

2.

Mating with single animals: F1, *cn bw**/*CyO.513*(Female, single line with mutation) x *Fs(2)D/CyO.513*(Male).

Mating with many animals: F2, *cn bw**/*CyO.513*(Male/Female, line with mutation), *cn bw**/*Fs(2)D*(Male/Female, another line with mutation), *Fs(2)D/CyO.513* (Male/Female)

3.

Missing and weakness:

1) The 'maternal effect locus' here is not an absolute distinction. It will just almost lethally influence the embryo and there are some unusual alleles of deficiencies will also reduce the zygotic viability.

2) Most phenotypes will be determined by several loci because various genes are involved in the pathways. A lot of information will be lost if only single mutation was considered.

3) Not all active genes in embryogenesis are merely related to maternal effect. Those taking effect both zygotically and maternally will be detected in these female sterile screens.

4) There could be some other dependent patterning pathways in the embryo cannot be easily detected because all contributing genes are also required for the survival of the zygote.

5) This was not designed for dominant loci of maternal effect.

Strength and benefits:

1) The appropriate usage of *CyO.513*. The introduction of the balancer facilitated the selection of heterozygotes in F1 and F2.

2) The introduction of *Fs(2)D*. Because of it, all heterozygotes after F2 will be subsequently represented as sterile but mutated homozygotes were selected.

4.

To improve the screening, RNA-seq can be implemented for the description of molecular phenotype. To compare the expression level and gene association network (microRNA can also be included), we can identify the genes which are considerably affected and associate them with phenotypes.

However, I would prefer reverse genetics. With the help of whole genome sequencing, we can easily design and introduce mutations into specific genes using CRISPR-Cas9. All we need to do first is to build the mutations library of sgRNAs to cover almost every gene we are interested in from the *Drosophila* genome. Then, introduce the mutations of groups of mutations into zygotes with proper balancer to build the lines for testing. After the intersection, test the embryos of interesting lines and sequence them for the confirmation of mutation or just label them beforehand. So, we can get the association of mutations of genes with their phenotypes.