**Muller, H.J. Insertion of Foreign Chromosome into Homozygous Host Stock** (Stocks f and h need not contain any visible mutants)

(1) For substituting $1^f$ for $1^h$ in $1^h;2^h;3^h;4^h$ stock

$P_1$ f ♂♂ X ClB/+;T(2-3)S Cy/+,+;$ey^D$/+ ♀♀

$F_1$ $1^f$/ClB;$2^f$,$3^f$/T(2-3)S Cy;$4^f$/$ey^D$ ♀♀ X h ♂♂

$F_2$ one ♂ $1^f$;$2^h$,$3^h$/T(2-3)S Cy;$4^h$/$ey^D$ X ClB/+;Cy/+;CC D/+;$ci^D$/+ ♀♀
(save for remating!) (Different markers; Inversions)

$F_3$ Father (remated) X $1^f$/ClB;$2^h$/Cy;$3^h$/CC,D;$4^h$/$ci^D$ ♀

$F_4$ $1^f$;$2^h$;$3^h$;$4^h$ completely homozygous ♀♀ X ♂♂

(2) For substituting $2^f$ for $2^h$ in $1^h;2^h;3^h;4^h$ stock

$P_1$ f ♀♀ X Pm/+;H/+;$ey^D$/+ ♂♂ AND h ♂♂ X ClB/+;Cy/+; CC,D/+;$ci^D$/+ ♀♀

$F_1$ $1^f$;$2^f$/Pm;$3^f$/H;$4^f$/$ey^D$ ♂♂ X $1^h$/ClB;$2^h$/Cy;$3^h$/CC,D;$4^h$/$ci^D$ ♀♀

$F_2$ one ♂ $1^h$;$2^f$/Cy;$3^h$/H;$4^h$/$ey^D$ X $1^f$/ClB;$2^f$/Cy;$3^f$/CC,D;$4^f$/$ci^D$ ♀♀
(Recombination; save!)

$F_3$ Father X $1^h$/ClB;$2^f$/Cy;$3^h$/CC,D;$4^h$/$ci^D$ ♀♀

$F_4$ $1^h$;$2^f$;$3^h$;$4^h$ completely homozygous ♀♀ and ♂♂

(3,4) Analogous schemes apply for 3 and 4 (selecting appropriate $F_2$ ♂)

If desired, foreign autosome may first be made like that in homozygous stock except in regard to particular region. Method for this is shown below in scheme for combining invisible genes in the same chromosome.

**Muller, H. J. Insertion of Desired Genes into Attached X's**

$P_1$ y ♀♀ X T(1-4)$B^S$ ♂♂ (Austin T; left part X attached to 4; rt end X carrying "Bar-super")

$F_1$ y/XR,$B^S$ ♀♀ X m $g^2$ sd f ♂♂ (Let m, $g^2$, sd, f be the genes to be inserted)

$F_2$ (Crossing-over) y·$B^S$/m $g^2$ sd f ♀ X m $g^2$ sd f ♂♂

$F_3$ (Crossing-over) m $g^2$ sd f·$B^S$/m $g^2$ sd f ♀ X + ♂♂

$F_4$ (Crossing-over) m $g^2$ sd f Y ♀

In earlier method, used by L.V. Morgan et. al, genes were inserted by crossing-over, from separate X-chromosome containing them, into attached X's, in triploids.

**Muller, H. J. Combination of Invisible Genes**

Nearly same methods apply for getting desired chromosome combinations of recessives that always must be worked with in the heterozygous condition.

1. Two invisibles in different autosomes (a in 2; b in 3)

$P_1$ a/2 ♂ X S/+;CC,D/+ ♀ AND b/3 ♂ X Cy/+;CC,Dfd/+ ♀

$F_1$ a/S;CC,D/+ ♀ X Cy/+;b/CC,Dfd ♀

$F_2$ a/Cy;b/CC,D ♂ X a/Cy;b/CC,D ♀
(balanced stock, if recessives have low viability or low fertility)

$F_3$ a/a;b/b ♂ and ♀ (if homozygotes are viable and fertile)
(for chromosome 4, markers $ci^D$ and $ey^D$ may be used in same way.

2. Getting combination of invisibles in 1 and 2 (a in 1;b in 2)

$P_1$ a/1 ♀ X Cy/+ ♂ AND b/2 ♂ X ClB/+;Pm/+ ♀.
(double mated) y;Pm/+ ♀

$F_1$ a;Cy/+ ♂ (double mated) X ClB/+;b/Pm ♀ and Xy;b/Pm ♀

$F_2$ a/ClB;b/Cy ♀ X a;b/Cy ♂ (balanced $\not{S}$)

$F_3$ a/a;b/b ♀ and a;b/b ♂ (if homo. viab. and fert.)

. Analogous scheme using CC;D and CC,Dfd or Mc-2 for 3 applies for chromosomes 1 and 3.

3. Getting combination of two invisible genes (a and b) in same chromosome (in known positions). Let map order be a, $v_1$, $v_2$, b where $v_1$ and $v_2$ are visible genes between a and b.

$P_1$ a/+ X $v_1$ $v_2$ AND b/+ X $v_1$ $v_2$

$F_1$ a++/+$v_1$ $v_2$ ♀ X $v_1$ $v_2$ ♂ AND ++b/$v_1$ $v_2$ + ♂ x $v_1$ $v_2$ ♂

$F_2$ (by crossing-over) a + $v_2$/+ $v_1$ $v_2$ X $v_1$ + b/$v_1$ $v_2$ +
(chance of losing a or b from ♀ obviable by interpolating cross of ♂ by individual containing inversions)

$F_3$ a + $v_2$ +/+ $v_1$ + b ♀ X $v_1$ $v_2$ ♂

$F_4$ (by crossing-over) a++b/$v_1$ $v_2$ X $D_1$ v ♀
(where $D_1$ is dominant linked and where v is either $v_1$ or $v_2$)

$F_5$ a b/$D_1$ v ♂ X C,$D_2$ ♀ (where $D_2$ is a different linked dominant)
(lethal when homozygous, and C is an inversion preventing crossing-over).

$F_6$ a b/C,$D_2$ ♂ and ♀ (balanced $\not{S}$)

$F_7$ a b/a b ♂ and ♀ (homozygous $\not{S}$)

Combination of one invisible with one visible is correspondingly simpler.

<u>Muller, H. J. and C. B. Bridges</u>
<u>Balanced Stocks</u>

Given "<u>1</u>", a gene (or combination of linked genes) with low or no viability or fertility when homozygous in ♂ or ♀ (or both, and "C" a genetic complex that is non-perpetuating or lethal when homozygous, that contains gene rearrangements which prevent crossing-over between "<u>1</u>" and "C" and that preferably contains a dominant marker, then the most usual balanced stock for maintenance of "<u>1</u>" without selection is:

<u>1</u>/C ♂ X <u>1</u>/C ♀

<u>1</u>/<u>1</u> (fails to perpetuate ); C/C (dies); <u>1</u>/C and C/<u>1</u> (continue as before.

. To construct simple balanced autosomal stocks (<u>1</u>/C)
Given <u>1</u> a/+ (or <u>1</u>/a) ♂, where a = recessive marker and M = a dominant linked marker subject to crossing-over. Then:

$P_1$ M/+ X a/a

$F_1$ M/a ♀ X a/a ♂

$F_2$ M a/a ♀ X <u>1</u> a/+ ♂ (or <u>1</u>/a ♂)

$F_3$ M a/<u>1</u> a ♂ X C/+ ♀ (or M a/<u>1</u> X C/+ ♀ )

$F_4$ <u>1</u> a/C ♂ and ♀ (or <u>1</u>/C ♂ and ♀ )

The more used complexes represented above by "C" are as follows: