Perspectives

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THE FOUNDATIONS OF GENETIC FINE STRUCTURE: A RETROSPECTIVE FROM MEMORY

THE theory of the gene as formulated by T. H. MORGAN and his associates posited the chromosome to be a linear array of genes, each occupying a fixed position on the chromosome. The frequency of meiotic crossing over between linked genes measured the distance between them. Crossing over was deemed to be intergenic and, with normal disjunction, allelic genes invariably segregated into separate gametes.

The seminal experiments presaging the conclusion that meiotic crossing over is not exclusively intergenic and that allelic genes can segregate into the same gamete originated in 1939 in C. P. (PETE) OLIVER's Drosophila laboratory located in the basement of the Zoology Building at the University of Minnesota. The evidence for intragenic crossing over was simple. From females heteroallelic for mutations at either of two loci, lozenge (lz) eye on the X chromosome and Star (S) eye on chromosome II, phenotypically wild-type progeny were recovered. That the mutant alleles were not simply at different, closely linked loci was indicated by the fact that flies heteroallelic for two different recessive mutations had a mutant phenotype. The reversions to wild type were unique because they were invariably associated with polarized marker exchange. The association of reversion with exchange effectively excluded back mutation as the process involved. But unequal crossing over á la Bar eye could not be excluded because the reciprocal crossover products were not identified. Nevertheless, at the time, intragenic crossing over was a revolutionary concept. It was genetic dogma that the gene was inviolate with respect to crossing over; genetic mapping via meiotic crossing over defined the chromosomal limits of the gene and was intergenic.

The identification of the crossover reciprocal to the wild-type revertant, crucial to a demonstration of equal intragenic crossing over, was a tortuous process at each locus. The task of describing the Star story is best left to ED LEWIS, whose cytogenetic study of this region provided the basis of his doctoral dissertation

at the California Institute of Technology (LEWIS 1945). In the narrative that follows I shall describe the lozenge case, for here I have first-hand knowledge. Before doing so, I should emphasize that the demonstrations of intragenic crossing over at the S and lz loci represent contrasting methods of how science happens. In the first case, LEWIS believed that S and its functional allele asteroid (ast, then s^r or Star recessive) occupied separate but contiguous chromosomal sites and therefore should be separable by crossing over. He set out to do this by looking for wild-type recombinants. The lz situation is a classical example of the BATESON-BRIDGES prescription to treasure one's exceptions; the discovery of wild-type recombinants associated with crossing over was serendipitous. The details follow.

In the summer of 1939 I was a graduate student in Zoology at the University of Minnesota, completing an MA thesis under PETE OLIVER's supervision. The Sixth International Congress of Genetics was to convene in Edinburgh at the end of the summer and PETE planned to attend. During his absence, he left me in charge of the lab with a number of tasks. Make the fly medium, clean the vials and bottles, transfer the stocks and, if there was time, find out why homozygous lz females were poorly fertile. (My stipend was $16 per month for the three summer months, which was not very much even for those days. But I was living at home and $16 would pay for trolley fare and lunches. Moreover, because I was making the Drosophila medium, I could eat any left-over bananas.) PETE OLIVER's interests were in X-ray mutagenesis following MULLER's great discovery; PETE was a MULLER student at the University of Texas. His doctoral dissertation had been on the radiation dose-response curve in Drosophila melanogaster and his interest in X-ray mutagenesis had continued. PETE's research philosophy was unambiguous and direct: select a multiple allelic series and go to work. He was particularly interested in two X-ray-induced lz alleles, lz^s (lozenge-spectacle) and lz^g (lozenge-glossy), both in the

X chromosome inversion $\Delta 49$. Each caused poor fertility in homozygous females, although hemizygous males were fully fertile.

So, I undertook the task of finding out why *lozenge* females were poorly fertile. A simple, direct approach was to dissect them to determine the effect of the mutations on internal genitalia. This turned out to be rewarding. Homozygous lz^s and lz^g and heteroallelic lz^s/lz^g were indistinguishable anatomically; all lacked spermathecae and parovariae. I concluded that this was responsible for the reduced fertility and looked no further. [This conclusion is probably wrong. Some 30 years later, in a stock of another allele, lz^{34k}, I found a recessive third-chromosome suppressor. The homozygous suppressor shifts all the phenotypic elements of the lz^{34k} phenotype–eyes, dorsal claws and female fertility–toward wild type. Yet the females lack spermathecae and parovariae (BENDER and GREEN 1960). HARVEY BENDER, then a postdoc, did a histological study of the ovaries of lz^{34k} females with and without the suppressor and concluded that reduced female fertility was associated with the onset of a syndrome causing the oocytes to degenerate. The suppressor delays the onset of the syndrome for several days (BENDER and GREEN 1962). I believe that the entire question of female fertility and lz mutations needs reexamination.]

Subsequent to PETE OLIVER's harrowing return from the Edinburgh Congress, which was marred by the onset of World War II and the torpedoing of ships carrying a number of Congress attendees, a series of experiments was designed to measure the fertility of lz females. The number of eggs, egg hatch, and number of adults were determined for lz^s and lz^g homozygotes and lz^s/lz^g heterozygotes. The order of fertility was $lz^s/lz^g > lz^g/lz^g > lz^s/lz^s$. Such results were not very exciting, but PETE OLIVER found an exception which was. He found occasional lz^+ phenotypes among the progeny of lz^s/lz^g females. Several facts could be established immediately. The lz^+ flies were not contaminants. because they had the appropriate marker genes and the $\Delta 49$ inversion. They were in all probability not back mutations, for no such types were found in the progeny of lz^s or lz^g homozygotes. Crossing over was involved in some way because each exception arose in association with the same unidirectional marker exchange (OLIVER 1940; OLIVER and GREEN 1944). In those days nobody thought of gene conversion as a possibility.

Two crucial points were established directly. The occurrence of the lz^+ flies was not a fluke; they could be recovered regularly from lz^g/lz^s females, always with the same unidirectional marker exchange. In all phenotypic details, including female fertility, the lz^+ flies were wild type. To be sure, crossing over was involved, but was it equal or unequal? The answer

might come from finding the reciprocal crossover product. What would its phenotype be? At the time this was PETE OLIVER's problem. I had a doctoral dissertation to complete, involving mutations at the *vestigial* (*vg*) wing locus and utilizing segmental aneuploidy.

The search for the reciprocal crossover product was fruitless. PETE tested a number of appropriate crossovers involving the outside markers without success. At one point there was great hope that the elusive type had been found, but it turned out to be a mutation of the *glass* (*gl*) locus on the third chromosome.

By the time my doctoral dissertation was completed in 1942, little additional progress had been made. The complementary crossover type was still an unknown. In the interim, PETE OLIVER was occupied with other matters and could spend little time on the lz problem. CHARLES DIGHT, an eccentric physician with an abiding interest in eugenics, had willed to the University of Minnesota funds to establish an Institute for the study of human genetics. PETE OLIVER became the first director of the Dight Institute and was preoccupied with its establishment and with research in human genetics. The organization of the Institute plus the entry of the United States into World War II put the lz problem on the back burner until the war was over. (OLIVER later returned to his roots and joined the faculty of the University of Texas. For many years he was Chairman of the Zoology Department; he is now retired and lives in Austin.)

After completing my dissertation I spent about four years in the U.S. Army. I recall that in the fall of 1945, while waiting in the Philippines for my medical unit to join the occupation army, I mulled over the future–specifically, what Drosophila research to pursue. Among potential research problems that I outlined, the *lozenge* problem was paramount. The issue of the reciprocal crossover product remained unresolved. Was reversion to lz^+ unique to lz^s/lz^g females, or could other heteroallelic combinations generate the same results? This question had been put to OLIVER on more than one occasion but he was reluctant to investigate any other lz alleles until the lz^s-lz^g problem had been solved. Furthermore, these alleles were within the $\Delta 49$ inversion and were therefore useless as testers of other lz alleles carried in chromosomes with the normal sequence.

In the fall of 1946, following discharge from the Army, I joined the Department of Zoology at the University of Missouri. I had spent the summer working in L. J. STADLER's cornfield. The ensuing four-year association with STADLER, one of the real giants of genetics (ROMAN 1988), was a rewarding, exhilarating experience. He provided the intellectual retreading that I needed after four years of military life.

The Zoology Department included a Drosophila

laboratory organized by A. B. GRIFFIN, a Drosophila cytogeneticist from the University of Texas who later moved to The Jackson Laboratory. I returned to Drosophila research and undertook two problems. One was a continuation of the lz work; the second was a biochemical study of eye color mutants in Drosophila. For the lz problem I turned to the unanswered question, does reversion to wild type occur with any other heteroallelic combinations? I had a number of lz mutations, both spontaneous and X-ray-induced, from a number of sources, all in chromosomes with the normal sequence. Phenotypically, these fell into two classes: those essentially like lz^s and those more or less like lz^g. I undertook a systematic study of combinations of lz^g-like with lz^s-like. (I was busy teaching general zoology 14 hours per week and trying to do some biochemistry. Therefore, most of the progeny scoring was done by my wife, an experienced fly-pusher who had completed a Master's degree under PETE OLIVER's guidance.)

This strategy produced mixed results: some heteroallelic females produced wild-type recombinants, others did not. Paralleling the lz^s/lz^g results, lz^{36}/lz^{46} females produced lz^+ progeny associated with polarized crossing over. This was expected because lz^{36} is a phenotypic mimic of lz^s, and lz^{46} of lz^g. In contrast, lz^{36}/lz^{BS} females–lz^{BS} is a lz^g mimic–produced no lz^+ flies among about 6000 progeny when at least five would have been expected. Now we had two questions to answer: why no lz^+, and why still no reciprocal crossover product? There was only one obvious experiment: what happens in lz^{BS}/lz^{46} females? We set up the crosses and, after the first progeny were scored, my wife left for Minneapolis to spend the winter vacation, December 1948, with her family. I remained behind and, being temporarily free of teaching, could score the remaining experiments. Two new results were obtained. First, lz^+ progeny appeared and marker exchange placed lz^{BS} at the same site as lz^{36} despite its phenotypic similarity to lz^g. Second, in two separate vials I saw, stuck in the medium, a male whose phenotype was neither lz^{BS} nor lz^{46} but was identical to lz^s and whose markers were those expected in the hoped-for reciprocal recombinant.

I fished each male out of the medium, hoping that at least one could be bred after drying out. To my disappointment, neither survived. Nonetheless, I was now confident that the long-sought reciprocal crossover had been found. If obtained once, it could be gotten again. Upon repeating the experiment, some 15,000 progeny yielded nine lz^+ and six lz^s-like flies. All had the appropriate marker combination and all six lz^s-like males produced progeny.

The proof that the lz^s-like flies did in fact carry the coupled lz^{BS} lz^{46} mutations on the X chromosome was straightforward. Crossovers between this and a wild-type chromosome should separate the two. When the experiment was done, both lz^{BS} and lz^{46} progeny were recovered with the predicted marker exchange.

The recovery of the coupled lz^{BS} lz^{46} crossover made it clear why all attempts to recover the complementary crossover product among the progeny of lz^g/lz^s females had failed: the recombinants were phenotypically indistinguishable from lz^s. It might be noted that ED LEWIS had a comparable problem identifying the crossover complementary to the wild-type product of S/ast females. It turned out that $S +/+ +$ and $S ast/+ +$ are also phenotypically indistinguishable.

By this time ED NOVITSKI, chromosome engineer par excellence, joined the Missouri Drosophila group as a postdoc. He volunteered to extract lz^g and lz^s from the $\Delta49$ inversion. (For reasons still not clear, neither mutant could be freed from the inversion by a double crossover.) He exploited the enhanced crossing over in triploid females and thereby succeeded in inserting the mutations into chromosomes of normal sequence (NOVITSKI 1950).

With lz^g in a chromosome of normal sequence, it was now mapped with respect to both lz^{BS} and lz^{46}. To make a long story short, lz^g mapped to a third site (GREEN and GREEN 1949). Eventually, 17 lz mutations were mapped and each could be be unambiguously assigned to one of the three identified lz sites (GREEN and GREEN 1956). Somewhat later, the mutation lz^k, first described as an *almondex* allele, turned out to be a lz allele and mapped to a fourth site (GREEN 1961). The lz gene is about 0.1 cM long and is separable into four equidistant, discontinuous sites. Proceeding from telomere to centromere, the four are identified by the mutations lz^{BS}–lz^k–lz^{46}–lz^g.

Ironically, the lz locus, the first to reveal the subdivisibility of the gene, has lagged in molecular analysis. The lz crossover problem went on the back burner during World War II; lz gene structure remains on the back burner. The nature of the phenotypic discontinuity revealed by the four mutation classes is ripe for analysis by modern methods.

The study of intragenic crossing over and genetic fine structure in *D. melanogaster* is a saga of brute-force genetic analysis at a time when selection techniques were not available. The Drosophila research provided the intellectual foundation for the elegant genetic fine structure analysis of the *rII* locus in bacteriophage T4 (BENZER 1955). All told, studies of genetic fine structure forced a rethinking of the concept of the indivisible gene and demonstrated that there is more than one type of gene organization. Gene analysis at the molecular level has reinforced this point of view.

M. M. GREEN
Department of Genetics
University of California
Davis, California 95616

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