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PAPER

PATHOLOGY AND BIOLOGY

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Variation in Developmental Time for Geographically Distinct Populations of the Common Green Bottle Fly, *Lucilia sericata* (Meigen)*

ABSTRACT: Time between death and discovery of remains, or postmortem interval (PMI), can be assessed using blow fly maggot age. Forensic entomologists rely on published, often nonlocal, species-specific developmental tables to determine maggot age. In a series of common garden experiments, we investigated the developmental rate variation between populations of *Lucilia sericata* collected from Sacramento, CA, San Diego, CA, and Easton, MA at 16°C, 26°C, and 36°C. For the 16°C trial the time measurement started at egg hatch, while for the higher temperatures the experiment began at oviposition; the wandering stage signified the endpoint for all experiments. The distribution of developmental times differed significantly (ANOVA, $p < 0.001$) between the three populations within each temperature treatment. We discovered that regional variation of developmental times within a blow fly species exists. This study demonstrates the importance of assembling local population-specific developmental tables when estimating larval age to determine PMI.

KEYWORDS: forensic science, forensic entomology, postmortem interval, Calliphoridae, *Lucilia sericata,* larvae, blow fly development

The period between death and discovery of remains, or postmortem interval (PMI), significantly aids in the reconstruction of events surrounding a death. Forensic entomologists can estimate a form of minimum PMI, called the period of infestation, using the age of the oldest cohort of blow fly maggots found on the remains. Maggots appear to lengthen in a continuous manner during growth, developing at a predictable, species-specific temperature-mediated growth rate. Thus, within limits, their age may be estimated from length, thereby providing a minimum estimate of elapsed time since death occurred. However, it seems reasonable to postulate that fly strains will adapt to the temperature regimes and other conditions of climate prevalent in their local environment, and this may alter their rate of development. We postulate that to take better advantage of a shorter active season, widely distributed blow fly species inhabiting more northerly climates should have a lower minimum developmental temperature threshold than those occurring in more southerly or warmer climates. Such a lowered threshold would cause the temperature-mediated growth rate curve to shift to the left. Thus, we expect cold-adapted maggots would develop faster than warm-adapted maggots at low temperatures, and warm-adapted maggots should develop more rapidly at high temperatures (Fig. 1).

Estimation of the period of infestation from maggot age requires growth rate reference data. These exist for many forensically significant species (1–8) and for many agricultural pests as well (9–11). Yet, reference data from different studies present dissimilar developmental rates for the same species (12–14). Developmental

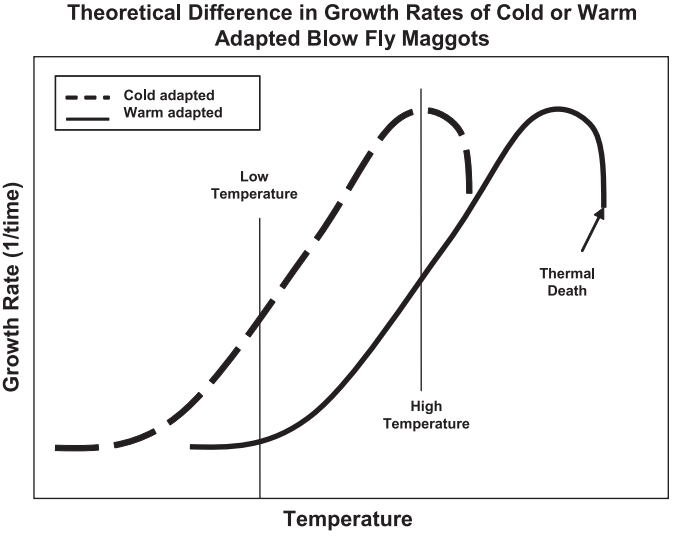

FIG. 1—*Larval growth rate as a function of temperature: We postulated that the population of flies from the cooler climate will exhibit a reduced lower temperature threshold, creating a left shift of the curve. If we compare the development at a temperature approaching the lower threshold we expect to find that the cold-adapted population, which is in the linear portion of the curve, develops faster than the warm-adapted population, whose development is lagging behind. Additionally, the warm-adapted population will develop faster at the high temperature because they are still on the linear part of the curve, whereas the cold-adapted population has reached their threshold causing further development to slow or cease.*

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time varies between populations of other ectothermic organisms also, such as fish (15), amphibians (16,17), and other insects (18,19). Blow fly populations may exhibit different developmental rates as a consequence of dissimilar rearing or experimental method, but alternatively, local conditions may select different developmental rates in geographically separated populations of the same blow fly species.

It may be that maggots from more northerly populations of the green bottle fly, *Lucilia sericata* (Meigen) develop more rapidly at lower temperatures than more southerly populations. Accordingly, we established laboratory populations from isolated regions representing latitudinal extremes within the continental United States. From a series of common garden experiments, we reared cohorts of first- and second-generation maggots from each population at three temperatures and compared their developmental rates. Specifically, we sought to determine whether different populations developed at significantly different rates at any of the three temperature conditions chosen.

Materials and Methods

Fly Colonies

We established laboratory populations of green bottle flies starting with field-collected eggs from three isolated regions representing latitudinal extremes within the continental United States. These include Sacramento, CA (global positioning system (GPS) coordinates: 38.6757N, -121.3763W), Easton, MA (GPS: 42.0481N, -71.071W), and San Diego, CA (GPS: 32.7159N, -117.1296W). Average seasonal temperatures for these three regions can be found in Table 1.

We sampled each location near the beginning of the most active fly season. Accordingly, the Sacramento population was collected in early April, 2007 and the Massachusetts and San Diego populations were collected in mid-May, 2007. In each location, we set out five to ten small bowls, each containing a 100-g piece of beef heart or a large rat, protected by chicken wire. These were retrieved when four or more egg masses (≥1000 eggs total) had accumulated on the carrion. Hatching maggots were reared in a 250-mL cup containing approximately 150 g of beef heart, sitting on rice hulls, which served as a pupation substrate.

We sorted and identified emerging adult flies at 0°C (20). Identifications were verified by Michael Niemela (Associate Public Health Biologist, CDPH-CID-DCDC), at the University of California Bohart Museum of Entomology, where pinned voucher samples of flies from each population were deposited. We maintained live cohorts of flies in wire cages at approximately 26°C and 16:8 light:dark cycle provided with sugar and water *ad libitum*. To promote ovarian development, we additionally provided previously frozen beef heart for the first three days postemergence.

TABLE 1—*Average seasonal temperatures of experimental regions.**

	Sacramento Area High–Low Averages (°F)	San Diego Area High–Low Averages (°F)	Boston Area High–Low Averages (°F)
Winter (Dec–Feb)	55.9–39.4	66.1–50.0	39.0–24.7
Spring (Mar–May)	72.1–47.1	68.1–56.6	56.4–40.7
Summer (Jun–Aug)	90.5–56.6	75.1–65.3	79.6–63.3
Fall (Sep–Nov)	76.5–49.8	73.6–60.3	62.0–47.0

*Data obtained from the National Weather Service.

Determination of Experimental Cohort Size

We chose to limit the number of maggots per cohort to avoid the modification of temperature by maggot aggregations. In preliminary experiments, we counted out groups of 50, 100, 250, or 500 blow fly eggs and placed each group into a small bowl with 1 g of previously frozen beef heart per egg. The inoculated meat bowls were maintained at 25°C. A data logger (Onset, HOBO U12) simultaneously recorded the ambient temperature and temperatures within each bowl every 10 min. This experiment was conducted once.

Experimental Trials

We experimented only with first- or second-generation eggs and maggots. To obtain eggs, we placed 50 g of warm (30–34°C), previously frozen beef heart in a plastic cup partially covered with a damp paper towel in each cage of flies. We examined the meat every 30 min for eggs. Using a damp paintbrush, we collected and counted batches of 100 eggs; pilot studies demonstrated that 100 maggots did not measurably raise their mass temperature above ambient. We then placed each batch into a 250-mL opaque cup together with 125 g of finely chopped beef heart and secured it within a rearing chamber specifically designed to minimize the

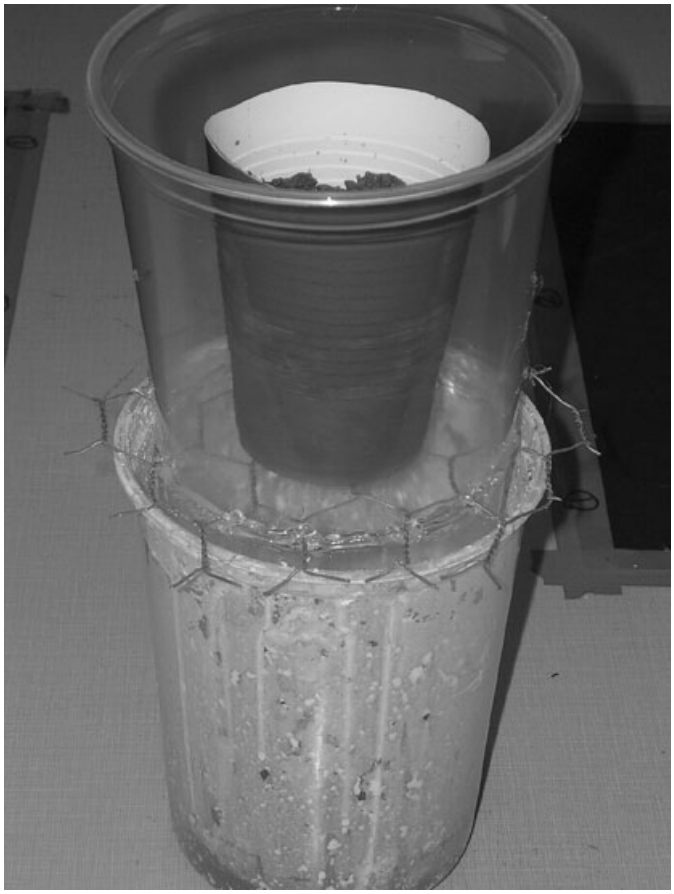

FIG. 2—*Rearing chamber for* Lucilia sericata*: the top contains a 250-mL cup filled with inoculated ground beef heart, on a chicken wire floor. When maggots complete feeding they enter their wandering stage, crawl out of the meat cup, and fall into the bottom container, which contains 5 cm of sand and is coated with Fluon (ICI United States, Inc.) to prevent escape. We periodically counted wandering stage maggots that had accumulated in the sand, without disturbing those still feeding in the top container.*

disturbance of maggots (Fig. 2). Rearing chambers to be maintained at 26°C or 36°C were immediately moved to incubators. In initial experiments at 16°C eggs did not hatch, thus we maintained these eggs at 24–26°C until hatching, then immediately moved them to a 16°C incubator. Each incubator (16°C and 26°C; Precision Scientific, Chennai, India, 36°C; National Labnet Co., Edison, NJ) maintained a relative humidity of 65–75%, and a 24-h day light cycle. Ambient temperature and the internal temperature of one cup in each incubator were recorded at 30-min intervals, using a data logger (Onset, HOBO U12).

Experiments were run using three temperatures with each of the three fly colonies for a total of nine temperature-location treatments. Each treatment was replicated in 12 chambers with *c.* 100 maggots per chamber, for a total of 1200 maggots per treatment.

After a short period (16°C: 7 days, 26°C: 3 days, 36°C: 2 days), we checked every 4–6 h for wandering third-stage larvae by sifting the sand in the bottom of the rearing chamber (Fig. 2). Once wandering commenced, we sifted at 3-h intervals. Only those maggots that actually exited the feeding cup were counted.

Analysis

We generated Kaplan–Meier survival function curves to examine the feeding probability of surviving maggots as a function of time (Egret for Windows v. 2.0; Cytel Software Corporation, Cambridge, MA). Kruskal–Wallis one-way analysis of variance (ANOVA) was used to compare the three collection sites' (conditional on temperature) distribution of developmental times; when significant ($p < 0.05$), *post hoc* Mann–Whitney tests were used to see which sites significantly differed from each other (StatXact 8.0; Cytel Software Corporation). We used time between egg hatch and wandering as the developmental time at 16°C, and time between oviposition and wandering at 26°C and 36°C.

Results

First we determined the greatest number of maggots feeding together that did not raise their aggregate temperature. Cups holding 50 and 100 maggots maintained a temperature within 0.5–1.0°C of the ambient temperatures, whereas data from cups holding 250 and 500 maggots illustrate temperature spikes more than 4°C above ambient during the maggots' third instar stage of development. Thus, for all further experiments we used cohorts of 100 maggots.

The distribution of developmental times significantly differed between the three locations at each temperature (ANOVA, $p < 0.001$) (Figs. 3–5). The Sacramento and San Diego populations differed at 36°C (Mann–Whitney, $p = 0.0016$), as the Sacramento and Massachusetts populations ($p = 0.0003$). The San Diego and Massachusetts populations did not differ at 36°C ($p = 0.20$). At 26°C, maggot developmental rates between each pair of populations differed from each other ($p < 0.0001$). Similarly, at 16°C, development rates also differed between each pair of populations ($p < 0.0001$). Overall, the distribution of developmental times between each pair of populations differed at each temperature, except for San Diego and Massachusetts at 36°C.

Larval development times, expressed as the mode, can be found in Table 2. These data suggest that the San Diego population developed more rapidly than Sacramento and Massachusetts populations at 16°C, but more slowly at 36°C. At 26°C, Sacramento and San Diego populations developed at similar rates, whereas the Massachusetts population lagged behind.

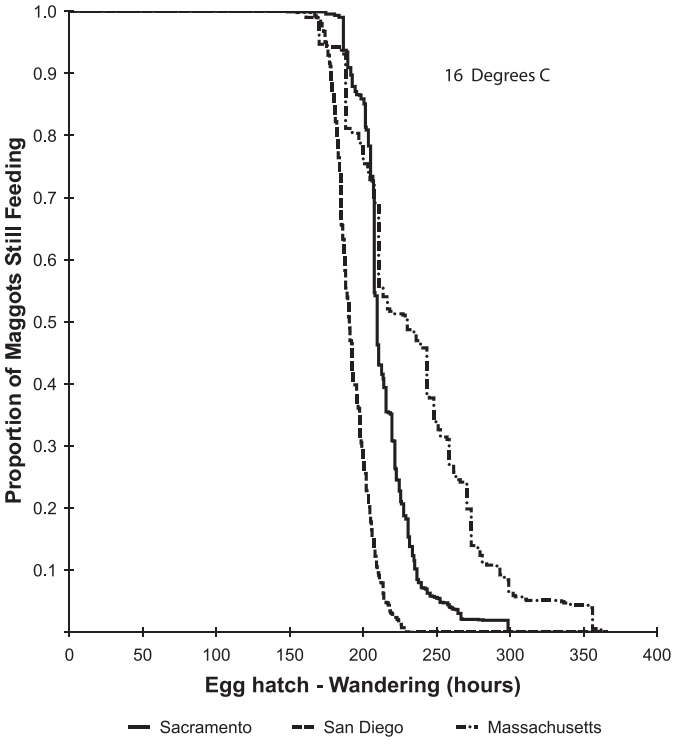

FIG. 3—*Kaplan–Meier curve of 16°C experimental trial.*

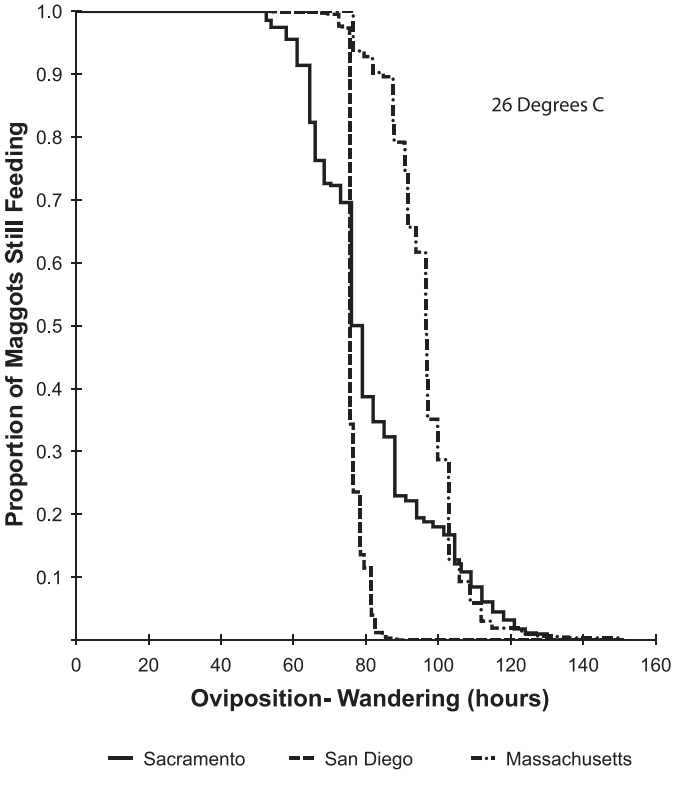

FIG. 4—*Kaplan–Meier curve of 26°C experimental trial.*

Discussion

The distribution of developmental times for the three green bottle fly populations differed from one another at each temperature, except for San Diego and Massachusetts at 36°C. Currently,

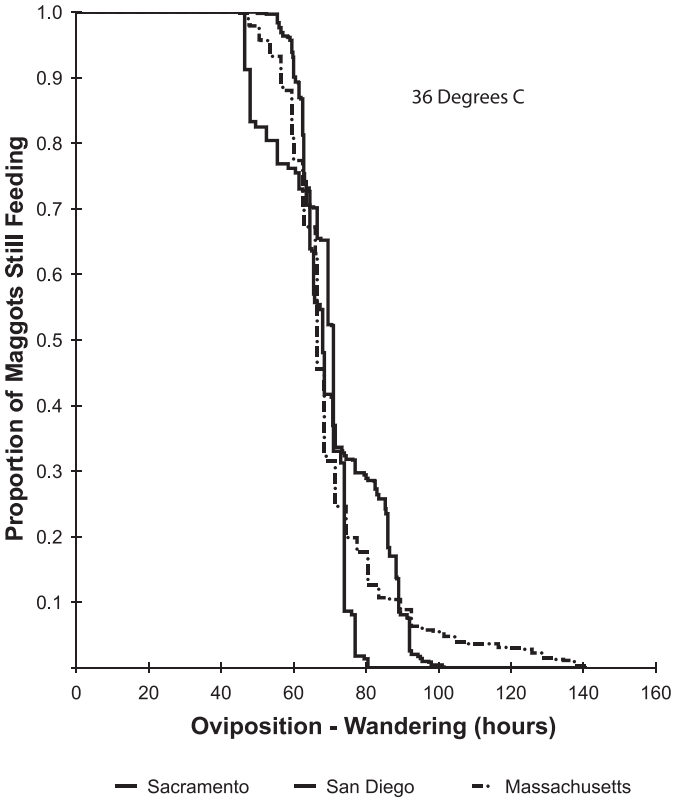

FIG. 5—*Kaplan–Meier curve of 36°C experimental trial.*

TABLE 2—*Larval development time in hours.*

	16°C Trial*		26°C Trial†		36°C Trial†	
Population	Mode	Std. Dev.	Mode	Std. Dev.	Mode	Std. Dev.
Sacramento	207.5	20.27	76.0	17.03	64.5	11.38
San Diego	185.0	12.66	75.5	2.45	74.0	10.19
Massachusetts	210.5	44.78	102.8	9.80	66.5	15.60

*Development time = egg hatch − wandering stage.
†Development time = oviposition − wandering stage.

TABLE 3—*Example of potential % error using 16°C developmental data.**

	Actual Population		
	Sacramento (%)	San Diego (%)	Massachusetts (%)
Reference data used			
Sacramento	0	−12.20	1.40
San Diego	10.80	0	12.11
Massachusetts	−1.40	−13.80	0

*Percent error calculated using the modes derived from experimental data: % error = ([mode of population − mode of reference data used]/mode of population) × 100.

estimates of maggot age often depend on reference development data from nonlocal populations, yet these findings suggest that such estimations should derive from reference data obtained from local fly populations for minimum PMI estimates. For example, if we were to use modes to approximate the percent error in a time of infestation estimate when drawing on reference data derived from a distant population, applying the 16°C data we present here, percent error could be as high as −13.80% (Table 3).

It has been suggested that intraspecies growth rate variation may exist because of disparities in developmental rate determined in various studies (1–5). Yet, each of these studies used unique rearing methods and laboratory conditions, rendering accurate comparison of results between studies ambiguous. Because of logistical constraints (available equipment, rearing medium, fly collection locations, etc.), we were unable to precisely reproduce the conditions of any previous studies and are therefore unable to accurately compare our results to those of other studies.

We mistakenly expected that the population from the cooler climate (MA) would develop faster in a low-temperature common garden experiment than warm-climate populations (Sacramento & San Diego) because of the adaptation to the colder temperatures and shorter active season. This finding would have typified the thermal adaptation models of organisms that reside in contrasting habitats (15–17). As this occurs in other ectothermic organisms such as fish and amphibians, we expected flies to develop similarly.

Although developmental time differed between populations, the trend did not adhere to this model. However, the thermal adaptation model only takes temperature into account. It may be that differences in development time we observed derived from adaptations to other environmental constraints that differ between these regions, in addition to temperature. For example, the topography, humidity, photoperiod, and plant ecology of Massachusetts greatly differ from that of California habitats. Although we have not identified actual modifiers of developmental time, we have ascertained that regional variation does exist.

Inhabiting a wide range of environments, the green bottle fly remains an ideal model for this kind of study. Other blowflies, such as *Lucilia cuprina*, for example, have a much more restricted climactic distribution (21). It is reasonable to suggest that the more widely distributed species may exhibit greater variation between distant populations than those that are limited to one climactic zone. Similar experiments to this one, on a more climactically restricted species, might contrast this idea.

Under natural conditions, the number of maggots feeding together is usually less restricted. As the larvae begin actively feeding and generating metabolic heat, the temperature inside their microenvironment, the maggot mass, often climbs high above the ambient temperature (22,23). It may be that ambient temperature is insignificant when a large maggot mass is present. Maggot masses greater than 20 cm^3 exhibit internal temperatures independent of the ambient temperature (24). Thus, an investigation on developmental variation between populations under more natural conditions is necessary.

Each population exhibited variation in developmental time within each temperature treatment. The majority of the maggots often left the meat cups in very large groups usually within 10–12 h of each other. In some cases, small groups of 10–15 maggots would remain in the cup for several days after the majority had left. At 16°C, a few maggots from the Massachusetts population remained in the cup for 2.9 days after most of the maggots had left. Interestingly, these "independent wanderers" usually left the cup after the mass departure, not before, so we suggest that they had actually stopped feeding and entered the wandering stage, even though they did not leave the cup. Because only maggots actually exiting the cup were counted as wandering, this introduced some error into the experimental method. The few that wandered early usually preceded the large groups by only 3–6 h. Thus, we conclude that the mode is the most accurate measurement to determine developmental time because it eliminates the error associated with delayed wandering behavior.

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