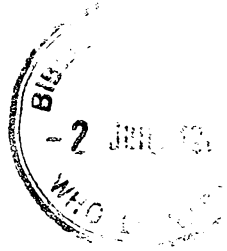


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STUDIES ON THE AGE COMPOSITION OF ANOPHELES
CULICIFACIES GILES AT DIFFERENT PHASES OF THE
DEVELOPMENT OF RESISTANCE TO DDT IN PANCHMAHALS
DISTRICT OF GUJARAT STATE, INDIA

by

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Introduction

In the world-wide malaria eradication programme based on the use of residual insecticide, it is considered that one of the most important effects of the residual spraying is to reduce the longevity of the vector to less than the extrinsic period of the plasmodium, resulting in interruption of malaria transmission. The study of the age composition of the vector population in areas which have been treated with the insecticide is therefore of great value.

The determination of the accurate age of mosquitos has engaged the interest of mosquito workers for almost half a century. Several methods have been used by a number of workers (e.g. Perry, 1912; Mer, 1932; Davidson, 1955; Gillies, 1954 and 1956 and Gillett, 1957). These and other methods, however, have some limitations in estimating the actual age of mosquitos comprising the parous group. The Russian work on this subject (Polovodova, 1949 and Detinova, 1949) as reviewed by Gillics (1958) and fully reported by Detinova (1959) showed that it is possible to determine the age of the individual mosquito with more precision. "Polovodova's method" was based on the finding that each time an egg was shed from an ovariole, a thickening or a follicular dilatation was formed. The largest number of these on any terminal pedicle thus indicated the number of times an egg-batch was deposited. Bertram & Samarawickrema (1958) utilized Polovodova's method for estimating the age of Mansonioides mosquitos. Additional work has been done by Martinez Palacios (1960) and by Hamon and his colleagues (1961).

Area, spray history and development of resistance

In the present studies Polovodova's method has been used for the recognition of the age composition of A. culicifacies Giles, a major malaria vector in Panchmahals district of Gujarat State. This district which is situated about 350 miles north of Bombay city (Fig. 1) has been under intensive residual DDT spraying for the last 11 years. It is particularly significant to note that A. culicifacies is at present DDT-resistant in the district, as well as in a number of nearby places in Gujarat and Maharashtra States where residual DDT spraying has been applied for about the same length of time. In September 1959, an increased tolerance to DDT in A. culicifacies was first detected by Rahman et al. (1959) in the village Sindbhai Metha of Panchmahals district, and in the village Motipura of the same district by Luen & Shalaby (1961). The latter authors reported the development of DDT resistance in various places of the district.

General plan and course of age-grouping studies

The original intention of these studies was to use Polovodova's method to study the effect of the spray round on longevity. The studies were extended to see what effect resistance might have on age-grouping.

The studies of the age composition of A. culicifacies were undertaken during June, July and August 1959, before any increase of tolerance to DDT was detected; during January to May 1960, when such a tolerance to DDT was apparently increasing; and during June 1960, October 1960 to April 1961 and August to September 1961, when resistance to DDT was confirmed. The significance of this study, therefore, lies in evaluating the change in the age composition of the A. culicifacies population in relation to its development of resistance to DDT.

Material and methods

Wild caught females were collected during early morning hours from various places in Panchmahals district of Gujarat State. The age-grouping studies and the susceptibility tests have been carried out on material obtained from the

same places in the district. Ovaries of 1910 specimens were dissected and examined; this figure comprises 318 females taken between June and August 1959; 809 between January and May, during June and between October and December 1960; and 783 between January and April and during August and September 1961.

Polovodova's method for age-determination was followed. Accordingly, each specimen was dissected in a drop of physiological saline on a glass microscope slide, using two very fine needles. The ovaries are dissected and examined under 10 X eye-pieces and 3 X objective of the dissecting binocular microscope. The legs and wings are removed and the mosquito held in position with the aid of one needle at the thorax, whereas the tergite and sternite of the seventh abdominal segment are bent with the second needle. Larger incisions are usually required for mosquitos with ovary stages IV and early V. This procedure exposes the whole reproductive system, which then can be pulled out easily. Difficulties encountered with surface tension are met by moving one of the ovaries towards the periphery of the saline drop. The tracheae and tracheoles of the ovary may then be removed by gently pulling them with one needle. During this process the ovarian sheath is torn carefully, thus freeing the ovarioles in the physiological saline. The ovary is then held onto the slide with one needle, while with the other needle the follicles are very gently pulled out. During the pulling process, the follicular dilatations are counted making sure that the terminal pedicle remains attached to the ovariolar tube. The terminal pedicle often breaks, preventing the detection of the total number of the follicular dilatations. It is therefore necessary to examine 10 to 15 follicles per ovary of each specimen.

Tests on the susceptibility levels were made concurrently with the age-grouping studies. From September 1959 to September 1961, intensive susceptibility tests with DDT have been carried out at monthly intervals except for a period of three months in 1960 (March, April and May) and a period of two months in 1961 (May and June). The A. culicifacies utilized for the tests were fully fed, uninjured females collected during the morning hours. The tests were performed according to WHO procedure.

Results

The results are represented in Tables I to VI and Figures 2 and 3.

It will be seen from Table 1 and Figure 2 that a total of 318 A. culicifacies females were dissected for age determination during the months of June, July and August of 1959. In addition to the nulliparous females encountered during these three months, the June dissections revealed females completing one and two gonotrophic cycles; the July dissections revealed females completing one, two and three gonotrophic cycles, while in August one female (1.13%) completing four gonotrophic cycles was found in addition to females belonging to the age-groups uniparous, biparous and triparous.

During 1960, females totalling 809 were dissected over a period of nine months (Table II and Figure 2). An average of 23.38 per cent. were nulliparous, 67.24 per cent. had one dilatation uniparous, 8.77 per cent. had two dilatations. No females completing more than three gonotrophic cycles were encountered. It should be noted that females with three dilatations were only encountered during January, March and April. It was noticed that the proportion of biparous females had declined since the month of April, while the proportion of females of the uniparous age-group had increased since the month of March. Also, a noticeable decline in the proportion nulliparous had taken place since the month of March.

During 1961, females numbering 783 were dissected over a period of six months. An average of 20.94 per cent. were nulliparous, 74.97 per cent. were uniparous and 4.09 per cent. had two dilatations biparous. No females completing more than two gonotrophic cycles were encountered. In August, out of 182 females dissected 80.22 per cent. were parous, all of them uniparous (Table III and Figure 2).

It is important to point out that until February 1960 the A. culicifacies mortalities at 4.0 per cent. DDT concentration ranged between 91.0 per cent. and 65.0 per cent. giving LC_{50} 's ranging between 1.4 per cent. and 2.5 per cent. From June 1960, to September 1960, mortalities at the 4.0 per cent. DDT concentration ranged between 45.0 per cent. and 20.0 per cent., giving LC_{50} 's greater than 4.0 per cent. (Table V).

The records of dissections during 1959, prior to the detection of and increased tolerance to DDT show that A. culicifacies were living long enough to complete four gonotrophic cycles (Tables I and IV and Figs. 2 and 3). On the other hand after DDT tolerance began to increase only 1.08 per cent. were found to complete three gonotrophic cycles and no older females were found. Out of 1123 dissected after the resistance to DDT was confirmed no females with more than two follicular dilatations were encountered and 4.82 per cent. only were found to complete two gonotrophic cycles (Table IV and Figure 3).

Discussion

The present investigation was primarily concerned with the effect of the DDT spray rounds on the age compositions of A. culicifacies in Panchmahals district. Therefore, the records of dissections obtained during 1959, are of particular importance.

It should be noted that the June dissections were made just before the first round of DDT spraying of the year, while those of July and August coincided with the first and second rounds of spraying. It was therefore to be expected that the dissections during July and August would reveal physiologically young and nulliparous females and perhaps a few which might be able to complete one gonotrophic cycle at the most. These would in all probability be the ones which were able to escape contact with DDT. Nevertheless, there appeared to be no significant difference in the proportions nulliparous to parous between the June dissections and those of July and August. On the contrary, females which completed one, two, three and four gonotrophic cycles were found after the application of the insecticide.

It would be reasonable to consider that the nulliparous A. culicifacies are those which are less than six days of age. Those with one follicular dilatation are six to nine days old, those with two dilatations are nine to 12 days old, those with three dilatations are 12 to 14 days old and those with four dilatations are 14 to 16 days old, depending upon the important factors of temperature and the relative humidity (Table VI). Evidently, therefore, A. culicifacies females found

during and after the rounds of DDT spraying have been surviving beyond the epidemiologically dangerous age and should be capable of transmitting the malaria parasite, in the absence of other factors like change in behaviour or irritability.

The investigations showed that the spray coverage in the district could be generally evaluated as satisfactory. Accordingly, it seemed likely that during that period the DDT spraying operations were possibly ineffective in reducing the longevity of the individual A. culicifacies. This was thought to be due to the possible development of physiological DDT resistance in the A. culicifacies in the district, and/or to higher irritability on contact with DDT sprayed surfaces causing them to leave the deposit prematurely, or to a behaviouristic avoidance without contact with the DDT deposits.

The disclosure of the lowered susceptibility of A. culicifacies to DDT in September 1959 at Motipura village in the district was of much interest. The LC_{50} encountered ranged between 2.3 per cent. and 2.5 per cent. DDT (Luen & Shalaby, 1961) while the previous most recent was 0.84 per cent. in 1958 at Potgaon village in Thana district of Bombay State (Patel et al., 1958). It is also important to stress that in June 1960 confirmed resistance to DDT had been revealed in the district and which prevailed thereafter (Table V).

The results obtained on age-grouping during January and February 1960, indicate that the proportions nulliparous did not show a marked variation from those encountered in June, July and August 1959. The most significant changes, however, occurred in the physiological age of the individual mosquitos among the parous group of the population. Females of the age-groups uniparous and biparous were encountered throughout the records of dissections in 1960 and 1961. It was only during the months January, March and April 1960, that relatively few females were found with three follicular dilatations. It is to be emphasized that no females out of 1197 dissected during a period of 11 months, from May 1960 to September 1961, were found with more than two follicular dilatations.

It is also important to note that the proportions of the uniparous females A. culicifacies had increased since March 1960 and showed a more or less gradual rise in the following months reaching as high as 85.0 per cent. in November 1960.

The second point of importance is the noticeable decline in the proportion of biparous females in the A. culicifacies population since April 1960. This decline has prevailed in the records of dissections in 1960 and 1961 and never exceeded 8.65 per cent. as encountered in December 1960 (Tables II and III and Figure 2).

On the whole, then, and from the data presented the obvious decline in the proportion nulliparous, with a consequent rise in the proportion parous in the A. culicifacies population demonstrated since March 1960, was mainly because of the rise in the proportion of the females which had succeeded to complete one gonotrophic cycle only rather than to a general lengthening in the physiological age of the individual mosquitos. These records therefore, seem to suggest that the A. culicifacies population has acquired a shorter life span compared to their life span in the earlier part of 1960 or during June, July and August 1959, a phenomenon that occurred since May 1960 only and prevailed thereafter.

It was thought that this marked change in the age composition of A. culicifacies population in Panchmahals district might be seasonal. Pal (1943) stated that the range for maximum longevity in A. culicifacies is when the temperature ranges between 25°C and 30°C and when the relative humidity is between 60 per cent. and 80 per cent. However, the consistency of the pattern of the age composition during the period from May 1960 until September 1961, irrespective of the weather factor (Table VI), throws doubt as to the weather being a responsible factor. Admittedly, we still know little of the factors influencing the physiological age of individual mosquitos or of the relationship between those factors and the total survival rates of the mosquito population.

It should be stressed that since resistance to DDT had developed in this species, the records of dissections showed only females which survived to complete one gonotrophic cycle, and a significantly low ratio of females which survived to complete two gonotrophic cycles; 4.82 per cent. only out of 1123 females dissected were found to have two dilatations and no older females were found since that time. The apparent association of the shortening in the physiological age of the individual A. culicifacies with the development of DDT resistance in the population is of interest. The only available information on the subject is that of Abedi & Brown (1960) and Brown (1960) who also found that the resistant genotypes in a laboratory

bred Malayan strain of Aedes aegypti have a lower survival rate than the susceptible ones in normal conditions. It should be pointed out that the development of DDT resistance in the A. culicifacies population in Panchmahals district was not accompanied or followed up, by an increase in the transmission of malaria as assessed by epidemiological methods. The examination of 3967 blood slides during pre- and post-spraying surveys in three talukas of this district during the latter half of 1960, showed no transmission of malaria taking place. This survey also revealed a decrease of the parasite reservoir compared with one year before (Luen, 1961). The examination during 1961 of 41 000 blood slides in the district has shown no positive blood slides (Patel, 1961). As a result, three questions immediately arise: (1) What is the relationship between the development of insecticide resistance in a mosquito population and its age composition? (2) Are the DDT-resistant A. culicifacies shorter lived than the susceptible ones? and (3) If so, would DDT resistance therefore, affect the vectorial capacity of A. culicifacies?

Despite the data presented in this paper with evidence which lends support to the observation that the DDT-resistant A. culicifacies are short lived, they by no means prove that the females of the older age-groups have completely disappeared from the population. Other factors or behavioural conditions, which are presently unknown, might also account for the absence of the females of the older age-groups since resistance to DDT had developed. Whatever the relationship between the development of the DDT resistance in A. culicifacies and its longevity may be, the findings presented in this paper are of interest and the problem deserves full attention and further investigations.

Summary

Age determination by Polovodova's method based on the dissection of the ovary and searching for the presence of follicular dilatations has been carried out on 1910 female A. culicifacies in Panchmahals district of Gujarat State, India.

The age-grouping was performed before and after DDT resistance was detected and confirmed in the population of A. culicifacies.

Females numbering 318 had been dissected during the period June to August 1959 prior to the detection of increased tolerance. The June dissections yielded nulliparous, uniparous and biparous females. The proportions nulliparous showed no marked variation before and after the application of the insecticide.

A total of 469 females were dissected during the period January to May 1960, during which increased DDT tolerance had been observed. A decline in the proportions nulliparous had taken place since March. A consequent rise in the proportions parous was ascribed mainly to the noticeable rise in the proportion of the age-group uniparous. The ratios of the age-group biparous had demonstrated a marked decline during April and May.

Females totalling 1123 were dissected during the periods June 1960, October 1960 to April 1961 and August to September 1961, when the LC_{50} DDT rose to greater than 4.0 per cent. and DDT resistance had been confirmed. The ratios of the biparous females remained markedly low, never exceeding 8.65 per cent. as found in December 1960. The proportions nulliparous declined considerably. A consequent rise in the proportions parous continued to be restricted to the increase in the ratio of the age-group uniparous. In November 1960, the proportion parous was 92.0 per cent., 85.0 per cent. of which were uniparous females and 7.0 per cent. were biparous.

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Last but not least, the author extends his sincere appreciation to His Highness the Maharaja of Baria. His unreserved generous assistance in extending all facilities in the area of investigations during the long period of these studies is deeply appreciated.

EDITOR'S COMMENTS

The unusual findings reported in this paper may well prove of significance in the wider sphere of entomology in malaria eradication. However, the interpretation of these figures is closely bound up with the validity of the methods used by the author for sampling the mosquito population. This sampling was restricted to habitations or mixed dwellings, and there is a possibility therefore (as pointed out by the author) that with the development of resistance to DDT on the part of A. culicifacies the older females might show an increasing avoidance of indoor resting places, and thus be excluded from the sample. It is difficult to say how far the age grouping of these samples is truly reflective of the age grouping of the population as a whole. The implications however are of sufficient interest to make it worth while to extend the sampling spectrum to include a range of outdoor resting places, and to collections on human or animal bait.

A further point is that in the interpretation of age grouping in terms of longevity one must consider not only the number of dilatations but also the duration of the gonotrophic cycle. The author tacitly assumes that the duration of the cycle has - allowing for seasonal variations - remained uniform throughout the period of observations. This assumption may well be correct, but again it would be worth dealing with this possible variable by confirming whether this is indeed the case, or whether the duration of the cycle has perhaps changed significantly in the resistant population. Before deciding whether the resistant population is "short-lived" it would be well to consider the possibility that a significant increase in the duration of the gonotrophic cycle could counterbalance the effect of a decrease in physiological age.

For these and other reasons it would appear that a comparative study of laboratory strains of the two populations would be a logical follow-up of this stimulating field study.

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TABLE I. AGE COMPOSITION OF A. CULICIFACIES IN PANCHMAHALS DISTRICT
OF GUJARAT STATE DURING JUNE, JULY AND AUGUST 1959

Date (Month)	Total dissected	Percent. per gonotrophic cycles					
		0	1	2	3	4	5 or more
June 1959	84	34.54(29)	63.10(53)	2.38(2)	-	-	-
July 1959	137	31.40(43)	56.2(77)	7.3(10)	5.1(7)	-	-
August 1959	97	36.00(35)	49.48(48)	10.30(10)	3.09(3)	1.13(1)	-
Totals/Averages	318	33.65(107)	56.00(178)	7.00(22)	3.14(10)	0.21(1)	-

Note: The figures in brackets are the numbers of females found per gonotrophic cycle.

TABLE II. AGE COMPOSITION OF A. CULICIFACIES IN PANCHMAHALS DISTRICT
OF GUJARAT STATE DURING 1960

Date (Month)	Total dissected	Percent. per gonotrophic cycles					
		0	1	2	3	4	5 or more
January 1960	73	37.00(27)	45.2(33)	15.0(11)	2.8(2)	-	-
February 1960	80	38.75(31)	48.75(39)	12.5(10)	-	-	-
March 1960	88	22.72(20)	60.23(53)	16.0(14)	1.05(1)	-	-
April 1960	154	28.00(43)	65.60(101)	5.10(8)	1.30(2)	-	-
May 1960	74	24.3(18)	67.6(50)	6.10(6)	-	-	-
June 1960	70	22.8(16)	71.5(50)	5.7(4)	-	-	-
October 1960	66	15.1(10)	81.87(54)	3.03(2)	-	-	-
November 1960	100	8.0(8)	85.0(85)	7.0(7)	-	-	-
December 1960	104	15.4(16)	75.95(79)	8.65(9)	-	-	-
Totals/Averages	809	23.38(189)	67.24(544)	8.77(71)	0.63(5)	-	-

Note: The figures in brackets are the number of females found per gonotrophic cycle.

TABLE III. AGE COMPOSITION OF A. CULICIFACIES IN PANCHMAHALS DISTRICT OF GUJARAT STATE DURING JANUARY, FEBRUARY, MARCH, APRIL, AUGUST AND SEPTEMBER 1961

Date (Month)	Total dissected	Percent. per gonotrophic cycles					
		0	1	2	3	4	5 or more
January 1961	100	21.0(21)	73.0(73)	6.0(6)	-	-	-
February 1961	104	14.5(15)	77.8(81)	7.7(8)	-	-	-
March 1961	87	17.24(15)	74.71(65)	8.05(7)	-	-	-
April 1961	94	20.21(19)	75.54(71)	4.25(4)	-	-	-
August 1961	182	19.78(36)	80.22(146)	-	-	-	-
September 1961	216	26.9(58)	70.0(151)	3.1(7)	-	-	-
Totals/Averages	783	20.94(164)	74.97(587)	4.09(32)	-	-	-

Note: The figures in brackets are the numbers of females found per gonotrophic cycle.

TABLE IV. AGE COMPOSITION OF A. CULICIFACIES BEFORE, DURING AND AFTER THE DETECTION OF THE DEVELOPMENT OF TOLERANCE/RESISTANCE TO DDT IN PANCHAMAHALS DISTRICT OF GUJARAT STATE

Susceptibility status of <u>A. culicifacies</u>	Dates of dissections	Total dissected	Percent. per gonotrophic cycle					
			0	1	2	3	4	5 or more
Before tolerance to DDT was detected	June to August 1959	318	33.65(107)	56.00(178)	7.00(22)	3.14(10)	0.21(1)	-
During increased tolerance to DDT	January to May 1961	469	29.63(139)	58.85(276)	10.44(49)	1.08(5)	-	-
During resistance to DDT	June 1960, October 1960 to April 1961 and August to September 1961	1123	19.05(214)	76.13(855)	4.82(54)	-	-	-

Note: The figures in brackets are the numbers of females found per gonotrophic cycle.

TABLE V. SUSCEPTIBILITY TREND OF A. CULICIFACIES AT MOTIPURA VILLAGE
IN PANCHMAHALS DISTRICT OF GUJARAT STATE DURING 1959, 1960 and 1961

Susceptibility status	Dates of tests	No. of tests series	Range of temperature during exposures (°C)	Range of relative humidity during exposures (%)	Percent. mortalities at DDT concentrations				Control	LC ₅₀	
					0.25%	0.5%	1%	2%			4%
Tolerant to DDT	September 1959 to February 1960	5	18 to 27.5	40 to 94	1.12 to 9.0	5.0 to 20.0	14.0 to 37.5	30.0 to 64.0	65.0 to 91.0	0.0 to 5.3	1.4% to 2.5%
Resistant to DDT	June 1960 to April 1961 and July 1961 to September 1961	14	21 to 31	34 to 93	-	-	0.0 to 15.0	2.5 to 26.2	20.0 to 45.0	0.0 to 3.0	>4.0%

Spray history of the locality

1950)	2 cycles DDT at 56 mg/sq. ft	1960)	2 cycles DDT at 112 mg/sq. ft
1951)		1961)	
1952)		1953)	
1956)	1 cycle DDT at 112 mg/sq. ft	1954)	2 cycles DDT at 112 mg/sq. ft
1957)		1955)	
		1958)	2 cycles DDT at 112 mg/sq. ft
		1959)	

TABLE VI. AVERAGE MONTHLY RECORDS OF THE TEMPERATURE AND THE RELATIVE HUMIDITY DURING THE PERIOD SEPTEMBER 1959 TO SEPTEMBER 1961

Month	Temperature		Relative Humidity	
	Max. (°C)	Min. (°C)	Max. (°C)	Min. (°C)
September 1959	30.0	25.0	99.9	81.5
October 1959	27.0	25.3	92.8	66.6
November 1959	26.0	22.6	81.0	45.9
December 1959	22.0	20.0	85.0	42.0
January 1960	21.0	18.7	69.3	39.0
February 1960	26.3	21.6	59.4	31.3
March 1960	27.5	23.5	59.6	34.3
April 1960	37.5	33.5	43.5	21.0
May 1960	35.2	32.5	56.5	31.5
June 1960	33.0	30.5	84.8	72.5
July 1960	31.7	30.0	88.4	77.4
August 1960	28.4	27.9	95.0	79.3
September 1960	29.0	26.8	87.0	69.7
October 1960	28.8	23.6	75.0	35.4
November 1960	27.2	21.5	60.4	32.1
December 1960	24.6	19.7	69.3	45.6
January 1961	24.0	18.2	60.8	37.5
February 1961	24.2	18.2	62.0	30.5
March 1961	29.4	20.7	64.4	23.0
April 1961	33.0	25.3	50.0	25.3
May 1961	37.7	30.4	78.1	30.8
June 1961	35.8	28.8	83.7	42.3
July 1961	29.5	26.0	89.7	76.6
August 1961	28.6	26.3	91.7	79.4
September 1961	27.5	24.3	83.4	84.3

FIG. 1
MAP OF INDIA, WITH THE SHADED PART OF GUJARAT STATE INDICATING
THE DISTRICT OF PANCHMAHALS



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FIG. 2
 AGE-GROUPING CARRIED OUT DURING 1959, 1960 AND 1961 OF *A. GULCIFICACIES* IN RELATION
 TO ITS DEVELOPMENT OF TOLERANCE/RESISTANCE TO DDT IN PANCHMAHALS DISTRICT

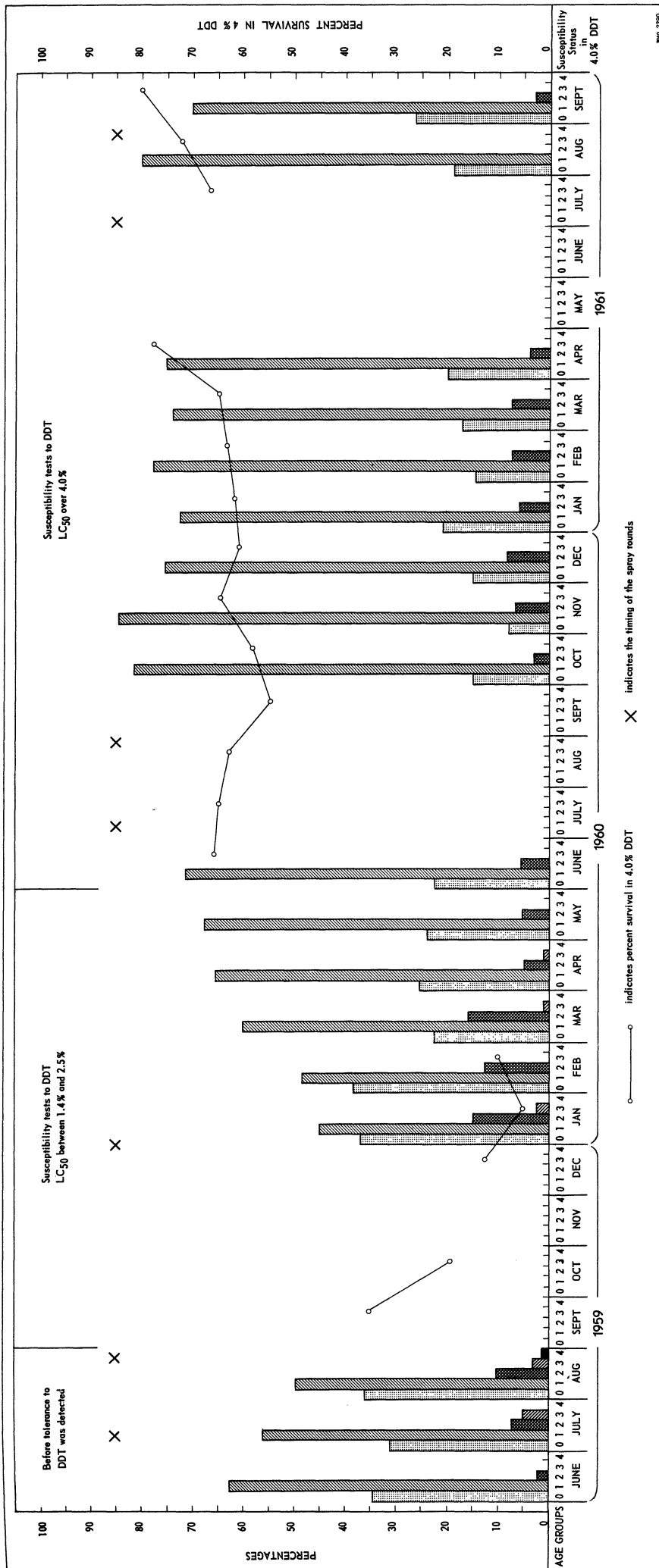
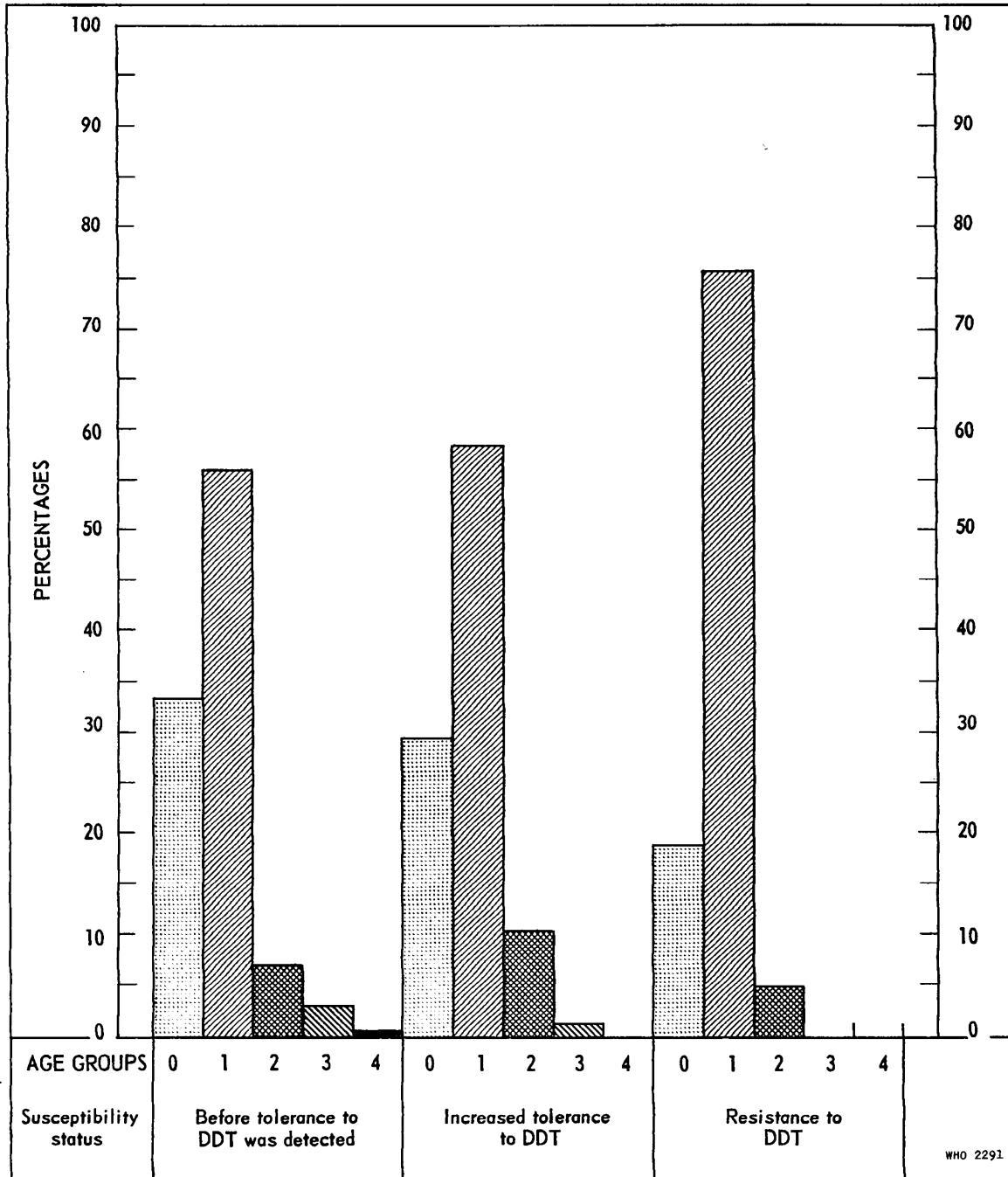


FIGURE 3

AGE COMPOSITION OF *A. CULICIFACIES* IN PANCHMAHALS DISTRICT BEFORE, DURING AND AFTER THE DETECTION OF TOLERANCE/RESISTANCE TO DDT



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