Developmental variation among Cochliomyia macellaria *Fabricius (Diptera: Calliphoridae) populations from three ecoregions of Texas, USA*

Charity G. Owings, Cliff Spiegelman, Aaron M. Tarone & Jeffery K. Tomberlin

International Journal of Legal Medicine

ISSN 0937-9827

Int J Legal Med
DOI 10.1007/s00414-014-1014-0

Your article is protected by copyright and all rights are held exclusively by Springer-Verlag Berlin Heidelberg. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Springer

Author's personal copy

Int J Legal Med
DOI 10.1007/s00414-014-1014-0

ORIGINAL ARTICLE

Developmental variation among *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) populations from three ecoregions of Texas, USA

Charity G. Owings · Cliff Spiegelman · Aaron M. Tarone · Jeffery K. Tomberlin

Received: 20 October 2013 / Accepted: 23 April 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract Forensic entomologists rely on published developmental datasets to estimate the age of insects developing on human remains. Currently, these datasets only represent populations of targeted insects from specific locations. However, recent data indicate that populations can exhibit genetic variation in their development, including signatures of local adaptation demonstrated by regionally distinct plastic responses to their environments. In this study, three geographically distinct populations of the secondary screwworm, *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae; College Station, Longview, and San Marcos, TX, USA), a common blow fly collected from human remains in the southern USA, were reared in two distinct environments (cool 21 °C, 65 % relative humidity (RH); and warm 31 °C, 70 % RH) over 2 years (2011 and 2012) in order to determine differences in development time and mass. Significant differences in immature and pupal development time, as well as pupal mass, were shown to exist among strains derived from different populations and years. For immature development times, there was evidence of only an environmental effect on phenotype, while genotype by environment interactions was observed in pupal development times and pupal mass. College Station and San Marcos populations exhibited faster pupal development and smaller pupal sizes in the cooler environment relative to the Longview population, but showed an opposite trend in the warm environment. Rank order for College Station and Longview populations was reversed across years. Failure to take genetic variation into consideration when making such estimates can lead to unanticipated error and bias. These results indicate that genetics will have little impact on error when working with Texas genotypes of *C. macellaria* at ~30 °C and 70 % RH, but will have a more meaningful impact on error in postmortem interval estimates with this species in cooler, drier environments.

Keywords Phenotypic plasticity · Forensic entomology · Blow fly · Decomposition ecology

Introduction

After death of a person or companion animal, insects such as blow flies (Diptera: Calliphoridae) can utilize the remains as a resource for their offspring [1]. Knowledge of the developmental biology of these flies can be used to estimate the length of time between colonization and discovery of the decedent, which is referred to as the postcolonization interval [2] or minimum postmortem interval (m-PMI) [2–4]. Laboratory-derived datasets, however, may not always accurately reflect ontogenetic patterns across all geographic genotypes or populations of a species [5–7]. This variation is a point of concern when applying such data to death investigations, as estimates of the m-PMI could vary significantly based on locale and thus prove less reliable in a legal context. While it is now acknowledged that genetic and/or population differences are potentially important components of error in an m-PMI estimate, little is known about the conditions that may dictate when they are most likely to affect (or not affect) casework.

Potential explanations for variation in arthropod development across studies include genetic drift and/or selection of traits that favor local adaptations [8], as well as restricted

Electronic supplementary material The online version of this article (doi:10.1007/s00414-014-1014-0) contains supplementary material, which is available to authorized users.

C. G. Owings (✉) · A. M. Tarone · J. K. Tomberlin
Department of Entomology, Texas A&M University, College Station, TX, USA
e-mail: charityowings@gmail.com

C. Spiegelman
Department of Statistics, Texas A&M University, College Station, TX, USA

Published online: 09 May 2014

Author's personal copy

sampling of wild flies, which might not represent the true genetic variation of the species [9, 10]. Locally optimal phenotypes may even change as environmental shifts occur due to changes in climate (e.g., seasonal fluctuations in temperature) [11] or resource availability [12]. Additionally, the diverse biological responses of conspecific populations may be the result of phenotypic plasticity (the ability of a single genotype to produce multiple phenotypes when exposed to different environments [13–15]). These variations may also manifest themselves as genotype by environment (G × E) interactions when responses change across environments and can be considered locally adaptive if exhibited in a phenotype that affects organismal fitness [8]. Given these effects, it will be critical for forensic entomologists to dissect, and ultimately account for, the roles of such factors on m-PMI estimates made with blow fly developmental data.

Recent studies have focused on this phenomenon in regard to forensically relevant insects, particularly in relation to growth substrate, temperature, and geographic distribution of blow flies [16–18]. The implications of developmental variation lie in estimating the m-PMI with published datasets. If conspecific blow fly populations diverge from published data, but are treated as equals by the investigator, accuracy of the estimation is diminished. These errors have the potential for drastic outcomes, such as a faulty verdict in a court of law.

The goal of our research was to explore the variable responses of three populations of the secondary screwworm *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) in two constant, but distinct, environments (distinguished by temperature and relative humidity, RH). The secondary screwworm was selected as a model organism as it is one of the dominant blow flies found on decomposing human remains in TX, USA (Tomberlin, unpublished data).

Materials and methods

The methods used in this research were modified from Gallagher et al. (2010) and Tarone et al. (2011). Experiments were conducted during August and October 2011 and May 2012, which allowed for the examination of phenotypic variation across locations and years. Furthermore, the initial experiment (August 2011) examined phenotypic responses within and between populations reared in distinct environments and varying larval densities. This initial density study allowed us to determine the appropriate larval density for the primary phenotypic variation study.

Collection sites Distinct ecoregions were chosen in order to maximize the potential for variation between conspecific populations [19]. Collection locations within each ecoregion included College Station, TX, USA (30° 37′ 40.72″ N, 96° 20′ 03.86″ W) in the subtropical and temperate Post Oak Savannah ecoregion [20]; Longview, TX, USA (32° 30′ 02.53″ N, 94° 44′ 25.76″ W) in the humid subtropical Pineywoods [21]; and San Marcos, TX, USA (29° 52′ 59.79″ N, 97° 56′ 29.02″ W) in the humid subtropical Edwards Plateau [22] (Table 1; all % humidity data were obtained from www.wunderground.com). As complete weather data were not available for San Marcos during the 2011 collection periods, data from New Braunfels, TX (approximately 29 km SW of San Marcos) were utilized. Each ecoregion included three collection sites separated by a distance of 8.0–16.0 km. Collections were designed to span spatially (at least three different collection sites per ecoregion per collection time) and temporally (several collections at each ecoregion site over a period of days or weeks) in order to reduce drift effects of blow fly populations in each area [9, 10] and increase genetic heterogeneity of experimental colonies.

Insect collections *C. macellaria* eggs, larvae, and adults were collected from baits that included road kill (RK), euthanized pigs, and/or an aged beef liver (ABL) bait between April and July at each collection site using the following methods (see Supplemental Table 1 for an overview of all baits used and species collected in each geographic region). Multiple baits were used at each site in most collecting periods, while in some instances only one bait was used. The use of several types of resources, as well as collecting over several geographic regions and time intervals, increased the likelihood of collecting adults and/or larvae at each site, allowed for 1,500 individuals per population and environment to be assessed in each trial of this study, and greatly increased the likelihood of observing variation within Texas samples. Adults were collected with an aerial net, while several groups of approximately 100 eggs and/or larvae were hand collected from one or many of the resources previously described (Supplemental Table 1). Adult flies, constituting generation zero (G_0), were held in 30.48×30.48×30.48 cm Lumite screen collapsible cages (BioQuip, Rancho Dominguez, CA) and returned to the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility, Texas A&M University, College Station, TX. Batches of eggs or larvae were placed on approximately 100 g of beef liver, which rested within a 946-ml Kerr mason jar (Hearthmark, LLC, Daleville, IN) that contained approximately 45 g of vermiculite. Jars containing field-collected eggs or larvae were covered with a breathable Wypall® paper cloth (Kimberly-Clark Global Sales, LLC, Roswell, GA), returned to the laboratory, and held at approximately 27 °C and a 14:10 L/D cycle until needed for an experiment. Larvae collected in the field were reared to adulthood and also constituted G_0.

Fly rearing and colony maintenance Adult flies were maintained in cages within a rearing room at approximately 27 °C and a 14:10 L/D cycle. Adult flies were provided an ad libitum

Author's personal copy

Table 1 Weather data for each ecoregion in TX, USA during sampling periods

Region	April–July 2011				March–May 2012			
	Min. temp. (°C)	Max. temp. (°C)	Mean % humidity	Total precip. (mm)	Min. temp. (°C)	Max. temp. (°C)	Mean % humidity	Total precip. (mm)
College Station	17.4–25.1	31.1–37.7	59.0–62.5	0.0–85.6	14.5–20.3	24.6–31.3	69.0–75.0	14.2–220.0
Longview	14.9–25.3	29.1–38.7	56.9–66.7	19.8–102.9	12.8–18.3	24.9–30.6	68.4–72.2	75.4–120.9
San Marcos	15.7–23.0	31.2–37.9	43.1–50.5	0.0–51.8	–	–	–	–

50:50 mixture of Pure Sugar (Great Value®, Wal-Mart Stores, Inc., Bentonville, AR) and Cultured Buttermilk Powder (SACO Foods, Madison, WI), as well as deionized water (dH_2O). Each cage of adult flies was also provided with an 88.7-ml white plastic bath cup (Great Value®, Wal-Mart Stores, Inc., Bentonville, AR) daily, which contained a single Kimwipe® (Kimberly-Clark Global Sales, Inc., Roswell, GA) soaked in bovine blood. When eggs were needed to maintain colonies, an 88.7-ml cup containing approximately 15 g of beef liver was placed inside each cage of flies to induce oviposition. The liver was partially covered with a Kimwipe dampened with dH_2O in order to provide cover for flies ovipositing on the substrate and to keep the substrate moist.

Preliminary density study An initial pilot study was implemented in August 2011 to determine the appropriate density at which to conduct the primary phenotypic variation study. Replicates for the pilot study contained 50, 100, or 150 larvae/50 g liver, with five replicates per density of each environment-population treatment. Results from the pilot study were used to determine that each replicate for the primary phenotypic variation study (October 2011 and May 2012) should contain 100 larvae/50 g liver, with 15 replicates (1,500 larvae per population) per environment-population treatment.

Rearing container design Replicates were held within a rearing container specifically designed to minimize contact with larvae (Fig. 1) [17]. The top of the apparatus consisted of a Dart 32DN05 907-g translucent plastic deli cup (The WEBstaurant Store Food Service Equipment and Supply Company, Lancaster County, PA) with the bottom removed and replaced with chicken wire, which allowed dispersing larvae to descend into the bottom container. A 266-ml red Solo® opaque plastic feeding cup containing liver and larvae was placed on the wire floor of the top container. The bottom of the apparatus consisted of an identical, but intact, deli cup coated with Fluon® (Insect-a-Slip; BioQuip, Rancho Dominguez, CA) and containing approximately 575 g of sand for pupation.

Experimental design Adults from the G_4 and G_5 generations were used for the October 2011 experiment, while G_2 generations were used for the May 2012 experiment. Experiments were conducted with generations $< G_{10}$ in order to minimize the likelihood of losing genetic variation over time [23]. Oviposition was induced using the method outlined above. Observations were made hourly until egg masses were noted, at which time masses were collected, placed on approximately 5 g of beef liver in a separate cup, labeled, covered with one Kimwipe held in place with a rubber band, and placed arbitrarily inside either cool (21 °C, SEM ± 0.02, 65 % RH) or warm (31 °C, SEM ± 0.04, 70 % RH) environmental incubators (136LLVL Percival®, Percival Scientific Inc., Perry, IA) at a 14:10 L/D cycle. The chosen environmental combinations reflect ecological interests in understanding performance in ecoregions that vary by temperature and humidity. This process continued hourly until approximately 3,000 eggs from

Fig. 1 Specialized rearing container designed to segregate feeding larvae from wandering larvae in order to nondestructively sample pupae [17]

Author's personal copy

each population were attained. Observations of eggs in the incubators were made hourly until approximately 50 % of the eggs hatched. A camel hair paintbrush moistened with dH_2O was used to transfer 100 of the newly emerged larvae into a Kimwipe-lined 266-ml red Solo® opaque plastic feeding cup containing 50 g of fresh beef liver. This feeding cup was placed within the specialized rearing container (Fig. 1), which was then randomly assigned to the either warm or cool environmental chamber. All three October 2011 populations (College Station, Longview, and San Marcos) were successful in producing the necessary amount of eggs (3,000 per population) required to begin the phenotypic variation experiment. However, only two populations from the May 2012 trial (College Station and Longview populations) were successful in this regard.

Experiments were conducted in incubators under previously described conditions with each of the three blow fly populations in the cool and warm environments for a total of six environment-population treatments. Both incubators contained a data logger fit with air, water, and soil temperature probes (Onset® HOBO U12-006 with Onset® TMC6-HD sensors, Onset Co., Pocasset, MA). Three levels within the incubator were attached with a probe to record temperature and humidity every 10 min.

Each replicate container was rotated to a randomized position (via random assignment generator) inside the incubator after each observation period. Observations were made every 12 h until 3rd instar larvae were noted, at which time observations switched to every 8 h. Sand was sifted using a #18 stainless steel 1.00 mm mesh screen sieve (VWR International, LLC, Radnor, PA) during each observation time once 3rd instar larvae in the wandering stage were observed. Pupae were placed individually into 30-ml Jetware® medicine cups (Jetware, Hatfield, PA) containing approximately 2 cm of sand, capped with a breathable lid, labeled, and returned to the appropriate incubator for 24 h. Pupae were individually weighed using an Adventure-Pro AV64 Ohaus® scale (Ohaus Corporation, Pine Brook, NJ). Preliminary experiments indicated high Spearman's correlation between mass and length (r=0.85; P<0.01) and mass and width (r=0.95; P<0.01) of pupae, so length and width measurements were omitted from the current study. Pupae were returned to their individual containers and placed in the appropriate incubator, with observations made every 8 h until adult emergence. Adults were euthanized by placement in a −20 °C deep freezer and then placed in an oven at 55 °C for 24 h in order to measure dry mass. The following data were obtained for each individual specimen: developmental time (h) from egg to pupa (referred to here as the *immature stage*, egg stage + larval stage), pupal mass (mg), development time (h) from pupa to adult (referred to here as the *pupal stage*), and adult mass (mg). Total development time (h; immature + pupal stage) was also calculated for each individual, as this collective information may be useful to investigators when making an estimation of the m-PMI.

Statistical analyses Data were analyzed using JMP 9 software (JMP 2009). Data for the preliminary density experiment were approximately normally distributed and were explored using a full-factorial analysis (ANOVA). The factors used in the density experiment were environment, population, and density, while the only phenotype (response variable) recorded was percent survival. All interactions up to an order 3 were used. The factors used in the primary phenotypic variation experiment were year, environment, and population, with interactions of up to 3 being used. Data for this experiment were not approximately normally distributed and could not be appropriately transformed. Thus, Friedman ANOVA tests and Wilcoxon paired comparisons were used to test for differences between populations, environments, and years (P<0.05). Mean values were obtained for all phenotypes.

Results

Fly populations exhibited significantly different development times (P≤0.05) and masses (P≤0.05) between the two environments (cool and warm). Genetic variation, environmental effects (plasticity), and genetic variation in plasticity (G × E interactions) were observed for development times and mass. For the purpose of this paper, only mean immature and pupal development times, as well as mean pupal mass, will be presented (ANOVA values Table 2; phenotypic values Table 3). ANOVA values for all phenotypes, including across years, are found in Supplemental Table 2, while minimum, maximum, mean, and median values for every minimum and mean phenotype measured are given in Supplemental Table 3.

Density Environment significantly impacted percent survival (P<0.01). Population, density, and all two- and three-way interactions between environment, population, and density were not significant predictors of survival. The density of 100 larvae/50 g liver typically had the greatest survival rates and was used for further experimentation.

Immature development (egg stage + larval stage) Environment and population were significant predictors (P<0.01) of immature development time in 2011 and 2012 (Table 2). Comparing across years, year, environment, and a three-way year-environment-population interaction effect were important predictors of immature developmental progress (P<0.01; Supplemental Table 2). In 2011, the Longview population exhibited shorter immature development times in both environments when compared to the two other populations (Fig. 2a, Table 3). In contrast, the 2012

Author's personal copy

Table 2 ANOVA values for all phenotypes

Phenotype	Model	Source	df	Mean square	*F* ratio	Prob. > *F*
2011 mean immature dev. time	Friedman ANOVA	Model	5	8,745.58	75.48	**<0.0001**
		Error	80	115.87		
		Total	85			
	Effect tests	Environment	1	39,190.95	338.23	**<0.0001**
		Population	2	1,884.49	16.26	**<0.0001**
		Env. × pop.	2	199.96	1.73	0.1846
2011 mean pupal dev. time	Friedman ANOVA	Model	5	8,854.10	81.17	**<0.0001**
		Error	80	109.09		
		Total	85			
	Effect tests	Environment	1	39,322.01	360.46	**<0.0001**
		Population	2	643.37	5.90	**0.0041**
		Env. × pop.	2	1,606.84	14.73	**<0.0001**
2011 mean pupal mass	Friedman ANOVA	Model	5	1,032.63	1.73	0.1379
		Error	80	597.93		
		Total	85			
	Effect tests	Environment	1	174.59	0.29	0.5905
		Population	2	125.18	0.21	0.8116
		Env. × pop.	2	2,359.66	3.95	**0.0232**
2012 mean immature dev. time	Friedman ANOVA	Model	3	4,737.98	70.17	**<0.0001**
		Error	56	67.52		
		Total	59			
	Effect tests	Environment	1	13,500.00	199.94	**<0.0001**
		Population	1	707.27	10.48	**<0.0001**
		Env. × pop.	1	6.67	0.10	0.7545
2012 mean pupal dev. time	Friedman ANOVA	Model	3	5,079.84	103.24	**<0.0001**
		Error	56	49.20		
		Total	59			
	Effect tests	Environment	1	13,500.00	274.36	**<0.0001**
		Population	1	1,344.27	27.32	**<0.0001**
		Env. × pop.	1	395.27	8.03	**0.0064**
2012 mean pupal mass	Friedman ANOVA	Model	3	2,432.56	12.73	**<0.0001**
		Error	56	191.02		
		Total	59			
	Effect tests	Environment	1	4,437.60	23.23	**<0.0001**
		Population	1	2,693.40	14.10	**0.0004**
		Env. × pop.	1	166.67	0.87	0.3543

All boldfaced values indicate significance at $P \leq 0.05$

College Station population consistently exhibited shorter development times than Longview in both environments. Overall, 2011 populations generally had longer developmental times compared to those found in 2012.

Pupal development Significant predictors of pupal development time for both years included environment, population, and two-way environment-population interactions (all $P<0.01$; Table 2). Significant predictors across years were year ($P=0.012$), environment ($P<0.01$), two-way interactions between environment and population ($P=0.021$) and both environment and population with year (both $P<0.01$), and a three-way year-environment-population interaction ($P<0.01$). In 2011, pupal duration showed the following order in the cool environment (from shortest to longest): College Station, San Marcos, Longview (Fig. 2b, Table 3). In the warm environment, however, the Longview and San Marcos populations exhibited similar durations, while the College Station population took longer to develop. The 2012 College Station population exhibited longer pupal durations than the Longview population in the cool

Author's personal copy

Table 3 Mean values for phenotypes by year, environment, and population

Year	Phenotype	Environment	Population	N	Mean	±SD
2011	Mean imm. dev. time	Cool	College Station	15	286.78	9.87
2011	Mean imm. dev. time	Cool	Longview	15	269.74	22.76
2011	Mean imm. dev. time	Cool	San Marcos	15	286.44	32.29
2011	Mean imm. dev. time	Warm	College Station	13	145.60	9.41
2011	Mean imm. dev. time	Warm	Longview	14	127.87	6.37
2011	Mean imm. dev. time	Warm	San Marcos	14	138.16	7.29
2012	Mean imm. dev. time	Cool	College Station	15	222.50	18.14
2012	Mean imm. dev. time	Cool	Longview	15	231.80	13.66
2012	Mean imm. dev. time	Warm	College Station	15	120.10	13.18
2012	Mean imm. dev. time	Warm	Longview	15	136.00	13.35
2011	Mean pupal dev. time	Cool	College Station	15	178.99	12.94
2011	Mean pupal dev. time	Cool	Longview	15	203.02	12.34
2011	Mean pupal dev. time	Cool	San Marcos	15	189.18	9.64
2011	Mean pupal dev. time	Warm	College Station	13	87.64	3.40
2011	Mean pupal dev. time	Warm	Longview	14	85.94	1.82
2011	Mean pupal dev. time	Warm	San Marcos	14	84.86	3.82
2012	Mean pupal dev. time	Cool	College Station	15	169.70	13.59
2012	Mean pupal dev. time	Cool	Longview	15	140.70	6.44
2012	Mean pupal dev. time	Warm	College Station	15	89.80	2.39
2012	Mean pupal dev. time	Warm	Longview	14	88.90	1.76
2011	Mean pupal mass	Cool	College Station	15	30.75	6.52
2011	Mean pupal mass	Cool	Longview	15	34.62	4.96
2011	Mean pupal mass	Cool	San Marcos	15	31.58	4.92
2011	Mean pupal mass	Warm	College Station	13	34.57	7.24
2011	Mean pupal mass	Warm	Longview	14	31.23	4.39
2011	Mean pupal mass	Warm	San Marcos	14	34.71	6.11
2012	Mean pupal mass	Cool	College Station	15	37.40	4.35
2012	Mean pupal mass	Cool	Longview	15	33.90	6.80
2012	Mean pupal mass	Warm	College Station	15	33.30	4.20
2012	Mean pupal mass	Warm	Longview	15	27.50	4.13

environment, although no discernible differences were identified in the warm environment (Fig. 2b, Table 3). When comparing across years, both 2011 Longview and College Station populations had longer pupal duration times than in 2012 in the cool environment and shorter times in the warm environment.

Pupal mass Two-way environment-population interactions were determined to only be significant for 2011 (P=0.023; Table 2). Environment and population were significant predictors of mean pupal mass only in 2012 (both P<0.01). Comparing 2011 and 2012 shows that population (P=0.024), environment (P<0.01), a two-way interaction between them (P=0.024), and a two-way interaction between each of these and year (both P<0.01) were significant predictors for mean pupal mass (Supplemental Table 2).

No difference for pupal mass in the cool environment was determined between populations for either year. Although the 2011 Longview population exhibited the largest pupae in the cool environment, it also possessed the smallest pupae in the warm environment (Fig. 2c, Table 3). However, such interactions were not observed in 2012, where the Longview population displayed smaller pupae than the College Station population in both environments. There was no remarkable change in pupal size within populations across years.

Discussion

Phenotypic ranges observed for *C. macellaria* overlapped and fell within those given in previous studies from College Station, TX, USA [24], and Gainesville, FL, USA [25]. However, it is clear that a range of ontogenetic variation is possible at different life stages. Development time of the early immature stage is highly variable between datasets, which may indicate that rearing substrates used in these three studies

Author's personal copy

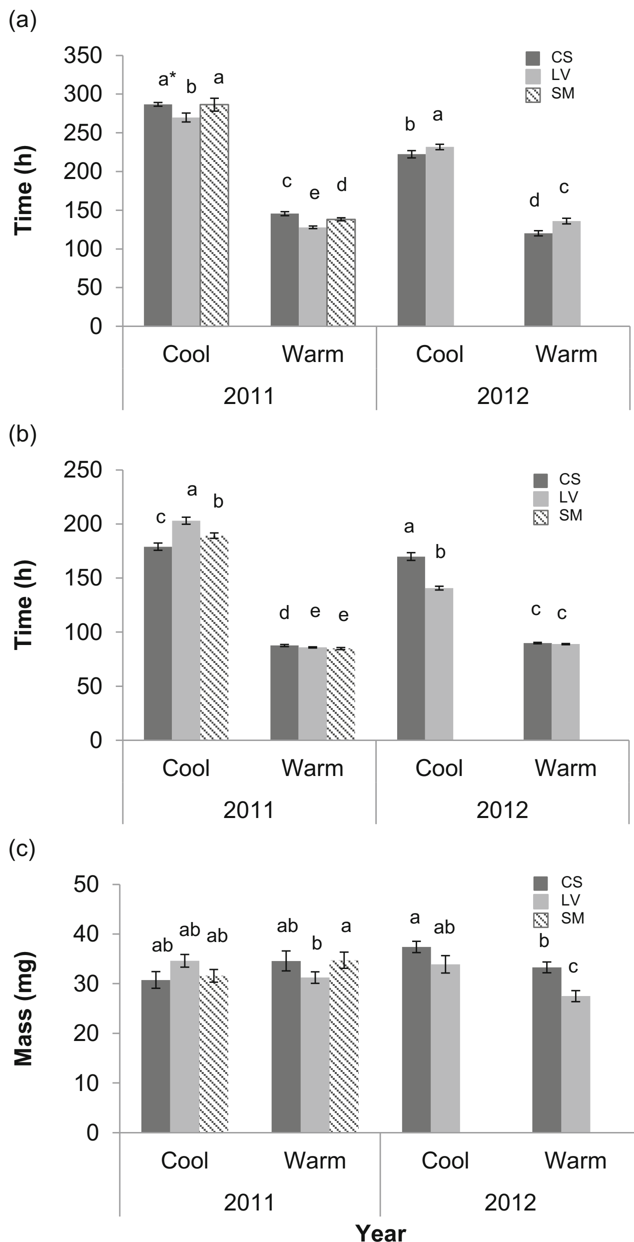

Fig. 2 Paired comparison plots for *C. macellaria* mean immature development time on beef liver (**a**), mean pupal development time (**b**), and mean pupal mass (**c**), ±SEM, at 21 °C, 65 % RH (*cool*) and 31 °C, 70 % RH (*warm*) for 2011 and 2012. $N=15$ for all 2011 populations at 21 °C, 65 % RH and all 2012 populations in both environments; $N=13$ for 2011 College Station (*CS*) population, $N=14$ for 2011 Longview (*LV*) and San Marcos (*SM*) populations at 31 °C, 70 % RH. *Columns with different letters* indicate significant difference ($P\leq0.05$)

(porcine, equine, and bovine tissues) have a drastic impact on larval development. Tissue type has been shown to significantly affect larval mass and length of *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae) [26], larval development time of *Calliphora vomitoria* Linnaeus (Diptera: Calliphoridae) [27], and larval development time, larval size, and adult size of *Lucilia sericata* Meigen (Diptera: Calliphoridae) [28]. Although Boatright and Tomberlin (2010) did not observe a tissue effect on College Station *C. macellaria* development [24], tissue type may be an important growth factor for the Longview and San Marcos *C. macellaria* populations observed in the present study.

Phenotypic variation observed here for *C. macellaria* contrasts with published data for *L. sericata* as both Gallagher et al. (2010) and Tarone et al. (2011) demonstrated that *L. sericata* larvae reverse rank orders between warm and cool environments. *C. macellaria* larvae responded similarly, and *L. sericata* differently, across environments. These differences indicate that populations used in the *L. sericata* studies may have been locally adapted to specific ecoregions for the larval stage, unlike *C. macellaria*. Taken together, these data suggest that population effects in blow flies are likely to be highly species specific, although further studies of these and other blow fly species should be performed in order to determine if phenotypic responses to specific environments affect fitness.

Local adaptations between *C. macellaria* populations may have occurred in the pupal stage with regard to development time and size (Fig. 2b, c), although fitness was not measured in this study. 2011 College Station and San Marcos populations grew faster in the pupal stage and were smaller than Longview in the cool environment, whereas the 2012 College Station population grew slower and larger than Longview in the same environment. Larger pupae did correspond with larger adults (Supplemental Table 3). Such increased body size could be beneficial in drought situations (such as the exceptional drought experienced in Texas in 2011) as larger bodies may correlate to female fecundity [29, 30] and have been shown to correlate with increased surface area of wings, which could potentially aid in dispersal of the fly from a suboptimal resource [31].

Although care was taken in designing this study, the larval density chosen for these experiments may have affected developmental patterns as the above conditions most likely do not reflect the optimal density for each population under natural circumstances [32]. It has been hypothesized that density could be a contributing factor influencing phenotypic variation in carrion-breeding flies [33, 34], and several laboratory studies support this notion [35–37]. However, density does not seem to account for the trend seen in the immature stage of larvae here, as the fastest developers tended to exhibit larger pupae, which contradicts previous studies [36, 37].

This study highlights the importance of seasonal and yearly comparisons as population dynamics are clearly subject to change and are well established in other studies of flies [38–40]. Particularly, the yearly differences observed here could be explained by the severe Texas drought in 2011, which may have constituted a large selection pressure on Texas populations by altering the quality or quantity of resources available for colonization (as similar conditions have been recorded for the congener *Cochliomyia hominivorax*

Author's personal copy

Coquerel (Diptera: Calliphoridae) [41]). In addition, more variation was observed in one environmental condition (cool environment) in this study (Fig. 2), which has also been documented for other forensically relevant species, such as *L. sericata* [17] and *Chrysomya megacephala* Fabricius (Diptera: Calliphoridae) [16]. The emerging picture illustrated by this study and others [17, 18, 33] suggests that genetic variation appears to contribute to error more in some environments than in others. Given this, larval age estimations based on *C. macellaria* development in environments similar to the warmer treatment should work well if case specimens experienced similar conditions during life. However, predictive accuracy may decline as a result of error by genetic variation if case specimens are collected in cooler/drier seasons or climates. This does raise the possibility that entomological evidence may be subject to a large degree of genetically derived error when the evidentiary samples are exposed to atypical ecological conditions.

When making an age estimate of insects in a forensic investigation, the forensic entomologist ultimately makes the assumption that development of the insects in question does not diverge from published data being used for that species. Regardless of drift, selection, or sampling in this study, we showed that genetic differences among strains lead them to diverge from published data and that this alone should encourage the forensic entomologist to take a conservative approach when estimating the ages of case specimens. It will be necessary, however, to determine a link between fitness in the focal populations and the phenotypes tested in this research before local adaptation can be invoked (in addition to sampling many populations from each ecoregion). The statistical analyses in this study indicate that genotype and its interaction with the environment are important in explaining only the observed differences among the naturally derived populations in the two specific environments tested. These results raise the need to determine if there is a link between these traits and fitness in *C. macellaria*, though it is also worth pointing out that these phenotypes frequently are linked to fly fitness [42–44].

Conclusion

We demonstrated that *C. macellaria* strains derived from populations in distinct geographic locations within a single state significantly differed in development time and size. Such differences indicate the application of blow fly development data from one region to another could result in error when estimating the m-PMI. In other words, conspecific development data cannot be universally applied. A quantitative demonstration by Gallagher et al. (2010) showed that the use of nonlocal data for *L. sericata* at 16 °C could generate up to −13.80 % error in development-based estimations of the m-PMI. Clearly, similar research in this specific field is required to better understand variation in wild blow flies and how such population differences can impact real-world death investigations. Therefore, the statistically based conclusions offered here should be considered tentative until such work is performed [45]. Possible avenues of future research include examining the impact of fluctuating environments, longevity and fecundity of adults, varying larval densities, rearing substrates, and moisture levels of pupal substrate, seasonal and yearly replicates, and minimum geographic distance required between populations in order to observe distinct phenotypic responses.

The occurrence of developmental variation in carrion insect species is a pressing issue that cannot be ignored under that *Daubert* standard [2, 46], which sets the criteria for admission of scientific evidence in a court of law (*Daubert vs. Merrell Dow Pharmaceuticals*, 509 U.S. 579 (1993)). In order to comply with the last requirement of *Daubert* (known error rate), the forensic entomologist must be able to estimate the sources of developmental variation exhibited by a species. Taking a quantitative genetic approach to evaluating developmental variation enables researchers to evaluate the comparative roles of environment and genetics on error. Such studies are valuable to practitioners of forensic entomology both in providing context to m-PMI estimates (addressing *Daubert*) and in guiding future areas of research (such as the identification of genotypes expected to develop in different ways). In approaching research in this manner, the forensic entomology community can address cultural, legal, and scientific expectations of the field.

Acknowledgments The authors would like to extend their gratitude to the Texas A&M University Department of Entomology for providing the funds to make this project possible. C.G.O. thanks all of the members of the F.L.I.E.S. Facility at Texas A&M University for their assistance, support, and patience during this research.

References

1. Payne JA (1965) A summer carrion study of the baby pig *Sus scrofa* Linnaeus. Ecology 46(5):592–602
2. Tomberlin JK, Mohr R, Benbow ME, Tarone AM, VanLaerhoven S (2011) A roadmap for bridging basic and applied research in forensic entomology. Ann Rev Entomol 56(1):401–421
3. Amendt J, Amendt C, Campobasso E, Gaudry C, Reiter H, LeBlanc M (2007) Best practice in forensic entomology: standards and guidelines. Int J Legal Med 121(2):90–104
4. Amendt J, Krettek R, Zehner R (2004) Forensic entomology. Naturwissenschaften 91(2):51–65
5. Langerhans RB, Chapman LJ, Dewitt TJ (2007) Complex phenotype-environment associations revealed in an East African cyprinid. J Evol Biol 20(3):1171–1181
6. Relyea RA (2002) Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. Ecol Monogr 72(4):523–540
7. Wilbur HM, Collins JP (1973) Ecological aspects of amphibian metamorphosis. Science 182(4119):1305–1314

Author's personal copy

8. Conner JK, Hartl DL (2004) A primer of ecological genetics. Sinauer Associates, Sunderland, Mass
9. Picard CJ, Wells JD (2009) Survey of the genetic diversity of *Phormia regina* (Diptera: Calliphoridae) using amplified fragment length polymorphisms. J Med Entomol 46(3):664–670
10. Picard CJ, Wells JD (2010) The population genetic structure of North American *Lucilia sericata* (Diptera: Calliphoridae), and the utility of genetic assignment methods for reconstruction of postmortem corpse relocation. Forensic Sci Int 195(1–3):63–67
11. Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, van Kleunen M (2010) Plant phenotypic plasticity in a changing climate. Trends Plant Sci 15(12):684–692
12. Funk JL (2008) Differences in plasticity between invasive and native plants from a low resource environment. J Ecol 96(6):1162–1173
13. Gilbert SF, Epel D (2009) Ecological developmental biology: integrating epigenetics, medicine, and evolution. Sinauer Associates, Sunderland, Mass
14. Piersma T, van Gils JA (2011) The flexible phenotype: a body-centered integration of ecology, physiology and behaviour. Oxford biology. Oxford University Press, Oxford, New York
15. DeWitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. Trends Ecol Evol 13(2):77–81
16. Hu Y, Yuan X, Zhu F, Lei C (2010) Development time and size-related traits in the oriental blowfly, *Chrysomya megacephala* along a latitudinal gradient from China. J Therm Biol 35(7):366–371
17. Gallagher MB, Sandhu S, Kimsey R (2010) Variation in developmental time for geographically distinct populations of the common green bottle fly, *Lucilia sericata* (Meigen). J Forensic Sci 55(2):438–442
18. Tarone AM, Picard CJ, Spiegelman C, Foran DR (2011) Population and temperature effects on *Lucilia sericata* (Diptera: Calliphoridae) body size and minimum development time. J Med Entomol 48(5):1062–1068
19. Gould FW, Hoffman GO, Rechenthin CA (1960) Vegetational areas of Texas. vol 492. Texas Agricultural Experiment Station
20. National Oceanic & Atmospheric Administration NES, Data and Information Service (2011-2012) Annual climatological summary: College Station Easterwood Field, TX US. National Climatic Data Center, Federal Building, Asheville, NC
21. National Oceanic & Atmospheric Administration NES, Data and Information Service (2011-2012) Annual climatological summary: Longview East Texas Regional Airport, TX US. National Climatic Data Center, Federal Building, Asheville, NC
22. National Oceanic & Atmospheric Administration NES, Data and Information Service (2011) Annual climatological summary: New Braunfels Municipal Airport, TX US. National Climatic Data Center, Federal Building, Asheville, NC
23. Briscoe DA, Malpica JM, Robertson A, Smith GJ, Frankham R, Banks RG, Barker JSF (1992) Rapid loss of genetic variation in large captive populations of *Drosophila* flies: implications for the genetic management of captive populations. Conserv Biol 6(3):416–425
24. Boatright SA, Tomberlin JK (2010) Effects of temperature and tissue type on the development of *Cochliomyia macellaria* (Diptera: Calliphoridae). J Med Entomol 47(5):917–923
25. Byrd JH, Butler JF (1996) Effects of temperature on *Cochliomyia macellaria* (Diptera: Calliphoridae) development. J Med Entomol 33(6):901–905
26. Kaneshrajah G, Turner B (2004) *Calliphora vicina* larvae grow at different rates on different body tissues. Int J Legal Med 118(4):242–244
27. Ireland S, Turner BD (2006) The effects of larval crowding and food type on the size and development of the blowfly, *Calliphora vomitoria*. Forensic Sci Int 159(2–3):175–181
28. Clark K, Evans L, Wall R (2006) Growth rates of the blowfly, *Lucilia sericata*, on different body tissues. Forensic Sci Int 156(2–3):145–149
29. Tammaru T, Kaitaniemi P, Ruohomäki K (1996) Realized fecundity in *Epirrita autumnata* (Lepidoptera: Geometridae): relation to body size and consequences to population dynamics. OIKOS 77(3):407–416
30. Briegel H (1990) Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti*. J Insect Physiol 36(3):165–172
31. Reigada C, Godoy WAC (2006) Larval density, temperature and biological aspects of *Chrysomya megacephala* (Diptera: Calliphoridae). Arq Bras Med Vet Zoo 58(4):562–566
32. Rivers DB, Thompson C, Brogan R (2011) Physiological trade-offs of forming maggot masses by necrophagous flies on vertebrate carrion. B Entomol Res 101(5):599–611
33. Tarone AM, Foran DR (2006) Components of developmental plasticity in a Michigan population of *Lucilia sericata* (Diptera: Calliphoridae). J Med Entomol 43(5):1023–1033
34. Goodbrod JR, Goff ML (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(3):338–343
35. So P, Dudgeon D (1989) Variations in the life-history parameters of *Hemipyrellia ligurriens* (Diptera: Calliphoridae) in response to larval competition for food. Ecol Entomol 14(1):109–116
36. Shahein A (1986) Effect of intraspecific competition between larvae of *Calliphora erythrocephala* (MEIG.) (Calliphoridae, Diptera). Beitr Entomol (Berlin) 36(2):233–235
37. Martínez-Sánchez A, Smith KE, Rojo S, Marcos-García MA, Wall R (2007) Geographic origin affects larval competitive ability in European populations of the blow fly, *Lucilia sericata*. Entomol Exp Appl 122(2):93–98
38. Dobzhansky T, Pavan C (1950) Local and seasonal variations in relative frequencies of species of *Drosophila* in Brazil. J Anim Ecol 19(1):1–14
39. Dobzhansky T (1948) Genetics of natural populations XVI. Altitudinal and seasonal changes produced by natural selection in certain populations of *Drosophila pseudoobscura* and *Drosophila persimilis*. Genetics 33(2):158–176
40. Dobzhansky T (1943) Genetics of natural populations IX. Temporal changes in the composition of populations of *Drosophila pseudoobscura*. Genetics 28(2):162–186
41. Krafsur ES, Hightower BG (1979) Field tests of sterile screwworm flies, *Cochliomyia hominivorax* (Diptera: Calliphoridae), against natural populations in three coastal areas of Mexico. J Med Entomol 16(1):33–42
42. Reeve MW, Fowler K, Partridge L (2000) Increased body size confers greater fitness at lower experimental temperature in male *Drosophila melanogaster*. J Evol Biol 13(5):836–844
43. Santos M, Ruiz A, Quezada-Díaz JE, Barbadilla A, Fontdevila A (1992) The evolutionary history of *Drosophila buzzatii* XX. Positive phenotypic covariance between field adult fitness components and body size. J Evol Biol 5(3):403–422
44. Blanckenhorn WU (1998) Adaptive phenotypic plasticity in growth, development, and body size in the yellow dung fly. Evolution 52(5):1394–1407
45. Berk R, Brown L, Zhao L (2010) Statistical inference after model selection. J Quant Criminol 26(2):217–236
46. Tomberlin JK, Benbow ME, Tarone AM, Mohr RM (2011) Basic research in evolution and ecology enhances forensics. Trends Ecol Evol 26(2):53–55