

Review Article

Investigation of Insect Chromosomes Using Some Cytogenetic Methods

Safa Salah Salman⁽¹⁾ and Dhefaf Radhi Mahdi^{*(2)}

(1). Department of Biology, Faculty of Education, Al-Iraqia University. Iraq.

(2). Department of Biology, Faculty of Science, Tikrit University, Iraq.

(*Corresponding author: Dr. Dhefaf Radhi Mahdi. E-Mail: dhefaf.radi@tu.edu.iq).

Received: 01/10/2020

Accepted: 20/10/2020

Abstract

Chromosomes are the secret of the transmission of the characteristics of parents to children, and this applies to all plants, animals and single cells. Chromosomes carry genes, and determine the type and characteristics of the next generation. There are some insects that scatter and leave their eggs, and others lay their eggs in tree trunks or in dead animal tissues or stick eggs on the back of the male after intercourse. And cockroaches and grasshoppers lay their eggs closed in a spongy substance that forms a mass of eggs, and few types of insects do not depend on fertilization, as unfertilized eggs lay half the number of chromosomes to multiply and grow as if fertile, and their females do not need to mate, and this is common in aphids and small insects that feed on juice plants, especially in the spring, when food juice is abundant, and when food is scarce in summer, they resort to sexual reproduction. In sum, it can be said that most insects make sure that their eggs are close to a source of food after they lay it.

Keywords: Chromosome number, Insect, Cytogenetic Methods, classification, Cytogenetic variants.

Introduction:

The list of organisms by chromosome number describes the chromosome or numbers of chromosomes in the cells of plants, animals, pioneers, and various other organisms (Chambers, 1917). This number (the number of chromosomes in the cell), as well as the visual appearance of the chromosome, is known as the nuclear pattern, and can be found by looking at the chromosomes through a microscope. Attention is paid to its length, centromere position, range pattern, any differences between sexual chromosomes and any other physical properties (Carlson, 1952; Nicklas and Staehly, 1967). The preparation and study of nuclear patterns is part of cellular genetics (Nicklas, 1967). Biological diversity is the occurrence of multiple and varied phenotypes of organisms, and this term is sometimes used to explain that different morphologies can arise from the same genotype, while the term genetic polymorphism is used by molecular biologists differently to describe some mutations or differences in The genotype is such as the nucleotide polymorphisms which may not always correspond to the phenotype. Polymorphism and its diversity is common in nature and is associated with biological diversity, genetic diversity and adaptation, and usually leads to the maintenance of a variety of different forms in a diverse environmental community, and polymorphism may be the most common example of

it, and other examples include various blood types, human hemoglobin and different types of butterflies and insects (Nicklas and Koch, 1969; Nicklas and Ward, 1994). Polymorphism results from different evolutionary processes according to the theory of evolution as does any aspect of the species, it is ultimately a genetic method and is modified by natural selection as the genetic makeup of the individual determines his external form, and the term polymorphism also indicates the emergence of more than two different types of The structural and functional aspect, and despite the general use, the term variety of shapes is very broad, but in biology it refers to a specific meaning, especially when we talk about two apparent forms only, noting also that this definition deletes the characteristics that are constantly different such as weight despite It contains inheritable components, but polymorphism deals with forms in which the difference is binary and clear, and it is common to use the phrase "morph" or "polymorphism" to refer to geographical and spatial differences, but this use is completely wrong because geographical differences may lead to Variation in formal characteristics occurs, but true polymorphism occurs within single clusters (Li and Nicklas, 1995; Nicklas, 1983).

This term was used for the first time to describe the visible morphological differences, but it is now used to describe the invisible differences also, such as blood types, for example, which can be detected by laboratory analysis, and rare differences or genetic mutations are not classified as multiple forms, because this classification requires that The frequency of the formal trait is too high to be caused by new genetic mutations, and if we want to use the language of numbers, it must be greater than 1% although this percentage is much higher than any normal rate of genetic mutations (Nicklas, 1988; Nicklas and Koch, 1972).

Definition of Cytogenetic:

Cytogenetic, is a branch of genetics concerned with the study of genetic phenomena and how chromosomes relate to the behavior of the cell, especially its behavior during equal division and meiosis without the need for DNA extraction through the use of optical microscopy (Zhang and Nicklas, 1995; Nicklas *et al.*, 1989). The techniques used include stereotyping of cellular nuclei, analysis G-banded chromosomes, and other genetic cellular domain techniques, as well as molecular cellular genetics such as fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH) (Zhang and Nicklas, 1995).

Scientific classification:

The basic scientific classification was that the six-legged division consists of four main groups: insects, glove-tailed, silverfish with two thorns, and conical-head, where the last three groups were classified as belonging to the "inner jaw" group (in Latin: Entognatha) because Its mouthparts are internal (Nicklas, 1977; Paliulis and Nicklas, 2000). This classification has changed dramatically, especially after the advancement of methods of studying the evolution of animals, where it became possible to determine their ancestors more accurately, in addition to the development of studies in genetics. One theory has recently emerged that postulates that hexapods are multiracial, in which it appears that an endopods group has an evolutionary pathway different from that of insects (Church *et al.*, 1986).



Figure 1. Some insects (dancing fly, long-beaked fruit weevil, mole cricket, German wasp, imperial gum moth, and shrew bug) (Forer and Koch, 1973)

The section of exopterygote (Latin: exopterygote) can be divided from Neoptera to arthropods (Orthopteroida) and Hemipteroid (Latin: Hemipteroida) (Camenzind and Nicklas, 1968). It can also be called the exopteryoid and exopteroid. There are about 5,000 species in the order of Odonata (Latin: Odonata), 2,000 species of mantis, 20,000 species of grasshoppers and their relatives, 170,000 butterflies and moths, 120,000 flies, 82,000 true bugs, 360,000 species of beetles, and 110,000 bees. And ants. Since most of the ancient scientific classifications of different people have now emerged as being multi-ethnic in fact, it is best when classifying insects to avoid using words such as class, above rank, and suborder, and focus on the ranks instead. The following list represents the best agreed upon scientific compilations of insects (Ault *et al.*, 2017; Felt *et al.*, 2017).

Chromosome Chemistry and Familial Propensity:

The chromosome is chemically composed of two nucleic acids, namely, deoxyribonucleic DNA, Ribonucleic acid, and certain types of proteins. The increase in the number of sex cells, the formation

of the small nucleus, the increase in the central assembly of the chromosomes and the increase in the rate of chromosomal changes represented by the chromosomal and chromatid fracture and chromosomal segments, annular chromosome, and types of chromosomal abnormalities

A- The anomaly that occurs in autosomes

B- The anomaly in the sex – chromosomes (Golding and Paliulis, 2011).

Each of these two sections is further classified into two main types, depending on the type of anomaly, namely, numerical chromosomal abnormalities and structural chromosomal abnormalities (Doan and Paliulis, 2009).

Where the Canadian scientist Murray Barr discovered in 1949, by chance, the existence of a nuclear body inside insect neurons, which he called the nuclear follower, and indicated that its existence is limited to female cells, not male cells, and thus was considered a useful body for the purposes of distinguishing between male and female (Paliulis, and Nicklas; 2004). In 1951 Barr named the nuclear dependent, sex-chromatin, and later named Par's particle after his discoverer. Then it became clear that a bar particle could be seen in the cells of most tissues and in many types. Bar's particle is located closely to the inner surface of the nuclear membrane and reaches a diameter of about one micron and can be clearly observed through swabs taken from human skin or from the mucous layer of the lining of its mouth and when preparing the traditional laboratory report, it writes female sex chromatin or what is written as a positive particle Barr minus. It has been shown that only one chromosome, and not more, will be active, regardless of the increase in the number of X chromosomes present in the cell (<http://micromanipulator.scholar.bucknell.edu>, 2018). Therefore, the slight phenotypic differences between individuals with an abnormal sex chromosome number and normal individuals are due to the activity of extra chromosomes before the inactivity of those chromosomes. With X-chromatin tests, it is very useful in the field of diagnosing disorders of the sex chromosomes, and it is worth noting that the Parasome can be one of the X-chromosomes - meaning that its origin is either from the father or from the mother and it is ineffective from the genetic point of view (Ellis, 1962).

Cytogenetic Variants:

In previous cytogenetic studies on males of two types of flour beetles, which are the rusty flour beetle, *castaneum. T* and *confusum. T*, to show the imprint of the nucleus and to know the physical and sexual chromosomal shapes. With scissors and pasted onto graph paper and with a unit of measure, that is, the number of squares occupied by the chromosome that was, it was affixed to the graph paper and this represents the length of the chromosome in millimeters, then the units were converted to the micrometer m (which represents the true length of the chromosome) (Ellis and Begg, 1981; Powell, 1953; Alsop and Zhang, 2004). The difference between the sizes of the sex chromosomes X and Y is clearly present in the family Tenebrionidae, as the Y chromosome is smaller than the sex chromosome X, It has been shown that only one chromosome, and not more, will be active, regardless of the increase in the number of X chromosomes in the cell (Zhang and Nicklas, 1996). Therefore, the slight phenotypic differences between individuals with anomalies in the sexual chromosome number and normal individuals are due to the activity of extra chromosomes before the inactivity of those chromosomes (Jockusch and Ober, 2004). There are tests known as X-chromatin tests that are very useful in the field of diagnosing disorders of sex chromosomes, and it is worth noting that the Parasome can be one of the X-chromosomes - meaning that its origin is either from the father or from the mother and is ineffective from the genetic point of view (Dudley, 1998).

Genetic Crossing:

It is a type of genetic transmission or cell division that may occur in somatic cells during the direct mitosis phase in both sexual and asexual creatures, where the study of genetic linkage and crossing over in non-sexual creatures is one of the ways to understand genetic mutations because it is the only source to follow Genetic transmission by binding and crossing over in a single cell. In addition, the phenomenon of crossing over can lead to the expression of recessive or asymmetric genes in an individual, and this expression has important implications for studying tumors and recessive lethal genes (Drake, 1988; Biewener, 2003). The process of homologous linking or crossing over occurs mainly between sister chromatids after the stage of replication (but before cell division), and this pattern of homologous crossing between sister chromatids is usually genetically silent (i.e., recessive characteristics) (Ikawa *et al.*, 2004). Non-sister about 1% compared to crossing over in sister chromatids in the direct division stage (Richard *et al.*, 2007). The phenomenon of genetic crossing over was detected by the detection of the point mutation in the *Drosophila melanogaster* named *Drosophila* insect, where this double detection or mosaic was observed in the *Drosophila* insect (*D. melanogaster*) in early in 1925 AD, but Dr. Curt Stern in 1936 AD explained in detail this phenomenon as a result of direct division. Before the discovery of Dr. Styrone, scientists assumed that the phenomenon of the point mutation occurred because some genes have the ability to get rid of the chromosome that is on it, but After this discovery, many experiments clarified what might happen during direct division in the cell cycle and the mechanism of the phenomenon of transit and genetic linkage (Mill, 1975).

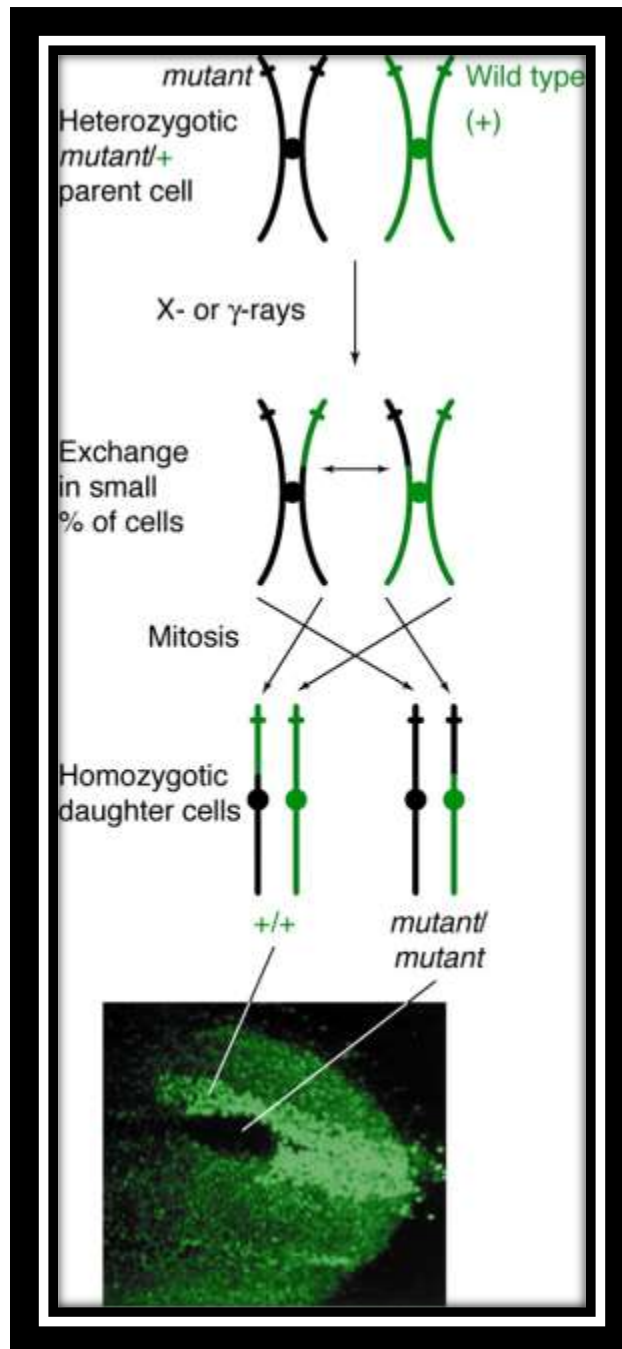


Figure 2. Illustration of a homologous link in an individual asymmetric chromatid (Linsenmair, 1976).

Crossing over can occur at any stage of mitosis but can be clearly observed in individuals who are heterogeneous in specific traits. If crossing over between non-sister chromatids occurs in a specific site, this crossing over affects the traits carried in those genes at the same site, and then both Homologous chromosomes will be chromatids that contain the genetic traits resulting in a phenotype, and the resulting phenotype depends on the way in which the chromosomes are lined up in the interphase stage just before division (Gullan, 2005). If these chromatids occur and contain different alleles on the same side of the row, they will appear. The resulting cells are heterogeneous and thus undetectable despite the occurrence of crossing over in the chromatin, but if the chromatids containing the same alleles are lined up on the same side, the resulting cells will be homogeneous at this site, resulting in the discovery

of a point mutation where one of his cells displays the recessive phenotype. Homozygous while the other cell contains the homozygous wild type phenotype, and if those resulting cells continue to replicate and divide, then the phenomenon of the point mutation will continue in the cell. Mo, which results in a new phenotype, which is the heterogeneous phenotype (Barnes *et al.*, 2001). It is known that genetic crossing or mutations occur in the *Drosophila* insect and some fungi that reproduce sexually and in human cells naturally, as it may allow the expression of recessive and carcinogenic genes naturally and thus prepare the cell for division and formation of the cancerous tumor, and the cell may produce a homogeneous mutation for the tumor-bearing gene. Cancerous and this leads to the same result (Chown, (2004).

Genetic Control:

Genetic methods have been applied to control insects, but in most cases these methods are still under development, and the first genetic applications are the extermination of the modern world spiral fly (Merritt *et al.*, 2007; Eisemann *et al.*, 1984). In this program, the males were sterilized by exposure to gamma rays and released at rates that exceed the natural non-radioactive males present in the local clan for several generations in order to compete with the natural males, which leads to a decrease in the reproductive capacity of the number of insects present in nature depending on the percentage of sterile males that are released (Tracey *et al.*, 2003). If the ratio of 1: 1 (sterile males: natural males) and sterile insects were fully competitive, the reproductive capacity of insects present in nature decreased by 50%. If the ratio was 9: 1, the reproductive capacity of insects in nature decreased by 90%. This technique has been used successfully on the island of Curaçao, and its success was helped by the fact that the female mated only once. This technique is called sterile male release technique (Nevo and Coll, 2001).

The success of this experiment encouraged if it was applied to many other insect pests, including medical and veterinary pests. The cypress fly *Chrysomya* the sarcophaga meat fly (Salt, 1961; Lee and Jr, 1989). These studies included models for the clans to be controlled to determine the percentage of sterile males to be released, and the genetic changes to develop strains that produce males continuously and strains that contain cytoplasmic sterility factors. The cause of infertility in sterile males is due to the presence of lethal mutation dominant mutations, which are nuclear changes or mutations that lead to the death of the zygote or zygote, meaning that these mutations occur in the germ cell that unites with the other germ cell in the fertilization process (Lloyd, 1984; Cator *et al.*, 2009). In general, these mutations do not prevent the maturation of the affected cell and its transformation into a gamete, nor do they prevent gametes from forming the zygote, but rather they work to stop the growth of the zygote until maturity, meaning that the dominant lethal mutations are not fatal to the treated cells, but they are fatal to the zygote after its formation (Briscoe and Chittka, 2001). Death often occurs before the blastoderm phase and during the early cleavage stages. All researchers agreed that the dominance of the deadly mutation is due to a fracture of the chromosome and the failure of this chromosome to fuse (Hristov, 2005). On the other hand, radiation sterilization causes sperm to become idle or stop producing (Bennet-Clark, 1998; Miklas, 2001).

Conclusion:

The study of insect cytogenetics is still in the survey period. Further nuclear patterns analysis for taxonomic review of several groups of species in order. Until now, classical chromosome techniques remain one of the methods for collecting data relevant to systemic entomology. These methods give an

additional impetus to a detailed karyological study of specific insect populations, especially those that have a major impact on humanity.

References:

- 1-Chambers, R. (1917). Microdissection studies II. The cell aster: a reversible gelation phenomenon. *Journal of Experimental Zoology*. 23(3): 483-505.
- 2-Carlson, J.G. (1952). Microdissection studies of the dividing neuroblast of the grasshopper, *Chortophaga viridifasciata*. *Chromosoma*. 5(3): 199-220.
- 3-Nicklas, R.B.; and C.A. Staehly (1967). Chromosome micromanipulation. I. The mechanics of chromosome attachment to the spindle. *Chromosoma*. 21(1): 1-16.
- 4-Nicklas, R.B. (1967). Chromosome micromanipulation. II. Induced reorientation and the experimental control of segregation in meiosis. *Chromosoma*. 21(1): 17-50.
- 5-Nicklas, R.B.; and C.A Koch (1969). Chromosome micromanipulation. 3. Spindle fiber tension and the reorientation of mal-oriented chromosomes. *Journal of Cell Biology*. 43(1): 40-50.
- 6-Nicklas, R.B.; and S.C. Ward (1994). Elements of error correction in mitosis: microtubule capture, release, and tension. *Journal of Cell Biology*. 126(5): 1241-1253.
- 7-Li, X.; and R.B Nicklas (1995). Mitotic forces control a cell-cycle checkpoint. *Nature*. 373(6515): 630-632.
- 8-Nicklas, R.B. (1983). Measurements of the force produced by the mitotic spindle in anaphase. *Journal of Cell Biology*. 97(2): 542-548.
- 9-Nicklas, R.B. (1988). The forces that move chromosomes in mitosis. *Annual Review of Biophysics and Biophysical Chemistry*. 17: 431-449.
- 10-Nicklas, R.B.; and C.A. Koch (1972). Chromosome micromanipulation. IV. Polarized motions within the spindle and models for mitosis. *Chromosoma*. 39(1): 1026.
- 11-Zhang, D.; and R.B. Nicklas (1995). The impact of chromosomes and centrosomes on spindle assembly as observed in living cells. *Journal of Cell Biology*. 129(5): 1287-1300.
- 12-Nicklas, R.B.; G.M. Lee; C.L. Rieder; and G. Rupp (1989). Mechanically cut mitotic spindles: clean cuts and stable microtubules. *Journal of Cell Science*. 94(Pt 3): 415-423.
- 13-Zhang, D.; and R.B. Nicklas (1995). Chromosomes initiate spindle assembly upon experimental dissolution of the nuclear envelope in grasshopper spermatocytes. *Journal of Cell Biology*. 131(5): 1125-1131.
- 14-Nicklas, R.B. (1977). Chromosome distribution: experiments on cell hybrids and in vitro. *Philosophical Transactions of the Royal Society of London B*. 227(955): 267-276.
- 15-Paliulis, L.V.; and R.B. Nicklas (2000). The reduction of chromosome number in meiosis is determined by properties built into the chromosomes. *Journal of Cell Biology*. 150(6): 1223-1232.
- 16-Church, K.; R.B. Nicklas; and H.P. Lin (1986). Micromanipulated bivalents can trigger mini-spindle formation in *Drosophilamelanogaster* spermatocyte cytoplasm. *Journal of Cell Biology*. 103(6): 2765-2773.
- 17-Forer, A.; and C. Koch (1973). Influence of autosome movements and of sex-chromosome movements on sex-chromosome segregation in crane fly spermatocytes. *Chromosoma*. 40(4): 417-442.

- 18-Camenzind, R.; and R.B. Nicklas (1968). The non-random chromosome segregation in spermatocytes of *Grylotalpa hexadactyla*. A micromanipulation analysis. *Chromosoma*. 24(3): 324-335.
- 19-Ault, J.G.; K.D. Felt; R.N. Doan; A.O. Nedo; C.A. Ellison; L.V. Paliulis (2017). Co-segregation of sex chromosomes in the male black widow spider *Latrodectus mactans* (Araneae, Theridiidae). *Chromosoma*. 126(5): 645-654.
- 20-Felt, K.D.; M.B. Lagerman; N.A. Ravidia; L. Qian; S.R. Powers; L.V. Paliulis (2017). Segregation of the amphitelicly attached univalent X chromosome in the spittlebug *Philaenus spumarius*. *Protoplasma*. 254(6): 2263-2271.
- 21-Golding, A.E.; and L.V. Paliulis (2011). Karyotype, sex determination, and meiotic chromosome behavior in two pholcid (Araneomorphae, Pholcidae) spiders: implications for karyotype evolution. *PLoS One*. 6: e24748.
- 22-Doan, R.N.; and L.V. Paliulis (2009). Micromanipulation reveals an XO-XX sex determining system in the orb-weaving spider *Neoscona arabesca* (Walckenaer). *Hereditas*. 146(4): 180-182.
- 23-Paliulis, L.V.; and R.B. Nicklas (2004). Micromanipulation of chromosomes reveals that cohesion release during cell division is gradual and does not require tension. *Current Biology*. 14(23): 2124-2129.
- 24-Biology Micromanipulator. DIY High Precision Micromanipulator. <http://micromanipulator.scholar.bucknell.edu> (2018).
- 25-Ellis, G.W. (1962). Piezoelectric micromanipulators. *Science*. 138(3537): 84-91.
- 26-Ellis, G.W.; and D.A. Begg (1981). Chromosome micromanipulation studies. *Mitosis/Cytokinesis*. Zimmerman, A. M., Forer, A. Academic Press. 155-179.
- 27-Powell, E.O.A. (1953). Micro forge attachment for the biological microscope. *Journal. Royal Microscopical Society*. 72(4): 214-217.
- 28-Alsop, G.B.; and D. Zhang (2004). Microtubules continuously dictate distribution of actin filaments and positioning of cell cleavage in grasshopper spermatocytes. *Journal of Cell Science*. 117(Pt 8): 1591-1602.
- 29-Zhang, D.; and R.B. Nicklas (1996). Anaphase' and cytokinesis in the absence of chromosomes. *Nature*. 382: 466-468.
- 30-Jockusch, E.L.; and K.A. Ober (2004). Hypothesis testing in evolutionary developmental biology: a case study from insect wings. *Journal of Heredity*. 95 (5): 382-396.
- 31-Dudley, R. (1998). Atmospheric oxygen, giant Paleozoic insects and the evolution of aerial locomotor performance. *Journal of Experimental Biology*. 201 (8): 1043-1050.
- 32-Drake, V.A. (1988). The Influence of Atmospheric Structure and Motions on Insect Migration. *Annual Review of Entomology*. 33: 183-210.
- 33-Biewener, A.A. (2003). *Animal Locomotion*. Oxford University Press. ISBN 0-19-850022-X.
- 34-Ikawa, T.; O. Hidehiko; H. Sugihiko; K. Takahiro; and C. Lanna (2004). Distribution of the oceanic insects Halobates (Hemiptera: Gerridae) off the south coast of Japan. *Entomological Science*. 7 (4): 351-357.
- 35-Richard, W.M.; K.W. Cummins; and M.B. Berg (2007). *An introduction to the aquatic insects of North America*. Kendall Hunt Publishers.

- 36-Mill, P.J. (1975). Jet-propulsion in an isopteran dragonfly larvae. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*. 97 (4): 329–338.
- 37-Linsenmair, K. (1976). Das "entspannungsschwimmen" von *Velia* and *Stenus*. *Naturwissenschaften*. 50: 231.
- 38-Gullan, P.J. (2005). *The Insects: An Outline of Entomology* Oxford: Blackwell Publishing. ISBN 1-4051-1113-5.
- 39-Barnes, R.S.K.; P. Calow; P. Olive; D. Golding; and J. Spicer (2001). *Invertebrates with Legs: the Arthropods and Similar Groups. The Invertebrates: A Synthesis*. Blackwell Publishing. Page168. ISBN 0632047615.
- 40-Chown, S.L. (2004). *Insect physiological ecology*. New York: Oxford University Press. ISBN 0198515499.
- 41-Merritt, R.W.; K.W. Cummins; and M.B. Berg (2007). *An introduction to the aquatic insects of North America*. Kendall Hunt Publishing Company. ISBN 0-7575-4128-3.
- 42- Eisemann, C.H.; W.K. Jorgensen; D.J. Merritt; M.J. Rice; B.W. Cribb; P.D. Webb; and M.P. Zalucki (1984). Do insects feel pain? — A biological view. *Cellular and Molecular Life Sciences* 40: 1420-1423
- 43-Tracey, J.; W. Daniel; R.I. Wilson; G. Laurent; and S. Benzer (2003) painless, a *Drosophila* gene essential for nociception. *Cell* 113: 261-273.
- 44- Nevo, E.; and M. Coll (2001). Effect of nitrogen fertilization on *Aphis gossypii* (Homoptera: Aphididae): variation in size, color, and reproduction, *J. Econ. Entomol.*, 94: 27–32.
- 45-Lee, R.; and E. Jr (1989). Insect Cold-Hardiness: To Freeze or Not to Freeze. *BioScience*. 39 (5): 308–313.
- 46-Salt, R.W. (1961). "Principles of Insect Cold-Hardiness". *Annual Review of Entomology*. Lethbridge, Alberta, Canada: Canada Agriculture Research Station. 6: 55.
- 47-Cator, L.J.; B.J. Arthur; L.C. Harrington; R.R. Hoy (2009). Harmonic convergence in the love songs of the dengue vector mosquito. *Science*. 323 (5917): 1077–1079.
- 48-Lloyd, J.E. (1984). Occurrence of Aggressive Mimicry in Fireflies. *The Florida Entomologist*. 67 (3): 368–376.
- 49-Briscoe, A.D.; and L. Chittka (2001). The evolution of color vision in insects. *Annu. Rev. Entomol.*, 46: 471–510.
- 50-Hristov, N.I. (2005). Sound strategy: acoustic aposematism in the bat–tiger moth arms race. *Naturwissenschaften*. 92: 164–169.
- 51-Bennet-Clark, H.C. (1998). Size and scale effects as constraints in insect sound communication. *Phil. Trans. R. Soc. Lond. B*. 353: 407–419.
- 52-Miklas, N. (2001). The Influence of Substrate on Male Responsiveness to the Female Calling Song in *Nezara viridula*. *Journal of Insect Behavior*. 14 (3): 313–332.