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Biodiversity study of arthropods collected on rat carrion in Yaounde, Cameroon: first study on forensic entomology in Central Africa

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Abstract

The first investigation of arthropods associated with carrion in Cameroon was carried out within the campus of the University of Yaounde I (Cameroon) from 17thJanuary to 3rd April 2008. Carcasses of rats (*Rattus norvegicus* Berkenhout, 1769 var WISTAR*)* were exposed to colonization by the local fauna of arthropods. The invading organisms were collected daily during the study period. 2287 individuals of arthropod belonging to 3 classes, 16 orders, 37 families and 7 subfamilies were identified. The insects assessed were mainly Diptera, Coleoptera and Acari. This study illustrates the high diversity of the necroentomofauna in Cameroon and provides an insight approximation into the succession pattern of invading insect and a weekly estimation of the time of death.

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Introduction

During judicial inquiry following the discovery of corpses, the determination of the time of death is a very important issue for the legal authorities (Catts & Goff,, 1992). Such estimation is more difficult to establish when the cadaver has reached an advanced stage of decomposition. In these cases, the entomological evidence can be one of the few sources of certainty. This scientific discipline named forensic entomology (Wolff *et al.*, 2001) is the use of information obtained from the study of insects collected on the cadaver to solve crime (Catts & Goff, 1992; Moretti *et al.*, 2008). The insects sample on the crime scene can be used to determine the time elapsed since death or post-mortem interval (Smith, 1986). These insects can also be used to determine the cause of death such as poisoning, illicit traffic and the movement of the corpse after death (Anderson & Sherah, 1996; Goff *et al.*, 1997). The process of decay of organic material is highly complex and is influenced by numerous interrelated factors such as macroclimate and microclimate, availability and accessibility of insects to the carcasses (Braack, 1981). For intervals greater than three (3) days, forensic entomology can be more accurate in determining post-mortem interval than others traditional techniques, and sometimes is the only available method (Anderson & VanLaerhoven, 1996). Therefore, it reveals necessary to study the necrophagous entomofauna of each region. Except in South Africa where, the succession of the insects on carcasses in the Kruger national park were studied (Braack, 1981 and 1987), the history of research relevant to forensic entomology is known (Williams & Villet, 2006) and the influence of clothing and wrapping on carcass decomposition and arthropod succession have been explored (Kelly *et al.*, 2009), and in Nigeria where the arthropods associated with mammalian carcasses in Rivers State were examined (Okiwelu *et al.*, 2008), in general, carrion-feeding insects are not well known in Africa. In Cameroon, no information is available regarding these organisms. Therefore, the systematics, ethology and ecodynamics of the necrophagous entomofauna has to be elucidated.

This paper presents preliminary results of research on the arthropods which are associated with carrion in Yaounde, in order to create a Cameroonian database on forensic entomology.

Materials and methods

The present study was carried out in four sites which are 200 m apart, with similar ecological conditions in the campus of the University of Yaounde I (11°33'01"E, 3°51'35"N) Cameroon. 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 annual mean rainfall is 1600 mm and the average annual temperature fluctuates between 19°C to 33°C (Suchel, 1987). The landscape of this part of the city is characterized by the presence of *Elaeis guineensis* (Arecaceae) and *Musa* sp. (Musaceae).

Sixteen carcasses of laboratory bred rats (*Rattus norvegicus* Berkenhout, 1769 var WISTAR) each weighing about 210g were used as models in our investigation. Four rats were sacrificed by strangulation on January 17th, 2008 and immediately placed in one cage (120cm x 120cm x 120cm) per site. The cages were covered with a 5cm mesh to allow colonization of carcasses by insects, while preventing scavengers' attacks (Sukontason *et al.*, 2003). The cages were visited during 20 minutes for sampling purposes three times per day, during the first and second stages of decomposition i.e. from Day 1 to Day 4 according to Wolff *et al.* (2001). Then, arthropod sampling was undertaken once daily at 12:00 h. This exercise was stopped on the 3rd of April 2008 when only animal bones remained.

During the sampling periods, carcasses pictures were taken, observations of physical modifications due to decay were made, and the ambient air temperature was recorded using a mercury column thermometer (Miras *et al.*, 1998; Catts & Haskell, 1990; Bharti & Singh, 2003; Amendt *et al.*, 2006; Gaudry *et al.*, 2007). The thermometer was held inside the cage 5cm above ground.

Flying insects were caught with a hand net of 1 mm mesh, then brought back to the laboratory and sprayed with 70 per cent ethyl alcohol to asphyxiate them. After 10 minutes, the asphyxiated insects were preserved in 70 per cent ethyl alcohol for further taxonomic identification. Larvae and pupae were collected from carcasses using flexible forceps, then divided into two sub samples: the first one preserved inside tubes containing 70 per cent ethyl alcohol for further identification and the other reared in the laboratory until emergence of adults flies. After the emergence, insects were fed with honey during the two first days, then captured and preserved for taxonomic identification.

The identification of the specimens was partially completed using keys proposed by Delvare & Alberlenc (1989) for families, and by Smith (1986), Claudio & Cátia (2008), Prins (1982), Couri (2007), Regina (2002) for genera and species. Other specimens were determined at the Zoology Laboratory of Rhodes University, South Africa. The trophic categories were those proposed by Smith (1986) and Magaña (2001) in Martinez *et al.* (2007). Adding to their proposal, we consider the type of the mouth parts and the ecology of insect for the classification of the trophic categories.

Results

A total of 2287 arthropod specimens were collected during the realization of this research. Except Arachnida (Acari and Araneida orders) and Myriapoda (Chilopoda and Diplopoda orders), Hexapoda (Diptera, Coleoptera, Lepidoptera, Dermaptera, Hemiptera, Hymenoptera, Dictyoptera, Collembola, Homoptera, Orthoptera, Psocoptera and Thysanura orders) were both mainly sampled amongst which Diptera, Coleoptera and Hymenoptera revealed the more diversified orders with 16, 9 and 5 families on the one hand and 19, 10 and 10 species respectively on the other hand (Table 1).

The trophic categories were mainly made up of predators, necrophagous and omnivorous with 1277, 362 and 294 individuals respectively, and secondary of phytophagous, saprophagous, opportunistic, parasitoids, hematophagous and incidentals with 131, 125, 85, 8, 3 and 2 individuals.

No noticeable morphological changes were observed on the carcasses during the fresh stage. Late in day 2, maggots started emerging from eggs, odours were faintly noticeable in the immediate (50cm) surroundings of the carcasses. Calliphoridae was the only insect family found. *Lucilia* sp. started laying eggs in the natural orifices (nose, mouth and eyes) on day 1; it was followed on day 2 by Calliphorinae and *Chrysomya* sp.

From day 3 to day 4 (bloated stage) swelling and deflation of the carrion began. The rat smelt even 5m far away. In addition to Calliphoridae, Muscidae, Heleomyzidae and Fanniidae occurred on the body at day 3; Drosophilidae appeared at day 4. At the same day, Coleopteran families (Staphylinidae, Histeridae and Trogidae) were recorded. Maggots continued to feed, grow, and finally extend the abdominal region of the rat carrion. Amount of Phaoniinae (phytophagous) also occurred at that period.

During the decayed stage (day 5 to day 9) inwards were completely consumed; the outpouring of the decomposition fluid caused the maggot migration from cadavers to the ground. Calliphoridae number decreased significantly from days 5 to 8. Eight new families were collected at this stage: Sarcophagidae and Silphidae (day 5), Sepsidae, Phoridae and Cleridae (day 6), Lauxaniidae and Scarabaeidae (day 7), Ephydridae (day 8). Later on, one individual of Culicidae was caught on day 9. This putrefaction stage ended with the complete degradation of viscera and the migration of maggots.

In the dried stage (day 10 to day 73) decay stopped. Only cartilage, bones, nails, teeth and skin remained. The skin started to split on day 10 while hair and bones were already delocalized. Odours were almost imperceptible. Dermestidae, Sphaeroceridae, Mordellidae, Ptiliidae, Piophilidae, Sciaridae, Haplozetidae, Staphylinidae (Tachyporinae) and Cecidomyiidae were found at day 10, 11, 13, 15, 21, 36, 41, 42 and 73 respectively.

During the skelotonized stage (day 74 to 77), only dried pieces of skin and bones were observed. No new family was recorded but there was a decrease of those which consume the tough and dried material: Ptiliidae (91%), Dermestidae (46%) and Cleridae (94%).

According to the trophic categories, the arthropod fauna was classified as necrophagous, saprophagous, phytophagous, predators, omnivorous, opportunistic, hematophagous, parasitoids and incidental (table 2).

At the fresh stage, the first trophic categories to invade the corpse were necrophagous (67 individuals) and a few predators (17 individuals). The number of these populations doubled at the second post mortem stage. One parasitoid individual was also censued within this stage. These groups were joined by saprophagous, phytophagous and very few omnivorous. Except predators and omnivorous whose numbers increased, those of the other present trophic categories (necrophagous, saprophagous and phytophagous) decreased during the decayed stage. The new trophic categories recorded at this stage were opportunistic and one individual of hematophagous. During the dried stage, the over- population of necrophagous, saprophagous and opportunistic stimulated that of predators (1062 individuals) and omnivorous (149 individuals). During the skelotonized stage, the disappearance of necrophagous, saprophagous and phytophagous provoked a drastic decrease of predators (≈ 91%) and opportunistic (≈ 95%) and a moderated one of omnivorous (≈ 34%) (Table 2).

The statistical analysis (x^2) was done manually with a calculator and the result showed that the number of a given trophic category varied significantly ($p < 0.001$) amongst phases.

Discussion

The sequence of the different stages recorded during our study (fresh, bloated, decayed, dried and skeletonized) is similar to that observed by Martinez *et al.* (2007) in the high altitude plains in Colombia and Moretti *et al.* (2008) in a secondary forest in the city of Campinas, São Paulo State (Brazil), although the durations of their stages were shorter. Conversely, Braack (1981) and Kelly *et al.* (2009) recorded four stages (fresh, bloated, active decayed and advanced decayed) in South Africa. The differences between our results and those obtained by these last authors are explained by the climatic variation i.e. equatorial with annual mean rainfall of 1600 mm and average annual temperatures in Yaounde (Suchel, 1987), tropical semi-arid with annual mean rainfall of 438 mm in the Kruger National Park (Braack, 1981; Kelly *et al.*, 2009). Our findings are likely comparable to those observed by Moretti *et al.* (2008) and Velásquez (2008) also from small carcasses, but distant from that of Kelly *et al.* (2009) on bigger carcasses.

As Tullis & Goff (1987), Anderson & VanLaerhoven (1996), Wolff *et al.* (2001), Wyss & Cherix (2006), Martinez *et al.* (2007), Okiwelu *et al.* (2008) and Velásquez (2008), we equally mentioned that Diptera (mostly necrophagous and saprophagous) were the predominant insects invading carrion during the first, second and third stages of decomposition. Their number gradually decreased while that of predators (mostly Coleoptera and Hymenoptera) progressively increased.

Table 1. Inventory of arthropods associated with carrion in Yaounde (Cameroon) during the dry season (January 17th to April 3rd, 2008).

Taxonomy					Trophic category	State of decomposition (period in days)															Total (%)
						F (0-2)			B (3-4)			D1 (5-9)			D2 (10-73)			S (74-77)			
Class	Order	Family	Subfamily	Species		E	I	A	E	I	A	E	I	A	E	I	A	E	I	A	
Hexapoda	Diptera	Calliphoridae	Calliphorinae		Necroph			2			1										3 (0,13)
			Chrysomyinae	*Chrysomya* sp.				31	√												31 (1,36)
			Luciliinae	*Lucilia* sp.		√		34	√		122			9			23				188 (8,22)
		Muscidae	Unidentified	*Ophyra* sp.					√		2										2 (0,09)
			Phaoniinae		Phytoph						101			20		√	10				131 (5,73)
		Sarcophagidae	Sarcophaginae		Necroph									9			23				32 (1,40)
		Fanniidae	Unidentified								4						17				21 (0,9)
		Sphaeroceridae	"														8				8 (0,35)
		Sepsidae	"											6			26				32 (1,40)
		Piophilidae	"														8				8 (0,35)
		Phoridae	"											7			16				23 (1,00)
		Cecidomyiidae	"														1				1 (0,04)
		Drosophilidae	"								6			7							13 (0,57)
		Anthomyiidae	"		Saproph						7			9			31				47 (2,06)
		Heleomyzidae	"								47			3			3				53 (2,32)
		Ephydridae	"											6			6				12 (0,52)
		Sciaridae	"														3				3 (0,13)
		Lauxaniidae	"											6			4				10 (0,44)
		Culicidae	"		Hematoph									1			2				3 (0,13)
	Coleoptera	Trogidae	"		Predat						1						1				2 (0,09)
		Silphidae	"											3							3 (0,13)
		Dermestidae	"														56			30	86 (3,76)
		Staphylinidae	Tachyporinae														5				5 (0, 20)
		Cleridae	Unidentified											3			96			6	105 (4,60)
		Scarabaeidae	"											1			3				4 (0,17)
		Mordellidae	"		Opport												22				22 (0,96)
			Aleocharinae								10			28			230				268 (11,72)
		Histeridae	Unidentified		Predat						3			7			97				107 (4,68)
		Ptiliidae	"														554			52	606 (26,5)
	Lepidoptera	Tineidae	"														2			5	7 (0,30)
	Dermaptera	Unidentified	"											1							1 (0,04)
	Hemiptera	Anthocoridae	"		Incidental												1				1 (0,04)
		Aphididae	"														3				3 (0, 13)
		Cicadidae	"										1								1 (0,04)
	Hymenoptera	Chalcididae	"		Predat			4			4			7							15 (0,66)
		Unidentified	Myrmicinae	*Monomorium* sp.													1				1 (0,04)
		Unidentified		*Pheidole* sp.				11			5			2			9			1	28 (1,22)
		Unidentified		*Tetramorium* sp.							1										1 (0,04)
		Formicidae	Formicinae	*Paratrechina longicornis*				2			3			13			7			8	33 (1,44)
				Oecophylla longinoda							2										2 (0,09)
			Dolichoderinae														6				6 (0,26)
		Proctotrupidae	Unidentified								3										3 (0,13)
		Braconidae	"		Parasitoids															5	5 (0,22)
		Scelionidae	"								1						2				3 (0,13)
	Dictyoptera	Blattidae	"		Omniv									6			13			7	26 (1,14)

	Collembola	Unidentified	"																	3					3 (0,13)
	Homoptera	Unidentified	"									4				23				35				20	82 (3,59)
	Orthoptera	Gryllidae	"																	18				12	30 (1,31)
	Psocoptera	Unidentified	"																					1	1 (0,04)
	Thysanura	Unidentified	"		Opport															5					5 (0,22)
Arachnida	Acari	Haplozetidae	"		Omniv															60				36	96 (4,20)
	Araneida	Unidentified	"													14				17				22	53 (2,32)
Myriapoda	Chilopoda	Unidentified	"		Opport											17				28				2	47 (2,06)
	Diplopoda	Unidentified	"													1				4				1	6 (0,26)
Total (%)						84 (3,67)				327 (14,29)				209 (9,14)				1459 (63,80)				208 (9,10)			2287 (100)

Necroph = necrophagous; phytoph = phytophagous; saproph = saprophagous; hematoph = hematophagous; predat = predator; opport = opportunistic; omniv = omnivorous; F = fresh, B = Bloated, D1 = Decayed, D2 = Dried, S = skelotonized.

Table 2. Number of specimens per trophic category and morphological phase.

Trophic categories	Phase					Total	Percentage (%)	P value
	Fresh	Bloated	Decayed	Dried	Skelotonized			
Necrophagous	67	135	38	122	0	362	15.83	0.001
Saprophagous	0	54	24	47	0	125	5.47	"
Phytophagous	0	101	20	10	0	131	5.73	"
Predator	17	32	64	1062	102	1277	55.84	"
Omnivorous	0	4	43	149	98	294	12.85	"
Opportunist	0	0	18	64	3	85	3.72	"
Hematophagous	0	0	1	2	0	3	0.13	undetermined
Parasitoids	0	1	0	2	5	8	0.35	"
Incidental	0	0	1	1	0	2	0.08	"
Total	84	327	209	1459	208	2287	100	0.001

Similarly to Martinez *et al.* (2007) and Bharti & Singh (2003), we noticed that *Lucilia* sp. and *Chrysomya* sp., were the first species to lay eggs on carrion, although these authors also recorded respectively Sarcophagidae on the one hand and Muscidae i.e. (*Dasymorellia seguyi*, *Fannia* sp. and *Limnophora* sp.) on the other hand. The difference can be explained by the specificity of necroentomofauna of each region (Wolf *et al.*, 2001) or the type of ecosystem (anthropizied area or not). Velásquez (2008) argued that the taxonomic composition of the colonizing entomofauna varies between ecosystems, since it is influenced by the environmental parameters of the study site. The large number of individuals of Calliphoridae, Muscidae and Heleomyzidae (122, 101 and 47 individuals respectively) at the bloated and putrefied stages is the main justification of the disappearance of abdominal organs due to the feeding activity of maggots, the outpouring of the cadaver liquid and maggot migration to the ground (Anderson, 2000; Benecke, 2002 and Mansfield, 2003). During the skelotonized stage, the numbers of predator (Ptiliidae) reduces significantly but remains relatively high due to the availability of preys. This finding supports that of Wolf *et al.* (2001).

Conclusion

This work presents the first results obtained from the study of the assemblages of arthropods on rat carrion in Central Africa. The process of the carrion decomposition showed five stages: fresh, bloated, decayed, dried and skelotonized. The carrion arthropods fauna is numerically made up of three major groups: Diptera, Coleoptera and Acari. According to the feeding behaviour, nine throphic categories are recognized: necrophagous, sarcophagous, phytophagous, predator, omnivorous, opportunistic, hematophagous, parasitoid and incidental.

The evolution of the number of these different groups showed a clear synchronization between preys and predators. The increasing (the decreasing) of prey number stimulates that of the predators. Morphological changes of the carrion and the entomofauna composition henceforth make it possible to estimate the time of death at least at a weekly level in case of advanced stage of decomposition.

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