GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES
(**Published By**: Global Institute for Research & Education)
www.gifre.org

Wonder Model organism for Forensic Entomology and Genetic Studies - *Megaselia scalaris* –Its Life Cycle, Breeding Methods and Wing Mutants

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Abstract

Megaselia is a genus of small flies, belonging to the family Phoridae, it is commonly called as "Scuttle fly". *Megaselia scalaris*, the "scuttle fly" a little insect about 2-3mm long, is an excellent organism to study genetic mechanisms. The general principles of, sex determination, molecular and developmental genetics may all be admirably demonstrated by using the scuttle fly *Megaselia scalaris*. The life cycle of *Megaselia scalaris* is short and completes in about three weeks. Embryonic development, which follows fertilization and the formation of the zygote, occurs within the egg membrane. Egg develops into larva, which eats and grows and at length becomes pupa. The pupa, in turn develops in to adult. The duration of these stages varies with the temperature. *Megaselia* cultures ought to be kept in room temperature where the temperature does not range below 21- 31^{0}C. They bred on jaggery medium, agar-based diets such as chocolate and blood agar and animal tissues such as liver or meat are commonly being used as food source because it provides sufficient nutrient for larval growth .The male and the female are differentiated (under the microscope) based on their size, markings on their abdomen and presence of sex combs following anesthetization with ether. Various wing mutants were isolated from *Megaselia scalaris* like vestigial wing, wave wing, curly wing, hook wing, which are resembled to the wing mutants of *Drosophila melanogaster.*

Key words: *Megaselia scalaris*, Phoridae, Genetics, Life cycle, Breeding, Wing mutants.

1. Introduction

Megaselia is a genus of small flies which belongs to the family Phoridae, commonly called as "Scuttle fly". *M.scalaris* has been heavily used in genetic research and it is a common model organism for developmental, bioassay, medical and forensic investigation (Disney, R.H.L. (2008). The Phoridae family has about 225 genera and over 2,500 species are known in the world fauna (Borgmeier and Prado, 1975).In the entire genus of *Megaselia Rondani* includes around 1,400 species widely distributed in the tropic and subtropic areas (Costa *et al*., 2007). *Megaselia scalaris* can be a parasitoid of insects of agronomic, veterinary and medical importance (Ulloa and Hernandes, 1981; Rocha *et al*., 1984; Harrison and Gardner, 1991; Costa *et al*., 2007). "Scuttle fly", is a cosmopolitan species and has been recorded in many forensic cases worldwide (Disney 1994, 2008). This species belongs to the family Phoridae and can be easily distinguished from other forensically important species by having thick costal vein, humpbacked appearance and frequently running in an erratic manner (Disney, R.H.L.1994).This flies are very diverse in appearance, behavior (Miller, 1979), and breeding habitat. Other distinctive features for this species includes having a shorter and broader sixth tergite on female adult and a single strong bristle on left side of epandrium on male adult (Disney & Sinclair 2008). *M. scalaris* and other phorid species are generally smaller compared to other forensically important species such as blow flies or flesh flies. This feature provides advantage for *M. scalaris* to enter enclosed environments as this species were usually found indoors. In forensic entomology, *M. scalaris* was frequently discovered on bodies in human premises and could be used as the reference to estimate time of death or post mortem interval (PMI) (Campobasso et al. 2004; Reibe & Madea 2010; Thevan *et al.* 2010). This review gives details on lifecycle of *Megaselia scalaris*, Methods of breeding and its importance in genetic and forensic entomology studies.

2. Why *Megaselia scalaris*?

Megaselia scalaris is a "scuttle fly", of kind that accumulates around broad spectrum of food source including decaying organic matter (Disney 2008). It is one of the most valuable organisms in biological research, particularly in genetics, forensic investigation (Greenberg and Wells, 1998) and developmental biology (Disney, R.H.L. (2008). *Megaselia scalaris*; a little insect about 2-3mm long is an excellent organism to study genetic mechanisms and the general principles of sex determination (Traut, 1994), molecular and developmental genetics. May all be admirably demonstrated by using the "scuttle fly".

Megaselia scalaris are important in the study of forensic entomology because evidence derived from the lifecycle and behavior of these flies is useful in both medico criminal and abuse/neglect cases and is admissible in court. *Megaselia scalaris* are small in size; this allows them to locate carrion buried within the ground and to locate bodies concealed in coffins. They may travel 0.5 m in a four-day period. Flies lay their eggs on carrion to provide food for the hatched larvae. Often, *Megaselia scalaris* may be the only forensic entomological evidence available if the carrion is obstructed or concealed in a place that is hard for other insects to reach (Greenberg, B; Wells, JD 1998). Larger flies are not always able to reach the carrion. Calculations involving "*M. scalaris*" can result in an insect colonization time that can be used for a postmortem interval, which may help to estimate the time of death (Greenberg, B; Wells, JD, 1998).

Megaselia scalaris are classified in a secondary forensic role because they prefer older decaying carrion (Greenberg, B; Wells, JD, 1998). *Megaselia scalaris* is also involved in cases of myiasis. The larva has been known to

cause myiasis in man and animals (Haider 1956).Specimens of *M. scalars* collected from a corpse may be useful in forensic investigations. Due to its relatively small size (~ 2-3 mm), M. scalaris was the only insect found breeding within a tightly sealed corpse (Greenberg and Wells, 1998). Toxicological analysis of pupal cases of this fly species was used to detect concentrations of amitriptyline and its metabolite, nortriptyline, in a corpse by Miller *et al*. 1994). *Megaselia scalaris* larvae found on a body can be used in court as a tool to show "time of death" or "time of neglect." (Benecke, M; Josephi, E; Zweihoff, R, 2004). *M.scalaris* and its hundreds of related species have been extensively studied for decades and there is extensive literature available.

M.scalaris has been used in genetic studies (Burisch, 1963; Manix, 1964). Sex determination in *M. scalaris* was resumed in the 1980s; a wild-type strain from Mainx's cultures had survived and was subsequently called 'Wien'. The three chromosome pairs were labelled cytogenetically with X ray- induced translocations but could not safely be assigned to the formel linkage groups because all previously isolated phenotypic marker strains had been lost. A few phenotypic markers were newly isolated and mapped to the respective chromosomes in crosses with translocations strains (Johnson *et al*. 1988; Traut *et al*. 1994). But only one, *ge* from chromosome 3, was a 'good' marker, i.e. a stably expressed and easily recognisable one. Besides phenotypic markers, RAPD markers were defined for all the chromosomes (Traut 1994).

The phorid fly *Megaselia scalaris* is a laboratory model for the turnover and early differentiation of sex chromosomes. The sex chromosomes are 'homomorphic' (Traut *et al*. 1990; Wolf *et al*. 1994; Traut *et al*. 2001). But display early signs of sex chromosome differentiation: a low level of molecular differences between X and Y. The male-determining function (M), maps to the distal part of the Y chromosome's short arm. In the laboratory cultures, new Y chromosomes with no signs of a molecular differentiation arise at a low rate, apparently by transposition of M to these chromosomes. Primary signal of the downstream, homologue of the Drosophila doublesex (*dsx*) is part of the sex-determining pathway while Sex-lethal (*Sxl*), though structurally conserved, and is not.

In *Megaselia*, X-chromosomal genes have active counter parts on the Y chromosome. Heterozygotes of sex-linked recessive alleles are wild type, no matter whether the wild-type allele is in the X or the Y chromosome. This holds for sex chromosomes from each of the three linkage groups (Mainx 1964). Although this does not prove the absence of dosage compensation, it may suggest that dosage compensation is not needed in *M. scalaris*.

2.1. It is an ideal organism for several reasons

1. "Scuttle flies" are hardy with simple food requirements and occupy little space.
2. The life cycle completes in about 10 days at 22-24^{0}c.
3. "Scuttle flies" produce large number of offspring's to allow sufficient data to be collected. Examination and collection of data is very easy because the flies can quickly and easily immobilize for examination.
4. Many types of hereditary physical variations can be recognized with low-power magnification.
5. Megaselia has small number of chromosomes (3pairs), (2n=6).
6. The genome is relatively small (approximately 540 mega bases).

3. Life cycle of *Megaselia scalaris*

3.1 Stages and duration

Embryonic development, which follows fertilization and the formation of the zygote, occurs within the egg membrane. The egg develops into larva, which eats and grows and at length becomes pupa. The pupa, in turn develops into an adult (Fig. 1). The duration of these stages varies with the temperature. At 22^{0}C-24^{0}C, the first instar lasts 1-2 days, the second instar larvae for 1-2 days, and the third for 3-4 days before pupation and a further 1-2 days before pupation." (Disney 1994). The pupal life at 22-24^{0}C is about 5 days, whereas at 28^{0}C about 8 days. Thus at 24^{0}C the life cycle may be completed in about 10 days, but at 28^{0}C about more than 20 days are required. The optimal culture temperature is 28^{0}C.Under optimal conditions emergence of male's starts on the 18th day and females on the 20th day after placing the parents together (Manix, 1964). Megaselia cultures ought to be kept in room temperature where the temperature does not range below 21^{0}C or above 31^{0}C. Continued exposure to temperatures above 31° C may result in sterilization or death and at low temperatures the viability of flies is impaired and life cycle prolonged.

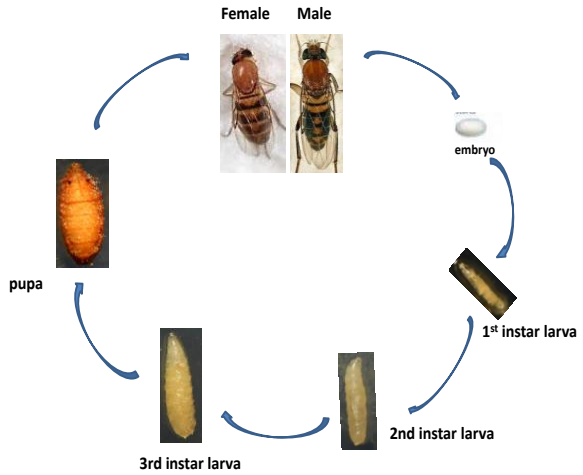

Figure 1: **Lifecycle of *Megaselia scalaris***

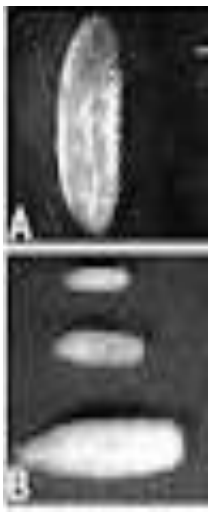

Fig.2 (a) Egg, (b) All larval instars

Source: Department of Biology, University of Kentucky.

3.2 The Egg

The egg of *Megaselia scalaris* is about 0.5-1 of a millimeter long. An outer investing membrane called as chorion, is opaque and shows a pattern of hexagonal markings. Penetration of spermatozoa into egg occurs through a small micropyle in the conical protrusion at the anterior end. Many numbers of sperms may enter an egg, through normally fertilization. The spermatozoa have been stored by the female since the time of mating. Immediately after the sperm enters, the reduction (meiotic) division occurs and the pronucleus is formed. The sperm and the egg nucleus come into position and form the zygote nucleus, which further divides to form the first two cleavage nuclei; this is the initial stage of the development of embryo. Soon eggs may be laid by the mother shortly after they are penetrated by the sperm, or they may be kept in the uterus during the early stages of embryonic development (Demerec M, Kaufman P),(Fig.2).

3.3 The Larval Stages

The larva , after hatching from the egg , undergoes two molts, so that the larval period consist of three stages (instars).The final stage , or third instar may attain a length of about 4 millimeters The body is cylindrical and tapers toward the head . Integument is composed dorsally and laterally of short spinous processes (peg like pro- cesses; Liu & Greenberg 1989). The larvae are such intensely active and voracious feeders that the culture medium in which they are crawling become heavily channeled and furrowed (Demerec M, Kaufman P). The larva has 12 segments. The body wall is soft and flexible and consists of the outer non cellular cuticula and the inner cellular epidermis. A great number of sense organs are spread regularly over the whole body (Fig.2).

The larvae are quite transparent. Their fat bodies, in the form of long whitish sheets, the black coiled intestine, and the yellowish malpighian tubules, as well as the gonads embedded in the fat body can easily be distinguished in the living larva when it is observed under the light source. The dorsal blood vessel is the circulatory organ of the larva. The larval muscles, segment ally arranged, are transparent but can be made visible when the larva is fixed in hot water. During the development, larva contains a number of primitive cell complexes called imaginal discs, which are the primordial for later imaginal structures (Demerec M, Kaufman P; 1996), (Milislav D).

The primary mechanism by which the larva grows is molting. At each molt the entire cuticle of the insect, including many specialized cuticular structures, as well as the mouth armature and the spiracles, is shed and has to be rebuilt again. During the process of each molt, therefore many reconstruction processes occur, leading to the formation of characteristic structures of the ensuing instar. The growth of the internal organs proceeds gradually and seems to be rather independent of the molting process, which may mainly affects the body wall. The organs such as Malpighian tubes, muscles, fat body, and intestine grow by an increase in cell size; the number of cells in the organ remains constant. The organ discs, on the other hand, may grow chiefly by cell multiplication; the size of the individual cells remains about the same. Therefore, in general one might say that purely larval organs are grown by an increase of cell size, whereas the presumptive imaginal organs grow by cell multiplication (Demerec M, Kaufman P; 1996), (Milislav D).

3.4 The Pupa

A series of developmental steps by means of which the insect passes from the larval into the adult organism is called "metamorphosis". The most extreme changes in this transformation process take place during the pupal stage.

Shortly before pupation the larva leaves the food and usually crawls onto the sides of the culture bottles, looking for a suitable place for pupation, and finally comes to rest. The larva going to be very sluggish, it everts its anterior spiracles, and becomes motionless. Soon the larva shortens and appears to be somewhat broader, thus gradually acquiring its pupal shape. Due to muscular action the shortening of the larval cuticle occurs and form the case of the puparium. Pupation takes place after puparia formation by the muscular contraction. A typical pupa with the head, thorax, and abdomen is thus shaped. Now pupa lies within three membranes: The first one is outer membrane, the puparium: second one is intermediate membrane, the prepupal cuticle; and the third one is inner membrane, the newly secreted pupal cuticle (Demerec M, Kaufman P; 1996), (Milislav D).

Now metamorphosis involves the destruction of certain larval tissues and organs (histolysis) and the organization of adult structures from primitive cell complexes called imaginal discs. However, it must be realized that some larval organs are transformed into their imaginal state without any very drastic change in their structure. The time duration and extent of these transformation processes vary greatly for the different organs involved. The organs of the larva which are completely histolyzed during metamorphosis are the salivary glands, the intestine, the fat bodies and apparently the muscles. All these new organs are either formed from imaginal disc cells already present in the larva or from cells which come visibly into being in the course of pupal reorganization. The Malpighian tubules are relatively little altered during metamorphosis but nevertheless they undergo some of the changes in their structural composition. The similar situation seems to prevail in the brain, which is not completely histolyzed. From their appropriate imaginal discs the differentiation of eyes, extremities, mouthparts, antennae, and genital apparatus occurs, which were already present in the larval stage and which undergo histogenesis during pupal development. From imaginal disc cells the body wall of the imaginal head, thorax, and abdomen is also formed. The body wall of head and thorax is formed by the combined effort of all the imaginal discs in this region, each of which contributes its part (Demerec M, Kaufman P; 1996), (Milislav D).

The characteristic feature of the *Megaselia scalaris* pupa contains intersegmental spines along the dorsal and lateral segments and sculpture of the pupal respiratory horn of this puparia may be useful in future studies and this is also going to be helpful for distinguishing it from the other closely related species. Such examination of puparia has been the entomological evidence in forensic investigation, as documented by Erzinclioglu (2000), Liu and Greenberg (1989).

3.5 Adult stage

When metamorphosis is complete, the male *Megaselia scalaris* fly matures more quickly than the female pupa; males emerge two days prior to the females. Adult *Megaselia scalaris* reproduce by means of oviposition. The females lay large eggs for their size due to the extended incubation period of the eggs (Disney 1994), (Fig. 3).

Figure 3: **Female and Male adult *Megaselia scalaris* flies**
Source: Department of Genetics, Indian Academy, Center for Research & Post Graduate Studies.

Features to determine the sex of adult fly (Fig. 3)
Size of adult and features

The female is generally larger than the male. Other distinctive features for this species includes having a shorter and broader sixth tergite on female adult and a single strong bristle on left side of epandrium on male adult (Disney & Sinclair 2008).

Shape of abdomen

The tip of the abdomen is more elongated in females, when compare to males.

Markings on the abdomen

Alternating dark bands can be seen on the entire rear portion of the female and male. On the abdomen of female have six dark distant v shape bands. In males also have six dark bands but not distant.

Appearance of sex comb

We present here the first report of *M. scalars* sex comb. In these flies on every tarsal segment of the fore leg contains a sex comb. Each sex comb contains more than 12 dark bristles, (Fig.4).

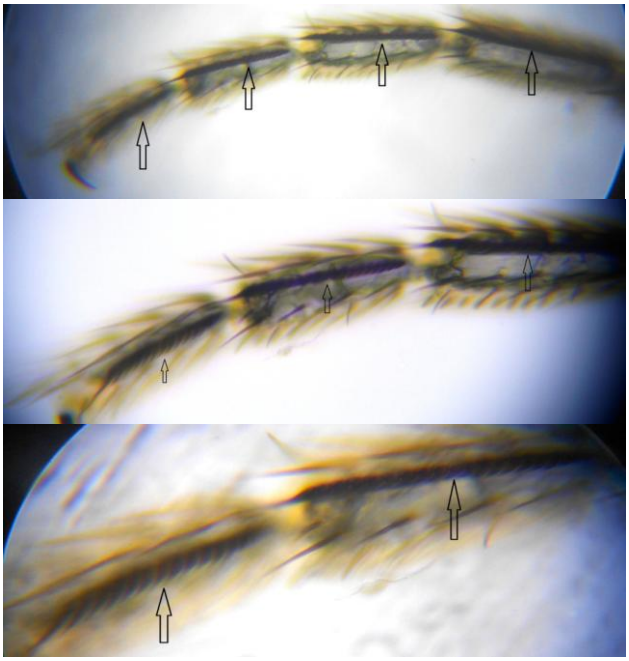

Figure 4: **Sex combs in a male fly**
Source: Department of Genetics, Indian Academy, Center for Research & Post Graduate Studies.

External genitalia on abdomen

External genitalia is located at the tip of the abdomen, the female ovipositor is pointed. The claspers of the male are very darkly pigmented, circularly arranged, and located just ventral to the tip (Fig.5).

4. Methods of breeding *Megaselia scalars*

Megaselia scalars are found in around broad spectrum of food source including decaying organic matter (Disney 2008). And since yeast is present wherever fermentation is in progress, it is believed that yeast constitutes an important part of their diet. *Megaselia scalaris* may be raised on any fermenting medium. The different types of medium commonly used for breeding of *Megaselia scalaris* include jaggery medium, agar-based diets such as blood agar and chocolate agar (Biery *et al*. 1979). Cornmeal agar (Harrison & Cooper 2003), deer blood agar (Tumrasvin *et al*. 1977) and agar mixed with snail tissue extract (Idris *et al*. 2001). In forensic entomology laboratory procedure, animal tissues such as liver or meat are commonly being used as food source because it provides sufficient nutrient for larval growth (Davies &

Ratcliffe 1994; Grassberger *et al*. 2003; Grassberger & Reiter 2001). However, the use of decomposing animal tissues often brings unpleasant odour and unsterile (Sherman & Tran 1995).Sucrose dextrose medium and maltose corn medium. The composition of the food predominantly includes sugar, yeast extract, dextrose and corn flour. They can be bred in glass or culturing bottles to obtain large numbers of the progeny (Fig. 5). And most often crosses and experiments are set up in glass vials.

Figure 5: **Breeding *Megaselia Scalaris***
Source: Department of Genetics, Indian Academy, Center for Research & Post Graduate Studies.

5. Wing mutants of *Megaselia scalaris*

We present here the first report of *M. scalars* wing mutants. While working in the lab with *M. scalaris* various wing mutants were isolated like vestigial wing, wave wing, curly wing, hook wing, which resembled the wing mutants of *Drosophila melanogaster* (Fig.6).

Figure 6: *Megaselia scalaris* wing mutants
Source: Department of Genetics, Indian Academy, Center for Research & Post Graduate Studies.

Scientists who study *Megaselia scalaris* attribute the species' diversity to its ability to be competitive in almost every habitat. Much research about the genetics of *Megaselia* over the last 50 years has resulted in a wealth of reference literature. Its ease of handling and it has short reproductive cycle. Also, the offspring are produced in large numbers which provides statistically significant data and phenotypic mutant changes are easily recognizable under the microscope.

6. Acknowledgment

I would like to thank Department of Applied Genetics, Indian Academy, Center for Research & Post Graduate Studies Bangalore-43, India for continuous support and encouragement throughout this research work.

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