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EFFECT OF TEMPERATURE, HEAT-SHOCK AND GENETIC COMBINATIONS ON THE *rosy* LOCUS IN *DROSOPHILA*

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ABSTRACT

The aim of the present investigation was to study the developmental genetic effects of temperature, heat-shock and genetic combinations on *rosy (ry)* locus in *Drosophila*. *rosy* proved to be one of the most important genes in *Drosophila* because *rosy* eye-coloured flies, from the homozygous viable *rosy*^{506}/*rosy*^{506}, were the background M cytotype strain used in genetic transformation via P-element mediated method. So, the research was focused on determining the suitable developmental time of gene expression. The approach of temperature sensitivity as well as the gene-dose dependent interaction technique were applied in this respect.

As it revealed from the results, the lethal phase analysis for [*ry506/Df(3)ry81*], [*oho-31/+;ry*^{506}/+], [*oho-31/+;Df(3)ry81/+*], [arm^{k_2} / ry^{506} /+] and [arm^{k_2} /+ ;*Df(3)ry81/+*] genetic combinations were shown to take place during larval stage of *Drosophila*. Temperature sensitivity studies also showed that the most effective and proper time of gene expression starts as early in the development as in fertilized eggs and lasts up to the end of the third larval instar. Heat-shock experiments and data about the formation of white puparium indicated that the products of the *rosy* gene expression are mostly needed during the first 40→100 hrs of the development of the insect. Results about the (oho-3,ry) and (arm,ry) genetic interactions suggested that the products of these three genes have to be supplied in sufficient concentrations to maintain the normal phenotype of the animal. Overall, the *rosy* locus, as a factor on the third chromosome, seemed to have an effect on the level of both *arm* and *oho-31* gene expression in *Drosophila*. These findings have relevance to possible future applications of these methods in very important genetic loci in insects and mammalian.

INTRODUCTION

Development is the cumulative effect of dynamic changes in gene expression in different cells within an organism. At present, several techniques exist that allow an examination of gene expression in *Drosophila* through heat-sensitivity and genetic interactions (Yeh *et al*, 1995; Mamon *et al*., 1998; Wang *et al*., 1999; Bouley *et al*., 2000). The potential to genetically reach this goal constitutes one of the major reasons to study these approaches in *Drosophila* (Homyk *et al*., 1986; Botas *et al*., 1982). The *rosy* locus, on the third chromosome of *Drosophila*, is the structural gene of xanthine dehydrogenase (XDH) which is not synthesized in the adult eye, but is transported there. That is because the enzyme levels climb from low levels in the zygote to a peak at puparium formation and then drop down in the adult fly (Hoffman and Corces, 1984; Samson and Wegnez, 1988). *rosy* eye-colored flies were used as a background (M cytotype) strain for introducing P elements to obtain transformed animals and to study its contribution in

sustantial new variations for the *Drosophila* important traits (Tiveron *et al*., 1991; Torkamanzehi *et al*., 1992; Maitra *et al*., 1996; Dombrowski *et al*., 1998; Sepp and Auld, 1999; Maitra *et al*., 2000).

On the other hand, *armadillo (arm)* is a homozygous embryonic lethal mutation in *Drosophila* with segmentation defects by time of germ-band shortening (Nusslein-Volhard and Wieschaus, 1980). The *arm* gene was found to be implicated in some genetic mechanisms with other genes in *Drosophila* (Fitzsimons *et al*., 1999; McCartney *et al*., 1999; Hatini *et al*., 2000; Uren *et al*., 2000). *arm* protein have a very highly conserved *motif* now known as *arm* motif which is important in cell-to-cell communications (Riggleman *et al*., 1989).

Genetic analysis of *Drosophila melanogaster* has led to the identification of more than 50 genes in which homozygous mutations cause tumors in different tissues of organisms body (Gateff, 1978; Torok *et al*., 1993). Most of these mutations affect genes controlling tissue overgrowth and they were selected by their recessive lethal effects. This is why they are designated as tumor suppressor genes (Mechler and Strand, 1990). o*ho-31* is one example of this kind of genes which was recovered in a genetic screen designed for identifying genes located on the *Drosophila* second chromosome (Torok *et al*., 1993). The *oho-31* gene was later cloned and sequenced and its protein was found to have a motif known as *arm* motif. The latter was first identified in the segment polarity gene *armadillo. oho-31* gene showed to be involved in nuclear protein import (Torok *et al*., 1995; Weis *et al*., 1996; Ollmann *et al*., 2000).

In *Drosophila,* gene-dose-dependent interactions as well as temperature sensitive (ts) lethal mutation approaches have been used extensively as means of experimental manipulation of gene expression. This allows one to delineate the developmental stages when specific essential gene products are required. Also facilitates determining both the spatial and temporal pattern of gene activity during development (Homyk *et al*., 1986). Moreover, by altering the duration of exposure to the restrictive temperature, it would be easy to follow the effects of a given (ts) mutation on specific developmental processes (Tsuruhara *et al*., 1990). Taken the above mentioned statements into consideration, the present study attempts to find out the developmental genetic role played by the gene *rosy* in itself and in its combinations with both *arm* and *oho-31* genes in *Drosophila*.

MATERIALS AND METHODS

I- Culture conditions:

Drosophila melanogastor strains and crosses were maintained at 25°C±1°C either in bottles or in vials. The standard *Drosophila* medium consisted of cornmeal, molase, agar and yeast. Propionic and acetic acids were added to the medium in rational quantities as inhibitors of mold growth. 18°C and 29°C were used as the restrictive and permissive temperatures, respectively unless stated elsewhere (Hamada, 1995). This work was done at the Genetics Department, Faculty of Agriculture, Mansoura University through 1999 and 2000.

II- *Drosophila melanogaster* strains:

Five different *Drosophila* strains were used in this investigation. They were *Oregon-R (Ore-R)* as a Wild Type stock, *rosy*^{506}*(ry*^{506}*)* as homozygous viable allele on the third chromosome, *Df(3)ry81/TM3Sb* as a deficiency in the *rosy* locus, *oho-31/CyO* as a ressesive lethal (tumor suppressor) mutation on the second chromosome and *armadillo* (*arm*) as an embryonic lethal mutation on the X chromosome affecting the segmented body pattern. Most of these strains were kindly provided by Prof. Dr. Istvan Kiss at Szeged, Hungary. For a detailed description of the strains, see Lindsley and Zimm, 1992.

III- Genetic crosses:-

a, The main genetic cross for the *rosy* locus was done as following:-

1- Males *ry506/ry506* ⊗ *Df(3)ry81/TM3Sb* Females

2- Females *ry506/ry506* ⊗ *Df(3)ry81/TM3Sb* Males

and the resulting offspring flies of both directed crosses should fill into two categories: *Stubble-haired* flies (*Sb*), with the genotype *ry506/TM3Sb*; and Wild type-like-haired flies (*Sb*$^{+}$), with the genotype *ry*^{506}*/Df(3)ry*^{81}. The latter is the important genotype and appears as *Sb*$^{+}$ flies.

b, The control cross in this case was:

Males or Females Wild Type (+/+) ⊗ *Df(3)ry81/TM3Sb* *Females or Males*

↓

+/Df(3)ry^{81} **+** *+/TM3Sb*

* Look for *Sb*$^{+}$ flies among *Sb* ones.

c, Another series of crosses were carried out in the aim of obtaining variant doses of the *rosy* gene in combination with other genes. The crosses were done as following:-

1- *ry506/ry506* ⊗ *oho-31/CyO*

+/+; *ry506/ry506* ⊗ *oho-31/CyO*; +/+

↓

$$\left[\left(\frac{oho-31}{+}+\frac{CyO}{+}\right);\left(\frac{ry^{506}}{+}\right)\right]^{Offsprin}_{[1:1\ \mathbf{and}\ 1:2]}$$

* Look for *Cy*$^{+}$ *ry*$^{+}$ males and females?

2- +/+; *Df(3)ry81/TM3Sb* ⊗ *oho-31/CyO*; +/+

↓

$$\left[\left(\frac{+}{oho-31}+\frac{+}{CyO}\right);\left(\frac{Df(3)ry^{81}}{+}+\frac{TM3Sb}{+}\right)\right]^{[1:1;1:2;2:1;2:2]}_{Offspring}$$

* Look for *Cy*$^{+}$ *Sb*$^{+}$ males and females?

3- +/Y;*ry506/ry506* ⊗ $\frac{arm^{K_2}}{FM7B}$; +/+ Females

↓

$$\left[\left(\frac{+}{arm^{k_2}}+\frac{+}{FM7B}+\frac{arm^{k_2}}{Y_{dies}}+\frac{FM7B}{Y_{dies}}\right);\left(\frac{ry^{506}}{+}\right)\right]^{\mathbf{offspring}}_{[1:1\ \mathbf{and}\ 1:2]}$$

* Look for B^{+}rosy^{+} females?

4- +/Y; *Df(3)ry81/TM3Sb* $\otimes \frac{arm^{k_2}}{FM7B}$;+ /+ Females

$$\left[\left(\frac{+}{arm^{k_2}}+\frac{+}{FM7B}+\frac{arm^{k_2}}{Y_{dies}}+\frac{FM7B}{Y_{dies}}\right);\left(\frac{Df(3)ry^{81}}{+}+\frac{TM3Sb}{+}\right)\right]^{offspring}_{[1:1,1:2,2:1,2:2]}$$

* Look for B^+Sb^+ Females?

IV- Developmental analysis:

a); Analysis of the lethal period:-

The lethal phase analyses both for the *rosy* locus alone and for the *rosy* gene in combinations with arm^{k_2} and *oho-31* genes were performed according to Hamada, 1995. The whole procedure can be summarized as follows:-

1- Eggs from fertilized females belonging to a specific genotype, were collected, counted and kept at the suitable temperature.
2- After 2 days which allowed complete hatching, the unhatched eggs were counted and the number of hatched larvae was then registered.
3- The number of formed pupae was also counted at the proper time.
4- Number of eclosed flies was determined as found.

The lethal phase analysis was also done for the control cross.

b); Temperature shift studies:-

The temperature sensitive period for all the genotypes under study was done as follows:-

1- Eggs were collected every 12 hrs or 4 hrs intervals at 25°C.
2- Eggs were kept for different times at 29°C or 18°C before shifting down or up, respectively.
3- Then eggs were kept on the proper temperature for the rest of the development.
4- Shift-up experiments define the beginning of the lethality or gene interaction, whereas, shift-down defines the end of it (Homyk *et al*., 1986).

c); Heat-shock experiments:-

Eggs were collected in vials from the proper cross (at 12 hrs intervals) at 25°C and then shifted up to 29°C for three days. The vials containing eggs and/or larvae were heat-shocked for one hour at 37°C then returned immediately to 18°C. After 1 hrs at 18°C, heat-shocked again for another one hour at 37°C then returned back to 18°C and so on in one experiment. In the other experiment, the vials with eggs and/or larvae were heat-shocked for 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 hrs. at 37°C and then returned back to 29°C until the time of flies eclosion (Hoffman and Corces, 1984).

d); White puparium:-

By the end of third larval instar of *Drosophila* development, white puparium formation takes place. It was counted both at 29°C and 18°C every 4 or 6 hrs starting from the time of forming the first one in every cross (Hamada, 1995).

RESULTS AND DISCUSSION

I- Analysis of the lethal period:-

a) Lethal phase analysis for *ry506/Df(3)ry81* genotype:-

As shown in Table 1, the majority of lethality occurred during the larval period, however, some lethality did happened causing embryonic and pupal death. As the cross, yields theoretically, *Sb* and Sb^+ flies and knowing that *Sb* flies (in this case: *ry506/TM3Sb*) are mostly genetically viable, the larval lethality has to be due to the Sb^+ flies (*ry506/Df(3)ry81*, in this case). In addition, larval lethality seemed to be a little bit higher than expected but this may be caused by a lower than normal viability of the *Sb* flies. The 15.6% embryonic lethality could also mean that the lethality of *ry506/Df(3)ry81* gradually starts at the end of embryonic phase and lasts up to the end of larval stage.

Table 1. Lethal Phase Analysis for *ry506/Df(3)ry81*.

Specimens				
Notes	**Fertilized eggs**	**Larvae**	**Pupae**	**Adults**
Number	4672	3943	2294	2233
%	100	84.4	49.1	47.8

1- Only fertilized females were involved in the experiment.

b), Lethal phase analysis for *rosy* gene as combined either with *arm* or *oho-31* genes at different temperatures:-

In general, data in Table 2 showed that temperature affected all the genetic combinations causing lethality which took place during larval stage. The larval lethality was always higher at 29°C than at 25°C and 18°C. This result could mean that the higher temperature (29°C) is restrictive to the timing of gene action for all tested genetic combinations. Testing the eclosed adult flies, especially from crosses 2 and 4, revealed that there were no survivors belonging to [(*oho-31*/+); (*Df(3)ry81*/+)] or [(arm^{k_2}/+); (*Df(3)ry81*/+)] genotypes, respectively, among the offspring flies. Therefore, it could be deduced that larval lethality resulted only from these combinations.

The results of lethal phase analysis (Tables 1 and 2), suggest that larvae neither from (*ry506/Df(3)ry81*) genotype nor from [(*oho-31*/+); (*Df(3)ry81*/+)], [(arm^{k_2}/+)], [(arm^{k_2}/+);(*Df(3)ry81*/+)] genetic combinations could pupariate. This was in accordance with the expectations based on that the temperature sensitive period corresponds to the larval and may be embryonic expression periods of the tested genes. These findings are in agreement with Riggleman *et al*., 1989; Tiveron *et al*., 1991; Yeh *et al*., 1995; Wang *et al*., 1999 and Carney and Bender, 2000).

II- Temperature sensitivity studies:

a) Shift up-Shift down experiments:

1- Shift down (29°C→18°C):-

The temperature sensitivity of the (*ry506/Df(3)ry81*) genotype was tested by applying shift up-shift down experiments. As shown in Table 3, there was a cold sensitive period for *ry506/Df(3)ry-81* to 18°C which started as early in development as the fertilized egg and lasted up to the third-fourth day. So, this shift down experiment defines the end of cold sensitivity period of the *rosy* gene as the fourth day on 29°C. However, such effect was not found in the control experiment using ORE-R as can be shown also in Table 3. Also data represented a peak of gene activity in the 5th day of development. From the above mentioned results it can be concluded that the time of gene action (expression) for the *rosy* gene could be between the 3rd and 6th day of development at 29°C.

Table 2. Lethal phase analysis for *rosy* gene combinations with arm^{k_2} and *oho-31* genes at different temperatures.

1 Crosses	2 % Expected lethality	Temperature °C	Specimens							
			Fertilized eggs		Larvae		Pupae		Adults	
			Number	%	Number	%	Number	%	Number	%
1	50%	18	1264	100	1121	88.7	763	60.4	748	59.2
		25	2618	100	2395	91.5	1683	64.3	1667	63.7
		29	970	100	828	85.4	563	58.0	550	56.7
2	25%	18	2060	100	1793	87.0	1460	70.9	1417	68.8
		25	2873	100	2533	88.2	2086	72.6	2066	71.9
		29	1064	100	909	85.4	729	68.5	705	66.3
3	50% in females	18	1815	100	1053	58.0	615	33.9	602	33.2
		25	2344	100	1376	58.7	815	34.8	792	33.8
		29	886	100	508	57.3	288	32.5	275	31.0
4	50% in females	18	2365	100	546	23.1	260	11.0	238	10.1
		25	2917	100	700	24.0	335	11.5	303	10.4
		29	1211	100	270	22.3	125	10.3	118	9.7

1- For 1,2,3 and 4 see Materials and Methods.

2- % Expected lethality of the transheterozygous genotype of the two genes.

Table 3. Shift down of *Df(3)ry^{81} crossed both to rosy^{506}* and ORE-R flies at 29°C.

Time (in days)	*Sb*$^+$ flies	*Sb* flies	Total	*Sb*$^+$/*Sb*	% *Sb*$^+$
1	0 (630)	314 (613)	314 (1243)	0 (1.03)	0 (50.7)
2	0 (640)	286 (509)	286 (1149)	0 (1.25)	0 (55.7)
3	15 (516)	375 (353)	390 (869)	0.04 (1.46)	3.8 (59.4)
4	118 (406)	227 (372)	345 (778)	0.52 (1.09)	34.2 (52.2)
5	357 (410)	219 (353)	576 (763)	1.60 (1.16)	62.0 (53.7)
6	220 (397)	183 (323)	403 (720)	1.20 (1.23)	54.6 (55.1)
7	79 (888)	97 (721)	176 (1609)	0.81 (1.23)	44.9 (55.2)
8	304 (814)	380 (748)	684 (1562)	0.80 (1.09)	44.4 (52.1)

**** Sb$^+$: Either (ry506/Df(3)ry81) or (+/Df(3)ry^{81}) genotype***

**** Sb: Either (ry^{506}/TM6Sb) or (+/TM6Sb) genotype***

**** Number in brackets belong to the ORE-R cross as control experiment.***

2- Shift-up (18°C→29°C):-

Data from Table 4 showed that 18°C is not favourable by the (*ry506/Df(3)ry81*) genotype even if the *Drosophila* eggs were raised on 18°C for one day then shifted up to 29°C till complete development. Likewise, the percentage of the (*ry506/Df(3)ry81*) flies seemed to be lower than expected (50%). A transient period of gene activity between the 9th and 11th day on 18°C was noticed. Besides, there was a more or less stable period of gene activity between days 3 up to 8. Data also showed that the activity of the *rosy* gene dropped down to zero starting from the 12th day on. From these results it can be concluded that the proper time of gene action corresponded well with the previous data in Table 3. This conclusion could be understood well if days of development both at 29°C and at 18°C are converted into 25°C (Ashburner and Thompson, 1978).

Table 4: Shift up of (*ry506/Df(3)ry81*) genotype at 18°C.

Time (in days)	Sb^+	*Sb*	Total	Sb^+/Sb	Sb^+%
1	283	485	768	0.58	36.85
2	330	613	943	0.54	34.99
3	203	525	728	0.39	27.88
4	242	718	960	0.34	25.21
5	167	503	670	0.33	24.93
6	222	699	921	0.32	24.10
7	186	620	806	0.30	23.08
8	137	540	677	0.25	20.24
9	98	552	650	0.18	15.08
10	61	738	799	0.08	7.63
11	7	614	621	0.01	1.13
12	0	430	430	0	0
13	0	401	401	0	0
14	0	362	362	0	0
15	0	428	428	0	0

Sb^+: ry506/Df(3)ry81. ***Sb: ry^{506}/TM6Sb***

3- Shift up-down-up and shift down-up-down:-

Data about this type of shift are present in Table 5. As shown, shifting down to 18°C as a restrictive temperature, 96→120 hrs showed the lower Sb^+/Sb ratios as well as the lower Sb^+%. It was (0.09 and 7.89%), (0.06 and 6.12%) and (0.06 and 5.66%), respectively.

Converting the time of 96, 108 and 120 hrs, we would reach to the conclusion that the proper time of *rosy* gene action could be between 3rd-6th days of its development at 29°C.

On the other hand, shift down-up-down data showed that one day at 29°C after 7 days at 18°C was not enough to rescue the *ry506/Df(3)ry81* genotype (Sb^+ =1.48%). Whereas, increasing the time on permissive temperature led to the increasing of Sb^+% up to more than 26%. Again, this type of data confirms that the proper developmental time of the *ry506/Df(3)ry81* genotype lies between the third and sixth day at 29°C.

Table 5:Shift up-down-up and shift down-up-down of the *rosy* locus in *Drosophila.*

Type of shift	Temp. °C	Time (in hrs)	*Sb*$^+$	*Sb*	Total	*Sb*$^+$/*Sb*	*Sb*$^+$%
Up-down-up	1	48	110	272	382	0.40	28.80
		60	90	231	321	0.39	28.04
		72	35	91	126	0.38	27.78
	29	84	66	252	318	0.26	20.75
		96	3	35	38	0.09	7.89
		108	3	46	49	0.06	6.12
		120	3	50	53	0.06	5.66
Down-up-down	2	24	4	265	269	0.01	1.48
		48	43	310	353	0.13	12.18
		72	58	317	375	0.18	15.47
	18	96	81	272	353	0.30	22.95
		120	99	281	380	0.35	26.05

1: Eggs were shifted up to 29°C for 3 days before shifting down to these different times.
2: Eggs were shifted down to 18°C for 7 days before shifting up to these different times.

b) Heat-shock experiments:-

As mentioned earlier, the (*ry506/Df(3)ry81*) genotype behaves badly at 18°C as a restrictive temperature for it. So, in the heat-shock experiment, when the heat-shocked eggs (at 37°C) returned down to 18°C, no *Sb*$^+$ offspring flies were eclosed at all. This means that the heat-shock has no rescue effect on *ry506/Df(3)ry81* genotype which is very sensitive to 18°C during larval-pupal stages.

Table (6) shows the results obtained form another experiment in which heat-shocked eggs were returned back to 29°C until the adulthood stage. As it revealed, applying heat-shock at 37°C for one hour and 3 hours were favourable by the *rosy* locus for *Sb*$^+$ survival % and for *Sb*$^+$/*Sb* ratio, respectively if the development was completed at 29°C.

Table 6. Heat-shock of (*ry506/Df(3)ry81*) for different intervals at 37°C.

Time (hrs) at 37°C	*Sb*$^+$ flies	*Sb* flies	Total No. of flies	Dead purpae	Total	*Sb*$^+$/*Sb*	% S*	*Sb*$^+$ %	*Sb*%
0.5	43	60	103	411	514	0.72	20.04	8.37	11.67
1	58	52	110	385	495	1.12	22.22	11.72	10.50
1.5	42	32	74	362	436	1.31	16.97	9.63	7.34
2	26	50	76	427	503	0.52	15.11	5.17	9.94
2.5	47	95	142	446	588	0.49	24.15	7.99	16.16
3	58	35	93	439	532	1.66	17.48	10.90	6.58
3.5	47	121	168	504	672	0.39	25.00	6.99	18.01
4	49	122	171	428	599	0.40	28.55	8.18	20.37

***S%: Survival percentage of the flies after heat-shock.**

c) White puparium:-

It is well known that the puparium formation is the first major event of metamorphosis in *Drosophila melanogaster* which begins with "white puparium". So, the percentage of daily formed white puparium yielded form the cross: (*ry506/ry506* ⊗ *Df(3)ry81/TM3Sb*) was calculated both at 29°C and 18°C. As shown in Table 7, the puparium formation started at the age of 96 hrs (after egg laying) and lasted up to the age of 164 hrs when reared at 29°C. However, at 18°C white puparium started at 180 hrs and lasted up to 264 hrs. At 29°C, there was a peak of white puparium formation at the age of 119 hrs (5 days), but such a sharp peak was not found at 18°C. Instead, there was a flattened (curved) one between the age of 209 till 221 hrs which is the counterpart of the peak of 119 hrs at 29°C. In conclusion, the result insures the idea that the most suitable time of *rosy* gene expression should be (practically) at the end of the third larval instar especially at the beginning of white puparium formation, i.e., the product of the *rosy* gene is mostly needed at that time of *Drosophila* development. These results are in agreement with Kiss *et al*., 1988; Mamon *et al*., 1998; Maitra *et al*., 2000.

Table 7:White puparium formation of the (*ry506/ry506* ⊗ *Df(3)ry^{81}*) genetic cross at 29°C and 18°C.

Temp.	Time (in hrs)	*Sb*$^+$ flies	*Sb* flies	Total	*Sb*$^+$ %	*Sb*%	% Total	*Sb*$^+$/*Sb*
29°C	96	24	2	26	4.33	0.36	4.69	12.00
	103	40	17	57	7.22	3.07	10.29	2.37
	109	48	31	79	8.66	5.60	14.26	1.55
	119	156	125	281	28.16	22.56	50.72	1.23
	126	70	51	121	12.64	9.20	21.84	1.37
	139	15	24	39	2.71	4.33	7.04	0.63
	149	16	15	31	2.89	2.71	5.60	1.07
	164	9	11	20	1.62	1.99	3.61	0.82
	Total	378	176	554	68.23	31.77	100	
18°C	180	1	11	12	0.11	1.24	1.35	0.09
	185	2	17	19	0.23	1.91	2.14	0.12
	195	24	58	82	2.71	6.55	9.26	0.41`
	209	83	151	234	9.37	17.04	26.41	0.55
	221	70	170	240	7.90	19.19	27.09	0.41
	235	65	120	185	7.34	13.54	20.88	0.54
	245	37	47	84	4.18	5.30	9.48	0.79
	256	7	15	22	0.79	1.69	2.48	0.47
	264	0	8	8	0	0.90	0.90	0
	Total	289	597	886	32.62	67.38	100	

III- Dose-dependent interaction between "*rosy*" locus and both *arm* and *oho-31* genes in *Drosophila*:-

Applying the method of gene-dose titration (Botas *et al*., 1982) for the *rosy* locus, produced animal flies which carried one or two doses of the *rosy* gene as well as one or two doses of the wild-type allele of the other tested gene. The (1:1) and other ratios of dose variants were scored for viability and morphological malformations.

Table 8 represents data dealing with this point. In general, as in table 8, the viability of all combinations representing the dose (1:1) showed the lowest viability percentage especially if the *Df(3)ry*^{81} was used in the genotype. 18°C seemed to be more deleterious than 29°C where the viability dropped down to zero. The combination also affected other ratios but in different ways. The morphological malformations percent was almost very near to 100% in the (1:1) class, however it was varieing through the other ratios.

The above results suggest the idea that there is a genetic interaction both between (*rosy* and *arm*) and (*rosy* and *oho-31*) genes where they regulate each other. These findings are in agreement with Kiss *et al*., 1988; Hatini *et al*., 2000; Uren *et al*., 2000. It is obvious in all the above results of genetic interaction that the change in the phenotype is a threshold phenomenon at the same time. A change in the dose ratio can cause phenotypic alterations only if the normal concentration of the gene product is deficiently near to the minimum threshold level. In such a case, the concentration supplied by one dose may drop under the threshold and does not maintain the normal phenotype any more. Hence, the products of these three genes have to be found in normal concentrations to fullfil the phenotype of a character.

So, the method of gene-dose titration, especially in the (1:1) dose, is suggested to be a quick effective tool for a survey of direct inductive interactions between the developmental genes.

This kind of study can open a new dimension in developmental genetics and will certainly contribute to the understanding of the roles these specific genes play in *Drosophila* development.

Table 8. Dose-dependent interaction between the *rosy* locus and both *oho-31* and *arm* genes.

Cross	°C	Item	% Viability and (% malformation) of offspring)			
+/+;*ry506/ry506* ⊗ *oho-31/CyO*; +/+		Category	Cy+		Cy	
		Dose	1:1		1:2	
	29		12% (95%)		88% (4%)	
	18		1% (100%)		30% (2%)	
+/+;*Df(3)ry81/TM3Sb* ⊗ *oho-31/CyO*; +/+		Category	Cy^{+}*Sb*$^{+}$	Cy^{+}*Sb*	Cy*Sb*$^{+}$	Cy*Sb*
		Dose	1:1	1:2	2:1	2:2
	29		2% (100)	10(2%)	80%(1%)	98%(0.3%)
	18		0(0)	5%(30%)	45%(0)	80%(2%)
+/Y;*ry506/ry506* ⊗ *arm*^{k_2} */FM7B;* +/+		Category	B^{+}		B	
		Dose	1:1		1:2	
	29		3%	(100%)	65%	(3%)
	18		0	(0)	25%	(1%)
+/Y;*Df(3)ry81/TM3Sb* ⊗ *arm*^{k_2} */FM7B;*+/+		Category	B^{+}*Sb*$^{+}$	B^{+}*Sb*	B*Sb*$^{+}$	B*Sb*
		Dose	1:1	1:2	2:1	2:2
	29		0(0)	18%(70%)	34%(2%)	91%(0.5%)
	18		0(0)	10%(95%)	28%(3%)	78%(1.5%)

*** Numbers in brackets show the % malformation.**

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تأثير كل من درجة الحرارة والصدمة الحرارية والتوليفات الوراثية على الموقع الوراثى (*rosy*) فى حشرة دروسوفيلا

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يهدف هذا البحث إلى دراسة التأثيرات الوراثية والتكوينية لكل من درجة الحرارة والصدمة الحرارية والتوليفات الوراثية على الموقع الجينى (*rosy*) فى حشرة الدروسوفيلا حيث يعتبر هذا الموقع الوراثى ذا أهمية خاصة وذلك لكون الحشرات ذات التركيب الأليلى المتماثل *ry506/ry506* كانت تمثل السلالة التى استخدمت لأول مرة كمهد (أرضية) لنقل الجينات إليها عن طريق استخدام P-element بالتكنيك المعروف باسم P-element mediated transformation وبذلك فهى أولى السلالات التى تم لها تحول وراثى بهذه الطريقة. وبذلك أيضا يدور البحث حول التركيز على تحديد الوقت الملائم والمناسب لاستحداث نشاط وتعبير هذا الجين. وللوصول الى هذا الهدف فقد تم تطبيق نظام الحساسية للحرارة وكذلك نظام تكنيك التفاعل الجينى المعتمد على الجرعة.

وقد بينت النتائج المتحصل عليها من خلال تحليل الطور المميت سواء للتركيب الجينى (*ry506/Df(3)ry81*) أو لجميع التوافيق الوراثية تحت الدراسة وبالذات مع الجين *oho-31* وكذلك مع الجين *arm* أن هذا الطور المميت دائما يكون فى مرحلة اليرقة من التكوين. كما بينت تجارب الحساسية للحرارة أن التعبير الجينى للموقع (*rosy*) يبدأ مبكرا فى مرحلة البيضة المخصبة ثم يستمر حتى يصل الى نهاية الطور اليرقى الثالث.

أيضا اتضح من دراسة الصدمة الحرارية وكذلك تكوين "العذراء البيضاء" أن الحشرة تحتاج لنواتج التعبير الجينى للموقع (*rosy*) فى خلال 40-100 ساعة الأولى من التكوين.

وقد أوضحت النتائج المتحصل عليها من التفاعل الجينى بين الموقع (*rosy*) والجين (*oho-31*) وكذلك بين الموقع (*rosy*) والجين (*arm*) أن نواتج التعبير الجينى للجينات الثلاثة لابد وأن تتوافر بتركيزات كافية من أجل التكوين الطبيعى للكائن. علاوة على ذلك اتضح أن الجين rosy له تأثير على مستوى النشاط والتعبير الجينى لكلا الجينين تحت الدراسة.

هذه النتائج المتحصل عليها تلقى الضوء على امكانية تطبيق مثل هذه الطرق على مواقع وراثية ذات أهمية خاصة سواء فى الحشرات أو فى بعض الكائنات الأخرى كما أنها تفتح بعدا جديدا فى الوراثة التكوينية وبذلك فهى تساهم فى فهم أوضح للدور الذى تلعبه مثل هذه الجينات فى التكوين والتطور.