MEDICO-LEGAL RELEVANCE OF CADAVER ENTOMOFAUNA FOR THE DETERMINATION OF THE TIME SINCE DEATH

22

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I. Present state and trends in development of criteria of determining the time of death in late post mortem period

The optimum criteria of determining the time of death in late post mortem period can be developed as a result of comprehensive study of the following processes :

First of all, regular changes occur directly in the tissues and organs of a cadaver and, on the whole, completely depend on the specific properties of a particular cadaver.

Analysis of over 50 Soviet papers devoted to the study of dynamics of post mortem processes in tissues and organs occurring later than 6 days or more after the moment of death has shown that :

a) practically all organs and tissues were studied using biophysical, biochemial, histological, histochemical, neurohistological, spectrographic, spectrophotometric, physical, photometric, chemical, chromotographic, cytological and electronic microscopy methods;
b) studies were carried out mainly on isolated organs;
c) temperature conditions of experiments, when accurately specified, did not encompass the complete range of environment temperatures;
d) the majority of studies were done under artificially created conditions, which ensured only the putrefaction process;
e) studies of different tissues and organs by the same method naturally give dissimilar results;
f) the duration of supposed practical application of proposed methods varies considerably depending on the tissues and organs to be studied and on the experimental conditions;

g) studies related to dynamics of investigated processes in dead tissues under varying environmental conditions were not completed, and therefore, the regularities controlling the progress of said processes in time were not determined.

Thus presently available materials of research work on post mortem changes occurring directly in tissues and organs of a cadaver, as a rule carried out during 3-4 years studies for theses, do not allow to reconstruct an integral picture of cadaver decomposition in its dynamics as consistent with the environmental conditions altered in time. Criteria based on the study of this group of cadaver changes as a rule require initial normal rate of one or the other parameter to be known and, therefore, at the present stage normally are within the limits of laboratory studies and hardly suitable for wide practical application.

Secondly, there are processes regularly accompanying post mortem changes in tissues and organs of a cadaver, but developing according to their own specific laws independent of the properties of a particular cadaver. These are processes such as decay of radioactive isotopes which entered an organism during its lifetime; cadaver biological decomposition (micro-organisms, mould fungi, insects); destruction and revival plants of cadaver bed.

Difficulties in practical utilization of the results of studies of the first direction have forced experts in forensic medicine to apply once more to entomology, the fundamentals which, for the determination of the time of death, were developed by M. Bergeret, P. Brouardel, J.P. Megnin, C.P. Yovanovitch as early as the 19th century.

Recent monographs of applied character appeared in England (K.G. Smith, 1986), in Belgium (M. Leclercq, 1978) and in Filand (P. Nuorteva, 1977). This direction is successfully developed in Austria (C. Reiter, 1983 a,b, 1984 a,b), in Belgium (M. Lezclercq et al., 1985, 1988), in USA (W.C. Rodriguez et al., 1982, 1983, 1985; T.R. Adkins, 1988; E.P. Catts, 1988, B. Greenberg, 1988; R.D. Hall, 1988, N.H. Haskell et al, 1988; M. Lee Goff, 1988; C.L. Meek, 1988 and B. Morris, 1988), in Finland (P. Nuorteva, 1987), in Japan (K. Nishida et al., 1984, 1986), in Italy (F.J. Introna and B.M. Altamura et al., 1987) and in many other countries. Two bibliographic articles on forensic entomology have appeared (Meek et al., 1983, Vincent et al., 1985).

Analysis of said editions proved forensic entomology to be an independent branch of applied entomology and a promising trend in solving the problem of identifying the time of death.

A scientific basis for using entomological data in forensic medicine comprises :

a) existence of necrobiont insects in Nature, which utilize cadaver tissues and pass the major part of their life cycle on cadavers;

b) relative constancy and specificity of cadaver entomofauna in a particular geographical region comprising widely spread predominating species;

c) compliance of species composition of cadaver entomofauna to the degree of its tissues decomposition and to its location;

d) seasonal alterations of predominant necrobiont insect species;

e) beginning of insect activity in Spring and its end in Autumn as a result of transition to diapause condition due to changes in temperature and light-time interval, the values thereof being dependent on geographical regions and being specific for each species;

f) regulation of the number of generations per vegetative period and of species life cycle duration by strictly definite thermal parameters peculiar to the species;

g) long preservation of insect chitine cuticles in Nature.

At the present stage of development of forensic entomology the main efforts are concentrated on the research on biology of flies. There leads can be distinguished :

a) identification of species composition of fly association as well as of factors affecting the formation of the above composition;

b) study of changes in characteristics of larvae growth at different temperatures aimed at working out methods of determining larvae development periods;

c) determination of thermal values, regulating insect development, the obtained data to be further applied for a calculation technique used to retrospectively determine the time when insects invaded a cadaver.

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Table 1 : Distribution of subjects of inquiry according to biotope, season, cadaver species and period of observations.

N	Biotope	Nb of cadavers total	Nb of observat. total	Animals species				
				dog	cat	rabbit	suckling pig	others
1	Mixed forest	12	79	3	1	-	8	-
2	Underwood	11	195	5	2	4	-	-
3	Bushes	11	131	6	3	1	1	-
4	Clearing	15	106	2	3	2	2	6
5	Hill slope	12	287	11	-	-	1	-
6	Field	34	1046	20	5	8	1	-
7	Ravine	18	607	15	-	-	-	3
8	Grass swamp	17	650	10	1	5	-	1
9	Sphagnum swamp	12	355	9	3	-	-	-
10	Others biotopes*	11	35	3	5	-	-	3
11	Under--wood**	23	903	9	5	8	1	-
12	Sphagnum swamp, clearing	27	198	-	12	15	-	-
13	River shore, clearing	6	36	4	2	-	-	-
14	Bushes clearing	2	36	-	2	-	-	-
	Total	211	4664	97	44	43	14	13

* Biotopes : town garden, river shore, canyon - 2000 m, room;

** hanged cadavers.

Beginning of experiment, months						Period of experiment, days (up to)				
3-4	5	6	7	8	9	25	50	100	150	200 and over
-	-	-	2	10	-	7	-	1	-	-
-	7	1	3	-	-	3	-	4	-	4
3	-	-	5	3	-	4	3	1	1	2
1	-	-	5	7	-	10	1	3	1	-
1	-	-	4	1	6	-	7	4	-	1
3	8	4	12	-	7	5	8	6	6	9
2	1	10	3	-	2	3	2	-	9	4
3	1	10	1	-	2	-	2	2	10	3
5	2	4	-	1	-	-	1	4	6	1
-	3	2	2	2	2	9	2	-	-	-
4	1	13	-	1	4	-	5	1	9	8
-	-	27	-	-	-	27	-	-	-	-
-	2	-	-	4	-	-	4	-	2	-
-	-	2	-	-	-	-	-	-	-	2
22	25	75	37	29	23	68	39	26	44	34

2. Material and methods

2.1. Material and methods of fly studies

Studies were carried out in 1971-1983 in the Leningrad region and Kaunas region of Lithuanian SSR on 211 subjects of which 197 had been purposefully delivered to the site of experiment and 14 found in natural environment. There were 100 cadavers of big animals, mainly dogs), 106 of medium-size ones (cats, rabbits, sucking-pigs), 5 of small animals (moles, pigeons, kittens). One hundred ninety cadavers were obtained from the vivarium of Kirov Military Medical Academy after clinical studies and 7 animals were killed directly before the experiment.

Subjects were placed in 13 biotopes of the above regions; nine of them are the principal ones : mixed forest, mixed underwood, bushes, clearing, hill slope, field, ravine, grass and sphagnum swamps.

180 subjects were placed on soil surface, 23 were at the height of about I am from the ground; 7 subjects buried into the ground at different depths and 1 subject placed in unheated service room. Qualitative composition of subjects, their distribution of biotopes and starting date of experiments, as well as distribution of number of observations and experiment duration are given in Table I.

All the studies are subdivided into 3 groups.

1. Test runs 1 to 10 are devoted to the study of cadaver entomofauna and effect of biotope conditions on cadaver decay. They were carried out in the principal biotopes, with a particular test run corresponding to each one.

2. Runs carried out to study the effect of particular cadaver location (hanged and buried) on insects, as well as the effect of additional agents on cadaver tissues (clothes impregnated with chemicals, effect of flame).

- Run n° 11 : 23 subjects, 903 observations - underwood (hanged);
- Run n° 12 : 27 subjects, 198 observations - sphagnum swamp, clearing (clothing simulation);
- Run n° 13 : 6 subjects, 36 observations - river shore, clearing (buried);
- Run n° 14 : 5 subjects, 58 observations - sphagnum swamp, clearing busches (action of flame).

3. Using subjects of 14 principal runs, a number of additional questions were solved, which did not involve any special treatment of cadavers leading to changes in initial cnditions of tests. These studies can be subdivided into 6 subgroups :

- Specificity of tissue decay in cadavers placed under snow in early spring was studied on 22 subjects - 1191 observations;
- Specificity of cadaver tissue decay in autumn season was studies on 21 subjects - 454 observations;
- Temperature conditions of cadavers were studied on 56 subjects - 4472 measurements;
- Role of ants in cadaver decay was studied on 8 subjects - 60 observations;
- Changes in cadaver remains and its bed vegetation were studied on 52 subjects - 1748 observations;
- Study of necrophages fly phenology on 45 subjects in 1979-1980 in Leningradian region.

In all test runs, except run n° 13 cadaver inspection was done daily during the first week and later 2 - 3 times a week. Totally 4664 observations were made, from 1 to 68 observations per subject. In the course of observations, insect composition, their life stages, cadaver tissue condition, presence and characters of injuries by vertebrate changes in bed vegetation, temperature condition of cadaver and ambient air, air humidity were registered. Results of observations and measurements were recorded in a diary.

2.2. Material and methods of laboratory studies

Study of fly development periods was carried out at the laboratory of experimental entomology of the zoological Institute of the Academy of Sciences of the USSR using special photo-thermostats featuring automatic control of day length and temperature (temperature deviation +/- 0,5° C).

Periods of development of 17 fly species of 5 families at different constant temperatues were determined experimentally (Table 2). Some of the species were collected from animal cadavers in nature by the author (Table 2, NN I, 2, 8-12, 17, 18), while the others were given by laboratory colleagues E.B. Vinogradova and K.B. Zinovjeva. living flies collection of 8-10 species was kept at toom temperature in a specially designed photostat, automatically maintaining

Table 2 : Number and brief specification of laboratory tests carried out on flies

N	Family, Species	Total number of tests	T° parameters calculated	Conditions of keeping imago	
				Length of day(H)	T° (°C)
	CALLIPHORIDAE				
1	Calliphora vicina	43	x	20	20-23
2	Calliphora vomitaria	19	x	20	20
3	Calliphora uralensis	18		20	20
4	Lucilia sericata*	1	x	-	-
5	Lucilia caesar	9			19 24
6	Lucilia hirsutula	18		10-20	20
7	Lucilia illustris	9		19	24
8	Protophormia terranovae	37	x	20	20-25
9	Chrysomya albiceps	18	x	20	20-25
10	Phormia regina	8		x	20 25
	MUSCIDAE				
11	Muscina assimilis	19	x	20	20
12	Muscina stabulans	16	x	19	20
	SARCOPHAGIDAE				
13	Boettcherisca septentrionalis	12	x	20	20-23
14	Parasarcophaga argyrostoma	18		14-20	20-24
15	Parasarcophaga semenovi	14		14-19	20-24
16	Parasarcophaga similis	12		17-19	20-24
	PIOPHILIDAE				
17	Piophila foveolata	19	x	18-19	18-20
	PHORIDAE				
18	Phorida sp.	11		18-19	18-20
	TOTAL :	300	10	-	-

* calculations cited based on data by I.V. KOZHANCHIKOV, 1961.

continuation Table 2

N	Number of tests under specified temperature and light conditions for keeping preimagonal life stages (day length / temperature)							
	12 / 12.5	12-16 / 15	12 / 17	12-20 / 20	20 / 23	10-20 / 25	16-20 / 27	20 / 30-35
CALLIPHORIDAE								
1	8	4	2	11	16	1	1	-
2	2	-	3	5	4	1	4	-
3	-	1	-	4	13	-	-	-
4	-	-	-	-	-	-	-	-
5	-	3	-	4	2	-	-	-
6	-	4	-	9	-	5	-	-
7	-	4	-	2	3	-	-	-
8	3	2	6	9	6	7	4	-
9	5	-	-	4	-	3	3	3
10	-	1	-	2	-	2	2	2
MUSCIDAE								
11	-	-	4	8	5	1	1	-
12	3	3	-	4	-	2	4	-
SARCOPHAGIDAE								
13	-	-	2	5	5	-	-	-
14	-	4	1	5	8	-	-	-
15	-	6	-	4	4	-	-	-
16	-	7	-	2	3	-	-	-
PIOPHILIDAE								
17	6	-	4	4	3	2	-	-
PHORIDAE								
18	1	-	2	2	4	2	-	-
	28	39	24	84	76	26	19	4

given day length. Flies lived in compartments of 15 x 15 x 30 cm size and 30 x 30 x 15 cm size made of gauze of fine-mesh plastic fabric. They were fed meat, sugar and water. Larvae were fed by meat and kept in 0,5 - 1,0 litre jars filled by 1/2 - 1/3 with slightly humidified sawdust. Jar necks were covered with thick cloth or with a polyethylene cover having wire insert and an opening to let a thermometer in. The distribution of species according to the conditions in which they were kept and number of repetitions is given in Table 2. Totally, 300 tests were made at constant conditions for keeping the flies.

3. Biological decomposition of cadaver - General.

In a moderate climatic zone of the USSR, decomposition of tissues in a nonburied cadaver is a combined process embracing both destruction byd birds and animals and biological decomposition (microbes, mould fungi, insects). Micro-organisms and insects take part in the decomposition of all the cadavers. Presence of mould fungi and algae is observed after the end of fly larvae nutrition in 30% of cases. This is observed for the cadavers which started to decay in Spring, in 55% of cases, in Summer, 35% of cases, and in Autumn, none. The latter effect is accounted for by the fact that fly larvae continue to develop till the very frosts and till cadavers are covered with an icy crust, secreting a substance preventing the development of mould fungi (Zvereva, 1985). Damage by birds and animals are observed in 26% of the cases in the 1st year of decay and in 20% of the remains in the 2nd year. In Spring, 67% of cadavers are damaged, in Summer, 17%, in Autumn, 26% (Nainys, Marchenko, 1987).

Basing on predominant and representative for a given time period activity of one or other necrobiont subgroup, the process of cadaver decomposition is subdivided into 5 stages* and each stage corresponds to a certain degree of cadaver tissue decay.

The 1st stage - early microbial decomposition - follows the autolytic processes and lasts till fly egg-laying and larvae emergence. Average duration from May to September is 3 days (1-5 days).

* The division into periods differs from the earlier one proposed by Marchenko, 1978.

The 2nd stage - active decomposition of cadaver by insects - starts from fly larvae emergence, goes on simultaneously with microbial decay and ends upon the termination of larvae development which destroy major part of soft cadaver tissues. Average duration from May to September : 22 days (8-65 days).

The 3rd stage - advanced decomposition of cadaver by insects - starts from fly larvae pupation and is mainly done by beetle larvae, which destroy almost all the soft tisues left. Microbial decay continues and is supplemented by mould fungi activity. Duration varies from 12 to 504 days.

The 4th stage - microbial decomposition of cadaver - starts from the moment the beetle larvae leave the remains of cadaver and ends when the skeleton breaks into separate bones. Duration varies from 27 to 976 days.

The 5th stage - bony tissues disintegration - was not completed in the course of 4-years observation period.

There are no doubt about the effect of meteorological factors on biological decomposition of cadaver, however, up to now, this problem has not been specially studied. Let us make an attempt to narrow this gap by investigating cadaver biological decay period VS (Table 3), 7 meteorological factors under Leningrad region conditions (Table 4) relationship. Correlation analysis between average duration of individual cadaver decay stages and average values (of several years) of meteorological factors for the starting decade of each stage was executed (Table 5).

The duration of individual stages of biological decay in cadaver is considerably affected by meteorological factors. The duration of four stages was found to be inversely proportional to total radiation, air and ground surface temperature; duration of the 1st, 3rd and 4th stages - to solar radiation period; of the 1st stage also to the amount of precipitations. Duration of the 1st, 3rd and 4th stages and the 3rd and 4th were found to be directly proportional to the number of days with precipitation and to relative air humidity respectively. No pronounced affect of relative air humidity, number of days with precipitation and amount of precipitation on the duration of the 2nd stage was observed. This is connected with fly larvae ability to maintain the humidity of their life environment at a certain level through parenteral nutrition (Marchenko, Nainys 1987).

Table 3 : Duration of cadaver biological decay stages for the Leningrad region

MONTH	DECADE	MEAN DURATION OF DECAYS, DAYS				
		I	II	III	IV	I-III
MARCH	I	57.2				124.7
	II					107.0
	III	47.0				
APRIL	I	35.5				37.0
	III		30.7			
MAY	I		30.2			
	II		23.0	31.7		
	III	3.0	15.1	34.2	77.6	
JUNE	I	3.25	20.3	33.5	77.3	
	II	2.1	17.2	70.7	73.5	
	III			57.6		
	I-III					93.0
JULY	I	3.0	14.0	54.3		
	II	3.0		52.7		35.0
	III	2.6	12.9	17.0	26.5	
	I-III					111.5
AUGUST	I	3.0		25.8		
	II	2.5	14.8	14.3		40.0
	III		20.3	6.0		
	I-III				132.0	
SEPTEMBER	I			12.0		
	II	2.9	32.4			63.0
	I-III		27.3		598.3	
OCTOBER	I			426.5		
	II			492.3		
	III			411.5		
NOVEMBER	I-III					887.0

Table 4 : Decade average values (data of several years) of meteorological factors for the Leningrad region.

MONTH	DEC.	DECADE AVERAGE VALUES OF METEOROLOGICAL FACTORS						
		Period of solar rad.,H.	Total radiat. kcal/cm2	Air temper. °C	Soil surface °C	Relative air humid.%	Precipi -tation quant.	Precipi -tation quant.
MARCH	I	2.8	1.134	-6.4	-6.3	80.7	11.0	6.2
	II	4.1	1.651	-4.4	-6.1	77.7	10.0	6.5
	III	5.0	2.086	-1.9	-2.5	76.3	11.0	6.1
APRIL	I	4.6	2.196	0.6	1.0	74.9	12.0	6.0
	III	6.7	3.122	5.4	6.7	68.9	13.0	5.3
MAY	I	8.1	3.794	7.7	10.1	65.3	16.0	4.4
	II	8.4	4.158	9.5	13.4	64.1	16.0	5.0
	III	8.9	4.429	11.7	15.0	61.9	16.0	4.2
JUNE	I	9.3	4.623	13.5	18.6	64.4	21.0	3.5
	II	8.7	4.558	14.8	19.2	65.8	22.0	4.4
	III	8.4	4.376	16.2	20.2	69.3	22.0	5.6
	I-III	244.1	13.45	14.8	19.3	66.3	65.0	13.6
JULY	I	8.2	4.254	17.4	20.3	72.6	21.0	5.4
	II	8.1	4.201	18.0	21.0	71.3	22.0	4.6
	III	8.2	4.074	18.0	21.0	72.1	22.0	5.3
	I-III	265.0	13.49	17.8	20.8	71.4	65.0	15.3
AUG.	I	7.5	3.544	17.4	20.0	74.6	28.0	5.3
	II	7.1	3.196	16.4	18.2	76.8	28.0	5.0
	III	5.7	2.561	14.5	16.2	78.2	28.0	6.5
	I-III	209.5	9.950	16.0	18.1	76.3	84.0	16.8
SEPT.	I	5.4	2.245	12.8	14.3	79.8	22.0	5.7
	II	4.2	1.790	10.8	11.6	81.3	22.0	6.5
	III	3.5	1.384	8.8	8.5	81.4	22.0	6.0
	I-III	131.3	5.590	11.1	11.5	80.2	66.0	18.3
OCT.	I	2.5	0.946	6.2	6.5	82.8	19.0	6.6
	II	1.9	0.754	4.8	4.7	83.4	19.0	6.5
	III	1.5	0.506	2.9	2.4	85.7	18.0	7.9
NOV.	I-III	220.0	0.67	-0.5	-1.0	87.1	48.0	23.9

Table 5 : Duration of cadaver biological decay stages VC, decade average values (data of several years) of meteorological factors (Leningrad) relationship. Statistical data.

METEORO-LOGICAL FACTORS	EXPONENT	CADAVER BIOLOGICAL DECAY STAGE I	II	III	IV	I-III
Period	r	-0.708	-0.565	-0.874	-0.978	-0.617
solar	R	-0.067	-0.149	-0.013	-0.274	-0.483
radiation	P	<0.01	>0.05	<0.01	<0.01	<0.05
Total	r	-0.695	-0.589	-0.830	-0.967	-0.610
radiation	R	-0.037	-0.091	-0.007	-0.014	-0.127
	P	<0.02	<0.05	<0.01	<0.01	<0.05
Air	r	-0.683	-0.857	-0.844	-0.938	-0.905
temperature	R	-0.326	-0.484	-0.031	-0.019	-0.262
	P	<0.01	<0.01	<0.01	<0.02	<0.01
Soil	r	-0.864	-0.931	-0.881	-0.969	-0.862
surface	R	-0.381	-0.632	-0.031	-0.024	-0.292
temperature	P	<0.01	<0.01	<0.01	<0.01	<0.01
Relative	r	0.435	0.207	0.669	0.911	0.219
air	R	0.117	0.205	0.025	0.020	0.047
humidity	P	>0.1	>0.1	<0.01	<0.05	>0.1
Precipitations	r	-0.751	-0.388	-0.417	-0.711	-0.818
quantity	R	-0.197	-0.256	-0.009	-0.025	-0.155
	P	<0.01	>0.1	>0.1	>0.1	<0.01
Precipitations	r	0.759	0.305	0.674	0.934	0,355
number of days	R	0.032	0.039	0.004	0.010	0.011
	P	<0.01	>0.1	<0.01	0.02	>0.1

Average period of cadaver skeletization varies from 205 to 829 days (54-1074 days) depending of month when decay started. In 65% of cases skeletization occurs the same year as a cadaver reached the stage when it was detected in March-June. Extensive variability in the duration of individual decomposition stages of cadavers being under identical conditions makes it impossible to use the average data on their duration in order to determine the time of death (Marchenko, 1987; Nainys, Marchenko, 1987).

The effect of cadaver location (biotope) on the duration of decay stages is conditioned by the possibility and the effect of meteorological factors on cadaver tissues and feeding necrobionts. In a moderate climatic zone, primary importance is attribuated to the conditions of cadaver environment heat exchange, particularly to the absorption of part of solar radiation by the cadaver. It should be noted in this connection that assuming cadaver temperature to be equal to environment temperature would be a wrong thing to do, especially if the cadaver is exposed to the effect of solar radiation or of a source of heat. The duration of cadaver tissues decay in different biotopes at different seasons of the year is determined by the character of radiation regime. In Spring and Autumn decay progresses quicker on the slopes oriented perpendicularly to sunrays and more slowly in horizontal areas of the country.

Hanged cadavers decompose slower than the ones lying on the ground, because of higher convective heat transfer and mummification of the surface layers of tissues. When a cadaver remains in natural environment since March, the average duration of the 1st stage is 63 days, 2nd stage 46 days, 3rd stage 311 days. Since May the average duration of the 1st stage is 2 days, 2nd stage is 40 days, 3rd stage 75 days.

Further at the 3rd and 4th stages, depending on the time the cadaver remained into natural environment, the tissue decomposition is abruptly decelerated and mummified remains preserve the form of a body in a hanged position up to 3 years.

The influence of cadaver mass on its decay period depends on meteorological factors involving changes in tissues condition by making them more or less suitable for necrobiont feeding. Relevance of each of the above factors depends on month and biotope.

Clothes on a cadaver do not delay insects invasion, though the decay period will be somewhat longer. If clothes are stained with combustibles,

Table 6 : Time of insect invasion and character of soft tissues decay in cadaver, clothes stained with various contaminants being simulated (Marchenko, 1980 a)

TYPE OF DIRT ON CLOTHES	N.OF THE RUN	TIME WHEN INSECTS STARTED TO INVADE CADAVER (DAY)		DECOMPOSITION OF CADAVER SOFT TISSUES		
		insolated event	mass event	complete	partial	absent
GASOLINE	1	5	8	+		x
	2	5	9		+	x
	3	-	4	x +		
KEROSENE	1	2	5		+	x
	2	5	8			x +
	3	-	4	x +		
DIESEL	1	2	8	+	x	
FUEL	2	5	9	+	x	
	3	5	7	+	x	
NITROCEL-	1	2	9			x +
LULOSE	2	2	8			x +
ENAMEL	3	4	7		x +	
LUBRICANT	1	2	5	x +		
	2	2	5			x +
	3	4	5	+	x	
LACQUER	1	2	8			x +
S-4	2	8	11		+	x
	3	6	7	x +		
OIL PAINT	1	2	5	x +		
	2	-	5		x	x
	3	4	6	x +		
REMAINS	1	-	5	x +		
OF BURNT	2	5	8		x +	
CLOTHES	3	4	6	x +		
CLEAN	1	2	5		x +	
CLOTHES,	2	2	5		+	x
REFERENCE	3	-	4	+		x
NO CLOTHES	1	-	5	x +		
REFERENCE	2	2	5		x +	
	3	-	4	x +		

(x) cadaver tissues condition at the moment the clothers were taken off on the 14th day;
(+) cadaver tissues condition on the 23rd day.

lubricants and paintwork materials, the time before insects invade the cadaver will be approximately doubled, and in 47% of cases decomposition is perceptibly postponed (Table 6). Meteorological factors such as solar radiation, precipitation etc. produce a considerable effect on character and durability of dirt influence. Rain and higher air humidity contribute to the transfer of chemicals into cadaver tissues thus increasing their repellent effect. Solar radiation and increased air temperature tend to evaporate and dry out substances on clothes, thus preventing the transfer of chemicals into tissues, which results in accelerated insect invasion (Marchenko, 1980 a).

The leading part in biological decay of a cadaver is played by necrobiont insects. Based on taxonomic and ecological features, cadaver entomofauna can be divided into two groups :

1. Cadavericole entomofauna properly. It comprises necrobiont insects, for which the cadaver is a permanent environment for life and development. Accoring to trophic specialization they are subdivided into necrophages and entomophages.
2. Arbitrary entomofauna of cadaver. It comprises the species for which a cadaver is not a constant scene of life and development. This group is subdivided into polyphages, entomophages and necro-entomophages.

Properly cadavericole entomofauna is the most important component of biological decay of a cadaver. The leading position is held by representatives of Diptera order, families : Calliphoridae, Muscidae, Sarcophagidae, Helomizidae, Phoridae and Piophilidae. The second order by relevance is Coleoptera with horny forewings, families : Silphidae, Dersmestidae, Histeridae ans Staphylinidae (Marchenko, 1980 b).

The process of cadavericole fauna formation in a man's living quarters is characterized by a certain specificity. First of all this is connected with the steady microclimate maintained in the living quarters, favouring round-the-year insect activity. Secondly, this depends on whether necrophage insects did have or not access to the room with the cadaver, on the one hand, and depends on whether there existed in the room already formed complex of obligate synanthropic species of insects capable of round-the-year development. Thirdly, species composition of such a complex varies depending on the intrusion of new insect species, brought in with food stuffs, or the fact of disinsectization measures being taken. The composition of insect species can be dissimilar even in the rooms of the same house (Marchenko, Skrizhinsky, 1985).

Table 7 : Average periods of mummification of individual parts of cadaver in relation to month and biotope in USSR North-West climate conditions, days.

BIOTOPE	MONTH			
	4	5	6	9
FIELD	66.0	-	15.7	30
SWAMP	47.5	35.0	23.6	-
RAVINE	36.0	-	20.5	-
UNDERWOOD	-	-	25.0	-
AVERAGE FOR 4 BIOTOPES	49.8	35.0	21.2	30.0

Table 8 : Changes of vegetation condition in cadaver bed area : average periods, days.

TIME OF THE YEAR	NUMBER OF CADAVER	PERIOD OF GREEN COLOUR FADING TO YELLOW	COMPLETE DESTRUCTION OF PLANTS IN CADAVER BED AREA	
			After flies appeared on cadaver	After fly larvae emerged
SPRING	10	14.4	28.6	23.1
SUMMER	9	8.4	10.9	8.0
AUTUMN	17	8.7	23.8	19.0

In 12% of cases partial mummification of cadavers was observed. The average periods of cadaver parts mummification as a function of biotope and month, when the cadaver got into nature conditions, are presented in Table 7.

An insignificant formation of grave wax (in 1-1,5 years on an average) was noted in 4% of cases, more frequently on "autumnal" cadavers.

A cadaver and products of its decomposition produce both mechanical and chemical effect on vegetation at underlying ground. Mechanical effect, apart from broken plants, is manifested as the colour of plants being altered from green to different shades of yellow, which is connected with a change of plant respiratory pigments. As can be seen from Table 8, in Spring, colour changes two times more slowly than in Summer or Autumn. Chemical effect is mainly connected with the period of fly larvae development on a cadaver, because their parenteral digestion leads to an abrupt increase of liquid fraction of decomposition products, and chemical reactions rate grows due to metabolic heat release (temperature of larvae mass is 45-49°C). On the average, the chemical burning out of plants occurs by the 7th day from the beginning of larvae development. Dynamics of vegetation revival on cadaver bed is presented in Table 9. On the 2nd year, a vegetation billow is formed around the cadaver bed, which looks distinctly unlike the surrounding plants. A sort of bed "de camouflage" occurs. Revival of vegetation on the cadaver bed properly starts on the 2nd or 3rd year and ends only on the 4th year (Marchenko, Nainys, 1986).

The above data may be of importance for the determination of the season, when the cadaver "got" to the place, where it was discovered.

4. Biology of flies and their significance in forensic medicine

At the present development stage of forensic entomology, the main efforts are made to study the biology of flies. The reason for it consists in the fact that flies are the first to invade a cadaver, and this is done quickly and simultaneously, so that their species composition accurately corresponds to the degree of cadaver tissues decomposition and alters in a regular pattern during decomposition process (Kaib, 1974). Flies are predominant in quantity and consume the major parts of tissues.

Three directions of studies on necrobiont flies species can be distinguished.

1. Identification of the species composition of necrobiont flies community and of factors affecting the formation of the said composition.

This was and is successfully developed by C.F. Porta (1929), M.E. Fuller (1934), G.F. Bornemissza (1957), H.B. Reed (1958), J.A. Payne and D.A. Crossley (1966), P. Nuorteva (1972, 1977, 1987), I. Hansky (1976), I. Hanski and S. Kuusela (1977), M. Leclercq (1978), M. Leclercq and G. Brahy (1985), R.S. Putman (1978). S. Kuula and I. Nanski (1982), Ph. Vernon (1983), K.G.V. Smith (1986), A.L. Ozerov (1988). A. Ozerov has found out, while studying necrobiont flies in the woods of the southern part of the Far East of the USSR :

- necrobiont flies community comprises 128 species of 16 families and is divided into obligate necrobionts (26 species of 5 familes) and facultative necrobionts (102 species of 16 families);
- the structure of flies community depends on the weight and type of cadaver, the degree of its decomposition, season of the year, biotope condition, climatic factors;
- the variety of species reduces as cadaver weight decreases;
- periods of egg-laying into cadaver (larvae emergence) are connected with certain stages of its tissues decay and were identified for 72 species;
- flies of anthropogenetic biocenoses lay eggs into the cadaver (or larvae emerge) for a limited period of time and only once;
- the wider is the variety of species composition collected from a cadaver, the higher is the probability of obtaining data to be efficiently used for expertize.

The above findings completely comply with the results of studies of cadaver entomofauna in the North-West region of the USSR.

2) Study on the variation of larvae growth parameters at different temperatures (length of the body, weight) aimed at developing a method for determining the period of their development.

The second direction is developed in Japan (Nishida) and in Austria (Reiter). It is based on registering the length and weight of fly larvae as they grow. Thus, C. Reiter (1984a) gives data on the length variation of *Calliphora vicina r.-D.* larvae at the 6.5 - 30°C. range of temperature; the data are presented in Table 5. Based on these data he proposed an isomegalodiagram for practical application. Studies, carried out by K. Nishida et al. (1986) in Japan, on 7 fly species at different temperatures, resulted in a statistically true dependence of length and weight of larvae VS development period, which allowed to make diagnostic tables. Independently of each other, scientists point out that the

increase of length and weight of larvae changes to their decrease from the moment feeding is over prior to pupation. Development at temperatures higher than the optimum ones leads to an increased duration of development, delayed pupation or larvae death. In the study of flies development on dead bodies, a process of larvae mass self-heating was observed owing to metabolic heat release and results in an up to 50% shortening of the development period in a certain part of pre-imago stages compared to the time predicted according to the environment temperature data (Marchenko, 1973, 1975, 1988; Zvereva, Marchenko, 1987). The method proposed by C. Reiter and K. Nishida attracts by its simplicity, but requires thorough practical testing, especially in connection with the above observations. It is effective only till the pupation moment, which is a significant disadvantage. From the above paper by K. Nishida et al. (1986) one can utilize data on development periods of pre-imaginal stages and how they correspond to each other on cadavers.

3. Determination of thermal parameter values regulating insects development, to be further used in calculation technique for retrospective detrmination of the time of insect invasion into a cadaver.

The third direction is being successfully developped by P. Nuorteva (1977), M. Leclercq (1978), K.G.V. Smith (1986) and F.J. Introna et al. (1987). The direction is being developed in the USSR and comprises a calculation technique for retrospective determination of the time, when insects started to develop on a cadaver, this technique being based on thermal parameters regulating insect development (Marchenko, 1973, 1975, 1978, 1988, 1989); Marchenko, Vinogradova, 1984; Nainys, Marchenko, Kazak, 1982).

Beginning of Summer activity and reproduction of flies is predominantly regulated by temperature. In a moderate climatic zone fly reproduction starts in Spring when twenty-four hours temperature is stabilized at 10-15° level and ends in Autum, when temperature drops below 10-13°C. (Vinogradova, Marchenko, 1984). Thermal parameters regulating the development of 9 fly species are given in Table 10; their meaning is considered in the next section (Marchenko, 1988). For the species cited in Table 10 and for *Lucilia sericata Mg.*, there were calculated development periods from egg to puparium and to imago emergence an hourly development indices at temperatures from 11° to 30° C., which are presented in Table 11. Egg development period for *Calliphora vicina R.-D.*, according to C. Reiter (1984A), is 2.83 days at 11°C., 1.21 days at 15°C., 0.95 days

Table 9 : Periods of cadaver bed vegetation restitution

CHARACTER OF REVIVED VEGETATION	NB OF STUDIED CADAVER BED	PERIOD OF VEGETATION REVIVAL, years 1	2	3	4
Grass around cadaver bed	36	9	26	33*	no observation
Formation of vegetation billow around cadaver	36	4	15	21	no observation
Grass in cadaver bed area :	36				
complete restitution		0	2	6	12
partial revival		0	22	30	24
no revival		36	2	0	0
Moss in cadaver bed area	6				
complete restitution		0	0	0	4
partial revival		0	3	5	2
no revival		6	3	1	0

* In 3 cases, no grass spreading around cadaver bed was observed.

Table 10 : Thermal parameters (°C), regulating necrobiont fly development

N	FLY SPECIES	LOWER DEVELOPMENT THRESHOLD	HEAT CONSTANT OF SPECIES	SUM OF EFFECTIVE TEMPERATURES TO DEVELOP FROM EGG TO PUPARIUM
1	Calliphora vicina R.-D.	2.0	388.0	191.0
2	Calliphora vomitaria L.	3.0	472.0	213.0
3	Protophormia terranovae R.-D.	7.8	251.0	191.0
4	Lucilia sericata Mg*	9.0	207.0	-
5	Chrysomya albiceps Wd.	10.2	186.0	123.0
6	Phormia regina Mg	11.4	148.0	101.0
7	Muscina stabulans Fll.	7.2	269.0	139.0
8	Muscina assimilis Fll.	7.9	240.0	102.0
9	Boettcherisca septentrionalis Rohd.	7.8	279.0	117.0
10	Piophila foveolata Mg.	6.4	434.0	278.0

* according to I.V. Koshanchikov, 1961

Table 11 : Development periods (days) and nourly indices of fly development from egg to puparium and from egg to imago emergence.

DEVELO-PMENT TEMP. °C	DEVELOP. PERIOD FROM EGG TO : PUPARIUM	IMAGO	INDEX x 10^{-4}	DEVELOP.PERIOD FROM EGG TO : PUPARIUM	IMAGO	INDEX x 10^{-4}
	PROTOPHORMIA TERRAENOVAE			CALLIPHORA VICINA		
11	50.0	78.4	5.312	21.2	43.1	9.664
12	38.1	59.8	6.972	19.1	38.8	10.738
13	30.8	48.3	8.632	17.4	35.3	11.812
14	25.8	40.5	10.293	15.9	32.3	12.886
15	22.2	34.9	11.952	14.7	29.8	13.960
16	19.5	30.6	13.616	13.6	27.7	15.034
17	17.4	27.3	15.273	12.7	25.9	16.108
18	15.7	24.6	16.937	11.9	24.3	17.182
19	14.3	22.4	18.952	11.2	22.9	18.256
20	13.1	20.6	20.256	10.6	21.6	19.329
21	12.1	19.0	21.918	10.0	20.4	20.403
22	11.3	17.7	23.580	9.6	19.4	21.477
23	10.5	16.5	25.237	9.1	18.5	22.551
24	9.9	15.5	26.899	8.7	17.6	23.626
25	9.3	14.6	28.588	8.3	16.9	24.699
26	8.8	13.8	30.215	7.9	16.2	25.773
27	8.3	13.1	31.879	7.6	15.5	26.847
28	7.9	12.4	33.548	7.3	14.9	27.920
29	7.5	11.8	35.192	larvae death		
30	7.2	11.3	36.873			
	CHRYSOMYA ALBICEPS			PHORMIA REGINA		
11	larvae death			lower development threshold		
12	"			168.3	246.6	1.689
13	43.9	66.3	6.289	63.1	92.5	4.504
14	32.4	48.8	8.535	38.8	57.0	7.319
15	25.6	38.6	10.781	28.0	41.1	10.135
16	21.2	32.0	13.027	22.0	32.2	12.950
17	18.1	27.3	15.273	18.0	26.4	15.785
18	15.8	23.8	17.520	15.3	22.4	18.581
19	14.0	21.1	19.766	13.3	19.5	22.804
20	12.6	18.9	22.012	11.7	17.2	24.211
21	11.4	17.2	24.258	10.5	15.4	27.027
22	10.4	15.7	26.504	9.5	13.9	29.842
23	9.6	14.5	28.750	8.7	12.7	32.657
24	8.9	13.4	30.997	8.0	11.7	35.472
25	8.3	12.5	33.243	7.4	10.9	38.288
26	7.8	11.7	35.489	6.9	10.1	41.103
27	7.3	11.0	37.735	6.5	9.5	43.918
28	6.9	10.4	39.981	6.1	8.9	46.734
29	6.5	9.8	42.227	5.7	8.4	49.549
30	6.2	9.4	44.474	5.4	7.9	52.364

Continuation Table 11 : Development periods (days) and hourly indices of fly development from egg to puparium and from egg to imago emergence.

DEVELO--PMENT TEMP. °C	DEVELOP. PERIOD FROM EGG TO : PUPARIUM	IMAGO	INDEX x 10^{-4}	DEVELOP.PERIOD FROM EGG TO : PUPARIUM	IMAGO	INDEX x 10^{-4}
	CALLIPHORA VOMITARIA			LUCILIA SERICATA		
11	26.6	59.0	7.062	103.5		4.025
12	23.7	52.4	7.944	69.0		6.038
13	21.3	47.2	8.826	51.8		8.051
14	19.4	42.9	9.710	41.4		10.064
15	17.7	39.3	10.593	34.5		12.077
16	16.4	36.3	11.475	29.6		14.090
17	15.2	33.7	12.358	25.9		16.103
18	14.2	31.5	13.241	23.0		18.111
19	13.3	29.5	14.124	20.7		20.128
20	12.5	27.8	15.007	18.8		22.141
21	11.8	26.2	15.889	17.3		24.154
22	11.2	24.8	16.772	15.9		26.167
23	10.6	23.6	17.655	14.8		28.180
24	10.1	22.5	18.538	13.8		30.193
25	9.7	21.5	19.420	12.9		32.206
26	9.3	20.5	20.303	12.2		34.219
27	puparium death			11.5		36.231
28				10.9		38.244
29				10.4		40.257
30				9.9		42.270
	MUSCINA ASSIMILIS			MUSCINA STABULANS		
..11	32.9	77.4	5.381	larvae death		
..12	24.9	58.3	7.118			
..13	20.0	47.1	8.854	24.0	46.4	8.983
..14	16.7	39.3	10.590	20.4	39.5	10.532
..15	14.4	33.8	12.326	17.8	34.5	12.081
..16	12.6	29.6	14.062	15.8	30.6	13.630
..17	11.2	26.4	15.798	14.2	27.4	15.179
..18	10.1	23.8	17.534	12.9	24.9	16.728
..19	9.2	21.6	19.270	11.8	22.8	18.277
..20	8.4	19.8	21.006	10.4	21.0	19.841
..21	7.8	18.3	22.743	10.1	19.5	21.375
..22	7.2	17.0	24.479	9.4	18.2	22.924
..23	6.7	15.9	26.215	8.8	17.0	24.473
..24	6.3	14.9	27.951	8.3	16.0	26.022
..25	6.0	14.0	29.687	7.8	15.1	27.571
..26	5.6	13.3	31.423	7.4	14.3	29.120
..27	5.3	12.6	33.159	7.2	13.6	30.669
..28	5.1	11.9	34.895	6.7	12.9	32.218
..29	4.8	11.4	36.631	6.3	12.3	33.767
..30	4.6	10.9	38.368	6.1	11.8	35.315

Continuation Table 11 : Development periods (days) and hourly indices of fly development from egg to puparium and from egg to imago emergence.

DEVELO--PMENT TEMP. °C	DEVELOP. PERIOD FROM EGG TO : PUPARIUM	IMAGO	INDEX x 10^{-4}	DEVELOP.PERIOD FROM EGG TO : PUPARIUM	IMAGO	INDEX x 10^{-4}
	BOETTCHERISCA SEPTENTRIONALIS			PIOPHILA FOVEOLATA		
11	36.6	103.3	4.032	diapause		
12	27.9	75.4	5.525			
13	22.5	59.4	7.019			
14	18.9	49.0	8.512			
15	16.3	41.6	10.016			
16	14.3	36.2	11.499			
17	12.7	32.0	12.992	diapause		
18	11.5	28.8	14.482	24.0	37.4	11.136
19	10.4	26.1	15.979	22.1	34.4	12.096
20	9.6	23.8	17.473	20.4	31.9	13.056
21	8.9	22.0	18.966	19.0	29.7	14.016
22	8.2	20.4	20.459	17.8	27.8	14.976
23	7.7	19.0	21.953	16.8	26.1	15.937
24	7.2	17.8	23.446	15.8	24.7	16.897
25	6.8	16.7	24.940	15.4	23.4	17.818
26	6.4	15.8	26.433	14.2	22.1	18.817
27	6.1	14.9	27.927	13.5	21.1	19.777
28	5.8	14.2	29.420	12.9	20.1	20.737
29	5.5	13.5	30.913	12.3	19.2	21.697
30	5.3	12.9	32.407	11.8	18.4	22.657

at 19°C. and 0.86 days at 22.5°C. In the article by Marchenko (1980b) data on development periods at constant temperatures for another 8 fly species are given.

In the study of *Protophormia terraenovae R.-D.* development on dead bodies, a process of larvae mass self-heating was observed owing to metabolic heat release.

While studying cadaver decomposition by insects under natural conditions of the North-West European part of the USSR in 1971, thermometry of 17 cadavers (256 measurements) confirmed, that temperature increases to 45°C. in cadaver tissues (Marchenko, 1973). It was established that this fact is connected with the development of fly larvae mass. The fact was already described in the literature (Reed, 1958; Payne, 1965). For all the big animals (6 subjects) and for part of medium-size ones (5 subjects), temperature increase to 18-45°C. was registered inside the cadavers in the mass of fly larvae, air temperature being 4-15°C. The measurements were made at 9-11 h. pm when

Table 12 : Temperature characteristic of fly larvae development on cadaver (biotope - hill slope)

DATA	AIR TEMPERATURE / CADAVER TEMPERATURE				
	HOUR WHEN TEMPERATURE WAS MEASURED				
	7 am	11 am	2 pm	7 pm	10 pm
MAY					
3	0.5/ 6.0	-	-	3.0/ 7.0	-
4	5.5/ 4.0	11.5/ 4.5	11.0/ 9.0	10.0/10.5	4.5/ 9.0
5	4.5/ 7.0	8.5/ 9.5	9.5/ 8.0	9.0/ 9.5	7.0/ 9.0
6	5.5/ 5.0	11.5/ 6.0	10.0/11.5	8.0/12.5	7.0/10.0
7	8.0/ 9.5	-	17.0/11.5	-	3.5/ 7.0
8	15.0/11.0	-	11.0/14.0	-	12.0/10.0
9	11.0/13.5	18.0/16.0	17.0/19.0	14.0/19.0	10.5/16.0
10	8.0/12.0	12.0/12.0	12.0/12.0	9.0/13.0	10.0/13.5
11	8.5/ 6.5	9.0/ 7.0	9.0/ 7.0	8.0/17.0	6.5/ 9.5
12	4.0/17.0	9.0/19.0	9.0/19.0	8.5/17.0	5.0/15.5
13	2.0/ 7.0	7.5/11.5	7.5/11.5	7.0/14.0	3.5/10.0
14	9.0/31.0	14.0/39.0	14.0/39.0	8.5/44.0	5.0/18.0
15	7.0/31.5	13.0/45.0	13.0/45.0	15.5/43.5	-
16	9.0/38.0	7.5/40.0	7.5/40.0	9.5/44.0	-
17	-	15.5/40.0	15.5/40.0	-	8.0/44.5
18	8.5/37.5	14.0/41.0	14.0/41.0	12.0/40.0	-
19	10.0/30.0	13.5/36.0	13.5/36.0	14.5/39.0	7.5/39.5
20	-	15.5/40.0	15.5/40.0	-	8.0/38.0
21	-	3.0/25.0	3.0/25.0	2.0/11.0	-
22	1.0/ 6.0	3.0/ 6.0	3.0/ 6.0	4.0/ 7.0	0.0/ 8.0
23	2.0/ 6.0	11.0/11.0	11.0/11.0	10.0/10.0	2.0/ 7.0
24	7.0/10.0	12.0/12.0	12.0/12.0	13.0/14.5	8.0/ 9.0

any heating due to direct solar radiation was excluded. Temperature range at cadavers surface was 4-13°C.

In 1980 the ascertained fact was the subject of a special study on 39 dog cadavers. 4216 measurements of their temperature 5 times per day (7.00, 11.00, 14.00, 19.00 and 22.00) were made, air temperature was registered at the same time. In Table 12 results of measurements on one subject are presented as examples.

According to the observation of temperature conditions of 21 cadavers during Spring-Summer season, maximum temperature in the mass of developing *Protophormia terraenovae* larvae amounted to 30-49°C.

In Spring self-heating of feeding larvae mass was distinguished by a great stability in cadavers located on soil surface (variation factor of maximum temperatures CV = 6.0%) if compared to hanged cadavers (CV = 16.4%) for which heat exchange was intensified to turbulence effects. This is reflected by a statistically true difference (P = 0.999) of average maximum temperatures : for cadavers on the ground they are 44.4°C, for hanged ones - 36°C (Table 13, lines 1 and 2).

Table 13 : Characteristic of average maximum temperatures stability in the mass of fly larvae living on cadaver as a function of season and cadaver location

N	n	X	o	CV
1	12	44.4	2.67	6.0
2	4	36.0	5.89	16.4
3	4	45.5	2.52	5.5
4	16	29.6	6.82	23.0

Note : *N1 : Spring, cadavers are on soil surface,*
N2 : Spring, hanged cadavers,
N3 : Summer, cadavers on soil surface,
N4 : Autumn, cadavers on soil surface.

Summer group of 4 cadavers lying on the soil surface vas characterized by average maximum temperature 45.5°C (CV = 5.5%). No statistically true difference between maximum temperatures of Spring and Summer cadavers was found (P = 0.788), which is due to predominance of the same larvae species, namely *Pr. Terraenovae*. Maximum temperature level is attained 2 or 3 days after larvae emergence : it keeps up for 6 to 20 days and then decreases in 2-4 days. Average time period during which temperature in the larvae mass was maintained at the highest level varied from 18.2 days (in Spring) to 12.0 days (in Summer) for the cadavers on the soil surface and 12.7 days (in Spring) for hanged cadavers, evidencing great instability in all the above cases (CV - 20.3%, 11.7% and 21.2% respectively).

Of 18 cadavers observed in Autumn, selfheating effect was registered on 16 cadavers lying on the ground; this process was not noticed for 2 hanged cadavers. At the time mainly *Calliphora vomitoria* and *Calliphora vicina* developed on cadavers; average maximum temperature equal to 29.6°C was lower than in the case of *Pr. terraenovae* larvae during Spring-Summer period. Truth of difference between Autumn group and Spring & Summer groups is 1.0. Instability of Autumn self-heating process was found to be the highest - CV = 23%. This is accounted for by fly species peculiarities and also by the fact that in Spring there is a constant increase of solar radiation intensity and air temperature, while in Autumn they decrease.

The time of expected fly emergence was calculated for 45 dead bodies based on air temperature data and then compared to actual values. In all cases flies started to emerge before the calculated time. In 21 of 26 cases under study the maximum peak of flies emergence also came earlier than expected. The greatest acceleration of development is observed on open land, the lowest one - at closed, shadowy places and especially on hanged cadavers due to higher heat transfer. The intensity of heat release depends on the season, biotope and conditions of cadaver heat exchange with the environment. In Nature, the warming up of the mass of feeding larvae helps to maintain a favourable microclimate for their development and supports their survival during temporary periods when environment temperature drops to 0°C; it reduces by up to 50% the development period of a part of preimagal stages compared to the period predicted based on environment temperature only.

The regularities of the process of warming up, taking place in the mass of feeding fly larvae, were establised for 7 fly species, of Calliphoridae, Muscidae, Sarcophagidae and Piophilidae families. For *Protophormia terraenovae R.-D.*, larvae environment temperature value of 10°C was determined, which is the starting point of food substrate heating process. In the study of *Chrysomya albiceps Wd.* and *Protophormia terraenovae R.-D.* development in laboratory conditions, the sums of effective temperatures which larvae could have by the moment of pupation, were calculated.

When calculation was made according to ambient air temperature, the sum of effective temperatures for the said species amounted to 87°C and 81°C respectively. The same parameter determined from larvae mass temperature was 112°C and 178°C respectively. The later values are closer to the sum of effective temperatures needed for development from egg to pupation. So *Chrysomya albiceps Wd.* requires 123°C (we have 112°C), and *Protophormia terraenovae R.D.*

requires 191°C (we have 178°C). Consequently, practical application of the method for retrospectively determining the time of insects settling in a cadaver based on air temperature alone may result in certain errors.

Experiments carried out on *Chrysomya albiceps Wd.*, *Protophormia terraenovae R.-D.* and *Musca domestica L.* have shown a direct connection between the rate of temperature increase in the mass of feeding larvae and their quantity, age and conditions of heat exchange with the environment. It was also confirmed that larvae development is speeded up due to metabolic heat release (Zvereva, 1984; Marchenko, 1985, 1988; Zvereva, Marchenko, 1987).

Presented data on necrobiont flies biology will be useful to make a correct evaluation of their development in each particular case.

5. Method of retrospective determination of the starting moment of insects development on a cadaver.

Prior to discussing the method definitions explaining the meaning of individual thermal parameters regulating insects development periods will be gven.

Lower development threshold is the lowermost limit value of environment temperature, above which a progressive development of species becomes possible. The threshold value is specific for each species. At temperatures below this threshold value cold torpidity is observed till certain limit, then the insects die.

Effective temperature is the temperature value which directly involves progressive development of species. At each specific time interval for a given species it is the difference between the environment temperature value and the lower threshold value of this particular species. As a rule, average twenty-four-hour effective temperature is used.

Heat constant of species is the sum of effective temperatures a species needs to develop from egg to emergence of adult insect (imago). The value of heat constant of species, as well as lower threshold of development is specific for each species and is not changed depending on the geographical region. heat

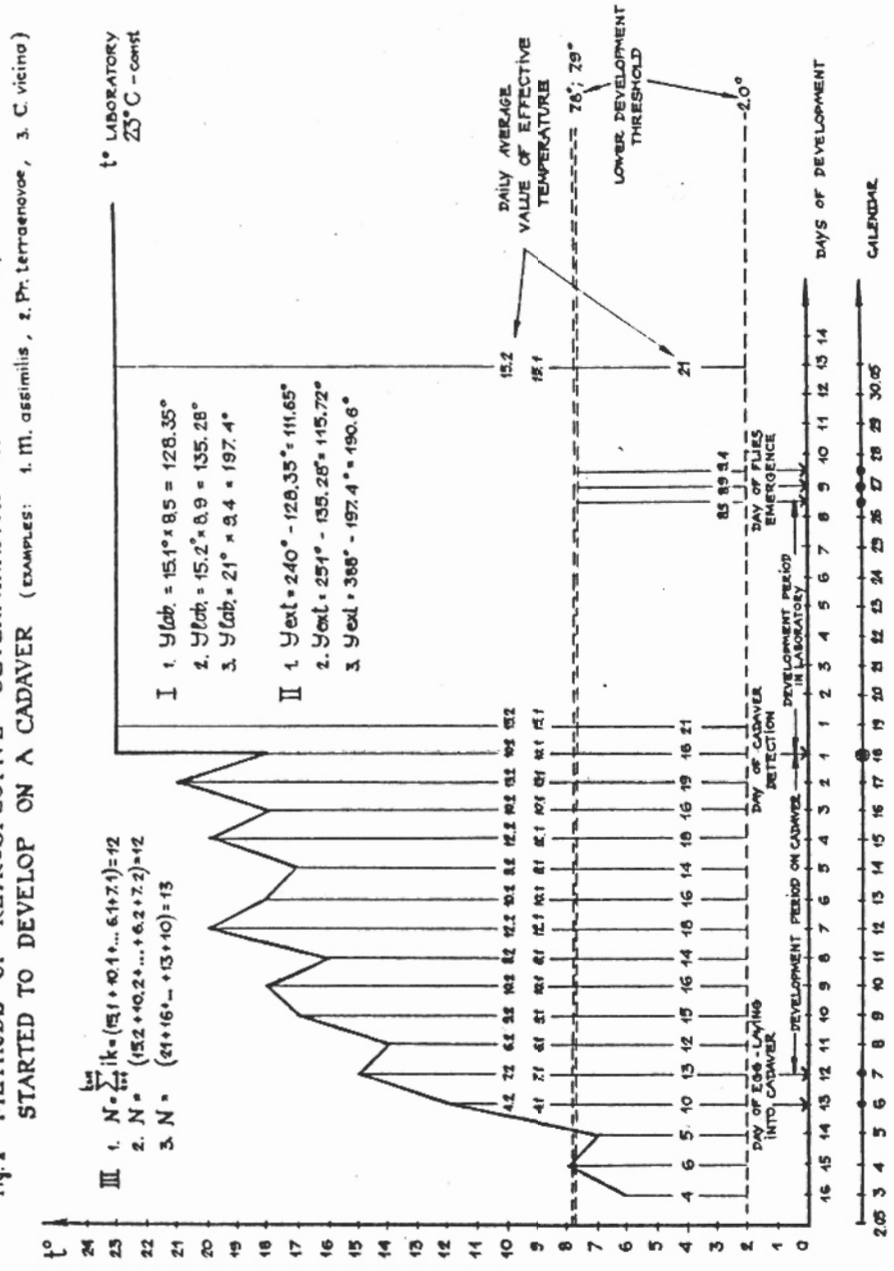

Fig. 1 METHODS OF RETROSPECTIVE DETERMINATION OF THE DATE, WHEN INSECTS STARTED TO DEVELOP ON A CADAVER (EXAMPLES: 1. M. assimilis, 2. Ph. terraenovae, 3. C. vicina)

constant of species governs the development period under specific conditions of environment and area of this species.

The sum of effective temperatures necessary for the development from egg to pupa (puparium) is a constant value for each species, exactly like the heat constant is.

Development index is that part of development which a species undergoes per unit of time (hour, day) at a given temperature. Development from egg to imago is conventionally assumed to be a unit. Development index is determined for a specific temperature value by dividing the unit into development period at given temperature value, which remained constant during the total development period from egg to imago. Thus, the development index is a specific value for each species, varying with temperature value. When development indices are applied, calculations of effective temperatures can be avoided when determining species development period under specific conditions.

The parameters listed above led to development of a method of retrospective determination of the moment, when insects started to develop on a cadaver, which is based on the said development indices and effective temperatures, and to adopt it for practical application in forensic medicine. The essence of the method consists in breeding pre-imaginal insects stages found on a cadaver till imago emergence in the laboratory. If "grown up" insects, emerged from pupae, are found on a cadaver, one can directly start from point 3 of calculations (see below). The same is done when pupae (puparii) of those species are found, for which the life cycle is well studied. Having determined experimentally the stage of development in the laboratory, the stage of development on a cadaver before the insects were collected from it is identified. From temperatures observed in nature during the days preceeding the discovery of the cadaver, the probable date of a given species invasion into the cadaver is determined by fairly simple calculations.

Initial data to make calculations using thermal parameters for *Calliphora vicina R.-D.*, *Protophormia terraenovae R.-D.* and *Muscina assimilis Dlln.* are given in Fig. 1. In the same Fig., an example of calculations using effective temperatures is presented. The procedure of calculations with development index is given below.

1. The stage of development undergone by the insects in laboratory is determined ($Y_{lab.}$)

a) at constant temperature in laboratory : $Y_{lab.} = it$, where :

i - development index, t - development time, hours, days

$Y_{lab.1} = 0.062916 \times 8.5 = 0.534786$,
where 0.062916 is twenty-four-hours development index for M. assimilis at 23°C;

$Y_{lab.2} = 0.0605688 \times 8.9 = 0.53906232$,
where 0.0605688 is twenty-four-hours development index for Pr. terraenovae at 23°C;

$Y_{lab.3} = 0.0541224 \times 9.4 = 0.50875056$,
where 0.0541224 is twenty-four-hours development index for C. vicina at 23°C;

b) at varying temperature in the laboratory, the calculations are made in the same way, as in point 3 :

$$Y_{lab.} = \sum_{j=1}^{n} ij,$$

where j is time (hours, days).

2. The stage of development which took place on the cadaver is determined ($Y_{ext.}$).

$Y_{ext.} = 1 - Y_{lab.}$
$Y_{ext.} = 1 - 0.0534786 = 0.465214;$
$Y_{ext.} = 1 - 0.53906232 = 0.4609377;$
$Y_{ext.} = 1 - 0.50875056 = 0.4912494.$

3. The duration of insect development on a cadaver is determined (N).

$$N = \sum_{K=1}^{K=n} iK = (i_1 + i_2 + i_3 = \ldots + i_n)$$

where K is the number of sum members corresponding to the time of development on the cadaver; $(i_1 + i_2 + i_3 = \ldots + i_n)$ respective values of twenty-four-hour temperatures of development index.

$N_1 = (0.0420816 + 0.0379152 + 0.0420816 + 0.0504144 + 0.0379152 + 0.0420816 + 0.0504144 + 0.0337488 + 0.0295824 + 0.0379152 + 0.025416 + 0.0295824 + 0.17832 = 0.476232) = 13$ days;

$N_2 = (0.0406488 + 0.0366552 + 0.0406488 + 0.0486144 + 0.0366552 + 0.0406488 + 0.0486144 + 0.0326784 + 0.0286848 + 0.0366552 + 0.0247032 + 0.0286848 + 0.0167328 = 0.4606248) = 13$ days;

$N_3 = (0.0412368 + 0.0386592 + 0.0412368 + 0.0463896 + 0.0386592 + 0.0412368 + 0.0463896 + 0.0360816 + 0.033504 + 0.0386592 + 0.0309264 + 0.033504 + 0.0257712 = 0.4922544) = 13$ days.

4. The calender date when insects started to develop on a cadaver is determined.

In our example it is May 6-7. The given example of two calculation alternatives proves that the method itself is sufficiently simple and does not require special training of a medico-legal expert, whereas the determination of insect species is a fairly complicated task calling for serious training and practical skills.

The proposed method was experimentally checked through solving "blind" problems, based on the temperature parameters of fly development. First, 9 problems to determine probable emergence data were solved for *Pr. terraenovae and C. vicina*, egg-laying dates, the periods of retention in each temperature condition during the development being known. From the calculation results presented in Table 14, in 5 cases the date in summer was determined precisely, in other cases the deviation amounted to +/- 2 days.

Table 14 : Results of testing predection accuracy for dates of Calliphoridae fly emergence

NN	ACTUAL DATA					CALCULATED DATA	
N	T° AT THE START OF - DEVEL. (1)	DATE EGGS WERE LAID (°C)	EMER-. GENCE DATE OF FLIES (2)	ACTUAL DEVEL. PERIOD, (days)	DATA OF FLY EMER-GENCE	DEVIA-TION OF CALCULATED DATA FROM ACTUAL DATA (days)	
				PROTOPHORMIA TERRAENOVAE			
1	4	14.5	12.03	10.04	30	10.04	0
2	4	14.5	12.03	11.04	31	10.04	1
3	4	14.5	12.03	12.04	32	10.04	2
4	4	20.0	16.03	17.04	33	17.04	0
5	4	20.0	21.04	27-28.05	37-38	25.05	-2
6	4	20.0	23.04	27-28.05	35-36	27.05	0
7	4	20.0	28.04	2.06	36	31.05	-2
				CALLIPHORA VICINA			
8	2	20.0	28.03	14.04	18	14.04	0
9	3	20.0	28.03	17-18.04	21-22	18.04	0

1. Number of changes of temperature condition (number of rearrangements)
2. Start of development.

Statistical evaluation of the difference between the actual period of fly development and the theoretically calculated one was made through the application of X^2 correspondence criterion. In 9 tests $X^2 = 0.31$ ($P = 1.0$). It means that practically complete compliance is observed between the actual data on development duration and their theoretical prediction.

Later on, the task became more complicated. The experimentor received from the laboratory personnel data on starting date and mass ending of fly emergence, and also data on dates and temperature conditions variation, which changed at random simulating conditions of natural environment. The aim was to determine egg-laying date. Altogether 35 problems were solved for three fly species : *Chrysomya albiceps, Protophormia terraenovae, Calliphora vicina* (Calliphoridae).

Results obtained from solving the problems were statistically assessed using X^2 criterion. Accuracy evaluation of the method for retrospective determination of the starting date of 3 fly species development is given in Table 15.

Table 15 : Evaluation of accuracy of method for retrospective determination of starting date of calliphoridae development under laboratory conditions using chi-squared criterion.

N	FLY SPECIES	CALCULAT. MADE BASED ON START/MASS EMERGENCE	Nb OF CALCULA TIONS	CORRES- PONDENCE CRITER.	PROBABILI-LITY OF GIVEN VALUE
			n	X_2	P_{X^2}
1	Chrysomya albiceps	start	8	1.47	0.99
		mass	6	0.1	1.0
2	Protophormia terraenovae	start	6	1.18	0.95
		mass	9	0.95	1.0
3	Calliphora vicina	start	10	2.10	0.99
		mass	17	1.88	1.0
4	Total for the three species	start	24	4.75	1.0
		mass	32	2.93	1.0

Correctness of the proposed method is fully confirmed for all three species ($P = 1.0$). A slightly lower prbability of X^2 criterion ($P = 0.95$) was obtained for *Pr. terraenovae* when calculations were made based on the beginning of emergence. on the whole, for the three species, X^2 criterion probability is 1.0 if calculated from the beginning of emergence. To avoid any possible inaccuracies, practical calculations should be made for all the species found on a cadaver.

The result obtained using the proposed method corresponds to the maximum possible development time of a species under specific conditions. The development can be accelerated by metabolic heat release in the course of fly larvae development.

The time when insects started to develop on a cadaver does not always coincide with the time of death, just as the place where a cadaver was discovered is not always the one where death occurred.

6. Methods of making entomological and botanic studies while examining the place of cadaver detection

Successful application of entomological data in the investigation practice depends on the correct collection of insects on the place where the cadaver was found, and their timely delivery alive for entomological study. Only a qualified expert can properly and reliably collect cadaver fauna at the discovery place and properly register parameters influencing the vital activities of insects. To accomplish this task, an investigator may cooperate with an entomologist from a local sanitary and epidemiological inspection unit (in USSR) or with a medico-legal expert, participating in the examination of the cadaver and the place where it was found.

To collect and deliver insects to the laboratory one should prepare beforehand a set of tools (pincers, spoons, spatulas, compass, thermometer or better a thermograph) and containers, designed to transport live insects. Containers should meet stringent requirements. They should be glass or plastic jars (cases) sealed with perforated lids of lids having fine-mesh metallic wires, or covered with dense cloth, which can be secured to the neck by rubber rings. It is strictly forbidden to use polyethylene bags or rubber instead of cloth. A jar should be filled half-height with sawdust or sand.

In the course of cadaver examination, it is necessary to collect as many species of insects and other living organisms as possible from different spots, noting their precise location on the cadaver remains and not only the area should be taken into consideration, but also the depth from which they they were taken.

Special attention will be paid to collecting fly life stages, as it is the earliest, most numerous and well-studied component of cadaver entomofauna. Flies are caught by a conventional chemical test tube, a sitting insect is covered with it. Several flies can be put into the same test tube with cotton wool interlayers between. Young flies just emerged from puparii are necessarily collected separately. They differ from the "grown-up" ones by folded or yet not completely unfolded wings of, if the wings are already unfolded, by a whitish shade and tendency to fly. Huge armies of coloured flies on cadaver remains and adjacent objects point to recent mass emergence of flies from puparii; some 20-30 of such flies should be caught. Young flies are to be placed separately from the mature ones and a label accurately specifying the date and hour of collection is to be provided.

Laid eggs will be transported separately in jars on a piece of nutrient substrate or wet soil, to prevent their drying.

Fly larvae will be taken from different spots on the cadaver, since they tend to be grounded species to species, and all age groups will be collected which can be visually distinguised by their length and thickness of body. Eggs and larvae are delivered to the laboratory alive, so that flies can be raised from them. This helps to identify species and is an obligatory component of calculation technique discussed earlier. In parallels, a portion of larvae is preserved in 70° ethyl alcohol; formaline is undesirable.

Puparii (pupae), just as their chitine cuticles are taken from different parts of the cadaver after fly emergence and also from the ground under the cadaver (pieces of furniture, slits in the floor etc.), since larvae of the majority of fly species pupate in the soil with the exception of *Protophormia terraenovae R.-D.* and small species of Phoridae and Piophilidae families; and are grouped according to size and resemblance in appearance. They are transported in jars on slightly wet sawdust or sand. In the laboratory puparii are raised to flies.

It is important to fix the interrelation between different life stages of flies on cadavers, because this may prove to be helpful in determining the multiplicity of fly invasion, which is especially essential when a suspicion exists of cadaver habing been moved.

Each sample of fly life stages should amount to at least 30-50 items from different spots on the cadaver itself, cadaver bed and bed soil at 25-30 cm depth. In building, samples from pieces of furniture and slits in the floor will be taken.

Beetles are collected with pincers, then killed by ether and put into test tubes apart from other insects. If beetles or their larvae are not killed, then each specimen should be put into separate jars or test tubes with a cork plug permeable to air (Beetles gnaw through the cloth). When samples are taken from a mummidied cadaver, especially when *Dermestidae* are present or their larvae (covered with thick bristles (hairs), one should remember that they are perniciously affected by rotten meat and moisture. Nutrient substrate should be dried. In the dust around a mummified cadaver large amounts of very small insects (1-1,5 mm) can be found, therefore, samples should be also taken, closed tightly and delivered to entomologists for study.

Beetle pupae found in the ground under a cadaver at depths of 30-40 cm are distributed between the jars according to places where they were taken and

powdered with soil. A portion of larvae and pupae specimens is fixed in alcohol. If it is impossible to collect insects from soil during examination, 3-4 soil samples 0.3 x 0.3 x 0.4 m in size should be taken for further treatment in the laboratory.

It is necessary to preserve part of the collected insects in order to carry out studies, if some insects taken alive died in the course of transportation or wrong handling in the laboratory. Jars containing live insects are not to be exposed to direct sunrays or left near sources of heat.

During the examination, interrelation between insect location and clothes position, their dirty patches and damages, zones of cadaver tissue injuries should be recorded. If individual parts of cadaver show some kind of preservation condition, factors entailing this type of should be determined at the place, since they may affect the vital activity of insects.

When inspecting the cadaver bed in natural environment, it is advisable along with insect collection to pay attention to vegetation conditions. The protocol of inspection of the incident place should contain the distinctions of cadaver bed vegetation from that around it (species composition, colour, density, shoot richness), presence of area with dead plants and relation to cadaver projection and to released cadaver decay products and fly larvae location, revival of vegetation on the cadaver bed. Samples of plants and their remains are fixed between strong paper sheets or flooded with 96° ethyl alcohol. Branches and grass used to hide the cadaver should be picked up as well.

Attention is to be paid to the character of cadaver damages by rodents and birds, or whether it was torn asunder by bigger animals.

Examination of the place is suplemented by a more detailed characteristic of locality, which includes : description of vegetable and animal worlds; soils, relief, water courses, living and service houses, evaluation of slight conditions, especially illumination with direct sunrays, and light variation during the day, humidity, regime of temperatures, particularly in the layer near the ground, cadaver tissue temperature (fly larvae mass), soil water level and its probable variation, a possibility of cadaver location being flooded with rain or melted snow, probable snow-retention time.

If a room is the place where death occurred, it is necessary to ensure at least a twenty-four-hour fixation of air temperature by means of thermograph, condition of windows and air vents (open-closed) should be noted, presence of

ventilation openings, smoke stacks, refuse shafts and also the distance between cadaver and heat sources.

While working at the place where cadaver was found, it is advisable to find an explanation for all the distinctions between the development of cadaver fauna and that which would be expected according to the characteristics of locality, meteorological conditions, season and cadaver tissue states.

Decision to make entomological expertise should be taken right at the incident place and collected material should be sent to the entomologist by special delivery. Any delay in delivery can render the entomological study a useless task. Formal warrant approving such expertise may be sent later.

Table 16 : Intervals of necrobiont fly development periods vs. environment temperature relationship, days.

DEVELOPMENT TEMPERATURE 0°	DEVELOPPEMENT PERIOD FROM EGG TO PUPARIUM	FROM EGG TO FLY EMERGENCE
11	21.2-50.0	43.1-103.5
12	19.1-38.1	38.8-69.0
13	17.4-30.8	35.3-51.8
14	15.9-25.8	32.3-42.9
15	14.7-22.2	29.8-39.3
16	13.6-19.5	27.7-36.3
17	12.7-17.4	25.9-33.7
18	11.9-15.7	23.0-31.5
19	11.2-14.3	20.7-29.5
20	10.6-13.1	18.8-27.8
21	10.0-12.1	17.3-26.2
22	9.6-11.3	15.9-24.8
23	9.1-10.6	14.8-23.6
24	8.7-10.1	13.8-22.5
25	8.3- 9.7	12.9-21.5
26	7.9- 9.3	12.2-20.5
27	7.6- 8.3	11.5-15.5
28	7.3- 7.9	10.9-14.9
29	7.5.	10.4-11.8
30	7.2.	9.9-11.3

Moreover, an entomologist should receive : a warrant, a copy of the protocol of incident place inspection, a copy of incident place layout

photographs, a copy of the medico-legal autopsy in the morgue (not a medico-legal conclusive statement), hourly meteorological reports for 1-2 months preceding the date of cadaver discovery (temperature, humidity, precipitations, time of sunrise and sunset), temperature measurements in the building.

To plan investigation activities before the results of the entomological expertise are obtained, the following averaged periods of fly development on a cadaver can be used (Table 16).

Conclusion

Using the results of entomological expertise one should not forget that the time when insects started to develop on a cadaver does not necessarily coincide with the moment of death, and the place where cadaver was found may not be the one where death occurred. It is inadmissible to make conclusoins about the time of death, based on the degree of cadaver tissue decomposition or cadaver skeletization.

Entomological studies are the base for solving the following problems :

a) to determine the season when cadaver "got" to the place where it was found;
b) to identify the time of death or the time the cadaver "got" to place where it was discovered;
c) to establish the fact of the cadaver being moved;
d) to identify the initial location of the cadaver or the place where it was hidden (by means of investigational simulation on experiment).

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EVOLUTION ET ETAT ACTUEL DE L'ENTOMOLOGIE MEDICO-LEGALE EN ESPAGNE

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Introduction

La détermination de la date de la mort est un problème compliqué que doit résoudre le médecin légiste, mais elle a également une importance primordiale au point de vue criminologique.

A partir d'un point de vue méthodologique, la bibliographie médico-légale comprend une importante description des différents modes de preuves permettant de déterminer la date de la mort, notamment en présence d'un cadavre récent.
Des preuves de natures physique, chimique, histologique, sont constamment employées dans la littérature médico-légale.

Mais lorsque la phase de rigidité cadavérique est dépassée et que commence la phase de putréfaction, le chronothanato-diagnostic devient plus difficile, compte tenu des nombreuses réactions qui peuvent avoir lieu dans un organisme en décomposition et de leur grande variabilité biologique.

On peut dire qu'à ce stade, la méthode entomologique atteint son seuil de validité optimale. Après la systématisation de cette méthode que fit Megnin à la fin du siècle dernier, de nombreux auteurs ont écrit et fait des recherches sur les descriptions et classifications de ce qu'il appela "lès escadrons des travailleurs de la mort".

Notre propos consiste à passer en revue l'évolution et les investigations réalisées en Espagne jusqu'à nos jours dans ce domaine.