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STUDIES ON INSECTICIDE-RESISTANT ANOPHELINES

1. Chromosome Arrangements in a Dieldrin-Selected Strain of *Anopheles atroparvus*

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SYNOPSIS

The authors present the results of a cytological examination, carried out by the technique developed by Frizzi, of the larvae of a normally susceptible and a dieldrin-selected strain of *Anopheles atroparvus*, both laboratory-reared. A very high percentage (77.4) of heterozygous inversions was observed in the larvae of the 28th generation of the dieldrin-resistant strain, whereas only 20.8% occurred in the normally susceptible strain, in which the standard chromosomal arrangement predominated (74.2%). These results are similar to those obtained recently by other workers in the case of larvae of the 12th generation of a DDT-selected strain of *A. atroparvus* (74.8% heterozygous inversions observed).

As part of an extensive research programme on laboratory-developed strains of insecticide-resistant anophelines, some of the strains reared in our laboratory were submitted to a cytological examination by the method developed by Frizzi (1953) and later employed successfully by D'Alessandro, Frizzi & Mariani (1957) on DDT-selected *Anopheles atroparvus* and by Frizzi & Holstein (1956) and Holstein (1957) on normal and resistant *A. gambiae*, respectively.

In view of the interest in the dieldrin resistance found in *A. gambiae* in Nigeria (Elliot & Ramakrishna, 1956; Davidson, 1956, 1958) and *A. quadrimaculatus* in the USA (Mathis et al., 1956), we are prompted to present here the results of the cytological examination of a dieldrin-selected strain of *A. atroparvus*.

Description of the Strains

Susceptible reference strain

This strain originates from a laboratory colony of *A. atroparvus* maintained since 1935 at the malariotherapy centre of the Istituto Superiore di Sanità and established from ovipositions of numerous anophelines captured

in the zone of Ferrara, Italy. To this colony were added some ovipositions of *A. atroparvus* captured at the same locality in 1946, before the initiation of the antimalaria campaign with DDT in the area.

The larvae are reared in earthenware bowls (upper diameter, 24 cm; height 10 cm; depth of water, 8 cm; volume of water, approximately 1.5 litres; surface area of water, approximately 380 cm^2) at a room temperature of 30°C and a water temperature of 25°C.

The LC_{50} of dieldrin for adult females of the reference strain, as determined by a modification of the Busvine & Nash method (Busvine & Nash, 1953) in which large tubes (20 females per tube) were used,[1] was 0.14% in 1956 and was practically unchanged after two years (0.18% in 1958).

The LC_{50} of dieldrin for fourth-stage larvae of the susceptible strain was 0.016 parts per million (p.p.m.). These tests were carried out in low rimmed rectangular enamelled metal basins (volume of water, 1 litre; surface area, 20 cm × 25 cm; depth, 2 cm; number of larvae per basin, 20). The insecticide was added in solution in 1 ml of ethanol. Each concentration was tested in 5 replicates.

Dieldrin-selected strain

The method of selection with dieldrin consisted in treating the larvae in the first and third stages with small doses of the insecticide, increasing in each generation. Many times, however, it was found necessary to return to smaller selecting doses than those already reached, or even sometimes to suspend selection completely for a generation, because of the high mortality of the larvae on the days immediately following the treatment or because the adults hatched from treated larvae exhibited physiological disturbances, such as being unable to feed on guinea-pigs or laying very few eggs.

The larval and adult LC_{50}'s were as follows:

	Larval LC_{50} (p.p.m.)	*Adult LC_{50} (%)*
Parent strain	0.016	0.14 (0.18)
Dieldrin-selected strain, 15th generation .	0.025	0.28
Dieldrin-selected strain, 28th generation .	0.045	0.35

In the 28th generation, the larval LC_{50} was thus three times the normal value and the adult LC_{50} was 2.5 times the normal.

Results of the Cytological Examination

Early fourth-stage larvae of the 28th generation of the dieldrin-selected strain from batches which were reared without exposure to the insecticide in this generation, and of the susceptible reference strain, were transferred

[1] This modification will be described in detail in a later paper.

from the breeding-rooms to a room kept at 15°C, so that the period during which batches of larvae could be examined was protracted. No larval mortality was noted as a consequence of the transfer to the lower temperature.

Following the Frizzi technique, the left arm of the third chromosome was examined in 131 larvae of the susceptible strain and 147 of the dieldrin-selected strain. The following results were obtained:

Chromosomal arrangement	*Number of larvae of susceptible strain*	*dieldrin-selected strain*
Standard	89	25
Heterozygous	25	106
Inverted homozygous	6	6
Illegible *	11	10
Total	131	147

* Though technically sound preparations, no sure conclusion as to chromosomal arrangement could be arrived at.

Distribution of Chromosomal Arrangements among Legible Preparations

	Susceptible strain		*Dieldrin-selected strain*	
	number	*%*	*number*	*%*
Standard	89	74.2	25	18.2
Heterozygous	25	20.8	106	77.4
Inverted homozygous	6	5.0	6	4.4
Total	120		137	

It is possible to infer from these results that in the dieldrin-selected strain there occurs a very high percentage (77.4%) of heterozygous inversions. Interestingly enough, Holstein (1957) found in dieldrin-resistant *A. gambiae* a total of 38.4% heterozygous inversions (sum of heterozygous inversions in second right, third right and third left arms), but has not yet reached a conclusion as to whether all or only a part of these chromosomal arrangements result from dieldrin resistance or whether some are due to different geographical races.

As regards DDT-resistant *A. atroparvus*, D'Alessandro, Frizzi & Mariani (1957) found, for three strains selected by different modes of exposure, 51.4%, 63.7% and 50.8% heterozygous inversions already in the third generation selected, and recently (1958) reported for the 11th and the 12th generation of a DDT-selected strain 67.5% and 74.8% heterozygous inversions, respectively.

The high percentage of heterozygous inversions in the dieldrin-selected strain contrasts with the low percentage (20.8) of heterozygotes found in our susceptible reference strain, in which the standard arrangement predominated (74.2%). These results agree with the recent findings for the susceptible parent strain of D'Alessandro, Frizzi & Mariani (1958) (standard, 80.73%; heterozygous, 19.27%). In the earlier work of the same authors (1957), however, the parent strain had been found to contain

46% standard, 47% heterozygous and 7% inverted homozygous chromosomal arrangements.

In our search for chromosomal rearrangements, which was confined to the left arm of the third chromosome, we noted the three following types of heterozygous inversions (for numeration of zones, see Canalis et al., 1954):

	Heterozygous inversion involving segment between zones	*% occurrence among heterozygotes in dieldrin-selected strain*
" Big ring "	44-47/48	41.8
" Small ring near terminal end " .	46-47/48	20.2
" Small ring near centre-base " .	44-46	38.0

Some Technical Observations

1. While working with the Frizzi technique, it was found to be of the utmost importance that the larvae used for the cytogenetic examination be reared at a *very low* density. We used to rear 100 larvae in the earthenware bowls containing approximately 1.5 litres of water (see detailed description above). Food should be offered copiously. Larvae reared under these conditions present only a low percentage of " illegible " (Frizzi & Holstein, 1956) preparations. Immediately before dissection, a number of larvae should be transferred to a beaker with distilled water, to remove from them the scum of the breeding medium.

2. The reagents—fixative, stain and decolorizing agent—should be prepared in bulk a few weeks before use, to allow them to stabilize (most probably there will be formation of ethyl acetate and water in the fixative, and solvation in the decolorizing agent). If the fixative is still unsatisfactory after the elapse of such a period (excessive spreading of the drop put on the salivary glands before they are teased out, and excessive evaporation), addition of up to 10% water will remedy the situation.

3. It was found very expedient to seal the preparations immediately after squashing with a varnish (nail varnish was found to be satisfactory); in this way it was possible to avoid going to the trouble of carrying out the lengthy procedure involved in making a permanent preparation (Darlington & La Cour, 1950), since the varnish-sealed preparations remained unchanged and well legible for at least ten days.

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Work is now in progress on the investigation of the chromosomal arrangements of several highly DDT-resistant strains of *A. atroparvus*, selected by different modes of exposure (adults only, larvae only, both larvae and female adults), with a view to determining the effect of mode of selection on chromosomal arrangements.

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RÉSUMÉ

Poursuivant les études cytologiques d'insectes résistants aux insecticides — selon la méthode mise au point par Frizzi — les auteurs ont observé les modifications chromosomiques d'une souche d'*Anopheles atroparvus* soumise à la sélection par la dieldrine, et l'ont comparée à une souche normale; ces deux souches faisaient partie d'un élevage de laboratoire.

Des inversions chromosomiques hétérozygotes ont été observées dans 77,4% des larves de la 28e génération, contre 20,8% dans la souche normalement sensible. Ces résultats sont du même ordre que ceux d'autres auteurs, obtenus chez des larves de 12e génération d'une souche d'*atroparvus* soumise à la sélection par le DDT.

Les auteurs indiquent les trois types d'inversion observés et rendent les chercheurs attentifs à certains détails de technique importants.

REFERENCES

Busvine, J. R. & Nash, R. (1953) *Bull. ent. Res.*, **44**, 371

Canalis, A., De Negris, U., Frizzi, G. & Sepulcri, P. (1954) *Le specie del gruppo* "maculipennis" *identificate col metodo citogenetico dai focolai larvali in zona trattata con D.D.T. nelle provincie di Rovigo e Venezia*, Venezia

D'Alessandro, G., Frizzi, G. & Mariani, M. (1957) *Bull. Wld Hlth Org.*, **16**, 859

D'Alessandro, G., Frizzi, G. & Mariani, M. (1958) *Riv. Parassit.*, **19**, 67

Darlington, C. D. & La Cour, L. F. (1950) *The handling of chromosomes*. 3rd ed., London, p. 126

Davidson, G. (1956) *Nature (Lond.)*, **178**, 705

Davidson, G. (1958) *Bull. Wld Hlth Org.*, **18**, 579

Elliot, R. & Ramakrishna, V. (1956) *Nature (Lond.)*, **177**, 532

Frizzi, G. (1953) *Bull. Org. mond. Santé*, **9**, 335

Frizzi, G. & Holstein, M. (1956) *Bull. Org. mond. Santé*, **15**, 425

Holstein, M. (1957) *Bull. Wld Hlth Org.*, **16**, 456

Mathis, W., Schoof, H. F., Quarterman, K. D. & Fay, R. W. (1956) *Publ. Hlth Rep. (Wash.)*, **71**, 876