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ROLES OF FRESH AND DECOMPOSED LIVER ON ASPECTS OF REPRODUCTION OF BLOWFLY *CHRYSOMYA CHLOROPYGA* (WIED.) (DIPTERA: CALLIPHORIDAE)

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ABSTRACT

This study compared the effects of fresh and decomposed liver on aspects of reproduction of the blowfly, *Chrysomya chloropyga* to determine the possibility of multiple generations of the blowfly on a single cadaver. The total development time from egg to adult were 8.75 and 8.50 days in the flies fed on fresh and decomposed liver respectively and were not significantly different (p=0.58) from each other. The sex ratio (male: female) was 1:1 on both diets. There was a significant difference in the survival of adults fed fresh and decomposed liver (p=0.001, 0.001). There was no significant difference (p=0.362, 0.029) in the longevity of flies fed fresh and decomposed liver. Age at first egg laying on fresh and decomposed liver were 15.00 and 7.25 days respectively on fresh and decomposed liver. Fecundity of 175.6 eggs recorded in the flies fed on fresh liver was not significantly different (p=0.804) from that (200.8 eggs) of flies on decomposed liver. Mean weights of male and female flies maintained on both diet increased from the first day to tenth day only. However, it was not significantly different (p=0.130, 0.576) from each other throughout the period of exposure. Protein concentration was significantly higher in females fed fresh liver than those maintained on decomposed liver at ages 0 to 25 days. In conclusion, decomposed liver was as effective as fresh liver in supporting reproduction of the *C. chloropyga* with a better performance on decomposed liver. The study also demonstrated a strong indication for more than one generation of flies on a sizeable cadaver.

Key Words: Blowfly, *Chrysomya*, Decomposition, Forensic entomology, Reproduction

INTRODUCTION

Chrysomya chloropyga a typical carrion fly is known to cause myiasis in both man and animals and the short life cycle of the blowfly makes it useful for studies in Forensic Entomology (Archer and Elgar, 2003). The blowfly maggots are commonly found on corpses and its consistent developmental time is extremely helpful to establish a post mortem interval (PMI) (Anderson, 2000; Sukontason, 2004; Tarone and Foran, 2008; Zurawski *et al*., 2009). Carrion flies are often the first to visit carcass when majority of flesh still remains (Archer, 2002). This pattern of visit continues until late decay when the carcass is nearly or completely skeletonized. Very little explanation has been provided on the reason for this. In an effort to explain this Archer and Elgar (2003) submitted that the continued attraction well after the blowflies have ceased to oviposit on the carrion is an indication that a decomposed carcass remains a suitable source of protein for vitellogenesis. Huntington *et al.* (2008) further explained that decomposition of carcasses is a vital process in every terrestrial ecosystem because carcasses release water, energy, and nutrients. Therefore there is presumably a great reduction in the volume of attractive chemicals released after flesh has decayed but nevertheless, flies present at late decomposition stages may simply be responding to residual decay odours emanating from the carcass or body. Estrada *et al*. (2009) observed that beef and liver serve as efficient diet for breeding larvae of necrophagous dipterans and Huntington and Higley (2010) reported that both the fresh and decomposed stages had similar effect on the ovarian development of the fly but the extent to which fresh and decomposed carcass support the reproductive development of the blowfly *Chrysomya chloropyga* is yet to be determined hence the use of the fresh and decomposed liver to study aspects of reproduction of the blowfly.

MATERIALS AND METHODS

Adult *Chrysomya chloropyga* from laboratory stock (collected from the refuse dump site and reared in the laboratory) were used for this study. Decomposed cow liver was obtained from fresh cow liver purchased from the abattoir by exposing it in a plastic bowl covered with muslin cloth and left for seven days at room temperature (25°C± 2°C). It was thereafter kept in the freezer (-20 °C). Fresh liver was also kept in the freezer until use.

Experimental Procedure

All experiments were carried out in cages (40 x 30 x 30 cm^3) at 28±2 °C and 75±5% relative humidity. Three Petri dishes containing water-soaked cotton wool, fresh or decomposed liver, and 21 g of sugar respectively were put in the cages. All experiments were carried out in quadruplicate

Life Cycle and Sex Ratio of Newly Ecdysed Adults on Fresh and Decomposed Liver

Two batches (200 eggs per batch) of freshly laid eggs were separately placed on fresh and decomposed liver in two separate Petri dishes and thereafter put in two separate cages. The eggs which hatched into larvae were monitored for their development until adult emergence. The development of the larvae which hatched from the eggs was monitored by recording the number of days spent on each stage before metamorphosing on the fresh and decomposed liver until adult emergence.

Survival and Longevity

Two batches (20 males and 20 females) of newly emerged flies were separately put in two cages per batch and provided with fresh and decomposed liver respectively. Survival and longevity of males and females were determined by taking daily mortality records of the flies.

Life-time Fecundity of Females

Two batches (20 males and 20 females) of newly emerged flies were separately put in two cages per batch and provided with fresh and decomposed liver respectively. Eggs deposited were removed and counted on daily basis until all the flies died. The age of fly at first egg laying was also recorded on the fresh and decomposed liver respectively.

Adult Weights of Males and Females

Twenty pairs of newly emerged males and females each were placed in two cages with each provided with fresh and decomposed liver respectively and maintained as described earlier. The growth rates of these flies were determined by recording the individual weights of the flies on a Mettler weighing balance at five-day interval until the death of the population.

Protein Content Determination of Flies

Thirty (30) newly emerged pairs of males and females each were put in two separate cages with one provided with fresh liver and the other with decomposed liver. At days 0, 5, 10, 15, 20, 25, four males and four females were removed from each of the cages, freeze killed at -18 °C. Each of the flies was first rinsed with 1 ml cold PBS (0.1M Potassium Buffered Saline), homogenized using 1 ml of the buffer with mortar and pestle for 30 seconds and centrifuged twice at 6000 rev per minute for 10 minutes. The supernatant was then used for subsequent determination of whole body protein content according to Lowry *et al.* (1951).

Data Analysis

Data generated were analyzed using Statistical Package for Social Sciences (Version 21), both descriptive and inferential statistics were used, Paired T-test was done to compare the mean duration of life cycle and sex ratio at emergence of adults fed fresh and decomposed liver; fecundity between female fed fresh and decomposed liver; weight of male and female fed fresh and decomposed liver; and protein content of male and female fed fresh and decomposed liver. While Analysis of Variance was used to check if there was significant difference in the survival rates and longevity of adults fed fresh and decomposed liver; compare the weight of male and female fed fresh and decomposed liver at different ages; and compare protein content at different ages between male and female fed fresh and decomposed liver.

RESULTS

Duration of Various Stages in the Life cycle of *C. chloropyga* on Fresh and Decomposed Liver

The mean durations (days) of the development of egg, larval instars, pupa and adult stages of *C.*

chloropyga maintained on fresh and decomposed liver are shown in table 1. There was no significant difference in the mean duration of each stage of development on fresh and decomposed liver (8.75 ± 0.30 and 8.50 ± 0.37 days; t = 0.567, p = 0.58).

Table 1: Life Cycle Duration of Various Stages of *C. chloropyga* Maintained on Fresh and Decomposed Liver

Stage of development	Mean duration (days)	
	Fresh	**Decomposed Liver**
Egg	1.00 ± 0.0	1.00 ± 0.0
First instar	1.00 ± 0.0	1.00 ± 0.0
Second instar	1.00 ± 0.0	1.00 ± 0.0
Third instar	2.25 ± 0.25	2.0 ± 0.41
Pupa	3.50 ± 0.29	3.50 ± 0.29
Egg-Adult	8.75 ± 0.30	8.50 ± 0.37

Sex Ratio at Emergence and Maximum Longevity of Male and Female *C. chloropyga* Fed Fresh and Decomposed Liver

Results show that there was no significant difference in the mean number of males and females that emerged on the fresh liver (t = 0.660, p = 0.556) as well as on decomposed liver (t = 2.166, p = 0.119). Also, there was no significant difference in the number of males maintained on fresh liver and on decomposed liver (t = 0.394, p = 0.720) as well as females maintained on fresh liver and on decomposed liver (t = 0.515, p = 0.642) (Table 2).

Table 2: Sex Ratio at Emergence and Maximum Longevity of Male and Female *C. chloropyga* Fed Fresh and Decomposed Liver

Sex	Mean Sex ratio (± S.E)		Maximum longevity (±S.E)	
	Fresh	**Decomposed**	**Fresh**	**Decomposed**
Male	88.00 ± 41.85	67.50 ± 10.85	41.25 ± 6.81	50.50± 11.64
Female	80.75 ± 31.30	58.25 ± 13.00	46.00 ± 5.11	62.25 ± 5.51
Approximate sex ratio	1:1	1:1		

Findings show that there was no significant difference in the mean longevities of males and females fed fresh liver (41.25 ± 6.81 and 46.00 ± 5.11 days; f= 0.311, p = 0.597). Mean longevity of males fed decomposed liver was not significantly different from females fed decomposed liver (50.50 ± 11.64 and 62.25 ± 5.51 days F= 0.833, p = 0.397). Females fed decomposed liver lived significantly longer than females fed fresh liver (t = -3.922, p = 0.029).

Survival Rate of Male and Female *C. chloropyga* Maintained on Fresh and Decomposed liver

Survival patterns of males maintained on fresh and decomposed liver as shown in figure 1 reveal that percentage survival of male and female maintained on fresh liver was stable at 100 percent up to day 17 compared to male population that suffered mortality after 24 hr of exposure. There was significant difference in the survival of male and female from day 0 to 85 (F = 8.287, p = 0.01) (F = 51.904, p = 0.01). Females on fresh and decomposed liver survived between 90 and 100 percent from days 0 to 25 and thereafter suffered continuous mortality up to days 62 and 54 on fresh and decomposed liver respectively (Figure 2). There was significant difference in the survival of females on fresh and decomposed liver ($F = 10.125$, $p = 0.002$).

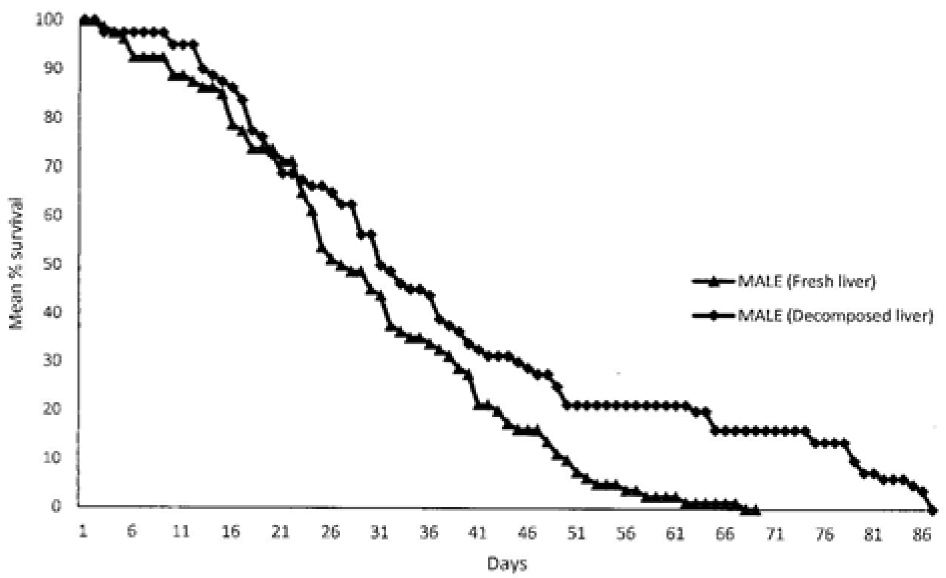

Figure 1: Mean Percent Survival of Male *C. chloropyga* Fed Fresh and Decomposed Liver

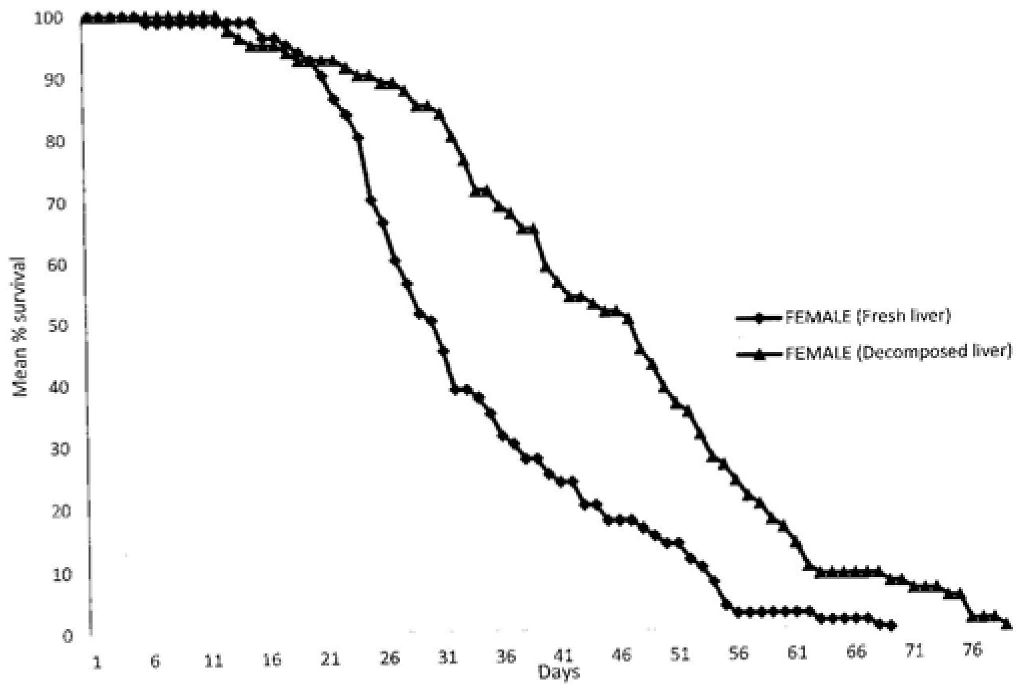

Figure 2: Mean Percent Survival of Female *C. chloropyga* Fed Fresh and Decomposed Liver.

Age of Fly at First Egg laying and Fecundity of Female *C. chloropyga* Fed Fresh and Decomposed Liver

Table 3 shows the mean age at first egg laying of female *C. chloropyga* fed fresh and decomposed liver. Mean days of first egg laying on fresh and decomposed liver were significantly different (15.00 ± 1.779 and 7.25 ± 0.75 days; $t = 6.990$, $p = 0.01$). On the other hand the mean number of eggs laid by females fed fresh and decomposed liver were not significantly different (175.57 ± 10.69 and 200.78 ± 11.25; $t = 0.250$, $p = 0.804$).

Table 3: Age of Fly at First Egg laying and Fecundity of Female *C. chloropyga* Fed Fresh and Decomposed Liver.

Diet	Age at first egg laying (days) (Mean ± S.E)	Egg number (Mean ± S.E) (Fecundity)
Fresh liver	15.00±1.779	175.57 ± 10.69
Decomposed liver	7.25±0.75	200.78 ± 11.25

Adult Weights of Male and Female *C. chloropyga* Fed Fresh and Decomposed Liver at Different Days of Development

Males and females increased in weight on fresh and decomposed liver from days 0 to 10 (Table 4). Thereafter the weights fluctuated with continuous decrease up to day 50. In comparing the mean weights of adult males fed fresh and decomposed liver as well as females fed fresh and decomposed liver, there was no significant difference (t =1.547, p = 0.130) (t =-0.564, p = 0.576).

Table 4: Weight (mg) of Adult Male and Female *C. chloropyga* Fed Fresh and Decomposed Liver at Different Days of Development

Age (Days)	Mean weight (mg) (± S.E)			
	Fresh liver		Decomposed liver	
	Male	Female	Male	Female
0	59.00±1.29^{abc}	53.25±11.64^{ab}	66.00±3.16^{cd}	63.00±8.70^{abcd}
5	64.00±8.12^{abc}	72.50±5.68^{ab}	70.50±2.22^{d}	82.50±0.96^{cd}
10	79.00±5.75^{c}	78.00±4.24^{ab}	73.50±2.36^{d}	92.50±0.96^{d}
15	71.00±7.23^{bc}	82.00±4.08^{abc}	71.50±4.57^{d}	80.00±5.89^{bcd}
20	61.50±6.85^{abc}	62.00±2.45^{ab}	49.50±7.97^{bcd}	60.00±7.79^{abc}
25	41.00±5.45^{a}	60.00±4.08^{ab}	38.00±1.83^{abc}	59.50±3.78^{abc}
30	44.50±3.50^{ab}	55.25±2.29^{ab}	34.25±6.49^{ab}	54.50±3.20^{abc}
35	47.00±4.73^{ab}	60.50±8.51^{ab}	42.00±6.38^{abcd}	61.00±6.35^{abcd}
40	37.50±5.91^{a}	58.50±10.59^{ab}	38.75±6.63^{abc}	51.50±5.50^{ab}
45	41.50±6.06^{a}	53.00±5.90^{ab}	29.25±15.3^{ab}	44.75±5.59^{a}
50	37.00±6.04^{a}	48.00±4.90^{a}	52.00±0.0^{cd}	45.75±8.93^{a}

Mean values followed by the same letter along the same column are not significantly different (p>0.05) by Tukey test.

Protein Concentration of Male and Female *C. chloropyga* Fed Fresh and Decomposed Liver

Protein concentrations of whole body homogenate of male and female flies fed on fresh and decomposed liver fluctuated with age of flies. Protein concentrations were generally higher in females than in males on both fresh and decomposed liver (Table 5). There was general increase in protein concentrations from days 0 to 5 for males and females in fresh and decomposed liver. There was no significant difference in the mean protein concentrations of males maintained on fresh and decomposed liver (t = -1.813, p = 0.088) but there was significant difference in the mean protein concentration of females maintained on fresh and decomposed liver (t = 2.861, p=0.011).

Table 5: Protein Concentrations (mg/ml) of Male and Female *C. chloropyga* Fed Fresh and Decomposed Liver at Different Ages

Ages (Days)	Protein content (Mean ± SE)			
	Fresh		Decomposed	
	Male	**Female**	**Male**	**Female**
0	7.07±1.49	6.21±1.83	4.52±0.57	5.31±1.00
5	8.42±2.32	8.77±2.48	5.81±0.41	7.01±1.43
10	5.08±1.58	6.77±2.84	3.30±1.18	5.44±0.88
15	8.09 ± 0.11	10.78 ± 0.65	4.21 ± 1.31	8.74 ± 2.94
20	4.18 ± 0.93	5.74 ± 2.73	6.21 ±1.65	3.68 ±0.93
25	4.51 ± 2.59	7.17 ± 1.28	4.13 ± 1.07	4.30 ± 1.90

DISCUSSION

The study showed that there was no significant difference in the mean development time from egg to adult on fresh and decomposed liver demonstrating the effectiveness of the two types of diets in the rearing and maintenance of the blowfly *C. chloropyga*. The mean duration of between 8.50 and 8.75 days obtained for *C. chloropyga* agrees with the work of Fathy *et al.* (2008) who reported 8-9 days duration for *C. albiceps* under field condition using a whole carcass. A shorter life cycle duration of 6.55 days had been reported by Queiroz (1996) on *Lucilia sericata* using an artificial diet. A longer life cycle of 13.89 days on artificial protein diet was reported by Rueda *et al.* (2010). Nuorteva (1977) had earlier reported life cycle duration of 23-28 days for *Lucilia* under field conditions.

The life cycle duration obtained in this study is not unexpected as Tachibana and Numata (2001) reported that cow liver is highly proteinous and maximally support the development of larvae as compared to artificial protein. Richards *et al.* (2013) equally observed that there was no significant difference between fresh and decomposed liver treatment in the *Lucilia vicina* which is consistent with the result of this study in which fresh and decomposed liver equally support the development of the blowfly. The sex ratio of emerged àdults on both fresh and decomposed liver were approximately 1:1 indicating the suitability of fresh and decomposed liver in the rearing of the blowfly.

Rocha and Perodini (2000) reported a sex ratio of 1:1 in *Bradysia matogrossensis*. Tabadkani *et al.* (2012) also reported a sex ratio of 1:1 in the predatory gall midge (*Aphidoletes aphidimyza*) when the females were exposed to either high or low densities of males. Martin-Vega and Baz (2013) however reported female-biased sex ratio in a mark and capture experiment with species of Calliphoridae, Muscidae, and Sarcophagidae using carrion baited traps under field conditions. Inadequate diet and nutritionally deficient diets during larval development often results in sex ratios that are skewed in favour of males or females. Sex ratio of 1:1 for adult *C. chloropyga* even on decomposed liver demonstrates its suitability for the propagation of balanced sex ratio in the blowfly.

The survival curves of males and females either together or in isolation by sex on fresh and decomposed liver followed the same pattern throughout the study period. It is known that development and survival of insects are affected by several factors including nutrition (Vrba *et al.*, 1983) and a medium deficient in one or more required substance may promote death at larval moult or decrease the number of insects pupating (Hogan, 1972). In this study, there was absence of significant difference in the development time from egg to adult, including survival on both diets, although mortality seems to be higher in males than in females either on fresh or decomposed liver. There was no significant difference in the survival of males and females indicating the suitability of fresh and decomposed liver in the development of the reproductive stages of *C. chloropyga*.

Female population was stable at 95 and 100% for 19 days in comparison with three days for male population, demonstrating that fresh liver was better utilized by females than males which suffered continuous mortality within few days after exposure. On decomposed liver, up to 80% of females survived for about 29 days as against male population at 15 days. Difference in survival time of males on fresh and decomposed liver at 70, 50 and 20% were 3, 6, 9 days respectively with males on decomposed liver outliving males on fresh liver. Also the difference in survival time of females at 70, 50, and 20% were 10, 16 and 14 days respectively in favour of females on decomposed liver, indicating that decomposed liver was better utilized for the survival of the blowfly than fresh liver. The maximum longevity of males maintained on fresh and decomposed liver was 41 and 51 days respectively while females maintained on fresh and decomposed liver lived for 46 and 62 days respectively. Abou-zeid *et al*. (2003) reported longevity of 43.4 days in *L. cuprina* reared on Tilapia fish.

Rueda *et al.* (2010) and Abou-zeid *et al*. (2003) reported that the mean number of eggs laid by female blowflies was 184.51 eggs under field conditions. This is consistent with the 175.57 and 200.78 eggs for females on fresh and decomposed liver respectively, obtained in this study. Blowflies are more attracted to carrion than decomposed organic matter in the environment. It is however quicker to obtain protein in decaying carcass because abundant liquid decomposition products are present (Huntington and Higley, 2010) resulting in the females accessing more protein in decaying than in fresh liver as observed by Cangussu and Zucoloto (1997) in the medfly *C. capitata*. Perez *et al.* (2000) also reported that increase in protein concentration increased fertility in the fruit fly *Drosopilla melanogaster* as well as in *Ceratitis capitata* (Pereira, 2001).

Another reason for the attraction could be the fact that carcass at early and late decay produce different chemicals and sulphurous compounds which are highly attractive to the carrion fly (Ashworth and Wall, 1995), suggesting that chemicals produced in late decay seems to favour increased fertility in *C. chloropyga.* The ability to recognize chemical signature is useful in finding suitable substrates for their offspring (Ullyett, 1950) so as to maximize their survival. Therefore, any larva that fails to obtain enough food becomes small-sized adult (Stoffolano, *et al*., 2000). Johansen *et al.* (2014) reported that *Calliphora vicina* was strongly dependent on carcass-emitted volatiles when locating and evaluating a carcass. They further reported that *C. vicina* females were highly attracted to carcasses in the earliest stages of decay confirming why females of *C. chloropyga* fed decomposed liver started laying eggs as early as six days after the commencement of feeding. Their study, therefore demonstrates that decomposed liver supported the reproduction of *C. chloropyga* better than fresh liver. *C. chloropyga* fed decomposed liver were heavier than those fed fresh liver with an initial increase in the weight of males and females up to day 15 on fresh and decomposed liver. Gray and Bradley (2003) reported significant increase in body mass of the mosquito *Culex tarsalis* from emergence up to day 12. It appeared that the newly emerged adults fed significantly immediately after emergence to strengthen body parts for subsequent adult activities including mating and egg production.

Protein concentration of whole body of male and female *C. chloropyga* fluctuated with age with the highest protein concentration at 15 days old for male and female fed fresh and decomposed liver. This is consistent with the body weights of male and female which reached the highest weight at 15 days of age in this study. Protein seems to play a significant role in the body building of male and female *C. chloropyga* as observed by Arrese *et al.* (2001).

In conclusion, from this research decomposed liver supported the development of the blowfly, *C. chloropyga* better than fresh liver, although not significant. This investigation indicates that a cadaver in the field will support multigenerational colonization by the blowfly provided the cadaver is sizeable and not removed from site.

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