# Notes

## Effect of DDT Selection Pressure on the Frequency of Chromosomal Structures in *Anopheles atroparvus* *

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During the course of studies on the development of resistance to the chlorinated insecticides in anophelines, we were able to establish that considerable variations in the degree of susceptibility to DDT were shown by different specimens of a strain of *Anopheles atroparvus* bred in our laboratory for the purpose of these experiments, and not previously subjected to selective insecticide treatment (parent strain). Tests were carried out at varying intervals, in accordance with the Busvine & Nash method.[a]

In addition, tests using the method described by Mariani[b, c] proved that in an appreciable proportion of the population of the strain the susceptibility to DDT was clearly lower. In fact, a series of tests showed that 40 minutes' contact with a surface treated with 2 g/m² of DDT killed 78% of the insects, whereas 95 minutes were needed to obtain 100% deaths. Obviously, some of the individuals of the strain—that is, the less susceptible individuals—do not follow the statistical pattern of behaviour that is characteristic of populations with homogeneous susceptibility.

The heterogeneity of the strain in the matter of DDT resistance led us to study the subject from the point of view of chromosome structure, by means of cytological examination using the Frizzi technique.[d]

These studies enabled us to establish the fact that in the population in question there are two different arrangements in the left arm of the third chromosome. These arrangements involve a segment which lies between zone 44 and the limits of zone 47-48, and they may be present in three different forms—standard, inverted homozygous, heterozygous—the standard and heterozygous types being more frequent than the inverted homozygous one.

The discovery of this heterogeneity in the initial experiments led us to compare the frequency of the various types of arrangement in a non-treated strain with that in strains being subjected to selective DDT treatment, in order to see whether there was any difference in distribution.

The strains treated with DDT were all derived from the parent strain. They were treated with the insecticide in various ways and may be classified as follows:

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[a] Busvine, J. R. & Nash, R. (1953) *Bull. ent. Res.*, **44**, 371
[b] Mariani, M. (1950) *Mem. Soc. ent. ital.*, **29**, 5
[c] Mariani, M. (1954) *Boll. Soc. ent. ital.*, **84**, 67
[d] Frizzi, G. (1953) *Bull. Wld Hlth Org.*, **9**, 335

*Strain I.* Only larvae (usually at stages 1 and 3) are treated. They are immersed for 15 minutes in an aqueous colloidal solution of DDT, in a concentration of 1 mg/litre (pp'-isomer).

*Strain II.* The eggs are allowed to hatch out in water containing 4 $\mu$g DDT/litre and remain there throughout the larval stage, i.e. from hatching until the pupal phase is reached.

*Strain III.* Only the adults undergo DDT selective treatment—that is, we rear the egg-masses of females which have resisted one hour's exposure to surfaces treated with 3% DDT solution (Busvine & Nash method) or more than one hour's exposure to surfaces treated with 2 g/m$^2$ DDT (Mariani method).

*Strain IV.* Both larvae and imagos are subjected to selective DDT treatment; thus the individuals of this group undergo a combination of the treatments applied to Strains II and III.

At the present time we are using the third generation of strains I, II and III.

Details of the degrees of susceptibility to DDT of these strains will be given in a subsequent note; here, we will do no more than say that the first—preliminary—tests seem to show a marked reduction in susceptibility.

The investigations regarding the frequency of the various chromosome arrangements were carried out on the parent strain and on strains I, II and III only, in view of the scarcity of strain IV material.

Two groups of tests were performed, as follows:

1. *Examination of the frequency of the various chromosome arrangements in the parent strain and in the " treated " strains (I, II and III)*

Stage-4 larvae were selected at random from various egg masses and observed for one month. They represented approximately 20% of the population bred, i.e., 185 larvae from the parent strain, 185 from strain I, 190 from strain II and 191 from strain III.

The distribution of the chromosome arrangements found, expressed as percentages, is given in Table I.

TABLE I. DISTRIBUTION OF CHROMOSOME ARRANGEMENTS (STANDARD, INVERTED HOMOZYGOUS AND HETEROZYGOUS) IN THE VARIOUS STRAINS

| Chromosome arrangement | Parent strain | | Strain I 3rd generation | | Strain II 3rd generation | | Strain III 3rd generation | |
|---|---|---|---|---|---|---|---|---|
| | number | % | number | % | number | % | number | % |
| Standard | 85 | 46.0 | 77 | 41.6 | 47 | 24.7 | 63 | 33.0 |
| Heterozygous | 87 | 47.0 | 95 | 51.4 | 121 | 63.7 | 97 | 50.8 |
| Inverted homozygous | 13 | 7.0 | 13 | 7.0 | 22 | 11.6 | 31 | 16.2 |
| Total | 185 | | 185 | | 190 | | 191 | |

The data in Table I show the frequency of the standard arrangement as compared with that of the heterozygous and inverted homozygous arrangements, as well as the relative frequencies as between the heterozygous and inverted homozygous arrangements. The probability of finding similar frequencies, by pure chance, in specimens of a homogeneous population was calculated on the basis of the $\chi^2$ test.[e] Comparison was made in all cases between the frequencies of two arrangements in various strains (Table II). Differences in frequency giving a $\chi^2$ value above 3.86 are considered as significant and not as purely fortuitous. In fact, with only one degree of freedom, variations giving a $\chi^2$ value equal to 3.86 can only be found with a probability of 0.05 (5%). As the $\chi^2$ value increases, there is less probability of finding, by pure chance, differences which are equal to, or more marked than, those observed.

TABLE II. COMPARISON OF FREQUENCY OF CHROMOSOME ARRANGEMENTS IN THE VARIOUS STRAINS

| Comparison between : | | $\chi^2$ value |
|---|---|---|
| Standard arrangement | Heterozygous arrangement | |
| (untreated strain) | (strain I) | 0.74 |
| ,, | (strain II) | 16.45 |
| ,, | (strain III) | 3.38 |
| Standard arrangement | Inverted homozygous arrangement | |
| (untreated strain) | (strain I) | 0.05 |
| ,, | (strain II) | 8.48 |
| ,, | (strain III) | 106.59 |
| Heterozygous arrangement | Inverted homozygous arrangement | |
| (untreated strain) | (strain I) | 0.03 |
| ,, | (strain II) | 0.27 |
| ,, | (strain III) | 4.23 |

2. *Examination of the frequency of the three arrangements in stage-4 larvae of the parent strain after treatment with various concentrations of DDT*

The aim of this group of experiments was to confirm, under different conditions, what appeared to follow from the first group of tests, i.e., the greater frequency of the inverted arrangement in specimens subjected to selective DDT treatment.

To this end, stage-4 larvae, chosen at random from the parent strain, were exposed for 15 minutes in water containing a decreasing series of DDT concentrations.

[e] Snedecor, G. W. (1946) *Statistical methods applied to experiments in agriculture and biology*, 4th ed., Ames, Iowa, p. 198

The mortality of the larvae was determined during the 24 hours following treatment and, in order to avoid any alteration in the chromosome structure (which happens 5-6 hours after death), the larvae were examined as soon as they died. Nevertheless, it was not possible to examine every specimen.

The number of larvae treated, and their mortality and survival rates in the presence of various concentrations, are shown in Table III.

TABLE III. MORTALITY AND SURVIVAL RATES OF STAGE-4 LARVAE EXPOSED FOR 15 MINUTES TO VARIOUS DDT CONCENTRATIONS

| Concentration | Number of individuals treated | Deaths | | Survivals | |
|---|---|---|---|---|---|
| | | number | % | number | % |
| 2 μg/ml | 423 | 292 | 69 | 131 | 31 |
| 1 μg/ml | 254 | 151 | 59 | 103 | 41 |
| 0.5 μg/ml | 210 | 107 | 51 | 103 | 49 |

The frequency of the various chromosome arrangements in the dead and surviving larvae treated with DDT is shown in Table IV. The number of individuals treated, and their behaviour, would seem to show that the frequency of the different forms is relatively constant.

The figures in Table IV all give a $\chi^2$ value of less than 3.86 when the comparison is made between the standard and heterozygous types. The number of larvae showing the inverted homozygous arrangement is too small to permit of statistical comparison, but if the $\chi^2$ value is calculated on the basis of the chromatids, it is possible to include also the few inverted homozygotes. Thus, in the second and third experiments, $\chi^2$ is equal to 3.37 and 5.07 respectively, which latter value would confirm a differential selectivity in favour of the inverted arrangement. This group of experiments gives further support to the assumption that the inverted homozygous and heterozygous combinations show more resistance in the DDT selective test.

**Discussion.** As foreseen, the parent strain of *A. atroparvus*, the subject of our investigations, shows a considerable degree of heterogeneity in its susceptibility to DDT. Moreover, as will be seen from the information communicated in the present note, this strain is also heterogeneous from the cytogenetic point of view, for it contains individuals with different chromosome structures (standard, inverted and heterozygous).

Thus it would clearly be of interest to determine if there exists any correlation between the heterogeneous response to DDT and the above-mentioned cytogenetic heterogeneity. The results of the experiments carried out for this purpose seem to supply an affirmative answer, in the sense that the individuals with an inverted chromosome arrangement, whether homozygous or heterozygous, are less susceptible to DDT.

This conclusion seems to be justified by the fact that in the strains submitted to selection by exposure to DDT and, in particular, as regards strain II (for which the selective treatment was exposure to small and conti-

**TABLE IV. FREQUENCY OF CHROMOSOME ARRANGEMENTS IN LARVAE EXPOSED FOR 15 MINUTES TO VARIOUS DDT CONCENTRATIONS**

| Type of arrangement | Larvae examined and % after DDT treatment (2 μg/ml) | | | | Larvae examined and % after DDT treatment (1 μg/ml) | | | | Larvae examined and % after DDT treatment (0.5 μg/ml) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | deaths | | survivals | | deaths | | survivals | | deaths | | survivals | |
| | number | % | number | % | number | % | number | % | number | % | number | % |
| Standard | 30 | 50 | 37 | 42 | 40 | 56 | 25 | 40 | 29 | 50 | 15 | 29 |
| Heterozygous | 30 | 50 | 50 | 56 | 31 | 44 | 36 | 57 | 29 | 50 | 32 | 63 |
| Inverted homozygous | — | — | 2 | 2 | — | — | 2 | 3 | — | — | 4 | 8 |
| Total | 60 | | 89 | | 71 | | 63 | | 58 | | 51 | |
| | $\chi^2 = 0.8$ | | | | $\chi^2 = 3.09$ | | | | $\chi^2 = 3.58$ | | | |

nuous doses of DDT), the frequency of the inverted arrangement is significantly higher than in the parent strain. The same conclusion may be drawn from the data with regard to the frequency of the above-mentioned arrangements in larvae that survived exposure to DDT, particularly those surviving the 0.5% concentration, which, since it is the least drastic treatment, reveals the phenomenon more clearly.

The increase in the inverted homozygotes in the first experiment (strains II and III) could also be considered as an indication of the greater probability of the appearance of this type, in the subsequent combinations, in relation to the greater number of heterozygotes. But this is negated by the fact that there are more inverted homozygotes in strain III than in strain II, even though this latter shows a definitely higher number of heterozygous arrangements. Another factor is the slight increase in the inverted homozygotes in the second group of experiments.

It might be argued that the heterogeneous behaviour of the parent strain in the presence of DDT may be caused by the fortuitous contact of the generations bred in the laboratory with some DDT-contaminated food, or with DDT in the original breeding-place. It is also possible that the selective process itself may have brought the cytogenetic heterogeneity to light, revealing a chromosome mutation, but one so rare in nature that it was not observed in any of the thousands of larvae of the same species during the preparation of the chromosome map, or in successive tests up to 1951.

The greater resistance to DDT on the part of the heterozygotes as compared with that of the standard homozygotes could be explained on the assumption that the DDT-resistance factors are localized in the segment in which the inversion is observed. There being no interchange, such factors would be transmitted *en bloc* in the case of the heterozygotes, whereas in the standard type the more frequent interchange in this segment would create various genetic combinations involving the resistance factors and, therefore, bring about a greater dispersion of them. Among the data in support of this theory are results of experiments with other insects—in particular *Drosophila*—showing that certain ecological conditions favour heterozygous chromosomes, precisely because of the combination of favourable factors introduced by heterozygous inversion.

Although the possibility of mere coincidence cannot be excluded, it nevertheless seems significant that *Anopheles sacharovi*, which has become resistant to DDT in Greece and the Lebanon,[f] presents the same inverted homozygous arrangement in the left arm of the third chromosome, by an extension which is the same, or almost the same, as in our inverted homozygous strain of *atroparvus*.

It is to be hoped that research will be extended to include the other species of *Anopheles* showing chromosome polymorphism, such as *A. gambiae*, *A. freeborni*, *A. quadrimaculatus*, etc.

On the basis of this preliminary note a programme of research has been established with the aim of going further into the question of the relationship between cytogenetic constitution and resistance. Research in this direction is already proceeding.

[f] Expert Committee on Malaria (1954) *Wld Hlth Org. techn. Rep. Ser.*, **80**, 30