IV. RESULTS

A. Selection of susceptible and refractory strains:

Two selection trails were carried out during the present work, the first trail was based on a controlled brother-sister mating (previously described in the text). The second trail was based on the results obtained from the first one which led to the appearance of lethal genes, and terminating the selected strains in F_9 generation. Accordingly, a cousin-cousin mating method (Previously described in the text) of selection was followed in the second trail.

1. The first trial

Results of selection to produce susceptible and refractory strains of *Culex pipiens* infected with *Wuchereria bancrofti* by full sib-mating throughout the nine selected generations are shown in tables (1 and 2).

Table (1): Selection of a susceptible Culex pipiens strain (El-Kashish) to Wuchereria bancrofti infection.

Generations	Number of microfilariae per 53 mm^3 blood	Selection for susceptibility		
		Number of ♀♀ dissected after 12 days	Number of infective mosquitoes*	% infective females
P	85	102	96	94.12
F_1	60	137	120	87.59
F_2	75	31	25	80.65
F_3	50	24	18	75.00
F_4	8	53	20	37.76
F_5**	--	--	--	--
F_6	121	59	53	89.83
F_7	130	23	21	91.30
F_8	112	10	8	80.00
F_9	--	--	--	--

* Mosquitoes with L_3 larvae.

** Inbreeding for colonization.

Table (2): Selction of a refractory Culex pipiens strain (El-Kashish) to Wuchereria bancrofti infection.

Generations	Number of MF. per 53 mm^3 blood	Selection for Refractoriness		
		Number of ♀♀ dissected after 12 days	Number of refractory ♀♀ *	% refractory ♀♀
P	85	102	6	5.88
F_1	60	(Inbreeding	for	colonization)
F_2	75	6	1	16.67
F_3	50	2	1	50.00
F_4	8	3	2	66.67
F_5	--	--	--	--
F_6	121	59	6	10.17
F_7	130	8	1	12.50
F_8	112	7	4	57.14
F_9	--	--	--	--

* Mosquitoes without L_3 larvae.

The data in table (1) show that, the percentages of infective females of susceptible strain are ranged from 75 to 94.12 with a mean 85.5 but with the exception of F_4 generation due to very low level of microfilariae. Moreover this mean becomes 79.5% including the susceptibility of F_4 generation. The low susceptibility in F_4 due to the lower number of microfilariae in the volunteer patient during feeding the mosquitoes on blood. In the susceptible strain, the susceptibility rate increased from 87.59 in F_1 generation to 91.30 in F_7. The number of females decreased during selection due to high mortality and led to loss of the susceptible strain in F_9 generation.

The results of table (2) show that, the percentage of refractory females increasd from 5.88 in the parental generation to 66.67 in F_4 generation. Mortality increased till loss of all females in F_5 generation. The refractory females of susceptible strain were used in F_6 generation. The percentage of refractory females increased from 10.17 in F_6 to 57.14 in F_8 generation and the number of females decreased due to high mortality and again led to loss of the refractory group in F_9 generation.

In F_5 generation, both susceptible and refractory groups were not tested for filarial infection but inbree-

ding for colonization. In F_9 generation, all egg batches laid by females in the two groups did not hatch although they have mated with males. Several trails to refeed them with blood were failed and then all females were died.

In this selection, twelve virgin females were mated with one male as described previously in the text.

The extrinsic incubation period for development of L_3 larvae, was 12 days after feeding the females on the infecting blood meal.

1. 1. Effect of lethal genes during selection:

The selection of susceptible and refractory strains of Culex pipiens to filarial infection, using full sib-mating method, a high mortality of pupae was observed (tables 3 and 4) due to accumulation of deleterious genes as a result of the close inbreeding.

The effect of lethal genes on survival rates of a selected susceptible strain of Cx. pipiens to W. Bancrofti infection is shown in table (3) and Fig. (5).

Table (3): The effect of lethal genes on survival rates of a selected susceptible strain of Culex pipiens to W. bancrofti infection

Generations	Number of microfilariae per (53 mm^3) blood	Susceptible strain		
		Number of ♀♀ fed on microfilaraemia	Number of ♀♀ survived during incubation period	(%)
Parent	85	110	102	(92.73)
F_1	60	157	137	(87.26)
F_2	75	46	31	(67.39)
F_3	50	48	27	(56.25)
F_4	8	85	55	(64.71)
F_5*	--	--	--	--
F_6	121	73	59	(80.82)
F_7	130	39	22	(56.41)
F_8	112	11	4	(36.36)
F_9	--	--	0	(0.00)

* Inbreeding for colonization.

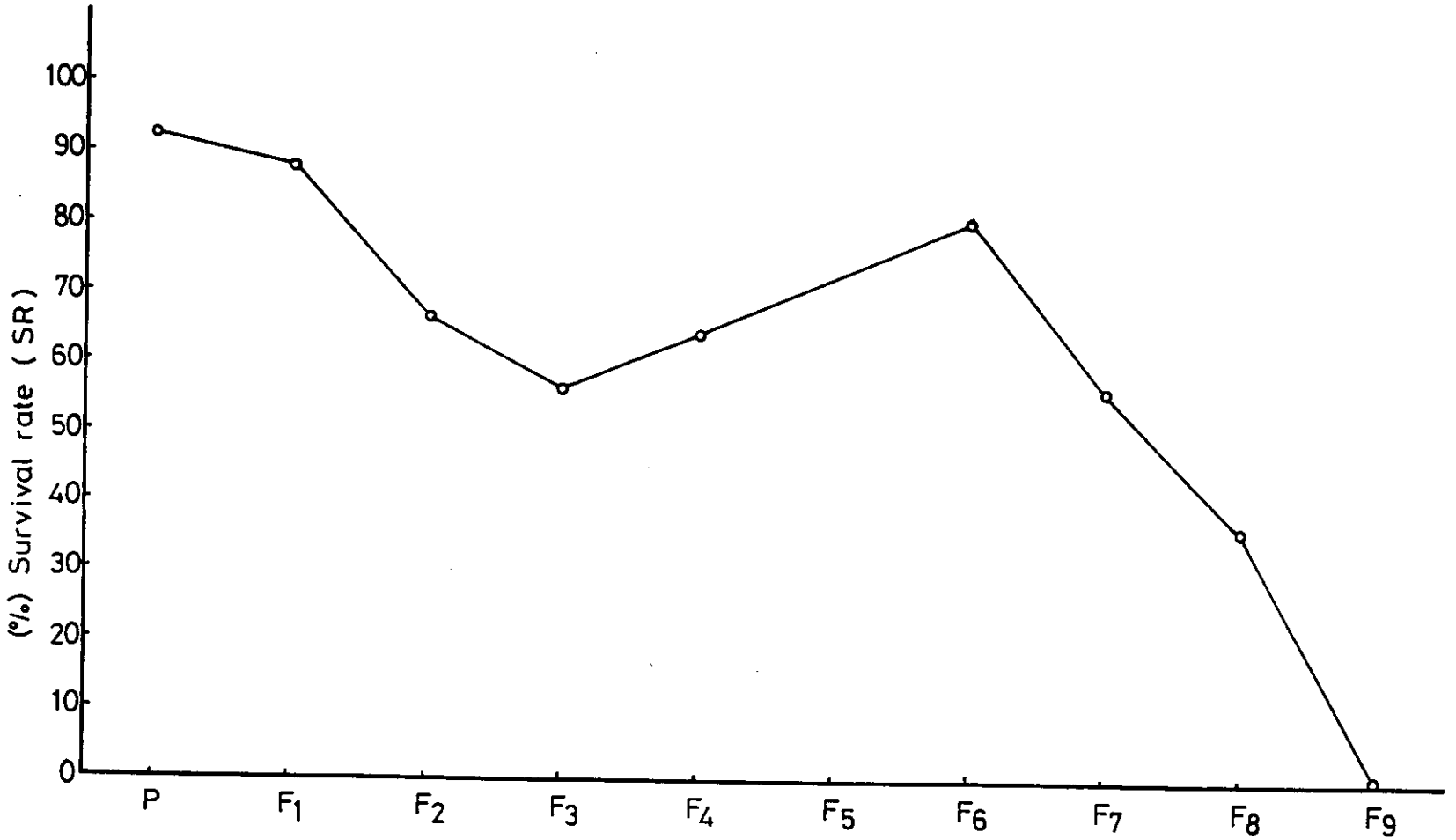

100
90
80
70
60
50
40
30
20
10
0
(%) Survival rate (SR)
P
F1
F2
F3
F4
F5
F6
F7
F8
F9
Generations of susceptible strain

Fig. (5)

The data in table (3) indicate that the survival rate (SR) decreased from 92.73 in parent to 36.36 in F_8 generation, after which the selected population disappeared totally and no living individual mosquito was obtained in F_9 generation. The survival line in Fig. (5) shows a primary decrease till F_3 generation, then raised again slightly till F_6 due to inbreeding for colonization by mass-mating to get on a high number of adults, then started to decrease till to zero level at F_9 generation.

The effect of lethal genes on survival rates of a selected refractroy strain of Cx. pipiens to W. bancrofti infection is shown in table (4) and Fig. (6).

Table (4): The effect of lethal genes on survival rates of a selected refractory strain of Culex pipiens to W. bancrofti infection

Generations	Number of Microfilariae per (53 mm^3) blood	Refractory strain		
		Number of ♀♀ fed on microfilaraemia	Number of Survived ♀♀ during incubation period	(%)
Parent	85	110	102	(92.73)
F_1	60	(inbreeding for colonization)		
F_2	75	17	8	(47.06)
F_3	50	5	2	(40.00)
F_4	8	4	3	(75.00)
F_5	--	--	0	(0.00)
F_6	121	--	--	--
F_7	130	16	8	(50.00)
F_8	112	8	1	(12.50)
F_9	--	--	0	(0.00)

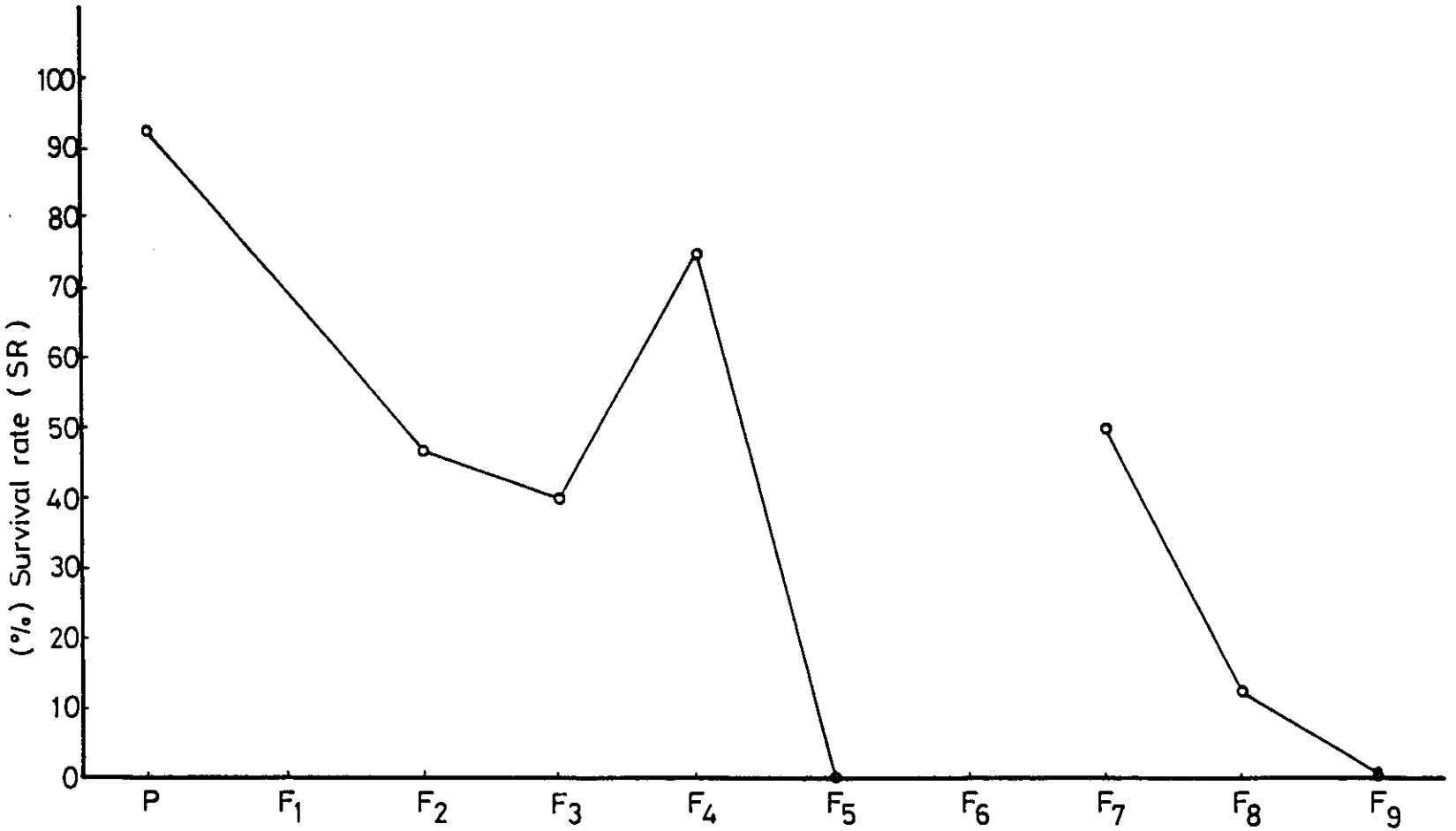

100
90
80
70
60
50
40
30
20
10
0
(%) Survival rate (SR)
P
F1
F2
F3
F4
F5
F6
F7
F8
F9
Generations of refractory strain

Fig. (6)

It is clear from the data of table (4) that the survival rate (SR) decreased from 92.73 in parent to zero in F_5 generation and then no living individual mosquito was obtained from the selected population. The refractory females of susceptible strain were used in F_6 and have 50.0 survival rate in F_7 generation, then decreased to 12.50 in F_8 where most pupae were died. The decrease of survival adults continued till became zero in F_9 generation and the selected population disappeared again. It is obvious here that the effet of lethal genes is high because the number of survival females are very low. The survival line in Fig. (6) shows a decrease till F_3 generation, then raised again slightly in F_4, then decreased till to zero level in F_5 generation. In F_7 generation, the survival rate (SR) decreased again till to zero level in F_9 generation for the second time.

A comparison between susceptible and refractory strains for the effect of lethal genes on the survival rates is shown in table (5).

Table (5): Comparison between susceptible and refractory strains of Cx. pipiens to W. bancrofti infection for the effect of lethal genes on survival rates.

Generations	Number of microfilariae per (53 mm^3)	Survival rate (%)	
		Susceptible strain	Refractory strain
F_1	60	87.26	(inbreeding for colonization)
F_2	75	67.39	47.06
F_3	50	56.25	40.00
F_4	8	64.71	75.00
F_5	--	(inbreeding for colonization)	0.00
F_6	121	80.82	--
F_7	130	56.41	50.00
F_8	112	36.36	12.50
F_9	--	0.00	0.00

Data of table (5) indicate that the population mortality of refractory strain is higher than the susceptible one. Accordingly, the survival rates of refractory strain are less than that of the susceptible one. In refractory strain, the survival rates are decreased twice to zero as in F_5 and F_9 generations but in susceptible strain, the survival rates are decreased to zero level only in F_9 generation. It is clear from the data that the effect of lethal genes is higher in the refractory strain than the susceptible one.

2. The second trial

In the second trial, 7 generations were studied and originated from the same breeding site of the strain used in the first trial. Results of selection to produce susceptible and refractroy strains of Culex pipiens to Wuchereria bancrofti are presented in tables (6 and 7).

Table (6): Selection of a susceptible Culex pipiens strain (El-Kashish) to Wuchereria bancrofti infection.

Generations	Number of microfilariae per (53 mm^3)	Selection for susceptibility		
		Number of ♀♀ dissected after 12 days	Number of infective * mosquitoes	% infective females
F_1	18	39	19	48.72
F_2	41	39	23	58.97
F_3	110	50	38	76.0
F_4	19	52	22	42.31
F_5	50	53	39	73.58
F_6	80	32	29	90.63
F_7	85	33	30	90.91**

* Mosquitoes with L_3 larvae.

** +ve rank correlation (r = 0.714).

Table (7): Selection of a refractory Culex pipiens strain (El-Kashish) to Wuchereria bancrofti infection.

Generations	Number of microfilariae per (53 mm^3)	Selection for Refractoriness		
		Number of ♀♀ dissected after 12 days	Number of refractory ♀♀ *	% Refractory ♀♀
F_1	18	39	20	51.28
F_2	41	36	19	52.78
F_3	110	52	18	34.62
F_4	19	45	27	60.00
F_5	50	43	20	46.51
F_6	80	28	7	25.00
F_7	85	14	6	42.86**

* Mosquitoes without L_3 larvae.

** + ve rank correlation (r = 0.5).

The selection used during this trial was started with only one mother female. Data in table (6) indicate that percentage of infective females in the susceptible group of F_1 generation was (48.72), and increased to (90.91) in F_7. It is obvious from the results of this table that the susceptibility of Culex pipiens to Wuchereria bancrofti increased through the seven selected generations.

The results of table (7) show that the percentage of refractory females was increased from 51.28 in F_1 to 60.0 in F_4 generation. The percentages of refractory females were ranged between 25.0 to 60.0 with a mean (44.72) through the seven selected generations. The statistical analysis enhances the increase of refractoriness in Culex piupiens females to Wuchereria bancrofti parasite during this selection.

B. Experimental human filaria transmission by Culex pipiens using capillary feeding technique:

The difficulty of testing the potentiality of Culex pipiens in transmitting Wuchereria bancrofti, led to the development of the capillary tube feeding technique (previously described in the text) for differentiation between the transmitter and non-transmitter groups of Culex pipiens under laboratory conditions.

The selection trials of both groups for all generations were based on this technique, and the susceptible individual females tested are added to susceptible mosquitoes from the refractory group.

1. The first trial

The results of testing seven generations of Culex pipiens for Wuchereria bancrofti transmission showing the transmitter and non-transmitter females, are presented in table (8).

Table (8): Transmitters and non-transmitters of susceptible females of Culex pipiens infected with Wuchereria bancrofti.

Generations	Number of females tested	Transmitters (T)			Non-transmitters (NT)			T/NT
		No. of ♀♀	Grand Total L_3 larvae	Average L_3/♀	No. of ♀♀	Grand total L_3 larvae	Average L_3/♀	
F_1	63	35	315	9.0	28	128	4.57	1.25
F_2	30	9	79	8.78	21	114	5.43	0.43
F_3	19	0	0	0.0	19	58	3.05	0.0
F_4	21	0	0	0.0	21	32	1.52	0.0
F_5*	--	--	--	--	--	--	--	--
F_6	53	8	71	8.88	45	271	6.02	0.18
F_7	28	4	48	12.0	24	308	12.83	0.17

* In F_5, inbreeding for colonization.

The data of table (8) indicate that, the average number of infective stage of filaria (L_3) per transmitter female in F_1 generation is significantly more than the non-transmitter female ($P < 0.01$). In F_2, the transmitters harbour significantly more L_3 larvae than the non-transmitters ($P < 0.05$). In F_3 and F_4 generations, no transmitter females are found while in F_6 and F_7 generations, no significant difference between transmitter and non-transmitter females for the average number of L_3 larvae per female. It may be evident from the ratio between T (transmitters) and NT (non-transmitters) that the number of transmitter females are less than that of non-transmitters except in the F_1 generation.

The ejected and non-ejected L_3 larvae per transmitter female are presented in table (9).

Table (9): The ejected and non-ejected L_3 larvae per transmitter female of Cx. pipiens to W. bancrofti infection.

Generations	Ejected L_3 larvae			Non-ejected L_3 larvae		
	Number of females	No. of L_3 ejected in capillary tube	Average $L_3/♀$	Number of females	No. of L_3 non-ejected in capillary tube	Average $L_3/♀$
F_1	35	153	4.37	35	162	4.63
F_2	9	18	2.0	9	61	6.78
F_3	0	0	0.0	0	0	0.00
F_4	0	0	0.0	0	0	0.00
F_5*	--	--	--	--	--	--
F_6	8	23	2.88	8	48	6.00
F_7	4	16	4.00	4	32	8.00

* In F_5 , inbreeding for colonization.

Data of table (9) show that there is no significant difference between the ejected and non-ejected L_3 larvae per transmitter female of Cx. pipiens in F_1 generation while in the 2 nd generation, the non-ejected L_3 larvae are significantly more than the ejected L_3 larvae ($P < 0.03$). In F_3 and F_4 generations , no transmitter females are found and in F_6 generation, the average number of non-ejected L_3 larvae per female is higher than that the ejected larvae ($P < 0.01$). In addition, there is no significant difference between the ejected and non-ejected L_3 larvae in F_7 generation.

A comparison between the percentages of ejection and non-ejection of infective larvae for the transmitter females is shown in table (10).

Table (10): Comparison between the percentages of ejection and non-ejection for the transmitter females of Cx. pipiens infected with W. bancrofti.

Generations	Transmitters	
	Ejection (%)	Non-ejection (%)
F_1	48.57	51.43
F_2	22.78	77.22
F_3	0.00	0.00
F_4	0.00	0.00
F_5*	--	--
F_6	32.39	67.61
F_7	33.33	66.67

* In F_5, inbreeding for colonization.

The data of table (10) indicate that, there is no significant difference between the ejection and non-ejection percentages of infective larvae in the first generation but in F_2, the non-ejection percentage is significantly higher than the ejection one ($P < 0.0001$) In the 3 rd and 4 th generations, no transmitter females are found. The non-ejection percentages in F_6 and F_7 generations are significantly higher than the ejection ones ($P < 0.001$ and $P < 0.01$) respectively.

A comparison between the presence of L_3 larvae in different body regions of transmitter and non-transmitter females of Cx. pipiens after dissection is presented in table (11).

Table (11): Comparison between the presence of L_3 larvae in different body regions of transmitter and non-transmitter females of Cx. pipiens infected with W. bancrofti.

Generations	Transmitters								Non-transmitters							
	Proboscis		Head		Thorax & abdomen		Total non-ejected L_3 larvae (L_3/♀)		Proboscis		Head		Thorax & abdomen		Total non-ejected L_3 larvae (L_3/♀)	
	Total L_3	(L_3/♀)	Total L_3	(L_3/♀)	Total L_3	(L_3/♀)			Total L_3	(L_3/♀)	Total L_3	(L_3/♀)	Total L_3	(L_3/♀)		
F_1	12	(1.2)	57	(2.9)	93	(3.9)	162	(4.6)	17	(2.1)	47	(2.1)	64	(4.0)	128	(4.6)
F_2	10	(2.5)	22	(2.8)	29	(4.8)	61	(6.8)	12	(1.2)	32	(2.3)	70	(3.9)	114	(5.4)
F_3	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	5	(1.0)	53	(2.9)	58	(3.1)
F_4	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	3	(1.0)	29	(1.4)	32	(1.5)
F_5*		--		--		--		--		--		--		--		--
F_6	4	(1.0)	20	(2.9)	24	(3.4)	48	(6.0)	9	(1.3)	61	(3.1)	201	(4.9)	271	(6.02)
F_7	3	(3.0)	8	(2.7)	21	(5.3)	32	(8.0)	2	(1.0)	16	(1.6)	290	(12.6)	308	(12.8)

* In F_5, inbreeding for colonization.

It is clear from the data of table (11) that in F_1 generation, the number of L_3 larvae per female present in the proboscis of transmitters is not significantly different than that in the non-transmitters. In addition, in F_2, the proboscis of transmitter female is significantly harbours higher L_3 larvae than that in the non-transmitter female ($P < 0.03$). The proboscis of non-transmitters in F_3 and F_4 have not L_3 larvae while no transmitter females are found in those generations. In F_6 generation, there is no significant difference between the proboscis of transmitters harbouring L_3 larvae and that in non-transmitters. In F_7, only one transmitter female has 3 L_3 larvae in its proboscis while two non-transmitter females, each harbours one L_3 larva.

With regard to the number of L_3 larvae per female present in the head of transmitter and non-transmitter groups in the same table, no significant difference was found betwen them in all generations.

Similarly, there is no significant difference between the number of L_3 larvae per female present in thorax and abdomen for the two groups in the selected generations.

In comparing the total number of non-ejected L_3 larvae per female for transmitter group with that of

non-transmitter one, it is found that no significant difference between the two groups in all generations.

The percentages of different body regions of transmitters and non-transmitters of Cx. pipiens harbouring the infective stage of filarial larvae are presented in table (12).

Table (12): Percentages of the different body regions of transmitters and non-transmitters of Cx. pipiens harbouring the infective stage of filarial larvae.

Generations	Transmitters			Non-transmitters		
	Proboscis (%)	Head (%)	Thorax & abdomen (%)	Proboscis (%)	Head (%)	Thorax & abdomen (%)
F_1	28.57	57.14	68.57	28.57	78.57	57.14
F_2	44.44	88.89	66.67	47.62	66.67	85.71
F_3	0.0	0.0	0.0	0.0	26.32	94.74
F_4	0.0	0.0	0.0	0.0	14.29	100.0
F_5*	--	--	--	--	--	--
F_6	50.0	87.50	87.50	15.56	44.44	91.11
F_7	25.0	75.00	100.0	8.33	41.67	95.83

* In F_5, inbreeding for colonization.

2. The second trial

The results of testing seven generations of Culex pipiens for Wuchereria bancrofti transmission showing the transmitter and non-transmitter females, are presented in table (13).

Table (13): Transmitters and non-transmitters of susceptible females of Culex pipiens infected with Wuchereria bancrofti.

Generations	Number of females tested	Transmitters (T)			Non-transimitters (NT)			T/NT
		No. of ♀♀	Grand Total L_3 larvae	Average L_3/♀	No. of ♀♀	Grand total L_3 larvae	Average L_3/♀	
F_1	19	0	0	0	19	36	1.89	0.0
F_2	39	0	0	0	39	80	2.05	0.0
F_3	72	19	274	14.42	53	271	5.11	0.36
F_4	40	3	10	3.33	37	54	1.46	0.08
F_5	62	7	42	6.00	55	156	2.84	0.13
F_6	50	2	26	13.00	48	208	4.33	0.04
F_7	39	3	18	6.00	36	114	3.17	0.08

The data of table (13) indicate that no transmitter females are found in the 1 st and 2 nd generations. It is clear from the data that the average number of L_3 larvae per female in transmitters is significantly higher than that in non-transmitters for all selected generations. In F_3, the average L_3 larvae per female in transmitter group is significantly higher than that in non-transmitter group ($P < 0.0001$). Similarly, in F_4 and F_5 is significantly higher than that in non-transmitter group ($P < 0.0002$). The number of L_3 larvae per female of transmitter group in F_6 and F_7 generations is significantly higher than that in non-transmitter group ($P < 0.002$, $P < 0.05$) respectively. It is clear from the ratio of T (Transmitters) and NT (Non-transmitters) that the number of transmitter females are less than that of non-transmitters in all selected generations.

The ejected and non-ejected L_3 larvae per transmitter female, are presented in table (14).

Table (14): The ejected and non-ejected L_3 larvae per transmitter female of *Cx. pipiens* to *W. bancrofti* infection.

Generations	Ejected L_3 larvae			Non-ejected L_3 larvae		
	Number of females	No. of L_3 ejected in capillary tube	Average $L_3/♀$	Number of females	No. of L_3 non-ejected in capillary tube	Average $L_3/♀$
F_1	0	0	0.0	0	0	0.0
F_2	0	0	0.0	0	0	0.0
F_3	19	65	3.4	18	209	11.6
F_4	3	4	1.3	2	6	3.0
F_5	7	19	2.7	6	23	3.8
F_6	2	18	9.0	2	8	4.0
F_7	3	5	1.7	3	13	4.3

It is obvious from the data of table (14) that no transmitter females are found in the 1 st and 2 nd generations while in F_3, the average number of non-ejected L_3 larvae per female is significantly higher than the number of ejected L_3 larvae ($P < 0.001$). There is no significant difference between the ejected L_3 larvae per female and the non-ejected L_3 in the other generations (F_4 - F_7).

A clear comparison between the percentages of ejection and non-ejection of infective larvae for the transmitter females, is presented in table (15).

Table (15): Comparison between the percentages of ejection and non-ejection for the transmitter females of Cx. pipiens infected with W. bancrofti.

Generations	Transmitters	
	Ejection (%)	Non-ejection (%)
F_1	0.0	0.0
F_2	0.0	0.0
F_3	23.72	76.28
F_4	40.00	60.00
F_5	45.24	54.76
F_6	69.23	30.77
F_7	27.78	72.22

The data of table (15) indicate that no transmitter females are found in the 1 st and 2 nd generations. In F_3, the percentage of non-ejection is significantly higher than the ejection percentage of infective larvae ($P < 0.0001$). There is no significant difference between ejection and non-ejection percentages in the 4 th and 5 th generations. In F_6, the ejection percentage of L_3 larvae is significantly higher than the non-ejection one ($P < 0.02$) but in F_7 generation, the non-ejection percentage is significantly higher than the ejection one ($P < 0.02$).

A comparison between the presence of L_3 larvae in different body regions of transmitter and non-transmitter females of Cx. pipiens after dissection, is presented in table (16).

Table (16): Comparison between the presence of L_3 larvae in different body regions of transmitter and non-transmitter females of Cx. pipiens infected with W. bancrofti.

Generations	Transmitters							Non-transmitters						
	Proboscis		Head		Thorax & abdomen		Total non-ejected L_3 larvae (L_3/♀)	Proboscis		Head		Thorax & abdomen		Total non-ejected L_3 larvae (L_3/♀)
	Total L_3	(L_3/♀)	Total L_3	(L_3/♀)	Total L_3	(L_3/♀)		Total L_3	(L_3/♀)	Total L_3	(L_3/♀)	Total L_3	(L_3/♀)	
F_1	0	(0.0)	0	(0.0)	0	(0.0)	0 (0.0)	2	(1.0)	21	(1.4)	13	(1.6)	36 (1.9)
F_2	0	(0.0)	0	(0.0)	0	(0.0)	0 (0.0)	7	(1.0)	17	(1.1)	56	(1.9)	80 (2.1)
F_3	19	(1.7)	77	(5.1)	113	(7.1)	209 (11.6)	26	(1.4)	86	(2.6)	159	(3.3)	271 (5.1)
F_4	0	(0.0)	1	(1.0)	5	(2.5)	6 (3.0)	3	(1.0)	13	(1.0)	38	(1.4)	54 (1.5)
F_5	4	(1.3)	12	(2.0)	7	(1.8)	23 (3.8)	21	(1.2)	42	(1.3)	93	(2.2)	156 (2.8)
F_6	0	(0.0)	2	(2.0)	6	(3.0)	8 (4.0)	18	(1.1)	82	(2.5)	108	(3.2)	208 (4.3)
F_7	0	(0.0)	4	(2.0)	9	(4.5)	13 (4.3)	1	(1.0)	27	(1.5)	86	(2.8)	114 (3.2)

The obtained results of table (16) revealed that no transmitter females in the 1 st and 2 nd generations are found. In F_3 and F_5 generations, there is no significant difference between the average number of L_3 larvae per female present in the proboscis of transmitters and non-transmitters. In the remainder generations, there are no L_3 larvae in the proboscis of tramsmitter females.

With regard to head, the number of L_3 larvae per female in transmitters of F_3 and F_5 is significantly higher than that of non-transmitters ($P < 0.01$ and $P < 0.05$) respectively but no difference between them in F_7 generation.

The number of L_3 larvae per female present in the thorax and abdomen of transmitters is significantly higher than present in the non-transmitters of F_3 and F_4 generations ($P < 0.0002$ and $P < 0.02$) respectively while no difference between them in the remainder generations.

It is observed in F_3 generation that the total number of non-ejected L_3 larvae per female of transmitters is significantly higher than present in the non-transmitters ($P < 0.0002$) but no significant difference between them in the remainder generations.

A comparison between the presence of L_3 larvae in proboscis, head, thorax and abdomen, and total non-ejected

L_3 larvae of transmitter and non-transmitter females is shown in figures (7, 8, 9 & 10). It is indicated from Fig. (7), that the average number of L_3 larvae per transmitter female present in the proboscis for all generations is zero except in F_3 and F_5. Moreover, in the non-transmitter females, the proboscis harbours more L_3 larvae in all generations than in the transmitter females except in F_3 and F_5.

It is evident from Fig. (8) that the average number of L_3 larvae per transmitter female present in the head is zero as in F_1 and F_2 generations while in F_3, the transmitter female harbours more L_3 larvae than the non-transmitter one. Generally in other generations, the transmitter female harbours more or less higher L_3 larvae in the head than the non-transmitter female.

The obtained results in Fig. (9) indicated that the average number of L_3 larvae per transmitter female in thorax and abdomen is zero as in F_1 and F_2 generations. While in F_3, F_4 and F_7, the transmitter female harbours higher L_3 larvae in the thorax and abdomen than non-transmitter female. Moreover in F_5 and F_6, the transmitter female harbours lower L_3 larvae in the thorax and abdomen than non-transmitter one.

Results of Fig. (10) revealed that the total non-ejected L_3 larvae per transmitter female in F_1 and F_2

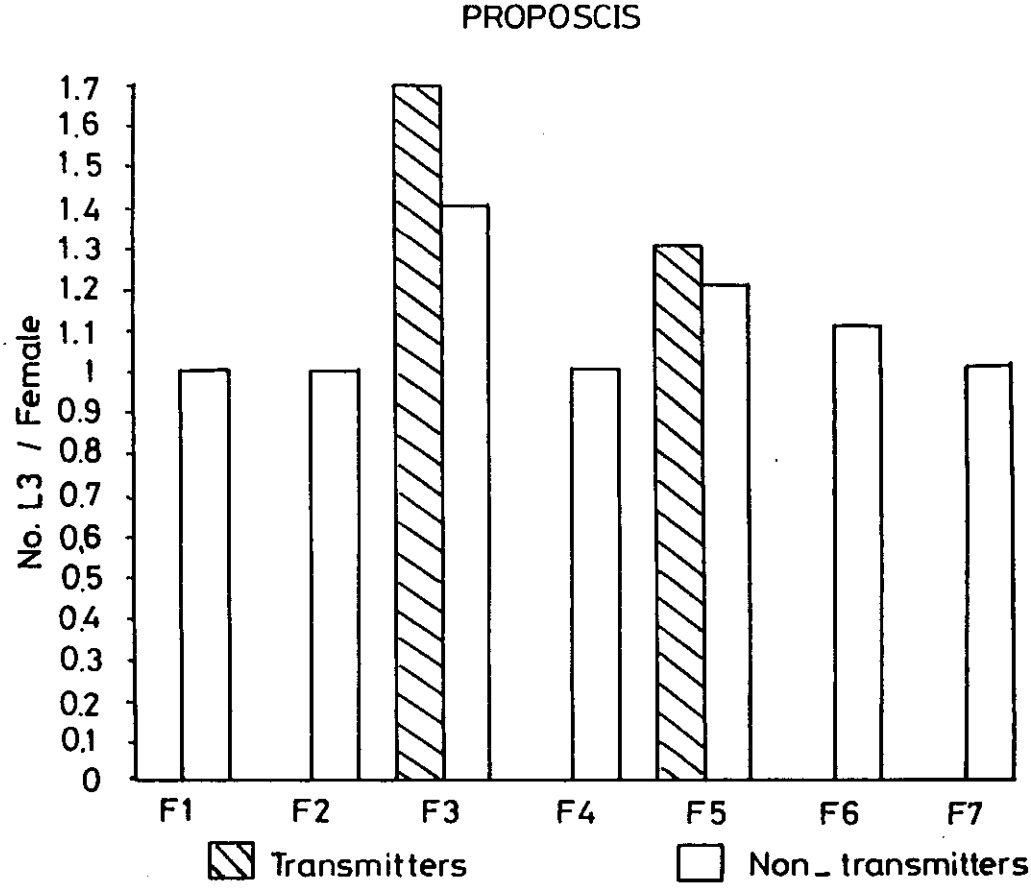

PROPOSCIS
No. L3 / Female
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
F1
F2
F3
F4
F5
F6
F7
Transmitters
Non_transmitters

Fig.(7)

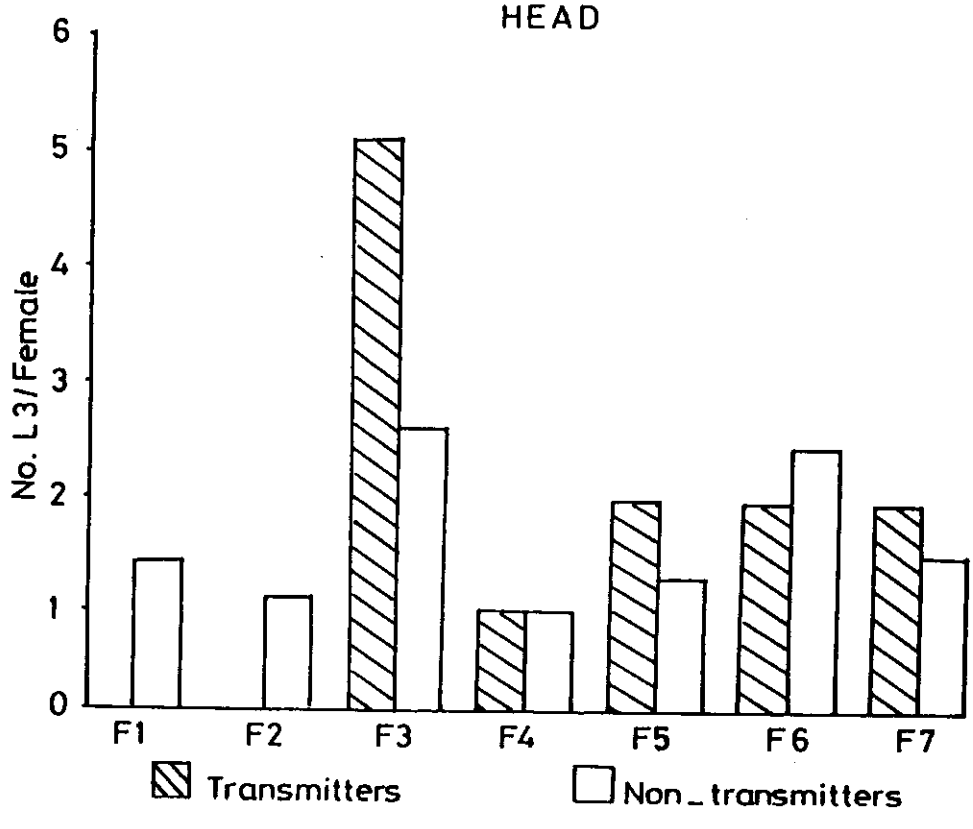

HEAD
No. L3/Female
6
5
4
3
2
1
0
F1
F2
F3
F4
F5
F6
F7
Transmitters
Non_transmitters

Fig. (8)

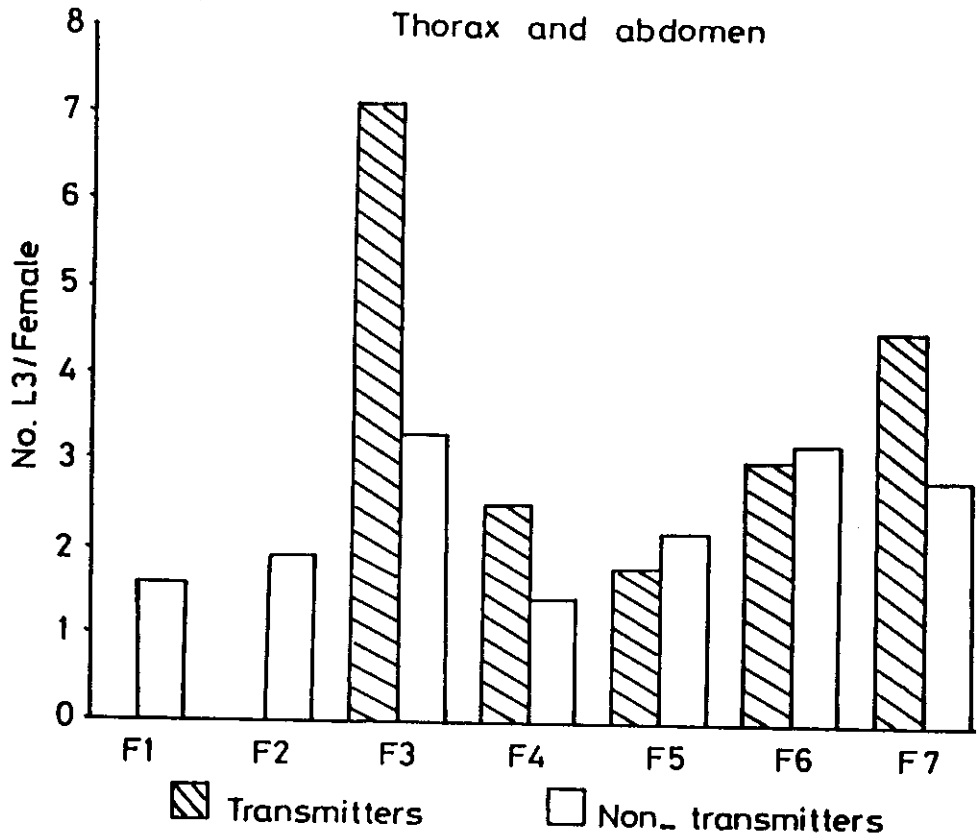

Thorax and abdomen
No. L3/Female
0
1
2
3
4
5
6
7
8
F1
F2
F3
F4
F5
F6
F7
Transmitters
Non_ transmitters

Fig. (9)

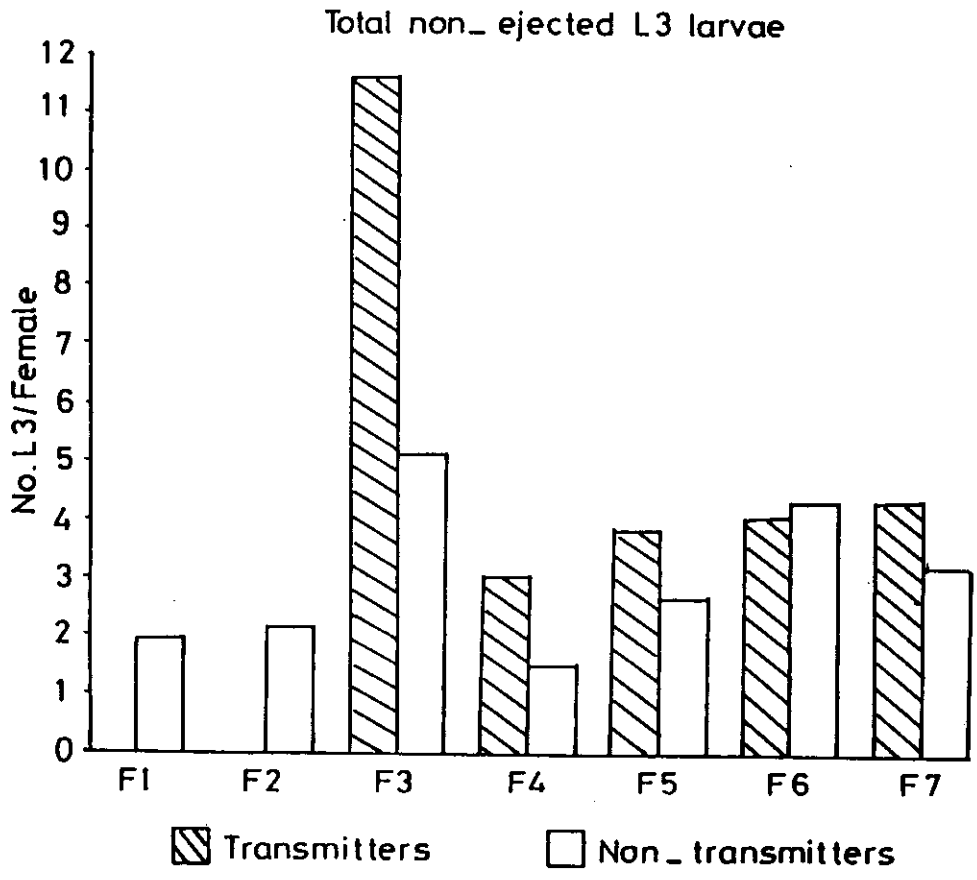

Total non_ ejected L3 larvae
No. L3/Female
0
1
2
3
4
5
6
7
8
9
10
11
12
F1
F2
F3
F4
F5
F6
F7
Transmitters
Non_ transmitters

Fig. (10)

is zero. In F_3, F_4, F_5 and F_7, the total number of non-ejected L_3 larvae per transmitter female is more than that in non-transmitter one. On the other hand, in F_6, the total non-ejected L_3 larvae per transmitter female is lower than that in non-transmitter one.

The percentages of different body regions of transmitters and non-transmitters of Cx. pipiens harbouring the infective stage of filarial larvae are presented in table (17).

Table (17): Percentages of the different body regions of transmitters and non-transmitters of Cx. pipiens harbouring the infective stage of filarial larvae.

Generations	Transmitters			Non-transmitters		
	Proboscis (%)	Head (%)	Thorax & abdomen (%)	Proboscis (%)	Head (%)	Thorax & abdomen (%)
F_1	0.00	0.00	0.00	10.53	78.95	42.11
F_2	0.00	0.00	0.00	17.95	38.46	74.36
F_3	57.89	78.95	84.21	35.85	62.26	90.57
F_4	0.00	33.33	66.67	8.11	35.14	72.97
F_5	42.86	85.71	57.14	32.73	58.18	78.18
F_6	0.00	50.00	100.00	33.33	68.75	68.75
F_7	0.00	66.67	66.67	2.78	50.00	86.11

Data of table (17) indicate that there are no transmitter females in the 1 st and 2 nd generations. In F_3, the percentages of proboscis harbouring infective larvae in transmitters are significantly higher than that in non-transmitters ($P < 0.05$) but no difference between them in F_5 generation. In the remainder generations, the percentages of proboscis harbouring L_3 larvae in transmitters are zero.

It is obvious from the data of the same table, that there is no significant difference between the percentages of head harbouring L_3 larvae in transmitter and non-transmitter females for all selected generations.

Data of table (17) showed that there is no significant difference between the percentages of thorax and abdomen harbouring L_3 larvae in transmitter and non-transmitter groups in all generations.

It was observed that the period for transmission of infective larvae (L_3) by Cx. pipiens using capillary feeding technique in the two trials of selection is 13 days. this period is the extrinsic incubation period for transmission while the extrinsic incubation period for development of L_3 larvae was 12 days post infection of mosquitoes.

C. Mode of inheritance of susceptibility and refractoriness of Cx. pipiens to Wuchereria bancrofti:

Reciprocal crosses were made between the selected susceptible and selected refractory strains and a part of the two reciprocal (F_1) hybrids was tested for the ability to support infections of W. bancrofti and they were left to give separate F_2 progenies. The rest of both F_1 hybrids were reciprocally backcrossed to the susceptible and refractory parents. The F_2 offspring and the eight possible backcrosses were exposed to infection of W. bancrofti to determine the different genotypes composing the population. The results are summarized in table (18).

Table (18): Data that showing the inherited susceptibility of Cx. pipiens to W. bancrofti following crosses and backcrosses between susceptible and refractory strains.

Cross no.	Parents ♂♂	Parents ♀♀	No. of mosquitoes dissected after 12 days	-	+	% infective	No. of L_3 larvae per infective mosquito
1	RR	RR	43	20	23	53.49	2.74
2	SS	SS	53	14	39	73.58	3.46
F_1 hybrids:							
3	RR	SS	15	1	14	93.33	5.07
4	SS	RR	18	5	13	72.22	3.77
F_2 offspring:							
5	F_1 (RR♂♂ X SS♀♀)	F_1 (RR♂♂ X SS♀♀)	17	6	11	64.71	3.82
6	F_1 (SS♂♂ X RR♀♀)	F_1 (SS♂♂ X RR♀♀)	31	11	20	64.52	2.15
Backcrosses to RR stock:							
7	RR	F_1 (RR♂♂ X SS♀♀)	15	10	5	33.33	1.4
8	RR	F_1 (SS♂♂ X RR♀♀)	9	5	4	44.44	2.0
9	F_1 (RR♂♂ X SS♀♀)	RR	14	9	5	35.71	1.2
10	F_1 (SS♂♂ X RR♀♀)	RR	5	3	2	40.0	1.0
Backcrosses to SS stock:							
11	SS	F_1 (RR♂♂ X SS♀♀)	22	6	16	72.73	2.13
12	SS	F_1 (SS♂♂ X RR♀♀)	22	7	15	68.18	not tested
13	F_1 (RR♂♂ X SS♀♀)	SS	6	3	3	50.0	2.33
14	F_1 (SS♂♂ X RR♀♀)	SS	5	1	4	80.0	2.0

RR = refractory strain

SS = susceptible strain.

- = Mosquitoes without L_3 larvae.

+ = Mosquitoes with L_3 larvae.

Data in table (18) showed that on crossing RR males x SS females almost all the progeny (14 out of 15 or 93.33%) were susceptible to infection with W. bancrofti. However, from the reciprocal cross of SS males x RR females, a high percentage of the progeny was susceptible (13 out of 18 or 72.22%). It was found that there is no significant difference between the two reciprocal crosses but the selected susceptible strain (SS males x SS females) was significantly higher ($P \leq 0.02$) than the selected refractory strain (RR males x RR females). From these results, it would appear that the susceptibility to infection with W. bancrofti is almost dominant and refractoriness is recessive. The results of reciprocal crosses suggested that cytoplasmic inheritance was not involved because if inheritance was through factors carried in the cytoplasm, one would expect that the offspring of crosses 3 and 4 to be much more different like their respective female parents but the reciprocal crosses here had not significant difference although the parents significantly different ($P \leq 0.02$). Accordingly, there was no maternally cytoplasmic inheritance involved.

As expected, the backcrosses to the RR strain showed a generally lower susceptibility than those of SS strain and both F_2 groups were, on average, intermediate (Table 18). The backcrosses to SS gave no higher suscepti-

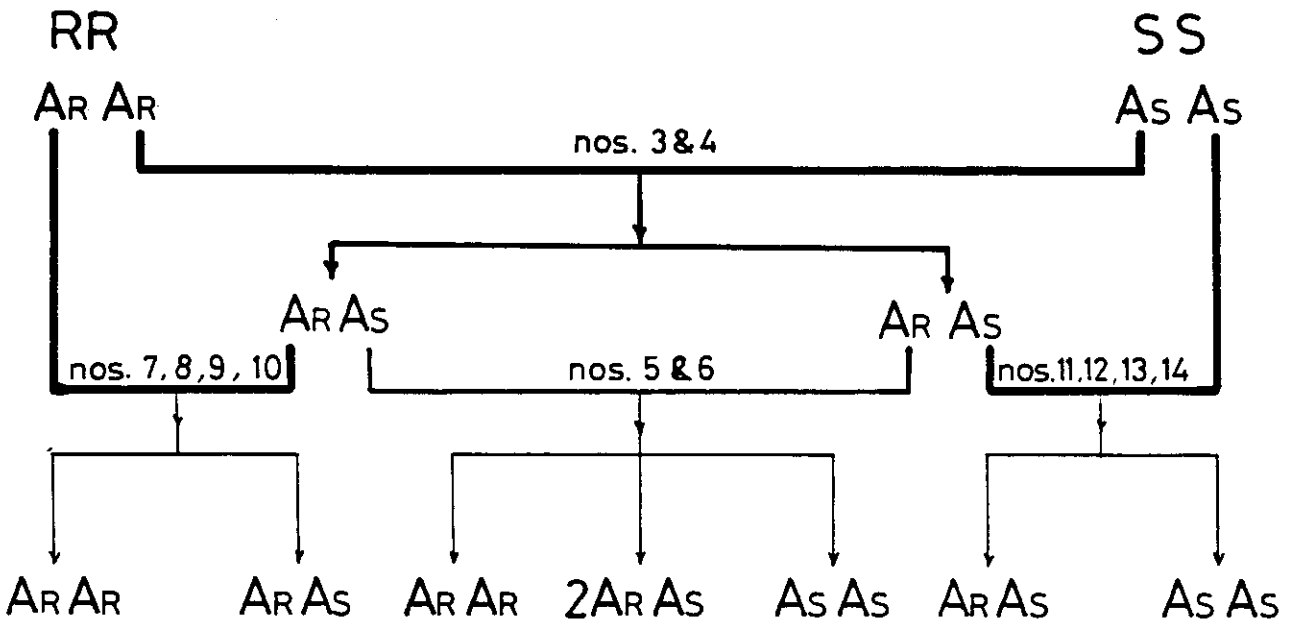

RR
SS
AR AR
AS AS
nos. 3&4
AR AS
AR AS
nos. 7, 8, 9, 10
nos. 5 & 6
nos. 11, 12, 13, 14
AR AR
AR AS
AR AR
2AR AS
AS AS
AR AS
AS AS

Fig. (11)

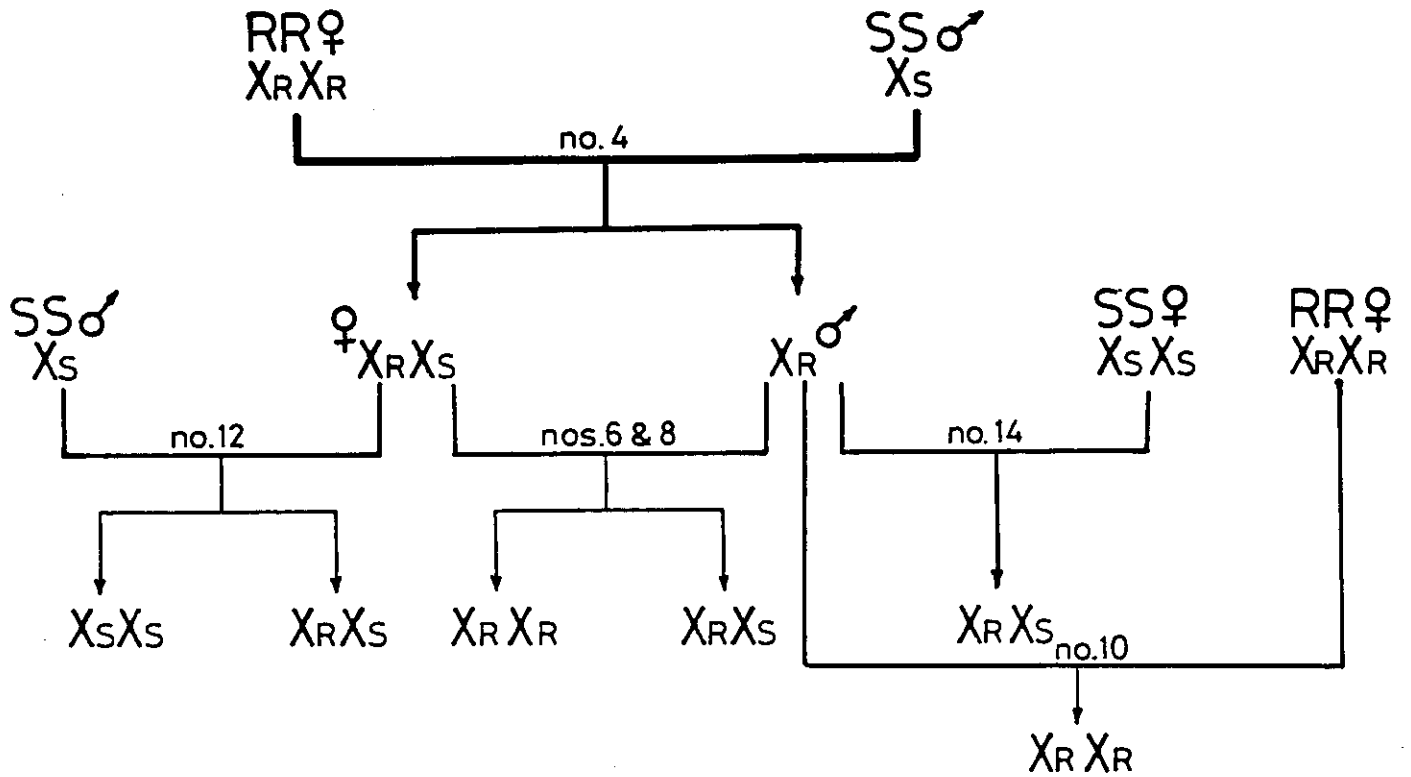

RR♀
XRXR
SS♂
XS
no. 4
SS♂
XS
♀XRXS
XR♂
SS♀
XSXS
RR♀
XRXR
no.12
nos.6 & 8
no.14
XSXS
XRXS
XRXR
XRXS
XRXS
no.10
XRXR

Fig. (12)

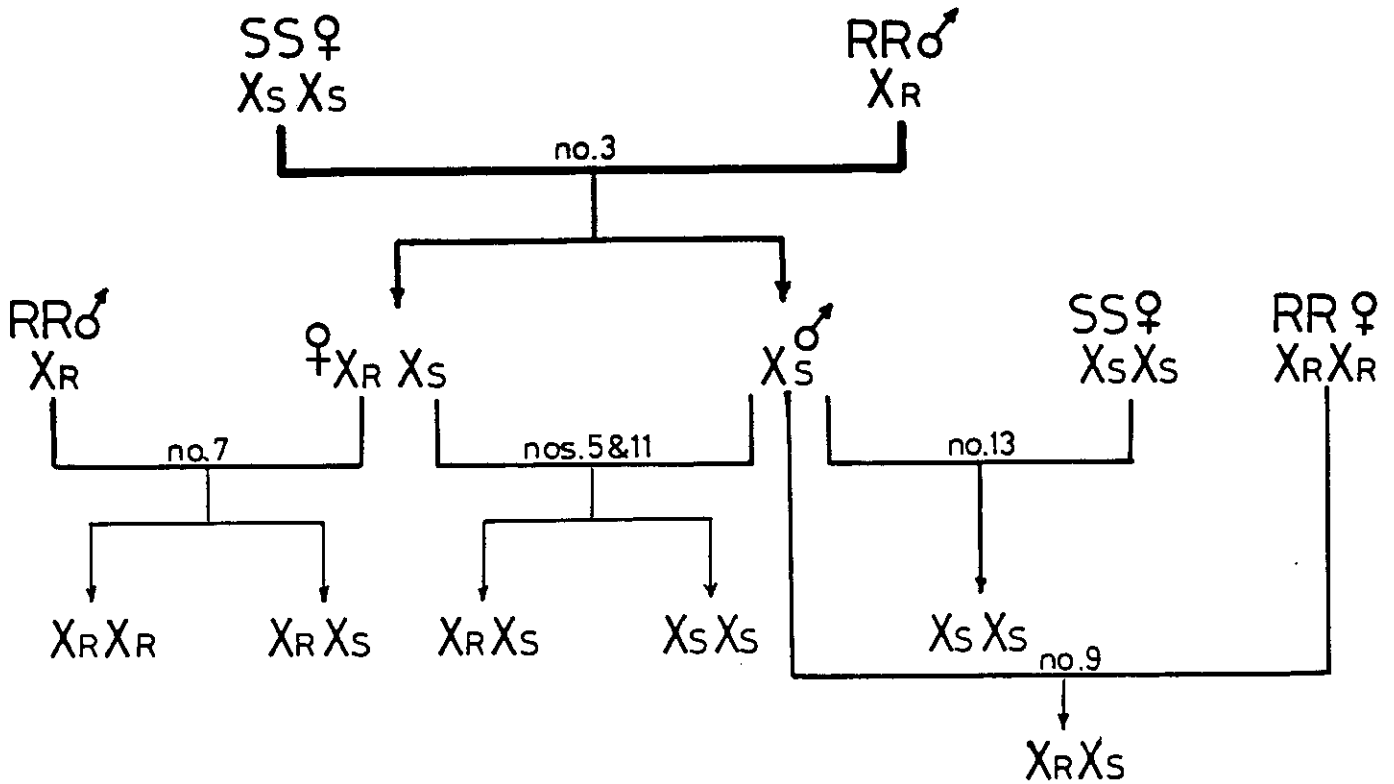

SS♀
XS XS
RR♂
XR
no.3
RR♂
XR
♀XR XS
XS♂
SS♀
XS XS
RR♀
XR XR
no.7
nos.5&11
no.13
XR XR
XR XS
XR XS
XS XS
XS XS
no.9
XR XS

Fig. (13)

bility than in the F_1 hybrids. In addition, there was generally a difference in the number of L_3 larvae per infective mosquito between the proportion of mosquitoes positive for infection and showed the intensity of infection in positive mosquitoes. This difference was higher in the backcrosses to SS than in that to RR and also higher in crosses 2 and 3 which had high infective percentages than in crosses 1 and 4 with lower infective percentages.

Figure (11) showed the autosomal composition of the progeny of cross numbers 3-14, while Figs. (12 and 13) showed the X-chromosome composition of the female progeny of these crosses. The autosomal composition of all backcrosses to RR males or females was the same, and similarly for all backcrosses to SS and for the two F_2 generations. However, the F_1 males inherit only one X chromosome (from their female parent) and pass this to all their female progeny. Thus the F_1 males from the two reciprocal crosses produce female progeny with different X chromosome compositions. On comparing the observed percentage susceptibility with that expected on the hypothesis of control of susceptibility/refractoriness by a single gene on the X chromosome or an autosome, the average susceptibility of the two reciprocal crosses was estimated (Table 19).

Table (19): Observed precentage susceptibilities from the reciprocal crosses, F_2 generations and backcrosses and expectations where refractoriness is dependent on a partially recessive genetic factor (heterozygote showing 82.78% susceptibility). Expectations are shown if the factor is either sex linked or autosomal

Crosses	Cross number	Actual (%)	If sex-linked (%)	If Autosomal (%)
F_1 generations:				
RR♂♂ X SS♀♀	3	93.33	82.78	82.78
SS♂♂ X RR♀♀	4	72.22	82.78	82.78
F_2 generations:				
Intercrossing F_1 (RR♂♂ X SS♀♀)	5	64.71	78.18	73.16
Intercrossing F_1 (SS♂♂ X RR♀♀)	6	64.52	68.14	73.16
Backcrosses to refractory stock:				
RR♂♂ X F_1♀♀ (RR♂♂ X SS♀♀)	7	33.33	68.14	68.14
RR♂♂ X F_1♀♀ (SS♂♂ X RR♀♀)	8	44.44	68.14	68.14
F_1♂♂ (RR♂♂ X SS♀♀) X RR♀♀	9	35.71	82.78	68.14
F_1♂♂ (SS♂♂ X RR♀♀) X RR♀♀	10	40.0	53.49	68.14
Backcrosses to susceptible stock:				
SS♂♂ X F_1♀♀ (RR♂♂ X SS♀♀)	11	72.73	78.18	78.18
SS♂♂ X F_1♀♀ (SS♂♂ X RR♀♀)	12	68.18	78.18	78.18
F_1♂♂ (RR♂♂ X SS♀♀) X SS♀♀	13	50.0	73.58	78.18
F_1♂♂ (SS♂♂ X RR♀♀) X SS♀♀	14	80.0	82.78	78.18

Results in table (19) show that the average was 82.78% and this percentage could be used as the expected susceptibility of heterozygotes for the supposed single gene. The observed and expected susceptibilities in table (19) were almost in agreement with the sex-linked gene and not to the autosomal gene.

D. Evaluation of the influence of different media on the ejection of L_3 larvae:

Ejection of L_3 larvae from the infective females during filaria transmission was tested under different stimulating media. Seven different media were tested. The obtained results are presented in tables 20 and 21, as well as graphically illustrated in Fig. (14).

Table (20): Effect of different media on the stimulation of ejection (infective larvae) of infected females Cx. pipiens with W. bancrofti by using capillary feeding technique.

Types of media	Number of females tested		
	No. of infected mosquitoes with L_3	Transmitters	Non-transmitters
1- Air	20	--	20
2- Water	20	4	16
3- Blood	20	7	13
4- Sweat	20	13	7
5- Sugar solution	20	5	15
6- (Sweat+blood)	20	8	12
7- (Sugar soln.+ blood)	20	6	14

Data in table (20) indicate that ejection of L_3 larvae of W. bancrofti was highly stimulated by sweat (13 transmitters) while air, females failed to eject L_3 larvae. The 2 nd preferred medium is sweat plus blood (8 transmitters) then blood (7 transmitters) while the 4 th, 5 th and 6 th media are (sugar solution + blood), sugar solution and water respectively. The females were ejected only fluid in air and it did not make any stimulation for ejection of L_3 larvae. This fluid may be saliva or is most probably haemolymph.

Through the dissection of infected females, after capillary feeding, the number of non-ejected L_3 larvae in the proboscis, head, thorax and abdomen, as presented in table (21).

Table (21): Effect of different media on the ejection of L_3 larvae per female Cx. pipiens infected with W. bancrofti.

Types of media	No. of ejected L_3/♀	No. of non-ejected L_3/♀		
		Proboscis (n)	Head (n)	Thorax & abdomen (n)
1. Air	--	1.7 (6)	1.3 (14)	1.6 (10)
2. Water	2.0	1.4 (10)	1.8 (10)	2.9 (12)
3. Blood	2.6	1.0 (3)	1.7 (8)	2.5 (6)
4. Sweat	3.8	1.0 (3)	1.0 (8)	1.5 (8)
5. Sugar solution	2.4	1.3 (4)	1.8 (12)	2.9 (9)
6. (Sweat + blood)	3.6	1.2 (5)	1.6 (8)	1.3 (4)
7. (Sugar soln. + blood)	3.5	1.5 (6)	3.6 (3)	1.3 (18)

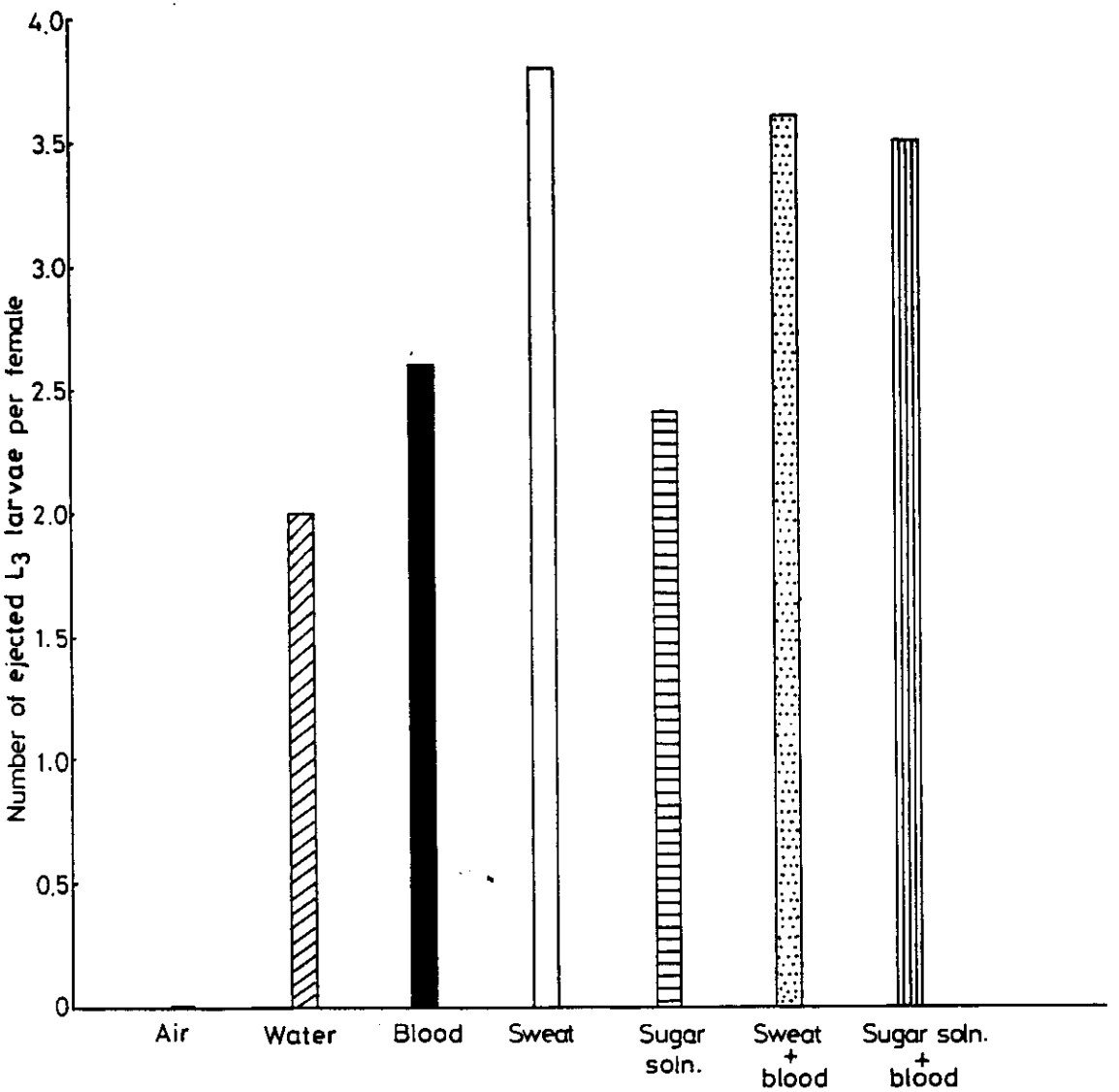

4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Number of ejected L_3 larvae per female
Air
Water
Blood
Sweat
Sugar soln.
Sweat + blood
Sugar soln. + blood
Type of media

Fig.(14)

E. Biochemical differentiation between susceptible and refractory groups of Cx. pipiens to W. bancrofti infection:

Diferentiation between susceptible and refractory strains of Cx. pipiens to W. bancrofti infection made by two types of electrophoretic techniques. Starch-gel electrophoresis was used for characterization of enzymes and SDS polyacrylamide gel electrophoresis for the separation of protein subunits and the estimation of their molecular weights.

1. Characterization of enzymes:

Data were recorded immediately after staining. Using the technique described previously, zymograms of the studied mosquito species appeared as follows:

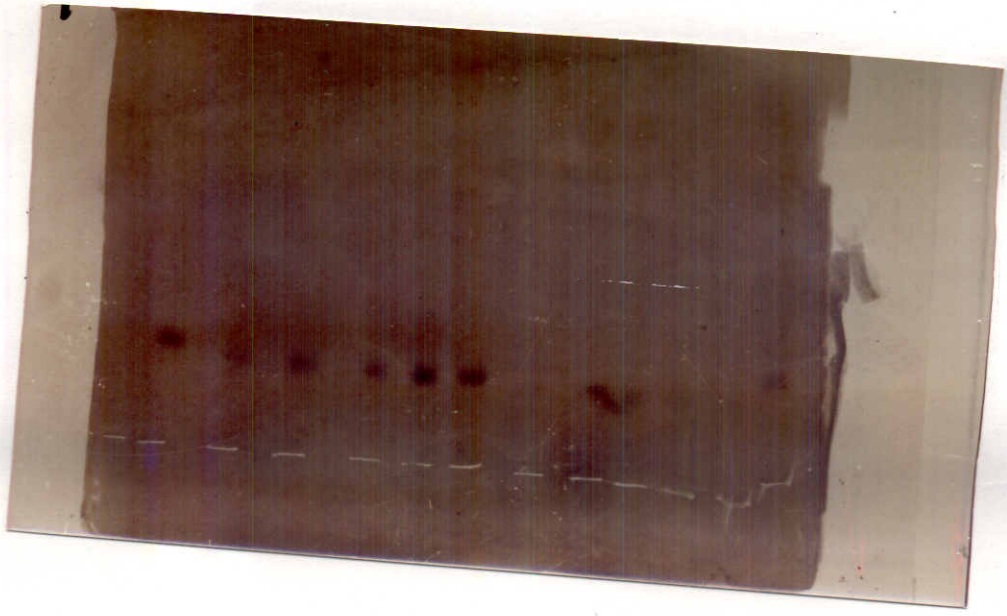
1. 1. α-glycerophosphate dehydrogenase, α-GPD.

Analysis of zymogram for α-glycerophosphate dehydrogenase proved that no variation was detected between susceptible and refractory samples. All samples of the two strains showed that all allozymes had equal migration rates. The individual homogenates in susceptible and refractory strains showed (distinctly) 3 bands for all samples.

Fig. 15: Aldehyde oxidase patterns of susceptible and refractory strains.

(a) Concentrated bands (dark staining) of susceptible strain and no bands detected in the refractory strain.

(b) Concentrated bands (dark staining) of susceptible strain and undetectable bands in the refractory strain.



(a)

(b)

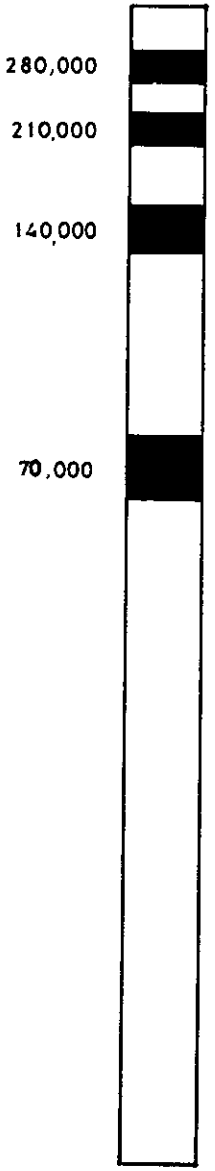
Fig. (15)

by studing Me zymogram. All individual hemogenates in the two strains showed one zone of activity and all allozymes had equal relative mobility.

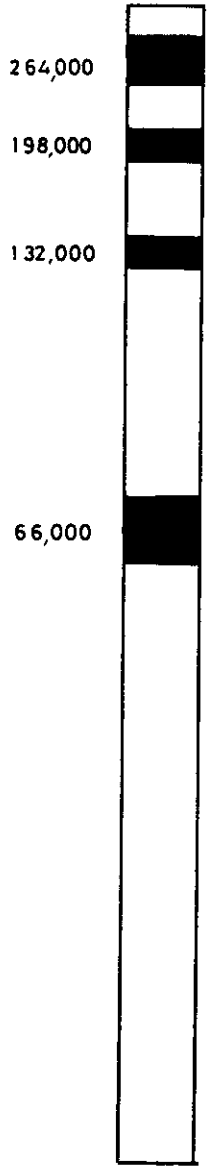
2. Characterization of protein subunits:

Molecular weights of the obtained protein subunits were calculated using the standard known proteins as a comparative measurment. Three standard proteins were used to establish the standard protein curve, namely; Hemocyanin, Albumin and Hemoglobin (Fig. 16). From the obtained protein fractions, the logarithmic slope representing these fractions was illustrated (Fig. 17).

The resulting separated protein subunites in the susceptible and refractory strains as represented by five generations only (F_1, F_2, F_3, F_4, F_7) which are illustrated in Figs. (18-22).



Hemocyanin



Albumin (Bovine)

Fig. (16)

Hemoglobin (Bovine)

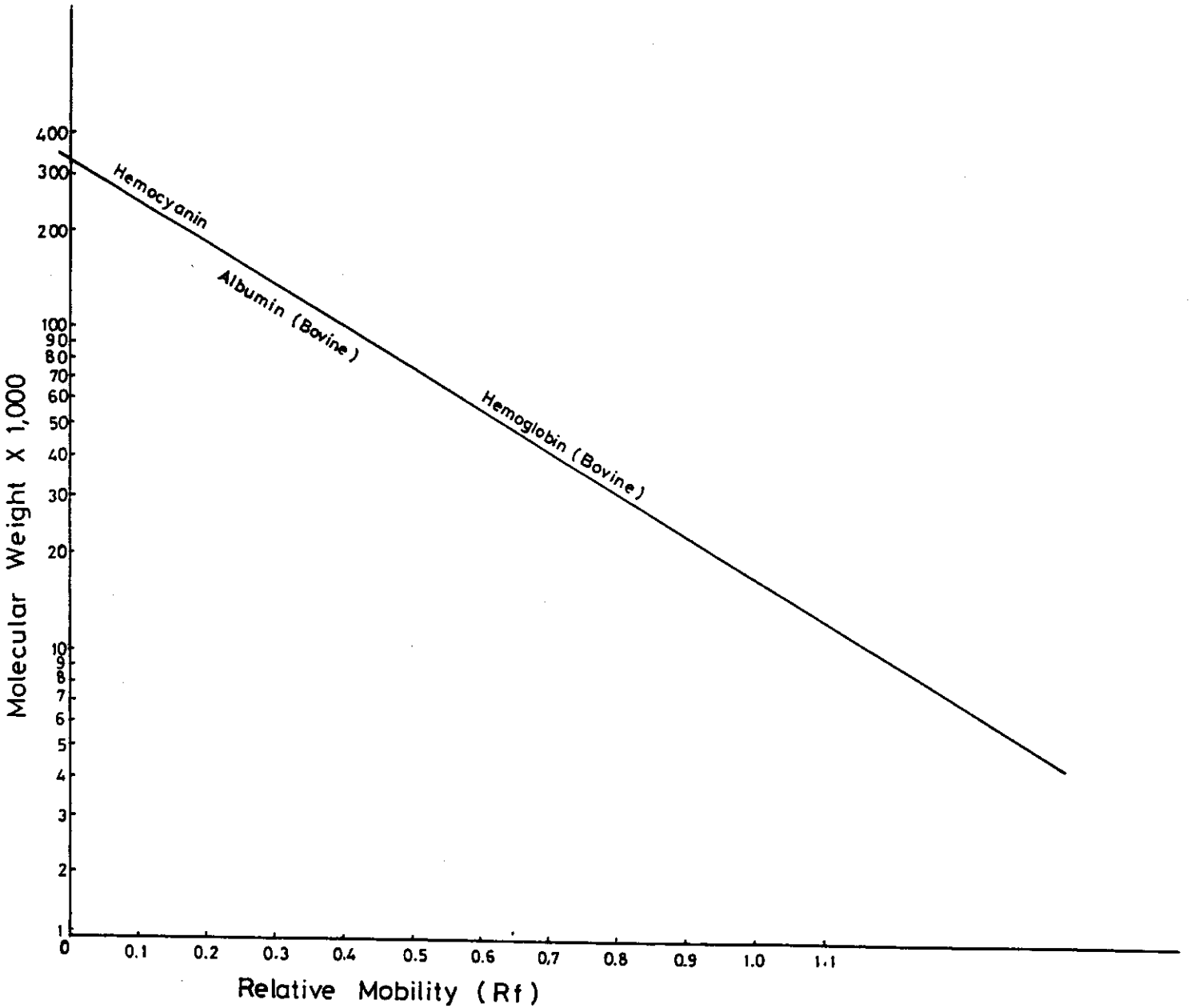

Molecular Weight X 1,000
400
300
200
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
0
Hemocyanin
Albumin (Bovine)
Hemoglobin (Bovine)
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
Relative Mobility (Rf)

Fig. (17)

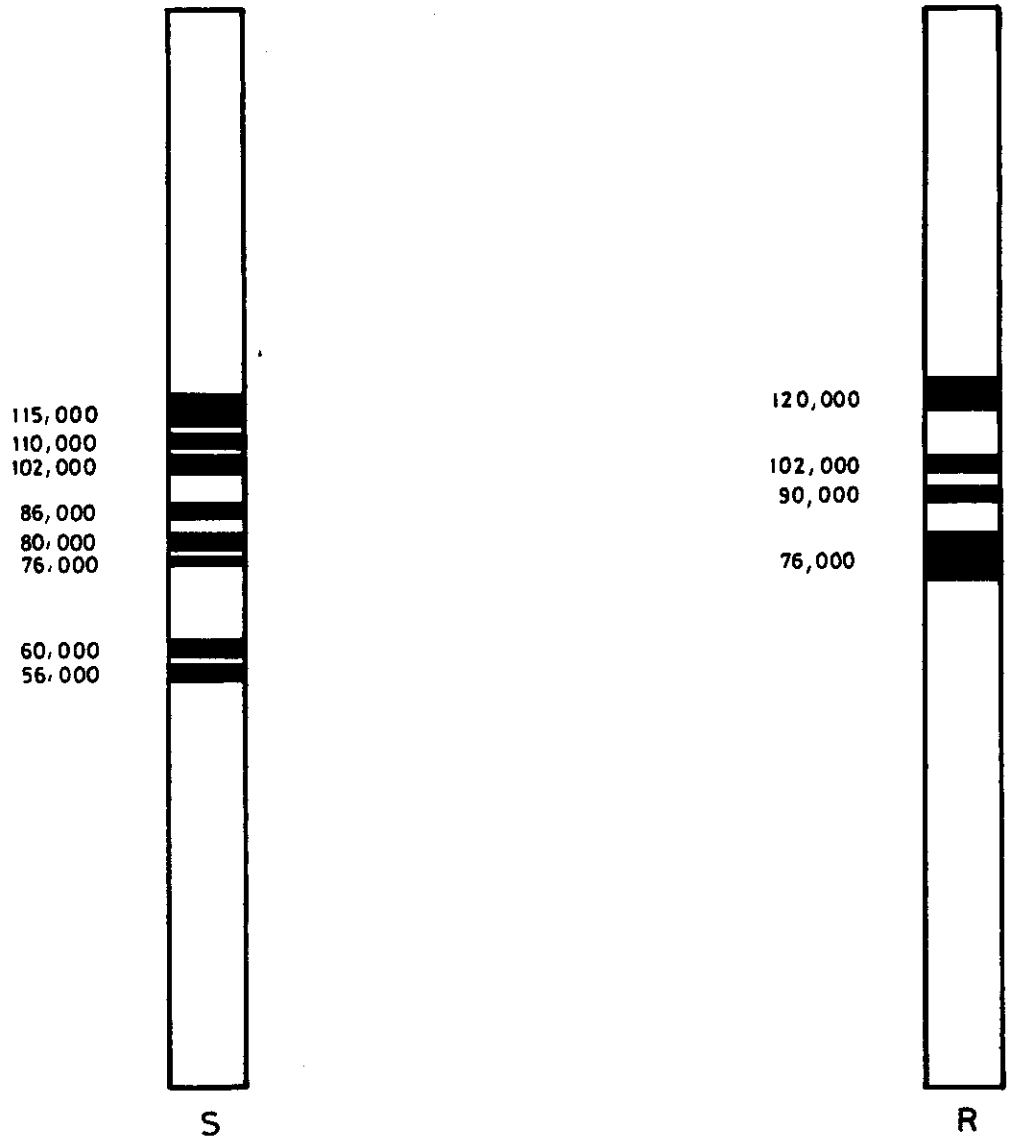

115,000
110,000
102,000
86,000
80,000
76,000
60,000
56,000
S
120,000
102,000
90,000
76,000
R

Fig. (18)

2. 1. (F_1) Subunits

It is clear from Fig. (18) that in F_1 generation, eight developed protein fractions were recognized in the susceptible group while only four were observed in the refractory group. The estimated molecular weights indicate similarity of 2 bands in both the susceptible and refractroy groups namely; 102,000 and 76,000 dalton while the other bands in the two groups are different.

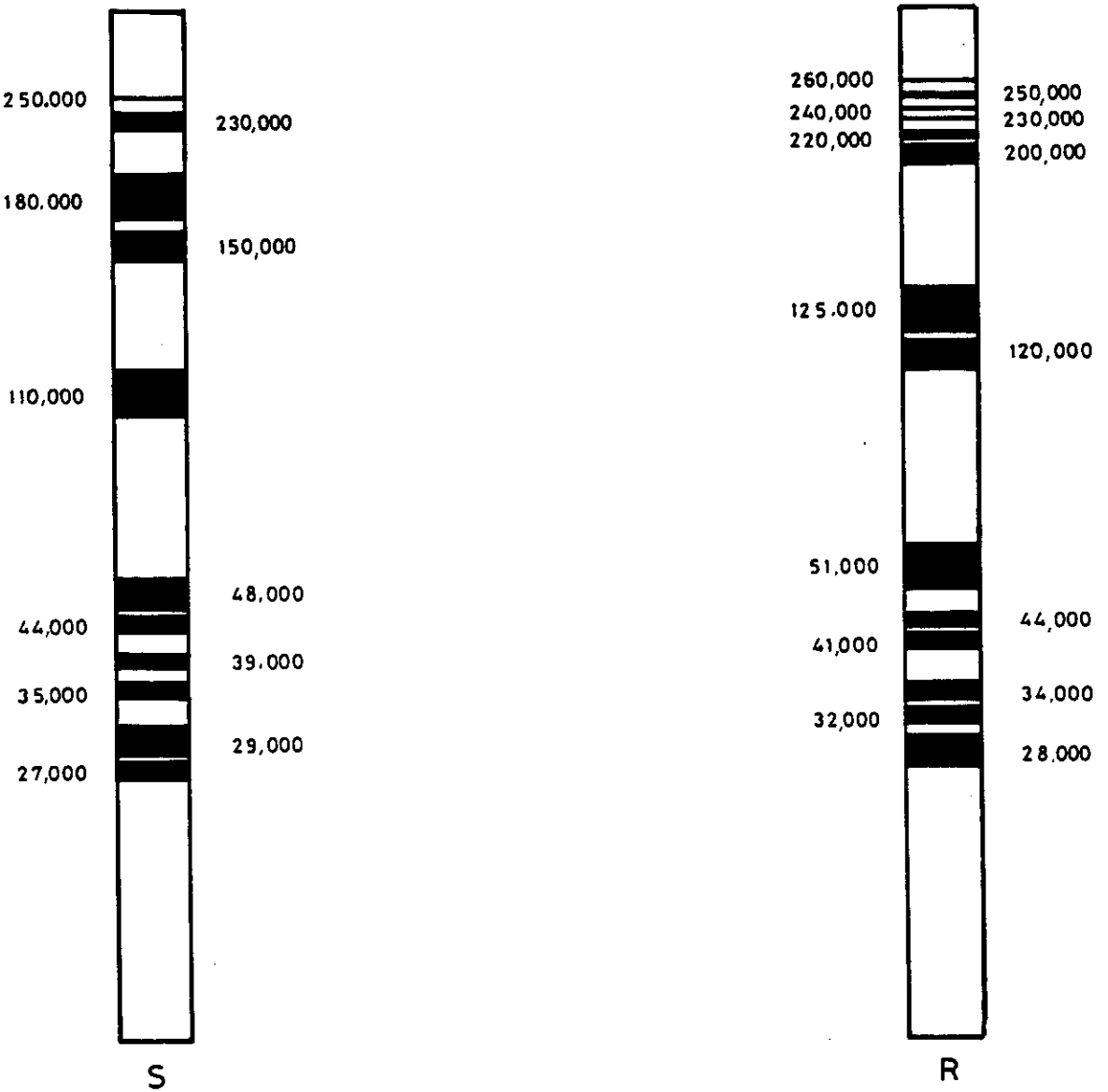
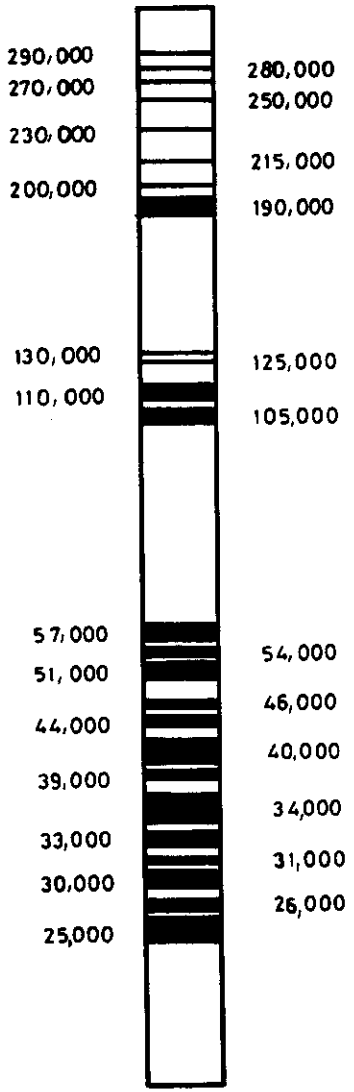

250.000
230,000
180.000
150,000
110,000
48,000
44,000
39.000
35,000
29,000
27,000
S
260,000
240,000
220,000
250,000
230,000
200,000
125.000
120,000
51,000
44,000
41,000
34,000
32,000
28.000
R

Fig. (19)

2. 2. $\underline{(F_2)}$ Subunits

The separated protein subunits of the second generation (F_2) for susceptible and refractory groups are represented as stained bands on the electrophoretic gel, as illustrated in Fig. (19). Eleven developed protein fractions were recognized in the susceptible group while 14 bands were observed in the refractory group. There are 3 bands in both the susceptible and refractory groups have the same molecular weights that are 250,000 , 230,000 and 44,000 dalton but the other bands in the two groups are different.


290,000
270,000
230,000
200,000
130,000
110,000
57,000
51,000
44,000
39,000
33,000
30,000
25,000
280,000
250,000
215,000
190,000
125,000
105,000
54,000
46,000
40,000
34,000
31,000
26,000
S

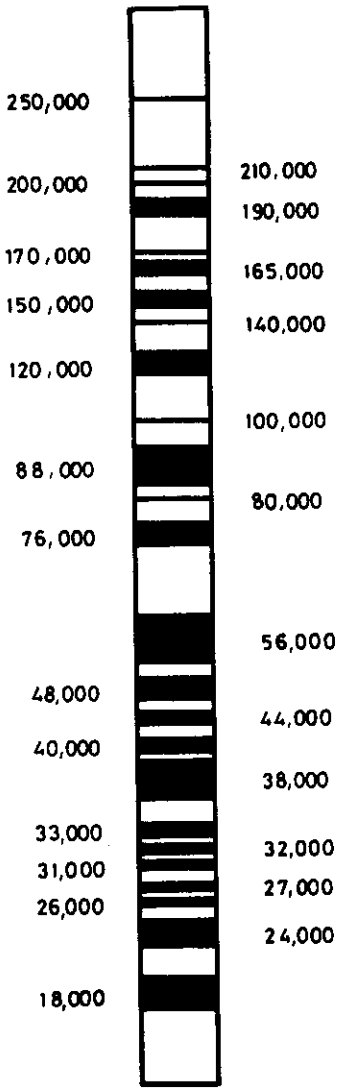
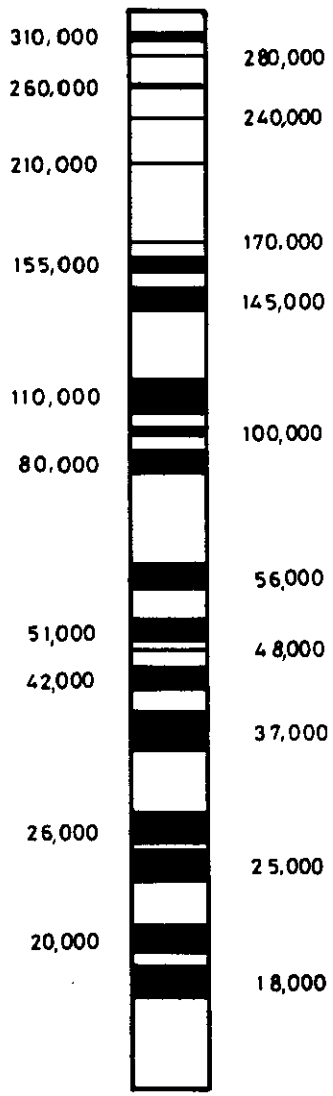

250,000
200,000
170,000
150,000
120,000
88,000
76,000
48,000
40,000
33,000
31,000
26,000
18,000
210,000
190,000
165,000
140,000
100,000
80,000
56,000
44,000
38,000
32,000
27,000
24,000
R

Fig. (20)

2. 3. (F_3) Subunits

Electrophoretic run of the 3 rd generation (F_3) for susceptible and refractory groups resulting separated protein subunits as indicated by stained protein bands are presented in Fig. (20).

From Fig. (20) that 25 developed protein bands were recognized in both the susceptible and refractory groups. The estimated molecular weights indicate similarity of 8 bands in both susceptible and refractory groups which are; 250,000 , 200,000 , 190,000 , 44,000 , 40,000 , 33,000 , 31,000 and 26,000 dalton while the other bands in the two groups are different.


310,000
260,000
210,000
155,000
110,000
80,000
51,000
42,000
26,000
20,000
280,000
240,000
170,000
145,000
100,000
56,000
48,000
37,000
25,000
18,000
S

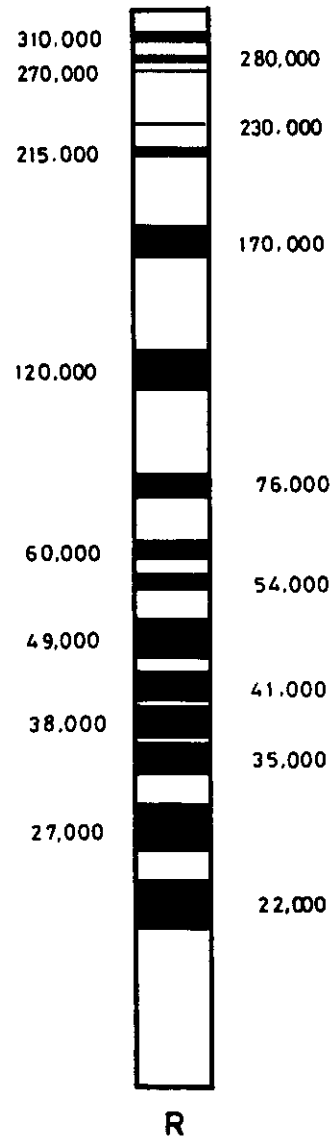
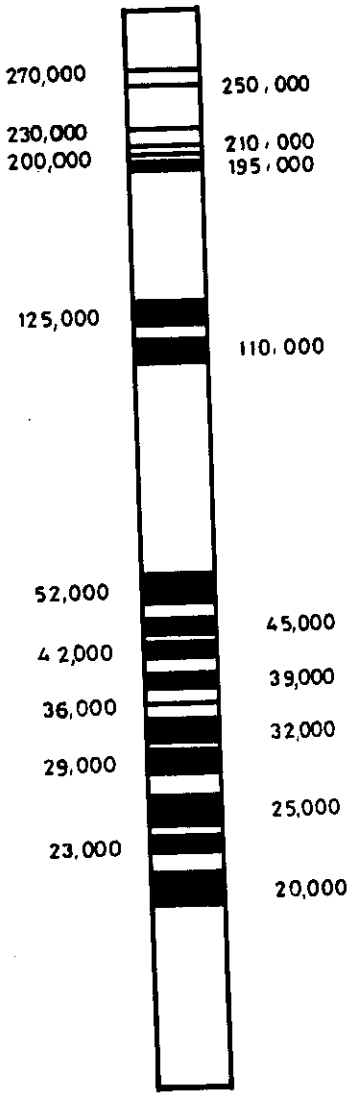

310.000
270.000
215.000
120.000
60.000
49.000
38.000
27.000
280,000
230.000
170.000
76.000
54.000
41.000
35.000
22,000
R

Fig. (21)

2. 4. (F_4) Subunits

The resulting separated protein subunits of the 4 th generation (F_4) for the susceptible and refractory groups as represented by stained protein bands in the electrophoretic gel are illustrated in Fig. (21).

Figure (21) showed that 20 protein fractions were observed in the susceptible strain but 16 fractions were recognized in the refractory strain. There are 3 bands in both susceptible and refractory strains have the same molecular weights that are; 310,000 , 280,000 and 170,000 dalton while the other bands in the two strains are different.


270,000
250,000
230,000
210,000
200,000
195,000
125,000
110,000
52,000
45,000
42,000
39,000
36,000
32,000
29,000
25,000
23,000
20,000
S

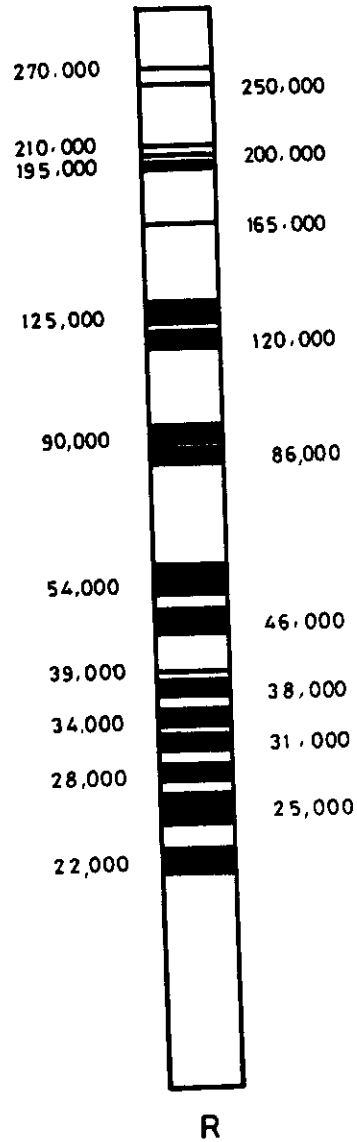

270,000
250,000
210,000
200,000
195,000
165,000
125,000
120,000
90,000
86,000
54,000
46,000
39,000
38,000
34,000
31,000
28,000
25,000
22,000
R

Fig. (22)

2. 5. (F_7) Subunits

It is obvious from Fig. (22) that in F_7 generation, 18 developed protein bands were observed in the susceptible strain while 19 bands were recognized in the refractory strain. The estimated molecular weights indicate similarity of 8 bands in both susceptible and refractory groups namely; 270,000 , 250,000 , 210,000 , 200,000 , 195,000 , 125,000 , 39,000 and 25,000 dalton while the other bands in the two groups are different.

The estimated molecular weights of protein subunits in all generations of susceptible and refractory groups were arranged and segregated to specify the proteins of both groups. Results are presented in table (22).

Table (22): The estimated molecular weights of proteins specific for susceptible and refractory groups.

Susceptible strain		Refractory strain	
Molecular weights	generations	Molecular weights	generations
20,000	F_4-F_7	22,000	F_4-F_7
23,000	F_7	24,000	F_3
29,000	F_2-F_7	28,000	F_2-F_7
30,000	F_3	38,000	F_3-F_4-F_7
36,000	F_7	41,000	F_2-F_4
37,000	F_4	46,000	F_7
42,000	F_4-F_7	49,000	F_4
45,000	F_7	88,000	F_3
52,000	F_7	90,000	F_1-F_7
57,000	F_3	120,000	F_1-F_2-F_3-F_4-F_7
105,000	F_3	140,000	F_3
110,000	F_1-F_2-F_3-F_4-F_7	165,000	F_3-F_7
115,000	F_1	220,000	F_2
145,000	F_4		
155,000	F_4		
180,000	F_2-F_7		
290,000	F_3		

Data of table (22) indicate that there are 17 proteins in the susceptible strain and are not present in the refractory one while 13 proteins are found in the refractory strain and are not repeated in the susceptible group. In addition, there is a specific protein repeated in all generations of the susceptible strain, which has a molecular weight 110,000 dalton. Accordingly, there is a specific protein repeated in all generations of the refractory strain, which has a molecular weight 120,000 dalton.

In addition, it was found that there is a relationship between proteins in the susceptible and refractory groups. there are 39 common proteins present in the susceptible and refractory groups.