

gles will not develop, although when placed in medium without Actinomycin, these bristles will grow if done at the proper developmental time. Possible explanations of the very low concentrations needed for inhibition were also presented. Further experiments have given more information on this phenomenon.

Whole brain complexes (cerebral hemispheres, optic lobes and attached eyes) were dissected 25 1/2 hr after puparium formation and placed in approximately 1 ml Schneider's medium containing Actinomycin D (Merck, Sharp & Dohme) to 0.075 µg/ml. They were removed with a fine pipette after 5 min and washed twice in 2 ml each of Schneider's medium without the antibiotic. They were then placed in hanging drop cultures containing no inhibitor as described in Hanly, Fuller and Stanley (1967) and observed for a 72 hr period. Appropriate controls were also cultured.

Eyes with attached optic lobes, but without cerebral hemispheres, competent to form ommatidial bristles (27 1/2 hr after puparium formation) were also dissected and treated in like manner. Eyes alone were similarly tested by dissecting at 28 1/2 hr after puparium formation. Controls in these cases consisted of one side of a complex while experimentals were the opposite side.

In all cases examined, ommatidial bristles developed in the controls while none appeared in the treated eyes (approximately 100 cultures). Other developmental processes occurring in the eye and brain during this time (growth of the ommatidia, apparent deposition of pigment granules, extension of the outer optic glomerulus, and growth of the optic nerve) did not seem to be affected by the antibiotic treatment. Subsequent deposition of normal in vitro amounts of corneal material was also apparently not affected. These results indicate that RNA synthesis necessary for developmental functions other than ommatidial bristle synthesis has occurred prior to or occurs after the pulses of Actinomycin D; or that the DNA locus or loci responsible for bristle synthesis are particularly sensitive to low concentrations of the inhibitor.

Cultures were also established in order to determine the sensitivity of optic lobes with attached eyes over different time periods of development. These tissues were dissected, with appropriate controls, every half hour from 27 1/2 through 33 hr. Eye-optic lobe complexes dissected at 29 1/2 hr following puparium formation and treated with Actinomycin D developed very short, fine bristles. Those dissected and treated at 30 hr had slightly longer and larger bristles. Each subsequent half hour produced longer bristles until normal-sized bristles were produced in cultures of eyes dissected at 31 hr. Bristles normally appear in vivo between 32 and 33 hr at 26°C following puparium formation. These results indicate that at least some DNA-dependent RNA necessary for ommatidial bristle development is synthesized approximately 2 1/2 - 3 hr before its apparent function is seen. Fristrom (personal communication) and others have calculated a "half-life" of some messenger-type RNA molecules in *Drosophila* to be between 2 1/2 and 3 1/2 hr. Furthermore, it appears that for normal bristle development, continued RNA synthesis is necessary for approximately 1 1/2 hr. This could be a developmental "maintenance" process.

During the course of these experiments it was noted that if concentrations of Actinomycin D of 0.05 µg/ml were used, or if the exposure period used was less than 5 min, some ommatidial bristles would develop. They were, however, usually abnormal: irregular, short and stubby, multiple bristles from single sockets, or bent and crooked. Many of these appeared similar to those in the singed phenotype.

References: Hanly, E.W., C.W. Fuller and M.S. Stanley 1967, The morphology and development of *Drosophila* eye. I. In vivo and in vitro pigment deposition. *J. Embryol. exp. Morphol.* 17:491-499; Hanly, E.W. and W.H. Hemmert 1967, Morphology and development of the *Drosophila* eye. II. In vitro development of ommatidial bristles. *J. Embryol. exp. Morphol.* 17:501-511.

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Mitra, N. and A.S. Mukherjee. University of Calcutta, India. Continuous <sup>3</sup>H-TdR labeling pattern as the beginning of replication cycle: further evidence.

The results of the present experiment throw some light on the controversy regarding the type of labeling pattern at the initiation of the DNA replication cycle in the polytene chromosomes of *Drosophila melanogaster*. Ninety-hour old, late third instar larvae were fed for 2 hr on mitomycin C mixed in the medium at a conc. of 0.02 ml/gm of food (conc. 2 mg/ml). Excised salivary glands were subjected to autoradiography after a 20 min incubation in 200 µCi/ml of <sup>3</sup>H-

TdR (Sp. Act. 6000 mCi/mM; BARC; Trombay). DNA replication patterns were analysed from autoradiograms prepared after a 25 day exposure by categorizing the patterns into 2C (light continuous), 3C (heavy continuous), 3D (heavy discontinuous), 2D (medium discontinuous), 1D (light discontinuous) types after Rodman (1968). It was seen that in comparison with the control, the treatment series showed a significant reduction in the overall labeling frequency in both sexes. When a comparison of the absolute frequencies (amongst all nuclei examined, both labeled and unlabeled) of the continuous and discontinuous types is made, it is seen that though MC affects both types of patterns, the effect is more pronounced on the former. It has been shown earlier that inhibitory action of MC on DNA synthesis (Szybalski and Iyer, 1964) is restricted mainly to cells which are about to enter or have just entered into a new replication cycle (Doi et al., 1967). Our data, in the light of this action of MC, gives further proof of our earlier postulation (Lakhotia and Mukherjee, 1970) that the continuous type of pattern and not the discontinuous type is at the initiation of the DNA replication cycle.

References cited: Doi, O., S. Takai, Y. Aoki, H. Higashi and G. Kosaki 1967 *Gann* 58:125; Lakhotia, S.C. and A.S. Mukherjee 1970 *J. Cell Biol.* 47:18; Rodman, T. 1968 *Chromosoma* 23: 271; Szybalski, W. and V.N. Iyer 1964 *Fed. Proc.* 23:946.

Paik, Y.K. and K.C. Sung. University of Hawaii, Honolulu, Hawaii. Chromosomal polymorphism in Hawaiian populations of *D. immigrans*.

In most cases, *Drosophila* flies adjust themselves to new habitats, where environmental stresses are different, through the formation of races or ecotypes by selection. This formulation, however, appears not to fit the populations of all species of *Drosophila*, especially

those which are geographically very widespread or cosmopolitan. *Drosophila immigrans* represents the latter though displaying remarkable ability to colonize diverse natural habitats on the islands of Hawaii. Unfortunately, our ideas of what constitutes such a successful colonization of this species are much too vague and are purely speculative at present, despite the fact that solution of the problems posed is biologically very important.

As an initial step toward attacking the problems, we have analyzed the inversion polymorphisms of this species on the islands of Hawaii. Preliminary report of part of this work is presented in this communication.

The population samples presented here were taken from the islands of Oahu and Hawaii, Hawaii. The Oahu samples were from three different habitats: the first population sample (OT-70) was taken in late November, 1970, in Mt. Tantalus - moist forest - at 1500-1700 foot levels. A second collection (OT-71) was taken in mid-January, 1971. Another sample (OM-70) was collected in Mt. Mauna Kapu - dry forest - at 2100-2300 foot levels in late October, 1970 and a second collection (OM-71) in early January, 1971. The final sample (OP-71) was collected in mid-March, 1971, in Mt. Puu Keaau - cactus forest - at 100-1000 foot levels.

The Hawaii samples were taken in early April, 1971, in Hawaii Volcanoes National Park (HS-) and in Kilauea Forest Reserve (HK-) - a virgin rain forest. In the first collecting area samples were taken at six elevations along Mauna Loa Strip Road extending from 3000 to 6700 feet; in Kilauea Forest a sample was collected at an altitude of approximately 5300 feet. In Tables 1 and 2 are summarized the data on chromosomal polymorphisms obtained from these populations by "egg sample" technique.

Table 1. Frequencies (in per cent) of inversion heterozygotes in the Oahu populations (N, total number of larvae examined, one larva per line).

Sample	N	Heterozygous Inversions						Total Het. Inversions
		A	B	C	A+B	A+C	B+C	
OT-70	83	25.3	2.4	1.2	-	-	-	28.9
OT-71	120	18.3	3.3	0.8	0.8	-	-	23.3
OM-70	53	24.5	1.9	-	1.9	1.9	-	30.2
OM-71	158	22.8	6.3	1.3	2.5	1.3	1.3	35.4
OP-71	54	18.5	5.6	-	3.7	1.9	-	29.6
All samples	468	22.0	4.3	0.9	1.7	0.9	0.4	29.9

A, B, and C denote different inversions on the 2nd chromosome; and these are identical with or similar to those described by Brncic (1955).