Perspectives

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Forty Years Ago in GENETICS: The Unorthodox Mating Behavior of Bacteria

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IN October, 1953 GENETICS published the first detailed report of a sex difference in *Escherichia coli*. This is a fitting time to look back at those years of simultaneous excitement and confusion when the genetics of bacteria was just beginning to be understood.

Looking at recent papers on bacterial genetics, I am surprised to still recognize the names of some of the markers and strains of *E. coli* K-12. So much has happened since that exciting time. *E. coli* is well on its way to becoming one of those few organisms for which we know the whole genome, incredible progress from what was understood around 1950 when we knew only a few linkages for less than a dozen markers.

Starting in 1941, bacteria had become my major interest and in 1948 I gave a paper at the International Congress of Genetics in Stockholm on cross resistance to radiation and nitrogen mustard in *E. coli* based on work done earlier in Milan with NICCOLO VISCONTI (CAVALLI and VISCONTI 1948). Italy was then, and for several decades, a scientific desert with a few oases. After much search I was lucky to have found one of these oases, with ADRIANO BUZZATI-TRAVERSO as my professor. In 1948 I received a scholarship from the Italian National Research Council to work with KENNETH MATHER at the John Innes Horticultural Institution, then at Merton and directed by C. D. DARLINGTON. This was the first time I was able to go abroad, a major success in post-war Italy, and I enjoyed enormously drinking directly at one of the original fountains of genetic and statistical knowledge.

It was in that eventful summer of 1948 that I had the surprise, immediately after introducing myself to R. A. FISHER at the Stockholm International Congress, of being offered a job in his laboratory. Very probably FISHER was one of the first readers of the GENETICS paper by JOSHUA LEDERBERG (1947) to be entirely convinced by it. The "Pope" of bacteriophage, MAX DELBRÜCK, who listened to the first communication by LEDERBERG at the famous 1946 Cold Spring Harbor Symposium, was initially skeptical of the *E. coli* K-12 crosses. SALVA LURIA, who was also present, tried to repeat LEDERBERG's experiments using *E. coli* B, but failed. Several years later ENRICO CALEF and I tested *E. coli* B and found it to be self-sterile but able to cross with *E. coli* K-12.

FISHER was immediately enthusiastic about K-12 genetics. He obviously was not scared by what JIM WATSON (1968) in The *Double Helix* called the "rabbinical complexity" of JOSHUA's papers. FISHER's main experimental interest was crossing over and gene mapping, which he studied mostly in mice. His laboratory was saturated with these smelly animals and his garden was full of various experimental plants, including MENDEL's peas. His hope was that *E. coli* would become an excellent organism for the study of crossing over.

I immediately accepted FISHER's offer and started working in Cambridge (at 44 Storey's Way, the address of the old Genetics Department) on October 1, 1948. My laboratory was carved out of the tea room and I must confess that I chose the equipment not so much on the basis of price or reliability as simply on early availability. In any case, there was very little difference in prices and reliability was hard to guess; moreover, a bacteriological laboratory at that time required only simple equipment. K-12 strains were sent by the LEDERBERGS and I began to make crosses in February, 1949.

It was perfectly easy to repeat the original experiments; people did not believe them because they did not try to duplicate them. But the skepticism around me was incredible. To classical bacteriologists, we (the very few bacterial geneticists could be counted on one or at most two hands) were lunatics. Bacteriologists had been taught that bacteria have no nucleus or chromosomes and besides, very few of them had clear ideas of Mendelism and, in particular, of recombination. Geneticists, such as D. G. CATCHESIDE and GUIDO

PONTECORVO, working with fungi were not so skeptical. GUIDO had developed a procedure for selecting recombinants in Ascomycetes very similar to the prototroph technique that permitted LEDERBERG to show bacterial recombination, mixing different mutant strains on a medium in which neither parent could grow but a recombinant would. On GUIDO's invitation, I went to Glasgow in 1950 and gave a demonstration of bacterial recombination to bacteriologists. At the time, it was customary to wash suspensions of the parent bacteria three times before plating them on minimal medium (without the nutritional supplements necessary for growth of the mutant strains), and the many bacteriologists who came to see had to stand for a long time during these simple but lengthy operations. I also kept them at some distance for fear of contaminations. They came back later to see the cultures after they had grown.

Recombination as observed at the beginning had a very low frequency. It stopped being rare when I found a mutant strain which I called Hfr for "high frequency of recombination." I found it accidentally in 1949 while I was selecting mutations resistant to nitrogen mustard and radiation. The first two resistant mutants, which had undergone a rather heavy treatment in the process of selecting for resistance to nitrogen mustard, proved to be exceptional in their mating behavior. One was Hfr and it showed immediately its remarkable mating ability, which was higher than that of normal crosses by a factor of 1000 or more. I repeated the experiment two more times before believing it. The other mutation, as I later proved, was an F^- (self-sterile) mutation of an F^+ (fertile) strain.

Hfr was especially interesting but the biology of mating was difficult to understand. There was nothing to be seen microscopically on a plate or in mixed cultures; no distinguishable zygotes were formed. It was only in 1954 that LEDERBERG first proved by micromanipulation experiments that when mating took place there was something–an invisible thread–holding a male and a female together in a drop of saline, though at some distance from each other. Electron microscopy was for a long time negative or unclear. The genetics of the Hfr crosses were difficult to understand, not surprising in retrospect.

I wanted to publish only when I felt that I understood the phenomenon, and thus I published nothing about the finding of Hfr except for a short mention in an Italian journal (CAVALLI 1950). A year or so later, another independent Hfr turned up spontaneously in an old culture in Great Britain and was studied by W. HAYES. The two Hfrs are still around and are called by our two names or simply their initials, HfrC and HfrH. Much later, many more independent Hfrs were obtained, each with unique properties.

FISHER had planned to make bacterial genetics a major part of his research program, but despite his impassioned protestations the program was eliminated. It is ironic that FISHER, who pioneered both in blood groups (especially the Rh factor) and bacterial genetics, was unable to obtain support for sustained research in either area. It was not clear if I was going to keep my Cambridge position.

In this situation of uncertainty I was glad to accept an interesting offer to return to Milan in 1950, back to the laboratory of the Istituto Sieroterapico Milanese where I had started working in 1945 after the end of the war. Although this was a pharmaceutical firm, I was able to continue my genetic research on a part-time basis. I undertook the examination of other fertility mutations, which proved easier. The original K-12 strain is capable of mating with itself, but at a low frequency. I found several independent mutants that had lost this capacity to mate with themselves. To show this, I had to develop new biochemical mutants that would make it possible to test if a strain could or could not mate with itself. Self-sterile strains are called F^-. F^+ is the original K-12 strain, fertile at a low rate with all F^- strains and at an even lower rate with itself. Hfr is a mutation of F^+ which has a high frequency of recombination. While the progeny of $F^+ \times F^-$ crosses were consistently F^+ (except with some special F^- strains, a phenomenon that I never got around to publishing), those of Hfr $\times$ F^- were consistently F^-. But a very brief mixture of F^+ and F^- cells, allowing contact between them, could pass the F^+ property to F^- cells with high probability and without detectable recombination.

While I was doing these experiments I was in correspondence with JOSHUA LEDERBERG and wrote to him about these findings. He and ESTHER LEDERBERG had obtained very similar results, and we decided to publish them together. One joint paper was sent to GENETICS (LEDERBERG, CAVALLI and LEDERBERG 1952), the other to the *Journal of General Microbiology* (CAVALLI, LEDERBERG and LEDERBERG 1953). The LEDERBERGS and I had never met, and it was a strange but pleasant experience to write papers with people known only through air mail. We finally met when I was able to go to Madison, Wisconsin, in 1954, thanks to a Rockefeller Fellowship that allowed me to work with them for three months.

In England I had met BILL HAYES. I happened to give him the first *E. coli* K-12 strains and to show him the crossing and scoring techniques in the practicals of a course which was held at Cambridge. BILL and I also corresponded, though more rarely. He once wrote me the following on F^+ and F^-, about which I had written him: "I guess one can pass the F^+ property

by infection to an F^-." Both the LEDERBERGs and I had independently found this, and I hastened to write to him that he would find the experiment works quite well. He later told me that he was quite shocked when he received my answer because meanwhile he also had done the experiment. It was planned that the HAYES paper would appear in the same issue of the *Journal of General Microbiology* as ours, and it did (HAYES 1953).

In 1952 I had a student of KENNETH MATHER, JOHN L. JINKS, as a guest in my Milan laboratory. It was clear at the time that the Hfr × F^- cross yielded F^- progeny, as I said above. But we now found that the cross of HfrC with an F^- did generate some Hfr progeny, clearly linked to a galactose marker which only rarely segregated. The results could be summarized by saying that F is an infectious particle which could be easily transmitted by cell-to-cell contact and which showed no indication of linkage to other markers, but in some conditions would become irreversibly part of the bacterial chromosome at a specific site, losing the capacity to infect by cell-to-cell contact but acquiring the property of high-frequency recombination. We communicated this finding to the Bellagio 1953 International Congress of Genetics together with the first information on recombination and fitness (CAVALLI-SFORZA and JINKS 1954). The latter study was one of the original purposes of my work in FISHER's department. For example, all possible parental combinations of three markers were tried to test for effects of markers on viability. Until the mechanism of fertility became clear, research on *E. coli* recombination proved completely frustrating. JIM WATSON, who was then at Cambridge, spent a few days in Milan in 1952 to see my recombination data. He was convinced that a three-chromosome theory could explain the observations. He offered to write a paper together with HAYES and me on this theory, which he later published with HAYES (WATSON and HAYES 1953), but I was not persuaded by the theory and declined.

It became progressively more clear that there were some phenomena that could be interpreted on the basis of breaks and that a specific chromosome region was contributed only by female (F^-) parents. This made the results of recombination difficult to understand. It took a long time for JINKS and me to agree on the formal interpretation of recombination data, and it was only in 1956 that we were able to publish a joint manuscript. The formal interpretation of detailed recombination results (CAVALLI-SFORZA and JINKS 1956) is, I think, correct to this day, and a *tour-de-force* of recombination analysis. It showed how difficult it would have been to use the *E. coli* recombination system for the quantitative study of crossing over that FISHER was hoping to do. Nevertheless, FISHER followed with great interest and full open-mindedness the unexpected results that were coming out of bacterial crosses, and was more flexible than I in accepting the unorthodox behaviors of bacteria.

Up to this time bacterial genetics had been the province of a very small group. The stage was now set for a full-scale attack on *E. coli* and many joined in the fray. Phenomena that had seemed mysterious were soon understood, and *E. coli* became the best known species and the geneticist's favorite organism.

My position in the Istituto Sieroterapico Milanese was far from ideal for keeping up with the explosion of research on *E. coli* genetics. Beginning in 1952 I started flirting with human genetics while lecturing part time at the University of Parma, and I slowly left bacteria. The last Petri dish I touched must have been in 1960, working with JOSHUA and ESTHER LEDERBERG at Stanford on the effects of streptomycin on the phenotype of bacterial mutants, a very interesting phenomenon that we, as well as LUIGI GORINI, independently observed. Conversion to human genetics provided a completely different outlet for my scientific interests, replacing work at the laboratory bench with statistical and theoretical analysis, along with trips to such places as Africa to study human populations in their native habitats.

LITERATURE CITED

CAVALLI, L. L., 1950 La sessualità nei batteri. Boll. Ist. Sieroter. Milano **29:** 281–289.

CAVALLI, L. L., J. LEDERBERG and E. M. LEDERBERG, 1953 An infective factor controlling sex compatibility in *Bacterium coli*. J. Gen. Microbiol. **8:** 89–103.

CAVALLI, L. L., and N. VISCONTI, 1948 Variazioni di resistenza agli agenti mutageni in *Bacterium coli*. II. Azotoiprite. Ric. Sci. **18:** 1569–1574.

CAVALLI-SFORZA, L. L., and J. L. JINKS, 1954 Observations on the genetic and mating system of *E. coli* K-12. Atti IX Congr. Int. Genet. Caryol. Suppl. **1954:** 967–969.

CAVALLI-SFORZA, L. L., and J. L. JINKS, 1956 Studies on the genetic system of *E. coli* K-12. J. Genet. **54:** 87–112.

HAYES, W., 1953 Observations on a transmissible agent determining sexual differentiation in *Bacterium coli*. J. Gen. Microbiol. **8:** 72–88.

LEDERBERG, J., 1947 Gene recombination and linked segregation in *Escherichia coli*. Genetics **32:** 505–525.

LEDERBERG, J., L. L. CAVALLI and E. M. LEDERBERG, 1952 Sex compatibility in *Escherichia coli*. Genetics **37:** 720–730.

WATSON, J., 1968 *The Double Helix*. Atheneum, New York.

WATSON, J. D., and W. HAYES, 1953 Genetic exchange in *Escherichia coli* K-12; evidence for three linkage groups. Proc. Natl. Acad. Sci. USA **39:** 416–426.