ORIGINAL ARTICLE

Temperature-dependent appearance of forensically useful flies on carcasses

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Abstract Flies are frequently used for postmortem interval (PMI) estimations. These estimates are usually based on the age of larval or pupal specimens. However, the age defines only the minimum PMI. In order to move forensic entomology further, a method useful for the estimation of an interval preceding insect appearance on a corpse called the pre-appearance interval (PAI) is needed. Recently, it was demonstrated that the PAI of several carrion beetles is closely related to the temperature prevailing throughout this interval. Hence, it was postulated to estimate PAI from temperature. In order to check premises for using this approach with flies, a test of the relationship between adult or oviposition PAI and temperature was made for nine species of European flies. Data on PAI originated from pig carcasses decomposing under various temperatures. Adult PAI of *Hydrotaea dentipes*, *Hydrotaea ignava*, *Hydrotaea similis*, *Phormia regina*, and *Stearibia nigriceps* and oviposition PAI of *S. nigriceps* were exponentially related to temperature. Only *S. nigriceps* revealed a close relationship, demonstrating solid premises for PAI estimation from temperature alone. Adult and oviposition PAI of *Calliphora vomitoria* and adult PAI of *Hydrotaea pilipes* were not related to temperature. Adult and oviposition PAI of *Lucilia sericata* and *Lucilia caesar* responded similarly, with an abrupt and large increase in a narrow range of low temperatures and no response in a broad range of high temperatures. Probably, different mechanisms form the basis for the response of PAI to temperature in flies colonizing carcasses shortly after death and flies colonizing carcasses later in the decomposition process.

Keywords Forensic entomology · Postmortem interval · Pre-appearance interval · *Lucilia* · *Phormia* · *Calliphora* · *Hydrotaea* · *Stearibia*

Introduction

Postmortem interval (PMI) may be estimated from the development or the succession of insects on corpses [1–5]. Entomological estimates are most frequently based on the age of larval or pupal specimens sampled from a corpse [1–5]. However, the age of an insect alone may define only the minimum PMI, as it gives information just about a development interval. Unfortunately, the age of an insect gives no information about an interval which precedes the appearance of a given specimen on a corpse which is named the pre-appearance interval (PAI) or the pre-colonization interval [6]. In the case of early arriving insects (e.g., blowflies), the minimum PMI is usually close to the actual PMI, but in the case of middle and late arriving insects, it defines actual PMI with the intrinsic and frequently very high inaccuracy. This is probably the main reason why middle and late arriving insects are rarely used for PMI estimation. However, even with the early arriving insects, the minimum PMI may be far from the actual PMI, as was exemplified by results of European, early spring carrion studies in which blowflies colonized carcasses with a delay of several days [7, 8]. Accordingly, in order to move forensic entomology further, a robust method for PAI estimation is needed.

In casework, an estimate of PAI may be reached through a qualitative evaluation of factors which delay colonization of carcasses by insects or through a reference to results of case-relevant pig carrion studies. Due to the lack of transparency

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and inapplicability to middle and late arriving insects, the first approach is inadequate for routine use. The second approach is, however, clearly impractical because it needs unattainable quantity of data from pig carrion studies. Recently, a novel approach was proposed. It was demonstrated that adult or larval PAI of some forensically important insects (particularly beetles) are closely related to the temperature prevailing in the corpse surrounding throughout the PAI [9–12]. Hence, it was postulated to estimate PAI from temperature, and several methods useful for that purpose were initially tested [9, 10]. This approach has several advantages. First, previous works demonstrated that models of the relationship between PAI and temperature give accurate estimates for cases from different habitats, seasons, and biogeographic areas [9, 10]. These results suggest that PAI may be estimated using case-specific temperature data and a species and stage-specific model for the relationship between PAI and temperature. Second, temperature is an elementary factor, and as such, it may be easily combined with other elementary factors (e.g., air humidity), if multifactor models for the relationship will be needed. Third, temperatures are the only case-specific data required by the method, and they may be gathered with well-defined and easily applicable protocols for measurement and retrospective correction [13–15].

In order to use the temperature approach to PAI estimation, the relationship between PAI and temperature should be close. It was found that several forensically important species of beetles meet this requirement [11]. However, no previous work tested the relationship in forensically important flies, insects which are most frequently used for the estimation of PMI [16, 17]. There are flies which colonize carcasses immediately after death, as for example Calliphoridae or Sarcophagidae, but there are also flies which colonize corpses later in the decomposition process, e.g., Muscidae, Fanniidae, or Piophilidae [8, 18–22]. A temperature method for the estimation of PAI would be particularly useful in the case of the latter group of flies. In order to calibrate the method for these flies, a much better insight into the relationship between PAI of carrion flies and temperature is necessary. Hence, in this article, the relationship is tested for several forensically important European species of flies.

Materials and methods

Experimental design

In order to collect PAI data from a broad range of temperatures, carcasses were separated in time and space. The study was divided into six placements: 18 April (four pigs), 15 June (six pigs), 4 July (six pigs), 21 July (four pigs), 16 August (four pigs), and 30 August (six pigs) of 2011. In each placement, half of carcasses were exposed in forest habitats (hornbeam–oak forest, alder forest, and birch forest) and half in open habitats (xerothermic grasslands, sparse clumps of young birches in grasslands, and edges of grasslands and pine–oak or birch forests). This design assumes that the effect of season or habitat on PAI reduces itself to the effect of temperature specific for that season or habitat.

The experiment was conducted in the Biedrusko Military Range (Western Poland, Europe). In order to decrease migrations of immature insects and joint attraction by carcasses, they were far away from each other by at least 50 m (the distance between particular pigs was, in most cases, much larger, e.g., "open" and "forest" carcasses by about 2–3 km).

Table 1 Ranges in PAI and temperature for particular species

Family	Species	Stage	N	PAI range [days]	Temperature range [°C]
Calliphoridae	*Calliphora vomitoria* (Linnaeus, 1758)	A	25	0.2–9.4	13.0–23.3
		E	19	0.2–26.3	13.3–21.6
	Lucilia caesar (Linnaeus, 1758)	A	26	0.2–3.2	14.0–33.7
		E	26	0.2–3.4	13.5–33.7
	Lucilia sericata (Meigen, 1826)	A	15	0.2–5.2	14.0–25.1
		E	14	0.4–12.2	15.4–25.1
	Phormia regina (Meigen, 1826)	A	25	1.2–8.4	14.8–25.5
Muscidae	*Hydrotaea dentipes* (Fabricius, 1805)	A	15	0.3–11.0	13.2–24.4
	Hydrotaea ignava (Harris, 1780)	A	16	2.2–24.3	12.5–23.8
	Hydrotaea pilipes Stein, 1903	A	21	2.3–12.3	13.8–20.6
	Hydrotaea similis Meade, 1887	A	20	0.3–11.0	12.8–19.5
Piophilidae	*Stearibia nigriceps* (Meigen, 1826)	A	23	3.4–23.3	12.3–23.5
		E	22	8.3–37.3	13.3–22.4

A adult stage, *E* egg stage

Carcasses

In total, 30 domestic pig carcasses of similar mass (mean= 23 kg; range=11.4–56.7 kg) were bought from a local pig farm. Pigs were killed at about 6 A.M. (a blow to the base of the skull) and, after 1 to 3 h, were exposed in the field. Carcasses were laid down on a metal grating and were protected with welded wire mesh.

Sampling of flies

Flies were sampled three times during the first day (after exposition, i.e., 1–3 h postmortem and then 4–6 and 10–11 h postmortem), two times a day for the following 5 days (between 10 A.M. and 1 P.M. and between 4 and 6 P.M.), and then once a day for the rest of the study (usually between 11 A.M. and 2 P.M.). The sampling was more frequent during the first days postmortem, as we wanted to increase the accuracy of PAI determinations for the early arriving flies. Two researchers performed the morning inspections, whereas the afternoon inspections were made by one person. The former lasted about 30 min and the latter about 15 min.

Samples included pitfall trap, aerial sweep net, manual and soil collections. Two traps (diameter=16 cm, height=17 cm, 50 % ethylene glycol) were buried dorsally and ventrally to the carcass. Adult flies were collected with a swatting technique, performed twice using large aerial sweep net (diameter=55 cm). Manual sampling focused on the surface of a carcass and soil under and near the carcass. It lasted about 5 min. Trap, net, and manual collections were taken at every inspection. Samples were preserved in 70 % ethanol. Soil sampling started after termination of bloating and was made for the subsequent 2–3 weeks (every second day during the morning inspections). Samples (about 700 ml) were manually screened for larger insects and, afterwards, were put into the Tullgren funnels for 2 days.

Flies were determined using keys for identification [23–26] and collections of authors. In order to time oviposition, eggs were sampled for rearing purposes. Larvae were kept on pork in 20 °C until adults emerged. Rearing containers were filled with vermiculite (Calliphoridae) or sand (Piophilidae).

Temperature measurements

Because carcasses were exposed on the ground, ground-level temperature was measured. HOBO U23 Pro v2 2× External Temperature Data Loggers (Onset Computer Corporation, Bourne, MA, USA) were used with sensors positioned on the ground (one dorsally and one ventrally to the carcass). Sensors were not protected in any way (debris, plants, etc. were carefully removed). Logging started at 6 A.M. of the first day and 5-min logging intervals were used. Temperatures

relevant for every PAI were averaged separately for each sensor and then a single mean was calculated.
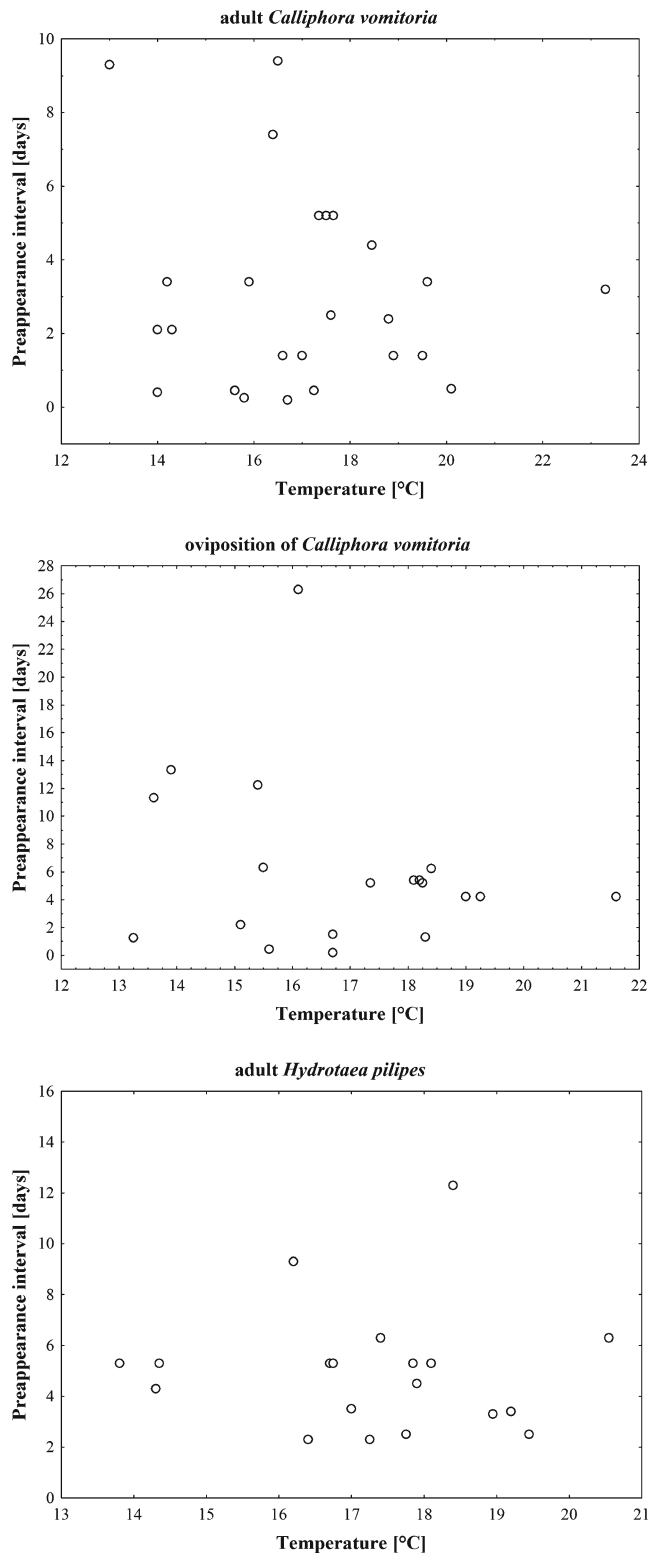

Fig. 1 The PAI and ground-level temperatures averaged for the duration of PAI for *C. vomitoria* and *H. pilipes*

Data analyses

The relationship between PAI and temperature was tested separately for the adult PAI and the oviposition PAI. The adult PAI is an interval from the moment of death until the arrival of first adult insect of a given species. The oviposition PAI is an interval from the moment of death until the appearance of the first eggs of a given species. The relationship between the oviposition PAI and temperature was analyzed for *Calliphora vomitoria*, *Lucilia caesar*, *Lucilia sericata* (Calliphoridae), and *Stearibia nigriceps* (Piophilidae). As for the other species, there were only singular observations which precluded any robust analyses.

The relationship was modeled from at least 14 observations (Table 1). Data sets usually covered a broad range of temperatures (Table 1). In order to check for colinearity, scatter plots were made. As expected, for most species, the relationship was exponential with a clear representation for the minimum PAI. Consequently, the exponential

model with asymptote displaced from zero was fitted $(PAI = c + e^{(b_0 + b_1 \times \text{temperature})}$, where c is "the minimum PAI," e is "the base of the natural logarithm," b_0 and b_1 are parameters responsible for the shape of the curve). Because the estimate of c regularly differed from the observed minimum PAI, models with c fixed a priori were also tested. Graphs are given for models with estimated c, equations for both types of models. Parameters were approximated with the Levenberg–Marquardt procedure.

For species which revealed no relationship, only scatter plots are given. For species with statistically insignificant relationship, scatter plots and models with estimated c are presented. For the other species, both types of models and lower temperature (LT) thresholds are given. LT thresholds were determined using the method of the least coefficient of variation in accumulation of degree-days [11]. For all analyses, the 5 % level of significance was accepted. Calculations were made using Statistica 9.1 (StatSoft, Inc., 2010).
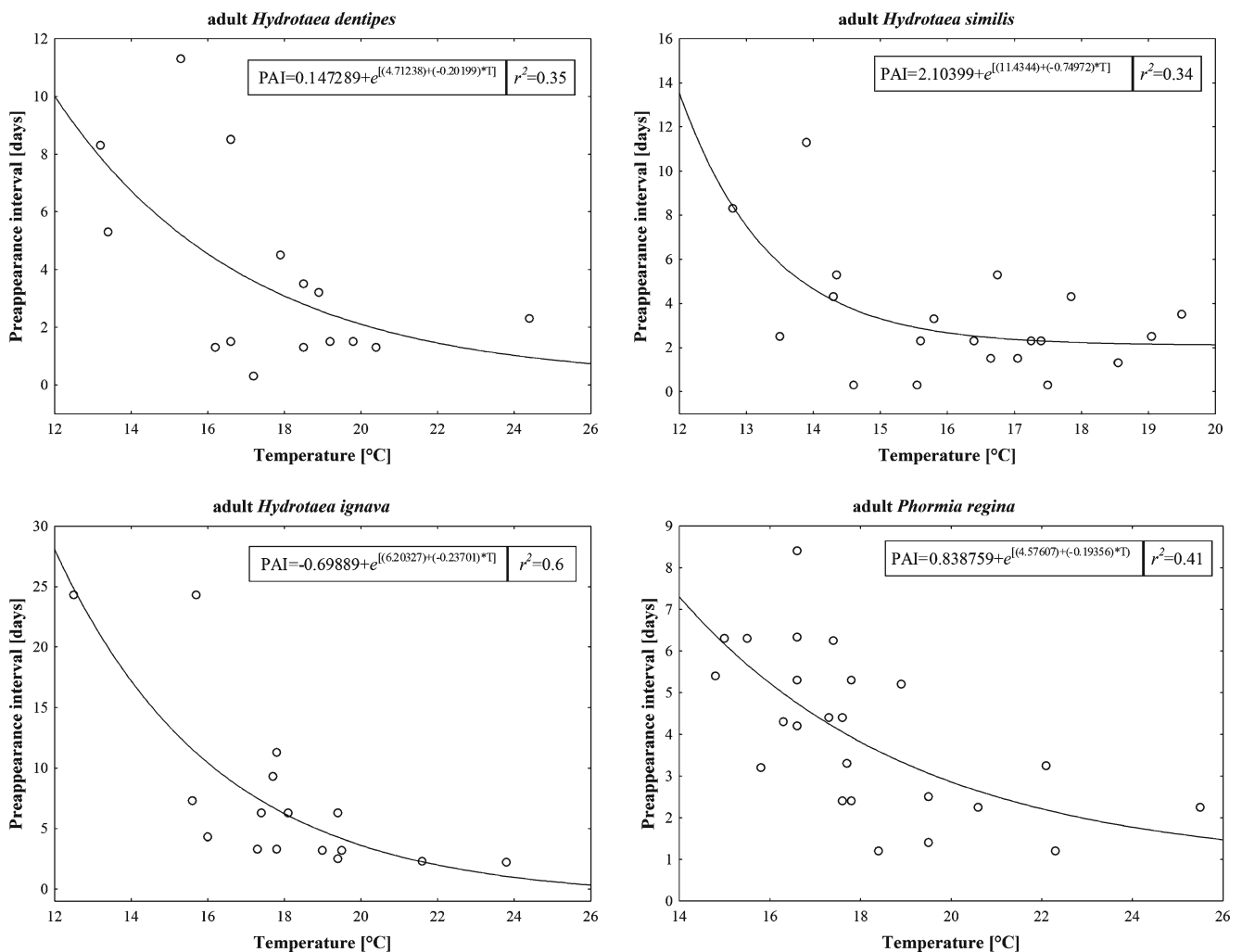

Fig. 2 The relationship between the PAI of selected Muscidae and Calliphoridae and ground-level temperatures averaged for the duration of PAI (*T*)

Results

Adult and oviposition PAI of *C. vomitoria* and adult PAI of *Hydrotaea pilipes* showed no relation to temperature at all (Fig. 1). Adult PAI of *Hydrotaea dentipes*, *Hydrotaea ignava*, *Hydrotaea similis*, and *Phormia regina* were exponentially related to temperature with a clear representation of the minimum PAI, however, with no statistical significance (nonlinear regression, *t* test for b_1, $P = 0.47$, 0.11, 0.19, and 0.3 respectively; Fig. 2). Adult and oviposition PAI of *S. nigriceps* were exponentially and significantly associated to temperature with evident representation of the minimum PAI (nonlinear regression, *t* test for b_1, $P < 0.001$; Fig. 3). LT thresholds for *S. nigriceps* were 11.3 °C for the adult PAI and 11.4 °C for the oviposition PAI (Table 2).

Adult and oviposition PAI of *L. caesar* and *L. sericata* responded similarly to temperature (Fig. 4). In a high temperature range, PAI was more or less constant and unrelated to

Table 2 LT thresholds and accumulations of degree-days (ADD) for the adult and oviposition PAI of *S. nigriceps*

PAI	LT [°C]	ADD				Coefficient of variation in ADD [%]
		Mean	N	Standard error	Range	
Adult	11.3	39.29	23	2.32	23.2–64.3	28.32
Oviposition	11.4	75.57	22	3.07	45.1–109.3	19.04

temperature (Fig. 4). Under daily average temperatures above 17 °C, the oviposition of *L. caesar* was always recorded within the first 12 h postmortem, whereas in the case of *L. sericata*, it started, in each case, with temperatures above 18 °C within the first 35 h after death (Fig. 4). In a low temperature range, adult and oviposition PAI of *L. caesar* responded erratically to the temperature with some cases near the minimum PAI and some cases largely above the minimum PAI (Fig. 4). In the case of *L. sericata*, the response of PAI to low temperature was similar, although less erratic (Fig. 4). For both species and both kinds of PAI, the low temperature change in PAI may be represented by a near-vertical line (Fig. 4).

Discussion

Previous results indicate that an exponential decrease in PAI with an increase in temperature is a general model for the relationship between PAI and temperature in carrion insects [11]. Current results partly support this proposition. For most species of flies, PAI decreased exponentially with an increase in average daily temperatures. A clear exception was only adult and oviposition PAI of *L. caesar* and *L. sericata*, in which case lack of response in a high temperature range was followed by an abrupt and large increase in PAI in a narrow range of low temperatures. In order to understand this unusual response, one should look at factors governing the attraction of insects to carcasses.

Carrion insects respond to volatile organic compounds emitted from a decomposing carcass [27–32]. For example *L. sericata* and *L. caesar* are attracted by oligosulfides, in particular dimethyl disulfide [27, 29], which is formed early in carrion decomposition from the sulfur-containing amino acids [33, 34]. These results indicate that the emission of relevant attractants is the most important precondition for the appearance of insects on a carcass. Consequently, it was suggested that processes in which attractants are generated depend on temperature, and as a result, PAI for most carrion insects is also temperature-dependent [9, 10]. Previous [9–11] and current models for *S. nigriceps*, *P. regina*, and species of *Hydrotaea* support this hypothesis. However, the unusual pattern of PAI response to temperature, as recorded for both
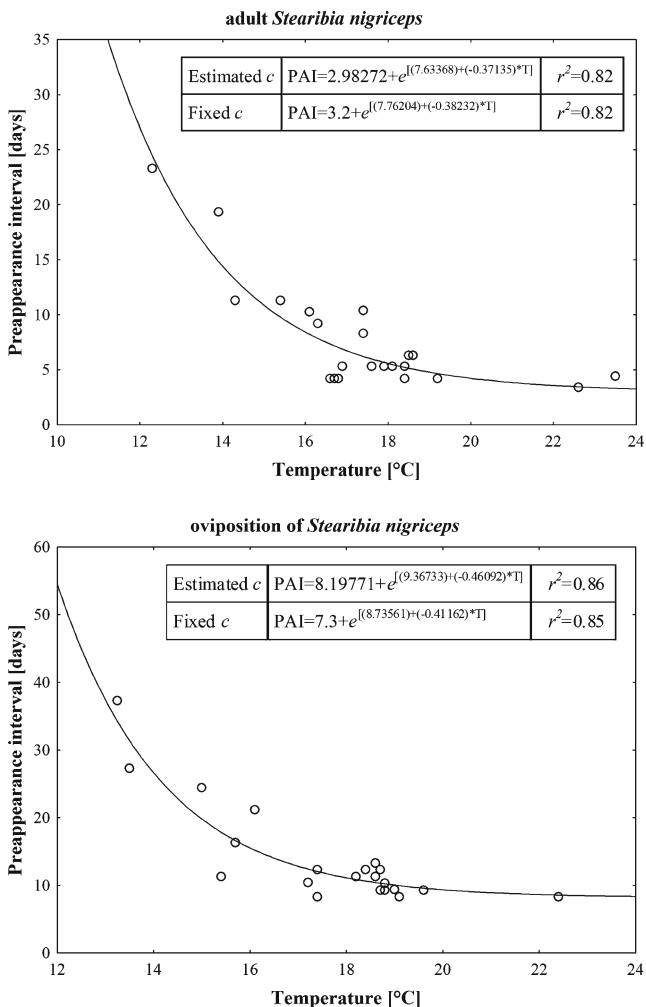
adult *Stearibia nigriceps*

Estimated c	PAI$=2.98272+e^{[(7.63368)+(-0.37135)*T]}$	$r^2=0.82$
Fixed c	PAI$=3.2+e^{[(7.76204)+(-0.38232)*T]}$	$r^2=0.82$

oviposition of *Stearibia nigriceps*

Estimated c	PAI$=8.19771+e^{[(9.36733)+(-0.46092)*T]}$	$r^2=0.86$
Fixed c	PAI$=7.3+e^{[(8.73561)+(-0.41162)*T]}$	$r^2=0.85$

Fig. 3 The relationship between the PAI of *S. nigriceps* and ground-level temperatures averaged for the duration of PAI (*T*). Graphs represent models with estimated *c*
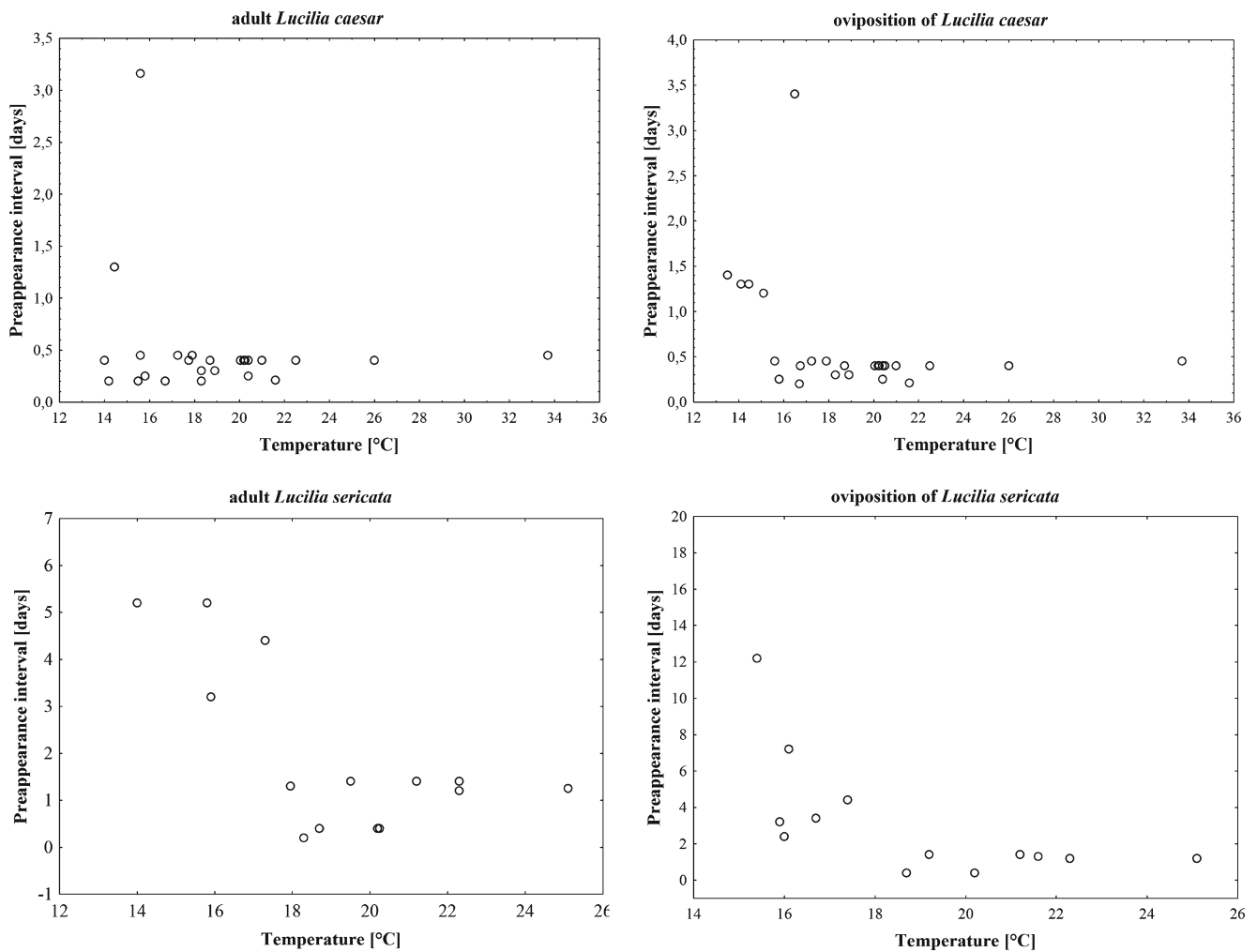
Fig. 4 The PAI and ground-level temperatures averaged for the duration of PAI for *L. caesar* and *L. sericata*

species of *Lucilia*, indicates that the processes-oriented hypothesis may not be valid for all carrion insects.

Apart from the presence of VOCs, there are two classes of factors which may potentially affect PAI: factors related to the release of attractants and factors related to the activity of insects (for a broad discussion of factors affecting colonization of carcasses by flies, see [35]). The first group is composed of barriers of any kind for attractants. However, they are irrelevant for the current results; hence, they will not be discussed here. The second class are abiotic mediators of insect activity, e.g., air temperature or humidity [36, 37], repellents [38–40], or the time of the day [41–45] and biotic mediators of insect activity, e.g., group oviposition effects [46] or ovarian development status. From this point of view, air temperature seems to be particularly important for the current results. It was found that ambient air temperature is a positive predictor for visitation on non-odor sticky traps by adult *Lucilia* flies [47] and for blowfly oviposition on a beef liver bait [37]. These results directly demonstrate that forensically relevant aspects of blowfly activity are mediated by temperature. Species of *Lucilia* have high

temperature thresholds for the activity, as was recently exemplified by Richards et al. [36] for South African *L. sericata*, which revealed the onset of coordinated muscle activity (i.e., standing) at a temperature of about 21 °C. It seems that European *Lucilia* flies also have high thresholds for the activity. As for oviposition, it was suggested that *L. sericata* normally needs carcass surface temperatures above 30 °C to start this behavior [48]. It was also demonstrated that the oviposition rate of *L. sericata* increases with average daily temperature, and temperature at which this rate is 0 is about 11 °C [49]. These results indicate that adult *Lucilia* flies have high temperature threshold for the activity (flying, locating a corpse, etc.), probably about 20 °C, and even higher threshold for oviposition. Temperatures below these thresholds will result in longer adult or oviposition PAI due to the lack of relevant activity of flies. Hence, we hypothesize that abrupt and erratic increase in PAI, as recorded in a low temperature range for *L. sericata* and *L. caesar*, resulted simply from ambient air temperatures being below the threshold for the relevant activity. In order to check whether this was true, the raw temperature data were inspected.

Interestingly, a similar pattern of temperature change was present in a majority of "high PAI" carcasses, i.e., an interval of low temperatures (approximately equal in length to PAI) followed by a substantial temperature increase before the first sampling of a given species and stage. Summarizing, current results corroborate the view that the PAI response to temperature in those insects which visit carcasses early in the decomposition process is better explained by the activity-oriented hypothesis than the processes-oriented hypothesis. The latter hypothesis is superior in the case of middle and late arrivers.

The strength of the relationship was generally weaker than expected. Apart from *S. nigriceps*, all the other species revealed at most weak premises for the estimation of PAI from temperature. When one compares current models with those for beetles [11], it is evident that the temperature-based estimation of PAI is more promising for beetles than flies. Differences between these models resulted, to some extent, from the methods used in this study. The resolution of sampling must have resulted in higher inaccuracy of PAIs in the early arrivers (flies) than in the late arrivers (beetles). Moreover, techniques of sampling, as being less effective for flies than beetles, must have given further inaccuracies of the PAI for flies. However, these methodical factors may not explain all the differences. It is suggested that factors related to the biology of flies and beetles or the nature of processes in which relevant attractants are produced may be more important. Anyway, further studies are needed.

Summarizing, only *S. nigriceps* revealed solid premises for estimating adult or oviposition PAI from temperature alone. In the case of the other species, temperature alone is a poor predictor for PAI, which suggests that models useful for the estimation of PAI in most carrion flies will be more complex. Therefore, future works should focus on other abiotic or biotic factors which may affect PAI in forensically useful flies.

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