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Synthesis of a ring third chromosome and its use in inserting markers into In(3R)C.

Despite the early successes of Muller (1918 Genetics 3:422-499) and Sturtevant (Carnegie Inst.Wash.Publ. 421:1-27), it is difficult to insert markers into In(3R)C through double crossovers even with the aid of the interchromosomal effect of heterologous rearrangements. After scoring on the order of 100,000 flies in unsuccessful attempts to insert Tubby into In(3R)C with the aid of C(1)M3 and CyO, I decided to abandon the direct approach and look for a more practical method. I have now accomplished this marker insertion with the aid of a ring third chromosome.

The Upper portion of Figure 1 shows the synthesis of the ring, R(3C)S1, from In(3LR)P88+(3R)C and a structurally normal chromosome. In(3LR)P88+(3R)C/+ females are mated to LS(3)P88/DS(3)P88 males (see Genetics 99:75-97 for a description of the LS and DS notation) to recover the LS(3)P88/DS(3)P88, IN(3R)C--+ constellation. R(3)S1 is generated by a crossover in the 92E-100F regions of the DS(3)P88, In(3R)C--+ chromosome; the ring is duplicated for 89C to 92D and deficient for terminal chromatin in 61A and 100F. The duplication provides a convenient marker for identifying putative ring derivatives. It can be used to cover a deficiency that would otherwise cause lethality.

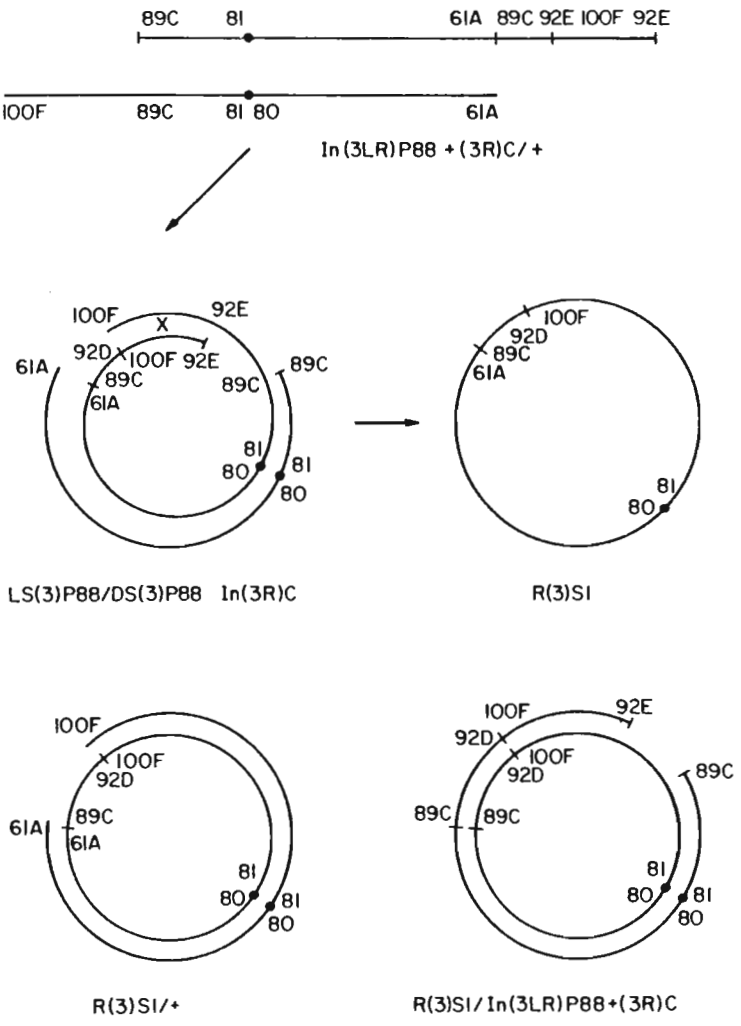

Fig. 1. Synthesis of R(3)S1 and the predicted pairing configurations for R(3)S1/+ and R(3)S1/In(3LR)P88+(3R)C.

To recover the ring, LS(3)P88/DS(3)P88, In(3R)C, bx^{34e} e--Tb ca females were mated to Df(3R)P47/Dp(3:3)MRS, Sb--+ males (Df(3R)P47 extends from 89D to 92A, while Dp(3:3)MRS is derived from Tp(3)MRS and has 87D to 93C inserted at 71B-C). Sb^+ offspring were then presumably R(3)S1/Df(3R)P47. Most of the Sb^+ offspring proved to be sterile, but it was possible to recover and maintain an R(3)S1, Tb ca chromosome. The ring structure was verified cytologically from metaphase figures from larval ganglia, and genetic confirmation was provided by transferring both Df(3R)P47 into the ring from a standard sequence chromosome and Tb ca from the ring into In(3LR)$Ubx^{UL}P88^{R}$+(3R)C.

The lower portion of Figure 1 illustrates the expected configuratio of R(3)S1 heterozygotes for either a standard sequency of In(3LR)P88+(3R)C chromosome. Double crossovers readily occur in females of either genotype so that markers can be transferred from a standard sequence chromosome to R(3)S1 to In(3LR)P88+(3R)C or from the inversion to the ring to the structurally normal chromosome. (Actually, In(3LR)$Ubx^{UL}P88^{R}$ is used instead of In(3LR)P88 since In(3LR)P88/R(3)S1 is lethal; In(3LR)$Ubx^{UL}P88^{R}$ also has the advantage of carrying sbd^2 as a marker.)

Newly recombined R(3)S1 chromosomes commonly induce sterility. R(3(S1/Df(3R)P47 progeny, identifiable as having minor phenotypic abnormalities characteristic

of the ring, from the ring synthesis are usually sterile or viturally so; moreover, those animals which are virtually sterile--at most 2 progeny--have sterile or nearly sterile progeny. R(3)S1, Df(3R)P47+ progeny from R(3)S1/Df(3R)P47 mothers also show this pattern of sterility. The few crossovers which are completely fertile show no indication of sterility in subsequent generations.

Because of this sterility problem and because of the lowered viability of R(3)S1+ due to hyperploidy, it is desirable to selectively recover ring-bearing progeny when inserting markers into the ring from a standard sequence chromosome. This can be accomplished by crossing R(3)S1, Df(3R)P47/marker females to Df(3R)P47/Dp(3;3)MRS, Sb--+ males to recover recombinant R(3)S1 chromosomes (with or without the marker) over Df(3R)P47 and later test for presence of the marker. To transfer markers from the ring to In(3R)C, it is convenient to recover an $In(3LR)Ubx^{UL}P88^{R}+(3R)C$, sbd^2 chromosome with the marker from R(3)S1, marker/$In(3LR)Ubx^{U}$ +(3R)C, sbd^2 ss Ubx^{U} mothers: sbd^2 provides a marker for the inversion, and absence of the Ubx^{U} phenotype identifies a crossover.

Stocks of (1) R(3)S1, Df(3R)P47, ca/In(3R)C, Sb cd Tb ca,
(2) Df(3R)P47/Dp(3;3)MRS, Sb--+,
and (3) $In(3LR)Ubx^{U}+(3R)C$, sbd^2 ss Ubx^{U} cd/$T(2;3)ap^{Xa}$ are available from the Pasadena stockcenter.

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Transferring markers to or from autosomal inversions.

Markers can be transferred from a standard sequence chromosome into a large pericentric inversion by double crossing over. For inversions of moderate length--on the order of 10 numbered divisions--such double crossovers can be exceedingly rare and are prohibitively rare for small inversions. It is, however, possible to transfer markers from one inversion to another through a sequence of selected single crossovers. $InA^{L}B^{R}$/InB females are crossed to $InB^{L}A^{R}/InA^{L}B^{R}$ males to selectively recover recombinant $InA^{L}B^{R}$ and $InB^{L}A^{R}$ chromosomes (it is assumed that $InA^{L}B^{R}$/"+" and $InB^{L}A^{R}$/"+" are lethally aneuploid genotypes). InA and InB are then reconstituted by crossing $InA^{L}B^{R}/InB^{L}A^{R}$ females to structurally normal males. The reconstituted InA and InB chromosomes are frequenctly double crossover chromosomes, so that markers may be transferred from one inversion to the other via this sequence of crosses.

Transferring markers from a structurally normal chromosome to moderate length or smaller inversions is accomplished by first transferring the markers into a large inversion, then transferring the markers from the large inversion to the smaller one. As an example, the following sequence of crosses was used to insert se, h^2, rs^2, and th into In(3L)P:

P_1 C(1)M4, y^2; In(3L)C90/se h^2 rs^2 th st cp in ri p^{p} females were crossed to se h^2 rs^2 th st cp in ri p^{p} males.

C(1)M4 is present in this cross to increase crossing over. In(3L)C90 is a large pericentric inversion with 62B and 80 breaks. In(3L)C90, se h^2 rs2 th st was recovered in the P_2. A balanced stock of C(1)M4, y^2; In(3L)C90, se h^2 rs^2 th st/In(3L)P, Me h D^3 was then constructed.

P_4 C(1)M4, y^2; In(3L)C90, se h^2 rs^2 th st/In(3L)P, Me h D^3 females were crossed to $In(3L)C90^{L}P^{R}+(3R)P18$, Ubx $e^4/In(3L)P^{L}C90^{R}$ males,

to recover $In(3L)C90^{L}P^{R}$, se h^2 rs^2 th st/$In(3L)P^{L}C90^{R}$ (recognizable as being Me^{+} and Ubx^{+}) and $In(3L)P^{L}C90^{R}$, se h^2 $D^3/In(3L)C90^{L}P^{R}+(3R)P18$, Ubx e^4. These two genotypes were crossed to each other to produce a

C(1)M4, y^2; $In(3L)C90^{L}P^{R}$, se h^2 rs ^{2}th st/$In(3L)P^{L}C90^{R}$, se h^2 D^3 stock.

P_6 C(1)M4, y^2; $In(3L)C90^{L}P^{R}$, se h^2 rs^2 th st/$In(3L)P^{L}C90^{R}$ females were crossed to: th st cp in ri p^{p} males.

A few $th_2st^{+}{}_2$offspring (In(3L)P, se h^2 rs^2 th) offspring were produced and a stock of In(3L)P, se h^2 rs^2 th was then established.