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This Week's Citation Classic

Edgar R S & Lielausis I. Temperature-sensitive mutants of bacteriophage T4D: their isolation and genetic characterization. *Genetics* **49**:649-62, 1964. [Division of Biology, California Institute of Technology, Pasadena, CA]

The isolation of temperature-sensitive mutants of bacteriophage T4D is described. The mutants grow at 25°C but unlike the wild-type strain, cannot grow at 42°C. It is shown that the 382 mutations studied are located in 37 genes widely scattered over the genome. [The *SCI*® indicates that this paper has been cited over 105 times since 1964.]

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"When I learned that this paper was a 'Citation Classic' I must admit I was pleasantly surprised. I believe it was cited often because it demonstrated a relatively simple method for studying essential genes in virtually any organism. The method quickly found favor in the study of other bacteriophages and many animal viruses as well. Today temperature sensitive lethals are routinely employed in many genetic systems.

"I have already described my recollections of how this work was started.[1] Independently R.H. Epstein in T4 and Alan Campbell in lambda had already discovered the class of suppressor-sensitive lethals called *ambers* which are now known to be nonsense mutations. (R.H. Epstein's paper about their isolation and characterization in T4, originally planned as a comparison to our paper, in fact was never completed!) It was during a conversation at Cold Spring Harbor with Alan Campbell that the notion of the general class of Conditional Lethals occurred to us (I at first called them facultative). We also surmised that temperature sensitive and other types of conditional mutants should exist. We both went home at the end of the summer, he to Rochester, I to Caltech, to test the idea further.[2]

"I recall retrieving an old copper-lined water-jacketed incubator from basement storage because our standard incubators in use at that time did not have adequate heat capacity. At first I told no one that I was looking for temperature sensitive lethals. I was afraid skeptics in the lab would talk me out of looking for them! But after the first success the hard work began. I was interested in identifying all the essential genes of T4, and the job of isolating and characterizing thousands of mutants occupied Ilga Lielausis (my technician) and me a number of years. It did not take long for the law of 'diminishing returns' to set in and I can remember trying to think of a good answer to the question I heard too often, 'How many new genes have you found since I saw you last?'

"It is surprising that no one had thought of exploiting temperature sensitive mutations earlier. They had been discovered in the 1930s and were even used in a test of the one gene-one enzyme theory.[3] Yet somehow no one had made the connection between the well known Horowitz and Leupold work and the use of such mutants for other purposes. I know I didn't.

"I do believe that great impetus comes to science from new ways of revealing things. Sucrose gradients and SDS-polyacrylamide gels are obviously such ways, but I think one can make the case that temperature sensitive mutations are also a 'device for seeing' and I am happy to have contributed to popularizing their use."

1. **Edgar R S.** Conditional lethals. *Phage and the origins of molecular biology.* (Cairns J, Stent GS & Watson JD, eds.) Cold Spring Harbor. NY: Cold Spring Harbor, p. 166-70, 1966.
2. **Campbell A.** Sensitive mutants of bacteriophage λ. *Virology* **14**:22-32, 1961.
3. **Horowitz N H & Leupold V.** Some recent studies bearing on the one gene-one enzyme hypothesis. *Cold Spring Harbor Symp. Quant. Biol.* **16**:65-72, 1951.