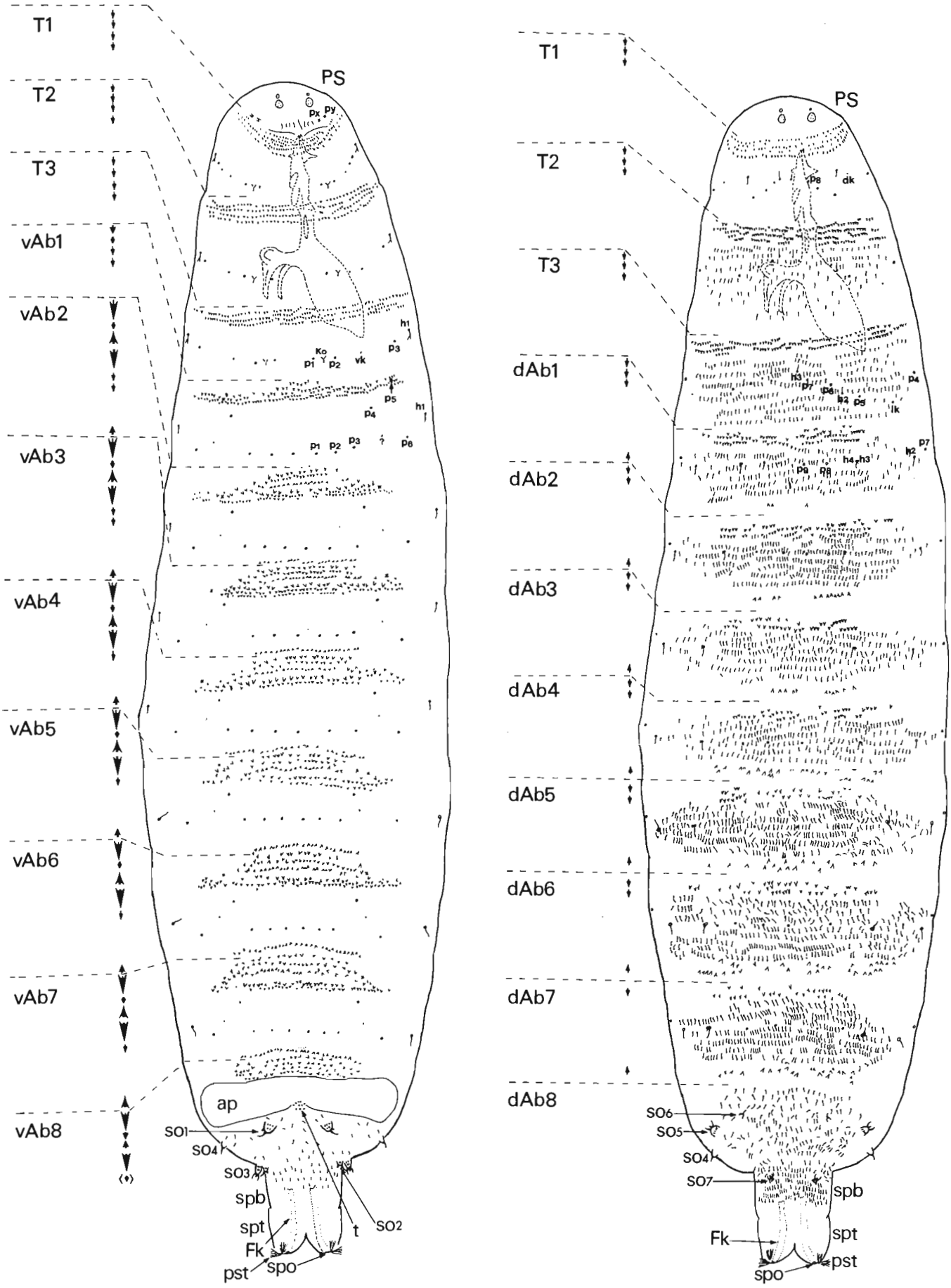
References: Goodwin, G.H. and E.W. Johns 1983, Chromosomal non-histone proteins: Hnilica, L.S. (ed.). Racobaton: CRS Press Inc. 69-91; Goodwin, G.H and M. Bustin 1988, The HMG proteins and their genes, Architecture of Eukaryotic genes, Ch. 8 VCH Publ., Weinheim:187-205; Laemmli, U.K. 1970, Nature 227:680-685; Schroeter, H., G. Maier, H. Ponstingl, and A. Nordheim 1985, The EMBO J. 4:3867-3872; Mukherjee, A.S., F. Schneeweiss and L.E. Feinendegen 1990, In: Colloquium on Realm of Differentiation (March 2-3, 1990), JNU, New Delhi; Chatterjee, R.N., D.K. Dube and A.S. Mukherjee 1981, Chromosoma, 82:515-523; Umiel, N. and W. Plaut 1973, J. Cell Biol. 46:139-144; Weisbrod, S. and H. Weintraub 1979, Proc. Natl. Acad. Sci. 76:630-634.

Kuhn, D.T., M. Sawyer, J. Ventimiglia and Th. E. Sprey. University of Central Florida, Orlando, and Zoological Laboratory, University of Leiden, the Netherlands.
Cuticle morphology changes with each larval molt in *D. melanogaster*.

Caudal structures on the 1st instar cuticle are difficult to study due to the tiny size of the larva at this developmental stage. The same morphological features can easily be analyzed during the large 3rd instar stage. Results presented here detail the dorsal and ventral ectodermal features of the wild type 1st, 2nd and 3rd instar larva, with special emphasis placed on features seen on the 3rd instar larva, especially the sense organs that appear on abdominal segments 8, 9 and 10.

The Canton-S stock was maintained at room temperature, approximately 24°C, on a standard *Drosophila* medium with tegosept added to suppress mold growth. Cuticles of 1st through 3rd instar larvae were prepared for phase-contrast

Figure 1. Composite camera lucida drawing of the 1st instar larva. The ventral view is on the left and dorsal view on the right. Segment borders are identified by dashed lines. The orientation of both the spinules on the dorsal side and the denticle bands on the ventral side is shown by direction of the arrowheads. ap, anal pad; dAb1-dAb8, dorsal abdominal segments 1 through 8; dk, dorsal *kolbchen*; Fk, Filzkorper; h1-h4, hair 1 through 4; KO, Keilin organ; lk, lateral kolbchen; p1-p9, papillae 1 through 9; px-py, papillae x and y; pst, posterior stigmatophore hairs; S01-S07, sense organs 1 through 7; spb, stigmatophore base; spo, spiracular opening; spt, stigmatophore top; T1-T3, thoracic segments 1 through 3; vAb1-vAb8, ventral abdominal segments 1 through 8; vk, ventral *kolbchen*.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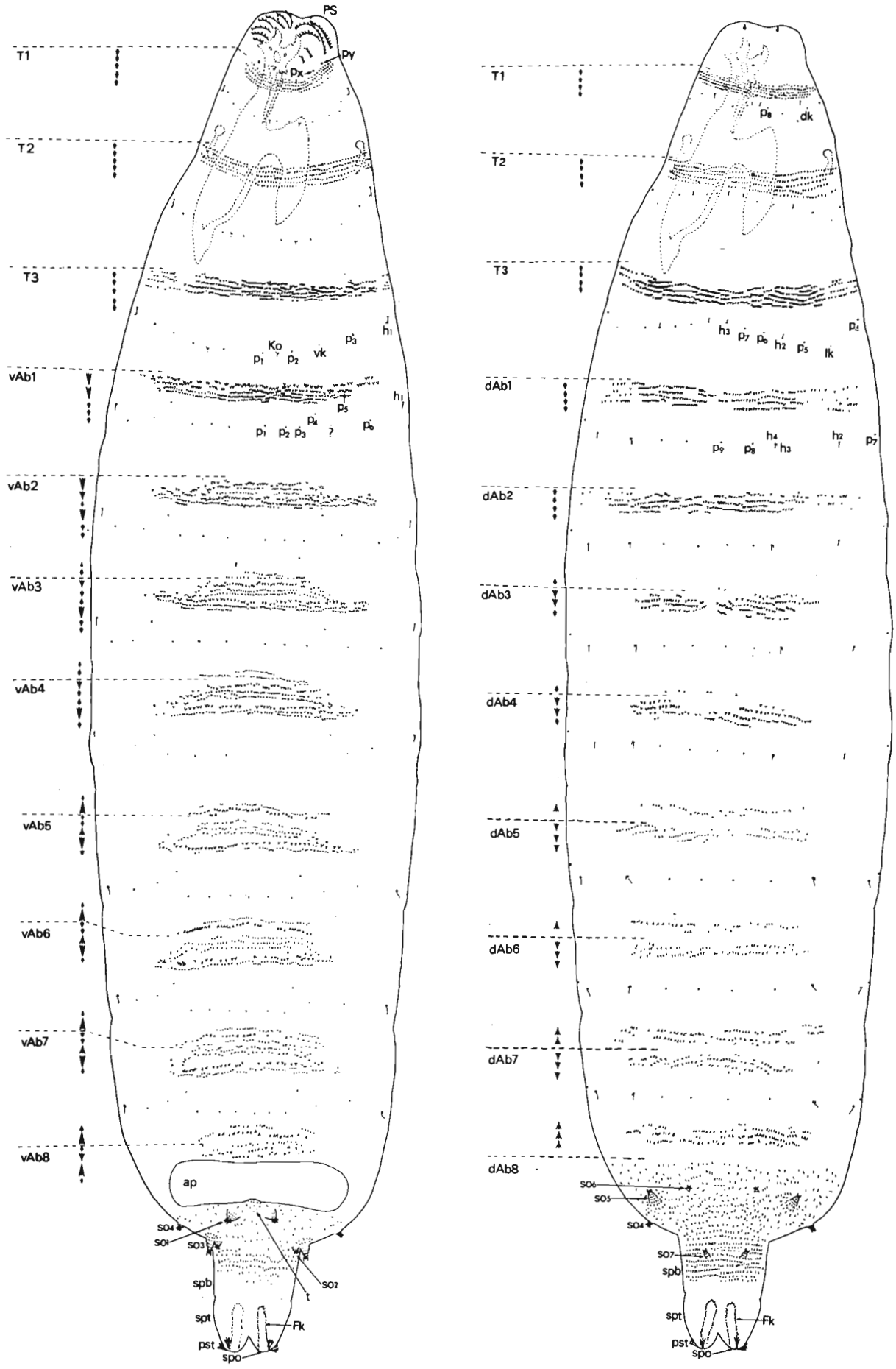

Figure 2. Composite camera lucida drawing of a 2nd instar larva. Left figure shows the ventral structures and the right dorsal structures.

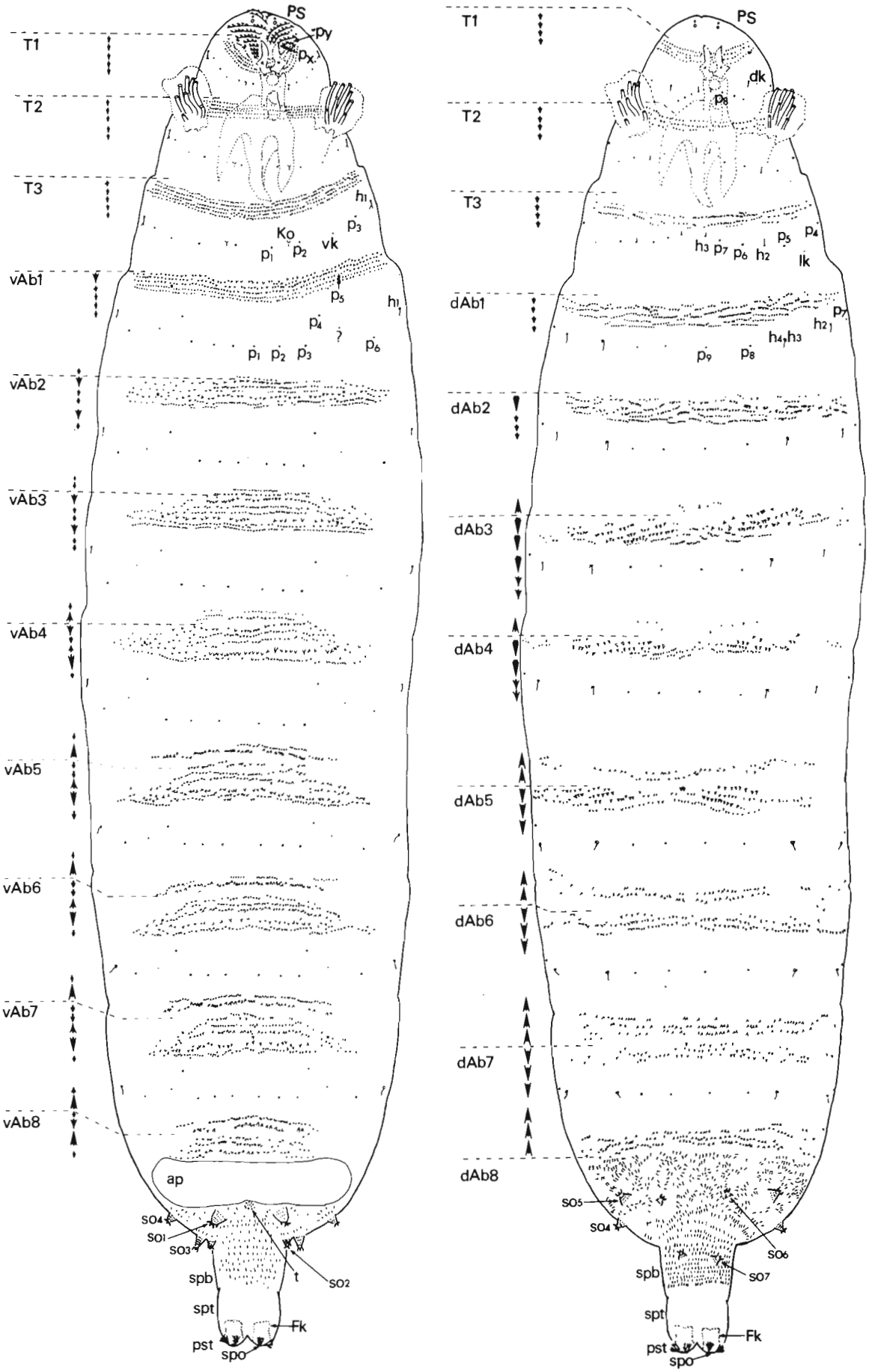
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Figure 3 (previous page). Composite camera lucida drawing of a 3rd instar larva. Drawing on the left gives ventral structures with dorsal structures given on the right. Direction and orientation for ventral denticle and dorsal spinule rows, along with relative size differences shown by arrow heads. Dotted lines give segment borders. Ventral, lateral and dorsal hairs in area of naked cuticle drawn but not identified. By combining dorsal and ventral patterns each segment has a unique pattern, especially in regard to rows of dorsal spinules.

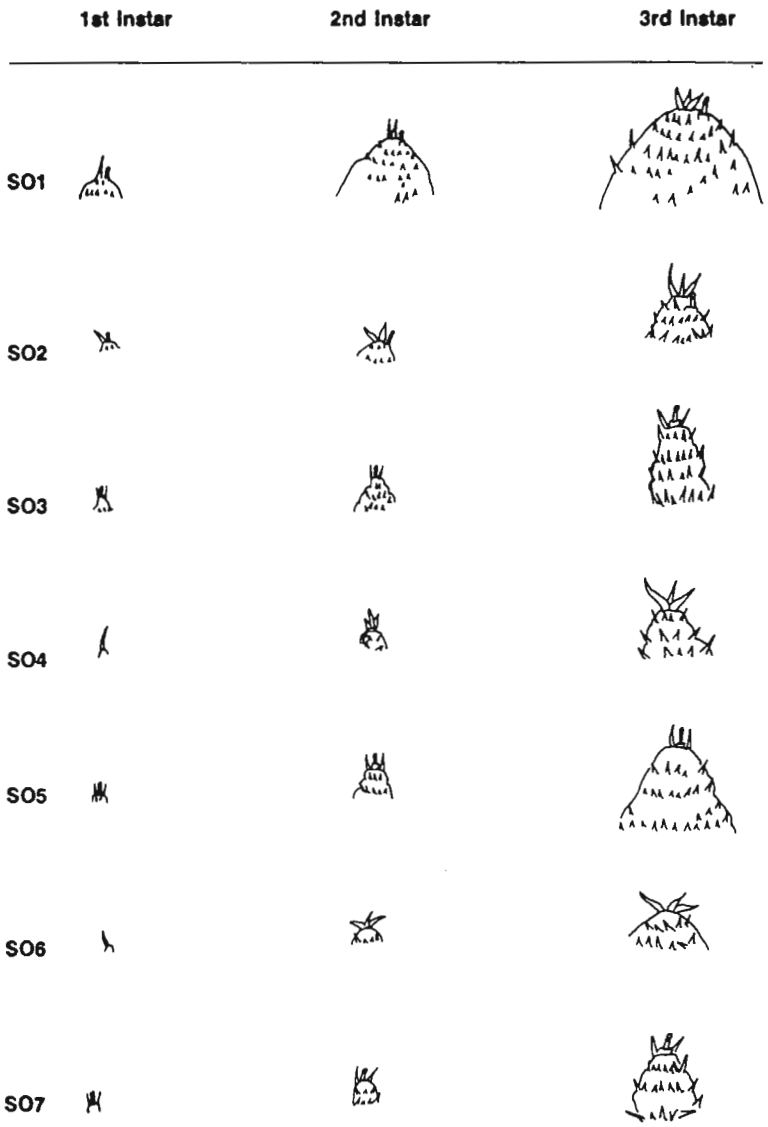


Figure 4. Camera lucida drawings of caudal sense organs in 1st, 2nd and 3rd instar larvae. Shown also are the relative size differences of the sense organs between the different larval stages, along with the average number of rows of hairs or spinules surrounding each cone.

microscopy following a modification of the procedure of Van der Meer (1977). Larvae were cleared in Nesbitt's fluid for up to 1 1/2 hours and small holes poked in them with a sharp tungsten needle. Each larva was then transferred to a microscope slide in a drop of Hoyer's solution and coverslipped. Identification and segmental identity of caudal cuticular structures followed the published results of Lohs-Schardin *et al.* (1979), Sato and Denell (1986), Whittle *et al.* (1986), Jurgens (1987), Turner and Mahowald (1979) and Hertweck (1931).

Camera Lucida drawings (Figures 1, 2 and 3) depict the cuticular features found on the dorsal and ventral sides of typical wild type larvae. The drawings are not meant to be exact, since some features have been enhanced to make them more easily recognized, while some minor features have been omitted. At least 20 larvae were examined in detail for each stage of the life cycle in order to produce the most typical patterns. Relative size differences between the instars sense organs (SO1-SO7) are shown in Figure 4, along with sense organ details.

Ectodermal features on the *D. melanogaster* 1st instar larva have been well documented. However, those of the 2nd instar have not, nor has much attention been given to the 3rd instar cuticle. Prominent features on 2nd and 3rd instar larvae differing from those of the 1st instar are: 1) loss of hairs on dorsal abdominal segment 1 (A1) through A7 by the 2nd instar stage; 2) replacement of the hair sensillum on SO1, SO2, SO4 and SO6 by several spike-like sense cells in 2nd instars; and 3) presence of broad based sense cells that resemble leaflets in 3rd instar larvae.

Acknowledgments: Work supported by NSF Grant DBM-8811383 and Amer Cancer Soc, Fla Div and NATO Grant 930/83.

References: Hertweck, H., 1931, Z Wiss Zool 139:559; Jurgens, G., 1987, Roux's Arch Dev Biol 196:141; Lohs-Schardin, M., C. Cremer, 8 C. Nusslein-Volhard, 1979, Dev Biol 73:239; Sato T. and R.E. Denell, 1986, Dev Biol 116:78; Turner, F.R. and A.P. Mahowald, 1977, Dev Biol 68:96; Van der Meer, J., 1979, DIS 52:160; Whittle J.R.S., S.Y.K. Tiong, and C.E. Sunkel, 1986, J Embryol Exp Morph 93:153.

Kroczynska, B., A.N. Lobanov and O.P. Samarina. Institute of Molecular Biology, Academy of Sciences, Moscow, Russia. On the molecular mechanism of *gypsy* and unidentified element-induced mutations at the *yellow* locus of *Drosophila melanogaster.*

The *yellow* (*y*) locus of *Drosophila* controls the pattern of pigmentation of larval and adult cuticular structures. It is transcribed into 1.9-kb mRNA under the control of, at least, four cis-acting elements (Biessman, 1985; Campuzano *et al.*, 1985;Geyer and Corces, 1987). In addition, many different genes are involved in its trans-regulation, and there appear to be nearly forty zones on the body surface where *y* sequence is differentially expressed (Nash, 1976). Mutations at the *y* locus may result in the total loss of pigmentation or in mosaic pigmentation patterns (Green, 1961; Nash and Yarkin, 1974). Some of these latter mutations appear to be super-unstable (Georgiev and Elugin 1990; Georgiev *et al.*, 1991).

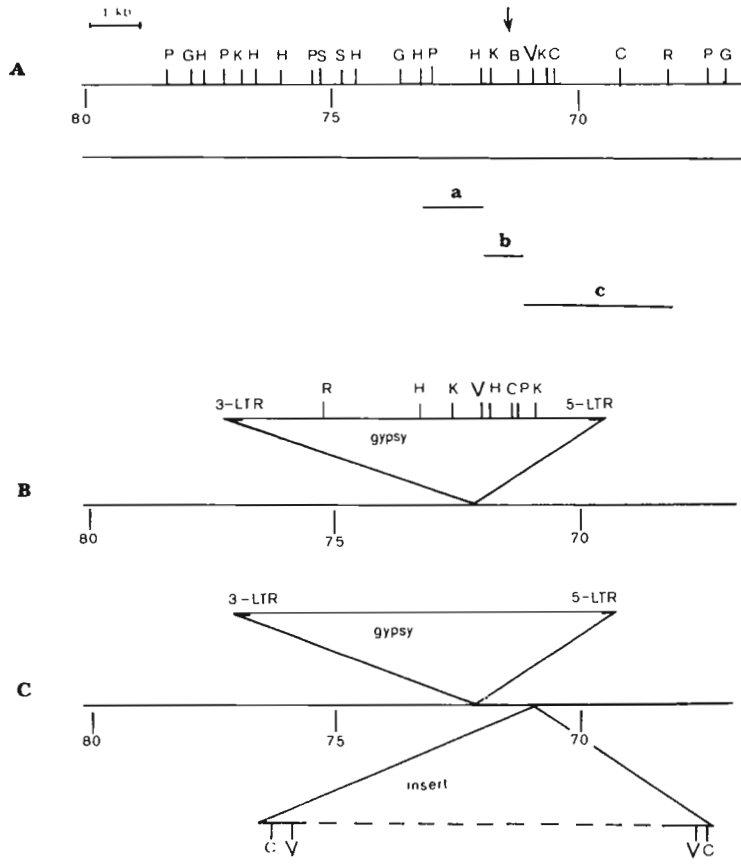
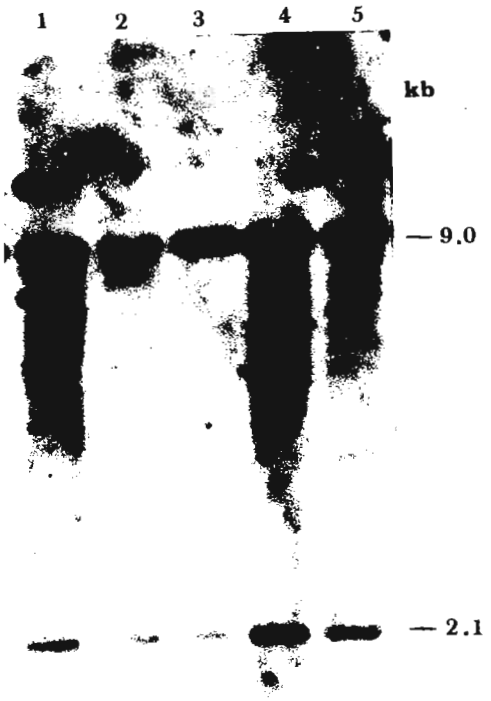


Figure 1. Schematic description of probes used for hybridization analyses and insertions found within the *yellow* locus of *D. melanogaster* mutant lines. Panel A: Restriction map of the *yellow* locus (Canton S stock). P, *Pst*I; G, *Bgl*II; H, *Hind*III; K, *Kpn*I; S, *Sal*I; B, *Bam*HI; V, *Pvu*II; C, *Cla*I; R, *Eco*RI; arrow, transcription start site. Thin lines below the map correspond to clone lambda-sc133 and probes a, b and c, as indicated. Panel B: Location of the gypsy element within the *yellow* locus of the strain y^2 sc^+ w^{aG} (gypsy restriction sites are indicated. Panel C: Location of the gypsy element and unidentified insertions within the *yellow* locus of super-unstable mutants. The dashed line corresponds to inserts of various length (4-25 kb, see text) while the indicated restriction sites are common to all the inserts.

We have attempted to search for DNA sequence features which may be responsible for super-unstable mutations at the *y* locus. All the mutant lines were of a local origin (Institute of General Genetics USSR Acad. Sci., Moscow) and were derived from the strain *y sc w* which contains a gypsy (mdg 4) insertion 700 bp upstream from the *y* coding sequence (see Figure lB). This insertion is known to influence expression of the *y* gene in body and wings but not in bristles (Modolell *et al.*, 1983; Judin and Lukine 1988). The mutant phenotypes are listed in Table 1.

To locate the breakpoints of the insertions or deletions responsible for mutant phenotypes, the genomic DNA of adult flies was digested with selected (see figure legends) restriction endonucleases and, after electrophoresis and blotting (Southern, 1975), hybridized to specific DNA probes. The probes chosen correspond to *y* locus segments (a, b, and c) shown in Figure 1 and were derived from the clone lambda sc133 (a generous gift of Dr. J. Modolell). The probes a and c do not discriminate between the DNA blots compared (Figures 2 and 3). However, the use of the probe b has indicated that, in addition to the gypsy element (present also in the initial strain), the mutant lines contain a novel insertion within the *y* locus (Figure 4). The size of this unidentified insert is different for various mutants and may be estimated roughly as 4, 7, 15 and 25 kb for y^{2bs} y^{+ms} y^{2s} and y^{+Ds} respectively.

Further restriction analyses (Figures 5 and 6) indicate that all these inserts are similar with respect to their 3 - and 5 -end sequences, while their central regions are different. They seem to be located within the sequence separating the



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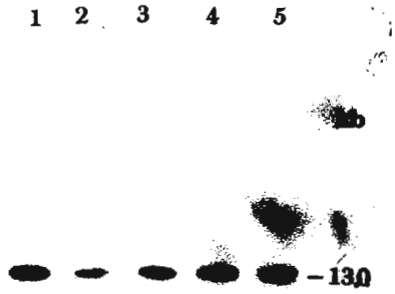

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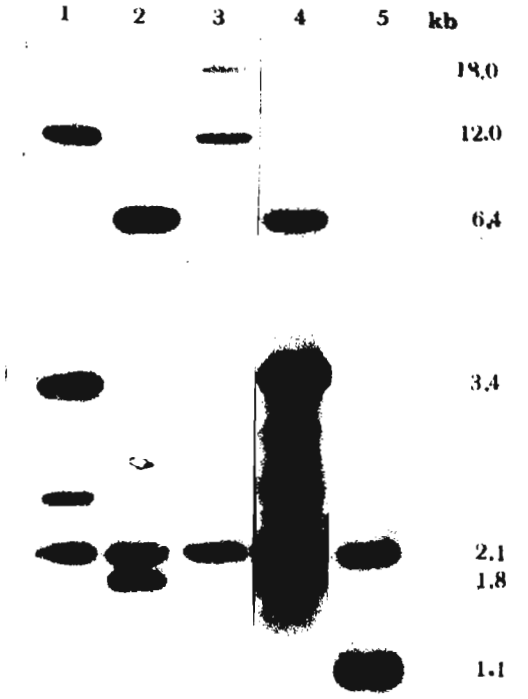
Figure 2. Hybridization of *Kpn*I-digested genomic DNAs to probe a. The lanes correspond to DNAs of various *D. melanogaster* strains, as follows: 1, y^{2s}; 2, y^{+ms}; 3, y^{+Ds}; 4, y^{2bs}; 5, $y^{2}\ sc^{+}\ w^{aG}$.

Figure 3. Hybridization of *Bam*HI-digested genomic DNAs to probe c. The lane numbers are the same as in Fig. 2.

Figure 4. Hybridization of *Kpn*I-digested genomic DNAs to probe b. The lane numbers are the same as in Fig. 2.

Figure 5. Hybridization of *Pvu*II-digested genomic DNAs to probe b. The DNAs compared were from the indicated strains.



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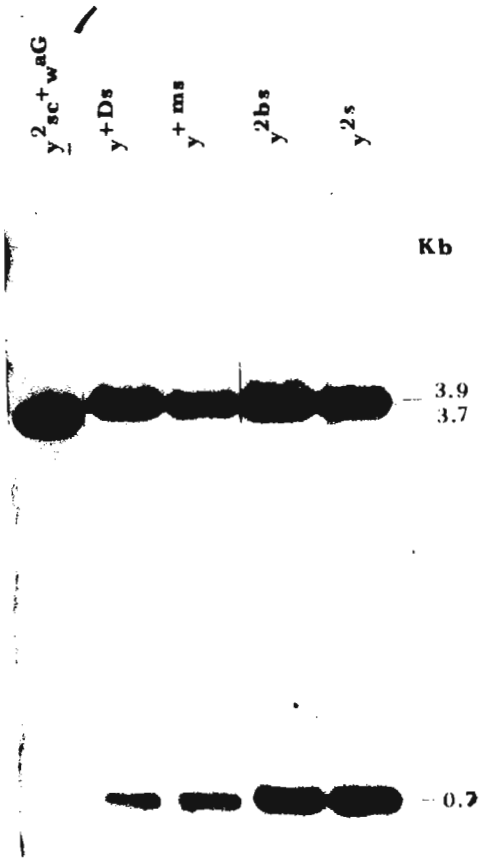


5

Table 1. Description of y phenotypes of *D. melanogaster* super-unstable mutants (general cuticule structures).

Strain	Body	Wing	Thorax bristles	Abdominal bristles	Wing bristles
y^{2s}	+	+	+++++	+++++	+++++
y+Ds	++++++	++++++	+++++	+++++	+++++
y+ms	++++	++++	+++++	+++++	+++++
y^{2bs}	+	+	+	+	+

Number of + shows the degree of pigmentation (five + = normal pigmentation).

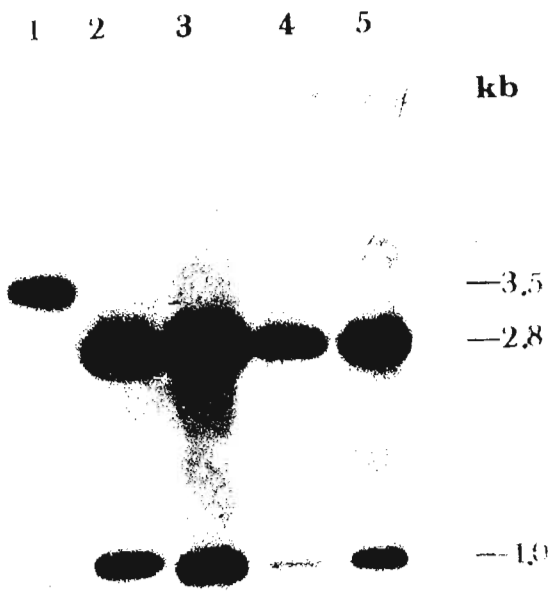


Figure 6. Hybridization of *Cla*I-digested genomic DNAs to probe b. Lane key: 1, $y^2\ sc^+\ w^{aG}$; 2, y^{2s}; 3, y^{+Ds}; 4, y^{+ms}; 5, y^{2bs}.

gypsy element from the transcription start site as it is schematically shown in Figure 1C. A relation of these insertions to the super-instability of the mutations observed remains to be elucidated.

References: Biessman, H. 1985, PNAS 82:7369-7373; Campuzano S., L. Carromolino, C.V. Cabrera, M. Ruiz Gomes, R. Villares, A. Boronat and J. Modollel 1985, Cell 40:327-338; Georgiev, P.G. and V.A. Elugin 1990, Genetica (USSR) 26(9):1564-1572; Georgiev, P.G., V.A. Elugin and M.V. Kozicina 1991, Genetica (USSR), in press; Geyer, P.K. and V.G. Corces 1987, Gene Dev. 1:996-1004; Green, M.M. 1961, Genetics 46:1385-1388; Judln, B.F. and K.A. Lukina 1988, Genetica (USSR) 24(5):867-877; Modolell, J., W. Bender and M. Meselson 1983, PNAS 80:1678-1681; Nash, W.G. 1976, Dev. Biol. 48:336-343; Nash, W.G. and R.J. Yarkin 1974, Genet. Res. 24:19-26; Southern, E.M. 1975, J. Mol. Biol. 98:503-517.

Escalante, A. and M. Benado. Department of Ecology and Evolutionary Biology, IJC Irvine, USA; Departamento de Estudios Ambientales, Universidad Simon Bolivar, Caracas, Venezuela; Departamento de Biologia Celular y Genetica, Universidad de Chile, Santiago. Morphometric variation in *Drosophila venezolana*.

D. venezolana belongs to the cactophilic *D. martensis* cluster, a taxon restricted to northern S. America (Wasserman et al., 1983).

Test-crosses suggest that within *D. venezolana* 2 races may exist along an E-W axis in mainland Venezuela (Cerda, 1982). The tests indicate that these putative races have diverged genetically. In this note, we analyze in *D. venezolana* morphometric differences between E and W populations, and a third insular population in the Caribbean, that exhibits partial reproductive isolation with the mainland ones (Cerda, pers. com.), to test whether these partially isolated populations have diverged in morphology. The null hypothesis is that there are no morphometric differences between the populations.

Material and Methods

The populations studied are shown in Figure 1. Localities 1 (Coro, E race) and 2 (Guaca, W race) are described in Benado *et al.*, 1983. Population 3, Los Roques, is located in the Gran Roque, the largest island in the Los Roques Archipelago in the Caribbean.

The strains analyzed were kept in the laboratory several years, and outcrossed with freshly captured flies a few times before the experiment was started.

The 9 morphometric characters examined are indicated in Figure 2. Sample size was determined by standard statistical techniques. 25 males and 25 females were randomly chosen from at least 2 culture bottles. Measurements were made on freshly killed material, using a WILD M3 stereomicroscope with a micrometric ocular. A Discriminant

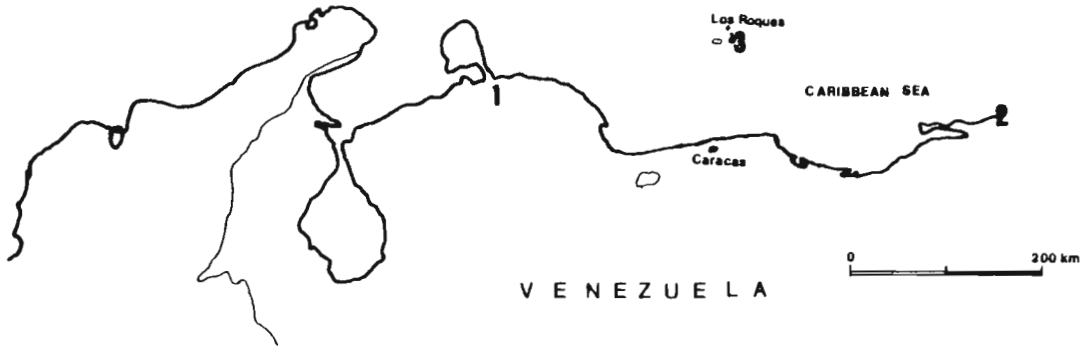


Figure 1. Localities. 1, Coro; 2, Guaca; 3, Los Rocques.

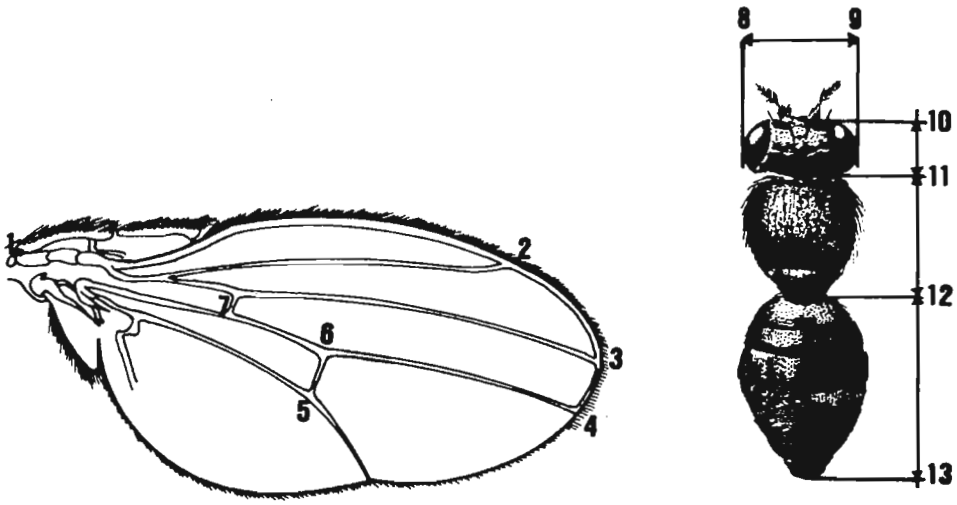
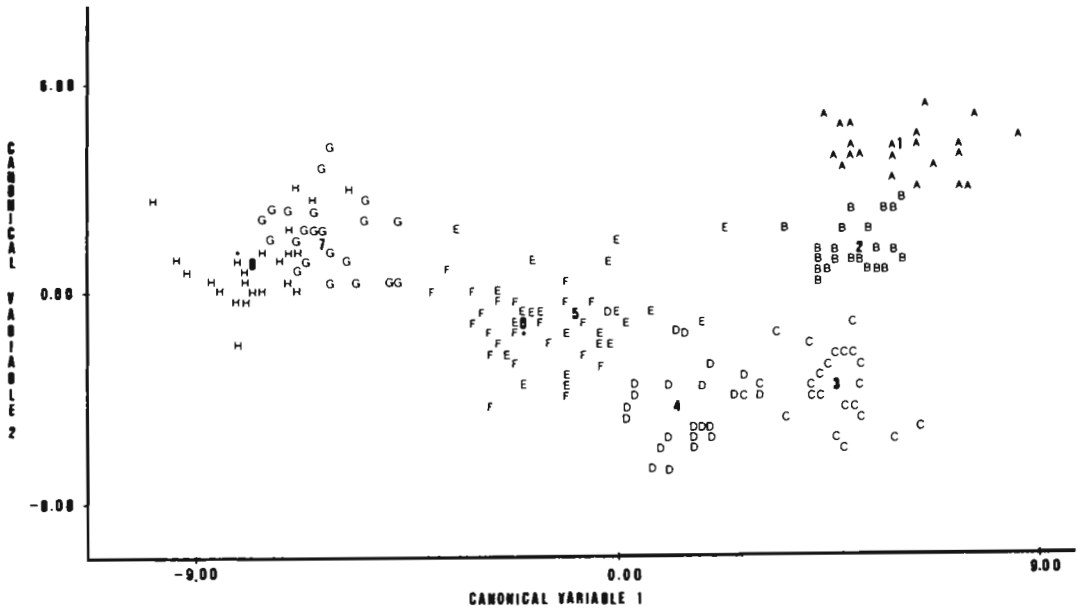


Figure 2. Characters measured. HD, 10-11; EY, 8-9; TH, 11-12; AB, 12-13; C1, 6-7; C2, 5-6; WG, 1-2; CB, 2-3; An, 3-4.

Figure 3. Canonical coordinates for 4 *Drosophila* populations. 1 to 8: centroids. Overlap is indicated by *. See Table 3.



Multivariate Analysis was used to compare the populations (BMDP, stepwise procedure, alpha=0.05). We used a population from *D. starmeri, D. venezolana*'s sibling, as an outgroup standard.

Results and Discussion

Basic statistics are given in Tables 1 and 2 for males and females, respectively. The stepwise discriminant procedure allowed for 8 characters in the discriminant functions. C1 was the first one to be included, and TH was left out. The classification matrix is given in Table 3 and the canonical coordinates are shown in Figure 3. The percentage of correct classifications ranged from 94% to 100% within *D. venezolana*. Furthermore, only a single individual of *D. starmeri* was misclassified as *D. venezolana*. We conclude that the races of *D. venezolana* are morphologically distinct entities, clearly distinguishable from each other and from *D. starmeri*. These results agree with those of Budnik *et al.* (1991), who found morphological quantitative differences among laboratory stocks of *D. subobscura*. Divergence in morphology was also found in *D. simulans* and *D. melanogaster* populations inhabiting the isolated Ogasawara Islands in Japan (Watada *et al.*, 1986). Our results are consistent with these findings, in the sense that geographical isolation seems to be correlated with morphological divergence.

Acknowledgments: We are grateful to E. Pannier, who helped with the drawings. Funded by CONICIT, SI-1206, Decanato de Investigaciones, U. Simon Bolivar, and OAS.

References: Budnik, M., L. Cifuentes and D. Brncic, 1991 Heredity 67;29-33; Cerda, H. G., 1982 Unpublished Thesis, Universidad Simon Bolivar, Venezuela; Wasserman, M., A. Fontdevila and A. Ruiz 1983 Annals of the Entomological Society of America 76:675-677; Watada, M., S. Ohba and Y.N. Tobare 1986 Japanese Journal of Genetics 61:469-480.

Table 1. Statistics for 4 *Drosophila* populations (males). M: mean (mm); CV: coefficient of variation (%). DSG; *D. starmeri* (Guaca). *D. venezolana*: DVG (Guaca), DVC (Coro), DVR (Los Roques). See Figure 2.

		POPULATION			
CHARACTER		DSG	DVG	DVR	DVC
HD	M	0.35	0.30	0.34	0.36
	CV	2.70	3.29	3.70	5.90
EY	M	0.87	0.81	0.99	0.94
	CV	3.76	4.25	1.55	4.73
TH	M	0.81	0.81	0.98	0.90
	CV	4.35	4.66	3.24	4.49
AB	M	0.99	1.13	1.45	1.19
	CV	5.97	3.79	3.85	6.84
C1	M	0.78	0.75	0.82	0.79
	CV	0.81	0.68	1.15	1.37
C2	M	0.44	0.41	0.45	0.44
	CV	2.24	2.52	1.33	1.98
WG	M	1.81	1.78	2.00	1.91
	CV	2.15	2.63	0.91	1.78
CB	M	0.15	0.13	0.17	0.15
	CV	6.10	2.23	6.73	4.89
AN	M	0.14	0.15	0.19	0.17
	CV	4.31	6.80	5.45	4.25

Table 2. Statistics for 4 *Drosophila* populations (females). See Table 1.

		POPULATION			
CHARACTER		DSG	DVG	DVR	DVC
HD	M	0.37	0.32	0.37	0.37
	CV	2.77	3.38	1.90	2.71
EY	M	0.87	0.81	0.99	0.94
	CV	3.76	4.25	1.55	4.73
TH	M	0.81	0.81	0.98	0.90
	CV	4.35	4.66	3.24	4.49
AB	M	0.99	1.13	1.45	1.19
	CV	5.97	3.79	3.85	6.84
C1	M	0.80	0.76	0.82	0.79
	CV	0.79	0.62	1.11	0.69
C2	M	0.44	0.44	0.45	0.44
	CV	2.00	2.14	1.28	2.08
WG	M	1.86	1.79	2.00	1.90
	CV	1.69	1.47	0.91	1.23
CB	M	0.15	0.14	0.18	0.15
	CV	5.81	3.88	6.29	4.14
AN	M	0.15	0.15	0.19	0.17
	CV	7.39	2.32	4.96	3.42

Table 3. Classification matrix for 4 *Drosophila* populations.GRP: groups. %: % correct classifications. TOT: total. A, B: *D. starmeri* (males, females) Guaca. C, D: *D. venezolana* ( )Guaca. E, F: *D. venezolana* ( ) Coro. G, H: *D. venezolana* ( )(Los Roques).

		NUMBER OF CASES INTO GROUPS							
GRP	%	A	B	C	D	E	F	G	H
A	96	24	1	0	0	0	0	0	0
B	96	0	24	0	0	0	0	1	0
C	100	0	0	25	0	0	0	0	0
D	100	0	0	0	25	0	0	0	0
E	96	0	0	0	0	24	1	0	0
F	88	0	0	0	0	3	22	0	0
G	72	0	1	0	1	1	0	18	4
H	84	0	0	0	0	0	0	4	21
TOT	92	24	26	25	26	28	23	23	25

Ismat Ara Ali, M.A. Hossain and M.A. Salam. University of Rajshahi, Rajshahi, Bangladesh. Effect of actinomycin-D on the frequency of methyl methanesulfonate induced autosomal recessive lethal mutations in *D. melanogaster*.

Table 1. Autosomal recessive lethal mutations (ARLM) following treatment with methyl methanesulfonate (MMS) (1.0 mM) and actinomycin-D (ACM-D) in *D. melanogaster*.

Cross *	Brood **	No. of Chromosomes tested	No. of lethal	% of ARLM ± S.E.
	I	897	2	0.21 ± 0.11
Control ♂	II	752	1	0.12 ± 0.12
X	III	1037	3	0 29 ± 0 .01
Control ♀	IV	846	1	0.11 ± 0.11
	I - IV	3532	7	0.18 ± 0.04[a]
	I	832	92	11.07 ± 0.08[l]
MMS ♂	II	965	87	9.01 ± 0.11[l]
X	III	870	33	3.77 ± 0.12m
Control ♀	IV	884	15	1.70 ± 0.02[m]
	I - IV	3551	227	6.39 ± 2.19[b]
	I	785	15	1.91 ± 0.5[l]
ACM- D ♂	II	897	14	1.56 ± 0.7[l]
X	III	866	12	1.37 ± 0.8[l]
Control ♀	IV	837	10	1.19 ± 0.09[l]
	I - IV	3385	51	1.51 ± 0.15[a]
	I	858	61	7.10 ± 0.12[l]
MMS+ACM-D ♂	II	866	37	4.27 ± 0.12 [lm]
X	III	842	17	2 03 ± 0. 08[m]
Control ♀	IV	893	8	0.89 ± 0.08[m]
	I - IV	3459	223	3.57 ± 1.37[b]
	I	972	70	7.23 ± 0.16[l]
MMS ♂	II	1033	46	4.45 ± 0. 07[lm]
X	III	854	17	1.99 ± 0.00[lm]
ACM-D ♀	IV	862	8	0 93 ± 0. 08[m]
	I - IV	3721	141	3.65 ± 1.40[b]
	I	853	62	7.27 ± 0.09[l]
ACM- D ♂	II	819	36	4.40 ± 0.11[lm]
X	III	827	16	1.95 ± 0.06[m]
MMS ♀	IV	854	7	0.80 ± 0.04[m]
	I - IV	3352	121	3.60 ± 1.43[b]

* Brood means indicated by the same letter do not show significant difference among crosses. ** Means indicated by the same letter do not elicit significant difference within broods of the same cross.

It has long been reported that the genetic damage induced by both physical and chemical agents can be modified in some situation by treatment with antibiotics (Mukherjee, 1965; Proust *et al.*, 1972; Salam, 1986). Actinomycin-D (ACM-D) is known for its ability to modify genetic effects induced by various agents. ACM-D was found capable of reducing the frequency of X-ray induced mutations in *Drosophila* (Burdette, 1961). Proust *et al.* (1972) showed that ACM-D, besides reducing the frequency of X-ray induced recessive lethals also reduced the frequency of translocations. Similar reduction of recessive lethal mutations induced by chemical mutagens have already been reported (Salam, 1986).

The aim of the present study is to assess whether ACM-D would be able to modify the MMS-induced second chromosome recessive lethal mutations.

Oregon-K males and the Curly Lobe/Plum (*CyL/Pm*) females were used for the experiment. Before treatment all the flies were starved for 6 hours. Duration of treatment was restricted for 24 hours at 25°C. Treatment with MMS was made by placing a piece of moistened filter paper with 1 ml MMS solution of 1.0 mM concentration in the starved flies containing vials. Treatment with ACM-D solution (1.5 mg ACM-D + 2 mg sugar + 100 ml distilled water) was made by keeping in vials a piece of properly drenched filter paper in ACM-D solution. Brood pattern analysis has been made with four 3-day broods for the detection of induced mutations at stage specific times of spermatogenesis (Fahmy and Fahmy 1961). For the detection of second chromosome recessive lethal mutation the classical *CyL/Pm* method was used. Results pooled for the experiments are tabulated in the table.

The present study revealed that ACM-D reduced the MMS-induced autosomal recessive lethal mutations in all the broods.

Acknowledgment: Authors are grateful to the Bangladesh University Grants Commission, Dhaka for financial assistance.

References: Burdette, W.J. 1961, Science 133:40; Fahmy, O.G. and M.J. Fahmy 1961, Genetics 46:361-372; Mukherjee, M. 1965, Genetica 51:363-367; Proust, J.P., K. Sankaranarayanan and F.H. Sobels 1972, Mutat. Res. 16:65-76; Salam, M.A. 1986, DIS 63:118.

Muñoz, Enzo R. Comisión Nacional de Energía Atómica, Buenos Aires, Argentina. A test for detecting maternal nondisjunction in *Drosophila melanogaster*.

A testing scheme has been developed that allows detection of meiotic nondisjunction of the X chromosomes in *Drosophila* females, that saves work and time because only one fourth of the expected regular progeny survives.

Treated females of the genotype *y w f Df(1)n20/y w f Df(1)n49* that are heterozygous for two non overlapping deficiencies are mated to males *y Df(1)n23/B*^{S} Y *y*$^{+}$ with a deficiency partially allelic to both deficiencies present in the

females. The marked Y chromosome of these males, besides *Df(1)n23*, covers one of the female's deficiencies [*Df(1)n20*] but not the other. Thus, from the preceeding cross only *y w f Df(1)n20/B*^{S} Y *y*$^{+}$, that is "*w f B*^{S}" males representing one fourth of the expected regular progeny survives, since the other classes namely *y w f Df(1)n20/y Df(1)n23* females, *y w f Df(1)n49/y Df(1)n23* females and *y w f Df(1)n49/B*^{S} Y *y*$^{+}$ males die.

Nondisjunctional females (XXY) are easily identifiable because they are "*w f B*^{S}," XXX females are "y" and appear in the usual low frequency, and the two other classes, XO and YO are lethal.

Male nondisjunction gives rise to "*y B*" females and lethal males. The treated females come from the cross of the following stocks: a) *y w f Df(1)n20/FM6(l); y w f Df(1)n20/y*$^{+}$ Y *mal*^{106}. b) *y w f Df(1)n49/FM6(l);y w f Df(1)n49/y*$^{+}$ Y *mal*^{106}. The lethal in the *FM6* chromosome is not covered by the *y*$^{+}$ Y *mal*^{106} duplication. The males mated to the treated females come from a stock that is also balanced with the *FM6* lethal chromosome.

The three deficiencies employed were induced by 3 MeV neutrons and genetically mapped in Section 20 of the X chromosome, their breakage points being: 1) deficiency n23: distal, to the right of lethal 114; proximal, between lethal x-4 and R-9-18. 2) deficiency n20: distal, between lethals P-19 and Q-463; proximal, between lethal 3 Des and "*bb*". 3) deficiency n49: distal, between "extra organs" (*eo*) and "wings apart" (*wap*); proximal, between lethals x-3 and P-19. For the map of the proximal end of the X chromosome see Lifschytz and Falk (1969) and Schalet and Lefevre (1976). Before these three deficiencies were definitively named, there appeared in the literature as 17-123, 17-274 and 17-439, respectively, a transitory designation used in the laboratory.

Since the complementing deficiencies in the treated females are extremely close, viable crossover products are rare.

Due to the fact that with this scheme all nondisjunctional females are recovered but only one fourth of the expected regular progeny survive, to calculate the frequency of X-nondisjunction the recovered regular males have to be multiplied by 4. Also, to obtain a more accurate estimation of the actual frequency of nondisjunction, exceptional females should be multiplied by 4 to take into account the other nondisjunctional products (XXX, XO and YO) (Grell et al., 1966; Traut, 1970).

$$\% \text{ N.D.} = \frac{4\ (\text{exc } ♀)\ \text{x}\ 100}{4\ (\text{reg } ♂ + \text{exc } ♀)} = \frac{\text{exc } ♀\ \text{x}\ 100}{\text{reg. } ♂ + \text{exc } ♀}$$

In the control series thus far run, we have obtained 0.067% of X-nondisjunction (200,000 regular males recovered) that is within the limits observed in other stocks.

References: Grell, R.F., E.R. Muñoz and W.F. Kirschbaum 1966, Mutation Research 3:494-502; Lifschytz, E. and R. Falk 1969, Mutation Res. 1:147-155; Schalet, A. and G. Lefevre 1976, The Genetics and Biology of Drosophila, M. Ashburner and E. Novitski (eds.) pp. 847-902; Traut, H. 1970, Mutation Res. 10:125-132.

Shinde, S.L., V.R. Karekar and V.G. Vaidya. M.A.C.S. Research Institute, Pune, India. Fecundity trend of dark-reared *D. melanogaster* strains routinely used in mutagenicity assessment.

Daily and life-time trends of fecundity are known in *Drosophila* cultures maintained on a constant light-dark cycle (see Ashburner and Thompson, 1978). Under this condition, it is established that the life-time fecundity trend is characterized by initial sudden rise that follows gradual decline. The daily rhythmicity of egg laying shows a peak around evening. However, it is not known whether such trend and rhythmicity exists in the flies reared under constant dark condition.

Some mutagen-sensitive strains of *D. melanogaster* are maintained in our laboratory in dark at 25°C in a constant airflow incubator supplemented with a moisture tray. We wanted to know peak periods of fecundity in such dark-reared flies so as to standardize a routine strategy for procuring synchronously laid eggs, the ultimate aim being to get developmentally synchronized larvae for acute and chronic treatment by a test mutagen.

Initially, the life-time trend of fecundity was studied in repair-deficient *zeste* strain *C(1)DX, y f*/Y/*sc z w*$^{+(TE)}$ *mei-9*^{a}, maintained under constant dark condition. The entire brood analysis was carried out using inbred parental pool of 60 females and 40 males both of which were isolated as virgins. The mated females were allowed to lay eggs for 24 hrs in 200 ml (half-pint) milk bottles containing 40 ml of normal agar-yeast medium with jaggery-wheat flour. These were then transferred every day into the next set of bottles to continue egg laying. Serial transfer of parents was continued until survival of females in the parental pool was ended. In our culture set-up maintained at 25°C, it takes exactly 9 days to complete development from egg to fresh adults. The number of adults in the progeny was scored from each bottle to determine the number of fertilized eggs laid on consecutive days (Figure 1). The rate of egg laying is seen to be

dramatically higher between days 7 and 23 with a peak at day 12 and gradual decline thereafter. Nearly 75% of the eggs were laid by parents aged between 6 to 24 days and are obviously the best choice for collecting large numbers of eggs.

Later, the daily trend of fecundity within a 24 hr period was studied in the following four dark-reared strains: *zeste* repair-deficient, *C(1)DX, y f/Y/sc z* $w^{+(TE)}$ *mei-9*^{a}; *zeste* repair-proficient, *C(1)DX, y w f/Y/sc z* w^{+UR} *sn*; wing hair mutants *mwh/Y/flr*^{3}*/TM3 Ser*; and *Basc,* sc^{8} sc^{S1} w^{a} *B*. The pattern of egg laying was studied daily in each of the above strains by collecting parental pools of isolated virgins comprising 60 females and 40 males each. The parents were allowed to mate and remain in the same bottle until they attained the age of five days. This corresponds to the sudden rise in egg laying. During this period, the parental pools (maintained in duplicate for each strain) were transferred serially after every two hours into fresh bottles containing 20 ml medium. The transfers were made up to completion of 48 hours in dim red light. We thus procured samples of eggs laid at intervals of two hours during two successive days. The adults emerging in each successive bottle were scored to determine the number of fertilized eggs laid per hour.

The daily trend of fecundity was more or less the same in all the four mutagen-sensitive strains used (Figure 2). All the four strains showed a distinct peak of egg laying in the evening between 6 to 10 pm. Remarkable decline in the fecundity was observed in all the four strains in the early hours of morning. Almost 70% of eggs are laid within 10 hours between 2 pm and 12 am with the exception of repair-proficient *zeste* strain wherein this period is shifted between 10 am and 8 pm.

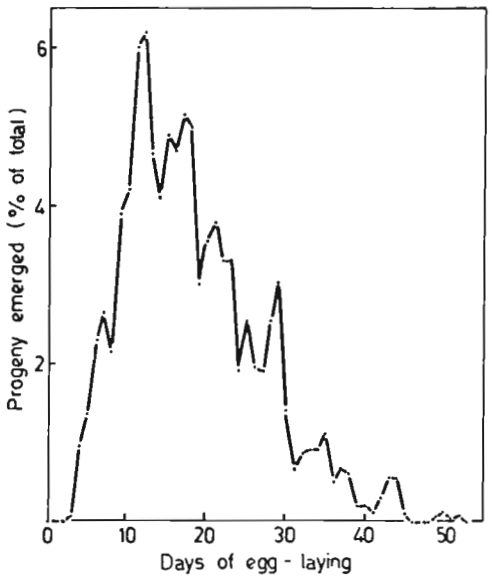


Figure 1. Life-time fecundity in repair-deficient *zeste* strain of *D. melanogaster* reared under constant dark conditions.

The life-time trend and daily rhythm of fecundity in the above dark-reared *D. melanogaster* strains are largely similar to the overall pattern known for *Drosophila* cultures maintained on a constant light-dark cycle (see Ashburner and Thompson, 1978), which includes the burst of egg laying at dusk (Elgin and Miller, 1978).

Using mated females of 6 to 24 days, the peak hours of egg laying in the evening can be effectively used for collecting large numbers of developmentally synchronized larvae. Alternatively, as a routine, one can set a dusk to dawn schedule for mass harvesting of eggs.

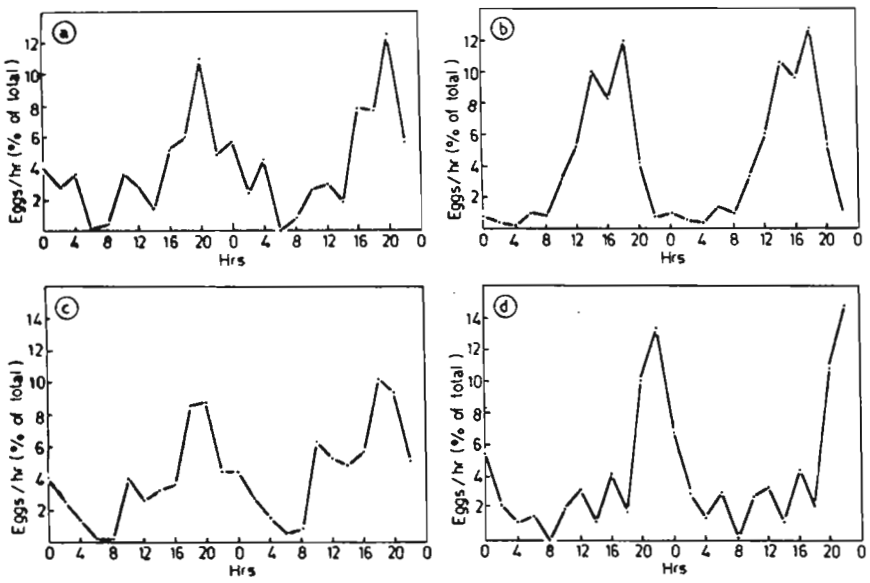


Figure 2. Daily rhythm of egg laying in different mutagen-sensitive *D. melanogaster* strains reared under constant dark conditions: (a) *zeste* repair-deficient, (b) *zeste* repair-proficient, (c) *Basc* and (d) wing hair mutants.

Acknowledgments: We are thankful to Dr. K. Fujikawa (Takeda Chemical Industries Ltd., Osaka, Japan), Dr. U. Graf (Institute of Toxicology, Schwerzenbach, Switzerland), Prof. M. Sanjeeva Rao (Osmania University, Hyderabad, India) and Umeå *Drosophila* Stock Center (University of Umeå, Sweden) for making available the *D. melanogaster* strains used in this study. Mrs. Parvatibai Agharkar Fellowship to V.R.K. is gratefully acknowledged.

References: Ashburner, M. and J.N. Thompson 1978, Genetics and Biology of Drosophila (Ashburner and Wright, eds.), Academic Press, London, vol. 2a:1-109; Elgin, S.C.R. and D.W. Miller 1978, in: Genetics and Biology of *Drosophila* (Ashburner and Wright, eds.), Academic Press, London, vol. 2a:112-121.

Shinde, S.L., V.R. Karekar and V.G. Vaidya. M.A.C.S. Research Institute, Pune, India. Optimum saline concentration for extraction of *Drosophila melanogaster* larvae from the medium.

For extraction of *Drosophila* larvae from the medium, different saline concentrations in terms of molarity or percentage are reported in the literature. For extraction of the larvae of mutagen-sensitive strains, 20X saline is generally used (Graf, Frei, Kägi, Katz and Würgler 1989). During our studies, which needed a large number of healthy and viable larvae of mutagen-sensitive *D. melanogaster* strains for use in a treatment protocol, we found that the survival of the larvae is affected by the use of 20% saline. This resulted in uncertainty in determining sublethal doses of a test mutagen after chronic or acute exposure of saline extracted larvae. We therefore decided to find out optimum saline concentration for successful larval extraction that can give considerable yield of viable larvae. The larvae were grown in 40 ml of normal agar-yeast medium containing jaggery and wheat flour (Godbole, Kothari and Vaidya 1971); the cultures were maintained at 25 ± 0.5 °C. To determine survival of the larvae, *zeste* somatic eye mutation assay strain *C(1)DX,y f/Y/sc z* $w^{+(TE)}$ *mei-9*^{a} was used. Effect on the yield of larvae was determined by using larvae of wing spot assay strains, *mwh* and *flr*^{3}*/TM3, Ser.*

Table 1. Effect of different saline concentrations on the survival and the yield of *D. melanogaster* larvae extracted from the medium

Saline concentration	Duration in saline (min)	NUMBER OF		Percent survival	NUMBER OF LARVAE		Percent yield
		larvae grown	adult survivors		Initial	extracted	
0.5 M (2.9 %)	10	360	325	90.28	650	352	54.15
1.0 M (5.8 %)	10	360	308	85.56	900	667	74.11
2.0 M (11.7 %)	10	360	297	82.50	900	804	89.33
3.0 M (17.5 %)	10	360	274	76.11	750	688	91.73
4.0 M (23.5 %)	10	360	263	73.06	800	705	88.13
5.0 M (29.2 %)	10	360	246	68.33	650	570	87.69
2.5 % (0.43 M)	10	360	357	99.17	550	244	44.36
5.0 % (0.86 M)	10	360	335	93.13	450	274	60.89
10.0 % (1.71 M)	10	360	312	86.67	450	369	82.00
15.0 % (2.57 M)	10	360	275	76.39	450	383	85.11
20.0 % (3.42 M)	10	360	260	72.22	1400	1274	91.00
25.0 % (4.28 M)	10	360	238	66.11	750	652	86.93
10 % (1.71 M)	4	360	390	88.61	800	321	40.76
10 % (1.71 M)	8	360	320	88.89	1500	951	63.40
10 % (1.71 M)	12	360	280	77.78	650	592	91.08
10 % (1.71 M)	16	360	272	75.56	450	362	80.44
10 % (1.71 M)	20	360	228	63.33	450	363	80.67
10 % (1.71 M)	24	360	152	42.22	600	508	84.67

The saline extraction of the larvae was carried out by using following variations in the protocol for ready comparison with previous usages: (a) 6 different molar saline concentrations, (b) 6 different percent saline concentrations and (c) 6 different intervals of exposure to saline. Within 10 min duration, larvae in the medium were extracted from the bottles by 3 pours each of 50 ml saline. These were then separated from adherent medium debris in a large (1000 ml) separating funnel and finally removed on muslin cloth filter of 80 mesh by a wash of distilled water. The extracted larvae were rinsed and maintained in *Drosophila* Ringer's solution (Ransom 1982) either until count of the surviving larvae or until their transfer into the vials containing 5 ml medium to check the survival. The viability of saline extracted larvae was estimated by counting number of adults emerged. The initial number of larvae existing in each bottle prior to saline extraction was estimated approximately by actual count of number of eggs laid in each bottle and by its tally with the number of adults emerging from unextracted bottles. The yield was estimated by counting total number of extracted larvae.

We observed that larvae do not float out on the surface in 0.5 M, 1 M, 2.5% and 5% saline. They tend to remain suspended in saline which is polluted by a mixture of medium debris. In 2.0 M, 3.0 M, 10%, 15% and 20% saline, larvae float out effectively, increasingly quicker in higher concentrations. In these, the mixing of the debris is much reduced except occasional presence of bits and pieces. In 4.0 M, 5.0 M and 25% saline, larvae float out easily, much quicker than in all the other concentrations used. In these, the medium debris is almost absent but for the presence of large pieces

and chunks of the medium. Sometimes, even entire medium base detaches itself and floats out. Based on the observations and the results obtained in Table 1, the following inferences were drawn : (1) Extraction with the lower saline concentrations (0.5 M, 1.0 M, 2.5% and 5%) perturbed the yield by presence of excessive food particles. The survival of the extracted larvae is better than in higher saline concentrations used. (2) Optimum survival of the extracted larvae, so also the yield was observed in 2 M and 10% saline. (3) In remaining saline concentrations, the yield is not affected but survival is increasingly reduced with increase in the concentrations. (4) Optimum exposure time to the saline appears to be between 8-12 min considering both the survival and the yield. (5) Finally, it is concluded that 2 M and 10% saline concentrations are optimum for successful extraction of larvae within an exposure duration of about 10 min. Alternatively, for isolating *Drosophila* larvae one may use procedure of sucrose density separation (Nöthiger 1970).

Acknowledgments: Mrs. Parvatibai Agharkar fellowship to V.R.K. is gratefully acknowledged. We are thankful to Dr. K. Fujikawa, Takeda Chemical Industries, Ltd., Osaka, Japan and Dr. U. Graf, Institute of Toxicology, University of Zürich, Schwerzenbach, Switzerland, for making available the strains of *D. melanogaster* for these studies.

References: Godbole, N.N., R.M. Kothari and V.G. Vaidya 1971, DIS 46:58-59; Graf, U., H. Frei, A. Kägi, A.J. Katz and F.E. Würgler 1989, Mutation Res. 222:359-373; Nöthiger, R. 1970, DIS 45:177; Ransom, R. 1982, A Handbook of *Drosophila* Development, p 1-29.

Shinde, S.L., V.R. Karekar and V.G. Vaidya. M.A.C.S. Research Institute, Pune, India. Mutagenicity of antifungal preservative methyl p-hydroxybenzoate in *zeste* eye spot test of *Drosophila melanogaster*: a preliminary report.

Methyl p-hydroxybenzoate (MPHB), also known as Nipagin M, Methyl Chemosept, Methyl Parasept, Tegosept M and more popularly as Methylparaben, is a pharmaceutically ubiquitous preservative (Hamilton et al. 1990). It is used as a preservative in foods, beverages and cosmetics. Its spermicidal (Bao-Liang et al. 1989), nematicidal (Maheshwari and Anwar 1990) and vasodilator (Hamilton et al. 1990) roles are known. It is also commonly used in *Drosophila* culture medium to prevent mold and fungal growth (Ashburner and Thompson 1978; Ashburner 1989). It has been reported to induce germ cell aneuploidy in female *D. melanogaster* using the free inverted X chromosome system (Costa et al. 1988). We report here its ability to induce somatic cell mutations in the repair-deficient system of *zeste* eye spot test.

For growing both the stock as well as the experimental cultures of mutagen-sensitive strains, we have been using normal agar-yeast medium containing jaggery and wheat flour (Godbole et al. 1971). During last two years when the cultures suffered a fungal attack, we started supplementing the medium initially by 0.625% MPHB. Fungal growth was thus controlled but the developmental span of larval and pupae stages was extended by 3 to 4 days. Such delay in emergence of adults caused by MPHB is also reported by Wette (1954).

Further we observed that, on addition of MPHB, the medium solidified into a more or less elastic sap thereby posing a problem to larval penetration. This leads to considerable larval mortality. In order to avoid the delay in emergence and also the larval mortality, we finally settled to a reduced supplementation of MPHB (0.0625%) as a routine. Later, while conducting mutagenicity assessment studies using the repair-deficient *zeste* strain, we noticed a sudden rise in spontaneous mutation frequency. This was also evident in routine check on spontaneous mutation levels. We therefore examined whether somatic mutations are induced by MPHB using repair-deficient *D. melanogaster* strain *C(1)DX, y f/Y/sc z* $w^{+(TE)}$ *mei-9*^{a}. The *zeste* eye spot test system was used, which has been successfully employed in detecting mutagens (Rasmuson et al. 1978, 1984; Fujikawa Kauai et

Table 1. Somatic mutations induced in *zeste* eye spot test system of *D. melanogaster* with and without incorporation of MPHB in the culture medium. The occurrence of exceptional males (§) with red spot(s) comprising 4 or more ommatidia on *zeste* colored eye is considered as a measure of induced somatic mutations.

	Number of		Sex ratio	Number of exceptional	Mutation	Net induced mutations by
	females	males	♂/♀	males(§)	frequency (%)	MPHB (%)
Spontaneous	3119	3335	1.07	59	1.77	
2 mM MPHB	3742	4546	1.22	147*	3.23	}
						} 1.39
Solv. Control	3584	4030	1.12	74	1.84	}
4 mM MPHB	4820	5794	1.20	245*	4.23	}
						} 2.48
Solv. Control	5692	5994	1.05	105	1.75	}

* Significant with respect to control (P = 0.01)

al. 1985) and which is based on the interaction between the *zeste* mutation and the duplicated *white*$^+$ locus on the X chromosome (Rasmuson 1984; Mitchell and Combes 1984; Gubb et al. 1985).

In the present study, 2 mM (0.0304%) and 4 mM (0.0609%) concentrations of MPHB were chosen for the testing, which include a concentration compatible to the one routinely used by us and one below it. Entire brood analysis was carried out using inbred parental pool of 80 females and 60 males. The larvae were treated with the above MPHB concentrations by chronic exposure. Males from the resulting progeny were scored for induced somatic mutations according to the procedure of Fujikawa et al. (1985). The appropriate negative control and solvent control (ethanol) were maintained. The number of exceptional males, which showed presence of red spots comprising four or more ommatidia on *zeste* colored eyes, was considered in determining the frequency of induced somatic mutations.

The spontaneous mutation frequency and MPHB-induced mutation frequency are given in Table 1. The treatment with both 2 mM and 4 mM MPHB showed significant rise over the respective solvent control. Although only two concentrations of MPHB were used, the large sample size enables us to draw self-evident conclusion that MPHB induces somatic mutations in the *zeste* eye spot test system. Comparison between the frequency of exceptional males in untreated (negative control) and ethanol treated (solvent control) cultures clearly shows that the dose of ethanol used as a solvent in the present study has no mutagenic effect by itself.

After having detected mutagenic potential in MPHB, we have discontinued its use in the medium of mutagen-sensitive *D. melanogaster* strains. Commonly, in formulations of *Drosophila* media 0.15% or 0.18% MPHB is added routinely (Ashburner and Thompson 1978). These are obviously much higher concentrations than those used in the present study. On the basis of this preliminary report, it is felt that the MPHB should be incorporated as an antifungal agent in culture media with adequate caution, at least in cultures of mutagen-sensitive *D. melanogaster* strains.

Acknowledgments: Mrs. Parvatibai Agharkar Fellowship to V.R.K. is gratefully acknowledged. We are thankful to Dr. K. Fujikawa, Takeda Chemical Industries Ltd., Osaka, Japan for making available the *D. melanogaster* strain used in this study. Thanks are also due to our colleague Dr. S.M. Ghaskadbi for helpful suggestions.

References: Ashburner, M. 1989, in: *Drosophila*, A Laboratory Manual, Cold Spring Harbor Laboratory Press; Ashburner, M. and J.N. Thompson 1978, in: Genetics and Biology of *Drosophila* (Ashburner and Wright, eds.), Academic Press, London, vol. 2a:1-109; Bao-Liang, S., L. Hai-Ying and P. Dun-Ren 1989, Contraception 39:331-336; Costa, R., M. Zordan, A. Tavella and A.G. Levis 1988, DIS 67:16; Fujikawa, K., H. Ryo and S. Kondo 1985, in: Progress in Mutation Research (Ashby and de Serres et al., eds.), Elsevier Science Publishers, Amsterdam, vol. 5:319-324; Godbole, N.N., R.M. Kothari and V.G. Vaidya 1971, DIS 46:58-59; Gubb, D., J. Roote, S. McGill, M. Shelton and M. Ashburner 1986, Genetics 112:551-575; Hamilton, J.T., Y. Zhou and A.W. Gelb 1990, Anesthesiol. 73:1252-1257; Maheshwari, D.K. and M. Anwar 1990, J. Phytopathol. 129:159-164; Mitchell, I. deG. and R.D. Combes 1984, in: Mutagenicity Testing: a Practical Approach (Vennitt and Perry, eds.), IRL Press, Oxford, pl49-185; Rasmuson, A. 1984, Ph.D. Thesis, Umea Univ., Sweden; Rasmuson, B., A. Rasmuson and J. Nygren 1984, in: Handbook of Mutagenicity Test Procedures (Kilbey et al., eds.), Elsevier Science Publ., Amsterdam, p603-613; Rasmuson, B., H. Svahlin, A. Rasmuson, I. Montell and H. Olofsson 1978, Mutation Res. 54:33-38; Wette, R. 1954, DIS 28:166-167.

Hoelzinger, Dominique and W. W. Doane. Department of Zoology, Arizona State University, Tempe, AZ 85287-1501. The *Amy* locus in *Drosophila hydei*.

The *Amy* (alpha-amylase) structural gene family in *Drosophila hydei* contains at least two functional *Amy* gene copies per genome, based on biochemical/genetic studies of amylase isozymes (Doane, 1968; Doane et al., 1975). This is not unusual since two or more functional copies of the gene are known to comprise the *Amy* family in several other species of *Drosophila*, as deduced from either electrophoretic and/or genetic analyses (e.g. Kikkawa 1964; Bahn, 1967; Doane, 1969) or from transient assays of the amylase isozymes produced by exogenous *Amy* genes in somatically transformed host larvae (Hawley et al., 1990).

Unlike other *Drosophila* species, however, the *Amy* family in *D. hydei* is thus far unique in that the alpha-amylases it produces fall into two distinct classes that differ widely in their electrophoretic mobilities (Doane and Abraham, 1974; Doane et al., 1975). The slow class of isozymes is expressed primarily in the midgut of both sexes, as is typical of other species. The other class, by contrast, displays a very rapid mobility and is male-specific, its activity being expressed only in the sex glands (paragonia) of mature male flies. Paragonia-specific alpha-amylases have not been reported for other *Drosophila* species, nor have they been detected in a number of species whose paragonia were specifically tested for them (Doane, unpublished data). As reported earlier (Doane and Abraham, 1974; Doane et al., 1975), the slow class of midgut amylase isozymes is also active in mature male paragonia. This suggests that the regulatory mechanism for tissue-specific expression of alpha-amylase in the paragonia of *D. hydei* probably evolved prior to the *Amy* duplication that gave rise to the fast-migrating amylase class.

Two electrophoretic variants of alpha-amylase, AMY-7 and AMY-8, were used in genetic recombination studies that placed the *Amy* locus of *D. hydei* in linkage group V between *cn* and *vg*, 6 cM centromere-distal to *cn* (Doane, 1968; Doane et al., 1975). These variants belong to the slow-migrating classes of amylase isozymes and are produced by respective alleles Amy^7 and Amy^8. Prior cytogenetic studies (Berendes, 1963) had tied linkage group V to chromosome 5, the homolog of chromosome 2R in *Drosophila melanogaster*. [*Amy* is located at 54A on the cytological map of 2R in *D. melanogaster* (Gemmill et al., 1985).] Since no variants are known for the fast-migrating, male-specific alpha-amylase expressed only in paragonia, the *Amy* copy producing it remained to be located. Here we report results of *in situ* hybridization experiments that bear on its position in the genome by identifying the site of the *Amy* locus in *D. hydei*.

Polytene chromosome squashes were prepared from salivary glands of late third instar larvae of a Zurich strain of *D. hydei* which is homozygous for Amy^7 (Doane, 1968; Doane et al., 1975). These were hybridized *in situ* using a biotinylated probe prepared from cDNA clone (gift of D. A. Hickey) containing sequences from the proximal *Amy* gene of an Oregon-R strain of *D. melanogaster*. The probe was labeled with biotin-dUTP by nick translation (BRL Bio-Nick Labeling System). Standard protocols for *in situ* hybridization of biotinylated DNA to polytene chromosomes were used (Ashburner, 1989), except that denaturation of chromosomes was done by placing the squashes in 70% neutral formamide in 2 X SS-PE buffer (phosphate-EDTA) for 5 min at 70°C and by then running them through an ethanol series at 0°C. Conditions of high stringency were used during hybridization and subsequent washes.

The *Amy* probe hybridized to a single site in polytene chromosome 5 of *D. hydei*. According to the revised map of this chromosome (Ananiev and Barsky, 1982), we identified the site of hybridization as being located in the centromere-distal portion of region 107D. This implies that the putative duplication associated with the male-specific amylase variant in this species resides within the same locus as the *Amy* copy that produces midgut amylase activity. Thus, it appears that the *Amy* gene family in *D. hydei*, which consists of at least two functional copies of *Amy*, is situated within a single, complex locus in section 107D of chromosome 5.

References: Ananiev, E.V. and V. Barsky 1982, Chromosoma (Berl.) 87:239-24; Ashburner, M. 1989, *Drosophila*: A Laboratory Manual. Cold Spring Harbor Lab. Press; Bahn, E. 1967, Hereditas 58:1-12; Berendes, H.J. 1963, Chromosoma (Berl.) 14:195-206; Da Lage, J.-L, M.-L. Cariou, and J.-R. David 1989, Heredity 63:67-72; Doane, W.W. 1968, DIS 43:143; Doane, W.W. 1969, J. Exp. Zool. 171:321-342; Doane, W.W. and I. Abraham 1974, IB 7:38; Doane, W.W., I. Abraham, M.M. Kolar, R.E. Martenson, and G.E. Deibler 1975, In: Isozymes: Current Topics Biol. Med. Res., Vol. IV, pp. 585-607, Academic Press, NY; Gemmill, R.M., J.N. Levy, and W.W. Doane 1985, Genetics 110:299-312; Hawley, S.A., R.A. Norman, C.J. Brown, W.W. Doane, W.W. Anderson, and D.A. Hickey 1990, Genome 33:501-508; Kikkawa, H. 1964, Jap. J. Genet. 39:401-411.

Offenberger, M. and A.J. Klarenberg. Zoologisches Institut der Universität, München, Germany. Baiting of *Drosophila* with plants in which decay was artificially induced.

Our knowledge about natural breeding and feeding sites of *Drosophila* is still limited. In particular in Europe scarcely any attention has been paid to decaying-herbage-breeders. Therefore the *Drosophila* fauna near Munchen was surveyed during the summer of 1990 and 1991 with baits, using plants in which decay was induced (Offenberger and Klarenberg, 1992). For this purpose fresh leaves and stalks of the most frequent local plant species were gathered from a deciduous flood plain forest near the river Isar in southern Bavaria. To facilitate the start of the decay 120-150 grams of each plant species was deep-frozen overnight (-20°C). The plant material was thawed next morning and returned to the forest. The baits were placed on the forest floor in the shade. where they were open to infection with naturally occurring bacteria and yeasts. Attracted flies were collected by sweeping with a net. After 9 days the baits were returned to the laboratory, where newly hatched flies were collected. This new kind of bait attracted *Drosophila*, especially members of the *quinaria* species group and their hymenopteran parasites. *D. limbata, D. phalerata* and *D. kuntzei* composed 59.4% of the total of attracted flies. Of the emerged flies 65.8% belonged to the *quinaria* species group. Here *D. limbata* proved to be a true decaying-herbage-breeder like *D. quinaria* and *D. magnaquinaria* in North America (James et al. 1988; Kibota and Courtney 1991). Although *D. phalerata*, a fungus specialist (Burla and Bächli 1968; Shorrocks 1982), was attracted in high numbers (macro-fungi were scarce), only few individuals emerged from the plant baits. Other numerous species were *D. testacea, Scaptomyza pallida, D. subobscura, D. immigrans* and *D. fenestrarum*. The suitability of the baits was confirmed by finding the natural breeding sites of these *Drosophila* species in the field by collecting naturally decaying plant material. For *Drosophila*, the plant breeding substrates of the genera *Arum, Heracleum, Cirsium* and *Petasites,* were earlier recorded in Japan, Europe and North America (Kimura et al. 1977; Schatzmann 1977: Grimaldi and Jaenike 1983; van Alphen et al. 1991). In addition we found that plant species of the genera *Allium, Angelica, Aeqopodium* and *Impatiens* were also used as a natural breeding

substrate. Fermenting banana-baits, which attract high numbers of *Drosophila* (Carson and Heed 1983) proved less productive in attracting decaying-herbage-breeding *Drosophila*.

References: van Alphen et al. 1991, Oecologia 87:325-329; Burla and Bächli 1968, Vjschr. Naturf. Ges. Zurich 113:311-336; Carson and Heed 1983, Methods of Collecting *Drosophila*. In: The Genetics and Biology of *Drosophila*, Vol. 3d (Ashburner, M., H.L. Carson, J.N. Thompson); Grimaldi and Jaenike 1983, J. NY. Ent. Soc. 91:83-89; James et al. 1988, Evolution 42:626-630; Kibota and Courtney 1991, Oecologia 86:251-260; Kimura et al. 1977, 45:571-582; Offenberger and Klarenberg 1992, Oecologia, in press; Schatzmann 1977, Mitt. Schw. Ent. Ges. 50:135-148; Shorrocks 1982, The Breeding Sites of Temperate Woodland *Drosophila*. In: The Genetics and Biology of *Drosophila*, Vol. 3b (Ashburner, M., H.L. Carson, J.N. Thompson, eds.).

Graf, U., M. Badoux, and E. Holliger. Institute of Toxicology, Swiss Federal Institute of Technology and University of Zurich, CH-8603 Schwerzenbach, Switzerland. The carcinogen DAB is highly genotoxic in the improved high bioactivation cross of the *Drosophila* wing spot test (SMART).

In the wing Somatic Mutation And Recombination Test (SMART) of *Drosophila melanogaster* high bioactivation crosses have been developed which are characterized by an improved sensitivity to a number of promutagens and procarcinogens (Frölich and Würgler, 1989, 1990a,b, 1991; Graf et al., 1991; Graf and van Schaik, 1992). The high bioactivation strains contain chromosomes 1 and 2 from the DDT-resistant strain Oregon R(R) which possesses a high constitutive level of cytochrome P450 (Hällström and Blanck, 1985; Pacella, pers. comm.). So far, the high bioactivation crosses have only been tested with promutagens that were already known to be genotoxic to a greater or lesser extent in the standard cross of the wing spot test. Here we report for the first time results which were obtained with a compound that was negative in the standard cross but shows considerable genotoxic activity in the improved high bioactivation cross (Graf et al., 1991).

The well-known carcinogen p-dimethylaminoazobenzene (DAB, butter yellow, methyl yellow; CAS 60-11-7; IARC, 1975) was assayed according to standard procedures (Graf et al., 1984, 1989; Graf and van Schaik, 1992). DAB was obtained from Fluka (Buchs, Switzerland) and dissolved in 1% Tween-80 plus 3% ethanol. It was fed to larvae of the standard cross (*flr*^{3}/*TM3, Ser* females and *mwh* males) as well as to larvae of the improved high bioactivation cross (*ORR*; *flr*^{3}/*TM3, Ser* females and *mwh* males). This latter cross produces larvae which are heterozygous for the dominant *R* gene on chromosome 2 derived from the Oregon R(R) strain which confers high bioactivation capacity to these larvae. The trans-heterozygous progeny (*mwh* + / + *flr*^{3}) of both crosses were analyzed for the occurrence of single and twin spots on the wings (for technical details, see Graf et al., 1984). Twin spots are produced by mitotic recombination exclusively, whereas single spots can be the result of various types of mutational events as well as of mitotic recombination. The pooled data obtained in three separate experiments are shown in Table 1. Concentrations up to 10 mM could be analyzed with the standard treatment procedure (i.e., 48 h chronic feeding of 3-day

Table 1. Summary of results obtained in the *Drosophila* wing spot test with DAB.

Conc. (mM)	Number of wings	Spots per Wing (Number of Spots) Stat. Diagn. *			
		Small single spots (1-2 cells) m = 2.0	Large single spots (>2 cells) m = 5.0	Twin spots m = 5.0	Total spots m = 2.0
A. Standard Cross					
0	180	0.23 (42)	0.09 (17)	0.03 (6)	0.36 (65)
0.5	170	0.30 (51)-	0.09 (15)-	0.04 (6)-	0.42 (72)-
1.0	168	0.25 (42)-	0.05 (8)-	0.01 (2)-	0.31 (52)-
5	26	0.19 (5)-	0.04 (1)-	0.00 (0)-	0.23 (6)-
10	26	0.23 (6)-	0.04 (1)-	0.08 (2)i	0.35 (9)-
B. Improved High Bioactivation Cross					
0	160	0.26 (42)	0.03 (5)	0.03 (5)	0.32 (52)
1	80	0.41 (33)+	0.06 (5)i	0.03 (2)-	0.50 (40)+
5	60	1.07 (64)+	0.30 (18)+	0.25 (15)+	1.62 (97)+
10	48	1.35 (65)+	0.35 (17)+	0.23 (11)+	1.94 (93)+

* Statistical diagnoses according to Frei and Würgler (1988): + =positive; - =negative; i = inconclusive. m = multiplication factor. Kastenbaum-Bowman tests, one-sided. Probability levels: alpha = beta = 0.05.

old larvae). However, this treatment proved to be much more toxic to the larvae of the standard cross than to those of the improved high bioactivation cross. Less than 5% of the larvae of the standard cross survived the treatment with 5 and 10 mM DAB, respectively, whereas approximately 40% and 20% of the larvae treated with these same concentrations survived in the improved high bioactivation cross. With respect to the genotoxic effects, in the standard cross all four concentrations tested gave clearly negative results for all three categories of spots. In contrast, in the improved high bioactivation cross the two higher concentrations produced significantly increased frequencies of small single, large single and twin spots with a clear dose response. For illustration, the spot size distributions for single and twin spots obtained with 10 mM DAB in the high bioactivation cross are plotted in Figure 1. These results demonstrate that DAB is mutagenic and recombinagenic in wing somatic cells of larvae of this specific cross. DAB has already been tested in male germ cells of *Drosophila melanogaster* for sex-linked recessive lethals where it showed genotoxic activity after adult feeding (Parry and Sinclair, 1985). Our results obtained in the wing spot test confirm the genotoxicity of DAB in *Drosophila* and show that it is dependent on cytochrome P450-mediated metabolic activation. The results also demonstrate again the versatility and increased sensitivity of the improved high bioactivation cross for the detection of genotoxicity of promutagens and procarcinogens.

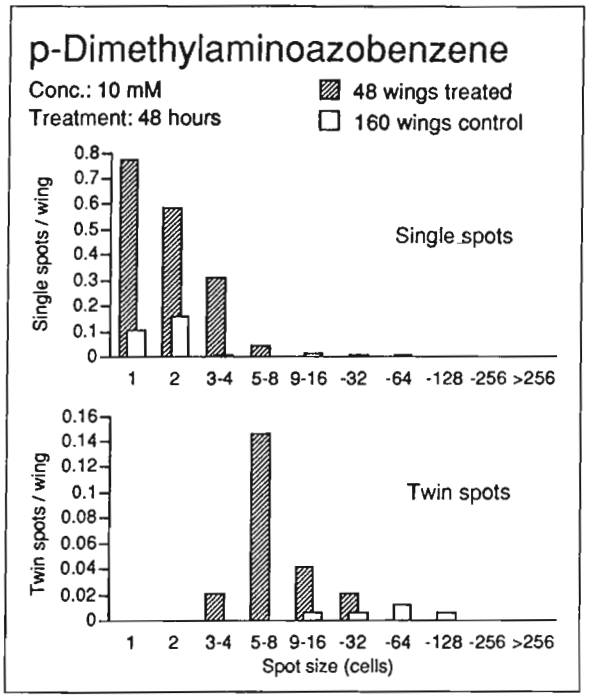


Figure 1. Spot size distributions obtained with the improved high bioactivation cross with 10 mM DAB.

References: Frei, H. and F.E. Würgler 1988, Mutation Res. 203:297-308; Frölich, A. and F.E. Würgler 1989, Mutation Res. 216:179-187; --- 1990a, Mutation Res. 234:71-80; --- 1990b, Mutation Res. 244:201-208; --- 1991, Dros. Inf. Serv. 70:246-247; Graf, U. and N. van Schaik 1992, Mutation Res. in press; Graf, U. et al. 1984, Environ. Mutagen. 6:153-188; --- 1989, Mutation Res. 222:359-373; --- 1991, Dros. Inf. Serv. 70:247-248; Hällström, I. and A. Blanck 1985, Chem.-Biol. Interact. 56:157-171; IARC 1975, Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 8, IARC, Lyon, pp. 125-146; Parry, J.M. and P.B. Sinclair 1985, Comparative Genetic Toxicology, The Second UKEMS Collaborative Study (J.M. Parry and C.F. Arlett, eds), VCH, Weinheim FRG, pp. 499-505.

Jayashankar, M. and S.N. Hegde. Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysore-570 006 India.
Interpopulation variation in morphometric characters of Indian *Drosophila ananassae*.

Morphological variation among natural populations of *Drosophila* are attributed to natural selection, depending on the pattern of geographical variation (David and Bocquet, 1975). Barnes and Kearsey (1970) studied variation in metrical characters in *Drosophila* populations. Mather (1953) and Thoday (1963) investigated the genetic variation and the opportunity of stabilizing selection on certain metrical characters. Kitagawa et al. (1982) studied the morphological differentiation qualitatively and quantitatively in *D. nasuta* sub-group. Smith and Zack (1979) have studied the heritability of phenotypic characters. Kuo and Larsen (1987) studied how genes and the environment control the size and shape of these phenotypic characters in *Drosophila*. This study of morphological traits in *Drosophila* reflects on the effect of environment on it.

D. ananassae is a domestic, cosmopolitan species with extensive chromosomal, allozyme and behavioural polymorphism. Studies on variation in morphological traits of this species is wanting. Hence *D. ananassae* populations collected from five different geographical areas of the Indian sub-continent, viz., Shivpuri, Meeraj, Sringeri, Mysore and Malai Mahadeswara Hills were used to study the morphological variations. Morphometric characters such as number of sternopleural bristles, lengths of femur, tibia, tarsus, wing length, wing width and the wing indices such as costal, 4V, 4C,

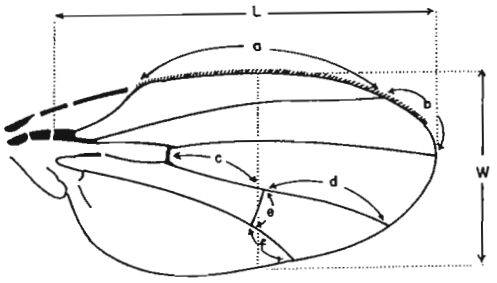


Figure 1. Wing indices measured for metric characters. a/b = Costal index; b/c = 4C index; d/c = 4V index; f/e = 5V index; WL/WW = ratio. * 1 unit = 100 u.

5V and wing length/wing width ratio were analyzed in both the sexes.

Figure 1 shows the dimensions from which different wing indices are measured and Tables 1 and 2 show the mean values with standard error and ANOVA. Lengths of femur, tibia, tarsus, wing and wing width were greater in females than the males, which clarifies the general assumption that the females are larger than the males. Wing length/wing width ratios of both the sexes show no significant variations among populations. Variation in tibia length and costal index were also insignificant. But all other characters analyzed were statistically significant. Females of Shivpuri, Sringeri and Malai Mahadeswara Hill populations had a larger number of sternopleurals than their males. These three collection spots are hilly regions. Costal index and 5V index of females of Shivpuri population were less than their respective males. Thus the morphometric characters of distant geographical populations of *D. ananassae* are polymorphic.

Acknowledgment: We wish to thank the University of Mysore and the chairman, Department of Zoology, Manasagangotri, Mysore.

References: Barnes B.W. and M.J. Kearsey, 1970, Heredity 25:1-21; David J.R. and C. Bocquet, 1975, Experimentia 15: 164-166; Kitagawa, O., K.I. Wakahama, Y. Fuyama, Y. Shimada, E. Takanashi, M. Hatsumi, M. Uwabo, and Y. Mita 1982, Jap. J. Genet. 57:133-141; Mather K. 1953, Symp. Soc. Expl. Biol. 7:66-95; Smith, J.N.M. and R. Zach, 1979, Evolution 33:460-467; Thoday, J.M. 1963, Proc. Xth Int. Cong. Genetics 3:533-540; Tea Kuo and E. Larsen 1987, Development Genetics 8:91-98.

Table 1. Metric characters for the males of *D. ananassae* (values are mean ± S.E.)

Populations	No. of sterno-pleurals	Femur£	Tibia£	I Tarsus£	Wing£ length	Wing£ width	Wing Indices: Costal index	4V index	4C index	5V index	WL/WW ratio
Shivpuri	6.12 ± 0.22	4.69 ± 0.04	4.14 ± 0.06	1.45 ± 0.02	16.84 ± 0.24	8.01 ± 0.10	1.50 ± 0.01	1.67 ± 0.01	2.38 ± 0.02	2.13 ± 0.02	2.10 ± 0.02
Meeraj	7.48 ± 0.22	5.02 ± 0.09	4.50 ± 0.08	1.60 ± 0.03	17.51 ± 0.15	8.12 ± 0.14	1.53 ± 0.04	2.28 ± 0.04	1.58 ± 0.05	1.93 ± 0.04	2.14 ± 0.03
Sringeri	6.36 ± 0.23	5.25 ± 0.07	4.56 ± 0.10	1.66 ± 0.02	18.51 ± 0.15	8.95 ± 0.10	1.51 ± 0.02	2.42 ± 0.02	1.69 ± 0.16	1.81 ± 0.03	2.06 ± 0.02
Mysore	7.04 ± 0.24	5.19 ± 0.16	4.39 ± 0.21	1.66 ± 0.06	18.91 ± 0.54	8.71 ± 0.25	1.40 ± 0.03	1.90 ± 0.15	2.71 ± 0.17	2.09 ± 0.09	2.17 ± 0.14
Malai Mahadeswara Hill	5.92 ± 0.14	4.91 ± 0.05	4.18 ± 0.05	1.61 ± 0.02	17.37 ± 0.31	8.15 ± 0.07	1.40 ± 0.11	1.76 ± 0.14	2.44 ± 0.16	2.01 ± 0.15	2.11 ± 0.11
F values	7.16*	3.3*	1.83*	8.63*	3.62*	7.8*	1.74	7.45*	6.25*	26.4*	0.43

* P < 0.01; ** P < 0.05; £1 unit = 100 μ; Df = 4, 120

Table 2. Metric characters for the females of *D. ananassae* (values are mean ± S.E.)

Populations	No. of sterno-pleurals	Femur£	Tibia£	I Tarsus£	Wing£ length	Wing£ width	Wing Indices: Costal index	4V index	4C index	5V index	WL/WW ratio
Shivpuri	6.44 ± 0.16	4.81 ± 0.01	4.18 ± 0.04	1.57 ± 0.03	19.11 ± 0.24	9.14 ± 0.07	1.38 ± 0.08	1.72 ± 0.03	2.55 ± 0.73	1.89 ± 0.03	2.01 ± 0.09
Meeraj	7.36 ± 0.43	5.39 ± 0.10	4.56 ± 0.08	1.73 ± 0.04	19.02 ± 0.36	8.96 ± 0.17	1.64 ± 0.03	1.58 ± 0.03	2.28 ± 0.05	1.88 ± 0.03	2.09 ± 0.02
Sringeri	7.00 ± 0.18	5.37 ± 0.04	4.69 ± 0.05	1.89 ± 0.02	20.19 ± 0.10	9.54 ± 0.08	1.55 ± 0.03	1.59 ± 0.03	2.39 ± 0.05	1.94 ± 0.03	2.12 ± 0.01
Mysore	6.20 ± 0.16	5.67 ± 0.12	5.23 ± 0.14	2.07 ± 0.06	21.50 ± 0.37	10.17 ± 0.30	1.51 ± 0.16	1.61 ± 0.10	2.42 ± 0.11	1.63 ± 0.09	2.12 ± 0.05
Malai Mahadeswara Hill	6.72 ± 0.18	5.11 ± 0.22	4.66 ± 0.07	1.81 ± 0.02	20.47 ± 0.22	9.77 ± 0.10	1.53 ± 0.07	1.69 ± 0.10	2.43 ± 0.13	1.80 ± 0.07	2.10 ± 0.13
F values	7.68*	4.4*	5.11*	13.52*	5.32*	15.6*	4.43*	5.75*	5.82*	20.71*	1.10

* P < 0.01; ** P < 0.05; £1 unit = 100 μ; Df = 4, 120

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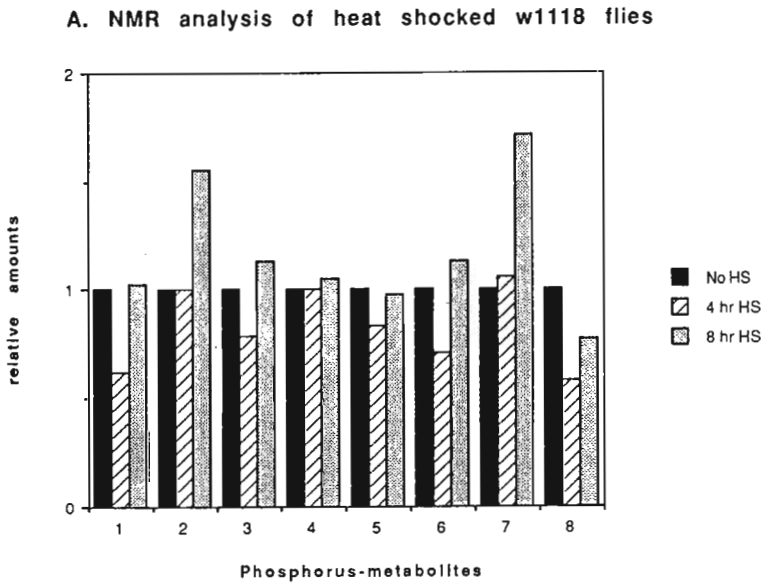
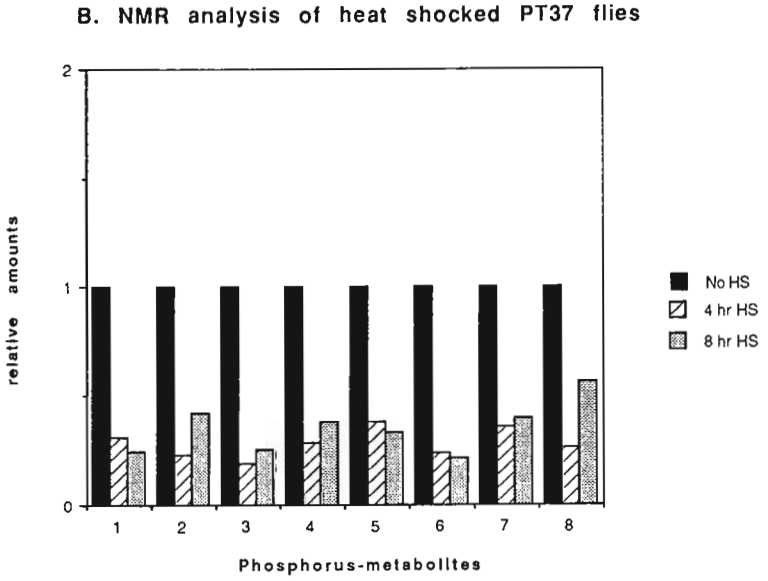
Levels of phosphorus-metabolites in adult *Drosophila* expressing pertussis toxin were determined by [^{31}P] NMR spectroscopy.

Pertussis toxin inactivates certain G-proteins by introducing an ADP-ribose group near the carboxyl-terminus of the alpha subunit (Ui, 1990). The major pertussis toxin substrate in *Drosophila* tissues is $G_{O\alpha}$. We introduced a pertussis toxin gene under control of the hsp70 heat shock promoter into the *Drosophila* genome of flies homozygous for w^{1118}. When heat shocked, transformed flies produce active pertussis toxin which ADP-ribosylates endogenous $G_{O\alpha}$. Pertussis toxin is expressed in photoreceptors, in the lamina of the eye and in epithelial cells lining the gut. Because $G_{O\alpha}$ is absent in photoreceptors, pertussis toxin does not affect the photoreceptor component of the *Drosophila* visual response. However, it abolishes photo-induced electrical transients that normally arise from the lamina where $G_{O\alpha}$ transcripts have been detected. Pertussis toxin expression also blocks embryonic development and shortens the lifetime of adult *Drosophila*.

While investigating the phenotypes of these transformed flies, it was necessary to determine the levels of NAD in adult extracts. Since NAD is a precursor for ADP ribose, we hypothesized that depletion of NAD might be the cause of the early death of adult flies following heat shock. We used [^{31}P] NMR spectroscopy to determine the levels of all acid-extractable phosphorus-metabolites which included nucleotide di- and tri-phosphates and pyridine nucleotides.

Transformed pertussis toxin flies (PT37) and w^{1118} flies were used in these experiments. Flies were counted and weighed. The flies were treated with or without a heat shock regime. They were then frozen, pulverized, and homogenized in cold 1:5 diluted 70% perchloric acid. Denatured proteins and lipids were pelleted by centrifugation at 32,000g for 1 hour at 4°C. The pH of the supernatant was adjusted to 9-10 by titration with 2 M KOH. The ClO_4 precipitate was removed by centrifugation at 32,000g for 15 minutes. The resulting supernatant contained the water soluble metabolites. Divalent cations were removed by passing the supernatant through a K^+-chelex-100 column pre-rinsed with 100mM KCl. A 1 ml wash with 100mM KCl was added to the eluate and was then lyophilized. The weights of the lyophilates were determined. Each lyophilate was resuspended in 1.6 ml of 20mM Tris buffer containing 50mM EDTA and 0.4 ml D_2O (20% total volume, 2 ml). The final pH was 9 (without correction for the deuterium ion).These methods were based on protocols from Glonek *et al.* (1982).





A vertical, high-resolution, narrow-bore spectrometer (GN500; field strength 11.75 T) operating at 202.4 MHz and a commercial 10 mm broad band probe were used to obtain spectra of the extracts. Data acquisition parameters were as follows: Spectra were obtained by averaging 500 transients for a total acquisition time of 2.35 h under relaxed conditions (60 μs pulse duration (α = 90°) and a 17 s pulse interval). Data size was 32 K and sweep width was $\pm$ 4202 Hz. Routine baseline correction and 3 Hz exponential multiplication were applied and peak assignments were made on the basis of chemical shift and by spiking with known standards. Endogenous phosphoarginine (PArg) was used as the frequency standard and was set to 0.5 ppm because PArg resonates at 0.5 ppm with phosphocreatine at 0.0 ppm.

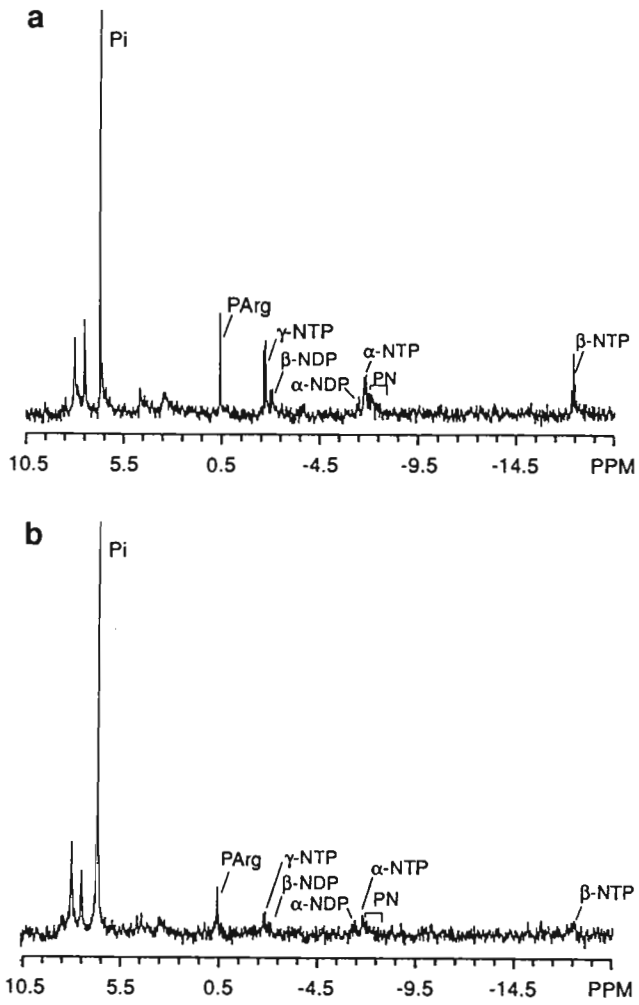


Figure 1 shows the [^{31}P] NMR spectra of acid extracts of w^{1118} (A) and PT37 (B) flies heat shocked for 8 hours at 37°C. Columns 1 through 8 represent beta-NTP, PN, alpha-NTP, alpha-NDP, beta-NDP, gamma-NTP, PArg, and Pi, respectively. Note that NTP, NDP, PN and PArg peaks have diminished in the spectrum of PT37 flies. Figure 2 shows the relative levels of phosphorus containing metabolites in w^{1118} (A) and PT37 (B) flies before and after heat shock. All values are normalized by weight of lyophilate prior to resuspension. 1U = 0.09 uM in a 2 ml volume. These weights were: w^{1118} No HS, 220 mg; w^{1118} 4 hr HS, 250 mg; w^{1118} 8 hr HS, 210 mg; PT37 No HS, 220 mg; PT37 4 hr HS, 270 mg; and PT37 8 hr HS, 210 mg. No heat shock: solid column, 4 hours heat shock: diagonal line column, 8 hours heat shock: shaded column. PArg: phosphoarginine, PCr: phosphocreatine, PN pyridine nucleotides, NTP: nucleotide triphosphate, NDP: nucleotide diphosphate.

The heat shock induced expression of pertussis toxin causes a decrease in all high energy phosphorus-metabolites, PArg, NTP, and NDP, and PN. The decrease in the pyridine nucleotide level is likely due to the consumption of NAD which serves as a substrate for the ADP-ribosylation of G_O, but the decrease in the level of the other nucleotides suggests that S1 expression may affect the flies in a more general manner. These effects are probably neurological in nature and are mediated by G_O. One such phenotype is the cessation of feeding behavior and consequent lack of nutrient uptake which is the subject of further investigations to fully characterize these flies.

The purpose of this report is to demonstrate a method in *Drosophila* that specifically determines all acid-extractable phosphorus-metabolites which are important for energy metabolism. The use of [^{31}P] NMR spectroscopy is a novel approach for determining these metabolite levels in *Drosophila*.

References: Glonek, T., S.J. Kopp, E. Kot, J.W. Pettegrew, W.H. Harrison, and M. Cohen 1982, [^{31}P] Nuclear magnetic resonance analysis of brain: the perchloric acid extract spectrum. J. of Neurochemistry 39: 1210-1219; Ui, M. 1990, Pertussis toxin as a valuable probe for G-protein involvement in signal transduction. In: ADP-ribosylating Toxins and G-proteins: Insights into Signal Transduction (Moss, J. and M. Vaughn, eds.) Washington, D.C., American Society for Microbiology, pp. 45-78.

Marinković,D., I.Kovać and V.Stojiljković. Faculty of Science, Belgrade, Yugoslavia. Adaptive variation in a multilocus system.

We succeeded to analyse by starch gel electrophoresis allozymic variation for nine polymorphic gene-enzyme systems in each of 3 x 100 *D. melanogaster* male individuals, with extremely fast, intermediate, and extremely slow preadult development (see also, Marinkovic et al., 1987). In three studied systems (6Pgdh, Pgm and Acph) we have determined three allelic forms, and in other six - only two forms, which makes a possibility of producing about 80,000 genotypes.

If some of allelic forms were neutral at nine loci, we would expect that a majority (or all) of 300 studied individuals may have a unique genotype. However, a total of 137 genotypes were discovered, 85 of them being unique, 36 repeatable only 2-3 times, 10 of them 4-7 times, and 6 of them being present in even 74 individuals. The most frequent genotype, found in 17 individuals, was homozygous at all loci for the commonest alleles. No one individual was found to be heterozygous at nine studied loci.

Table 1 shows that highly significant differences exist among three developmental groups of individuals (F, M, S) as of the presence of 14 most frequent genotypes. Among the fastest developed flies, the genotypes no. 1, 2, 4, 6, 7, 11 are present in 31 out of 100 studied individuals. In intermediate group the genotypes no. 1, 2, 3, 5, 10, 12 are present in 35 individuals. Among flies with the slowest preadult development, the genotypes no. 1, 2, 3, 9, 13 are present among 21 out of 100 studied individuals.

The obtained results suggest that neutral hypothesis could not be applied in a well studied multilocus system, which is a reality in complex life phenomena. The absence of the knowledge about the constitution of gene combinations which are determining complex developmental processes, could not be taken as a basis of application of neutral hypothesis, which is frequently done in studies of life history phenomena.

Table 1.

	Genotypes									Phenotype			
	2nd chrom.			3rd chrom.					Ist chr.				
	αGpd	Adh	Hex	Sod	Pgm	Est	Odh	Acph	6Pgd	F	M	S	Total
1/	1	1	1	1	1	1	1	1	1	5	9	3	17 ind
2/	1	F/S	1	1	1	1	1	1	1	6	5	4	15 "
3/	1/93	F/S	1	1	1	1	1	1	1	2	5	8	15 "
4/	1	1	1	1	1	1/103	1	1	1	5	2	2	9 "
5/	1/93	1	1	1	1	1/103	1	1	1	3	5	1	9 "
6/	1/93	1	1	1	1/96	1	1	1	1	7	2	0	9 "
7/	1	F/S	1	1	1	1/103	1	1	1	4	1	2	7 "
8/	1	F/S	1/103	1	1	1/103	1	1	1	2	3	2	7 "
9/	1/93	F/S	1	1	1	1/103	1	1	1	3	1	3	7 "
10/	1	1	1/103	1	1	1	1	1	1	0	6	0	6 "
11/	1/93	1	1	1	1	1	1	1	1	4	1	1	6 "
12/	1	1	1	1	1/96	1	1	1	1	0	5	0	5 "
13/	1/93	1	1/103	1	1	1	1	1	1	0	2	3	5 "
14/	1/93	F/S	1/103	1	1	1	1	1	1	1	2	2	5 "
										42	49	31	122/300

Acknowledgment: Excellent assistance of Mrs. Lorrain Barr, and warm hospitality of Prof. F.J. Ayala when senior author spent his sabbatical at the University of Davis, California, is still very much appreciated.

References: Marinković, D., N. Tucić, A. Moya and F.J. Ayala 1987, Genetics 117: 513-520.

Meera Rao, P. and H.A. Ranganath. Department of Studies in Zoology, University of Mysore, Manasagangotri, Musore - 570 006, India. A catalogue of metaphase karyotypes of the members of the *immigrans* species group of *Drosophila*.

Cytotaxonomy of *Drosophila* has substantially shown that the karyotype of a species is of taxonomic value. The diploid chromosome number of a species is relatively a stable evolutionary phenotype. The *immigrans* species group is a heterogenous assemblage of species with marked morphological differentiation. Taxonomically, the species are grouped under five subgroups namely, *nasuta, immigrans, quadrilineata, hypocausta* and *lineosa* subgroups. Here is a catalogue of information of 34 known karyotypes of the *immigrans* species group of *Drosophila*.

Acknowledgment: The authors are grateful to The Chairman of the Department of Studies in Zoology for providing facilities and to the University Grants Commission, New Delhi for financial assistance.

Species	2n	Karyotype	Dot Chromosome	Sex Chromosomes	References
			Subgroup: lineosa		
D. lineosa	12	5R+1D	D	X = R	PRESENT STUDY
				Y = R	
D. argentostriata	12	5R+1D	D	X = R	Bock (1966);
				Y = R	Wakahama *et al.* (1983).
D. silivistriata	10	3R+1V+1D	D	X = R	Bock and Baimai (1967);
				Y = R	Wakahama *et al.* (1983).
			Subgroup: nasuta		
D. nixifrons	10	3R+1V+1D	D	X = R	Tan *et al.* (1949)
				Y = R	
D. n. nasuta	8	2R+1V+1D	D	X = R	Rajasekarasetty *et al.* (1979); Rangunath and Hägele (1982);
				Y = J	Ramachandra and Ranganath (1986);PRESENT STUDY
D. kepulauana	8	2R+1V+1D	D	X = R	Wilson *et al.* (1969);
				Y = R	Wakahama *et al.* (1983).
D. kohkoa	8	2R+1V+1D	D	X = R	Wilson *et al.* (1969);
				Y = R/J/SM	Wakahama *et al.* (1983).
D. pallidifrons	8	2R+1V+1D	D	X = R	Wilson *et al.* (1969);
				Y = R	Wakahama *et al.* (1983).
D. komaii	8	3R+1V	—	Y = SM	Kikkawa and Peng (1938).
D. spinofemora	8	2R+1V+1D	D	X = R	Wharton (1943).
D. setifemur	8	2R+1V+1D	D	X = R	cf. Mather (1962).
D. niveifrons	8	2R+1V+1D	LD	X = R	Wakahama *et al.* (1983)
				Y = J	
Taxon-F	8	2R+1V+1D	D	X = R	Wakahama *et al.* (1983)
				Y = J	
D. s. sulfurigaster	8	2R+1V+1D	D	X = R	Wilson *et al.* (1969); Wakahama *et al.* (1983);
				Y = J/SM	Ushakumari and Ranganath (1986).
D. s. bilimbata	8	2R+1V+1D	D	X = R	Patterson and Wheeler (1942);
				Y = R/J	Wakahama *et al.* (1983);
					Ushakumari and Ranganath (1986).
D. s. albostrigata	8	2R+1V+1D	D	X = R	Wilson *et al.* (1969); Wakahama *et al.* (1983);
				Y = R/SM/J	Ushakumari and Ranganath (1986).
D. s. neonasuta	8	2R+1V+1D	D	X = R	Nirmala and Krishnamurthy (1973);
				Y = J	Wakahama *et al.* (1983); Ushakumari and Ranganath (1986);
					PRESENT STUDY
D. pulaua	8	2R+1V+1D	D	X = R	Wilson *et al.* (1969); Wakahama *et al.* (1983);
				Y - R/J	Ushakumari and Ranganath (1986).
D. n. albomicana	6	2V+1D	LD	X fused to 3	Rajasekarasetty *et al.* (1979); Ranganath and Hägele (1982);
				Y fused to 3	Ramachandra and Ranganath (1986); PRESENT STUDY
			Subgroup: immigrans		
D. immigrans	8	2R+1V+1D	LD	X = R	Metz and Moses (1923); Ward (1949); Mather (1962);
				Y = R/J/M/SM	Wakahama *et al.* (1983); Gupta and Kumar (1986);
					PRESENT STUDY.
		1R+1J+1V+1D	—	X = R	Emmens (1937);
				Y = R	Wharton (1943).
D. formosana	8	2R+1V+1D	LD	X = R	(cf.) Wakahama *et al.* (1983);
				Y = R	PRESENT STUDY
D. curviceps	8	2R+1V+1D	D	X = R	Okada and Kurokawa (1957);
				Y = J	Wakahama *et al.* (1983).
D. ruberrima	8	2R+1V+1D	D	X = R	(cf.) Wakahama *et al.* (1983)
				Y = R	
Taxon-C	8	2R+1V+1D	LD	X = R	Wakahama *et al.* (1983).
				Y = J	
D. tongpua	8	2R+1V+1D	LD	X = R	Lin and Tseng (1973).
			Subgroup: hypocausta		
D. rubida	8	2R+1V+1D	D	X = R	Mather (1960);
				Y = R	PRESENT STUDY
		2V+1R+1D	LD	X = M	Mather (1962).
D. pararubida	8	2R+1V+1D	D	X = R	Mather (1961);
				Y = R	PRESENT STUDY.
		2V+1R+1D	D	X = M	Mather (1962).
D. hypocausta	8	2R+1V+1D	D	X = R	Pipkin (1956);
				Y = R	Wakahama *et al.* (1983)
					PRESENT STUDY
D. xanthogaster	8	2R+1V+1D	D	X = M	Lin and Tseng (1973).
D. nasutoides	8	2R+1V+1v	M	X = R	Wheeler *et al.* (1973).
				Y = R	
D. neohypocausta	6	2R+1V	Not	X = R	Wakahama *et al.* (1983);
			present	Y = R	PRESENT STUDY
			Subgroup: quadrilineata		
D. quadrilineata	12	4R+1V+1D	D	X = M	Wakahama *et al.* (1983).
				Y = R	
		5R+1D	D	X = R	Gupta and Kumar (1986).
				Y = R	
D. hexastriata	8	2R+1V+1D	D	X = R	Tan *et al.* (1949).
				Y = R	
D. annulipes	6	1V+1R+1J	Not	X = M	Lin *et al.* (1974).
			present	Y = M	
		2V+1R	Not	X = R	Wakahama *et al.* (1983).
			present	Y = R	

D = Dot chromosomes, lD = long dot chromosomes, R = Rod chromosomes, J = Submetacentric chromosome, v/SM = Small metacentric, V/M = Metacentric.

Bhadra (nee Pal), M., U. Bhadra and R.N. Chatterjee. University of Calcutta, India. $MgCl_2$ induced activity pattern of 93D and other puff sites in *Drosophila melanogaster.*

Kroeger (1963, 1966) noted that NaCl and KCl at concentrations above the isotonic level induce puffing at many sites in salivary gland chromosomes of diptera. However, no systematic study on effects of various concentrations of Mg^{2+} ions on the puffing activity pattern of *D. melanogaster* have been carried out. In the present report, the results of the study on effect of high molar Mg^{2+} ions on puffing activity pattern of the polytene chromosome of *D. melanogaster* have been presented.

For the present investigation, salivary glands from third instar larvae of *D. melanogaster* were dissected out by hand in *Drosophila* Ringer (pH 7.2) incubated either in Ringer or in Ringer containing 96 mM $MgCl_2$ for 10 minutes. Thereafter, the glands were incubated for 10 minutes in ^{3}H-uridine (500 uCi/ml; Sp. activity 15,200 mCi/mM; obtained from BARC, Trombay). Cytological preparations were then made and processed for autoradiography.

Results show that *in vitro* treatment with 96 mM $MgCl_2$ induced puffs at a number of sites viz., 2AB, 10BC, 47BC, 63BC, 87A, 87C, 90BC, and 93D. Observation reveals that among these puff sites, the 93D puff is highly induced (Figure 1). The puffing activity index and ^{3}H-uridine labeling (mean grain number) are shown in Table 1. It may be noted that the 63BC, 87A, 87C, and 93D puffs are the heat shock puffs. This may imply that *in vitro* incubation of salivary glands of *D. melanogaster* at high concentration of Mg^{2+} reproduced, at least partially, the changes in gene activity attributed to heat shock. Furthermore, in comparing the changes in the puffing pattern resulting from $MgCl_2$ and heat shock treatments, it appears that only a few loci respond to both agents, whereas a number of other loci respond to either one of them. It appears, therefore, that different agents affect different gene families. However, these gene families may share a number of loci. On the basis of these observations, it may be suggested that different agents should have different consequences for the physiological machinery of the cell.

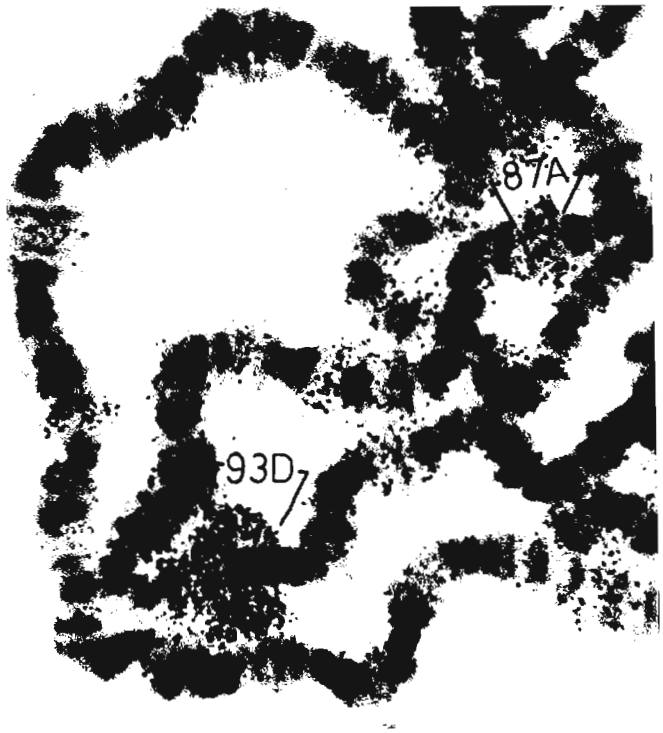


Figure 1. Photomicrograph showing ^{3}H-uridine labeling pattern on different puff sites of salivary gland chromosomes after treatment with 96 mM $MgCl_2$. Note the intense labeling on 93D puff site.

Acknowledgment: This work is financially supported by "Lady Tata Memorial Trust" to M. Bhadra (nee Pal).

References: Kroeger, H. 1963, Nature (Lond.) 200:1234; Kroeger, H. 1966, Expt. Cell Res. 41:64.

Table 1. Data showing puffing activity indices and ^{3}H-uridine labelling pattern in the 8 puff sites of *D. melanogaster* in normal and after *in vitro* treatment of salivary glands in 96 mM $MgCl_2$.

Puff sites	No. of nuclei examined	Average puffing activity value in Ringer control	Average puffing activity value in 96 mM $MgCl_2$ treatment	Mean grain number with S.E. Ringer control	Mean grain number with S.E. 96 mM $MgCl_2$ treatment
2AB	30	1.22	1.75	22.19 ± 3.17	31.47 ± 4.92*
10BC	27	1.48	2.47	14.17 ± 3.78	27.71 ± 3.27*
47BC	30	1.07	2.02	24.18 ± 4.52	39.91 ± 5.72
63BC	30	1.17	1.91	17.37 ± 2.47	31.71 ± 5.13*
87A	30	1.28	1.93	11.72 ± 2.29	52.19 ± 6.19*
87C	29	1.01	1.09	9.32 ± 2.92	14.72 ± 2.08
90BC	28	1.11	1.78	19.31 ± 3.23	41.44 ± 3.14*
93D	30	1.21	3.21	21.49 ± 2.81	108.19 ± 8.12*

*Significantly different from respective controls, $P < 0.05$

Bhadra, U., M. Bhadra (nee Pal) and R.N. Chatterjee. University of Calcutta, India. The influence of whole arm trisomy on larval denticle distribution pattern of *Drosophila melanogaster*.

In *D. melanogaster*, trisomy causes reduction in the expression of linked and unlinked structural genes of the genome (Devlin *et al.*, 1988). It has been suggested that inviability of trisomic individuals in late developmental stages (pupal stage) is due in part to the reduction in expression of many autosomal structural genes (Devlin *et al.*, 1984, 1988). In order to determine if trisomy causes phenotypic abnormalities in larval morphology due to the repression of gene product levels, we have examined the structural morphology of third instar larvae trisomic for entire chromosomal arm. In this report, preliminary results on the effect of autosomal trisomy on the number and the distribution pattern of denticles on the ventral setal belt of third instar larvae are presented.

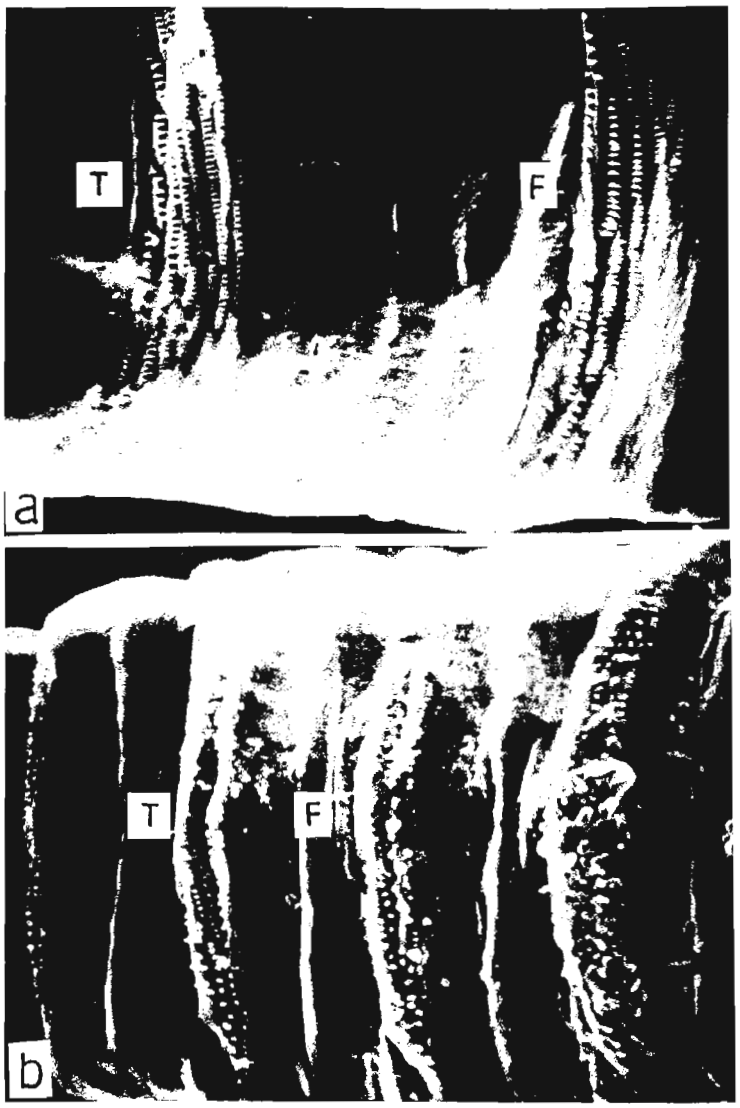


Figure 1. Ventral view of third instar larvae showing distribution of denticles on setal belt. (a) Female trisomic for 2L, (b) Female trisomic for 3L. Note the increase in denticle number in 2L trisomic larvae. T - Third thoracic, F - First abdominal setal belt.

For this series of investigations, the wild type strain Oregon R^+ and compound chromosome stocks C(2L) SHI^+; F(2R) *bw*/F(2R) *bw* and C(3L) VGI *ru st*, F(3R) VDI e^s/F(3R) VDI e^s were used. Individuals trisomic for the left arm of either chromosome 2 or chromosome 3 were produced as described by Fitz-Earle and Holm (1977). The trisomic larvae were fixed in gluteraldehyde (10-12%, pH 7.2) for 3 hours and subsequently passed through ascending grades of ethanol and stored in 70% ethanol. Thereafter, the larvae were processed for scanning electron microscopic study as described by Ford (1986). The larvae were then observed under a Phillips 501B Scanning Electron Microscope.

Observation reveals that the larvae carrying duplicated left arm of either chromosome 2 or chromosome 3 were smaller in size in contrast to their diploid sibs. When the number and distribution pattern of denticles on third thoracic and first abdominal setal belts of third instar larvae of trisomic individuals were examined, a clear asymmetry in the distribution of denticles was noted in comparison to control. Furthermore, the distribution pattern of denticle was not identical in the two trisomic larvae. Figure 1 presents the denticle distribution pattern in the trisomic larvae of *D. melanogaster*. As it appears from the photomicrograph, the number of denticles in 2L trisomic larvae was much higher than that in 3L trisomic larvae. The results of the present investigation have therefore revealed that trisomy for 2L or 3L in *D. melanogaster* has striking effects on denticle distribution patterns and formation of the ventral setal belt in third instar larvae.

Acknowledgment: This work is financially supported by "Lady Tata Memorial Trust" to M. Bhadra (nee Pal).

References: Devlin, R.H., T.A. Grigliatti and D.G. Holm 1984, Chromosoma (Berl). 91:65; Devlin, R.H., D.G. Holm and T.A. Grigliatti 1988, Genetics 118:87; Fitz-Earle, M and D.G. Holm 1978, Genetics 89:499; Ford, S.C. 1986, DIS 63:52.

Kane, J. and H.H. Fukui. Minehaha Academy and University of Minnesota, Minneapolis, MN USA. The maximum age at which a male *Drosophila melanogaster* can produce progeny.

The study was carried out to measure the maximum age at which a male fly can produce progeny in *D. melanogaster*. This type of data is necessary for experiments in which chromosomes are recovered from old flies.

An inbred strain (JWC-3) was chosen to study because mean virgin male longevity for this strain was the shortest of the inbred strains studied in our laboratory (35.3 ± 0.78 S.E.). Inbred lines were derived from a large random-mating laboratory population ("LF350", Weber and Diggins, 1990) by 50 generations of half-sib mating. The flies were subjected to three generations of density control (200 ± 25 eggs/a half pint bottle). To ensure virginity, the bottles were cleared and eclosed flies were collected at 4 to 5 hour intervals for twelve hours. Flies were anesthetized with CO_2 briefly to sex them. Throughout the experiment, flies were reared and kept in 24°C dark incubator with standard yeasted-cornmeal-molasses-agar-medium. Flies were inspected once a day, and deaths were recorded at the inspection. Flies were tested at 20, 30 or 31, 35 or 36, and 44 to 47 days post-emergence.

Male flies were kept in groups of 10 to 15 individuals in 8-dram vials and transferred twice a week during the aging stage. On test days, four three-day old virgin females from the outbred base population were introduced into a 4-dram vial with one test male. Flies were kept in the vial for four days. On the fifth day, males were transferred to a fresh 4-dram vial without females and transferred twice a week to a fresh vial until death. Females were kept three more days in the egg-laying vials then discarded. Larval movement was used to check progeny production. An analysis of variance was used to compare mean longevity between the fertile and non-fertile males by using SAS version 5.0 General Linear Models procedure (SAS Institute, 1985).

Table 1. Age specific male fertility measured as the proportion of flies producing progeny. The flies were given four virgin females for four days at each age group.

Age	N	Fertile males (%)
20	18	17 (94.4)
30 / 31	23	10 (43.5)
35 / 36	16	3 (18.8)
44 to 47	4	0 (0.0)

Over 90% of males aged 20 days were able to produce progeny. However, fertility fell rapidly as age progresses; none of the four males aged 44 to 47 days produced progeny (Table 1). The difference in longevity between producers and non-producers was not significant at $P = 0.05$ level at the first two age classes. It is important to point out that those flies which lived more than 50 days all produced at the age 30 or 31 days. If the objective is to recover the chromosomes from the longest lived flies, only flies which survive to the age of 30 days need to be given mates.

Acknowledgment: J.K. was supported by NSF Young Scholars Program at Univ. Minnesota. H.H.F. is supported by NIH grants on "Oldest-old Mortality" to J.W. Curtsinger and J. Vaupel (P01 AG08761).

References: SAS Institute, Inc., 1985, SAS User's Guide: Statistics, Version 5, SAS Institute Inc., Cary, NC; Weber, K.E. and L.T Diggins 1990, Genetics 125:585-597.

Kane, J. and H.H. Fukui. Minehaha Academy and University of Minnesota, Minneapolis, MN USA. Virgin male general locomotor activity variation among four inbred strains *Drosophila melanogaster* that vary in longevity.

During analyses of virgin male longevity in four inbred strains of *D. melanogaster*, it was noted that one of the strains showed a much higher general locomotor activity level than the others. The current study was designed to quantify this observation.

Virgin males from four inbred strains of *D. melanogaster* were tested for their general locomotor activity level. The base population from which these strains were derived was maintained as a large random-mating laboratory population ("LF350", Weber and Diggins, 1990). Mated females were drawn from the base population to initiate lines which were inbred for 50 generations by half-sib mating. All of the flies were reared and kept on standard yeasted-molasses-cornmeal-agar-medium in a dark incubator at 24.0°C. The flies were sexed when they were 0- to 5-hr post-emergence under light CO_2 anesthesia to ensure their virginity, and matured to 3-day old on the day of testing as groups of 20 flies per 8-dram vial. Test flies were brought out from the incubator 20 min before testing began. The procedures for evaluating general locomotor activity level followed those of Cobb et al. (1987). A single fly (not anesthetized) was aspirated into a 10 x 10 x 1 cm chamber made of plexiglas with transparent plexiglas lid. The outer surface of the lid was marked with 100 one cm squares. Each fly was allowed to "settle" into the new environment for 90 sec. Flies were observed for 60 sec, recording the number of squares entered. To minimize environmental effects, the following procedures were used: 1) The four lines were tested alternatively; 2) Each strain was tested in all nine

chambers with equal frequency to randomize "box effects"; 3) Lighting was uniform; 4) Chambers were cleaned with 70% alcohol immediately after each observation, then they were left for at least 30 min before reuse; and 5) Time of testing and room temperature were recorded every hour of the testing period. An ANOVA using strain, day of testing, time of testing, temperature and their interactions as sources of variation was performed by using SAS Version 5.0 General Linear Models procedure (SAS Institute, 1985). Means were compared by using the Tukey multiple comparison test at $P = 0.05$ level (Zar, 1984 p. 186).

Table 1. Mean general locomotor activity levels with standard errors, and the results of the Tukey multiple comparison test. The results of an ANOVA to evaluate strain and environmental effects.

A) Mean general locomotor activity levels.

Strain	N	Mean	S.E.	Tukey test[a]
2	100	20.7	2.2	A
3	99	18.1	2.3	A
1	100	22.1	2.3	A
4	100	46.8	3.2	B

[a]Strains sharing a letter designation do not differ significantly at $P = 0.05$.

B) ANOVA table.

Source	df	MS	F
Model	47	2509	4.64***
I)Strain	3	7412	13.70***
2)Chamber	8	1587	2.93
3)Date	2	9179	16.96***
1 • 2	24	423	0.78
1 • 3	6	939	1.74
2 • 3	4	870	1.61
Error	351	541	
Total	398		

***Significant at $P < 0.001$.

Table 2. Mean longevity with standard errors of the four inbred lines studied. The Tukey multiple comparison test was used to compare the means.

Strain	N	Mean	S.E.	Tukey test[a]
2	155	33.9	1.1	A
3	233	35.3	0.8	A
1	216	46.1	0.8	B
4	236	52.5	1.1	C

[a]Strains sharing a letter designation do not differ significantly at $P = 0.05$.

The activity level difference is significant between strain-4 and the other strains (Table 1A). Furthermore, the mean activity level of strain-4 is very similar to that of an outbred population of *D. melanogaster* reported by Cobb et al. (1987, Figure 1), suggesting that inbreeding depression affected the lines to different degrees. Two environmental factors affected the behavior; day of testing and temperature (Table 1B). The time of day on which flies were tested and the chambers used are not significant. None of the interactions among these factors are significant, indicating environmental variation affected the behavior of each strain in the same directions (Table 1B).

The mean post-emergence virgin male longevities of these four strains are summarized in Table 2. The data were collected two generations before the behavioral assay as a part of full-diallel analysis (Fukui and Curtsinger, in prep.). The mean longevity of strains 1 and 4 are significantly different from strains 2 and 3 as well as between themselves. Therefore, virgin males from the longest lived strain (strain-4) showed the highest level of general activity in this experimental design. The Pearson product-moment correlation coefficient (r) between these two traits is 0.84. However, this is not significant at $P = 0.05$ level because of the large standard error (0.39).

No correlation between longevity and general activity level was found in outbred populations by Le Bourg et al. (1984) and Le Bourg (1987). However, a positive correlation between these traits was found when 3-day old females from the lines selected for postponed senescence and the control lines were compared (Service, 1987). There seems to be a positive correlation between these traits in the current study also, though more inbred strains must be tested to confirm this. Thus, high general activity in early life may be correlated with elongated longevity when: 1) These traits are differentiated by either selection (directionally) or inbreeding depression (randomly); and 2) Correlation is measured between lines. Rose (1984) pointed out that inbreeding induces positive genetic correlations between fitness components. The results cannot, therefore, be extrapolated to the base population. The results do show that a long-lived genotype can have highest activity at least in one life stage, contrary to an expectation of "rate of living" theory, which proposes that less active organisms live longer (Sohal, 1976).

Acknowledgment: J.K. was supported by NSF Young Scholars Program at Univ. Minnesota. H.H.F. was supported by NIH grants on "Oldest-old Mortality" to J.W. Curtsinger and J. Vaupel (P01 AG08761).

References: Cobb, M., K. Connolly, and B. Burnet 1987, Anim. Behav. 35:705-713; Le Bourg, E. 1987, Exp. Gerontol. 22:359-369; Le Bourg, E., F.A. Lints and C.V. Lints 1984, Exp. Gerontol. 19:205-210; Rose, M.R. 1984, Am. Nat. 123:565-569; SAS Institute, Inc., 1985, SAS User's Guide: Statistics, Version 5.0, SAS Institute Inc., Cary, NC; Service, P.M. 1987, Physiol. Zool. 60:321-326; Sohal, R.S. 1976, Interdiscip. Topics Gerontol. 9:25-40; Weber, K.E. and L.T Diggins 1990, Genetics 125:585-597; Zar, J.H. 1984, Biostatistical Analysis (2nd ed.), Prentice-Hall, Inc., NJ.

Shyamala B.V. and H.A. Ranganath. Department of Studies in Zoology, Manasagangotri, University of Mysore, Mysore- 570 006, India. Metaphase karyotypes of the members of the *montium* species subgroup of *Drosophila*.

The study of karyotypic differentiation among taxonomically closely related species provides an insight into the still intriguing problem of chromosomal change during evolutionary change. John (1981) feels that the immense variety of, and the striking variation in,the karyotypes of the extant forms provide suggestive evidence that chromosome change has played and continues to play a major role in the evolutionary change.

Perusal of relevant literature has revealed that Clayton and Wheeler (1975), Baimai (1980), Clayton and Guest (1986) and Lemeunier *et al.* (1986) have provided some information as to the karyotypic composition of some species of the *montium* subgroup of *Drosophila*. Even though Lemeunier et al. (1986) have claimed that the karyotypes of 55 of the 79 species of the *montium* subgroup are known, the scrutiny of the catalogues compiled by Clayton and Wheeler (1975), Baimai (1980) and Clayton and Guest (1986) plus the present study reveals information about 37 species. It is as follows:

Sl. No.	Species	CHROMOSOMES: Large autosomes	X	Y	4th	References
1.	*D. agumbensis*	2V	R	J	D	Prakash & Reddy,1978
		2V	R	R	MD	Present Study
2.	*D. anomelani*	2V	R	V	D	Reddy & Krishnamurthy, 1973
		2V	R	R	D	Present Study
3.	*D. auraria*	2V	R	R	D	Kikkawa & Peng, 1938
		2V	J	J	D	Baimai, 1980
4.	*D. baimai*	2V	J	LJ	D	Baimai, 1980
5.	*D. barbarae*	2V	J	LJ	D	Baimai, 1980
6.	*D. biauraria*	2V	R	R	-	Bock & Wheeler,1972
7.	*D. bicornuta*	2V	R	R	D	Bock & Wheeler, 1972
8.	*D. birchii*	2V	J	LJ	D	Baimai, 1980
9.	*D. bocki*	2V	J	LV	D	Baimai, 1980
10.	*D. bocqueti*	2V	J	SR	D	Lemeunier & Ashburner (unpublished)
11.	*D. davidi*	2V	R	J	D	Tsacas, 1975
12.	*D. dominicana*	2V	J	MV	SV	Baimai,1980
13.	*D. jambulina*	2V	J	MV	D	Baimai,1980
		2V	R	J	SR	Singh & Gupta, 1979
		2V	R	LJ	SV	Present study
14.	*D. kanapiae*	2V	R	R	-	Bock & Wheeler, 1972
15.	*D. khaoyana*	2V	R	R	-	Bock & Wheeler, 1972
16.	*D. kikkawai*	2V	R	R	R	Ward, 1949
		2V	R	J	LV/R	Present study
17.	*D. lactecornis*	2V	J	R	LD	Baimai, 1980
18.	*D. leontia*	2V	J	LV	D	Baimai, 1980
19.	*D. lini*	2V	J	SJ	R	Baimai, 1980
20.	*D. mayri*	2V	R	R	D	Mather & Dobzhansky,1962
		2V	J	LJ	D	Baimai, 1980
21.	*D. montium*	2V	R	R	R	Kikkawa, 1936
22.	*D. mysorensis*	2V	R	M	D	Present study
23.	*D. nagarholensis*	2V	R	SR	D	Prakash & Reddy,1980
		2V	R	SR	D	Present study
24.	*D. nikananu*	2V	J	MV	D	Baimai, 1980
25.	*D. orosa*	2V	L	LJ	D	Baimai, 1980
26.	*D. parvula*	2V	R	R	-	Bock & Wheeler,1972
27.	*D. pennae*	2V	L	SV	LD	Baimai, 1980
28.	*D. pseudomayri*	2V	V	LJ	D	Baimai, 1980
29.	*D. punjabiensis*	2V	J	R	D	Baimai, 1980
30.	*D. quadraria*	2V	R	R	-	Bock & Wheeler,1972
31.	*D. rhopalca*	2V	R	J	D	Present study
32.	*D. rufa*	2V	R	R	D	Kikkawa & Peng,1938
33.	*D. sequyi*	2V	J	LJ	D	Baimai, 1980
34.	*D. serrata*	2V	R	R	D	Mather, 1956
		2V	J	SV	LD	Baimai, 1980
35.	*D. truncata*	2V	R	SM	LJ	Present study
36.	*D. tsacasi*	2V	R	R	D	Burla, 1954
37.	*D. vulcana*	2V	R	J	D	Bock & Wheeler,1972

Where, V = metacentrics; R = acrocentrics; J = submetacentrics; D = dots; M = medium; L = large and S = small

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Stanić, S. and D. Marinković. Faculty of Science, University of Kragujevac, and University of Belgrade, Yugoslavia. Intrinsic and acquired mating behavior of *Drosophila*.

In a set of experiments we obtained an indication that the newly eclosed *Drosophila* flies, when surrounded by older individuals, are able to verify their reproductive behavior, which later affects their own mating efficiency (see also, Stanić and Marinković, 1991).

The newly eclosed flies, F_2 or F_3 progenies of a few hundreds of wild ancestors have been split in two groups. In one group (A) the females and males (separately, ten flies in each test-tube with food medium), have been surrounded for three days by a large number of older individuals in a jar, being able to verify their behavior, i.e., their courtships, matings, and other activities. In another group (B) the test-tubes with males and females have not been surrounded by older flies. The mating success of these two groups of flies was compared in 35 replicates, by placing ten males in each test-tube with ten females, and watching them for one hour (Table 1). It turns out that females and males which have been surrounded by older individuals are reproductively more active than those which did not have such a surrounding at the onset of their development.

In another set (Table 2) of 48 experiments we compared the matings between young flies which were first allowed three days to verify the surrounding of older individuals (A1) by all sensory means, (A2) only by vibration and olfactory means, (B) without such contacts and stimuli. It comes out that vibration plus olfactory stimuli could be of crucial importance in *Drosophila melanogaster* in a verification of its biological surrounding, which may contribute to the increase of mating success of young individuals.

Table 1.

No. mated pairs	1	2	3	4	5	6	7	8	9	10	$\bar{X} \pm SE$	t-test
No. replicates (A)		2	0	0	4	4	9	5	9	2	7.2 ± 0.2	3.46 (p< .01)
(B)	2	1	3	4	4	6	6	2	4	3	5.9 ± 0.3	

Table 2.

	A1	A2	B
No. mated pairs	364	363	329/ 480/1h
Per cent of matings	75.8 ± 2.0	75.6 ± 2.1	68.5 ± 1.8

Table 3.

	A	B
Total time before mating	6 min. 2 sec.	13 min. 2 sec.
From courtship to mating	2 min. 4 sec.	5 min. 27 sec.

A shortening of the time before reproductive contacts has also been determined in young flies which have grown in the surrounding of older individuals. In 75 tests for matings, with 1 male and 3 females from (A) group surrounded and (B) non-surrounded by older flies, the mean time before the start of first mating was as shown in Table 3. This again gives rise to an increase of fitness of such young individuals, in comparison with those which have grown without their natural biological surrounding.

References: Stanić, S. 1990, M.Sc. thesis, Fac. Sci. Belgrade; Stanić S., and D. Marinković 1991, Arch. Biol. Sci. 42: 7p.

Thompson, J.N., jr. and A.D. Preston, Department of Zoology, University of Oklahoma, Norman, OK. The *Drosophila* segmentation gene *hairy* responds to a late postembryonic gradient in the wing.

One model for defining positional information proposes that cells respond to the concentration of a specific morphogen distributed in a gradient during development. Most of the recent work on morphogenetic gradients in *Drosophila melanogaster* has focused upon genes acting in early embryogenesis. One of these genes, the recessive mutant *hairy* (*h*), is an example of a "pair rule" mutation (Ingham et al., 1985) in that it causes the loss of homologous elements in alternate segments along the anteroposterior axis. It is active in distinct stripes in the early embryo, and its transcription rate is sensitive to cellular position. The *h* transcripts accumulate in the anterior region and the stripes of *h* RNA are broader ventrally than dorsally (Ingham et al., 1985; Ingham, 1988). Quantitative analysis of *h* expression on the adult wing has shown us that this gene also responds to gradients in postembryonic development. Specifically, it affects the type of sense organ differentiation that occurs along a proximal-distal gradient in the ventral surface of the developing wing. The expression of *h* provides a sensitive marker for the contour of this gradient and evidence for developmental thresholds.

Drosophila bristle patterns offer excellent experimental systems for analyzing developmental mechanisms (Held, 1991). The mutant *hairy* causes extra sense organs to be formed on the body surface. In the light microscope, they can be seen on both surfaces of the wing and appear to be of three different kinds, based upon the relative growth of the bristle-forming cell (trichogen) and associated socket cell (tormogen). The campaniform sensillae have a small dome surrounded by a socket, while the bristles have a long hair projecting from the socket. Organs of intermediate size also occur.

These three organ types are not randomly distributed on the *h* wing. Campaniform sensillae are preferentially formed on the proximal end of the L3 vein, bristles form nearer the tip, and the intermediate organs are found between them (Thompson et al., 1985). Furthermore, the point of transition from campaniform sensillae to bristles is

temperature sensitive, in that the distribution shifts proximally at 29°C and distally at 18°C.

These observations are consistent with a model in which the values along a proximal-distal morphogen gradient cause increasing amounts of growth by trichogen cells. But evidence from genetic mosaics shows that the L3 vein and associated sensillae form autonomously in the dorsal surface of a normal wing (Garcia-Bellido, 1977). There must therefore be two components to the system determining sensillae placement: a gradient-based system that specifies relative position on the wing and an autonomously-expressed response to that information.

The separate components of the system can be identified when the dorsal and ventral wing surfaces are analyzed separately. To quantify *hairy* expression precisely, we used the scanning electron microscope to measure sense organ sizes on each side of the wing. We recorded the length of the projection from the base of the socket to the top of the dome or tip of the bristle. Wings were scored from 10 *hairy* females that had been raised at 25±1°C. The left-hand wings were removed and mounted on SEM plugs to show the dorsal surface; right-hand wings were mounted to show the ventral. Plugs were sputter-coated with gold-palladium and examined in an ETEC Autoscan scanning electron microscope fitted with a KEVEX image analysis system for storing selected fields. Sense organs were measured at 1000x, 2000x, or 4000x, depending upon their size. A 1000-mesh copper TEM grid was used as a size standard. Overlapping Polaroid micrographs were taken of each wing at 200x or 250x for determining the distances between sensillae along the vein.

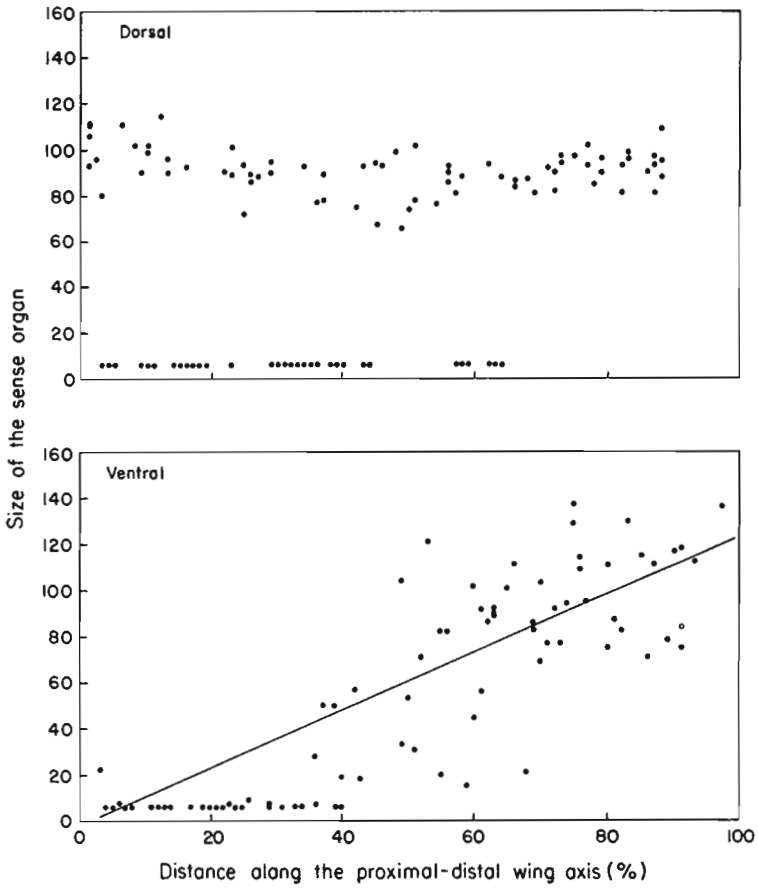

Figure 1. Size of the L3 wing vein sense organs in *hairy*. Top, dorsal surface; Bottom, ventral surface. (See text for discussion of regression.)

On the ventral surface of the wing, there is a clear transition in size that is associated with position along the L3 vein (Figure 1). Beyond about 40% of the distance from the anterior crossvein to the wing tip, the size of the sense organs grows steadily. In contrast, the dorsal surface has only campaniform sensillae and large bristles; there is no transition in sensilla size.

If we consider only the data from ventral sense organs that have some degree of "bristle" growth, a regression of size on proximal-distal position (Y = -2.27 + 1.24X) intersects the Y axis near 0. In other words, the distal to proximal transition in bristle size reaches zero (i.e., essentially the size of a campaniform sensilla dome) at the crossvein. Since the organs that form in the most proximal 35-40% of the wing are all campaniform sensillae, this suggests that a threshold exists at about a third of the distance from the crossvein to the wing tip. Presumably the amount of morphogen in this region of the gradient is not high enough to cause the sensillae to begin growing into bristles. Beyond this threshold, the amount of growth in directly proportional to the distance from the crossvein.

The mutant *hairy*, therefore, provides an example of a gene active during larval to adult metamorphosis having a transcription pattern that is dependent upon its position along the proximal-distal axis of polarity. Its expression may serve as a sensitive marker of the shape and activity of that developmental gradient.

Acknowledgments: We thank William Chissoe and Greg Strout of the S.R. Noble Electron Microscopy Laboratory for technical assistance. This work was supported by grant number 3664 from the Oklahoma Center for Advancement in Science and Technology.

References: Garcia-Bellido, A. 1977, Wilhelm Roux's Arch. 182:93-106; Held, L.I., Jr. 1991, BioEssays 13:633-640;

Ingham, P.W., K.R. Howard, and D. Ish-Horowicz 1985, Nature 318:439-445; Ingham, P.W. 1988, Nature 335:25-34; Thompson, J.N., jr., W.E. Spivey, and D.K. Duncan 1985, Experientia 41:1346-1347.

El Amrani, A., N. Cadieu, and J.C. Cadieu. Université P. Sabatier 31062, Toulouse, France, U.R.A. au C.N.R.S.n° 664. Impact of *Adh* gene alleles and their genetic background upon the choice by *Drosophila* between unequally alcoholized media as well as on the larval development.

Ethanol tolerance is characteristic of *Drosophila melanogaster*, due to ADH (alcohol dehydrogenase) enzyme (McKenzie and Parsons 1972; David and Van Herrewege 1983). This enzyme is maintained by the *Adh* gene, whose several alleles differ by their activity level. In the present work, we compared the effect of the alleles Fast (F, fast ethanol detoxification), Slow (S, slow detoxification) and null (no detoxification). These alleles were included in two different genetic backgrounds: "Colmar" and "Moulis". Four strains were available: Moulis SS, Moulis FF; Colmar FF and Colmar Adh^{null}.

We first studied the choice behavior, by adults of the four strains, between unequally alcoholized media. In a second step, larval development in these strains was investigated on the same substrates.

In order to study the choice, 300 flies from the four strains were introduced (by samples of 100 animals from the same strain) into an apparatus where they could choose between vials containing either corn flour medium without alcohol or the same medium added with: 1.5 vs 3, 6 or 9 p.100 ethanol. In this choice procedure with correction, the flies were free to move from one medium to the other until their capture 24 h later.

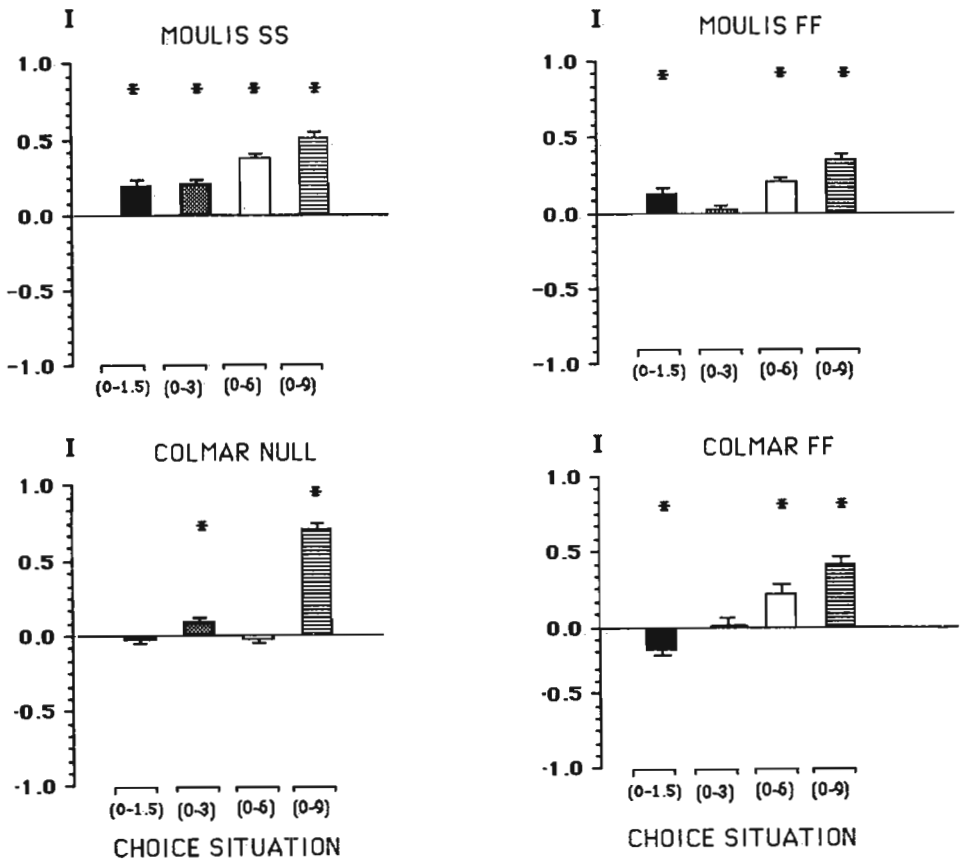

Figure 1. Active choice of four strains *Drosophila melanogaster* between the medium without alcohol and an alcoholized medium (1.5-3-6 or 9% ethanol). I: measure indicates the preference of the flies: if $0 < I < +1$, the flies prefer the medium without alcohol; if $-1 < I < 0$, the flies prefer an alcoholized medium; if $I = 0$, no preference is detectable.

Choice performances were rated by the index I as (A - B)/(A + B), where A is the number of flies caught on the alcohol-free medium and B is the number caught on the alcoholized medium.

The outcome of a variance analysis (Factors: strain, choice situation) complemented by a Students t test, reveals that (Figure 1):

- In the Moulis strains, SS flies avoid any ethanol dilution; this aversive effect is weaker in the FF genotype bearers, which seem unaffected by the 3 percent dilution.

- In the Colmar strains, FF flies are attracted by the two weaker dilutions, whereas the two stronger ones (6% and 9%) are aversive. Adh^{null} samples are strongly repelled by the highest 9 p 100 dilution; however, they do not exhibit any coherent choice behavior

between unalcoholized medium and any of the three other levels of alcoholization. Adult choice between unalcoholized and differently alcoholized media hence depends on an interaction between their *Adh* genotype and the genetic background.

The second part of this experimentation has been devoted to the impact of ethanolization on larval developmental success. Two pairs of parents from the same strain were put for 5 days into vials containing the same corn-medium, either alcohol-free or added with one of the four concentrations of ethanol used for studying the imaginal choice behavior. The number of emergents from each vial has been counted and the results submitted to a variance analysis (factors: strain, choice situation) complemented by the Students t test reveals that (Figure 2):

- Despite a high variation in the number of emergences per vial this number globally decreases when ethanolization in the medium increases.

- A significant decrease in the offspring number, when alcohol concentration increases, appears from the 3 p 100 dilution in both Moulis strains (FF and SS) as well as in the Colmar strain Adh^{null}. Whereas this decrease may only be stated for 6 and 9% in the Colmar FF strain.

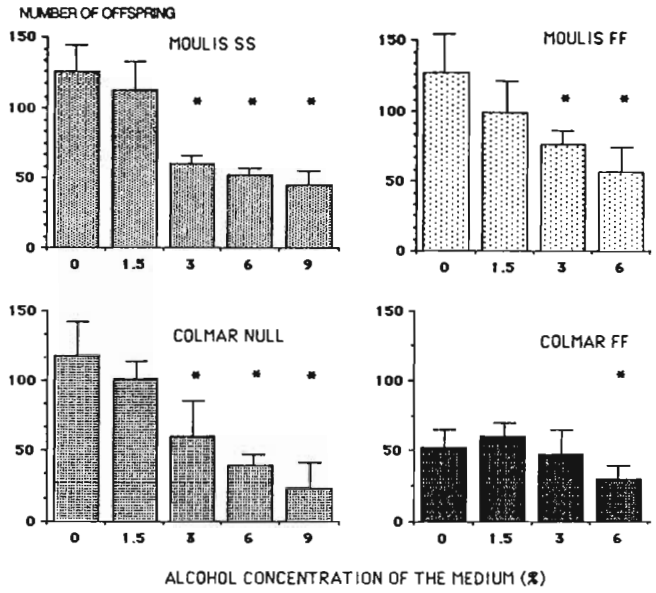

Figure 2. Total number of offspring obtained in four strains of *Drosophila melanogaster* when the alcohol concentration in the rearing medium is systematically varied. The star (*) indicates a significant difference.

This high ethanol tolerance during preimaginal development, in the Colmar FF strain, agrees with the strong preference found in adults for the weakly (1.5 p. 100) alcoholized medium, which discriminates this strain from the three other ones. The Colmar Adh^{null} strain, however, shows no clear preference, in its choice behavior, for the weakly alcoholized media, and is not disturbed in its development by such concentrations.

Such a correlation between adult active choice and preimaginal developmental success is by no means so clear in the two other (Moulis) strains: these flies (FF as well as SS) are able to tolerate the weakest ethanol concentration (1.5 p 100) during their preimaginal development; they nevertheless avoid it, as adults, in the active choice situation.

Briefly, developmental success is not always predictive of the active choice performance in adults. Likewise, the *Adh* genotype does not always fit either with the active choice of the flies or with their developmental success. On the other hand, the differences between the contrasted genotypes SS and FF are only revealed at low ethanol concentrations. Moreover the impacts of the alleles Fast and Slow differ according to their genetic background.

References: David, J.R. and J. Van Herrewege 1983, Comp. Biochem. Physiol. 74A:283-288; McKenzie, J.A. and P.A. Parsons 1972, Ecologia, 10:373-388.

Mohler, J. and G. Vasilakis. Barnard College, New York, NY, USA. A block in early head involution in *daeh* mutant embryos.

We have analyzed the developmental pathology of *daeh* mutant embryos, which have a defect in head involution. The *daeh* mutation appears to be contained on a variant *TM3* balancer chromosome. Most *TM3* chromosomes, in particular those used by Jurgens et al. (1984) in their screen for embryonic mutants, have a homozygous cold-sensitive embryonic semi-lethality with nearly normal cuticular morphology. We obtained this variant *TM3* chromosome, showing the *daeh* head involution defect, from B. Levis in a strain carrying a P(*w,ry*)A insertion in the *unkempt* locus in 94E (Levis et al., 1985) and has been maintained in a number of our induced-transposon loss strains.

Embryos homozygous for *daeh* develop cuticle with a range of defects in the structure of the internal head skeleton. Individuals with a weaker defect possess the posterior portion of the cephalopharyngeal skeleton (dorsal and ventral arms and posterior pharyngeal wall, which have been manually inverted out of the thoracic cuticle in Figure 1a), but more anterior structures are malformed and are coalesced on the anterior external surface of the larva. More extreme individuals possess an open head phenotype at the anterior end, where only portions of the cephalopharyngeal skeleton have cuticularized, and portions of the dorsal thoracic epidermis is missing (Figure 1b).

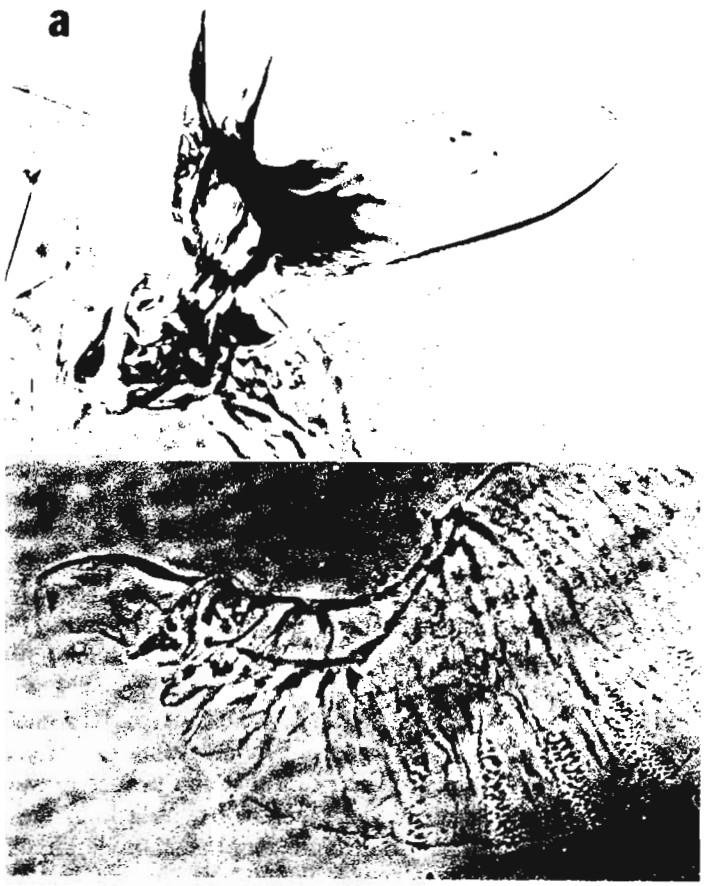

Figure 1. Cuticular phenotype of homozygous *TM3, daeh* individuals. a) weak defect, the internal head skeleton has been expelled mechanically from the thoracic cuticle. b) strong defect.

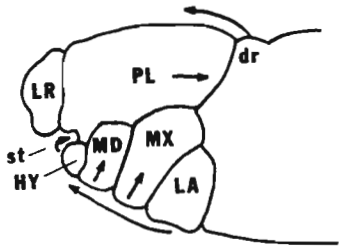

Figure 2. A diagram of the head segments of a stage 12 embryo showing the initial movements of each of the various primordia during head involution. (segments: HY = hypopharyngeal, LA = labial, LR = labral (clypeolabrum), MD = mandibular, MX = maxillary, PL = procephalic lobe; landmarks: dr = dorsal ridge, st = stomodeum).

These phenotypes of *daeh* suggest a block in the process of head involution; embryogenesis of *daeh* embryos was followed to determine at which stages of head involution development became abnormal. The first movements of head involution are shown in Figure 2. The ventral movements begin during stage 13, when the hypopharyngeal primordium involutes into the stomodeum and the labial segment primordium migrates anteriorly to displace the mandibular and maxillary lobes, which move laterally away from the ventral mid-line. During stages 14 and 15, the labial and mandibular primordia migrate over the ventral and lateral edges of the stomodeum ultimately lining the ventral and lateral portions of the pharynx. The dorsal movements begin at the beginning of stage 14, when the dorsal fold, formed from the fusion of the dorsal ridges from either side, extends anteriorly over the procephalic lobe, which retracts underneath.

No morphological abnormalities were observed in *TM3, daeh* embryos until stage 13 at the onset of head involution. Figure 3a shows the position of the hypopharyngeal and mandibular lobes in stage 13, which have been labeled by whole mount *in situ* hybridization (Tautz and Pfiefle, 1989) of CNC DNA to segment specific RNA (Mohler et al., 1991). The hypopharyngeal lobe has failed to invaginate over the stomodeum and the mandibular lobe is more lateral and anterior than normal at this stage (Figure 3b shows the normal relative positions of the hypopharyngeal and mandibular lobes in a slightly older stage 13 embryo). The hypopharyngeal lobe subsequently migrates anteriorly along side the mandibular lobe, so that at stage 15 the mandibular and hypopharyngeal primordia form a continuous ring around the ventral and lateral surfaces of the clypeolabrum, with a variable degree of involution of hypopharyngeal and mandibular tissues into the pharynx (not shown). On the dorsal side at stage 14, the dorsal fold often, but not invariantly, fails to form and to extend over the suprapharyngeal ganglion. Instead the suprapharyngeal ganglion retracts over, rather than under, the dorsal ridge, such that the larval brain after stage 15 lies outside the dorsal thoracic epidermis (Figure 3c). Thus, *TM3, daeh* embryos appear to be defective in both the ventral and dorsal processes of head involution from the time of the first ventral movements.

Linkage of the *daeh* mutation to the *TM3* chromosome was determined by a-HRP staining of the embryos. In most *Drosophila* strains, a-HRP labeling specifically stains neurons and a few other tissues, including the garland gland (Jan and Jan, 1982). In embryos homozygous for *TM3*, however, a-HRP fails to react with neurons, but continues to stain

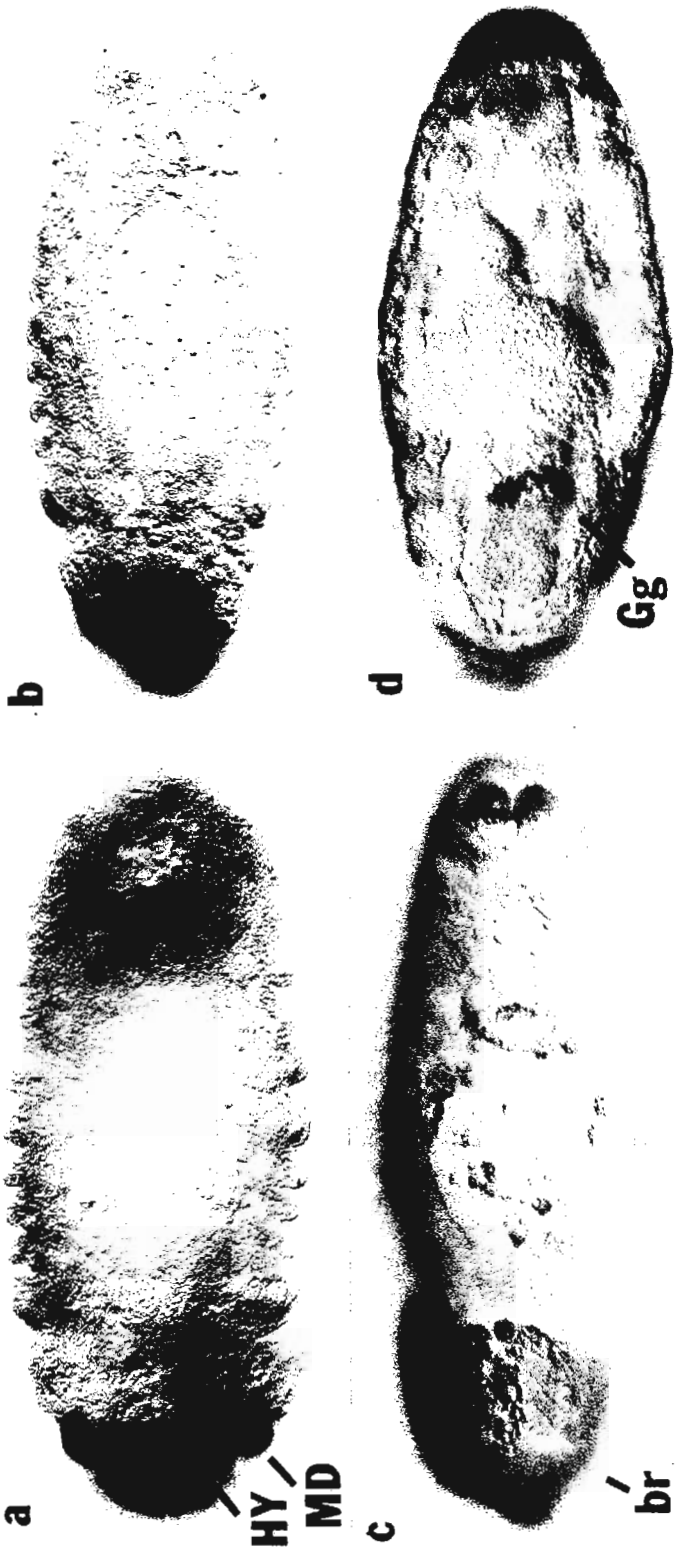

Figure 3. Embryonic phenotype of *TM3, daeh*. a,b) Effect of *TM3, daeh* on hypopharyngeal involution (stage 13); the hypopharyngeal primordia and mandibular segment have been stained by hybridization to CNC RNA. a) *TM3, daeh*, b) wild-type, c) Dorsal head involution defect at stage 15, d) Expression of the a-HRP determinant in the nervous system (stage 17) in an individual with an extraepidermal brain. (MD = mandibular lobe, HY = hypopharyngeal lobe, br = brain, Gg = Garland gland).

the garland gland (Snow et al., 1988). Analysis of homozygous *daeh* embryos, identified by the extraepidermal location of the suprapharyngeal ganglion, only the garland gland was stained by a-HRP in the manner specific to *TM3* homozygotes (Figure 3d). Embryologically normal sibling embryos usually demonstrated staining of both the nervous system as well as the garland gland.

The head involution defect of *TM3, daeh* embryos suggests that the proper movement of hypopharyngeal lobe, which is the first tissue to involute over the stomodeum, plays a pivotal role in the initiation of head involution. In the *TM3, daeh* embryos the hypopharyngeal lobe fails to involute and the retention of this primordium at the ventral edge of stomodeum appears to block the subsequent involution of the labial segment, disrupting the entire process of head involution. It will be intriguing to discover how this relates to other events in the involution of the ventral segments and how the ventral processes are coupled with the dorsal processes of head involution.

References: Jan, L. Y. and Y.N. Jan 1982, Proc. Natl. Acad. Sci. USA 79:2700-2704; Jürgens, G., E. Weischaus, C. Nüsslein-Volhard, and H. Kluding 1984, Roux Arch. Dev. Biol. 193:283-295; Levis, R., T. Hazelrigg, and G.M. Rubin 1985, Science 229:558-561; Mohler, J., K. Vani, S. Leung, and A. Epstein 1991, Mech. Dev. 34:3-10; Snow, P.M., N.H. Patel, A.L. Harrelson, and C.S. Goodman 1987, J. Neurosci. 7:697-715; Tautz, D. and C. Pfiefle 1989, Chromosoma 98:81-85.

Dytham, C., B. Shorrocks and R. Cooper. University of Leeds, UK. Coexistence in large caged populations of *Drosophila* using a microcosm of a fruit market.

Seven species of *Drosophila* have been recorded from the wholesale fruit market in Leeds, UK (*D. busckii, D. funebris, D. hydei, D. immigrans, D. melanogaster, D. simulans, D. subobscura*), where they exploit damaged or rotting fruit and vegetables (Atkinson and Shorrocks 1977; Rosewell 1986). Such resources are both patchy and ephemeral (Shorrocks et al. 1979; Hanski 1981), as are many other sites exploited by *Drosophila* such as fungi, flowers and sap flows (e.g., Kimura et al. 1977). We have developed large cages in which the characteristics of the fruit market environment can be reproduced in a controlled way.

Grapes were chosen as a natural substrate for this system as they are small, but not prone to desiccation, are available all year round and are known to be exploited by several species of *Drosophila* (Capy et al. 1987).

Each microcosm consisted of a metal (Dexion) frame within which was suspended a nylon mesh bag 188 x 44 x 75 cm. Four sleeves evenly spaced along one side of the bag, 22 cm in diameter, allowed easy access to the cage without losing flies. The grapes were placed on trays containing two plastic scouring pads placed on top of each other, the uppermost having had 18 holes punched in it which allowed the grapes to be placed upright and evenly spaced (Cooper 1990). The scouring pads prevented movement of larvae between grapes. The trays were arranged in a grid of 18 trays by 4.

Grapes were prepared by cutting off a small portion of flesh at the stipe in order to 'damage' the fruit. Each grape was then washed and dipped in a weak solution of bakers yeast. The grapes were introduced to the cage on a weekly basis in an eight week cycle with nine trays of grapes being added each week (a total of 162 grapes).

Preliminary experiments using the system described above were carried out in a general purpose laboratory where light, temperature and humidity were not controlled. Samples of 100 individuals (even sex ratio) of six of the seven species mentioned above (not *D. busckii*) were released into a single cage. Numbers were estimated every four weeks using mark-release-recapture techniques. All populations except *D. immigrans* had increased after 8 weeks, but by 20 weeks the system had reduced to just *D. melanogaster, D. simulans* and *D. funebris* (in very small numbers). Populations of 4000 to 7000 each of *D. melanogaster* and *D. simulans* were maintained for 40 weeks.

Table 1. Estimated maximum and mean population sizes achieved in grape microcosms.

Species	Max Est.Pop.	Mean Est.Pop.
D. funebris	<100[a]	—
D. hydei	2200	2200
	1207[b]	446[b]
D. immigrans	3597[b]	1801[b]
D. melanogaster	52711	13891
D. simulans	56090	12925
	30526[c]	—
D. subobscura	14053	6635
D. virilis	1284[b]	574[b]

[a] = in cage containing *D. funebris, D. hydei, D. immigrans* and *D. virilis*
[b] = in cage containing *D. hydei, D. immigrans* and *D. virilis*
[c] = in cage containing *D. simulans* and a genetically modified strain of *D. melanogaster* with neomycin resistance and LacZ genes inserted.

Later experiments were carried out in a set of 12 microcosms housed in a controlled environment room under constant light at 20°C and 70% relative humidity. Illumination was provided by 120 cm, 36W fluorescent light tubes suspended 30 cm above each cage. Single species and mixed species microcosms were set up using all the market species as well as *D. virilis* (a species which is common in British breweries). Single species cages of *D. busckii, D. funebris* and *D. immigrans* proved unsuccessful, with little or no increase in population size. The maximum and mean population sizes for all species are shown in Table 1.

It is clear that very large mixed populations of *D. simulans* and *D. melanogaster* can be maintained for long periods in these fruit microcosms as can three species systems of *D. hydei, D. immigrans* and *D. virilis*. Such microcosms are presently being used to monitor the fate of genetically modified strains of *D. melanogaster* in competition with other species of *Drosophila*.

Acknowledgment: This work was supported by the NERC and DoE.

References: Atkinson and Shorrocks 1977, Oecologia 29:223-232; Capy et al. 1987, Acta Oecologica 8:435-440; Cooper 1990, PhD Thesis, University of Leeds; Hanski 1981, Oikos 37:306-312; Kimura et al. 1977, Knotyu, Tokyo 45:571-582; Rosewell 1986, PhD Thesis, University of Leeds; Shorrocks et al. 1979, J. Anim. Ecol. 48:899-908.

Dytham, C. and S. McNamee. University of Leeds, UK. Differentiating *Drosophila melanogaster* and *D. simulans*.

Although the external genitalia of the males of these sibling species allow for easy discrimination, the females are often left unassigned to species. Several previous attempts have been made to provide simple

rules for distinguishing females. Basden (1954) stated that if cheek (measured from the bottom of the eye to the bottom of the head) was wider than the widest point of the first tibia then the fly was *D. melanogaster* and if narrower *D. simulans*. Frydenberg (1956) stated that in *D. simulans* the width of the cheek is less than one sixth the greatest diameter of the eye and greater than one sixth in *D. melanogaster*.

Samples of flies were anesthetized with CO_2, frozen and stored at -70°C, then pinned and measured within three hours of defrosting. A dissecting microscope at x50, equipped with a digitiser tablet, was used to take measurements. In total, 11 linear variables; thorax length, tibia width and 9 head variables (including eye height, eye width, cheek width, "forehead" width) were recorded. None of the variables constituted a subsection of any other. In addition, 10 angles were calculated using the digitised points. These were four internal eye angles (front, back, top and bottom), four internal head angles and two angles describing the "forehead".

Table 1. Mean values of some linear variables by species and sex. All measurements in mm.

	D. melanogaster		*D. simulans*	
	Males	Females	Males	Females
Thorax length	0.915	1.064	0.903	1.044
Tibia width	0.083	0.083	0.079	0.081
Eye height	0.448	0.491	0.462	0.507
Eye width	0.358	0.387	0.365	0.410
Check width	0.076	0.087	0.045	0.050
Forehead width	0.055	0.064	0.034	0.033
Eye top margin	0.032	0.038	0.022	0.025

Flies used were taken from stock cultures which had both been started using collections made in a wholesale fruit market in Leeds in summer 1991. Forty individuals of each species were measured (20 females and 20 males). Data were analyzed using the SAS statistical package (SAS Institute Inc. 1982). None of the data were scaled to adjust for size before analysis.

Using thorax length as a simple size measure, females were considerably larger than males and *D. melanogaster* was slightly, but not significantly, larger than *D. simulans* (Table 1). *D. simulans* of both sexes have larger eyes (eye height and eye width) and smaller heads (head height only), and therefore considerably smaller eye margins. Frydenberg (1956) used a ratio of one sixth (cheek width to eye height) to identify species. All individuals exceeding this ratio were *D. melanogaster*, but 37.5% were misclassified as *D. simulans*. Better discrimination can be achieved at this site using a ratio of one eighth, where all females and 90% of males are correctly assigned to species. Using Basden's (1954) method of discrimination proved totally ineffective as although all *D. simulans* are correctly identified, 62.5% of *D. melanogaster* were misclassified.

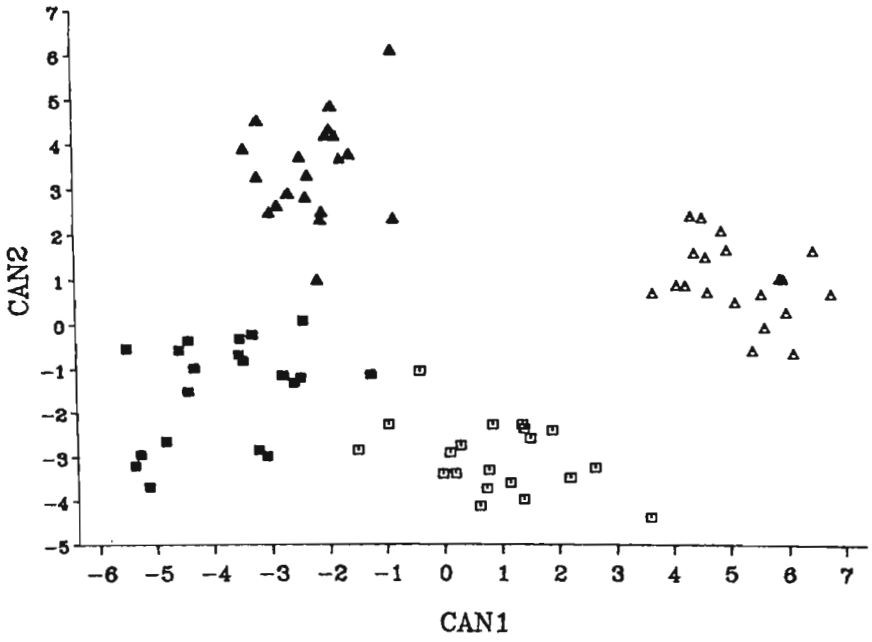

Figure 1. Two dimensional plot of first two canonical variates (CAN1 and CAN2). Filled shapes *D. melanogaster*, open *D. simulans*. Triangles females, squares males.

Canonical variate analysis (CVA) was used to determine if a discriminant function could be constructed to identify unknown individuals. CVA is a multivariate technique which constructs a series of orthogonal axes by weighting independent variables such that the differences between groups are maximised. In this case species were discriminated on axis 1 (CAN1) and sex on axis 2. Using this technique each of the four groups (species and sex) were highly significantly different from all others ($P < 0.0001$) and there was no overlap between groups (Figure 1). Unidentified flies from this locality could be very accurately assigned to species (and sex) using this technique.

References: Basden, E.B. 1954, Trans. Roy. Soc. Edin. 62:602-654; Frydenberg, O. 1956, Entomologiske Meddelelser 27:249-294; SAS Institutes 1982, SAS Cary, NC.

Del Pino, F.C.[1] **and H.A. Martinez**[2]. [1] Dpto. Agro-ind. Fac. RR.NN.U del Bio-Bio. Campus, Chillán, Chile, and [2]Dpto. Biologia Celular Y Genética, Fac. Medicina, U. de Chile, Santiago. Double row of teeth in mandibular hooks of *D. melanogaster* larvae.

To consider mandibular traits of *D. melanogaster* larvae as phenotypic features to explain taxonomic, genetic and behavioral phenomena, it is necessary to know intimately their structure. In the present communication, observations made on third instar *D. melanogaster* larvae, both at the level of the optic (O.M.) and the scanning electron microscope (S.E.M.), are reported, using standard methods. The mandibular hooks in S.E.M. showed a double row of teeth, interdental pieces and a palate.

Figure 1. Mandibular structure of *D. melanogaster*. (arrow points to mandibular hooks)(O.M., 330x).

Figure 2. Mandibular hooks (S.E.M., 1800x).

Figure 3. T, teeth; IP, interdental pieces; and P, palate. (S.E.M., 4500x).

Singh, B.K. and N.S. Negi. Department of Zoology, Kumaun University, Nainital, India. Further report on the Drosophilid fauna of Uttarakhand region.

About 250 Drosophilid species have been reported so far from different regions of Indian subcontinent, which is very meager in comparison to the world fauna (Parshad and Paika 1964; Gupta 1969, 1970, 1971, 1972, 1973, 1974 a,b; Singh and Gupta 1974, 1977 a,b,c, 1979; Singh and Bhatt 1988; Singh and Negi 1989).

In continuation with our study of the Drosophilid fauna of Uttarakhand region, collections were made from different geographical regions. The Uttarakhand region includes Kumaun and Garhwal regions which are the hilly areas of the state Uttar Pradesh. The Kumaun region includes three border districts viz. Nainital, Almora and Pithoragarh while as Garhwal region includes five border districts viz. Dehra Dun, Chamoli, Tehri Garhwal, Pauri Garhwal and Uttarkashi of the state Uttar Pradesh.

A total of about 14087 flies were collected and identified with the help of their head, thorax and abdomen. The Table 1 shows the Genus, Subgenus, Number and Collection Locality of these flies. Besides the known species some species viz. *Drosophila analspinii* (*Drosophila*), *Drosophila bishtii* (*Drosophila*), *Drosophila paunii* (*Drosophila*), *Drosophila painii* (*Drosophila*), *Amiota bandes* and *Paraleucophenga neojavanaii* were described as new species and *Gitona distigma* was recorded for the first time from India.

Acknowledgments: This work was supported by the Ministry of Science and Technology, Department of Science and Technology, Govt. of India to Dr. B.K. Singh.

References: Gupta, J.P. 1969, Proc. Zool. Soc. (Calcutta) 22:53-61; ----, 1970, Proc. Ind. Nat. Sci. Acad. (B) 36:62-70; ---- 1971, Amer. Midl. Natur. 86(2):493-496; ---- 1972, Orient. Insects 6(4):491-494; ---- 1973, DIS 50:112; ---- 1974a, Indian Biologist V (3):7-30; ---- 1974b, J. Ent. (B) 43(2):209-215; Parshad, R. and I.J. Paika 1964, Res. Bull. Punjab Univ. 15:225-252; Singh, B.K. and J.P. Gupta 1974, Ind. J. Zoot. 15(i):23-26; ---- 1977a, Orient. Insects 11(2):237-241; ---- 1977b, Ent. Month. Mag. Oxford 113:71-78; ---- 1977c, Proc. Zool. Soc. 30:31-38; ---- 1979, Entomon 4(2):167-172; Singh, B.K. and M. Bhatt 1988, Oriental Insects (22):147-161; Singh, B.K. and N.S. Negi 1989, Proc. Zool. Soc. (Calcutta) 40:19-26.

Table-1: Showing genus, subgenus, species, number and collection locality

Genus,Subgenus and species	No of flies	Locality
Genus-*Drosophila*		
Subgenus-*Sophophora*		
1. *D. bilasciata*	2,225	Bhowali, Nainital, Almora
2. *D. jambulina*	1,251	Nainital, Pithoragarh
3. *D. kikkawai*	1,521	Nainital, Pithoragarh
4. *D. malerkotliana*	409	Almora, Nainital
5. *D. melanogaster*	956	Srinagar, Nainital, Almora
6. *D. nepalensis*	550	Pithoragarh, Nainital, Tehri
7. *D. suzukii indicus*	159	Pauna, Garhwal
8. *D takahashii*	650	Nainital, Almora
9. *D. vulcana*	125	Tanakpur, Nainital
Subgenus-*Drosophila*		
10. *D. immigrans*	950	Nainital, Pithoragarh
11. *D. lacertosa*	458	Nainital, Maikoti
12. *D. nainitalensis*	250	Nainital, Almora
13. *D. analspinii* New species	20	Sleepy hollow, Nainital
14. *D. paunii* New species	15	Pauna, Garhwal
15. *D. painii* New species	10	Paines, Nainital
16. *D. bishtii* New species	25	Nainital
17. *D. repleta*	384	Garhewal, Nainital
18. *D. sulfurigaster*	525	Nainital, Pithoragarh
19. *D. nasuta*	659	Nainital, Tehri
Subgenus-*Dorsilopha*		
20. *D. busckii*	319	Pauri Garhwal, Nainital
Subgenus-*Scaptodrosophila*		
21. *D. coracina*	436	Nainital, Almora
22. *D. chandraprabhiana*	225	Pithoragarh, Srinagar
Genus-*Leucophenga*		
23. *L. bellula*	95	Nainital, Almora
24. *L. quttiventris*	85	Neinital, Pithoragarh
25. *L. neolecteusa*	54	Nainital, Almora
26. *L. interrupta*	76	Pauri Garhwal
Genus-*Paraleucophenga*		
27. *P. neojavanaii* New species	5	Nainital
Genus-*Lissocephala*		
28. *L. parasiatica*	65	Nainital, Pithoragarh
Genus-*Scaptomyza*		
29. *S. himalayana*	36	Almora, Pithoragarh
Genus-*Zaprionus*		
30. *Z. indianus*	1565	Nainital, Almora
Genus-*Gitona*		
31. *G. distigma* New record	5	Maikti, Chamoli
Genus- *Amiota*		
32. *A. bandes* New species	7	Nainital
Total =	14,087	

Paik, Y.K., C.G. Lee, B.H. Cha and H.S. Yim. Hanyang University School of Medicine, Seoul, Korea.
Prevalence of incomplete P elements in natural Q and M strains of *D. melanogaster* in Korea.

Our earlier studies of *in situ* and Southern hybridizations (DIS-70) revealed that BamHI-fragment-containing P elements were well conserved in all or absolute majority of Q and M strains of Korean *D. melanogaster*. Nevertheless, no P factors active for potential GD sterility were found in the Korean strains which were so far analyzed. In order to see if their P element copies have internal deletions, (O'Hare and Rubin, 1983) these strains were further analyzed at generation 20 with two biotin-labeled probes (4.7kb BamHI fragment and 0.7kb PstI fragment of plasmid pπ25.1).

As seen in Table 1, all or almost all of the 185 isofemale lines (M+Q types) were found to contain multiple copies of incomplete P elements in which the central regions are deleted, together with only a few copies of complete elements retaining the PstI-fragment in the central portion. In fact, copy number of the P elements detected with BamHI-fragment probes on their polytene chromosomes ranged from 20 to 50, and the total number of hybridization sites scored with the PstI-fragment, from 1 to 9. Our result is somehow comparable with the previous findings reported from several geographic populations in Asia and Europe (Sakoyama et al., 1985; Black et al., 1987; Anxolabehere et al. 1985; Gamo et al,,1990).

To confirm the results, we surveyed the P elements by the Southern blot analysis of genomic DNAs prepared from 50 selected

Table 1. Copy numbers per chromosome arm of the members of the P element family detected in 185 isofemale lines from a natural population of *D. melanogaster* in Taenung, surburb of Seoul

Type of P element	Chromosome arm					
	X	2L	2R	3L	3R	Total
Complete	313 (1.69)	83 (0.45)	144 (0.78)	80 (0.43)	113 (0.61)	733 (3.69)
Incomplete	1537 (8.31)	876 (4.74)	1266 (6.79)	1047 (5.66)	1462 (7.85)	6168 (33.34)
Total	1850 (10.00)	969 (5.18)	1400 (7.57)	1127 (6.09)	1565 (8.46)	6901 (37.30)

The copy number of complete P element was estimated by counting the number of bands which were detectable with both the BamHI and PstI probes at the same site of the salivary chromosome Incomplete P elements are those which were hybridized only with the BamH1. Figures in parenthesis denote average numbers of the element per strain

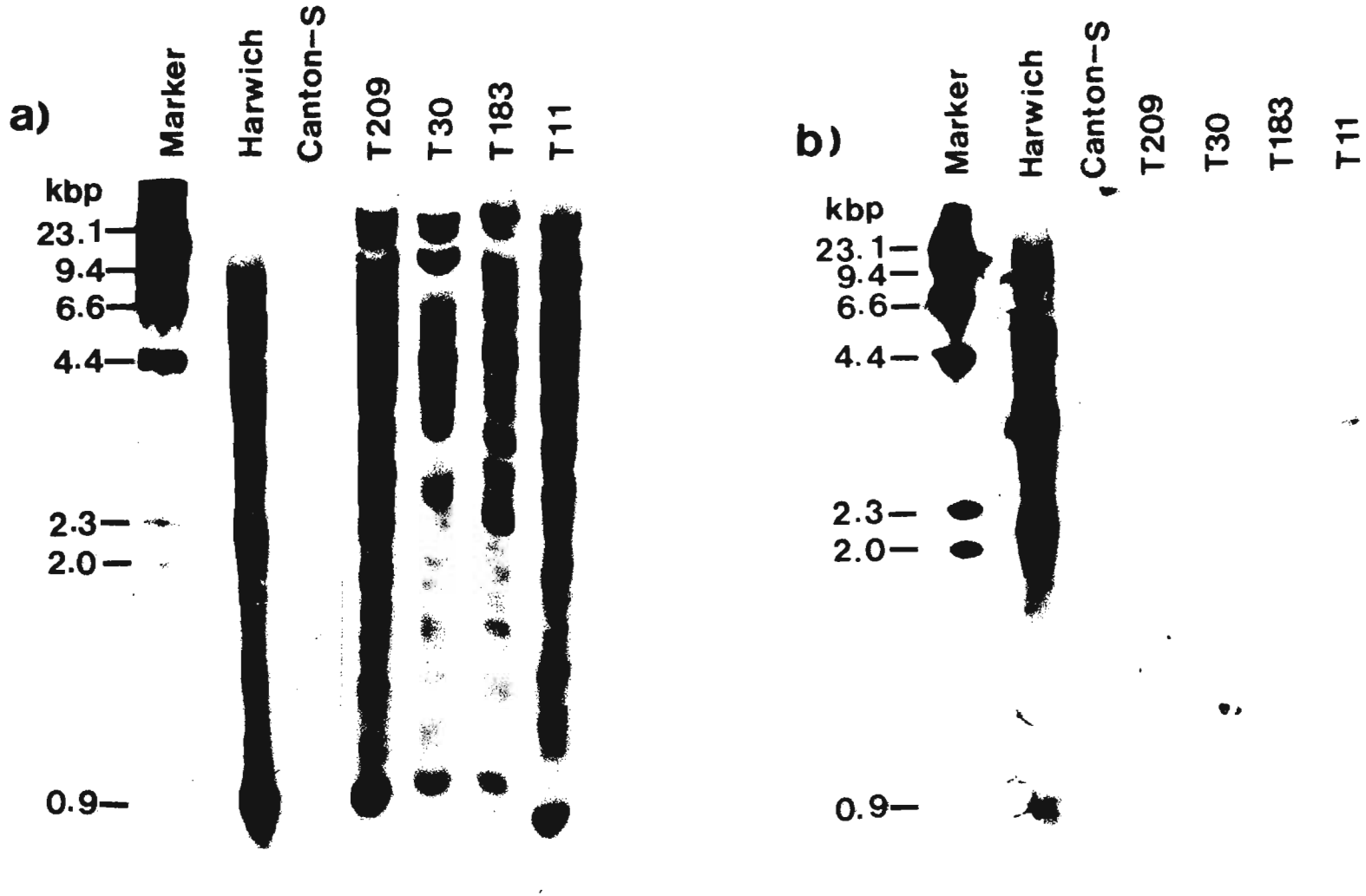

Figure 1. Photographs of HindIII digests of three Q and one M' strains in Tables 2 and 3. Approximately 10 ug of total genomic DNA was used per lane and the biotin-labeled 4.7 kb BamHI fragment (a) and the 0.7 kb PstI fragment (b) were used as the probes. Lane T11, M strain in Table 3; lanes T30, T183 and T209, Q strains in Table 2.

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Table 2. Numbers per genome of P element family detected by Southern blot analysis in 15 Q strains derived from a wild population of *D. melanogaster* in Taenung

Line	Number of hybridization sites to BamH1 fragment	Pst1 fragment	% GD sterility A cross	A* cross
5	21(42)	4(5)	0	0
10	21(45)	6(5)	0	0
21	21(46)	6(n)	0	0
30	22(43)	2(5)	0	0
74	25(36)	8(7)	0	0
76	21(48)	3(n)	0	0
86	21(38)	6(5)	0	0
113	21(49)	4(n)	0	0
141	20(47)	2(n)	0	0
151	24(48)	5(8)	0	0
169	18(51)	3(5)	0	0
183	22(45)	4(7)	0	0
195	26(39)	4(5)	0	0
209	23(40)	9(4)	0	0
232	22(48)	6(6)	0	0
Mean	2l.87(44.67)	4.73(5.64)		
S.D.	2.00(4.48)	2.02(1.21)		

In the estimation of copy numbers of P element on Southern filters, the strong and very strong bands are counted as composite bands made of two and three normal bands respectively. The total number of in situ hybridization bands scored on polytene chromosomes is given in parenthesis. The crosses A and A*(Kidwell 1979) denote an estimate of P factor activity and the cytotype, respectively.(n) not tested with the probe.

Table 3. Numbers per genome of P element family detected by Southern blot analysis in 35 M strains derived from a wild population of *B* in Taenung

Line	Number of hybridization sites to BamH1 fragment	Pst 1 fragment	% GD sterility A cross	A*cross
1	20(46)	4(6)	0	83.33
8	17(43)	5(6)	0	93.57
11	23(35)	3(n)	0	97.92
15	20(43)	1(n)	0	31.25
23	20(45)	4(n)	0	70.83
25	19(42)	4n)	0	97.92
67	21(32)	2(n)	0	10.42
83	24(47)	3(n)	0	100
84	20(64)	3(5)	0	100
91	19(45)	3(4)	0	100
100	21(43)	2(5)	0	66.67
116	23(46)	3(n)	0	58.33
125	24(48)	2(5)	0	72.92
130	23(40)	2(5)	0	52.08
136	22(51)	2(n)	0	93.75
139	24(44)	5(n)	0	22.92
150	22(37)	4(n)	0	31.25
158	25(53)	1(n)	0	31.25
160	19(37)	3(6)	0	10.42
161	20(37)	3(n)	0	52.08
163	19(47)	1(n)	0	100
164	26(47)	3(n)	0	100
171	26(44)	2(n)	0	66.67
176	19(41)	3(5)	0	10.42
181	24(54)	1(2)	0	14.58
187	24(48)	5(n)	0	16.67
208	23(45)	3(n)	0	95.83
212	17(45)	5(n)	0	41.67
216	19(48)	1(3)	0	100
217	17(40)	3(n)	0	22.92
221	20(58)	2(3)	0	100
223	21(51)	2(5)	0	100
227	18(39)	3(3)	0	62.5
229	18(50)	3(n)	0	37.5
233	24(41)	2(5)	0	100
Mean	21.17(44.71)	2.71(4.47)		
S.D.	2.62(5.77)	1.20(1.36)		

See the footnote of Table 2 for a detailed explanation.

lines. Tables 2 and 3 summarize the results obtained. For comparison, the total number per polytene chromosome of *in situ* hybridization sites with the two probes is given in these tables, with the results of GD sterility tests also included in the last column. As shown in the tables, all the lines have many DNA sequences hybridized with the BamHI fragment (Figure 1a), whereas they show only a few weak bands having homology to the PstI fragment (Figure 1b).

Therefore, combining the data obtained by the two probes, we conclude that almost all P elements from Q and M (by definition M') strains of current populations of *D. melanogaster* in Korea are incomplete elements in which the central regions are lost. DNA sequence analysis of these incomplete P elements is in progress.

References: Sakoyama, Y., T. Todo, S. Ishiwa-Chigusa, T. Honjo and S. Konda, 1985, Proc. Natl. Acad. Sci. USA 82:6236-6239; O'Hare, K. and G.M. Rubin 1983, Cell 34:25-35; Black, D.M., M.S. Jackson, M.G. Kidwell and G.A. Dover 1987, EMBO J. 6:4125-4135; Anxolabehere, D., D. Nouard, G. Periquet and P. Tchen, 1985, Proc. Natl. Acad. Sci. USA 82:5418-5422; Gamo, S., M. Sakajo, K. Ikeda, Y.H. Inoue, Y. Sakoyama and E. Nakashima-Tanaka, 1990, Jpn. J. Genet. 65:277-285; Kidwell, M.G. 1979, Genet. Res. Camb. 33:205-217.

Pandey, M. and B.N. Singh. Zoology Department, Banaras Hindu University, Varanasi, India. Pupation height of F_1 hybrids obtained from reciprocal crosses between *Drosophila bipectinata* and *Drosophila malerkotliana*.

A number of studies on pupation site preference in various species of *Drosophila* have been carried out by numerous investigators (Sokal et al., 1960; Markow, 1979; Sameoto and Miller, 1968; Bauer and Sokolowski, 1985; Casares and Carracedo, 1987; Singh and Pandey, 1991). From the results of these studies, it is evident that there are intra- and interspecific variations in

Table 1. Mean pupation height (mm) of *D. bipectinata* and *D. malerkotliana*.

D. bipectinata U.L. strain			*D. malorkotliana* Nagaland strain		
Replicate number	Pupation height ±SE (mm)	N	Replicate number	Pupation height ±SE (mm)	N
1	0.7 ± 0.09	10	1	0.5 ± 0.07	10
2	0.8 ± 0.07	10	2	1.3 ± 0.09	10
3	0.8 ± 0.08	10	3	0.8 ± 0.07	10
4	0.4 ± 0.06	9	4	0.9 ± 0.07	10
5	0.8 ± 0.09	10	5	1.6 ± 0.08	10
6	1.0 ± 0.34	10	6	1.3 ± 0.09	10
7	0.4 ± 0.06	10	7	1.3 ± 0.08	10
8	1.0 ± 0.08	10	8	1.2 ± 0.09	10
9	0.7 ± 0.09	10	9	1.0 ± 0.05	10
10	0.9 ± 0.07	9	10	1.2 ± 0.06	10
Mean	0.7 ± 0.07	98	Mean	1.1 ± 0.09	100
Female 0.66	Male 0.77		Female 1.3	male 1.2	

N = total number of pupae

Table 2. Mean pupation height (mm) of F_1 hybrids obtained from reciprocal crosses between *D. bipectinata* and *D. malerkotliana*.

D. bipectinata ♀ x *D. malerkotliana* ♂			*D. malerkotliana* ♀ x *D. bipectinata* ♂		
Replication number	Pupation height ± SE (mm)	N	Replication number	Pupation height ± SE (mm)	N
1	2.8 ± 0.22	10	1	1.6 ± 0.20	10
2	2.1 ± 0.27	10	2	0.2 ± 0.06	10
3	5.0 ± 0.29	10	3	1.8 ± 0.31	10
4	1.0 ± 0.13	10	4	0.2 ±0.01	10
5	0.2 ± 0.01	9	5	0.5 ± 0.09	8
6	2.0 ± 0.22	9	6	1.6 ± 0.21	9
7	0.5 ± 0.09	10	7	2.4 ± 0.21	9
8	1.9 ± 0.23	9	8	0.2 ± 0.01	10
9	0.8 ± 0.12	9	9	0.7 ± 0.05	10
10	0.9 ± 0.12	9	10	0.5 ± 0.07	9
Mean	1.7 ± 0.04	95	Mean	1.0 ± 0.03	95
Female 2.1	Male 1.5		Female 1.0	Male1.0	
Average of reciprocal crosses		1.34 ± 0.17			

N = total number of pupae

pupation height (Markow, 1979; Singh and Pandey, 1991). Furthermore, pupation height is influenced by various biotic and abiotic factors such as sex, larval development time, larval density, diet water content, temperature and light (Sokal et al., 1960; Mensua, 1967; Markow, 1979; Bauer, 1984; Casares and Carracedo, 1987; Pandey and Singh, 1991). Pupation site preference is also affected by genetic factors (Sokal, 1966; De Souza et al., 1968; Markow, 1979; Bauer and Sokolowski, 1985; Casares and Carracedo, 1986). Bauer and Sokolowski (1985) studied genetic component of variation in two prepupal behaviours, path length and pupation height for strains of *D. melanogaster* derived from natural populations. The strains isogenic for second and third pairs of chromosomes were constructed and crossed to produce F_1 progeny. Pupation height of F_1 individuals was intermediate between the original strains and was significantly different from both original stocks which provides evidence that pupation height is under polygenic control. The reciprocal crosses did not differ from each other indicating that all non-autosomal factors are not significant.

Pupation site preference has also been studied in two sibling species, *D. melanogaster* and *D. simulans* and their hybrids (Manning and Markow, 1981). *D. melanogaster* prefers to pupate in the dark and *D. simulans* in the light. The F_1 individuals obtained from reciprocal crosses between *D. melanogaster* and *D. simulans* were also tested for pupation preference. The F_1 hybrids of *D. melanogaster* female x *D. simulans* male cross tended to be intermediate between the two parental species and the F_1 hybrids of the reciprocal cross were even more photopositive than their *D. simulans* mothers. This led Manning and Markow (1981) to conclude that genes controlling light dependent pupation site preference in these species are sex-linked.

D. bipectinata and *D. malerkotliana* are closely related and sympatric species. Sexual isolation between these two species has been examined and it has been found that they show incomplete sexual isolation. Mating has been found to be nonrandom due to preferential mating between females and males of the same species (Singh et al., 1981, 1982; Singh and Chatterjee, 1991). By using several wild strains of these species, pupation site preference has been studied and it has been found that *D. malerkotliana* pupates higher than *D. bipectinata* (Singh and Pandey, 1991). Furthermore, there is significant variation in pupation height among different strains of *D. bipectinata*. The present note describes the results of studies on pupation height of F_1 hybrids obtained by making reciprocal hybridization between *D. bipectinata* and *D. malerkotliana*.

During the present study, one strain of each of two species was chosen: *D. bipectinata* - U.L. originated from flies collected from Unchawa Lodge near Udai Pratap College, Varanasi; *D. malerkotliana* - Nagaland originated from flies collected from Nagaland. All the strains were cultured on simple culture medium containing agar, dried yeast, brown sugar, maize powder, nipagin, propionic acid and water. All the experiments were conducted in a room maintained at

approximately 24°C temperature with 60-80 percent relative humidity and 12:12 hr light and dark cycle. Virgin females and males of each species were collected and aged separately for 3-5 days. Reciprocal crosses between *D. bipectinata* and *D. malerkotliana* were made in food vials by using 10 females and 10 males. After a few days flies were transferred to petri dishes containing thin layers of food medium for egg laying for 48 hr. Since two species were crossed to produce hybrids, less number of eggs were obtained. In order to get sufficient number of eggs, repeated transferring of flies to petri dishes was done. After larval eclosion, 10 first instar larvae were removed and carefully seeded in a food vial (100 mm length x 25 mm diameter). At the end of pupation the height of pupae was measured. For each cross 10 replicates were carried out. To detect sex differences in pupation height, each pupa after measurement of height was transferred to a food vial and after adult emergence its sex was noted. Data on pupation height of parental strains were taken from Singh and Pandey (1991).

Table 1 shows mean pupation height of *D. bipectinata* and *D. malerkotliana*. The pupation height of *D. malerkotliana* is higher than that of *D. bipectinanta* as difference in pupation height of two species is significant ($t=2.91$, $df=18$, $p<0.01$). Mean pupation height of F_1 hybrids resulting from reciprocal crosses between two species is given in Table 2. Mean pupation height of hybrids obtained from *D. bipectinata* female x *D. malerkotliana* male is 1.7 mm. In the opposite cross, mean pupation height of hybrids is 1.0 mm. Although there is difference in pupation height of F_1 hybrids in reciprocal crosses, the difference is not significant ($t=1.34$, $df=18$, $p>0.10$). The average pupation height of F_1 hybrids (reciprocal crosses combined) is 1.34 mm which is higher than pupation height of parental species. When average pupation height of hybrids is compared with pupation height of parental species, difference is not significant (1.34 vs 1.1 - $t=0.54$, $df=28$, $p>0.20$; 1.34 vs 0.7 - $t=1.55$, $df=28$, $p>0.10$). Furthermore, pupation height of females and males also do not differ significantly for hybrids as well as parental species.

Thus it is concluded that there is no difference in pupation height of F_1 hybrids in reciprocal crosses. Furthermore, F_1 hybrids also do not differ from parental species with respect to pupation height.

References: Bauer, S.J. 1984, DIS 60:58; Bauer, S.J. and M.B. Sokolowski 1985, Can. J. Genet. Cytol. 27:334-340; Casares, P. and M.C. Carracedo 1986, Genetica 70:17-22; Casares, P. and M.C. Carracedo 1987, Beh. Genet. 17:523-535; De Souza, H.L., A.B. Da Cunha and E.P. Dos Santos 1968, Am. Nat. 102:583-586; Manning, M. and T.A. Markow, 1981, Beh. Gene. 11:557-563; Markow, T.A. 1979, Beh. Genet. 9:209-217; Mensua, J. 1967, DIS 42:76; Pandey, M. and B.N. Singh 1991, submitted; Sameoto, D.D. and R.S. Miller 1968, Ecology 49:177-180; Singh, B.N. and S. Chatterjee 1991, Genome 34: in press; Singh, B.N., Y.N. Dwivedi and J.P. Gupta 1981, Ind. J. Exp. Biol, 19:898-900; Singh, B.N. and M. Pandey 1991, Ind. J. Exp. Biol. 29:926-929; Singh, B.N., O.P. Singh and J.P. Gupta 1982, Experientia 38:237-238; Sokal, R.R. 1966, Univ. Kansas Sci. Bull. 46:697-715; Sokal, R.R., P.R. Erhlich, P.E. Hunter and G. Schlager 1960, Ann. Ent. Soc. Amer. 53:174-182.

Pandey, M. and B.N. Singh. Banaras Hindu University, Varanasi, India. No evidence of sterility in interstrain crosses of *Drosophila ananassae*.

Intraspecific hybrid sterility is known in certain species of *Drosophila* (for references see Kidwell, 1983). It is of special interest because it is concerned with the process of speciation. Recently two major types of intraspecific hybrid sterility have been discovered in *Drosophila melanogaster*. When flies from certian strains are crossed, the offspring may display dysgenic traits (Picard and L'Heritier, 1971; Hiraizumi, 1971; Kidwell and Kidwell, 1975). Hybrid dysgenesis has been defined as a syndrome of correlated genetic traits that is spontaneously induced in hybrids between certain mutually interacting strains usually in one direction only (Kidwell and Kidwell, 1976). The most important abnormal genetic trait in hybrids is manifestation of sterility. Besides this, the other abnormal traits include lethal mutations, chromosomal aberrations, male recombination and distorted segregation at meiosis. The role of mobile genetic elements in hybrid dysgenesis has been identified (see Bergliano and Kidwell, 1983). There are two systems responsible for hybrid dysgenesis in *D. melanogaster*. They are: (i) I-R system (SF sterility) and (ii) P-M system (GD sterility).

Drosophila ananassae, a cosmopolitan and domestic species, is of common occurrence in India. It is characterized by many unusual genetic features (Singh, 1985). The most unusual feature of this species is the presence of spontaneous male recombination in considerable frequency (Singh and Singh, 1988). In order to search for the syndrome of hybrid dysgenesis in *D. ananassae*, fertility of 14 wild laboratory stocks derived from ecogeographically different localities in India which have spent varying number of generations in the laboratory, and their hybrids produced by making reciprocal crosses, was studied. Table 1 shows the details of stocks used in fertility tests.

All the stocks were cultured on standard food medium and virgin females and males were collected and aged in small batches separately for 3-4 days. To obtain F_1 flies, all these stocks were crossed with each other by making reciprocal crosses. The females and males of the same stock were also mated. In this way 182 interstrain crosses and 14

intrastrain crosses were made. From F_1 progeny, 5 females and 5 males were transferred to a fresh food vial. After 5 days, the same set of flies were transferred to a fresh food vial. In this way, 5 replicates were made and each replicate with three sets of vials. Thus in each cross, there were 15 vials for counting the F_2 progeny. In few cases, less than 15 vials were made due to less number of F_1 flies obtained. When all the F_2 flies hatched in these vials, they were counted to score the fertility of F_1 flies. The average number of F_2 flies/vial was calculated and a comparison among different crosses as well as original stocks was made with respect to fertility. There is a large amount of data of the fertility tests so it is not given here. It is evident from the results in fertility in any of the crosses involving 14 stocks of *D. ananassae*. However, the average number of flies/vial varied in different stocks as well as different crosses. In several crosses, F_1 hybrids were superior in fertility to the parental stocks. Futhermore, there was considerable variation in fertility of F_1 flies between reciprocal crosses in several interstrain crosses. In some crosses, the F_1 hybrid flies produced fewer flies than the original stocks. Thus it is concluded that the present results provide no evidence for intraspecific sterility in *D. ananassae* and we have failed to find the syndrome of hybrid dysgenesis in *D. ananassae* although Duttagupta and Manna (1988) reported the presence of dysgenic traits in *D. ananassae*.

Table 1. Strains of *Drosophila ananassae* used in fertility test.

Abbreviation	Place of origin	Year of collection
JM	Jammu	1987
MD-90	Madras	1990
MD-84	Madras	1984
PU	Puri	1984
MR	Madurai	1984
PAT-90	Patna	1990
PAT-86	Patna	1986
VN	Varanasi	1984
SG	Siliguri	1989
CA	Calcutta	1985
BOMB	Bombay	1985
KK-90	Kanniya Kumari	1990
KK-84	Kanniya Kumari	1984
SH	Shillong	1984

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References: Berglianao, J.C. and M.G. Kidwell, 1983, in: Mobile Genetic Elements (J.A. Shapiro, ed.), Academic Press; Duttagupta, A.K. and N. Manna, 1988, DIS 67: 34-35; Hiraizuma, Y., 1971, PNAS 68:268-270; Kidwell, M.G., 1983, in: The Genetics and Biology of *Drosophila* (M. Ashburner, H.L. Carson and J.N. Thompson Jr., eds.), Academic Press; Kidwell, M.G. and J.F. Kidwell, 1975, 1975, Nature 253:755-756; Kidwell, M.G. and J.F. Kidwell, 1976, Genetics 84:333-351; Picard, G. and P. L'Heritier, 1971, DIS 46:54; Singh, B.N., 1985, Nucleus 28:169-176; Singh, A.K. and B.N. Singh, 1988, Genome 30:445-450.

Parkash, R., Shamina and Indu. Dept. of Biosciences, M.D. University, Rohtak. Clinal variation at *Adh* and *Est-6* loci in Indian populations of *D. melanogaster*.

Colonizing species such as *D. melanogaster* populations offer excellent material for microevolutionary studies. Most studies on allozymic polymorphism had been made on U.S. and Australian populations of *D. melanogaster* while Asian populations remain unexplored. Thus, it was considered worthwhile to characterize the extent of genic divergence at the polymorphic *Adh* and *Est-6* loci in latitudinally varying (13°.4'N to 33°N) Indian natural populations of *D. melanogaster*. Homogenates of single individuals were applied to 12% starch slab gels (each gel accommodated 15 samples) and were analyzed electrophoretically at 250V and 30 mA at 4°C for 4 hours. Each gel was sliced into three and slices were stained for ADH and EST-6 gene-enzyme systems. The isoelectrophoretic thermoresistent (tr) and thermosusceptible (ts) variants were examined in species individuals by heat treating the enzymes *in situ* in the starch gel slices for 12 minutes at 42°C for ADH and at 56°C for 12 minutes in case of EST-6.

The data on observed genotypes, sample size, allelic frequencies, heterozygosity values and application of G-test for fit to Hardy-Weinberg expectations at polymorphic *Adh* and *Est-6* loci in *D. melanogaster* are given in Table 1. The extent of clinal variation at *Adh* and *Est-6* loci was found to be significantly higher (3% with 1° latitude; r = > 0.90) and revealed significant deviations from Hardy-Weinberg equilibrium at both the loci in Indian populations of *D. melanogaster*. The genotypic as well as allelic frequency patterns at *Adh* and *Est-6* loci revealed significant interpopulation heterogeneity, on the basis of contingency chi-square test among Indian populations of *D. melanogaster*. The data on Wright's fixation index (F_{ST}) revealed significant genic divergence at *Adh* and *Est-6* loci in *D. melanogaster* populations. Indian populations of *D. melanogaster* revealed occurrence of widespread heat stability polymorphism in addition to electrophoretic variation at polymorphic *Adh* and *Est-6* loci (Table 2). In *D. melanogaster* populations, the $Est\text{-}6^S$ (tr) allelic frequency increased positively with latitude while $Est\text{-}6^F$ (tr) and Adh^S (tr) allelic frequency was negatively correlated with increasing latitude. The statistical ("t" test) comparison of electrophoretic versus cryptic variation revealed significant increase in heterozygosity at *Adh* locus while no significant deviation was found at *Est-6*

Table 1. Data on *Adh* as well as *Est-6* genotypes, allelic frequencies, heterozygosities(obs./exp.), Wright's coefficients (f) and G-values for log-likelihood X^2 test for fit to Hardy-Weinberg expectations in six Indian natural populations of *Drosophila melanogaster*

Locus/Populations	Obs. genotypes			Sample	Allelic freq.		Het.	f	G-values
	FF	SS	FS	size	F	S	obs./exp.		
Adh									
Tirumala	12	113	23	148	.16	.84	.15/.26	.42	20.30*
Nagpur	16	48	18	82	.30	.70	.21/.42	.47	18.51*
Bhopal	21	15	18	54	.56	.44	.33/.49	.32	5.83*
Rohtak	62	13	28	103	.74	.26	.27/.38	.29	8.54*
Saharanpur	78	12	26	116	.78	.22	.22/.34	.34	11.72*
Dalhousie	90	10	25	125	.82	.18	.20/.29	.32	10.92*
Est-6									
Tirumala	94	0	46	140	.83	.17	.33/. 28	-.18	9.19*
Nagpur	24	36	50	110	.45	.55	.45/.49	.08	0.74*
Bhopal	7	26	39	72	.37	.63	.54/.46	-.17	2.03
Rohtak	6	48	69	123	.33	.67	.56/.44	-.27	9.76*
Saharanpur	4	36	52	92	.33	.67	.56/.44	-.27	8.32*
Dalhousie	4	58	41	113	.22	.78	.36/.34	-.06	0.60

The latitudinal locations include: Tirumala(13°.40'N); 2. Nagpur(21°.09'N); 3.Bhopal (23°.16'N); 4. Rohtak (28°.94'N); 5. Saharanpur (29°.58'N); 6. Dalhousie (33°N). *Significant at 5% level.

locus in *D. melanogaster* populations.

Biochemical properties of ADH and EST-6 allozymes in *D. melanogaster* have suggested that temperate or cooler places could favor Adh^F and $Est\text{-}6^S$ alleles while tropical or warm places would select Adh^S and $Est\text{-}6^F$ allelic variants. The observed clinal patterns at *Adh* and *Est-6* loci in Indian populations of *D. melanogaster* are in agreement with the known maximum catalytic activity of $Est\text{-}6^F$ at 25-30°C, and higher thermostability of Adh^S variant. Hence, observed higher allelic frequencies of $Est\text{-}6^F$ and Adh^S in the south Indian populations of *D. melanogaster* could be favoured by tropical environment. The occurrence of clinal variation across diverse biogeographical regions cannot be explained on the basis of stochastic process (genetic drift) and/or gene flow. Since the continental populations of *D. melanogaster* differ significantly in their evolutionary history as well as ecogeographical conditions, the existence of parallel clinal allelic frequency changes at *Adh* and *Est-6* loci provide strong evidence for the action of latitudinally related environmental gradients.

Table 2. Patterns of cryptic allelic frequencies at polymorphic loci (*Adh* & *Est*) on the basis of post-electrophoretic heat denaturation technique in six Indian natural populations of *D.melanogaster*

Populations	Adh-F		Adh-S		H/H'	$Est\text{-}6^F$		$Est\text{-}6^S$		H/H'
	tr	ts	tr	ts		tr	ts	tr	ts	
A. *D.melanogaster*										
Tirumala	-	.16	.55	.29	.27/.59	.61	.22	.14	.03	.28/.56
Nagpur	.05	.25	.37	.33	.42/.69	.45	-	.55	-	.49/.49
Bhopal	.26	.30	.17	.27	.49/.74	.30	.07	.47	.16	.46/.66
Rohtak	.34	.40	.09	.17	.38/.69	.03	.30	.49	.18	.44/.64
Saharanpur	.37	.41	.07	.15	.33/.67	-	.33	.47	.20	.44/.63
Dalhousie	.49	.33	.07	11	.30/.63	.06	.16	.63	.15	.34/.55

F and S represent fast and slow electromorphs respectively; tr = thermoresistant and ts = thermosusceptible; H and H' are heterozygosity values on the basis of electrophoresis and due to post-electrophoretic heat denaturation technique respectively.

Parkash, R., J.P. Yadav and Shamina. Dept. Biosc., M.D. University, Rohtak - 124001, India. Latitudinal variation at *Adh* locus in *Zaprionus indianus*.

Allopatric populations of *D. melanogaster* have revealed latitudinal cline at *Adh* locus and Adh^F allelic frequency was found to correlate with latitude (David *et al.*, 1989). However, there is complete lack of information about the allozymic genetic structure at the *Adh* locus in various cosmopolitan as well as colonizing drosophilids from Asia. Thus, the present investigation was undertaken to examine the nature and extent of electrophoretic and cryptic variation at *Adh* locus in twelve geographical natural populations of *Z. indianus* which is a colonizing species throughout the Indian subcontinent.

The data on observed genotypes, sample size, allelic frequencies, heterozygosity values, Wright's coefficients and application of G-test for fit of Hardy-Weinberg expectations at polymorphic *Adh* locus in *Z. indianus* are given in Table

1. The range of high heterozygosity values observed at the *Adh* locus were in agreement with allelic frequency distribution patterns. The population samples from Bangalore revealed deviations from the Hardy-Weinberg expectations. The Adh^F frequencies increased significantly with increasing latitude (>1% with 1° latitude; Figure 1) and revealed significant correlation as well as regression coefficient (r = 0.95; b = 0.01). Significant genotypic as well as allelic frequency heterogeneity patterns were observed at the *Adh* locus, on the basis of contingency chi-square test among Indian populations of *Z. indianus*. The data on Wright's fixation index (F_{ST}) revealed moderate genic differentiation (F_{ST} = 0.026) at this locus.

Table 1. Distribution of *Adh* genotypes, allelic frequencies, heterozygosities (obs./exp.), Wright's coefficients (f) and G-values for log-likelihood X^2 test for fit to Hardy-Weinberg expectations in twelve Indian natural populations of *Zaprionus indianus*

Populations	Latitudes	Obs. genotypes			Sample	Allelic freq.		Het.	f	G-values
		FF	SS	FS	size	F	S	obs./exp.		
Ernakulam	10°N	4	30	18	52	.25	.75	.35/.37	.05	0.30
Bangalore	12°.58'N	24	66	42	132	.34	.66	.32/.45	.29	11.04*
Tirumala	13°.40'N	8	26	30	64	.36	.64	.47/.46	-.02	0.04
Hyderabad	17°.20'N	17	35	36	88	.40	.60	.41/.48	.15	1.87
Nagpur	21°.16'N	18	30	38	86	.43	.57	.44/.49	.10	0.84
Bhopal	23°.16'N	14	20	29	63	.45	.55	.46/.49	.06	0.32
Jaipur	26°.55'N	12	21	39	72	.44	.56	.54/.49	-.10	0.73
Rohtak	28°.94'N	23	25	47	95	.49	.51	.49/.50	.02	0.01
Roorkee	29°.52'N	22	27	59	108	.48	.52	.54/.50	-.08	1.0
Dehradun	30°.19'N	20	24	48	92	.48	.52	.52/.50	-.04	0.88
Chandigarh	30°.43'N	21	18	42	81	.52	.48	.52/.50	-.04	0.12
Jammu	32°.74'N	29	20	45	94	.55	.45	.48/.49	.02	0.11

Data on contingency X^2 analysis include: Inter-populational genotypic heterogeneity = 17.26; allelic heterogeneity = 48.67*.
*Significant at 5% level.

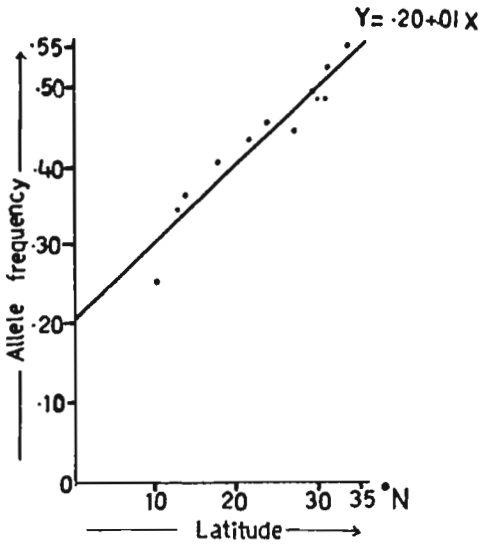

Figure 1. Relationship of Adh^F allelic frequency with latitude in twelve Indian natural populations of *Zaprionus indianus*.

The present observations on clinal variation at the *Adh* locus in *Z. indianus* further validate and support the hypothesis that occurrence of parallel or complementary latitudinal clines among different colonizing drosophilids as well as across continents provide strong evidence of natural selection maintaining such allozymic variation (David *et al.*, 1986; David *et al.*, 1989; Oakeshott *et al.*, 1982). The observed patterns of genotypic as well as allelic heterogeneity and clinal variation at the *Adh* locus in *Z. indianus* populations are in agreement with its habitat-generalist or broad-niche characteristics. Since the north-south transect of the Indian sub-continent represents an array of climatically as well as ecologically variable habitats, the clinal patterns of genic polymorphism at *Adh* locus could be adaptively maintained by natural selection acting at this locus.

References: David, J.R., H. Mercot, P. Capy, S.F. McEvey and J. Van Herrewege, 1986, Genet. Sel. Evol. 18:405-416; David, J.R., A. Alonso Moraga, F. Borai, P. Capy, H. Mercot, S.F. McEvey, A. Munoz, Serrano and S. Tsakas, 1989, Heredity 62:11-16; Oakeshott, J.G., J.B. Gibson, P.R. Anderson, W.R. Knibb, D.G. Anderson and G.K. Chambers, 1982, Evolution 36:86-96.

Parkash, R., J.P. Yadav, and M. Vashist. Dept. Biosc., M.D. University, Rohtak - 124001, India. Allozymic variation in six Indian populations of *D. busckii*.

Various cosmopolitan *Drosophila* species populations constitute excellent material for microevolutionary studies. Except the sibling species pair of *D. melanogaster* and *D. simulans*, the studies on biogeography, adaptive morphological, physiological, ecological traits and allozymic variation in other cosmopolitan *Drosophila* species are almost lacking. There is little

information on *D. busckii* which is the only known cosmopolitan species of the subgenus Dorsilopha. *D. busckii* occurs in North Indian regions during December-February. Isofemale lines were established for populations sampled from Chandigarh (latitude, 30°.43'N; longitude, 76°.54'E), Saharanpur (latitude, 29°.58'N; longitude, 77°.23'E), Roorkee (latitude, 28°.94'N; longitude, 76°.38'E), Darjeeling (latitude, 27°.03'N; longitude, 88°.18'E), Hasimara (latitude, 26°.42'N; longitude, 89°.22'E). Homogenates of single individuals were applied to a 12% horizontal starch gel slab (with a capacity of about 15 samples) and were run electrophoretically at 250 V and 30 mA at 4°C for 4 hrs. The gel slices were stained for different enzymes.

Multiple zones of activity occur for three enzymes (APH, MDH and EST) while all other enzymes are represented by a single zone. The banding patterns at different zones are independent of each other. Gel slices stained for esterases revealed five zones of activity. The EST-2 and EST-5 zones are represented by segregating single-band variants and two-band patterns while other non-segregating EST zones are monomorphic. However, a single polymorphic zone each for AO, ODH, MDH-1, ME and APH-3 is represented by segregating single-band variants and three band patterns. Complex ACPH in *D. busckii* include three-banded and five-banded patterns. ACPH being a dimeric molecule, the occurrence of triple-banded patterns in long term inbred laboratory strains could only be interpreted on the basis of a duplicate locus. However, this suggestion needs to be verified by dissociation-reassociation of ACPH isozymes. A single monomorphic zone of alpha-GPDH is represented by two-banded patterns while the single ADH zone depicted segregating patterns of two-banded and three-banded phenotypes in single individuals.

The data on distribution of allelic frequencies at ten polymorphic loci in *D. busckii* are given in Table 1. Data on genetic indices (number of alleles and allelic frequencies) for diallelic loci (ACPH, EST-5, ADH, ODH, MDH-1, ME) and triallelic loci (EST-2, APH-3, and AO) revealed similarity in genic diversity patterns in six natural populations of *D. busckii*. The observed inter-populational

Table 1. Allelic frequency distribution patterns at ten polymorphic loci in six Indian natural populations of *D. busckii*

Locus	Alleles	Chandigarh	Saharanpur	Roorkee	Rohtak	Darjeeling	Hasimara
Est-2	F	.24	.26	.28	.31	.27	.28
	M	.47	.49	.45	.40	.46	.48
	S	.29	.25	.27	.29	.27	.24
	N	99	80	92	108	98	89
Est--5	F	.45	.43	.47	.44	.48	.45
	S	.55	.57	.53	.56	.52	.55
	N	94	80	100	90	98	89
Aph-3	F	.12	.10	.12	.13	.09	.11
	M	.35	.36	.32	.34	.33	.34
	S	.53	.54	.56	.53	.58	.55
	N	102	80	92	89	113	97
Acph	F	.06	.05	.08	.11	.04	.07
	S	.94	.95	.92	.89	.96	.93
	N	146	106	80	103	108	100
Adh	F	.07	.05	.0	.06	.04	.07
	S	.93	.95	1.0	.94	.96	.99
	N	104	80	95	100	102	100
Ao	F	.52	.51	.52	.48	.53	.51
	M	.03	.0	.0	.0	.01	.01
	S	.45	.49	.48	.52	.46	.48
	N	116	60	68	124	102	100
Odh	F	.34	.38	.36	.32	.35	.33
	S	.66	.62	.64	.68	.65	.67
	N	100	85	60	110	102	100
α–Gpdh	F	1.0	.975	1.0	1.0	.99	1.0
	S	.0	.025	.0	.0	.01	0
	N	100	80	95	100	102	100
Mdh-1	F	.64	.55	. 59	.60	.62	.58
	S	.36	.45	. 4 1	.40	.38	.42
	N	136	76	80	102	102	100
Me	F	.26	.32	.29	.35	.28	.33
	S	.74	.68	.71	.65	.72	.67
	N	92	66	60	104	102	100

F, M, and S represent fast, medium and slow electromorphs respectively. N = sample size.

Table 2. Data on contingency X^2 analysis of inter-populational genotypic frequencies as well as allelic frequencies and analysis of Wright's fixation index (F_{ST}) at ten polymorphic loci in six Indian natural populations of *D. busckii*

Locus	Contingency Chi-square values: genotypic freq.		Allelic freq.		F_{ST}
Est-2	38.16*	(18)	5.68	(10)	.002
Est-5	18.18*	(06)	1.29	(05)	.001
Aph-3	53.72*	(18)	3.17	(10)	.001
Acph	7.16	(06)	10.38	(05)	.008
Adh	50.49	(05)	20.76*	(05)	.018
Ao	17.37	(12)	16.59	(10)	.002
Odh	26.48*	(06)	1.89	(05)	.002
α -Gpdh	0.12	(02)	18.01*	(05)	.017
Mdh-1	17.68*	(06)	4.07	(05)	.003
Me	12.86*	(06)	5.19	(05)	.004

Degrees of freedom given in parenthesis; * Significant at 5% level.

heterogeneity in terms of genotypes as well as allelic frequencies have been shown in Table 2. Significant genotypic heterogeneity is observed at *Est-2*, *Est-5*, *Aph-3*, *Adh*, *Odh*, *Mdh-1* and *Me* loci. Significant allelic frequency heterogeneity is observed at the *Adh* and *alpha-Gpdh* loci. Previous electrophoretic data on *D. busckii* are limited to a single study which revealed low amounts of genic variation (i.e. P = 0.166 and H = 0.044) in laboratory maintained stocks of US populations of this species. The present observations on the extent of polymorphic loci (P = 0.52 for all the populations), allelic frequencies; and heterozygosity (H_o/H_e = 0.213/0.235) in *D. busckii* from northern India differ significantly from those on US populations. The low amount of genic variation (H = 0.044) reported for US laboratory populations of *D. busckii* could be due to extensive inbreeding in 2-10 year old cultures as well as operation of genetic drift resulting in fixation of alleles at most loci. The electrophoretic analysis of six Indian natural populations of *D. busckii* have revealed homogeneity of allozymic frequencies, i.e. the most common allele was found to be the same everywhere and its frequency was almost similar at most of loci in different populations. The data on the basis of Nei's (I) values also showed high genetic similarity between different populations. The data on Wright's Fixation Index (F_{ST}) also revealed that there was very little genetic differentiation (Table 2). The homogeneity of patterns of genic variation at most polymorphic loci of *D. busckii* can be explained on the basis of gene flow. This is expected to affect all the polymorphic loci similarly. An alternative explanation would be the action of balancing natural selection in maintaining the observed genic variation patterns in *D. busckii*. The occurrence of lower levels of allozymic variation in Indian populations of this species supports that populations of *D. busckii* could be adapted to its narrow niche-width in terms of climatic adaptation and food resources. Further detailed analysis of the global populations of this cosmopolitan species are needed to ascertain genetic structure in terms of its colonizing potential.

Sharma, M., S. Sharma and R. Parkash. Dept. Biosc., M.D. University, Rohtak - 124001, India. Patterns of ACPH allozymic variation in *D. nepalensis*.

Allozymes revealed by gel electrophoretic technique constitute useful markers to examine the role played by microevolutionary processes in modifying the genetic architecture of species population. The present paper reports the temporal patterns of electrophoretic

Table 1. Data on temporal distribution of allelic frequencies, heterozygosity and G-values for the log-likelihood X^2 test for Hardy-Weinberg expectations; and extent of post-electrophoretic cryptic variation at *Acph* locus in three natural populations of *D. nepalensis*

		1988			1989			1990		
		Roorkee	Dehradun	Jammu	Roorkee	Dehradun	Jammu	Roorkee	Dehradun	Jammu
Acph	F'	—	—	.01	—	.015		—	—	—
	F	.93	.93	.90	.91	.899	.919	.90	.96	.89
	M	.06	.07	.07	.06	.067	.057	.06	.04	.08
	S	.02	—	.02	.03	.019	.014	.04	—	.03
	N	64	90	134	150	134	105	142	81	106
Het.H_o/H_e		.16/.15	.13/.12	.17/.18	.17/.17	.49/.19	.11/.15	.02/.19	07/.08	.21/.19
G-values		.85	.86	3.95	2.73	3.91	6.67	3.28	.27	2.56

Achp (*D. nepalensis*)

	Jammu				Dehradun			
	1988		1990		1988		1990	
	tr	ts	tr	ts	tr	ts	tr	ts
F'	—	.01	—	—	—	—	—	—
F	.73	.17	.72	.17	.75	.18	.76	.20
M	—	.07	—	.08	—	.07	—	.04
S	—	.02	—	.03	—	—	—	—
Total freq.	.73	.27	.72	.28	.75	.25	.76	.24
H & H'	.14	.43	.19	.44	.13	.40	.08	.38
n_e & n_e'	1.23	1.76	1.20	1.79	1.14	1.68	1.08	1.63
n_e'/n_e	1.4		1.5		1.5		1.51	

F', F, M and S represent faster, fast, medium and slow electromorphs respectively; N= sample size; H and n_e denote degree of heterozygosity and effective number of alleles based on electrophoretic variation; H' and n_e' are the similar values based on post-electrophoretic heat stability variation (tr=thermostability; ts=thermosensitive electromorph).

variation at the *Acph* locus in natural population samples of *D. nepalensis.* Homogenates of single individuals from isofemale lines of *D. nepalensis* were applied to 12% starch and electrophoresed at 250 V and 30 mA at 4°C for 4 hours. Each starch gel slab accommodated about 12 samples and the gel slices were stained for acid phosphatase. The application of heat denaturation technique involved post-electrophoretic thermal treatment of the enzyme *in situ* in the starch gel slice (at 54°C for 12 $\pm$ 1 minutes in case of acid phosphatase). However, the respective control gel slices were pretreated at 37°C.

The data on distribution patterns of allelic frequencies, observed and expected heterozygosity and G-values for log-likelihood X^2 test for Hardy-Weinberg equilibrium for the *Acph* polymorphic locus in *D. nepalensis* are given in Table 1. The patterns of genic variation include occurrence of a common allele and a few rare alleles at the *Acph* locus in *D. nepalensis*. The electrophoretic analysis at polymorphic loci in natural population samples taken in consecutive years from different sites have revealed persistence of common, less frequent as well as rare alleles in the natural populations sampled in different years. The data on distribution of thermoresistant (tr) and thermosensitive (ts) alleles, allelic frequencies, heterozygosity and effective number of alleles (n_e') at *Acph* locus in the yearly population samples of *D. nepalensis* are given in Table 1. The occurrence of cryptic variation has resulted in significant increase in heterozygosity as well as effective number of alleles. The patterns of allelic frequencies at various loci (samples collected in 1990) have been found to be very similar to those of the population sampled in 1988 and/or 1989. The yearly data on allelic frequency patterns revealed no deviation on the basis of student's "t" test. The yearly population samples revealed homogeneity patterns in allelic frequencies and heterozygosity. The present observations have revealed temporal stability of allelic frequencies at *Acph* locus in *D. nepalensis*. Our results concur with earlier reports of temporal constancy at phosphoglucomutase (PGM) and persistence of alcohol dehydrogenase (ADH) cryptic variation in *D. melanogaster.* Present results reveal homogeneous genic variability patterns (electrophoretic and cryptic) at *Acph* polymorphic locus in yearly populations of *D. nepalensis* and this may be interpreted due to the action of balancing natural selection.

Parkash, R., S. Sharma and M. Sharma. Dept. Biosc., M.D. University, Rohtak - 124001, India. Clinal allozymic variation at *Acph* locus in *D. ananassae* populations.

Microevolutionary processes are intimately related to the extent of genetic variability occurring in natural populations. The aim of the present investigation is to compare the level of genic variability at the *Acph* locus in ten latitudinally varying populations of *D. ananassae* through standard gel electrophoresis alone as well as coupling it with heat denaturation technique. The population samples of *Drosophila ananassae* were collected from ten latitudinally varying sites (Dehradun, Rohtak, Phuntsholing, Bagdogra, Hasimara, Bhopal, Calcutta, Nagpur, Bangalore and Cochin). The wild caught female individuals of *D. ananassae* were maintained as isofemale lines on the *Drosophila* medium and were kept at 25 $\pm$ 1°C in the laboratory. The homogenates of single individuals were subjected to 12% starch gel and were run electrophoretically at 250 V and 30 mA at 4°C for 3.5 hrs and the gel slices were stained for acid phosphatase (ACPH) activity. The application of heat denaturation technique involved post-electrophoretic heating the ACPH enzyme *in situ* in starch gel at 57°C for 15 $\pm$ 1 min.

The genotypic distribution patterns at *Acph* locus were found to be characteristically large due to occurrence of some rare alleles. The data on the comparative distribution of allelic frequencies in all the ten natural populations of *D. ananassae* have been shown in Table 1. The *Acph-1* locus has shown one common allele ($Acph^{100}$) in addition to one less frequent ($Acph^{95}$) and three rare alleles ($Acph^{90}$, $Acph^{105}$ and $Acph^{85}$) in the populations analyzed. However, the three rare *Acph* alleles were observed in a few populations only (Table 1). Some of the species populations have depicted unique alleles and the frequencies of certain alleles have varied in distantly located populations.

The data on the heat stability polymorphism at the polymorphic *Acph* locus in ten geographical populations of *D. ananassae* from different parts of India have been represented in Table 1. The data on the basis of standard electrophoresis have been represented in terms of heterozygosity (H) and effective number of alleles (n_e) while cryptic variability patterns have been shown by the patterns of distribution of isoelectrophoretic thermoresistant (tr) and thermosensitive (ts) allelic frequencies, heterozygosities (H') and effective number of alleles (n_e'). The *Acph* locus is represented by one frequent and other less frequent alleles on the basis of standard electrophoresis alone. However, the $Acph^{100}$ allele in all the natural populations has revealed isoelectrophoretic variants while the less frequent alleles $Acph^{105}$, $Acph^{95}$ and $Acph^{90}$) depicted limited amount of cryptic variation at this locus in some of the south Indian populations only. However, the patterns of cryptic variability of the most common *Acph* allele were found to be nearly uniform in all the populations analyzed. The present data concurred with earlier reports that electromorphs might not represent homogeneous electrophoretic variants. The extent of genic variation with respect to number of alleles and

Table 1. Data on *Acph* allelic frequencies, heterozygosity and effective number of alleles (n_e) on the basis of electrophoretic analysis alone and post-electrophoretic heat denaturation test in ten Indian natural populations of *Drosophila ananassae*

Populations	Cochin	Bangalore	Nagpur	Calcutta	Bhopal	Hasimara	Bagdogra	Puntsholing	Rohtak	Dehradun
Latitude:	9°.58'N	12°.58'N	21°.09'N	22°.34'N	23°.16'N	27°.40'N	26°.41'N	27°.40'N	28°.58'N	30°.43'N
longitude:	76.17E	77.38E	79.09E	88. 24E	77.36E	89.22E	88.19E	90.10E	76.53E	78.09E
N:	125	107	102	100	106	121	122	112	63	126
105 tr	—	.01	—	—	—	—	—	—	—	—
105 ts	.04	.01	—	—	—	—	—	—	.01	—
100 tr	.43	.50	.48	.55	.52	.59	.57	.57	.58	.57
100 ts	.24	.21	.20	.24	.17	.15	.16	.14	.15	.15
95 tr	.13	.16	.21	.21	.24	—	—	—	.19	.18
95 ts	.05	.07	.05	—	—	.23	.22	.22	—	—
90 tr	—	.02	—	—	—	.03	.05	.07	—	—
90 ts	.08	.02	.05	—	.06	—	—	—	.06	.10
85 ts	.03	—	—	—	—	—	—	—	.01	—
H	.51	.45	.46	.33	.45	.40	.42	.44	.43	.44
H'	.73	.71	.68	.59	.63	.57	.60	.61	.60	.62
H'- H	.22	.26	.22	.26	.18	.17	.18	.17	.17	.18
ne	2.06	1.81	1.84	1.50	1.80	1.66	1.72	1.78	1.75	1.77
ne'	3.7	3.43	3.08	2.46	2.72	2.55	2.50	2.54	2.50	2.58
ne'/ne	1.87	1.89	1.67	1.64	1.50	1.41	1.45	1.43	1.44	1.46

N=Sample size; H and n_e = heterozygosity and effective number of alleles based on electrophoresis alone; H' & n_e' are the same based on post-electrophoretic heat stability variation. tr = thermoresistant; ts = thermosensitive.

heterozygosity at the polymorphic *Acph* locus is highly significant on the basis of heat denaturation technique. The heat stability polymorphism at the *Acph* locus has resulted in 20 to 40 percent increase in the effective number of alleles at this locus in latitudinally varying populations.

The common occurrence of heat stability polymorphism in all the populations suggest that natural selection might be responsible for the maintenance of such cryptic genic variation. Temperature constitutes an important component of the environment, and empirical data exist on the adaptive correlation of biochemical properties of allozymic (allelic isozymes) variants with habitat temperature in some organisms. Thus, it can be suggested that in clinally varying heterogeneous environments of the Indian subcontinent, tr and ts acid phosphatase variants may confer adaptive advantage to *D. ananassae* individuals which occur in its natural habitat during all seasons of the year.

Parkash, R., M. Vashist and J.P. Yadav. Dept. Biosc., M.D. University, Rohtak - 124001, India. Isoelectrophoretic variation at *Acph* locus in *D. ananassae* populations.

Application of sequential electrophoresis and heat denaturation technique has revealed detection of cryptic or hidden isoelectrophoretic variation at few loci. Thus, the present investigation was undertaken to examine the nature and extent of electrophoretic and cryptic variation at the *Acph* locus in three natural populations of *D. ananassae*. Population samples of *D. ananassae* were collected from three sites, Bagdogra and Hasimara (West Bengal); and Phutsholing (Bhutan) in January, 1988. Homogenates of single individuals from isofemale lines were applied to 12% starch gels and electrophoresed at 250 V and 30 mA at 4°C for 4 hours. Each starch gel slab accommodated about 15 samples and the gel slices were stained for acid phosphatase. The heat denaturation technique of Trippa *et al.* (1980) was followed. After electrophoresis, each gel was cut into two slices, one of which served as control and was incubated in a 37°C water bath and the other gel slice was incubated in a 57°C water bath for 15± 1 minutes. The comparison of heat treated with the control gel slice revealed differential thromostability patterns, i.e. some electrophoretic variants could be classified either as temperature resistance (tr) or temperature sensitive (ts), while some individuals showed isoelectrophoretic variants having similar mobility but differing in their thermostability property. Such characterization was followed for all the isofemale lines.

A total of 6 ACPH electrophoretic phenotypes were observed in three population samples of *D. ananassae* which are governed by three autosomal codominant alleles ($Acph^S$, $Acph^M$, $Acph^F$) in order of increasing electrophoretic mobility. The data on distribution of ACPH genotypes, allelic frequencies, observed and expected heterozygosity, Wright's fixation index (f) and log-likelihood X^2 tests for fit to Hardy-Weinberg expectations at the polymorphic *Acph* locus in *D. ananassae* population are given in Table 1. The $Acph^F$ and $Acph^S$ represent the most common (0.72 to 0.74) and the rare alleles (0.03 to 0.07) respectively in all the populations analyzed (Table 1). The two population samples

Table 1. Distribution of observed and expected *ACPH* genotypes, allelic frequencies, heterozygosities, G-values for the log-likelihood X^2 test for Hardy-Weinberg expectations & Wright's coefficient (f) in three natural population samples of *D. ananassae*

Populations	*ACPH* genotypes						Sample	Allelic freq.			Het.	Wright's	G-value
	FF	MM	SS	FM	FS	MS	(N)	F	M	S	o/e	coeff. (f)	
Bagdogra													
(o)	68	8	4	38	4	0	122	.73	.22	.05	.34/.42	.18	22.81*
(e)	64.84	5.96	0.31	39.30	8.89	2.70							
Hasimara													
(o)	56	6	2	34	2	0	100	.74	.23	.03	.36/.40	.10	13.15*
(e)	54.16	5.29	0.09	34.04	4.44	1.38							
Phuntsholing													
(o)	50	4	2	32	8	2	98	.72	.21	.07	.43/.44	.02	3.5 n.s.
(e)	50.1	4.49	0.49	29.99	9.95	2.98							

FF, MM, and SS represent genotypes homozygous for fast (F), medium (M) and slow (S) allelic variants while FM, FS and MS represent heterozygous genotypes. o and e refer to observed and expected values. Wright's coefficient (f=1-obs. het./exp. het).
*Significant at 5% level

Table 2. Data on *ACPH* allelic frequencies, heterozygosities, effective number of alleles (n_e) based on electrophoresis alone* and heat denaturation test in three natural populations of *D. ananassae*

	Thermostability of *ACPH* alleles in three natural populations								
	Bagdogra			Hasimara			Phuntsholing		
ACPH electromorphs	*	tr	ts	*	tr	ts	*	tr	ts
F	0.729	0.568	0.161	0.74	0.592	0.148	0.715	0.572	0.143
M	0.221	—	0.221	0.23	—	0.23	0.214	—	0.214
S	0.050	0.050	—	0.03	0.03	—	0.071	0.071	—
Total tr & ts alleles		0.618	0.382		0.622	0.378		0.643	0.357
H and n_e		0.418	1.718		0.40	1.666		0.438	1.779
H' and n_e'		0.60	2.50		0 574	2.347		0.606	2.538
Increase in variation n_e'/n_e		1.455			1.408			1.426	

H & n_e degree of heterozygosity and effective number of alleles based on electrophoretic variation; H' and n_e' are the same based on post-electrophoretic heat stability variation, tr = thermoresistant; ts = thermosensitive.

from Bagdogra and Hasimara (West Bengal), with similar levels of heterozygosity, have revealed significant deviations from Hardy-Weinberg expectations while the population sample from Phutsholing is in agreement with the expected values. The values of Wright's coefficient (f) in the two population samples (Bagdogra and Hasimara) indicate deficiency of heterozygotes at this locus while this is not so in case of the third population from Phutsholing.

The data on the distribution of thermoresistant (tr) and thermosensitive (ts) alleles, allelic frequencies, heterozygosities and effective number of alleles (n_e') are shown in Table 2. The most frequent $Acph^F$ allele revealed two isoelectrophoretic variants (tr and ts) with polymorphic frequencies in all the populations analyzed. However, $Acph^M$ and $Acph^S$ represent thermosensitive (ts) and thermoresistant (tr) variants respectively, and these did not reveal any isoelectrophoretic variants. The application of heat denaturation technique has resulted in the increase in heterozygosity (H'-H = 0.17) as well as effective number of alleles (n_e'/n_e = 1.45) at the *Acph* locus (Table 2). However, there is no differentiation with respect to distribution patterns of tr and ts alleles in the population samples analyzed. All population samples have depicted a common pattern of the distribution of thermoresistant (tr) and thermosensitive (ts) alleles (Table 2). The cryptic isoelectrophoretic variation has not changed the overall pattern of allelic variation at the *Acph* locus in *D. ananassae*. The uniform pattern of cryptic and electrophoretic variation at the *Acph* locus can be argued both on the basis of action of some type of natural selection at this locus or on the basis of migration of flies through human transportation.

Yadav, J.P., M. Vashist and R. Parkash. Dept. Biosc., M.D. University, Rohtak - 124001, India. Patterns of genic homogeneity in Indian populations of *D. immigrans*.

Except some Hawaiian and Korean populations of *D. immigrans*, the information about the genetic structure of this cosmopolitan species is lacking (Steiner *et al.*, 1976). Thus, ten Indian natural populations of *D. immigrans*, collected along 12° longitudinal and 6° lati-

tudinal ranges, were analyzed electrophoretically for allozymic variation of eight gene-enzyme systems. The populations of *D. immigrans* were bait-trapped from various geographical sites. The homogenates of single individuals were analyzed electrophoretically in 12% starch gels at 250 V and 30 mA at 4°C for four hours. The gel slices were stained for eight different gene-enzyme systems (Steiner and Joslyn, 1979). The genetic basis of banding patterns was interpreted from segregation ratios of electrophoretic phenotypes of the parents as well as F_1 and F_2 progeny of several genetic crosses.

Table 1. Data on the distribution of allelic frequencies in ten natural populations of *Drosophila immigrans*

Locus	Electro-morphs	Jammu	Chandigarh	Dehradun	Saharanpur	Risikesh	Rohtak	Darjeeling	Dhulabari	Phuntsholing	Hasimara
Est-1	F	1.0	1.0	.95	1.0	1.0	1.0	.92	1.0	1.0	.93
	S	0	0	.05	0	0	0	.08	0	0	.07
	N	65	92	102	80	74	82	57	100	94	68
Est-2	F	.76	.75	.81	.87	.85	.83	.80	.82	.75	.72
	S	.24	.25	.19	.13	.15	.17	.20	.18	.25	.28
	N	85	114	152	112	74	82	78	100	95	115
Est-6	F	1.0	1.0	1.0	1.0	. 96	1.0	1.0	1.0	1.0	.95
	S	0	0	0	0	.04	0	0	0	0	.05
	N	85	114	102	80	74	82	57	100	94	80
Acph	F	.06	.04	.06	.07	.08	.02	.07	0	.05	.03
	S	.94	.96	.94	.93	.92	.98	.93	1.0	.95	.93
	N	121	80	81	186	146	86	108	152	94	111
Mdh-1	F	.27	.25	.24	.20	.15	.17	.19	.14	.196	.18
	S	.73	.75	.76	.80	.85	.83	.81	.86	.804	.82
	N	107	72	72	50	111	86	103	106	112	102
Odh	F	.78	.76	.75	.80	.82	.79	.77	.73	.76	.75
	S	.22	.24	.25	.20	.18	.21	.23	.27	.24	.25
	N	90	80	99	65	104	81	9 2	117	101	107
Ao	F	.63	.65	.59	.66	.64	.62	.61	.60	.61	.59
	S	.37	.35	.41	.34	.36	.38	39	. 40	.39	. 41
	N	114	86	114	89	106	85	98	103	99	119

In Hasimara population F'=.04. F', F and S refer to faster, fast and slow electromorphs respectively. N = sample size.
Jammu: latitude = 32°.74'N, longitude =75° E; Chandigarh: latitude =30° .43'N, longitude =76 .54'E; Dehradun: latitude = 30°. 19 ' N, longitude =76°. 38' E; Saharanpur: latitude = 29°. 58 ' N, longitude =77°. 23'E; Risikesh: latitude=29°.58'N, longitude =78°.13'E; Rohtak: latitude = 28°.94'N longitude =76°. 38'E; Darjeeling: latitude =27°.03'N, longitude = 88°. 18'E; Dhulabari: latitude = 26° .88'N, longitude = 88°. 19'E; Phuntsholing: latitude=26°. 86'N, longitude = 89°. 22'E, Hasimara: latitude = 26°. 42'N, longitude = 89°. 22'E

The data on the distribution of allelic frequencies at polymorphic loci are given in Table 1. The most allelic variants as well as their frequency patterns at polymorphic loci were found to be almost similar in all the ten natural populations. The species populations were characterized by occurrence of two common alleles at *Est-2, Mdh-1, Odh* and *Ao* loci. The *Acph* locus revealed one common and one rare allele in all the populations except Dhulabari and Hasimara (Table 1). Except few populations, *Est-1* and *Est-6* loci were monomorphic in most of them. The data on allelic frequency for diallelic loci (*Acph, Est-2, Ao* and *Odh*) revealed homogeneity in genic diversity patterns in all the ten natural populations. However, geographical variation was observed at *Mdh-1* locus in the Indian populations of *D. immigrans*. The observed inter-populational heterogeneity in terms of genotypes as well as allelic frequencies are given in Table 2. Significant genotypic heterogeneity among populations was observed at *Est-2* locus and lesser at *Mdh-1* and *Adh* loci. Significant allelic frequency heterogeneity was observed at the *Acph-1, Est-1* and *Est-6* loci, while lesser geographical variation was found at *Est-2* and *Mdh-1* loci. The observed F_{ST} values at various

Table 2. Data on contingency X^2 analysis of inter-populational genotypic frequencies as well as allelic frequencey and analysis of Wright's fixation indices (F_{ST}) at seven polymorphic loci in ten Indian populations of *D.immigrans*

	Contingency chi-square values		
Locus	Genotypic freq.	Allelic freq	F_{ST}
Est-1	3.05(3)	83.86*(9)	.051
Est-2	61.32*(10)	27.35*(9)	.012
Est-6	0.36(2)	65.55*(9)	.055
Acph	10.92(11)	105.42*(18)	.010
Mdh-1	19.51*(10)	20.20*(9)	.009
Ao	13.84(10)	7.06 (9)	.006
Odh	19.65*(10)	4.01 (9)	.002

Degrees of freedom are given in the parenthesis;
*Significant at 5% level.

polymorphic loci were found in the range of .002 to .055 and thus revealed little differentiation at these loci.

The electrophoretic analysis of ten Indian natural populations of *D. immigrans* revealed patterns of homogeneity in allozymic frequency, i.e., the most common allele was found to be the same everywhere, and its frequency was found to be almost similar at most of the loci in different populations of *D. immigrans*. The homogeneity patterns of genic variation at most polymorphic loci and modest clinal variation at the *Mdh* locus in populations of *D. immigrans* can be explained on the basis of gene flow which is expected to affect all the polymorphic loci similarly. An alternative explanation could be the action of balancing natural selection in maintaining the observed genic variation patterns in *D. immigrans*.

The comparison of genetic variability indices among allopatrically distributed Indian, Korean and Hawaiian populations of *D. immigrans* revealed occurrence of genic differentiation at some of the loci. Thus, Indian populations of *D. immigrans* seem to be genetically uniform and adapted to almost similar climatic conditions occurring in the north and north-eastern Indian localities while allopatric populations of different continents might have adapted through genic differentiation at several loci.

References: Steiner, W.W.M. and D. Joslyn 1979, Mosquito News 39:35-54; Steiner, W.W.M., K.C. Sung and Y.K. Paik 1976, Biochem. Genet. 14:495-506.

Rabossi, A. and L.A. Quesada-Allué. I.I.B. "Fundacion Campomar", (1405) Buenos Aires, Argentina.
Drosopterin-like pteridine in *Ceratitis capitata*.

A group of six pteridines called drosopterins was described in *Drosophila melanogaster* (Dorsett *et al.*, 1979). Outside the *Drosophila* genus, similar pigments were only found in *Anagasta* (Needham, 1978). Ziegler and Feron (1965) analyzed the eye pigments of *Ceratitis capitata* and could not detect the presence of drosopterins. We recently found that extracts from adult eyes of this insect contain an orange pigment (Rabossi, 1991) which is described here as being similar to a *Drosophila* drosopterin.

Drosophila and *Ceratitis* eye pigments were extracted with solvent C: n-propanol: 3.5% NH_4OH (2:1), according to Wilson and Jacobson (1977), separated in thin layer chromatography (TLC) sheets of cellulose and located under UV light (lambda = 254 nm). Individual spots were eluted from the plates with solvent C (see above) and analyzed in a Jasco spectrofluorometer to obtain its absorbance profile.

Table 1. Qualitative analysis of *Drosophila melanogaster* drosopterin and *Ceratitis capitata* pigment M.

		TLC Relative mobility[a]		Max. Absorbance
		Solvent A	Solvent B	Wavelength (nm)
Drosophila m. drosopterins	$.d_1$	0.20 (0.09)	0.04 (0.03)	
	$.d_2$		0.17 (0.11)	414
	$.d_3$		0.27 (0.19)	
Ceratitis c. pigment M		0.20 (0.10)	0.20 (0.13)	412

(a) Unbracketed figures indicate the relative mobility respective to that of Biopterin (R_b) and figures between brackets show the mobility respective to solvent front (R_f). Solvent A: n-butanol: acetic acid: water (20: 3: 7); Solvent B: 2-propanol: 2% Ammonium acetate (1 : 1).

The flourescence color under U.V. light of *C. capitata* substance M spots was orange, identical to that of *D. melanogaster* drosopterin. Table 1 shows that both pigments showed identical relative mobility in Solvent A and that three *Drosophila* drosopterins, d1, d2 and d3, were resolved by chromatography in solvent B. *Ceratitis* M pigment showed similar intermediate mobility as d2 drosopterin. Table 1 also shows that maximum absorbance wavelength of the putative *Ceratitis* drosopterins. In these flies both pigments appear at equivalent times during development (i.e. early pharate adult stage) (Hadorn and Mitchell, 1951).

From the above data, that are consistent with previous studies in *Drosophila* by Descimon and Barial (1966), it can be postulated that *Ceratitis* pigment M seems to be a pteridine closely related or identical to a member of the *Drosophila* drosopterin family.

References: Descimon, H. and M. Barial 1966, J. Chromatog. 25:391-397; Dorsett, D. *et al.* 1979, Biochemistry 18:2596-2600; Needham, A.E. 1978, pp. 233-308 in Biochemistry of Insects (Rockstein, M. ed.), Acad. Press, N.Y.; Rabossi, A. 1991, M.Sc. Dissertation, FCEyN, Univ. Bs. Aires; Wilson, T.G. and K.B. Jacobson 1977, Biochem. Genetics 15:307-319; Ziegler, I. and M. Féron 1965, Z. Naturforschg. 20 b:318-322.

Ruiz, D.G. Universidad Austral de Chile, Valdivia, Chile. Aggregation rates in laboratory strains of *Drosophila melanogaster*.

The aggregated oviposition of several species of *Drosophila* has been reported in laboratory and nature by different investigators. The causal factor commonly invoked to explain this aggregation pattern lies in the organism's response to the environmental heterogeneity, such as substrate's texture and color, temperature, humidity, intensity of light, chemical compounds, density, and senescence of females.

However, experiments of selection for high and low aggregation intensity in *Drosophila melanogaster* determined that these preferences were under genetic control (Ruiz and del Solar 1986). Furthermore, hybridization of the selected lines showed that this behavior possesses an additive polygenic basis with some genes showing dominant effects (Ruiz and del Solar 1991).

Another approach to the analysis of genetic systems is to verify whether two or more strains differ in the mean phenotypic expression of a behavioral trait. In order to check this assumption I evaluated the gregarious rate of seven laboratory strains of *Drosophila melanogaster*: *B*(1-57.0), *Cy*(2-6.1), *dp*(2-13.0), *e*(3-70.7) and *tx*(3-91.0).

In a population cage containing 25 tubes (2.0 cm diameter; provided with Burdick culture medium), arranged in 5 by 5 rows and columns, 25 four-five day old females of one strain were placed to oviposite. After 24 h, the females were removed and the number of eggs in each tube was counted.

Aggregation intensity was measured statistically by the variance-mean ratio. In an aggregated distribution, the population variance exceeds the population mean (Pielou 1977). Thirty replicates for each of the strains were made.

Table 1 shows the mean and standard error of the thirty replicates of each strain. The strains exhibited different degrees of aggregated spatial distribution for oviposition, as confirmed by ANOVA ($F = 15.45$, $P < 0.001$). *Ebony* was the most aggregated strain, whereas *Bar* was the less aggregated one. To statistically differentiate any single strain from each other, the Least Significant Difference (LSD) method was carried out. It was found that *Bar, dumpy, ebony* and *taxi* are significantly different in the aggregation intensity for oviposition.

Table 1. Mean and standard error of the aggregation rate of seven laboratory strains of *Drosophila melanogaster*

Strain	Mean	S.E.	
* Bar	10.7	0.8	
white	16.1	1.6	
Curly	12.7	1.7	
* dumpy	29.0	1.8	F= 15.45, P < 0.001
vestigial	15.4	2.3	
* ebony	39.7	2.7	
* taxi	18.0	1.9	

*Different significantly, P< 0.05 according to LSD.

At present, these *Drosophila* strains have been used in the diallele cross to uncover the different components of the genotypic variance of the aggregated oviposition.

References: Pielou, E.C. 1977, Mathematical Ecology, John Wiley and Sons, N.Y.; Ruiz, G. and E. del Solar 1986, Austr. J. Biol. Sci., 39:155-160; Ruiz, G. and E. del Solar 1991, Evol. Biol. (in press).

Benado, M. and E. Weir. Departamento de Biologia Celular y Genetica, Facultad de Medicina, Universidad de Chile and Departamento de Estudios Ambientales, Universidad Simon Bolivar, Caracas, Venezuela. Oviposition choice in the cactophilic *Drosophila martensis*.

Drosophila martensis is a cactophilic insect that lives in Colombia and Venezuela (Benado et al., 1984). Field studies have shown that this *Drosophila* exploits the fermenting tissues of several columnar and prickly pear cacti, the columnar *Subpilocereus repandus* being its major host (Table 1; Benado, 1989). In this note, we study in the laboratory host preference for oviposition of *D. martensis,* using several Cactaceae on which the fly feeds in nature. We test two related hypotheses: I) the fly does not choose hosts, and II), *S. repandus* is the major host for oviposition.

Material and Methods

We used a strain of *D. martensis* from Guaca (Benado et al., 1984), kept in the laboratory on standard medium for several years. Four cacti species were used for the oviposition-choice trials: *Acanthocereus tetragonus* (1), *Pilosocereus lanuginosus* (2), *Stenocereus griseus* (3), and *S. repandus* (4). We fermented fresh tissue samples with innocula from the same species collected in nature. Afterwards, 15 g each of fresh and fermenting tissues were diluted in 270 ml of water. 25 ml of this solution was diluted, again, in 200 ml of water, and 4 g of agar were added; after warming, 1 ml of propionic

Table 1. Emergences of *D. martensis* in the field (Benado , 1989; Benado et al., 1984).

Substrate	1	2	3	4	other
# rots	7	38	74	35	91
# flies	<5	20	—	4640	369

Substrates: 1: *Acanthocereus tetragonus*; 2: *Pilosocereus lanuginosus*; 3: *Stenocereus qriseus*; 4: *Subpilocereus repandus* .

Table 2 . Oviposition choice (# of eggs) in *D. martensis* .

						AGED									
D	1					2					3				
S	1	2	3	4	N	1	2	3	4	N	1	2	3	4	N
R															
1	0	0	7	0	7	127	79	64	0	270	0	1	278	85	364
2	128	0	47	3	178	0	24	14	109	147	340	0	0	217	557
3	63	87	0	0	150	0	33	71	0	104	133	0	0	372	505
4	90	0	0	0	90	132	0	0	33	165	169	58	0	33	260
5	78	0	44	16	138	211	2	50	0	263	33	16	0	58	107
6	142	0	3	0	145	359	0	0	0	359	0	0	1	11	12
7	0	0	0	0	0	493	48	0	0	541	84	35	41	0	160
8	119	23	1	0	143	14	57	91	36	198	0	115	0	15	130
9	142	0	0	0	142	143	43	0	0	186	27	623	0	0	650
N	762	110	102	19	993	1479	286	290	178	2233	786	848	320	791	2745
						FRESH									
R															
1	54	49	86	0	189	34	0	0	0	34	214	42	16	31	303
2	137	35	0	0	172	238	76	71	0	385	84	0	0	0	84
3	25	0	0	0	25	113	65	0	0	178	83	33	0	0	116
4	54	0	0	11	65	422	44	0	36	502	121	0	0	50	171
5	39	0	0	0	39	6	0	0	0	6	0	0	0	0	0
6	59	275	26	46	406	452	23	0	0	475	45	30	34	31	140
7	21	42	0	0	63	127	27	0	0	154	17	0	0	0	17
N	389	401	112	57	959	1392	235	71	36	1734	564	105	50	112	831

R: replicate; S: substrate (Table 1); N: total eggs; Aged: aged substrate; Fresh: fresh substrate; D: days, 1, 2, 3.

Table 3. Non parametric Q-test for oviposition differences between substrates. Symbols as for Table 1.

substrate	2	4	day
AGED MEDIUM			
1	2.94*	3.17**	1
1	2.70*		2
substrate	3	4	day
FRESH MEDIUM			
1		2.74*	1
1	3.33**	3.44**	2
1	2.74*		3

* $p < 0.05$; ** $p < 0.01$

acid was added. The resulting solution was poured into one of the cells of a Petri dish 9 cm D X 1.5 cm H, divided into 4 pieces of equal area. The media were distributed in 3 different sequences (1, 2, 3, 4; 1, 3, 2, 4; 1, 3, 4, 2), to minimize the influence of cactus distribution on the dish on oviposition choice. The Petri dishes were put into a plastic box with a mesh, 24 cm W X 27.7 cm L X 13.5 cm H. Two experimental protocols were followed: either the same plate was reintroduced into the box after egg counting (aged media experiments, AM), or an identical fresh one replaced the old plate (fresh media experiments, FM). Eggs were counted on 3 consecutive days. 30 males and 60 females, 7-10 days old, were introduced in the box in each trial. The experiments were carried out at 28°C with a 12 h dark/light cycle. Data were analyzed with non-parametric ANOVAs and non-parametric multiple comparisons (Q-test, Zar 1984).

Results and Discussion

Egg laying is given in Table 2. Among-substrate and among replicate variation was high. However, there was a clear choice for *A. tetragonus*. Non-parametric ANOVAs detected significant differences among substrates for days 1 and 2 for the AM, and for days 1, 2, and 3 for the PM. Q-tests indicated that significant differences for oviposition existed between *A. tetragonus* and several other Cactaceae (Table 3).

The high preference shown for *A. tetragonus* suggests that the fly oviposits on this cactus in nature. However, very few individuals have been found emerging from this columnar (Table 1). Either egg-to-adult viability in the field is poor, or the cactus is less available than other Cactaceae. The frequency of rots for *A. tetragonus* is about 3% (Table 1), indicating a lower availability when compared with the other cacti. On the other hand, *A. tetragonus* is smaller than other columnar cacti (pers. observ.) and, consequently, may be a more ephemeral resource. It is interesting that in the laboratory *D. martensis* does not choose substrates when eggs are already laid on them (day 3, Table 2), and that it oviposits on substrates where the fly is not found in the field (*Stenocereus griseus,* Tables 1 and 2). These findings make more complex the interpretation of rearing records, widely used when studying cactophilic niches.

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References: Benado, M. 1989, Ecotropicos 2:45-48; Benado, M., A.

Fontdevila, H. Cerda, G. Garcia, A. Ruiz and C. Montero 1984, Biotropica 16:120-124; Zar, J.H. 1989, Biostatistical Analysis, 2nd ed., Prentice Hall, N.J.

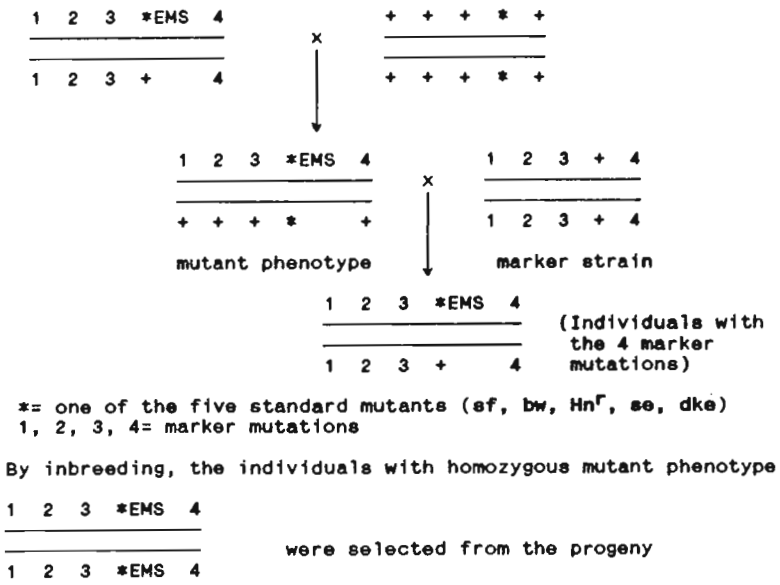
Millán, J.M. and C. Nájera. Departament de Genética, Universitat de Valencia, Dr. Moliner, 50, 46100 Burjassot (València) Spain. Mutagenesis at five pteridine pathway loci in *Drosophila melanogaster* with the alkylating agent ethyl-methanesulfonate.

The ability of EMS for inducing mutation in *Drosophila* has been well established (Epler, 1966; Jenkins, 1967; Patton and Sullivan, 1978; Reynolds and O'Donell, 1988).

In this report we have analyzed the frequency of mutation for five eye colour mutations affecting the pteridine pathway (*sf, se, bw, dke* and Hn^r).

The mutations were obtained through 4 experiments by EMS mutagenesis of *D. melanogaster* males, homozygous for second chromosomes marked with *al b cn sp* or for third chromosomes marked with *st ri ss*.

So, 3 days males were fed in 40 mM EMS solution in 5% sucrose for 24 hours at room temperature according to the procedure of Lewis and Bacher (1968). The treated males were allowed 24 hours to recover from the EMS treatment. Each male was placed in a vial containing standard medium and were crossed with 6 or 8 virgin mutant female carriers of standard alleles of one of the five studied mutations. The males were transferred to fresh virgin females every two days, each transfer constituting a brood. This type of brood analysis allows us to sample premeiotic, meiotic and postmeiotic sperm cells. The first three broods (0 to 6 days) sample postmeiotic cells. The fourth brood from the F_1, individuals with mutant genotype were isolated according to the mutant isolation scheme at the right.



The number of offspring observed, the number of mutants obtained for each mutation and the proportion of mosaics and complete mutants for each experiment can be observed in Table 1.

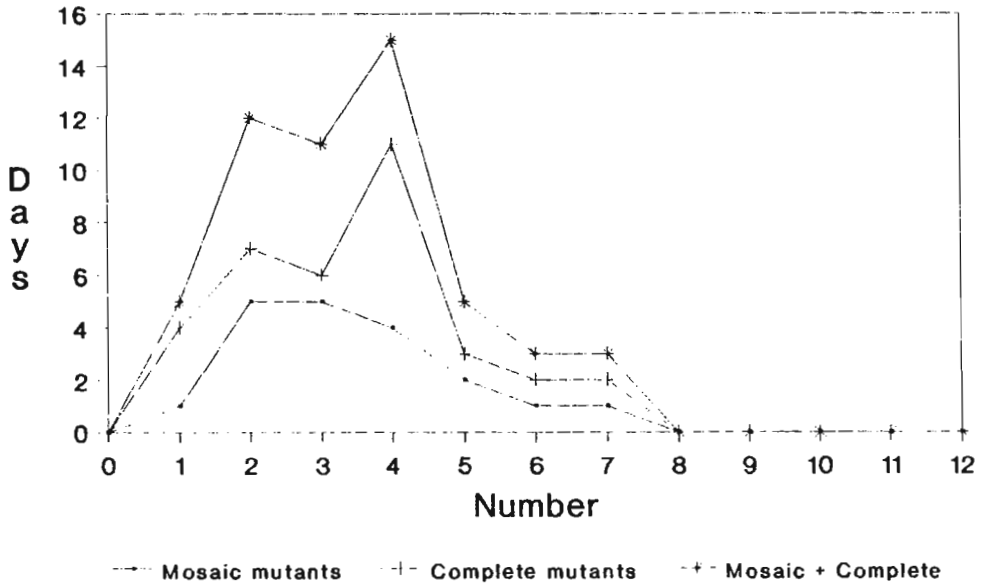


FIGURE 1.

Table 1. Induction of five pteridine pathway mutations by EMS

1st EXPERIMENT

Mutation	Number of F1 flies screened	Number of mosaics	Number of complete mutants
se	4812	0	0
sf	8419	1	4
bw	5713	3	2
TOTAL	19018	4	6

2nd EXPERIMENT

Mutation	Number of F1 flies screened	Number of mosaics	Number of complete mutants
se	3941	1	0
sf	4103	0	1
bw	4668	2	7
Hrf	1531	0	0
TOTAL	14243	3	8

3rd EXPERIMENT

Mutation	Number of F1 flies screened	Number of mosaics	Number of complete mutants
se	23445	0	0
sf	7058	1	3
bw	8273	0	0
Hrf	4886	0	0
dke	1441	0	0
TOTAL	50331	1	3

4th EXPERIMENT

Mutation	Number of F1 flies screened	Number of mosaics	Number of complete mutants
Hrf	33530	5	7
dke	31598	7	11
TOTAL	65128	12	18

Table 2. Mosaic, complete and total frequencies for each one of the five mutants.

Mutation	n♀ flies screened	Mosaics		Completes				Total	
		n♀	freq.	n♀	freq.	recov.	freq.	n♀	freq.
se	32198	1	3.1×10^{-5}	0	0	0	0	1	3.1×10^{-5}
sf	19654	2	1.1×10^{-4}	8	4.1×10^{-4}	4	2.0×10^{-4}	10	5.1×10^{-4}
bw	18654	4	2.1×10^{-4}	9	4.8×10^{-4}	1	5.3×10^{-5}	13	6.9×10^{-4}
Hrf	39947	5	1.3×10^{-4}	7	1.8×10^{-4}	1	2.5×10^{-5}	12	3.0×10^{-4}
dke	31598	7	2.2×10^{-4}	11	3.5×10^{-4}	1	3.2×10^{-5}	18	5.7×10^{-4}

freq. (frequency); recov. (recovered)

Table 3. Percentage of mutations appeared during the first 4 days versus the subsequent 4.

Mutation	First 4 days			Subsequent 4 days		
	mos	com	tot	mos	com	tot
sf	100.00	87.50	90.00	0	12.50	10.00
dke	100.00	81.82	88.89	0	18.18	11.11
bw	75.00	100.00	92.31	25.00	0	7.69
se	100.00	0	100.00	0	0	0
Hrf	40.00	85.71	66.67	60.00	14.29	33.33
Total	78.95	88.57	85.19	21.05	11.43	14.81

mos (mosaics); com (complete mutants); tot (totals: mosaics + completes)

The number of total F_1 flies screened for each mutation grouping the four mutagenesis experiments, the number of mosaics obtained for each strain, its frequency, the number of total mutants and the number recovered from them, their frequencies and the total frequencies of mutation for each one of the five mutations are shown in Table 2.

Considering separately the results for each one of the five mutations:

safranin From the 39308 chromosomes tested, 10 phenotypically mutant individuals were found in the F_1 from which 4 inheritable recessive mutations were isolated. 20% of the phenotypic mutants could be classified as mosaics.

brown 37308 chromosomes were tested and 10 phenotypically mutant individuals were found from which only one could be isolated. 30.7% of the phenotypic mutants were mosaics.

dark-eye 63198 chromosomes were tested and 18 phenotypically mutant individuals were found in the F_1 from which only one could be recovered.

Henna recessive 79894 chromosomes were tested and phenotypically mutant individuals were found in the F_1 from which only one could be recovered. 41.6% of the F_1 mutants could be classified as mosaic.

sepia From the 64396 chromosomes tested only one individual was classified as mosaic for this mutation and could not be recovered.

Figure 1 shows the order of appearance of the different mutants through the time. In Table 3 can be observed that the percentage of the mutations appearing during the four first days (85.19%) is significantly higher than the percentage of the mutations appearing during the four following (14.81%). So, the most striking aspect of the EMS mutagenesis is its exclusive action on postmeiotic sperm cells.

No cryptic mutants were found for any of the five mutations.

References: Epler, J.L. 1966, Genetics 54:31-36; Jenkins, J.B. 1967, Genetics 57:783-793; Lewis, E.B. and F. Bacher 1968, D.I.S. 43:193; Patton, D.R. and D.T. Sullivan 1978, Biochem. Genet. 16:855-865; Reynolds, E.R. and J.N. O'Donell 1988, Genetics 119:609-617.

Millán, J.M. and C. Nájera. Departament de Genètica, Universitat de Valencia, Dr. Moliner, 50, 46100 Burjassot (Valencia) Spain. Pigment patterns in mutants affecting the pteridines pathway in *Drosophila melanogaster.*

Two classes of pigments contribute to the final colour of *Drosophila* eyes: the ommatins and the pterins.

The pathway of pteridines is not yet understood but the information available was summarized in a hypothetical pathway proposed by Ferré *et al.* (1986).

In order to obtain the chromatographic pattern of seven *D. melanogaster* EMS-induced mutants (4 *sf*, 1 *dke*, 1 *Hn*^{r}, 1 *bw*), the method of Ferré *et al.* (1986) was used. All the compounds were measured after separation by thin layer chromatography and semiquantitative pteridin patterns were obtained and compared to the chromatographic pattern of each standard mutant. In Table 1 are reflected the results. *bw2MN* and *dke3MN* have chromatographic patterns very different to the standard patterns of the same mutants. *sf2MN* and *sf6MN* have the same chromatographic pattern and *sf4MN* and *sf5MN* have a very similar one but different from the other two. *Hn*^{r}*3MN* has a decrease of drosopterins in respect to its standard mutant with lack of aurodrosopterin, isoxanthopterin and pterin.

Table 1. Semiquantitative estimation of pteridines in eye colour induced mutants of *Drosophila melanogaster*

Strain	NDP	ADP	DP	SP	ADHP	IXP	PTE	BP
Or-R	+	+	+	+	+	+	+	+
bwst	0	0	0	0	0	0	0	0
bw2MN	-1	-1	-2	+	-1	+	0	+3
dkest	-1	+	-1	+	+	+	-1	+
dke3MN	+	-1	-1	-1	+	-1	-1	+1
Hn^{rst}	-1	-2	-1	+2	+1	-1	-1	+
Hn^{r}3MN	-2	0	-2	+1	+1	0	0	+1
sfst	+	+	-1	-1	+	+	+	+
sf2MN	+	+	+	-1	+	-1	+	+1
sf4MN	-1	-1	-1	-1	+	-2	+	+1
sf5MN	-1	-1	-1	-1	+	-1	-1	+1
sf6MN	+	+	+	-1	+	-1	+	+1

+ amount approximately equal to Or-R ; +1, +2, +3 increasing amounts; -1,- 2 decreasing amounts; 0 not detected; MN induced mutants; st standard mutants NDP, neodrosopterin; ADP, aurodrosopterin; DP, drosopterin; SP, sepiapterin; ADHP, acetyldihidrohomopterin; IXP, isoxanthopterin; PTE, pterin; BP, biopterin

Table 2. Quantitative estimation of the red pigment of eye colour induced mutants of *Drosophila melanogaster*

Strain	Absorbance at 480 nm	%with respect to Or - R
bwst	0.010 ± 0.001	1.44 ± 0.13
bw2MN	0.101 ± 0.004	22.52 ± 0.42
dkest	0.481 ± 0.006	72.92 ± 0.91
dke3MN	0.230 ± 0.008	51.22 ± 0.63
hn^{rBt}	0.177 ± 0.003	26.82 ± 0.41
Hn^{r}3MN	0.103 ± 0.004	22.94 ± 0.15
sfst	0.280 ± 0.007	42.48 ± 1.03
sf2MN	0.238 ± 0.005	53.01 ± 0.74
sf4MN	0.183 ± 0.005	40.76 ± 0.98
sf5MN	0.182 ± 0.007	40.53 ± 0.84
sf6MN	0.242 ± 0.007	53.90 ± 0.72

Table 3. Quantitative estimation of isoxanthopterin in male bodies of different induced mutants of *Drosophila melanogaster*

Strain	Fluorescence	% with respect to Or - R
bw2MN	49.6 ± 0.1	91.85 ± 0.27
dke3MN	40.7 ± 0.2	76.32 ± 0.73
Hn r3MN	1.0.± 0 3	1.90 ± 0.04
sf2MN	45.2 ± 0.4	86.10 ± 0.78
sf4MN	24.0 ± 0.2	45.71 ± 0.81
sf5MN	35.7 ± 0 7	67.94 ± 0.91
sf6MN	31.9 ± 0.2	60.90 ± 0.82

The induced mutants were analyzed for their total drosopterin levels according to the method of Real *et al.* (1985). In Table 2 can be observed the quantitative estimation of the red pigment (absorbance at 480 nm) and the relative percentage with respect to the wild strain Or-R, the same as these values for the same standard mutants. *bw2MN* has a level of pteridines higher than the standard mutant, approximately 20% of the Or-R level. In *dke3MN* and *Hn*^{r}*3MN* is clear the decrease of the level of pteridines in respect to the standard mutants and in *sf* mutants, there are two alleles (*sf2MN* and *sf6MN*) with a level of pteridines similar to the

standard mutant level and the other two with a decreased level.

In Table 3 is reflected the quantitative estimation of the isoxanthopterin in the induced mutants according to the method of Silva and Mensua (1987). The most striking is the practically lack of isoxanthopterin in *Hn^{r}3MN* mutant strain.

References: Ferré, J., F.J. Silva, M.D. Real, and J.L. Ménsua 1986, Biochem. Genet. 24:545-569; Real, M.D., J. Ferré, and J.L. Ménsua 1985, D.I.S. 61:198-199; Silva, F.J. and J.L. Ménsua 1987, D.I.S. 66:166.

Kim A.I.[1], B.M. Khudaybergenova[1], E.S. Belyaeva[2], M.M. Aslanian.[1] [1]M.V. Lomonosov Moscow University, Dept. of Genetics, Moscow, Russia; [2]Inst. of Molecular Genetics, Moscow, Russia. Transposition of mobile element *gypsy* and mutational changes in genetically unstable mutator strain of *D. melanogaster*.

Autonomous transpositions of *gypsy* and *hobo* were shown earlier to occur in genetically unstable MS strain while the distribution of 12 other families (retrotransposons, retroposons, transposons) of mobile elements was invariant (Kim *et al.* 1990). It was suggested that the spontaneous transpositions of *gypsy* and/or *hobo* might be the reason of genetical instability in the MS (increase of the frequency of morphological mutations and chromosomal aberrations, appearance of unstable mutations, etc). Our experiments demonstrated the participation of *gypsy* insertions in the mutation induction in the loci of insertions. The subject of the present paper is the comparison of behavior of both *gypsy* and some other retrotransposons during the mutational changes which occur in the MS and its derivatives.

The distribution of *gypsy*, *412* and *mdg1* was studied in the X chromosomes of the MS, of visible mutants obtained from the MS - ct^{13K} and f^{14K}, and of their revertants by *in situ* hybridization. All studied chromosomes were maintained intact (non-crossover) by crossing of males of above strains with females carrying attached X chromosomes *C(1)RM, y w f* / Y. Male progeny were analyzed genetically for carrying previous or new mutations by *in situ* hybridization for the localization of mobile elements.

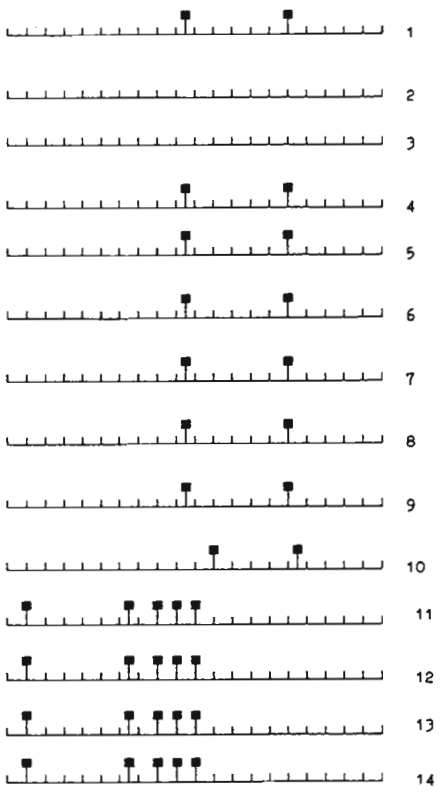


Figure 1. Localization of *gypsy* in the X chromosomes of studied stocks. 1, f^{14K}; 2, $f^{14K+(1)}$; 3, $f^{14K+(2)}$; 4-6, non-inverted siblings for 2; 7-9, non-inverted siblings for 3; 10, $ct^{13K+(1)}$; 11, ct^{13K}; 12-14, non-inverted siblings for 11.

The results obtained have demonstrated that the mutations studied - ct^{13K} and f^{14K} - were caused by insertions of *gypsy* because we have observed the hybridization of *gypsy* labeled probe with 7B and 15 EF of X chromosomes respectively (Figure 1). Localization of *gypsy* copies for the mutants was different both in number of copies and in their distribution and differed from that in the MS. At the same time the distribution of *mdg1* and *412* in both mutant chromosomes was identical and coincided with that for the MS. Thus one may conclude that during mutational changes autonomous transpositions of only *gypsy* but not other studied elements, take place. In the study of revertants two independent back mutants of f^{14K} and one of ct^{13K} were analyzed. The distribution of *mdg1* and *412* was shown to be unchanged (Figures 1 and 2). However, the pattern of *gypsy* distribution in the X chromosomes of the revertants differed greatly from initial mutants and each other. Both number of *gypsy* hybridization sites and their distribution were changed. Thus the process of mutational changes of f^+ (MS) -----> f (mutant) -----> f^+ (revertant) (the speculations for *ct* are the same) involved the mobilization and transposition of *gypsy* only, but not other elements (*mdg1* and *412* for example). The analysis of the X chromosomes of non-mutated sibling males appearing in the same vial with revertants detected no changes in the localization pattern of the investigated mobile elements (Figures 1 and 2). For each revertant three non-reverted stocks were studied.

Our findings confirmed the autonomous transposition of *gypsy* but not other retrotransposons (*mdg1*

and *412*) in the genetically unstable MS strain. Transpositions of *gypsy* do not occur in all germ cells of MS-individuals and its mutants. Our results suggest several ways or types of mobile element transposition in the *D. melanogaster* genome. They can move by way of simultaneous coordinated transpositions of different families as it was documented for stocks with fitness changes (Belyaeva *et al.*, 1982P and/or during transposition explosions in stocks similar to ct^{MR2} system (Gerasimova *et al.*, 1983). The alternative possibility may be the movement of single or few families of elements as we have described for the MS-system (Kim and Belyaeva 1986; Kim *et al.*, 1990) and as it has been detected by Lim (1988) for *Uc* chromosome, Biemont (1987) for inbred stocks of natural origin and Georgiev for element *Stalker* (1990).

Figure 2. Localization of mdg1 (dark square) and 412 (*) in the X chromosomes of the studied stocks. Distribution of the elements in all strains is identical.

References: Belyaeva E.S., Pasyukova E.G., Gvozdev V.A. *et al.*, 1981, Genetica (USSR) 17:1566-1580; Biemont C., Aouar A., Arnault C. 1987, Nature 329:742-744; Georgiev P.G. 1990, Dokl. Acad. Nauk (USSR) 310:470-474; Gerasimova T.I., Mizrokhi L.J., Georgiev G.P. 1984, Nature 309: 714-716; Lim J.K. 1988, Proc. Natl. Acad. Sci. (USA) 856:9153-9157; Kim A.I., Belyaeva E.S. 1986, Dokl. Acad. Nauk (USSR) 289:1248-1252; Kim A.I., Belyaeva E.S., Aslanian M.M. 1990, Mol. Gen. Genet. 224:303-308.

Millán, J.M. and C. Nájera. Departament de Genètica, Universitat de Valencia, Dr. Moliner, 50, 46100 Burjassot (Valencia) Spain. Viabilities and developmental times in natural and EMS-induced eye colour mutants of *Drosophila melanogaster*.

In the present report we have analyzed viability and developmental time of 23 *D. melanogaster* mutant strains. 16 of them (7 *sf*, 3 Hn^r, 3 *se*, 2 *dke*, 1 *bw*) were laboratory strains coming from captures carried out by Nájera and Ménsua (1988) in three different natural populations (a cellar, a vineyard and a pine-wood) in spring and in autumn of the same year. The other 7 (4 *sf*, 1 *dke*, 1 Hn^r, 1 *bw*) were isolated from four mutagenesis experiments with EMS. Viability and developmental time were also analyzed for all the transheterozygous combinations of the five mutations (*sf, se,* Hn^r, *dke* and *bw*). For viability testing one hundred eggs were placed in each vial and a total of ten replicates were made in each case. For developmental time testing, the formula $n_i \times i / n_t$ was used, where n_i = total flies emerged in day *i* and n_t = total flies emerged in the vial. A total of ten replicates were also made in each case.

In Table 1 (next page) can be observed the analysis of variance of the mean viabilities for all mutant strains (natural and induced) viability of EMS-induced mutants is similar to that of the natural ones. With regard to the transheterozygous genotypes, viability is, in general, higher than viability of the parental strains except for *se* mutants.

Table 2 (next page) shows the analysis of variance of the mean developmental time of all mutants (natural and induced) for each one of the five mutations grouped by underlining the means that do not differ significantly. A high variability and a trend to increase the mean developmental time for the induced mutants can be observed.

With regard to the transheterozygous genotypes, a tendency to decrease the mean developmental time with respect to the parental strains for *se* transheterozygotes and a tendency to increase the mean developmental time with respect to the parental strains for *sf* transheterozygotes can be observed.

Table 3. Comparison of the viabilities of the induced mutants at 25°C and 16°C

Strain	df	t
sf2MN	18	1.96 ns
sf4MN	18	8.32***
sf5MN	18	10.44***
sf6MN	18	4.25 ***
Hn^r3MN	18	5.55***
dke3MN	17	0.19 ns
bw2MN	18	- 4.27***

df = Degrees of freedom;
t = Values of t test ; *** = p < 0.001

Table 4. Comparison of the viabilities of the induced mutants versus its heterozygotes with Or-R.

Strain	df	t
sf2MN	18	10.02 ***
sf4MN	18	1.45 ns
sf5MN	18	6.35 ***
sf6MN	18	7.29 ***
Hn^r3MN	18	25.28 ***
dke3MN	16	6.06 ***
bw2MN	18	25.98 ***

df = Degrees of freedom; t = Values of t test ; *** = p < 0.001

The viability of the induced mutants was studied at two different temperatures: 25° and 16°C. All the strains except *bw2MN* have higher viability at 25° although in *dke3MN* and *sf2MN* strain differences were not significant (Table 3).

The viability of heterozygous combinations of the induced mutants with Or-R was also studied and in all cases heterozygotes have a higher viability although in the *sf4MN* strain differences were not significant (Table 4).

References: Nájera, C. and J.L. Ménsua 1988, Genet. Sel. Evol. 20:25-36.

Table 1. ANOVA of the mean viabilities of natural and induced mutant strains

SAFRANIN

Source of variation	df	SS	MS	F
Among groups	10	10.3222	1.0332	77.9162***
Within groups	99	1.3286	0.0134	
Total	109	11.6508		

Mean	Strain
-0.5381	I9B0
-0.3740	23BP
-0.3297	51B0
-0.2170	2MN
-0.1171	2P0
0.0675	17V0
0.0839	5MN
0.1574	6MN
0.2962	1VP
0.3853	16VP
0.4046	4MN

HENNA RECESSIVE

Source of variation	df	SS	MS	F
Among groups	3	9.1080	3.0360	167.8797***
Within groups	36	0.6510	0.0181	
Total	39	9.7590		

Mean	Strain
-0.9028	63B0
-0.7370	28BP
-0.4645	3MN
0.3397	20VP

SEPIA

Source of variation	df	SS	MS	F
Among groups	2	0.1752	0.0876	4.0697*
Within groups	27	0.5811	0.0215	
Total	29	0.7563		

Mean	Strain
0.1517	3V0
0.2890	85B0
0.3305	33BP

DARK-EYE

Source of variation	df	SS	MS	F
Among groups	2	0.8306	0.4153	6.3681**
Within groups	26	1.6956	0.0652	
Total	28	2.5262		

Mean	Strain
-0.2391	58B0
0.0801	3MN
0.1426	1BP

BROWN

Source of variation	df	SS	MS	F
Among groups	1	0.6553	0.6353	60.0009***
Within groups	18	0.1996	0.0106	
Total	19	0.8259		

df = Degrees of freedom; SS = Sums of squares; MS = Mean squares;
F = Values of F test; * = $p < 0.05$; ** = $p < 0.01$ ***; = $p < 0.001$
C = cellar , V = vineyard, P = pine-wood, A = autumn, S = spring,
MN = induced mutants

Table 2. ANOVA of the mean developmental times of natural and induced mutant strains

SAFRANIN

Source of variation	df	SS	MS	F
Among groups	10	79.7010	7.9701	8.5018***
Within groups	99	92.8088	0.9375	
Total	l09	172.5099		

Media	Strain
11.2307	2P0
11.3951	I9B0
11.6437	6MN
11.6923	16VP
11.9979	1VP
12.0157	23BP
12.0661	51B0
12.5795	5MN
12.7000	4MN
13.1343	2MN
14.3179	17V0

HENNA RECESSIVE

Source of variation	df	SS	MS	F
Among groups	3	36.9976	12.3325	34.9413***
Within groups	28	9.8826	0.3529	
Total	31	46.8802		

Media	Strain
10.3889	63B0
10.5990	28BP
12.3135	3MN
13.0496	20VP

SEPIA

Source of variation	df	SS	MS	F
Among groups	2	3.5275	1.7638	8.7295**
Within groups	27	5.4553	0.2020	
Total	29	8.9828		

Media	Strain
11.5528	3V0
12.0559	85B0
12.3868	33BP

DARK-EYE

Source of variation	df	SS	MS	F
Among groups	2	10.2697	5.1349	17.0275***
Within groups	25	7.5395	0.3016	
Total	27	17.8092		

Mean	Strain
11.8772	58BO
11.9619	1BP
13.2164	3MN

BROWN

Source of variation	df	SS	MS	F
Among groups	1	10.2588	10.2588	21.8066***
Within groups	18	8.4680	0.4704	
Total	19	18.7267		

df = Degrees of freedom; SS = Sums of squares; MS = Mean squares;
F = Values of F test; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$
C = cellar , V = vineyard, P = pine-wood , A = autumn , S = spring ,
MN = induced mutants