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# On the Prospects of Using Balanced Sex-linked Lethals for Insect Pest Control

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**Summary.** A new method is suggested for controlling species of insect pests in which the female is heterogametic. This method, involving the use of balanced lethals on the Z chromosome, causes the death of females in the embryonic stage. The method has already been tested in practical sericulture for the production of entirely male progeny of the silkworm. The method requires the construction of two strains of the pest, one carrying two balanced non-allelic but closely located lethals on the Z chromosome, and another with two other pairs of lethals of the same type. In the hybrid progeny from the crosses between the two strains, 100% of the female embryos would die, thus making it possible to release only males without any laborious procedure for sex discrimination. In the progeny from the crosses between the released males and females from the natural population, again 100% of females would die, but the males would survive and when they mated – 62.5% of the female progeny would die. This rate would decline to 34.4 and 16.6% in the sons and grandsons respectively. The repeated release of hybrid males would lead to a progressive increase, with each successive generation, in the percentage of female mortality in the natural population until its total extinction.

**Key words**: Insect pest control – Z chromosome – Sex linked lethals

As early as 1939 A.S. Serebrovsky (1940, 1971) first set forth the theoretical framework for the control of insect pests by genetic means through the release into natural populations of specially constructed insect pest strains carrying various genetic aberrations, primarily, chromosomal translocations. According to his scheme, the released insects or their progeny would show reduced fertility after cross matings to the individuals from the natural population, thus contributing to the reduction of the pest numbers of the natural population.

Since then the possibility of using genetic methods for the control of insect pests has been extensively studied by many investigators (Curtis 1968; Curtis and Hill 1971; Robinson 1976; Whitten and Foster 1975).

At present, genetic methods of pest control, in the broad sense of the term, are based on the reduction of fertility in natural pest populations by introducing material into their genetic system which is either incompatible or damaged to such an extent that the zygotes formed by the fusion of genetically changed and normal gametes fail to develop. Among the factors inducing various genetic disorders or sterility, the most convenient and effective one is ionizing radiation.

A special advantage of genetic methods of pest control is that their application has no adverse effect on the biosphere, and insects could probably not evolve resistance to factors reducing population fertility. Despite this, genetic methods have as yet not found wide application. Since the striking demonstration of the eradication of the screwworm fly, *Cochliomya hominivorax*, from a vast area in the USA by the release of $\gamma$-irradiated sterile insects of this species, not a single example has been reported of other successful applications of genetic methods on a large scale (Bushland 1971). With regards to other insect pests, the genetic methods either have not been applied at all or have been applied on such a small scale that sufficiently reliable data could not be obtained (Butt et al. 1970; Jermy and Nagy 1971; Proverbs 1970, 1971, 1974; Proverbs et al. 1977; White et al. 1976). Such inadequate use of genetic methods is due to a number of purely technical and economic factors. According to most investigators, beginning with Serebrovsky (1971), genetic methods will be more effective if only male individuals are released (Whitten and Foster 1975). This conclusion comes from the following considerations. Firstly, this will enable one to give the males optimum doses of nuclear irradiation.

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This is not always possible when both sexes are simultaneously subjected to radiation since males and females show differential radiosensitivity. Secondly, males released without females can be encouraged to disperse rapidly and widely (Proverbs 1970). Thirdly, the expenses of the method will be considerably reduced, as the numbers of males required to be released in order to produce the necessary effect can be reduced by half. Fourthly, in some species (fruit flies, mosquitoes) released females are themselves harmful. Standard procedures of male release usually involve the rearing in captivity of fairly large numbers of insects, their separation into males and females, male sterilization and release.

Some insects, Lepidoptera in particular, have rather lengthy larval periods which makes their rearing complicated and expensive. The collection of the reared insects is also a laborious procedure, and mass separation of males and females with subsequent elimination of females and release of males presents practical difficulties (Pal 1974; Whitten 1969). This is especially well known to sericulturists who, after decades of an unsuccessful search for mechanical means of determining sex in the silkworm, which is necessary in the production of hybrids, succeeded in solving the problem only after turning to genetic methods, namely, the construction of strains with sex-marked eggs (Strunnikov 1975; Strunnikov and Gulamova 1969, 1971; Tazima 1941, 1951).

Sterilization of insects by chemical or physical methods most often adversely affects the male competitiveness, thus appreciably reducing the efficiency of control measures. Another serious disadvantage of methods based on male sterilization: they do not combine a maximal effect in reducing pest reproduction in the $F_1$ generation with an effect in the following generations (delayed effect). The two effects are, in fact, inversely related: the higher the death rate in the first generation, the less the genetic load to be found in the following generations. Thus, for example, in the case of 100% death in the $F_1$ progeny from matings by sterilized males, the delayed effect is nil. On the other hand, to attain a high level of sterility in the second and subsequent generations the death rate in the first generation must be relatively low. It is obvious that the most promising pest control methods should avoid these drawbacks, so that the technical and economic possibilities for their application would be considerably increased.

These requirements are in many respects fulfilled in the new method we suggest here (Strunnikov 1976). This method is referred to as 'Z-lethal' since it is based on a deleterious effect of sex-linked lethals (lethals on the Z chromosome). It stems from a method developed earlier by the author for producing entirely male offspring in the silkworm in practically unlimited numbers (Strunnikov 1969 and 1975). A special strain, balanced by two lethals

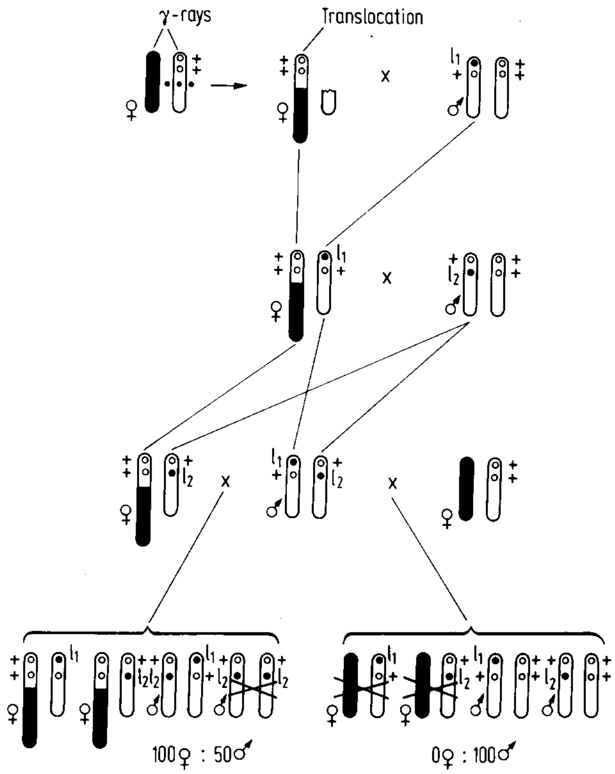


Fig. 1. Construction of silkworm strains balanced by two nonallelic Z-lethals. W chromosome is shown by a dark colouration; Z chromosome and its translocated fragment, by light colouration

by means of the translocation of a fragment of the Z chromosome onto the W chromosome, has been constructed for this purpose (Fig. 1). Using ionizing radiation a fragment of the left end of the Z chromosome was broken off and transferred to the W chromosome. The remaining part of the broken chromosome was substituted for the normal Z chromosome. These chromosomal rearrangements eventually resulted in the appearance of a new form in which, in addition to the Z and W chromosomes typical of females, there was a paired region of the Z chromosome, as in males. Despite such sex-chromosome constitutions, these individuals were functionally female. In these, female lethals carried on the left end of the Z chromosome were covered by the corresponding normal alleles in the translocated region of the Z chromosome. These females therefore survive and transmit the lethals to their sons. These translocation females were successively crossed first with males bearing one lethal, then – in the following generation – with males heterozygous for another lethal. Thus we succeeded in constructing a new line balanced by two lethals. Matings within this line produce viable females in all successive generations, but half of the males (these receiving the same two lethals) die. The surviving ones have two nonallelic lethals: one in each of their Z chromosomes. These males, outcrossed to females of any other strain bearing no chromosome rearrangements, produce entirely male progeny. In fact, the sex ratio is usually 99.9 ♂ : 0.1 ♀ because of crossing-over between the two Z chromosomes. Each of the males produced in the $F_1$ generation has one Z lethal from the father so that when crossed to normal females they produce progeny with a sex ratio of 2 ♂ : 1 ♀. In sericultural practice this method proved quite satisfactory for producing entirely male broods. It might also be used for insect pest control. The female mortality of the $F_1$ would make it possible to release males without a laborious procedure for sex discrimination, and the release of males, each carrying one lethal, would produce progeny with a sex ratio of 2 ♂ : 1 ♀. It might also be successfully used in combination with partial male sterilization by physical or chemical means. However, for the control of insect pests it would be desirable to have a method allowing one to induce 100% female lethality in two successive generations. The 100% female mortality in the $F_2$, i.e. in the progeny of the released males, would be more effective for the suppression of the reproduction of the pest. This aim could be achieved by constructing two, instead of one, strains balanced by several recessive Z-lethals (Fig. 2).

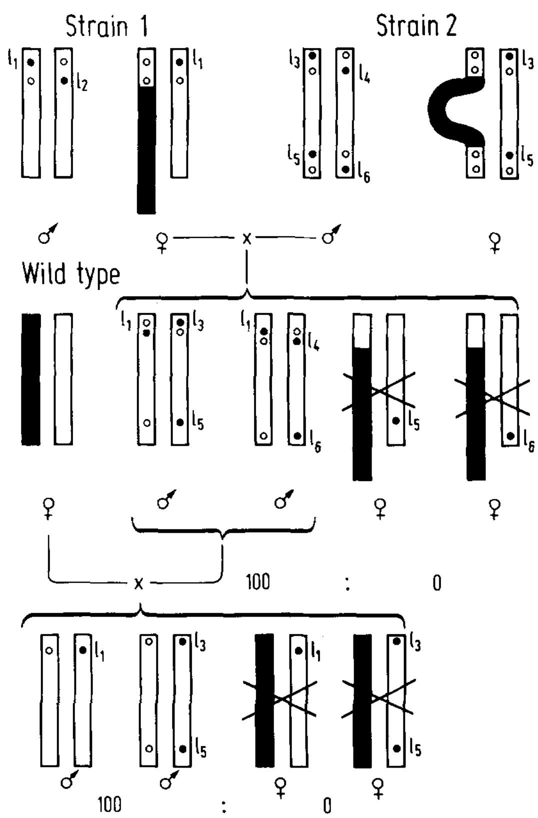


Fig. 2. Construction of two strains of Lepidoptera balanced by six nonallelic Z-lethals. W chromosome is shown by dark colouration; Z chromosome and its translocated fragment, by light colouration. Lethal loci are shown as closed circles and the corresponding open circles represent loci of their normal alleles

Strain 1 would have to be balanced by two non-allelic lethals, $l_1$ and $l_2$, located in the left end of the Z chromosome, which had little or no crossing-over between them. The procedure for constructing such a strain is shown in Figure 1: the balanced condition is attained by translocating the left end of a Z chromosome carrying normal alleles onto the W chromosome. Strain 1 breeds true, producing both male and female progeny.

Using the translocation females of strain 1 as starting material, another fragment of Z chromosome, its right end this time, is translocated onto the W chromosome using the procedure described above. As in strain 1, two non-allelic lethals, $l_3$ and $l_4$, are located on the left end of the Z chromosome with little or no crosing-over between them and are balanced under the cover of the translocation of the left end of the Z chromosome. These lethals are not allelic with the lethals $l_1$ and $l_2$ of strain 1 but must be located near their loci. Then, under the cover of the translocation of the right end of the Z, another pair of non-allelic and closely linked lethals, $l_5$ and $l_6$, are held in balance. Strain 2 will normally breed true giving the progeny with a sex ratio of 4 ♀ : 1 ♂.

The construction of two such strains in various Lepidoptera pest species seems to present no difficulties in view of the fact that strains with translocations of various fragments of autosomes or the Z chromosome into the W chromosome have been experimentally produced in the silkworm. The task of producing sex-linked lethals presents even less difficulties. In the silkworm it is possible to obtain up to 3-5% males heterozygous for a newly induced Z-lethal in the progeny of $\gamma$-irradiated insects. The work now in progress on the breeding of balanced lethal strains of the codling moth *Laspeyresia pomonella* has already yielded several Z-lethals (Litvinova 1976).

From the hundreds of lethals produced it is not difficult to select firstly the lethals which in the heterozygous state does not affect male viability, and subsequently from these, to select closely linked pairs which are covered by translocations of corresponding segments of Z chromosome. Thus, one of our commercial silkworm strains has two translocations onto the W chromosome which in no way reduces its viability. By the method described this strain could easily be converted into a strain balanced by two pairs of Z-lethals (Fig. 2), but since there was no need for such a strain the work has not been carried out.

Now we shall consider the prospects for the eradication of natural pest populations which follow from the possibility which has now been pointed out of producing 100% male progeny for two generations in succession.

Matings are made within the strains to increase their number; one generation before a release is scheduled strain 1 females are crossed to strain 2 males. Since the females of strain 1, unlike those of strain 2, have no translocation of the right and of the Z chromosome onto the W chromosome, all the hybrid females will die in the egg stage due to the effect of the uncovered lethals $l_5$ and $l_6$. All the male eggs are viable and will develop into males balanced by the lethals on the left end of the Z chromosome. The males can have the following combinations of the balanced lethals: $l_1/l_4$, $l_1/l_3$, $l_2/l_3$, $l_2/l_4$ (Fig. 2 shows only two of the combinations). In addition, each of the father's Z chromosomes has another lethal, $l_5$ or $l_6$, at the right end. According to our scheme these males are to be released. The presence of two balanced non-allelic lethals in the left end of the Z chromosome of these released males again provides 100% mortality of female eggs in the hybrid progeny resulting from the crosses of these males with females from natural populations. All the males survive and each has one or two lethals which ensures 62.5% mortality of their daughters, assuming free recombination between the lethals at opposite ends of the Z chromosome. The percentage of female mortality will then decrease by approximately half in the progeny of each successive generation of males (34.4, 16.6, 8.4%, etc.).

The reduced pest reproduction due to the death of the females and father-to-daughter transmission of embryonic lethals causing only female lethality gives the proposed method a special advantage. The mortality of all females in the $F_1$ progeny from the crosses between the females from natural populations and the released balanced lethal males is combined in this method with male-to-male transmission of the selective female mortality in the second and subsequent generations in the natural population. Because of this, if repeated releases are made, female mortality in each generation is composed of the deaths caused by lethals from the newly released males and lethals inherited from males released in the preceding generations. Thus, the repeated release of two-lethal males is expected to lead eventually – over several generations – to eradication of the pest species.

In order to demonstrate the principal efficiency of the method we have made a theoretical calculation of a change in the pest numbers produced by the release of balanced lethal males in a ratio of 10:1 to those in the natural population.

The same numbers of males should be released in all subsequent generations despite the decrease in the natural population numbers. We also proceeded from the assumption that use of other means of pest control would keep the reproduction coefficient of the insect near 1.

As seen from Table 1, under such conditions the numbers of females in the natural population will theoretically be reduced by a factor of 21 after the first release, by a factor of 833 after the second one, and, after the third release, practically no females will be found. The high efficiency of the method is indicated by the relative increase in the numbers of lethal carrying males in the natural population. After the first release the number of lethal carrying males exceeds that of the normal ones by 20 times, but in the following generation the difference becomes 100-fold. One should remember that half of the daughters of males carrying one lethal die at the embryonic stage. In addition to the high efficiency in reducing pest reproduction, the proposed method has some additional advantages over the known genetic control methods. Of these, the most important ones are as follows:

1) it enables one to release only males while avoiding the extremely laborious and inaccurate procedure of mass sex separation at the pupal stage
2) it also makes unnecessary the procedure of male sterilization, using either x-radiation or chemosterilants
3) with these two above-mentioned procedures excluded, the collection of insects at the pupal stage becomes unnecessary, which makes it possible to release males at earlier stages, including even the egg stage. No mass rearing of the pest insect in the laboratory would be needed; it could be substituded by rearing in 'semi-captivity': on plants or other feeding substrates in a suitable enclosure or cage which could be opened at the time of the release of male moths
4) the method provides the release of fully viable and competitive males carrying balanced lethals: this is con-

**Table 1.** Expected changes in population numbers produced by release of balanced lethal males

| Generation | No. of balanced lethal males released in each generation | Expected number in natural population | | | No. of females as percentage of initial number |
|---|---|---|---|---|---|
| | | Males inheriting one lethal | Normal males | Females | |
| 1 | 2 | 3 | 4 | 5 | 6 |
| 1 (startling) | 1,000,000 | 0 | 50,000 | 50,000 | 100.00 |
| 2 | 1,000,000 | 47,620 | 2,380 | 2,380 | 4.76 |
| 3 | 1,000,000 | 2,321 | 59 | 59 | 0.12 |
| 4 | | 59 | ~ 0 (0.072) | ~ 0 (0.072) | ~ 0 |

firmed by a great amount of data obtained from both experimental work with the silkworm and in practical sericulture. Moreover, the two strains of the pest species can be matched genetically in such a way that the hybrid males for release produced by the crosses between them will show strong heterosis.

5) the method proposed can be readily combined with other pest control methods which allows one to reduce markedly the pest numbers shortly before the release. The fact is extremely important since the suggested Z-lethal method would be especially effective in conditions of markedly reduced pest numbers when widely used chemical methods are ineffective.

There is no need for a detailed discussion of possible applications of the Z-lethal method. Each pest insect has its own pecularities which are to be taken into account when one is planning a concrete program of integral pest control.

It might be desirable to use the proposed method in combination with partial male sterilization obtained by use of a chemosterilant mixed with the food. Because of the somewhat lower doses of chemicals required for a given level of sterilization there is less chance that the competitiveness of the treated males would be affected than when a chemosterilant is relied upon for all the sterilizing effect. In some cases it may prove more expedient to use one strain balanced by two lethals only (Fig. 1), while in other cases introduction of two strains, each of them having different translocations of Z chromosome and balanced by different Z-lethals, may produce a stronger effect.

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