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Comparison between insects gathered on a death corpse from the study site and insects obtained by rearing larvae within the laboratory under natural environmental conditions

Abstract

Forensic entomology is the use of insect and others arthropods in solving crime. During this purpose, forensic entomologists use carrion insect communities to produce evidence in case of murder, suicide, neglect, accident or poaching, since they are natural witnesses of the crime scene. This offers of insects as physical evidence during legal procedures are use worldwide except in Africa where the documentation of this domain is poor. The present study was to compare the diversity of necrophagous insect gathered at the study site and that of the insect obtain from the rearing of thier larvae in the laboratory under natural environmental conditions. Adult flies were identified to species level. Overall, 3414 adult flies were obtained both from the census on the field and from the rearing in the laboratory. These flies belong to 3 classes of insects namely, Insect, Arachnida and Myriapoda with 3343, 70 and 1 individual respectively, distributed amongst 9 orders, 30 families, 20 genus and 27 species.

Keywords: forensic entomology, necrophagous insect, larvae, Cameroon

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Introduction

Nowadays, Forensic entomology or medico-legal entomology is one of the modern means of investigations essential for strengthening criminal justice systems.[1,2] This discipline also named medico-criminal entomology deals with the use of results gathered from the study of insects and other arthropods collected on and around the crime scene in solving crime. The community of insects retrieved from a corpse or carcass is used in forensic investigations primarily to develop an estimate of when the person or animal died; a period commonly termed the post mortem interval (P.M.I.)[3] which is the time lapse since death. During the progression of corpse decay, the microenvironment of the corpse will undergo change; simultaneously, the composition of the insect community will also undergo changes Hu Guoliang et al.,[4,5] This decaying process varies in response to the effect of multiple influencing parameters such as biotic and abiotic factors. These biotic factors are mostly related to the cause and manner of death and the characteristics of the animal body such as body mass, age, gender, injuries, or medical treatments whereas the abiotic conditions consist of moisture, clothing, concealment, weather, hygrometry, insect activity, temperature and humidity.[6–8] The two last factors are the most significant influencing the rate of decomposition.[9]

During criminal affairs, forensic entomologists use carrion insect communities to produce evidence in case of murder, suicide, neglect, accident or poaching,[10] since they are natural agents of decomposition involved in nutrient recycling.[11–13] They are also important in human and veterinary medicine as transmitters of pathogens and myasis agents (Rognes, 1991), and in forensic entomology as indicators of post mortem interval.[1,9] Insects are also good promising candidates as animal feed ingredients, especially blow flies[12] whose adult and larval stages can be used as animal food.[14–17] Amongst the different ways in which insects are used, the outputs of carrion insect communities have been explored quite intensively worldwide except in Africa in general and particularly in Cameroon where only some few papers are available.[9,18–20] Up to date, nothing has been written on the comparison between the necroentomofauna gathered at the study site and that of those emerging from the rearing of their larvae in the laboratory under normal atmospheric conditions.

To sidestep this lack, the present study was designed using ten carcasses of Rats *Rattus norvegicus*, Berkenhout, 1769 Var Wistar within the campus of the University of Yaounde 1. The purpose of this pilot paper is to emphasize the relationship between necrophagous insects collected on the field and their counterparts emerging from the rearing of their larvae in the laboratory under natural ambient air conditions and provide knowledge on corpse insect communities related to that of their larvae in the Central African Sub region. It has been proven that insects are the most diverse group of animals, estimated at over 5.5 million species worldwide,[21] there is an immense population of unexplored or under-explored species that may bring new perspectives to science.[14]

Materials and methods

Study site

The present study was carried out on the campus of the University of Yaounde I (11°33'01"E, 3°51'35"N) Cameroon. Yaounde is a city located in the heart of the Central African Sub-region, at about 250 KM from the Atlantic Ocean. The area is 304 KM^2 with some valleys and mountains having between 700 and 1200m of altitude.[22] The climate is equatorial and characterized by four distinct seasons: a short rainy season (April to June), a long rainy season (September to mid November), a long dry season (mid November to March) and a short dry season (July to August). The mean annual rainfall is 1600 mm and the average annual temperature fluctuates between 19°C to 33°C.[23,24] The landscape of this part of the city is characterized by the presence of *Elaeis guineensis* (Arecaceae) and *Musa* sp. (Musaceae).

Animals and experimental setup

Rats[9] where obtained from the Physiology laboratory of the faculty of science of the University of Yaoundé 1, alive and in very good health. Ten of them, each weighing about 210g were euthanized with carbondioxide, killed by slaughter according to ethical rules and immediately placed on top of a 10cm layer of sterilized soil put inside a rectangular plastic box (10cm x 20cm x 15cm). The whole setup was then deposited inside a wooden cage (120cm x 120cm x 120cm) protected with a 5cm mesh to allow colonization of carcasses by insects, while preventing scavenger attacks.[25] These carcasses were left to decay under natural environmental conditions up to the skeletonized stage. Every day, the cage was visited three times (08:00GMT, 12:00GMT and 16:00GMT), for 20 minutes and carcasses examined for sampling purposes, during the first and second stages of decomposition i.e. from Day 1 to Day4 according to Wolff et al.[26] Then, arthropod sampling was undertaken once daily at 12:00 GMT.

During carcass examination, flying insects were sampled simultaneously with the monitoring of environmental conditions. The insect communities were caught with the help of an insect hand held net and killed by vaporizing with 70% alcohol, then picked with flexible forceps to keep inside 70% alcohol within a plastic container for further identification. The carcass survey continued and when all the larvae migrated inside the soil beneath eight days after death, they were removed and placed on top of another layer of sterilized layer of soil as written above for the continuation of the experiment. The other soil together with these larvae was carried to the laboratory for rearing under natural environmental parameters. All harvested necroentomofauna were identified to various levels of taxonomy using a binocular stereomicroscope and various dichotomic keys.[19,27–30]

Data analysis

The categorization of taxa was evaluated according to the modified formula of Dajoz[31] where the rate of occurrence (C) of each taxa is: C= Ni/N*100 where Ni is the number of occurrence of a given taxa during the survey and N is the total number of all the individual census during the whole experiment. The results are analyzed as: C > 25% = consistent species, 5% ≤ C ≤ 25% = accessories species and C < 5% = rare species.

Results

During the experimental time period, 3414 adult flies were obtained both from the census on the field and from the rearing in the laboratory within the natural ambient air conditions. These flies belong to 3 classes of insects namely, Insect, Arachnida and Myriapoda with 3343, 70 and 1individual respectively, distributed amongst 9 orders, 30 families,20 genus and 27 species (Table 1).

Table 1 Synoptic table showing the overall number of individuals gathered both on the field and at the laboratory during the entire experiment

Classes	Orders	Families	Genus/Species
			Hemipyrelliafernandica (Macquart, 1855)
			Chrysomyasp. (Robineau-Desvoidy, 1830)
		Calliphoridae	Undeterminated
			Luciliasp. (Robineau-Desvoidy, 1830)
			Luciliacuprina (Wiedemann, 1830)
			Chrysomyaputoria (Wiedemann, 1830)
			Chrysomyaalbiceps (Wiedemann, 1819)
			Chrysomyalaxifrons (Villeneuve, 1814)
			Hemipyrelliasp. (Towsend, 1918)
		Drosophilidae	Undeterminated
		Heleomyzidae	Undeterminated
		Muscidae	Muscasp. (Linnaeus, 1758)
			Ophyrasp. (Robineau-Desvoidy, 1830)
			Atherigonasp. (Rondani, 1856)
			Hydrotaeasp.(Robineau-Desvoidy, 1830)
		Fanniidae	Undeterminated
	Diptera	Sepsidae	Sepsissp. (Fallén, 1810)
		Phoridae	Undeterminated
		Anophelidae	Undeterminated
		Lauxanniidae	Undeterminated
		Sarcophagidae	Sarcophagaafrica(Wiedemann, 1824)
			Sarcophagasp. (Meigen, 1826)
		Staphylinidae	Undeterminated
		Silphidae	Undeterminated
		Histeridae	Undeterminated
	Coleoptera	Cleridae	Undeterminated
		Formicidae	Pheidolesp. (Wilson & Taylor, 1967)
			Myrmicariaopaciventris (Emery, 1893)

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Table 1 Continued...

Classes	Orders	Families	Genus/Species
			Odontomachus troglodytes (Santschi, 1914)
			Tetramoriumsp. (Santschi, 1924)
			Cardiocondylaemeryi (Menozzi, 1930)
			Tapinomaluteum (Emery, 1895)
			Plagiolepisbrunni (Mayr, 1895)
			Lepisiotasp. (Santschi, 1926)
		Chalcididae	Undeterminated
		Ponerinae	Odontomachussp. (Smith, 1863)
		Scelionidae	Undeterminated
	Hymenoptera	Braconidae	Paramesiussp. (Westwood, 1832)
		Braconidae	Coelalysianigriceps(Szépligeti, 1911)
			Undeterminated
		Pteromalidae	Spalangiasp. (Latreille, 1805)
	Dictyoptera	Blattidae	Undeterminated
Insecta	Hemiptera	Aphididae	Undeterminated
		Undeterminated	Undeterminated
	Orthoptera	Gryllidae	Undeterminated
	Diplopoda	Undeterminated	Undeterminated
Myriapoda	Chilopoda	Chilopodae	Undeterminated
Arachnida	Araneida	Undeterminated	Undeterminated

Adult flies collected on the field

2062 adult's flies were gathered at the study site. The most abundant order consisted of Diptera with 1309 (63,48%) individuals, dominated by Calliphoridae with 648 flies(31,43%) (Table 2).Within Diptera, the family Calliphoridae was followed successively by Sepsidae and Muscidae with populations of 254 (12,32%) and 183 (8,87%), respectively. This order was followed by the Hymenoptera families Formicidae and Chalcididae with 250 (12,12%) and 147 (7,13) respectively. Then came the Coleopterans with 136 (6,60%) individuals of Staphylinidae. These three orders comprised 1618 individuals representing 78,47% of the field collection. Some of the samples gathered from the field were identified up to the genus/species level. These ones are organized successively as follow: 254 (12,32%) flies of *Sepsis* (Fallén, 1810), 150 (7,27%) *Chrysomya putoria* (Wiedemann, 1830), 142 (6,89%) *Chrysomya albiceps* (Wiedemann, 1819), 132 (6,40%) *Hemipyrellia fernandica* (Macquart, 1855), 122 (5,92%) *Lucilia* (Robineau-Desvoidy, 1830) and 112 (5,43%) *Ophyra* (Robineau-Desvoidy, 1830).

Adult flies gathered from the larvae rearing at the laboratory

1352 were collected after the emergence of the adults flies from the rearing of the larvae in the laboratory under normal atmospheric conditions (Table 2). Coleopterans with 758 (56,07%) individuals represented the most abundant group with Dermestidae and Staphylinidae numbered respectively 697 (51,55%) and 50 (3,70%) specimens. Then, Diptera included 594 (43,93%) with 507 (37,50%) specimens of Calliphoridae and 46 (3,40%) specimens of Muscidae. All the adults flies obtained in the laboratory belong to the above two orders only. The abundance of the emerged insects are organized as follow: 221 (16,35%) adult of *Hemipyrellia fernandica* (Macquart, 1855), 107 (7,91%) *Chrysomya putoria* (Wiedemann, 1830), 101 (7,47%) *Chrysomya albiceps* (Wiedemann, 1819), 36 (2,66%) *Hydrotaea* (R-D, 1830) and 35 (2,59%) *Lucilia* (R-D, 1830).

Adult flies sampled from the field and their counterparts from the rearing of larvae at the laboratory

The analysis of the entomofauna census on the field and their counterparts emerging from the rearing larvae in the laboratory looks slightly similar according to the variation of their abundance (Table 2). The most abundant specimens caught at the field was genus *Sepsis* (Fallén, 1810) with 254 (12,32%) individuals while *Hemipyrellia fernandica* (Macquart, 1855) were the most abundant on the field with 221 (16,35%) individuals. The second and the third highest number of insects collected at both sites were *Chrysomya putoria* (Wiedemann, 1830) containing 150 (7,27%) and 101 (7,91%) from the field and the laboratory, respectively. These flies were followed successively by *Chrysomya albiceps* (Wiedemann, 1819) hosted 142 (6,89%) and 101 (7,47%); *Hemipyrellia fernandica* (Macquart, 1855) with 132 (6,40%) and *Hydrotaea* [36 (2,66%)] came in the fourth position from the field and the laboratory respectively. The fifth position was occupied by *Lucilia* (R-D, 1830) at both sites with 122 (5,43%) and 35 (2,59%) respectively. 112 (5,43%) specimens of *Ophyra* (R-D, 1830) were caught on the field even though none of them emerged as imago within the laboratory.

The slight difference noted about the abundances maybe explained by the fact that some species are part of the necroentomofauna mainly only for feeding (e.g.: *Sepsis* sp., *Ophyra* sp., *Pheidole* sp., *Musca* sp. and *Odontomachus troglodytes* (Santschi, 1914) while some are using carcasses as food and reproduction ground (e.g.: *Hemipyrellia fernandica* (Macquart, 1855), *Chrysomya putoria* (Wiedemann, 1830), *C. albiceps* (Wiedemann, 1819) and *Lucilia* sp. (R-D, 1830). The data analysis illustrated that the most consistent ($C > 25\%$) species belongs to the orders of Diptera and Coleoptera with 55,74% and 27,91% respectively making a total of 83,65% of the entomofauna counted during the experiment. The accessory species with occurrence (C) ($5\% \leq C \leq 25\%$) are made up of only Hymenoptera (11,98%) and the rare species are Araneida, Hemiptera, Diplopoda, Orthoptera, Dictyoptera and Chilopoda with 2,05%, 1,87%, 0,15%, 0,12% and 0,03 chronologically (Table 3).

Table 2 Descriptive statistics according to species and area of flies sampling

Species	Field		Laboratory			
	Ni	%	Ni	%	Ni	%
Atherigona sp. (Rondani, 1856)	3	0,15	1	0,07	4	0,12
Cardiocondyla emeryi (Menozzi, 1930)	9	0,44	0	0,00	9	0,26
Chrysomya albiceps (Wiedemann, 1819)	142	6,89	101	7,47	243	7,12
Chrysomya laxifrons (Villeneuve, 1814)	14	0,68	3	0,22	17	0,50
Chrysomya putoria (Wiedemann, 1830)	150	7,27	107	7,91	257	7,53
Chrysomya sp. (R-D, 1830)	6	0,29	19	1,41	25	0,73
Hemipyrellia fernandica (Manquart, 1855)	132	6,40	221	16,35	353	10,34
Hemipyrellia sp. (Towsend, 1918)	0	0,00	22	1,63	22	0,64
Hydrotaea sp. (R-D, 1830)	0	0,00	36	2,66	36	1,05
Lepisiota sp. (Santschi, 1926)	23	1,12	0	0,00	23	0,67
Lucilia cuprina (Wiedemann, 1830)	9	0,44	0	0,00	9	0,26
Lucilia sp. (R-D, 1830)	122	5,92	35	2,59	157	4,60
Musca sp.(Linnaeus, 1758)	68	3,30	9	0,67	77	2,26
Myrmicaria opaciventris (Emery, 1893)	4	0,19	0	0,00	4	0,12
Odontomachus troglodytes (Santschi, 1914)	45	2,18	0	0,00	45	1,32
Ophyra sp. (R-D, 1830)	112	5,43	0	0,00	112	3,28
Paramesius sp. (Westwood, 1832)	6	0,29	0	0,00	6	0,18
Paratrechina sp. (Motschulsky, 1863)	13	0,63	0	0,00	13	0,38
Pheidole p.(Westwood, 1839)	95	4,61	0	0,00	95	2,78
Plagiolepis brunni (Mayr, 1895)	12	0,58	0	0,00	12	0,35
Sarcophaga africa (Wiedemann, 1824)	0	0,00	8	0,59	8	0,23
Sarcophaga inaequalis (Austein, 1909)	0	0,00	1	0,07	1	0,03
Sarcophaga sp. (Meigen, 1826)	0	0,00	5	0,37	5	0,15
Sepsis sp. (Fallén, 1010)	254	12,32	2	0,15	256	7,50
Tapinoma luteum (Emery, 1895)	16	0,78	0	0,00	16	0,47
Tetramorium sp. (Mayr, 1855)	33	1,60	0	0,00	33	0,97
Undetermined	794	38,51	782	57,84	1576	46,16
General Total	**2062**	**100,00**	**1352**	**100,00**	**3414**	**100,00**

Legend: Ni=Abundance and %=Percentage.

Table 3 Occurrences of different taxa gathered during the experimental period

	Field		Laboratory			
Orders	**Ni**	**%**	**Ni**	**%**	**Total Ni**	**Total %**
Araneida	70	3,39	0	0,00	70	2,05
Chilopoda	1	0,05	0	0,00	1	0,03
Coleoptera	195	9,46	758	56,07	953	27,91
Dictyoptera	4	0,19	0	0,00	4	0,12
Diplopoda	5	0,24	0	0,00	5	0,15
Diptera	1309	63,48	594	43,93	1903	55,74
Hemiptera	64	3,10	0	0,00	64	1,87
Hymenoptera	409	19,84	0	0,00	409	11,98
Orthoptera	5	0,24	0	0,00	5	0,15
General Total	2062	100,00	1352	100,00	3414	100,00

Discussion

To the best of our knowledge, this study is the first published data worldwide, regarding the comparison between field necroentomofauna and their counterparts emerging from the rearing of larvae within the laboratory. The estimation of post mortem interval is a key aspect of forensic entomology given that it helps to solve criminal affairs during legal procedures in court. During our experiment, carcasses were evaluated every day for 20 minutes and after their migration, the larvae where carried to the laboratory for rearing, similar to the methods adopted by Pittner et al.[32] during their field study to evaluate PMI estimation methods for advanced decomposition stages at forensic research filed in northern Germany,[9,18–20] while conducting forensic entomology research in Yaounde, Cameroon.

In accordance with Matuszewski et al.,[33] the data on abundances of various insects in necroentomofauna assemblages in the present study site showed the domination of the orders Diptera and Coleoptera. These two taxa were sampled from the field and from the individuals emerging from the rearing in the laboratory showing that although the necroentomofauna involved in the carcass decay varied between biogeographical regions, ecological guilds are consistent and

functioned in a very consistent way worldwide as emphasized by the above authors.

Order diptera

The order Diptera was the most abundant and diverse taxa and the species sampled were distributed amongst the families Calliphoridae, Muscidae, Sarcophagidae, Anophelidae, Drossophilidae, Fanniidae, Heleomyzidae, Lauxanniidae, Micropezidae, Phoridae, Sciaridae and Sepsidae. These findings are in accordance with the result obtained by Dao et al.,[34–43] This similarity can be explained by the same/unique behavioral habits of diet and reproduction noted on necroentomofauna around the world independent of areas. The diversity within family mainly the Calliphoridae was *Hemipyrellia fernandica* (Macquart, 1855), *Chrysomya putoria* (Wiedemann, 1830), *Chrysomya albiceps* (Wiedemann, 1819), *Chrysomya* sp. (Robineau-Desvoidy, 1830), *Lucilia cuprina* (Wiedemann, 1830), *Lucilia* sp. (Robineau-Desvoidy, 1830), *Chrysomya laxifrons* (Villeneuve, 1814) and *Hemipyrellia* sp (Towsend, 1928). This high diversity looks like that of Shiuh-Feng Shiao and Ta-ChuanYeh (2008), Silahuddin *et al*. (2015) and can be the consequence of the high egg-laying behavior of the females firstly and the well-developed sensorial and olfactive organs located within their antennas.

Nevertheless the families gathered during our experiment look like those obtained in many countries, the species diversity varied within families in countries like Côte d'Ivoire, Egypt, Algeria, Namibia and South Africa where these researchers sampled the species *Calliphora vicina*, *C. vomitoria*, *Chrysomya marginalis, C. megacephala*, *C. rififacies*, *Protophormia terranovae*, *Lucila Caesar*, *Fannia caricularis*, *Sarcophaga carnaria*, *S. haemorrhoidalis*, *S. tibalis*, *S. inzi*, *S. exuberans*, *Liosarcophaga emmrichiana*, *Hermetia illucens*, *Piophila casei*, *Hydrotaea sinigera*, *Haematobia minuta*, *Chrysomya chloropyga*, *Drosophilia* spp, *Musca domestica*. This results are in line with those of Villet[10,44] in South Africa and can be due to the fact that organisms' geographical distributions will rarely coincide over the spatial scale of a continent like Africa where abiotic parameters vary significantly within the Sub-region and even within a country; even if necroentomophagous insects such as blowflies are mobile. This is apparently largely due to differences in physiological tolerance to climatic variables such as relative humidity and temperature.

Order coleoptera

The order Coleoptera was the second most abundant taxa and the species sampled belong to the following families: Cleridae, Histeridae, Silphidae and Staphylinidae contrary to some studies where in addition to the above families, this order was also composed of Nitidulidae, Scarabaeidae, Catopidae, Ptinidae, Trogiidae and Carabidae. This variation can be the consequence of the permanent amelioration of faunistic inventory sampling methods during experiments with time. This idea is supported by Baz et al.,[45] Gotelli et al.,[46] Woodcock et al.,[47] and Tews et al.[48] who highlight that faunistic inventory is generally incomplete due to the fact that the number of inventoried species continues increasing with sampling efforts (1). It can also be the result of the study areas with proper climatic parameters since the above authors set up their research where temperature oscillated from 3,3 to 43°C and total rainfall of 451 to 521 mm (2) and finally the type of insect trapping substrat as they used carcasses of Pig (*Sus scrofa domesticus*).[49]

Other arthropods

In addition to the taxa mentioned above, carcasses were also colonized by other arthropods namely Bostrichidae, Phoridae, Ptiliidae, Sarcophagidae, Sciaridae and Sepsidae. Surprisingly, most of them were absent from the field specimens but only recorded from the adult flies emerging from the rearing within the laboratory. This output which is in accordance with the results of Maisonhaute and Forbes (2020) can be due to interspecific competition which only allowed the above mentioned families time to lay eggs and quickly escape from the carcasses avoiding being eaten by predators. Maybe some species mainly dry eaters such as Coleopterans first visited the carcasses for egg laying to procreate before coming later for feeding. This may be the reason why they were absent from the field species list and only appeared after laboratory larvae rearing even thought their abundance was low as registered by the op.cit authors.[50]

Overall, the main types of the insects assessed on cadaver during this research have also been sampled in Africa.[9,10,18–20,33,35,36,39,41,43,44,51–63] This parallelism of the results maybe a consequence of the cosmopolitan, easy adaptation to various biotopes and abiotic determinants that govern the development of various necrophagous flies.

Conclusion

The present study maybe amongst the first published data on the comparison between field necroentomofauna and its counterparts emerging from the rearing of their larvae within the laboratory under natural environmental conditions. The outcome of this work illustrated the overall diversity of this specific type of entomofauna, with the collection of 2062 insects from the field and 1352 emerging from larvae reared in the laboratory making a total of 3414 flies distributed amongst 30 families, 20 genus and 27 species. Nevertheless, this research work has only been performed one time at one location within a unique habitat/ecosystem in Cameroon, the results are similar to those obtained in other countries with differences due to biotic and abiotic variations within species and location/study sites. This antagonism observation confirms the importance/emergence of conducting local studies and replicates to maximize information's on necroentomofauna for the wellbeing of future investigations by researchers, lawyers, law officers, law enforcement agents, and criminal investigators. It also provides a base line for students with respect to the use forensic entomology to elucidate various types of crime within the city of Yaounde.

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None.

Conflicts of interest

The author declares there is no conflict of interest.

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