Alonso, C. University of Nijmegen, The Netherlands. The effect of gibberellic acid on the development of D. hydei.

The effect of injection into larvae of D. hydei of various concentrations of gibberellic acid ($GA_3$) on the chromosomal puffing pattern, the moment of puparium formation and the number of flies emerging from pupae was investigated.

It was observed that $GA_3$ can induce a new puff, 4-72B, in 15% of the larvae injected at 140 hours following oviposition. This puff can be observed 9 - 10 hours after injection. If $CA_3$ is injected at 153 h. a new puff develops within 3 - 4 hours at region 2-21B. These puffs have never been observed in normal development and are absent in animals injected with the solvent (Ringer's). The frequency with which puff 2-21B occurs depends on the concentration $GA_3$ applied, but never reaches a 100%. Apart from the newly induced puffs, $GA_3$ appears to affect the occurrence and size of puffs which are characteristic for the period prior to puparium formation. Injection of 1.5 µg/larva delays the appearance of the ecdysone-specific puff 4-78B and inhibits its full development. The same applies for puff 4-77BC. Mixtures of ecdysone and $GA_3$ injected into intermoult larvae gave a similar effect, a delayed development of the puffs 4-78B, and 4-77BC as compared with larvae injected with ecdysone only ($2.10^{-1}$ µg/larva).

Also puparium formation was delayed if $GA_3$ was injected at 153 h. or later (pup. formation occurs normally around 160 h.). The delay was increased with increasing concentration of $GA_3$.

The percentage of flies emerging from pupae developed from $GA_3$ injected larvae decreased considerably with increasing $GA_3$ concentration. Injection of 6 µg/larva resulted in 54% lethality.

Schalet, A.* University of Connecticut, Storrs, Connecticut. Two modified crossover-selector systems of general application to fine structure analysis.

Scheme 1 has been used in a fine structure analysis of the vermilion locus, but is applicable to other X-linked and autosomal loci. For the v locus a cross of the following type was used:

♀♀ (A) $\frac{l1 + v^x\ l3 + X}{(B) + l2\ v^y + l4}$ ♂♂ (C) $\frac{+\ l2\ v\ l3 +}{(D) - + v + - Y}$ ; $bw^D$

From this cross the only ♂♂ that survive are 1/2 of the crossovers between the outside lethals, l1 & l4. The only ♀♀ that survive, aside from non-disjunctionally produced individuals, are 1/2 of the crossovers between the inside lethals, l2 & l3. If $v^x$ is located to the left of $v^y$, then $v^+$ recombinant ♂♂ will survive. If $v^x$ is located to the right of $v^y$, then $v^+$ recombinant ♀♀ will survive.

Chromosome (B) was introduced into the cross thru parental ♂♂ carrying a Y chromosome that covered the region from l2 thru l4 (Schalet DIS 44: 123). This chromosome, as well as chromosome (D), was derived from the $v^+Y$ $y^+$ chromosomes synthesized by Chovnick, DIS 43: 170. The presence of the $y^+$ region on the Y, chromosome (D), with its Hw effect, and the partial suppression of the $bw^D$ phenotype in ♀♀ carrying a Y chromosome, permitted the detection of XXY ♀♀. Such ♀♀ appeared at an estimated rate of 1 for every 1,500 regularly produced zygotes. Linkage relationships determined from other crosses were as follows: ras--l1, 0.1; l1--l2, 0.4; l2--v, 0.2; v--l3, 0.7; l3--l4, 2.0. Note that the value of 0.7 for the interval between ras and v is closer to the value of 0.59 reported by Lefevre, DIS 45: 40, than the standard value of 0.2.

In the table below the total number of zygotes sampled has been calculated on the basis that each regularly produced ♀ represented approximately 1/444 of the number of eggs laid, (2/1,000 & 7/1,000)/4. Although only 1/2 of the eggs laid represent sampled chromosomes, this scheme provides the advantage that for any two potentially separable alleles, whatever their left-right orientation, only a single cross is required. Consequently, each allele need be inserted in or induced on only one of the two types of lethal bearing chromosomes.

| Test | $v^+$/Total | Order | Test | $v^+$/Total | Order |
|---|---|---|---|---|---|
| 2/1 | 0/307,000 | | 1/E1 | 1♂/330,000 | 1-E1 |
| 1/k | 0/890,000 | | 36f/E1 | 1♀/460,000 | E1-36f |
| 36f/65c | 0/250,000 | | 2/E1 | 3♂♂/167,000 | 2-E1 |
| 1/36f | (Green) | 1-36f | 36f/2 | 1♀/195,000 | 2-36f |
| 48a/36f | (Barish & Fox) | 48a-36f | 36f/k | 2♀♀/350,000 | k-36f |

All $v^+$ individuals were fertile and the recombinant chromosomes proved to carry $v^+$ and the appropriate non-selective lethal markers, except that the 3♂♂ from 2/El were not tested for l3. However, all 3 chromosomes did carry dy which was derived from chromosome (A) of the parental ♀♀. The recovery of $v^+$ individuals in crosses 1/El and 36f/El indicates that there are 3 separable sites in the order 1-El-36f. The El allele was indiced by EMS and is unsuppressed by $su^{51c15}$-v. Note that the data in the table present additional examples of the separability of unsuppressed alleles, 36f/El, and the separability of suppressed and unsuppressed alleles, 1/El, 2/El, 2/36f and k/36f. The separability of the left-positioned suppressible alleles, 1, 2 and k has not been achieved.

Scheme 2 was originally devised for a fine structure analysis of the maroon-like locus, but was abandoned in favor of Glassman's purine selector system. The basic scheme is not only applicable to sex-linked visible loci, but an important variation may also be used for any sex-linked lethal locus where an analysis is not facilitated by the existence of temperature sensitive mutants. The basic scheme has recently been utilized by Finnerty and Chovnick (Genetical Research 1970) to recover maroonlike double mutant recombinants. For any visible mutant, m, consider a cross of the type:

$$♀♀ \frac{l1\ m^x\ +}{+\ m^y\ l2}\ ;\ \frac{\text{Ins, Sb or Ubx}}{l(3)26,\ \text{Sb or Ubx}}\ X\ ♂♂\ T(1;3),\ \text{cu kar ry}^{26}\ l(3)26\ \text{Sb Ubx}$$

The translocation used was one selected for good viability and fertility from among a number of X-ray-induced rearrangements between a wild-type X chromosome and a third chromosome carrying the indicated markers. In addition to the zygotic lethality produced by paternally derived aneuploid chromosomal combinations, all ♀ euploid zygotes, aside from those produced by maternal X chromosome non-disjunction, die because of the lethality associated with homozygosity for l(3)26, Sb or Ubx. All euploid ♂ zygotes die except those derived from a lethal-free chromosome produced by crossovers, including those between $m^x$ and $m^y$, in the small region between the tightly linked lethal markers. For loci in which the double mutant cannot be immediately recognized, it is necessary, at least in the initial stages of the analysis, to make a companion cross in which the distribution of the X chromosome markers is l1 $m^y$ +/+ $m^x$ l2. In addition to the availability of 2 closely linked lethals, it is of course necessary to have a duplication, preferably on the Y, for the region that covers at least one of the lethals in order to introduce into the cross one of the tested alleles thru the ♂ line.

Welshons and Von Halle (Genetics 1962) have used a very effective selector system for the Notch locus to separate alleles that behaved as non-complementary recessive lethals. Their scheme, essentially: ♀♀ $N^x/N^y$;Ins, $DpN^+$ l1 l2/+ X ♂♂ $fa^{no}$/Y;l1/l2, was facilitated because the viable $fa^{no}$ was lethal in the combination $fa^{no}$/N. A variation of scheme 2 can be applied to any sex-linked lethal locus, m, as follows: ♀♀ $m^x/m^y$;$Dpm^+$ Ly Sb/In(3)$Ubx^{130}$ X ♂♂ T(1;3), cu kar $ry^{26}$ l(3)26 Sb Ubx/Ly. Aside from the sex-linked lethal alleles being tested, there are two new elements added to scheme 2. The dominant mutant Lyra behaves as a recessive lethal. The Ly Sb third chromosome carries a duplication for the region of interest. In principle, the production and detection of such a duplication should offer no problems. Again ♀♀ die as in the basic scheme 2, and all ♂♂ with the $Dpm^+$ chromosome die because they will be homozygous for Ly. The only survivors of the cross will be ♂♂ derived from a crossover, conversion or back-mutation event at the m locus.

Finally, it is recognized that the type of scheme just discussed may be dispensed with, for the most part, where the expense of the purine selector system is not a prime consideration. Since purine will kill maroonlike and rosy mutants, and $mal^+$ Y chromosomes are available, the following type of selector system is possible for a sex-linked lethal locus: ♀♀ $m^x$ mal/$m^y$ mal;$Dpm^+$ ry/In(3)$Ubx^{130}$ X ♂♂ mal/$mal^+$ Y;ry. However, it should also be emphasized that further, relatively simple variations of scheme 2 alone, or in combination with the purine system, may be used for a fine structure analysis of autosomal lethal loci.

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