Components of Developmental Plasticity in a Michigan Population of *Lucilia sericata* (Diptera: Calliphoridae)

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ABSTRACT Forensic entomologists rely on laboratory growth data to estimate the time of blow fly colonization on human remains. Several data sets exist for the development of the common blow fly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), and although they generally describe similar rates of preadult development, all vary. Such differences could be explained by genetic variation, environmental (rearing) variation, or both. In the study presented here, flies from a single population were reared under variable conditions of food moisture, substrate type, substrate freshness, and sampling, to determine the effect each had on developmental time. Cohorts were tested in a single incubator at a single temperature and humidity, to eliminate effects of undesired environmental variation. Fly developmental times were significantly influenced by multiple laboratory rearing treatments; food moisture, transferring postfeeding larvae to fresh substrate, and destructive sampling affected different stages of development. Developmental times ranged from 329 to 505.5 h, covering the spectrum of variation observed in published data sets. Growth was then compared with larval development on rat carcasses under the same environmental conditions, establishing a link between laboratory-controlled growth and development on carrion. Cohorts raised on rats matured to adulthood between 333 and 337 h, which was best mimicked by the fastest growth treatment observed under laboratory conditions. The large environmental influence on development observed in this study could affect forensic entomology casework and accentuates the need for a standardized means of rearing flies in a laboratory setting that is relevant to decomposition on a corpse.

KEY WORDS Calliphoridae, *Lucilia sericata*, forensic entomology, ecological genetics, life history

Forensic entomologists rely on published data of blow fly development to estimate the time since initial colonization of remains, thus extrapolating a postmortem interval (PMI) (Catts and Haskell 1990). PMI estimates based on entomological evidence have been widely and successfully presented in legal proceedings; however, the laboratory study of blow fly development, on which these estimates are founded, has never been standardized. Because of this, entomologists may use different blow fly developmental data sets, which can lead to variable PMI predictions. Furthermore, a lack of scientific standardization has the potential to call into question the overall accuracy of entomological evidence (Saks and Koehler 2005).

Prominent examples of differing laboratory rearing methods and resultant data sets can be found for the widely distributed blow fly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) (Kamal 1958, Greenberg 1991, Anderson 2000, Grassberger and Reiter 2001). These data sets all present a developmental time scale from egg to adult. Kamal (1958) recorded only the duration of each developmental stage, whereas Grassberger and Reiter (2001) and Greenberg (1991) also measured the length of maggots until pupation, and Anderson (2000) measured crop length throughout development. Each of these studies used different fly-rearing techniques, varying in the quality and type of food, the quality of pupation substrate, and the destructiveness of sampling. Likewise, the authors measured fly development at different temperatures and reported development data in assorted ways (e.g., minimum, average minimum, mode, and maximum growth). The resulting picture of *L. sericata* development is clouded, with relatively small differences in minimum developmental time among all studies, whereas Anderson (2000) characterized a notably longer minimum developmental time at temperatures similar to the others. Unfortunately, direct comparison of these studies is impossible, because experimental conditions and genetic background of the flies varied among them. Furthermore, even though the data sets were generated with a goal of relating larval development to PMI estimates on corpses, no attempt was made to tie laboratory-established growth rate data to ecologically relevant larval development on carrion.

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Developmental time is a quantitative trait that is expected to vary due to both genetic and environmental factors (Mackay 2001; Conner and Hartl 2004). Understanding genetic and environmental effects on quantitative traits is best accomplished by altering one variable while keeping all others constant, and a limited number of such experiments have been conducted in a forensic entomological context. For example, Kaneshrajah and Turner (2004) demonstrated that *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae) reared under otherwise constant conditions showed variable growth when raised on different organs, and Wells and Kurahashi (1994) indicated that differences in rearing protocols were the likely source of discrepancies regarding developmental times of *Chrysomya megacephala* (Diptera: Calliphoridae). Likewise, high-density rearing conditions that increase maggot mass temperatures were shown to shorten developmental times of *C. megacephala* (Goodbrod and Goff 1990). Recently, *L. sericata* was found to exhibit variable growth patterns depending on the species and tissue type on which cohorts were raised (Clark et al. 2006). Certainly, it seems that rearing conditions can have a major impact on the developmental timing of calliphorids.

Just as environmental factors influence calliphorid development, intraspecific differences have the potential to produce variation in fly developmental times. Ecological genetics is replete with cases demonstrating the effects of genetic background on quantitative traits (for review, see Mackay 2001; Conner and Hartl 2004). Developmental variability has been documented in many fly species, including strains of *Drosophila* (Diptera: Drosophilidae) (Johnson and Schaffer 1973, Oudman et al. 1991, Hoffmann and Harshman 1999, Parsch et al. 2000), *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae) (Feder et al. 2003), and *Scathophaga stercoraria* L. (Diptera: Scathophagidae) (Blanckenhorn 2002). Because each *L. sericata* study referenced above was conducted on different populations, it is impossible to separate the effects of environment and genetics on fly development. Potentially, any (perhaps all) differences among *L. sericata* studies could be explained by genetic variation among strains; however, this would only be demonstrated if each strain was raised using the same experimental protocol. Unless standard rearing conditions are adopted, such comparisons are impossible.

The potential influence of the environment and genetics on quantitative traits, and in particular developmental time, led to the hypotheses tested herein that *L. sericata* growth is plastic with respect to rearing conditions and that fly development on carrion will best be predicted by a specific combination of laboratory conditions that affect this plasticity. Temperature and humidity are already known to affect developmental time (Greenberg 1991, Anderson 2000, Grassberger and Reiter 2001) and mortality (Wall et al. 2001) in this species, so these conditions were held constant to investigate the effects of other rearing variables. Likewise, the flies in these experiments originated from the same source population, allowing genetic differences to be largely ruled out as a source of developmental variation. By changing the exposure of a single strain of *L. sericata* to specific environmental conditions, several questions related to the hypotheses were addressed. In particular, 1) Do laboratory rearing conditions affect the developmental time of *L. sericata*? 2) Are any developmental differences caused by laboratory rearing conditions large enough to explain the variation observed among published growth data on this species? and 3) Does growth generated under laboratory conditions accurately reflect larval development of *L. sericata* on a carcass?

Materials and Methods

Fly Collection and Rearing. *L. sericata* adults were collected from the Michigan State University campus in East Lansing, MI, throughout spring, summer, and fall 2004 and were used to establish a general population cage of ≈200 flies. Species identification was done using multiple keys, two independent identifications, and by comparing the DNA sequence of a 798-bp region of the mitochondrial cytochrome oxidase I gene to published sequences on the National Center for Biotechnology Information Web site by using the BLAST link. Forward primers for DNA amplification were GATCAGTAGTAATTACAGCT and TAATATTGCTCATGGAGGAG and reverse primers were TTGACTTTTTAATATCTTAG and CCTAAGAAATGTTGAGGGAAG. Polymerase chain reactions were run for 35 cycles by denaturing at 95°C for 30 s, annealing primers at 50°C for 30 s, and extending amplicons for 1 min at 72°C. Sequences were generated on a CEQ 8000 capillary electrophoresis system, by using a CEQ DTCS QuickStart kit and the manufacturer's suggested protocols (Beckman Coulter, Fullerton, CA).

Experimental rearings were carried out between January and March 2005. To minimize the loss of genetic variation during this period, the population was expanded to three cages of >100 individuals, from which 20–50 migrants were transferred as pupae to the other cages each generation. Generations were allowed to overlap until the cage required cleaning, which was done monthly while the next generation was in the juvenile form.

Cages of adult flies were provided water and honey ad libitum. Beef liver was supplied as a protein source 1 d before oviposition. On days that eggs were collected, fresh liver was placed into a cage in the late morning to mid afternoon. Cages were checked every 15–30 min until oviposition was observed. Approximately 250–1,000 eggs (one to four egg masses) were removed 1 h after the first observation of oviposition. The egg masses were immediately transferred to fresh liver and placed into a 1-liter glass canning jar (Alltrista, Muncie, IN), with a breathable cloth screwed on as a lid. Jars were placed into a temperature-controlled incubator at 25 ± 0.5°C with a photoperiod of 12:12 (L:D) h. A beaker filled with water was kept in

Table 1. Treatment types for the 37 liver-fed cohorts

Meat	Destructive	Transfer	Substrate under	Substrate	Paper towel
FMD: 33	No: 32	No: 24	No: 33	Sand: 14	No: 25
NFMD: 4	Yes: 5	Yes: 13	Yes: 4	Vermiculite: 23	Yes: 12

Meat: fresh meat daily (FMD) or not (NFMD). Destructive (sampling): 12 individuals removed from the cohort at each sampling time. Transfer: 125 postfeeding third instars were transferred to 500 ml of fresh pupation substrate. Substrate under: food was placed on top of a substrate or at the bottom of an empty jar. Substrate: pupation substrate used. Paper towel: received FMD placed on a moist paper towel.

the incubator, which provided a relative humidity of 25 ± 4%.

Several treatments were examined to assess the influence of rearing variables on the developmental time of specific immature stages and on total immature developmental time (Table 1). These considered the freshness of food, moisture of food, type of pupation substrate used, orientation of the substrate with respect to food, transfer of larvae to fresh pupation substrate, and destructiveness of sampling. The influence of meat freshness was tested by providing cohorts with 40 g of liver every day (fresh meat daily [FMD]) or 120 g of liver every third day (no fresh meat daily [NFMD]). Paper towel treatments received fresh meat daily, which was placed on a moist paper towel (FMDPT). The influence of pupation substrate was examined by providing either clean sand (Fairmount, Wedron, IL) or vermiculite (Therm-O-Rock West, Chandler, AZ) to jars containing postfeeding third instars. The influence of food orientation with respect to pupation substrate was tested by either placing meat on top of the substrate at the egg stage or by placing the substrate on top of meat when larvae reached the postfeeding third instar. Fresh pupation substrate was tested by removing 125 postfeeding third instars from individual cohorts and placing them into a jar with 500 ml of fresh pupation substrate. The transfer treatments were taken from cohorts with far more that 300 individuals in the jar, meaning larval density was much greater in untransferred than transferred treatments. Destructive sampling was assessed by permanently removing or not removing 12 individuals from a cohort each day.

Experimental cohorts were checked approximately every 24 h, except jars with eggs, which were checked every 0.5 h until they hatched, and pupae, which were observed throughout the day until eclosion occurred. Length measurements were taken throughout larval development, incorporating the 12 most mature larvae in all treatment groups (either the largest maggots or postfeeding maggots lacking blood in their crops). Ruler-measured lengths of the maximum body extension (to the nearest 0.5 mm) were determined using a stereomicroscope for first instars (because of their small size) or by eye for all other stages. Advances in developmental stage were recorded to the closest 15 min; however, given that most animals were observed once per day, developmental time variation of less than 1 d was indistinguishable from sampling time variation. All experiments were conducted in the same temperature controlled incubator, with jars rotated within the incubator daily.

Development of larvae on mammal carcasses was performed using three Sprague-Dawley rats from breeding colonies at Michigan State University, sacrificed by CO_2 asphyxiation within 2 d of egg placement on the body. The rats weighed ≈500 g and were in excess of the feeding needs of individual cohorts (larvae used approximately half of the carrion before the postfeeding stage). An egg mass collected in the manner detailed above was placed along the mouth of the rat. Rat carcasses were set in an open plastic bag, which was placed into a Styrofoam container with an opening cut from the lid. A screen was fitted between the container and the lid to prevent escape of postfeeding larvae. Animals were reared at 25 ± 0.5°C and 25 ± 4% RH, with maggot length and the duration of developmental stages recorded as described above. Larvae from rat treatments were transferred to sand substrates to pupate.

Statistical Analyses. Owing to unbalanced data (Table 1), Multiple analysis of variance (MANOVA) could not be used; thus, analyses of variance (ANOVAs) were examined using type III ANOVAs (Scheiner and Gurevitch 2001). This approach removes the variance from variables other than the one of interest and compares the variance remaining to the dependent variable. ANOVA and regression statistics were performed with the R statistical package (R Development Core Team 2004) at $\alpha < 0.05$ significance.

Developmental times in hours and accumulated degree-days (ADD), including standard deviations, were calculated for every significant treatment type and for rat cohorts. ADD was calculated using a base temperature of 10°C.

Graphs of larval growth were produced using the R statistical package. Curves were plotted by nonlinear quantile regression using smoothing parameters that yielded curves comparable to published data from Greenberg (1991), Wells and Kurahashi (1994), and Grassberger and Reiter (2001). Treatments in the comparisons include FMD cohorts that were transferred to fresh pupation substrate, FMDPT cohorts that were transferred to fresh pupation substrate, and NFMD cohorts that were not transferred to new pupation substrate. The plots included average and 95% confidence intervals (CIs), from the day flies hatched until the first day pupae were noted, which were then compared with averages of larval growth on rats. Data from Grassberger and Reiter (2001) also were compared with larval development on rats, because that study included growth at 25°C. For these

Table 2. Average developmental times ± SDs in hours and ADD for significant rearing treatments of *L. sericata*

Treatment	Egg	First instar	Second instar	Third instar	Postfeeding	Pupa	Total
FMD	21.74 ± 2.72	26.56 ± 2.18	26.05 ± 4.59	40.02 ± 12.55	76.04 ± 19.85	177.8 ± 26	368.2 ± 41.63
	(13.6 ± 1.7)	(16.6 ± 1.36)	(16.28 ± 2.87)	(25.01 ± 7.85)	(47.52 ± 12.41)	(111.1 ± 16.25)	(230.1 ± 26.02)
NFMD	21.75 ± 2.1	25.25 ± 0.87	36.44 ± 12.49	58.75 ± 12.7	98.5 ± 17.71	208.3 ± 58.93	448.9 ± 38.8
	(13.59 ± 1.31)	(15.78 ± 0.54)	(22.77 ± 7.81)	(36.72 ± 7.94)	(61.56 ± 11.07)	(130.2 ± 36.83)	(280.6 ± 24.25)
Paper towel	22.5 ± 2.24	25.75 ± 1.98	26.13 ± 3.37	23.88 ± 1.4	68.06 ± 13.31	175.6 ± 15.91	341.9 ± 16.85
	(14.06 ± 1.4)	(16.09 ± 1.24)	(16.33 ± 2.11)	(14.92 ± 0.87)	(42.54 ± 8.32)	(109.7 ± 9.95)	(213.7 ± 10.53)
No paper towel	21.38 ± 2.77	26.74 ± 2.13	27.68 ± 7.59	50.76 ± 6.12	83.46 ± 21.87	183.8 ± 36.53	393.8 ± 49.25
	(13.36 ± 1.73)	(16.71 ± 1.33)	(17.3 ± 4.74)	(31.73 ± 3.82)	(52.16 ± 13.67)	(114.9 ± 22.83)	(246.1 ± 30.78)
Transfer	22.02 ± 3	26.25 ± 2.25	25.87 ± 2.84	37.6 ± 13.37	59.88 ± 15.33	175 ± 12.2	346.6 ± 24.35
	(13.76 ± 1.88)	(16.41 ± 1.4)	(16.17 ± 1.78)	(23.5 ± 8.36)	(37.43 ± 9.58)	(109.4 ± 7.62)	(216.6 ± 15.22)
No transfer	21.59 ± 2.46	26.51 ± 2.07	27.89 ± 7.79	44.45 ± 13.58	88.53 ± 15.62	184.4 ± 37.78	393.4 ± 50.04
	(13.5 ± 1.54)	(16.57 ± 1.3)	(17.43 ± 4.87)	(27.78 ± 8.49)	(55.33 ± 9.76)	(115.3 ± 23.61)	(245.9 ± 31.2)
Destructive	21.7 ± 1.89	26.65 ± 2.33	28.7 ± 10.22	52.85 ± 9.8	92.2 ± 9.72	243.2 ± 41.23	465.3 ± 41.5
	(13.56 ± 1.18)	(16.66 ± 1.46)	(17.94 ± 6.39)	(33.03 ± 6.12)	(57.63 ± 6.08)	(152 ± 25.77)	(290.8 ± 25.94)
Not destructive	21.75 ± 2.75	26.38 ± 2.11	26.94 ± 5.94	40.35 ± 13.58	76.32 ± 21.16	171.4 ± 14.22	363.2 ± 31.72
	(13.59 ± 1.72)	(16.49 ± 1.32)	(16.84 ± 3.71)	(25.22 ± 8.49)	(47.7 ± 13.22)	(107.1 ± 8.89)	(227 ± 19.82)
Rat	19.67 ± 1.04	30.67 ± 1.53	23.5 ± 1.73	24.33 ± 0.58	60.67 ± 12.29	175.7 ± 11.58	334.5 ± 2.18
	(12.29 ± 0.65)	(19.17 ± 0.95)	(14.69 ± 1.08)	(15.21 ± 0.36)	(37.92 ± 7.68)	(109.8 ± 7.24)	(209.1 ± 1.36)

ADD was calculated using a base temp of 10°C. Values are displayed parenthetically. Third instar, the feeding portion of the stage; postfeeding, the nonfeeding portion of the third instar.

analyses, a locally weighted sum of squares (lowess) curve was plotted through the estimates by using R.

Results

Species Identification. Morphological identification of flies indicated that all were *L. sericata.* To confirm identification, a 798-bp mitochondrial cytochrome oxidase I sequence (National Center for Biotechnology Information accession no. DQ062660) was obtained from a collected adult fly. A BLAST search showed it was identical to a cytochrome oxidase I sequence from an *L. sericata* population in Ontario, Canada (accession no. L14947). The closest 13 National Center for Biotechnology Information gene sequences were from *L. sericata*, with a maximum difference of 4 bp (<1%), confirming the species identification.

Developmental Plasticity. The prepupation period for this fly population (reared at 25°C) ranged from 145 to 264.5 h (6–11 d), whereas the duration of egg to adult was 329–505.5 h (14–21 d), with all data given in *Appendix* 1. Throughout the experiment replicate treatments followed synchronized growth trajectories during the feeding stages, with a small number of individuals lagging. In contrast, postfeeding larvae within a treatment advanced to pupation gradually over a week. Eclosion also took place over a week.

Developmental times for stages and treatments are given in *Appendix* 1 and are summarized in Table 2 (using both hours and ADD). Linear models showed that development among treatments did not exhibit statistical differences in the shortest stages—the egg or the first two instars (a single exception is detailed below)—nor did these stages significantly influence total developmental time (data not shown). In contrast, the feeding portion of the third instar ($F = 18.52$, df = 1, $P = 0.00013$, $R^2 = 0.35$), the postfeeding stage of the third instar ($F = 27.67$, df = 1, $P < 0.0001$, $R^2 = 0.44$), and pupation ($F = 53.59$, df = 1, $P < 0.0001$, $R^2 = 0.62$) significantly affected overall developmental times.

Substrate type and its placement had no significant effect on development during any stage. Other treatments examined (Table 1) significantly impacted developmental time (Fig. 1; Table 2), whereas the stage at which that impact occurred differed. FMD accelerated development compared with treatments that received supplements every third day during the feeding portion of the third instar ($F = 12.19$, df = 1, $P = 0.0015$), although it was also a significant variable in the duration of the second instar ($F = 8.336$, df = 1, $P = 0.0072$). Accordingly, the two treatment types that developed in 14–16 d were FMD. FMDPT also resulted in faster growth during the feeding portion of the life cycle compared with treatments without paper towels ($F = 206.8$, df = 1, $P < 0.0001$). Moist paper towels were not necessary for the most rapid overall development, given that the fastest recorded time from egg to eclosion was from an FMD-transferred treatment (329 h; cohort 14 in *Appendix* 1); however, they promoted consistently faster development (Fig. 1). Once feeding ceased, the moisture of food did not contribute to developmental variation (postfeeding third instar: $F = 0.8439$, df = 1, $P = 0.37$ for FMD and $F = 1.677$, df = 1, $P = 0.21$ for FMDPT); however, transferring larvae to fresh substrate significantly shortened the amount of time spent as postfeeding third instars ($F = 17.59$, df = 1, $P = 0.00022$). The results indicate that handling larvae during the study did not impede development.

Destructive sampling did not influence larval stages, but it significantly increased the pupal stage ($F = 49.13$, df = 1, $P < 0.0001$). Finally, variables were assessed together to determine their relative influence on total immature development. Each had significant effects on total developmental time (FMD: $F = 4.644$, df = 1, $P = 0.039$; FMDPT: $F = 8.019$, df = 1, $P = 0.0079$; transfer to fresh substrate: $F = 4.454$, df = 1, $P = 0.043$; and destructive sampling: $F = 26.14$, df = 1, $P < 0.0001$).

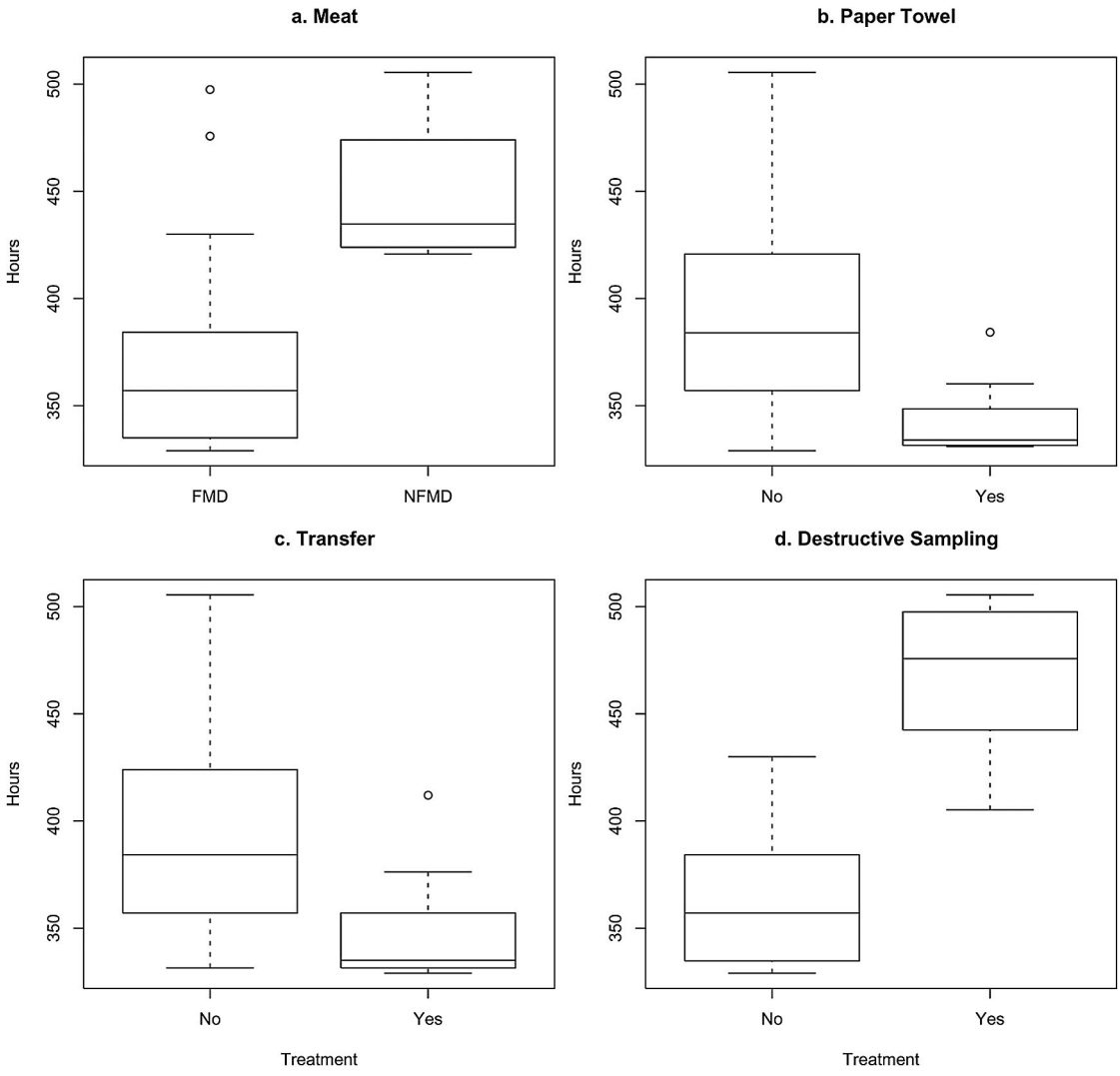

Fig. 1. Developmental variation among *L. sericata* cohorts by treatment type. Boxplots of total developmental time (hours) for each of the 37 liver-fed cohorts. The line within the box represents the median developmental hours, the box represents the developmental times between the 25th and 75th percentiles, and the "whiskers" (outermost lines) represent the 5th and 95th percentiles. (a) Fresh meat daily or no fresh meat daily (FMD versus NFMD). (b) Paper towel (moist paper towel placed under meat). (c) Transfer: transfer of larvae to fresh substrate for pupation. (d) Destructive (removal of 12 individuals each day). Note that treatments were in combination with other treatment types (Table 1) that had significant effects on developmental time. For example, the two outliers in the FMD boxplot (a) are those that also were destructively sampled.

Development on Carrion. The prepupal growth of larvae on rats was compared with the statistically significant experimental treatments as well as growth observed by Grassberger and Reiter (2001) (Figs. 2 and 3). The results displayed in Fig. 2 show that the shape and rate of larval growth curves for FMDPT treatments most closely matched the three cohorts reared on rats.

Figure 3 displays the growth of larvae during the first 3 d of development, when growth rate is relatively constant. A linear regression demonstrated different rates of growth among treatments, which were 0.20, 0.10, 0.12, 0.21, and 0.23 mm/h, for rat, NFMD, FMD, FMDPT, and Grassberger and Reiter (2001), respectively, with R^2 values of 0.92, 0.77, 0.90, 0.95, and 0.99. The regression model showed that length varied significantly with age ($F = 7099$, df = 1, $P < 0.0001$), whereas the effect of treatment types on length was also statistically significant ($F = 281.8$, df = 4, $P < 0.0001$), as was the interaction between age and treatment type ($F = 155.0$, df = 4, $P < 0.0001$).

Figure 4 compares the development of the flies reared on rats to development of liver-fed treatments in this study. Cohorts on rats developed in a manner that was most similar to the observed maximal devel-

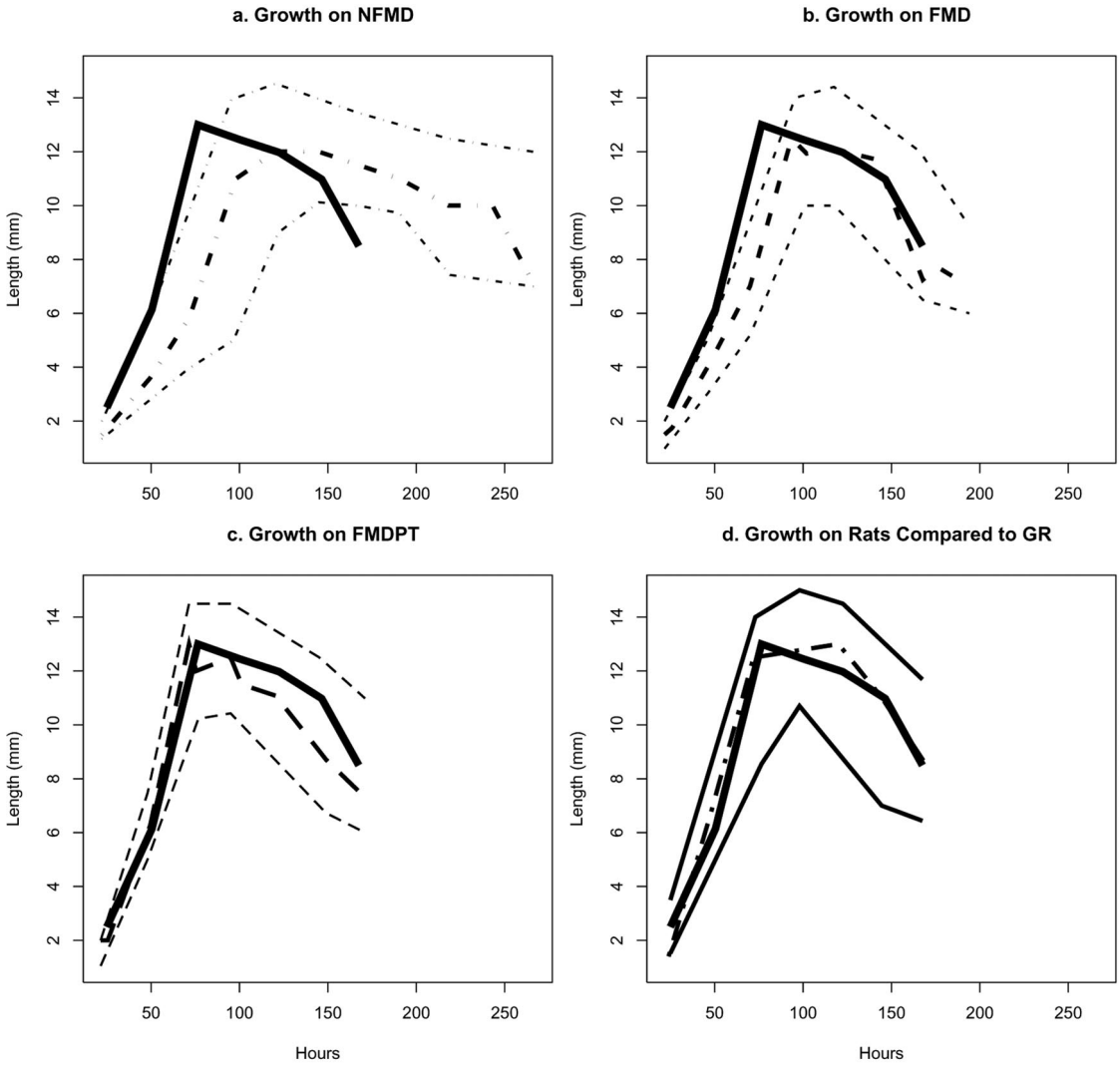

Fig. 2. Growth curves of *L. sericata* on liver versus growth on rat carrion. Nonlinear quantile regression curves created from the lengths of maggots in daily collections of each treatment type. (a) Meat added every third day, no moist paper towel, larvae were not transferred to fresh substrate to pupate. (b) Fresh meat daily, no moist paper towels, larvae transferred to fresh substrate to pupate. (c) Fresh meat daily, moist paper towel used, larvae transferred to fresh substrate to pupate. (d) Locally weighted sum of squares curve of data estimated from Grassberger and Reiter (2001) plotted against larval growth on rat carrion. Numbers of cohorts plotted for each treatment were three, four, six, and six for rat, NFMD, FMD, and FMDPT respectively. The solid line on each curve is the 50th percentile plot from cohorts raised on rats. Treatments are shown as dashed lines, with the thicker dashed line representing the 50th percentile and the thinner lines representing the 97.5th and 2.5th percentiles (95% CIs). Confidence intervals for the rat cohorts are present in d.

opment of liver-reared flies (i.e., FMDPT and some FMD treatments), with developmental times between 333 and 337 h (≈14 d). Furthermore, growth on rat carcasses was much less variable than the growth of liver treatments.

Discussion

Environmental Components of Variation in Development of *L. sericata*. *L. sericata* is a widely distributed species of great forensic importance. Several authors have examined different fly populations reared under various environmental conditions, and perhaps not surprisingly, the developmental times differ from one another, with Kamal (1958), Greenberg (1991), and Grassberger and Reiter (2001) estimating faster minimum developmental times than Anderson (2000). This variability could result from genetic differences among populations, but could equally result from dissimilarity in the conditions under which the animals were reared. Furthermore, none of the authors compared the laboratory growth of flies to that on actual

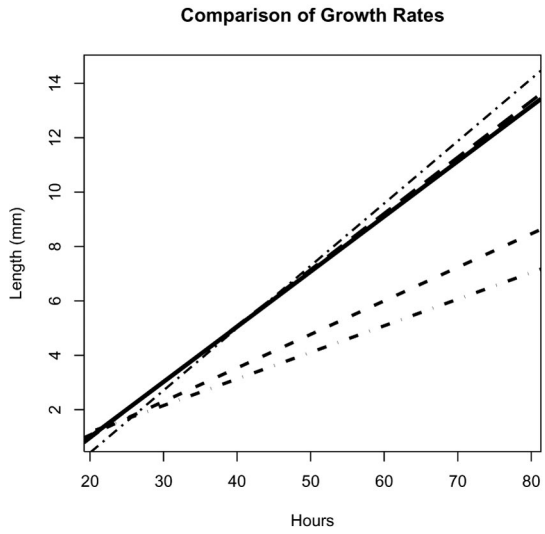

Fig. 3. Linear growth of *L. sericata* on liver versus growth on rats. Regression lines of the same treatments displayed in Fig. 2, for the first three days of growth—the linear phase of development. Line types used to indicate treatments are the same as in Fig. 2.

carcasses. In the current study, designed to estimate variation in developmental rates resulting from environmental differences, a single population of *L. sericata* was grown under laboratory conditions that mimicked those used in the previous studies, and these treatments were compared with larval development on carrion.

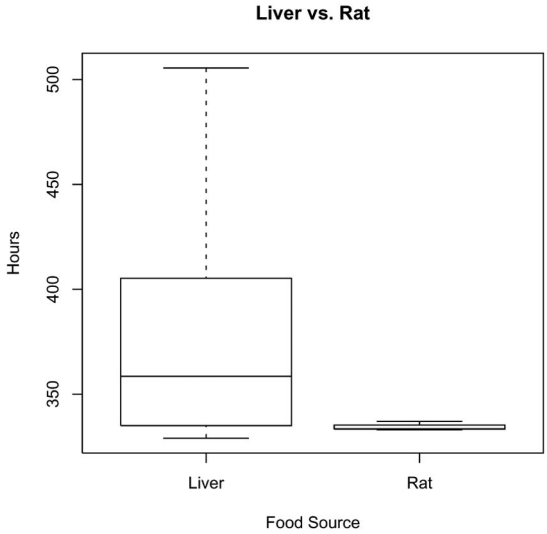

Fig. 4. Developmental times of *L. sericata* cohorts raised on liver versus rat carrion. Comparison of total developmental hours produced by the 37 liver-fed cohorts in this study to the development of the three rat-fed cohorts. Developmental time on rats was much less variable than growth on liver, with a developmental time most similar to the fastest growing liver-fed cohorts. Boxplot design is as in Fig. 1.

Given the minimum developmental times of the treatments detailed here, the fastest fell within the standard errors for *L. sericata* reared at 22°C by Greenberg (1991) and Grassberger and Reiter (2001), and is close to the mode reported by Kamal (1958), which is a common forensic entomology resource. Likewise, the slowest minimum developmental time for flies in this study was longer than the developmental minimum at 23.3°C found by Anderson (2000). This indicates that environmental variation alone can potentially explain all differences in developmental rates detailed in previous studies.

Results of these experiments demonstrate that variation in food moisture and pupation substrate have a significant influence on the growth of *L. sericata;* variation in rearing conditions generated a developmental difference of up to 7.4 d. Most notably, treatments designed to maintain meat moisture during feeding shortened the development of larvae. FMDPT treatments significantly shortened the feeding portion of third instars and produced a much smaller developmental range (Figs. 1 and 2). These results accentuate the importance of considering food moisture when rearing fly larvae. Grassberger and Reiter (2001) provided larvae with fresh liver daily, resulting in a similar growth rate at 25°C. Other studies have included moist sawdust, paper towels, or wood chips underneath meat (Kamal 1958, Goodbrod and Goff 1990, Anderson 2000), which would be expected to hold moisture. Interestingly, moist paper towels changed the life history table of FMD treatments toward the Greenberg (1991) estimate of third instar duration, which is approximately 1 d shorter than that of Grassberger and Reiter (2001). Unfortunately, Greenberg (1991) was vague about how flies were raised, so it is unclear what other factors could be involved, but food moisture may play a role in the differences in third instar developmental time observations between these authors.

Transferring postfeeding larvae to a fresh substrate for pupation significantly shortened the time spent at this larval stage. The postfeeding portion of blow fly larval development is generally variable (Wells and Kurahashi 1994), and *L. sericata* is exceptional among blow flies for wandering far from its food to pupate (Anderson 2000). This may mean that *L. sericata* searches for a specific set of environmental cues for pupation, making the postfeeding stage susceptible to disturbance. The conditions that produced the fastest growth in this study yielded a postfeeding stage duration of 2 to 3 d. Kamal (1958) provided sawdust with food, and observed a mode postfeeding duration of 90 h at 26.7°C, with a minimum of 48 h and a maximum of 192 h. His mode observation is similar to untransferred treatments in this study, which lasted a day longer than transferred cohorts. Greenberg (1991) reported an average postfeeding time of 108 h at 22°C, whereas Grassberger and Reiter (2001) reported 94 h at 20°C and 87 h at 25°C (the temperature at which this research was conducted). With little information on rearing conditions described by Greenberg (1991), the shorter times reported in the latter study are hard

to explain, but Grassberger and Reiter (2001) reared their flies with dry sawdust in jars, which may have resulted in the shorter average duration, given that the treatment seems similar to the transfer treatments in the research presented here.

There is little information in the literature that helps explain the developmental variability between transferred and untransferred postfeeding larvae found in the current study. Three plausible explanations for this phenomenon are density of individuals in each cohort, moisture differences between old and fresh substrates, and difference in odor between the treatments. Larval density seems unlikely to have had much influence on developmental time. Several treatments that were transferred to sand had larvae that had congregated on the substrate surface, and these densely packed cohorts still pupated in a timely manner. However, a lack of moisture and odor are both plausible agents behind the accelerated onset of pupation in transferred larvae. The sensitivity of larvae to moisture during feeding (outlined above) indicates that moisture is a potential cue for the cessation of feeding, with maggots actively searching out wet areas (tissues) while feeding, and reversing this behavior when heading toward pupation. Likewise, blow flies are attracted to odors associated with decay (Catts and Haskell 1990, Chaudhury et al. 2002, Hall et al. 2003); thus, it might be advantageous to be attracted to putrefying odors during feeding, followed by a prepupation move away from such odors.

Destructive sampling was found to be unimportant in larval development, yet was the only significant variable affecting the duration of pupation. The delay in pupation most likely resulted from the elimination of the earliest individuals to form a puparium, which were destructively sampled (removed) by necessity. Given these findings, studies of pupal developmental rates that require destructive sampling should consider its effects.

Other Potential Sources of Variation. The data presented demonstrate the effects of differing rearing treatments on this population of *L. sericata.* It should be noted however, that although most variation in growth existed among treatments, within-treatment variation also was observed. A portion of this could be explained by unmeasured environmental factors, because only a small number of rearing modifications were tested. Certainly, factors not considered in this study are likely to impact developmental differences.

Likewise, although environmental conditions were found to be highly significant in the development of *L. sericata,* genetic variation among fly populations used in different studies could potentially be just as important in understanding developmental variability. It is necessary to remember that each publication mentioned above outlined the development of flies that originated from a different ecogeographical region. There is precedence for population effects on the development of blow flies and several related species (Johnson and Schaffer 1973, Greenberg 1991, Oudman et al. 1991, Hoffmann and Harshman 1999, Parsch et al. 2000, Blanckenhorn 2002, Ames and Turner 2003, Feder et al. 2003). Genetic makeup is likely to affect other populations of blow flies, although these have been largely untested. Genetic differences, including potential interactions between genotype and environment, may be important sources of developmental variation when comparing populations of *L. sericata.*

Optimal Rearing Condition Using Liver and Growth on Rat Carrion. One might expect that blow flies have evolved to develop fastest under natural conditions of carrion decomposition. If this is the case, the fastest growth rate obtained in laboratory rearings would be expected to mimic the growth of flies living on carrion at the same temperature. In the current study, *L. sericata* development on rat carcasses was most similar to flies reared under high moisture conditions (Fig. 4). This finding helps address concerns raised by Kaneshrajah and Turner (2004) and Clark et al. (2006) who observed a significant effect of tissue type on the growth of *C. vicina* and *L. sericata,* respectively. Kaneshrajah and Turner (2004) were critical of rearing flies on liver, because it seemed to delay development. This delay was similar to slower developing treatments observed on desiccated liver in the current study, suggesting that larval rearing should take place on nondesiccated substrates to best mimic growth on a corpse.

Applications to Forensic Entomology. *L. sericata* development is plastic, at a level that alone could explain differences in the species' published developmental times. This finding highlights two important factors that need to be considered when estimating a PMI based on blow fly development. First, the discrepancies among development data sets can potentially be explained, in toto, by differences in laboratory rearing protocols used to develop such timetables. Accordingly, establishment of a common set of rearing conditions, which best relate to growth on carrion, is critical if direct comparisons are to be made among data sets, and if these data sets are to be used in legal proceedings. Second, because forensic entomologists use a quantitative trait (developmental rate) and decomposition ecology to make PMI estimates, researchers conducting studies on developmental time must aim to address the effects of both genetics and environment on their findings. By doing so, the forensic community can achieve a greater understanding of how important each of these factors is to forensic entomology.

A final consideration regarding entomological evidence involves its legal use in general. In the wake of judicial decisions that place a far greater emphasis on systematic analyses, known error rates, and statistical probabilities (see Daubert v. Merrell Dow Pharmaceuticals, 509 U.S. 579 [1993] and KumhoTire Co. v. Carmichael, 526 U.S. 137 [1999]), forensic scientists are under increasing pressure to conduct research, present legal analyses, and draw conclusions in a methodical and scientifically replicable way, while relying less on generalized knowledge and personal experience. Forensic entomology, although based on sound scientific principles, can currently be included

among an assemblage of forensic disciplines that may be called into question with regard to repeatability and standardized techniques (Saks and Koehler 2005). Efforts to establish calliphorid laboratory rearing protocols that best portray fly development on cadavers, and to standardize those techniques for future research, are central to meeting the demands of Daubert and Kumho. Such endeavors are necessary if forensic entomological evidence is to be routinely accepted in courts of law.

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References Cited

Ames, C., and B. Turner. 2003. Low temperature episodes in development of blow flies: implications for postmortem interval estimation. Med. Vet. Entomol. 17: 178–186.

Anderson, G. S. 2000. Minimum and maximum development rates of some forensically important Calliphoridae (Diptera). J. Forensic Sci. 45: 824–832.

Blanckenhorn, W. 2002. The consistency of quantitative genetic estimates in field and laboratory in the yellow dung fly. Genetica 114: 171–182.

Catts, E. P., and N. H. Haskell. 1990. Entomology and death: a procedural guide. Joyce's Print Shop, Inc., Clemson, SC.

Chaudhury M. F., J. B. Welch, and L. A. Alvarez. 2002. Responses of fertile and sterile screwworm (Diptera: Calliphoridae) flies to bovine blood inoculated with bacteria originating from screwworm-infested animal wounds. J. Med. Entomol. 39: 130–134.

Clark, K., L. Evans, and R. Wall. 2006. Growth rates of the blowfly, *Lucilia sericata*, on different body tissues. Forensic Sci. Int. 156: 145–149.

Conner, J. K., and D. L. Hartl. 2004. A primer of ecological genetics. Sinauer, Inc., Sunderland, MA.

Feder, J. L., S. H. Berlocher, J. B. Roethele, H. Dambroski, J. J. Smith, W. L. Perry, V. Gavrilovic, K. E. Filchak, J. Rull, and M. Aluja. 2003. Allopatric genetic origins for sympatric host-plant shifts and race formation in *Rhagoletis.* Proc. Natl. Acad. Sci. U.S.A. 100: 10314–10319.

Goodbrod, J. R., and M. L. Goff. 1990. Effects of larval population density on rates of development and interactions between two species of *Chrysomya* (Diptera: Calliphoridae) in laboratory culture. J. Med. Entomol. 27: 339–343.

Grassberger, M., and C. Reiter. 2001. Effect of temperature on *Lucilia sericata* (Diptera: Calliphoridae) development with special reference to the isomegalen- and isomorphen-diagram. Forensic Sci. Int. 120: 32–36.

Greenberg, B. 1991. Flies as forensic indicators. J. Med. Entomol. 20: 565–577.

Hall, M. J., R. A. Hutchinson, R. Farkas, Z. J. Adams, and N. P. Wyatt. 2003. A comparison of Lucitraps and sticky targets for sampling the blowfly *Lucilia sericata.* Med. Vet. Entomol. 17: 280–287.

Hoffmann, A. A., and L. G. Harshman. 1999. Desiccation and starvation resistance in *Drosophila:* patterns of variation at the species, population and intrapopulation levels. Heredity 83: 637–643.

Johnson, F. M., and H. E. Schaffer. 1973. Isozyme variability in species of the genus *Drosophila.* VII. Genotype-environment relationships in populations of *D. melanogaster* from the Eastern United States. Biochem. Genet. 10: 149–163.

Kamal, A. S. 1958. Comparative study of thirteen species of sarcosaprophagous Calliphoridae and Sarcophagidae (Diptera) I. Bionomics. Ann. Entomol. Soc. Am. 51: 261–270.

Kaneshrajah, G., and B. Turner. 2004. Calliphora vicina larvae grow at different rates on different body tissues. Int. J. Legal Med. 118: 242–244.

Mackay, T.F.C. 2001. The genetic architecture of quantitative traits. Annu. Rev. Genet. 35: 303–339.

Oudman, L., W. Van Delden, A. Kamping, and R. Bijlsma. 1991. Polymorphism at the *Adh* and alpha *Gpdh* loci in *Drosophila melanogaster:* effects of rearing temperature on developmental rate, body weight, and some biochemical parameters. Heredity 67: 103–115.

Parsch, J., J. A. Russell, I. Beerman, D. L. Hartl, and W. Stephan. 2000. Deletion of a conserved regulatory element in the *Drosophila Adh* gene leads to increased alcohol dehydrogenase activity but also delays development. Genetics 156: 219–127.

R Development Core Team. 2004. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (http://www.R-project.org).

Saks, M. J., and J. J. Koehler. 2005. The coming paradigm shift in forensic identification science. Science (Wash., D.C.) 309: 892–895.

Scheiner, S. M., and J. Gurevitch. 2001. Design and analysis of ecological experiments, 2nd ed., pp. 112–113. Oxford University Press, New York.

Wall, R., Pitts, K. M., and K. E. Smith. 2001. Pre-adult mortality in the blowfly *Lucilia sericata.* Med. Vet. Entomol. 15: 328–334.

Wells, J. D., and H. Kurahashi. 1994. *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) development: rate, variation, and the implications for forensic entomology. Jpn. J. Sanit. Zool. 45: 303–309.

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Appendix 1. Treatments and duration of the immature life cycle and individual stages from all cohorts of *L sericata*

Cohort	Meat	Destructive	Transfer	Substrate under	Substrate	Paper towel	Egg	First instar	Second instar	Third instar (feeding)	Postfeeding	Pupa	Total hours	Total days
1	NFMD	No	No	No	Vermiculite	No	21.5	26	47	69.5	100.5	162.5	427	17.79
2	NFMD	Yes	No	No	Vermiculite	No	22.5	24.5	25.25	48	98.25	224	442.5	18.44
3	FMD	Yes	No	No	Vermiculite	No	20	30	21.75	47.25	94.5	284	497.5	20.73
4	FMD	No	No	No	Vermiculite	No	24	27	28	45	98	162.25	384.25	16.01
5	FMD	No	No	Yes	Vermiculite	No	21	29.5	22.75	49	99.25	187.5	409	17.04
6	FMD	No	Yes	No	Vermiculite	No	24	27	28	45	48	163.25	335.25	13.97
7	NFMD	No	No	No	Vermiculite	No	19	26	47.5	47.5	119.25	161.5	420.75	17.53
8	NFMD	Yes	No	No	Vermiculite	No	24	24.5	26	70	76	285	505.5	21.06
9	FMD	Yes	No	No	Vermiculite	No	22.5	26.5	23.75	52	91.5	189	405.25	16.89
10	FMD	No	No	No	Vermiculite	No	25	25.25	23.75	51	118	187	430	17.92
11	FMD	No	Yes	No	Vermiculite	No	25	26.25	23.75	51	94	192	412	17.17
12	FMD	Yes	No	No	Vermiculite	No	19.5	27.75	46.75	47	100.75	234	475.75	19.82
13	FMD	No	No	No	Vermiculite	No	18.5	28	23.5	52.25	95.25	140	357.5	14.9
14	FMD	No	Yes	No	Vermiculite	No	18.5	28	23.5	52.25	43.75	163	329	13.71
15	FMD	No	No	Yes	Vermiculite	No	21	25.5	22.5	53	93	160	375	15.63
16	FMD	No	No	No	Sand	No	15	30.75	23.75	48	95.5	163	376	15.67
17	FMD	No	Yes	No	Sand	No	15	30.75	23.75	48	71.75	187	376.25	15.68
18	FMD	No	No	No	Sand	Yes	18.5	29.5	25.5	25	69	164	331.5	13.81
19	FMD	No	Yes	No	Sand	Yes	18.5	29.5	25.5	25	48	185	331.5	13.81
20	FMD	No	Yes	No	Sand	Yes	24	26.75	28	21.5	72.25	162.5	335	13.96
21	FMD	No	No	No	Sand	Yes	24	26.75	28	21.5	72.25	162.5	335	13.96
22	FMD	No	No	Yes	Sand	No	19	27.75	24.75	47.75	93.75	188	401	16.71
23	FMD	No	No	Yes	Sand	No	22	28	25	47	72.25	162.75	357	14.88
24	FMD	No	No	No	Sand	No	24	27.25	24	48.25	65	168.5	357	14.88
25	FMD	No	Yes	No	Sand	No	24	27.25	24	48.25	65	168.5	357	14.88
26	FMD	No	No	No	Vermiculite	Yes	22	24	29.5	23.5	71.75	162.25	333	13.88
27	FMD	No	Yes	No	Vermiculite	Yes	22	24	29.5	23.5	71.75	162.25	333	13.88
28	FMD	No	Yes	No	Vermiculite	No	21.75	24	28.25	50.25	47	163.25	334.5	13.94
29	FMD	No	Yes	No	Sand	No	23	23.5	28.25	50.75	46.75	167.75	340	14.17
30	FMD	No	Yes	No	Sand	Yes	21.5	24.25	29.75	25.75	70.5	188.5	360.25	15.01
31	FMD	No	No	No	Vermiculite	No	21.75							
32	FMD	No	No	No	Sand	No	23							
33	FMD	No	No	No	Sand	Yes	21.5							
34	FMD	No	Yes	No	Vermiculite	Yes	24.5							
35	FMD	No	Yes	No	Vermiculite	Yes	24.5							
36	FMD	No	No	No	Vermiculite	Yes	24.5							
37	FMD	No	No	No	Vermiculite	Yes	24.5							
38	Rat	No	Yes	No	Sand	No	20.5							
39	Rat	No	Yes	No	Sand	No	20							
40	Rat	No	Yes	No	Sand	No	18.5							

(Continued)

Appendix 1. Continued

First instar	Second instar	Third instar (feeding)	Postfeeding	Pupa	Total hours	Total days
24	28.25	50.25	65.25	166.5	356	14.83
23.5	28.25	50.75	94.25	164.25	384	16
24.25	29.75	25.75	70.5	212.5	384.25	16.01
25	22	23.5	50.25	185.75	331	13.79
25	22	24	49.5	186	331	13.79
25	22	23.5	95.5	168	358.5	14.94
25	22	24	75.5	167.5	338.5	14.10
29	22.5	24	68.5	172.5	337	14.04
31	25.5	24	67	166	333.5	13.9
32	22.5	25	46.5	188.5	333	13.88

The minimum developmental times of each stage and the minimum total developmental time for cohorts of *L. sericata* are given. Also listed are the combinations of variables that each cohort experienced. All times are reported in hours (to the closest quarter hour) except the total developmental time, which is reported in hours and days. Minimum developmental times ranged from 329 to 505.5 h for liver-fed cohorts (cohorts 1–37) and from 333 to 337 h for rat-fed cohorts (cohorts 38–40). Labels are as in Table 1.