

Table 2. Seasonal changes in the number of individuals collected at the Jyozankei timberyard in northern Japan.

| Month | May | Jun | Jul | Aug | Sep | Oct |
|----------------------|-----|-----|-----|-----|-----|-----|
| <i>D.ezoana</i> | 13 | 1 | -- | -- | -- | -- |
| <i>D.moriwakii</i> | 138 | 12 | 1 | -- | -- | -- |
| <i>D.okadai</i> | 25 | 3 | -- | -- | -- | -- |
| <i>Chy.caudatula</i> | 7 | -- | 103 | 3 | -- | -- |
| <i>Chy.costata</i> | -- | -- | 4 | 4 | 3 | -- |
| <i>Chy.fuscimana</i> | -- | -- | 74 | 27 | 8 | 1 |
| <i>Chy.distincta</i> | -- | -- | 42 | 4 | -- | -- |

of this group. *D.lummei* was described from Finland (Hackman 1972), who remarks that it has an extensive eastward distribution. To date in Japan, the collection records of *D.lummei* are almost restricted to timberyards in the western part of Tohoku, although the detailed information is still poor. Furthermore, as mentioned above, some logs at the SK timberyard are imported from USSR. These facts suggest that the Tohoku district might be a marginal zone for the distribution of *D.lummei*, or that its SK individuals might have colonized with logs from the Eurasian Continent. In the *robusta* group and the genus *Chymomyza*, *D.lacertosa* and *Chy.caudatula* were the most widely distributed species, respectively.

To study seasonal fluctuations of drosophilids at a timberyard, periodical collections were made at weekly intervals from May 5 to November 3 in 1979, at Jyozankei near Sapporo. The *virilis* group, the *robusta* group and *D.bifasciata* have been collected exclusively in spring (Table 2).

This is due to the desiccation of logs during the summer, so that these flies would withdraw into surrounding forests in this season. Compared to these species, the adults of *Chymomyza*, except for *Chy.caudatula*, began to appear about two months later, which would depend upon differences in the life cycles between the species. Like a majority of drosophilids distributed in the Holarctic region, the *virilis* group and the *robusta* group spend the winter in the adult stage, while *Chy.costata* and *distincta* are known to commit larval diapause (Watabe & Beppu 1977; Enomoto 1981; Lumme & Lakovaara 1983). Therefore, the adults of *Chymomyza* appear in late June to early July in Hokkaido. Judging from the phenological data, it seems that *Chy.fuscimana* would be also a larval diapause species.

At last, an excess of *Chymomyza* males in the samples taken is remarkable. This has relation to behavior of males. The males of *Chymomyza* appeared twice in a day, morning and evening, on the cut end of logs, and exhibited complex and species-specific courtships, e.g., active walking, wing scissoring and foreleg swinging. On the other hand, most females usually remained resting near the edge of logs. Therefore, the males could be captured more effectively in the collection using the insect net or an aspirator. Such a bias of the sex ratio is not true in the natural populations of *Chymomyza*, of course.

References: Enomoto, O. 1981, *Low Temp. Sci. Ser. B* 39:21-29; Hackman, W. 1972, *Notul. Ent.* 52:89-92; Kimura, M.T., M.J. Toda, K. Beppu & H. Watabe 1977, *Kontyu* 45:571-582; Lumme & Lakovaara 1983, in: *Genetics and Biology of Drosophila 3d* (Ashburner et al., eds.) 171-220; Watabe, H. & K. Beppu 1977, *J. Fac. Sci. Hokkaido Univ. Ser. IV* 20:611-620.

Williamson, R.L. and A.D. Riggs. Beckman Research Institute (City of Hope), Duarte, California USNA. 5-Aza-2'-deoxycytidine does not cause recessive lethal mutations in *Drosophila melanogaster*.

In mammalian cells, 5-aza-2'-deoxycytidine (5-AzaCdR) at relatively low concentrations (less than 10 μ M) will often efficiently cause somatically heritable changes in cellular phenotype (reviewed in Riggs & Jones 1983). This analog is thought to function by preventing the formation of 5-methylcytosine. Present evidence suggests that 5-AzaCdR is not a significant mutagen for mammalian cells in culture (Landolph et al. 1982; Jones 1984; Olsson & Forchhammer 1984; Kerbel et al. 1984; Delers et al. 1984), but nevertheless, may be a carcinogen (Carr et al. 1984). *Drosophila* has been reported not to contain detectable 5-methylcytosine (Urieli-Shoval et al. 1982), although a recent report indicates that *Drosophila* may contain this modified base at very low levels (Achwal et al. 1984). It is possible that only the germ cells of the adult contain 5-methylcytosine. For these reasons, we thought it would be interesting to see if 5-AzaCdR was toxic, mutagenic, or would affect fertility in *Drosophila*. Our test for the mutagenic effect of 5-AzaCdR was modified from the standard design of Muller (1928), but used a lethal-bearing FM6 chromosome, designated I(FM6), in place of CLB. Virgins for the cross were derived from Canton-S male and I(1)J1/I(FM6) parents.

Feeding experiments with 5-AzaCdR were unsuccessful; therefore, Canton males were injected with 0.1 μ l of *Drosophila* Ringers solution (Ephrussi & Beadle 1936), either with or without 5 mg/ml 5-AzaCdR (Sigma). Solutions were made fresh prior to injection.

DADE 5- μ l Accupettes that had been drawn to fine needles with a pipette puller were used for the injections. A graph-paper scale was glued to a short segment of fine polyethylene tubing and a lengthwise

wedge of the tubing was cut out so that it could be clipped onto the pipette and used as a movable scale to measure the volume of the injection. Injections were made in the right side of the ventral abdomen, medial to the 5th or 6th sternites. No leakage of fluid was observed after injection. No toxic effects were seen, and fertility was not obviously affected.

To ensure that all stages of meiosis were assessed, the treated males were placed with fresh +/- (FM6) virgins (3 to 6 days old) on 3 successive occasions lasting 3 days each, and on a fourth occasion lasting 5 days. The females that had been mated were subsequently transferred to new bottles and allowed to lay additional eggs for a time equal to the times that they had been with the males. An exception was the second 5-AzaCdR-treated brood whose females were inadvertently discarded prior to the second egg lay.

Lethal mutations, detected by the absence of adult males, were subsequently made heterozygous with FM6 balancers and confirmed in the fourth generation.

Results: Lethal mutations/chromosomes tested

| Broods | 1 | 2 | 3 | 4 | Total | % |
|-------------|-------|-------|-------|-------|--------|------|
| Treatments: | | | | | | |
| Control | 0/311 | 0/371 | 1/233 | 0/227 | 1/1142 | 0.09 |
| 5-AzaCdR | 0/431 | 0/260 | 0/229 | 1/233 | 1/1153 | 0.09 |

Our results indicate that injections that should lead to an initial concentration greater than 2 mM 5-AzaCdR in the haemolymph are relatively nontoxic and do not cause mutations in the germ line. This apparent lack of mutagenicity agrees with the studies on mammalian cell culture. Since our results are

negative, they are not unambiguously interpretable. For example, the metabolism of 5-AzaCdR may be different in *Drosophila* than it is in mammalian cells.

We do not plan to pursue this work further at this time.

References: Achwal, C.W., P. Ganguly & H.S. Chandra 1984, *EMBO J.* 3:263-266; Carr, B.I. & A.D. Riggs 1984, *Carcinogenesis* 5:1583-1590; Delers, A., J. Szpirer, C. Szpirer & D. Saggiaro 1984, *Mol. Cell. Biol.* 4:809; B. Ephrussi & G.W. Beadle 1936, *Amer. Nat.* 70:218; P. Frost, R.G. Liteplo, T.P. Donaghe & R.S. Kerbel 1984, *J. Exp. Med.* 159:1491; P.A. Jones 1984, in: *DNA Methylation: Biochemistry and Biological Significance* (Razin et al. eds.), Springer-Verlag, New York; Landolph, J.R. & P.A. Jones 1982, *Cancer Res.* 42:817; L. Olsson & J. Forchhammer 1984, *Proc. Nat. Acad. Sci. USA* 81:3389; A.D. Riggs & P.A. Jones 1983, *Adv. Cancer Res.* 40:1; Urieli-Shoval, S., Y. Gruenbaum, H. Cedar & A. Razin 1982, *FEBS Letters* 146:148.

Züst, B. and J. Wüest. Université de Geneve, Switzerland. The tumorous-head mutant of *Drosophila melanogaster*: examination by scanning electron microscopy of eye imaginal discs.

In flies of the homoeotic mutant tumorous-head (*tuh*) of *Drosophila melanogaster* head structures are partially replaced by abdominal, genital or undefined "amorphous" outgrowths (Newby 1949; Postlethwait et al. 1972; Kuhn et al. 1981). Kuhn and collaborators described the aldehyde oxidase (aldox)-staining

patterns in the eye imaginal discs of *tuh* larvae (Kuhn & Cunningham 1976; Kuhn & Walker 1978) and found it likely that "the changes in aldox distribution correlate with the transformed portion of the disc affected by the homoeotic mutation". These transformed portions of *tuh* discs, isolated and cultured in vivo, differentiated autonomously (Kuhn et al. 1979). In this study *tuh* eye discs with visible abnormal regions were analysed by scanning electron microscopy (SEM).

Imaginal eye discs of third instar larvae of a *tuh* strain (Arizona State University strain; kindly provided by D.T. Kuhn) were dissected in Ringer's solution to which 2% glutaraldehyde in 0.1M cacodylate buffer (pH 7.3) was slowly added. They were fixed in pure 2% glutaraldehyde for 2h at room temperature, then washed, and in some cases stored up to 5 days in 0.1M cacodylate buffer (pH 7.3) at 4°C. Postfixation was done in 2% aqueous OsO₄ for 1h. The osmium-fixed discs were stored in 70% ethanol and later prepared for SEM. Eye discs of *D.melanogaster* Colmar strain served as controls.

Results and Discussion. 52 eye discs carrying transformed areas have been grouped according to size of their outgrowths: 14 small (27%; Fig. A: arrow), 14 medium (27%; Fig B1: arrow) and 24 large ones (46%; Fig. D1. Fig. E). Some of these areas had uniform shape (20/52 = 38%; Fig. A1), others appeared lobed (26/52 = 50%; Fig. D1), or twisted (6/52 = 12%; Fig. E). No correlation between size and shape has been found. Certain of these abnormal growths appeared clearly delimited from their unaltered supporting tissue (Fig. D1), while in others no clear limit could be detected (Fig. A1, B1). In the 52 discs analysed, 43 growths were located in the central region of the disc (region 2: giving normally rise to many of the eye facets), one in each lateral part of the disc (region 1 = ventral and 3 = dorsal) and in 7 cases the region could not be determined (for more details on the eye disc regions (see Kuhn & Walker 1978).