CHAPTER 2.3.

GENETIC BASIS OF THE STERILE INSECT TECHNIQUE

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SUMMARY

The use of the sterile insect technique (SIT) for insect control relies on the introduction of sterility in the females of the wild population. This sterility is produced following the mating of these females with released males carrying, in their sperm, dominant lethal mutations that have been induced by ionizing radiation. The reasons why the SIT can only be effective when the induced sterility in the released males is in the form of dominant lethal mutations, and not some form of sperm inactivation, are discussed, together with the

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relationship of dominant lethal mutations to dose, sex, developmental stage and the particular species. The combination of genetic sterility with that induced by radiation is also discussed in relation to the use of genetic sexing strains of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) in area-wide integrated pest management (AW-IPM) programmes that integrate the SIT. A case is made to lower the radiation dose used in such programmes so as to produce a more competitive sterile insect. Increased competitiveness can also be achieved by using different radiation environments. As well as radiation-induced sterility, natural mechanisms can be recruited, especially the use of hybrid sterility exemplified by a successful field trial with tsetse flies *Glossina* spp. in the 1940s. Modern biotechnologies are having some impact on the SIT, especially regarding the introduction of markers for released flies and the construction of genetic sexing strains, as well as other applications such as molecular sterility. Nevertheless, it is concluded that using a physical process, such as radiation, will always have significant advantages over genetic and other methods of sterilization for the large-scale application of the SIT.

1. INTRODUCTION

E. F. Knippling realized in the 1930s (Lindquist 1955) that, if male insects could be sterilized genetically without affecting their ability to mate, then they could be used to introduce a genetic load into a wild population in the field that would lead to its suppression or even eradication. For some time, geneticists were aware that X-rays could induce mutations in insects (Runner 1916; Muller 1927), but it was not until A. W. Lindquist showed a publication by H. J. Muller (1950) to Knippling that applied entomologists realized the great potential it offered (Baumhover 2001, 2002). The results from the first experiments to sterilize the New World screwworm *Cochliomyia hominivorax* (Coquerel) were published in 1951 (Bushland and Hopkins 1951). This demonstration, that X-rays could indeed induce sterility, was the first small step on the way to the eradication of this important livestock pest in the southern states of the USA, and then in Mexico and all the countries of Central America, as well as Panama (Vargas-Terán et al., this volume). A permanent barrier of sterile insects has been established in eastern Panama to prevent the reinvasion of the pest from South America. Baumhover (2001, 2002) provided a historical account of the early days of the screwworm eradication programme, and Klassen et al. (this volume) describe the sterile insect technique (SIT), in general, from an historical perspective.

During the first field trials of sterile screwworms in Curaçao, the genetic basis of sterility was poorly understood, but it was realized that sterility resulted from the induction of dominant lethal mutations in the irradiated sperm (Bushland and Hopkins 1951; LaChance et al. 1967). At that time the level of understanding of the genetics of the screwworm led Bushland to comment that (quoted by LaChance 1979):

. . . we eradicated screwworms from Curaçao and the south-eastern United States without knowing how many chromosomes it had.

Prior to the adoption of radiation to sterilize insects, and following the discovery in the early years of the Second World War that mustard gas and other chemicals can produce mutations and affect fertility in *Drosophila* (Oehlkers 1943; Beale 1993), chemical mutagens were extensively evaluated (Borkovec 1966). However, difficulties relating to toxicity, handling, and residues were considerable, and so radiation has usually been the method of choice. Even though field trials with chemosterilized *Anopheles albimanus* Wiedemann mosquitoes in El Salvador were successful (Breland et al. 1974), it is unlikely that today such releases could be carried out.

2. STERILITY REQUIREMENTS FOR SIT APPLICATION

It is very important that the word “sterility” be precisely understood in terms of its use in the SIT. The word “sterility” describes one of many possible end points of the reproductive process, but it can cover a multitude of causal factors. The following definitions of sterility were taken at random from three biological dictionaries:

- Structural or functional inability to reproduce,
- Involuntary total inability to reproduce,
- Any complete or partial failure to produce functional gametes or viable zygotes.

These definitions cover genetic, physiological, morphological or even “psychological” factors, which can lead to a final end point of sterility, and clearly many of these manifestations would not be useful for sterility in area-wide integrated pest management (AW-IPM) programmes. For the SIT to be effective, females of the wild population in the field have to be permanently prevented from reproducing, and any factor(s) transferred by the released male that accomplishes this would, in fact, be sufficient. True genetic sterility in released male insects requires: (1) production of viable sperm, (2) their transfer to the wild female during mating, (3) their use in fertilization of eggs, and (4) the inability of the fertilized zygote to complete development to a fertile adult. Thus, an irradiated male insect must be able to carry out all the functions of a normal fertile insect — it must produce fully functional sperm that succeed in fertilizing eggs and initiating the development of fertilized eggs.

In the SIT, the radiation-induced sterility is actually produced in the generation following the release of the males, i.e. with the death of the embryo, larva, pupa or adult, or the production of F_1 adults that themselves produce gametes that result in zygotes that do not develop. A male insect that cannot mate, is aspermic, or that transfers non-functional sperm, could be classed as sterile, but males with any of these defects would probably not be effective for the SIT.

The International Plant Protection Convention (IPPC 2017) adopted the following definition of a sterile insect:

An insect that, as a result of a specific treatment, is unable to reproduce.

and the following definition of the SIT:

Method of pest control using area-wide inundative release of sterile insects to reduce reproduction in a field population of the same species.

Irradiated males must also be able to transfer the appropriate accessory gland fluids during mating, ensuring that female behaviour corresponds to that following mating with a fertile male. In some insects, this female post-mating response involves temporary or permanent refractoriness to further mating, and a change in female behaviour. In *Drosophila* sp. the peptides transferred in the accessory fluid, that are involved in the female post-mating behavioural changes, have been well studied (Chen 1996), and it has even been possible to sterilize females by the ectopic expression (i.e. gene expressed in all tissues) of a transgene which codes for the sex peptide (Aigaki et al. 1991). In fact, a male that only transferred accessory gland fluids, and which could elicit the correct post-mating female response, could theoretically “sterilize” the female. In the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), it has been shown that irradiated sterile males produce the same kind of change in wild female

behaviour, from mating to oviposition, as do fertile males (Jang et al. 1998; Jang 2002).

In species where females remate (von Borstel and Knipling 1960), sterility for use in the SIT must be efficiently induced in sperm without affecting sperm function and its capacity to compete with other sperm, and exert its effect only after fertilization of the female egg. Dominant lethal mutations are such sterility factors. They are readily induced in all chromosomes by irradiation, and they have little effect on the phenotype of the sperm, at least at the doses usually used for the SIT (Bakri et al., this volume).

Lethality occurs when the haploid nucleus, carrying such a mutation or mutations, is combined with a normal haploid nucleus, resulting in the death of the early embryo at the moment when the genetic information required for normal development is absent or incorrect (Muller 1927). In addition, cell division can become asynchronous and lead to the death of the zygote.

3. DOMINANT LETHAL MUTATIONS

The mechanisms by which these mutations cause lethality in Diptera in the developing zygote are now well documented (LaChance 1967; Smith and von Borstel 1972); they are illustrated in Figure 1.

Figure 1. Schematic representation of the fate, during cell division in the embryo, of chromosomes with radiation-induced dominant lethal mutations, leading to the accumulation of serious imbalances in the genetic information of subsequent daughter cells.

The primary lesion leading to a dominant lethal mutation is a break in the chromosome, in this case, induced by radiation. When a break is induced in a chromosome in mature sperm, it remains in this condition until after the sperm has entered an egg. (This was shown very elegantly by mating queen bees to irradiated

males, and then measuring fertility after storage of the sperm in the spermatheca for one year; there was no difference in the level of sterility in the same queen tested one year later (Lee 1958)). Following fusion, nuclear divisions begin, and a break in a chromosome can have drastic effects on the viability of the embryo as development proceeds. During early prophase the broken chromosome undergoes normal replication, but during metaphase the broken ends can fuse leading to the formation of a dicentric chromosome and an acentric fragment. The acentric fragment is frequently lost, while the dicentric fragment forms a bridge at anaphase leading to another chromosomal break. This whole process then repeats itself, leading to the accumulation of serious imbalances in the genetic information of the daughter cells. The accumulation of this genetic damage finally leads to the death of the zygote.

If two different chromosomes are broken they can also rejoin in the way depicted in Fig. 1. These chromosomes produce the same problems for the dividing cells as those formed by a break in a single chromosome, by undergoing incorrect fusion and leading to the *breakage-fusion-bridge cycle* (McClintock 1941). In this way dominant lethal mutations can cause cell death, and the accumulation of genetic imbalance in the developing zygote leads to lethality.

3.1. Dose Response for Dominant Lethals

Dose-response curves for the induction of sterility by radiation are generally developed using measurements of hatchability of eggs, deposited either by irradiated females mated with non-irradiated males, or by non-irradiated females mated with irradiated males. The implicit assumption is that most dominant lethal mutations exert their effect during early embryonic development. Data from *Drosophila* sp., and other dipteran species containing chromosomes with monokinetic centromeres, have shown that this is the case (Demerec and Fano 1944; Catcheside and Lea 1945; Lee 1958; Tantaway et al. 1966; Franz 2000), making egg hatch an appropriate stage to evaluate. In insect species with holokinetic chromosomes, many dominant lethal mutations exert their lethal effects only at later developmental stages (Marec et al., this volume).

Dose-response curves for dominant lethal mutations can be developed by irradiating insects with increasing doses of radiation and calculating the percentage egg hatch. The curves tend to show a characteristic shape, depending on the cell type and stage irradiated. The shape of the curve can be used to infer information about the types of initial chromosomal lesion producing the lethal effect. In irradiated sperm, there is an approximately linear relationship between the dose and the induction of dominant lethals at low doses, but at higher doses there is a noticeable departure from linearity, i.e. it tends to saturate at higher doses, and approaches 100% sterility asymptotically. This is due to the induction of multiple lethal events in the same cell, but only one is needed to cause the egg to die. The shape of the curve, as well as indicating the underlying causal factors of dominant lethality, should help in selecting a dose that will be used to sterilize insects for release. Fig. 2 shows the dose-response curves for the house fly *Musca domestica* L. and the large milkweed bug *Oncopeltus fasciatus* (Dallas). The difference in the shape of the curves is due to the fact that the latter species has holokinetic chromosomes (section 3.4.), and this results in the need for much higher doses of radiation for sterilization.

Figure 2. Dominant lethal dose-response curves for *Musca domestica* and *Oncopeltus fasciatus*. (Figure from Lachance and Graham 1984, reproduced with permission from Elsevier.)

Assuming that dose-response curves have been developed for both sexes, that both sexes are released, and that close to full sterility is required, then the minimum radiation dose chosen must lead to full sterility in females. Any residual fertility in released females can be extremely damaging, as this will actually contribute to the reproductive potential of the wild population. For example, a residual fertility of 2% in irradiated females, following a sterile:wild release ratio of 100:1, will actually double the number of insects in the next generation. Residual fertility in males is of less importance; it simply reduces the rate at which the population is suppressed.

These simplistic assumptions ignore the impact of any density-dependent regulation in the population. The full impact that the decision — on the dose to be used, based on the shape of the curve (Fig. 2) — has on the effectiveness of the SIT is described below (section 4.).

3.2. Dominant Lethals and Sex

Male and female insects normally differ considerably in their sterility response to radiation, primarily because sperm are haploid post-meiotic cells, whereas mature eggs are pre-meiotic. Eggs usually develop in the female to metaphase I, and then are arrested until oviposition when meiosis is completed with the production of a single

pro-nucleus and three polar bodies. Mature eggs in the female are much more tolerant of radiation than are the earlier oocytes, with the consequence that a radiation dose which induces full sterility in mature eggs often leads to the cessation of oogenesis at a later date. This can lead to an increase in the lifespan of females as energy is redirected away from reproduction to survival (White and Hutt 1970; Hafez and Shoukry 1972). In most cases this is of no consequence, but it can be important if the sterile females that are released are themselves damaging in some way. In tsetse, for example, it is essential to remove sterile females from the released insects as their increased lifespan might enable them to be efficient vectors of disease (Dean and Clements 1969; Abd-Alla et al. 2013).

In addition to this basic difference between male and female germ cells, there are also species-related differences in the overall sensitivity of male and female insects to radiation sterilization. In most species, females are more sensitive than males to radiation sterilization, and therefore it is possible to identify the sterilizing dose for the SIT based on the acceptable dose for males (section 4); use of this dose will ensure that all released females are fully sterile. In the minority of species where the female is more tolerant (Haynes et al. 1977; Crystal 1979), the sterilizing dose for females must be used, and this can compromise the competitiveness of the sterilized males. These patterns of differential sensitivity of germ cells in males and females are important to investigate when the radiation dose for the SIT is identified.

3.3. Dominant Lethals and Developmental Stage

To maximize genetic damage to mature germ cells and minimize somatic damage, insects should be irradiated as late as possible in the development pathway, ideally as fully differentiated adults. In the adult stage, insects are most tolerant to somatic damage because somatic cell division is at a minimum. However, even in a fully differentiated insect, certain somatic cells, especially in the gut, continue to divide, and sterilizing radiation doses can compromise viability and hence the fitness of the treated insect (Flint et al. 1966).

In spite of these biological relationships, operational considerations in implementing the SIT often play the major role in determining the stage at which insects are irradiated. The operational decision is based on identifying the most convenient stage for radiation, from the point of view of mass-rearing, handling and release protocols. In most insects, this is the pupal stage, and consequently radiation is applied to pupae as late in development as possible. In New World screwworm and Mediterranean fruit fly AW-IPM programmes applying the SIT, pupae are irradiated two days before emergence, enabling the shipment of irradiated pupae to fly emergence and release facilities far from the production plant, where adults emerge and are fed and processed for release.

In AW-IPM programmes integrating the SIT for some other species, adult insects have to be irradiated. In Lepidoptera, larvae generally pupate within a cocoon that attaches itself to a substrate, and in these cases it is impractical to irradiate this stage, and thus teneral adults are irradiated (Proverbs et al. 1975; Boersma 2021). In tsetse, before release, adult males are fed blood containing a trypanocide and irradiated at 3-4 days of age (Dyck et al. 2000; Vreysen et al. 2000). In general, operational factors tend

to be decisive in determining the developmental stage that is irradiated (Bakri et al., this volume).

3.4. *Dominant Lethals and Species*

The class Insecta comprises 31 orders (Wheeler et al. 2001), and pest species are found in most major orders. This very diverse class exhibits widely different sensitivities, both within and between orders, to the induction of dominant lethal mutations (Bakri et al. 2005; IDIDAS 2020), and to the somatic effects of radiation (Willard and Cherry 1975). Probably there are many factors that contribute to these differences, including the centromere structure (Smith and von Borstel 1972), the degree of chromosome condensation (Israelewski 1978), radiation repair (Beatty and Beatty 1967), and the chromosome environment (Thoday and Read 1947). This is certainly not an exhaustive list, and other factors, as yet unidentified, could still be important.

Insects can, however, be divided into two main groups which show major differences in sensitivity to the induction of dominant lethals, and for which experimental evidence is available that identifies the underlying biological and genetic factors responsible. Orders such as Diptera, Hymenoptera, and Coleoptera can be classed as radiation-sensitive, while orders such as Lepidoptera and Homoptera are more radiation-tolerant. A major difference between these two groups of insects is that the former group has a localized centromere (monokinetic), while the latter has a diffuse centromere (holokinetic) (Bauer 1967), and this difference plays a major, although not exclusive, role in radiation sensitivity (Tothová and Marec 2001). Lepidoptera also do not show the classical breakage-fusion-bridge cycle that is a characteristic of dominant lethals induced in Diptera. It appears that lepidopteran chromosomes can tolerate telomere loss without the drastic effects that this has on chromosomes in other orders. Discussions on this phenomenon are found in Bakri et al. (this volume) and Marec et al. (this volume).

4. APPROPRIATE RADIATION DOSE FOR THE SIT: IS 100% STERILITY REQUIRED?

The word “sterile” in the acronym “SIT” is generally perceived to imply a requirement for full sterility, and hence a radiation dose is sought that achieves 100% sterility. However, because there are varying levels of sterility that can be induced, the word “sterile” is not an absolute term, and often it is not required that released insects are fully sterile. As described above, the relationship of radiation dose to dominant lethal induction in the mature sperm of insects approximates to linearity at low radiation doses, but not at high doses, producing an asymptotic approach towards full sterility (Fig. 2). This curve is used as a decision-making tool to identify the radiation dose appropriate for a specific insect in a specific AW-IPM programme using the SIT. The fact that the curve approaches 100% sterility asymptotically makes it difficult to select the dose that gives the required full sterility. The equation used to describe the curve predicts that, in theory, given a large enough sample of eggs, it will always be possible to find one that hatches, and hence the goal of full sterility in eggs will never actually be met (although these may not reach the adult stage). However, in practical terms, a

dose is chosen which prevents the hatch of more than 99% of eggs in a large sample. This decision is often taken solely on the basis of the dominant lethal-induction curve; the effects of radiation on somatic tissues, and hence on the final ability of the insect to introduce sterility into wild females in the field, are generally ignored.

In fact, the goal of attempting to achieve full sterility in treated insects can seriously compromise their competitiveness in the field. This follows from the shape of the dose-response curve. At high doses, increasing amounts of radiation are required for proportionally smaller increases in sterility. LaChance and Graham (1984) described the dominant lethal-induction curve for several insect species, and, using equations derived from their curves, it can be shown that, for example, to decrease egg hatchability from 2 to 1%, an 11% increase in dose is required. This marginal increase in sterility, obtained at the expense of a disproportionate increase in the radiation dose, can have major negative effects on the competitiveness of the released insects. Since the shape of the curve relating somatic damage to dose is not known, some assumptions have to be made in arriving at this conclusion.

The proportion of wild females rendered sterile by a given number of males released depends on both their sterility and their success in competing with wild males to mate with wild females. Therefore, to optimize the balance between somatic fitness and genetic sterility, it is in the interest of programmes applying the SIT to choose radiation doses that maximize the genetic load introduced into the wild populations. This means that chosen optimal radiation doses to achieve this objective may give sterility levels well below 100% (Toledo et al. 2004; Rull et al. 2012).

A word of caution is required here. It is known that, in *Drosophila* spp., males receiving substerilizing radiation doses can recover fertility over time (Luning 1952), and it is important to evaluate this aspect if lower doses of radiation are used. Nevertheless, currently all operational programmes consistently err on what may be considered the side of caution, and use radiation doses that induce almost 100% sterility in the treated insects. Unfortunately, high levels of somatic damage, and hence lower sterile-male performance, normally result from such high doses.

5. RADIATION IN DIFFERENT ENVIRONMENTS

Usually radiation is the final treatment that insects receive at the mass-rearing facility before being transported for release in the field. Any technique that reduces somatic damage induced by the treatment would be advantageous. Mass-produced insects are expensive, and need to function as well as possible in the field. The amount of genetic damage produced by radiation, both in reproductive and somatic tissues, is related to the environment in which the tissue finds itself, in particular, the oxygen tension. It is well known that treatment in low-oxygen tension, e.g. irradiating in nitrogen or hypoxia, reduces radiation damage (O'Brien and Wolfe 1964; Bakri et al., this volume).

However, if low-oxygen tension gives the same protective effect for somatic tissue (competitiveness) and sperm (sterility), then there would be no net gain. To achieve the same level of sterility as irradiation in air, irradiation in nitrogen would have to be at a higher dose. Nevertheless, the differential protection afforded to somatic cells by irradiation in nitrogen is related to the fact that somatic tissue is diploid and still

undergoing cell division, whereas sperm are fully differentiated and haploid. This means that damage induced in somatic cells can manifest itself during the further life of the insect, whereas damage induced in sperm cells is only realized following fertilization of eggs in the wild females.

The positive effects on competitiveness of irradiation in nitrogen have been demonstrated for several insect species (Hooper 1971; Curtis and Langley 1972; Hallinan and Rai 1973; LaChance and Richard 1974; Economopoulos 1977). Two successful fruit fly AW-IPM programmes integrating the SIT were carried out in Australia using pupae irradiated in nitrogen (Hooper 1971; Fisher et al. 1985; Fisher 1996), and this approach is still being used for Mediterranean fruit fly programmes in that country (Fisher 1997). At present, no other operational programmes irradiate insects in nitrogen, whereas holding pupae in airtight containers to achieve hypoxia before irradiation is a common practice (Bakri et al., this volume). López-Martínez and Hahn (2012) demonstrated the benefit of this simple practice by showing that exposure to gamma radiation under anoxic conditioning results in lower lipid and protein oxidative damage in males at sexual maturity.

6. COMBINATION OF RADIATION AND GENETIC STERILITY

Genetic sexing strains are now being used in almost all programmes that use the SIT for the Mediterranean fruit fly (Robinson et al. 1999; Caceres et al. 2004; Franz et al., this volume). Since these strains are constructed using male-linked translocations, they are semi-sterile (Laven 1969). To obtain a more competitive insect, it has been suggested (Steffens 1982, 1983) that this genetically contrived sterility be combined with a lower dose of radiation-induced sterility. At lower doses of radiation, the overall sterility of males from a genetic sexing strain is, of course, higher than that of males from a normal strain. However, as the radiation dose increases, the contribution from genetic sterility gets progressively less, and eventually disappears as the sterility increases. For a male with a normal karyotype, and a male carrying a translocation, the radiation dose close to full sterility is the same. Nevertheless, the use of males produced from a genetic sexing strain offers the opportunity to seriously re-examine the radiation strategy of Mediterranean fruit fly programmes, at least those that aim at suppression, to maximize the genetic load introduced into wild populations.

7. HYBRID STERILITY

When hybrids are formed between closely related species, or even between some populations of the same species, sterility is observed. The sterility phenotype can include the total absence of viable progeny, the production of hybrids of both sexes with varying levels of sterility, or the production of a unisexual sterile F₁ generation that is usually male. Examples of all these are given below.

This array of different hybrid phenotypes has a corresponding wide range of underlying genetic and cytoplasmic causes, which in many cases overlap to produce a very complex phenotypic picture. The causes of hybrid sterility can be grouped roughly into those factors that act on the nuclear genome of the insects themselves, and

those that are maternally (cytoplasmic) inherited. Many nuances and interactions are possible within these two major groups. Currently the picture is far from complete.

An early paper by Haldane (1922) reviewed cases of classical hybrid sterility in many different animal groups, including a large number of lepidopteran species. His analysis showed that there was preferential sterility or inviability in the hybrids of the heterogametic sex. This observation has become known as *Haldane's Rule*, and has largely stood the test of time (Orr 1997). For pest insects, this means that, for example in Lepidoptera where the female is heterogametic, the major hybrid effects would be seen in the resulting female hybrids, whereas in Diptera where the male is heterogametic, the F₁ males will be more affected.

7.1. *Cytoplasmic Incompatibility in Culex pipiens L.*

Mosquitoes of the genus *Culex* are important vectors of filarial worms and some arthropod-borne viral diseases such as arboviruses. As early as 1938, Marshall (1938) showed that certain crosses of populations of *Culex pipiens* from England and France failed to produce progeny. In the 1950s Laven (1967a) carried out a worldwide survey of compatibility among many different populations of this complex. He showed that this phenomenon was cytoplasmic in origin, and that incompatibility could be uni- or bi-directional. The causative agent was a rickettsia-like bacterial symbiont; its removal by antibiotic treatment abolished the sterility phenotype (Yen and Barr 1973). The symbiont has been classified as *Wolbachia pipientis* Hertig, and is widely distributed in arthropods, with up to 76% of insect species so far examined showing evidence of infection (Jeyaprakash and Hoy 2000). It has a wide variety of effects on arthropod reproduction (Bourtzis and O'Neill 1998), and has been implicated in maintaining the viability of filarial worms that cause river blindness.

In insects, females infected with *Wolbachia* can successfully reproduce with males that are uninfected, but the reciprocal cross is sterile. This, coupled with the maternal inheritance of the infected state, enables the bacterium to spread through a population and carry with it any other factor that is exclusively maternally inherited (Pettigrew and O'Neill 1997; Curtis and Sinkins 1998). Releasing *Wolbachia*-infected males into a naive population would be equivalent to the release of radiation-sterilized males.

In 1967, an experiment to use cytoplasmic incompatibility to suppress a small isolated population of *Culex fatigans* Wiedemann was carried out in Okpo, a small village near Rangoon (Laven 1967b). Over a period of 4 months, each day about 5000 infected or incompatible males were released into a population estimated to fluctuate between 2000 and 10 000 mosquitoes. After 4 months of releases, all the remaining egg rafts collected were sterile. Unfortunately, the arrival of the monsoon season prevented any further observations. Curtis et al. (1982) carried out a much larger field trial. Although releases of incompatible males did reduce the population build-up, it was not possible to increase the percentage of sterile egg rafts above 70%. It was concluded that immigration of fertilized females from outside the village caused the stagnation in the numbers of sterile egg rafts observed.

The use of this approach for suppression requires that exclusively males of the incompatible strain be released; any females that are co-released would be compatible with the males and would lead to establishment of the *Wolbachia* strain in the wild

population and its loss for suppression purposes (Augustinos et al., this volume; Lees et al., this volume). To solve the problem of error-free sexing, as well as escaping females, a low dose of radiation can be given since female mosquitoes are generally more sensitive than males (Arunachalam and Curtis 1985; Bourtzis et al. 2016).

7.2. Hybrid Sterility in Tsetse

The first evidence that sterile hybrids are formed by crossing different tsetse species was provided by Corson (1932), who obtained progeny from mass-matings involving female *Glossina morsitans centralis* Machado and male *G. swynnertoni* Austen, but as he did not make observations on mating, he concluded that it was due to parthenogenesis. Potts (1944) repeated these experiments, and concluded that true hybrids were obtained as evidenced by the morphology of the hybrid male genitalia. He also was able to backcross F₁ females and produce progeny. Vanderplank (1944) confirmed these observations, but noted that, when no choice of mates was offered, inter-specific crosses took place as readily as the intra-specific crosses. By catching and identifying each member of copulating pairs, Jackson (1945) confirmed that this was true also in the field. Extensive work by Curtis (1972), Rawlings (1985), Gooding (1997a, b, 2000), and Gooding and Krafur (2005) expanded the knowledge of tsetse hybridization phenomena, and indicated how this might, or might not, be used to develop methods of pest population suppression. Interspecific crosses in tsetse produce sterile male hybrids and partially sterile female hybrids, and there is a strong asymmetry in the sterility phenotype of the reciprocal crosses. In some crosses no F₁ progeny are produced. The male hybrids can copulate with and inseminate females, but the hybrid sperm cannot always succeed in fertilising eggs. For other crosses, Curtis (1972) showed that, following multiple mating of females, the two types of sperm were equally competitive. However, Gooding (1992) showed that, in multiple-mated females of the *G. morsitans* subspecies, the sperm from the conspecific male was used preferentially. The inability of hybrid males to fertilize females, and the preferential use of conspecific sperm, would be serious handicaps to the use of hybrid sterility for pest suppression.

Hybrid sterility in tsetse appears to be mediated by both genetic and maternally inherited factors. A very complex picture has emerged, with many factors interacting, each with differing contributions to the total picture (Doudoumis et al. 2013). Apart from the expected chromosomal and genic interactions, two interesting observations have been made. Firstly, the fertility of reciprocal crosses between the different taxa show high levels of asymmetry. This is reminiscent of phenotypes induced by *Wolbachia* symbionts, and it is known that many tsetse species carry *Wolbachia* (Chen et al. 1999; Abd-Alla et al. 2013). Secondly, it appears that in some hybrid crosses there is a form of interaction between the mother and hybrid offspring that determines whether a pregnancy will be successfully completed. (Tsetse flies reproduce by adenotrophic viviparity, where the fertilized egg hatches into a larva that is fed within the female by milk glands, and a mature third-instar larva is produced every 9–10 days). Results by Olet (2001) have shown that females mated to males of a different taxon, though initially sterile, can as they get older begin to produce viable progeny.

Using hybrid sterility for tsetse control would involve either the release of fertile males into a non-compatible population, or the release of F_1 sterile males into a population of either of the parental species. The use of F_1 males has the disadvantage that two taxa have to be maintained in the laboratory, and that males and females from the respective taxa have to be obtained to set up the appropriate cross. In addition, the release population must be sexed to prevent the release of semi-sterile F_1 females, and the F_1 males need to be good inseminators. For the release of fertile males of one taxon into a wild population of a second taxon, only the release population must be sexed (however 100% accuracy is required).

In a large field experiment, Vanderplank (1947) eliminated a population of *G. swynnertoni* from the Shinyanga area, Tanzania, by releasing into it fertile *G. m. centralis* from Kondoa-Irangi, Tanzania (Klassen et al., this volume). About 140 000 *G. m. centralis* pupae were released over a 7-month period, and the population of *G. swynnertoni* fell, presumably due to the reduced fertility of the hybridized *G. swynnertoni* females and the subsequent matings with sterile hybrid F_1 males. *G. swynnertoni* was eradicated, but *G. m. centralis* did not become established because of the harsh climatic conditions, and therefore the area remained tsetse-free for some time. These field trials were carried out before it was known to applied entomologists that sterility could be readily induced by radiation, and clearly demonstrated the potential of hybrid sterility to suppress tsetse flies. They were carried out before mass-rearing of these species was possible, and all the flies released, both males and females, were from field-collected pupae (there was no way to separate the sexes). Recent improvements in tsetse mass-rearing procedures and sex-separation methods (Opiyo et al. 2000; Dowell et al. 2005) should encourage a re-examination of this form of pest suppression for tsetse.

7.3. *Heliothis* Hybrids — an Exception to Haldane's Rule

Laster (1972) demonstrated that, when *Heliothis subflexa* (Guenée) females and *Heliothis virescens* (F.) males are hybridized, sterile male and fertile female progeny are produced, and the hybrid females continue to produce sterile male and fertile females through many generations of backcrossing to *H. virescens* males. Since females are heterogametic, they would be expected to suffer more from hybridization, so novel factors may be involved. Hybrid males can mate and transfer a spermatophore, but no eupyrene sperm reach the female spermathecae, even though they are produced in the testis. However, as the number of backcross generations increases, the ability of the males to produce eupyrene sperm decreases. This pattern of hybrid sterility suggests a very strong maternal component, but treatment of the moths with agents known to be lethal for *Wolbachia* failed to remove the sterility syndrome (LaChance and Karpenko 1981, 1983). An analysis of mitochondrial biogenesis in hybrid males failed to identify elements of protein synthesis or transport as being the cause of the sterility, but sperm mitochondria were implicated in the phenomenon (Miller and Huettel 1986). Such hybrid males would unlikely be effective "sterile males" in the field. Nevertheless, field trials on the island of St. Croix were conducted using the release of backcross males (Proshold 1983; Proshold et al. 1983), and temporary sterility could be demonstrated in the target population.

7.4. *Anopheles gambiae* Complex

The *Anopheles gambiae* Giles complex in Africa consists of seven sibling species, and crosses between every combination led to hybrid males, with some crosses producing no females (Davidson et al. 1967). The sterility is due to chromosomal interactions, and cytoplasmic factors have not been implicated. Davidson (1969) carried out a successful series of laboratory cage experiments to evaluate the use of these sterile males, and was able to demonstrate the induction of the expected levels of sterility in target mixed-sex populations. However, a field trial (Davidson et al. 1970) failed to demonstrate any substantial degree of mating between released sterile hybrid males and wild females. Pre-mating isolation mechanisms played a decisive role in the failure, illustrating the behavioural constraints that underlie the use of hybrid sterility in the field.

8. TRANSFORMATION AND MOLECULAR STERILITY

The successful demonstration of transformation in *Drosophila melanogaster* Meigen (Rubin and Spradling 1982) encouraged the development of similar systems in other insects. A review by Häcker et al. (this volume) illustrates the current state of modern biotechnology for introducing foreign genes into pest insects, including classical transformation technologies via transposable elements, and site-specific genome modification techniques using recombinases. The use of molecular tools in support of the SIT involves mainly the development of marker and genetic sexing strains (Bourtzis and Hendrichs 2014; Franz et al., this volume).

Also, molecular sterility systems have been developed that might be used to produce both an elimination of females from the insects to be released, and sterility induced by matings with the released males (Heinrich and Scott 2000; Thomas et al. 2000; Häcker et al., this volume). Some of these rely on conditional lethality in F₁ females that are produced following the mating of released transgenic males with wild females. To date, some transgenic strains are available of several pest insects, although what is known is giving some cause for concern in two areas related to the technology itself, namely stability of the insertion and expression of the transgene. Currently, there are insufficient data to predict if transgenic strains will retain the appropriate expression patterns of the constructs under industrial mass-rearing over many generations, and following their large-scale release in the field. This will be particularly relevant for systems based on molecular sterility where permanent and absolute sterility of the system is essential. It will be difficult to predict the long-term stability of transgenes, and hence their expression in the transformed strains. This problem could be magnified once fertile laboratory transgenic strains are released into a wild population, the genomic diversity of which is unknown.

There is also the slightly disconcerting idea that a transgene, once released in a fertile insect, cannot be “recalled” or destroyed. This applies particularly to self-sustaining strategies, which leave an “ecological footprint” because they are intended to persist indefinitely in the target population and may invade other populations (Alphey 2014). The integration of transgenic technology with the SIT, as part of self-limiting strategies, would be an option to reduce risk, since radiation-induced sterility

would prevent vertical transmission of the transgene. Public concern related to genetically modified organisms needs to be addressed, and a regulatory framework is required, so that available transgenic insect strains are properly field-tested.

9. CONCLUSIONS

Due to the extensive studies on the radiation biology of *Drosophila* sp. carried out in the 1950s and 1960s, there is a very good understanding of the genetic basis of radiation-induced sterility in that species. However, even though the underlying mechanisms are known and are probably of wide relevance, there still are many unexplained observations, especially in regards to the different dose-response curves found for different species, even within the same order (Bakri et al. 2005). For one type of mutation, i.e. specific locus mutations, there is a very good correlation between the dose-response curve and the DNA content of the haploid genome (Abrahamson et al. 1973); these studies ranged from bacteria to man. There is as yet insufficient information on genome size, in a large enough number of insect species, to assess whether such a simple relationship holds for dominant lethal induction. In addition, in species with holokinetic chromosomes, the mechanism(s) of dominant lethal induction has/have still to be fully explained (Marec et al., this volume).

Radiation is usually one of the last procedures that insects undergo before leaving mass-rearing facilities for release in the field, and it is important that it be applied in a way that minimizes its detrimental effects on insect competitiveness. Firstly, it is essential that the dosimetry of the radiation source be checked to ensure that all the insects receive the required minimum dose, and that none is unnecessarily overdosed (Bakri et al., this volume). Secondly, a dose should be chosen that maximizes the level of introduced sterility in the wild females in the field, i.e. both the sterility level in the released males and their competitiveness has to be taken into account. Thirdly, numerous studies have shown that irradiation in nitrogen or hypoxia can provide protection against the detrimental somatic effects of radiation.

Currently, modern biotechnology tools are applied extensively in support of the SIT to develop marker and genetic sexing strains (Häcker et al., this volume). Furthermore, the development of molecular methods to sterilize pest insects, by the release into the field of fertile insects carrying transgenes, has made considerable progress, in spite of the many unknowns inherent in such systems. Several of these strains are now available, and some have undergone an initial evaluation under mass-rearing scenarios or in open-field trials (Carvalho et al. 2015). Overall, however, their wider adoption has so far been quite limited in view of the negative public opinion on transgenic technology, and the regulatory requirements and approvals that are required in most countries for their application.

Using biological/molecular methods to sterilize insects is quite different from using a physical process, such as radiation. In the former, the essential biological interaction required to generate the sterility phenotype is subject to biological variation in both components, and is therefore unpredictable in the long term. A physical process such as radiation is not subject to this variation, and hence, in some ways, is an ideal methodology. Insects cannot become resistant to ionizing radiation as it is used in the SIT (Whitten and Mahon, this volume).

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