# GENETIC INFLUENCES OF YEAST AND TWO PLANT HORMONES ON *DROSOPHILA MELANOGASTER*

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## ABSTRACT

The genetic effects of two plant hormones, gibberellin (GA3) and ethephon with the effects of two yeast strains on *D. melanogaster* wild type flies were studied in four different experiments. The doses of GA3 were 2, 5 and 10 mg/l and those of ethephon were 0.02, 0.05 and 1.0 mg/l. In the first experiment, hormone-treated yeast strains were applied to *D. melanogaster* flies. In the second one, *Drosophila* flies were treated with hormones and normal yeast strains. In the third experiment, *Drosophila* flies were treated with hormones (without yeast) and in the fourth experiment, standard *Drosophila* flies (free of inversions) were treated with hormones (without yeast). Inversions *In(2L)Ns*, *In(2L)Cy*, *In(2R)Ns*, *In(3L)P*, *In(3L)M*, *In(3R)P*, *In(3R)Mo* and *In(3R)C* were detected. Some inversions decreased with the application of ethephon or GA3 while *In(3R)Mo* was not observed in the highest concentrations in the second and third treatments where either GA3 or ethephon were applied in the $10^{th}$ generation. The fourth treatment showed that the standard stock had the lowest thorax (24.9), and wing (57.50) lengths at the highest doses of GA3. Using the ethephon middle dose, the fourth treatment also had the lowest thorax length (25.4) and wing length (57.34) at the highest dose of ethephon. The dose 10 mg/l of GA3 gave the highest lethal load (0.51±0.01) at the second treatment in the $5^{th}$ generation where M5 yeast strain was applied. The application of ethephon highest dose in the third treatment gave the highest lethal load (0.60±0.01) in the $10^{th}$ generation. Plant hormones were found to be harmful to flies.

***Key words:*** *Drosophila*, yeast, gibberellin, ethephon, population genetics, inversions, body size, lethal load.

## INTRODUCTION

The environmental pollutants that may affect the life and biology of different organisms are exceeding daily in our life. One of these is the group of plant hormones which is widely used to enhance the growth of many plants. Now, there is considerable

interest in the roles of plant-derived compounds such as bioflavonoids. Flavonoids are secondary metabolites synthesized inside the plants and they are daily consumed by humans, and different organisms (Middelton and Kandaswami, 1993 and Schramm *et al.* 1998). Human consumption of flavonoids has been estimated to be in the range of 1 g/day (Kuhnau, 1976) and is increasing daily. This increasing rate of flavonoid consumption is for many reasons. One reason is the pursuing of increased plant flavonoid content with the intent of improving disease resistance in plants and plant nodulation efficiency (Dixon *et al.* 1996). Another reason is the use of flavonoids as food additives since they can reduce food spoilage (Zeiger, 1993).

Although flavonoids inhibit pathology in a variety of experimental systems, many investigators reported the harmful effects of flavonoids (Austin *et al.* 1992; Bjeldanes and Chang, 1977; Ono and Nakane, 1990 and Watson, 1982). Another drastic effect of flavonoids is the ability to alter the synthesis and degradation of cell signaling molecules such as cyclic nucleotides, and they could affect meiotic processes. Meiotic non-disjunction in humans is the leading cause of human prenatal death and leads to syndromes such as Down's (Sherman *et al.* 1994).

*Drosophila* was found to be able to maintain positive epistatic relationships among loci within gene arrangements that developed as the species adapted to a heterogeneous environment (Schaeffer *et al.* 2004). However, the precise genetic causes of the adaptive value of inversions remain uncertain (Puig *et al.*, 2004). There was also negative impact on the *Drosophila* populations studied near nuclear power plants in Mexico (Pimentel *et al.*, 2004).

The genetic activities of gibberellin and ethephon as environmental pollutants on *D. melanogaster* natural flies were measured as well as the combined effect of these two hormones and two yeast strains through four different experiments. These effects were estimated using the chromosomal inversion frequencies, body size and lethal load of treated flies.

## MATERIALS AND METHODS

### *Drosophila* population:

Samples of a natural population of *Drosophila* flies were collected in nature at the Faculty of Agriculture Farm, Kafr EL-

Sheikh, Egypt. Males of *D. melanogaster* were separated from those of *D. simulans* and progeny test was conducted to select for *D. melanogaster* offspring of wild females. The ordinary commeal-molasses-agar mixture was used in this study.

Standard flies, free of inversions in the second chromosome, were used in this study. To produce this standard stock, the balancer stock; Curly Lobe/Plum (*CyL/Pm*), was used. At first, standard males were mated to virgin females of the *CyL/Pm* stock. Homozygous second chromosome flies that don't carry any inversion that could be obtained after three matings, were screened to be sure that they do not carry any inversion.

The *CyL/Pm* stock was used to detect the lethal effect of gibberellin and ethephon. The balancer stock was used as the same manner described before except the treated males were used.

**Yeast strains:**

Two *Saccharomyces cerevisiae* wild type strains were used in this study, M1 (LBC 1341) and M5 (LBC 254).

Yeast medium ingredients were 0.05% yeast extract, 2% peptone and 2% glucose. Media were solidified by the addition of 1.5% agar.

**Gibberellin (Gibberellic acid GA3):**

Molecular formula $C_{19}H_{22}O_6$, was used in the form of gibberellic acid 90%. This hormone was used in concentrations of 2, 5, and 10 mg/l (Watson, 1982).

**Ethephon [(2-chloroethyl) phosphoric acid]:**

Molecular formula $C_2H_6ClO_3P$, was used in the form of ethephon 50%. Three concentrations were used according to Thomson (1992); 0.02, 0.05, and 1.0 mg/l. The plant hormones were added to the cold medium.

**Experimental procedure:**

*Drosophila melanogaster* flies were divided into the following four experimental groups.

**Exp. I: Feeding of *Drosophila* on yeast treated with hormones:**

*Drosophila* flies were fed on two hormone-treated strains of yeast (30 vials for each).Vials, each containing 5 ml of optimal medium, were inoculated with 0.1 ml samples of either *S. cerevisiae* M1 or M5 suspension, which were prepared in such a way as to give equal weight / ml of each. These flies were also

treated with three doses of each of the two hormones [15 vials for each of gibberellin (GA3) and ethephon for each yeast strain].

**Exp. II: Treatment of *Drosophila* with hormones and untreated yeast strains:**

*Drosophila* flies were treated first with the doses of the two hormones [30 vials for each hormone], then the two normal yeast strains were added (15 vials for each of M1 and M5 for each hormone).

**Exp. III: Treatment of *Drosophila* with hormones (without yeast):**

*Drosophila* flies were treated with the two hormones [15 vials for each of (GA3) and ethephon] without yeast.

**Exp. IV: Treatment of standard *Drosophila* flies (free of inversions) with hormones (without yeast):**

The standard *Drosophila* flies were treated with the two hormones [15 vials for each of GA3 and ethephon] without yeast.

**Cytological studies:**

Salivary gland chromosomes were studied in squash preparation of salivary gland dissected from third instar larvae and stained with aceto-lactic orcein. The observed inversions were identified using salivary chromosome maps Lindsley and Grell (1967). Estimation of the inversion frequencies was based on cytological analysis of ten larvae for each vial.

**Characters measured:**

To study the effect of the two plant hormones and yeast on the body size character, thorax and wing lengths were chosen. The two traits were measured under an ordinary microscope using an ocular micrometer.

**Data analysis:**

The Chi-square distribution ($\chi^2$) was used to test for inversion significance according to Spiess (1977).

Genetic load due to lethals (L) has been calculated in the following manner; according to Chung (1962). The chance of surviving a lethal (1-x) and the load (L) are defined as:

$$1 - x = e^{-L} \text{ or } L = - \ln (1 - x),$$

where x represents the proportion of the lethal chromosomes in the homozygous condition. The variance of the estimated L was

calculated as $Vx/(1-x)^2$ where Vx is the empirical variance of the mean of x within populations.

Analysis of variance was carried out to test the significance of differences between yeast and between hormones.

## RESULTS AND DISCUSSION

### 1- Inversion frequencies and distribution:

Eight different paracentric inversions; *(2L)Ns, (2L)Cy, (2R)Ns, (3L)P, (3L)M, (3R)P, (3R)Mo, (3R)C*, have been detected in the natural collected population of *D. melanogaster* which was used as control. These gene arrangements were found only in heterozygous condition in the $F_1$ of the wild collected flies. The inversions were distributed on the second and third chromosomes.

### 1-1-Hormone treatments and Hormone-treated yeast:

Yeast strains were treated first with each of the two hormones then they were applied to *Drosophila* flies. The effects of this treatment after 10 generations are presented in Table (1) and Figure (1a).

The treatments of GA3 and GA3 treated M1 yeast strain caused inversions *(2L)Cy* and *(2R)Ns* to be significantly decreased, from the control, by 55% and 61%, respectively at 10 mg/l. Inversion *(3R)Mo* was not observed at any concentration. When M5 GA3-treated-yeast strain was applied, *In(2L)Cy* and *In(2R)Ns* encountered highly significant decrease of 68% and 74%, respectively from the control at 10 mg/l. Inversion *(3R)Mo* significantly decreased, from the control, by 85% (10 mg/l).

When *Drosophila* flies were treated with M1 ethephon-treated yeast strain, inversions *(2L)Cy* and *(3R)Mo* significantly declined, from the control, by 68% and 85%, respectively, at 1.0 mg/l. Inversion *(3L)M* was not observed at the highest ethephon dose. The most affected inversions with the application of ethephon-treated M5 yeast strain were *(2L)Cy*, which encountered highly significant decrease of 68%, *(2R)Ns*, *(3R)P* and *(3R)Mo* which significantly decreased, from the control, by 62%, 87% and 85% (1.0 mg/l), respectively.

### 1.2 Treatment of *Drosophila* with GA3, ethephon and untreated yeast:

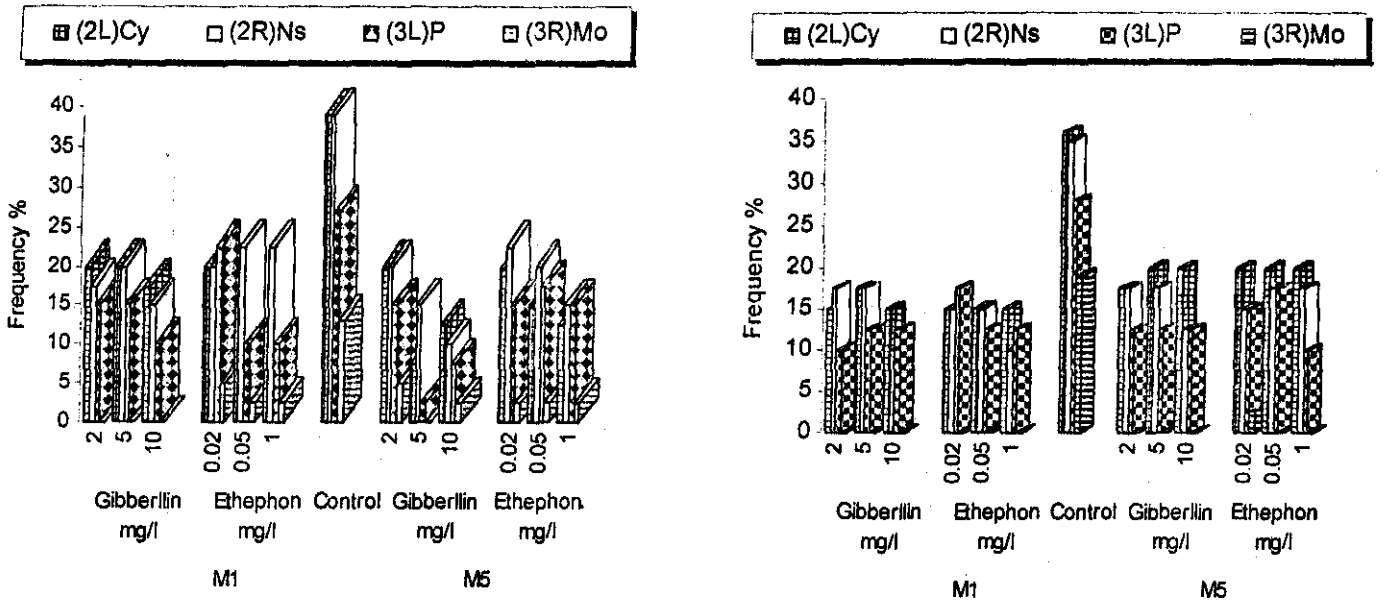
The data in Table (2) and Figure (1b) show that after GA3 treatment with untreated-M1 yeast strain, inversions *(2L)Cy* and

*(2R)Ns* significantly decreased, from the control, by 58% and 71%, respectively (10 mg/l), *In(3R)Mo* was not detected also with untreated-M5 application. The application of untreated-M5 gave the only significant decrease 77% to *In(2R)Ns*.

Table (1): Effects of gibberellin and ethephon treated-yeast strains on the chromosome inversion frequencies (as percentages) of *D. melanogaster* in the 10th generation.

| Gibberellin (GA3) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Inversion | M1 | | | M5 | | | Control |
| | 2 mg/l | 5 mg/l | 10 mg/l | 2 mg/l | 5 mg/l | 10 mg/l | |
| *(2L)Ns* | 7.5 | 5 | 7.5 | 5 | 2.5 | 5 | 3 |
| *(2L)Cy* | 20 | 20 | 17.5* | 20 | 17.5* | 12.5** | 39 |
| *(2R)Ns* | 17.5* | 20 | 15* | 20 | 15* | 10** | 39 |
| *(3L)P* | 15 | 15 | 10 | 15 | 17.5 | 12.5 | 27 |
| *(3L)M* | 10 | 2.5 | 5 | 10 | 2.5 | 7.5 | 9 |
| *(3R)P* | 5 | 10 | 5 | 10 | 5 | 7.5 | 19 |
| *(3R)Mo* | - | - | - | 5 | - | 2.5* | 17 |
| *(3R)C* | 22.5 | 22.5 | 22.5 | 12.5* | 25 | 15 | 31 |
| Ethephon | | | | | | | |
| Inversion | M1 | | | M5 | | | Control |
| | 0.02mg/l | 0.05mg/l | 1.0 mg/l | 0.02mg/l | 0.05mg/l | 1.0 mg/l | |
| *(2L)Ns* | 5 | 5 | 5 | 7.5 | 5 | 5 | 3 |
| *(2L)Cy* | 20 | 15* | 12.5** | 20 | 15* | 12.5** | 39 |
| *(2R)Ns* | 20 | 22.5 | 22.5 | 22.5 | 20 | 15* | 39 |
| *(3L)P* | 22.5 | 10 | 10 | 15 | 17.5 | 15 | 27 |
| *(3L)M* | 5 | 10 | - | 7.5 | 2.5 | 7.5 | 9 |
| *(3R)P* | 5 | 10 | 5 | 10 | 7.5 | 2.5* | 19 |
| *(3R)Mo* | 5 | 2.5* | 2.5* | 2.5* | 2.5* | 2.5* | 17 |
| *(3R)C* | 25 | 12.5* | 20 | 22.5 | 15 | 17.5 | 31 |
| Total no. of examined chromosomes | | | | | | | |
| | 40 | 40 | 40 | 40 | 40 | 40 | 100 |

Table (2) shows that when ethephon and untreated-M1 yeast strain were applied, the most affected inversions were *(3L)M* and *(3R)Mo* where *In(3L)M* was not observed at the highest concentration and *In(3R)Mo* was not observed at any concentration. With ethephon and untreated-M5 yeast strain, only *In(3R)Mo* was not observed at 0.05 mg/l and 1.0 mg/l. Third chromosome




(a) (b)

Figure (1).Effects of gibberellin and ethephon and hormone-treated-yeast strains (a) and untreated yeast strains (b) on the chromosome inversion frequencies of *D. melanogaster* in the $10^{th}$ generation.

inversions decreased in frequency through the middle and highest ethephon doses.

Table (2): Effects of GA3, ethephon and the untreated-yeast strains on the chromosome inversion frequencies (as percentages) of *D. melanogaster* in the 10th generation.

| Gibberellin (GA3) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Inversion | M1 | | | M5 | | | Control |
| | 2 mg/l | 5 mg/l | 10 mg/l | 2 mg/l | 5 mg/l | 10 mg/l | |
| *(2L)Ns* | 7.5 | 10 | 7.5 | 5 | 5 | 5 | 12 |
| *(2L)Cy* | 15* | 17.5 | 15* | 17.5 | 20 | 20 | 36 |
| *(2R)Ns* | 17.5 | 17.5 | 10* | 17.5 | 17.5 | 12.5* | 35 |
| *(3L)P* | 10* | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 28 |
| *(3L)M* | 7.5 | 5 | 5 | 5 | 7.5 | 5 | 12 |
| *(3R)P* | 15 | 17.5 | 15 | 5 | 5 | 2.5 | 11 |
| *(3R)Mo* | - | - | - | - | - | - | 18 |
| *(3R)C* | 15 | 17.5 | 15 | 17.5 | 15 | 15 | 25 |
| Ethephon | | | | | | | |
| Inversion | M1 | | | M5 | | | Control |
| | 0.02mg/l | 0.05mg/l | 1.0 mg/l | 0.02mg/l | 0.05mg/l | 1.0 mg/l | |
| *(2L)Ns* | 7.5 | 10 | 7.5 | 5 | 5 | 7.5 | 12 |
| *(2L)Cy* | 15* | 15* | 15* | 20 | 20 | 20 | 36 |
| *(2R)Ns* | 15* | 15* | 10* | 15 | 17.5 | 17.5 | 35 |
| *(3L)P* | 17.5 | 12.5 | 12.5 | 15 | 17.5 | 10* | 28 |
| *(3L)M* | 2.5 | 2.5 | - | 7.5 | 5 | 2.5 | 12 |
| *(3R)P* | 15 | 15 | 15 | 5 | 2.5 | 2.5 | 11 |
| *(3R)Mo* | - | - | - | 2.5 | - | - | 18 |
| *(3R)C* | 15 | 15 | 15 | 20 | 25 | 17.5 | 25 |
| Total no. of examined chromosomes | | | | | | | |
| | 40 | 40 | 40 | 40 | 40 | 40 | 100 |

**1.3- Treatment of *Drosophila* with GA3 and ethephon (without yeast):**

Table (3) and Figure (2) show that with the application of GA3, the most frequent inversions; *(2L)Cy* and *(2R)Ns*, significantly decreased, from the control, by 72% and 73%, respectively (10 mg/l). Only *In(3R)Mo* exhibited a severe elimination from the base population.

Ethephon application did not score any significantly decrease in any inversion, from the control. Inversions *(3R)Mo* was not observed at any concentration.

Table (3): Effects of gibberellin and on ethephon the chromosome inversion frequencies (as percentages) of *D. melanogaster* in the 10th generation.

| Inversion | Gibberellin (GA3) | | | | Ethephon | | | |
|---|---|---|---|---|---|---|---|---|
| | 2mg/l | 5mg/l | 10mg/l | Control | 0.02mg/l | 0.05mg/l | 1.0mg/l | Control |
| *(2L)Ns* | 6.25 | 8.75 | 6.25 | 11 | 8.75 | 8.75 | 5.0 | 11 |
| *(2L)Cy* | 10.0 | 10.0 | 8.75* | 31 | 15.0 | 12.5 | 10.0 | 31 |
| *(2R)Ns* | 11.25 | 10.0 | 8.75* | 33 | 12.5 | 12.5 | 11.25 | 33 |
| *(3L)P* | 12.5 | 11.25 | 11.25 | 27 | 11.25 | 10.0 | 10.0 | 27 |
| *(3L)M* | 3.75 | 6.25 | 5.0 | 8 | 7.5 | 5.0 | 8.75 | 8 |
| *(3R)P* | 5.0 | 5.0 | 5.0 | 13 | 2.5 | 5.0 | 3.75 | 13 |
| *(3R)Mo* | -- | -- | -- | 13 | -- | -- | -- | 13 |
| *(3R)C* | 15.0 | 10.0 | 15.0 | 31 | 15.0 | 11.25 | 15.0 | 31 |
| Total no. of examined chromosomes | | | | | | | | |
| | 80 | 80 | 80 | 100 | 80 | 80 | 80 | 100 |

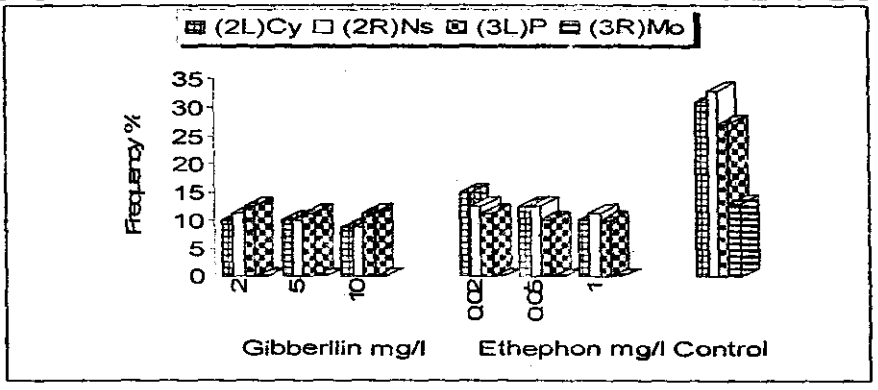



Figure (2). Effects of gibberellin and on ethephon the chromosome inversion frequencies of *D. melanogaster*.

**1.4- Treatment of the standard stock of *Drosophila* with GA3 and ethephon:**

The data in Table (4) show that with GA3 application, the second chromosome inversions emerged but they maintained low frequencies. The data also show that when ethephon was used, *In(2L)Ns* increased to 3.75% (1.0 mg/l), *In(2L)Cy* to 10% (0.05 and 1.0 mg/l) and *In(2R)Ns* to 12.5% (1.0 mg/l).

These results revealed that *Drosophila* flies needed these inversions when they were treated with ethephon hormone to maintain their survival. This may be due to the selection advantages in favor for the genes included inside these inversions.

Table (4): Effects of gibberellin and ethephon on the second chromosome inversion frequencies of *D. melanogaster* standard stock in the 10th generation.

| Inversion | Gibberellin (GA3) | | | | Ethephon | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 mg/l | 5 mg/l | 10 mg/l | Control | 0.02mg/l | 0.05mg/l | 1.0mg/l | Control |
| | % | % | % | % | % | % | % | % |
| *(2L)Ns* | 3.75 | 2.5 | 5.0 | - | 2.5 | 2.5 | 3.75 | - |
| *(2L)Cy* | 5.0 | 6.25 | 8.75 | - | 3.75 | 10.0 | 10.0 | - |
| *(2R)Ns* | 3.75 | 7.5 | 8.75 | - | 2.5 | 6.25 | 12.5 | - |
| Total no. of examined chromosomes | | | | | | | | |
| | 80 | 80 | 80 | 100 | 80 | 80 | 80 | 100 |

Plant hormones are very useful to maintain plant life cycle, however, it has been recently discovered that the extensive use of hormone extracts, as a plant growth enhancer, may cause harmful effects on human and animal life

The results showed that the application of both gibberellin or ethephon affected the most frequent inversions; *(2L)Cy, (2R)Ns, (3L)P* and *(3R)C*. These four inversions faced a severe reduction more than the half of their observed frequencies in the control flies. The most affected inversion was *(3R)Mo* which was eliminated from the populations.

These alterations in gene arrangements have the ability to cause a tremendous reduction in fly fitness. The fly needs these inversions to keep certain genes intact together. Flies use these inversions to cope with the surrounding environment and utilize the available food components. The reduction in inversion frequency caused by the elimination of inversions like *In(3R)Mo* let the flies imbalance with the environment and end with their death.

Brown (1980) and Watson (1982) were the first researchers who introduced the mutagenic activity of flavonoids and other plant hormones to the research community. Watson (1982) revealed that flavonoids can be considered mutagenic compounds to *D. melanogaster* which caused high frequency of lethal males.

Selection can act in favor or against certain inversion. Adaptive changes in nature occur by a variety of mechanisms, and *Drosophila*'s chromosomal inversion is one of the first studied examples. However, the precise genetic causes of the adaptive value

of inversions remain uncertain (Puig *et al.*, 2004). *Drosophila* inversions have emerged as suppressors of recombination to maintain positive epistatic relationships among loci within gene arrangements that developed as the species adapted to a heterogeneous environment (Schaeffer *et al.* 2004). Megeed *et al.* (1997) pointed out that selection acted against *In(2L)NS* in *Drosophila* natural flies after exposing the flies to monosodium glutamate. The rate of evolution depends on the ability to detect selection at several genes rearrangements (Mousset *et al.*, 2003).

The genetic effects of GA3 and ethephon were profound especially after the application of higher doses. These severe effects resulted in the elimination of one cosmopolitan inversion; *(3R)Mo* and in the decreasing and selecting against the other inversions; *(2L)Cy, (2R)Ns* and *(3R)P*. Selection against the advantage of some inversions means that flies do not need any of the genes located inside the disappeared inversions.

The effect of treated-yeast strains did not differ from those of untreated-yeast strains. The M1 strain gave more effects when it was treated with GA3 than M5 strain.

Flavonoid plant pigments are an integ al part of the human diet Although potentially negative mitotic effects of flavonoids were observed, investigation into genetic effects of flavonoids has been neglected as well as their effects on *Drosophila* chromosomal inversions. As flavonoids affect cell signaling and DNA replication, and because the flavonoid content of the human food supply is being increased, determining the effects of flavonoids on chromosomal inversion fidelity is important. The mutagenic activity of flavonoids were detected (Bjeldanes and Chang, 1977; Maruta *et al.*, 1979; Brown, 1980; and Watson, 1982)..

Carver *et al.* (1983) indicated that flavonols induced chromosomal aberrations in Chinese hamster ovary cells. Mitchell *et al.* (1993) found that plant flavonoids inhibited the wandering stage larvae of *D. melanogaster*. Baker *et al.* (1998) found that naringenin, quercetin and kaempferol inhibited estrogen inding to AFP in rats. A significant effect was observed on the number of offspring; $F_1$ and $F_2$ generations of flies raised on a quercetin diet than flies raised on a control diet (Schramm *et al.*, 1998).

Since there are no any other documented studies on the genetic effects of plant hormones on *Drosophila* chromosomal inversions, we may compare the results of this study with those of Carver *et al.* (1983) on Chinese hamster where they reported a significant increase of chromosomal aberrations by quercetin, kaempferol and galangin. The results of this study clearly showed the negative effects of gibberellin and ethephon on inversion frequencies in *D. melanogaster*. Some cases of inversion reductions were observed as *In(2L)NS* or *In(3L)M* and even the elimination of *In(3R)Mo*. As plant hormones may be considered as food additives in the dietary of human or animal food, the results of this study can be compared with those of Megeed *et al.* (1997) who found that glutamtic mono-sodium affected the frequencies of chromosomal inversions and resulted in the elimination of *In(2R)NS* in *D. melanogaster*.

The reduction of inversion frequencies may be harmful to *Drosophila* flies since these flies need the genes intact within the inversions and would end with the decrease of heterozygosity conditions of the base population. When heterozygosity is reduced in a population, this population will be less homeotic or co-adapted to the surrounding environment. Plant hormones are useful to maintain plant life cycle, however, it has been recently discovered that extensive use of hormone extracts as a plant growth enhancer may cause harmful effects on human and animal life.

**2- Body Size :**

**2.1-Hormone treatments and Hormone-treated yeast:**

The data in Table (8) show that the GA3 dose of 10 mg/l caused a reduction in both thorax length (29.23) and wing length (63.44) compared with 2 mg/l which gave the highest values (31.17 and 65.44) for thorax and wing, respectively.

The dose of 1.0 mg/l of ethephon had the lowest values ((28.60 and 64.10) for thorax and wing lengths, respectively.

The data clearly indicated that the observed measures of thorax and wing lengths decreased by concentration increase.

**2-2-Treatment of *Drosophila* with GA3, ethephon and untreated yeast:**

When GA3 was used with the untreated yeast strains, it reduced thorax and wing lengths to 26.43 and 57.42, respectively, 10 mg/l,

while the control gave values of 28.83 and 64.43 for thorax and wing lengths, respectively (Table 8).

These reductions were more than those of the treated-yeast in the former experiment. When ethephon was used with the untreated yeast strains, it caused a reduction in both thorax and wing lengths to 27.25 and 59.36, respectively, at 1.0 mg/l, compared with the control (Table 8).

**2.3-Treatment of *Drosophila* with GA3 and ethephon (without yeast):**

In the third treatment, 2 mg/l had the highest value of thorax length (28.56) whereas the concentration of 5 mg/l had the highest value of wing length (63.66). On the other hand, the concentration of 10 mg/l had the lowest values (26.56 and 59.86) for the thorax and wing lengths, respectively (Table 8). These results showed that GA3 application alone had lower effect than that when it was applied with the untreated yeast.

The ethephon dose of 0.02 mg/l had the highest value of thorax length (26.88) whereas the concentration of 0.05 mg/l had the highest value of wing length (63.10). On the other hand the concentration of 1.0 mg/l had the lowest values (25.70 and 57.74) for the thorax and wing lengths, respectively (Table 8).

These results showed that ethephon application was not affected by either the treated or the untreated yeast strains.

**2.4-Treatment of the standard stock of *Drosophila* with GA3 and ethephon:**

The dose 10 mg/l of GA3 had the lowest values (24.90 and 57.50) for thorax and wing lengths, respectively (Table 8). The data revealed that the observed measures of thorax and wing lengths decreased by the increase in GA3 concentration.

Ethephon dose 1.0 mg/l had the lowest value of wing length (57.34) whereas the dose 0.05 mg/l had the lowest value of thorax length (25.40), while the dose 0.02 mg/l had the highest values (27.50 and 59.54) for the thorax and wing lengths, respectively. These results agree with those in the former experiment.

The results revealed that GA3 application alone with untreated yeast and its application to the standard flies gave the highest reduction in both thorax and wing lengths than any other treatment.

Table (8) : Effects of GA3 and ethephon on wing and thorax lengths of *D. melanogaster*.

| Plant Hormone | Doses (mg/l) | Hormone-treated yeast | | Hormone treatment with untreated yeast | | Hormone treatment without yeast | | Hormone treated *Drosophila* standard stock | |
|---|---|---|---|---|---|---|---|---|---|
| | | Thorax | Wing | Thorax | Wing | Thorax | Wing | Thorax | Wing |
| GA3 | 2 | 31.17±0.21a | 65.44±0.39a | 27.58±0.24a | 59.53±0.46a | 28.56±0.32a | 63.12±0.69 a | 26.90±0.36a | 59.98±0.64a |
| | 5 | 30.25±0.21b | 65.24±0.39a | 27.81±0.24a | 59.71±0.46a | 28.08±0.32 a | 63.66±0.69 a | 26.14±0.36a | 58.10±0.64 ab |
| | 10 | 29.23±0.21c | 63.44±0.39 b | 26.43±0.24b | 57.42±0.46b | 26.56±0.32b | 59.86±0.69 b | 24.90±0.36b | 57.50±0.64b |
| Ethephon | 0.02 | 29.75±0.21a | 67.64±0.39a | 28.34±0.24a | 60.82±0.46a | 26.88±0.32a | 62.10±0.69 a | 27.50±0.36a | 59.54±0.64a |
| | 0.05 | 28.94±0.21b | 64.66±0.39b | 28.06±0.24a | 59.47±0.46a | 26.68±0.32a | 63.10±0.69 a | 25.40±0.36b | 57.64±0.64b |
| | 1.0 | 28.60±0.21b | 64.10±0.39b | 27.25±0.24b | 59.36±0.46a | 25.70±0.32b | 57.74±0.69 b | 25.46±0.36b | 57.34±0.64b |
| Control | | 32.66±0.12 | 70.30±0.22 | 28.83±0.14 | 64.43±0.27 | 29.37±0.16 | 64.39±0.34 | 29.70±0.18 | 65.06±0.32 |

Table (9). The pooled analysis of variance of the effects of different sources and their interactions on both thorax and wing lengths in *D. melanogaster* natural population.

| Source of Variance | D.F. | Thorax length | | Wing length | |
|---|---|---|---|---|---|
| | | M.S. | F | M.S. | F |
| Hormones (H) | 2 | 2006.5855 | 17238.7070** | 8723.4951 | 1363.1739** |
| Doses (D) | 6 | 784.3461 | 6738.3685** | 3242.6645 | 506.7138** |
| Treatments (T) | 3 | 1522.1713 | 13077.0730** | 6132.7089 | 958.3256** |
| Yeast Strains (Y) | 2 | 364.1132 | 3128.1203** | 1678.2534 | 262.2517** |
| Replicates ® | 4 | 820.2398 | 7046.7337** | 2062.4518 | 322.2883** |
| H X T | 6 | 150.1025 | 1289.5404** | 186.3117 | 29.1139** |
| H X Y | 4 | 1053.3439 | 9049.3462** | 1159.6233 | 181.2081** |
| D X Y | 12 | 142.1561 | 1221.2723** | 20.4132 | 3.1899** |
| D X T | 18 | 56.3624 | 484.2131** | 106.2861 | 16.6088** |
| Error | 2642 | 0.1164 | | 6.3994 | |

The results from the pooled analysis of variance, Table (9), show that there are significant effects on thorax and wing lengths.

Results summarized in Table (10) show that there was no significant difference between both hormones on thorax and wing lengths, the two hormones significantly decreased both thorax and wing lengths of *D. melanogaster* natural flies. The results from Table (9) also show that there were highly significant effects of the six different doses on both thorax and wing lengths. Data in Table (10) show that the two highest doses of GA3 and ethephon significantly decreased thorax length. These two doses were significantly differed from the other doses.

This trend was also true for the effects of both GA3 and ethephon on wing length. While the effects of both higher doses of the two hormones were significantly different from the control, they were not significantly different from each other.

The results also showed that there was significant difference between the effects of the two yeast strains (Table, 9). Also, data in Table (10) presented that the second strain had more significant decreasing effect than the first yeast and the control. The second strain significantly decreased the thorax length of *D. melanogaster* natural flies. The second strain had more severe significant effect on decreasing the wing length (61.32) than the first strain.

The fourth experiment gave severe significant reduction to thorax length (from 30.52m, control, to 27.50) comparing to the other treatments (Table, 10). The second experiment gave similar results with the third experiment.

The second and fourth experiments had more effects on reducing wing length to 61.06 and 60.95, respectively (Table, 10), than the the first experiment (66.85) and third one (62.89).

The results showed that there were highly significant effects for the interactions between hormones and yeast strains, between concentrations and yeast strains and between hormones or their concentrations and the four different experiments (Table, 9). While *Drosophila* body size was found to be significantly affected by the two hormones, the two yeast strains and the four experiments, it was supportive to find that their interactions were also significant.

Although there was a significant reducing effect between the two yeast strains on both thorax and wing lengths, they did not have

a significant effect on thorax or wing lengths when they were treated first with both hormones.

**Table (10).The mean effects of GA3, ethephon and two yeast strains on *D. melanogaster* thorax and wing lengths.**

| Thorax length | | | | | | Wing length | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Hormones** | | | | | | | | | | | |
| 1 | | 2 | | Control | | 1 | | 2 | | Control | |
| 27.99±0.0934 | | 27.88±0.0891 | | 30.52±0.0990 | | 61.36±0.1818 | | 61.60±0.1844 | | 66.87±0.1573 | |
| M. S. | | L.S.D. (0.01) | | | | M. S. | | L.S.D. (0.01) | | | |
| 2006.5855** | | 0.3425 | | | | 8723.4951** | | 0.6377 | | | |
| **Yeast Strains** | | | | | | | | | | | |
| 1 | | 2 | | Control | | 1 | | 2 | | Control | |
| 29.36±0.0821 | | 27.88±0.0961 | | 28.94±0.0547 | | 63.15±0.1793 | | 61.32±0.2482 | | 64.11±0.1560 | |
| M.S. | | L.S.D (0.01) | | | | M.S. | | L.S.D (0.01) | | | |
| 364.1132** | | 0.3678 | | | | 1678.2534** | | 0.6876 | | | |
| **Concentrations** | | | | | | | | | | | |
| Gibberellin (mg/l) | | | Ethephon (mg/l) | | | Gibberellin (mg/l) | | | Ethephon (mg/l) | | |
| 2 | 5 | 10 | 0.02 | 0.05 | 1.0 | 2 | 5 | 10 | 0.02 | 0.05 | 1.0 |
| 28.70± 0.1087 | 28.22± 0.1087 | 27.04± 0.1087 | 28.55± 0.1087 | 27.85± 0.1087 | 27.25± 0.1087 | 62.36± 0.3029 | 61.73± 0.3029 | 59.98± 0.3232 | 62.90± 0.3030 | 61.71± 0.3325 | 60.20± 0.3035 |
| **Control** | | | | | | | | | | | |
| 30.52±0.0666 | | | | | | 66.87±0.1573 | | | | | |
| M.S. | | L.S.D (0.01) | | | | M.S. | | L.S.D (0.01) | | | |
| 784.3461** | | 0.5840 | | | | 3242.6645** | | 1.0905 | | | |
| **Treatments** | | | | | | | | | | | |
| 1 | 2 | 3 | 4 | | | 1 | 2 | 3 | 4 | | |
| 30.62± 0.0696 | 27.99± 0.0696 | 28.06± 0.0948 | 27.50± 0.0963 | | | 66.85± 0.1578 | 61.06± 0.1740 | 62.89± 0.2546 | 60.95± 0.2778 | | |
| M.S. | | L.S.D.(0.01) | | | | M.S. | | L.S.D.(0.01) | | | |
| 1522.1713** | | 0.3381 | | | | 6132.7089** | | 0.6338 | | | |

Many investigators reported this effect of yeast on *Drosophila*. Dobzhansky and Levene (1955) found that exposure of *D. pseudoobscura* flies to different species of yeasts and different temperatures gave significant variable variability of homozygous and heterozygous flies. El-Helw and Ali (1970) assured the significant effect of two different yeast species on *Drosophila* flies. Also, Moreover, McDonal and Ayala (1974) exposed *D. pseudoobscura* natural flies to different yeast types and these flies

retained more genetic variability than other factors like varying food or light.

Also, Good and Tatar (2001) showed the relationship between the adult dietary yeast programs with the mortality rate and life span of *Drosophila* flies. Ueda and Kidokoro (2002) found that there were aggressive behaviors of *Drosophila* flies when they were fed on food containing yeast than when they were on food did not contain yeast.

These different results assured the relationships between *Drosophila* flies and yeast. The changes of yeast strains strongly affect the behavior, mortality rate, life span, reproduction, chromosomal inversions and even the size of *Drosophila* flies. The results of this study approved this kind of relationship. The application of different yeast strains significantly affected the frequencies of chromosomal frequencies and *Drosophila* body size.

The decreasing effects of GA3 or ethophon on thorax and wing lengths, reported here, were presented before by Bjeldanes and Chang (1977) who reported that quercetin was mutagenic and Maruta *et al.* (1979) who found that quercetin and Kaempferol were proved to have mutagenic activities.

**3- Lethal Load:**

**3.1- Gibberellin treatments:**

The data in Table (11) show that the highest estimate of lethal load was found when M5 yeast strain was treated first with 5 mg/L of GA3 in the fifth generation (0.57±0.03). There was not any significance between the three GA3 concentrations when they were applied to the M1 yeast strain. On the other hand, when M5 yeast strain was applied, there were significant differences between the three concentrations. In the tenth generation, the highest lethal loads were recorded when M5 yeast strain was exposed to both the 5 and 10 mg/l doses, but they were not significant.

In the second experiment there was not any significant record in both generations. The highest estimate of lethal load was recorded in the fifth generation after the application of 2 mg/l (0.56 ± 0.01) and M1 yeast strain.

There was only one case of significance in the fifth generation of the third experiment.

**Table (11): Lethal load estimates, their standard errors and the mean square values in *D. melanogaster* natural population after the treatment with GA3 and ethephon and two yeast strains.**

| Plant hormone | Doses (mg/l) | Hormone-treated yeast | | | | Hormone treated *Drosophila* with untreated yeast | | | | Hormone treated *Drosophila* without yeast | | Hormone treated *Drosophila* standard stock | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | generation | | | | generation | | | | generation | | Generation | |
| | | 5th | | 10th | | 5th | | 10th | | | | | |
| | | M1 | M5 | M1 | M5 | M1 | M5 | M1 | M5 | 5th | 10th | 5th | 10th |
| GA3 | 2 | 0.51±0.02 | 0.51±0.01 | 0 44±0.03 | 0.43±0.03 | 0.56±0.01 | 0.49±0.02 | 0.45±0.02 | 0.41±0.00 | 0.45±0.01 | 0.47±0.03 | 0.44±0.03 | 0.43±0.03 |
| | 5 | 0.46±0.02 | 0.57±0.03 | 0.44±0.01 | 0.48±0.03 | 0.53±0.02 | 0.54±0.01 | 0.49±0.03 | 0.44±0.04 | 0.42±0.01 | 0.46±0.04 | 0.47±0.04 | 0.47±0.04 |
| | 10 | 0.49±0.02 | 0.47±0.01 | 0.47±0.03 | 0.48±0.04 | 0.50±0.03 | 0.51±0.01 | 0.50±0.03 | 0.50±0.03 | 0.48±0.02 | 0.41±0.03 | 0.45±0.03 | 0.48±0.03 |
| M.S | | 0.0382 | 0.1217* | 0.0148 | 0.0033 | 0.099 | 0.0076 | 0.0032 | 0.1043 | 0.0044 * | 0.003 | 0.0149 | 0.0032 |
| LSD (0.05) | | 0.1341 | | | | | | | | 0.0194 | | | |
| Ethephon | 0.02 | 0.50±0.02 | 0.47±0.03 | 0.49±0.08 | 0.47±0.03 | 0.49±0.03 | 0.48±0.02 | 0.43±0.01 | 0.42±0.02 | 0.48±0.02 | 0.44±0.03 | 0.44±0.03 | 0.44±0.04 |
| | 0.05 | 0.52±0.02 | 0.52±0.02 | 0.51±0.02 | 0.48±0.03 | 0.44±0.02 | 0.54±0.02 | 0.44±0.03 | 0.46±0.03 | 0.51±0.01 | 0.51±0.04 | 0.51±0.04 | 0.50±0.04 |
| | 1.0 | 0.49±0.02 | 0.56±0.01 | 0.51±0.03 | 0.51±0.02 | 0.51±0.03 | 0.53±0.02 | 0.50±0.04 | 0.55±0.01 | 0.51±0.01 | 0.60±0.01 | 0.57±0.05 | 0.50±0.04 |
| M.S | | 0.0161 | 0.0919 * | 0.0068 | 0.0013 | 0.1136 | 0.1033 | 0.0078 | 0.2261** | 0.0021 | 0.2404 * | 0.0076 | 0.0067 |
| LSD (0.05) | | | 0.0342 | | | | | | 0.0397 | | 0.0538 | | |
| Control | | 0.42 ± 0.004 | | 0.42 ± 0.004 | | 0.42 ± 0.004 | | 0.41 ± 0.003 | | 0.44±0.013 | 0.41±0.01 | 0.40±0.01 | 0.41±0.03 |

The highest scores of the lethal load were recorded in both the fifth generation of the third experiment and the tenth generation of the fourth experiment with the application of 10 mg/l (0.48±0.03). The highest lethal load in the fourth experiment was recorded for the highest dose of 1.0 mg /l in the fifth generation (0.57 ± 0.05, Table 11).

**3.2- Ethephon treatments:**

The dose of 1.0 mg / l gave the highest significant lethal load (0.56 ± 0.01) in the fifth generation comparing with the other doses in both generations for the first experiment (Table 11). In the second treatment, the dose of 1.0mg /l also gave the highest and highly significant lethal load (0.55 ± 0.01) in the tenth generation comparing with the other doses in both generations. In the third treatment, also, this higher dose of ethephon (1.0 mg /l) gave the. highest and significant lethal load (60 %) in the tenth generation. The highest lethal load in the fourth experiment was recorded for the highest dose in the fifth generation (0.57 ± 0.05). Watson (1982) found that quercetin and kaempferol gave sex–linked lethal in *Drosophila*. This finding, along with other studies which confirmed the mutagenic activity of plant hormone (Austin *et al* 1992; Sherman *et al* 1994 and Schramm *et al* 1998) confirmed our results.

Plant hormones were found to be harmful to flies in changing the frequencies of chromosomal inversions even caused the elimination of one inversion; In *(3R) Mo*. They also caused reduction in flies body size and they were lethal

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## التأثيرات الوراثية للخميرة واثنان من الهرمونات النباتية علي *الدروسوفيلا ميلانوجاستر*

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### الملخص العربي

درست التأثيرات الوراثية اثنان من الهرمونات النباتية هما الجبريلين والايثيفون وكذلك تأثير سلالتين من الخميرة على الحشرات البرية *للدروسوفيلا ميلانوجاستر* في أربعة تجارب. تم استخدام ثلاثة تركيزات من الجبريلين هى 2, 5, 10 ملجم/لتر وثلاثة من الايثيفون هى 0.02، 0.05، 1.0 ملجم/لتر. في التجربة الأولي تم تغذية الدروسوفيلا على سلالتي الخميرة المعاملة بأحد الهرمونين . في التجربة الثانيى تم معاملة الدروسوفيلا بالهرمونين ثم اضافة سلالتي الخميرة العادية الغير معاملة , وفي التجربة الثالثة تم معاملة الدروسوفيلا بالهرمونين بدون الخميرة . وفي التجربة الرابعة تم معاملة سلالة قياسية من *الدروسوفيلا ميلانوجاستر* خالية من أى انقلاب على الكروموسوم الثاني بالهرمونين بدون الخميرة . وجدت الانقلابات الكروموسومية *(3R)C, (3R)Mo, (3R)P, (3L)M, (3L)P و(2R)Ns, (2L)Cy, (2L)Ns* . وجد ان تكرار بعض الانقلابات قد انخفض بإستخدام الجبريلين أوالايثيفون بينما لم يلاحظ الانقلاب *(3R)Mo* في الجيل العاشر بعد استخدام الجرعات العالية من الهرمونين في التجربتين الثانية والثالثة . أظهرت التجربة الرابعة أن السلالة القياسية احتوت اقل القياسات لطولي الصدر (24.9) و الجناح (57.50) عند الجرعة العالية من الجبرلين . أظهرت ايضا التجربة الرابعة اقل القياسات لطول الصدر (25.4) عندما استخدمت الجرعة المتوسطة للايثيفون ، واقل القياسات للجناح بعد استخدام أعلي جرعة للايثيفون (57.34) . أعطت الجرعة 10 مجم/ ل للجبرلين اعلي حمل مميت (0.51±0.01) عند التجربة الثانية وفي الجيل الخامس وباستخدام سلالة الخميرة . اعطت الجرعة العالية للايثيفون اعلي حمل مميت في التجربة الثالثة في الجيل العاشر (0.60±0.01). وقد وجد أن الهرمونات النباتية لها تأثير ضار علي *الدروسوفيلا* .