WORLD HEALTH
ORGANIZATION

ORGANISATION MONDIALE
DE LA SANTÉ

WHO/Mal/263
WHO/Insecticides/109

11 May 1960

STUDIES ON INSECTICIDE-RESISTANT ANOPHELINES

VI. SEX OF LARVAE AND CHROMOSOME ARRANGEMENT IN A. ATROPARVUS

by

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In two previous studies of this series, namely, Parts 1 and 2 (Mosna et al., 1958, 1959) the percentage of heterozygous inversions in the left arm of the third chromosome was determined in several DDT-resistant and a dieldrin-selected strain of A. atroparvus. A greatly increased percentage of heterozygous inversions was found in strains selected by larval pressure (R/dieldrin - 77.4%, RL - 55.3%, RLAF - 54.0%), while in a highly DDT-resistant strain developed by adult exposure, only a low percentage of heterozygotes (RAFM - 24.4%), comparable to that of normal and semi-normal reference strains (Sens. Roma - 18.4%, Sens. Hamb. - 12.5%, Romania - 14.2%) was found.

It became, moreover, evident that the increase in frequency of heterozygous inversions in strains selected by larval selection could not be related to the degree of resistance in the larval state (Mosna et al., 1959). To sum up, it could be concluded that only larval selection, irrespective of degree of resistance reached, enhanced the percentage of heterozygous inversions in A. atroparvus.

It was therefore deemed quite probable that larval selection with DDT or dieldrin brought about, apart from changes in susceptibility, also some other modifications in the treated strains. Indeed, the prevalence of heterozygous inversions might be a symptom of these changes. It was through this reasoning that we were led to investigate chromosomal arrangements in male and female larvae.

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[1] This is the sixth and last paper of the series of communications indicated in the list of references by an asterisk. The investigation was financially assisted by the World Health Organization.

The strains R/dieldrin, RL, RAFM and Sens. Roma have been described in detail in Parts 1 and 2 of this series. The breeding of the various strains of anophelines developed by Dr E. Mosna and which have been investigated through studies described in the first five parts of this series, was reduced from the fortieth generation onwards to a maintenance level (i.e. a few bowls per strain). Consequently also the selection doses in strains submitted to larval pressure were drastically reduced; thus, R/dieldrin in the maintenance state was selected with 0.0005 p.p.m. in the first larval stage and 0.002 p.p.m. in the third larval stage (previous doses 0.004-0.005 and 0.05 p.p.m. dieldrin, respectively) and RL with 0.0012-0.004 p.p.m. DDT in first and 0.2-0.3 p.p.m. in third larval stage (instead of 0.01-0.02 and 1.25 p.p.m. previously).

The present work had to be done on biological material derived from the maintenance culture (44th to 47th generation). The larvae examined were taken from untreated bowls.

1. Determination of the Sex of Larvae and Cytological Examination

(a) Determination of sex in the intact larva. There was first sought a method by which larvae could be sexed with 100% accuracy and the following approaches were tried:

Colour. Jones (1957), working with a comparatively small number of larvae, found that in A. quadrimaculatus 92% to 100% of heavily pigmented larvae ("black") developed into female adults, from 70-100% of the least pigmented larvae ("white") into males and the intermediate group into either sex. We first checked the applicability of these findings to our laboratory culture of A. atroparvus (the colour of the larvae, as it is well known, being influenced by the colour of the background and the type of food, see e.g. Achundow, 1928; Corradetti, 1930; 1934). Very small samples divided by colour and sex were used, and verified from the adult hatchings (Table 1).

TABLE 1. PRELIMINARY DATA ON COLOUR AND SEX IN LARVAE OF A. ATROPARVUS

Strain	Colour of larvae	No. used	Adults	
			Numbers	Per cent.
RAFM	dark	30	29♀♀ + 1♂	96.7% ♀♀
"	light	23	6♀♀ + 17♂♂	73.9% ♂♂
"	intermediate	41	18♀♀ + 23♂♂	43.9% ♀♀ + 56.1% ♂♂
R/dieldrin	dark	46	40♀♀ + 6♂♂	87.0% ♀♀

This was in agreement with Jones' findings in *A. quadrimaculatus*. Since, however, a 100% distinction was being sought, we looked for other methods (see below). At any rate we followed up during this study the colour of larvae, the sex of which was determined with 100% accuracy by the methods described below. Out of 471 larvae we found the following (Table 2):

TABLE 2. COLOUR AND SEX OF A. ATROPARVUS LARVAE

Colour of larvae	Sex of larvae	
	Numbers	Per cent.
dark	154 ♀♀ + 27 ♂♂	85.1% ♀♀ + 14.9% ♂♂
intermediate	78 ♀♀ + 74 ♂♂	51.3% ♀♀ + 48.7% ♂♂
light	41 ♀♀ + 97 ♂♂	29.7% ♀♀ + 70.3% ♂♂

This was again in agreement with Jones' findings.

Imaginal antennal discs. Live fourth stage larvae of *A. quadrimaculatus* could be sexed with a 100% accuracy by examination of antennal discs. The method was best adapted to freshly moulted fourth stage larvae, while second and third stage larvae could not be sexed due to the difficulty of seeing the discs at these stages (Jones, 1957). We were unable to apply successfully this method in third and fourth stage larvae, though we did not use freshly moultad fourth instars, i.e. larvae with unpigmented head-capsules).

Gonads. The gonads in larvae of A. maculipennis have been studied by Imms (1908). The male gonads, situated in the sixth abdominal section, are short and somewhat globular, while the female gonads, occupying both the fifth and sixth abdominal segments, are relatively longer and fusiform. In both sexes the genital rudiments are invested exteriorly by a tunic. According to Frizzi (1947) the two sexes are distinguished easily under a binocular microscope. In the males, the testes, which are situated dorsally in the fourth but last abdominal segment, have an ovoidal form and their tunic has a pale yellow colour. The testes can thus be seen well through the chitin (Fig. 1A). We have used this method for third and fourth stage larvae and have found it to be most convenient. It should be mentioned, however, that our larvae were reared in lightly coloured earthenware bowls and under good conditions of light and were therefore never heavily pigmented. Larvae are best examined dorsally or laterally and males are easily recognized.

The determination of sex by examination of gonads was later confirmed independently by cytological examination (X-chromosome, see below), done by a second person.

(b) Cytological examination. The larvae were then dissected and the salivary glands were teased out for cytological examination following the technique outlined by Frizzi (1953). The particulars examined were (1) X-chromosome for independent determination of sex. The sex chromosome in the salivary gland of females has a better defined linear structure than that in male larvae, while in the other four chromosomes the structure is of equal compactness and clearness in both sexes. In the homozygous X-chromosome of female larvae the structure appears as compact as in the autosomes, while in the homozygous male sex chromosome the structure appears relaxed and the lines are much finer and diffuse. In the female the same structures are present but are always much clearer (Frizzi, 1947; 1947a; 1947b). These differences are easily distinguished after some experience (Fig. 1B, 1C), but they are not always as obvious as in the diagram. Also examined was (2) the left arm of the third chromosome (see Parts 1 and 2 of this series).

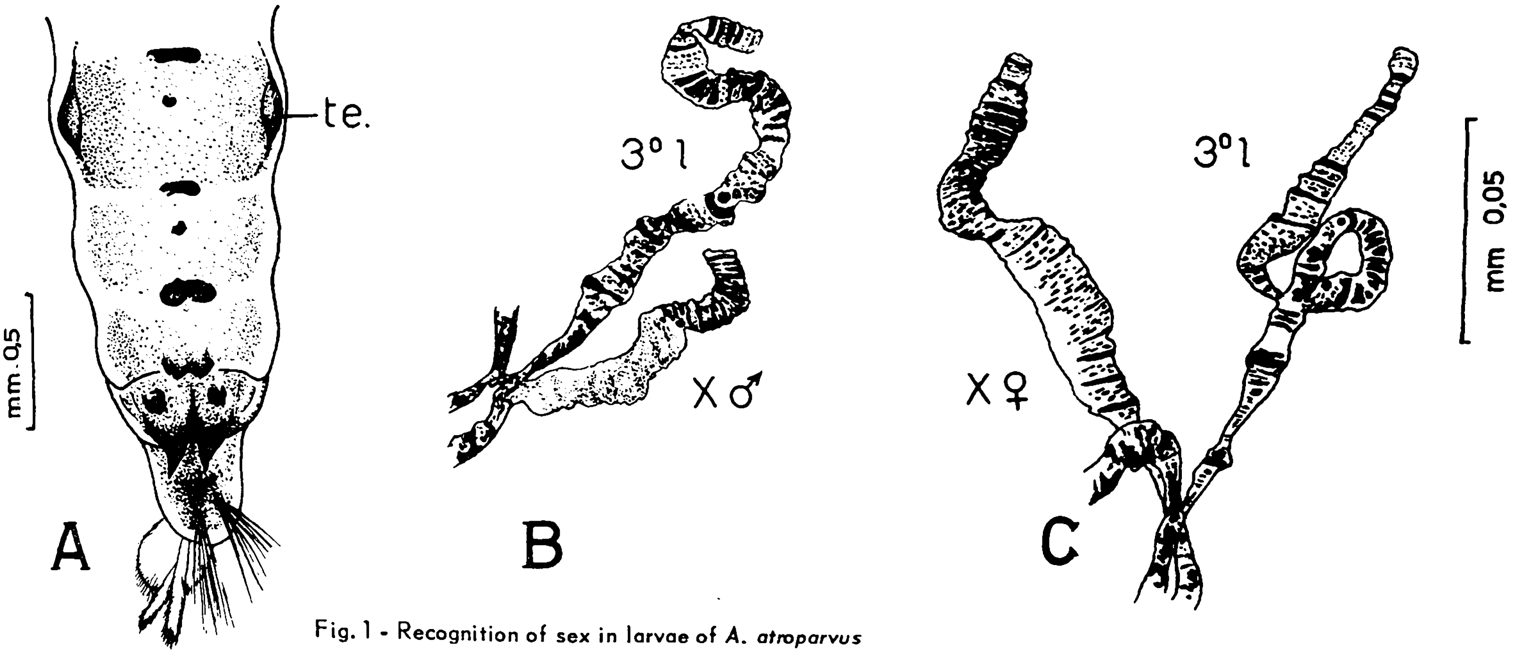

Fig. 1 - Recognition of sex in larvae of *A. atroparvus*

A - testicles (te) in the male larva

B - X-chromosome from salivary glands of male larva

C - X-chromosome from salivary glands of female larva

In Figures B and C (free-hand drawings) there is also reproduced the third arm of the left chromosome (3° 1) which is normal homozygous in B and heterozygous in C.

Figure 1 - Reconnaissance du sexe chez les larves d' *A. atroparvus*

A - testicules (te) de la larve mâle

B - chromosome X des glandes salivaires de la larve mâle

C - chromosome X des glandes salivaires de la larve femelle

Dans les figures B et C (dessin à main levée) on a également reproduit le troisième bras du chrosome gauche (3° 1), qui est homozygote normal en B et hétérozygote en C.

(c) Results of cytological examinations of sexed larvae

Strain R/dieldrin. As a first step, 183 unsexed larvae of this strain were examined only as regards the arrangement of the left arm of the third chromosome.

Of these 65 (35.5%) were heterozygotes
111 (60.7%) were normal homozygotes
7 (3.8%) were inverted homozygotes

The percentage of heterozygotes found in the strain R/dieldrin of the maintenance stock, to which there were administered only very low selection doses (see above), was much lower than that of the heavily selected 28th generation (77.4%). Even if the relaxation of selection pressure is not complete, there is thus a strong lowering of percentage of heterozygotes. This seems to be the case also on complete relaxation of dieldrin pressure in A. gambiae which through larval selection had reached a tolerance level of seven times (Jackson, 1958). On the other hand, the partial relaxation of pressure has not led so far in R/dieldrin to a drop in resistance. The larval LC_{50}, determined again in enamelled metal basins (cf. details of method in Part 1), has remained constant at 0.045 p.p.m. (last determination in generation 48). Here there is again evidence that an increase in the percentage of heterozygotes is not bound to degree of resistance, but is rather a byproduct of larval selection pressure.

Of 155 larvae which were examined both as to sex and chromosome arrangement, the following was found:

Of 155 larvae examined 54 (34.8%) were heterozygotes
95 (61.3%) were normal homozygotes
6 (3.9%) were inverted homozygotes

Of the 155 larvae 65 (41.9%) were females
90 (58.1%) were males

Of the 65 females 50 (76.9%) were heterozygotes
13 (20.0%) were normal homozygotes
2 (3.1%) were inverted homozygotes

Of the 90 males 82 (92.1%) were normal homozygotes
4 (4.45%) were heterozygotes
4 (4.45%) were inverted homozygotes

Of the 54 heterozygotes	50 (92.6%) were females
	4 (7.4%) were males
Of the 95 normal homozygotes	82 (86.3%) were males
	13 (13.7%) were females

It is thus noted that in R/dieldrin, which was selected through dieldrin larval pressure, the majority of the females were heterozygotes, while nearly all the males were normal homozygotes.

Strain RL. Also in this strain the resistance level, notwithstanding partially relaxed selection pressure, remained practically constant (1.0 p.p.m. DDT using the method of Wharton, 1956, see Part 2 of the series). Nevertheless, relaxation of pressure brought about also here some drop in percentage of heterozygotes.

Of 120 larvae examined	48 (40.0%) were heterozygotes
	64 (53.3%) were normal homozygotes
	8 (6.7%) were inverted homozygotes
Of the 120 larvae	66 (55.0%) were females
	54 (45%) were males
Of the 66 females	46 (69.7%) were heterozygotes
	12 (18.2%) were normal homozygotes
	8 (12.1%) were inverted homozygotes
Of the 54 males	52 (96.3%) were normal homozygotes
	2 (3.7%) were heterozygotes
Of the 48 heterozygotes	46 (95.8%) were females
	2 (4.2%) were males
Of the 64 normal homozygotes	52 (81.25%) were males
	12 (18.75%) were females

Also in this strain selected with DDT in the larval stage, a greater proportion of the females were heterozygotes, while again nearly all the males were normal homozygotes.

Strain RAFM. The chromosomal arrangement of this strain, according to sexes, was of high interest, since it was a very resistant strain (in the adult stage) developed through exposure of adults to DDT and containing no enhanced percentage of heterozygotes. It was expected that its females would be in part heterozygotes and in part normal homozygotes, while its males would be probably normal homozygotes only. This proved indeed to be the case.

Of 100 larvae examined	23 (23%)	were heterozygotes
	76 (76%)	were normal homozygotes
	1 (1%)	were inverted homozygotes
Of the 100 larvae	67 (67%)	were females
	33 (33%)	were males
Of the 67 females	22 (32.8%)	were heterozygotes
	44 (65.7%)	were normal homozygotes
	1 (1.5%)	were inverted homozygotes
Of the 33 males	32 (97.0%)	were normal homozygotes
	1 (3.0%)	were heterozygotes
Of the 23 heterozygotes	22 (95.7%)	were females
	1 (4.3%)	were males
Of the 76 normal homozygotes	32 (42.1%)	were males
	44 (57.9%)	were females

Strain Sens. Roma. This strain, which was the parent and normal reference strain, was expected to follow the same pattern as RAFM.

Of 96 larvae examined	15 (15.6%)	were heterozygotes
	77 (80.2%)	were normal homozygotes
	4 (4.2%)	were inverted homozygotes
Of the 96 larvae	75 (78.1%)	were females
	21 (21.9%)	were males
Of the 75 females	15 (20.0%)	were heterozygotes
	56 (74.7%)	were normal homozygotes
	4 (5.3%)	were inverted homozygotes

Of the 21 males	21 (100%) were normal homozygotes
Of the 15 heterozygotes	15 (100%) were females
Of the 77 normal homozygotes	21 (27.3%) were males
	56 (72.7%) were females

The results of the cytological examination of sexed larvae were evaluated statistically. The X^2 and P values, when frequency of the heterozygous and the normal homozygous chromosomal arrangements are compared in the two sexes in different strains, are presented in Table 3, and for chromosomal arrangement in the females of different strains (RL and R/dieldrin against Sens. Roma and RAFM, respectively) in Table 4.

TABLE 3. FREQUENCY OF HETEROZYGOUS AND NORMAL HOMOZYGOUS CHROMOSOMAL ARRANGEMENTS IN FEMALE AND MALE LARVAE OF DIFFERENT STRAINS OF A. ATROPARVUS

Strain	Sex of larvae	Chromosomal arrangement				X^2	P
		Heterozygous		Normal homozygous			
		No.	%	No.	%		
R/dieldrin	♀	50	76.9%	13	20.0%	87.8	< 0.001
	♂	4	4.45%	82	92.1%		
RL	♀	46	69.7%	12	18.2%	65.2	< 0.001
	♂	2	3.7%	52	96.3%		
RAFM	♀	22	32.8%	44	65.7%	11.3	< 0.001
	♂	1	3.0%	32	97.0%		
Sens. Roma	♀	15	20.0%	56	74.7%	5.30	< 0.05
	♂	0	0%	21	100%		

TABLE 4. COMPARISON BETWEEN DIFFERENT STRAINS OF HETEROZYGOUS AND NORMAL HOMOZYGOUS CHROMOSOMAL ARRANGEMENTS IN FEMALE LARVAE OF *A. ATROPARVUS*

Strains compared	Chromosomal arrangement				X^2	P
	Heterozygous		Normal homozygous			
	No.	%	No.	%		
RL	46	69.7%	12	18.2%	41.6	<0.001
Sens. Roma	15	20.0%	56	74.7%		
RL	46	69.7%	12	18.2%	25.9	<0.001
RAFM	22	32.8%	44	65.7%		
R/dieldrin	50	76.9%	13	20.0%	45.3	<0.001
Sens. Roma	15	20.0%	56	74.7%		
R/dieldrin	50	76.9%	13	20.0%	27.6	<0.001
RAFM	22	32.8%	44	65.7%		

2. Chromosomes in Adults of A. atroparvus

The next investigation was based on the examination of chromosomes in the adults of our strains following the procedure described by Mariani & Bruno-Smiraglia (1957) for adults of *A. atroparvus*. Preparations were made from the nurse cells of the ovarioles or the Malpighian tubes in females and from Malpighian tubes in males. Numerous attempts were made with blood-fed females of different age, with mosquitos of both sexes fed on sucrose and with females kept on sucrose and then for several days on water. In all our preparations the chromosomes were not legible. This might have been due to the adults not having been on a special feeding schedule required for Mariani's and Bruno-Smiraglia's method.

It would have been also of great interest to compare the respective LC_{50}'s for DDT and dieldrin of male and female adults of strains RL and R/dieldrin with those of RAFM and Sens. Roma. Up to 69.7% and 76.9% of the females in strains RL and R/dieldrin had the heterozygous arrangement and nearly all the males in these strains were normal homozygotes, while in RAFM only 32.8% and in Sens. Roma only 20% of the females were heterozygous. If the heterozygous arrangement is related to resistance, one should expect the ratio LC_{50} - adult ♀♀ : LC_{50} - adult ♂♂ to be considerably greater in RL and R/dieldrin than in RAFM and Sens. Roma. Unfortunately, the data on tests conducted with male adults during the 40 generations of development of these strains by Mosna, Palmieri et al. (1959) were too few.

It goes without saying that on determining the percentage of heterozygous inversions in strains of A. atroparvus, especially in strains selected in the larval stage (especially if preparations from both larvae and adults are considered) (D'Alessandro, Frizzi & Mariani, 1958; D'Alessandro & Mariani, 1958), the sex of the specimens examined must be taken into account.

3. Influence of Selection on Sex Ratio and the Respective Susceptibility of Male and Female Larvae of Resistant Strains

Although we were unable to pursue these problems experimentally, some supporting data from earlier work and from the literature are discussed here.

(a) Sex ratios in adults of resistant strains selected in different modes. Some incomplete data on female/male ratio in adults of our strains during the earlier generations of selection could be assembled from the records of the breedings kindly put at our disposal by Dr Mosna.

The sex ratio of A. atroparvus in captivity (strain Sens. Roma) has been followed up for two years (1935-1936) at the Malariatherapy Centre of the Istituto Superiore di Sanità, and the percentage of females ranged from 44 to 48% (E. Mosna, personal communication).

Shidrawi (1957) noted that in Aedes aegypti the total number of males exceeded that of the females, whereas Qutubuddin (1953) in a very extensive study found that the ratio was equal in C. fatigans. Qutubuddin also reviewed and critically discussed numerous earlier papers on sex ratio of mosquitos in captivity. The male/female ratio in adult populations of mosquitos strongly influences the propensity of females for a blood meal and the mating activity in A. quadrimaculatus (Terzian & Stahler, 1949, 1954).

Only few recent data are available on the sex ratio in the Sens. Roma strain. The RAFM strain (selected with DDT in both sexes of the adult state), followed up through more than 20 generations (Table 4) had less than 50% females. The same was found to be true for two generations of a strain RAF (selection with DDT of adult females only), which was discarded after the second generation (Table 3).

Continuous selection pressure in the larval state seems to favour a slight preponderance of females in the adult state. Records on 20 generations of RLAF (Table 7), 15 generations of RL (Table 8) and the scanty data available for R/dieldrin (Table 5) seem to support such a statement. The strains in which there were available records on 15 generations or more (RAFM on one hand and RLAF and RL on the other hand) were compared and the data (sums of females and males) were evaluated statistically in Table 9.

TABLE 5. SEX RATIO IN VARIOUS STRAINS OF A. ATROPARVUS

Strain	Generation	Date	Pupae	Adults			Percentage of females
				♂♂	♀♀	Total	
Sens. Roma		15/3/56 - 17/4/56	438	236	202	438	46.11%
		10/4/56 - 10/5/56		1 282	1 070	2 352	45.49%
RAF	F_1	22/4/56 - 10/5/56	1 744	946	719	1 665	43.18%
	F_2	16/5/56 - 12/6/56	2 929	1 345	1 233	2 578	47.80%
R/dieldrin	F_5	10/5/56 - 21/6/56	728	265	286	551	51.90%
	F_6	28/6/56 - 13/7/56	551	155	211	366	57.65%

TABLE 6. SEX RATIO IN THE RAFM STRAIN OF A. ATROPARVUS

Generation	Beginning and end of generation	Pupae	Adults			Percentage of females
			♂♂	♀♀	Total	
F_1	18/3/56-9.4.56	3 539	1 816	1 521	3 337	45.48%
F_2	11/4/56-14/5/56	4 647	2 137	1 932	4 069	47.48%
F_3	Records incomplete					
F_4	" "					
F_5	3/6/56-13/7/56		3 096	2 709	5 805	46.66%
F_6	9/7/56-8/8/56	6 152	2 992	2 343	5 335	43.91%
F_7	1/8/56-1/9/56	9 242	4 215	3 367	7 582	44.40%
F_8	26/8/56-29/9/56	3 374	1 736	1 606	3 342	48.05%
F_9	17/9/56-23/10/56	7 907	4 117	3 500	7 617	45.94%
F_{10}	9/10/56-10/11/56	7 013	3 297	2 942	6 239	47.15%
F_{11}	2/11/56-30/11/56	4 081	1 836	1 745	3 581	48.72%
F_{12}	25/11/56-19/12/56	5 244	2 551	2 295	4 846	47.35%
F_{13}	17/12/56-12/1/57	4 053	1 745	1 923	3 668	52.48%
F_{14}	8/1/57-7/2/57	6 029	2 731	2 666	5 597	47.63%
F_{15}	2/2/57-6/3/57	6 892	2 929	2 624	5 553	47.25%
F_{16}	25/2/57-1/4/57	9 715	4 572	4 012	8 584	46.73%
F_{17}	26/3/57-21/4/57	6 254	2 990	2 842	5 832	48.73%
F_{18}	19/4/57-14/5/57	4 761	1 924	1 704	3 628	46.96%
F_{19}	10/5/57-1/6/57	3 911	1 911	1 568	3 479	45.07%
F_{20}	29/5/57-24/6/57	4 276	1 991	1 563	3 554	43.97%
F_{21}	20/6/57-18/7/57	5 881	2 730	2 504	5 234	47.84%
F_{22}	10/7/57-2/8/57	5 175	2 880	1 829	4 709	38.84%
F_{23}	27/7/57-24/8/57		2 635	2 176	4 811	45.22%
F_{24}	19/8/57-15/9/57	5 839	3 037	2 413	5 450	44.27%
F_{25}	14/9/57-14/10/57	7 075	3 644	2 831	6 475	43.72%
Total			63 512	54 615	118 127	46.23%

TABLE 7. SEX RATIO IN THE RLAF STRAIN OF A. ATROPARVUS

Generation	Beginning and end of generation	Pupae	Adults			Percentage of females
			♂♂	♀♀	Total	
F_7	13/3/56-27/4/56	1 569	755	771	1 526	50.52%
F_8	8/4/56-27/5/56	1 560	745	805	1 550	51.94%
F_9	8/5/56-29/6/56	1 627	639	790	1 429	55.28%
F_{10}	5/6/56-13/7/56	1 423	560	708	1 268	56.19%
F_{11}	4/7/56-24/8/56	2 890	1 295	1 310	2 605	50.29%
F_{12}	14/8/56-26/9/56	2 537	1 136	1 147	2 283	50.24%
F_{13}	9/9/56-20/10/56	3 595	1 655	1 446	3 101	46.63%
F_{14}	30/9/56-10/11/56	4 226	1 734	2 001	3 735	53.57%
F_{15}	25/10/56-27/11/56	2 514	1 039	1 259	2 298	54.74%
F_{16}	18/11/56-27/12/56	2 486	1 020	1 295	2 315	51.61%
F_{17}	16/12/56-23/1/57	2 573	945	1 099	2 044	53.76%
F_{18}	16/1/57-5/3/57	2 900	1 167	1 240	2 407	51.51%
F_{19}	14/2/57-8/4/57	4 166	1 748	1 638	3 386	48.38%
F_{20}	16/3/57-25/4/57	2 922	1 360	1 599	2 959	54.03%
F_{21}	14/4/57-20/5/57	2 318	867	1 048	1 915	54.77%
F_{22}	15/5/57-12/6/57	1 715	463	616	1 079	57.08%
F_{23}	7/6/57-7/7/57	2 487	941	867	1 808	47.95%
F_{24}	2/7/57-19/7/57	1 583	391	425	816	52.08%
F_{25}	Records incomplete					
F_{26}	27/8/57-30/9/58	1 990	577	613	1 190	51.57%
F_{27}	21/9/57-14/10/57	1 693	584	595	1 179	50.46%
Total			19 621	21 272	40 893	52.01%

TABLE 8. SEX RATIO IN THE RL STRAIN OF A. ATROPARVUS

Generation	Beginning and end of generation	Pupae	Adults			Percentage of females
			♂♂	♀♀	Total	
F_7	18/3/56-17/4/56	664	311	317	628	50.31%
F_8	22/4/56-13/5/56	358	123	143	266	53.70%
F_9	18/5/56-15/6/56	640	148	314	462	67.09%
F_{10}	28/6/56-13/7/56	665	273	309	582	53.09%
F_{11}	Records incomplete					
F_{12}	22/8/56-19/9/56	1 000	516	460	976	47.13%
F_{13}	24/9/56-13/10/56	684	284	277	561	49.37%
F_{14}	18/10/56-5/11/56	1 451	577	661	1 238	53.35%
F_{15}	4/11/56-25/11/56	1 292	517	678	1 195	56.73%
F_{16}	5/12/56-22/12/56	965	248	391	639	61.18%
F_{17}	17/12/56-25/1/57	1 342	381	459	840	54.64%
F_{18}	19.1.57-5/3/57	2 665	1 008	1 253	2 261	55.41%
F_{19}	13/2/57-10/4/57	2 976	1 284	1 307	2 591	50.44%
F_{20}	16/3/57-23/4/57	3 431	1 577	1 601	3 178	50.37%
F_{21}	14/4/57-14/5/57	1 667	760	832	1 592	52.26%
F_{22}	9/5/57-4/6/57	1 062	291	343	634	54.10%
Total			8 298	9 345	17 643	52.96%

TABLE 9. SEX RATIO IN SELECTED STRAINS OF A. ATROPARVUS

Strain	Sex				χ^2	P
	♂♂		♀♀			
	No.	%	No.	%		
RAFM	63 512	53.77%	54 615	46.23%	470.4	<0.001
RLAF	19 621	47.99%	21 272	52.01%		
RAFM	63 512	53.77%	54 615	46.23%	278.9	<0.001
RL	8 298	47.04%	9 345	52.96%		

Whether this slight shift in sex ratio is due to the toxic effect of the insecticides on the larvae cannot be said at present (see also below, in the section dealing with the relative susceptibility of male and female larvae to insecticides). The shift might be due also to an indirect effect of DDT, such as its influence on the microflora and microfauna in the breeding bowls. Laird (1958) has studied attentively the modifications caused by DDT in infusions in which A. atroparvus was raised. He noted that species representation and the relative order of appearance of microscopic animals and plants was the same in larval media with and without the insecticide, but that the rate of succession was more rapid in DDT-treated infusions. The DDT-induced initial larval mortality brought about a rapid acceleration of infusion development. Since the food supply was therefore better in the DDT-treated medium, these differences strongly influenced adult viability from treated and control cultures. That indeed larval food conditions may modify the sex ratio of the adults has been shown by Herms (1928) in Lucilia sericata. Underfed larval populations always yielded a preponderance of males, while exceptionally well-fed populations yielded a preponderance of females. Herms was of the opinion that the larval females required more nourishment than males for their development and therefore perished in greater numbers when subjected to a starvation diet. Interestingly enough there was some indication that in the mosquito Theobaldia incidens the tendency was reversed.

As regards dieldrin applied in the larval stage, there is the protracted toxic effect through further stages to be taken into account. A delayed mortality due to dieldrin, setting in before, during and after pupation and at or after emergence of the imagos, has been described for Aedes aegypti by Shidrawi (1957) and for anophelines by various authors (cf. e.g. Kuhlow, 1957; Mosna et al., 1958). Jones (1959) found that even a 5-minute exposure of pupae of A. quadrimaculatus to low concentrations (0.5 p.p.m.) of dieldrin effected a striking kill during the first three days of adulthood.

The phenomenon of delayed toxicity was taken into consideration in recording sex ratio of generation F_{37} of R/dieldrin (Table 10). On summing up live and dead females and males, it is noted that females constitute 54.66% of the total. Also, DDT may bring about delayed toxicity as demonstrated by the data of the exceptionally vigorously selected 39th generation of RLAF (Table 10).

TABLE 10. DELAYED MORTALITY AND ITS INFLUENCE ON THE SEX RATIO OF TWO STRAINS OF A. ATROPARVUS

Strain	Generation	Date	Pupae		Adults						Total percentage of ♀♀
					♂♂		♀♀		Total (l + d)		
			Live	Dead	Live	Dead	Live	Dead	♂♂	♀♀	
R/dieldrin	F_{37}	9/10/58-4/11/58	2 587	543	546	520	741	543	1 066	1 284	54.66%
RLAF	F_{39}	11/10/58-16/11/58	4 162	1 916	831	294	1 039	313	1 125	1 352	54.58%

We checked the number and sex of adults obtained from 6 bowls (untreated of the R/dieldrin strain from the 44th generation). There were 592 ♂♂ and 656 ♀♀, i.e. 52.57% females.

The delayed toxic effect of dieldrin in anophelines has been the subject of a special study by Rehm et al. (1958). It is interesting to note that by summing up the data in Table 3 of the work of Rehm et al., it is again found that females are in preponderance through larval selection of A. stephensi with dieldrin (Table 11).

TABLE 11. SEX RATIO IN A. STEPHENSI EXPOSED TO DIELDRIN IN THE FOURTH LARVAL INSTAR (AFTER REHM ET AL., 1958)

Larvae	Adults						Total percentage ♀♀
	♂♂		♀♀		Total	(l+d)	
	Live	Dead	Live	Dead	♂♂	♀♀	
Control	58	4	56	4	62	60	49.18%
Dieldrin-treated	91	278	268	209	369	477	56.33%

(b) Relative susceptibility to insecticides of male and female larvae. Shidrawi (1957) considered the possibility that similarly to adult *Aedes aegypti* also in the larval stage there might exist differences between the two sexes in susceptibility to insecticides. Jones (1957a) investigated the response of the two sexes towards DDT in pre-imaginal stages of a normal strain of *A. quadrimaculatus*. He adopted a short time exposure method (between 1 minute and 75 minutes for larvae and 30 to 60 minutes for pupae). The insects were then transferred for observation into clean water and LC_{50} were calculated. Female and male larvae of the same chronological age[1] ("regulated" fourth stage larvae - according to Jones) had the same levels of DDT susceptibility, while after pupation females were considerably more tolerant than males. In a later work with the same strain, Jones (1959) noted that male pupae were evidently more sensitive also towards dieldrin than females.

We have conducted several tests with strain RL and DDT using Wharton's (1956) method, slightly modified as previously described (Mosna et al. 1959). Sexed third stage larvae (examination of gonads and division prior to exposure) of "unknown chronological age" (cf. Jones 1957a) were used. Several replicates showed no difference in LC_{50}'s between male and female larvae (1.0 p.p.m. DDT). It might be that this holds true only for the short time exposure method of Jones and the 24 hours' exposure used by us. It might not be so for longer exposure times and for

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[1] The larval life of male mosquitos is significantly shorter than that of females, as demonstrated in *C. fatigans* (Qutubuddin, 1953) and *A. quadrimaculatus* (Terzian & Stahler, 1949; Jones 1957a).

selection throughout the greater part of larval life, as was done in the breedings of strains RL, RLAF and R/dieldrin (addition of insecticide in first and third larval stage - Mosna et al. 1958, 1959).

It would be of interest to investigate whether the higher frequency of the heterozygous chromosomal arrangement in larvae of *A. atroparvus* surviving a DDT exposure (D'Alessandro, Frizzi & Mariani, 1957) is also accompanied by an enrichment of the surviving population in females.

SUMMARY

Several procedures of sexing larvae of *Anopheles atroparvus* were tried (colour, imaginal antennal discs, and gonads). The last method proved to be practical.

Sexed larvae were examined as to chromosomal arrangement (left arm of third chromosome) and the previous sex determination (gonads) was confirmed by study of the X-chromosome. It was found in all the four strains examined (R/dieldrin, RL, RAFM, Sens. Roma) that either the majority (in R/dieldrin and RL) or all the heterozygotes (in RAFM and Sens. Roma) were females. The female larvae examined were made up as follows: *R/dieldrin* - 76.9% heterozygotes + 23.1% normal and inverted homozygotes; *RL* - 69.7% heterozygotes + 30.3% normal and inverted homozygotes; *RAFM* - 32.8% heterozygotes + 67.2% normal and inverted homozygotes; *Sens. Roma* - 20% heterozygotes + 80% normal and inverted homozygotes.

Chromosomes in adults were not legible. Some considerations on the influence of different modes of selection on sex ratio and the susceptibility of male and female anopheline larvae are appended.

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