Journal of Entomology

ISSN 1812-5670

Academic Journals Inc.

www.academicjournals.com

Journal of Entomology 11 (5): 291-298, 2014
ISSN 1812-5670 / DOI: 10.3923/je.2014.291.298

Mass Rearing and Life Table Attributes of Two Cyclorrhaphan Flies, *Lucilia sericata* Meigen (Diptera: Calliphoridae) and *Musca domestica* L. (Diptera: Muscidae) under Laboratory Conditions

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ABSTRACT

Life cycles of insects play a fundamental role in their subsequent activities. This study was designed to establish a colony of two medically important flies in the laboratory to construct their life tables and to compare baseline parameters of their survival. Adult blowflies, *Lucilia sericata* were collected from different slaughterhouses and meat processing plants. Adult house-flies *Musca domestica* were collected from different poultry farms and dairy plants. Both species were kept in rearing cages at 28±2°C average temperature, 50±5% relative humidity and 12 h photoperiodicity. Baseline parameters of their life cycles were recorded and life tables were developed for both species. Total survival rate were measured as 73.76±2.47 and 72.83±1.98% for *L. sericata* and *M. domestica,* respectively. Net reproduction rates for *L. sericata* and *M. domestica* were obtained as 3.398 and 2.206, respectively. The survival curve of both species was classified as type IV. The results confirmed the efficiency of applied diets for optimum development of both species. Based on these results, it could be concluded that both populations may be reared simultaneously to reduce extra labour and costs.

Key words: *Lucilia sericata, Musca domestica*, myiasis, blowflies, life table, house-flies

INTRODUCTION

The cyclorrhaphan flies, such as house-flies and blowflies (e.g., greenbottles) are major causes of concern in public health. The latter, *Lucilia sericata* as an insect species with worldwide distribution, belongs to the family Calliphoridae (Jang *et al*., 2013). Adult greenbottles are mostly metallic or coppery green (about 10 mm long). They frequently live on filthy substrates such as carrion, excreta, decaying materials and wastes, as well as wounds (Youssefi *et al*., 2012).

The house-fly, *Musca domestica* (Diptera: Muscidae) is another medically important species having almost worldwide distribution. House-flies are medium-sized (about 6-9 mm long) and non-metallic. They defecate on food sources and frequently regurgitate their food. The attraction of this insect to forage on food sources is very important since the size of adults is characterized by the amount of food consumed in the larval (or maggot) stages (Duarte *et al*., 2013).

Maggot therapy is the medical use of live maggots (fly larvae) for cleaning non-healing wounds. Maggots have been used to help treat wounds for thousands of years. Nowadays this method is accepted as a part of modern medicine (Scavee *et al*., 2003). Approximately 15% of the total number of diabetic patients will develop one or more wounds in their bodies, in 15-25% of them finally the amputations are done (Sherman, 2003).

Maggot therapy is a biological dressing that has different mode of actions. The maggots secrete powerful proteolytic enzymes that break down and liquefy dead tissues which they, then ingest (Casu *et al*., 1996). Healthy tissue is not affected by the maggots although their enzymes can cause excoriation or maceration (Acton, 2007). In sufficient numbers, maggots are able to eliminate a wide range of wound infections, including methicillin-resistant *Staphylococcus aureus* (MRSA) (Bexfield *et al*., 2004), due to the antimicrobial nature of their secretions and their ability to ingest and destroy bacteria as they pass through their gut (Thomas *et al*., 1999; Huberman *et al*., 2007). Maggots can help reduce malodorous wounds and there is evidence to suggest that their secretions stimulate the development of fibroblasts cells (Wolff and Hansson, 2005; Van der Plas *et al*., 2008). Following successful employment of *L. sericata* larvae in treating antibiotic-resistant wound infections and infected surgical wounds, the World Health Organization (WHO) introduced maggot therapy as a suitable alternative method (Sherman *et al*., 1995; Firoozfar *et al*., 2011).

About 30 countries are now using maggot therapy with about 60 centers in North America, 400 in the United Kingdom and more than 140 in Germany (Service, 2012).

Facultative myiasis flies are usually used for maggot therapy. So far, ten species of flies from family Calliphoridae, Muscidae and Sarcophagidae have been applied in maggot therapy (Mirabzade and Azma, 2004).

Since the microbial tolerance of insect gut is 1-10 million microbes, so the house-fly gastrointestinal tract should certainly be containing strong antibiotics. Furthermore considering that, this is not a true myiasis fly, so this can be used as a suitable species in maggot therapy (Mirabzade and Azma, 2004).

The reproductive cycles of arthropods play a fundamental role in their subsequent activities and special abilities. Therefore, it is necessary to use life tables to analyze and understand the impact on growth, survival, reproduction and population parameters. The main purpose of this study was to establish a colony of two medically important flies under laboratory conditions to construct their life tables and to compare baseline parameters of their life cycles.

MATERIALS AND METHODS

Rearing of *L. sericata* in the laboratory: Adult *L. sericata* were collected from different slaughterhouses and meat processing plants near Shiraz, the capital city of Fars province, South Iran. Beef and sheep meats were used as bait to attract blowflies, which were carefully captured using entomological nets immediately stored in glass jars and then transported in a polystyrene icebox to the laboratory. In each case, some information such as date, name of collecting site, temperature and humidity were recorded. Insect collections were conducted in the early hours of the morning when the sunlight was less intense than the rest of the day (Rueda *et al*., 2010). Collections of adult flies continued until a sufficient number of specimens for the colonization process had been captured. A total of 400 adult flies were transferred to an insectarium. The identification of adult *L. sericata* was carried out using the taxonomic keys of Calliphoridae (Carvalho and Ribeiro, 2000) and pictorial keys of department of health and human services (CDC), Atlanta (CDC, 2006). Also the 3rd larval instars of flies were identified using these keys.

Adults were kept in 45×45×45 cm cages at 28±2°C average temperature, 50±5% relative humidity and 12 h photoperiodicity. There was a plate with a cotton pad soaked in sugar solution (3%) in each cage to provide a carbohydrate source and water. An artificial diet which consisted of a mixed powder of MacConkey agar (50%) and glucose (50%) was prepared for adults feeding.

For females laying eggs, a thin layer (about 4-5 mm) of fresh mutton was used. There were scratches on the surface of the meat. A sheet of paper was placed in the incision to provide a place to lay eggs. All larval stages were fed by lean meat and water. Four days after the beginning of third larval stage, their feeding was stopped and they were transferred to a dry container filled with sterilized saw-dust and the corresponding counting was carried out. To prevent adults' escape, electric traps were also used in the insectarium (Spiller, 1966; Sherman and Wyle, 1996). Besides the feeding of larval stages with mutton, some of them were fed by beef. The results were not suitable and this process was discontinued.

Rearing of *M. domestica* in the laboratory: Adults were collected from different poultry farms and dairy plants. The collected flies were identified using pictorial key of (CDC, 2006). The collecting process and rearing conditions were conducted as the same method of *L. sericata*.

For laying eggs, a plate containing a few slices of apple (about 10-15 g) was placed in each cage. There were some deep scratches on the surface of apple slices to prepare the suitable condition for egg laying.

Early larval stages were fed only by apple and water. For later stages to improve the rearing condition, larvae were transferred to a dish containing chicken muck. Third larval stages became pupae in this media and they were transferred to cages.

Life table studies: The life tables were developed for two medically important cyclorrhaphan flies. For each species, 100 virgin adults born on the same day (80 females and 20 males) were released into separate cages and their mortalities were recorded daily. Also temperature and relative humidity of rearing room were monitored daily. After 3 days, prepared oviposition media were placed into the cages. The eggs on each oviposition medium were collected and the daily counting of the different life cycle stages was performed to compare the differences between the two examined species. Based on the life tables, mortality rates and survival curves were established (Rabinovich, 1980).

The net reproductive rate (R_0) was calculated for both species according to Southwood method (Southwood, 1978; Rueda *et al*., 2010).

RESULTS

Determination of life cycles of *L. sericata* and *M. domestica*: Life cycle durations of these two species were determined by taking this parameter over three succeeding filial generations (F_1-F_3) and recording the average time in days for the different stages in these species (Table 1).

Stage-specific mortality analyses on both species: Table 2 and 3 show the results for both species derived from the vertical life table, where ax is the result of the daily counting of the individuals observed at different life cycle stages and lx is the high number of survivors at each stage. Thus, lower survival rates from eggs to adults were observed for *M. domestica* in this rearing condition, showing significant differences from larvae I to adults. In dx (percentage of the original cohort that dies in each stage) it can be observed that mortality has a different behavior for these

Table 1: Life cycles of *Lucilia sericata* and *Musca domestica* under laboratory conditions

	Sample size		Duration (days)	
Developmental stage	*L. sericata*	*M. domestica*	*L. sericata*	*M. domestica*
Eggs	1105	747	0.50	0.83
Larvae I	967	718	0.80	1.87
Larvae II	902	649	1.28	2.29
Larvae III	878	600	2.50	2.08
Pupae	826	544	3.96	4.58
Male adults	369	246	28.30	23.05
Female adults	446	284	32.40	26.06

Table 2: Cohort life table for *Lucilia sericata* maintained in the laboratory

x	a_x	l_x	d_x	q_x	F_x	m_x	l_xm_x
Eggs	1105	1	0.125	0.125	-	-	-
Larvae I	967	0.875	0.059	0.067	-	-	-
Larvae II	902	0.816	0.021	0.026	-	-	-
Larvae III	878	0.795	0.047	0.059	-	-	-
Pupae	826	0.748	0.01	0.013	-	-	-
Adults	815	0.738	-	-	2054	4.605	3.398*

x: Life cycle stages, a_x: No. of individuals observed in each stage, l_x: % of the original cohort that survives at the beginning of each stage, d_x: % of the original cohort that dies in each stage, q_x: Mortality rate (average probability of death that an individual has), F_x: Eggs produced at each stage and m_x: Eggs produced per surviving individual at each stage and l_xm_x: Eggs produced per original individual in each stage. *The basic reproductive rate (R_0) = 3.398

Table 3: Cohort life table for *Musca domestica* maintained in the laboratory

x	a_x	l_x	d_x	q_x	F_x	m_x	l_xm_x
Eggs	747	1	0.038	0.038	-	-	-
Larvae I	718	0.962	0.093	0.097	-	-	-
Larvae II	649	0.869	0.066	0.076	-	-	-
Larvae III	600	0.803	0.075	0.093	-	-	-
Pupae	544	0.728	0.019	0.026	-	-	-
Adults	530	0.709	-	-	884	3.112	2.206*

x: Life cycle stages, a_x: No. of individuals observed in each stage, l_x: % of the original cohort that survives at the beginning of each stage, d_x: % of the original cohort that dies in each stage, q_x: Mortality rate (average probability of death that an individual has), F_x: Eggs produced at each stage and m_x: Eggs produced per surviving individual at each stage and l_xm_x: Eggs produced per original individual in each stage. *The basic reproductive rate (R_0) = 2.206

species showing the highest dx in eggs for *L. sericata* and larvae I stage for *M. domestica*. In qx, the average probability of death of the individuals for *L. sericata* is partly low except at eggs stage, that is very high, while for *M. domestica* this probability was high at the stages from larvae I to larvae III. In general, the survival of larval stages was of 90.8±2.6% for *L. sericata* (9.2% mortality), while for *M. domestica* it was of 83.57±1.57% (16.43% mortality). Total survival from eggs to adult stages was represented by a value of 73.76%±2.47 for *L. sericata* (26.24% mortality), while for *M. domestica* this parameter was of 72.83±1.98% (27.17% mortality).

Determination of net reproduction rate (R_0): According to Southwood (1978), the net reproduction rate (R_0) for *L. sericata* and *M. domestica* were obtained as 3.398 and 2.206, respectively (Table 2 and 3).

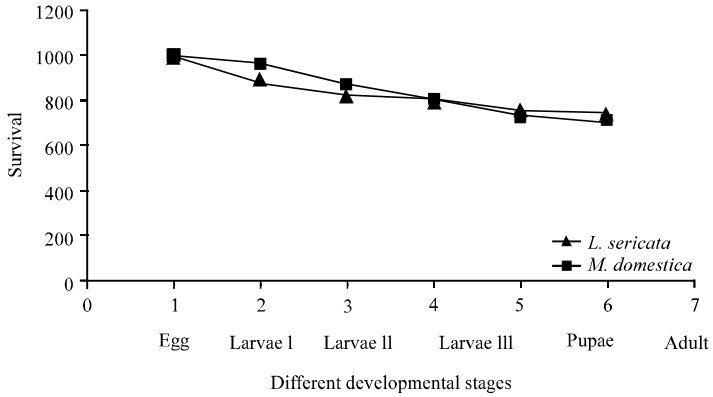

Fig. 1: Survival curve of *Lucilia sericata* and *Musca domestica* obtained from different developmental stages under laboratory conditions

Survival curves of *L. sericata* and *M. domestica* under laboratory condition: The survival curves of both species according to the classification of Rabinovich (1980) was a type IV curve, since it represented a population in which there was a "constant fraction" of individuals dying at each age intervals (Fig. 1).

DISCUSSION

To better understand the baseline parameters of life cycles of two medically important flies, their developments under experimental conditions were monitored. Results of this study showed that mortality of adults from field populations was more than the succeeding generations for both species. Hatching eggs and larval stage periods of field collected generation was also longer than the succeeding generations. It could be contributed to the induced stress caused by changes in environmental conditions.

The average duration of life cycle (from eggs to pupae) described in this research for *L. sericata* (9.04 days at 28±2°C and 50±5% relative humidity), is shorter than others in comparison to all data provided by previous researchers; 12-15 days at 22°C and 50% relative humidity (Kamal, 1958), 23-28 days under field conditions (Nuorteva, 1977), 32 days at 16°C and 20 days at 21°C (Anderson, 2000), 14 days at 27°C (Anderson, 2000), 26 days under natural environmental conditions (Usaquen and Camacho, 2004), 13.89 days at 22±1°C and 60±5% relative humidity (Rueda *et al*., 2010) and 2-3 weeks (Firoozfar *et al*., 2011).

The duration of adult emergence to oviposition (egg laying) was recorded as 5.5 days for *L. sericata* in this study, while this period was mentioned as 8.33-14 days in other similar studies (Firoozfar *et al*., 2011).

In the present study, the duration of hatching eggs, larval and pupal stages for *L. sericata* were calculated as 12, 110 and 95 h, respectively. These parameters were variously reported by other researchers. The duration of larval stage was reported as a wide range from 95-156 h by other studies (Grassberger and Christian, 2001; Rueda *et al*., 2010; Firoozfar *et al*., 2011). Survival time of adults for *L. sericata* was about 30 days in this study. This is almost the same as Rueda *et al*. (2010) results, while it has been reported up to 6 weeks by other researchers (Wolff and Hansson, 2005; Rueda *et al*., 2010).

In this study, the duration of hatching eggs, larval and pupal stages for *M. domestica* were calculated as 20, 130 and 110 h, respectively. These values were reported as 16-24 h, 3-4 days and 4-5 days for this species by Schoof (1964).

The average duration of life cycle for *M. domestica* was calculated as 11.65 days. These results indicated that *L. sericata* had shorter duration of life cycle in comparison to *M. domestica* at the same rearing conditions. Adult survival value was also longer for *L. sericata* than *M. domestica*. So establishment and rearing of *L. sericata* at the laboratory under mentioned conditions is more applicable than *M. domestica*.

At the first stages of insects' life cycle, the most important factor for survival is nutrients. Humidity and temperature are other important factors (Rueda *et al*., 2010). In the present study, feeding earlier stages (instar I and II) of *M. domestica* with slices of apple and water were used and also chicken muck was added for third larval stage to improve breeding conditions. Whereas a mixture of agar, yeast, powdered milk and boiled water were used in another study (Schoof, 1964).

In this study for adult feeding of both species, a mix powder of MacConkey agar (50%) and glucose (50%) were used, although powdered milk was used by other researchers (Kheirallah *et al*., 2007).

The *L. sericata* larvae tended to eat mutton more than beef. So earlier instars of this species didn't feed on beef and mortality occurred despite adequate food and water. One of the possible causes of this phenomenon could be the histochemical differences between the meats. The mutton is softer and wetter than beef, it could thus be more digestible for maggots.

In general, the comparison of the life tables for *L. sericata* and *M. domestica* indicate that the survival percentage was higher in the life table of *L. sericata*, while the mortality parameters had higher values in the life table of *M. domestica*, mainly in the first larval stages of the biological cycle.

The results confirm the efficiency of these diets for optimum development of both species under laboratory conditions in this research. In addition, no differences were observed in the survival curves, type IV corresponding to both species (Fig. 1), where a constant population of individuals dying at each time interval was clearly observed at all stages. This confirmed the previous reasoning on the effectiveness of this rearing method for both species.

Comparing the present results to the others indicate that whenever the temperature is higher during rearing period, the lifecycle of flies will be shorter under controlled laboratory conditions. So, it could be concluded that temperature is one of the most delicate key factors for flies rearing in the insectarium.

In conclusion, according to the results of this study and adaptation of both species under described conditions, to reduce the costs associated with mass rearing, efficient use of physical space at insectarium and increase the efficiency of rearing process, maintenance of both populations of *L. sericata* and *M. domestica* as colonized strains under controlled laboratory conditions could simultaneously be achieved. These flies could be used for maggot therapy, biological tests, bioassay for insecticide resistance objectives and even as a food source for rearing of other insectivorous species.

ACKNOWLEDGMENT

Authors wish to sincerely thank vice chancellor for study affairs at SUMS for full financial support of this research project via., contract No. 91-6129. This study was part of Mr. Vahid Saleh M.Sc. Thesis. We also would like to appreciate Mrs. Masoumeh Amin for her grateful helps in mass rearing flies.

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