Substrates	Drosophila ananassae			Drosophila malerkotliana			Drosophila bipectinata		
	Larva	Pupa	Adult	Larva	Pupa	Adult	Larva	Pupa	Adult
Ethanol	+	+	+	+	+	+	+	+	+
Methanol	+	-	+	+	+	+	+	-	+
Butanol	+	+	+	+	+	+	+	+	+
n-Propanol	+	+	+	+	+	+	+	+	+
2-Propanol	+	-	+	+	+	+	+	+	+
Benzyl alcohol	+	+	+	+	+	+	+	-	+
Allyl alcohol	+	+	+	+	+	+	+	-	+
Amyl alcohol	+	+	+	+	+	+	+	-	+
Cyclohexanone	+	-	+	+	+	+	+	-	+
Octanol	+	+	+	+	+	+	+	-	+

Presence (+) or absence (-) of activity for alcohol dehydrogenase enzyme in three species of Drosophila acting on a variety of alcohol substrates.

References: Ursprung, H. and J. Leone 1965, J. Exp. Zool. 160: 147; Jacobson, K.B., J.B. Murphy and F.C. Hartman 1970, J. Biol. Chem. 245: 1075; Sofer, W. and H. Ursprung 1968, J. Biol. Chem. 243: 3110; Ward, R.D. 1974, Biochem. Genet. 12: 449; Singh, R.S. 1976, Genetics 82: 507.

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In 1971, Van Valen and Van Valen (2) reported failure to find daily growth layers in D. melanogaster. However, in 1973 Van Valen (3) encouraged Drosophila workers to try the aging methods of Schlein and Gratz (4). By following these methods, we found limited areas of banding in thoracic muscle attachments (apodemes). As was shown in other Diptera (4), apodemes of Drosophila exhibit daily growth layers on regions of postmetamorphic growth. The apodemal growth layers are highly variable both in contrast and in maximum number. The best specimens show banding under transmitted light. Phase contrast microscopy improves the contrast, as does staining with Heidenhain's Hematoxylin. None of these methods, however, provide a reliable or repeatably good aging tool. After experimentation with a wide variety of techniques, stains, and counterstains, we developed the following simple and effective method: A fly with legs and head removed is placed into hot 4% KMnO4 for 5 minutes. After the fly is rinsed in distilled water, the apodemes are pulled from the thorax with forceps and placed into Paragon (5) or other water soluble mounting media under a cover slip. Nemarski differential interference contrast (DIC) microscopy shows areas of growth along the apodemes. The first, second, and third furca (ful, fu2, fu3) and the second thoracic phragma (phl, shown in Fig. 1) developed growth layers. However, the third furca shows the most distinct banding (Fig. 2).

The correspondence between fly age and growth band was tested using three homozygous strains of parthenogenetic D. mercatorum supplied by A. Templeton (6). The results are shown in Table 1. We analyzed this by a 3-way ANOVA with unequal subclasses using BMDP. Genetic differences between strains do not affect the banding ($F=0.68$, $P>.50$). Temperature, however, has an effect ($F=4.15$, $P<.05$). The banding was most distinct, and age correspondence closest, with a 22 to 14.5°C day to night temperature fluctuation. It may be important that the 22 to 14.5°C range is close to that experienced by the strains in nature. The actual fly age corresponded closely with the number of bands (F to fit the age effect = 48.89, $p<<.01$). Fifty percent of the flies from the 22 to 14.5° regime were correctly aged. The other 50% were only one day too high or too low. An experiment testing correlation between age and bands on 7-12 day old flies was not as successful. While a 12 day old fly was correctly identified once, most flies had a maximum of 8 growth bands.

The aging method is thus limited to young flies. Yet, the method is important because it permits age determination up to and into sexual maturity. The method is now being applied to ask a variety of questions about age structure of natural populations. In particular, we are interested in age-related dispersal. To date, 10 species have been successfully aged, including 8 repleta species, D. melanogaster, and D. mimica (a Hawaiian species).

Table 1. Comparison of actual age with estimated age in three strains of D. mercatorum maintained in three day:night temperature regimes.

Strain	Actual age (days)	Estimated age		
		22°C	22°-14.5°C	22°-5°C
O3-IM	1	2	2	4
	2	5	2	3
	4	6	3	5
	6	7	6	7
RSS18-IM	1	1	1	2
	2	5	3	3
	4	5	5	4
	6	7	6	6
S11-IM	1	2	2	2
	2	3	2	3
	4	5	5	6
	6	6	6	6

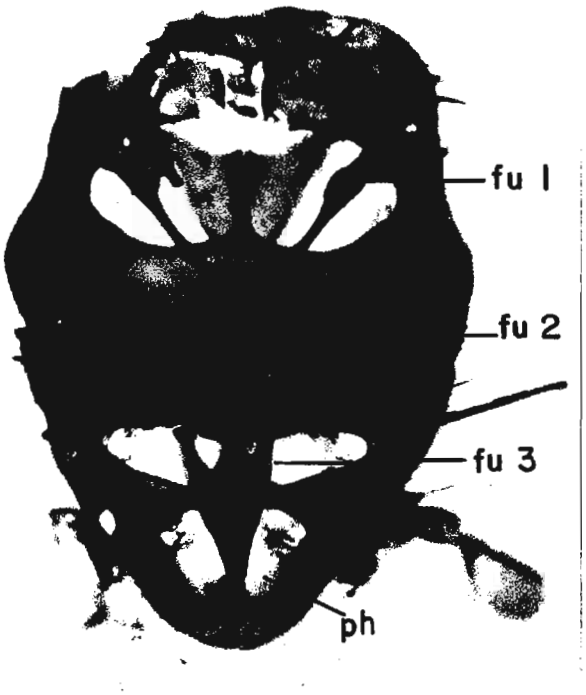

Fig. 1. Dorsal aspect of ventral thorax of D. mercatorum showing furcas 1,2,3 (ful,fu2, fu3) and thoracic phragma (ph).

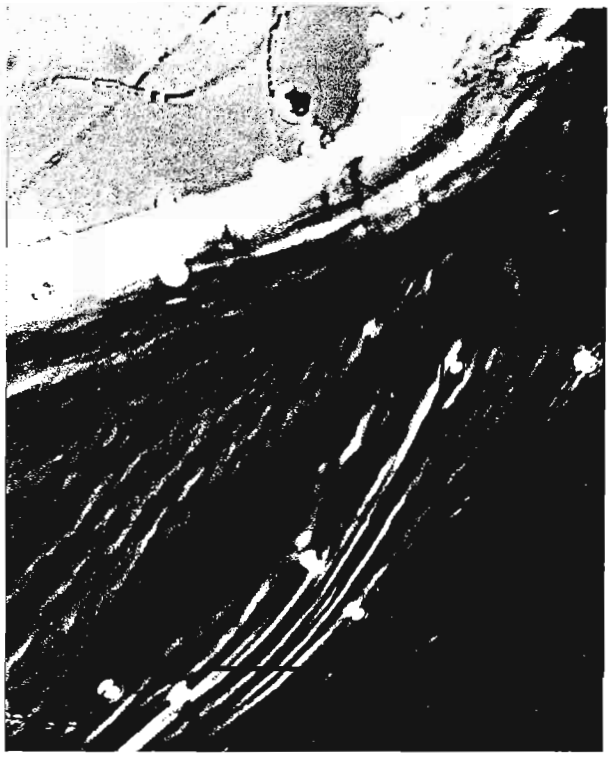

Fig. 2. 800X DIC micrograph of a portion of furca 3. 12 bands are visible beyond the eclosion layer (E).

References: (1) Supported in part by N.S.F. DEB-76-19879 to J.S. Johnston; (2) Van Valen, L. and P. Van Valen 1971, DIS 46:125; (3) Van Valen, L. 1973, DIS 50:110; (4) Schlein, Y. and N.G. Gratz 1972, Age determination of some flies and mosquitos by daily growth layers of skeletal apodemes, Bull. W.H.O. 47:71-76; (5) Paragon C. & C. Co., Inc., Bronx, NY 10454; (6) Templeton, A.R., H.L. Carson and C.R. Sing 1976, Genetics 82:527-542.