diploid set[4–6]. Since in the numerous different sections examined, never has more than one pair of these structures for diploid chromosome complement been observed, it is tempting to speculate that this structure could correspond to the nucleolar organizing region (NOR), which is visualized as a secondary constriction at the optical level. Moreover, the similarity between the characteristics of this structure found during meiotic division and those of the NOR described in A. cepa microspores[7], suggest that we are looking at the same region through the different stages of meiosis.

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Efficient methods for isolation of X-linked male sterile mutations in Drosophila melanogaster[1]

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Summary. Three different mating systems based on the production of virgin females in F_1 and the elimination of undesired males in F_2 are proposed for an efficient isolation of X-linked male sterile mutations in Drosophila melanogaster.

Male sterile mutations in Drosophila melanogaster provide a useful tool to study the control of sperm development at the gene and chromosomal level[3–5]. At least for the X chromosome, the large majority of mutations are known to interfere with spermatogenesis: in 180 from 192 mutant stocks tested, no mature motile sperm was found[6]. Some additional use of X-linked male sterile mutations can be inferred from the findings about the remaining 12 mutant stocks: they showed abnormalities in mating behavior[4], sperm transfer[5], acrosome reaction[5] or only a low number of weakly motile sperm was produced[6].

We are interested in mutations affecting the male accessory gland (paragonial) proteins. They should enable us to clarify the functional significance of these proteins. We assume that some of them are sterile and might belong to the group of mutations which interfere with sperm transfer. From the above data, only a very low frequency of such mutations could be expected. An economic screening system should facilitate our project.

Our system is based on Muller's standard procedure for detecting X-linked recessive lethals and sterile factors[7,8]. We found that it was desirable to eliminate or simplify the following time-consuming steps: first, the collection of virgin females in F_1, and second, the test for fertility with F_2 males bearing a mutagenized X chromosome. Figure 1 presents the mating scheme which has been realized by us. The Killer-prune system was utilized to produce virgin females in F_1: all flies which bear a Killer-prune (K-pn) allele and are homozygous or hemizygous respectively for prune (pn) die as larvae[9,10]. To simplify the test for fertility, we introduced a lethal factor into the Muller-5 (M-5) balancer. As a consequence, only those males which carry a mutagenized X chromosome emerge in the F_2. Thus, the test for fertility can easily be undertaken by transferring the offspring into fresh

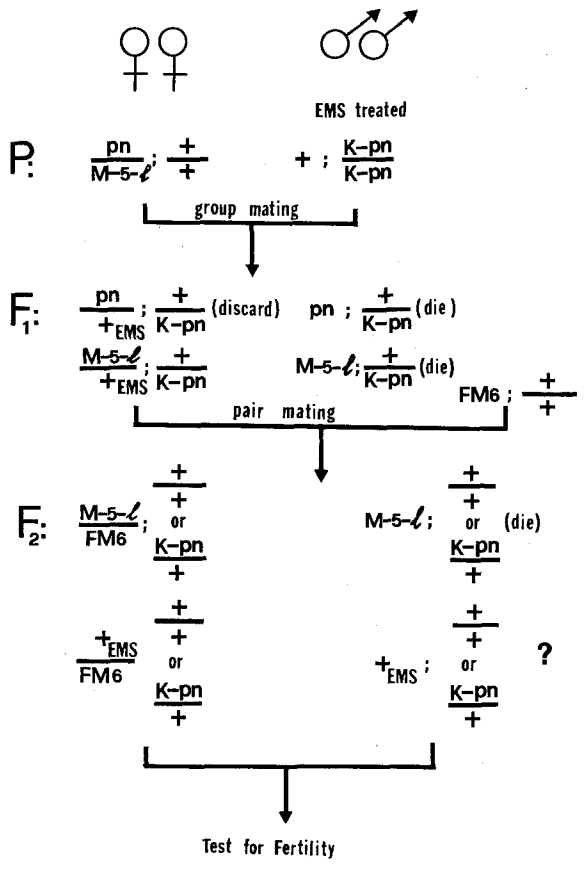

Fig. 1. Mating scheme 1 for isolation of X-linked male sterile mutations in Drosophila melanogaster. The Killer-prune system and Muller-5 balancers were combined in such a way that in F_1 all males died, and in F_2 those not bearing a mutagenized (EMS treated) X chromosome were eliminated. The test for fertility could be carried out simply by transferring all male-producing F_2 cultures into fresh vials and watching for larval growth survival. In case a mutation was found, $+_{EMS}$/FM6 females were crossed to FM6 males for maintaining the mutant stock. M-5-l, Muller-5 balancer (In(1)$sc^{s1}r sc^{8R}$+S, $sc^{s1}sc^{8}w^{a}$B) carrying a lethal. It was produced as follows: prune (pn) females were crossed to mutagenized M-5 males. F_1 females were set up in single cultures with pn males; FM6, First Multiple 6 (In(1)FM6, $y^{31d}sc^{8}$dm B); K-pn, Killer-prune.

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vials and watching for larval growth survival. The M-5-l balancer is principally a C1B chromosome[7] but it offers the advantage that fewer double crossing-overs occur, due to a larger extention of the inverted region[8]. The induced lethal should have a 100% penetrance. The M-5-l balancer was chosen among 20 different M-5-l chromosomes. In the progeny of the test cross (M-5-l/pn; + females x +; K-pn males) about 400 females but no males were found. FM6 provides a fully balanced system, since FM6/FM6 females are sterile due to dm; therefore separation of female genotypes becomes unnecessary. In addition, FM6 is a somewhat better crossing-over suppressor than M-5[11,12]. It is unnecessary to remove K-pn genes, since they do not interfere and will probably be lost in later generations.

As mutagenizing agent, ethyl methane sulfonate (EMS) was used[13]. Flies were routinely fed 10 ml of a 0.5% (v/v) EMS solution in 1% sucrose for 24 h, resulting in a lethal factor frequency of 28–37%. Among 9527 X chromosomes tested, 171 male sterile mutations were found (1.8%). With regard to mutations affecting the paragonial proteins, we were fortunate to find just one mutation. In flies bearing this mutation a main band (SDS polyacrylamide gel[14]) is reduced to a very low level. Alternative systems, not realized by us, are proposed in mating schemes 2 and 3 (figures 2 and 3). In mating scheme 2 pn and K-pn are substituted by maroonlike (mal). The mal system is based on the fact that the purine concentration in the medium can be adjusted to a level not tolerated by mal flies but tolerated by + flies[15–17]. Another possibility is the use of temperature-sensitive lethals[18] (figure 3). In general, among the EMS-treated X chromosomes bearing a single lethal, at least 10.7% can be expected to carry a heat-sensitive and 1.5–3% a cold-sensitive lethal[18,19]. Once realized, there is an advantage in this system due to the fact that in F_1 only 1 class of females will be produced; therefore separation of female genotypes becomes unnecessary.

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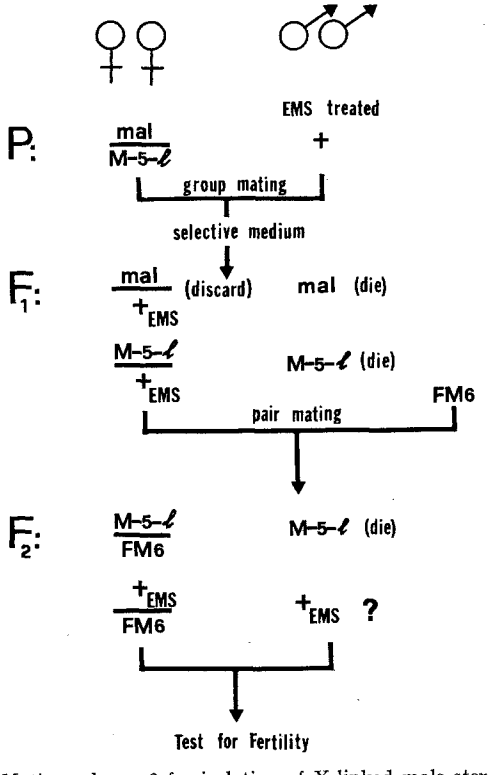

Fig. 2. Mating scheme 2 for isolation of X-linked male sterile mutations in Drosophila melanogaster. Instead of the Killer-prune system, the maroonlike (mal) system is proposed for the production of virgin females in F_1. According to Finnerty et al.[17], one would have to maintain parental flies for 2–3 days in half-pint bottles filled with standard food. Immediately after transfer, 1–2 ml of a 0.2% purine (Sigma P 6880) solution should be added homogeneously to the already growing culture. Abbreviations as in figure 1.

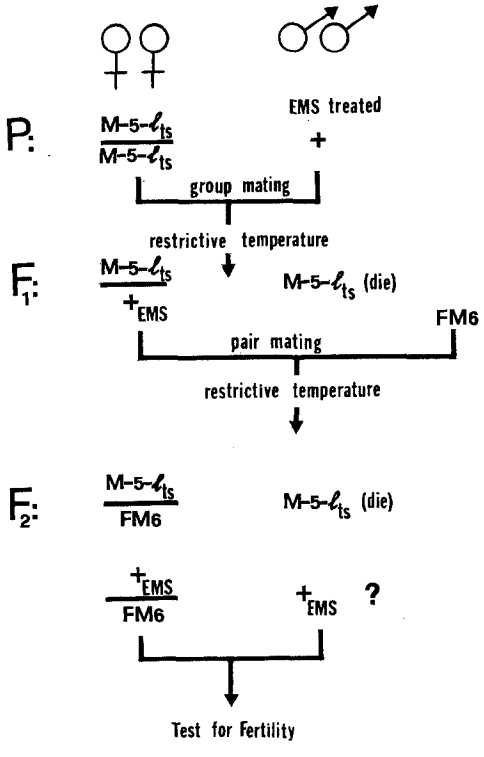

Fig. 3. Mating scheme 3 for isolation of X-linked male sterile mutations in Drosophila melanogaster. Muller-5 balancers carrying temperature-sensitive lethals are proposed for the production of virgin females in F_1, and for the elimination of undesired males in F_2. In contrast to mating schemes 1 and 2, only 1 class of females would be produced in F_1. M-5-l_{ts}, Muller-5 balancer carrying a temperature-sensitive lethal. Other abbreviations as in figure 1.