Van Valen, L. and P. Van Valen. University of Chicago and University of Illinois, Chicago, Illinois. A method that does not estimate age in Drosophila.

Perhaps the most important reason for the virtual lack of analytical ecology of Drosophila is the inability to determine individual post-teneral ages. Labeling techniques like that of Richardson et al. (1969) will help but are unsuitable for some purposes. Neville (e.g. 1967 a, b) and others have found daily growth layers in the cuticle of several insects and it seemed possible that they might occur in Drosophila.

D. melanogaster adults were kept at 25° for 6 or 7 hours in the day and at 15° at other times from eclosion. On the 15th to 20th day they were killed and the middle legs fixed in Kahle's fluid. Frozen sections of femora gave no results, probably because of the unfamiliarity of the technician with this method.

Standard embedding and sectioning produced useful sections which were stained with Mallory's or hematoxylin-eosin, or left unstained. No sets of layers were visible at magnifications to 1000X, for both thin and thick cuticle. A control using a wild-caught grasshopper did produce visible layers. It may be that electron-microscopic studies would detect layers in Drosophila, but a method more suitable for routine ecological work did not if layers are produced endogenously or by temperature.

We thank J. McLane for technical assistance and W.L. Doyle, D.B. Wake, E.B. Spiess and W.K. Baker for use of equipment. A grant from NSF supported this work.

References: Neville, A.C., 1967a, Adv. Ins. Physiol. 4: 213-286; Neville, A.C., 1967b Biol. Rev. 42: 421-441; Richardson, R.H., R.J. Wallace, S.J. Gage, G.D. Bouchey and M. Denell, 1969 Stud. Genet. (Univ. Texas) 5: 171-186.

Ehrman, L. and D.L. Williamson. Rockefeller University, New York, and Medical College of Pennsylvania, Philadelphia, Pennsylvania. Differential centrifugation and infectious hybrid sterility in D. paulistorum.

Matings between the D. paulistorum semispecies produce sterile male progeny (Perez-Salas, et al 1970). At least in some of the instances of sterility in this species-complex, transmissible symbionts or commensals are implicated. Genetically pure Mesitas males can sometimes be made sterile by injecting their mothers with material derived from Santa Marta X Mesitas sterile F_1 hybrid males (Ehrman and Williamson, 1969 and references therein). We have previously used homogenates of sterile hybrid male material centrifuged at low temperatures at approximately 1000X gravity; this results in only a crude homogenate. To try to purify the infectious "factor(s)" involved we attempted differential centrifugation in a sucrose solution using assorted speeds up to an including 100,000X gravity. Little or no purification of the self-reproducing entity (presumably a mycoplasma-like microorganism, see Kernaghan and Ehrman, 1970), resulted. But some of the data are worth recording:

1) The percentages of induced male sterility were higher in later broods, i.e., when the recipient females had longer intervals within which to incubate the infectious material injected into them;

2) The source of infectious material providing results closest to that of injected crude homogenates came from that pelleted at approximately 10,000X gravity. This was true in all broods surveyed for sterile males. At 10,000 gravity one may expect structures like mitochondria and/or mycoplasma to sediment;

3) The smallest percentage of induced male sterility occurred when the resuspended pellet from the highest speed, 100,000X gravity, was used to inject females whose sons were tested for sterility.

We realize that much more work remains to be done using the techniques of differential ultracentrifugation on this unique material. With Dr. R.P. Kernaghan of The State University of New York at Stony Brook, we plan to couple ultracentrifugation with the electron microscopy of suitable homogenates.

References: Ehrman, L. and D.L. Williamson, 1969, Genetics 62: 193-199; Kernaghan, R.P. and L. Ehrman 1970, Chromosoma 29: 291-304; Perez-Salas, et al, 1970, Evolution 24: 519-527.

Lee Ehrman is the recipient of USPHS Career Award 2K03 HD 09033-06. David L. Williamson acknowledges the support of NIH Grant AI 09615.