Katarzyna Frątczak-Łagiewska
Department of Animal Taxonomy and Ecology, Faculty of Biology, Adam Mickiewicz University in Poznań
Laboratory of Criminalistics, Faculty of Law and Administration, Adam Mickiewicz University in Poznań
katarzyna.fratczak@amu.edu.pl

Methods for the evaluation of the insect evidence age

Summary

The article presents the newest methods of assessment of entomological evidence used in forensic entomology. The authors outline the advantages and limitations of particular techniques and highlight those that can be routinely applied in forensic practice. Additionally, certain types of evidence are characterized, including the circumstances under which they may be useful.

Keywords forensic entomology, time of death, true flies, beetles

Introduction

Forensic entomology (entomoscopy) is the branch of entomology studying necrophilic insects for evidentiary purposes, i.e. to be used as evidence in the course of criminal proceedings. Necrophilic insects encompass the taxons that prefer cadavers as their feeding environments [1].

Entomological evidence involves living or dead insects or fragments thereof as well as traces of insect activities [2]. The insects relevant for forensic entomology, primarily the true flies (Diptera) and beetles (Coleoptera), undergo complete metamorphosis, which means that their life cycle includes the pupa stage. Thus, the development of these insects encompasses an embryonic phase occurring inside the egg and beyond embryonic phase that can be divided into three stages: larva, pupa and imago (adult, "perfect" insect). Consequently, entomological evidence makes up a diverse group of different developmental stages of insects representing various taxonomic ranks [2].

The fragments of insects primarily include empty puparia and molts, whereas the traces of activity are usually associated with damages of the cadavers [3] or with blood excreted by imagines of the true flies. Such excretions can be mistaken for case-related blood traces revealed at the site of cadaver discovery [4-6].

Entomological evidence is most frequently used to estimate the time elapsed between the moment of death and the discovery of the cadaver (the so called *postmortem interval*, PMI) [7]. The entomology-based PMI estimation methods focus on the age analysis of pre-imaginal individual insects (development-based method) or entire insect communities (succession-based method) secured from cadavers [1, 2].

The age of entomological evidence equals the duration of development of a particular individual, i.e. the time elapsed between laying eggs (or 1st stage larva) and securing evidence. Hence, the estimate value does not reflect the moment of death, but merely the minimum time elapsed from that moment. In order to determine the time of death more precisely, the minimum PMI can be complemented by estimation of the period preceding the appearance of a particular taxon on the cadaver. This period is referred to as PAI (*pre-appearance interval*) [2, 8, 9]. The newest methods for age estimation in forensic evidence secured on cadavers are presented below.

Eggs

In the case of true flies, the egg stage is the shortest developmental stage, making up about 5% of the complete development [10, 11]. Nevertheless, under certain circumstances, the eggs may constitute the only entomological evidence present at the cadaver discovery site. Moreover, the eggs are very useful in estimating the PMI value for cadavers discovered shortly after death. The insects are cold-blooded organisms, i.e. their body temperature and, in consequence, their pace of development, are dependent on ambient temperature. Therefore, lower temperatures facilitate longer prevalence of eggs on cadavers.

So far, there have been very few studies focusing on the egg age estimation. The authors of research articles on the development of particular insect species, usually refer only to the duration of the egg stage. Recently, a number of forms developing during the egg stage of the true fly Calliphora vicina Robineau-Desvoidy, 1830 (Calliphoridae) have been analyzed, resulting in the identification of 15 developmental phases, distinguishable under the microscope, based on which the egg age can be estimated [11].

Other methods allowing the estimation of the egg age are the molecular techniques based on gene expression [12]. Genes are differently expressed throughout the development process. This means that the activity can be switched on and off at different developmental stages. The expression level of some genes is predictable and temperature-dependent, which can be helpful in estimating the egg age [13]. Based on different gene expression patterns, the egg stage can be divided into shorter time periods, which can serve as genetic landmarks of the development process. This method enables age estimation of the eggs of the true fly *Lucilia sericata* (Meigen, 1826) (Calliphoridae) with accuracy of 2 hours [12]. Unfortunately, the practical use of the molecular methods entails certain drawbacks, such as requiring access to the laboratory with specialist equipment, being time-consuming and costly. Additionally, the entomological material must be secured in a proper manner such as to inhibit gene expression and preserve the expression products [12]. For the above reasons, the practical implementation of the molecular methods is very limited.

Larvae

The larval development of most insect species of practical use in forensic entomology can be divided into three stages, whereby the third stage is further split into two phases. During the first phase, the larva feeds intensively and thus grows rapidly, whereas during the second stage it does not acquire any nutrition (the so called *postfeeding* larva). Based on the rapid growth during the first two developmental stages and the beginning of the third stage, a method of larvae age assessment on the basis of their length and weight has been developed. Once these two indicators have been measured, they are compared against the developmental data obtained from a series of experiments addressing the development of a specific species [1]. The results are then plotted on isomegalen diagrams [14, 15], which contain larval growth curves. By knowing the temperature under which a particular insect has grown, the time elapsed between the initiation of the development and achieving a given size can be read off the diagram.

The age can be also estimated on the basis of the actual larval stage attained by individual insects. To this end, isomorphen diagrams are used, consisting of the curves representing key developmental milestones, i.e. hatching, first molt, second molt, pupation, imago appearance. The areas between the curves correspond to certain developmental stages, e.g. the area between the second molt curve and the pupation curve reflects the third-stage larva. Isomorphen diagrams also allow the estimation of time elapsed between the initiation of the development and achieving a given size under specified temperature conditions [14].

A far greater challenge is to estimate the age of the third-stadium larvae that do not acquire nutrition. The traditional age indicators such as body length and weight reach their maxima at this stage and subsequently they decrease slightly. Since age estimation of non-feeding larvae is highly problematic, forensic experts handling this type of entomological material are typically only able to determine the developmental stage of individual insects and estimate the time required to reach this stage [2]. Moreover, the duration of the *postfeeding* phase of the third larval stage can be dependent on environmental conditions. The non-feeding larvae move slightly away from their nutrition sources in order to undergo pupation, which occurs once the optimal site is found (away from predators, shadowed). The migration period can influence the total duration of the development and, in consequence, cause significant misestimations of the time of death [16]. This can be particularly important in the case of cadavers decomposing in enclosed areas with no favorable conditions for pupation. Under such conditions, the larvae preparing for pupation can migrate over a distance of even up to 30 meters [16]. In view of the above, the results of the studies carried out under laboratory conditions (optimal for the larvae) may not be representative of real casework. Considering the above mentioned limitations, the minimal PMI value determined on the basis of non-feeding larvae may be significantly underestimated. The age of such larvae can be more precisely estimated using the molecular methods, involving gene expression measurements [17].

Another method of age estimation consists in the analysis of hydrocarbons present on the chitin-rich cuticle constituting the insect's exoskeleton [18-21]. The composition of cuticular hydrocarbons changes over time, whereby the alterations can be modeled with the purpose of carrying out age estimation. The hydrocarbon composition data have been elaborated for the following species: *L. sericata* [19], *Aldrichina grahami* (Aldrich, 1930) [18], *Chrysomya rufifacies* Macquart, 1842 [20], *Calliphora vomitoria* (Linnaeus, 1758), *C. vicina* and *Protophormia terraenovae* (Robineau-Desvoidy, 1830) (Calliphoridae) [21].

Pupae

The pupae of beetles and true flies do not acquire nutrition and therefore, their size does not change. Hence, the traditional age indicators, e.g. body length or weight are not suitable for age estimation of the pupae. However, a precise estimation is particularly important as the pupal stage is the longest one, save the imago stage. Depending on the species, the true flies and beetles remain in the pupal stage between 35% and 69% of their pre-imaginal life [10, 25-28]. In practice, living pupae are transferred to the laboratory and bred under controlled conditions until maturity. Knowing the duration of the pupal stage in a given environment, the court expert can determine the age of individual insects by subtracting the time elapsed before reaching the imago stage from the duration of the pupal stage. This method will fail in the case of pupae preserved in alcohol or those that have died during breeding.

The pupae of true flies are exarate, i.e. having the legs and wings free and not glued to the body. They are enclosed by a barrel-shaped case called puparium. The color of puparium has earlier been considered relevant for age estimation. It was found, however, that it changes just hours after the formation of the puparium [13]. Hence, the newer methods base on observation of morphological features [26, 29, 30]. Dead pupae are stripped of their puparia and examined under a stereo microscope. Morphological changes may concern, inter alia, the color of eyes and leg bristles, color and shape of antennae, state of development of forehead bristles, presence of thoracic bristles, leg length and width. The values of these parameters are correlated with age expressed as *Accumulated degree days* (ADD) or *Accumulated degree hours* (ADH) [26]. The above terms refer to the number of heat units above a certain threshold temperature that an organism needs to accumulate in order to achieve a particular developmental stage [1].

An alternative approach is based on histological analysis consisting in observation of internal changes at the cellular (tissue) level [31]. The alterations are observed in histological specimens, under a light microscope. In simplified terms, the procedure consists in storing the pupae under specific conditions, preparing and staining the slices. The results obtained indicate that a combination of morphological and histological features is a much better age indicator than a morphological analysis alone [32]. A method allowing the simultaneous observation of changes in both external and internal structures is a computed tomography [32]. It provides the possibility of a simultaneous analysis of many samples, whereby each individual specimen can be viewed from three different perspectives, contrary to the traditional histological analysis, which only offers a single plane. The methods described above require the killing of insects. In consequence, their age cannot be estimated during breeding. The only method allowing in vivo age estimation is an optical coherence tomography (OCT) [33]. However, the OCT only offers the possibility to distinguish between older and younger pupae.

The pupal age can additionally be estimated by applying molecular methods based on gene expression [13, 25, 24, 25]. As in the case of eggs, differential gene expression can be used to divide the pupal stage into shorter periods equivalent of genetic landmarks of insect development. For example, 15 genetic landmarks have been characterized for the true fly *Calliphora vicina* [35].

There were numerous attempts to estimate the age by an analysis of volatile organic compounds released at different developmental stages. Additionally, a number of studies focused on the analysis of volatile compounds released postmortem by decaying carcasses [36-39]. Recent studies suggest that such compounds continue to be emitted throughout all developmental stages of the true fly [40] and they facilitate distinguishing between younger and older pupae. Unfortunately, this method is not suitable for precise age estimation. Moreover, certain genetic and environmental factors may potentially influence the composition and emission of volatile compounds. The impact of these factors has not been thoroughly studied, which prevents the use of this method even for the initial age estimation of pupae.

Adult insects

Adult insects are only rarely used for estimating the PMI by applying the development-based method, since so far only a few authors have focused on elaborating reliable methods of adult insect age estimation. The latest research conducted on 6 true fly species of the family Calliphoridae: *Chrysomya putoria* (Wiedemann, 1830), *Cochliomyia macellaria* (Fabricius, 1775), *C. rufifacies*, *P. terraenovae*, *C. vicina*, *C. vomitoria* revealed that hydrocarbon analysis can be an effective tool for age estimation in adult insects [21, 41, 42]. A correct age estimation of adult insects can be particularly important in the case of cadavers in an advanced state of decomposition found in enclosed areas, on which empty puparia as well as living and dead fly imagines are frequently revealed. In this case, the minimum postmortem interval can be determined by adding the age of living adult insects found in the surrounding area to the life span of a given species. Dead adult insects cannot be used for this purpose as the method for assessing the time elapsed from the moment of death has not been developed. At present, adult insects are used in connection with the succession-based method, which does not estimate the age of a particular individual, but rather, of the entire insect community present on the cadaver [1].

The age of adult insects can also be determined by pteridine analysis [43, 44]. Pteridines are the pigments constituting by-products of purine decomposition. As the insects age, pteridines tend to accumulate inside different parts of their bodies (e.g. eyes, head, wings), which renders them a potential aging marker for adult individuals [45]. A significant limitation of this method is the requirement for standardization of the results in order to account for the insect's body size. Assuming the same age, larger individuals accumulate more pteridines than smaller ones. Additionally, pteridine accumulation is gender-dependent, with better predictability in male individuals. Another method correlates pteridine quantities with wing wear. However, it does not account for insect activity which has a major impact on the wear and as such it is not recommended for age estimation in adult insects [45].

Finally, the age of adult insects can be estimated by using near infrared spectroscopy (NIRS) [45, 46]. This technique measures reflectance of infrared light off the insect cuticle for the purpose of detecting age-specific alterations. Compared to pteridine analysis, the NIRS method is more convenient, size- and gender-independent.

Fragments of insects

The most important fragments of insects for forensic entomologists include empty puparia and molts. These are usually found on cadavers in an advanced state of decomposition [22]. As in the case of larvae and adult individuals, age estimation is based on hydrocarbon analysis [22]. However, the analysis must be preceded by studies (also involving hydrocarbon analysis) aimed at the identification of a species from which the particular fragment originated. Each insect species has a unique hydrocarbon profile, constituting an important tool for species identification [45, 47]. The analysis yields a profile which is compared against all the other profiles stored in the database. If the database contains a searched species, the entire identification process takes merely a few hours. At present, hydrocarbon analysis performed on empty puparia allows distinguishing between the following 6 true fly species: *Aldrichina grahami*, *Chrysomya megacephala* (Fabricius, 1794), *C. rufifacies*, *L. sericata*, *Sarcophaga peregrina* (Robineau-Desvoidy, 1830) and *Sarcophaga crassipalpis* (Macquart, 1839) [48]. DNA barcoding can also be used for identification purposes, however, this approach is time-consuming and difficult to apply to empty puparia. The reason lies in a relatively low puparial DNA content which, over time, may undergo further degradation upon exposure to environmental factors.

Conclusions

Despite a growing number of novel techniques for estimating the age of entomological evidence, the classical methods based on breeding and morphological analysis with the use of a stereo microscope are still the most popular ones. The majority of the proposed age estimation methods have not found any practical application, primarily due to high costs, stemming from the need for access to laboratories with specialist equipment as well as time-consuming procedures.

Bibliography

1. Matuszewski S., *Katalog owadów przydatnych do ustalania czasu śmierci w lasach Polski* [Catalogue of insects useful in time of death estimation in forests of Poland], Part 1: *Wprowadzenie* [Introduction]. In „Problemy Kryminalistyki" 2010, vol. 267.
2. Matuszewski S., *Ekspertyza entomologiczna*, [Entomological expertise]. In Kała M., Wójcikiewicz J., Wilk D., *Ekspertyza sądowa* [Forensic expertise], Wolters Kluwer, 2016.
3. Baumjohann K., Benecke M., Trothschild M.A., *Schussverletzungen oder Käferfraß*, „Rechtsmedizin" 2014, vol. 24(2), p. 114-117.
4. Benecke M., Barksdale L., *Distinction of bloodstain pattern from fly artifacts*, „Forensic Science International" 2003, vol. 137(2-3), p. 152-159.
5. Durdle A., van Oorschot R., Mitchell J., *The morphology of fecal and regurgitation artifacts deposited by the blow fly Lucilia cuprina fed a diet of human blood*, „Journal of Forensic Sciences" 2013, vol. 58(4), p. 897-903.
6. Striman B., Fujikawa A., Barksdale L., Carter D.O., *Alteration of expirated bloodstain patterns by Calliphora vicina and Lucilia sericata* (*Diptera*: *Calliphoridae*) *through ingestion and deposition of artifacts*, „Journal of Forensic Sciences" 2011, vol. 56(1), p. 123-127.
7. Greenberg B., Kunich J.C., *Entomology and the law*: *flies as forensic indicators*, Cambridge University Press, Cambridge 2002.
8. Matuszewski S., *Estimating the preappearance interval from temperature in Creophilus maxillosus L.* (*Coleoptera*: *Staphylinidae*), „Journal of Forensic Sciences" 2012, vol. 57(1), p. 136-145.
9. Matuszewski S., *Estimating the pre-appearance interval from temperature in Necrodes littoralis L.* (*Coleoptera*: *Silphidae*), „Forensic Science International" 2011, vol. 212(1–3), p. 180-188.

10. Anderson G.S., *Minimum and maximum development rates of some forensically important Calliphoridae* (*Diptera*), „Journal of Forensic Sciences ” 2000, vol. 45, p. 824-832.
11. Martin-Vega D., Hall M., *Estimating the age of Calliphora vicina eggs* (*Diptera*: *Calliphoridae*): *determination of embryonic morphological landmarks and preservation of egg samples*, „International Journal of Legal Medicine” 2016, vol. 130, p. 845-854.
12. Tarone A.M., Jennings K.C., Foran D.R., *Aging blow fly eggs using gene expression*: *a feasibility study*, „Journal of Forensic Sciences” 2007, vol. 52(6), p. 1350-1354.
13. Boehme P., Spahn P., Amendt J., Zehner R., *Differential gene expression during metamorphosis*: *a promising approach for age estimation of forensically important Calliphora vicina pupae* (*Diptera*: *Calliphoridae*), „International Journal of Legal Medicine” 2013, vol. 127, p. 243-249.
14. Grassberger M., Reiter C., *Effect of temperature on Lucilia sericata* (*Diptera*: *Calliphoridae*) *development with special reference to the isomegalen- and isomorphen-diagram*, „Forensic Science International” 2001, vol. 120, p. 32-36.
15. Amendt J., Richards C.S., Campobasso C.P., Zehner R., Hall M.J., *Forensic entomology*: *applications and limitations*, „Forensic Science, Medicine, and Pathology” 2011, vol. 7(4), p. 379-392.
16. Mai M., Amendt J., *Effect of different post-feening intervals on the total time of development of the blowfly Lucilia sericata* (*Diptera*: *Calliphoridae*), „Forensic Science International” 2012, vol. 221, p. 65-69.
17. Baqué M., Amendt J., Verhoff M.A., Zehner R., *Descriptive analyses of differentially expressed genes during larval development of Calliphora vicina* (*Diptera*: *Calliphoridae*), „International Journal of Legal Medicine” 2015, vol. 129, p. 891-902.
18. Xu H., Ye G.Y., Xu Y., Hu C., Zhu G.H., *Age-dependent changes in cuticular hydrocarbons of larvae in Aldrichina grahami* (*Aldrich*) (*Diptera*: *Calliphoridae*), „Forensic Science International” 2014, vol. 242, p. 236-241.
19. Moore H.E., Adam C.D., Drijfhout F.P., *Potential use of hydrocarbons for aging Lucilia sericata blowfly larvae to establish the postmortem interval*, „Journal of Forensic Sciences” 2013, vol. 58(2), p. 404-412.
20. Zhu G.H., Ye G.Y., Hu C., Xu X.H., Li K., *Development changes of cuticular hydrocarbons in Chrysomya rufifacies larvae*: *potential for determining larval age*, „Medical and Veterinary Entomology” 2006, vol. 20(4), p. 438-444.
21. Roux O., Gers C., Legal L., *Ontogenetic study of three Calliphoridae of forensic importance through cuticular hydrocarbon analysis*, „Medical and Veterinary Entomology” 2008, vol. 22(4), p. 309-317.
22. Zhu G.H., Xu X.H., Yu J.-Y., Zhang Y., Wang J.-F., *Puparial case hydrocarbons of Chrysomya megacephala as an indicator of the postmortem interval*, „Forensic Science International” 2007, vol. 169(1), p. 1-5.
23. Zhu G.H., Yu X.-Y., Xie L.-X., Luo H., Wang D., Lv J.-Y., Xu X.-H., *Time of death revealed by hydrocarbons of empty puparia of Chrysomya megacephala* (*Fabricius*) (*Diptera*: *Calliphoridae*): *A field experiment*, „PLoS ONE” 2013, vol. 8(9), p. e73043.
24. Drijfhout F.P., *Cuticular hydrocarbons*: *a new tool in forensic entomology?*, In Amendt J., Campobasso C.P., Goff M.L., Grassberger M., *Current concepts in forensic entomology*, Springer, 2010, p. 179-203.
25. Zehner R., Amendt J., Boehme P., *Gene expression analysis as a tool for age estimation of blowfly pupae*, „Forensic Science International: Genetics Supplement Series 2” 2009, p. 292-293.
26. Brown K., Thorne A., Harvey M., *Calliphora vicina* (*Diptera*: *Calliphoridae*) *pupae*: *a timeline of external morphological development and a new age and PMI estimation tool*, „International Journal of Legal Medicine” 2015, vol. 129, p. 835-850.
27. Ridgeway J.A., Midgley J.M., Collett I.J., Villet M.H., *Advantages of using development models of the carrion beetles Thanatophilus micans* (*Fabricius*) *and T. mutilatus* (*Castelneau*) (*Coleoptera*: *Silphidae*) *for estimating minimum post mortem intervals*, *verified with case data*, „International Journal of Legal Medicine” 2013, vol. 128(1), p. 207-220.
28. Watson-Horzelski E.J., *Survival and time of development for Creophilus maxillosus* (*L.*) (*Coleoptera*: *Staphylinidae*) *at three constant temperatures*, „Coleopterists Bulletin” 2012, vol. 66(4), p. 365-370.
29. Barros-Cordeiro K.B., Báo S.N., Pujol-Luz J.R., *Intra-puparial development of the black soldier-fly*, *Hermetia illucens*, „Journal of Insect Science” 2014, vol. 14.
30. Ma T., Huang J., Wang J., *Study on the pupal morphogenesis of Chrysomya rufifacies* (*Macquart*) (*Diptera*: *Calliphoridae*) *for postmortem interval estimation*, „Forensic Science International” 2015, vol. 253, p. 88-93.
31. Davies K., Harvey M.L., *Internal morphological analyses for age estimation of blow fly pupae* (*Diptera*: *Calliphoridae*) *in postmortem interval*

estimation, „Journal of Forensic Sciences" 2013, vol. 58(1), p. 79-84.
32. Richards C.S., Simonsen T.J., Abel R.L., Hall M.J.R., Schwynd D.A., Wickleind M., *Virtual forensic entomology*: *Improving estimates of minimum post-mortem interval with 3D micro-computed tomography*, „Forensic Science International" 2012, vol. 220(1-3), p. 251-264.
33. Brown K., Harvey M., *Optical coherence tomography*: *Age estimation of Calliphora vicina pupae in vivo?*, „Forensic Science International" 2014, vol. 242, p. 157-161.
34. Ames C., Turner B., Daniel B., *Estimating the post-mortem interval* (*II*): *The use of differential temporal gene expression to determine the age of blowfly pupae*, „International Congress Series" 2006, vol. 1288, p. 861-863.
35. 3Zajac B.K., Amendt J., Horres R., Verhoff M.A., Zehner R., *De novo transcriptome analysis and highly sensitive digital gene expression profiling of Calliphora vicina* (*Diptera*: *Calliphoridae*) *pupae using MACE* (*Massive Analysis of cDNA Ends*), „Forensic Science International: Genetics" 2015, vol. 15, p. 137-146.
36. Dekeirsschieter J., Verheggen F.J., Gohy M., Hubrecht F., Bourguignon L., Lognay G., Haubruge E., *Cadaveric volatile organic compounds released by decaying pig carcasses* (*Sus domesticus L.*) *in different biotopes*, „Forensic Science International" 2009, vol 189, p. 46-53.
37. Dekeirsschieter J., Stefanuto P.-H., Brasseur C., Haubruge E., Focant J.-F., *Enhanced characterization of the smell of death by comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry* (*GCxGC-TOFMS*), „PLoS ONE" 2012, vol. 7(6), p. e39005.
38. Brasseur C., Dekeirsschieter J., Schotsmansc E.M.J., de Koningd S., Wilsonc A.S., Haubrugeb E., Focant J.-F., *Comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the forensic study of cadaveric volatile organic compounds released in soil by buried decaying pig carcasses*, „Journal of Chromatography A" 2012, vol. 1255, p. 163-170.
39. Forbes S.L., Perrault K.A., *Decomposition odour profiling in the air and soil surrounding vertebrate carrion*, „PLoS ONE" 2014, vol. 9(4), p. e95107.
40. Frederickx C., Dekeirsschieter J., Brostaux Y., Wathelet J.P., Verheggen F.J., Haubruge E., *Volatile organic compounds released by blowfly larvae and pupae*: *new perspectives in forensic entomology*, „Forensic Science International" 2012, vol. 219(1-3), p. 215-220.
41. Pechal J.L., Moore H., Drijfhout F.P., Benbow M.E., *Hydrocarbon profiles throughout adult Calliphoridae aging*: *A promising tool for forensic entomology*, „Forensic Science International" 2014, vol. 245, p. 65-71.
42. Braga M.V., Pinto Z.T., de Carvalho Queiroz M.M., Blomquist G.J., *Effect of age on cuticular hydrocarbon profiles in adult Chrysomya putoria* (*Diptera*: *Calliphoridae*), „Forensic Science International" 2016, vol. 259, p. e37-e47.
43. Zhu G.H., Ye G., Hu C., *Determining the adult age of the oriental latrine fly*, *Chrysomya megacephala* (*Fabricius*) (*Diptera*: *Calliphoridae*) *by pteridine fluorescence analysis*, „Insect Science" 2003, vol. 10(4), p. 245-255.
44. Zhu G.H., Ye G., Li K., Hu C., Xu H., *Determining the age of adult flesh flies*, *Boettcherisca peregrina*, *using pteridine fluorescence*, „Medical and Veterinary Entomology" 2013, vol. 27(1), p. 59-63.
45. Villet M.H., Amendt J., *Advances in entomological methods for death time estimation*, In Turk E.E., *Forensic Pathology Reviews*, Springer, 2011, p. 213-237.
46. Perez-Mendoza J., Dowell F.E., Broce A.B., Throne J.E., Wirtz R.A., Xie F., Fabrick J.A., Baker J.E., *Chronological age-grading of house flies by using near-infrared spectroscopy*, „Journal of Medical Entomology" 2002, vol. 39(3), p. 499-508.
47. Braga M.V., Pinto Z.T., de Carvalho Queiroz M.M., Matsumoto N., Blomquist G.J., *Cuticular hydrocarbons as a tool for the identification of insect species*: *puparial cases from Sarcophagidae*, „Acta Tropica" 2013, vol. 128, p. 479-485.
48. Ye G., Li K., Zhu J., Zhu G.H., Hu C., *Cuticular hydrocarbon composition in pupal exuviae for taxonomic differentiation of six necrophagous flies*, „Journal of Medical Entomology" 2007, vol. 44(3), p. 450-456.

Translation Rafał Wierzchosławski