INSECT LARVAE RECOVERED FROM DECOMPOSING PIG CARRIONS IN OKIJA, ANAMBRA STATE, NIGERIA

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ABSTRACT

Insect immature stages found on decomposing cadavers are source of information that assist forensic investigators elucidate the time of questionable death. Hence, insect larvae recovered from decomposing pig carrions as models to human cadavers in Okija (05°53.240"'N and 006°46.510"E) Anambra State, Nigeria were reared to adults in the laboratory by adopting a simple method to assist amateurs. Samples of the larvae were collected with camel hair (student's art) brush, 5ml spoon and blunt forceps. They were collected in batches and placed in rearing containers (9.0 cm depth and 6.5 cm width) and labeled accordingly. Each sampled batch was replicated thrice and reared under ambient temperature (28.6 ± 0.15 ºC) and relative humidity (68.5 ± 1.34 %). The reared insect larvae later emerged and they were taxonomically identified. The species were ascertained by a taxonomist at Insect Museum, Institute of Agricultural Research, Ahmadu Bello University, Zaria. The species include: Chrysomya albiceps, Chrysomya chloropyga, Chrysomya regalis, Isomyia dubiosa, Isomyia sp. (Calliphoridae), Sarcophaga inzi (Sarcophagidae), Chrysomyza africana (Ulidiidae), Musca domestica (Muscidae), Hermetia illucens (Stratiomyiidae), Dermestes frischii (Dermestidae) and Necrobia rufipes (Cleridae). The Calliphoridae first developed into adults at day 8, Sarcophagidae day 16, Ulidiidae, day 22, Muscidae, day 31, Stratiomyiidae, day 33, Dermestidae, day 38 and Cleridae, day 53. The probable reasons for the insect larvae to associate with the carrions were highlighted and their forensic importance discussed.

Keywords: Insect larvae, Carrions, Decomposition stages, Forensic science

INTRODUCTION

Insects as living creatures have adaptive potentials, to inhabit every aspect of the ecosystem. The insects that are associated with decomposing carrions are grouped as necrophagous, omnivorous, parasites/predators or incidentals (Smith, 1986). The necrophagous group specifically feed on the decomposing tissues of carcasses. Among this group are flies, beetles and their larvae.

Information about the size and the age of these larvae on decomposing carrion can be used to estimate when the carrion died and aid to identify the place of death; an entomological tool applied in forensic science (Hall, 2001). Larvae suspected to belong to flies are normally seen at the orifices of carrion within few hours of death, if allowed access and undisturbed while the carrion is bloating or decaying. Other groups of larvae suspected are those of beetles seen on the carrion when the soft tissues have dried out. Catts and Goff (1992) stated that for centuries, until in recent time in the developed world, larvae either dead or crawling in the orifices and wounds on dead bodies were considered disgusting elements of decay as soon as corpses were deposited on a table for autopsy. The larvae were most times washed away without any form of relevant information

deduced from them. However, the identity of the insects associated with decomposing carrions is the first essential step in estimating their ages (Amendt *et al*., 2004), especially their larvae.

Nigeria like other developing countries lack modern technology that can facilitate the identification of immature stages of necrophagous insects to species level. The challenge is one of the militating factors in the development of forensic entomology in these countries for legal use. Hence, morphological identification of the insects is only possible with the adults. Therefore, the study is geared towards collecting samples of larvae associated with decomposing pig carrions in Okija, Anambra State, Nigeria in view to rear them to adults and identify their species. This is because of the facts that adults can easily leave the carcass when disturbed while their larvae remain. The study will however, evolve simple and proper methodologies that are sequentially scientific, to rear larvae associated with carrions in Nigeria to adults with ease.

MATERIALS AND METHODS

Six white pigs (*Sus scrofa* Linn.) with mean weight of 24.8 ± 0.9 kg were used as a model for human corpse as recommended by Catts and Goff (1992). The pigs were purchased from a piggery at Umuogu-Okija and killed by 18.30 hours. The pigs were exposed immediately on a polyester sack and placed three meters apart in an open fallow plot of land between January and May, 2012. The fallow plot was located on 05°53.240N and 006°46.510E, at Ubahueze-Okija. Okija is a town in Ihiala Local Government Area of Anambra State, Nigeria. The vegetation in Okija is derived tropical savanna with patches of forest and palm trees which also characterize the plot. The topography of Okija is a combination of high and low lands, with Umuhu and Ubahueze in Ihite constituting the low lands. The temperature in Okija ranges from 26° C to 30° C with wet and dry seasons in a yearly cycle (Okija In-Home Club, 2010). The exposed pig carrions at the fallow plot were protected against vertebrate scavengers with wire mesh and guarded with cement blocks.

Insect Larvae Collection: The insect larvae found on the decomposing pig carrions were collected in batches with students' art brush and blunt forceps. The first batch of the larvae was collected on day 2, 3 and 4, when the carrions were bloating. The second batch was collected on day 5, 6, 7 and 8, when the carrions were actively undergoing wet decomposition. The third batch was collected on day 9, 10, 11, 12, 13, 14, 15 and 16 when the carrions were undergoing dry decomposition. The fourth batch of the insect larvae was collected when the body was undergoing skeletonization between day 17 and 30 at three days intervals.

All the daily collections of each batch of at least twenty insect larvae were replicated thrice and reared in a labeled container to adult stage in a simulated laboratory in Ubahueze-Okija. The rearing containers (9.0 cm depth and 6.5 cm width) were transparent; half filled with a mixture of wood saw dust and sandy soil. The mixture was heated for thirty minutes in oven at 60 °C to kill microbes and other micro-arthropods. Twenty (20 g) of crushed and deboned fresh Mackerel fish were introduce into each of the rearing containers as food substrate for the larvae. Muslin cloth was used as lids of the containers, tightly held with rubber bands. When the adult flies emerged in the containers, each of the containers was carefully introduced into a larger container (20.6 cm depth and 13.3 cm width) one-quarter filled with soap solution. The rubber band of the smaller container was gently removed, while the muslin cloth was still in place. The larger container was then covered with another muslin cloth and held with rubber band. Then the muslin cloth of the rearing container was carefully removed with forceps. The flies then escaped into the larger container and get drowned in the soap solution. The drowned flies were collected with forceps and preserved in 80 % ethanol. The adult beetles that emerged in the rearing containers were simply collected with forceps immediately the muslin cloth was removed and preserved in 70 % ethanol. The emerged adult flies and beetles were sorted to their taxonomic group and sent

to Insect Museum, Institute of Agricultural Research, Ahmadu Bello University, Zaria, Nigeria, for species identification using relevant taxonomic keys (Abajue *et al.,* 2013; 2014).

RESULTS

From the study, two insect orders: Diptera and Coleoptera in seven families consisting eleven insect species, emerged from the larvae recovered from the decomposing carrions and reared in the laboratory (Table 1). The first batch of the reared larvae emerged first as flies of *Chrysomya albiceps* (Wied.), *Chrysomya chloropyga* (Wied.), *Chrysomya regalis* (Rob-Desv.), *Isomyia dubiosa* (Villen), *Isomyia* sp. in the Calliphoridae family and *Sarcophaga inzi* (Curran) in the Sarcophagidae family. The second batch of the larvae emerged only as flies in the family of Calliphoridae; *Chrysomya albiceps* (Wied.), *Chrysomya chloropyga* (Wied.), *Chrysomya regalis* (Rob-Desv.), *Isomyia dubiosa* (Villen), *Isomyia* sp. The third batch of the larvae emerged still consists flies of Calliphoridae as *Chrysomya albiceps* (Wied.), *Chrysomya chloropyga* (Wied.), *Chrysomya regalis* (Rob-Desv.), *Isomyia dubiosa* (Villen), *Isomyia* sp., with two additional fly families; Ulidiidae - *Chrysomyza africana* (Hendel) and Stratiomyiidae - *Hermetia illucens* (Linn.). The fourth batch of the larvae emerged, also consist the Calliphoridae - *Chrysomya albiceps* (Wied.), *Chrysomya chloropyga* (Wied.), *Chrysomya regalis* (Rob-Desv.), Ulidiidae - *Chrysomyza africana* (Hendel), Stratiomyiidae - *Hermetia illucens* (Linn.), in addition to one fly family; Muscidae - *Musca domestica* (Linn.) and two beetle families; Dermestidae - *Dermestes frischii* (Kug.) and Cleridae - *Necrobia rufipes* (Deg.).

The development of the larvae into adults, in all the batches show that *S. inzi* only emerged once from the first batch of the larvae collected precisely on day 2. Other insects that only emerged once are *M. domestica*, *D. frischii* and *N. rufipes* from the fourth batch of the larvae. *Chrysomyza africana* and *Hermetia illucens* emerged twice, from the third and fourth batches of the larvae. The results consistently show that, *Chrysomya* species emerged from all the batches of the larvae. However, the genus *Isomyia* sharing the same family with *Chrysomya* also continued to emerge from the first batch to the third batch but not in the fourth batch. The first emergent dates of all the species from every batch were recorded counting from the day the carrions were exposed in the fallow plot. Thus, the species of Calliphoridae family developed into adult at day 8, Sarcophagidae at day 16, Ulidiidae at day 22, Muscidae at day 31, Stratiomyiidae at day 33, Dermestidae at day 38 and *N. rufipes* at day 53 respectively (Figure 1).

DISCUSSION

Dead animals including human beings begin to deteriorate, minutes after death. This is because of physiological changes which have abruptly stopped, leading to putrefaction of tissues. Evidence of this situation is the assemblage of insects and their larvae after some hours on the carrion, if allowed access. The composition of the insect larvae of this study validated most reports of adult insects associated with decomposing carrions in Nigeria (Usua, 2007; Ekanem, 2008; Ekanem and Dike, 2010; Ekrakene and Iloba, 2011; Abajue *et al*., 2013).

The emergent of these insect larvae recovered from the decomposing pig carrions as adults of: *C. albiceps*, *C. chloropyga*, *C. regalis, Isomyia dubiosa, Isomyia sp., S. inzi, C. africana, M. domestica, H. illucens, D. frischii, and N. rufipes*, clearly affirmed that they are truly necrophagous insects (Smith, 1986) as they can at least used the carrions as a food source and produce at least one generation of their progeny (Ekrakene and Iloba, 2011; Abajue *et al*., 2014). Some of these insects which their larvae were collected have been used as insects of forensic importance by earlier researchers such as (Goff and Odum, 1987; Lord *et al*., 1993; Goff and Win, 1997; Benecke, 1998; Greenberg and Wells, 1998). The insects of this study that are of forensic importance include the *Chrysomya* spp., *Sarcophaga sp., H. illucens* and *N. rufipes*. Other insect species obtained from the study, which have the same characteristics with the established insects of forensic importance, include *Isomyia dubiosa, C. africana* and *D. frischii*.

Table 1: Insects that emerged from the culturing process of larvae recovered from the decomposing pig carrions in Okija, Anambra State, Nigeria

Batches	Order	Family	Species
First Batch	**Diptera**	Calliphoridae	*Chrysomya albiceps* (Wied.)
			Chrysomya chloropyga (Wied.)
			Chrysomya regalis (Rob-Dev.)
			Isomyia dubiosa (Villen)
			Isomyia sp.
		Sarcophagidae	*Sarcophaga inzi* (Curran
Second Batch	**Diptera**	Calliphoridae	*Chrysomya albiceps* (Wied.)
			Chrysomya chloropyga (Wied.)
			Chrysomya regalis (Rob-Dev.)
			Isomyia dubiosa (Villen)
			Isomyia sp.
Third Batch		Calliphoridae	*Chrysomya albiceps* (Wied.)
			Chrysomya chloropyga (Wied.)
			Chrysomya regalis (Rob-Dev.)
			Isomyia dubiosa (Villen),
			Isomyia sp.
		Ulidiidae	*Chrysomyza africana* (Hendel)
		Stratiomyiidae	*Hermetia illucens* (Linn.)
Fourth Batch	**Diptera**	Calliphoridae	*Chrysomya albiceps* (Wied.)
			Chrysomya chloropyga (Wied.)
			Chrysomya regalis (Rob-Dev.)
		Ulidiidae	*Chrysomyza africana* (Hendel)
		Stratiomyiidae	*Hermetia illucens* (Linn.)
		Muscidae	*Musca domestica* (Linn.)
	Coleoptera	Dermestidae	*Dermestes frischii* (Kug)
		Cleridae	*Necrobia rufipes* (Deg.)

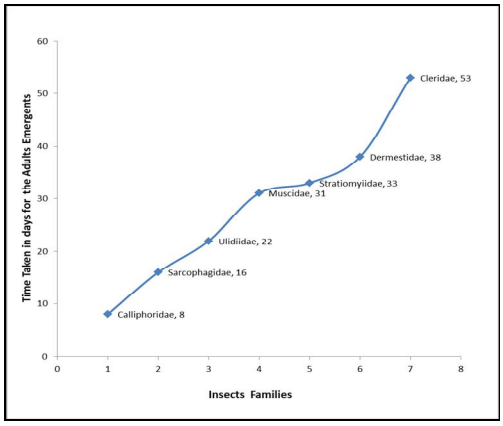

Figure 1: Time taken for each of the fly families to first emerge into adult in the laboratory

But the emergent of the Muscidae on day 31, do not represent the duration of its true life cycle. This is attributed to their in ability to lay eggs when the carrions are undergoing active decomposition because blowfly larvae have already colonized every part of the carrion body thus, depriving them early oviposition on the carrions' body until blowfly larvae have all dispersed for pupation. The probable reason for their exclusion from the previous studies may be, because of biogeoclimatic differences of these study locations. Though, have all being reported at the family level hence, indicating their ecological surrogates. Thus, the implicated larvae have shown that the emerged insects, visited the carrions as adults at least at one stage of the carrions decomposition. Hence, they found the carrions useful as food and exploiting resource for the production of at least one generation of their progeny.

The overall presence of these larvae at different stages of the carrions decomposition and at the different predictable time of adults emergent is viable tools in entomology, applied in forensic science. Therefore, the recovery of the larvae and their emerged adults, no doubt can be used to estimate when the carrions died.

Conclusion: The findings of this study were implicit and heuristic to conclude that; the carrions serve as a temporal micro community,

which the implicated flies and beetles use to at least complete one generation of their progeny. Hence, the collection of the larvae and their successful rearing to adults in the laboratory is an alternative means of identifying the necrophagous insects associated with decomposing carrions in view to use them estimate when an animal including human being died if the natural process of carrion decomposition is not intentionally altered.

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