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Laboratory colonization of *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) strain from Assiut, Egypt

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Abstract
The blowfly *Lucilia sericata* has a long history in medicine and its maggots have been used in wound healing. Considering the significance of maggot therapy, establishing an insectary for mass rearing of larvae is important. *L. sericata* sometimes determine its specific developmental events at precise timing. However, little is known about how the development timing and mass rearing of *L. sericata* are decided under effect of different food types. This study was carried out to examine effects of three different food types (liver, beef and meat) on the developmental timing and number of green bottle flies *L. sericata* from April to June 2015 for three generations. Hand catch and net trap baited with chicken viscera and cattle liver were used to collect adult flies from the field and sent to the laboratory for species identification using specific keys., The parental adult insects 40 flies (25 females and 15 males) were reared in cage (40X40X40 cm) at 30±4 °C average temperature, 80%±10 relative humidity and 12 h photoperiodicity in Al-Azhar University, Assiut. We found number of batches or egg masses and the rate of development of all stages of cultured of green bottle flies *L. sericata* increase toward type of diet of meat and green bottle flies favorite meat than liver and beef.

Keywords: Biology, rearing, green bottle flies *Lucilia sericata*, myiasis

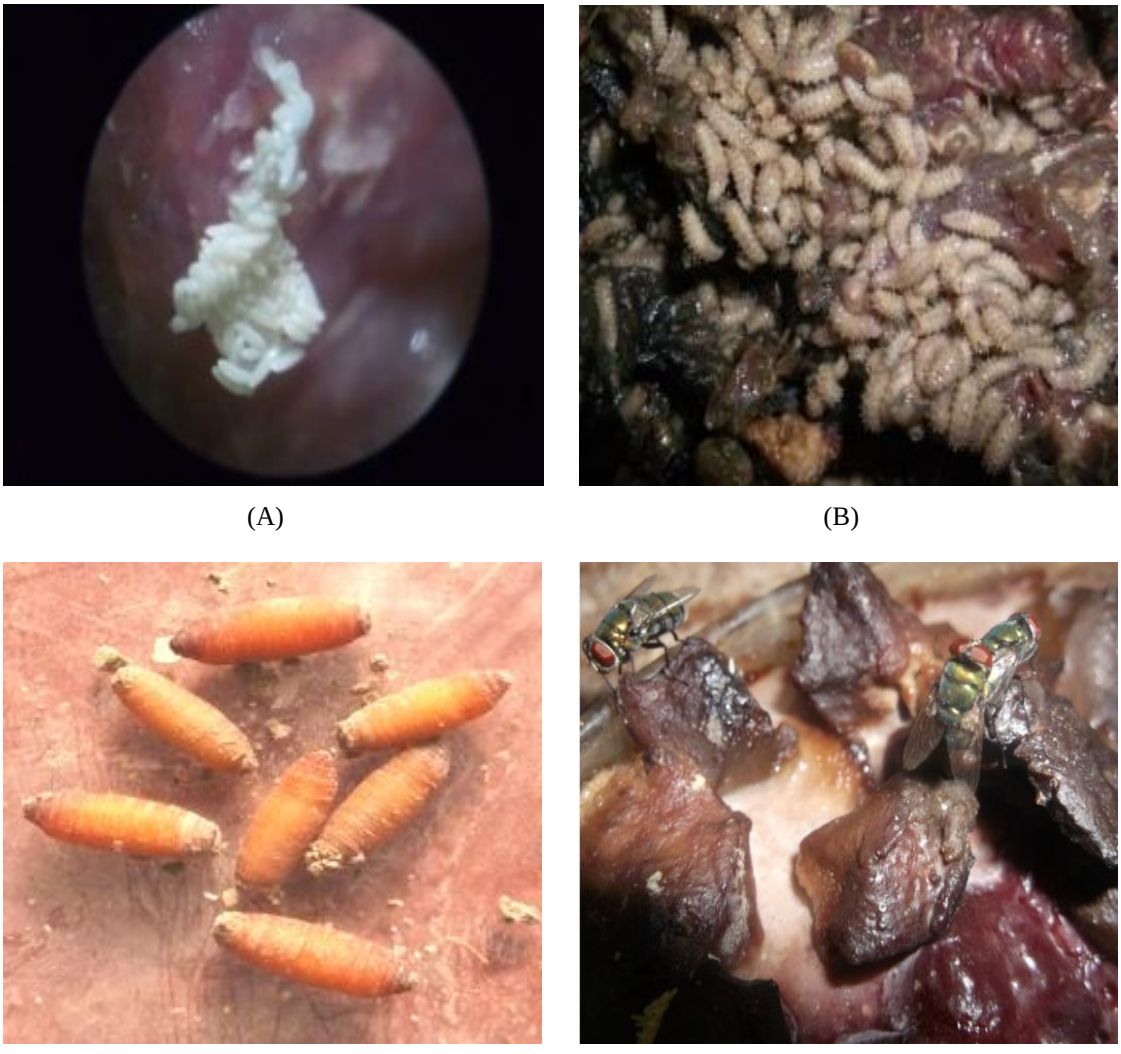
1. Introduction
The green bottle fly, *Lucilia sericata* (Meigen), formerly *Phaenicia sericata*, is a common visitor to carrion, feces, and garbage. *L. sericata* is also one of the most common species in the genus (Whitworth 2006) [1]. This blow fly is coastal in its distribution and refers warm and moist climates generally life cycle of blowfly includes four stages; egg, larval, pupal and imago. Three instars can be seen in the larval stage: 1st, 2nd and 3rd instars, where the latter is divided to feeding and post-feeding larvae (Day and Wallman 2008) [2]. The flies deposit egg directly on the food substrate to ensure a food supply for the hatching 1st instar larvae. The three instars can be distinguished by the number of respiratory slits at the posterior end of the larvae. The third instars stage lasts longer than the first two ones, and the post-feeding stage is a preparation phase for pupation (Reibe *et al*. 2010) [3]. About one third of the pre-adult development time is spent in the post-feeding larval stage (Greenberg 1991) [4]. Therefore, the larvae leave the food source to find a suitable place for pupation, emptying their gut (Arnott and Turner 2008) [5]. During pupal stage the imago develops within the pupal case till eclosion and takes about half of the time of the total development (Reibe *et al*. 2010) [3]. The female lays her eggs in meat, fish, and Animal corpses, infected wounds of humans or animals, and excrement. The larvae of this insect feed on most decomposing tissue. Larvae of *L. sericata* are facultative parasites, unable to ingest the vital tissue (Weil *et al*. 1933) [6]. Maggots of *L. sericata* have been used widely in several fields, for example Forensic needs - due to the well-known life cycle, the stage of the insect's development on a corpse is used to calculate a minimum period of colonization, so that it can used to aid in determining the time of death of the victim (Anderson 2011) [7]. Layer of loose, litter, damp soil was reported the main habitat for maggot growing and characterized as a facultative ectoparasites responsible for primary or secondary myiasis in humans and livestock (Zumpt 1965, Smith 1986, Hall and Wall 1995, Anderson, 2000, Grassberger *et al*. 2003) [8-12]. These species are considered as sinantropic species, i.e. it is in close relation with human settlements. They also feed on carrion and human feces, and breeds prolifically in carrion, making them medically, veterinary, sanitationarily,

and forensically important flies (Zumpt 1965, Grassberger *et al*. 2003) [8, 12]. Determination of postmortem interval (PMI) or the time between death and the discovery of a corpse is the most important application of forensic entomology. Blowfly is the first insect to arrive on a corpse where its larvae feed and breed effectively (Anderson 2001) [18], Dadour *et al*. 2001 [14], (Higley and Haskell 2010) [15]. Developmental rates of this fly are frequently used to estimate PMI in homicide investigations in the first few weeks after death. The rate of larval growth depends on its body temperature, which is directly affected by environmental conditions as ambient temperature and the heat generated by maggot aggregations (Slone and Gruner 2007) [16]. In addition, an important detail for PMI determination is that each species has its own temperature dependent growth rate. In the present work, rearing green bottle flies *L. sericata* on three diets, we compared number (eggs, larvae, pupae and adults) for three generations and calculated life cycle stages of each generations from April to June 2015 under laboratory conditions (like in corpse) at the faculty of science, Department of Zoology in Al Azhar university Assiut.

2. Materials and Methods

2.1 Sample collection

The samples were caught from different places including gardens, around livestock, in Al-Azhar University, Assiut. Adults were sent to the laboratory (Zoology Department) in faculty of science, in appropriately labeled tubes. Chicken viscera, cattle liver were used as bait for collecting adult stage of the fly in open area used hand catch by net was performed in March 2015. Beginning in morning and continued until a sufficient number of specimens for the colonization process had been captured. Information such as place and date of collection, sampling method and weather condition were recorded according to Hall (1995) [17].



(A) (B)

(C) (D)

Fig 1: Life cycle (A-egg mass B-Larvae C-Pupa D- Adult fly).

2.2 Rearing of flies in the laboratory

The experimental study was performed from April to June 2015. The adult forms were kept in 40 × 40 × 40 cm cages. The flies were maintained in the laboratory under controlled conditions of mean temperature of 30±4 °C, relative humidity of 80±10%, and daily light /dark period of 12 h. protected with an external net curtain to avoid the entry of other insect species. After laying eggs, the dead specimens were identified morphologically by using the taxonomic keys of (James 1947, Zumpt 1965, and Whitworth 2006) [18, 8, 1] The flies were fed on three diets (liver, meat and Beef), that were evaluated over three continuous generations and replicate of three cages for each food to F1, F2 and F3 generation. The flies were fed on diets were placed in the Petri dish and a carbohydrate-rich source 30% sucrose (Sherman 1996) [19]. Supervision of rearing cages was essential and larvae were isolated from rearing cages. Upon emerging, the adults were placed in new cages and provided

with essential food (Spiller 1996) [20]. Recording the time required for egg hatching, larval stage developments, and pupation, and total time for egg-eclosion was performed every three hours intervals for eggs and every six hours intervals for larvae and pupa. On each recording occasion, at least 10 individuals of each cages were checked by using a light microscope.

3. Results

The samples were caught from different places including gardens, around livestock, in Al Azhar University Assiut and were sent to the laboratory (Zoology Department) in Faculty of Science. In the first generation in March large numbers of flies were produced in the laboratory including males, and females consider as parents. We put totally 40 flies (15 males and 25 females) of each cages and repeated three replicates of each diet. Fig. 1 shows the life cycle of green flies which includes four distinct stages; egg, larval, pupal and imago. The recorded number of eggs, batch, and larvae stages I, II, III, pupae and adults of *L. sericata* during three generations in rearing laboratory 2015 are shown in table 1. Average numbers egg batches in (F1, F 2, F3) 5.7 batches of each cage and average number of eggs each cage 881.8 eggs all three generations of diet of liver. This average number of eggs hatching to1st instars 658.8 consider 100%, average numbers decrease during development to second instars to 91.2% and third instars to 81.5%. Meanwhile average number of pupa were 71.5% successfully to arrive to pupal stage and Average number of adults 64.7% (25.3% males: 39.3% females) from totally individuals hatching. While average numbers egg batches in (F1, F 2, F3) Average 9 egg batches contain average number of eggs1191.6 in all three generations of diet meat food. Average number of eggs hatched to 1025.1 individuals of 1st instars represented 100% and some individuals failed to arrive to second instars 957.7 represented 93.4% and as same third instars larval 875.2 individuals represented 85.3%. Also the average number of pupa were777.2 individuals represented 75.3% successfully to arrive to pupal stage and Average number of adults 693.4 represented 67.6% (223 individuals represented 21.7% males: 469.1 represented 45.7% females) from totally individuals hatching. We compare average numbers egg batches in (F1, F 2, F3) 5.1 egg batches contain average number of eggs 606 in all three generations of diet Beef food. Average number of eggs hatched to 536.3 individuals of 1st instars represented 100% and some individuals failed to arrive to second instars 472.4 individuals represented 88.8% and also third instars 402.1individuals 74.9%. The average number of pupa were 332.8 individuals represented 62.5% successfully to arrive to pupal stage and Average number of adults 290.1 represented 54.09% (103 individuals represented 19.2% males:186.4 represented 34.7% females) from totally individuals hatching. Determination of postmortem interval (PMI) or the time between death and the discovery of a corpse is the most important application of forensic entomology. Blow flies one flies is the first insects to arrive on a corpse where their larvae feed and breed effectively. Development rates of these flies are frequently used to estimate PMI in homicide investigations in the first few weeks after death. Since development of immature insects. The rate of larval growth depends on its body temperature, which is directly affected by environmental conditions as ambient temperature and the heat generated by maggot aggregations.

The duration of life stages of male and female *L. sericata* is given in table 2 We reared cultural under condition of Assiut city began second half month April and repeated three replicated, second generation in month May and also repeated to culture three replicated of each type of food and also third generation end May and first June and also repeated to culture three replicated of each type of food. The resulted showed in the Table 2 noticed duration of average egg period in three Cages (cultural) in first generation of liver food 28.3 ± 0.88 hours, second generation average duration egg period liver food 26.6 ± 0.33 hours and also third generation average egg period liver food 23.3± 0.88 hours. The duration of average egg period in three Cages (cultural) in first generation of meat food 24.6 ± 1.2 hours, second generation of meat food duration of average egg period in three Cages 23 ± 1 hours and also third generation of meat food duration of average egg period in three cages 22.3± 1.7hours. The duration of average egg period in three Cages (cultural) in first generation of beef food 29 ± 2.08 hours, second generation of beef food 27.3 ± 1.2 hours and also third generation of beef food duration of average egg period in three cages 26 ± 1.15 hours. The duration of average larval instars in three Cages (cultural) in first generation of liver food 116 ± 2 hours, the duration of average pupal instars in three Cages (cultural) in first generation of liver food 138.6 ±3.5 hours, and also the duration of average emerge adult flies in three Cages (cultural) in first generation of liver food 97.3 ± 0.88hours, and so on the Table 2 all generations and three types of foods**.**

Table 1: Number of eggs, batch, and larvae stages I, II, III, pupae and adults of *Lucilia sericata* during generations in rearing laboratory 2015

Generations	Cages	Egg batch batch(No)	Larval instars			Pupae	Adults		Total
			I	II	III		Male	Female	
F1Liver food	Cage1	4 (670)	550	500	445	400	140	220	360
	Cage2	2 (268)	210	180	150	125	40	75	115
	Cage3	5 (547)	460	440	415	385	150	200	350
F2Liver food	Cage1	4 (645)	585 543	543	500	445	165	248	413
	Cage2	5 (780)	700	640	555	476	173	257	430
	Cage3	5 (747)	690	640	575	483	182	277	459
F3Liver food	Cage1	8 (970)	740	685	610	540	175	285	460
	Cage2	7 (840)	775	710	640	565	190	285	475
	Cage3	12 (1500)	1220	1075	945	825	290	480	770
Average F1, F2and F3	9	5.7 (881.8)	658.8	601.1	537.2	471.5	167.2	258.5	426.3
			100%	91.2%	81.5%	71.5%	25.3%	39.2%	64.7%
F1Meat food	Cage1	7 (900)	740	670	565	467	170	255	425
	Cage2	7 (857)	740	680	607	530	156	300	456
	Cage3	4 (670)	594	532	466	385	112	225	337
	Cage1	8 (1020)	980	955	900	813	233	513	746

F2Meat food	Cage2	7 (964)	915	865	776	681	193	411	604
	Cage3	13 (1564)	1260	1165	1072	969	297	544	841
F3Meat food	Cage1	9 (1260)	1120	1075	1008	932	275	572	847
	Cage2	20 (2700)	2212	2095	1976	1800	461	1143	1610
	Cage3	6 (790)	665	583	507	418	116	259	375
Average F1, F2And F3	9	9 (1191.6)	1025.1	957.7	875.2	777.2	223	469.1	693.4
			100%	93.4%	85.3%	75.3%	21.7%	45.7%	67.6%
F1Beef Food	Cage1	3 (400)	320	277	233	163	43	77	120
	Cage2	5 (650)	540	459	378	303	84	178	262
	Cage3	4 (590)	545	466	397	331	91	197	288
F2Beef Food	Cage1	4 (570)	445	359	282	213	62	113	175
	Cage2	3 (440)	385	316	243	180	66	102	168
	Cage3	6 (730)	675	604	515	422	121	214	335
F3Beef Food	Cage1	4 (650)	598	543	477	425	165	233	398
	Cage2	8 (700)	653	619	551	476	148	277	425
	Cage3	9 (724)	666	609	543	483	153	287	440
Average F1, F2And F3	9	5.1(606)	536.3	472.4	402.1	332.8	103	186.4	290.1
			100%	88.8%	74.9%	62.5%	19.2%	34.7%	54.09%

Table 2: Duration of life stages of *Lucilia sericata* in the rearing laboratory 2015

Generation	Cages	Adult Male Female	Egg period Time (h) Mean±SEM	Larval stage Time (h) Mean ±SEM	Pupa stage Time (h) Mean ± SEM	Emergence to egg laying (h) Time (h) mean±SEM
F1Liver food	Cage1	15 25	30	114	139	97
	cage2	15 25	28 28.3 ± 0.88	120 116 ± 2	135 138.6 ±3.5	96 97.3 ± 0.88
	Cage3	15 25	27	114	14 2	99
F2Liver food	Cage 1	15 25	27	104	123	93
	Cage2	15 25	26 26.6 ± 0.33	96 100.6± 2.4	125 124±0.57	95 94.6 ± 0.88
	Cage3	15 25	27	102	124	96
F3Liver food	Cage 1	15 25	23	96	100	82
	Cage2	15 25	22 23.3± 0.88	90 94 ± 2	99 101 ±1.5	88 84.6± 1.8
	Cage3	15 25	25	96	104	84
Average F1,F2andF3	9	135 225	26.06 ±0.69	103.5 ±2.13	121.2 ± 1.85	92.1 ± 1.8
F1Meat food	Cage 1	15 25	23	102	121	90
	Cage2	15 25	27 24.6 ± 1.2	96 100 ±2	123 121±1.15	82 86.6±2.4
	Cage3	15 25	24	102	119	88
F2Meat food	Cage 1	15 25	22	96	101	76
	Cage2	15 25	25 23 ± 1	90 96 ±3.4	104 102.3±0.8	79 78.3 ± 1.2
	Cage3	15 25	22	102	102	80
F3Meat food	Cage 1	15 25	25	96	98	71
	Cage2	15 25	19 22.3 ±1.7	94 93.3 ±1.7	100 99.6 ±0.88	76 72.3 ± 1.8
	Cage3	15 25	23	90	101	70
Average F1, F2and F3	9	135 225	23.3 ± 1.30	96.4 ± 2.36	107.6±0.92	79.06 ± 1.80
F1Beef food	Cage 1	15 25	28	120	141	97
	Cage2	15 25	26 29 ± 2.08	120 120 ±0.00	140 140 ±0.57	100 98.6 ± 0.88
	Cage3	15 25	33	120	139	99
F2Beef food	Cage 1	15 25	29	102	124	97
	Cage2	15 25	25 27.3 ± 1.2	102 102 ± 0.00	125 125.3±0.88	93 96.3 ±1.7
	Cage3	15 25	28	102	127	99
F3Beef food	Cage 1	15 25	28	96	99	85
	Cage2	15 25	26 26 ± 1.15	102 98 ± 2	106 102.6 ±2.02	90 86.3 ± 1.8
	Cage3	15 25	24	96	103	84
Average F1, F2and F3	9	135 225	27.4 ± 1.47	106.6 ± 0.66	122.6 ±1.15	93.7±1.29

4. Discussion

L. sericata plays an important in the field of forensic science. The immature stages of the fly are used to estimate the minimum portion of the post-mortem interval, known as PMI, in a multitude of settings (Rueda *et al*. 2010) [21]. This study determined the minimum time of development and favorite type of food rearing of populations of *L. sericata* Meigen The period before oviposition is highly affected by temperature and humidity of the nurturing medium, therefore all pregnant female flies should be kept under controlled conditions of temperature and humidity. In this study we found that in general, the development duration of immature stages of *L. sericata* decreased steadily with increasing temperatures. In this study we found that the duration of *L. sericata* life cycle from egg hatching to adult eclosion were 14.5,13.3, and 11.74 days cultural feed in liver, while cultural feed in meat were 12.9,11.6,11.25 days and 14.93,13.6 and 12.22 days cultural feed in beef at Apirl,

May, and June respectively. These results are highly agree with that (Richards *et al* 2008) [22]. The duration of the life cycle described in this research for *L. sericata*, from egg to adult was 11–15 days which is more or less shorter in comparison to data provided by Rueda *et al*. (2010) (14 days), Anderson, (2000) [11]. (14 days at 27 °C), Usaquén and Camacho (2004) [23]. (26 days under natural environmental conditions), Nuorteva, (1977) [24]. (23–28 days under field conditions), and Anderson, (2000) [11] (32 days at 16 °C and 20 days at 21 °C), and somehow similar to the information by Kamal (1958) [25] (12–15 days at 22 °C and 50% relative humidity). This disparity between our results and other studies can be mainly explained by two factors; the higher temperature we used and the characterizations of the local populations we studied. It is proved that the development of fly larvae is temperature dependent, and in higher temperature the rate of development increases and duration of development becomes shortened. Variation has been observed in developmental time for geographically distinct populations (Richards *et al*. 2008; Gallagher *et al*. 2010) [22, 26]. It was clear that because fly unadult stages were temperature-dependent (Higley and Haskell 2009, Slone and Gruner, 2007) [27, 16] the rising of temperature caused accelerated development rate and decreasing development time. The results of this experiment is in agreement with the results of Day and Wallman in 2006) [2]. They stated that the growth rate of larvae fed on sheep liver was significantly lower than those grown on the chicken and beef flesh [19, 20]. According to these studies, food type is an essential factoron growth and development of green flies. However, many other factors play a role in development, including the food source and humidity (Tarone *et al*. 2006) [28]. We comparative three diet of food (meat, liver and beef of meat) in three generation April to June in Assiut, Egypt. We found *L. sericata* favorite meat food than liver and beef. They are often deposited in more batches or masses of eggs than liver and beef of meat. The occurrence of strain effects could necessitate population-specific data to calibrate PMI estimates. Unfortunately, this problem is further exasperated by the genotype-environment interactions in size and development time, which indicate that a universal adjustment among strains isn't possible, as each can respond differently to thermal changes. This challenge might be mitigated in part by considering evolutionary ecology theory (Tomberlin *et al*. 2011) [29], which seeks to explain phenotypic variation in terms of ecological effects (reviewed in Conner and Hartl 2004) [30]. Guiding principles behind the variation in development times in *L. sericata* and other forensically informative arthropods can most easily be elucidated by studying a series of populations, whereas ignoring population differences could lead to inaccurate PMI predictions based on single populations. One possible short-term method for developing a population-specific PMI would be to calibrate development times in published data with population-specific developmental information (e.g., is the population a slower developing population), thereby developing a posterior blow fly age estimate tailored to the population of interest (see Scheiner and Gurevitch 2001) [31].Clearly, it is impossible to determine the development times of all populations of blow flies in all environments, but it may be feasible to understand the factors influencing the evolution of forensically important by traits. In the long-term, factors that affect, or are predictive of, forensically informative blow fly phenotypes will need to be incorporated into PMI estimates (Wallman. and Day, 2006) [32]. For example, if flies develop more quickly at higher elevations than lower ones, integration of elevation data (including elevation-associated genetic polymorphisms) into PMI predictions could provide more accurate PMI estimates without requiring specific knowledge of the population in question. Elucidating such variables and incorporating them into PMI estimates will decrease error in age predictions made for by populations that differ from laboratory-based tables of development times. The identification of factors influencing developmental variation should continue to be a priority in forensic entomology research.

The variation between our results and other studies can be attributed by two possibilities namely differences in rearing conditions, with reference to the diet of immature stages, humidity and photoperiod, or because of the difference in populations which differed in geographical latitude. Variations have been observed in larval developmental time due to use of different kinds of diets in feeding Likewise, variations of developmental time of *L. sericata* were recorded for geographical populations, this result emphasizes on specific characterizations of regional developmental time of species.

5. Conclusion

Among the diets tested, growth and development parameters of *Lucilia sericata* showed significantly high values with Diet-meat. Thus Diet-meat can be used for mass rearing of blowfly larvae for several basic and applied studies.

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