

CHAPTER 5

MCCLINTOCK/STERN: GENETIC RECOMBINATION INVOLVES PHYSICAL EXCHANGE

Barbara McClintock in 1931 and then Curt Stern in 1933 demonstrated that the crossing-over process in meiosis actually involves a physical exchange of DNA. McClintock showed this through her experiments on corn, and Stern demonstrated this through his experiments on Drosophila.

THE MECHANICS OF RECOMBINATION

While recombination in meiosis provides one of the principal foundations of genetic analysis, sorting out how this recombination comes about has taken a long time. Even now, current journal articles contest the most basic aspects of the process. It has proven to be a difficult problem. The underlying mechanism is now understood at least in rough outline: although the principle of meiotic recombination is simple, the process itself is surprisingly complex.

In attempting to unravel how recombination occurs, scientists were first concerned with the physical nature of the process. No one had ever *seen* recombination. There was only Mendel's model, in which recombination takes place in a "black box," inferred indirectly by looking at the results. The first step in understanding the mechanisms of any process is to describe the physical events that occur. Understandably, the first physical investigations of recombination were at the chromosomal level, where events could be observed with the microscope.

As soon as it became apparent from Morgan's work the genes reside on chromosomes, the basic outlines of the problem became clear. If genes recombine, then chromosomes must do so-but how can chromosomes recombine their parts? F. A. Janssens studied sperm formation in amphibians, and in studying their chromosomes during the diplotene stage of meiosis, he noted frequent occurrence of chiasmata: again and again, chromosomes assumed "X" configurations, appearing as if they had crossed one another. Looking closely, Janssens saw that of the four filaments, two crossed each other and two did not. A simple hypothesis suggested itself: perhaps the *paternal* and *maternal* chromatids make contact, at intervals, and occasionally breakage and reunion occur, resulting in chiasma-and in recombinant chromosomes. His suggestion, the *chiasmotype theory*, was fundamentally correct-and was not accepted for fifty years.

MCCLINTOCK'S ZEA MAYS

The evidence came in 1931 from two experiments that are among the most lucid in genetics. Carried out completely independently, they used very much the same rationale. The first of these was H. B. Creighton and B. McClintock's work on corn (*Zea mays*). The logic of their experiment was to examine the recombination between two linked traits that were on a chromosome with unusual ends, ends that could be identified in the microscope. The ends served the function of visible "outside markers": when the traits recombine, do the chromosomal ends recombine too? The two genetic traits they chose to examine were the ones first studied in corn: colored (*C*) or colorless (*c*) aleurone layer in the endosperm, and starchy (*Wx*) endosperm. These two genes are on chromosome #9 in corn. A form of chromosome #9 was constructed that was cytologically unusual in two respects: it possessed a visible knob at the end of the short arm, and at the end of the other arm a segment of chromosome #8 was translocated so as to make it visibly longer.

Creighton and McClintock crossed a heterozygote of this chromosome as follows:



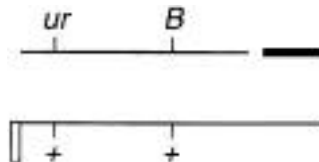
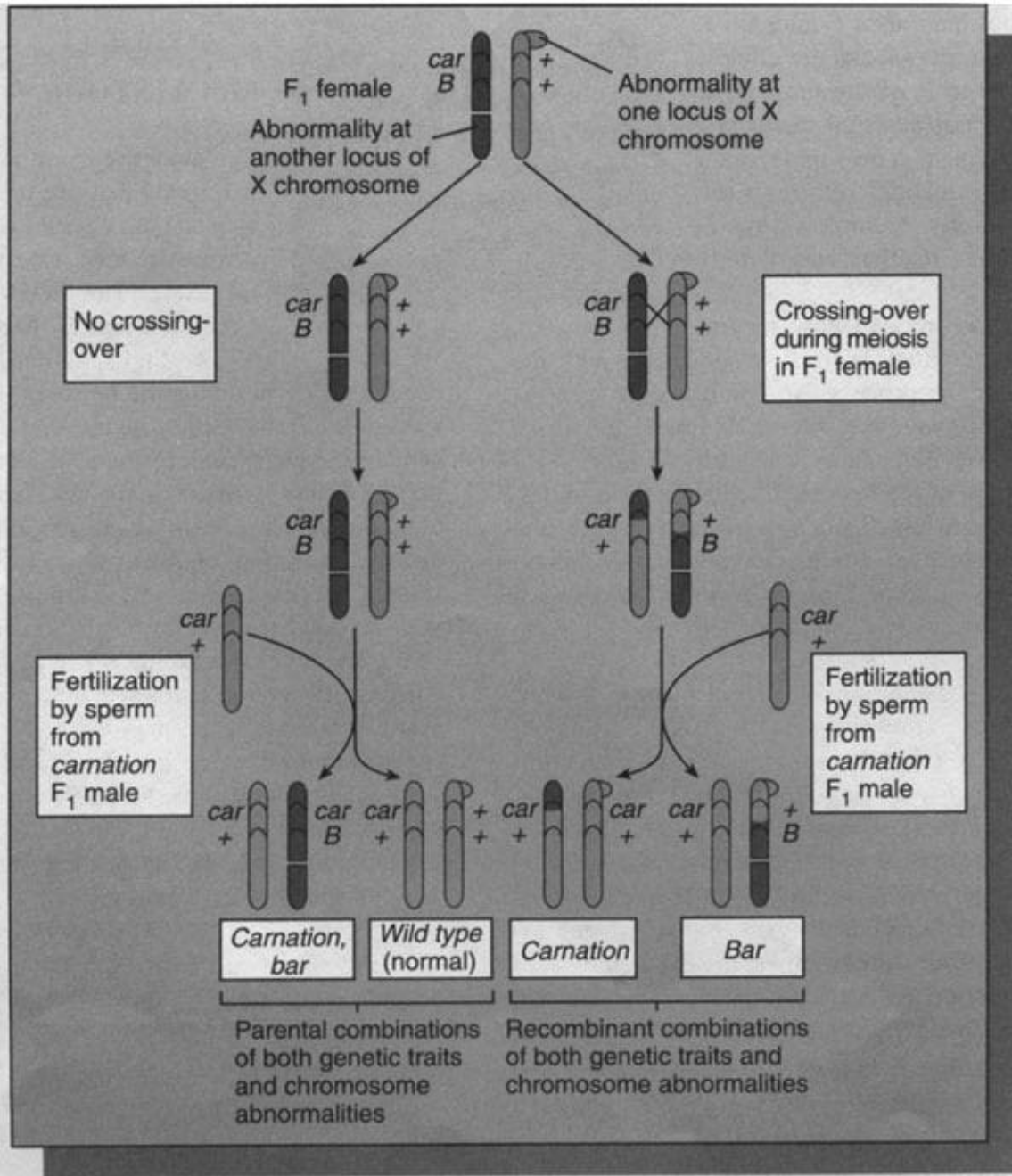
Of the progeny, 13 plants could be scored unambiguously. All 13 showed complete linkage between *C* and the knob, and 11 of the plants showed similar linkage between *Wx* and the translocation. Of these 11 plants, three showed recombination between *C* and *Wx*-and in every case the visible chromosome markers could be seen in the microscope to have exchanged too!

STERN'S DROSOPHILA MELANOGASTER

The second study carried out by Curt Stern involved the use of the fruit fly, where many more progeny could be conveniently analyzed. Indeed, Stern scored over 27,000 progeny, examining 364 of them cytologically. Looking at two sex-linked eye traits and cytologically abnormal X chromosomes, he obtained the same result as Creighton and McClintock: recombination of gene traits was always associated with recombination of visible chromosomal traits. Clearly, genetic crossing-over must involve a physical exchange of the chromosomes!

Stern set out to test whether or not genic crossing-over involved chromosomal crossing-over in the most direct possible way: by constructing a chromosome with visible abnormalities at each end. Recombinant chromosomes could be viewed through a microscope and therefore scored. Stern set up four different experiments, each involving two abnormalities in the X chromosome and two sex-linked traits. Each of the four experiments (we will describe only one of them here) gave the same result.

The two sex-linked traits used in one of Stern's experiments were *carnation eye* (*car*), a recessive eye color trait, and *Bar eye* (*Bar*), a dominant eye shape. The two X chromosome abnormalities were a portion of the Y chromosome attached to one end of the X chromosome and the far end of the X chromosome broken off and attached to tiny chromosome #4. In the cross Stern set up, both *car* and *Bar* were on a "broken" X, while the "Y-attached" X was wild-type. This produced female F₁ progeny that were heterozygous:



Recombination may occur in *Drosophila* females. To see whether chromosome exchange is correlated to genic cross-over, it was first necessary to be able to score the genic recombinants. How would this be done? With a test cross. A heterozygous fly was crossed to a fly recessive for both traits: *car* *+*.



Stern examined 8231 progeny of this cross for eye traits and examined 107 of them cytologically. There were a total of 73 recombinant progeny (*car, Bar*; or *+* *+*). Stern looked at three-quarters of these, 54 individuals, and as a control looked at 53 nonrecombinant progeny. What he found *without exception* was that genic recombinants were also chromosomal recombinants. All but one of the nonrecombinant gene progeny also showed a nonrecombinant chromosomal arrangement (the one deviant presumably represents the cross-over between *car* and the attached Y).

		<u>Parental</u>		<u>Recombinant</u>	
		<i>+, Bar</i>	<i>car, +</i>	<i>car, Bar</i>	<i>+, +</i>
Parental	Attached Y, broken	26	0	0	0
	<i>+</i> , <i>+</i>	0	26	0	0
Recombinant	<i>+</i> , broken	1	0	45	0
	Attached Y, <i>+</i>	0	0	0	9
Total (all progeny)		4001	4157	61	12