DEVELOPMENTAL PLASTICITY OF *COCHLIOMYIA MACELLARIA* FABRICIUS (DIPTERA: CALLIPHORIDAE) FROM THREE DISTINCT ECOREGIONS IN TEXAS

A Thesis

by

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ABSTRACT

Forensic entomology is a well-established science linking arthropod biology and ecology to legal investigations. Specifically, immature development on a decomposing corpse may give insight into the minimum time elapsed since death. Until recently, biological variation within a single species has been overlooked when estimating colonization events. Variation in the form of phenotypic plasticity, or the ability of a single genotype to produce multiple phenotypes under alternative stresses, has been documented in genetic and ecological literature and spans across all phyla. Taking this into account, different subpopulations of forensically pertinent insect species should also possess the ability to adapt to changing environments as geographic distribution increases. Thus, plastic responses of a species to alternative stresses may be measured in biological parameters, such as development time.

In this research, three geographically distinct strains of the blow fly *Cochliomyia macellaria* Fabricius (Diptera Calliphoridae) were reared in two distinct environments in order to measure development time, as well as pupal and adult masses. Strains exhibited genetic variance when compared to each other, and each strain exhibited variable responses across environments (phenotypic plasticity). Plasticity in the form of genotype by environment (GxE) interactions was also exhibited by *C. macellaria*, although consistent adherence to any single rule explaining ontogenetic trends was not apparent. This research supports the existence of intraspecific variation in a common blow fly of forensic importance. Results of this study will impact the forensic entomology

community by encouraging the generation of either strain-specific developmental datasets or statistical models to minimize variation caused by genetic, environment, or GxE effects in order to compare developmental data across strains.

DEDICATION

This thesis is dedicated to Mims and Granny, two of the strongest women I have ever known. I love you both.

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TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

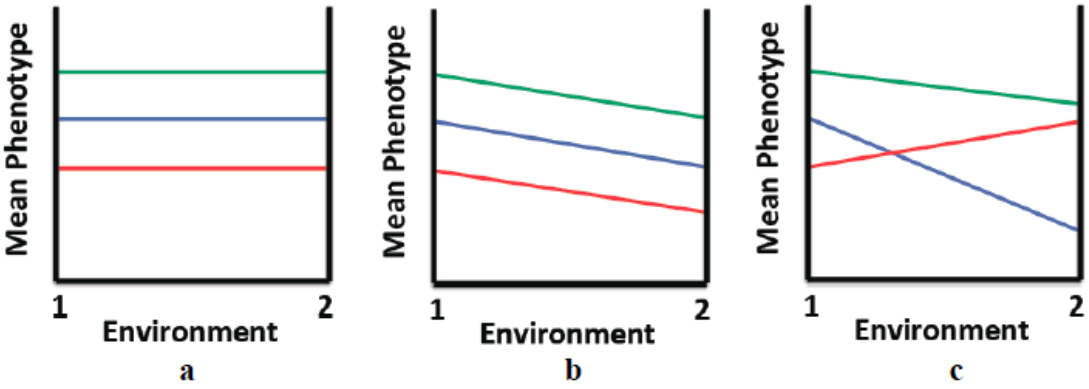
A population is defined as an integrated group of interbreeding individuals which, for the most part, do not come into contact with members of conspecific groups (Nicholson 1957). A population consisting of a single species may divide itself into distinct subpopulations as it expands across ecological and geographical boundaries. Barriers formed by both biotic and abiotic conditions must be overcome in order to maintain or increase fitness in a changing environment. As a species expands its geographical distribution due to factors such as population growth, competition, lack of resources, weather, and human transport, varying environmental conditions are likely to be encountered. Without adaptation to these environmental shifts, population expansion most likely will not take place in varied environments. Quantitative traits of populations inhabiting spatially or temporally distinct habitats may change over time in order to adjust to these fluctuating conditions (Nicholson 1957, Conner and Hartl 2004, Martínez-Sánchez et al. 2007). These traits can exhibit a wide spectrum of expression and often do not fall within distinct categories, thus requiring some form of measurement (Conner and Hartl 2004). These traits can be affected by phenotypic variation arising from genetics, the environment (plasticity), and corresponding interactions (i.e., genotype by environment, GxE).

Genotypes are specific alleles whose frequencies may change over time in response to environmental pressures and are phenotypically expressed (Conner and Hartl

2004). Genetic variation within populations can be driven by several factors, including natural selection, migration, genetic drift, and mutation. In this way, genes alone may be responsible for the present biology and behavior of populations inhabiting specific regions. More flexible biologies, however, may be advantageous to the proliferation of a species as shifting environments may require equally shifting phenotypes. For example, the wood frog tadpole *Rana sylvatica* LeConte has evolved the ability to manipulate its body size when in the presence of a predator (Relyea 2001). The presence of dragonfly (Odonata: Anisoptera) larvae in the environment induces a response in the developing tadpole to grow a shorter body and longer, more muscular tail for fleeing from and/or distracting the predator. Without this flexibility in phenotypic expression, *R. sylvatica* tadpoles may not be able to survive in certain environments. This phenomenon is called phenotypic plasticity, or the ability of a single genotype to produce different phenotypes in order to better adapt to a changing environment (DeWitt et al. 1998). Plastic phenotypes could be responsible for both variations among populations and their evolutionary responses to unstable environmental conditions (DeWitt et al. 1998, Terblanche 2006). This concept is illustrated by the Antarctic extremophilic green algae, *Chlamydomonas raudensis* ETL. UWO 241. Laboratory cultures were shown to respond differently at 8ºC and 1000 mM salinity than at 15ºC and 700 mM salinity(Pocock et al. 2011). Plastic phenotypes could be responsible for the success of the organism in both an optimal environment (approximately 8ºC and 1000 mM) and environments experiencing warming temperatures with decreased salinity.

Phenotypic variation can be expressed as the equation P = G + E + GxE, where P stands for phenotypic variation, G represents genetic variation, E is the variation due to a changing environment, and GxE represents the interaction between the genetic makeup of an organism and the environment in which it is exposed (Tomberlin et al. 2010). Phenotypic variation can be illustrated with the use of a reaction norm, which plots the phenotypic responses of different genotypes (y-axis) across two or more environments (x-axis) (Fig. 1a-c). The lines of the reaction norm therefore do not imply linearity, but simply indicate the difference between phenotypic responses of a single genotype. The spread between the phenotypic responses on the y-axes for a given environment represents genetic variation. For example, Fig. 1a illustrates genetic variation as there is a spread between all responses. However, because these lines are horizontal (responses are unchanging across environments), there is no plasticity present. If the genotypes do exhibit a different phenotypic response in each environment, plasticity is present (Fig. 1b). Slopes of the lines are the same in this case, indicating that all genotypes are responding similarly across environments. Finally, when genotypes exhibit different responses across environments as well as unequal slopes, there is an indication of GxE interactions (Fig. 1c).

Figure 1. Reaction norm plots for phenotypic variation, including genetic variance (a), plasticity due to environmental changes (b), and genotype by environment (GxE) interactions (c).



Natural selection aids in diversifying phenotypes by selecting for within-species, and even within-population, plasticity and local adaptation between distinct populations. The ability of conspecific populations to adapt to specific environments may be the product of interactions between specific genotypes and the environment. GxE interactions can represent genetic variation for phenotypic plasticity and allow genotypes to maintain fitness in continually changing environments (Sultan and Spencer 2002). For example, the collembolan *Orchesella cincta* Linnaeus (Entognatha: Collembola: Entomobryidae) displays temperature-induced GxE interactions on immature development and egg size when exposed to different temperatures in the laboratory, showing the existence of genetic variation within plasticity (Ellers and Driessen 2011). As an organism or population experiences environmental stresses, such as temperature, phenotypes that allow for adaptation to such stresses should increase total population fitness (Zhivotovsky 1996). Species exhibiting wide phenotypic variation are likely to encounter heterogeneous environmental pressures from distinct geographical regions

(Conner and Hartl 2004). These pressures shape individual phenotypes within populations and can lead to behavioral and phenotypic divergence within the same genotype.

Several evolutionary responses to environmental heterogeneity have been proposed, such as generalist phenotypes, phenotypic plasticity, or local adaptation and minimal migration (Tobler et al. 2008). Gene flow is restricted when populations exhibit local adaptation, thereby creating and maintaining heterogeneity in allele frequency. If several genotypes of conspecific populations show no variation in response to distinct environments, no measureable plasticity, or GxE, is present. However, if each genotype responds differently across distinct environments, a GxE interaction has occurred. These interactions play a prominent role in understanding how and why quantitative traits vary between populations. In order to test for GxE and plastic responses of conspecific populations, it is necessary to eliminate all but one variable to reduce the interactions of confounding factors. By keeping all but one variable constant, (i.e., temperature), differences in response, and thereby phenotypic plasticity and GxE interactions, among distinct populations can be quantified without concerns of interactions between independent variables. This may explain a majority of the variation exhibited by the model organism in question. Because replicating all climatic conditions in a laboratory is nearly impossible, Nicholson (1957) sought to measure a population's response to changing environmental conditions by exposing them to a direct consequence of climatic change: food supply. This was accomplished by setting up 10 cultures of *Lucilia cuprina* Wiedemann (Diptera: Calliphoridae) eight of which were exposed to varying amounts of

ground beef every day (0.05 g to 0.50 g). He determined that the number of individuals in the populations synchronized to the external factors (e.g., amount of food), demonstrating populations can adjust to changes in environmental conditions as well as cyclical changes.

Alternative phenotypes that correlate to reproductive success are needed in order to induce evolutionary change among populations in variable environments. Individuals exhibiting traits appropriate for local environments have a greater degree of likely success than those without the traits, resulting in increased fitness for that phenotype (Svanback et al. 2008). For example, the salt marsh planthopper *Prokelisia marginata* van Duzee (Hemiptera: Delphacidae) disperses in search of high nitrogen food sources as its native plant patches deteriorate over the course of a season (Denno 1994, Denno et al. 2000). These individuals exhibit well-developed flight muscles and large hind wings for dispersal. However, the sympatric congener *P. dolus* Wilson overcomes nitrogen limitation via compensatory feeding on nitrogen-poor plants (Huberty and Denno 2006). Individuals in this species are immobile with reduced or absent hind wings and an enlarged cibarial pump to facilitate increased feeding. The traits exhibited by these two species act to increase fitness for individuals within each population.

Quantitative physiological traits are often examined by researchers interested in understanding the response of an organism to its surroundings. Abiotic factors, such as temperature, can elicit specific developmental responses that may alter the duration of growth periods and corresponding body size. Being that insects are poikilothermic animals whose physiological processes are governed by ambient temperature (Sharper

and DeMichele 1977, Chapman 1998), they are excellent models for examining organismal responses to their environment. Their development from egg to adult, and each stage in between, is dependent upon specific temperature regimes. Development occurs within temperature ranges bookended by upper and lower thresholds and ceases when the lower threshold is not met, or the upper threshold is exceeded (Chapman 1998). Voss et al. (2009) observed through laboratory experiments that the parasitoid *Nasonia vitripennis* Ashmead (Hymenoptera: Pteromalidae) grew optimally at 30.56 ± 0.13ºC with thresholds at 11.05 ± 1.09 ºC and 36.57 ± 0.79ºC on the host *Lucilia sericata* Meigen (Diptera: Calliphoridae), and optimally at 31.81 ± 0.52ºC with thresholds at 9.60 ± 1.23ºC and 38.37 ± 1.17ºC on the host *Chrysomya rufifacies* Macquart (Diptera: Calliphoridae). Exposing *N. vitripennis* to temperatures beyond these thresholds decreased survival rate (Voss et al. 2009). As a population becomes more widely distributed throughout varying geographical regions, shifting temperature ranges may be encountered. Traits that allow individuals to successfully develop within these ranges will be selected for. When a specific phenotype for development rate is selected for in a specific region, it is expected that a genetically distinct and localized population will emerge (Sultan and Spencer 2002).

Varying environments may increase natural selection on biological traits of an organism due to interspecific interactions. Larvae of different blow fly species can coexist via resource partitioning based on size of the carcass, season, decomposition stage, and habitat (Denno 1975). For example, population distribution of *Ch. rufifacies* can overlap with *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) in North

America (Wells and Greenberg 1992a), while *Chrysomya albiceps* Wiedemann can co-occur with *C. macellaria* in Brazil (Faria et al. 2007). However, since both *Chrysomya* species are facultative predators in the larval stage, co-occurring species (e.g., *C. macellaria*) are predated upon and experience a decrease in abundance or are eventually removed (Wells and Greenberg 1992a, b, Wells and Greenberg 1992c, Faria et al. 1999). *Ch. rufifacies* significantly reduces the abundance of *C. macellaria* at various densities in the laboratory, and if left unhindered, the former species would drive the latter to extinction (Wells and Greenberg 1992c). This influence can select for faster development in the prey species in order to avoid being consumed. Laboratory studies have demonstrated that *L. sericata* can be outcompeted by *Calliphora vicina* Robineau-Desvoidy in the wild, as *C. vicina* showed greater survival rates when present in a mixed culture with *L. sericata* (Smith and Wall 1997). However *C. vicina* was not competitive enough to exclude *L. sericata* from the resource.

Intraspecific competition can also serve as a mechanism governing selection for phenotypic plasticity. Nicholson (1957) observed population size oscillations in *L. cuprina* over several generations due to intraspecific competition. The number of larvae present on a resource positively correlated with the number of adults present. And, as a consequence increased larval densities increased competition on the food source resulting in fewer and smaller adults. However, because there were fewer adults in the next generation, a lower number of eggs was deposited on the newly available resource. This, in turn, led to a greater number of surviving larvae and resulted in larger adults. Another study demonstrates that larval numbers of *L. sericata* increase on a resource (up

to 750 maggots/15 g liver), as well as when larval numbers of *C. vicina* increase on a similar resource (up to 500 maggots/15 g liver), subsequent adult size negatively correlates (Smith and Wall 1997).

Ranges in body size between populations may also be the result of phenotypic plasticity and environmental effects (Wilbur and Collins 1973). Resource limitation may align with biological trade-offs between body size and reproductive fitness within populations. Developing at a more rapid rate at the cost of reduced body size and fitness may be selected for in systems containing fleeting, or ephemeral, resources. *C. vicina* individuals reared at 16ºC exhibit larger body sizes when compared with individuals reared at 20-28ºC (Hwang 2009). Sexual variances in development times were present, as females were always larger than males except at 28ºC and were also found to develop significantly faster than males at 16ºC and 20ºC. In this study, larger size was correlated with higher fitness in *C. vicina* (Hwang 2009).

Ephemeral Resources

Carrion represents a quality resource whose occurrence is highly variable and fleeting (Elton and Miller 1954, Finn 2001, Yang 2006). Low occurrence and duration of carrion in most climates can be attributed to accelerated decomposition rates primarily determined by vertebrate and invertebrate scavengers (Archer 2003). Ephemeral resources are unique in that they are non-replenishing, rapidly exploited, and eventually exhausted by consumers (Elton and Miller 1954). Food supplies of this nature are

independent of the consumer's activity, making it virtually impossible to predict where and when the resource may occur as they exhibit patchy distributions spatially and temporally (Nicholson 1957). Therefore, animals must be highly adapted and efficient in locating ephemeral resources in order to optimize foraging. Blow flies represent the major insect family colonizing carrion as they are typically the first to locate and utilize such resources (Greenberg 1991, Horenstein et al. 2010, Anderson 2011). For example, several genera, including *Calliphora, Lucilia, Eucalliphora,* and *Cynomya*, have been documented arriving at exposed, freshly killed pigs hours after death (Anderson 2011). Early arrival and rapid development time give rise to the fact that blow flies are almost always the most abundant insect present on carrion (Payne 1965, Horenstein et al. 2010). For example, *Ch. albiceps*, accounted for 70% of the 24,710 dipterans collected on pig carcasses during a year-long study of carrion decomposition in Argentina (Horenstein et al. 2010). The high nutritive quality of carrion makes it a valuable protein source for egg development in adult blow flies and provides a suitable rearing substrate for resulting progeny (Beaver 1977, Belzer 1978, Huntington 2010). These qualities are exhibited by carrion through the duration of the decomposition process. Both fresh and decomposed animal tissues can act as a vitellogenic stimulus in *L. sericata*, as adults feeding on both tissues exhibit completely mature eggs (Huntington 2010). Thus, physiological barriers, such as decomposition of a protein source (carrion), for vitellogenesis are nonexistent for *L. sericata*.

Strong selection for rapid colonization and development allows blow flies to take advantage of fleeting resources. Such life-history constraints select for efficient time

management and energy usage in order to survive. For example, 550 observations were made of adults of the primary screwworm, *Cochliomyia homnivorax* Coquerel (Diptera: Calliphoridae) in outdoor enclosures, and they were documented to spend a majority of the day (75.7%) in quiescence, either resting, grooming, waiting for mating opportunities, or thermoregulating (Thomas 1991). These quiescent activities are of low cost to the flies as very little energy is expended. However, 10.4% of these observations encompassed predator attack and/or death of the fly during foraging behavior on flowers. Less time spent foraging not only conserved energy, it reduced the risk of predation as the fly may be exposed while searching for food. When appropriate (i.e., high-quality) resources are detected, however, blow flies will travel great distances in order to locate them. Anecdotal evidence suggests that some blow fly species may travel approximately 20 km in a single day to locate a resource (Greenberg 1991). In other words, blow flies forage when the benefits of energy gain from food sources outweigh the cost of energy output exhibited during seeking behavior, as well as the risk of predation.

Animals that preferentially consume high quality ephemeral resources may spend much of their lives consuming resources of low quality until a high ranking food item is located (Yang 2006). Some generalist vertebrates, such as the bearded pig *Sus barbatus* Müller (Artiodactyla: Suidae), the red spiny rat *Maxomys surifer* Miller (Rodentia: Muridae), and the long-tailed giant rat *Leopoldamys sabanus* Thomas (Rodentia: Muridae), will shift their diets in order to specialize on seeds or fruits during mast-fruiting episodes in which these items are highly abundant (Curran and Leighton 2000). Blow fly adults are not dependent upon carrion for survival, as carbohydrates provide

essential sustenance throughout an individual fly's lifetime (Mackley and Long 1983, Kentner 1990, Perez-Bañon et al. 2003). Carbohydrates supply short-term energy, maintaining internal systems until higher-quality foods are located (Hainsworth 1990) and are essential for high-energy activities, such as flying (Hudson 1958). Hudson (1958) determined that *P. regina* fed 1.0 M carbohydrate (glucose) led to complete assimilation in the gut and accounted for total energy expenditure during flights. Higher-quality food sources, on the other hand, may be stored for long term energy utilization pertinent to reproductive capabilities, and thereby increase the fitness of the individual (Hainsworth 1990).

Blow flies, as well as members of the dipteran families Muscidae, Sarcophagidae, Syrphidae, and Culicidae, act as pollinators of flowering plants (Perez-Bañon et al. 2003, Banziger and Pape 2004, Mitra et al. 2005). Nectar is consumed as a secondary food source by these flies in order to supplement their intermittent consumption of a primary resource, such as carrion, blood, dung, fungus, etc. (Mackley and Long 1983, Thomas 1991, Perez-Bañon et al. 2003). During a mark and recapture study of *C. homnivorax* by Mackley and Long (1983), blow flies were observed on 19 out of 23 flowering plant species in a 1.85 km^2 research plot in Mission, Texas. These flies were observed feeding, mating, and resting while on the flowers. *C. vicina* and *L. sericata* are known to frequently consume nectar from the plant *Medicago citrina* Font Quer Greuter (Fabales: Fabaceae), as they are some of the flower's most prominent pollinators (Perez-Bañon et al. 2003).

Blow Fly Diversity in Texas

Blow flies in Texas are represented by at least 11 species within 6 genera (Tenorio et al. 2003, Byrd and Castner 2010). The secondary screwworm, *C. macellaria*, is widespread throughout North America and can be found on carrion in Texas from late spring to late fall (Denno 1975, Kirkpatrick and Olsen 2007, Byrd and Castner 2010). *C. macellaria* is attracted to large carcasses including human remains, is usually the first blow fly to oviposit on a resource, and develops quickly, forming large larval masses (Byrd and Butler 1996, Tenorio et al. 2003). The hairy maggot blow fly, *Ch. rufifacies,* is an Old World species introduced to North America in the early 1980s (Wells and Greenberg 1992a, b). It has dispersed rapidly throughout the United States due to its tropical tolerance and aggressive nature. *Ch. rufifacies* has similar temporal occurrence as *C. macellaria,* with larvae being facultatively predaceous and cannibalistic (Goodbrod and Goff 1990). Larvae of these two species can coexist on the same resource; however competition does exist with *Ch. rufifacies* usually outcompeting and/or predating upon *C. macellaria*, ultimately reducing numbers of *C. macellaria* in many regions. Godoy et al. (1996) developed a model predicting that *C. macellaria* cannot enter into aperiodic, or chaotic, oscillations in population growth as opposed to *Ch. rufifacies* (Godoy et al. 1996). This aperiodic behavior is thought to stabilize spatially structured populations, such as the *Chrysomya* genus, and the lack thereof could be the reason *C. macellaria* populations may be declining. The oriental latrine fly, *Chrysomya megacephala* Fabricius, is an introduced species from the Old World and occurs mainly in south Texas

and on the coast, but has also been documented in central Texas (Byrd and Castner 2010, Sanford et al. 2010). This species will develop on both carrion and excrement, as the common name implies. *Ch. rufifacies* has been observed to displace *Ch. megacephala* on resources due to non-consumptive effects, even though *Ch. megacephala* usually arrives first (Byrd and Castner 2010). The black blow fly, *P. regina*, the blue bottle flies, *C. vicina* and *Calliphora livida*, and the shiny blue bottle fly, *Cynomya cadaverina* Robineau-Desvoidy are cold to moderate weather species that are found on carrion mainly in the late fall or early to late spring (Byrd and Allen 2001). *Phormia regina* is a producer of myiasis, or maggot infestation within a living organism, in sheep and other domestic animals. The genus *Lucilia* can be found in Texas in the spring and fall and includes the green bottle flies, *L. sericata, Lucilia coeruleviridis* Macquart, *Lucilia eximia* Wiedemann, and the bronze bottle fly *L. cuprina* (Byrd and Castner 2010). *L. coeruliviridis* was once one of the most common blow flies collected on carrion and human remains, but is now typically outcompeted by *Ch. rufifacies* (Byrd and Castner 2010). *L. cuprina* and *L. sericata* are attracted to both excrement and carrion, and can be associated with human dwellings, whereas *L. eximia* is attracted to excrement, carrion, and decaying fruit (Whitworth 2006). Members of this genus have also been adversely affected by the introduction and subsequent spread and domination of *Ch. rufifacies* in Texas. The cosmopolitan nature of *Ch. rufifacies* makes it a prime study specimen to observe under laboratory conditions. However, the larval morphology (i.e. spiny body that becomes embedded in a substrate) as well as pupation behavior (no dispersal) of this species makes it difficult to work with. Thus, *C. macellaria* is the most appropriate

candidate for this research due to its cosmopolitan nature and prevalence in forensic investigations in Texas (Tomberlin, personal communication).

Forensic Application

Forensic, or medicolegal, entomology is the application of entomological sciences to legal investigations (Catts and Goff 1992). Knowledge of insect morphology, physiology, and ecology, as well as the variability within these sub-disciplines, are utilized in order to understand the relationships of insects with human cadavers and decomposition (Tomberlin et al. 2011). As human remains are essentially animal carrion, they are readily located and colonized by necrophagous insects. Blow flies can locate remains hours after death (Greenberg 1991), making their arrival of great importance in estimating a minimum postmortem interval (m-PMI), or the minimum length of time elapsed from time of death to time of discovery (Byrd and Castner 2010). Since development rate of larval blow flies is dependent upon biotic as well as abiotic conditions, such as temperature, development studies are employed in order to determine the amount of time and thermal energy units required for maturation under certain temperature regimes (Gabre et al. 2005, Donovan 2006, Nelson et al. 2009).

Forensic entomologists utilize development studies of blow fly species in order to estimate the time of colonization (TOC) of a body. A TOC prediction can help narrow the m-PMI, as colonization precedes death in most cases. However, development studies of a single species, for example *C. macellaria* (Byrd and Butler 1996, Boatright and

Tomberlin 2010) cannot be accurately compared as they reveal inconsistent methods for obtaining development data. This variation is potentially due to a lack of standardization in rearing procedures (Tarone and Foran 2006), but may also be the result of fundamental differences in phenotypic responses between conspecific subpopulations. In other words, phenotypic plasticity and GxE responses of distinct populations may not have been considered in many developmental studies.

Distinct conspecific populations of blow flies inhabiting different geographic regions have been shown to exhibit variation in development rate (Grassberger 2002, Donovan 2006, Tarone et al. 2011). Gallagher et al. (2010) demonstrated that *L. sericata* populations from Sacramento, CA, San Diego, CA, and Easton, MA exhibited significantly different development times when exposed to 26ºC and 36ºC temperatures. Hwang and Turner (2009) also showed that developmental time, adult body size, and growth rate were significantly different in *C. vicina* populations inhabiting different urban heat-island regimes that were not spatially or temporally isolated (approximately 30 km apart). Unfortunately, the number of forensic entomology publications discussing phenotypic plasticity as a source of developmental variation is few. This leaves the forensic entomologist to utilize geographically isolated species data that may not correlate with species in other regions.

The implementation of the *Daubert* standards in recent years has placed significant pressure on forensic scientists to standardize research methods and validate findings in order for expert testimony and forensic evidence to be admissible in a court of law (Tomberlin et al. 2010). These standards seek to test the validity of forensic

science by making sure methods are testable, repeatable, accepted by the pertinent scientific community (peer-reviewed), contain a control, and contain a known error rate. Adhering to the *Daubert* standards is crucial in forensic entomology, as erroneous data, such as those obtained from geographically dissimilar development studies, can lead to faulty verdicts in a criminal trial.

Objectives and Hypotheses

The research objectives are as follows:

1. Determine the level of plasticity in development time exhibited by three geographically distinct populations of conspecific blow flies in Texas in response to temperature.

a) Measure the variance in development time from egg to pupa and egg to adult emergence of each population in the laboratory under two constant temperatures (low and high), and determine if they are significantly different from each other.

H_o: There is not a significant difference in variance of development time from egg to pupa and egg to adult emergence between the three populations.

H_a: There is a significant difference in variance of development time from egg to pupa and egg to adult emergence between the three populations.

Relevance of First Objective

Variability between populations due to genetic variance, plasticity due to changing environments, and/or GxE effects can account for significant differences in

development data for geographically dissimilar conspecific populations. Gallagher et al. (2010) determined that populations of *L. sericata*, in two regions of California and one region of Massachusetts had significantly different development times. Consequently, if a forensic entomologist from California used development data from Massachusetts, or even another region in California, for the green bottle fly, the TOC estimate could be erroneous and pose serious problems in a forensic investigation. It is reasonable to believe that similar developmental time variations exist between conspecific populations of blow flies in Texas. Therefore, it is my goal to explore the varying developmental responses exhibited by these distinct populations in order to apply the results to the current school of thought in forensic entomology.

2. Determine the level of plasticity in size exhibited by three geographically distinct populations of conspecific blow flies in Texas in response to temperature.

a) Measure the variance in pupal size and adult body size as it relates to temperature and sex in each population.

$\mathbf{H_o}$: Variance in pupal and adult body size as related to temperature and sex does not exist in each population flies.

$\mathbf{H_a}$: Variance in pupal and adult body size as related to temperature and sex exists in each population.

Relevance of Second Objective

It is pertinent for a forensic entomologist to understand all of the possible biological variations that can occur in forensically important insects as a result of environmental effects. Responses of blow flies to different environments (i.e. the two

temperatures in Objective 1a) can be shown not only through development time, but through the size of individuals as well. Sizes may differ according to population, temperature, or sex, as shown in Hu et al. (2011), in which *C. megacephala* males exhibited more plasticity in body size than females in response to temperature. This may have relevance in understanding the outcomes of natural selection, as faster developing larvae may become smaller adults, which, in turn, live shorter lives and produce fewer offspring. However, faster development time may allow for increased generations, which could potentially offset the negative effects of reduced fecundity. Whether an organism grows faster and produces less offspring, but rapidly produces multiple generations, or grows slower and produces more offspring at the expense of decreased generations depends, in part, on environmental cues. Larval size, particularly length, can also be used to estimate age in forensic entomology (Byrd and Butler 1997). If size varies as a result of plasticity, error may result when applying non-local data to another region.

If populations exhibit plastic responses to temperature, and temperature is the driver of development time and size (i.e. faster developers may be smaller), then the size of the pupa and adult fly could be an indirect response to changing environment (i.e. temperature). Published data regarding immature and adult size of forensically important species may only reflect a single population's response to a particular geographic region. Yet, forensic entomologists routinely utilize these data in order to make predictions regarding TOC or m-PMI, as the size of an insect collected at a crime scene may give insight into when the crime was committed. Ignoring potential variations in size of pupae and adults could lead to erroneous estimations in forensic investigations.

CHAPTER II

RESEARCH, RESULTS, AND DISCUSSION

Introduction

Medicolegal entomology is the application of arthropod evidence to a legal setting (Greenberg 1991, Catts and Goff 1992). After death, insects such as blow flies (Diptera: Calliphoridae) utilize vertebrate remains primarily as a resource for their offspring (Payne 1965). Blow fly developmental biology can lend insight into when they colonized remains (time of colonization [TOC]). This TOC estimation may provide further information regarding the minimum length of time between death and discovery of an individual (minimum postmortem interval [m-PMI]) (Tomberlin et al. 2011).

Forensic entomologists sometimes utilize faunal seres, or insect succession, on remains in order to predict the TOC (Schoenly et al. 1992, Schoenly et al. 1996). However, a more quantifiable method of estimation lies in comparing insect evidence collected from a crime scene to published development data for the corresponding species (Byrd and Castner 2010). Although forensic entomology represents a well-established and reliable method for estimating TOC, many assumptions are made concerning insect development that could produce less precise estimates. Errors in interpretation of development data may arise from the disregard of intraspecific variation and lack of standardization of methods. Biological variation between conspecific populations (e.g., the subpopulation of focus in a peer-reviewed development study

versus the subpopulation of insects found at a crime scene) is often not discussed, even though such differences may be significant. Furthermore, methods for rearing insects in development studies remain inconsistent, with variables such as feeding substrate, moisture content, temperature, and replication differing across studies (Wells and Greenberg 1992b, Byrd and Allen 2001, Boatright and Tomberlin 2010).

The notion of a species as an unchanging group of interbreeding organisms similar in biology and ecology is slowly dissolving as additional data are collected (Langerhans et al. 2007, Relyea 2002, Wilbur and Collins 1973). As alleles change temporally within populations, adaptations are selected that correspond to varying stresses experienced by a group of organisms (Conner and Hartl 2004). Environmental shifts may occur as change in climate (Nicotra et al. 2010) or resource availability (Funk 2008). Other factors resulting in such shifts include, but are not limited to, competition (Henry 2008), predation (Peluc et al. 2008), parasitism (Schwanz 2008, Welbergen and Davies 2012), and geographic range expansion (Schnell and Seebacher 2008). As a population expands its distribution, multiple overlapping geographic regions composed of smaller subpopulations will appear, each exploiting slightly different habitats along a geographic gradient (Terblanche 2006). Therefore, generalizations of characteristics indicative of an entire species may not account for geographic or other environmental complexities.

Alternative biological responses between conspecific strains may be the result of phenotypic plasticity, or the ability of a single genotype to produce various phenotypes under stress (DeWitt et al. 1998, Gilbert and Epel 2009, Piersma and van Gils 2011).

Although the phenotypes exhibited by strains may be significantly different in distinct environments, the overall response across the environments may be similar between strains. For example, one strain always exhibits a higher survival rate in both environments and another strain always exhibits a lower survival rate. Another form of plasticity occurs when responses between strains are not similar across environments. For example, strain A survives better in environment A but not environment B, while Strain B exhibits an opposite response. In this type of plasticity, the environment influences the expression of the genotype (genotype by environment interaction, GxE). The benefits of this evolutionary strategy are many, as a species may be more likely to thrive in multiple environments and exploit alternative resource patches. A species exhibiting plastic phenotypes may also be more suited to survive unfavorable and unpredictable circumstances, such as drought (Valladares et al. 2005, Richter et al. 2012).

Recent studies have focused on this phenomenon and its pertinence to forensic entomology, particularly in relation to growth substrate and geographic distribution of blow flies (Gallagher et al. 2010, Hu et al. 2010, Tarone et al. 2011). In these studies, development rates of conspecific blow fly subpopulations were found to be significantly different from each other when reared in distinct environments. The implications of plasticity between conspecific blow fly populations lie in estimating the TOC with published developmental data. If conspecific blow fly populations do not exhibit similar biology, but are treated as equals when forming this estimate, less accurate TOC, and

thus m-PMI, estimates may be made. These errors have the potential for drastic outcomes, such as a faulty verdict in a court of law.

The occurrence of genetic variance, phenotypic plasticity arising from environmental shifts, and GxE interactions in forensically important insect species is a pressing issue that cannot be ignored under that *Daubert* standard, which sets the standards for admission of scientific evidence in a court of law (*Daubert vs. Merrell Dow Pharmaceuticals,* 509 U.S. 579 (1993)). Under this court ruling, evidence and opinions expressed by an expert witness must be testable, repeatable, accepted by the scientific community (peer-reviewed), contain a control, and contain an estimated or known error rate. It is not reasonable to expect development studies to be conducted for each forensically important species under every potential set of environmental conditions. However, in order to comply with the last requirement of *Daubert* (known error rate), the forensic entomologist must be able to estimate the variation that a species can exhibit via phenotypic plasticity.

The goal of this research was to explore the variable responses of three conspecific populations, or strains, of the secondary screwworm *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) (Figure 2) in two constant, but distinct, environments. The secondary screwworm was selected as a model organism as it is one of the dominant blow flies found on decomposing human remains in Texas (Tomberlin, personal communication).

Figure 2. Pinned adult specimen of *Cochliomyia macellaria* Fabricius collected from College Station, Texas, USA.

Materials and Methods

The methods used in this research were modified from Gallagher et al. (2010) and Tarone et al. (2011). The experiments were conducted during August and October 2011, and May 2012. This allowed for the examination of phenotypically plastic responses as related to ecoregion and year effects. Furthermore, the initial experiment (August 2011) examined plasticity within and between strains reared at different temperatures and varying larval densities.

Collection Sites

Distinct ecoregions were chosen in order to maximize the potential for variation between conspecific strains (Texas Parks and Wildlife, www.tpwd.state.tx.us) (Figure 3). These ecoregions included the subtropical and temperate Texas Post Oak Savannah in central Texas (College Station), the humid subtropical East Texas Pineywoods in northeast Texas (Longview), and the humid subtropical Texas Edwards Plateau in southeast Texas (San Marcos). Each ecoregion included three collection sites separated by a distance of 8-16 km. Collections spanning across time and space were performed in order to reduce any drift effects that natural populations may have been experiencing, and helped increased heterogeneity of experimental colonies. Climate for the ecoregions included temperatures ranging between minimums of 16.0-23.0°C and maximums of 29.0-36.0°C, precipitation from minimums of 49.0-68.8 mm and maximums of 117.9-128.0 mm, and RH ranging between 39%-92% (Table 1). Annual weather data for College Station, Longview, and San Marcos from January 2011 to March 2012 can be found in the Appendix.

Figure 3. Map of Texas ecoregions. Stars indicate counties utilized in this study: Gregg (top right), Brazos (center right), and Hays (center left). http://www.lib.utexas.edu/geo/pics/ecoregionsoftexas.jpg.

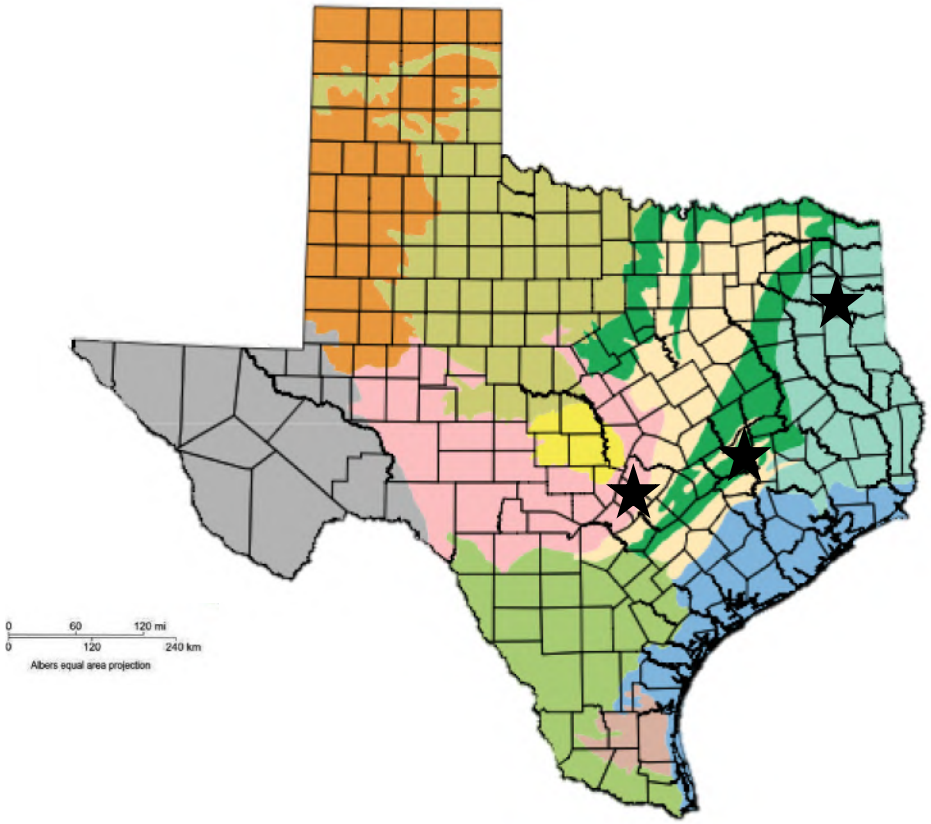


Table 1. Range of temperature (°C), precipitation (mm), and relative humidity (%RH) averages for College Station, Longview, and San Marcos, Texas.

Region	Average Minimum (°C)	Average Maximum (°C)	Mean Precipitation (mm)	Mean % RH
College Station	18.0-23.0	29.0-36.0	49.0-128.0	69.0-75.0
Longview	16.0-22.0	29.0-34.0	68.8-127.8	61.0-92.0
San Marcos	19.0-23.0	30.0-36.0	55.6-117.9	39.0-88.0

Insect Collections

C. macellaria eggs, larvae, and adults were collected from various decomposing resources (Table 2) between April and July (Table 3) at each collection site using the following methods. Adults were collected with an aerial net, while several groups of approximately 100 eggs and/or larvae were hand collected from carrion and liver bait. Adult flies, constituting generation zero (G_0), were held in 30.48 cm^3 Lumite screen collapsible cages (Bioquip, Rancho Dominguez, CA) and returned to the laboratory. Eggs and larvae were placed on approximately 100 g beef liver within 946 ml Kerr mason jars (Heathmark, LLC, Daleville, IN) containing approximately 45 g vermiculite. Jars containing larvae collected in the field were covered with a breathable Wypall paper cloth (Kimberly-Clark Global Sales, LLC, Roswell, GA) and returned to the laboratory. Jars containing larvae were held at room temperature in the Forensic Laboratory for Investigative Entomological Science (F.L.I.E.S.) Facility, Texas A&M University, College Station, Texas. Larvae collected in the field were reared to adulthood and also constituted G_0. Two collections each spanning approximately two weeks and within one month of each other, were made at each site for each year (2011 and 2012). Adult and immature flies from each collection were integrated into the laboratory colonies for use in the experiment.

Table 2. Baits utilized and blow fly species collected by collection date and region.

Region	Dates	Baits			Species
		Site 1	Site 2	Site 3	
College Station	May 22-24;27, 2011	500 g ABL	500 g ABL	500 g ABL	*Cochliomyia macellaria*
	June 3, 2011	1 pig 1 raccoon (RK) 1 snake (RK)			*Chrysomya rufifacies* *Lucilia spp.*
	May 20-22, 2011	500 g ABL 1 pig	500 g ABL	500 g ABL	*C. macellaria* *Ch. rufifacies*
	April 17-19, 2012	500 g ABL 1 pig	500 g ABL 1 pig	500 g ABL 1 pig	*C. macellaria* *Lucilia spp.*
	April 23-25, 2012	500 g ABL 1 pig	500 g ABL 1 pig	500 g ABL 1 pig	*C. macellaria* *Lucilia spp.*
	May 1, 2012	1 pig	500 g ABL	500 g ABL 1 pig	*C. macellaria* *Ch. rufifacies* *Lucilia spp.*
Longview	May 27-30, 2011	750 g ABL	750 g ABL	2 raccoons (RK)	*C. macellaria*
	June 11-12, 2011	1 pig 1 raccoon (RK) 1 armadillo (RK)	3 rats		*Ch. rufifacies* *Lucilia spp.*
	June 16-18, 2011	750 g ABL 1 pig	750 g ABL 3 rats	1 raccoon (RK) 1 armadillo (RK)	*C. macellaria* *Ch. rufifacies*
	April 12-16, 2012	750 g ABL 1 pig		500 g ABL 1 pig	*C. macellaria* *Ch. rufifacies* *Lucilia spp.*
	April 28-30, 2012	500 g ABL 1 pig	1 raccoon (RK)	500 g ABL 1 pig	*C. macellaria* *Ch. rufifacies* *Lucilia spp.*

Table 2 Continued

Region	Dates	Baits			Species
		Site 1	Site 2	Site 3	
San Marcos	June 2; 7-8, 2011	750 g ABL 1 pig	750 g ABL 1 deer (RK)	2 deer (RK)	*C. macellaria* *Ch. rufifacies*
	July 15; 25-27, 2011	750 g ABL 1 pig	750 g ABL 1 deer (RK) 1 feline (RK)	1 deer (RK)	*C. macellaria* *Ch. rufifacies*
	April 4, 2012	1 pig	1 pig 750 g ABL	1 deer (RK)	*C. macellaria* *Lucilia spp.*
	April 22, 2012	750 g ABL 1 pig		1 deer (RK)	*C. macellaria* *Ch. rufifacies* *Lucilia spp.*

Table 3. Weather data for each collection date per region.

Location	Date	Temp. Range (C°)	Mean Temp. (C°)	% RH Range	Mean % RH	Precipitation (mm)	Wind Range (km/h)	Wind Gust (km/h)
College Station	May 22, 2011	25.0-32.8	28.9	36.0-94.0	65.0	0.1	32.2-45.1	56.3
	May 23, 2011	24.4-32.2	28.3	41.0-93.0	67.0	0.0	32.2-51.5	61.2
	May 24, 2011	25.0-33.9	29.4	41.0-94.0	68.0	0.0	32.2-45.1	53.1
	May 27, 2011	21.1-36.7	28.9	29.0-93.0	61.0	0.0	20.9-40.2	59.5
	June 3, 2011	23.9-37.1	30.6	30.0-74.0	52.0	0.0	14.5-33.8	40.2
	July 20, 2011	25.0-37.2	31.1	35.0-94.0	65.0	0.0	14.5-29.0	37.0
	July 21, 2011	25.0-38.3	31.7	29.0-94.0	62.0	0.0	14.5-35.4	41.8
	July 22, 2011	25.6-38.3	32.2	31.0-91.0	61.0	0.0	16.1-35.4	45.1
	April 17, 2012	14.4-28.3	21.7	27.0-93.0	60.0	0.0	12.9-25.7	37.0
	April 18, 2012	12.8-27.2	20.0	26.0-93.0	57.0	0.0	4.8-12.9	24.1
	April 19, 2012	12.8-27.8	20.6	41.0-90.0	60.0	0.0	12.9-27.4	37.0
	April 23, 2012	13.3-25.6	19.4	24.0-60.0	37.0	0.0	11.3-25.7	33.8
	April 24, 2012	11.1-29.4	20.6	36.0-77.0	54.0	0.0	12.9-32.2	38.6
	April 25, 2012	16.1-31.7	23.9	48.0-75.0	75.0	0.0	20.9-40.2	49.9
	May 1, 2012	21.7-30.0	26.1	51.0-94.0	76.0	0.0	19.3-33.8	48.3
Longview	May 27, 2011	17.2-33.3	25.6	41.0-90.0	66.0	0.0	17.7-35.4	41.8
	May 28, 2011	23.9-35.6	30.0	26.0-82.0	54.0	0.0	27.4-45.1	56.3
	May 29, 2011	22.8-34.4	28.9	39.0-84.0	62.0	0.0	24.1-35.4	46.7
	May 30, 2011	22.8-34.4	28.9	41.0-93.0	67.0	0.0	20.9-35.4	46.7
	June 11, 2011	22.8-37.2	30.0	25.0-87.0	56.0	0.0	8.00-24.1	41.8
	June 12, 2011	21.7-37.2	29.4	29.0-84.0	57.0	0.0	11.3-22.5	27.4
	June 16, 2011	25.6-38.3	32.2	31.0-91.0	61.0	0.0	8.00-24.1	29.0
	June 17, 2011	24.4-37.8	31.1	38.0-88.0	63.0	0.0	6.40-25.7	35.4
	June 18, 2011	25.0-38.3	31.7	35.0-88.0	62.0	0.0	9.70-25.7	32.2
	April 12, 2012	14.4-25.6	20.0	59.0-87.0	73.0	0.0	12.9-24.1	29.0

Table 3 Continued

Location	Date	Temp. Range (C°)	Mean Temp. (C°)	% RH Range	Mean % RH	Precipitation (mm)	Wind Range (km/h)	Wind Gust (km/h)
Longview	April 13, 2012	18.9-31.1	23.3	58.0-100.0	79.0	0.0	16.1-33.8	41.8
	April 14, 2012	20.0-29.4	25.0	51.0-90.0	71.0	0.0	24.1-46.7	56.3
	April 15, 2012	15.6-28.3	21.1	67.0-97.0	82.0	0.3	24.1-32.2	46.7
	April 16, 2012	15.6-28.3	21.1	53.0-100.0	77.0	0.0	6.4-16.1	22.5
	April 28, 2012	18.9-30.0	24.4	48.0-93.0	71.0	0.0	9.3-35.4	48.3
	April 29, 2012	17.8-30.0	23.9	48.0-93.0	71.0	0.0	12.9-29	35.4
	April 30, 2012	22.2-30.0	26.1	55.0-90.0	73.0	T	16.1-32.2	40.2
San Marcos	June 2, 2011	17.8-35.6	26.7	25.0-68.0	46.0	0.0	9.70-29.0	40.2
	June 7, 2011	20.0-36.7	28.3	28.0-60.0	43.0	0.0	9.70-20.9	29.0
	June 8, 2011	22.8-35.6	28.9	30.0-69.0	50.0	0.0	16.1-33.8	38.6
	July 15, 2011	25.6-37.8	31.7	29.0-69.0	48.0	0.0	16.1-27.4	38.6
	July 25, 2011	23.9-38.9	31.1	25.0-69.0	47.0	0.0	14.5-22.5	29.0
	July 26, 2011	25.6-38.9	32.2	25.0-61.0	44.0	0.0	17.7-33.8	37.0
	July 27, 2011	23.9-38.9	31.1	26.0-69.0	48.0	0.0	16.1-27.4	37.0
	April 4, 2012	11.7-27.8	20.0	28.0-88.0	66.0	0.0	0.0-20.9	0.0
	April 22, 2012	8.9-30.6	20.0	24.0-93.0	46.0	0.0	8.0-25.7	0.0

Fly Rearing and Colony Maintenance

Adult flies were maintained within cages in a rearing room at approximately 27°C and a 14:10 L:D cycle. Adult flies were provided an *ad libitum* 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 a source of deionized water (dH_2O). Adult flies were also provided a single Kimwipe® (Kimberly-Clark Global Sales, Inc., Roswell, GA) soaked in bovine blood placed in an 88.7 ml white plastic bath cup (Great Value®,Wal-Mart Stores, Inc, Bentonville, AR) daily. Oviposition was induced by placing a 88.7 ml cup containing approximately 15.0 g beef liver inside each cage of flies. The liver was partially covered with a Kimwipe dampened with dH_2O. Observations were made hourly until egg masses were noted, at which time they were collected, placed on approximately 5.0 g beef liver in a separate cup, labeled, and placed inside incubators (136LLVL Percival® Percival Scientific Inc., Perry, IA) at a 14:10 L:D cycle and either 21°C (SEM±0.0289) and 65% RH or 31°C (SEM±0.03912) and 70% RH. This process continued until approximately 3000 eggs from each population were attained.

Rearing Container Design

An initial study was implemented in August 2011 to determine the appropriate density at which to conduct the plasticity study. Replicates for the density study

contained either 50, 100, or 150 larvae/50.0 g liver, with five replicates per density of each temperature-population treatment. Each replicate for the plasticity study (October 2011 and May 2012) contained 100 larvae/50.0 g liver, with 15 replicates per temperature-population treatment.

Replicates were contained within a rearing container specifically designed to minimize contact with the larvae (Figure 4). 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 to allow dispersing larvae to drop into the bottom container. The 266 ml red Solo® opaque plastic feeding cup containing liver and larvae was placed on this wire floor. The bottom of the apparatus consisted of an identical, but intact, deli cup containing approximately 575 g sand for pupation and coated with Fluon® (Insect-a-Slip; BioQuip, Rancho Domingo, CA) to prevent larval escape.

Figure 4. Specialized rearing container designed to segregate feeding larvae from wandering larvae in order to non-destructively sample pupae.

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 (Briscoe et al. 1992). Oviposition was induced and clutches

of eggs were collected using the methods outlined above. Eggs collected were placed on 5.0 g fresh beef liver in a plastic bath cup, covered with one Kimwipe held in place with a rubberband, and placed arbitrarily inside either incubator. Observations were made hourly until approximately 50% of the eggs hatched. Preliminary experiments indicated working with first instar larvae at a density of 100 larvae/50 g beef liver resulted in greatest survivorship. Using a camel hair paintbrush moistened with dH_2O, 100 of the newly emerged larvae were placed in a Kimwipe-lined 266 ml red Solo® opaque plastic feeding cup containing 50.0 g fresh beef liver and placed within a container previously described above. All three October 2011 strains were successful in producing the necessary amount of eggs required to begin the experiment. However, only two strains from the May 2012 trial (College Station and Longview) were successful in this regard.

Moisture has been shown to impact larval blow fly development (Tarone and Foran 2006). Therefore, percent moisture of liver was taken prior to, and at the conclusion of, the experiments. Wet mass was attained for five 1.0 g samples of fresh liver from each of five source bags. Each sample was placed onto a 2.54^2 sheet of aluminum foil (Great Value®,Wal-Mart Stores, Inc, Bentonville, AR) and baked in a Thelco® laboratory oven (Thermo Electron Corporation, Waltham MA) at 55ºC for 24 h, at which point dry mass was recorded using an Adventure-Pro AV64 Ohaus® scale (Ohaus Corporation, Pine Brook, NJ). A comparison of mass before and after drying in the oven provided an estimate of moisture content in the liver.

Experiments were conducted in incubators and under conditions previously described with each of the three blow fly strains for a total of six temperature-strain

treatments. Each temperature-strain combination during both trials had 15 replicates, each containing 100 larvae per container. Both incubators contained a hobo data logger Onset® HOBO U12-006 with Onset® TMC6-HD air, water, and soil temperature probes (Onset Co., Pocasset, MA) with one probe placed on each of three levels to record temperature every 10 min.

Each replicate 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 ($r = 0.8539$; $P < 0.0001$) and mass and width ($r = 0.9489$; $P < 0.0001$) of pupae, so length and width measurement were omitted from the current study. Pupae were returned to their individual containers and placed in the appropriate incubator. Pupae were observed every 8 h until adult emergence. Adults were killed by placement in a -20ºC deep freezer. Euthanized adults were placed in an oven at 55ºC for 24 h in order to measure dry mass. For each individual fly, sex, adult mass, pupal mass, developmental time from egg to pupa, and development time from

pupa to adult were recorded. Voucher specimens were placed in the Texas A&M University Insect Collection.

Analyses

Data were analyzed using JMP 9 software (JMP 2009). Mean, median, minimum, and maximum values were obtained for all development time data (oviposition to pupation time, pupation to eclosion time, and total development time), pupal and adult masses, and survival percentages. Data for the density experiment were normally distributed and were analyzed using full-factorial ANOVA. Data were not normally distributed and could not be appropriately transformed for development time, and pupal and adult mass. Thus, Friedman ANOVA tests and Wilcoxon paired comparisons were used to test for differences between strains and temperature ($P < 0.05$).

Results

Fly strains had significantly different development times ($P \leq 0.05$), masses ($P \leq 0.05$), and survival ($P \leq 0.05$) between the two environments.

2011

Density

Temperature significantly ($P < 0.0001$) impacted percent survival (Table 4). No significant two or three way interactions between strain, density, or temperature for survival were determined.

Table 4. ANOVA table for *C, macellaria* at three densities (50, 100, 150 larvae/50 g beef liver) at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Model	Source	Df	Mean Square	F ratio	Prob. > F
ANOVA	Model	17	1227.56	3.8556	**<0.0001***
	Error	72	318.39		
	Total	89			
Effect Tests	Temp.	1	15941.39	50.0693	**<0.0001**
	Strain	2	431.78	1.3562	0.2642
	Temp.XStrain	2	46.19	0.1451	0.8652
	Density	2	327.6	1.029	0.3626
	Temp.XDensity	2	789.05	2.4783	0.0910
	StrainXDensity	4	172.05	0.5404	0.7065
	Temp.XStrainXDensity	4	262.44	0.8243	0.5140

Immature development

Temperature and strain were significant variables ($P < 0.0001$) for all measures of immature development time (Table 5). However, interaction effects were not significant for any measures of the immature stage.

College Station had the longest mean and median development time at 31°C but was not significantly different from San Marcos for minimum and maximum duration at 31°C, nor any other measure at 21°C (Table 6; Figures 5, 6a-d). The Longview strain required the shortest mean immature development time at both temperatures and maximum and median duration at 21°C. It was not significantly different from San Marcos for minimum duration at 21°C or median duration at 31°C. San Marcos exhibited the greatest mean duration at 31°C and was not significantly different from either strain for maximum duration at 31°C.

Table 5. ANOVA table for 2011 *C. macellaria* immature development time on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Minimum Immature Development Time	Friedman ANOVA	Model	5	8630.99	70.15	**<0.0001***
		Error	80	123.03		
		Total	85			
	Effect Tests	Temp.	1	39308.56	319.50	**<0.0001**
		Strain	2	1672.23	13.59	**<0.0001**
		TempXStrain	2	97.53	0.79	0.4561
Maximum Immature Development Time	Friedman ANOVA	Model	5	8306.42	57.96	**<0.0001**
		Error	80	143.32		
		Total	85			
	Effect Tests	Temp.	1	39503.84	275.64	**<0.0001**
		Strain	2	870.94	6.08	**0.0035**
		TempXStrain	2	41.82	0.29	0.7477
Mean Immature Development Time	Friedman ANOVA	Model	5	8745.58	75.48	**<0.0001**
		Error	80	115.87		
		Total	85			
	Effect Tests	Temp.	1	39190.95	338.23	**<0.0001**
		Strain	2	1884.49	16.26	**<0.0001**
		TempXStrain	2	199.96	1.73	0.1846
Median Immature Development Time	Friedman ANOVA	Model	5	8596.56	68.67	**<0.0001**
		Error	80	125.18		
		Total	85			
	Effect Tests	Temp.	1	39164.09	312.85	**<0.0001**
		Strain	2	1389.36	11.10	**<0.0001**
		TempXStrain	2	656.815	2.62	0.0788

Table 6. Phenotype summaries for 2011 *C. macellaria* immature development time on beef liver at 21°C and 31°C.

Phenotype	Temperature (°C)	Strain	N	Minimum (h)	Maximum (h)	Mean (h)	Median (h)	SEM
Minimum Immature Development Time	21	College Station	15	205.50	269.50	239.10	243.75	5.22
Minimum Immature Development Time	21	Longview	15	191.75	277.75	218.62	205.75	6.82
Minimum Immature Development Time	21	San Marcos	15	198.75	269.75	228.35	222.75	6.21
Minimum Immature Development Time	31	College Station	13	108.75	168.25	132.44	134.75	4.97
Minimum Immature Development Time	31	Longview	14	100.75	138.75	107.89	100.75	3.30
Minimum Immature Development Time	31	San Marcos	14	103.75	142.25	123.50	120.75	3.32
Maximum Immature Development Time	21	College Station	15	301.50	445.50	368.63	367.50	9.27
Maximum Immature Development Time	21	Longview	15	295.75	391.75	335.05	336.75	6.51
Maximum Immature Development Time	21	San Marcos	15	278.75	422.75	359.62	358.75	9.53
Maximum Immature Development Time	31	College Station	13	136.75	189.25	158.42	156.25	3.97

Table 6 Continued

Phenotype	Temperature (°C)	Strain	N	Minimum (h)	Maximum (h)	Mean (h)	Median (h)	SEM
Maximum Immature Development Time	31	Longview	14	138.75	180.25	151.57	148.25	3.63
Maximum Immature Development Time	31	San Marcos	14	132.25	204.25	160.29	158.00	5.86
Mean Immature Development Time	21	College Station	15	272.13	306.92	286.78	283.33	2.55
Mean Immature Development Time	21	Longview	15	241.94	324.60	269.74	265.62	5.88
Mean Immature Development Time	21	San Marcos	15	228.38	344.54	286.44	293.97	8.34
Mean Immature Development Time	31	College Station	13	128.85	168.25	145.60	144.45	2.61
Mean Immature Development Time	31	Longview	14	119.86	141.86	127.87	128.44	1.70
Mean Immature Development Time	31	San Marcos	14	128.29	152.92	138.16	136.93	1.95
Median Immature Development Time	21	College Station	15	268.75	302.75	281.23	277.50	2.87
Median Immature Development Time	21	Longview	15	240.75	325.75	269.32	255.75	7.25
Median Immature Development Time	21	San Marcos	15	222.75	350.75	285.48	278.75	9.59
Median Immature Development Time	31	College Station	13	132.25	168.25	143.88	140.75	2.64
Median Immature Development Time	31	Longview	14	108.25	138.75	128.20	132.75	2.38
Median Immature Development Time	31	San Marcos	14	132.25	151.00	136.41	135.75	1.35

Figure 5. Reaction norm plots for *C. macellaria* immature development time on beef liver at 21°C and 31°C. 2011 (a-d), 2012 (e-h); minimum development time (in hours) (a, e), maximum development time (b, f), mean development time (c, g), median development time (d, h).

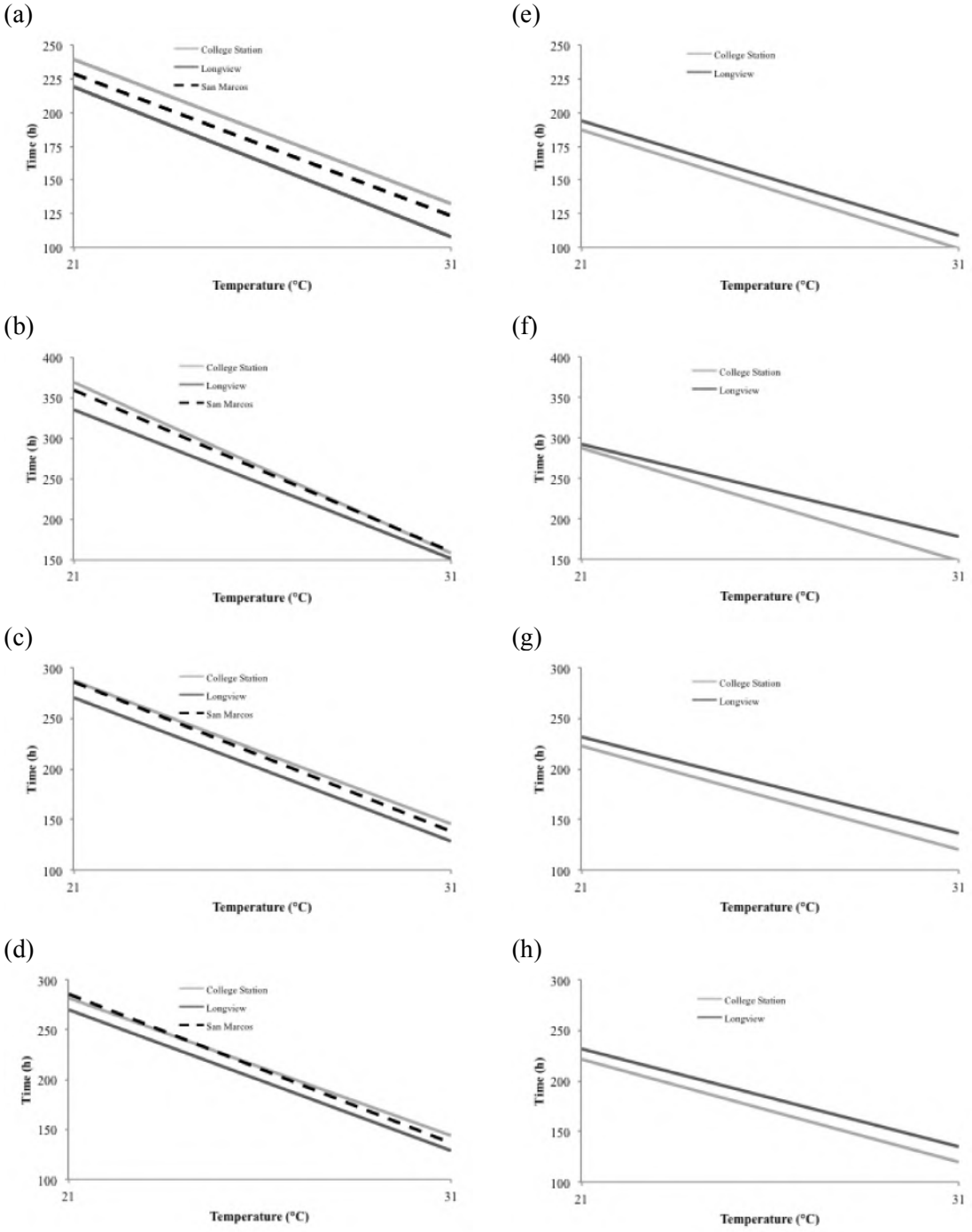
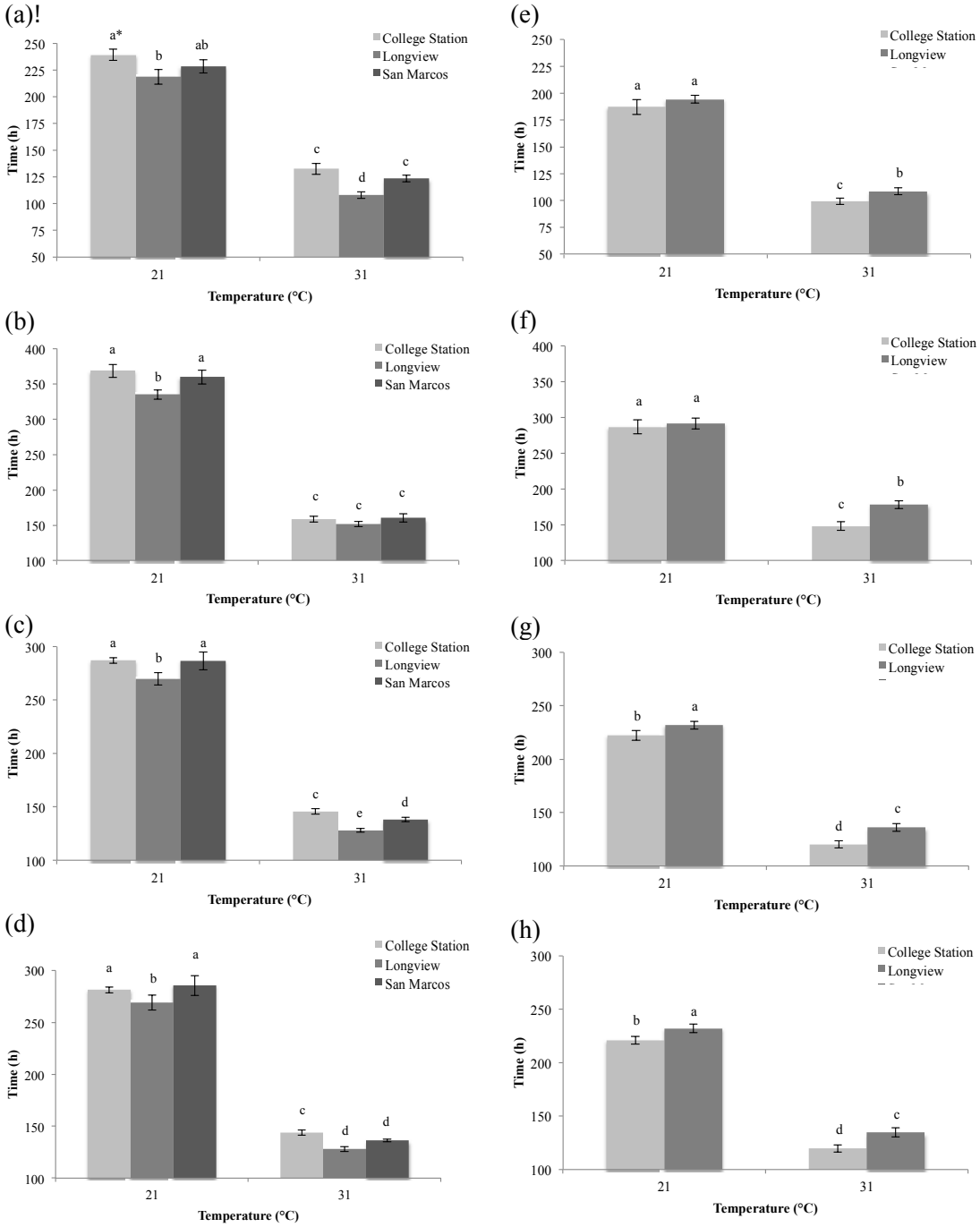


Figure 6. Comparisons between *C. macellaria* strains at 21°C and 31°C for immature development time ± SEM. N = 15 for all 2011 strains at 21°C, and all 2012 strains at both temperatures; N = 13 for 2011 College Station, N = 14 for 2011 Longview and San Marcos at 31°C. 2011 (a-d), 2012 (e-h); minimum development time (in hours) (a, e), maximum development time (b, f), mean development time (c, g), median development time (d, h). *Columns with different letters indicate significant difference ($P \leq 0.05$).



Pupal development

Temperature was a significant variable ($P < 0.0001$) for pupal development time, as was strain (minimum: $P = 0.0091$; maximum: $P = 0.0005$; mean: $P = 0.0041$; median: $P = 0.0029$) (Table 7). Two-way interactions were significant for all measures of pupal duration (minimum: $P = 0.0112$; maximum: $P = 0.0150$; mean: $P < 0.0001$; median: $P = 0.0002$) (Table 7).

College Station had the shortest maximum and mean duration at 21°C. It was not significantly different from San Marcos for maximum duration at 31°C, nor was it significantly different from either strain for minimum duration at 21°C (Table 8; Figures 7, 8a-d). No significant difference was determined for minimum pupal duration at 31°C. College Station was not significantly different from Longview for median duration at 31°C or both strains for maximum duration at 31°C. Longview exhibited an opposite trend, with the longest maximum, mean, and median duration for 21°C. Longview, however, was not significantly different from San Marcos for minimum, mean, and median duration at 31°C, nor was it significantly different from College Station for maximum and median duration at 31°C.

Table 7. ANOVA table for 2011 *C. macellaria* pupal development time on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Minimum Pupal Development Time	Friedman ANOVA	Model	5	8421.57	61.87	**<0.0001***
		Error	80	136.12		
		Total	85			
	Effect Tests	Temp.	1	39086	287.15	**<0.0001**
		Strain	2	678.42	4.98	**0.0091**
		TempXStrain	2	646.60	4.75	**0.0112**
Maximum Pupal Development Time	Friedman ANOVA	Model	5	8600.95	68.86	**<0.0001**
		Error	80	124.91		
		Total	85			
	Effect Tests	Temp.	1	39632.04	317.29	**<0.0001**
		Strain	2	1047.92	8.39	**0.0005**
		TempXStrain	2	553.18	4.43	**0.0150**
Mean Pupal Development Time	Friedman ANOVA	Model	5	8854.10	81.17	**<0.0001**
		Error	80	109.09		
		Total	85			
	Effect Tests	Temp.	1	39322.01	360.46	**<0.0001**
		Strain	2	643.37	5.90	**0.0041**
		TempXStrain	2	1606.84	14.73	**<0.0001**
Median Pupal Development Time	Friedman ANOVA	Model	5	8706.49	73.59	**<0.0001**
		Error	80	118.31		
		Total	85			
	Effect Tests	Temp.	1	39369.11	332.75	**<0.0001**
		Strain	2	743.57	6.28	**0.0029**
		TempXStrain	2	1147.76	9.70	**0.0002**

Table 8. Phenotype summaries for 2011 *C. macellaria* pupal development time on beef liver at 21°C and 31°C.

Phenotype	Temperature (C°)	Strain	N	Minimum (h)	Maximum (h)	Mean (h)	Median (h)	SEM
Minimum Pupal Development Time	21	College Station	15	136.00	168.00	147.73	152.00	2.45
Minimum Pupal Development Time	21	Longview	15	92.00	176.00	151.20	152.00	4.75
Minimum Pupal Development Time	21	San Marcos	15	128.00	168.00	149.87	144.00	3.17
Minimum Pupal Development Time	31	College Station	13	78.50	96.00	82.96	80.00	1.47
Minimum Pupal Development Time	31	Longview	14	72.00	88.00	77.36	80.00	1.30
Minimum Pupal Development Time	31	San Marcos	14	72.00	89.50	77.57	80.00	1.39
Maximum Pupal Development Time	21	College Station	15	160.00	240.00	213.33	216.00	5.39
Maximum Pupal Development Time	21	Longview	15	192.00	256.00	241.60	248.00	4.07
Maximum Pupal Development Time	21	San Marcos	15	200.00	272.00	236.27	232.00	4.34
Maximum Pupal Development Time	31	College Station	13	80.00	104.00	93.88	96.00	1.86
Maximum Pupal Development Time	31	Longview	14	88.00	104.00	97.57	96.75	1.55
Maximum Pupal Development Time	31	San Marcos	14	80.00	104.00	93.21	96.00	1.81
Mean Pupal Development Time	21	College Station	15	155.00	202.59	178.99	177.71	3.34
Mean Pupal Development Time	21	Longview	15	169.68	220.00	203.02	206.54	3.19
Mean Pupal Development Time	21	San Marcos	15	166.80	202.29	189.18	190.89	2.49
Mean Pupal Development Time	31	College Station	13	80.00	96.00	87.64	88.00	0.94

Table 8 Continued

Phenotype	Temperature (C°)	Strain	N	Minimum (h)	Maximum (h)	Mean (h)	Median (h)	SEM
Mean Pupal Development Time	31	Longview	14	82.16	89.29	85.94	85.91	0.49
Mean Pupal Development Time	31	San Marcos	14	80.00	94.30	84.86	83.93	1.02
Median Pupal Development Time	21	College Station	15	152.00	208.00	179.33	184.00	4.29
Median Pupal Development Time	21	Longview	15	168.00	216.00	204.27	208.00	3.39
Median Pupal Development Time	21	San Marcos	15	168.00	208.00	187.20	188.00	2.48
Median Pupal Development Time	31	College Station	13	80.00	96.00	87.50	88.00	0.97
Median Pupal Development Time	31	Longview	14	80.00	89.50	86.14	88.00	0.84
Median Pupal Development Time	31	San Marcos	14	80.00	96.00	85.39	86.38	1.31

Figure 7. Reaction norm plots for 2011 *C. macellaria* pupal development time on beef liver at 21°C and 31°C. 2011 (a-d), 2012 (e-h); minimum development time (in hours) (a, e), maximum development time (b, f), mean development time (c, g), median development time (d, h).

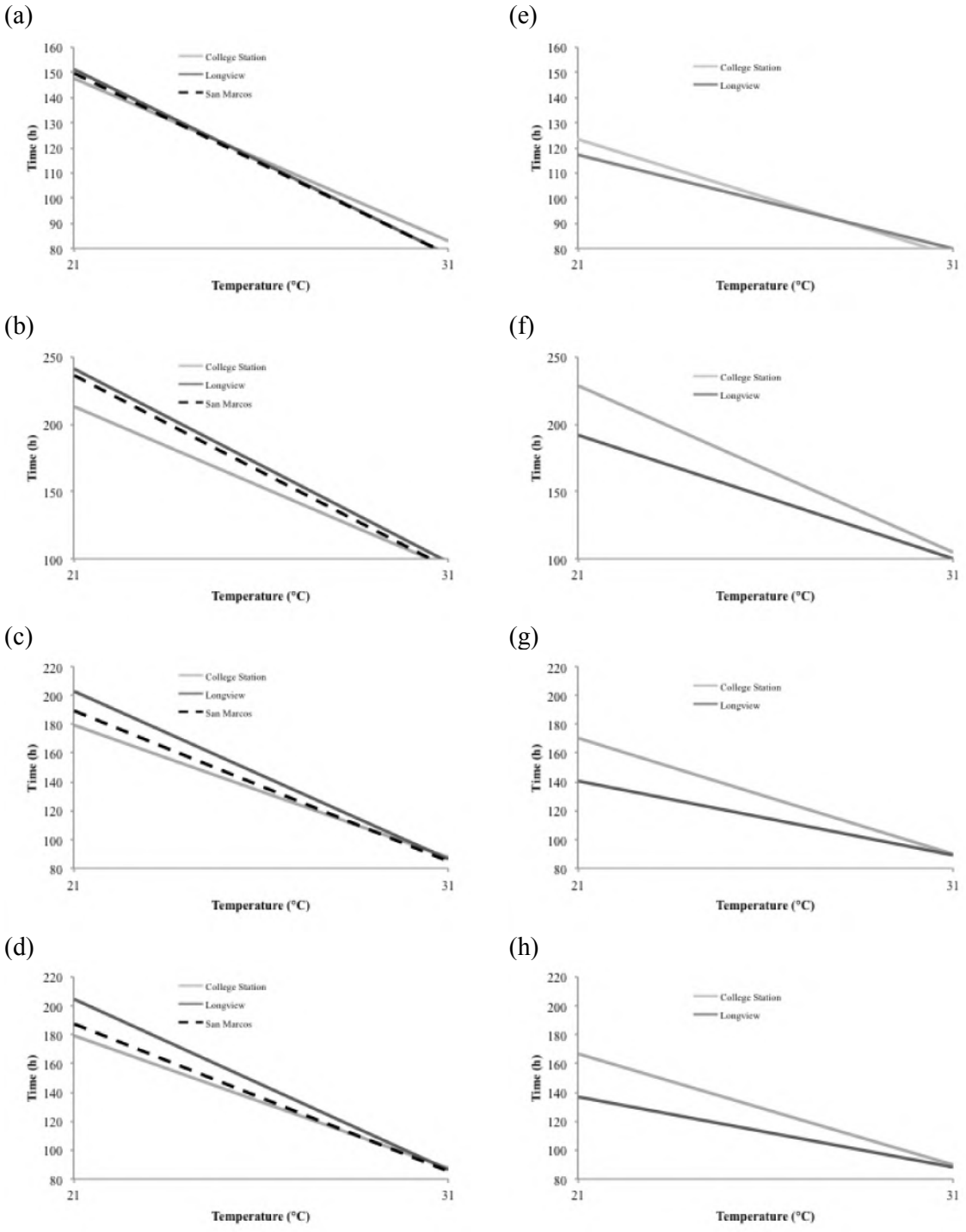
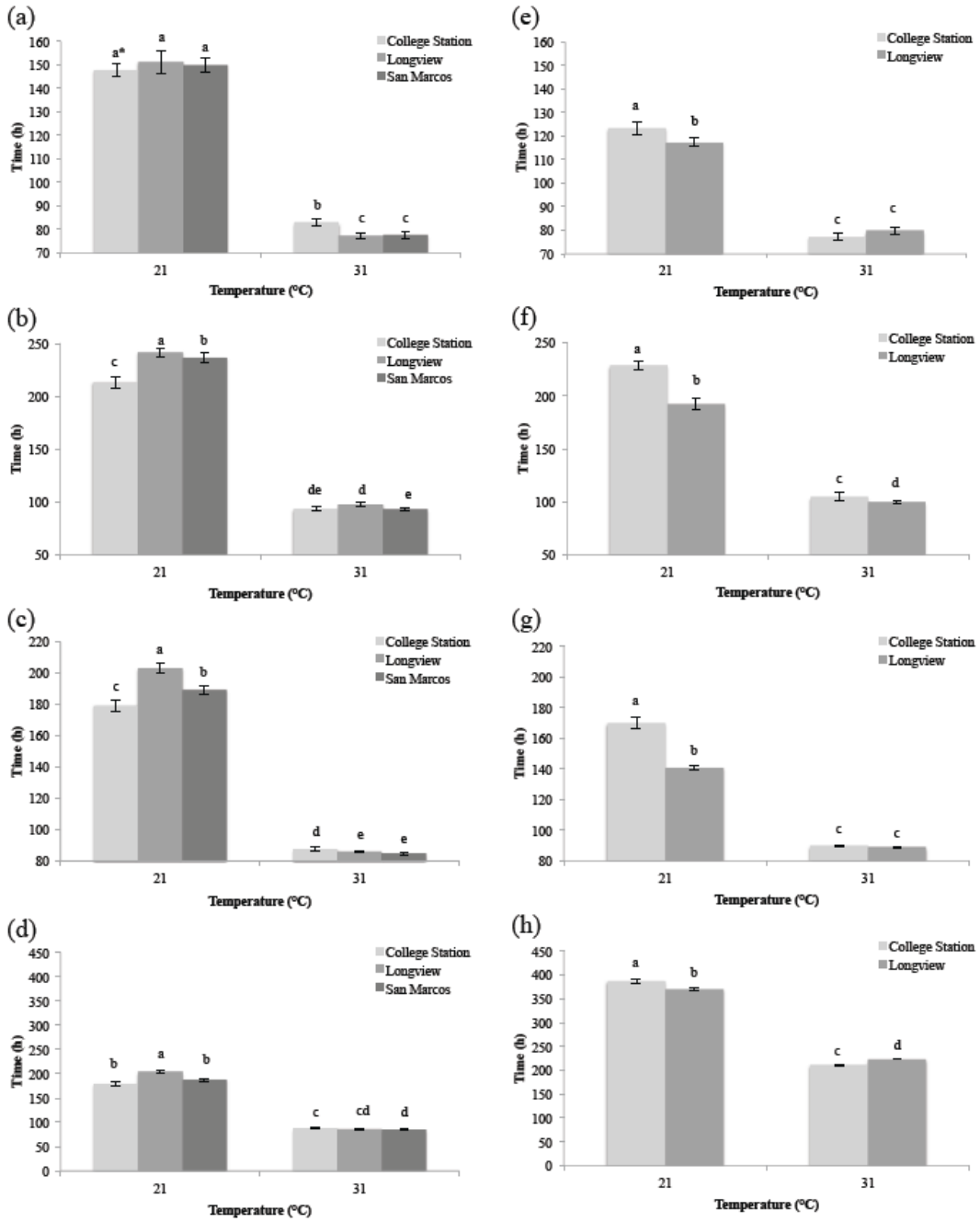


Figure 8. Comparisons between *C. macellaria* strains at 21°C and 31°C for pupal development time ± SEM. N = 15 for all strains at 21°C; N = 13 for 2011 College Station, N = 14 for 2011 Longview and San Marcos at 31°C; N = 15 for 2012 College Station, N = 14 for 2012 Longview at 31°C. 2011 (a-d), 2012 (e-h); minimum development time (in hours) (a, e), maximum development time (b, f), mean development time (c, g), median development time (d, h). *Columns with different letters indicate significant difference ($P \leq 0.05$).



Total development

Temperature was a significant variable for all measures of total development time ($P < 0.0001$). Strain was a significant variable for minimum ($P = 0.0136$) and mean ($P = 0.0352$) total duration (Table 9). Two-way interactions were determined to be significant for minimum ($P = 0.0006$), mean ($P < 0.0001$), and median ($P < 0.0001$) total duration.

College Station exhibited the shortest mean and median duration at 21°C (Table 10; Figures 9, 10a-d). College Station was not significantly different from either strain for minimum and maximum duration at 21°C. College Station exhibited the greatest mean and median duration at 31°C. It was not significantly different from San Marcos for minimum and maximum duration at 31°C. Longview and San Marcos exhibited the greatest mean and median duration at 21°C. Longview exhibited the shortest minimum and mean duration at 31°C. Longview was not significantly different from San Marcos for maximum and median duration at 31°C. San Marcos exhibited values for all other measures.

Table 9. ANOVA table for 2011 *C. macellaria* total development time on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Minimum Total Development Time	Friedman ANOVA	Model	5	8559.88	67.15	**<0.0001***
		Error	80	127.48		
		Total	85			
	Effect Tests	Temp.	1	39227.89	307.73	**<0.0001**
		Strain	2	578.55	4.54	**0.0136**
		TempXStrain	2	1048.64	8.223	**0.0006**
Maximum Total Development Time	Friedman ANOVA	Model	5	8112.22	52.18	**<0.0001**
		Error	80	155.46		
		Total	85			
	Effect Tests	Temp.	1	39456.66	253.81	**<0.0001**
		Strain	2	272.32	1.75	0.1801
		TempXStrain	2	185.40	1.19	0.3088
Mean Total Development Time	Friedman ANOVA	Model	5	8780.55	77.24	**<0.0001**
		Error	80	113.68		
		Total	85			
	Effect Tests	Temp.	1	39147.31	344.35	**<0.0001**
		Strain	2	396.67	3.49	**0.0352**
		TempXStrain	2	1785.42	15.70	**<0.0001**
Median Total Development Time	Friedman ANOVA	Model	5	8575.39	67.79	**<0.0001**
		Error	80	126.51		
		Total	85			
	Effect Tests	Temp.	1	39180.88	309.71	**<0.0001**
		Strain	2	140.30	1.11	0.3349
		TempXStrain	2	1504.64	11.89	**<0.0001**

Table 10. Phenotype summaries for 2011 *C. macellaria* total development time on beef liver at 21°C and 31°C.

Phenotype	Temperature (°C)	Strain	N	Minimum (h)	Maximum (h)	Mean (h)	Median (h)	SEM
Minimum Total Development Time	21	College Station	15	389.50	446.75	420.43	429.50	5.87
Minimum Total Development Time	21	Longview	15	347.75	477.75	425.58	424.75	8.89
Minimum Total Development Time	21	San Marcos	15	382.75	462.75	432.88	445.75	6.37
Minimum Total Development Time	31	College Station	13	191.75	256.25	222.44	216.75	4.98
Minimum Total Development Time	31	Longview	14	180.25	228.25	192.39	188.25	3.34
Minimum Total Development Time	31	San Marcos	14	188.75	229.25	208.79	208.50	3.34
Maximum Total Development Time	21	College Station	15	437.50	617.50	516.43	509.50	11.31
Maximum Total Development Time	21	Longview	15	487.75	549.75	515.22	511.75	4.81
Maximum Total Development Time	21	San Marcos	15	454.75	598.75	525.48	526.75	8.13
Maximum Total Development Time	31	College Station	13	228.25	262.75	246.40	244.75	3.14
Maximum Total Development Time	31	Longview	14	228.25	284.25	239.57	236.25	4.05
Maximum Total Development Time	31	San Marcos	14	212.75	255.25	241.54	246.25	3.85
Mean Total Development Time	21	College Station	15	415.75	491.43	459.17	457.34	5.18
Mean Total Development Time	21	Longview	15	450.32	506.44	473.44	471.08	3.99
Mean Total Development Time	21	San Marcos	15	429.14	522.99	473.73	477.99	6.22
Mean Total Development Time	31	College Station	13	216.38	256.25	234.22	233.25	2.75

Table 10 Continued

Phenotype	Temperature (°C)	Strain	N	Minimum (h)	Maximum (h)	Mean (h)	Median (h)	SEM
Mean Total Development Time	31	Longview	14	205.95	230.04	213.80	213.78	1.66
Mean Total Development Time	31	San Marcos	14	211.08	236.08	222.96	221.53	1.87
Median Total Development Time	21	College Station	15	410.50	487.75	455.40	457.50	5.54
Median Total Development Time	21	Longview	15	447.75	502.00	472.60	471.75	3.92
Median Total Development Time	21	San Marcos	15	430.75	534.75	474.02	477.75	6.92
Median Total Development Time	31	College Station	13	212.50	256.25	233.37	230.75	3.17
Median Total Development Time	31	Longview	14	208.50	228.25	214.89	212.75	1.45
Median Total Development Time	31	San Marcos	14	212.25	231.75	220.07	218.00	1.94

Figure 9. Reaction norm plots for 2011 *C. macellaria* total development time on beef liver at 21°C and 31°C.. 2011 (a-d), 2012 (e-h); minimum development time (in hours) (a, e), maximum development time (b, f), mean development time (c, g), median development time (d, h).

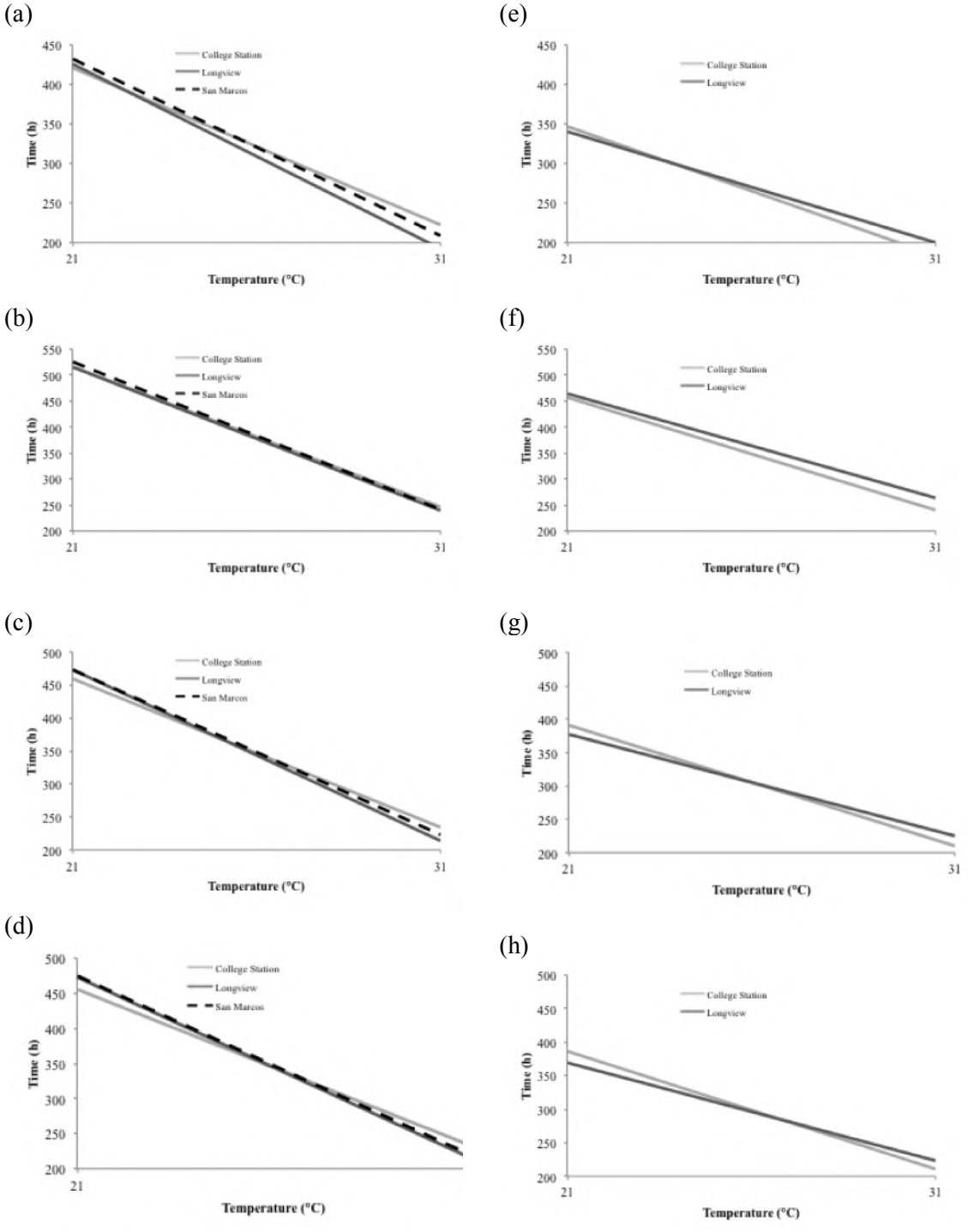
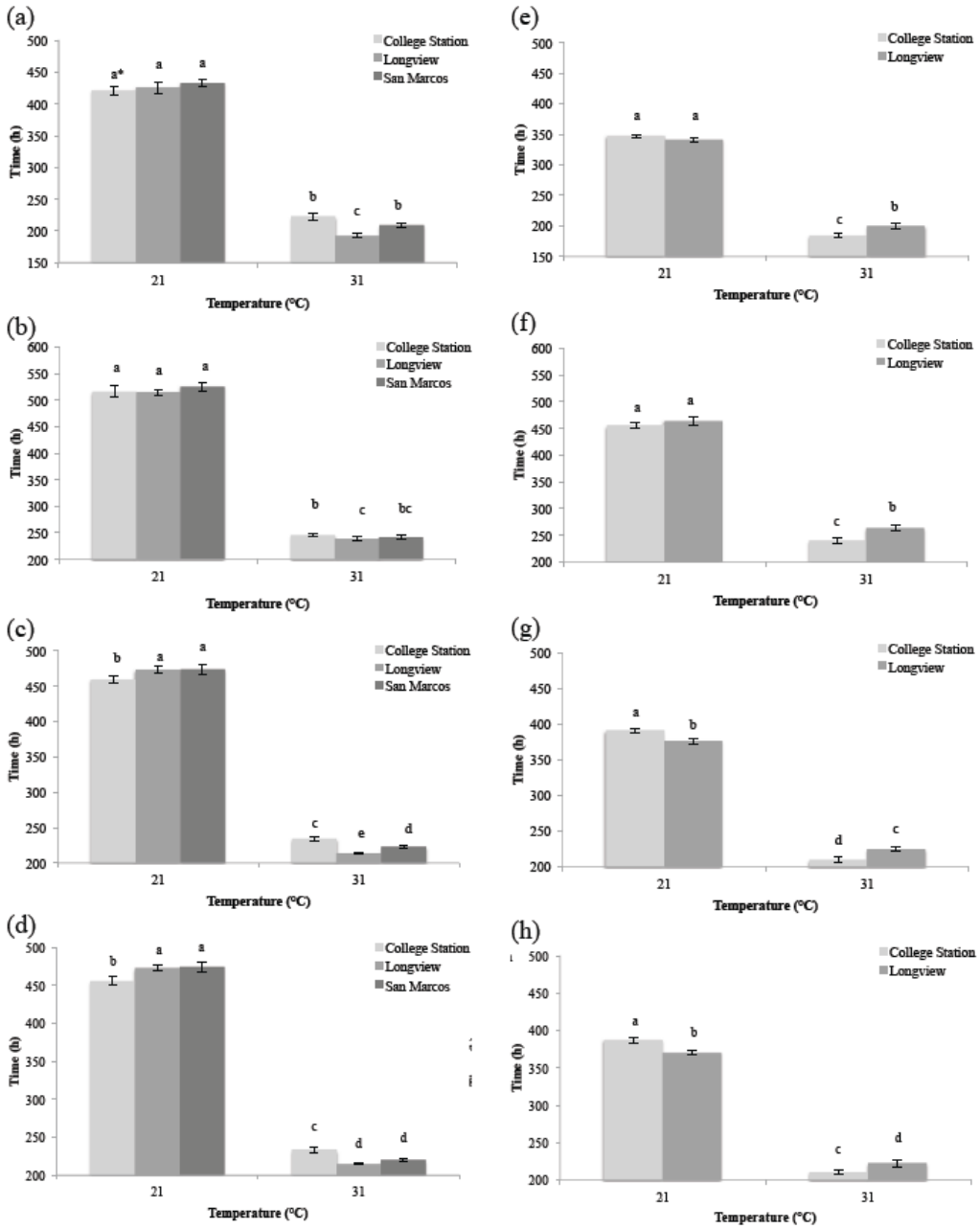


Figure 10. Comparisons between *C. macellaria* strains at 21°C and 31°C for total development time ± SEM. N = 15 for all strains at 21°C; N = 13 for 2011 College Station, N = 14 for 2011 Longview and San Marcos at 31°C; N = 15 for 2012 College Station, N = 14 for 2012 Longview at 31°C. 2011 (a-d), 2012 (e-h); minimum development time (in hours) (a, e), maximum development time (b, f), mean development time (c, g), median development time (d, h). *Columns with different letters indicate significant difference ($P \leq 0.05$).



Pupal Mass

Temperature was a significant variable for minimum (P = 0.0031) and maximum (P = 0.0045) pupal mass (Table 11). Two-way interactions were determined to be significant for mean (P = 0.0232) and median (P = 0.0179) pupal mass.

No significant difference for minimum and median pupal mass at either temperature, mean pupal mass at 21°C, or maximum pupal mass at 31°C was determined between strains. Longview and San Marcos had the greatest pupal mass at 21°C. San Marcos was not significantly different from College Station. Longview had the least mean pupal mass at 31°C (Table 12; Figures 11, 12a-d).

Adult Mass

Overall Friedman ANOVA values were only significant for minimum (P = 0.0210) and median (P = 0.0295) models. Temperature was a significant variable for median adult mass (P =0.0339). Strain was a significant variable only for minimum adult mass (P = 0.0081) (Table 13).

No significant differences were observed between strains for minimum adult mass at 21°C, maximum adult mass at both temperatures, mean adult mass at 31°C, or median adult mass at 31°C (Table 14; Figures 13, 14a-d). Longview had the smallest minimum adult mass at 31°C and significantly differed from College Station and San Marcos. At 21°C San Marcos had the smallest, and Longview the largest, mean adult

mass. However, neither was significantly different from College Station. San Marcos displayed a significantly smaller median adult mass than Longview at 21°C, but neither was significantly different from College Station.

Table 11. ANOVA table for 2011 *C. macellaria* pupal mass on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Minimum Pupal Mass	Friedman ANOVA	Model	5	1616.28	2.88	**0.0193***
		Error	80	561.45		
		Total	85			
	Effect Tests	Temp.	1	5225.68	9.31	**0.0031**
		Strain	2	1133.70	2.02	0.1395
		TempXStrain	2	396.14	0.71	0.4969
Maximum Pupal Mass	Friedman ANOVA	Model	5	1919.48	3.54	**0.0061**
		Error	80	542.50		
		Total	85			
	Effect Tests	Temp.	1	4636.93	8.55	**0.0045**
		Strain	2	1305.56	2.41	0.0966
		TempXStrain	2	1158.68	2.14	0.1248
Mean Pupal Mass	Friedman ANOVA	Model	5	1032.63	1.73	0.1379
		Error	80	597.93		
		Total	85			
	Effect Tests	Temp.	1	174.59	0.29	0.5905
		Strain	2	125.18	0.21	0.8116
		TempXStrain	2	2359.66	3.95	**0.0232**
Median Pupal Mass	Friedman ANOVA	Model	5	1037.25	1.74	0.1360
		Error	80	597.64		
		Total	85			
	Effect Tests	Temp.	1	138.55	0.23	0.6315
		Strain	2	2.06	0.01	0.9966
		TempXStrain	2	2527.86	4.23	**0.0179**

Table 12. Phenotype summaries for 2011 *C. macellaria* pupal mass on beef liver at 21°C and 31°C.

Phenotype	Temperature (°C)	Strain	N	Minimum (mg)	Maximum (mg)	Mean (mg)	Median (mg)	SEM
Minimum Pupal Mass	21	College Station	15	3.10	35.00	12.85	10.30	2.32
Minimum Pupal Mass	21	Longview	15	4.20	32.60	11.18	8.00	1.92
Minimum Pupal Mass	21	San Marcos	15	5.80	26.20	11.45	9.40	1.57
Minimum Pupal Mass	31	College Station	13	7.20	48.10	23.37	15.90	4.39
Minimum Pupal Mass	31	Longview	14	4.40	35.50	14.42	9.15	2.82
Minimum Pupal Mass	31	San Marcos	14	6.30	40.70	21.26	16.05	3.63
Maximum Pupal Mass	21	College Station	15	32.10	51.10	43.86	45.00	1.33
Maximum Pupal Mass	21	Longview	15	42.50	51.80	47.94	48.10	0.70
Maximum Pupal Mass	21	San Marcos	15	39.90	54.70	46.29	46.60	1.25
Maximum Pupal Mass	31	College Station	13	35.50	48.10	42.80	43.00	0.98
Maximum Pupal Mass	31	Longview	14	39.00	48.70	43.67	43.40	0.79
Maximum Pupal Mass	31	San Marcos	14	38.00	50.00	44.88	45.40	1.05
Mean Pupal Mass	21	College Station	15	20.03	40.01	30.75	33.47	1.68
Mean Pupal Mass	21	Longview	15	20.58	40.25	34.62	35.28	1.28
Mean Pupal Mass	21	San Marcos	15	16.25	36.44	31.58	31.91	1.27
Mean Pupal Mass	31	College Station	13	25.66	48.10	34.57	33.65	2.01
Mean Pupal Mass	31	Longview	14	24.65	37.65	31.23	29.49	1.17
Mean Pupal Mass	31	San Marcos	14	21.83	42.10	34.71	36.73	1.63
Median Pupal Mass	21	College Station	15	21.80	41.80	32.86	34.85	1.75
Median Pupal Mass	21	Longview	15	12.75	41.10	35.91	38.40	1.79
Median Pupal Mass	21	San Marcos	15	13.10	39.70	33.41	34.25	1.54
Median Pupal Mass	31	College Station	13	28.80	48.10	36.93	36.55	1.54
Median Pupal Mass	31	Longview	14	17.20	40.30	32.74	33.90	1.59
Median Pupal Mass	31	San Marcos	14	15.95	46.20	36.10	38.30	2.04

Figure 11. Reaction norm plots for 2011 *C. macellaria* pupal mass on beef liver at 21°C and 31°C. 2011 (a-d), 2012 (e-h); minimum mass (in mg) (a, e), maximum mass (b, f), mean mass (c, g), median mass (d, h).

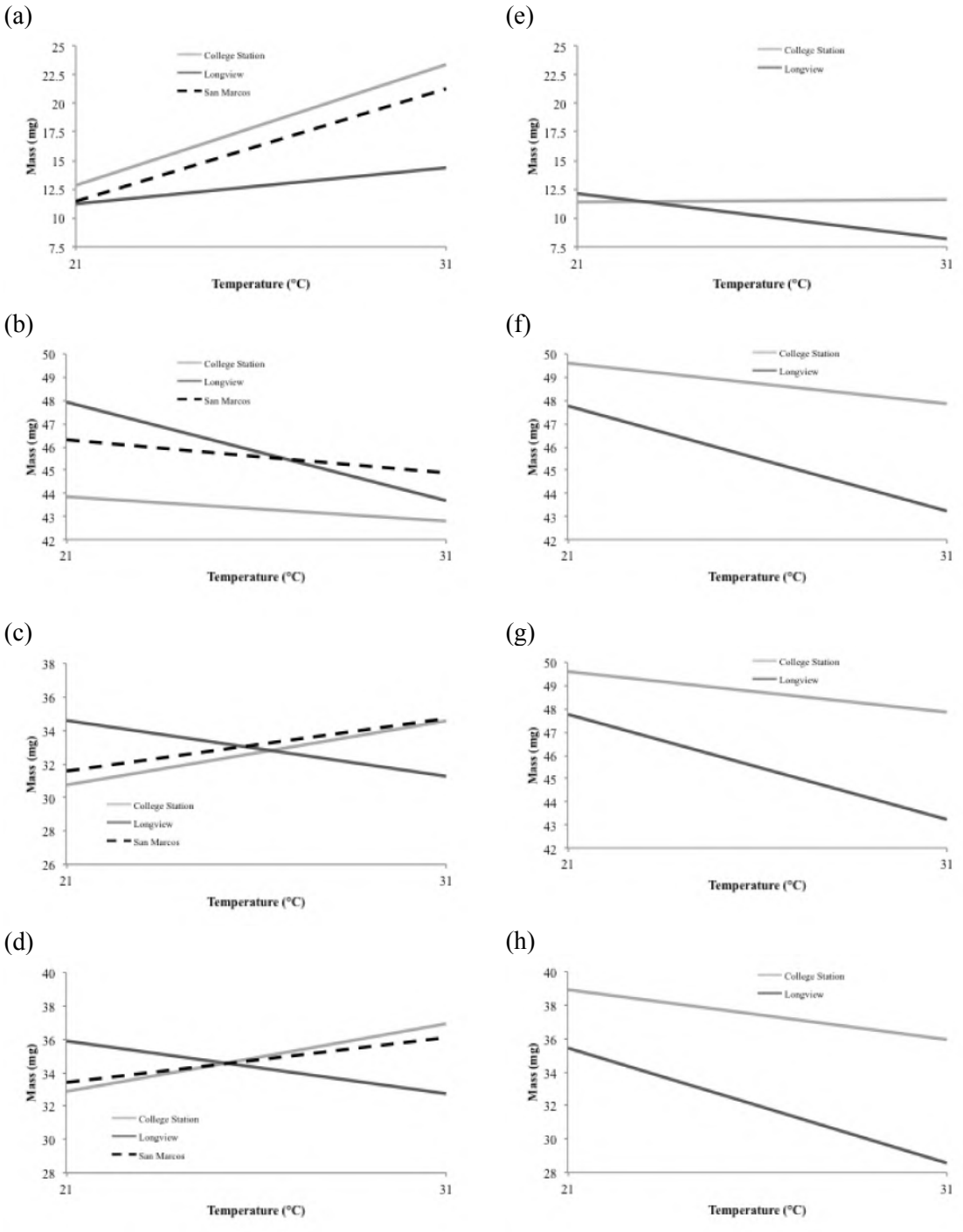


Figure 12. Comparisons between *C. macellaria* strains at 21°C and 31°C for pupal mass ± SEM. N = 15 for all strains at 21°C; N = 13 for 2011 College Station, N = 14 for 2011 Longview and San Marcos at 31°C; N = 15 for 2012 College Station, N = 14 for 2012 Longview at 31°C. 2011 (a-d), 2012 (e-h); minimum mass (in mg) (a, e), maximum mass (b, f), mean mass (c, g), median mass (d, h). *Columns with different letters indicate significant difference ($P \leq 0.05$).

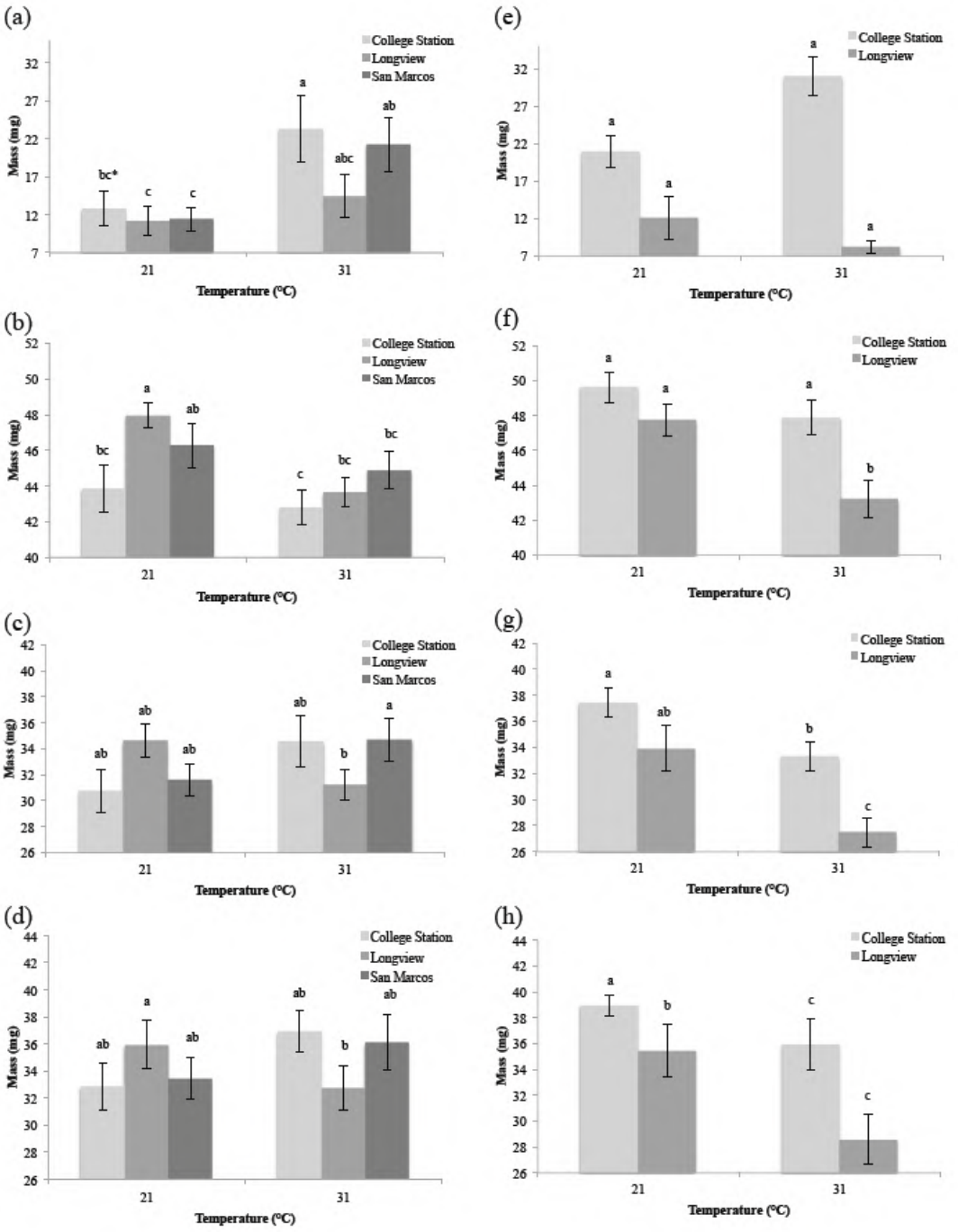


Table 13. ANOVA table for 2011 *C. macellaria* adult mass on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Minimum Adult Mass	Friedman ANOVA	Model	5	1592.50	2.83	**0.0210***
		Error	80	562.94		
		Total	85			
	Effect Tests	Temp.	1	300.45	0.53	0.4672
		Strain	2	2884.12	5.12	**0.0081**
		TempXStrain	2	1020.68	1.81	0.1698
Maximum Adult Mass	Friedman ANOVA	Model	5	827.84	1.36	0.2500
		Error	80	610.73		
		Total	85			
	Effect Tests	Temp.	1	2141.52	3.51	0.0648
		Strain	2	969.03	1.59	0.2110
		TempXStrain	2	10.59	0.02	0.9828
Mean Adult Mass	Friedman ANOVA	Model	5	995.80	1.66	0.1541
		Error	80	600.23		
		Total	85			
	Effect Tests	Temp.	1	663.06	1.10	0.2964
		Strain	2	489.34	0.82	0.4462
		TempXStrain	2	1685.11	2.81	0.0663
Median Adult Mass	Friedman ANOVA	Model	5	1498.79	2.64	**0.0295**
		Error	80	568.79		
		Total	85			
	Effect Tests	Temp.	1	2650.90	4.66	**0.0339**
		Strain	2	883.96	1.55	0.2177
		TempXStrain	2	1556.95	2.74	0.0708

Table 14. Phenotype summaries for 2011 *C. macellaria* adult mass on beef liver at 21°C and 31°C.

Phenotype	Temperature (°C)	Strain	N	Minimum (h)	Maximum (h)	Mean (h)	Median (h)	SEM
Minimum Total Development Time	21	College Station	15	389.50	446.75	420.43	429.50	5.87
Minimum Total Development Time	21	Longview	15	347.75	477.75	425.58	424.75	8.89
Minimum Total Development Time	21	San Marcos	15	382.75	462.75	432.88	445.75	6.37
Minimum Total Development Time	31	College Station	13	191.75	256.25	222.44	216.75	4.98
Minimum Total Development Time	31	Longview	14	180.25	228.25	192.39	188.25	3.34
Minimum Total Development Time	31	San Marcos	14	188.75	229.25	208.79	208.50	3.34
Maximum Total Development Time	21	College Station	15	437.50	617.50	516.43	509.50	11.31
Maximum Total Development Time	21	Longview	15	487.75	549.75	515.22	511.75	4.81
Maximum Total Development Time	21	San Marcos	15	454.75	598.75	525.48	526.75	8.13
Maximum Total Development Time	31	College Station	13	228.25	262.75	246.40	244.75	3.14
Maximum Total Development Time	31	Longview	14	228.25	284.25	239.57	236.25	4.05
Maximum Total Development Time	31	San Marcos	14	212.75	255.25	241.54	246.25	3.85
Mean Total Development Time	21	College Station	15	415.75	491.43	459.17	457.34	5.18
Mean Total Development Time	21	Longview	15	450.32	506.44	473.44	471.08	3.99
Mean Total Development Time	21	San Marcos	15	429.14	522.99	473.73	477.99	6.22
Mean Total Development Time	31	College Station	13	216.38	256.25	234.22	233.25	2.75

Table 14 Continued

Phenotype	Temperature (°C)	Strain	N	Minimum (h)	Maximum (h)	Mean (h)	Median (h)	SEM
Mean Total Development Time	31	Longview	14	205.95	230.04	213.80	213.78	1.66
Mean Total Development Time	31	San Marcos	14	211.08	236.08	222.96	221.53	1.87
Median Total Development Time	21	College Station	15	410.50	487.75	455.40	457.50	5.54
Median Total Development Time	21	Longview	15	447.75	502.00	472.60	471.75	3.92
Median Total Development Time	21	San Marcos	15	430.75	534.75	474.02	477.75	6.92
Median Total Development Time	31	College Station	13	212.50	256.25	233.37	230.75	3.17
Median Total Development Time	31	Longview	14	208.50	228.25	214.89	212.75	1.45
Median Total Development Time	31	San Marcos	14	212.25	231.75	220.07	218.00	1.94

Figure 13. Reaction norm plots for 2011 *C. macellaria* adult mass on beef liver at 21°C and 31°C. 2011 (a-d), 2012 (e-h); minimum mass (in mg) (a, e), maximum mass (b, f), mean mass (c, g), median mass (d, h).

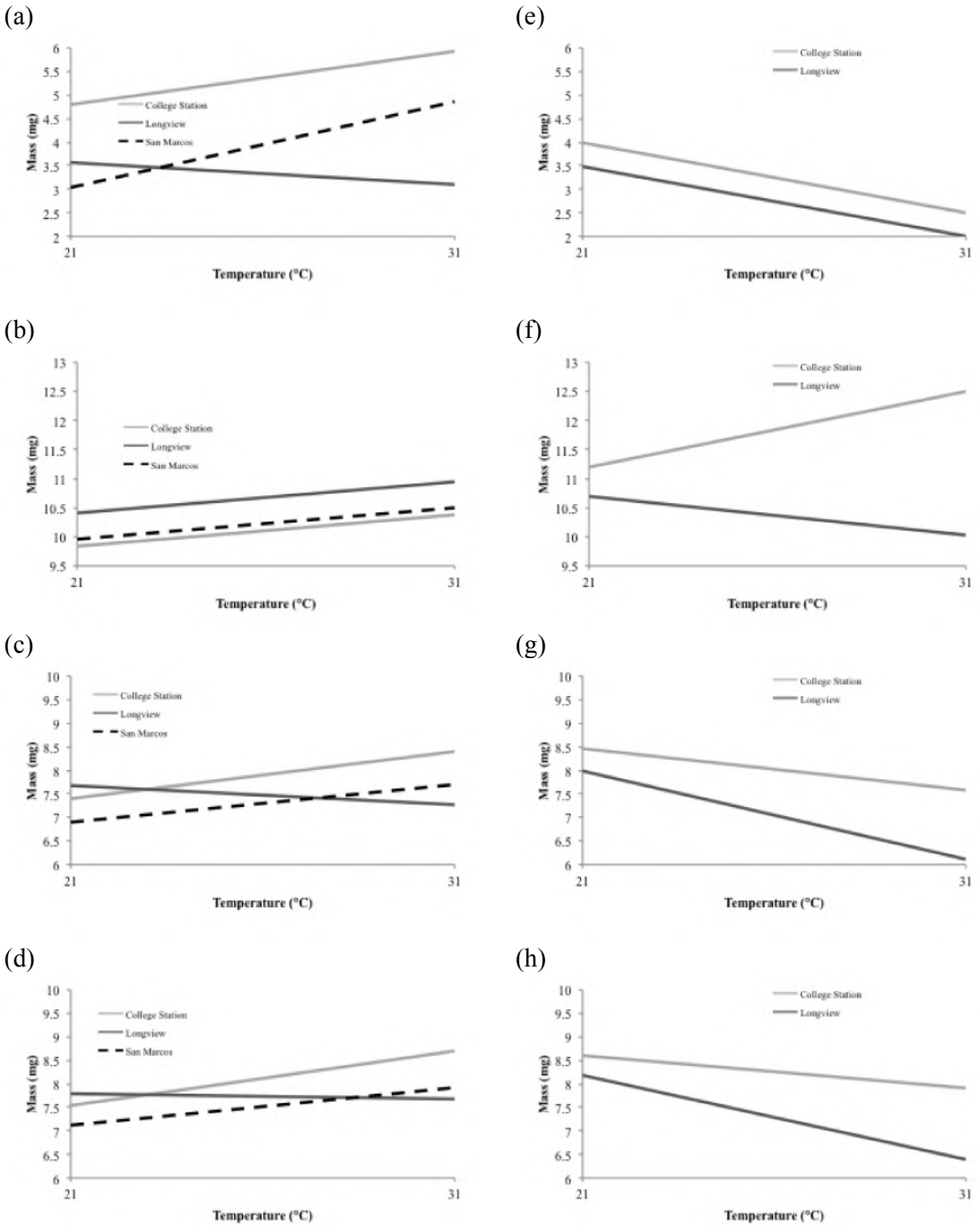
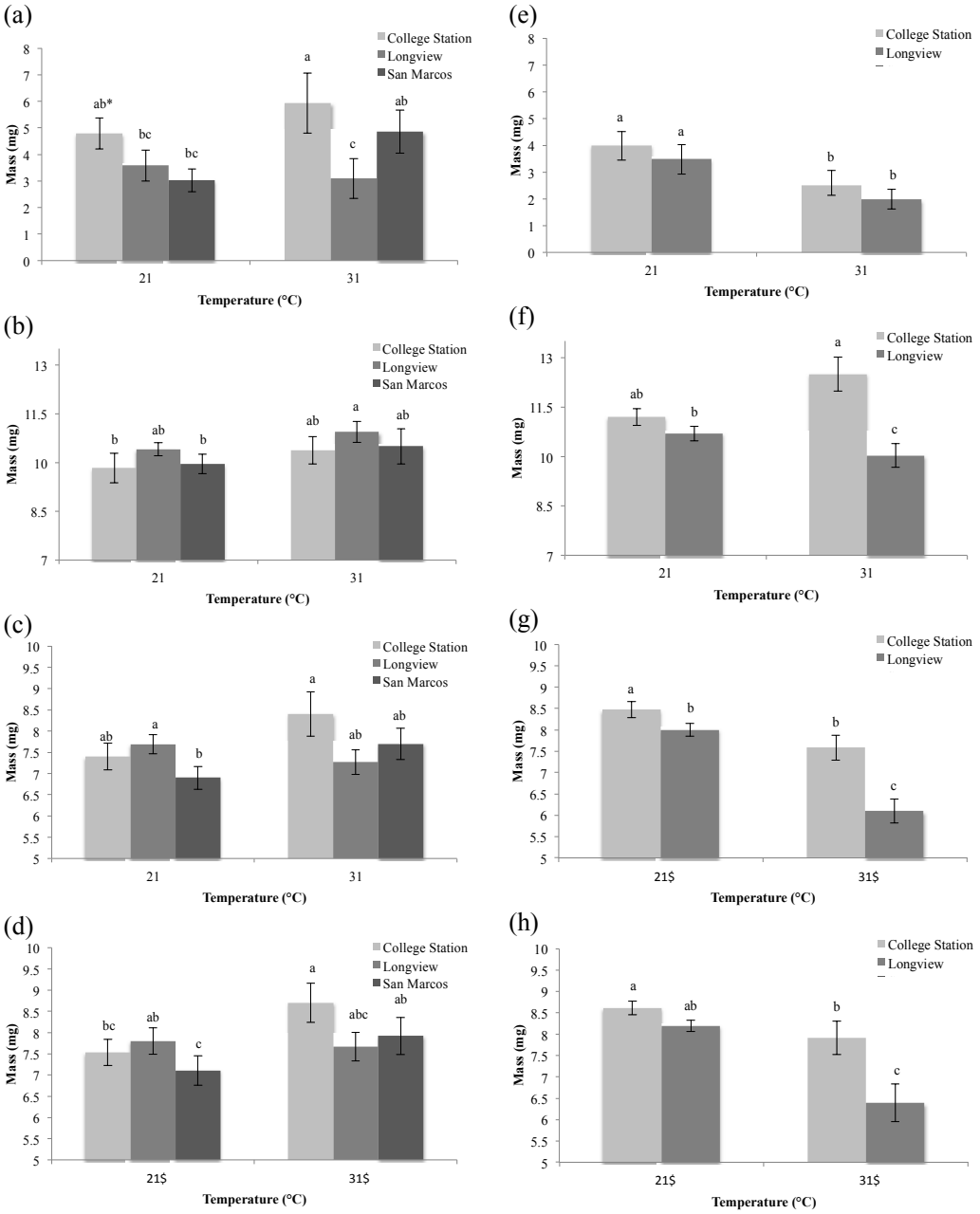


Figure 14. Comparisons between *C. macellaria* strains at 21°C and 31°C for adult mass ± SEM. N = 15 for all strains at 21°C; N = 13 for 2011 College Station, N = 14 for 2011 Longview and San Marcos at 31°C; N = 15 for 2012 College Station, N = 14 for 2012 Longview at 31°C. 2011 (a-d), 2012 (e-h); minimum mass (in mg) (a, e), maximum mass (b, f), mean mass (c, g), median mass (d, h). *Columns with different letters indicate significant difference ($P \leq 0.05$).



Survival

Temperature was a significant variable for larval and pupal survival ($P < 0.0001$). Strain was a significant variable for larval ($P < 0.0002$), but not pupal survival ($P=0.1860$) (Table 15). No significant interactions were determined for larval survival ($P=0.2005$). Significant interactions between survival percentage, strain, and temperature were determined for pupal survival ($P=0.0484$).

At both temperatures, College Station exhibited the lowest average larval survival percentage, which was significantly different from Longview but not San Marcos (Table 16; Figures 15, 16a-d). No significant difference in pupal survival was determined between strains reared at 31°C.

Table 15. ANOVA table for 2011 *C. macellaria* percentage of immature and pupal survival on beef liver at 21°C and 31°. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
% Immature Survival	Friedman ANOVA	Model	5	5246.12	15.68	**<0.0001***
		Error	80	334.59		
		Total	85			
	Effect Tests	Temp.	1	19158.78	57.26	**<0.0001**
		Strain	2	3247.71	9.71	**0.0002**
		TempXStrain	2	548.54	1.64	0.2005
% Pupal Survival	Friedman ANOVA	Model	5	4766.73	13.08	**<0.0001**
		Error	80	364.55		
		Total	85			
	Effect Tests	Temp.	1	20137.73	55.24	**<0.0001**
		Strain	2	626.21	1.72	0.1860
		TempXStrain	2	1146.74	3.15	**0.0484**

Table 16. Phenotype summaries for 2011 *C. macellaria* percentage of immature and pupal survival on beef liver at 21°C and 31°.

Phenotype	Temperature (°C)	Strain	N	Minimum (%)	Maximum (%)	Mean (%)	Median (%)	SEM
% Immature Survival	21	College Station	15	6.00	58.00	35.00	45.00	4.40
% Immature Survival	21	Longview	15	21.00	82.00	50.50	52.00	4.46
% Immature Survival	21	San Marcos	15	23.00	69.00	43.70	43.00	3.60
% Immature Survival	31	College Station	13	1.00	29.00	8.50	5.00	2.33
% Immature Survival	31	Longview	14	4.00	63.00	31.80	35.50	5.44
% Immature Survival	31	San Marcos	14	1.00	46.00	13.90	10.00	3.84
% Pupal Survival	21	College Station	15	33.30	87.00	63.10	66.70	4.26
% Pupal Survival	21	Longview	15	61.30	97.40	82.40	96.60	2.83
% Pupal Survival	21	San Marcos	15	60.00	94.20	77.00	78.30	2.46
% Pupal Survival	31	College Station	13	50.00	100.00	89.90	100.00	4.63
% Pupal Survival	31	Longview	14	77.80	100.00	92.30	93.20	1.78
% Pupal Survival	31	San Marcos	14	75.00	100.00	93.30	100.00	2.57

Figure 15. Reaction norm plots for 2011 *C. macellaria* percentage of immature and pupal survival on beef liver at 21°C and 31°. 2011 (a-b), 2012 (c-d); immature survival (%) (a, c), pupal survival (b, d).

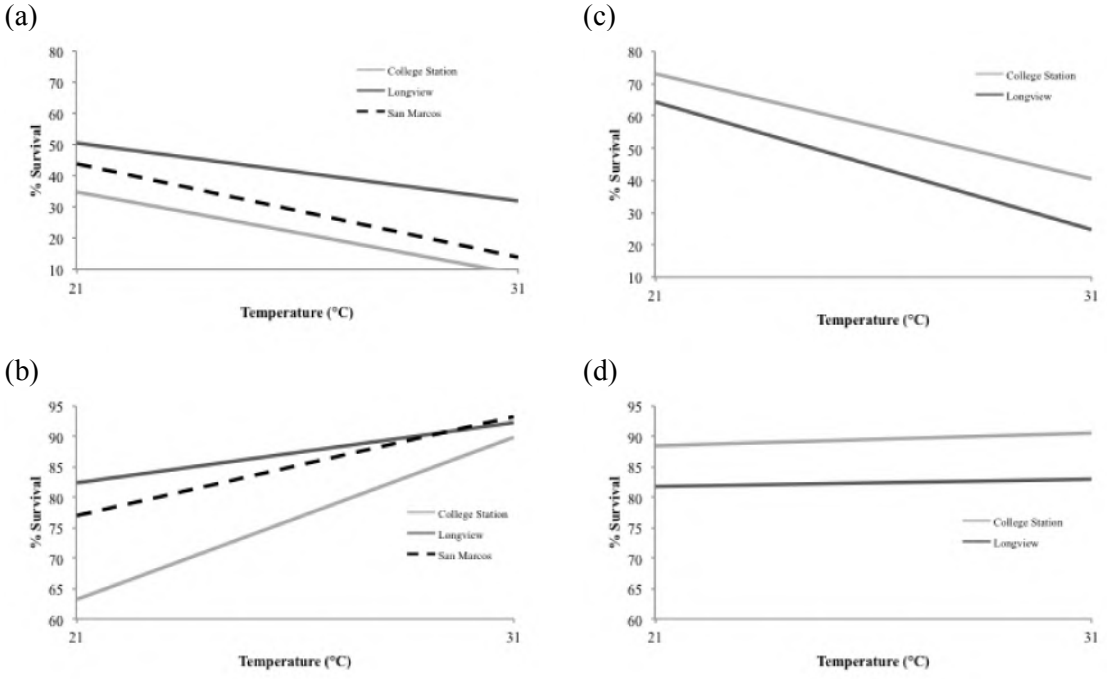
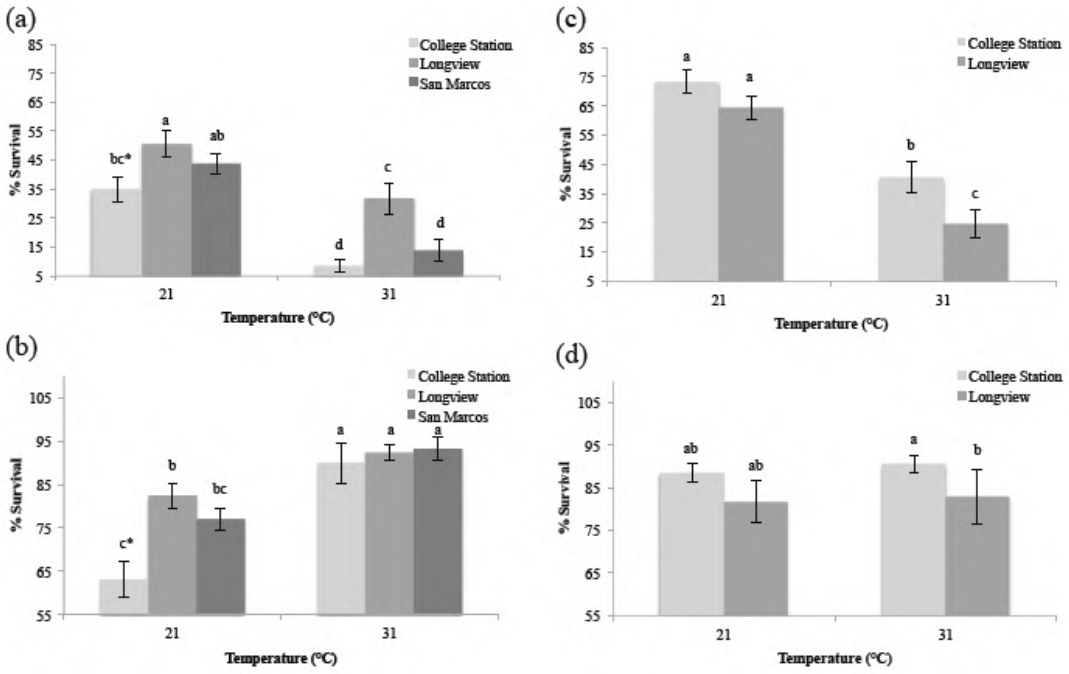


Figure 16. Comparisons between *C. macellaria* strains at 21°C and 31°C for immature and pupal survival ± SEM. N = 15 for all strains at 21°C; N = 13 for 2011 College Station, N = 14 for 2011 Longview and San Marcos at 31°C; N = 15 for 2012 College Station, N = 14 for 2012 Longview at 31°C. 2011 (a-b), 2012 (c-d); immature survival (%) (a, c), pupal survival (b, d). *Columns with different letters indicate significant difference ($P \leq 0.05$).



Sex

Significant differences were observed for every measure of all phenotypes as related to sex ($P < 0.0001$) (Table 17). Sex was a significant variable for pupal ($P < 0.0001$) and adult mass ($P = 0.0021$). No significant interactions were determined. Sex ratios can be found in Table 18 and Figure 17.

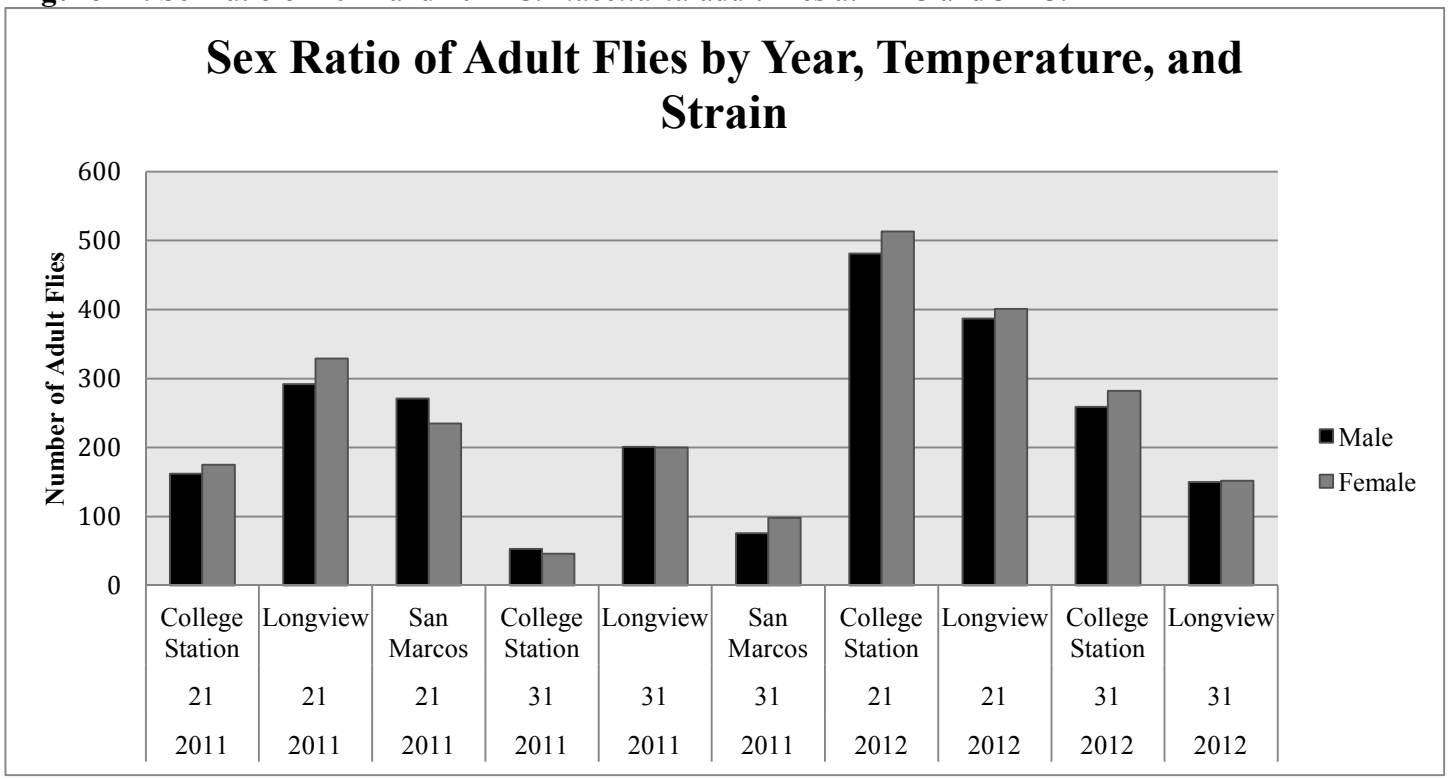
Table 17. ANOVA for 2011 *C. macellaria* sex at 21°C and 31°. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Immature Development Time	Friedman ANOVA	Model	11	49975364.00	398.36	**<0.0001**
		Error	2128	125454.02		
		Total	2139			
	Effect Tests	Sex	1	400884.48	3.20	0.0740
		Temp.XSex	1	141512.70	1.13	0.2883
		StrainXSex	2	114910.00	0.92	0.4003
		Temp.XStrainXSex	2	6181.48	0.05	0.9519
Pupal Development Time	Friedman ANOVA	Model	11	51697661.00	443.56	**<0.0001**
		Error	2128	116551.17		
		Total	2139			
	Effect Tests	Sex	1	12097.59	0.10	0.7474
		Temp.XSex	1	115948.14	0.99	0.3187
		StrainXSex	2	11984.13	0.10	0.9023
		Temp.XStrainXSex	2	60303.55	0.52	0.5961
Total Development Time	Friedman ANOVA	Model	11	48739225.00	369.67	**<0.0001**
		Error	2128	131843.83		
		Total	2139			
	Effect Tests	Sex	1	163176.23	1.24	0.2661
		Temp.XSex	1	418737.47	3.18	0.0749
		StrainXSex	2	133794.84	1.01	0.3627
		Temp.XStrainXSex	2	9961.10	0.08	0.9272
Pupal Mass	Friedman ANOVA	Model	11	5944273.00	16.84	**<0.0001**
		Error	2128	353058.00		
		Total	2139			
	Effect Tests	Sex	1	6264010.00	17.74	**<0.0001**
		Temp.XSex	1	43108.00	0.12	0.7268
		StrainXSex	2	60813.00	0.17	0.8418
		Temp.XStrainXSex	2	258510.00	0.73	0.4810
Adult Mass	Friedman ANOVA	Model	11	3516974.00	9.62	**<0.0001**
		Error	2128	365605.00		
		Total	2139			
	Effect Tests	Sex	1	3455141.00	9.45	**0.0021**
		Temp.XSex	1	10383.00	0.03	0.8662
		StrainXSex	2	34769.00	0.10	0.9093
		Temp.XStrainXSex	2	448493.00	1.23	0.2935

Table 18. Sex ratios for 2011 and 2012 *C. macellaria* adult flies at 21°C and 31°C.

Year	Temperature (C°)	Strain	Male	Female
2011	21	College Station	162	175
2011	21	Longview	292	329
2011	21	San Marcos	271	235
2011	31	College Station	53	46
2011	31	Longview	201	200
2011	31	San Marcos	76	98
2012	21	College Station	481	513
2012	21	Longview	387	401
2012	31	College Station	259	282
2012	31	Longview	150	152

Figure 17. Sex ratio of 2011 and 2012 *C. macellaria* adult flies at 21°C and 31°C.



Liver

Percent mass loss due to larval consumption and percent moisture were both significant variable ($P < 0.0001$) (Tables 19, 20). Percent mass loss at both temperatures for the three fly strains was significantly lower for controls than for all three strains. No significant difference in amount of liver consumed was determined between strains. Final percent moisture of liver provided to the strains at both temperatures was significantly lower than the control. No significant difference in final percent moisture was determined between strains at 21°C; however San Marcos exhibited a significantly lower moisture percentage than College Station and Longview at 31°C.

Table 19. ANOVA for mass loss percentage of liver given to *C. macellaria* strains for 2011 and 2012 at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Model	Source	Df	Mean Square	F ratio	Prob. > F
Friedman ANOVA	Model	11	28610.00	51.33	**<0.0001***
	Error	157	557.40		
	Total	168			

Table 20. ANOVA for moisture loss percentage of liver given to *C. macellaria* strains for 2011 and 2012 at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Model	Source	Df	Mean Square	F ratio	Prob. > F
Friedman ANOVA	Model	11	1954.07	45.33	**<0.0001***
	Error	177	43.11		
	Total	188			

2012

Immature Development

Temperature was a significant variable for all measures of larval development time ($P < 0.0001$), as was strain (minimum: $P = 0.0197$; maximum: $P=0.0416$; mean: $P < 0.0001$; median: $P < 0.0001$) (Table 21). A significant interaction was only observed for minimum larval duration ($P = 0.0197$).

No significant difference was observed between strains for minimum and maximum larval duration at 21°C (Table 22; Figures 5, 6e-h). College Station exhibited a statistically shorter duration than Longview for mean and median development times. College Station exhibited significantly shorter development times than Longview for all measures of immature development at 31°C.

Pupal Development

Temperature was a significant variable for all measures of pupal development ($P < 0.0001$), as was strain for maximum, mean, and median development times ($P < 0.0001$) (Table 23). Significant interactions were observed for minimum ($P = 0.0384$) and mean ($P < 0.0064$) pupal development times.

College Station exhibited significantly longer pupal duration than Longview for all measures at 21°C (Table 24; Figures 7, 8e-h). College Station also exhibited

significantly longer development times for maximum and median durations at 31°C. No statistical difference between strains was observed for minimum and mean duration.

Table 21. ANOVA table for 2012 *C. macellaria* immature development times on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Minimum Immature Development Time	Friedman ANOVA	Model	3	4616.20	62.35	**<0.0001***
		Error	56	74.04		
		Total	59			
	Effect Tests	Temp.	1	13380.27	180.71	**<0.0001**
		Strain	1	426.67	5.76	**0.0197**
		TempXStrain	1	41.67	0.56	0.4563
Maximum Immature Development Time	Friedman ANOVA	Model	3	4674.96	65.94	**<0.0001**
		Error	56	70.90		
		Total	59			
	Effect Tests	Temp.	1	13500.00	190.42	**<0.0001**
		Strain	1	308.27	4.35	**0.0416**
		TempXStrain	1	216.60	3.06	0.0860
Mean Immature Development Time	Friedman ANOVA	Model	3	4737.98	70.17	**<0.0001**
		Error	56	67.52		
		Total	59			
	Effect Tests	Temp.	1	13500.00	199.94	**<0.0001**
		Strain	1	707.27	10.48	**<0.0001**
		TempXStrain	1	6.67	0.10	0.7545
Median Immature Development Time	Friedman ANOVA	Model	3	4789.00	73.92	**<0.0001**
		Error	56	64.79		
		Total	59			
	Effect Tests	Temp.	1	13500.00	208.38	**<0.0001**
		Strain	1	866.40	13.37	**0.0006**
		TempXStrain	1	0.60	0.01	0.9237

Table 22. Phenotype comparisons for 2012 *C. macellaria* immature development times on beef liver at 21°C and 31°C

Phenotype	Temperature (C°)	Strain	N	Minimum (h)	Maximum (h)	Mean (h)	Median (h)	SEM
Minimum Immature Development Time	21	College Station	15	122.00	242.00	190.50	186.00	7.04
Minimum Immature Development Time	21	Longview	15	179.00	219.00	194.20	187.00	3.61
Minimum Immature Development Time	31	College Station	15	82.00	130.00	99.20	93.50	3.07
Minimum Immature Development Time	31	Longview	15	93.50	142.00	108.60	107.00	3.37
Maximum Immature Development Time	21	College Station	15	218.00	372.00	286.60	290.00	9.72
Maximum Immature Development Time	21	Longview	15	235.00	355.00	291.50	291.00	7.55
Maximum Immature Development Time	31	College Station	15	114.00	196.00	148.10	140.00	6.25
Maximum Immature Development Time	31	Longview	15	130.00	212.00	177.90	179.00	5.47
Mean Immature Development Time	21	College Station	15	184.10	263.20	222.50	226.70	4.68
Mean Immature Development Time	21	Longview	15	196.30	255.70	231.80	231.50	3.53
Mean Immature Development Time	31	College Station	15	102.50	140.00	120.10	123.00	3.40
Mean Immature Development Time	31	Longview	15	113.00	160.10	136.00	135.80	3.57
Median Immature Development Time	21	College Station	15	186.00	248.00	221.00	222.00	3.79
Median Immature Development Time	21	Longview	15	195.00	249.00	232.10	235.00	3.94
Median Immature Development Time	31	College Station	15	106.00	142.00	119.70	114.00	3.36
Median Immature Development Time	31	Longview	15	107.00	167.00	134.90	137.00	4.35

Table 23. ANOVA table for 2012 *C. macellaria* pupal development times on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. >F
Minimum Pupal Development Time	Friedman ANOVA	Model	3	4642.69	63.93	**<0.0001***
		Error	56	72.62		
		Total	59			
	Effect Tests	Temp.	1	13500.00	185.89	**<0.0001**
		Strain	1	101.40	1.40	0.2423
		TempXStrain	1	326.67	4.50	**0.0384**
Maximum Pupal Development Time	Friedman ANOVA	Model	3	5019.40	95.71	**<0.0001**
		Error	56	52.44		
		Total	59			
	Effect Tests	Temp.	1	13500.00	257.42	**<0.0001**
		Strain	1	1440.60	27.47	**<0.0001**
		TempXStrain	1	117.60	2.242	0.1399
Mean Pupal Development Time	Friedman ANOVA	Model	3	5079.84	103.24	**<0.0001**
		Error	56	49.20		
		Total	59			
	Effect Tests	Temp.	1	13500.00	274.36	**<0.0001**
		Strain	1	1344.27	27.32	**<0.0001**
		TempXStrain	1	395.27	8.03	**0.0064**
Median Pupal Development Time	Friedman ANOVA	Model	3	5280.56	137.33	**<0.0001**
		Error	56	38.45		
		Total	59			
	Effect Tests	Temp.	1	13500.00	351.08	**<0.0001**
		Strain	1	2306.40	59.98	**<0.0001**
		TempXStrain	1	35.27	0.92	0.3423

Table 24. Phenotype comparisons for 2012 *C. macellaria* pupal development times on beef liver at 21°C and 31°C.

Phenotype	Temperature (C°)	Strain	N	Minimum (h)	Maximum (h)	Mean (h)	Median (h)	SEM
Minimum Pupal Development Time	21	College Station	15	112.00	144.00	123.20	120.00	2.79
Minimum Pupal Development Time	21	Longview	15	104.00	128.00	117.30	120.00	1.86
Minimum Pupal Development Time	31	College Station	15	64.00	88.00	77.20	77.50	1.56
Minimum Pupal Development Time	31	Longview	14	64.00	86.00	79.90	80.00	1.65
Maximum Pupal Development Time	21	College Station	15	200.00	263.00	228.70	232.00	4.36
Maximum Pupal Development Time	21	Longview	15	168.00	259.00	192.10	184.00	5.81
Maximum Pupal Development Time	31	College Station	15	88.00	144.00	104.80	104.00	3.56
Maximum Pupal Development Time	31	Longview	14	93.00	112.00	99.70	98.50	1.37
Mean Pupal Development Time	21	College Station	15	150.10	190.10	169.70	168.90	3.51
Mean Pupal Development Time	21	Longview	15	131.40	154.00	140.70	138.40	1.66
Mean Pupal Development Time	31	College Station	15	86.30	95.00	89.80	89.20	0.62
Mean Pupal Development Time	31	Longview	14	85.00	92.80	88.90	88.90	0.47
Median Pupal Development Time	21	College Station	15	144.00	192.00	166.70	168.00	4.38
Median Pupal Development Time	21	Longview	15	128.00	160.00	137.30	136.00	2.40
Median Pupal Development Time	31	College Station	15	86.00	96.00	89.80	88.00	0.92
Median Pupal Development Time	31	Longview	14	86.00	92.00	87.90	88.00	0.45

Total Development

Temperature was a significant variable for all measures of total development time ($P < 0.0001$) (Table 25). Strain was a significant variable only for maximum total development ($P = 0.0176$). Significant interactions were observed for minimum ($P = 0.0109$) and median ($P = 0.0015$) total development times.

College Station exhibited a longer total development time than Longview for mean and median durations at 21°C (Table 26; Figures 9, 10e-h). No difference between strains was observed for minimum and maximum total development time. Longview exhibited significantly longer development times than College Station for all measures at 31°C.

Pupal Mass

Temperature was a significant variable for maximum ($P = 0.0040$), mean ($P < 0.0001$), and median ($P = 0.0004$) pupal mass (Table 27). Strain was a significant variable for maximum ($P = 0.0005$), mean ($P = 0004$), and median ($P < 0.0001$) pupal mass. Interactions were not significant for any aspect of pupal mass.

College Station exhibited significantly larger pupae than Longview for median pupal mass at 21°C; however, the strains were not statistically different at any other measure (Table 28; Figures 11, 12e-h). At 31°C, College Station exhibited significantly larger pupae than Longview for maximum and median pupal mass only.

Table 25. ANOVA table for 2012 *C. macellaria* total development times on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Minimum Total Development Time	Friedman ANOVA	Model	3	4672.38	65.78	**<0.0001***
		Error	56	71.03		
		Total	59			
	Effect Tests	Temp.	1	13500.00	190.05	**<0.0001**
		Strain	1	24.07	0.34	0.5629
		TempXStrain	1	493.07	6.94	**0.0109**
Maximum Total Development Time	Friedman ANOVA	Model	3	4666.42	65.40	**<0.0001**
		Error	56	71.35		
		Total	59			
	Effect Tests	Temp.	1	13500.00	189.20	**<0.0001**
		Strain	1	426.67	5.98	**0.0176**
		TempXStrain	1	72.60	1.02	0.3175
Mean Total Development Time	Friedman ANOVA	Model	3	4829.71	77.15	**<0.0001**
		Error	56	62.60		
		Total	59			
	Effect Tests	Temp.	1	13500.00	215.64	**<0.0001**
		Strain	1	13.07	0.21	0.6495
		TempXStrain	1	976.07	15.59	**0.0002**
Median Total Development Time	Friedman ANOVA	Model	3	4770.63	72.54	**<0.0001**
		Error	56	65.77		
		Total	59			
	Effect Tests	Temp.	1	13500.00	205.27	**<0.0001**
		Strain	1	77.07	1.17	0.2837
		TempXStrain	1	735.00	11.18	**0.0015**

Table 26. Phenotype summaries for 2012 *C. macellaria* total development times on beef liver at 21°C and 31°C.

Phenotype	Temperature (C°)	Strain	N	Minimum (h)	Maximum (h)	Mean (h)	Median (h)	SEM
Minimum Total Development Time	21	College Station	15	318.00	368.00	347.20	346.00	2.76
Minimum Total Development Time	21	Longview	15	313.00	355.00	340.90	347.00	3.15
Minimum Total Development Time	31	College Station	15	170.00	218.00	183.70	178.00	3.84
Minimum Total Development Time	31	Longview	14	172.00	228.00	199.90	195.50	4.63
Maximum Total Development Time	21	College Station	15	426.00	506.00	456.10	450.00	5.07
Maximum Total Development Time	21	Longview	15	387.00	530.00	46400	459.00	9.25
Maximum Total Development Time	31	College Station	15	218.00	292.00	240.50	234.00	5.87
Maximum Total Development Time	31	Longview	14	226.00	300.00	263.80	267.50	5.71
Mean Total Development Time	21	College Station	15	373.30	418.80	391.40	390.40	2.94
Mean Total Development Time	21	Longview	15	349.90	398.60	376.20	376.10	3.51
Mean Total Development Time	31	College Station	15	191.60	228.10	210.10	208.30	3.25
Mean Total Development Time	31	Longview	14	203.10	254.00	225.50	225.00	3.88
Median Total Development Time	21	College Station	15	370.00	419.00	386.70	386.00	3.85
Median Total Development Time	21	Longview	15	347.00	387.00	369.90	371.00	2.99
Median Total Development Time	31	College Station	15	194.00	228.00	210.90	210.00	3.36
Median Total Development Time	31	Longview	14	195.00	260.00	222.80	224.00	4.71

Table 27. ANOVA table for 2012 *C. macellaria* pupal mass on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Minimum Pupal Mass	Friedman ANOVA	Model	3	82.56	0.26	0.8535
		Error	56	316.92		
		Total	59			
	Effect Tests	Temp.	1	64.07	0.20	0.6547
		Strain	1	135.00	0.43	0.5166
		TempXStrain	1	48.60	0.15	0.6968
Maximum Pupal Mass	Friedman ANOVA	Model	3	1924.82	8.82	**<0.0001**
		Error	56	218.22		
		Total	59			
	Effect Tests	Temp.	1	1972.27	9.04	**0.0040**
		Strain	1	3024.60	13.86	**0.0005**
		TempXStrain	1	777.60	3.56	0.0643
Mean Pupal Mass	Friedman ANOVA	Model	3	2432.56	12.73	**<0.0001**
		Error	56	191.02		
		Total	59			
	Effect Tests	Temp.	1	4437.60	23.23	**<0.0001**
		Strain	1	2693.40	14.10	**0.0004**
		TempXStrain	35.2667	166.67	0.87	0.3543
Median Pupal Mass	Friedman ANOVA	Model	3	2257.00	11.26	**<0.0001**
		Error	56	200.43		
		Total	59			
	Effect Tests	Temp.	1	2884.27	14.39	**0.0004**
		Strain	1	3405.07	16.99	**<0.0001**
		TempXStrain	1	481.67	2.40	0.1267

Table 28. Phenotype summaries for 2012 *C. macellaria* pupal mass on beef liver at 21°C and 31°C.

Phenotype	Temperature (C°)	Strain	N	Minimum (mg)	Maximum (mg)	Mean (mg)	Median (mg)	SEM
Minimum Pupal Mass	21	College Station	15	2.90	28.60	11.40	7.80	2.11
Minimum Pupal Mass	21	Longview	15	1.70	35.00	12.10	6.80	2.89
Minimum Pupal Mass	31	College Station	15	5.10	37.40	11.60	8.10	2.55
Minimum Pupal Mass	31	Longview	15	2.50	16.20	8.20	8.10	0.87
Maximum Pupal Mass	21	College Station	15	45.50	58.70	49.60	49.10	0.88
Maximum Pupal Mass	21	Longview	15	39.90	54.60	47.80	47.50	0.91
Maximum Pupal Mass	31	College Station	15	37.40	52.40	47.90	49.40	0.99
Maximum Pupal Mass	31	Longview	15	33.70	49.70	43.20	43.40	1.06
Mean Pupal Mass	21	College Station	15	25.00	43.20	37.40	37.80	1.12
Mean Pupal Mass	21	Longview	15	16.40	44.40	33.90	34.00	1.75
Mean Pupal Mass	31	College Station	15	24.50	39.20	33.30	32.70	1.08
Mean Pupal Mass	31	Longview	15	21.30	35.30	27.50	27.30	1.10
Median Pupal Mass	21	College Station	15	32.70	43.90	38.90	38.90	0.80
Median Pupal Mass	21	Longview	15	9.80	44.00	35.40	37.10	2.04
Median Pupal Mass	31	College Station	15	14.00	45.00	35.90	36.80	1.98
Median Pupal Mass	31	Longview	15	15.70	37.40	28.60	37.40	1.93

Adult Mass

Temperature was a significant variable for all measures ($P < 0.0001$) except maximum adult mass ($P = 0.8464$) (Table 29). Strain was a significant variable for maximum ($P < 0.0001$), mean ($P < 0.0001$) and median ($P = 0.0002$) adult mass. Interaction effect tests were only significant for maximum adult mass ($P = 0.0046$).

College Station exhibited significantly larger adults than Longview only for mean adult mass at 21°C (Table 30; Figures 13, 14e-h). At 31°C, College Station exhibited significantly larger adults than Longview for maximum, mean, and median adult mass.

Survival

Temperature was a significant variable for larval ($P < 0.0001$), but not pupal, survival (Table 31). Strain was a significant variable for both larval ($P = 0.0133$) and pupal ($P = 0.0474$) survival, yet interaction effect tests were significant for neither.

At 21°C, larval nor pupal survival were not significantly different between strains (Table 32; Figures 15, 16e-h). At 31°C, however, College Station exhibited significantly higher survival percentages than Longview for both larvae and pupae.

Table 29. ANOVA table for 2012 *C. macellaria* adult mass on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Minimum Adult Mass	Friedman ANOVA	Model	3	1683.80	7.28	**0.0003***
		Error	56	231.14		
		Total	59			
	Effect Tests	Temp.	1	4611.27	19.95	**<0.0001**
		Strain	1	416.07	1.80	0.1851
		TempXStrain	1	24.07	0.10	0.7481
Maximum Adult Mass	Friedman ANOVA	Model	3	2022.47	9.50	**<0.0001**
		Error	56	212.99		
		Total	59			
	Effect Tests	Temp.	1	8.07	0.04	0.8464
		Strain	1	4200.07	19.72	**<0.0001**
		TempXStrain	1	1859.27	8.73	**0.0046**
Mean Adult Mass	Friedman ANOVA	Model	3	3155.22	20.72	**<0.0001**
		Error	56	152.31		
		Total	59			
	Effect Tests	Temp.	1	6000.00	39.39	**<0.0001**
		Strain	1	3110.40	20.42	**<0.0001**
		TempXStrain	1	355.27	2.33	0.1323
Median Adult Mass	Friedman ANOVA	Model	3	2708.11	15.36	<0.0001
		Error	56	176.26		
		Total	59			
	Effect Tests	Temp.	1	5041.67	28.60	**<0.0001**
		Strain	1	2774.40	15.74	**0.0002**
		TempXStrain	1	308.27	1.75	0.1914

Table 30. Phenotype summaries for 2012 *C. macellaria* adult mass on beef liver at 21°C and 31°C.

Phenotype	Temperature (C°)	Strain	N	Minimum (mg)	Maximum (mg)	Mean (mg)	Median (mg)	SEM
Minimum Adult Mass	21	College Station	15	1.60	6.70	4.00	4.90	0.51
Minimum Adult Mass	21	Longview	15	1.00	7.00	3.50	2.00	0.55
Minimum Adult Mass	31	College Station	15	1.00	8.00	2.50	1.40	0.57
Minimum Adult Mass	31	Longview	14	1.00	6.00	2.00	1.50	0.37
Maximum Adult Mass	21	College Station	15	9.90	13.90	11.20	11.40	0.25
Maximum Adult Mass	21	Longview	15	8.80	11.90	10.70	10.70	.022
Maximum Adult Mass	31	College Station	15	8.00	15.70	12.50	12.70	0.51
Maximum Adult Mass	31	Longview	14	7.40	13.00	10.00	10.00	0.36
Mean Adult Mass	21	College Station	15	6.70	9.90	8.50	8.40	0.19
Mean Adult Mass	21	Longview	15	7.40	9.00	8.00	7.70	0.15
Mean Adult Mass	31	College Station	15	5.20	9.50	7.60	7.60	0.29
Mean Adult Mass	31	Longview	14	4.10	7.80	6.10	6.10	0.28
Median Adult Mass	21	College Station	15	7.70	10.00	8.60	8.60	0.16
Median Adult Mass	21	Longview	15	7.30	9.00	8.20	8.10	0.13
Median Adult Mass	31	College Station	15	4.70	10.40	7.90	8.00	0.39
Median Adult Mass	31	Longview	14	3.00	8.10	6.40	7.00	0.44

Table 31. ANOVA table for 2012 *C. macellaria* immature and pupal survival on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
% Immature Survival	Friedman ANOVA	Model	3	3258.96	22.21	**<0.0001***
		Error	56	146.75		
		Total	59			
	Effect Tests	Temp.	1	8784.60	59.86	**<0.0001**
		Strain	1	960.00	6.54	**0.0133**
		TempXStrain	1	32.27	0.22	0.6410
% Pupal Survival	Friedman ANOVA	Model	3	481.18	1.63	0.1932
		Error	56	295.56		
		Total	59			
	Effect Tests	Temp.	1	4.27	0.01	0.9048
		Strain	1	1215.00	4.11	**0.0474**
		TempXStrain	1	224.27	0.76	0.3874

Table 32. Phenotype summaries for 2012 *C. macellaria* immature and pupal survival on beef liver at 21°C and 31°C

Phenotype	Temperature (C°)	Strain	N	Minimum (%)	Maximum (%)	Mean (%)	Median (%)	SEM
% Immature Survival	21	College Station	15	40.00	99.00	73.20	70.00	4.08
% Immature Survival	21	Longview	15	35.00	91.00	64.30	62.00	4.22
% Immature Survival	31	College Station	15	1.00	74.00	40.50	43.00	5.45
% Immature Survival	31	Longview	15	7.00	61.00	26.20	19.50	4.86
% Pupal Survival	21	College Station	15	76.30	100.00	88.50	88.50	2.09
% Pupal Survival	21	Longview	15	25.70	97.80	81.70	86.10	4.89
% Pupal Survival	31	College Station	15	71.40	100.00	90.60	91.70	2.01
% Pupal Survival	31	Longview	15	57.10	95.70	82.90	87.00	6.34

Sex

Sex was a significant variable for pupal mass (P = 0.0001) and adult mass (P = 0.0072) (Table 33). Temperature-sex interactions were significant for adult mass only. Strain–sex and temperature-strain-sex interactions were not significant for any phenotypes. Sex ratios can be found in Table 18 and Figure 16.

Table 33. ANOVA table for 2012 *C. macellaria* sex at 21°C and 31°. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Immature Development Time	Friedman ANOVA	Model	11	96255471.00	494.04	**<0.0001***
		Error	2652	194834.28		
		Total	2663			
	Effect Tests	Sex	2	121364.00	0.62	0.5365
		Temp.XSex	2	157575.00	0.81	0.4455
		StrainXSex	2	315640.00	1.62	0.1981
		Temp.XStrainXSex	2	21181.00	0.11	0.8970
Pupal Development Time	Friedman ANOVA	Model	11	106951329.00	710.78	**<0.0001**
		Error	2652	150469.86.00		
		Total	2663			
	Effect Tests	Sex	2	1037.00	0.01	0.9931
		Temp.XSex	2	72548.00	0.48	0.6175
		StrainXSex	2	35195.00	0.23	0.7915
		Temp.XStrainXSex	2	33386.00	0.22	0.8010
Total Development Time	Friedman ANOVA	Model	11	98629874.00	533.18	**<0.0001**
		Error	2652	184985.7.00		
		Total	2663			
	Effect Tests	Sex	2	20986.00	0.11	0.8928
		Temp.XSex	2	306151.00	1.66	0.1913
		StrainXSex	2	220978.00	1.19	0.3030
		Temp.XStrainXSex	2	31790.00	0.17	0.8421

Table 33 Continued

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Pupal Mass	Friedman ANOVA	Model	11	14860052.00	27.93	**<0.0001**
		Error	2652	531979.09		
		Total	2663			
	Effect Tests	Sex	2	4812788.00	9.05	**0.0001**
		Temp.XSex	2	566063.00	1.06	0.3452
		StrainXSex	2	380163.00	0.71	0.4895
		Temp.XStrainXSex	2	915890.00	1.72	0.1790
Adult Mass	Friedman ANOVA	Model	11	12726925.00	23.51	**<0.0001**
		Error	2652	541295.06		
		Total	2663			
	Effect Tests	Sex	2	2678207.00	4.95	**0.0072**
		Temp.XSex	2	1818434.00	3.36	**0.0349**
		StrainXSex	2	287292.00	0.53	0.5882
		Temp.XStrainXSex	2	393427.00	0.73	0.4835

Liver

Percentage mass loss due to larval consumption and percentage of moisture were significant variables ($P < 0.0001$) (Tables 19-20). Percentage mass loss at both temperatures was significantly lower for the controls compared to both strains. College Station exhibited a significantly higher percentage mass loss than Longview at 21°C. No significant difference between strains was observed at 31°C. Moisture percentage for the controls was significantly higher than either strain at both temperatures. No significant difference between strains was observed at either temperature.

Yearly Comparison

Immature Development

Year and temperature were significant variables for all measures (maximum: P = 0.0001; all others: P < *0*.0001) (Table 34). Strain was a significant variable only for minimum larval development time (P = 0.0304). Three-way interaction effects were significant for mean (P = 0.0016) and median (P = 0.0076) immature development time.

The 2011 College Station strain exhibited significantly longer immature developmental times than the 2012 strain for every measure at 21°C, and for all measures except maximum larval duration at 31°C. The 2011 Longview strain exhibited significantly longer larval development times than the 2012 strain for every measure at 21°C; however the 2012 strain exhibited a longer maximum and mean duration at 31°C.

Table 34. ANOVA table for 2011 and 2012 *C. macellaria* immature development time on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Minimum Immature Development Time	Friedman ANOVA	Model	7	26129.80	100.81	**<0.0001***
		Error	109	259.20		
		Total	116			
	Effect Tests	Year	1	17883.60	69.00	**<0.0001**
		Temp.	1	152564.70	588.63	**<0.0001**
		YearXTemp.	1	693.80	2.68	0.1047
		Strain	1	1247.20	4.81	**0.0304**
		YearXStrain	1	6598.40	25.46	**<0.0001**
		Temp.XStrain	1	9.50	0.037	0.8484
		YearXTemp.XStrain	1	790.40	3.05	0.0836
Maximum Immature Development Time	Friedman ANOVA	Model	7	23186.30	82.01	**<0.0001**
		Error	109	282.70		
		Total	116			
	Effect Tests	Year	1	4401.40	15.57	**0.0001**
		Temp.	1	142096.20	502.58	**<0.0001**
		YearXTemp.	1	9229.10	32.64	**<0.0001**
		Strain	1	0.92	0.00	0.9547
		YearXStrain	1	4139.20	14.64	**0.0002**
		Temp.XStrain	1	1439.10	5.09	**0.0261**
		YearXTemp.XStrain	1	509.10	1.80	0.1824
Mean Immature Development Time	Friedman ANOVA	Model	7	25417.50	133.78	**<0.0001**
		Error	109	190.00		
		Total	116			
	Effect Tests	Year	1	16059.90	84.53	**<0.0001**
		Temp.	1	144226.50	759.09	**<0.0001**
		YearXTemp.	1	3598.90	18.94	**<0.0001**
		Strain	1	646.20	3.40	0.0679
		YearXStrain	1	8738.80	45.99	**<0.0001**
		Temp.XStrain	1	125.30	0.66	0.4184
		YearXTemp.XStrain	1	1996.90	10.51	**0.0016**

Table 34 Continued

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Median Immature Development Time	Friedman ANOVA	Model	7	24844.10	117.62	**<0.0001**
		Error	109	211.20		
		Total	116			
	Effect Tests	Year	1	17695.60	83.78	**<0.0001**
		Temp.	1	141237.20	668.69	**<0.0001**
		YearXTemp.	1	2692.90	12.75	**0.0005**
		Strain	1	107.00	0.51	0.4782
		YearXStrain	1	7798.02	36.92	**<0.0001**
		Temp.XStrain	1	82.50	0.39	0.5334
		YearXTemp.XStrain	1	1561.10	7.39	**0.0076**

Pupal Development

Temperature was a significant variable ($P < 0.0001$) for all measures of pupal development (Table 35). Year was a significant variable for minimum ($P < 0.0001$), mean ($P = 0.0121$), and median ($P = 0.0062$) pupal durations. Strain was only a significant variable for median pupal duration ($P = 0.0214$). Three-way interactions were significant for all measures (minimum: $P = 0.0006$; maximum: $P = 0.0031$; mean: $P < 0.0001$; median $P = 0.0018$).

The 2011 College Station strain exhibited significantly longer minimum pupal development times than the 2012 strain at both temperatures. However, the 2012 strain exhibited significantly longer maximum, mean, and median pupal duration than the 2011 strain at 31°C. The 2011 Longview strain exhibited significantly longer pupal duration than the 2012 strain for all measures at 21°C. However, the 2012 Longview strain

exhibited significantly longer pupal times for minimum, mean, and median duration than the 2011 strain at 31°C.

Table 35. ANOVA table for 2011 and 2012 *C. macellaria* pupal development time on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Minimum Pupal Development Time	Friedman ANOVA	Model	7	22526.30	82.52	**<0.0001***
		Error	109	273.00		
		Total	116			
	Effect Tests	Year	1	10199.30	37.36	**<0.0001**
		Temp.	1	133616.00	489.48	**<0.0001**
		YearXTemp.	1	7504.30	27.49	**<0.0001**
		Strain	1	748.60	2.74	0.1006
		YearXStrain	1	608.10	2.23	0.1384
		Temp.XStrain	1	68.50	0.25	0.6175
		YearXTemp.XStrain	1	3423.50	12.54	**0.0006**
Maximum Pupal Development Time	Friedman ANOVA	Model	7	22149.60	72.69	**<0.0001**
		Error	109	304.70		
		Total	116			
	Effect Tests	Year	1	40.10	0.13	0.7176
		Temp.	1	136799.40	448.92	**<0.0001**
		YearXTemp.	1	5887.80	19.32	**<0.0001**
		Strain	1	21.70	0.07	0.7903
		YearXStrain	1	10912.00	35.81	**<0.0001**
		Temp.XStrain	1	9.20	0.03	0.8624
		YearXTemp.XStrain	1	2780.30	9.12	**0.0031**

Table 35 Continued

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Mean Pupal Development Time	Friedman ANOVA	Model	7	22620.60	121.57	**<0.0001**
		Error	109	186.10		
		Total	116			
	Effect Tests	Year	1	1210.50	6.51	**0.0121**
		Temp.	1	129263.00	694.73	**<0.0001**
		YearXTemp.	1	18763.90	100.85	**<0.0001**
		Strain	1	524.80	2.82	0.0959
		YearXStrain	1	3419.50	18.38	**<0.0001**
		Temp.XStrain	1	1018.30	5.47	**0.0211**
		YearXTemp.XStrain	1	4861.40	26.13	**<0.0001**
Median Pupal Development Time	Friedman ANOVA	Model	7	23651.20	111.06	**<0.0001**
		Error	109	213.00		
		Total	116			
	Effect Tests	Year	1	16587.00	7.79	**0.0062**
		Temp.	1	137019.80	643.44	**<0.0001**
		YearXTemp.	1	17079.00	80.20	**<0.0001**
		Strain	1	1160.40	5.45	**0.0214**
		YearXStrain	1	6017.10	28.26	**<0.0001**
		Temp.XStrain	1	1088.00	5.11	**0.0258**
		YearXTemp.XStrain	1	2178.00	10.23	**0.0018**

Total Development

Temperature and year were significant variables for all measures of total development (maximum total development: $P = 0.0012$; all others: $P < 0.0001$) (Table 36). Strain was not a significant variable for any measure. Three-way interactions were significant for all measures (maximum total development: $P = 0.0407$; all others: $P < 0.0001$).

The 2011 College Station strain exhibited significantly longer total development times for all measures (except maximum duration at 31°C) than the 2012 strain at both temperatures. The 2011 Longview strain exhibited significantly longer total development times for all measures at 21°C, but only maximum total development time at 31°C. No other measures were significant.

Table 36. ANOVA table for 2011 and 2012 *C. macellaria* total development time on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Minimum Total Development Time	Friedman ANOVA	Model	7	25453.70	126.07	**<0.0001***
		Error	109	201.90		
		Total	116			
	Effect Tests	Year	1	18019.50	89.25	**<0.0001**
		Temp.	1	144951.80	717.91	**<0.0001**
		YearXTemp.	1	3371.40	16.70	**<0.0001**
		Strain	1	549.30	2.72	0.1019
		YearXStrain	1	2692.20	13.33	**0.0004**
		Temp.XStrain	1	422.60	2.09	0.1509
		YearXTemp.XStrain	1	5507.60	27.28	**<0.0001**
Maximum Total Development Time	Friedman ANOVA	Model	7	22191.60	69.09	**<0.0001**
		Error	109	321.20		
		Total	116			
	Effect Tests	Year	1	3580.80	11.15	**0.0012**
		Temp.	1	137934.60	429.45	**<0.0001**
		YearXTemp.	1	9129.60	28.42	**<0.0001**
		Strain	1	554.70	1.73	0.1916
		YearXStrain	1	2419.80	7.53	**0.0071**
		Temp.XStrain	1	0.00	0.00	0.9984
		YearXTemp.XStrain	1	1378.40	4.29	0.0407

Table 36 Continued

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Mean Total Development Time	Friedman ANOVA	Model	7	24823.10	129.28	**<0.0001**
		Error	109	192.00		
		Total	116			
	Effect Tests	Year	1	15260.00	79.47	**<0.0001**
		Temp.	1	139177.50	724.84	**<0.0001**
		YearXTemp.	1	4904.20	25.54	**<0.0001**
		Strain	1	481.40	2.51	0.1162
		YearXStrain	1	2138.90	11.14	**0.0012**
		Temp.XStrain	1	473.50	2.47	0.1192
Median Total Development Time	Friedman ANOVA	Model	7	23854.30	112.47	**<0.0001**
		Error	109	212.10		
		Total	116			
	Effect Tests	Year	1	16551.10	78.04	**<0.0001**
		Temp.	1	135383.70	638.31	**<0.0001**
		YearXTemp.	1	4236.70	19.98	**<0.0001**
		Strain	1	463.50	2.19	0.1422
		YearXStrain	1	910.40	4.29	**0.0406**
		Temp.XStrain	1	479.20	2.26	0.1357
		YearXTemp.XStrain	1	6552.70	30.89	**<0.0001**

Pupal Mass

Year was a significant variable for minimum (P = 0.0026) and maximum (P = 0.0002) pupal mass (Table 37). Temperature was a significant variable for maximum (P < *0*.0001) and median (P = 0.0033) pupal mass. Strain was a significant variable for mean (P = 0.0268) and median (P = 0.0079) pupal mass. Three-way interactions were not significant for pupal mass.

The 2012 College Station strain exhibited significantly larger pupae for maximum, mean, and median pupal mass at 21°C and maximum pupal mass at 31°C

than the 2011 strain. The 2011 College Station strain exhibited significantly larger pupae than the 2012 strain for only for the minimum pupal mass at 31°C. There was no significant difference between the 2011 and 2012 Longview strains for any measure of pupal mass at either temperature.

Table 37. ANOVA table for 2011 and 2012 *C. macellaria* pupal mass on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Minimum Pupal Mass	Friedman ANOVA	Model	7	3940.34	2.36	**0.0275***
		Error	109	1667.03		
		Total	116			
	Effect Tests	Year	1	15867.05	9.52	**0.0026**
		Temp.	1	4053.22	2.43	0.1218
		YearXTemp.	1	3290.50	1.97	0.1629
		Strain	1	3852.69	2.31	0.1313
		YearXStrain	1	956.06	0.57	0.4505
		Temp.XStrain	1	480.37	0.29	0.5925
		YearXTemp.XStrain	1	1058.90	0.64	0.4272
Maximum Pupal Mass	Friedman ANOVA	Model	7	11200.00	9.67	**<0.0001**
		Error	109	1158.70		
		Total	116			
	Effect Tests	Year	1	17639.04	15.22	**0.0002**
		Temp.	1	28258.67	24.39	**<0.0001**
		YearXTemp.	1	32.40	0.03	0.8675
		Strain	1	1561.44	1.65	0.2482
		YearXStrain	1	23014.45	19.86	**<0.0001**
		Temp.XStrain	1	8283.04	7.15	**0.0087**
		YearXTemp.XStrain	1	34.33	0.03	0.8637

Table 37 Continued

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Mean Pupal Mass	Friedman ANOVA	Model	7	8104.71	5.59	**<0.0001**
		Error	109	1450.87		
		Total	116			
	Effect Tests	Year	1	253.81	0.17	0.6766
		Temp.	1	17584.95	12.12	**0.0007**
		YearXTemp.	1	10405.13	7.17	**0.0086**
		Strain	1	7315.44	5.04	**0.0268**
		YearXStrain	1	10681.03	7.36	**0.0077**
		Temp.XStrain	1	7595.28	5.24	**0.0241**
		YearXTemp.XStrain	1	1612.51	1.11	0.2941
Median Pupal Mass	Friedman ANOVA	Model	7	7802.47	5.40	**<0.0001**
		Error	109	1445.99		
		Total	116			
	Effect Tests	Year	1	831.73	0.58	0.4498
		Temp.	1	13079.73	9.05	**0.0033**
		YearXTemp.	1	6129.95	4.24	**0.0419**
		Strain	1	10585.23	7.32	**0.0079**
		YearXStrain	1	10188.79	7.05	**0.0091**
		Temp.XStrain	1	12158.79	8.41	**0.0045**
		YearXTemp.XStrain	1	568.33	0.39	0.5320

Adult Mass

Year was a significant variable for minimum (P = 0.0288) and maximum (P = 0.0142) (Table 38). Temperature was a significant variable for minimum (P = 0.0037), mean (P = 0.0005), and median (P = 0.0176). Strain was a significant variable for minimum (P = 0.0018), mean (P = 0.0035), and median (P = 0.0116) adult mass. Three-way interactions were significant only for maximum adult mass (P = 0.0446).

The 2012 College Station strain exhibited larger adults for maximum, mean, and median adult mass at 21°C and maximum adult mass at 31°C than the 2011 strain. The 2011 College Station strain exhibited larger adults than the 2012 strain only for the minimum adult mass at 31°C. No significant difference was observed between the 2011 and 2012 Longview strains for any adult mass measure at 21°C. However, at 31°C, the 2011 Longview strain exhibited significantly larger adults than the 2012 strain for maximum, mean, and median adult mass.

Table 38. ANOVA table for 2011 and 2012 *C. macellaria* adult mass on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Minimum Adult Mass	Friedman ANOVA	Model	7	7034.41	4.68	**<0.0001***
		Error	109	1502.50		
		Total	116			
	Effect Tests	Year	1	7372.93	4.91	**0.0288**
		Temp.	1	13242.82	8.81	**0.0037**
		YearXTemp.	1	7780.38	5.18	**0.0248**
		Strain	1	15337.54	10.21	**0.0018**
		YearXStrain	1	4220.62	2.81	0.0966
		Temp.XStrain	1	488.98	0.33	0.5695
		YearXTemp.XStrain	1	1320.40	0.88	0.3506
Maximum Adult Mass	Friedman ANOVA	Model	7	7055.63	5.03	**<0.0001**
		Error	109	1404.05		
		Total	116			
	Effect Tests	Year	1	8730.02	6.22	**0.0142**
		Temp.	1	1196.17	0.85	0.3580
		YearXTemp.	1	3177.87	2.26	0.1354
		Strain	1	4022.16	2.86	0.0934
		YearXStrain	1	21840.69	15.56	**0.0001**
		Temp.XStrain	1	3535.40	2.52	0.1155
		YearXTemp.XStrain	1	5794.77	4.12	**0.0446**

Table 38 Continued

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
Mean Adult Mass	Friedman ANOVA	Model	7	9434.38	7.07	**<0.0001**
		Error	109	1333.57		
		Total	116			
	Effect Tests	Year	1	1.61	0.00	0.9723
		Temp.	1	17421.60	13.06	**0.0005**
		YearXTemp.	1	20954.34	15.71	**0.0001**
		Strain	1	11889.86	8.92	**0.0035**
		YearXStrain	1	5594.62	4.20	**0.0429**
		Temp.XStrain	1	8786.40	6.59	**0.0116**
		YearXTemp.XStrain	1	86.29	0.06	0.7997
Median Adult Mass	Friedman ANOVA	Model	7	8067.93	5.90	**<0.0001**
		Error	109	1366.54		
		Total	116			
	Effect Tests	Year	1	2.17	0.00	0.9683
		Temp.	1	7937.99	5.81	**0.0176**
		YearXTemp.	1	23876.82	17.47	**<0.0001**
		Strain	1	9000.52	6.59	**0.0116**
		YearXStrain	1	6626.68	4.85	**0.0298**
		Temp.XStrain	1	7749.09	5.67	**0.0190**
		YearXTemp.XStrain	1	349.96	0.26	0.6138

Survival

Temperature was a significant variable for both larval and pupal survival ($P < 0.0001$) (Table 39). Year was a significant variable only for larval survival ($P < 0.0001$), and strain and three-way interactions yielded no significant results for larval or pupal survival.

The 2012 College Station strain exhibited significantly higher survival percentages compared to the 2011 strain for larval survival at both temperatures and pupal survival at 21°C. The 2012 Longview strain exhibited significantly higher larval

survival percentages than the 2011 strain at 21°C; however, the 2011 Longview strain exhibited the higher pupal survival percentages at 31°C.

Table 39. ANOVA table for 2011 and 2012 *C. macellaria* immature and pupal survival on beef liver at 21°C and 31°C. *indicates significance at $P \leq 0.05$.

Phenotype	Model	Source	Df	Mean Square	F ratio	Prob. > F
% Immature Survival	Friedman ANOVA	Model	7	17059.80	20.13	**<0.0001***
		Error	109	847.60		
		Total	116			
	Effect Tests	Year	1	28976.98	34.19	**<0.0001**
		Temp.	1	69145.55	81.58	**<0.0001**
		YearXTemp.	1	2444.60	2.88	0.0923
		Strain	1	1347.94	1.59	0.2100
		YearXStrain	1	18770.59	22.15	**<0.0001**
		Temp.XStrain	1	6.27	0.01	0.9316
		YearXTemp.XStrain	1	1881.51	2.22	0.1391
% Pupal Survival	Friedman ANOVA	Model	7	9382.79	7.31	**<0.0001**
		Error	109	1284.33		
		Total	116			
	Effect Tests	Year	1	780.75	0.61	0.4373
		Temp.	1	24925.00	19.41	**<0.0001**
		YearXTemp.	1	22324.51	17.38	**<0.0001**
		Strain	1	207.65	0.16	0.6884
		YearXStrain	1	10275.16	8.00	**0.0056**
		Temp.XStrain	1	6248.95	4.87	**0.0295**
		YearXTemp.XStrain	1	1271.40	0.99	0.3220

Sex

As sex was determined to be a significant variable for pupal mass in both 2011 and 2012, it was added into the model for comparison across years for each strain within each temperature, giving a 4-way Friedman ANOVA. Due to insufficient data, however, it was not possible to generate this model.

Discussion

Phenotypic variability was determined for the three strains of *C. macellaria* examined in this study. To date, this is the first study to examine plasticity of a forensically important species within a single US state. Expression of plastic responses was impacted by abiotic variables, as well as their interactions.

Phenotypic ranges observed for *C. macellaria* overlapped and fell within those given in previous studies from College Station, Texas, USA (Boatright and Tomberlin 2010), and Gainesville, Florida, USA (Byrd and Butler 1996). However, when comparing College Station strains from 2011 and 2012 to immature and pupal datasets generated for *C. macellaria* by Boatright and Tomberlin (2010), it is clear that a range of ontogenetic variation is possible at different life stages (Table 40). Examining Table 40 shows that the variation can range from 2-51% (2-62 hours) between the datasets, with the higher temperature showing less variation than the lower temperature. It would appear that tissue type does not play a significant role in developmental variation in the pupal stage at the higher temperature as datasets vary by < 5 hours. However, development time of the immature stage is highly variable between datasets, which may

indicate that tissue type has a much more drastic impact on larval, rather than pupal, development. Overall, two developmental datasets collected for the same species within the same region can exhibit drastic variability in the immature stage while showing little to no variation in the pupal stage. Variability in responses recorded across studies could be due to differences in experimental design (Tarone and Foran 2006). Tissue substrates used for larval growth were not consistent for all three studies, as Byrd and Butler (1996) and Boatright and Tomberlin (2010) both utilized porcine muscle tissue, whereas the current study utilized bovine liver. Although Boatright and Tomberlin (2010) found no difference in larval development between substrates, tissue type has been shown to significantly affect other parameters of blow fly development, including larval weight and length of *C. vicina* (Kaneshrajah and Turner 2004), larval development time of *Calliphora vomitoria* Linnaeus (Ireland and Turner 2006), and larval development time, larval size, and adult size of *L. sericata* (Clark et al. 2006). Given this, it is reasonable to assume that *C. macellaria* may also possess plasticity regarding ontogenetic response to various feeding substrates, and that the responses measured here reflect only a portion of its true potential to produce variable phenotypes.

Table 40. Comparison of immature and pupal development times of College Station strains between the current study and Boatright and Tomberlin (2010). The first number in the development columns represents the percent difference, while the number in parentheses represents the difference in hours.

		Owings 2012 College Station Data			
		% Difference in Immature Development Time (hours)		% Difference in Pupal Development Time (hours)	
	Temperature (°C)	2011 College Station	2012 College Station	2011 College Station	2012 College Station
Boatright and Tomberlin (2010)- Equine	21	-8.08 (23.20)	18.47 (41.1)	-13.24 (23.70)	-8.49 (14.40)
Boatright and Tomberlin (2010)- Porcine	21	-5.22 (15.00)	22.16 (49.3)	-17.87 (32.00)	-13.38 (22.70)
Boatright and Tomberlin (2010)- Equine	31	12.98 (18.90)	36.97 (44.4)	-3.12 (2.70)	-5.46 (4.90)
Boatright and Tomberlin (2010)- Porcine	31	25.21 (36.70)	51.79 (62.2)	-2.12 (1.90)	-4.57 (4.10)

Other potential sources of variation due to laboratory procedure are the utilization of either constant or cyclical temperatures, photoperiods, and destructive sampling. *Lucilia illustris* Meigen (Diptera: Calliphoridae) development time can decrease when exposed to fluctuating temperatures in comparison to constant temperatures (Hanski 1977). However, no significant difference was observed on the overall accumulated degree hours required for *C. vicina* development in a study comparing laboratory-reared strains at constant temperatures and strains raised in the field under natural conditions (Donovan 2006). The effects of photoperiod can also be significant, as an 18% decrease in larval development time and a 13% decrease in time to adult emergence has been documented for *Phormia regina* Meigen (Diptera: Calliphoridae) raised under constant light versus cyclic light (Nabity et al. 2007). Variability in quantitative traits has also been documented for *L. sericata*, as destructive sampling of larvae has been shown to significantly increase pupal development time by 30% (Tarone and Foran 2006). Overall, many different factors must be taken into account when comparing development data across studies, as laboratory maintenance, rearing procedures, and sampling regimes have not been standardized. For this reason, many of the differences between conspecific developmental datasets could be attributed to plasticity in response to the techniques utilized by the researcher.

Considerable temperature-induced plasticity was observed for all phenotypes in this study; however responses measured did not consistently follow traditional biological rules regarding effects of temperature on development time or body size. This is particularly evident for 2011 College Station and San Marcos pupal development (Figures 6, 7a-d), and pupal (Figures 10, 11a-d) and adult (Figures 12, 13a-d) size. The

temperature-size rule (TSR), or "*hotter is smaller"* hypothesis*,* states that regions experiencing higher temperatures should select for smaller body sizes and vice versa (Atkinson 1994, Atkinson and Sibly 1996). Alternatively, the *"hotter is better"* hypothesis predicts that organisms with higher optimal temperature regimes will consequently exhibit increased fitness, which could be reflected in a larger body size (Kingsolver and Huey 2008). A comprehensive review of both hypotheses by Kingsolver and Huey (2008) reveal that *"hotter is smaller*" is not as strongly supported by experimental data as other hypotheses (particularly "*hotter is better*"). Therefore, the TSR may not adequately explain size variation observed in the strains studied here. Overall, the *C. macellaria* strains in question for this study, particularly in 2011, exhibited no straightforward alignment with any one rule.

Immature Stage

The immature developmental stage (egg to pupa) showed solely genetic variability between strains for both years (Figure 4). Because pupal size is a direct consequence of the larval stage, it will be included in this section. However, pupal size exhibited genetic variance and GxE interactions rather than just genetic variance. The rank order for 2011 immature development time tended to be Longview<San Marcos, College Station and remained constant across environments (Figure 4a-d). This was coupled with a survival rank of College Station, San Marcos< Longview at 31°C (Figure 14a, d). Pupal sizes for College Station and San Marcos were 8-11% smaller than Longview at 21°C, giving a rank order of College Station<Longview, with nor significant

difference between San Marcos and Longview or San Marcos and College Station (Figure 10a-d). However, College Station and San Marcos exhibited 10% larger pupae than Longview at 31°C (Longview< San Marcos, no difference between College Station and San Marcos and College Station and Longview). This response clearly did not follow the TSR, as College Station and San Marcos pupae were 9-11% larger at the higher temperature than the lower temperature.

The rank order for immature duration was reversed in 2012, College Station<Longview, (Fig. 4e-h), which corresponded to a rank survival of Longview<College Station (Fig. 14e-h). Unlike the previous year, 2012 strains of College Station generally *did* follow the TSR, as larvae reared at 21°C exhibited 12% larger pupal and adult sizes than those reared at 31°C. However, College Station, which developed faster at the immature stage than Longview for most instances, exhibited 10% and 21% larger pupae at 21°C and 31°C respectively. Faster development and larger pupae at high temperatures has been determined for other blow fly species, including the green bottle fly, *L. sericata* (Tarone et al. 2011), and the oriental latrine fly, *Ch. megacephala* (Hu et al. 2010).

Genetic variability of immature development, as opposed to GxE interactions, in this study contrasts with published data for *L. sericata*. Gallagher et al. (2010) gives larval rank orders as San Diego, CA<Sacramento, CA<Easton, MI for 16 and 26°C, and the reverse for 36°C. Similarly, Tarone et al. (2011) gives rank order as Davis, CA<East Lansing, MI<Morgantown, WV at 20°C, and East Lansing<Morgantown<Davis for 33.5°C. Strains from the current study exhibited similar responses (similar slopes) across environments whereas the latter two studies exhibit distinctly different responses (non-

similar slopes) across environments. This difference in response between studies may indicate that strains used in the above studies were locally adapted to their specific regions, at least for the larval stage. This is not to say that the current study did not reflect GxE interactions, only that they were not determined for the larval stage.

Pupal Stage

The existence of local adaptations between strains was apparent for the pupal stage, as interactions between strains and environments were significant (Figures 6, 7; Table 7). College Station and San Marcos strains exhibited 7-10% more rapid pupal development than Longview at 21°C (College Station<San Marcos<Longview), and adult body size was 3-10% smaller than Longview (San Marcos<Longview; College Station not significantly different from San Marcos or Longview). In contrast, College Station and San Marcos exhibited 6-15% larger adults than Longview at 31°C, however, these differences were not significant. The rank order for pupal survival (survival to eclosion) was College Station< Longview at 21°C, however San Marcos was not significantly different from Longview or College Station, nor were any differences observed between strains for survival at 31°C. 2012 College Station exhibited 6% and 25% larger adults at 21°C and 31°C than Longview (Figure 12e-h). College Station consistently exhibited longer pupal durations than Longview, particularly at 21°C (Figures 6, 7e-h). For 2011 at least, it would appear that faster development leads to decreased survival to the adult stage and decreased survival at 21°C.

Adherence to the TSR by the strains examined in this study could be explained by a more temperature-sensitive development rate. The reverse of the rule may depend on a more temperature-sensitive biomass accumulation rate (Zuo et al. 2012). Taking this into consideration, temperature would seem to have a more noticeable effect on growth time for 2011 Longview and 2012 College Station, whereas a more drastic change in body size would be seen for 2011 College Station and San Marcos. Selection for this type of adaptation could be beneficial in drought situations (such as the exceptional drought experienced in 2011) as larger bodies have been shown to correlate with increased surface area of wings, which could potentially aid in dispersal of the fly from a suboptimal resource (Reigada and Godoy 2006). Increased body size may also correlate to female fecundity (Briegel 1990, Tammaru et al. 1996), which can vary within species of several insect orders (Honěk 1993).

Factors Affecting Development

Some strains exhibited rapid larval times and extended pupal times (Longview 2011, College Station 2012), while others exhibited the opposite response (College Station and San Marcos 2011, Longview 2012). Inherent differences between strains may be due to regional variation, as selection for faster or slower immature development time could be a strategy to combat various biotic and abiotic factors.

Biotic Factors

A closer examination of the different measures of immature development determined strains differed not only in mean duration, but minimum and maximum as well (Figures 4, 5). For example, minimum immature development time was significantly different between all 2011 strains at both temperatures, and maximum immature development times were significant only at lower temperatures for 2011 (Figures 4, 5). This difference could be attributed to each strain utilizing specific strategies to maximize assimilation without depleting energy reserves too rapidly (Hanski 1977). One potential strategy for carrion-breeding insects would be to "hedge bets" (Hopper 1999). The presence of both fast and slow developers within a larval cohort holds the potential for maximizing energy gain from carrion, as the duration of such an ephemeral resource is unpredictable (Elton and Miller 1954). Faster developers within a strain are more likely to reach their minimum threshold for transition into the next life stage before the resource is depleted (Rivers et al. 2011). Slower developers remain on the resource for a longer period of time in order extract nutrients for as long as possible. However, costs may be associated with faster development. For example, female seed beetles, *Stator limbatus* Horn (Coleoptera: Chrysomelidae), experienced a 4-10% decrease in body size, decreased fecundity, and smaller eggs all as a result of rapid development rate at increased temperatures (Stillwell and Fox 2005).

Rapid immature or pupal development may also be due to consumptive or non-consumptive effects (NCE). Immature stages of blow flies are vulnerable to attack by hymenopteran parasitoids in the families Braconidae (Reznik et al. 1992) and Encyrtidae

(Olton and Legner 1974), and predators such as rove beetles (Coleoptera: Staphylinidae), clown beetles (Coleoptera: Histeridae) (Byrd and Castner 2010) and fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (Wells and Greenberg 1994). Cammack et al. (2010) showed that dispersing 3rd instar larvae of *L. sericata* burrow into soil in order to escape parasitism by *Nasonia vitripennis* Ashmead (Hymenoptera: Pteromalidae) and that increased compaction of soil decreased burrowing depth (Cammack et al. 2010). This avoidance strategy could come at a cost, though, as burrowing requires energy. Cammack et al. (2010) speculated that, in the presence of *N. vitripennis*, pupation may occur sooner as energy is rapidly being expended, which would hasten the approach of the pupal stage. A now classic example of larval predation is exemplified by the hairy maggot blow fly, *Chrysomya rufifacies* Macquart, an invasive facultative predator of *C. macellaria* (Wells and Greenberg 1992a). *C. macellaria* displayed <25.5% survival when reared in various mixed cultures of *Ch. rufifacies* (Wells and Greenberg 1992b). Consequently, selection for a non-consumptive response by *C. macellaria* to *Ch. rufifacies* could be expected (Brown et al. 1999, Creel et al. 2007, Sheriff et al. 2009). Such a response could be *C. macellaria* having a more rapid immature development time resulting in avoidance of *Ch. rufifacies.* These types of biotic effects and resulting responses may explain the rapid larval stages of the 2011 Longview and 2012 College Station strains.

Blow fly larvae are most commonly associated with entire carcasses, rather than individual organs, and congregate in masses containing hundreds of thousands of individuals (Rivers et al. 2011). Given this, conditions in this study might not reflect the optimum density for each strain under natural circumstances. It has been hypothesized that density could be a contributing factor influencing plasticity in carrion-breeding flies

(Goodbrod and Goff 1990, Tarone and Foran 2006). Several studies concerning blow flies have shown that an increase in larval density will decrease development time and mean fecundity (So and Dudgeon 1989), pupal mass (Shahein 1986), size differences between sexes, and increase mortality (Martínez-Sánchez et al. 2007). Density does not seem to account for the trend seen in the immature stage, as the fastest developers tended to exhibit larger pupae and increased survival, which contradicts Shahein (1986) and Martinez-Sanchez, Smith et al. (2007). However, density may partially explain variability witnessed in the pupal stage, as the fastest developer (College Station) also exhibited decreased survival and adult size.

Variation observed between years may also be attributed to temporal segregation of fly strains (Picard and Wells 2009, Picard and Wells 2010). For example, Picard and Wells (2009, 2010) showed that adult blow flies of *P. regina* and *L. sericata* arriving at a decayed meat bait were genetically similar, however there was no geographic population structure for either species. This implies that temporal rather than spatial variation segregation may be responsible for variation observed both between and within species. This possibility, combined with drastic environmental shifts, makes it difficult to dissect if variation across years is random.

One source of variation witnessed between strains and years may have been due to the prevalence of a pathogenic fungus present in at least two of the ecoregions. This fungus was responsible for increased mortality of blow fly populations during the spring of 2012 when collections for this study were being made. The classification of this fungus is not known at this time, but is currently being cultured by the author in hopes of identification. It has been shown that the blow fly *P. regina* can die of infection by

Entomophthora bullata Thaxt (Phycomycetes: Entomophthorales) within 5 to 12 days after exposure (Kramer 1979). Further, the fungus *Metarhizium anisopliae* (Sordariomycetes: Hypocreales) has been shown in the laboratory to cause up to 70% mortality of *L. sericata* by contact with a surface treated with the fungus (Wright et al. 2004). Clearly, entomopathogenic fungi can play a crucial part in the population dynamics of blow flies and may have induced a bottleneck on infected populations of *C. macellaria* during spring 2012. As the survivors of this bottleneck constituted the experimental generations for the second year study, it is reasonable to assume that such drift effects could play a role in the variation witnessed in this study.

Abiotic Factors

Variation was determined to be solely genetic for the larval stage, and represents a plastic response of the phenotype rather than an adaptation to the environment (as seen in the pupal stage). The reversal of immature duration rank order, and consequently, rank order of survival, between years could be a result of an exceptional drought in 2011 (Table 1; Figures 18, 19), which may have altered the quality or quantity of resources available for colonization.

Figure 18. Maps of Texas from May 3, 2011 (left) to September 6, 2011 (right) indicating drought conditions (images from droughtmonitor.unl.edu).

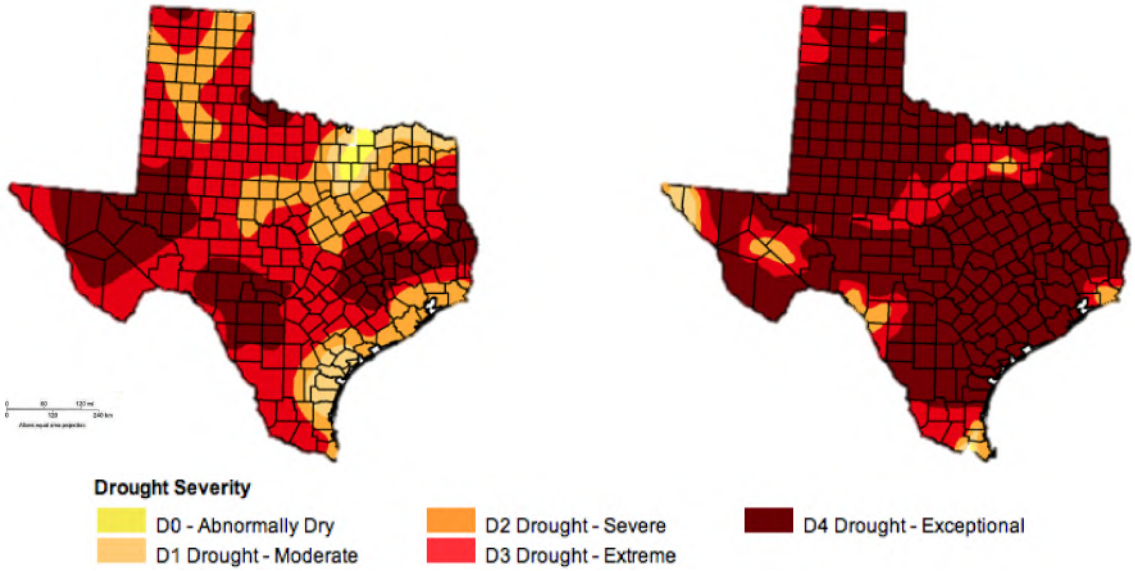
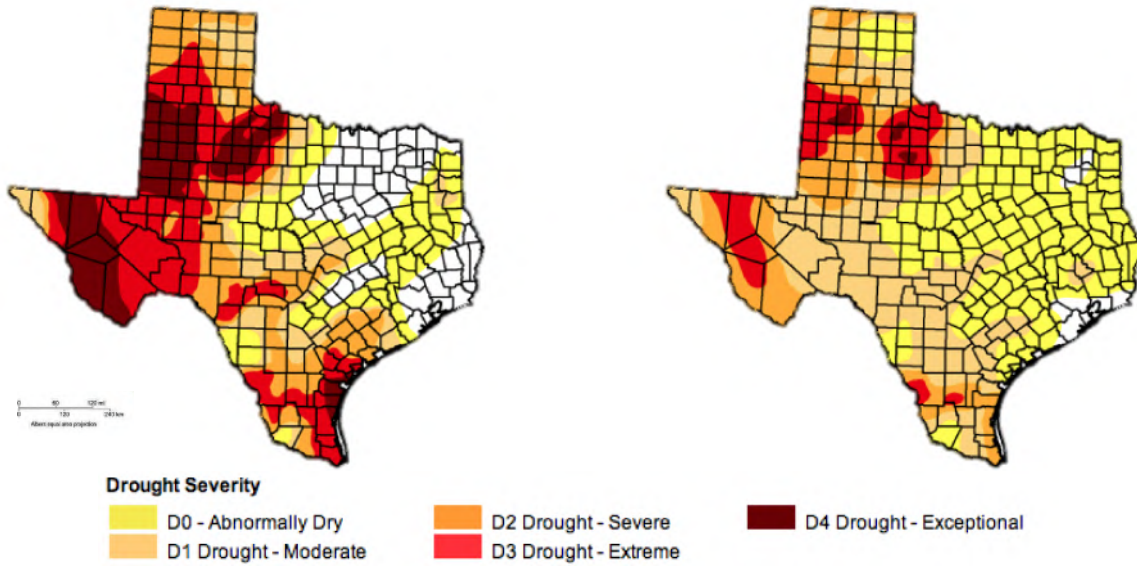


Figure 19. Maps of Texas from April 3, 2012 (left) to May 29, 2012 (right) indicating drought conditions (images from droughtmonitor.unl.edu).



Effects of drought on the primary screwworm, *C. homnivorax* a congener of *C. macellaria,* have been documented (Krafsur and Hightower 1979). Hot and arid

conditions may reduce the availability of suitable oviposition substrates (such as wounds of animals) due to desiccation, impact dispersal and pupation as soil may become dry and compact, and decrease probability of adult survival in test strains of *C. homnivorax* (Krafsur and Hightower 1979). These possible consequences from a drought on the congener of *C. macellaria* could be pertinent to this study, as similar conditions may have been experienced by *C. macellaria* in 2011. Such consequences of drought could be responsible for inducing a population bottleneck, which may also explain variability between ecoregions and years. Methods for collecting flies from each region were designed in order to reduce the impact of such a drift effect. However, it is possible that populations experiencing or having recently experienced a bottleneck may have been sampled for this study. Anecdotal evidence from researchers at the human decomposition facility in San Marcos (Forensic Anthropology Research Facility, FARF) states that blow fly larvae were dying in large numbers while on a resource (Aaron Tarone, Jeffery Tomberlin; personal communication). Whether the mortality witnessed was due to ambient temperature, maggot mass temperature, dry weather, or other factors remains unclear. However, it is clear that even though a suitable resource was available for larval development, larvae could not survive. This information, along with sparse numbers of adult flies collected in San Marcos at this time, support the assumption that blow flies, at least in this ecoregion, were experiencing a bottleneck effect.

Another possible source of variation could arise from the moisture of the feeding substrate (e.g., liver, carrion, human remains). Substrate moisture has been determined to influence the growth rate of *L. sericata* in the laboratory and has been hypothesized to aid in signaling termination of feeding as resource quality diminishes (Tarone and Foran

2006). These results may correspond to what was observed with the San Marcos strain, as it exhibited a significant 8% decrease in moisture for rearing substrate than the other strains at 31°C, which may align with the arid climate of San Marcos (Table 1). Most human and porcine remains at the decomposition facility in San Marcos (Forensic Anthropology Research Facility, FARF) do not skeletonize quickly; rather, they tend to mummify in approximately 10 days and remain in this state for 2-6 months (Parks 2011). Decomposition studies performed on porcine remains in College Station and Longview show that individuals will completely skeletonize within approximately two weeks in the summer months (personal observation). Hanski (1977) showed that nutrient assimilation in *L. illustris* larvae differs with temperature, suggesting an environmental effect on the larvae. This may be related to the observations of substrate moisture in the current study, as the efficiency of San Marcos to assimilate nutrients from moisture may differ in comparison to College Station and Longview. However, immature development of San Marcos was usually shorter or the same as College Station, yet longer than Longview (Figure 4a-d), suggesting that other mechanisms, such as density, may be driving this variation in substrate moisture.

Although certain biotic and abiotic pressures may have pressured College Station into exhibiting unique phenotypic responses (particularly in pupal mass during 2011) when compared to the other strains, one possible explanation lies in the location of the F.L.I.E.S. Facility at Texas A&M University in College Station. This laboratory maintains several colonies of various blow fly species, mainly *C. macellaria* and *Ch. rufifacies,* for ecological and molecular research. Colonies have been maintained in this laboratory for several years and escape is not uncommon. Therefore, the blow fly

populations in close proximity to the lab most likely exhibit distinct phenotypes, and may include several strains of mutants, from strains of blow flies naturally occurring in College Station. It has been shown that laboratory-reared colonies of wild-caught *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) will lose up to 86% genetic variability after approximately 30 generations in a laboratory culture (Briscoe et al. 1992). Although we have not quantified the amount of genetic variation being lost through culturing of blow flies in the FLIES Facility, it may be safe to assume there is a reduction in heterozygosity over time and that flies in close proximity to the laboratory may have decreased genetic variability as well. For this reason, distances of approximately 3 to 14 km from the laboratory were chosen for collection of wild flies. It has been documented that blow flies in South Africa may disperse up to 2.35 km/d, and 37.5 km in 17 days (Braack and Retief 1986), yet it is not known how far blow flies of this species may travel in the present region.

Forensic Implications

This study highlights the importance of seasonal and yearly comparisons as population dynamics are clearly subject to change. As discussed above, various biotic and abiotic factors, or combination thereof, may impact phenotypic responses of conspecific strains.

The amount of non-random variation in phenotypic responses (particularly regarding the pupal stage) changes across environments. Significant genetic variance between strains in the larval stage is enough to warrant strain-specific datasets for

individual species. However, the interactions between the environment and the strains seen in the pupal stage makes it much more difficult to apply developmental datasets to case populations as individual strains respond differently across environments. An average of 74% more variation in pupal development time was observed for the lower temperature in this study (Figures 6-7), which has also been documented for other forensically relevant species, such as *L. sericata (Gallagher et al. 2010)* and *Ch. megacephala* (Hu et al. 2010). This variability has strong implications for determining TOC in forensic entomology, as less variability in estimates could be determined by using developmental datasets for conspecific strains at higher temperatures, Plasticity at lower temperatures may result in inaccurate estimations if the developmental dataset being used is not specific to the geographic region in which larvae were collected. However, underestimation of TOC may occur when using development data from a higher temperature than field temperatures experienced by larvae for a given case.

I demonstrated that *C. macellaria* strains from different eco-regions 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 TOC. This is similar to what Tarone et al. (2011) suggested, in that the percentage of development time completed for one larva does not correlate to the same development duration (in hours) between two different strains. In other words, conspecific development data cannot be universally applied. A quantitative demonstration by Gallagher et al. (2010) showed using nonlocal data for *L. sericata* at 16°C could generate up to -13.80% error in development based estimations of the TOC. Although a validation

of the current work has not been executed, results similar to the findings of Gallagher et al. (2010) are anticipated.

Alternatives and extensions of this study could involve fluctuating temperatures, longevity and fecundity of adults, varying larval densities, various rearing substrates, varying moisture levels of pupal substrate, and seasonal and yearly replicates. Another interesting aspect that remains to be explored concerns the minimum distance required between strains in order to observe differences in phenotypic response. Can strains of blow flies inhabiting different ecosystems in the same geographic unit (e.g., ecoregion, county, town) respond differentially to the same environment? Or rather, do differences observed in phenotypic response correlate to measures of biodiversity of an ecosystem and not necessarily to the *distance* between ecoregions? Teasing apart subtleties within conspecific strains could lend valuable information, not only to the applied field of forensic entomology, but to ecology and evolutionary biology as well.

CHAPTER III

SUMMARY AND SUGGESTIONS FOR FUTURE RESEARCH

Phenotypic variation exists within *Cochliomyia macellaria* and can be represented as genetic variance, plasticity in response due to a changing environment, or genotype by environment interactions (GxE). Individual strains exhibit both types of variation, but each at a different life stage: genetic variance alone is reflected for the immatures, while GxE interactions account for pupal duration and size. Although genetic differentiation is present at high and low temperatures, increased variance between strains occurs at lower temperatures for most developmental phenotypes. An applied aspect of this research was to demonstrate intraspecific variability of a common blow fly species of forensic importance. Variability between strains of *C. macellaria* is apparent; however phenotypic responses in developmental duration, size, and survival do not align with any single rule concerning ontogeny.

My research will enable forensic entomologists to consider intraspecific variation as a source of error when forming time of colonization (TOC) estimates. Because the aim of this research was to demonstrate intraspecific variation within a single state, these data should be used with caution when estimating TOC in ecoregions other those examined in this study. These data, in conjunction with other development studies on *C. macellaria* (Byrd and Butler 1996, Boatright and Tomberlin 2010), could allow for more conservative estimates of colonization events. Specifically, duration and size datasets generated in this study can be potentially applied to forensic cases in ecoregions similar

to those examined in this study as we now have some idea of the variation that can be encountered as a result of geographic distribution within one state.

Several limitations were determined with this study. First, only constant temperatures and %RH were used to rear flies. In order to form more realistic datasets, incubators programmed for fluctuating temperatures and %RH should be implemented as natural populations of flies experience such oscillations in weather. Rearing strains in an outdoor environment would be even more realistic; however dissecting interactions influencing strain responses may be more challenging in that scenario. Second, density may have impacted responses measured in this study. A minor density study was performed prior to the main experiments presented here for the purpose of determining a time and cost-efficient number of larvae per gram of feeding substrate. As only three widely ranged densities were tested (50, 100, 150 larvae/ 50 g liver), little was gained regarding an optimal density. Clearly, a more in depth experiment would be valuable for this species as plasticity could be exhibited at varying densities across temperature treatments. Third, replication for this study could be considered a limiting factor, even though a satisfactory number of replicates per temperature-strain treatment were implemented. This study still falls short of recommendations set forth by the forensic entomology community on sufficient replication procedures (Michaud et al. 2012). In order to meet this ideal set of standards, each temperature would need to be replicated in separate incubators within the same temporal block, multiple comparisons would need to be made within each season, and then each season would need to be replicated for each year. In all practicality, this design presents a difficult task for a single researcher, as

resources, funding, and time are all limited. However, greater statistical power would be associated with such an endeavor.

It is highly recommended that a molecular component be incorporated into research similar to the present study. This would allow the quantifying of any genetic differences between strains, and may shed light on local adaptations. Advanced molecular techniques are now being applied to answer basic questions in forensic entomology, such as detecting full-sibling blow fly larvae to determine corpse relocation (Picard and Wells 2012) and estimating age via developmental gene expression (Tarone and Foran 2011). Molecular analysis would be the obvious next step for a study of this nature.

This study focused on the intraspecific variation of a common blow fly of forensic importance and shed light on the need for strain-specific development data. The endeavor of acquiring such specific datasets may seem like a daunting task for the forensic entomology community, and it is. However, there have been recent attempts at standardization of development predictors, which may minimize the effects of intraspecific variation on TOC estimates. Generalized Additive Models (GAMs) represent a statistical way to examine the value of quantitative developmental traits as predictors of larval age and allow for the generation of confidence intervals and error rates for TOC estimations (Tarone and Foran 2008). Tarone and Foran (2008) showed that development stage (particularly of pre-third instar larvae) of *L. sericata* was a more effective predictor of age than size measurements, and neither strain nor temperature were reliable predictors by themselves. This could potentially mean that variance due to GxE effects could be narrowed for comparison to non-local data. If such models prove

fruitful for age estimation while eliminating strain and temperature effects, then the generation of GAMs should be priority of the forensic entomology community in the future.

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APPENDIX

Appendix 1. Annual weather data for College Station, Longview, and San Marcos between January 2011 and March 2012.

Region	Date	Average Minimum Temperature (°C)	Average Maximum Temperature (°C)	Mean (°C)	Total Precipitation (mm)
College Station	January 2011	4.0	15.9	9.9	251.5
College Station	February 2011	6.0	19.2	12.6	320.0
College Station	March 2011	12.5	25.3	18.9	480.1
College Station	April 2011	17.4	31.1	24.2	614.7
College Station	May 2011	19.1	31.7	25.4	645.2
College Station	June 2011	23.8	36.8	30.3	769.6
College Station	July 2011	25.1	37.7	31.4	797.6
College Station	August 2011	25.6	39.9	32.7	830.6
College Station	September2011	21.1	35.9	28.6	726.4
College Station	October 2011	14.6	28.7	21.7	551.2
College Station	November 2011	10.3	23.5	16.9	429.3
College Station	December 2011	6.7	16.6	11.7	297.2
College Station	January 2012	6.8	19.7	13.3	337.8
College Station	February 2012	9.8	19.0	14.4	365.8
College Station	March 2012	14.5	24.6	19.6	497.8
Longview	January 2011	0.0	11.9	5.8	147.3
Longview	February 2011	2.7	15.8	9.2	233.7
Longview	March 2011	7.2	23.4	15.3	388.6
Longview	April 2011	13.4	28.6	21.0	533.4
Longview	May 2011	15.5	29.4	22.5	571.5
Longview	June 2011	23.0	36.4	29.7	754.4
Longview	July 2011	24.8	37.7	31.3	795.0
Longview	August 2011	25.5	40.4	33.0	838.2
Longview	September2011	18.4	34.4	26.4	670.6
Longview	October 2011	10.9	28.7	19.8	502.9
Longview	November 2011	7.3	21.9	14.6	370.8
Longview	December 2011	2.3	18.3	9.4	238.8
Longview	January 2012	1.6	19.9	10.7	271.8
Longview	February 2012	3.2	18.4	10.8	274.3
Longview	March 2012	10.9	25.2	18.1	459.7
San Marcos	January 2011	3.2	14.8	9.1	231.1
San Marcos	February 2011	6.1	18.9	12.2	309.9
San Marcos	March 2011	10.3	24.2	17.2	436.9
San Marcos	April 2011	12.7	13.3	23.3	591.8
San Marcos	May 2011	16.4	30.3	23.3	591.8
San Marcos	June 2011	22.7	36.7	29.7	754.4
San Marcos	July 2011	24.4	33.3	30.6	777.2

Appendix 1 Continued

Region	Date	Average Minimum Temperature (°C)	Average Maximum Temperature (°C)	Mean (°C)	Total Precipitation (mm)
San Marcos	August 2011	24.7	39.6	32.1	815.3
San Marcos	September2011	20.1	35.9	28.0	711.2
San Marcos	October 2011	14.0	28.2	21.1	535.9
San Marcos	November 2011	9.4	22.9	16.2	411.5
San Marcos	December 2011	6.1	14.9	10.5	266.7
San Marcos	January 2012	5.6	18.6	12.1	307.3
San Marcos	February 2012	2.2	22.2	13.3	337.8
San Marcos	March 2012	12.8	24.6	18.7	475.0