

GENETIC AND BEHAVIORAL INFLUENCES OF QUININE AND MONOSODIUM GLUTAMATE ON *Drosophila melanogaster*

AMIRA EL-KEREDY

Genetics Department, faculty of Agriculture, Tanta University, Egypt

D*rosophila* is widely used for the monitoring of the carcinogenicity, mutagenicity and toxicity.

Quinine and its d-isomer quinidine are cinchona alkaloids which have profound and lethal toxic side effects. Treatments of overdoses have included hemodialysis, Peritoneal dialysis, plasma exchange forced diuresis, and hemoperfusion. Many reports describe accelerated removal of quinine with successful outcomes. This study describes a case of quinine ingestion which was fatal despite hemoperfusion. Both human and animals feel a sense of aversion in bitter foods and drinks. But bitter coffee or tea become easy to drink by sweetener addition, because we feel the bitterness are suppressed (Goldenberg and Wexler, 1988).

The ability to learn may be regarded as one of the more remarkable products of biological evolution. Yet, our understanding of how changes in learning ability evolve remains rudimentary. In particular, they know almost nothing about the genetic and molecular nature of heritable variation in learning performance. This variation is the raw material of evolution. Thus, knowing which genes contribute to natural variation in learning ability would help us understand how differences in

learning ability and memory evolve among populations and species. It would also offer insights into the tradeoffs constraining the evolution of improved learning performance (Gerber *et al.*, 2009).

Studies in humans and several animal models (including *Drosophila*) have demonstrated that multiple training trials with rest intervals (spaced training) is most effective in producing long-term memory (LTM) (Tully *et al.*, 1994). A single trial, or even multiple training trials without rest intervals (massed training), usually only forms robust short-term memory (STM). However, there are some notable exceptions taste, aversion learning in rodents (and *Drosophila*), a single exposure of a tastant, followed by malaise (or salt exposure), leads to a long-lasting avoidance of the associated taste (Sugai *et al.*, 2007). In *Drosophila*, formation of aversive LTM requires 5-10 spaced training trials, pairing odor with punitive shock, with 15 min rest intervals (Tully *et al.*, 1994). Flies can also be trained with odor and a more ethologically relevant sucrose reward (Schwaezel *et al.*, 2003; Keene *et al.*, 2006; Kim *et al.*, 2007).

The impact of toxic substances on living organisms leads to severe damage which may reach their offspring's. These

toxic effects may be material secreted by plant in order to protect itself from insect attack. Micro-organisms and animals may secrete this article in the fruit, which dealt with human and another organism.

Example of toxic substances which are secreted by plant is quinine, which is extracted from the plant called *Cinchona officinalis* and used in treatment of malaria. It is also characterized by non preferred taste bitter (Tanimura and Ishimoto, 2003; Gerber and Stocker, 2007). The sense of taste is important because it allows animals to prefer edible and avoid and enhance taste substances. In addition, gustatory stimuli can be reinforcers they can induce memories for stimuli or actions that preceded them, such that the animal can yield well and avoid bad food. Monosodium glutamate (MSG) that are used as food enhancer in instant products such as soups, sauces or pizza. Presently, six additives are admitted in the European Union (EU): GLU (E620) and its sodium (E621), potassium (E622), calcium (E623), ammonium (E624) and magnesium (E625) salt. These food enhancers are not allowed to be added to milk, emulsified fat and oil, pasta, cocoa/chocolate products and fruit juice. Following the compulsory EU-food labeling law the use of 'enhancer' has to be declared and the name or E-number of the salt has to be given. GLU salts dissociate in the neutral area so that independent from origin and salt species free GLU is formed (Beyreuther *et al.*, 2006).

The deleterious effect of MSG on the brain and muscle of neonatal pig and

different animals (Stegink *et al.*, 1973; Oser *et al.*, 1975). MSG has been found to cause paralysis, headache and nausea in man and livestock (Hegarty, 1987), Megeed *et al.* (1997) studied the genetic effect of MSG on *Drosophila*, the genetic load, changes with the chromosome rearrangements, and changes in enzyme activity that may caused by MSG with the use of a natural population of *D. melanogaster*. They found that the cytological analysis showed that there were cases of selection for the advantage of certain inversions; 2L(NS), 3L(M), and 3R(P). One inversion; 2R(NS), which contains coding regions for glutamic acid, was eliminated from the basic population after treatment with MSG. Segregating gene arrangements in *D. subobscura*, hold together favorable combinations of alleles that interact epistatically (Santos, 2009).

In 1957 Lucas and Newhouse observed that suckling mice injected with MSG at 2.2 g/kg body weight daily or 14 days developed retinal lesions, a finding confirmed by other investigators in both mice and rats. Adult mice were more resistant to glutamate than the new born animal, and Lucas and Newhouse noted that glutamate injection of pregnant mice produced no observable abnormalities in the offspring.

Drosophila larva provides a numerically simple and genetically tractable model system in which to study the molecular and cellular basis of taste (Stocker, 2008; Gerber *et al.*, 2009; Ebbs and Amrein, 2007).

In mammals, taste perception is mediated by G-protein coupled receptors (GPCRs). On the one hand, sugars and amino acids interact with members of the T1R family, which form functional hetero- and homodimers (Zhao *et al.*, 2003), and on the other hand, bitter substances were detected by the T2Rs (Chandrashekar *et al.*, 2000)

Among the 68 gustatory receptors, expression patterns of only 15 Gr genes have been examined in detail in the larva, using the GAL4- UAS system (Colomb *et al.*, 2007). Thome and Amrein, 2008). The ligand specificity of each of the proposed umami receptors [taste metabotropic glutamate receptor 4, truncated metabotropic glutamate receptor 1, or taste receptor 1 (T1R1). The prototypic umami tastant monosodium glutamate (MSG) stimulates food intake in humans and other mammals and is widely used as a flavor enhancer in many cuisines in processed foods and in animal feed. (Maruyama *et al.*, 2006)

The larva, *Gr66a* is expressed in the larval terminal organ and putatively gustatory neurons along the pharynx (Colomb *et al.*, 2007).

Bitter-sensitive gustatory neurons typically seem to express more than one Gr gene. Not all *Gr66a*-positive neurons also express *Gr93a* (Lee *et al.*, 2009), as well as *Gr33a* (Moon *et al.*, 2009)

The millimolar concentration of glutamate in the adult brain is distributed in more than one cellular pool (Berl *et al.*, 1961; Berl and Clarke, 1983). Single neu-

rons were found that were tuned to respond to 0.001 M tannic acid, and represented a subpopulation of neurons that was distinct from neurons responsive to the tastes of glucose (sweet), NaCl (salty), HCl (sour), quinine (bitter) and monosodium glutamate (umami) (Critchley and Rolls, 1996). Taste in *Drosophila* is mediated by sensory bristles that reside on the proboscis, legs, wing, and genitalia (Stocker, 1994). Scott *et al.* (2001) have performed in situ hybridization and chemosensory gene experiments that reveal expression of these genes in both gustatory and olfactory neurons in adult flies and larvae.

MATERIALS AND METHODS

Drosophila population

1. Flies were collected from a natural population of *Drosophila* at the Faculty of Agriculture Farm, Tanta, Egypt.
2. The Curly Lobe/Plum (CyL/Pm) stock was used to detect the lethal effect of quinine (QUI) and monosodium glutamate (MSG).

Lethal load

Genetic load due to lethals (L) as expressed in lethal equivalents (Morton *et al.*, 1956) has been calculated in the following manner. The chance of surviving a lethal is $1 - x$ and the load L is 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 (Chung, 1962).

Larvae

Third-instar feeding-stage larvae from the *Drosophila* natural population I was collected normal natural population and Curly Lobe/Plum (CyL/Pm) wild-type, aged 5 days after egg laying were used. Flies were maintained on standard medium, in mass culture at 25°C, 60-70% relative humidity and a 14/10 hour light/dark cycle for behavior study. For each concentration of quinine and monosodium glutamate females were put individually in food vials to lay eggs at 18°C, for chromosome squashing to screen for the chromosome rearrangements. Inversions (homo/ heterozygous) were identified according to the standard chromosomal map of Lindsely and Grell (1967).

Pupa

In these experiments for behavior study we site the larvae after counted in the choice test for 3 days to become pupae, then recorded the number of pupae on either side of the dish, and calculated a gustatory preference index (PREF Pupariation). With the same way as his account of the larvae in the choice test.

Choice

In these experiments, the Petri dishes (with 90 mm inner diameter) were prepared and separated into two halves with a piece of overhead transparency, fill one side with only 1% agarose and the other side with 1% agarose was added with quinine hemisulfate or monosodium glutamate as a bitter or umami tastant,

with the following concentrations (0.2, 2.0 g/l QUI and 10, 22 g/l MSG); for the control condition, the medium was QUI or MSG free.

Fifteen larvae were placed in the middle of the dish and close the lid. The QUI or MSG-side is in half of the cases to the right and in the other half to the left, to balance for spurious effects of the experimental surround. Numbers of larvae were recorded on the two sides of the plate and calculate a gustatory preference index (PREF Gustatory) as follows:

$$\text{PREF}_{\text{Gustatory}} = \frac{\# \text{QUI} - \# \text{PURE}}{\# \text{TOTAL}}$$

Where # indicates the number of larvae on the respective side of the plate (El-Keredy *et al.*, 2012; Koning *et al.*, 2014).

Statistical analyses

The general linear models procedure of the SAS (1988) was utilized. Significant differences among means were determined by Duncan's multiple-range tests (Duncan, 1955).

RESULTS AND DISCUSSION

In the current experiments basically used the two concentrations of QUI (2.0 and 0.2 g/l) and two MSG concentrations (10 and 22 g/l) for ten generations to determine the effects of QUI and MSG toxicity on genetic load due to lethal.

The results in Table (1) revealed that the total number of second chromosome, average frequency of lethal and lethal load (L) for each QUI and MSG

concentration for ten generations. Higher and lower concentrations from QUI and higher concentration of MSG 22 g/L due to highly significant L 1.43, 1.23 and 0.94, respectively. These concentrations caused to higher lethal percentages; 80.67, 68.33 and 58.28, respectively. Comparing to the control the L 0.01 and lowest percentage 1.33.

So, quinine concentrations and higher concentration of MSG revealed a highly variation compared to the control. Toxicity of glutamate was studied in neuronal and neuroblastoma cells and the glutamate a dual effect, depending on concentration of glutamine in the culture medium (Simantov, 1989). Many toxic compounds are reported to taste bitter in humans, and are avoided by many animals, which have developed specialized cells to detect them (Glendinning, 1994). The accurate nucleus of the hypothalamus was particularly vulnerable to MSG-induced lesions in the infant mouse, rat, rabbit and a single immature rhesus monkey injected subcutaneously with doses of MSG ranging from 0.5 to 2.7 g/kg body weight (Olney and Sharpe, 1969).

Data in Table (2) concluded that in the 3rd generation the inversion 2L(Cy) on the chromosome two which decreased from 20% (control or natural population) to 6% (2.0 g/l) and 4% (0.2 g/l), respectively of quinine (QUI), on the other hand decreased to 4% (10 g/l) and did not change throughout the higher concentration (22 g/l) of monosodium glutamate (MSG) the same result was reached for the

higher concentration of MSG with other types of inversions except 3R (C) where completely disappeared (Fig. 5).

Inversion 2R(NS) did not show in (0.2 g/l) QUI but increased comparing with control and show a difference in the case of MSG. 3R(MO) on the chromosome three was eliminated from the higher concentration of QUI but inversion 3R(C) deleted from higher concentration of MSG. For 3L(M) was eliminated from quinine and monosodium glutamate concentration like the natural population except lower concentration of MSG (10 g/l) which appeared 2%.

Same result in natural populations of *D. subobscura* it is common to observe a large number of segregating gene arrangements in a given chromosome (Kirkpatrick and Barton, 2006). The results summarized in Table (3) in the 5th generation, the inversion 2L(Cy) in (Fig. 5) was decreased when treated with QUI concentrations from 18% (natural population) to 8% and when treated with MSG (10, 22 g/l) to 2%, while 2R(Ns) was eliminated in the fifth generation. For inversion 3L(P) decreased from 20% to 16% (2.0, 0.2 g/l) QUI respectively and 4% (MSG concentrations) compared with the control 30%.

Results presented in Table (4) the effects of QUI and MSG in the tenth generation on the inversion frequencies of *D. melanogaster*. Inversion 2R(Ns) in (Fig. 5) was eliminated from all concentrations except 0.2 g/l QUI. On the other side inversion 3L(M) in the third chromosome appeared only in the lower concentration

of QUI. Another trend was appeared for inversions, 3L(P) and 3R(C) which increased in its frequency for all concentrations of QUI and MSG comparing with the control. Only 3R(MO) decreased from 6% (2 g/l) to 12% (0.2 g/l) QUI and increased from 12% (10 g/l) to 20% (22 g/l) MSG.

The results of the cytological study, illustrated the effect of each of QUI and MSG on inversion frequencies compared to the control from the beginning of the third generation and its effect lasts until the tenth generation, extra chromosomes were appeared and others disappear as a result of this effect, and in particular the inversions 2L (Cy) and 2R (Ns) in the second chromosome and 3L (P, M) in the third chromosome (Fig. 5).

The most of gustatory receptor genes located on the left arm of chromosome 3 (Ueno *et al.*, 2001; Dahanuker *et al.*, 2001).

It is common to observe a large number of segregating gene arrangements in a given chromosome in natural populations of *D. subobscura*. Under the local daptation scenario (Kirkpatrick and Barton, 2006).

Quinine has major toxic effects on the nervous system including optic and auditory nerve damage secondary to both vascular and neural injury. Cinchonism, characterized by tinnitus, headaches, disturbed vision, and occasionally deafness and anaphylactic shock may be seen. Quinine causes an initial generalized stimula-

tion of the central nervous system leading to fever, delirium, and increased ventilatory rate which is followed by coma and respiratory depression (Goldenberg and Wexler, 1988).

Figures (1 and 2) show the effects of QUI and MSG in the fifth and tenth generations on the preference of larval *Drosophila*. The median of preference decreased from -0.037 (0.2 g/l) to -0.187 (2.0 g/l) of QUI in the fifth generation and from 0.21759 (0.2 g/l) to -0.222 (2.0 g/l) QUI in the tenth generation, the preference also decreased from -0.26377 (control) in the fifth generation to -0.5 (control) in the tenth generation.

quinine control result compatible with (El-Keredy *et al.*, 2012) but the difference in results is due to the dosage effect of quinine, which was placed in vials, in addition to the located in Petri dishes when conducting the test, so the concentration was doubly. *Drosophila* taste behavior and characterize a neural population that controls a specific subprogram of this behavior (Gordon and Scott, 2008).

For MSG concentrations, the preference decreased from 0.71428 (control) to 0.10688 (22 g/l) and 0.0666 (10 g/l) of MSG in the fifth generation. While in the tenth generation, the median of preference increased in the higher concentration. (Simantov, 1989) noted that neuronal activation regulates glutamate cytotoxicity, effect of chronic membrane depolarization and mechanism by which glutamine at high concentrations renders glutamate cytotoxic is yet unknown. Therefore ob-

served this special track deviation or preference of monosodium glutamate.

Behavioral and afferent nerve responses to glutamate are synergistically enhanced by the presence of 5-ribonucleotides, a characteristic feature of umami taste (Kuninaka, 1960). The implication is that umami responses may originate from more than a single type of receptor or receptor combination. Indeed, taste would not be unique in possessing such redundancy of receptors (Maruyama *et al.*, 2006). *Drosophila* larvae demonstrate that a given GR gene is expressed in one neuron in the larval terminal organ. Strains bearing two different GR promoter fusions reveal twice the number of expressing cells. Similar results are obtained in adult gustatory (Scott *et al.*, 2001). The positive selection has operated on some amino acids in extracellular domains, functional constraints against T2R genes are more relaxed in primates than in mice and this trend has culminated in the rapid deterioration of the bitter-tasting capability in humans (Go *et al.*, 2005).

Figures (3 and 4) represents the effects of QUI and MSG on the preference of pupal *D. melanogaster* in the fifth and tenth generations, the data show that the pupal preference decreased from -0.00625 (control) to -0.2631578 (0.2 g/l) and -0.35897 (2 g/l) of QUI in the fifth generation. The same thing in the tenth generation, the preference decreased from -0.5 (control) to 0.3589 (0.2 g/l) and -0.4605 (2 g/l) QUI. Also the pupal preference decreased from the fifth generation to tenth

generation in the control and concentrations of QUI. While the preference increased from -0.408 (control) to (22 g/l) of MSG in the fifth generation and - 0.27 (control) to -0.247 (22 g/l) MSG, in the tenth generation, the lower concentration of MSG similar the control. From the results presented note the significant difference in the effect of each of QUI and MSG on larvae and pupae *Drosophila* where it appears obvious difference.

The analysis of the pattern of GR gene expression by in situ hybridization demonstrates that a small number of GR genes is transcribed in either the proboscis or the antenna, suggesting that this family encodes chemosensory receptors involved in smell as well as taste (Scott *et al.*, 2001). All of the GR genes contain a signature motif in the carboxyl terminus that is also present within some members of the DOR gene family.

A full explanation of umami taste transduction may involve novel combinations of the proposed receptors and/or as-yet-undiscovered taste receptors (Maruyama *et al.*, 2006). Insect *Ors* and *Grs* might have distinct molecular properties and mechanisms of ligand recognition and/or signal transduction (Smadja *et al.*, 2009).

SUMMARY

Genetic and behavioral effects of both quinine and monosodium glutamate were studied on a natural population of *Drosophila melanogaster* from Tanta, Egypt. The main aim of this study was to

determine the long-term effects (toxicity) and short-term effects (choice) of quinine (QUI) and monosodium glutamate (MSG) on *D. melanogaster*. Two concentrations of quinine (0.2, 2.0 g/l) were used, and two concentrations of monosodium glutamate (10, 22 g/l).

Regarding long-term effects (toxicity) the genetic load was measured to be 1.23 and 1.43 for lower and higher of quinine concentrations, and 0.49 and 0.94 for monosodium glutamate concentrations, respectively. Cytological study revealed that there were different types of selection regarding the inversions 2L(Cy), 2R(NS), 3L(P), 3R(Mo) and 3R(C). Inversion 2R(NS) was eliminated from the basic population after treatment with quinine and monosodium glutamate concentrations in fifth and tenth generations.

Regarding short-term effects, this study used quinine as a case of a substance which humans report as "tasting bitter" and monosodium glutamate as "tasting umami". The dose-effect- behavioral functions (choice) for quinine and monosodium glutamate concentrations were showed. The influence of quinine on the preference was different in larva compared to pupa, while in monosodium glutamate case; there was no difference between larva and pupa.

The study focused on the genetics and behavioral effects the results showed correlation between toxicity and brief-access tests of bitter and umami tastants.

The results lay a foundation for genetic and behavior effects in genetic model organism. Increasing the concentration of quinine and monosodium glutamate increasingly the harmful effect on insects, larvae and pupae *Drosophila*, also represented in influencing the chromosomes (inversions of chromosomes) as well as behavior change as the results showed.

ACKNOWLEDGEMENTS

I am indebted and particularly grateful to my professor Prof. Bertram Gerber (Department Genetics of learning and memory, Magdeburg, Germany) where he has given me the work tools for behavior part and moral support. I would like to acknowledge Prof. Megeed M. (emeritus professor. Genetic Department, Agriculture Faculty, Kafrelsheikh University) to encourage me to do this search. Also i want to say thanks to my colleague Dr. Ola Galal (associate professor in the same department) for her help in cytological part.

REFERENCES

- Berl, S. and D. D. Clark (1983). The metabolic compartmentation concept, in Gfutamine, Glutamate and GABA in the Central Nervous System (Hertz L., Kvamme E., McGeer E. G., and Schousboe A., eds), p. 205-217. Alan R. Liss Inc., New York.
- Berl, S., A. Lajtha and H. Waelsch (1961). Amino acid and protein metabolism. V1. Cerebral compartments

- of glutamic acid metabolism. *J. Neurochem.* 7: 186-197.
- Beyreuther, K., H. K. Biesalski, J. D. Fernstrom, P. Grimm, W. P. Hammes, U. Heinemann, O. Kempfski, P. Stehle, H. Steinhart and R. Walker.(2006). Consensus meeting: monosodium glutamate – an update. *European Journal of Clinical*, 1-10
- Chandrashekar, J., K. L. Mueller, M. A. Hoon, E. Adler, L. Feng, W. Guo, C. S. Zuker and N. J. Ryba (2000). T2Rs function as bitter taste receptors. *Cell*, 100: 703-711.
- Chung, C. S. (1962). Relative genetic loads due to lethal and detrimental genes in irradiated populations of *Drosophila melanogaster*. *Genetics*, 47: 1489-1504.
- Colomb, J., N. Grillenzoni, A. Ramaekers and R. F. Stocker (2007). Architecture of the primary taste center of *Drosophila melanogaster* larvae. *J. Comp. Neurol.*, 502: 834-847.
- Critchley, H. D. and E. T. Rolls (1996). Responses of primate taste cortex neurons to the astringent tastant tannic acid. *Chem, Senses*, 1, 139: 135-145.
- Dahanuker, A., K. Foster, van der Goes W. M. van Naters and J. R. Carlson (2001). A Gr receptor is required for response of sugar trehalose in taste neurons of *Drosophila*. *Nat. Neurosci.*, 4: 1182-1186.
- Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, 11: 1-42.
- Ebbs, M. L. and H. Amrein (2007). Taste and pheromone perception in the fruit fly *Drosophila melanogaster*. *Pflugers Arch.*, 454: 735-747.
- El-Keredy, A., M. Schleyer, C. König, A. Ekim and B. Gerber (2012). Behavioural analyses of quinine processing in choice, feeding and learning of larval *Drosophila*. *PLoS One*, 7: e40525.
- Gerber, B., R. F. Stocker, T. Tanimura and A. S. Thum (2009). Smelling, tasting, learning: *Drosophila* as a study case. *Results Probl. Cell Differ.*, 47: 139-185.
- Glendinning, J. I. (1994). Is the bitter rejection response always adaptive? *Physiol. Behav.*, 56: 1217-1227.
- Go, Y., Y. Satta, O. Takenaka and N. Takahata (2005). Lineage-Specific loss of function of bitter taste receptor genes in humans and non-human primates. *Genetics*, 170: 313-326.
- Goldenberg, A. M. and L. F. Wexler (1988). Quinine overdose: Review of toxicity and treatment. *Clin. Cardiol.*, 10: 716-718.

- Gordon, M. O. and K. Scott (2008). Motor control in a *Drosophila* taste circuit. *Neuron*, 61: 373-384.
- Hegarty, M. P. (1987). Toxic amino acids in foods of animals and man. *Proc. Nutr. Soci. Australia*, 11: 73-81.
- Keene, A. C., M. J. Krashes, B. Leung, J. A. Bernard and S. Waddell (2006). *Drosophila* dorsal paired medial neurons provide a general mechanism for memory consolidation. *Curr. Biol.*, 16: 1524-1530.
- Kim, J. R., S. H. Jung, J. M. Regan and B. E. Logan (2007). Electricity generation and microbial community analysis of alcohol. *Bioresource Technology*, 98: 2568-2577.
- Kirkpatrick, M. and N. Barton (2006). Chromosome inversions, local adaptation and speciation. *Genetics*, 173: 419-434.
- Köning, C., M. Schleyer, J. Leibiger, A. El-Keredy and Gerber B. (2014). Bitter-Sweet Processing in Larval *Drosophila*. *Chem. Senses*, 1-17.
- Kuninaka, A. (1960). Studies on taste of ribonucleic acid derivatives. *J. Agric. Chem. Soc. Japan*, 34: 487-492.
- Lee, Y., S. J. Moon and C. Montell (2009). Multiple gustatory receptors required for the caffeine response in *Drosophila*. *Proc. Natl. Acad. Sci. USA*, 106: 4495-4500.
- Lindsey, D. L. and E. H. Grell (1968). Genetic variations of *Drosophila melanogaster*. Carnegie Institution of Washington Publ. 627, Washington, DC.
- Lucas, D. R. and J. P. Newhouse (1957). The toxic effect of sodium-L-glutamate on the inner layers of the retina. *AMA Arch. Ophthalmol.*, 58: 193-201.
- Maruyama, Y., E. Pereira, R. F. Margolskee, N. Chaudhari and S. Roper (2006). Umami responses in mouse taste cells indicate more than one receptor. *The Journal of Neuroscience*, 26: 2227-2234.
- Megeed, M. S., S. Dora and A. A. Ali. (1997). Genetic effects of monosodium glutamate on *Drosophila*. *Proceeding of the 7th International Conference of Environment Protection*, Alexandria, Egypt, p. 295-304
- Moon, S. J., Y. Lee, Y. Jiao and C. Montell (2009). A *Drosophila* gustatory receptor essential for aversive taste and inhibiting male-to-male courtship. *Curr. Biol.*, 19: 1623-1627.
- Morton, N. E., J. F. Crow and H. J. Muller (1956). An estimate of the mutational damage in man from data on consanguineous marriages. *Proc. Natl. Acad. Sci. US*, 42: 855-863.
- Olney, J. W. and L. G. Sharpe (1969). Brain lesions in an infant rhesus

- monkey treated with monosodium glutamate. *Science*, 166: 386-388.
- Oser, B. L., K. Morgareidge and S. Carson (1975). Monosodium glutamate studies in four species of neonat and infant animals. *Food Cosmet. Toxicol.*, 13: 7-14.
- Santos, M. (2009). Recombination Load in a Chromosomal Inversion Polymorphism of *Drosophila subobscura*. *Genetics*, 181: 803-809.
- SAS (1988). SAS/STAT User's Guide. Release 6.03 Edition. SAS Institute, Inc., Cary, NC.
- Scott, K., R. Brady, Jr. A. Cravchik, P. Morozov, A. Rzhetsky, C. Zuker, and R. Axel (2001). A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. *Cell*, 104: 661-673.
- Schwaerzel, M., M. Monastriotti, Friggi-Grelin F. Scholz, S. Birman and M. Heisenberg (2003). Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in *Drosophila*. *J. Neurosci.*, 23: 10495-10502.
- Simantov, R. (1989). Glutamate neurotoxicity in culture depends on the presence of glutamine: Implications for the role of glial cells in normal and pathological brain development. *J. Neurochemistry*, 52: 1694-1699.
- Smadja, C., P. Shi, R. K. Butlin and H. M. Robertson (2009). Large gene family expansions and adaptive evolution for odorant and gustatory receptors in the Pea Aphid, *Acyrtosiphon pisum*. *Mol. Biol. Evol.*, 26: 2073-2086.
- Stegink, L. D., L. J. Filer and G. L. Baker (1973). Monosodium glutamate metabolism in the neonatal pig: Effect of load on plasma, brain, muscle and spinal fluid free amino acid levels. *J. Nutr.*, 103: 1138-1145.
- Stocker, R. F. (1994). The organization of the chemosensory system in *Drosophila melanogaster*: a review. *Cell Tissue Res.*, 275: 3-26.
- Stocker, R. F. (2008). Design of the larval chemosensory system. In: "Brain Development in *Drosophila melanogaster*" (ed. Gerhard M. Technau), *Adv. Exp. Med. Bio.*, 628: 69-81.
- Sugai, G. (2007). Responsiveness-to-intervention: Lessons learned and to be learned. Keynote presentation at and paper for the RTI Summit, US Department of Education, Washington, DC.
- Tanimura, T. and H. Ishimoto (2003). Molecular neurophysiology of taste in *Drosophila*. *CMLS, Cell. Mol. Life Sci.*, 60: 001-09.

Thorne, N. and H. Amrein (2008). Atypical expression of *Drosophila* gustatory receptor genes in sensory and central neurons. *J. Comp. Neurol.*, 506: 548-568.10.1002/cne.21547.

Tully, T., T. Preat, S. C. Boynton and M. DelVecchio (1994). Genetic dissection of consolidated memory in *Drosophila*. *Cell*, 79: 35-47.

Ueno, K., M. Ohta, H. Morita, Y. Mikuni,

S. Nakajima, K. Yamamoto and K. Isono (2001). Trehalose sensitivity in *Drosophila* correlates with mutations in and expression of the gustatory receptor gene Gr5a. *Curr. Biol.*, 11: 1451-1455.

Zhao, G. Q., Y. Zhang, M. A. Hoon, J. Chandrashekar, I. Erlenbach, N. J. Ryba and C. S. Zuker (2003). The receptors for mammalian sweet and umami taste. *Cell*, 115: 255-266.

Table (1): Average frequency of lethal and genetic load due to lethal to quinine and monosodium glutamate.

Chemical material	Dose (g/l)	Total No. of chromosomes	No. of lethal	Percentage %	Lethal load (L)
Quinine (QUI.)	0.2	300	205	71.48a	1.23 ± 0.11a
	2.0	300	242	76.67a	1.43 ± 0.15a
Monosodium glutamate (MSG)	10.0	350	122	34.86c	0.49 ± 0.10c
	22.0	350	204	58.28b	0.94 ± 0.09b
Control	0.0	300	4.0	1.05d	0.07 ± 0.03d

Table (2): Effects of quinine and monosodium glutamate on the chromosome inversion frequencies of *D. melanogaster* in the 3rd generation.

Inversion	Quinine (g/l)				Monosodium glutamate (g/l)				Control	
	0.2		2.0		10		22			
Chromosome I	N	%	N	%	N	%	N	%	N	%
2 L (Cy)	2	4.0	3	6.0	2	4.0	10	20.0	10	20.0
2 R (Ns)	-	-	12	24.0	1	2.0	10	20.0	7	14.0
Chromosome III										
3L (P)	5	10.0	8	16.0	4	8.0	20	40.0	20	40.0
3L (M)	-	-	-	-	1	2.0	-	-	-	-
3R (MO)	7	14.0	-	-	2	4.0	10	20.0	10	20.0
3R (C)	7	14.0	31	62.0	4	8.0	-	-	12	24.0
Tot. No. of Chromosome examined	50		50		50		50		50	
	100				100				50	

N: indicates number of inversions detected.

Table (3): Effects of quinine and monosodium glutamate on the chromosome inversion frequencies of *D. melanogaster* in the 5th generation.

Inversion	Quinine (g/l)				Monosodium glutamate (g/l)				Control	
	0.2		2.0		10		22			
Chromosome I	N	%	N	%	N	%	N	%	N	%
2 L (Cy)	4	8.0	4	8.0	1	2.0	1	2.0	9	18.0
2 R (Ns)	-	-	-	-	-	-	-	-	8	16.0
Chromosome III										
3L (P)	8	16.0	10	20.0	2	4.0	2	4.0	15	30.0
3L (M)	-	-	-	-	-	-	-	-	-	-
3R (MO)	4	8.0	5	10.0	8	16.0	1	2.0	8	16.0
3R (C)	10	20.0	10	20.0	2	4.0	3	6.0	11	22.0
Tot. No. of Chromosome examined	50		50		50		50		50	
	100				100				50	

Table (4): Effects of quinine and monosodium glutamate on the chromosome inversion frequencies of *D. melanogaster* in the 10th generation.

Inversion	Quinine (g/l)				Monosodium glutamate (g/l)				Control	
	0.2		2.0		10		22			
Chromosome I	N	%	N	%	N	%	N	%	N	%
2 L (Cy)	12	24.0	9	18.0	5	10.0	8	16.0	6	12.0
2 R (Ns)	1	2.0	-	-	-	-	-	-	5	10.0
Chromosome III										
3L (P)	15	30.0	14	28.0	14	28.0	18	36.0	13	26.0
3L (M)	1	2.0	-	-	-	-	-	-	-	-
3R (MO)	6	12.0	3	6.0	6	12.0	10	20.0	9	18.0
3R (C)	18	36.0	17	34.0	18	36.0	21	42.0	12	24.0
Tot. No. of Chromosome examined	50		50		50		50		50	
	100				100				50	

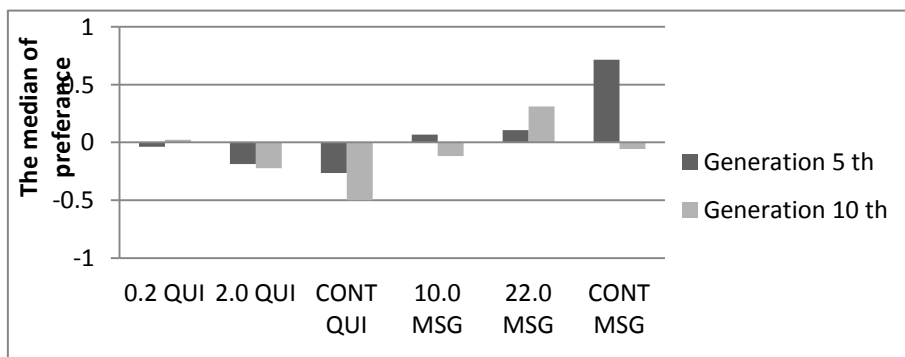


Fig. (1): Histogram of the median of preference for quinine and monosodium glutamate in 5th and 10th generations on larval *D. Melanogaster* after 8 minutes.

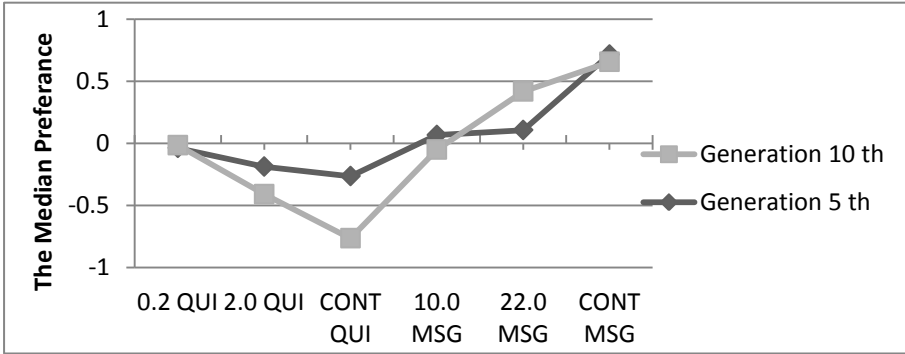


Fig. (2): Curves of the median of preference for quinine and monosodium glutamate in 5th and 10th generations on larval *D. melanogaster* after 8 minutes.

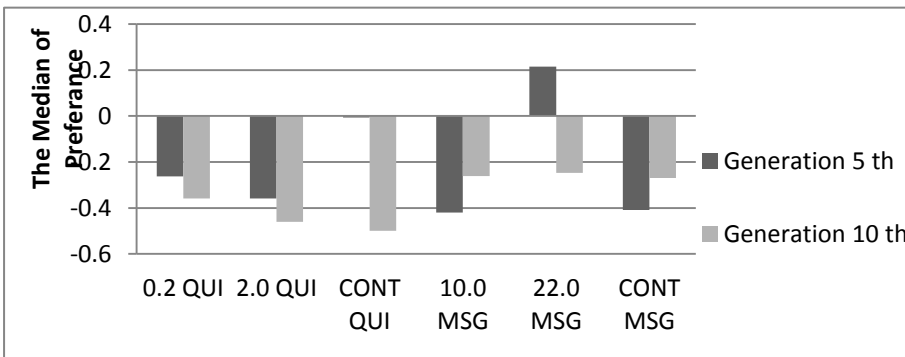


Fig. (3): Histogram of the median of preference for quinine and monosodium glutamate in 5th and 10th generations on pupal *D. melanogaster* after 8 minutes.

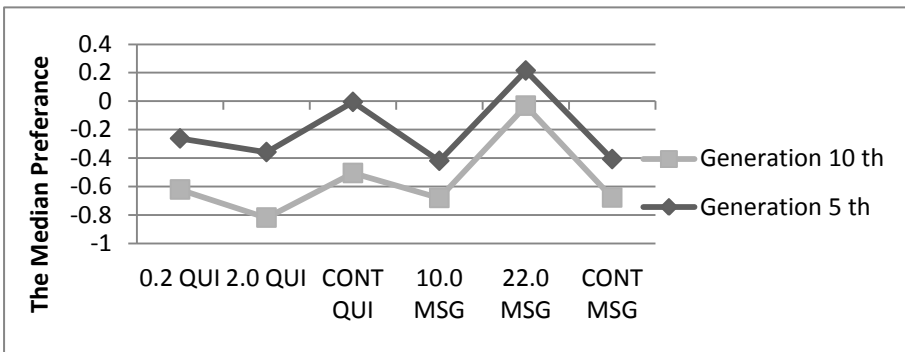


Fig. (4): Curves of the median of preference for quinine and monosodium glutamate in 5th and 10th generations on pupal *D. melanogaster* after 8 minutes.

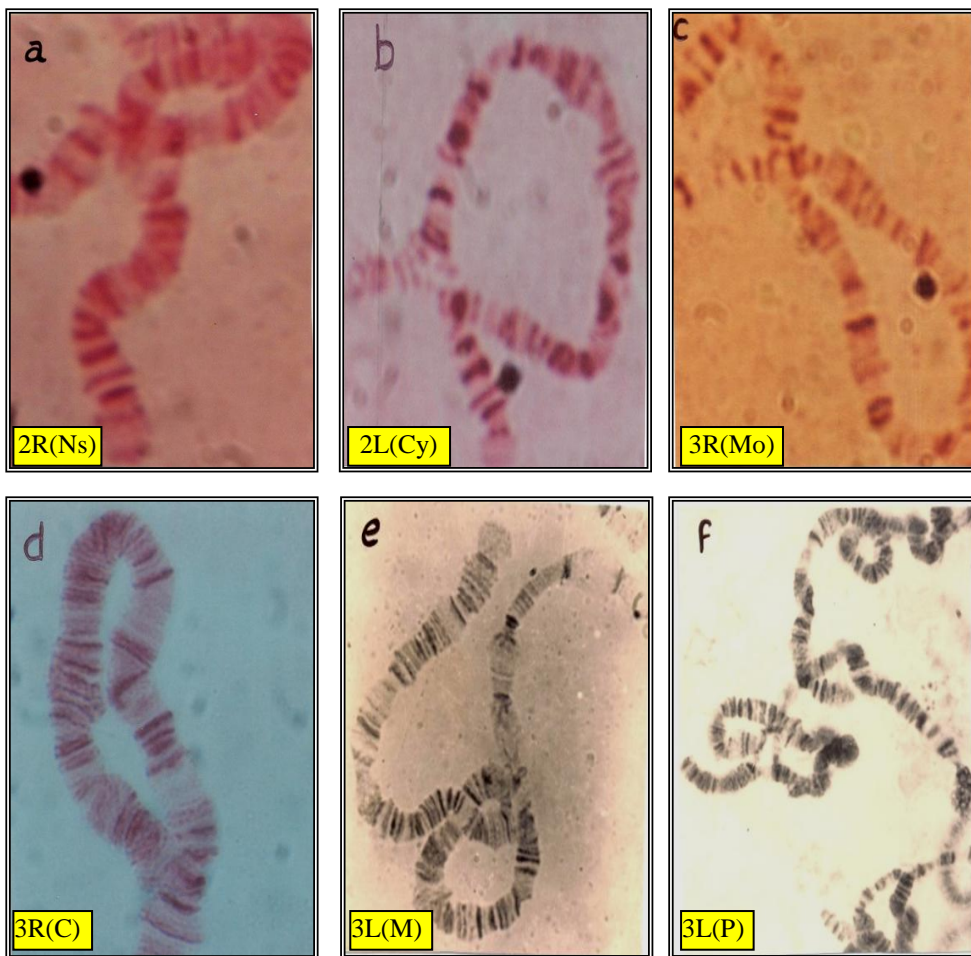


Fig. (5): Microphotographs of chromosomal inversions.