This Week's Citation Classic

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Hirota Y. The effect of acridine dyes on mating type factors in *Escherichia coli*. *Proc. Nat. Acad. Sci. US* 46:57-64, 1960.
[Department of Genetics, Stanford University Medical Center, Palo Alto, CA]

Acridine dyes irreversibly convert F^+ (male) clones of *E. coli* into stable F^- (female) forms directly without selective growth. Hfr (male) clones are resistant to the action. These results are accounted for by the dual nature of F, plasmid F, and chromosomal F. [The *SCI*® indicates that this paper has been cited in over 475 publications since 1961.]

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"This paper is an extension of my two previous publications[1,2] which were done when I had just started graduate research in Hideo Kikkawa's laboratory at the department of biology, Faculty of Science, University of Osaka, Japan. In these papers, I reported my discovery of an efficient genetic alteration of *E. coli* from F^+ (male) to F^- (female). When F^+ was grown under the presence of either cobaltus salt or acridine dye in the medium, a large fraction of F^+ bacteria was converted to F^- (this phenomenon is known as 'F elimination,' 'disinfection of F,' or 'acridine curing'). I correctly interpreted this phenomenon as follows: F (sex factor) in F^+ bacteria is an extrachromosomal state replicating autonomously, and these reagents specifically inhibit autonomous replication of extrachromosomal F but do not inhibit chromosomal DNA replication of the host (*E. coli*). Upon growth of the host, F is diluted out from the F^+ cell and F^- bacteria evolve. However, an alternate explanation was also possible: that is, spontaneously arised F^- by a rare mutational event is selected by these reagents.

"I needed to prove that my hypothesis was correct; however, I did not have enough experience to achieve my goal so I wanted to have the guidance of Joshua Lederberg at the University of Wisconsin. In 1959, Lederberg moved to Stanford University, and I followed him. He communicated my work to the January 1960 issue of the *Proceedings of the National Academy of Sciences of the USA*. From this work, I received the degree of Doctor of Science at the University of Osaka. A friend of mine said of my discovery and the previous great achievements in *E. coli* genetics by Lederberg and Jacob and Wollman,[3] 'An American discovered sexuality of bacteria, two Frenchmen enjoyed the bacterial sex, and a Japanese made bacteria enjoy two sexes.'

"My thesis work became a *Citation Classic* due to the nature of the work. It describes a simple method for strain construction of *E. coli*, and this organism has extensively been used in the fields of genetics, microbiology, molecular biology, and recombinant DNA technology. Also, it was the first clear demonstration of the existence of bacterial extrachromosomal factor, which was named plasmid.[4,5] Since publication of my work, the acridine curing technique has been applied to wide varieties of bacterial species to test for the presence or the absence of the other plasmid. It has been cited, in addition, because of its description of the methods to demonstrate direct conversion of F^+ to F^- without selective growth. It has often been cited in the textbooks of bacterial genetics."

1. **Hirota Y.** Artificial elimination of the F factor in *Bact. coli* K12. *Nature* 178:92, 1956.
2. **Hirota Y & Iijima T.** Acriflavine as an effective agent for eliminating F factor in *Escherichia coli* K12. *Nature* 180:655-6, 1957.
3. **Jacob F & Wollman E L.** *Sexuality and the genetics of bacteria*. New York: Academic Press, 1961. 374 p.
4. **Lederberg J.** Cell genetics and hereditary symbiosis. *Physiol. Rev.* 32:403-30, 1952.
5. **Bukhari A I, Shapiro J A & Adhya S L, eds.** *DNA: insertion elements, plasmids, and episomes*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1977. 782 p.