



An examination of the intrapuparial development of *Chrysomya albiceps* (Wiedemann, 1819) (Calliphoridae: Diptera) at three different temperatures

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Abstract

The determination of intrapuparial development periods and development times of insects with holometabolous metamorphosis is necessary both in terms of developmental biology and for minimum Post-mortem interval (PMI_{min}) calculations in forensic entomology. In this study, *Chrysomya albiceps* (Wiedemann, 1819), which is a cosmopolitan species and one of the most rapidly attracted to carrion, was studied. The focus was the intrapuparial development periods of this species at varying temperatures (20, 25, and 30 °C), knowledge which is used in forensic entomology for the estimation of Post-mortem interval (PMI) and is a type of black box. At the specified temperatures, pupae were collected and puparia were dissected hourly; thus, developmental periods were determined, and minimum and maximum starting times of these periods were calculated. With this research, a total of 20 periods, nine of which are new, were determined. The hourly determination of intrapuparial development periods at three different temperatures is the first in the world for this species.

Keywords *Chrysomya albiceps* · Development · Pupal stage · PMI_{min} · Temperature · Forensic

Introduction

Estimating the time of death in cases of murder or suspicious death constitutes an important step in the investigation. For this reason, forensic entomology is used in the resolution of events in many countries around the world [1]. In the minimum Post-mortem interval (PMI_{min}) estimation method, the larval and pupal stages of the holometabolous insect are the two main periods in time estimation. However, if the body was discovered a long time after death and decay has progressed, entomological succession data can also be used in Post-mortem interval (PMI) estimation. There are many studies by various researchers on this subject [2–8]. The larval stage is a development stage where morphological developments can be observed from the outside and allows the time to be estimated through these externally determined

data. Many researchers in this area have produced data on the larval stage of many species [9–21]. PMI_{min} can be determined by specific morphological changes such as larval age, larval length, and larval periods for many species, if environmental conditions are known [16]. This method is not suitable for intrapuparial periods because pupal development undergoes a metamorphic change without growth. However, the pupal stage constitutes about 50% of the immature developmental process, and, therefore, it is thought that pupal age can serve as an important tool in calculating the entomological post-mortem time [22].

The research of Greenberg [23] and Greenberg and Kunich [14] on *Phormia regina* (Meigen, 1826) are among the basic studies on this subject. In these studies, the starting times of 11 development periods of the pupae stage of *P. regina* at 22 °C and 29 °C were determined. Numerous studies have been conducted in different countries over the past decade. *Calliphora vicina* (Robineau-Desvoidy, 1830) was reared at 22 °C in the study by Brown [24], and *C. vicina* and *Lucilia sericata* (Meigen, 1826) were reared at 25 °C in the study by Zajac and Amendt [25]. In these studies, the pupal stage development of the related species was discussed using morphological and histological methods. In the study by Pujol-Luz and Barros-Cordeiro [26], the pupal development of *Chrysomya*

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albiceps females at 26 ± 1 °C was examined chronologically and morphologically, and a total of nine different periods were determined. Davies and Harvey [27], who used histological methods to determine the age of the pupa, also used *Calliphora vicina* and *Lucilia sericata* and carried out their rearing studies at 22 °C. Pasquerault et al. [28] and Sert and Ergil [29] investigated pupal age estimation of *Calliphora vomitoria* (Linnaeus, 1758); Brown et al. [30] investigated *Calliphora vicina* and Ma et al. [31] *Chrysomya rufifacies* (Macquart, 1843) in terms of external morphological characteristics and changes. Karabey and Sert [32] and Sert et al. [33] investigated the development of *Lucilia sericata* and *Sarcophaga argyrostoma* (Robineau-Desvoidy, 1830) respectively at 20 °C, 25 °C, and 30 °C. Differing from Greenberg and Kunich [14], nine new development periods were determined in the study by Karabey and Sert [32]. The studies of Karabey and Sert [32] and Sert et al. [33] differ from similar studies in that they perform hourly pupal dissection and thus monitor the developmental periods hourly. In 2018, Salazar-Souza et al. [34] studied the chronology of the pupal development of *Chrysomya albiceps* with a temperature cycle of 28 °C day/26 °C night. In addition to these studies, many researchers have investigated the use of the gene expression method as an age determination tool [22, 35–38]. In some more recent studies, Richards et al. [39] and Martín-Vega et al. [40] worked on determining the pupal age using the micro-computed tomography method.

The pupal dissection time interval is critical in determining the intrapuparial developmental periods. The determination of the phases in which morphological structures begin to form and end during the pupal development process

depends on dissection of the pupa in the shortest time intervals and recording the developments. In this study, dissection every hour, i.e., 24 times a day, allowed the detection of pupal development with precision. These data allowed for the accurate estimation of PMImin in the resolution of events. In addition to this study, in Turkey, Karabey and Sert [32] examined morphologically the pupal development periods of *Lucilia sericata* and the times of these periods at three different temperatures by dissecting hourly. Sert et al. [33] examined morphologically the pupal periods of *Sarcophaga argyrostoma* and the times of these periods by dissecting hourly at three different temperatures. Örsel [41], also examined morphologically the pupal periods of *S. argyrostoma* and the times of these periods by dissecting hourly at two different temperatures. Considering the dissection intervals of various studies, Pujol-Luz and Barros-Cordeiro [26] performed dissection every 3 h on the first day and every 6 h on the remaining days. Brown et al. [30] and Pasquerault et al. [28] performed dissection every 6 h, Ma et al. [31] performed dissection every 8 h, Zajac and Amendt [25] performed dissection every 24 h, and Richards et al. [39] performed dissection every 72 h. Studies that perform dissections 24 times a day by performing dissection hourly are only seen in the studies Karabey and Sert [32] and Sert et al. [33] (Table 1).

One of the methods used to determine the PMImin is the “Accumulated degree-day (ADD)” or “Accumulated degree-hour (ADH)” method. Since insects are poikilothermic, there is a proportionality between their growth rate and temperature. The reason for this is that there is a

Table 1 Dissection intervals and rearing temperatures of different studied species according to different researchers

	Species	Pupal sampling interval	Temperature
Present study	<i>Chrysomya albiceps</i>	1 h	20, 25, 30 °C
Zajac and Amendt [25]	<i>Calliphora vicina</i> <i>Lucilia sericata</i>	24 h	25 °C
Pujol-Luz and Barros- Cordeiro [26]	<i>Chrysomya albiceps</i>	3 h/6 h	26 ± 1 °C
Davies and Harvey [27]	<i>Calliphora vicina</i> <i>Lucilia sericata</i>	24 h	22 °C
Pasquerault et al. [28]	<i>Calliphora vicina</i> <i>Calliphora vomitoria</i> <i>Lucilia sericata</i>	6 h	22–24 °C
Brown et al. [30]	<i>Calliphora vicina</i>	6 h	22 °C
Ma et al. [31]	<i>Chrysomya rufifacies</i>	8 h	20, 24, 28, 32 °C
Richards et al. [39]	<i>Calliphora vicina</i>	72 h	23 °C
Martín-Vega et al. [40]	<i>Calliphora vicina</i>	6 h	$24 \text{ °C} \pm 0.8 \text{ °C}$
Salazar-Souza et al. [34]	<i>Chrysomya albiceps</i>	2 h (for the first 12 h) 12, 6, 18, 6, 18, 6, 18, 3 h (until adult emergence)	28 °C day/26 °C night
Örsel [41]	<i>Sarcophaga argyrostoma</i>	1 h	28, 32 °C
Karabey and Sert [32]	<i>Lucilia sericata</i>	1 h	20, 25, 30 °C
Sert et al. [33]	<i>Sarcophaga argyrostoma</i>	1 h	20, 25, 30 °C

correspondence between growth and the development of different stages called the physiological developmental energy budget. This budget is expressed in thermal units in degree-day ($^{\circ}\text{D}$) or degree-hour ($^{\circ}\text{H}$). Growth rate increases in direct proportion to the temperature increase between the upper and lower threshold temperatures that prevent development. Therefore, the energy budget is represented as the growth that varies depending on the temperature for hourly or each day. Thus, the accumulated degree hour (or day) indicates the time elapsed for the development stage at which the insect is at until gathered from the scene. This connection can be calculated with the equations $\text{ADH} = \text{time (hour)} \times (\text{temperature} - \text{base temperature})$ or $\text{ADD} = \text{time (day)} \times (\text{temperature} - \text{base temperature})$ [20, 42].

Chrysomya albiceps is also important for health since it causes myiasis in humans and livestock. It feeds on carrion and human excrement and multiplies rapidly in carrion [43–45]. Although it is a scavenger, this species differs from other European blowflies due to the facultative predatory behavior of its second and third instar larvae [44]. *Ch. albiceps*, one of the first colonists in the decomposition process [34], has also been identified on human bodies [44, 46–49].

In this study, the intrapuparial development periods and development times of *Chrysomya albiceps* were determined at three different temperatures: 20 $^{\circ}\text{C}$, 25 $^{\circ}\text{C}$, and 30 $^{\circ}\text{C}$. Regarding this species, differing from Greenberg and Kunich [14], nine different developmental periods and the minimum and maximum starting times of these periods were also determined. These findings regarding the species studied are new to the scientific world.

Materials and methods

In this study, the species *Chrysomya albiceps* was studied, samples of which was gathered from cattle liver left in different areas of Lefkoşa, Turkish Republic of Northern Cyprus and Hacettepe University Beytepe Campus, Ankara, Turkey in the morning, at noon and in the evening. Experimental studies were conducted in the Biocriminal Entomology Laboratory at Hacettepe University Biology Department. A binocular stereoscopic microscope and imaging system, the Leica MZ 16 A, were used for the identification of species, pupae examinations, and photographs, and the Sanyo MIR-253 refrigerated incubator was used for pupal development. The samples were identified using the adult species identification keys in Greenberg and Kunich [14] and the keys in Zumpt [43], and then they were taken into cages and rearing studies in the laboratory environment were begun.

Intrapuparial development was examined hourly from the beginning of pupariation to adult emergence, and the developmental differences between hours were determined. During the rearing experiments, 60 adult flies were kept in

adult cages of 10 \times 10 \times 10 cm. Cattle liver was added to the cages with a cotton soaked in mixture prepared with sugar, milk powder and water as nutrients to enable adult females to lay eggs. The milk powder-sugar mixture was prepared at a rate of 50–50% [18]. Each female laid about 180 eggs. After the oviposition was completed, the egg masses on the liver were separated according to the time they were laid and placed in different petri dishes containing cattle liver. The first larval instars were placed in a container of 8 cm depth \times 10 cm diameter filled with 4 cm sawdust, as groups of 50 larvae with 100 g of liver [19, 50]. Since the study was about the development of the pupal stage, the larvae were checked at regular intervals and reared in this way until they reached the post-feeding stage by replenishing their food when necessary. Samples that reached the post-feeding stage were separated from the others and the formation of the puparium was detected by the hourly controls. With the beginning of the formation of the puparium, the prepupa stage, which is considered to be the starting point of pupal development, had been reached. From this stage the puparium of randomly collected pupae were killed hourly by soaking in boiled water for 30 s and dissected immediately using a scalpel, forceps and steel pins [24]. At least five individuals were dissected hourly. However, this number was increased in order to accurately identify the transition stages. The puparium was dissected carefully, and the pupa was removed from inside, allowing the intrapuparial developmental periods to be examined. While photographing the dissected pupae, detailed photographs of the pupa were taken from the dorsal, ventral, and lateral directions. After these procedures, the pupae were placed in 80% ethyl alcohol along with their puparia and stored. During the study, a total of 4220 pupae were dissected.

Chrysomya albiceps is found in Ankara between the last week of May and the first week of November [51]. The optimum development temperature of the species is around 25–27 $^{\circ}\text{C}$ [52]. For this reason, while determining the intrapuparial development temperatures of this species, the optimum temperature and one below and one above this temperature were selected, and intrapuparial development was examined at 20 $^{\circ}\text{C}$, 25 $^{\circ}\text{C}$, and 30 $^{\circ}\text{C}$. Since the aim of this study was to determine the changes that occur in the pupal stage, the periods and calculations were given from the prepupa stage and the data related to the pre-pupal stage were not considered in this study.

Results

Samples of *Chrysomya albiceps* were reared at 20 $^{\circ}\text{C}$, 25 $^{\circ}\text{C}$, and 30 $^{\circ}\text{C}$, and the intrapuparial developmental periods and the minimum and maximum starting times

Table 2 Intrapuparial development periods (grouped according to general development phases of fly pupae), the minimum and maximum starting times of these periods, and relative development of *Chrysomya albiceps* at 20 °C, 25 °C, and 30 °C

General development phases	Period	20 °C			25 °C			30 °C		
		Min-max starting times	ADH	Relative development (% according to total development)	Min-max starting times	ADH	Relative development (% according to total development)	Min-max starting times	ADH	Relative development (% according to total development)
Prepupa phase	Prepupa	0–6th hours	0–58.8	0–3.6	0–4th hours	0–59.2	0–3.4	0–3rd hours	0–59.4	0–3.5
Cytocephalic pupa phase	Cytocephalic pupa	17–18th hours	166.6–176.4	10.2–10.8	11–12th hours	162.8–177.6	9.4–10.3	6–7th hours	118.8–138.6	6.9–8.1
	*Emergence of respiratory horns	18–21st hours	176.4–205.8	10.8–12.6	12–14th hours	177.6–207.2	10.3–12	7–9th hours	138.6–178.2	8.1–10.4
Phanerocephalic pupa phase	*Separation of respiratory horns laterally to the back of the compound eyes	23–25th hours	225.4–245	13.8–15	15–17th hours	222–251.6	12.8–14.5	10–12th hours	198–237.6	11.6–13.9
	Phanerocephalic pupa	25–27th hours	245–264.6	15–16.2	18–19th hours	266.4–281.2	15.4–16.2	13–14th hours	257.4–277.2	15–16.2
Pharate adult phase	*Prominence of stigmas on the abdomen	29–34th hours	284.2–333.2	17.4–20.4	20–24th hours	296–355.2	17.1–20.5	15–18th hours	297–356.4	17.3–20.8
	Beginning of pupal-adult apolysis	45–48th hours	441–470.4	27–28.8	36–38th hours	532.8–562.4	30.8–32.5	25–26th hours	495–514.8	28.9–30.1
Pharate adult phase	Completion of pupal-adult apolysis	58–62nd hours	568.4–607.6	34.8–37.2	39–41st hours	577.2–606.8	33.3–35	27–28th hours	534.6–554.4	31.2–32.4
	Segmented abdomen	66–69th hours	646.8–676.2	39.6–41.4	43–46th hours	636.4–680.8	36.8–39.3	29–31st hours	574.2–613.8	33.5–35.8
Pharate adult phase	*Recess formation in the middle of the abdomen	75–79th hours	735–764.4	45–47.4	46–48th hours	680.8–710.4	39.3–41	31–32nd hours	613.8–633.6	35.8–37
	*Formation of a longitudinal recessed band in the middle of the eye	80–83rd hours	784–813.4	48–49.8	53–55th hours	784.4–814	45.3–47	36–38th hours	712.8–752.4	41.6–43.9
Pharate adult phase	Pigmentation beginning on the posterior of the eye	85–89th hours	833–872.2	51.1–53.5	56–59th hours	828.8–873.2	47.9–50.4	40–42nd hours	792–831.6	46.2–48.6
	Complete pigmentation of the eye	90–93rd hours	882–911.4	54.1–55.9	61st–63rd hours	902.8–932.4	52.1–53.8	44–45th hours	871.2–891	50.9–52
Pharate adult phase	*Prominence of the ocelli	96–99th hours	940.8–970.2	57.7–59.5	65–69th hours	962–1021.2	55.6–59	48–51st hours	950.4–1009.8	55.5–59
	*Pinkish-red coloring of the eyes	100–105th hours	980–1029	60.1–63.1	71–74th hours	1050.8–1095.2	60.7–63.2	54–56th hours	1069.2–1108.8	62.4–64.7
Pharate adult phase	Bristle formation on the thorax	120–124th hours	1176–1215.2	72.1–74.5	78–82nd hours	1154.4–1213.6	66.7–70.1	55–58th hours	1089–1148.4	63.6–67.1
	Bristle formation on the abdomen	131–136th hours	1283.8–1332.8	78.7–81.7	86–89th hours	1272.8–1317.2	73.5–76.1	62–65th hours	1227.6–1278	71.7–75.1
Pharate adult phase	*Darkening of abdominal sutures	142–145th hours	1391.6–1421	85.3–87.1	95–97th hours	1406–1435.6	81.2–82.9	71–73rd hours	1405.8–1445.4	82.1–84.4
	*Beginning of the shedding of the transparent membrane surrounding the pharate adult	163–165th hours	1597.4–1617	97.9–99.1	114–115th hours	1687.2–1702	97.4–98.3	84–85th hours	1663.2–1683	97.1–98.3
Adult emergence	Adult emergence	165–168th hours	1617–1646.4	100	116–118th hours	1716.8–1746.4	100	86–87th hours	1702.8–1722.6	100

ADH values were calculated by using Marchenko [53] (Newly identified periods are indicated with asterisk (*))

of these periods were determined (Table 2). The determined intrapuparial development periods were grouped according to general development phases of fly pupae in Table 2. The relative development at all three temperatures according to the total development progress are given in Table 2 and Fig. 1. While determining the ADH, the basal temperature of 10.2 °C given for *Ch. albiceps* in Marchenko's study [53] was considered. The calculations were made according to this temperature and are given in Table 2. Within the scope of the study, nine new periods that were determined for the first time for *Ch. albiceps* are given in Table 3 for all three temperatures. Because the morphological changes seen in the pupa are the same for all temperatures, photographs of the periods are given for 20 °C, as an example.

Intrapuparial periods and development times at 20 °C

0–6th hours

This is the prepupa stage. The puparium is light brown, and the dorsal side has black spots in patches (Online resource 1a, 1b). It is soft because it has not hardened completely. When the puparium is dissected, the emerging pupa is in viscous form. (Online resource 1c).

7–12th hours

The pupa lost its viscous form in the first six hours. At the anterior end, the thoracic region of the pupa is attached to the cephalopharyngeal skeleton, and the posterior part is connected to the puparium where the posterior stigmas are. The cephalopharyngeal skeleton is visible anteriorly (Online resource 2a). After touching the posterior part of the pupa with the help of pin or forceps, the puparium is gently pulled out, and the area connected from the posterior is detached and released. However, since the anterior part is tightly attached to the puparium, this part ruptures when the puparium is pulled; therefore, when the puparium is dissected the pupa to be removed as a complete structure (Online resource 2b).

17–18th hours

At this hour, the pupa is no longer adjacent to the puparium. That is, both anterior and posterior ends are separated from puparium; therefore, when the puparium is dissected, the pupa can be pulled out as a complete structure. This period is called the cryptocephalic pupa. The head is not everted, and the thorax and the abdomen are not easily distinguishable

yet. The cephalopharyngeal skeleton is embedded, thus it is not visible at the anterior end. The pupa has an appearance resembling larval segments, narrowing posteriorly (Online resource 3).

18–21st hours

At the anterior end are respiratory horns which are the same color as the pupa (Online resource 4a, 4b). The wing (Online resource 4c) and leg buds are evident under the pupal skin. The legs extend anteriorly between the second and third sutures but not beyond the suture of the third of the segment-like rings.

23–25th hours

Respiratory horns attached to each other began to be pushed laterally. In addition, the respiratory horns became pigmented and light brown (Online resource 5a, 5b).

24th hour

From this time on, it is observed that the horns are pushed laterally, the gap between them gradually increases, and the positions of the horns change. The front part of the cephalopharyngeal skeleton becomes visible again through this opening (Online resource 6a, 6b).

25–27th hours

In the phanerocephalic pupa period, the head is now everted. The eyes are formed on the head. The respiratory horns are located in the middle of the eyes away from the anterior stigmas (Online resource 7a, 7b). The head, thorax, and abdomen borders are evident (Online resource 7c). The wings and legs are elongated towards the posterior end (Online resource 7d). There are lines on the abdomen in a circular structure resembling larval segments (Online resource 7d). In addition, a yellowish coloration is observed on the border of the abdomen and thorax.

29–34th hours

The segmented appearance in the abdomen seen in the previous hours decreases. The abdomen appears smooth (Online resource 8a). In addition, the stigmas on the abdomen become visible (Online resource 8b).

41st hour

The periphery of the respiratory horns creates a bright red spot similar to an eye (Online resource 9a, 9b).

45–48th hours

This is the beginning of the pupal-adult apolysis. The pupal skin begins to be discharged from the wing buds and legs (Online resource 10a, 10b).

58–62nd hours

The completion of the pupal-adult apolysis occurs. The pharate adult is completely covered with a transparent membrane which is tightly bounded around the pharate adult and is hard to remove (Online resource 11).

66–69th hours

The abdominal segments start to become visible (Online resource 12).

75–79th hours

A recess begins to form in the middle of the abdomen (Online resource 13).

80–83rd hours

In the middle of the eye, a longitudinal recessed band is formed (Online resource 14).

85–89th hours

The posterior of the eye begins to become pigmented (Online resource 15).

90–93rd hours

Pigmentation is evident throughout the eye. Eyes are light orange in color (Online resource 16).

96–99th hours

The eyes continue to gradually pigment and darken. The eye contour darkens in color. The ocelli become evident (Online resource 17a, 17b).

100–105th hours

The eyes become a pinkish-red color. The eye color is no longer the same as the color of the anterior stigmas (Online resource 18a, 18b).

105th hour

Thorax segment borders begin to become evident (Online resource 19a). The pigmentation in the eyes continues (Online resource 19b).

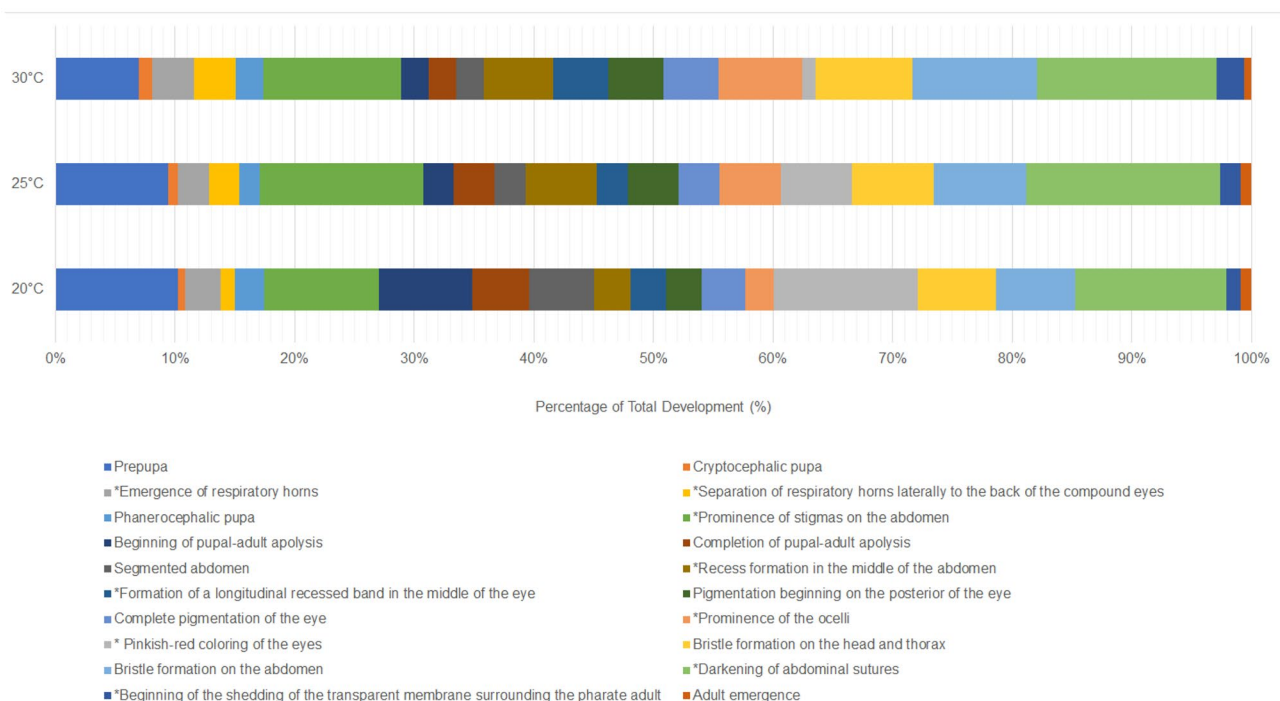


Fig. 1 The relative development according to the total development progress at three temperatures (newly identified periods are indicated with asterix (*))

Table 3 Progress of the newly identified periods, throughout the intrapuparial development at 20 °C, 25 °C, and 30 °C (number of boxes indicate the amount of change, 1: the least; 3: the most)

Hours			Periods								
20°C	25°C	30°C	Emergence of respiratory horns	Separation of respiratory horns laterally to the back of the compound eyes	Prominence of stigmata on the abdomen	Recess formation in the middle of the abdomen	Formation of a longitudinal recessed band in the middle of the eye	Prominence of the ocelli	Pinkish-red coloring of the eyes	Darkening of abdominal sutures	Beginning of the shedding of the transparent membrane surrounding the pharate adult
18-21	12-14	7-9	█								
23-25	15-17	10-12		█							
29-34	20-24	15-18		█ █	█						
75-78	46-48	31-32		█ █ █	█ █	█					
80-83	53-55	36-38		█ █ █	█ █	█ █	█				
96-99	65-69	48-51		█ █ █	█ █	█ █ █		█			
100-105	71-74	54-56		█ █	█ █	█ █		█ █	█		
142-145	95-97	71-73		█	█ █	█		█	█ █	█	
163-165	114-115	84-85			█				█ █ █	█ █	█

120–124th hours

Bristles on the edge of the eyes and the frons (Online resource 20a, 20b) and punctures on the thorax (Online resource 20c) become tanned and evident. Below the antennae, the rim around the mouth is pigmented (Online resource 20d).

131–136th hours

Bristles start to emerge on the abdomen. The bristles are only in the first segment of the abdomen and are situated fragmentally (Online resource 21).

135th hour

Except for the last abdominal segment, which is not visible from the dorsal side, there are bristles on all other segments, but those on the first segment are in a dense form (Online resource 22a, 22b).

142–145th hours

The abdominal sutures become darker in color (Online resource 23a, 23b).

157th hour

The connection between the respiratory horns and the anterior stigma is severed, and the transparent membrane surrounding the pharate adult begins to shed (Online resource 24).

163–165th hours

The adult is now very close to emergence. As the transparent membrane surrounding the pharate is shed completely, the extremities and structures become clearly visible (Online resource 25a). The respiratory horns are removed by the shedding of the transparent membrane, and the thoracic spiracle in the shape of an open-end tube remains in its place (Online resource 25b).

165–168th hours

Adult emergence occurs (Online resource 26a, 26b).

Discussion and conclusions

With this study, the aim was to determine the detailed morphological intrapuparial development periods and development times of *Chrysomya albiceps*, one of the species frequently used in post-mortem interval determination. For this, three different temperatures were used. In addition, the morphological periods of intrapuparial development, which were not seen in any study except Karabey and Sert [32] and Sert et al. [33], were determined by hourly dissections. The data obtained as a result of the dissections performed per hour, 24 times a day, prevented the changes that occurred in the developmental periods from being overlooked. In this study, nine different periods were determined in addition to the 11 developmental periods given in Greenberg and Kunich [14], which was taken as example methodologically. These periods were observed as follows: the emergence of respiratory horns, the separation of respiratory horns laterally to the back of the compound eyes, the prominence of stigmas on the abdomen, the recess formation in the middle of the abdomen, the formation of a longitudinal recessed band in the middle of the eye, the prominence of the ocelli, a pinkish-red coloring of the eyes, the darkening of abdominal sutures, and the beginning of the shedding of the transparent membrane surrounding the pharate adult.

Concerning the determination of the intrapuparial developmental periods of *Chrysomya albiceps*, there are also two similar studies by Pujol-Luz and Barros-Cordeiro [26] and Salazar-Souza et al. [34]. Although the aims of these two studies are the same as our research, some differences in their methodologies are reflected in the results. When the data of these three studies were compared, it was seen that some of the developmental periods (such as the cryptocephalic pupa period, the phanerocephalic pupa period, the eye color formation) were similar, but the development times of these periods are different from each other. Some developmental periods determined in our study were not found in the other two studies. The most important reason for this difference is that hourly pupae excision allows the beginning and the end of each developmental period and their times to be determined precisely.

In the study by Pujol-Luz and Barros-Cordeiro [26], they examined the intrapuparial development of *Chrysomya albiceps* females at 26 ± 1 °C by dividing the development into nine periods chronologically and morphologically. In our study, 20 different periods were determined because the findings were obtained hourly. Some of the findings of the 20 periods obtained from our study are also seen in the study

by Pujol-Luz and Barros-Cordeiro [26]. However, there are differences in this study that can be compared. The findings obtained at 25 °C, one of the temperatures studied in this study, were compared with the temperature 26 ± 1 °C in Pujol-Luz and Barros-Cordeiro [26]. According to our findings, the cryptocephalic pupa period was reached in 11–12 h and the phanerocephalic pupa period was reached in 18–19 h while the cryptocephalic pupa period was reached in six hours and the phanerocephalic pupa period was reached in nine hours in the Pujol-Luz and Barros-Cordeiro study [26]. In addition, some periods that we determined, such as the emergence of the respiratory horns, the separation of respiratory horns laterally to the back of the compound eyes, the prominence of stigmas on the abdomen, the recess formation in the middle of the abdomen, the formation of a longitudinal recessed band in the middle of the eye, and the prominence of the ocelli, could not be compared because they were not seen in the study by Pujol-Luz and Barros-Cordeiro [26]. The data on the formation of eye color in Pujol-Luz and Barros-Cordeiro [26] are not compatible with our data. In their study, the eye color turned yellow in 60 h, pink in 66 h, and red in 90 h. However, according to our findings, the color of the eyes turned yellow in 65–69 h, pink in 71–74 h, and red in 86–89 h (Online resource 20a, 20b). Again, in the same study, the time intervals given with some development periods spread over a wide period. For example, in the red eyes stage, it was stated that all periods of development such as the appearance of antenna, palpi, and ocelli; the pigmentation of all bristles; the formation of wings and darkening of the veins; the appearance of the external genital structure; the determination of the borders of sclerites; and the formation of the ptilinal sac are in the range of 66–90 h. Since it was not stated which stage occurred in which hour, its distinguishing feature is limited. Finally, adult emergence was between 116 and 118 h according to our findings, while adult emergence was observed at the 90th hour according to Pujol-Luz and Barros-Cordeiro [26].

In the other study, by Salazar-Souza et al. [34], in which the development was followed at an average temperature of 27 °C, they analyzed the developments by dividing the days into 18 and six hourly periods after the first day. Depending on this situation, the exact time of the development periods for each day was not determined. Therefore, the possibility of comparing both study data in hours is limited. In both studies, adult emergence time is one of the comparable periods. Salazar-Souza et al. [34] stated that adult emergence is seen in 99 h, but, in our findings, adult emergence occurred at the 105th hour according to the data at 27 °C calculated based on the data at 25 °C and 30 °C.

Although the data obtained from different studies provides important contributions to the field of forensic entomology, it suggests that the differences among the data between countries should be considered, especially in terms of PMImin calculations. There are examples of

differences in some biological data of cosmopolitan species across countries. For example, in Greenberg's study [54] in Chicago, USA, it was observed that *Lucilia sericata* laid a small number of eggs on rat corpses near a street lamp at night. During his two-year study, Greenberg found a nocturnal oviposition rate of approximately 33%. On the other hand, Sert et al. [55] determined in their study in Ankara, Turkey that *L. sericata* did not oviposit at night on the balcony of a house in the city. *L. sericata* populations in the North American and European continents appear to behave differently in terms of nocturnal oviposition. This situation is of great importance for PMI in calculations. It is thought that some morphological development period time differences between development periods of *Chrysomya albiceps* in the studies of Pujol-Luz and Barros-Cordeiro [26] and Salazar-Souza et al. [34] and our study may not be due to methodology differences and that cosmopolitan species may show different development times due to various environmental and evolutionary forces regionally (South America-Europe) as with nocturnal oviposition. This situation may lead to consequences that prevent the judicial system from making a correct decision. Therefore, it may be necessary to create regional data for each species.

Key points

- (1) Calliphorids are one of the first colonists found at death scenes and used in PMI in estimation. This study was carried out on one of the forensically important calliphorid species, *Chrysomya albiceps*.
- (2) Intrapuparial development of forensically important fly species is a major component in estimating PMI in, since pupal stage constitutes about half of the holometabolous life cycle.
- (3) Nine new intrapuparial development periods were identified with hourly dissections of the puparium at three different temperatures and minimum and maximum starting times of these period were detected.
- (4) This study is the first research which examines the process of intrapuparial development of *Chrysomya albiceps* with hourly dissections.

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Author contributions Conceptualization: OS; Investigation: OS, CE; Writing – Original Draft: OS, CE; Writing – review and editing: OS; Resources: OS; Supervision: OS.

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Data availability Available from the corresponding author on reasonable request.

Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors. “No animals except flies were used.”

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