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## The blowfly *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae) as a new forensic indicator in Central Europe

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**Abstract** During the summer months of the year 2001, six forensic cases (one is reported in the present paper), a pig carrion study in the city of Vienna (latitude 48°12'N, longitude 16°22'E) and several liver-baited traps north of Vienna, yielded large numbers of maggots of the blowfly *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae). Apart from some records from France, reports of *C. albiceps* from the palearctic region north of the Alps (i.e. north of a latitude of 48°N) have been scarce. Our findings provided an opportunity to derive developmental schedules for *C. albiceps* at five different constant temperatures (15, 20, 25, 30, 35°C). The minimal duration of development from oviposition to adult was inversely related to temperature, ranging from  $8.3 \pm 0.5$  days at 35°C to  $19.2 \pm 0.92$  days at 20°C. Although eggs hatched after  $1.9 \pm 0.16$  days at 15°C, larvae did not complete development and frequently died during the first instar stage. We also found a high mortality rate (up to 99%) of native *L. sericata* larvae caused by predation of *C. albiceps* larvae under laboratory conditions, indicating a high susceptibility of *L. sericata* to attack by *C. albiceps*. Apart from this, the current and possible future distribution of *C. albiceps* in Europe is discussed. The northward expansion of its range beyond southern Europe obviously decreases the value of *C. albiceps* in estimating the site of death, in that it is no longer exclusive to southern European regions. Moreover, the aggressive feeding behaviour of second and third instar larvae of *C. albiceps* could reset the post-mortem insect clock by clearing a corpse of all earlier arrivers.

**Keywords** *Chrysomya albiceps* · Calliphoridae · Forensic entomology · Development time · Post-mortem interval

### Introduction

Since the report by Mègnin (1894), synanthropic flies particularly calliphorids, have been recognised as the first wave of the faunal succession on human cadavers (Nuorteva 1977; Smith 1986). They are therefore the primary and most accurate forensic indicators of the post-mortem interval.

*Chrysomya albiceps* (W.) is normally a carrion breeder and is frequently involved in secondary myiasis in sheep, following an initial colonisation by *Lucilia sp.*, but cases of myiasis in man are not known (Zumpt 1965). The first-stage larvae feed on exudations of the decomposing flesh, but the second-stage and third-stage larvae are predaceous, feeding on other blowfly larvae (Del Bianco Faria et al. 1999). This behaviour may possibly lead to a decline in the population numbers of native species. The life history of *C. albiceps* has been summarised by Zumpt (1965).

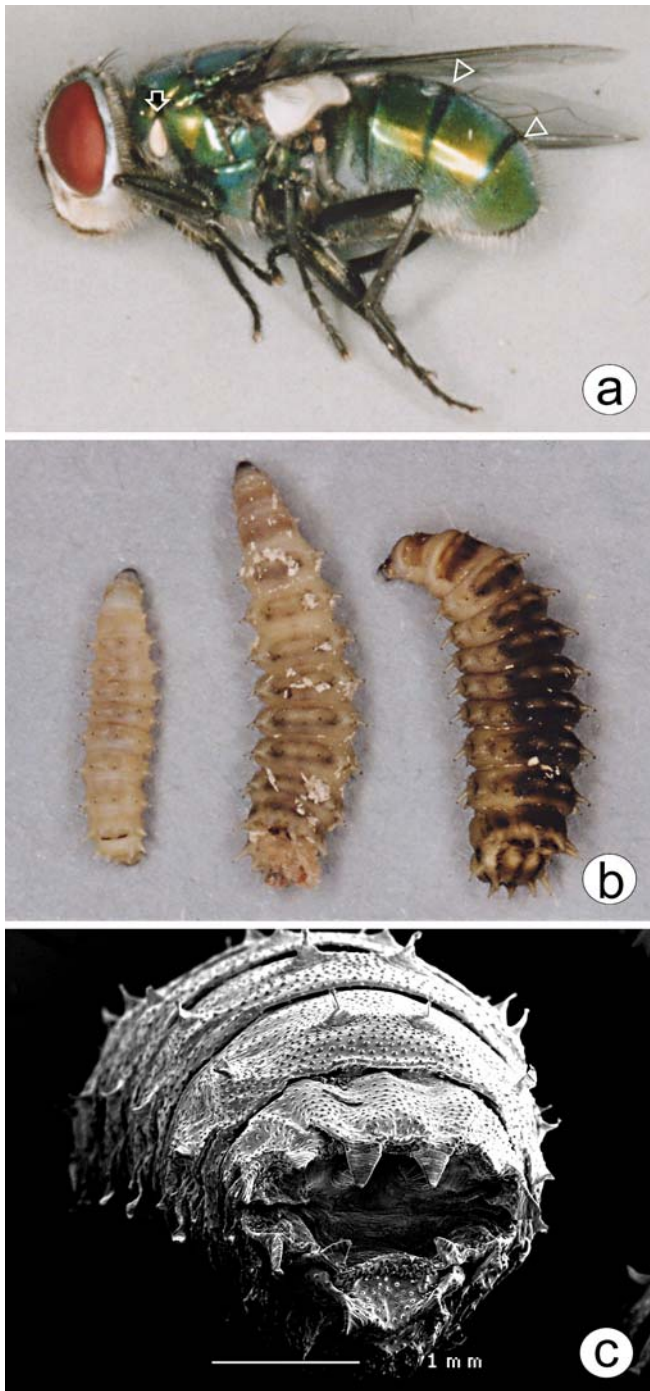
The larvae of *C. albiceps* (“hairy maggots”) are very similar to those of *C. rufifacies* (Macquart), and are morphologically very distinct from all other calliphoridae larvae, possessing prominent fleshy protrusions along their body (Fig. 1b) (Baumgartner and Greenberg 1984). The adults of this species have stout bodies and are brilliant green in appearance. The terminal edge of the abdominal segments is noticeably darker (Fig. 1a). Adults, larvae and pupae (Fig. 1c) can easily be distinguished from other carrion-associated fly species in central and northern Europe, even by investigators who are not specialised in forensic entomology.

*C. albiceps* is very common and abundant in southern Europe, afrotropical, oriental (from India to China) and neotropical regions (Central and South America) (Baumgartner and Greenberg 1984; Hall and Smith 1993). Before the case described in this report, no forensic case involving the blowfly *C. albiceps* (Wiedemann) as indicator

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**Fig. 1** a Adult *C. albiceps* with typical stout body, dark coloured terminal edge of abdominal segments (arrowheads), white face and white anterior spiracle (arrow), b *Chrysomya albiceps* larvae covered with fleshy protuberances ("hairy maggots"), note the increasing degree of pigmentation with age (from left to right), c scanning electron microscope picture of the posterior aspect of the pupal shell

for the post-mortem interval in central Europe had been published. Information on the developmental rate, the predatory behaviour and the possible geographic distribution of *C. albiceps* will provide background data for future forensic-entomological casework.

## Material and methods

### Rearing experiments

Eggs, larvae and adults of *C. albiceps* were collected from human cadavers and from pig carcasses in and around the city of Vienna during the fly-active period of the year 2001. Adults and larvae were identified using the morphological characters described by Crosskey and Lane (1993), Tantawi and Greenberg (1993b), Carvalho-Queiroz et al. (1997) and Wells et al. (1999).

The flies were held in an insectary at 22–25°C with approximately 60% relative humidity and a photoperiod of 14:10 (light: dark) hours. New adult flies were added from time to time. About 300 adult flies were kept in screen cages (40×30×30 cm) and fed a mixture of dry granular sugar, powdered milk and brewer's yeast. Water was supplied by inversion of a beaker filled with water on a Petri dish covered with a filter paper.

### Egg period under different constant temperature regimes

To study the time range of the egg period (i.e. time from oviposition to emergence of first instar larvae) under different constant temperatures, eggs were collected within 30 min of oviposition, using black 35-mm film cups filled with decaying beef liver. This provided a dark and moist environment preferred by the female adults for oviposition. The eggs were separated by soaking in sodium sulphite solution (1%). After shaking vigorously, the egg clusters were usually broken apart within 5 min. Eggs were spread on Columbia agar plates containing 5% sheep blood (BioMérieux) using a Pasteur pipette. The resulting egg-monolayer facilitated recognition of larval emergence and the moisture of the agar prevented the eggs from drying out, an important detail at higher temperatures. The agar plates were put in the incubator at one of the five selected temperatures (15, 20, 25, 30 and 35°C), and incubated plates were checked at 30-min intervals. For each temperature regime five plates at different times of day were prepared to ensure early recognition.

### Growth under different constant temperature regimes

Eggs were collected within 30 min of oviposition, samples of about 100 eggs were spread on 250 g raw beef liver, cut in slices approximately 1 cm thick, and subsequently transferred into plastic jars (25×25×7 cm) covered with a gauze net. Using this procedure, we achieved a more 2-dimensional and disseminated feeding behaviour, which is essential to prevent maggot mass formation. The bottom of the jars was covered with sawdust to provide a dry place for pupation. This is important because it is considered that pupation from larvae could be delayed under suboptimal conditions (Wells and Kurahashi 1994). However, we observed that if the substrate is relatively dry and not exposed to bright light, larvae of *C. albiceps* pupate on the surface.

The jars were then placed into a precision environmental chamber (KBK/LS 4330, Ehret, Germany) at one of the five temperature regimes (15, 20, 25, 30 and 35°C) with the relative humidity set to 65–70%. This procedure was repeated 10 times for each temperature regime and the mean temperature within the centre of actively feeding maggots was recorded twice daily using a digital thermometer.

Of the largest maggots, three were removed from the plastic jars twice daily. When the first maggots stopped feeding those in the migratory phase were removed for measurement purposes, until approximately 10% of the maggots had undergone pupation. Measuring the largest individuals (i.e. the oldest, before peak feeding) is regarded as common practice in forensic entomology (Byrd and Butler 1998).

Specimens were killed in hot water to prevent shrinkage, as might be the case with other killing and preservative solutions (Tantawi and Greenberg 1993a). Measurement followed immedi-

ately under a binocular microscope in 0.1 mm units using a vernier caliper.

#### Predation on local blowfly larvae

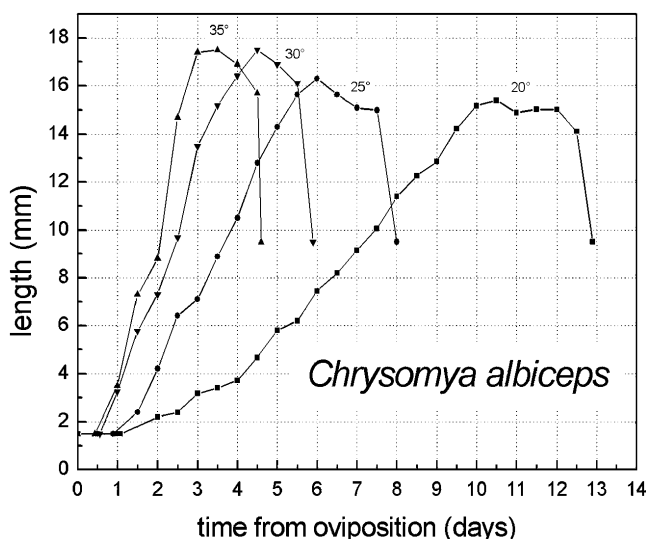
To assess the predatory potential of *C. albiceps* larvae on larvae of *Lucilia sericata*, one of the most frequent blowflies encountered in forensic-entomological cases in the Vienna area (Grassberger and Reiter 2001), we conducted the following in vitro experiment: early second instar larvae of *C. albiceps* and *L. sericata* (~5 mm in size) that had been reared in the laboratory were placed on rearing substrate in the ratios 1:1, 1:2 and 1:4 (representing 100:100, 50:100 and 25:100 larvae, respectively) in plastic jars (25×25×7 cm) containing a layer of 0.5 cm sawdust. For each ratio the experiment was replicated 3 times. As a rearing substrate, raw beef liver was provided in excess to avoid predation as a result of food shortage. The ambient temperature fluctuated between 23 and 26°C during the experiment.

After pupation, the pupae of *L. sericata* were removed from the sawdust and counted. Since *C. albiceps* frequently pupated in the remaining liver tissue, flies were allowed to hatch, and were counted after killing with ether.

## Results and discussion

#### Rearing experiments

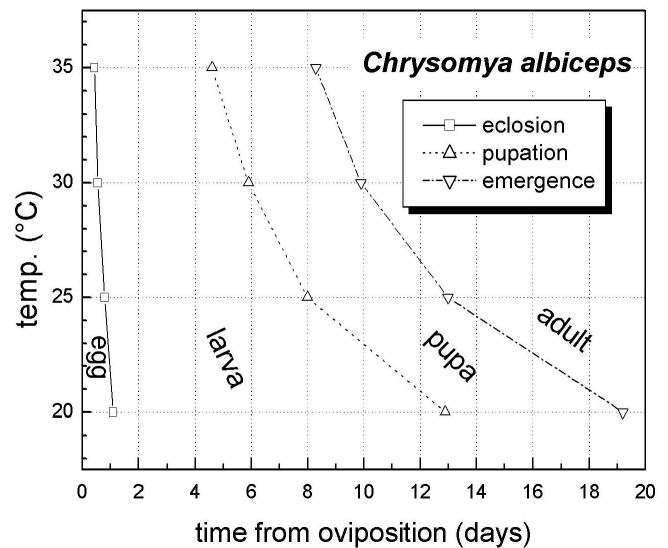
The means of the maximum measured lengths of all rearings were plotted against time for each of the constant temperature regimes (Fig. 2). In the centre of actively feeding third instar larvae the recorded temperature was sporadically 1–1.5°C above the desired temperature regime. The minimum duration of development from oviposition to pupation and from oviposition to eclosion (total immature development) at the five temperature regimes studied is presented in Table 1. In the laboratory, a constant temperature of 15°C stopped development of the first instar larvae.



**Fig. 2** Development of *C. albiceps* from oviposition to pupation at four different constant temperatures; for values (mean days  $\pm$  SD) see Table 1

**Table 1** Minimal developmental times (mean days  $\pm$  SD) of *C. albiceps* life stages at five constant temperature regimes

Temp. °C	Minimal developmental time from oviposition to:		
	Egg hatch	Pupation	Eclosion
15	1.9 $\pm$ 0.16	–	–
20	1.1 $\pm$ 0.09	12.9 $\pm$ 0.41	19.2 $\pm$ 0.92
25	0.8 $\pm$ 0.05	8.1 $\pm$ 0.21	13.0 $\pm$ 0.79
30	0.5 $\pm$ 0.03	5.9 $\pm$ 0.25	9.9 $\pm$ 0.61
35	0.4 $\pm$ 0.02	4.6 $\pm$ 0.22	8.3 $\pm$ 0.5



**Fig. 3** Isomorphen diagram for *C. albiceps*, showing all stages from oviposition to eclosion. Areas between lines represent identical morphological stages at various temperatures. Each line represents identical morphological changes of this holometabolous insect; for values (mean days  $\pm$  SD) see Table 1

Similar to the previously published isomegalen diagrams (Reiter 1984; Grassberger and Reiter 2001), all developmental data from oviposition to eclosion are represented in the isomorphen diagram. In this diagram, time from oviposition to eclosion is plotted against temperature, each line representing morphological changes. Areas between lines represent identical morphological stages of the blowfly *C. albiceps* (Fig. 3). This diagram is especially useful when post-feeding stage larvae or pupae are recovered from the corpse, a condition under which length is no longer a useful criterion of age.

#### Predation on local blowfly larvae

Table 2 shows the mortality rates of both species at the three different ratios. The mortality rate of *L. sericata* caused by predation of *C. albiceps* ranged from 57.6% ( $\pm$  4.5 SD) at a ratio of 4:1 (*L.s.:C.a*) to 99% ( $\pm$  0.1 SD) at a ratio of 1:1 indicating a high susceptibility of *L. sericata* to attack by *C. albiceps*. A similar vulnerability of other native blowfly larvae used in forensic post-mortem inter-

**Table 2** Mortality Rate of *L. sericata* and *C. albiceps* at various ratios ( $n = 3$  for each ratio)

Ratio (individuals) <i>L.s.:C.a.</i>	Mortality rate (mean±SD) %	
	<i>L. sericata</i>	<i>C. albiceps</i>
100:100	99.0 ± 1	10.3 ± 2.5
100:50	76.6 ± 8.7	14.0 ± 2
100:25	57.6 ± 4.5	5.3 ± 6.1
100:0 <sup>a</sup>	2.0 ± 2.6	–
0:100 <sup>a</sup>	–	7.3 ± 2.5

<sup>a</sup>Controls.

val estimates (e.g. *C. vicina*, *C. vomitoria*, *Ph. regina*, *P. terraenovae*, *L. caesar*) seems very likely, because of their similar biology. Biological invasions (i.e. migration of organisms into a new habitat) frequently cause severe changes in the structure of local communities (Hengeveld 1990). A pig carrion study conducted throughout summer 2001 showed that a cadaver can be almost monopolised by a single predatory fly species like *C. albiceps*, leaving only the well protected larvae of *Ophyra sp.* unharmed (Grassberger, unpublished data).

Del Bianco Faria et al. (1999) studied the larval predation by *C. albiceps* on native species in Brazil in the laboratory and found predation rates as high as 80%. They also cited the detailed work of Ulyett (1950) who found that *L. sericata* and *C. chloropyga* were eliminated when bred with *C. albiceps* at densities higher than 2,000 and 10,000 larvae per 140 g of meat, respectively.

However, it is difficult to draw conclusions about the impact of invaders on native species from simple laboratory experiments. We believe that experiments designed

to investigate this question should be performed in order to draw the right conclusions in future forensic entomological casework.

### Case description

On 4th August 2001 the caretaker of a house in the 10th district of the city of Vienna noticed an unpleasant smell emanating from an apartment on the third floor. Since nobody responded to his ringing and knocking, he reported this incident to the police who subsequently opened the door. Questioning of the neighbours revealed that the woman living in this apartment, a 56-year-old heavy drinker frequently had loud arguments with her boyfriend.

In the apartment, which was in an overall neglected and disorganised state, the remains of a woman were discovered under a large heap of clothing (Fig. 4). The body was in a bloated stage of decay with heavy maggot infestation in the face as well as on the partially skeletonised right arm and shoulder.

Entomological evidence found on and around the corpse was collected and preserved according to medico-legal standard procedures (Haskell et al. 1997).

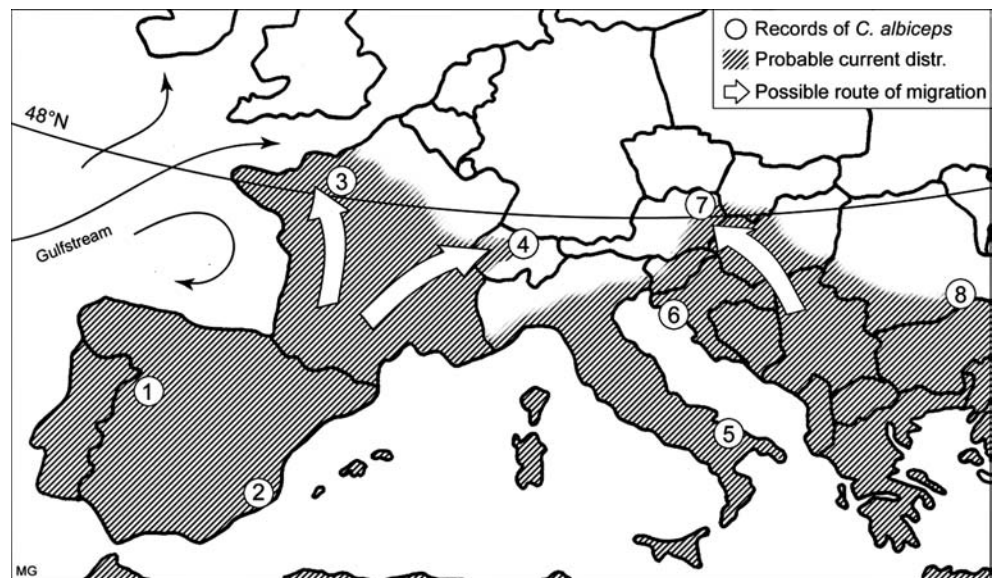
At autopsy at the Institute of Forensic Medicine of Vienna, multiple stab wounds in the ventral aspect of the trunk and one stab wound in the back, perforating several internal organs were described. The corpse showed general greenish discolouration, autolysis of internal organs and heavy maggot infestation as signs of advanced decay.

Large numbers of third instar hairy maggots (10–14 mm) and several pupae were found on the trunk in the clothing and on the head of the victim. Empty pupal cases of *C. albiceps* were not found at the crime scene. Two of the recovered pupae emerged in the early afternoon of the next day in the laboratory after incubation at 25°C. Near the corpse several empty pupal cases of *Muscina stabulans* (“false stable fly”) were found. A large number of larvae of *Piophilidae casei* (“cheese skipper”) were collected from the soiled clothing of the victim. Temperature readings obtained from the apartment and, from local weather stations revealed an almost constant temperature between 23 and 25°C over the last 3 weeks. The average minimal developmental time from egg to adult emer-

**Fig. 4** The victim's body was found on the evening of 4th August 2001 under a large heap of clothing in a bloated stage of decay with heavy maggot infestation



**Fig. 5** Previous records and probable current distribution of *C. albiceps* in the Palearctic region. The arrows indicate the possible migration route of this tropical and subtropical species. 1 Salamanca province, Spain (Martínez-Sánchez et al. 2000), 2 Murcia, Spain (Arnaldos and Romera 2001), 3 North of Paris and Channel coast (Erzinclioğlu 2000), 4 Zurich, Switzerland (Rognes 1997), 5 Bari, Italy (Introna et al. 1998), 6 Malinska, Croatia (Reiter 1999, unpublished results), 7 St. Valentin, Austria (Reiter 1988, unpublished results), Vienna, Hintersdorf and Retz, Austria (present study, 2001), 8 Bucarest, Romania (Nistorescu and Fabritius 2000, unpublished results)



gence of *C. albiceps* at 20°C and 25°C is 19.2 and 13 days, respectively (data from present study). According to Marchenko (2001), *Muscina stabulans* requires 17 days at 23°C and 15.1 days at 25°C to complete development. Therefore, it was estimated that oviposition of *C. albiceps* and *M. stabulans* took place somewhere between the 17th and 23rd of July, making it likely that the homicide took place around that time.

One day after the autopsy, the assailant reported himself to the police, and confessed to killing the victim with a kitchen knife on the evening of the 18th of July during a short domestic argument.

### Previous records of *C. albiceps* in the Palearctic region

*C. albiceps* is generally described as tropical and subtropical species which can be found in Africa, southern Europe, Arabia, India and, recently, Central and South America (Hall and Smith 1993). Nistorescu and Fabritius (unpublished results) found that after 30 years of absence, in the warm summer of 2000 in Bucarest (44°25'N, 26°06'E) Romania, 15.6% of all trapped *Calliphoridae* (11.3% of all trapped flies,  $n = 4,250$ ) were *C. albiceps*. Arnaldos and Romera (2001) recorded *C. albiceps* throughout the years 1996 and 1997 close to the city of Murcia (37°58'N, 1°07'W) in the southeast of the Iberian Peninsula (Spain). Martínez-Sánchez et al. (2000) described *C. albiceps* in the Salamanca province (Spain) (41°58'N, 5°40'W) and Introna et al. (1998) collected larvae of *C. albiceps* from a partly skeletonised decaying body in the month of September in a suburban area of the City of Bari (southern Italy) (41°07'N, 16°51'E). Reiter (unpublished data) collected adults from a trap near the town of Malinska (Island of Krk, Croatia) (45°07'N, 14°31'E) in July 1999.

A carrion succession study with pig cadavers conducted throughout the year 2001 along with six forensic entomological cases in and around the City of Vienna (Austria) (48°13'N, 16°19'E) revealed large numbers of *C. albiceps* larvae (Grassberger, unpublished data). Sev-

eral traps from various places in lower Austria (close to the border of the Czech Republic) baited with decaying beef liver also yielded large numbers of *C. albiceps* larvae throughout the summer of 2001.

The last time larvae of *C. albiceps* were recorded on the corpse of a homicide victim in Austria was in the year 1988 (Reiter, unpublished data). A similar far north record of this Mediterranean species has been described by Rognes (1997) who captured one female fly on a dead body in an apartment in Zurich (47°22'N, 8°32'E), Switzerland in October 1995. Apart from some records by the French Gendarmerie in the year 1996 (Erzinclioğlu 2000), reports of *C. albiceps* from the Palearctic region north of the Alps (i.e. north of a latitude of 48°N) have been scarce. The expansion of its range to the north of Paris and along the French channel coast during hot summers is probably facilitated by the mild climatic influence of the Gulfstream (Fig. 5).

### Conclusions

Developmental times of *C. albiceps* from the Vienna area were in general similar to those reported by Marchenko (2001) at temperatures between 20 and 30°C. Marchenko successfully reared *C. albiceps* at 13°C from egg to adult in 66.3 days, whereas larvae frequently died as second and third instars at 15°C in our study when maggot mass formation was prevented. However, larger differences do not necessarily have to be attributed to variation in experimental method (extrinsic factors). Geographic adaptation (intrinsic factors) could explain a difference in survival between *C. albiceps* from Russia and central Europe when reared at temperatures as low as 15°C. Carvalho et al. (2001) observed that *C. albiceps* larvae reared on rabbit tissues containing diazepam developed more rapidly than larvae from control colonies and that these differences were large enough to alter the estimate of post-

mortem interval based on fly development. Other drugs or toxic substances may also have a marked influence on the rate of development of *C. albiceps*, sufficient to alter post-mortem interval estimates (Gagliano-Candela and Aventaggiato 2001).

Our in vitro study on the predatory behaviour of *C. albiceps* on *L. sericata* clearly shows the dramatic negative effect of this facultative predator on other native dipteran species. After its introduction to the Americas in the last years along with other Old World species of blowflies, an apparent correlate of this biological invasion has been a sudden decline in the population numbers of *Cochliomyia macellaria*, a native species of the Americas (Del Bianco Faria 1999). *C. albiceps* along with *C. rufifacies* is rapidly expanding its range throughout the American continent (Baumgartner and Greenberg 1984). In the US, *C. rufifacies* has recently been described as far north as Knoxville, Tennessee (Shahid et al. 2000).

In the pig carrion study conducted during the summer of 2001 in the city of Vienna (Grassberger, unpublished data) females of *C. albiceps* began oviposition in the morning of the third day post-mortem. In the rural area north-west of Vienna *C. albiceps* began oviposition within a few hours after placement of decaying beef liver. These findings imply that that *C. albiceps* is among the early colonisers of a corpse when temperatures are favourable.

The large numbers of *C. albiceps* present and the consistency of such collections from July to September 2001 in the northern parts of Austria, suggest that this species is able to spread up to central and possibly even northern Europe from the Mediterranean basin during hot summers and compete with local species. However, one has to be careful when predicting shifts in the distribution of a species due to climate changes by simply relying on the 'climate envelope' approach (Porter 1995). The fault in this approach is that distributions of species also reflect the influence of interactions with other species. So predictions based on climate envelopes may be misleading if the interactions between species are altered by climatic change (Davis et al. 1998).

We believe that *C. albiceps* reached the northern parts of Europe not via the Alps but through the eastern and western parts of Europe, as indicated by the Zürich and Vienna records (Fig. 5). Because it is a rare species in central European fauna, we intend to monitor its presence in order to find out its future distribution, and whether it can be regarded as established north to a latitude of 48°N. The described northward expansion of its range beyond southern Europe obviously decreases the value of *Chrysomya albiceps* in estimating place of death, in that it is no longer exclusive to southern regions. However, since its abundance seems to be dependent on hot summers and immature development ceases at 15°C, some conclusions about the season of death can be made when pupal cases are found in association with human remains. Moreover, the aggressive feeding behaviour of second and third instar larvae of *C. albiceps* on local carrion-breeding larvae could reset the post-mortem insect clock by clearing a corpse of

all earlier arrivers. This is a crucial detail when estimating the post-mortem interval, based on entomological evidence.

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