INHERITED PARTIAL STERILITY AND CHROMOSOMAL REARRANGEMENTS IN SUCCEEDING GENERATIONS AFTER IRRADIATION OF *ADOXOPHYES ORANA* (LEPIDOPTERA, TORTRICIDAE) WITH SUBSTERILIZING DOSES OF X-RAYS AND FAST NEUTRONS

BY

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Male and female adult *Adoxophyes orana* F.v.R. were irradiated within 24 h of eclosion with doses of X-rays between 2.5 and 22.5 krad and with 3 krad fast neutrons. The treated moths were single pair mated with untreated moths and egg hatchability was assessed. The surviving F1 progeny and also succeeding generations were tested in a similar manner. Mortality of larval and pupal stages and sex ratios were also determined. Differences observed between the fertility of the F1 from irradiated males and from irradiated females led to the hypothesis that the time of meiosis with respect to the time of irradiation is of crucial importance in the expression and inheritance of the induced rearrangements. Consequences for differences in radiosensitivity between sexes and for the application of sub-sterile insects for practical control purposes are discussed.

In at least five lepidopteran species various aspects of inherited partial sterility have been studied in some detail while in another ten species its occurrence has been established (North & Holt, 1971b; Proshold & Bartell, 1972b). In those studies it was shown that after irradiation of male moths with substerilizing doses of X- or γ-rays the survival of the F1 is reduced, the developmental time is lengthened and the sex ratio shifts in favour of males. Also, F1-males of certain species do not transfer the normal complement of apyrene and eupyrene sperm. In general, the fertility of the F1 is below the fertility of the P-generation, and F1-females have a somewhat higher fertility than F1-males (Proverbs & Newton, 1962; Proshold & Bartell, 1972a, 1972b; North & Holt, 1968a, 1968b, 1971a, 1971b; Graham *et al.*, 1972; Cheng & North, 1972; Ashrafi *et al.*, 1972; LaChance *et al.*, 1973; Toba & Kishaba, 1973).

When female moths are irradiated with substerilizing doses it has been found that the F1 showed a fertility higher than that of the P-generation (Graham *et al.*, 1972; Proshold & Bartell, 1973). These authors also showed that there was a rapid disappearance of the sterility in the course of three generations of backcrossing. Bauer (1967), LaChance *et al.* (1970), and North & Holt (1968a) suggested that the basis of radiation-induced partial sterility was cytogenetic and resulted from chromosome rearrangements e.g. translocations, inversions and chromosome fragments.

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The reason why the F1 from irradiated females have a fertility above that of the P-generation and the F1 from irradiated males have a fertility below that of the P-generation, forms the basis of this report. Data were also obtained on the rapidity of the recovery in fertility following backcrossing. Finally, because irradiation with fast neutrons is reported to be superior to irradiation with X-rays in inducing sterility with a consequent low degree of somatic damage (Offori, 1971), moths were also irradiated with a substerilizing dose of fast neutrons.

The data were obtained in the framework of research for development of a genetic control method for *Adoxophyes orana* a key pest in an integrated control programme in apple orchards (De Jong *et al.*, 1971; Barel, 1973).

**Material and methods**

Experimental conditions and origin of the insects were essentially those reported earlier (Snieder *et al.*, 1973). Virgin females were irradiated with 0, 2.5 and 5 krad X-rays and 3 krad fast neutrons; virgin males with 0, 7.5, 10, 15 and 22.5 krad X-rays and 3 krad fast neutrons. The choice of these doses was based on dose-response curves for dominant lethality (Snieder *et al.*, 1973). Immediately after irradiation each moth was placed with one untreated moth of the opposite sex in a plastic box (250-650 cc) for mating and oviposition for 6 days. Resulting larvae were reared individually in 3-ml vials on a wheatgerm diet (Ankersmit, 1968). After pupal eclosion, at random chosen F1-moths were backcrossed in single pair matings. This procedure was followed for four generations, and % egg hatchability (= % eh), survival from first-instar larva to adult, and sex ratio in succeeding generations determined.

Squash preparations of the testes of L5-larvae in the F1- and B1-generation were made in 2% aceto-orcein and were examined for visible chromosomal rearrangements at a magnification of 1250 under phase-contrast.

**Results**

The progenies from males irradiated with 15 and 22.5 krad X-rays died out in the B1- and F1-generation, respectively. The progenies from the other treatments recovered their fertility after a few generations. In Fig. 1 the trend of the egg hatchability (% eh) in succeeding generations after irradiation is shown. Where P1-males were treated with 7.5 or 10 krad of X-rays the results have been bulked, as has the data from P1-females irradiated with 2.5 and 5 krad X-rays. This is justified by the fact that there seems to be, within these limits, no clear correlation between % eh in the P-generation (=related to dose of radiation) and the level and variation of the % eh in the following generations. Despite a wide variation in % eh of the control it is clear from Fig. 1 that the F1-generation from irradiated males possesses a fertility below that of the P-generation. However, the fertility of the F1 from irradiated females is somewhat higher than that of the P-generation. From Fig. 1 it follows also that the inherited partial sterility is nearly eliminated after three or four generations of outcrossing, independent of the type of radiation.
Following irradiation with X-rays the mortality between the first instar and adult stage and the sex ratio were determined in the different generations. We found (Table I) that the mortality was especially influenced in the F1- and B1-generation, and, the lower the % eh the higher the mortality. Mortality occurred in every development stage to almost the same extent. The sex ratio of the F1-adults was
Mortality and sex ratio in succeeding generations after irradiation with X-rays correlated with % egg hatchability. M: % mortality occurring between first instar and adulthood; S: sex ratio (δ/♀); I: irradiated in P-generation

<table>
<thead>
<tr>
<th>% eh</th>
<th>Control</th>
<th>Fl-generation</th>
<th>B1-generation</th>
<th>B2-generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>S</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>1—20</td>
<td>—</td>
<td>60 0.8</td>
<td>76 2.5</td>
<td>73 1.2</td>
</tr>
<tr>
<td>21—40</td>
<td>8</td>
<td>38 1.5</td>
<td>49 1.2</td>
<td>47 1.1</td>
</tr>
<tr>
<td>41—60</td>
<td>—</td>
<td>34 1.4</td>
<td>49 2.9</td>
<td>34 0.9</td>
</tr>
<tr>
<td>61—80</td>
<td>12±3</td>
<td>40 0.9</td>
<td>47 2.8</td>
<td>16 1.0</td>
</tr>
<tr>
<td>81—100</td>
<td>9±6</td>
<td>10 1.0±0.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table I

Markedly influenced following irradiation of parental males; however, the effect lasted only one generation. Irradiation of females had no effect on sex ratio. This difference can be explained by assuming that recessive lethals are induced on the X chromosomes of the homogametic males which are expressed in the heterogametic F1 female zygotes (Proshold & Bartell, 1970).

Combined with the % eh determinations of inherited partial sterility cytological analyses on the meiotic cells of F1- and B1-larval males were performed. In Table II the numbers of F1-larvae with analyzable M1-plates, their origin, and number of larvae with visible chromosomal rearrangements are summarized. Also data on cytological analyses of B1-lines are given in Table II. Almost 100% of the F1-males from irradiated males had chromosomal rearrangements. These rearrangements were in 80% of the cases very complex. However, the F1-males from irradiated females showed rearrangements in only about 25% of the cases and 30% of these were complex. Only about 10-20% of the B1-lines possessed visible chromosomal rearrangements.

In Fig. 2A the metaphase of the first meiotic division of untreated male larvae is represented: 29 or 30 bivalents can be discerned. All bivalents, except one, are very small and look very similar. The unusual bivalent is about twice as large as the other ones and is thought to consist of the sex chromosomes (Bauer & Lerche,

Table II

Summary of data sampled by cytological analysis of meiotic cells of F1- and B1-males. I: irradiated with X-rays

<table>
<thead>
<tr>
<th>Treatment in P-generation</th>
<th>F1-larvae with analyzable M1-plates</th>
<th>F1-larvae with visible chromosomal aberrations</th>
<th>B1-lines analysed</th>
<th>B1-lines with visible chromosomal aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>δ δ I</td>
<td>55</td>
<td>51</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>♀ ♂ I</td>
<td>42</td>
<td>10</td>
<td>17</td>
<td>1</td>
</tr>
</tbody>
</table>
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Fig. 2. Meiotic metaphase plates from fifth-instar male larvae of *A. orana*. A: M I-plate of an untreated larva; B, C and D: M I-plates from larvae with an irradiated set of chromosomes.
IRRADIATION OF ADOXOPHYES ORANA

1943). The chromosome configurations encountered in the meiotic cells with an irradiated set of chromosomes resembled chains consisting of different numbers of bivalents. The many differences in length of the chains in the cells of the same larvae are probably caused by variation in place and frequency of chiasmata (Bauer, 1967). Very occasionally a ring configuration was detected, while fragments were very often encountered. In Fig. 2B, C and D some examples are given of the variation in appearance of the chromosomal rearrangements.

DISCUSSION

These data obtained from irradiated A. orana adults on inherited partial sterility, on mortality in the developmental stages, on sex ratio distortion, and on visible chromosomal rearrangements are in close agreement with data on Trichoplusia ni (North & Holt, 1968a, 1968b, 1971a, 1971b), Heliothis virescens (Proshold & Bartell, 1972a, 1972b, 1973), Pectinophora gossypiella (Graham et al., 1972, LaChance et al., 1973) and many others. Therefore, it seems to be a special feature of the Lepidoptera, that the F1 from irradiated male moths is less fertile than from irradiated female moths. Further, the induced partial sterility is eliminated in a few generations coinciding with a quick disappearance of visible chromosomal rearrangements.

Both Cooper (1972), working on radiosensitivity of oogenesis of the grass mite, Siteroptes graminum (Reuter), and Murakami (1973), researching radiosensitivity of gametogenesis in Bombyx mori, reached the conclusion that meiosis is, genetically and mutagenetically, a very important stage. Moreover, it is believed that meiosis plays a crucial role in the explanation of our results. In pupal or adult males of Lepidoptera only postmeiotic sperm stages are present, while in adult females only meiotic or premeiotic stages are found (Virkki, 1965). For A. orana this is confirmed by E. Suomalainen (Department of Genetics, University of Helsinki, pers. comm.). This means that in the case of female irradiation, factors disturbing meiosis as well as dominant lethal factors show themselves as F1-egg mortality, while in the case of irradiating adult males, only dominant lethal factors cause F1-egg mortality but subsequently factors disturbing meiosis express themselves as mortality of B1-eggs. So, the surviving F1 of treated females is more fertile than the P-generation, while the fertility of the surviving F1 from treated adult males is in general lower than that of the P-generation depending on the ratio of dominant lethal factors to factors disturbing meiosis.

The results of LaChance et al. (1970) on Oncopeltus fasciatus may also fit in the picture. With this insect meiosis goes on in adult males and the average % eh of the F1 from irradiated males is indeed above the % eh of the treated P-generation. These data can perhaps also be explained as indications that the time of irradiation with respect to the meiotic process is very important for the expression of induced rearrangements.

The underlying mechanism should be the fact, that in insects with holokineti
chromosomes the diffuse or polykinetic organisation of the centromere enables induced fragments to function more or less as normal chromosomes until the meiotic divisions. Then, according to Virkki (1963) and Cooper (1972) factors such as chromosome pairing and co-orientation play an important part. During those stages most fragments without chiasmata or other orientating prerequisites will disturb the regular distribution of genetic information, thus leading to unbalanced gametes.

Also the visible chromosomal rearrangements, which Bauer (1967) and LaChance et al. (1970) called reciprocal translocations will lead to unbalanced gametes. But in our case at least 20% of the translocations were quite simple, comprising two or three bivalents. We think therefore that the rearrangements disappeared not because they caused complete sterility of the gametes, but because they occurred in cells with fragments.

If the foregoing is true, it is consequently not valid to deduce, from the hatching of F1-eggs produced by irradiated adult males and females, a measure of differences in radiosensitivity between the sexes. For that purpose equivalent meiotic stages should be irradiated in both sexes or otherwise a correction on F1-egg hatchability should be applied. Ashrafi et al. (1972) for Plodia interpunctella and Raun et al. (1967) for Ostrinia nubilalis using equivalent stages, established that the male sex is the sensitive one. Experiments are now underway to study the radiosensitivity of different embryonic and larval stages of A. orana to test the foregoing hypothesis.

Finally, the disappearance of almost all induced partial sterility after a few generations of backcrossing has important consequences for the application of sub-sterile insects for practical control purposes. Radiation doses should be used which completely sterilize the females, while the F1 of the irradiated males has also to be completely sterile. For A. orana this dose is somewhere in the range between 15 and 20 krad X-rays.

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ZUSAMMENFASSUNG

VERERBTE TEILSTERILITÄT UND CHROMOSOMENABERRATIONEN IN DEN FOLGEGENERATIONEN NACH BESTRAHLUNG VON ADOXOPHYES ORANA (LEPIDOPTERA: TORTRICIDAE) MIT SUBSTERILISIERENDEN DOSEN VON RÖNTGENSTRahlen UND SCHNELLEN NEUTRONEN

Falter von A. orana, Männchen und Weibchen, wurden bestrahlt mit substerilisierender Dosis von Röntgenstrahlen oder schnellen Neutronen innerhalb 24 Stunden nach dem Schlüpfen. Anschliessend wurden sie mit unbehandelten Faltern gekreuzt. Die folgende Generation wurde mit künstlicher Diät aufgezogen und danach rückgekreuzt mit Wildstammtypen, Paar für Paar. Dies wurde vier Generationen lang fortgesetzt. Die folgenden Parameter konnten für die auf die Bestrahlung folgenden Generationen bestimmt werden: 1) die Fertilität (= Schlüpf-
rate der Eier); 2) die Mortalität zwischen dem ersten Larvenstadium und der Imago; 3) das Geschlechtsverhältnis der Falter. Die Fertilität war niedriger bei der F1-Generation von bestrahlten Männchen als die der bestrahlten P-Generation, Hingegen war bei der F1-Generation von bestrahlten Weibchen die Fertilität höher als die der bestrahlten P-Generation. Dieser Befund ist unabhängig von der Strahlenart (Röntgenstrahlen oder schnelle Neutronen). Die Mortalität wurde in der F1- und B1-Generation beeinflusst, und zwar war sie umso höher, je niedriger die Schlüpfrate war. Das Geschlechtsverhältnis in der F1-Generation von bestrahlten Männchen war deutlich zugunsten der Männchen verschoben.


REFERENCES


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