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REVIEW

A global perspective of forensic entomology case reports from 1935 to 2022

Gengwang Hu[1] · Liangliang Li[1] · Yingna Zhang[1,2] · Shipeng Shao[1] · Yundi Gao[1] · Ruonan Zhang[1] · Yinghui Wang[1] · Yanan Zhang[1] · Yi Guo[1] · Chengtao Kang[1] · Jiangfeng Wang[1] · Yu Wang[1]

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Abstract

Forensic entomology case reports are the product of rapid development in the field, the widespread acceptance of the science and the application of forensic entomological knowledge. In this study, we retrospectively summarized information derived from 307 forensic entomology case reports from 1935 to 2022 from a global perspective. Our checklist of relevant information included insect species, specific indoor or outdoor preferences, preferred temperatures, and stages of body decomposition. Finally, a concept and calculation method for postmortem interval (PMI) estimation accuracy was proposed. There were 232 cases using insect developmental data and 28 cases using succession patterns to estimate PMI. A total of 146 species of insects were involved in the cases, of which 62.3% were Diptera and 37.7% were Coleoptera. Postmortem intervals were estimated from eggs in 4 cases, larvae in 180 cases, pupae in 45 cases, and puparia in 38 cases. The majority of cases were from June to October, and the average number of species mentioned in the cases was more at 15–30 °C. Considering the standardization of application, in the majority of cases, insect evidence was collected by other personnel and sent to forensic entomologists, there was a delay in the sampling, and the scene or meteorological data were directly used without correcting. Our data shows that there are still many shortcomings in the universality and standardization of forensic entomology in its practical application.

Keywords Forensic anthropology · Case reports · Necrophagous insects · PMI estimation accuracy · Decomposition stage

Introduction

The first recorded case of forensic entomology was reported by the world's earliest forensic monograph—"Washing Away of Wrongs" [1]. Sung Tz'u, the author of this book, had known about the bloodthirsty habits of flies as early as the 13th century and solved a case with it [2, 3]. The first forensic entomological case involving the estimation of the postmortem interval (PMI) was handled by French Doctor Bergeret, and described in 1855. He linked the life cycle of insects with the decomposition process of the corpse and made a tentative estimation of the PMI [4]. Until the early twentieth century, with the continuous increase in the number of publications related to forensic entomology [5–34], the science gradually attracted more and more scholarly interest, and its application in case investigation became more frequent [35–40], and has been accepted as a forensic tool by many countries [41–43]. Forensic entomology is undoubtedly in a period of rapid development and maturity. With the continuous progress of molecular sequencing, microscopic imaging, artificial intelligence, and other technologies, there will be more room for development and greater application value in the future [44].

Forensic entomology is a science that applies the theories and techniques of entomology and other natural sciences to study insects and other arthropods related to criminal events, so as to provide clues and evidence to various death-related information such as PMI, for judicial practice. In current studies on PMI estimation, forensic entomology mainly obtains basic application data through field succession and insect developmental studies [45]. In succession studies, human or animal carcasses are used for case simulation to observe and record body decomposition and

✉ Yu Wang
yuw@suda.edu.cn

1 Department of Forensic Medicine, Soochow University, Ganjiang East Road, Suzhou 215000, China

2 Department of Pathology, Shihezi University School of Medicine, Shihezi, Xinjiang, China

insect fauna [46–55]. The time range of death is estimated mainly based on the time when insects colonize and leave the carcasses. Insect developmental studies involve breeding insects under controlled conditions (such as temperature, humidity, photoperiod, and food source) in the laboratory, to obtain the relationship between insect age parameters such as developmental duration, body length, and thermal summation. Combined with the temperature of the case scene, the development time of the corresponding insects can be estimated using the maximum larval body length, pupal morphology, or other developmental events obtained in the case, allowing for the minimum postmortem interval (PMI_{min}) to be estimated [56–62]. In addition, studies on the morphology [63–68], physiology [69–73], ethology [74–78], and molecular biology [79–83] of insects can also obtain an in-depth understanding of insect morphological structure and functional mechanisms, aiding in species identification and explaining functions of biological systems, including the olfactory system of insects.

However, real cases are often complex. The dynamics and interactions of environmental conditions, body decomposition, and insect fauna will limit the application of insect evidence or affect the accuracy of estimation results [41, 84]. This is one of the reasons why the research content of forensic entomology, as an applied discipline, is obviously inconsistent with its application. In terms of research content, succession studies are mostly conducted on animal carcasses in a field-exposed environment, and its data is difficult to be directly applied to human corpses in other complex environments, such as burial, indoors, inside a vehicle, pond, or river. Development data are often obtained from incubators set to constant parameters, without considering the variable temperature conditions in the case, where insects are isolated from the effects of soil, intraspecific and interspecific competition, rainfall, etc. In terms of application, reports of individual cases can hardly reflect the great differences in the development of forensic entomology in different countries and regions, and whether the current procedures of forensic entomology in insect evidence collection, submission, and estimation are standardized. To further deepen our understanding of the influence or effect of these factors, it is necessary to comprehensively review and analyze case reports in forensic entomology, as they often contain successful examples or profound reflections on the estimation of PMI by experienced forensic entomologists or institutions, and can thus provide enlightenment and reference data for the application of similar cases in the future.

Given the sensitivity of some countries or forensic institutions to the publication of real case data, there are very few publications related to forensic entomology case reports [85]. According to bibliometric statistics, case reports only accounted for 3.3% of the total topics of forensic entomology from 1998 to 2017 [86]. Moreover, drawing meaningful conclusions is usually based on data sets collected in sufficient scale and time. So far, reports and studies on multiple cases (for example, the number of cases > 100) are limited to articles or reviews from forensic institutions in countries such as France [87], the United States [88], Germany [89], Switzerland [85], and Belgium [90], and there is no comprehensive review on global forensic entomology case reports.

We counted and analyzed various information disclosed in 307 forensic entomological cases involving insect evidence, and put forward an objective quantitative evaluation method for the accuracy of PMI estimation. To our knowledge, this is the first global review of case reports from 1935 to 2022 published.

Material and methods

Literature retrieval and screening strategy

The topics of "forensic entomology and case" and "legal entomology and case" were searched in three databases, Web of Science, Google Scholar, and PubMed. The full text of all the literature (including books) in the search results was viewed in turn. Initially, we eliminated the literature with no case reports. Secondly, we eliminated the literature that only mentioned the number of cases, without adding any specific information about each case, especially detailed information about the insect evidence. Through the above two steps, 126 pieces of literature were screened, including 307 cases of forensic entomology.

Statistics

The following information was recorded in Microsoft Excel 2019 in detail for later analysis. The statistical contents of this study included the following:

1. The latitude, continent, and country of the case scene.
2. The insect evidence mentioned in the cases, including the insects whose developmental data were used as well as other insects involved in the cases. For the insects whose developmental data were used, we also recorded the developmental stages (including eggs, first-instar larvae, second-instar larvae, third-instar larvae, pupa, and puparium). If the puparium of an insect species was collected it means that the insect has completed at least one generation of development on the corpse.
3. The decomposition stages of the corpses were the fresh stage, bloated stage, active decay stage, advanced decay stage, and skeletonized/dry/mummified stage.
4. The average ambient temperature during the decomposition of the corpses.
5. The environment in which the body was found was divided into two categories: indoor and outdoor. We

classified all cases occurring in buildings as indoor cases, while all cases including exposure, submersion, burial, or inside a vehicle were classified as outdoor cases.

6. Sex of the deceased.
7. Manner of death, including death by natural causes or disease, homicide, suicide, or accident.
8. The discovery time of the body.
9. The collection methods and locations of insect evidence. The collection methods included the following three situations: forensic entomologists collected at the scene of the case, other personnel collected insect evidence and sent it to forensic entomologists for analysis, and no description of the collection methods. The collection locations included collecting at and around the corpse at the scene of the case, collecting after the corpse had been removed to mortuaries, hospitals, funeral homes or other places, and collection locations not described.
10. The acquisition methods of temperature data include the direct use of meteorological data, direct use of data measured at the scene of the case, use of the above two data after correction, and the temperature acquisition methods not described.
11. The two most commonly disclosed time information, including the minimum postmortem interval (PMI_{min}) estimated by insect evidence (estimation methods include two categories, namely, using developmental data or using succession patterns, and some cases may have added pre-appearance interval PAI for correction) and the maximum postmortem interval (PMI_{max}), that is, the time from the last time when the deceased was proved to be alive to the time when the corpse was found. This is usually calculated based on feedback provided by the police or the family members and friends of the deceased, and the time is often longer than the actual time of death and the estimated PMI_{min}, as shown in Fig. 1.

Data processing and analysis

The time of body discovery was used to filter out cases that were reported more than once. The month information was extracted according to the time when the body was found, and the year information was extracted according to the time when the case was reported. Since the seasons in the northern and southern hemispheres are opposite, the months in the southern hemisphere were added or subtracted by 6 to correct to the same season as those in the northern hemisphere for data analysis. The total number of families, genera, and species mentioned were counted.

The estimation accuracy of the cases was calculated with the following formula: EAI (estimation accuracy index) = (PMI_{max}-PMI_{min}) /PMI_{max}, the lower the value, the higher the estimation accuracy.

Results

Case contribution

A total of 307 cases involving insect evidence were included in the study. The majority of cases occurred in low (n=119) and middle (n=156) latitude locations, and just a few cases occurred in high (n=22) latitude locations. As for the continent to which the cases belong, there were 144 cases from Europe (13 countries in total, the top three were Italy, Germany, and Spain), 72 cases from Asia (9 countries in total, the top three were China, Malaysia, and Saudi Arabia), 66 cases from North America (the USA and Canada), 13 cases from South America (Brazil and Colombia), 6 cases from Oceania (Australia), and 4 cases from Africa (Egypt and South Africa), as shown in Fig. 2. The cases from the twentieth century (n=81) all came from North America and Europe, and their contributions were roughly the same. At the beginning of the 21st century, many countries on other continents began to report cases (Fig. 3).

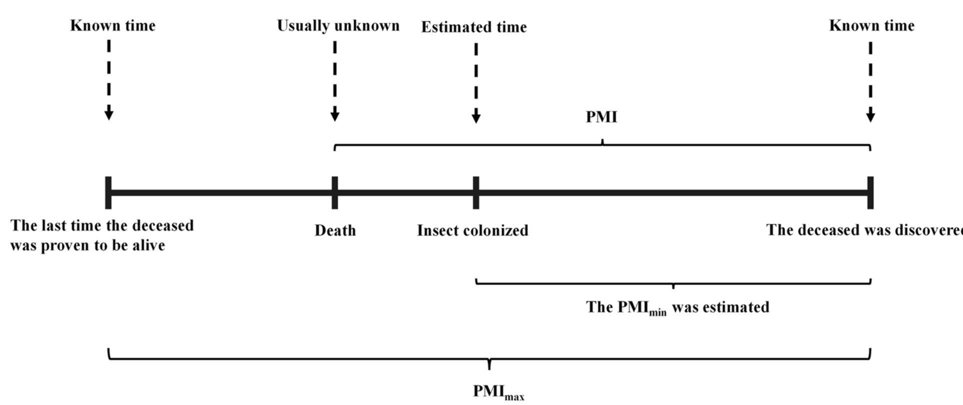


Fig. 1 Illustrations of PMI, PMI_{min}, and PMI_{max} in forensic entomology case investigations

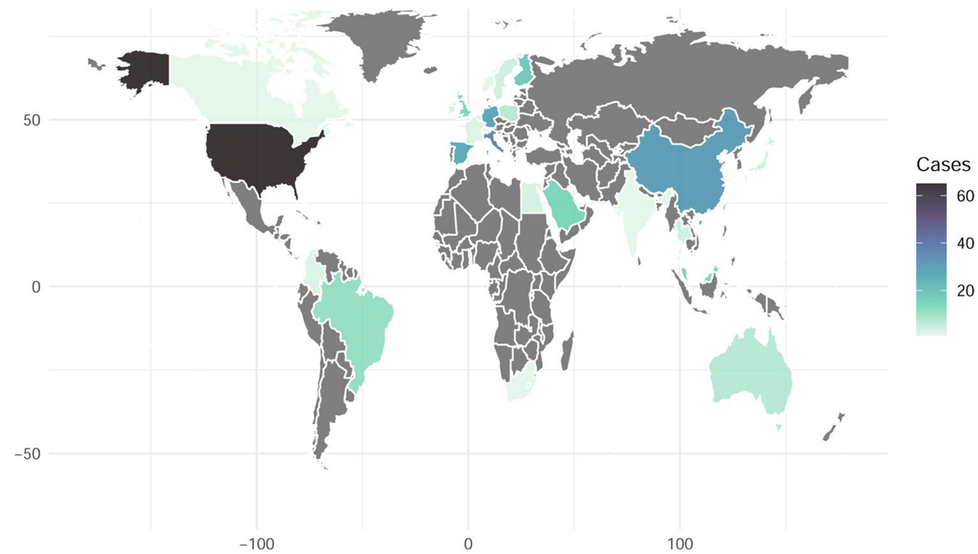


Fig. 2 Contribution of countries and regions to forensic entomology cases in this data set from 1935 to 2022

Insect species

The insect species involved are shown in Table 1. In cases where the PMI was estimated using developmental data, the insect species that were used as the estimation indicator (i.e., the earliest species colonizing corpses with the oldest immature stages) and their application times are also listed. Taxonomic information mentioned more than five times are listed in Table 2, including indoor and outdoor occurrence times, average PMI_{min} estimated with developmental data, the average temperature used to estimate $PMI_{min,}$ and corresponding insect developmental stages. Among them, there were 91 Dipteran species, mentioned 640 times and their developmental data were used 222 times. There were 55 Coleopteran species, which were mentioned 165 times and their developmental data were used 12 times. Among the Dipteran species of which developmental data were used, the top three species were: *Calliphora vicina* Robineau-Desvoidy, 1830, used 34 times (14.5%), *Chrysomya megacephala* (Fabricius, 1794) was reported 28 times (12.0%), and *Lucilia sericata* (Meigen, 1826) was reported 26 times (11.0%). In terms of specific continents, the main species were *C. vicina* (18.3%) and *L. sericata* (13.7%) from Europe, *C. megacephala* (17.2%) and *C. vicina* (12.0%) from North

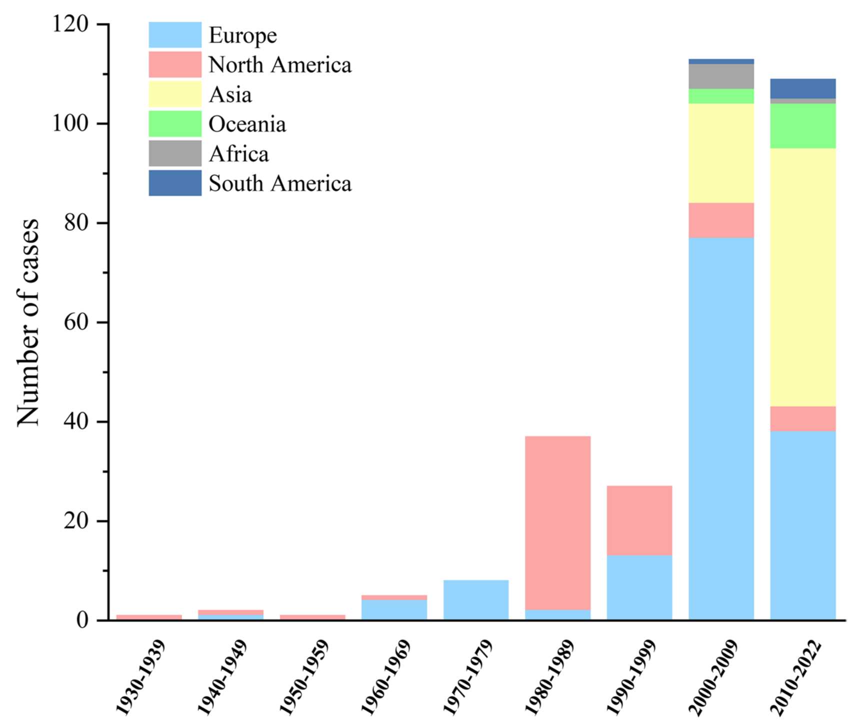


Fig. 3 The development of the number of case reports produced on six different continents from 1935 to 2022

Table 1 Species of Diptera and Coleoptera reported in forensic case reports from 1935 to 2022, including the number of times their developmental data were used and the number of times they were mentioned

Family/Species	N1	N2	Family/Species	N1	N2	Family/Species	N1	N2	Family/Species	N1	N2
Calliphoridae	157	354	*Hydrotaea leucostoma*		1	**Fanniidae**	2	24	**Staphylinidae**	1	36
Chrysomya megacephala *	28	50	*Hydrotaea similis*		1	*Fannia leucosticta* *	1	1	*Creophilus maxillosus* *	1	14
Chrysomya albiceps *	11	36	*Hydrotaea spinigera*		4	*Fannia scalaris* *	1	6	*Emus hirtus*		1
Chrysomya rufifacies *	9	36	*Hydrotaea capensis*		4	*Fannia manicata*		3	*Oxytelus* sp.		1
Chrysomya nigripes *	1	6	*Hydrotaea ignava*		4	*Fannia canicularis*		5	*Oxytelops tetracarinatus*		1
Chrysomya pinguis *	1	2	*Hydrotaea dentipes*		3	*Fannia trimaculata*		1	*Philonthus longicornis*		2
Chrysomya Putoria		2	*Hydrotaea* sp.		8	*Fannia pusio*		1	*Philonthus discoideus*		1
Chrysomya villeneuvi		3	*Haematobia irritans*		1	unidentified		7	*Philonthus* sp.		5
Chrysomya chloropyga		1	unidentified		11	**Otitidae**		1	*Philonthus jurgans*		1
Calliphora vicina *	34	55	**Sarcophagidae**	12	55	**Scenopinidae**		1	*Aleochara villosa*		1
Calliphora vomitoria *	4	13	*Sarcophaga argyrostoma* *	2	10	**Sphaeroceridae**		9	*Aleochara* sp.		3
Calliphora latifrons		1	*Sarcophaga caerulescens* *	1	2	*Leptocera caenosa*		1	*Thyreocephalus albertisi*		2
Calliphora coloradensis		1	*Sarcophaga bullata*		1	*Copromyza* sp.		1	*Anthobium atrocephalum*		1
Calliphora loewi		1	*Sarcophaga africa*		1	unidentified		7	unidentified		3
Calliphora spp.		4	*Sarcophaga protuberans*		1	**Ulidiidae**		2	**Cleridae**		31
Lucilia sericata *	26	54	*Sarcophaga tibialis*		1	*Physiphora alceae*		2	*Necrobia rufipes*		22
Lucilia silvarum *	4	4	*Parasarcophaga argyrostoma* *	1	1	**Heleomyzidae**		2	*Necrobia ruficollis*		4
Lucilia caesar *	4	8	*Parasarcophaga ruficornis* *	1	1	**Stratiomyidae**	11	19	*Necrobia violacea*		3
Lucilia illustris *	4	11	*Boettcherisca peregrina*	1	3	*Hermetia illucens* *	11	19	unidentified		2
Lucilia cuprina *	2	7	*Blaesoxipha plinthopyga*		2	**Dermestidae**	8	54	**Histeridae**		20
Lucilia ampullacea *	1	3	*Bercaea haemorrhoidalis*		3	*Dermestes maculatus* *	3	22	*Atholus rothkirchi*		1
Lucilia eximia *	1	1	*Peckia chrysostoma*		2	*Dermestes frischii* *	2	16	*Saprinus planiusculus*		1
Lucilia porphyrina		1	*Microcerella halli* *	1	1	*Dermestes ater* *	1	5	*Saprinus furvus*		1
Lucilia coeruleiviridis		1	unidentified*	5	26	*Dermestes haemorrhoidalis* *	1	2	*Saprinus lugens*		3
Lucilia spp.		3	**Piophilidae**	17	28	*Dermestes peruvianus* *	1	1	*Saprinus splendens*		3
Phormia regina *	11	23	*Piophila casei* *	1	11	*Dermestes undulatus*		1	*Saprinus chalcites*		1
Cochliomyia macellaria *	5	6	*Piophila megastigmata* *	1	3	*Dermestes Lardarius*		3	*Saprinus subnitescens*		1
Protophormia terraenovae *	6	12	unidentified*	1	7	unidentified		3	*Hister unicolor*		1
Aldrichina grahami *	1	2	*Piophila nigriceps*		5	**Silphidae**	3	17	*Hister striola*		1
Hemilucilia segmentaria *	1	2	*Piophila foveolata*		2	*Oxelytrum discicolle* *	1	1	*Carcinops pumilio*		1
Paralucilia fulvinota *	1	1	**Phoridae**	3	55	*Thanatophilus micans* *	1	1	*Margarinotus brunneus*		2
Sarconesia chlorogaster *	1	2	*Megaselia scalaris* *	9	20	*Thanatophilus mutilatus* *	1	1	unidentified		4
Cynomya cadaverina	1	2	*Megaselia abdita* *	4	9	*Thanatophilus rugosus*		2	**Nitidulidae**		6
Muscidae	20	90	*Megaselia rufipes* *	1	2	*Thanatophilus ruficornis*		1	*Nitidula carnaria*		1
Synthesiomyia nudiseta *	11	17	*Megaselia curtineura* *	1	1	*Oeceoptoma thoracica*		1	*Nitidula bipunctata*		1
Musca domestica *	4	12	*Megaselia spiracularis*		1	*Nicrophorus investigator*		1	*Nitidula flavomaculata*		1

Table 1 (continued)

Family/Species	N1	N2	Family/Species	N1	N2	Family/Species	N1	N2	Family/Species	N1	N2
Muscina stabulans *	4	15	*Dohrniphora cornuta* *	1	2	*Nicrophorus interruptus*		1	*Omosita* sp.		1
Muscina levida		1	*Triphleba nudipalpis* *	1	1	*Necrophorus vespilloides*		1	unidentified		2
Muscina assimilis	1	3	*Conicera similis*		1	*Necrodes littoralis*		2	**Leiodidae**		1
Muscina prolapsa		1	*Conicera tibialis*		1	*Silpha obscura*		1	*Catops morio*		1
Hydrotaea aenescens		4	unidentified		17	unidentified		4			

N1 represents the number of times the developmental data were used, and *N2* represents the total number of times the species were mentioned in this data set; The species marked with * are indicator species for PMI estimation, while the species not marked with * are the species mentioned in case reports but not used for PMI estimation; *sp.* indicates that the genus is identified but the species is not identified, *spp.* indicates that more than one species of the genus are not identified, *Unidentified* indicates that the genus is not identified, and families are shown in bold

America, and *C. megacephala* (36.0%) and *Chrysomya rufifacies* (Macquart, 1842) (12%) from Asia.

In addition, grouped by family, the percentage of Dipteran and Coleopteran species, whose developmental data were used, are shown in Fig. 4. Among them, the number of families in the advanced decay stage was the largest (9 families), while the fresh stage and bloated stage were the least (3 families). At the fresh stage, bloated stage, active decay, and advanced decay stage mainly Calliphoridae, Sarcophagidae, Phoridae, and Muscidae were used for developmental data, and in the skeletonized stage the Tabanidae, Piophilidae, and Dermestidae were used for developmental data more frequently than other families.

Application standardization evaluation

In terms of insect evidence collection methods, forensic entomologists personally collected insect evidence at the scene of only a small number of cases. Most cases employed non-professionals to collect insect evidence at the scene of the case or during autopsy and sent it to forensic entomologists for analysis. Another 122 cases did not provide enough information (Fig. 5).

As far as the collection location of insect evidence is concerned, the number of cases collected at and around the corpse at the scene is similar to the number of cases collected after the corpse had been transferred to mortuaries, hospitals, funeral homes, or other places, and 81 cases have not described this information.

Regarding temperature data, most cases used meteorological station data near the scene or temperature data measured at the case scene after the body was found to estimate the PMI_{min}. Only in a few of the cases, was data from both the case scene and the nearby meteorological station obtained for further calibration, and then PMI_{min} was estimated. In another 126 cases, this information was not given (Fig. 5).

Sex, location, and manner of death

Of the 260 cases in which the sex of the corpse was disclosed, more males than females were reported, with only five studies reporting both male and female corpses. A total of 301 cases disclosed the environment in which the bodies were found, most cases took place outdoors, including 152 cases (50.5%) exposed, 12 cases (4.0%) buried or sealed in some form (plastic bags, suitcases, etc.), 6 cases (2.0%) in water, 5 cases (1.7%) in vehicles, and 126 cases (41.9%) took place indoors.

Of the 231 cases in which the manner of death was disclosed, most cases were homicide, followed by natural or disease-related deaths, with only a few cases being due to drug or poison overdose (10.0%), suicide by hanging (9.1%),

Table 2 Indoor and outdoor occurrence times, average ambient temperature, average PMI_{min} estimated from developmental data, and developmental stages of important species in this data set

Order/ Family	N2	Out	In	T(+/-st.dev.)	PMI_{min}(+/-st. dev.)	1st	2nd	3rd	Pupae	Puparium	Order/ Family	N2	Out	In	T(+/-st.dev.)	PMI_{min}(+/-st. dev.)	1st	2nd	3rd	Pupae	Puparium
Calliphoridae	354	232	120	21.6°C (6.6)	10.9(13.8)	21	17	96	22	17	**Fanniidae**	24	15	9	18.7°C (4.1)	37.5(3.5)	-	-	-	1	1
-Chrysomya	136	91	45	23.4°C (5.1)	10.4(14.0)	2	4	31	10	8	-Fannia	24	15	9	18.7°C (4.1)	35.0	-	-	-	-	1
---*C. megacephala*	50	30	20	25.3°C (4.8)	6.3(4.6)	1	2	17	5	2	---*F. scalaris*	6	5	1	19.5°C (3.3)	35.0	-	-	-	-	1
---*C. albiceps*	36	23	13	21.1°C (5.3)	19.5(23.8)	1	2	6	3	4	**Piophilidae**	28	21	6	18.6°C (5.4)	75.7(42.0)	-	-	4	1	1
---*C. rufifacies*	36	29	7	24.1°C (3.3)	7.9(6.0))	-	-	6	1	2	-Piophila	28	21	6	18.6°C (5.4)	73.2(45.5)	-	-	-	-	-
---*C. nigripes*	6	4	2	26.1°C (4.5)	8.8	-	-	1	-	-	---*P. casei*	11	7	4	22.1°C (5.2)	76.5(43.5)	-	-	2	1	-
-Calliphora	75	39	34	15.9°C (6.6)	18.0(17.0)	4	6	21	5	2	---*P. nigriceps*	5	5	0	14.6°C (4.9)	-	-	-	-	-	-
---*C. vicina*	55	29	24	15.6°C (6.1)	13.5(13.6)	4	6	19	4	1	**Phoridae**	55	17	38	19.5°C (5.8)	32.8(33.7)	2	-	4	9	12
---*C. vomitoria*	13	9	4	15.9°C (9.0)	42.3(24.6)	-	-	2	1	1	-Megaselia	37	9	28	19.8°C (6.2)	33.2(34.6)	1	-	2	7	6
-Lucilia	93	65	27	22.6°C (5.9)	6.5(8.4)	11	4	27	2	1	---*M. scalaris*	20	4	16	23.0°C (3.9)	72.3(47.2)	-	-	1	2	1
---*L. sericata*	54	30	24	23.0°C (6.1)	6.4(6.4)	4	1	19	1	1	---*M. abdita*	9	2	7	13.2°C (6.4)	18.4(12.9)	1	-	1	4	5
---*L. caesar*	8	7	1	22.0°C (1.8)	3.3(3.2)	2	-	2	-	-	**Sarcophagidae**	55	20	33	23.6°C (5.9)	7.9(9.8)	3	1	8	2	-
---*L. illustris*	11	11	0	22.0°C (4.0)	1.5(1.3)	2	1	1	-	-	-Sarcophaga	16	4	12	22.3°C (5.0)	10.8(12.1)	2	-	5	1	-
---*L. cuprina*	7	5	2	27.5°C (8.6)	5.5(2.1)	-	-	2	-	-	---*S. argyrostoma*	10	2	8	20.8°C (4.6)	13.3(10.4)	-	-	2	1	-
-Phormia	22	17	5	25.0°C (7.6)	10.6(9.7)	1	-	4	2	2	**Sphaeroceridae**	9	9	0	15.5°C (2.1)	-	-	-	-	-	-
---*P. regina*	22	17	5	25.0°C (7.6)	10.6(9.7)	1	-	4	2	2	**Stratiomyidae**	19	17	2	23.4°C (4.8)	67.2(45.3)	6			2	3
-Protophormia	12	6	6	20.7°C (3.3)	15.8(11.2)	2	-	2	-	4	---*H. illucens*	19	17	2	23.4°C (4.8)	67.2(45.3)	6			2	3
---*P. terraenovae*	12	6	6	20.7°C (3.3)	15.8(11.2)	2	-	2	-	4	**Dermestidae**	54	33	21	24.6°C (6.5)	46.4(28.5)	7			1	-
-Cochliomyia	6	6	0	28.0°C (13.1)	7.2(4.7)	1	1	3	-	-	---*D. maculatus*	22	14	8	26.2°C (5.5)	39.0(12.5)	2			1	-
---*C. macellaria*	6	6	0	28.0°C (13.1)	7.2(4.7)	1	1	3	-	-	---*D. frischii*	16	12	4	22.8°C (9.2)	30.0(0)	2			-	-
- *Cynomya*	3	3	0	-	6.0(1.0)	-	-	1	-	-	---*D. ater*	5	3	2	26.5°C (4.2)	14.0	1			-	-
---*C. cadaverina*	2	2	0	-	6.0(1.0)	-	-	1	-	-	**Silphidae**	17	14	2	19.3°C (1.6)	14.3(5.1)	-	-	3	-	-
Muscidae	90	44	46	21.1°C (5.0)	10.2(10.5)	2	-	10	6	4	-Thanatophilus	5	4	1	20.8°C (1.8)	20.0	-	-	2	-	-

Table 2 (continued)

Order/ Family	N2	Out	In	T(+/-st.dev.)	PMI_{min}(+/-st. dev.)	1st	2nd	3rd	Pupae	Puparium	Order/ Family	N2	Out	In	T(+/-st.dev.)	PMI_{min}(+/-st. dev.)	1st	2nd	3rd	Pupae	Puparium
-Synthesiomya	18	5	13	21.6°C (5.7)	12.9(12.6)	1	-	4	5	1	**Staphylinidae**	36	34	2	18.4°C (3.9)	60.0	-	-	-	1	-
--*S. nudiseta*	17	4	13	21.1°C (5.7)	12.9(12.6)	1	-	4	4	1	-*Creophilus*	14	12	2	20.8°C (2.7)	60.0	-	-	-	1	-
-Musca	12	1	11	24.5°C (7.0)	6.6(4.9)	1	-	4	-	1	---*C. maxillosus*	14	12	2	20.8°C (2.7)	60.0	-	-	-	1	-
---*M. domestica*	12	1	11	24.5°C (7.0)	6.6(4.9)	1	-	4	-	1	-Philonthus	7	7	0	16.7°C (3.8)	-	-	-	-	-	-
-Muscina	20	11	9	19.8°C (4.1)	12.7(8.2)	-	-	2	1	2	**Cleridae**	31	22	9	22.0°C (6.9)	-	-	-	-	-	-
--*M. stabulans*	15	6	9	19.4°C (4.5)	12.7(8.2)	-	-	2	1	1	--*N. rufipes*	22	16	6	22.1°C (7.4)	-	-	-	-	-	-
---*M. assimilis*	3	3	0	22.7°C (2.4)	6.5(0.5)	-	-	-	-	1	**Histeridae**	20	17	3	19.6°C (4.4)	-	-	-	-	-	-
-Hydrotaea	29	20	9	20.1°C (3.6)	-	-	-	-	-	-	-Saprinus	10	8	2	19.6°C (4.4)	-	-	-	-	-	-
											Nitidulidae	6	6	0	16.3°C (1.7)	-	-	-	-	-	-

Out, outdoor; *In*, indoor; *T*, average ambient temperature; *1st*, first-instar larva; *2nd*, second-instar larva; *3rd*, third-instar larva

or drowning, CO poisoning and other deaths (8.6%), as shown in Fig. 5.

Decomposition stage

Among the cases that disclosed the decomposition stage (n=224), there were 23 cases in the fresh stage, 24 cases in the bloated stage, 29 cases in the active decay stage, 87 cases in the advanced decay stage, and 61 cases in the skeletonized/dry/mummified stage. In all cases of outdoor exposure, the median days of PMI_{min} for the corpses at each decomposition stage were 1.0 days in the fresh stage, 5.0 days in the bloated stage, 5.5 days in the active decay stage, 12.0 days in the advanced decay stage, and 90.0 days in the skeletonized/dry /mummified stage, respectively. In all cases where temperature, $PMI_{min,}$ and decomposition stage were mentioned, the total average ADD (PMI_{min} ×T) of the fresh stage was 40.3 degree days, the bloated stage was 111.9 degree days, the active decay stage was 135.3 degree days, the advanced decay stage was 305.8 degree days, and the skeletonized/dry/mummified stage was 1963.2 degree days.

Number of insect species

The average number of species mentioned in the 307 cases we examined was 3.0, among which an average of 2.7 species were mentioned in the cases using developmental data to estimate the PMI (individual species were not identified, only genera/families were mentioned), and an average of 5.1 species were mentioned in the cases using succession patterns. In terms of the decomposition stage, an average of 1.7 species were mentioned in cases reporting data from the corpses in the fresh stage, 2.4 species in cases of the bloated stage, 2.3 species in cases of the active decay stage, 2.9 species in cases of the advanced decay stage, and 3.3 species in cases of the skeletonized/dry/mummified stage. Outdoor exposure cases (n=152) mentioned an average of 3.0 species, indoor cases (n=126) mentioned an average of 2.4 species, and other cases (n=23) mentioned an average of 3.0 species. In addition, the influence of temperature on the number of species and cases is shown in Fig. 6. The average number of species mentioned in cases between 10–30 °C was the highest, and the number of cases was also the highest at this temperature range. The influence of months on the number of species and cases is shown in Fig. 7. The average number of species mentioned in cases during October and November was the highest, and the number of cases from June to October was relatively high, with the highest numbers occurring in July.

Postmortem interval

The 307 cases were divided into four categories based on the available PMI information: cases in which PMI_{max} was

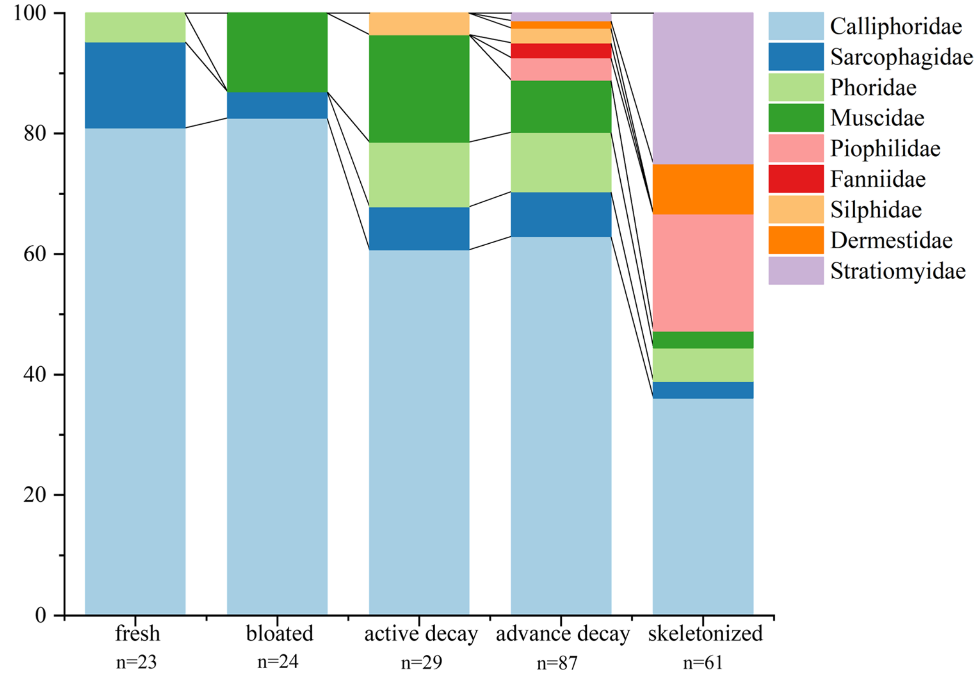


Fig. 4 The percentage of cases that used developmental data from Diptera and Coleoptera in the five decomposition stages

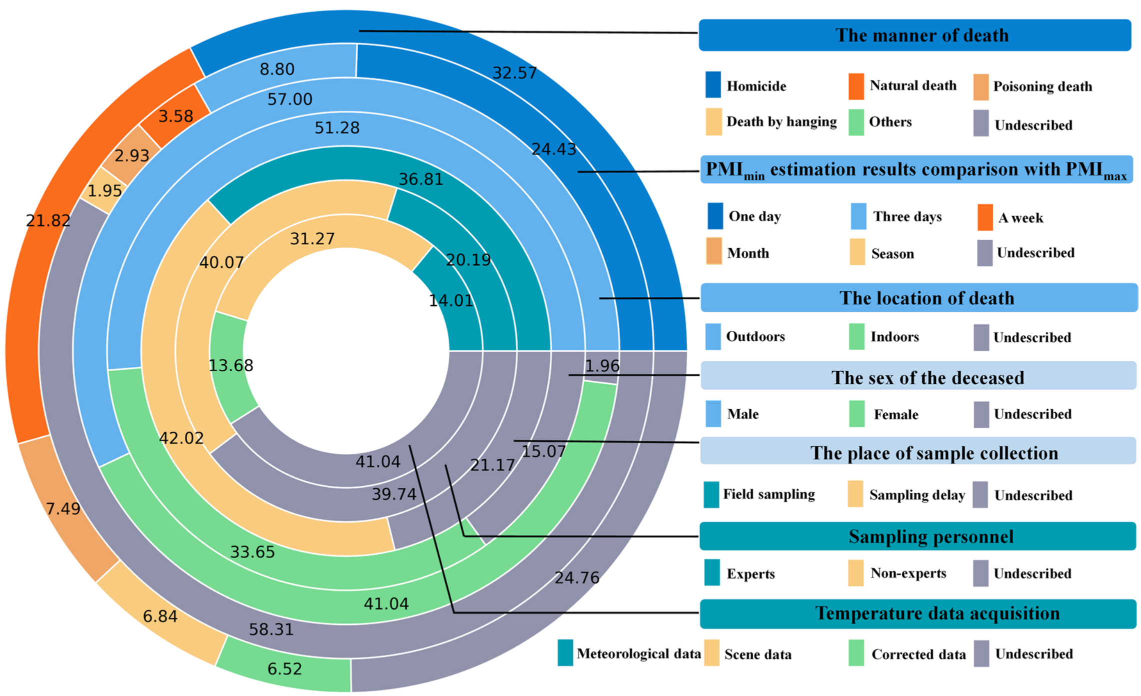


Fig. 5 The percentage of cases providing information about the manner of death, estimation error, location, sex, sampling place, sampling personnel, and temperature data acquisition (order from the outer ring to inner ring, numbers represent percentages) in this data set

disclosed and PMI$_{min}$ was estimated (n=129), cases in which only PMI$_{max}$ was disclosed and no PMI$_{min}$ was estimated (n=31), cases in which only PMI$_{min}$ was estimated but no PMI$_{max}$ was disclosed (n=131) and cases in which neither PMI$_{max}$ nor PMI$_{min}$ was disclosed (n=16).

Among the 31 cases where only PMI$_{max}$ was provided without a PMI$_{min}$ estimation, a few had PMI$_{max}$ for less than 1 month, while the majority had PMI$_{max}$ for greater than 1 month. In the cases with an estimated PMI$_{min}$ (n=260), among the 28 cases using succession patterns, a few had

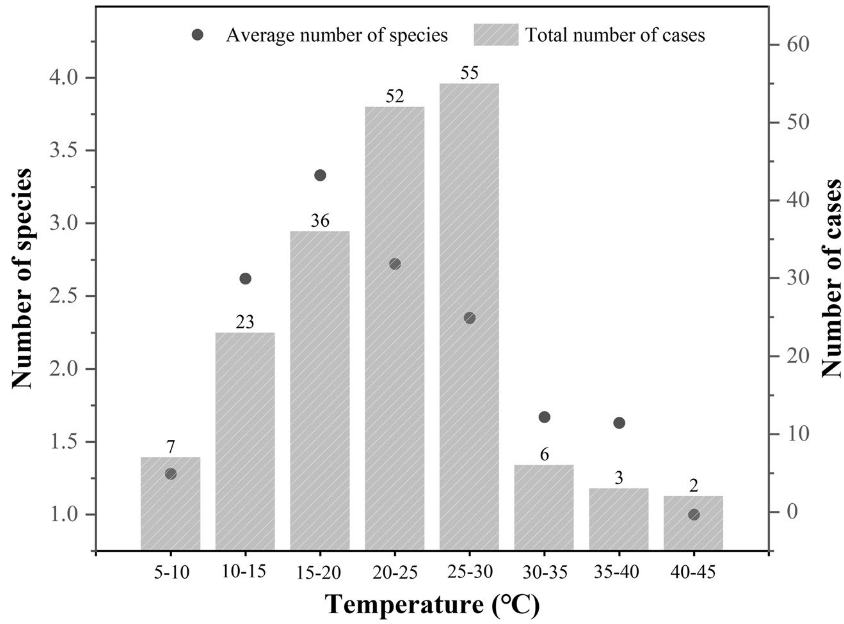


Fig. 6 Number of cases and average number of species mentioned in forensic case reports from 1935 to 2022 at different temperatures

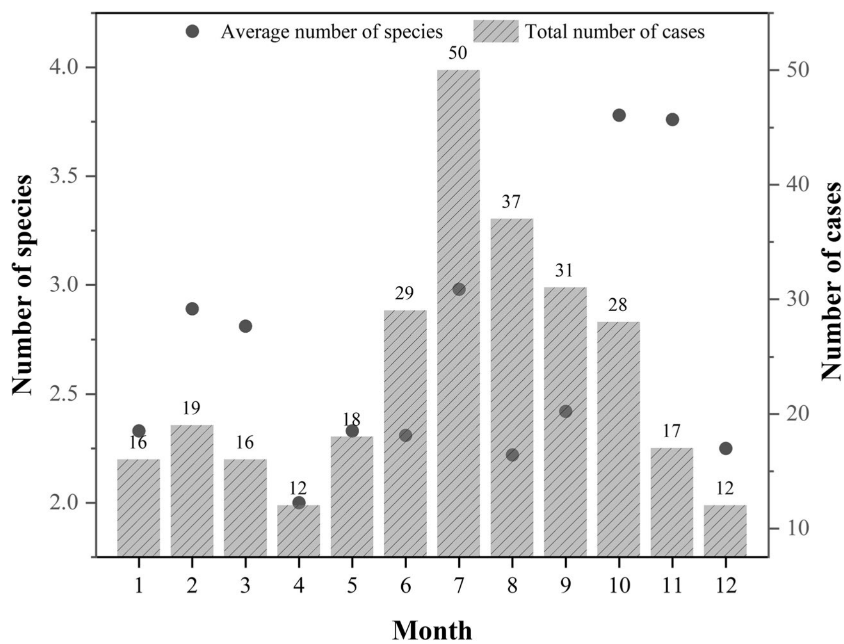


Fig. 7 Number of cases and average number of species mentioned in forensic case reports during different months from 1935 to 2022

PMI_{min} less than 2 months (the shortest was 13 days), while the majority had PMI_{min} greater than 3 months (the longest was 1 year and 3 months). Among the 232 cases using developmental data, PMI_{min} was mostly less than 1 month, and a few reported PMI_{min} greater than 1 month where the longest was 6 months, and the insect species was *Hermetia illucens* (Linnaeus, 1758), as shown in Fig. 8.

Comparing the real results with the estimated results, the difference in PMI_{max}-PMI_{min} values showed that 75 cases (58.5%) were accurate within 1 day, 27 cases (21.0%) were accurate within 3 days, 11 cases (8.5%) were accurate within 1 week, 9 cases (7.0%) were accurate to a month, and 6 cases (4.7%) were accurate to a specific season (Fig. 5).

Accuracy of estimation

Figure 9a shows a scatter plot of cases with PMI_{max} less than 3 months. The results show that the estimation error (i.e., PMI_{max}-PMi_{min}) increases with an increase in PMI_{max}, while the number of cases decreases. However, after the estimation error is converted into the EAI seen in Fig. 9b, the y value of its fitting curve lies between 0.1 and 0.2.

Fig. 8 Frequency of application of developmental data or succession patterns with PMI_{min} in forensic case reports from 1935 to 2022

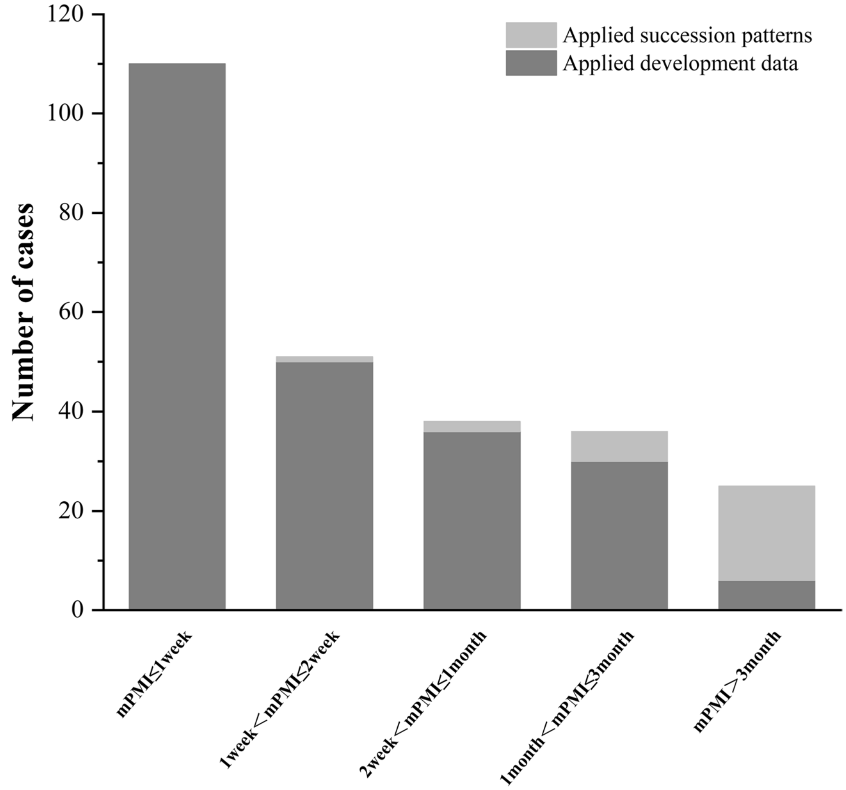
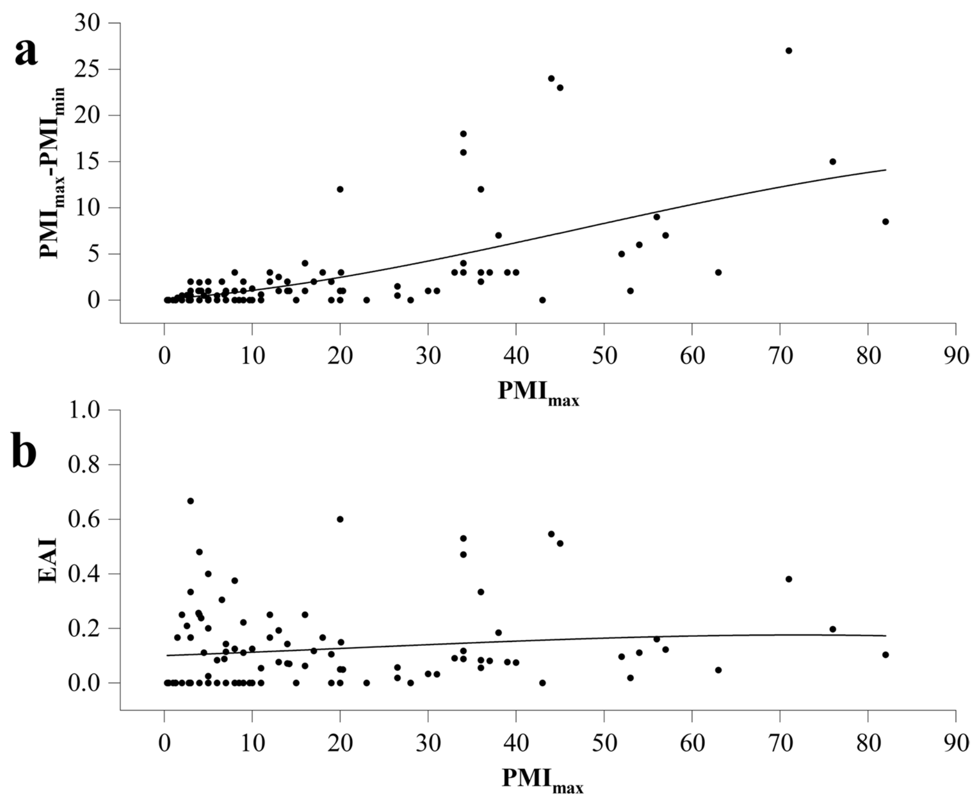


Fig. 9 Scatter plot and curve fitting of estimation error (**a**) and EAI (**b**) with PMI_{max} from forensic case reports dated 1935–2022



Among the 307 cases in this study, all cases containing both PMI_{max} and PMI_{min} information (n=129) had a total average EAI of 0.136. That is, for all cases in this dataset that were published to estimate the PMI using succession patterns or developmental data, the estimation error would increase by 0.136 days for each additional day after the death of the deceased. The EAI of developmental data was 0.135, which was better than that of succession patterns with an EAI of 0.144. The EAI of outdoor exposure (n=69) was 0.107, which was better than the indoor cases (n=49) with an EAI of 0.154. Other environments including burial and submersion in water or inside a vehicle (n=9) had the worst EAI, which was 0.272. In addition, the influence of temperature and month on the EAI is shown in Fig. 10. The EAI from June to December was generally better than that from January to May, and the EAI curve fitted with temperature shows that the PMI estimation accuracy is higher between 15 and 25 °C.

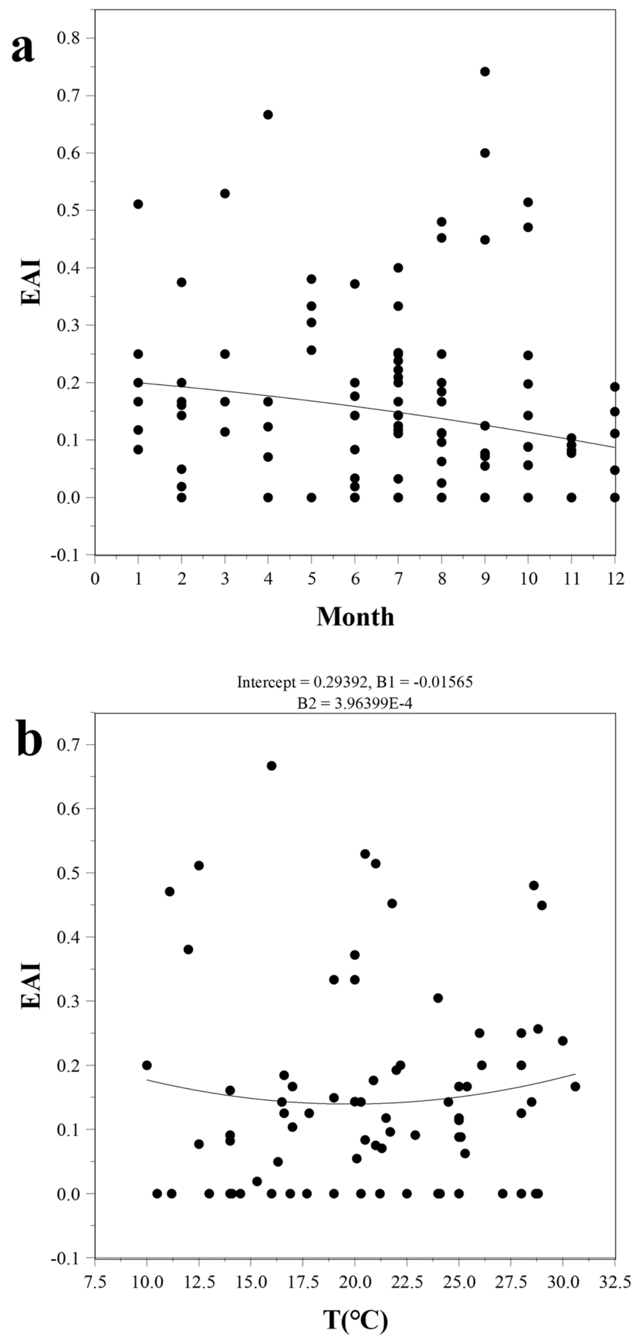


Fig. 10 Scatter plot and curve fitting of EAI in relation to the month of death (**a**) and reported ambient temperature (**b**) from forensic case reports from 1935 to 2022

Discussion

The proposal and use of EAI

Since the different cases have different PMIs, it is unreasonable to compare the accuracy directly by estimated differences in days or hours. For example, the case where the PMI_{min} of a corpse 10 days after death was estimated as nine days, compared with the case where the PMI_{min} of a corpse 100 days after death was estimated as 99 days, both have an error of 1 day, but the latter is more accurate. To further quantitatively compare the accuracy of different estimation results in different cases and evaluate the influence of different factors (such as ambient temperature and body location) on the accuracy of the estimation results, we put forward the concept and calculation method of estimation accuracy (daily average estimation error). Based on this, the influence of temperature, month, body location, estimation method, and other factors on the accuracy of the estimated PMI_{min} in this data set was quantitatively compared. If the sample size is large enough, this method can also evaluate the accuracy and applicable time range of different estimation methods (such as rigor mortis, the temperature of the corpse, vitreous humor, and insect evidence [91]) in the PMI estimation of different cases. It is also possible to establish an EAI curve for a facility or a specific factor (such as season, indoor/outdoor, poisoning) and compare it with the EAI curve for other facilities or factors, to improve the understanding of adverse factors and estimation weaknesses.

Of the 307 cases, 260 cases reported PMI estimations, with homicide accounting for only 34.6%, while natural death, suicide, and accidental death account for the majority of the cases. It is obvious that PMI is an important value not only for forensic investigators and police but also for family members. Interestingly, in the cases where PMI_{max} and PMI_{min} information were disclosed, the average EAI index was 0.102 in cases of blood loss caused by homicide or accident death (n=53), while only the EAI was 0.160 for the other types of cases (n=76). There are probably three possible explanations: Blood loss can accelerate the arrival and spawning of flies and enable a more accurate estimation of the PMI. Other types of death (such as burning, hanging, or poisoning) can delay the arrival, oviposition, or development of flies. The cases related to blood loss are mostly homicide criminal cases, which makes the whole forensic entomological investigation process more in-depth and meticulous and therefore the estimation of PMI more accurate.

In addition, even though the temperature changes in different countries or regions in the same hemisphere may vary slightly or sharply with different climates and latitudes, the overall trend of monthly variation remained grossly the same [92]. The analysis of this data set focuses on correlations and change trends rather than getting the exact values. Therefore, the fitting curve shown in Fig. 10a could reflect that the EAI of cases in the warmer summer and autumn months (i.e., June-November in the Northern Hemisphere) was generally better than that in the colder spring and winter months (i.e., December–May in the Northern Hemisphere). Figure 6 shows that warmer seasons have higher insect species numbers on corpses, which is expected as insects are more active in the warmer months. For cases with PMI_{max}, PMI_{min}, and temperature information (n=83), corresponding to the fitting curve in Fig. 10b, it can be seen that the EAI is higher in the temperature range of 15 to 25 °C, while the EAI will decrease at lower or higher temperatures. This is possibly due to the abundant insect evidence available in warm climates, and the fact that the data obtained from forensic entomology studies are mostly carried out at a suitable temperature, so the EAI is high.

With the development of insect larvae, the estimation error of the PMI will gradually increase, and it becomes more difficult to accurately estimate the PMI in cases where the PMI exceeds the developmental cycle of the first generation of flies [45, 93]. Figure 9a shows that the cases with large estimation errors begin to appear after 30 days, which supports this point. Most of these cases can correspond to the skeletonized stage in Fig. 4, and the evidence of Calliphoridae applied at this stage is mostly from the puparium left at the scene of the case, that is, the developmental cycle of the first generation of Calliphoridae is taken as PMI_{min}. In these cases, the estimated PMI_{min} values were often very different from PMI_{max}. However, even though the estimation error of some cases increased after 30 days, the fitting curve of EAI in Fig. 9b showed no significant increase, which could be due to the fact that among the cases with a longer PMI, the cases with a more accurate estimation of the PMI are more likely to be reported. Thus the actual EAI curve should be steeper after the flies' first generation's developmental cycle. In addition, in cases with a short PMI, there are still some large estimation errors, that is, a high EAI. This can be attributed to some scenes (such as indoor, burial, and submersion) that are difficult for flies to reach quickly after the death of the deceased [94–101], that is, the pre-appearance interval (PAI) is long [102]. Another reason is that PMI_{max} is the time when the deceased is confirmed to be alive, which may differ from the actual time of death, which also contributes to estimation errors.

Analysis of insect evidence

With the decomposition process, the insect evidence left on the corpse tends to increase (such as puparium, exuviae, and dead adults), and these pieces of evidences can sometimes be used as exclusion evidence. For example, the case reported by Lutz et al. [89] estimated that PMI_{min} was more than 6 months using the presence of Muscidae puparium and the absence of any evidence of Calliphoridae. Martínez-Sánchez et al. [103] estimated the PMI_{min} to be close to 1 year according to puparium of *H. illucens* found at the corpse scene combined with its colonization and oviposition preference. These investigations showed that the inclusion of non-living evidence can aid in the estimation of PMI_{min}.

The number of species mentioned in forensic case reports increased with the decomposition process. The average number of species mentioned outdoors was 3.0, which was higher than the indoor number of 2.4, which is consistent with the previous finding that species diversity on indoor corpses is reduced [94, 96, 98, 99, 104]. It is worth noting that the intra-puparial period of the flies accounts for nearly half of the developmental period of the immature stage [105–107], yet the number of cases that used pupae in the forensic analysis was very small compared with those including the third instar larvae of flies. Studies on the intra-puparial period need to be deepened, and attention paid to the insect evidence such as pupae/puparium at the case scene also needs to be emphasized [108]. In addition, to facilitate the comparison between cases, Lepidoptera, Hymenoptera, and other species present at corpse scenes were not included in this data set as insect evidence. These insects generally arrive randomly and are easily ignored, so they are rarely described in cases [45].

Forensic entomologists tend to estimate the PMI using insects at the presumed oldest developmental stage on the corpse [43, 88, 105, 109]. Therefore, the statistics of insects with developmental data applied in Table 2 can reflect the insects that arrived at the corpses earliest very well, among which more than two-thirds of the developmental data are from Calliphoridae. As can be seen from Table 1, the developmental data of *C. vicina*, *C. megacephala*, and *L. sericata* were used most frequently, and they were the three most important species of Calliphoridae in this data set. According to the average estimated PMI_{min} summarized in Table 2, it seems that *Chrysomya albiceps* (Wiedemann, 1819), *C. vicina*, *Calliphora vomitoria* (Linnaeus, 1758), and *Protophormia terraenovae* (Robineau-Desvoidy, 1830) from the Calliphoridae can usually be used to estimate cases with a long PMI (average estimated PMI_{min}>10 days). This is mainly because the above species are widely distributed and frequently reported in European countries with relatively low ambient temperatures throughout the year. *C. vicina* and *C. vomitoria*, especially, can develop at low temperatures [110, 111], and *C. vicina* is considered to be an active cryophilic species all year round [107, 112, 113]. *P. terraenovae* is considered to be a cold-resistant Calliphoridae species [99, 100, 114], while *C. albiceps* usually arrive at a corpse late because of their facultative predatory behavior [107, 112, 115–117]. Many other species were mentioned as being able to colonize corpses, but there is a lack of developmental data at the necessary temperatures to estimate the PMI [44], which indicates the importance and urgency of conducting relevant studies to obtain complete developmental data for these species.

Of the 307 cases in this study, only species of Muscidae and Phoridae were mentioned more indoors than outdoors. However, 9 species of Hydrotaea (Muscidae) were mentioned more outdoors than indoors, and their developmental data was not successfully applied to the cases in this data set despite their prevalence. As far as the Coleoptera are concerned, only species of Dermestidae and Cleridae were mentioned multiple times in indoor scene cases [118–121], while others, such as species of Staphylinidae, Silphidae, and Histeridae, rarely appear indoors [104, 122]. Excluding the cases in which the developmental data of the species were applied less than five times, we found *H. illucens* and *Megaselia scalaris* (Loew, 1866) were the species with the longest average PMI_{min} in outdoor and indoor Dipterans respectively [98, 123–130], while the average PMI_{min} of the species of Dermestidae was the longest in Coleopterans [111, 131, 132].

Evaluation and suggestion on application standards

Although forensic entomology has spread from North America and Europe to a total of 29 countries on six continents in the past two decades, all of them have added their own voices to the world in the form of case reports in which they apply this discipline to solve practical problems. However, there is a big gap in the development of forensic entomology among different countries, where most countries and regions have not even started [133]. In addition, as a forensic science, forensic entomology still lacks the ability to convince judges, litigants, and the public in court [109], and must therefore gradually help the public to establish the concept that insects are regarded as powerful evidence rather than accomplices in destroying other evidence.

From a practical perspective, the accurate recording of various environmental factors, such as temperature (from the time when the deceased was colonized by insects to the time when the insect evidence was collected) [53, 59, 134–137], as well as standard operating procedures such as comprehensive specimen collection, proper preservation and the timely delivery of insect evidence are all important conditions for improving the accuracy of PMI estimation [44, 45]. Importantly, it has been confirmed that the continuous development of live insect samples will alter the estimation of PMI_{min} during the process of transferring them from the corpses at the crime scene to an entomologist's laboratory or morgue [138]. Even though the collection of insect evidence at the scene of the case is often done by non-forensic entomologists, it is ideal for forensic entomologists to collect insect evidence on the scene in time, as this also standardizes the data. In addition, the temperature is different between the meteorological station and the case scene [139–145], even between indoor and outdoor conditions [100, 139, 146], rendering it necessary to make further corrections to account for these differences [45, 147–151]; therefore, ignoring these differences may lead to a serious deviation from reality in the estimation of PMI [89]. We evaluated the normalization of the two steps of insect evidence collection and temperature data acquisition based on each case description in an attempt to reflect the major deficiencies in current forensic entomology case processing. Figure 5 shows that, of the 307 cases, only 61 were collected by forensic entomologists, only 115 cases of insect evidence were collected without delay and only 40 were temperature data corrected. In addition, among the 146 species of necrophagous insects mentioned in this study, the developmental data of only 51 species were used in real cases, suggesting the importance for researchers to supplement and improve the developmental data of lesser-known necrophagous insects [152]. Therefore, the data from this study support the notion that forensic entomology still has a long way to go before becoming a standardized application in forensic investigations.

Limitations and benefits of case reports

It should be emphasized that the analysis results obtained from this study should not represent standard ecological investigation conclusions. First of all, countless cases involving insect evidence occur every year all over the world, but only a few investigators are aware of the usefulness of insect evidence [89], and even fewer cases involving insect evidence may be reported and published. Secondly, due to the lack of proficiency of the relevant personnel in the discovery, collection, and preservation of insect evidence and the lack of basic developmental and succession data, even though some insect evidence is included in investigations, it often does not add value to its full potential [44, 153, 154]. Finally, there is a certain subjectivity in case reports that needs to be clarified, which roughly includes the following four points:

1. There is a tendency to report cases with an accurate estimation of the PMI.
2. There is a tendency to report cases with special or rare species.

3. A tendency to report the first application cases in a certain area.
4. Different authors show different degrees of case information, and the sampling and recording (such as the evaluation of the decomposition stage) of persons in charge of different cases will be subjective [45].

The case is the most real and vivid reflection of the practicability of forensic entomology theory. Even though some of the above reasons will make it difficult for our data set to accurately describe the true values of various information found on the corpse scene, after the comprehensive analysis of this large relatively complete data set, there are still some rules to follow and it can provide valuable global information. Such as the contribution of published cases in different regions, the change of developmental data and succession patterns with the application proportion of the PMI, the distribution of different species indoors and outdoors, ambient temperature, postmortem interval, and the change rule of species number with temperature, month, and decomposition stage. Therefore, this retrospective study of the effect of these factors on the estimation of PMI can provide valuable information for forensic entomologists and caseworkers on the collection of insect information and the recording of actual cases and can direct future research.

Conclusion

We systematically reviewed the application of insect evidence in real forensic cases from 1935 to 2022, counted the total number of mentions of each species and the number of times their developmental data were used, and listed the insects that are currently focused on for research and application as well as other insects with future application potential. Through the analysis of the indoor, outdoor, and temperature distribution of different species, our understanding of the environmental preferences of specific species was deepened. The comprehensive analysis of information such as the decomposition stage, the number of mentioned species, temperature, and month, further explained the complex self-change and mutual influence laws among corpses, insects, and the environment, and put forward a simple and intuitive method that can quantitatively analyze various factors that affect the accuracy of PMI estimation. Through the standardization of the two key steps of insect evidence collection and temperature data acquisition before the PMI estimation, this study also showed that there is still an imbalance between the research achievements of forensic entomology and the breadth and depth of its application.

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Author contribution Conceptualization: Gengwang Hu and Yu Wang; Investigation, methodology, and software: Liangliang Li, Shipeng Shao, Yundi Gao, Ruonan Zhang, Yinghui Wang, Yanan Zhang, Yi Guo, Chengtao Kang; Writing—original draft preparation: Gengwang Hu, Yu Wang, Yingna Zhang, and Liangliang Li; writing—review and editing: Yu Wang and Jiangfeng Wang; supervision: Yu Wang and Jiangfeng Wang; funding acquisition: Yu Wang, Jiangfeng Wang, and Liangliang Li. All authors have read and agreed to the published version of the manuscript.

Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Informed consent Informed consent was obtained from all individual participants (or their relations) included in the study.

Conflict of interest The authors declare no competing interests.

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