

This Week's Citation Classic®

Schneider I. Cell lines derived from late embryonic stages of *Drosophila melanogaster*. *J. Embryol. Exp. Morphol.* 27:353-65, 1972.

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The development of three cell lines of *Drosophila melanogaster* is described. The primary cultures consisted of trypsinized fragments from 20- to 24-hour-old embryos. The characteristics of each cell line are given, and evidence is presented that one of the lines is derived, at least in part, from imaginal disc cells. [The *SCF*® indicates that this paper has been cited in over 215 publications.]

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Interest in the culture of insect cells was rekindled in the late 1950s to mid-1960s due largely to the efforts of T.D.C. Grace of Canberra, Australia, and K.R.P. Singh of Poona, India. In 1962 Grace reported the establishment of the first continuous insect cell line, derived from ovarian tissue of a large saturniid moth, *Antheraea eucalypti*.¹ Five years later Singh² established cell lines from two mosquito species, *Aedes albopictus* and *A. aegypti*; both are vectors of human pathogens. Singh indicated that mosquito embryos or first-instar larvae could serve as the source of the primary explants and, equally important, that fetal bovine serum could replace homologous or heterologous insect hemolymph as a medium supplement. Prior to 1967 conventional wisdom had dictated the use of insect serum as the supplement, a daunting prospect for those interested in working with insects having a total hemolymph volume of one to two microliters.

My first venture in insect culture centered on organ rather than cell culture and specifically attempted to document the timing of deposition of both ommochrome and pteridine pigments in cultured eye-antennal discs of *Drosophila melanogaster*. I started the initial project at the University of Chicago in 1958, but I soon dropped it in favor of a study of position-effect variegation for my doctorate. I returned to *Drosophila* organ culture during a postdoctoral fellowship at the University of Zurich and later at Yale University, where I finally had some measure of success resulting in a 1964 publication.³

In 1965 I joined the Walter Reed Army Institute of Research to set up an insect culture laboratory

and to explore the possibility of initiating cell lines from mosquito species to use as substrates for the culture of the insect stages of malaria parasites. Since this was the "pre-Singh" era, one of my first priorities was finding a source of insect hemolymph. Pupal stages of large moths such as *Antheraea* were most sought after as more than 1 ml of hemolymph could be obtained from a single pupa. Some of the largest of these moths were found outside the US. I signed numerous US Department of Agriculture and US Public Health import forms pledging to use only the pupal stage, and I assured these agencies that I would dispose of the pupae prior to adult emergence. Imagine my consternation then, when I opened the first shipment of some 400 *Antheraea* "pupae" and more than 50 adult moths emerged and began circling my laboratory with merry abandon. Fortunately, I was far more agile back then, but even so, it took the better part of the afternoon to capture every last individual.

Following Singh's technique I established several mosquito cell lines in the late 1960s and, given my background in developmental genetics, decided to find out if the technique could be extended readily to *Drosophila*. The answer was yes—using a medium, slightly revised, from that designed eight years previously for organ cultures. The three *Drosophila* lines were not the first to be established, but they were the first to be made available to interested investigators, even prior to the publication describing them.

I believe the paper has been cited so often for a number of reasons. The cell lines, especially line 2, have been very popular. (Over the years I have sent out more than 200 cultures to investigators throughout the world and still receive requests. In all likelihood more studies have been carried out with my line than with any other *Drosophila* line in existence,⁴ with the possible exception of the Kc line of G. Echaler and A. Ohanessian.⁵) The revised medium became commercially available in the early 1970s and was found to be suitable not only for *Drosophila* cultures but also for quite disparate purposes, such as the cultivation of leishmanial promastigotes for diagnostic assays.⁶ A successful effort was made to identify the source of at least some of the cells in the third line, and although the method used was not new, it attained considerable popularity thereafter for similar studies with either endogenous or exogenous hormones. The discussion section of the paper turned into a fairly comprehensive mini-review of dipteran cell culture up to that time, which probably enhanced interest in the paper. Incidentally, the manuscript was rejected by *Roux's Archives of Developmental Biology*, the first journal to which it was submitted!

1. Grace T D C. Establishment of four strains of cells from insect tissues grown *in vitro*. *Nature* 195:788-9, 1962. (Cited 400 times.)
2. Singh K R P. Cell cultures derived from larvae of *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.). *Curr. Sci.* 36:506-8, 1967. (Cited 260 times.)
3. Schneider I. Differentiation of larval *Drosophila* eye-antennal discs *in vitro*. *J. Exp. Zool.* 156:91-104, 1964. (Cited 145 times.)
4. Sang J H. *Drosophila* cells and cell lines. (Maramorosch K, ed.) *Advances in cell culture*. New York: Academic Press, 1981. Vol. 1. p. 125-82.
5. Echaler G & Ohanessian A. *In vitro* culture of *Drosophila melanogaster* embryonic cells. *In Vitro* 6:162-72, 1970. (Cited 255 times.)
6. Childs G E, Foster K A & McRoberts M J. Insect cell culture media for cultivation of New World *Leishmania*. *Int. J. Parasitol.* 8:255-8, 1978.