Forensic Science International 280 (2017) 44–48

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Quantifying pteridines in the heads of blow flies (Diptera: Calliphoridae): Application for forensic entomology

J.A. Cammack*, M.H. Reiskind, L.M. Guisewite, S.S. Denning, D.W. Watson

Department of Entomology, North Carolina State University, Raleigh, NC 27695-7613, United States

ARTICLE INFO

Article history:
Received 19 April 2017
Received in revised form 8 August 2017
Accepted 5 September 2017
Available online 12 September 2017

Keywords:
Chrysomya megacephala
Cochliomyia macellaria
Phormia regina
Spectrofluorometry
Indoor remains

ABSTRACT

In forensic cases involving entomological evidence, establishing the postcolonization interval (post-CI) is a critical component of the investigation. Traditional methods of estimating the post-CI rely on estimating the age of immature blow flies (Diptera: Calliphoridae) collected from remains. However, in cases of delayed discovery (e.g., when remains are located indoors), these insects may have completed their development and be present in the environment as adults. Adult fly collections are often ignored in cases of advanced decomposition because of a presumed little relevance to the investigation; herein we present information on how these insects can be of value. In this study we applied an age-grading technique to estimate the age of adults of *Chrysomya megacephala* (Fabricius), *Cochliomyia macellaria* (Fabricius), and *Phormia regina* (Meigen), based on the temperature-dependent accumulation of pteridines in the compound eyes, when reared at temperatures ranging from 5 to 35 °C. Age could be estimated for all species*sex*rearing temperature combinations (mean $r^2 \pm$ SE: 0.90 ± 0.01) for all but *P. regina* reared at 5.4 °C. These models can be used to increase the precision of post-CI estimates for remains found indoors, and the high r^2 values of 22 of the 24 regression equations indicates that this is a valid method for estimating the age of adult blow flies at temperatures ≥15 °C.

1. Introduction

Establishing the postcolonization interval (post-CI) (*sensu* Tomberlin et al. [1]), which is analogous to the minimum postmortem interval as defined by Amendt et al. [2], is a critical component of forensic investigations involving entomological evidence. Traditional methods of estimating the post-CI rely on the aging of immature flies collected from remains, using laboratory-generated, temperature-based development models. These methods have been used to estimate the development of many species of blow flies (Diptera: Calliphoridae) [3–8].

Forensic entomology cases often involve remains found indoors. For example, from 2013 to 2014, 66% of 127 cases involving entomological evidence investigated by the Harris County Institute of Forensic Science in Houston, TX were located indoors [9]. In Germany, over a ten-year period, entomological evidence was collected from 82% of 364 indoor cases [10]. Similarly, in New Zealand [11] and Hawaii [12], insects were collected in 74% of the 50 cases and in 40% of 35 cases, respectively, when the remains were found indoors. In such situations, the remains might not be discovered until after the initial colonizing larvae have completed development and emerged as adults [13,14]. A reliable method to age these adult flies is warranted, as traditional methods of estimating the post-CI are based on the duration of immature development and do not include the adults present at the scene. This omission is a critical gap in an entomological estimate of the post-CI and would greatly enhance the application of forensic entomology to cases involving indoor remains [15–17].

Goff [12] compared differences in the insect species present on remains that were found indoors versus those found outdoors in Hawaii. Species colonizing remains indoors include the house fly, *Musca domestica* L. (Diptera: Muscidae), and the calliphorids *Chrysomya rufifacies* (Macquart) and *Chrysomya megacephala* (Fabricius). Dead pigs placed in indoor and outdoor locations in Germany were colonized by the blow flies *Calliphora vicina* Robineau-Desvoidy and *Lucilia sericata* (Meigen); *C. vicina* comprised 75% of all insects collected indoors, and only 15% of those collected outdoors [18]. Similarly, six blow fly species were

* Corresponding author. Current address: Department of Entomology, Texas A&M University, 2475 TAMU, College Station, TX 77843-2475, United States.
E-mail address: jcammack_07@tamu.edu (J.A. Cammack).

http://dx.doi.org/10.1016/j.forsciint.2017.09.006
0379-0738/

collected from pig carcasses indoors in Raleigh, NC including *C. vicina*,*C. megacephala*, *Cochliomyia macellaria* (Fabricius), *Lucilia cuprina* (Wiedemann), *L. sericata*, and *Phormia regina* (Meigen) [19].

Numerous methods for aging adult flies have been developed, but most lack precision and few have application to forensic science [20]. Ellison and Hampton [21] found that cuticular bands on apodemes of *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) could be counted much like rings on a tree to determine fly age. However, this technique requires tedious dissection and worked only for flies less than 15 days old when temperatures fluctuated around 15 °C and did not exceed 21 °C. Techniques based on female reproductive physiology have been used for aging females of higher dipterans, but are imprecise and require tedious dissections; no methods for aging male flies based on reproductive physiology exist aside from separating mated from virgin males [22]. Mohr and Tomberlin [23] found that ovarian status of carcass-visiting females of *Cochliomyia macellaria*, *C. rufifacies*, and *P. regina* could be used to predict age of swine carcasses up to 3 days postmortem. However, use of such techniques is problematic as many blow fly species exhibit protandry, a phenomenon where males emerge prior to females [24], and some species, such as *C. rufifacies* lay single-sex egg batches [25], making it possible for only male flies to be collected at an indoor body-recovery scene. Therefore, a method of aging adult flies that is independent of reproductive status is needed.

Pteridines are byproducts of purine metabolism that are sequestered in the compound eyes and contribute to eye color, which increase at a temperature-dependent rate with fly age [26]. Pteridine accumulation curves have been generated using spectrofluorometry to successfully estimate age in several blow fly species. In *Chrysomya bezziana* Villenueve, pteridine fluorescence was significantly correlated (mean r^2: 0.94, range: 0.90–0.98) with age and sex at 20, 25, and 30 °C [27]. *L. sericata* exhibited similar multiplicative accumulation over time at 15.0, 20.0, 22.5, 27.5, and 32.5 °C for both sexes; however, the r^2 values were lower for *L. sericata* (mean r^2: 0.88, range: 0.76–0.94) [28]. In contrast to both *C. bezziana* and *L. sericata*, pteridines accumulate linearly in *C. hominivorax* at 25 and 30 °C (r^2: 0.90) [29]. Bernhardt et al. [30] raised *C. vicina* at constant (20 and 25 °C), and fluctuating temperatures (21.6 ± 5.7 °C) and generated models for males (r^2: 0.802) and females (r^2: 0.777). Although variable between species, pteridines provide a reliable estimate of adult fly age (explaining between 78–94% of variation), as long as size, sex, and the ambient temperature under which the insect was reared are known, all of which can be accurately estimated for entomological evidence.

In this study, we tested the hypothesis that pteridine accumulation is an accurate age marker and can be used to age adult flies of forensic importance. We tested this on three species of calliphorids that are commonly found colonizing remains located indoors: *C. megacephala*, *C. macellaria*, and *P. regina*. We varied adult rearing temperatures to represent a diversity of environmental conditions, and generated models for flies up to 30 days post eclosion.

2. Materials and methods

2.1. Identification, colony establishment, and rearing of blow fly species

Three species of blow fly; *C. megacephala, C. macellaria*, and *P. regina,* were selected for study because of their association with indoor remains [19]. To establish colonies, wild flies were collected either as adults visiting decomposing remains, or as larvae dispersing from decomposing remains (and identified post eclosion), and identified using the key of Whitworth [31]. Fly colonies were maintained at approximately 25.5 °C and 70% RH, on a 16L:8D cycle, and were provisioned with water, granulated sucrose, and powdered milk, ad libitum. Three to five days prior to when eggs were needed, flies were provided with a protein source (fresh beef liver) to stimulate egg production [32]. Eggs were collected within 2 h of when fresh beef liver was presented to the colonies, were transferred to a larval rearing medium, and allowed to complete development at 25.5 °C and 70% RH, on a 16L:8D cycle. For each species, adult flies were collected within 4 h of emergence from the puparia. Flies were separated into four groups of 17 age-discrete cohorts of a minimum of 10 individuals and reared in incubators (Fisher Scientific™ Isotemp™ BOD Refrigerated Incubators; ThermoFisher Scientific, Waltham, Massachusetts) on a 16L:8D cycle. We provided each cohort of flies with water and a mixture of granulated sugar and powdered milk. Rearing temperatures were chosen according to the temperatures at which each species is active in the wild. *Phormia regina*, is active throughout much of the year in North Carolina and was reared at approximately 5, 15, 25, and 35 °C (incubator set temperatures). *C. megacephala* and *C. macellaria* are active during warmer portions of the year in North Carolina, and were reared at approximately 15, 22, 28, and 35 °C (incubator set temperatures). Hobo® data loggers (H08-003-02, Onset Corporation, Bourne, MA, USA) recorded actual temperature and humidity inside each incubator. Cohorts of 10 flies were removed from the incubators at age = 0 day, 1 day, 2 days, and every subsequent 2 days until age = 30 days, and placed in a freezer (−20 °C) until cold anesthetized. The flies were then transferred to vials, which were wrapped in foil and stored protected from light at −20 °C. Flies that died prior to the scheduled date of sacrifice were removed on a daily basis to prevent analysis of a fly not killed at the correct age. All subsequent processing occurred in a darkroom with a red light (wavelength ~675 nm) to protect the pteridines from photo-degradation.

2.2. Quantifying accumulation of pteridines

Because pteridines are stored in the eyes of adult flies, fluorescence values were corrected for head capsule width to standardize (presented as relative fluorescence/HCW in tables and figures). Head width of each fly was measured to the nearest 0.5 mm, using an ocular micrometer on a Nikon® SMZ-2T dissecting microscope (Nikon Corporation, Melville, NY). The flies were decapitated after measurement, and the heads placed in 1.5 ml microcentrifuge tubes. To develop standard curves for pteridine fluorescence for each fly species, rearing temperature, and age, a modified version of published protocols [33,34] was used. Individual fly heads were homogenized with a pestle in 1.5 ml microcentrifuge tubes containing 250 μl of 50 mM pH 8.0 tris–HCl buffer. An additional 750 μl of buffer was added, and each sample centrifuged at 6000 rpm (2940 RCF) for 5 min in an Eppendorf® 5415C Micro Centrifuge (Eppendorf North America, Hauppauge, NY). An aliquot (200 μl) of supernatant from each sample was transferred into one well of a black, polystyrene, 96 well microplate (Whatman Inc., Florham Park, NJ). Spectrofluorometry was performed on a Molecular Devices FilterMax™ F5 Multi-Mode Microplate Reader (Molecular Devices, LLC, Sunnyvale, CA), with excitation set at 360 nm, emission at 450 nm, and integration at one second. Three standards (200 μl each) were run with each plate to ensure detector calibration and plate consistency: (1) 10 μg/ml pterin (Sigma–Aldrich, Inc., St. Louis, MO) in 1 M NaOH, (2) 50 mM tris–HCl buffer, and (3) distilled water. Relative fluorescence/head capsule width was plotted against chronological age (hours) to develop formulae for estimating fly age at each rearing temperature.

2.3. Statistical analyses

All statistical analyses were conducted in SAS® v 9.3 [35]. Accumulation rates of pteridines were compared across species, sex, and rearing temperatures, using PROC GLM. Because species and sex were significant factors in pteridine concentrations and can be easily determined in field-collected flies, we analyzed each species and sex separately. Furthermore, mean temperatures in an indoor setting can be known or estimated, so each temperature treatment was analyzed separately. To select which regression model was the best for predicting fly age, first, second, and third order models for each species, sex, and rearing temperature were compared using Akaike information criteria (AIC), and the models with the lowest AIC scores were chosen, and subsequent regression equations were generated using PROC REG.

3. Results

Pteridine accumulation was positively correlated with and highly dependent on temperature for each species (Fig. 1A–C) (*C. megacephala*: $F_{3,505} = 60.67$, $p < 0.0001$; *C. macellaria*: $F_{3,525} = 46.08$, $p < 0.0001$; *P. regina*: $F_{3,502} = 81.83$, $p < 0.0001$). Females and males of all three species accumulated pteridines at different rates across all temperatures tested, except for *P. regina* at the low and high temperatures (Table 1), and each species accumulated pteridines at different rates when compared by sex (♀: $F_{2,782} = 7.79$, $p = 0.0004$; ♂: $F_{2,761} = 36.64$, $p < 0.0001$). The differential rate of accumulation was most pronounced in *C. megacephala* at 26.26 and 34.64 °C.

AIC analyses supported regression models that were polynomial for all but males and females of *P. regina*, which were linear when reared at 5.4 °C. Pteridine fluorescence/HCW explained a large amount of variation (mean r^2: 0.90 ± 0.01) for all species*-sex*rearing temperature combinations for all but males and females of *P. regina* reared at 5.4 °C (Table 2).

4. Discussion

Quantifying pteridines in the heads of *C. megacephala*, *C. macellaria*, and *P. regina* is an effective method for estimating the age of adult flies. Based on this study, age can be estimated at temperatures above 15 °C. Being able to estimate the age of adult

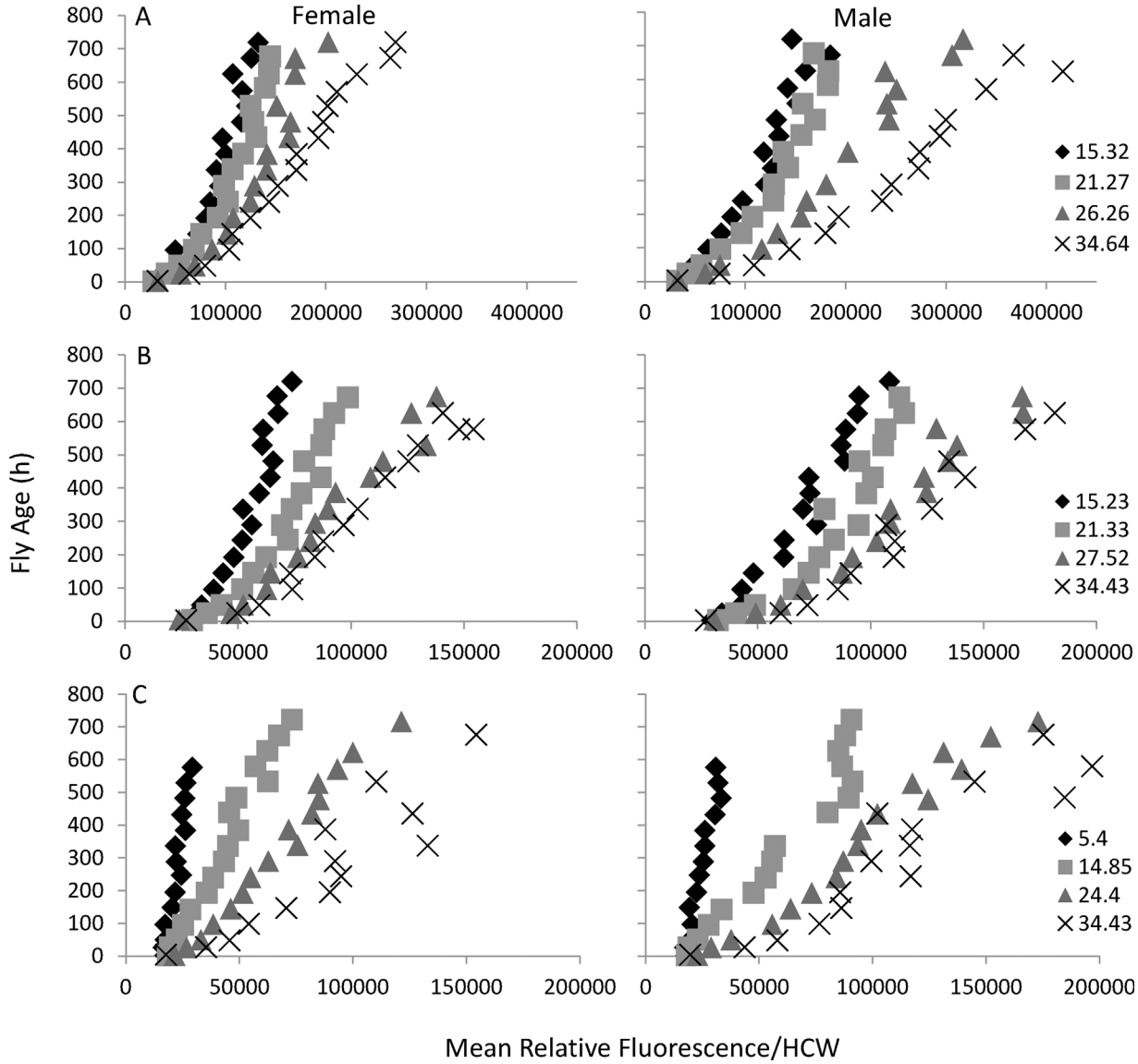

Fig. 1. Accumulation of pteridines in the heads of females and males of *Chrysomya megacephala* (A), *Cochliomyia macellaria* (B) and *Phormia regina* (C) when reared at four different temperatures.

Table 1
Test statistics for accumulation rates of pteridines when compared by sex for each species of blow fly.

Species	Temperature (°C)	F-statistic	p-Value
C. megacephala	15.32	$F_{1,152} = 18.54$	<0.0001
	21.27	$F_{1,146} = 16.01$	<0.0001
	26.26	$F_{1,108} = 47.42$	<0.0001
	34.64	$F_{1,91} = 98.08$	<0.0001
C. macellaria	15.23	$F_{1,155} = 62.26$	<0.0001
	21.33	$F_{1,149} = 11.08$	0.0011
	27.52	$F_{1,117} = 14.47$	0.0002
	34.43	$F_{1,95} = 8.88$	0.0037
P. regina	5.40	$F_{1,125} = 2.58$	0.11
	14.85	$F_{1,136} = 57.36$	<0.0001
	24.4	$F_{1,146} = 82.57$	<0.0001
	34.43	$F_{1,87} = 1.32$	0.25

flies collected at indoor body-recovery scenes when the remains are in a late stage of decomposition will increase the accuracy of entomology-based estimates of the post-CI. This study represents the third of its kind to quantify pteridines in the heads of blow flies for forensic purposes, and is the first to develop pteridine accumulation curves for Nearctic populations of blow flies. Most previous studies using these techniques on calliphorids have been done to assess population structure of species of veterinary and livestock importance.

In contrast to some previous studies on cyclorrhaphan dipterans (e.g., [20,29,33,36]) that found pteridines to accumulate linearly with fly age, in our study, maximum likelihood analyses indicated that non-linear models were the most accurate for estimating fly age for all but adults of *P. regina* reared at 5.4 °C. Zhu et al. [20] evaluated non-linear models, but the data were not presented, and their method for rejecting the non-linear models in favor of linear is unknown. Pteridines accumulated linearly in adults of *C. megacephala* from China [36], suggesting that populations of *C. megacephala* might differ between China and North Carolina. No prior studies have quantified pteridines in Nearctic populations of blow flies, and the differences found between our *C. megacephala* and those from China suggest that models developed on populations of other species in Australia [27,28], China [20,36], Germany [30], and Central America [29,37] might not accurately predict the age of forensically-important flies in other regions of the world.

Although statistical analyses indicated that females and males of *P. regina* did not accumulate pteridines at different rates when reared at 5.4 or 34.43 °C (see Table 2), separate regression models for each sex and rearing temperature are presented, as all prior studies quantifying pteridines in blow flies indicate that accumulation rates differ by sex (e.g., [27,28,37]). The low r^2 and relatively flat slope of the models developed for *P. regina* at 5.4 °C can be attributed to the lack of activity when reared at that temperature; pteridines are a by-product of purine metabolism, and metabolic activity of the flies was low as indicated by their lack of physical activity and low consumption of food and water. Although *P. regina* is present in North Carolina and much of the U.S. during the spring and fall when ambient temperatures can be near 5 °C, they are likely able to thermoregulate (e.g., via sun exposure) to increase activity.

Pteridines can be extracted from the heads of flies stored dry and at room temperature for up to 8 weeks [38] as long as they are stored protected from light. This ability should allow sufficient time following collection at a scene, for specimens to be transferred to the proper laboratory for analysis. Ideally, flies should be collected into vials or other small containers, the containers wrapped in foil to protect the specimens from light and prevent degradation of pteridines, and the flies killed by freezing and kept frozen until the specimens can be analyzed.

One potential concern regarding the application of this technique lies in the ability to distinguish between adults produced from the remains, and those that were attracted to the remains. Indoor body recovery scenes can contain hundreds to thousands of empty puparia and adult flies [14,17]. As pointed out by Bernhardt et al. [30], confirming that the majority of flies analyzed for pteridine fluorescence are of a similar age group could support the hypothesis that these flies were produced from

Table 2
Regression analyses and AIC scores of relative fluorescence/HCW against fly age (hours) for females and males of each species at different temperatures, where y = predicted age in hours and x = relative fluorescence of pteridines/HCW.

Species	Temperature (°C)	Sex	Regression model	r^2	n	AIC
C. megacephala	15.32	F	$y = 1.76E^{-7}x^2 - 6.86E^{-13}x^3 + 87.04$	0.85	74	672.67
		M	$y = 7.97E\text{-}8x^2 - 2.49E\text{-}13x^3 + 21.45$	0.86	82	722.65
	21.27	F	$y = -0.0059x + 1.11E^{-7}x^2 - 3.28E^{-13}x^3 + 91.40$	0.89	75	644.86
		M	$y = -0.0077x + 1.047E^{-7}x^2 - 2.77E^{-13}x^3 + 176.37$	0.84	75	671.98
	26.26	F	$y = -0.0059x + 7.97E^{-8}x^2 - 1.83E^{-13}x^3 + 122.28$	0.90	52	431.85
		M	$y = -0.0025x + 3.04E^{-8}x^2 - 5.07E^{-14}x^3 + 68.08$	0.97	60	449.24
	34.64	F	$y = -0.0023x + 3.72E^{-8}x^2 - 7.22E^{-14}x^3 + 39.52$	0.96	52	388.10
		M	$y = -0.00097x + 1.19E^{-8}x^2 - 1.39E^{-14}x^3 + 29.13$	0.96	43	315.18
C. macellaria	15.23	F	$y = -0.071x + 1.66E^{-6}x^2 - 1.012E^{-11}x^3 + 939$	0.84	90	818.24
		M	$y = -0.0092x + 2.78E^{-7}x^2 - 1.30E^{-12}x^3 + 73.47$	0.88	69	607.31
	21.33	F	$y = -0.027x + 5.54E^{-7}x^2 - 2.62E^{-12}x^3 + 388.92$	0.87	82	708.38
		M	$y = -0.024x + 3.80E^{-7}x^2 - 1.40E^{-12}x^3 + 447.05$	0.88	71	618.19
	27.52	F	$y = -0.010x + 2.11E^{-7}x^2 - 8.10E^{-13}x^3 + 127.69$	0.91	43	344.76
		M	$y = -0.0075x + 1.26E^{-7}x^2 - 3.85E^{-13}x^3 + 126.69$	0.92	78	633.94
	34.43	F	$y = -0.0055x + 1.14E^{-7}x^2 - 3.55E^{-13}x^3 + 69.98$	0.92	41	321.01
		M	$y = -0.0079x + 1.16E^{-7}x^2 - 3.16E^{-13}x^3 + 141.51$	0.96	58	413.27
P. regina	5.40	F	$y = -0.037x + 155.39$	0.53	71	682.57
		M	$y = -0.061x + 403.48$	0.57	58	553.33
	14.85	F	$y = 3.40E^{-7}x^2 - 2.67E^{-12}x^3 - 67.81$	0.91	93	782.07
		M	$y = 2.82E^{-7}x^2 - 1.68E^{-12}x^3 + 47.41$	0.88	47	416.18
	24.40	F	$y = 1.07E^{-7}x^2 - 5.35E^{-13}x^3 - 70.37$	0.94	71	559.41
		M	$y = 7.99E^{-8}x^2 - 2.83E^{-13}x^3 - 3.10$	0.95	79	622.71
	34.43	F	$y = 1.13E^{-7}x^2 - 4.54E^{-13}x^3 + 40.97$	0.80	44	388.90
		M	$y = -0.0038x + 7.81E^{-8}x^2 - 2.32E^{-13}x^3 + 78.54$	0.81	47	423.24

the remains; flies that were attracted to the remains would likely not fall into this same age group. Stable isotope analysis shows promise as an additional tool for distinguishing the larval food source (human vs. animal) of adult blow flies and their empty puparia [39].

The methodology used in the current study quantifies all pteridines in the heads of blow flies. The pteridines present in the heads of a number of species of dipterans have been identified, and different pteridines accumulate differently as adults of *Stomoxys calcitrans* (L.) (Diptera: Muscidae) [40] and *Anastrepha ludens* (Loew) (Diptera: Tephritidae) [41] age. Future work on quantifying pteridines should investigate the change in the ratios of these different pteridines as flies age, or quantify individual pteridines, as a way to increase the precision and accuracy of aging flies based on pteridine accumulation. The application of the developed models also should be tested on regional fly populations to determine the range of validity and application to forensic casework.

Acknowledgements

This work was partially funded by NIJ Grant 2010-DD-BX-0652. We would like to thank A.C. Cohen, R.M. Roe, K.L. Kreitlow, and J.K. Tomberlin for reviewing earlier versions of this manuscript.

References

[1] J.K. Tomberlin, R. Mohr, M.E. Benbow, A.M. Tarone, S. VanLaerhoven, A roadmap for bridging basic and applied research in forensic entomology, Ann. Rev. Entomol. 56 (2011) 401–421.
[2] J. Amendt, C.P. Campobasso, E. Gaudry, C. Reiter, H.N. LeBlanc, M.J.R. Hall, Best practice in forensic entomology—standards and guidelines, Int. J. Leg. Med. 121 (2007) 90–104.
[3] J.H. Byrd, J.F. Butler, Effects of temperature on *Cochliomyia macellaria* (Diptera: Calliphoridae) development, J. Med. Entomol. 33 (1996) 901–905.
[4] J.H. Byrd, J.F. Butler, Effects of temperature on *Chrysomya rufifacies* (Diptera: Calliphoridae) development, J. Med. Entomol. 34 (1997) 353–358.
[5] G.S. Anderson, Minimum and maximum development rates of some forensically important Calliphoridae (Diptera), J. Forensic Sci. 45 (2000) 824–832.
[6] J.H. Byrd, J.C. Allen, The development of the black blow fly, *Phormia regina* (Meigen), Forensic Sci. Int. 72 (2001) 79–88.
[7] S.A. Boatright, J.K. Tomberlin, Effects of temperature and tissue type on the development of *Cochliomyia macellaria* (Diptera: Calliphoridae), J. Med. Entomol. 47 (2010) 917–923.
[8] M. Flores, M. Longnecker, J.K. Tomberlin, Effects of temperature and tissue type on *Chrysomya rufifacies* (Diptera: Calliphoridae) (Macquart) development, Forensic Sci. Int. 245 (2014) 24–29.
[9] M.R. Sanford, Forensic entomology in the medical examiner's office, Acad. Forensic Pathol. 5 (2015) 306–317.
[10] C.L. Frost, H.R. Braig, J. Amendt, M.A. Perotti, Indoor arthropods of forensic importance: insects associated with indoor decomposition and mites as indoor markers, in: J. Amendt, M.L. Goff, C.P. Campobasso, M. Grassberger (Eds.), Current Concepts in Forensic Entomology, Springer, Dordrecht, Netherlands, 2011, pp. 93–108.
[11] W.M.I. Smeeton, T.D. Koelmeyer, B.A. Holloway, P. Singh, Insects associated with exposed human corpses in Auckland, New Zealand, Med. Sci. Law 24 (1984) 167–174.
[12] M.L. Goff, Comparison of insect species associated with decomposing remains recovered inside dwellings and outdoors on the island of Oahu, Hawaii, J. Forensic Sci. 36 (1991) 748–753.
[13] M. Benecke, E. Josepji, R. Zweihoff, Neglect of the elderly: forensic entomology cases and considerations, Forensic Sci. Int. 146S (2004) S195–S199.
[14] M.A. Parker, M. Benecke, J.H. Byrd, R. Hawkes, R. Brown, Entomological alteration of bloodstain evidence, in: J.H. Byrd, J.L. Castner (Eds.), Forensic Entomology: The Utility of Arthropods in Legal Investigations, CRC Press, Boca Raton, Florida, USA, 2010.
[15] Y.Z. Erzinçlioğlu, The entomological investigation of a concealed corpse, Med. Sci. Law 25 (1985) 228–230.
[16] Y.Z. Erzinçlioğlu, Areas of research in forensic entomology, Med. Sci. Law 26 (1986) 273–278.
[17] E.P. Catts, M.L. Goff, Forensic entomology in criminal investigations, Ann. Rev. Entomol. 37 (1992) 253–272.
[18] S. Reibe, B. Madea, How promptly do blow flies colonise fresh carcasses? A study comparing indoor with outdoor locations, Forensic Sci. Int. 195 (2010) 52–57.
[19] J.A. Cammack, A.C. Cohen, K.L. Kreitlow, R.M. Roe, D.W. Watson, Decomposition of concealed and exposed porcine remains in the North Carolina Piedmont, J. Med. Entomol. 53 (2016) 67–75.
[20] G.H. Zhu, G.Y. Ye, K. Li, C. Hu, X.H. Xu, Determining the age of adult flesh flies, *Boettcherisca peregrina*, using pteridine fluorescence, Med. Vet. Entomol. 27 (2013) 59–63.
[21] J.R. Ellison, E.N. Hampton, Age determination using the apodeme structure in adult screwworm flies (*Cochliomyia hominivorax*), J. Insect Physiol. 28 (1982) 731–736.
[22] Hayes, R. Wall, Age-grading adult insects: a review of techniques, Physiol. Entomol. 24 (1999) 1–10.
[23] R.M. Mohr, J.K. Tomberlin, Development and validation of a new technique for estimating a minimum postmortem interval using adult blow fly (Diptera: Calliphoridae) carcass attendance, Int. J. Leg. Med. 129 (2014) 851–859.
[24] M. Buck, Protogyny, protandry, and bimodal emergence patterns in necrophagous Diptera, Can. Entomol. 133 (2001) 521–531.
[25] D.L. Baumgartner, Review of *Chrysomya rufifacies* (Diptera: Calliphoridae), J. Med. Entomol. 30 (1993) 338–352.
[26] U. Patat, Über das Pterinmuster der Facettanaugen von *Calliphora erythrocephala*, Z. Vgl. Physiol. 51 (1965) 103–134.
[27] R. Wall, P.A. Langley, J. Stevens, G.M. Clarke, Age-determination in the old-world screw-worm fly *Chrysomya bezziana* by pteridine fluorescence, J. Insect Physiol. 36 (1990) 213–218.
[28] R. Wall, P.A. Langley, K.L. Morgan, Ovarian development and pteridine accumulation for age determination in the blowfly *Lucilia sericata*, J. Insect Physiol. 37 (1991) 863–868.
[29] D.B. Thomas, A.C. Chen, Age determination in the adult screwworm (Diptera: Calliphoridae) by pteridine levels, J. Econ. Entomol. 82 (1989) 1140–1144.
[30] V. Bernhardt, L. Hannig, R. Kinast, M.A. Verhoff, F. Rothweiler, R. Zehner, J. Amendt, Quantitative pteridine fluorescence analysis: a possible age-grading technique for the adult stages of the blow fly *Calliphora vicina* (Diptera: Calliphoridae), J. Insect Physiol. 98 (2017) 356–359.
[31] T. Whitworth, Keys to the genera and species of blow flies (Diptera: Calliphoridae) of America north of Mexico, Proc. Entomol. Soc. Wash. 108 (2006) 689–725.
[32] S.C. Rasso, G. Fraenkel, The food requirements of the adult female blow-fly, *Phormia regina* (Meigen), in relation to ovarian development, Ann. Entomol. Soc. Am. 47 (1954) 636–654.
[33] T.S. Mail, J. Chadwick, M.J. Lehane, Determining the age of adults of *Stomoxys calcitrans* (L.) (Diptera: Muscidae), Bull. Entomol. Res. 73 (1983) 501–525.
[34] M.J. Lehane, T.S. Mail, Determining the age of adult make and female *Glossina* morsitans morsitans using a new technique, Ecol. Entomol. 10 (1985) 219–224.
[35] SAS Institute, SAS v 9.3, SAS Institute Inc, Cary, NC, 2012.
[36] G.H. Zhu, G.Y. Ye, C. Hu, Determining the adult age of the oriental latrine fly, *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) by pteridine fluorescence analysis, Entomol. Sin. 10 (2003) 245–255.
[37] D.B. Thomas, A.C. Chen, Age distribution of adult female screwworms (Diptera: Calliphoridae) captured on sentinel animals in the coastal lowlands of Guatemala, J. Econ. Entomol. 83 (1990) 1422–1429.
[38] J. Perez-Mendoza, F.E. Dowell, A.B. Broce, J.E. Throne, R.A. Wirtz, F. Xie, J.A. Fabrick, J.E. Baker, Chronological age-grading of house flies by using near-infrared spectroscopy, J. Med. Entomol. 39 (2002) 499–508.
[39] V. Bernhardt, N. Scheid, T. Holderman, T. Schäfer, M.A. Verhoff, J. Amendt, *Same same, but different*! – Decoding the nutrition history of blow flies by isotope signature analysis of adult flies and their empty pupal cases, Presented at the 14th Meeting of the European Association for Forensic Entomology, 7–10 June 2017. Treviso, Italy, 2017.
[40] T.S. Mail, M.J. Lehane, Characterisation of pigments in the head capsule of the adult stablefly *Stomoxys calcitrans*, Entomol. Exp. Appl. 46 (1988) 125–131.
[41] N. Tomic-Carruthers, R. Mangan, R. Carruthers, Age estimation of Mexican fruit fly (Diptera: Tephritidae) based on accumulation of pterins, J. Econ. Entomol. 95 (2002) 1319–1325.