

Selection of strains of *Anopheles atroparvus* with different behaviour after contact with DDT *

by

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At the Congresses on Tropical Medicine in Rio de Janeiro, several investigators have insisted on the problems connected with the behaviour of *Anopheles* after contact with DDT. Though populations showing different responses to DDT could be distinguished by the Excito-Repellency Box Model OPS, nothing was known with regard to the cause of these differences.

To elucidate these problems we carried out a selection of *Anopheles atroparvus*, based on DDT-irritability (fig. 1). Escaping or not-escaping from a horizontally placed tube, lined with paper impregnated with 4 % DDT in Risella-oil, was chosen as criterion for selection.

The result of the selection is expressed by $\log \frac{\text{Percentage escaping fn}}{\text{Percentage escaping fo}}$. Thus each generation is compared with the original strain. The open dots give the results of the original strain at different moments, as compared with their mean value ; this mean of percentages escaping serves as baseline for fo. The drawn line gives the data of the separate generations and the dotted line gives the proceeding means over every five generations, by which the fluctuation of the separate generations has been eliminated.

The selection-experiment was started with a strain, originating from the Netherlands and bred for two years in the laboratory.

Selection-pressure was applied at two levels :

1st : a three times repeated response to the selection-criterion.

2nd : a single response to the selection-criterion.

The first procedure resulted in two strains A and B, respectively showing a high and low percentage of escaping.

The second procedure resulted in two strains, A' and B', which were shown to parallel the A and B strain only partly ; the extent of the selection is less than of A and B.

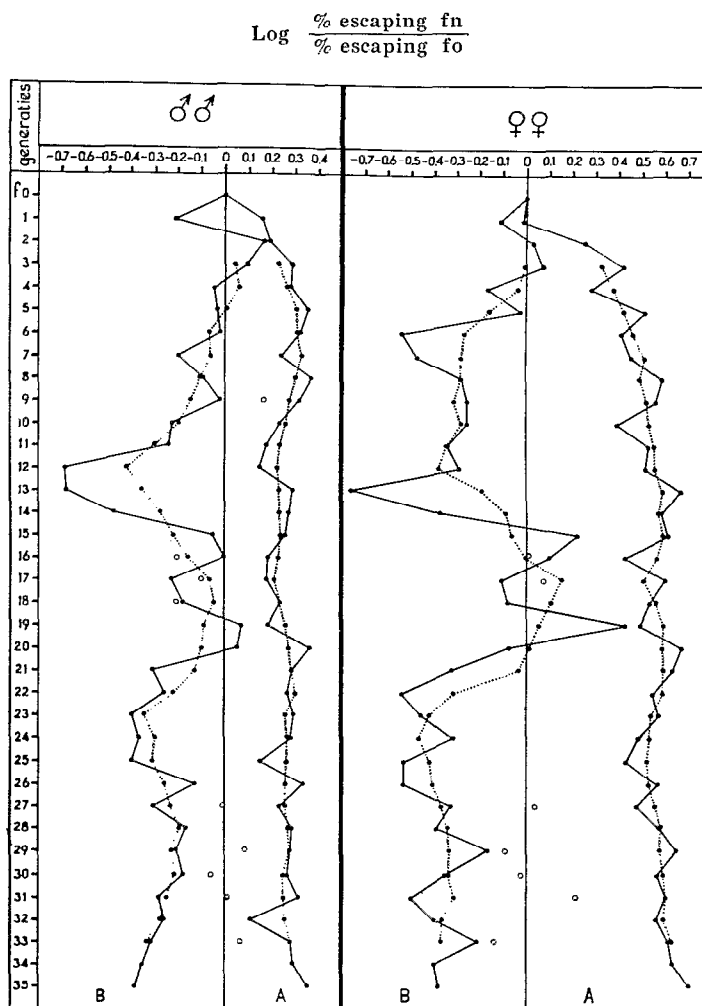
The final results of the selection have largely been obtained in the first six generations. The results of strain A are regular, whereas the results of strain B show a bias from the 12th to 23^d generation, without any known cause.

After relaxation from the selection-pressure, by breeding without selection, strain A dropped twice to the level of escaping of the original strain, but in the 32nd

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generation the strains A and B seem to have become stable with regard to escaping behaviour, as was indicated by relaxation at this moment and by the test results during the following 8 generations.



Open dots : the original strain.
 Drawn line : data of separate generations.
 Dotted line : the proceeding means over every five generations.

FIG 1. — Log $\frac{\text{percent escaping fn}}{\text{percent escaping fo}}$ of strain A and B and the original strain. Strain A, selected for escaping three times ; Strain B selected for not escaping three times.

We used strain A and B to analyse in detail the behavioural responses to contact with DDT³.

The time to first take-off, found in the WHO-irritability test was considered to indicate the irritability due to DDT. This was supported by the log-linear relationship with DDT-concentration shown in fig. 2. No differences in irritability have been

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shown between strain A and B, therefore we concluded that both strains A and B are equally irritated by DDT.

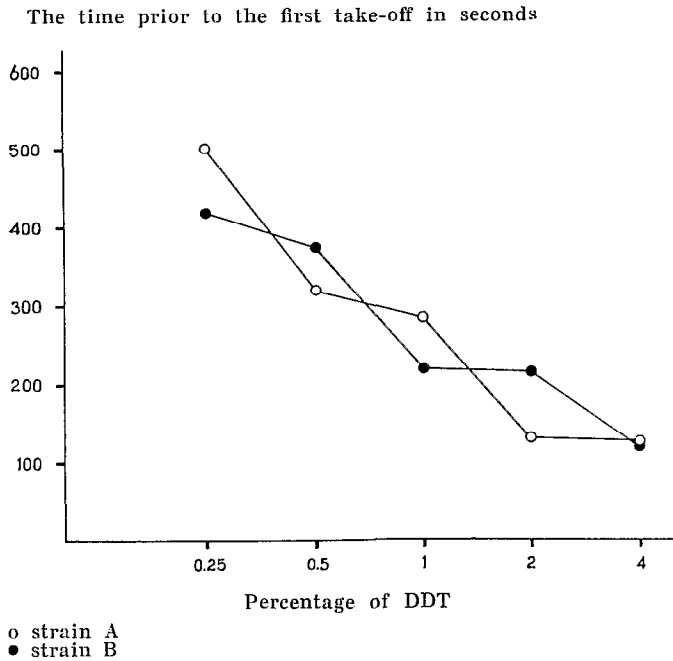


FIG. 2. — The relationship between the time prior to the first take-off and concentration of DDT in the WHO-irritability test.

Also in the WHO-irritability test we studied the flight activity (table 1). For flight activity we used two criteria :

- 1st : the number of take-off's during a testperiod of three minutes.
- 2nd : the total time flown in seconds during a testperiod of three minutes.

TABLE 1

Flight activity in the WHO-irritability test, expressed as :

1. number of take-off's in three minutes
2. total time flown in three minutes

	Strain A	Strain B
Number of take-off's	10.2	10.4
Total time flown in seconds	92.9	50.5
Number of females tested	20	20

The time flown can apparently discriminate between strain A and B, whereas the number of take-off's can definitely NOT.

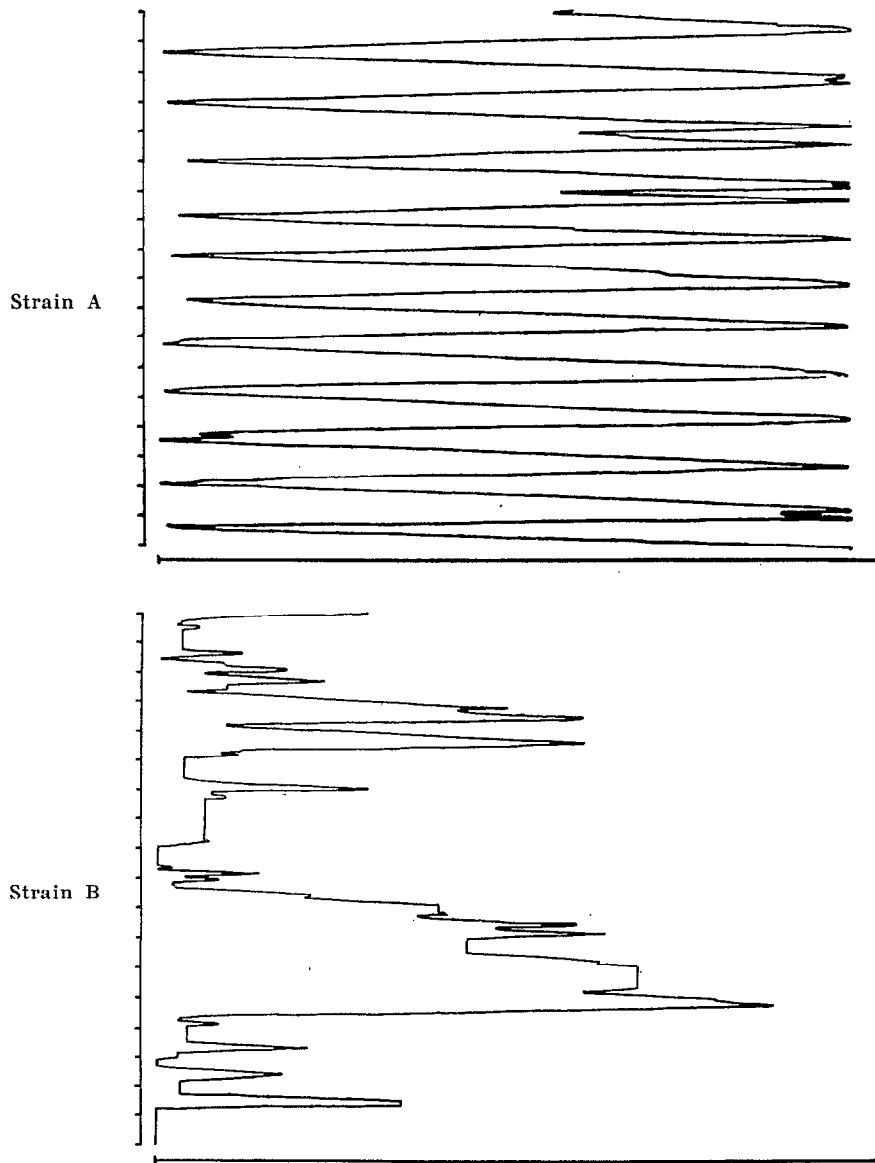
Since the total time flown is a direct criterion for flight activity and the number of take-off's an indirect one, we concluded that strain A is much more active than strain B.

Moreover it was proved that the flight patterns of both strains are quite different. We investigated these flight patterns of single mosquitoes within a long tube lined with DDT-impregnated paper. Recording was rendered possible by leaving a

narrow slit along the length of the tube uncovered. We followed by hand, with a pencil on moving paper, the position of a single mosquito. In this way we obtained a " Kinetogram " for each mosquito tested (fig. 3). These flight-patterns have been analysed for different characteristics (table 2). Whereas in strain A the percentage of flights, extending beyond half the length of the exposure-tube is high, these long flights are far less common in strain B. Moreover strain A flies faster and with less turns per unit of time than strain B.

We concluded that the flight patterns of strain A and B differ in three aspects :

1st : the average length of a separate flight.



Time is marked on the vertical scale with 5 seconds interval.

FIG. 3. — Flight patterns of single mosquitoes in a tube (46 cm long), expressed as kinetograms

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2nd : the speed of flight.

3^d : the number of turns per unit of time.

Selection has been towards a difference in escaping between strain A and B, and the results was expressed as percentage escaped mosquitoes. The need was felt however for a quantitative method of estimating the capacity to escape in single mosquitoes.

TABLE 2

Analysis of flight patterns of single mosquitoes in a tube (46 cm long).

	Strain A	Strain B
Percentage of flights extending beyond half the length of the exposure tube ...	47.9	20.3
Speed of flight in cm/second	15.5	12.2
Number of turns in 180 seconds total time flown	52.2	68.4
Number of females tested	25	25

This was realized by means of a special device, consisting of two WHO-irritability test funnels joined together. The pair of funnels, top-holes combined so as to make one escape-hole common to both sides, and their bases covered with DDT-impregnated paper, were illuminated with incident diffuse light (fig. 4). This test is referred to as the « Reflected Image ».

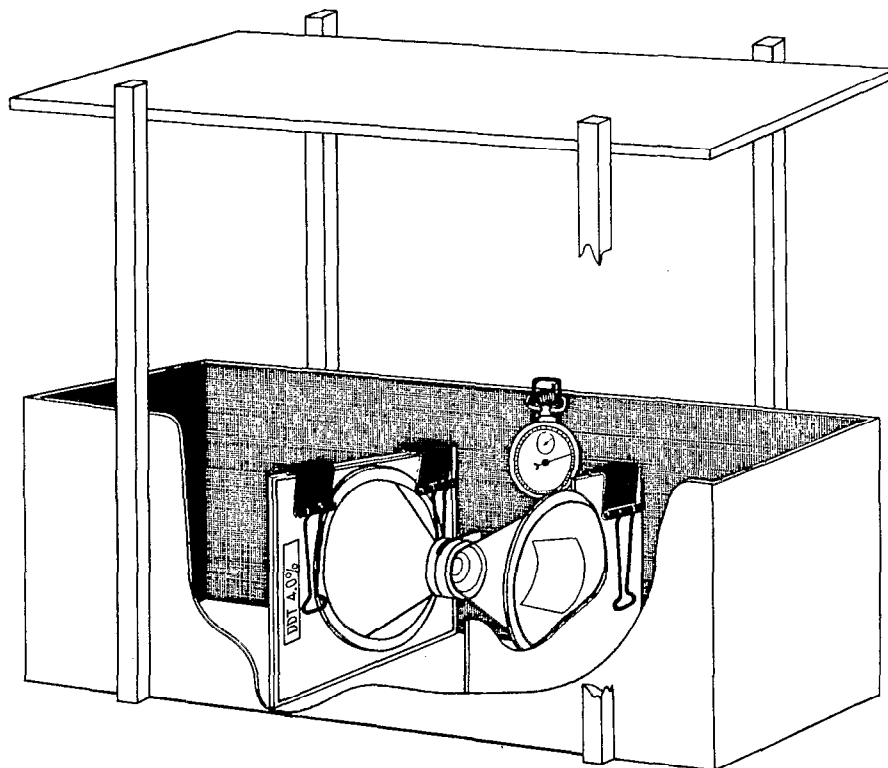


FIG. 4. — The « Reflected Image », a test apparatus, consisting of two joined WHO-funnels and a common escape-hole of variable diameter

Using the same diameter of the escape-hole, as in the selection-apparatus, mosquitoes of strain B did not escape at all, whereas mosquitoes of strain A did so repeatedly. This difference is not caused by a difference in time flown, as is demonstrated in fig. 5. Strain A and B have proved to differ not only in the number of escapes pro mosquito, but also in the number of arrivals just in front of the escape-hole. Therefore the capacity to pass through the escape-hole is better represented by :

" the number of successful escapes in relation to the number of arrivals of a mosquito just in front of the escape-hole ",

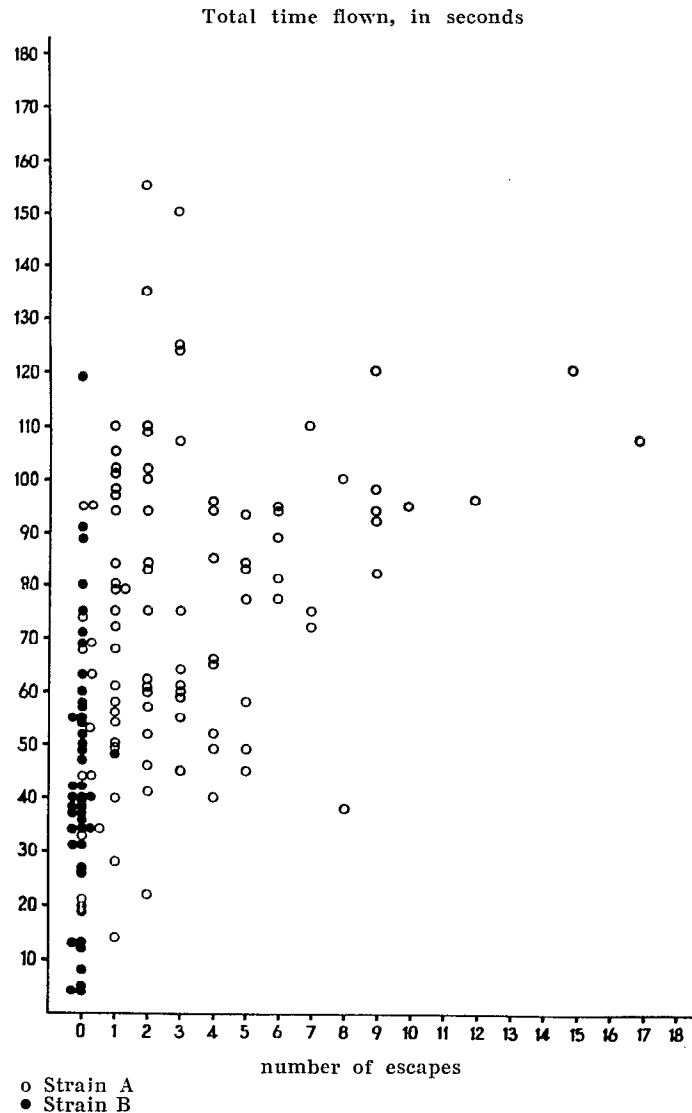


Fig. 5. — The escapes in the « Reflected Image », with 4 % DDT, diameter escape-hole : 11 mm

in other words : " the percentage of utilized arrivals ". This standard is independent of the duration of the test. This criterion has been tested for a range of diameters of the escape-hole (fig. 6). Strain A passes more easily through the escape-hole than

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strain B and the escaping of strain A is also much more influenced by the diameter of the escape-hole. We concluded that the difference in escaping is a result of two characteristics :

- 1st : Strain B does not penetrate into the narrow end of the exposure-chamber leading to the escape-hole as readily as does strain A.
- 2nd : The capacity to pass through the escape-hole is highly present in strain A and almost lacking in strain B.

It seems that this difference in capacity to pass the escape-hole is by far the most essential factor governing the difference in escaping of strain A and B.

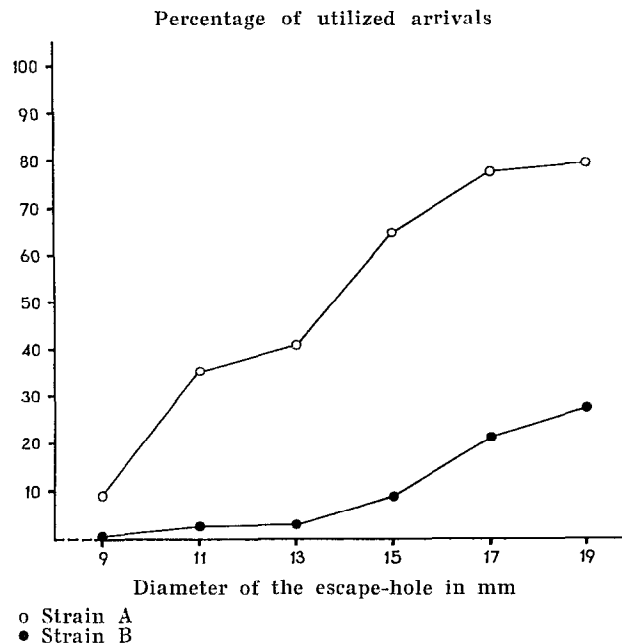


FIG. 6. — The capacity to pass the escape-hole expressed as percentage of utilized arrivals in the « Reflected Image », with 4 % DDT and different diameters of the escape-hole

The escaping percentages of strain A' and B', which are results of the selection-procedure based on only a single response to the selection-criterion, show a course, which is nearly the same as that of strain A and B represented in fig. 1, although deviation from the baseline is slightly less. We studied the flight patterns of the strains, resulting from both selection-procedures (table [3]).

It is concluded :

- 1st : Only the three times repeated selection for escaping has increased the percentage of flights extending beyond half the length of the exposure-tube, whereas the selection based on a single response did not increase this percentage.
- 2nd : Only the three times repeated selection for not escaping has diminished the number of arrivals, just in front of the escape-hole, in 180 seconds flown, whereas the selection based on a single response did not diminish this number.

We concluded, that both selection-procedures affected differently the two flight patterns, mentioned above.

Using the WHO-susceptibility test for adult mosquitoes we obtained a slight difference in 24 hours-mortality between strain A and B (fig. 7 a). This difference in mortality disappeared by topical application of DDT in acetone to glued, immobilized mosquitos (fig. 7 b). Strain A and B differ considerably in flight activity and

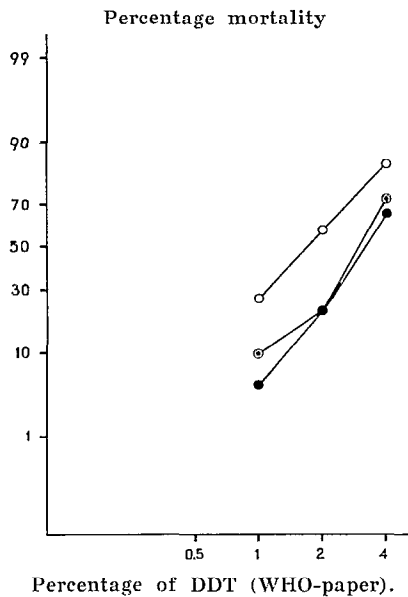
TABLE 3

Flight patterns after contact with 4% D.D.T. of two pairs of strains obtained by two different selection-procedures.

		Escaping		Original strain	Not escaping	
		Strain A selected for a three times repeated response	Strain A' selected for a single response		Strain B'	Strain B selected for a three times repeated response
« Long-tube » test.	Percentage of flights extending beyond half the length of the exposure-tube	47.9 (25)	26.3 (25)	27.9 (25)	17.8 (25)	20.3 (25)
« Reflected image » test.	Number of arrivals, just in front of the escape-hole, in 180 seconds flown	32.2 (50)	36.3 (50)	37.3 (50)	36.1 (50)	19.2 (50)

(Numbers of mosquitoes are in parentheses)

a) WHO-susceptibility test.



b) Topical application of pp-DDT on immobilized mosquitoes.

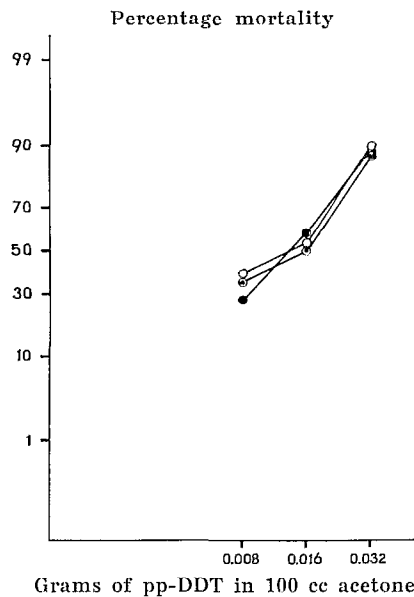


FIG. 7. — 24 Hours-mortality of the original strain (●), strain A (o), strain B (●) in tests with active mosquitoes (WHO-susceptibility test) and immobilized mosquitoes (topical application of pp-DDT to glued mosquitoes)

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because the WHO-susceptibility test for adult mosquitoes is influenced by flight activity, this difference accounts completely for the different 24 hours-mortality found with the WHO-susceptibility test.

Summarizing we can say, that strain A and B are exactly the same in their immediate reactions to DDT, *i.e.* irritability and susceptibility but that the irritability releases different behaviour-patterns in the strains tested.

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