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ORIGINAL ARTICLE

Effect of Intraspecific Larval Aggregation and Diet Type on Life-History Traits of *Dermestes maculatus* and *Dermestes caninus* (Coleoptera: Dermestidae): Species of Forensic Importance

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ABSTRACT

Introduction: Numerous studies have examined the effect of abiotic factors on the development and survival of *Dermestes* and their importance for forensic entomology. *Dermestes maculatus* is one of the most known beetle species associated with corpses and *D. caninus* has little biological information available and no case report records. To better understand the life-history traits of those species we evaluated the impact of diet type and intraspecific larval density. **Methods:** Adult beetles were collected from human remains and colonies were kept under controlled conditions (27.0 ± 1.0 °C, 55.0% RU, and 12:12 L:D) and F_1 generation was used to collect eggs. Newly emerged larvae were separated according to the treatments, being the combination of larval density (1, 15, 30 individuals), food (dried dog food or dried pork) and contact (with or without). We used factorial-ANOVA to test the individual and combined effect of both larval densities and diet on dependent variables, followed by post-hoc Tukey test. Pearson correlations were carried out to evaluate the relationship between larval parameters for each species in each treatment. **Results:** Pork-based diet positively affected species fitness, with larvae being ca. 1.1 (*D. caninus*) and 1.7 (*D. maculatus*) times bigger and heavier than in dog food. Diet type also impacted the development time for both species. **Conclusions:** Data generated through the current study serve as a foundation for potential application of this species as an indicator of time of colonization in relations to a minPMI. However, validation is still needed to determine the accuracy and precision of these calculations.

KEYWORDS: Forensic entomology, beetles, carrion fauna, insect diet

INTRODUCTION

Forensic entomology is defined as the application of insect science within legal investigations [1]. This science is commonly known for its medicolegal applications since necrophagous insects are often used to estimate the minimum period of insect activity (PIA) often related with myiasis or time of death [2]. As they typically colonize remains soon after death, Diptera species usually provide the entomological clues used to calculate time of colonization in the context of a minimum postmortem interval (min-PMI) [3]. However, beetles (Coleoptera) form a taxonomically and ecologically diverse part of carrion fauna assemblage [4] and may also be used for such calculations [5].

The duration of the immature stages of forensically important beetles is generally longer than flies, which makes these insects an ideal functional model for estimating min-PMI greater than one or two weeks [4]. Among the forensically important beetles, Dermestidae stands out as a cosmopolitan family with more than 1,460 described species that are usually associated with dry animal remains and stored animal products (e.g., leather, fur, skins, wool, and silk) [6,7]. *Dermestes* is a common genus associated with decomposing vertebrate remains [8,9]. Several studies examining *Dermestes* biology have been published using different diets [10], dried fish [11, 12], as well as artificial diets with bacon and fishmeal [13], beef [14]. However, there is limited information on select aspects of their biology allowing their use as min-PMI indicators [15,16].

Numerous studies have examined the effect of abiotic factors, such as temperature, humidity, and food substrate on the development and survival of *Dermestes* (see [16], for a review). Likely due to its forensic importance and cosmopolitan distribution, *Dermestes maculatus*, De Geer 1774, is the primary subject of most of these studies. Consequently, little is known about other *Dermestes* species that are associated with decomposing remains. Thus, a better understanding of the *Dermestes* life-history traits (e.g., larval length and weight, and development time) and survival on different food substrates and larval densities will allow for more accurate predictions as related to time of colonization (i.e., medico-legal and stored-products entomology).

Larval aggregation is common in necrophagous Diptera species such as blow flies (Diptera: Calliphoridae). Such behavior allows maggots to raise their own temperature to create an optimal development stage [17]. For example, studies demonstrated that development of these species is density-dependent [18]. In contrast, larval masses are not common in Coleoptera as many species are solitary. Thus, unlike blow fly (Diptera: Calliphoridae) larvae, the body temperature of beetle larvae is typically closer to that of the environment. [4]. Nevertheless, larvae of some beetle species have been shown to aggregate (personal observation). In cases where such aggregations occur, larval density may have bottom-up effects on development time and survival.

As previously mentioned, *D. maculatus* is a cosmopolitan species with extensive publication history on its biology and its relevance to forensic entomology [9,12,19], as this species is one of the beetle species most commonly associated with corpses. In contrast, and according to Magni *et al.* [16], *Dermestes caninus* Germar, 1824 (Coleoptera: Dermestidae) is a species with little biological information available, only a few records from carrion ecology studies [20, 21, 22, 23, 24], and no known records on forensic entomology case studies. This study aims to determine the impact of diet type and intraspecific larval density on life-history traits (i.e., larval length and weight, and development time to reach adult phase) of *D. maculatus* and *D. caninus*.

MATERIALS AND METHODS

Source of specimens and colonies establishment

Adult *D. maculatus* and *D. caninus* were collected from different human remains at the similar decomposition stage at Forensic Anthropology Research Facility (FARF) located at Texas State's Freeman Ranch, Texas, USA (29°56′1517″ N, 98°00′29.94″ W) (species confirmation through personal communication with A. Hermann). Adults of both species used in this study were brought to the Forensic Laboratory for Investigative Entomological Science (FLIES) at Texas A&M University for colony establishment. Colonies of each species were established using at least 20 individuals placed in a 6-L plastic container and fed *ad libitum* with dry dog food (Purina® Beneful Originals). Each container was maintained in a rearing chamber (Percival® Advanced Intellus) under controlled conditions (27.0 ± 1.0 °C, 55.0% RU, and 12:12 L:D). To keep the colonies healthy, containers were cleaned monthly, by removing excess frass and dead insects. The F_1 generation from each colony was used to conduct the experiment.

Experimental design

A random sample design with repeated measures was used to assess the effect of larval density and diet type on developmental and survival parameters of *D. maculatus* and *D. caninus*. Raw pork loin and dry dog food purchased from a local grocery store were used as the diets. To assess the moisture content of each, four 50 g replicates of each diet were placed in cups in a drying oven at 55°C for 30 h at which point weight loss was no longer experienced by the samples. The average moisture was 74% and 10% pork and dog food, respectively. Dried pork and dog food samples with moisture adjusted to 8% and 12% respectively were used in the study. The nutrient composition of a 30g portion of the raw pork diet was Protein 6.36 g, Fat 1.46 g, Calcium 0.04 g; nutrient composition of an equivalent portion of dog food was Protein 6.9 g, Fat 3.0 g, Calcium 0.3 g. Nutrient composition for raw pork loin was available through the National Nutrient Database for Standard Reference on USDA (United States Department of Agriculture) website and for dog

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food the information is available on the product bag or at Purina® Beneful website.

From the main colonies, three sets of 24 individuals (12 male and 12 female) were placed in 1-L containers for egg collection and held in an incubator at 27.0 ± 1.0 °C, 55.0% RU, and 12:12 L:D. The insects were left without food and water for 24 h and then we provided 30 g dried pork loin. Moist cotton pads were placed directly on the bottom of the container as an oviposition site and changed daily. Resulting eggs were placed in smaller plastic containers and kept in the incubator until their eclosion. The newly emerged larvae were placed in small rectangular (8.10 L x 15.72 W x 12.29 H cm) 236 ml plastic containers and separated according to the treatments, being the combination of larval density (1, 15, 30 individuals), food (dried dog food or dried pork) and contact (with or without - contact was included as a treatment in order to distinguish the effects of larval handling on survival rates.). Each container was provided 30 g of the assigned diet. Four replicates of each treatment were used: T1 - 1 larvae + dog food; T2 - 15 larvae + dog food; T3 - 30 larvae + dog food; T4 - 1 larvae + pork; T5 - 15 larvae + pork; T6 - 30 larvae + pork) for both species.

The life history traits were evaluated according to larval measurements: length (mm) and weight (g), and development time (days) from egg to adult emergency. Additionally, we analyzed the survivorship of each species in each treatment. For contact treatments, the largest 20% of larvae were measured every 48 h until the pre-pupal or pupae stage. Individuals were returned to their assigned replicate after each measurement. For the length measurements, we used the total distance between head and the last abdominal segment. Length was determined with a 0.1 mm scale ruler, while weight was measured using a laboratorial scale (Ohaus® Adventure Pro AV64) with four decimal places. For our data analysis we used the average values for each replicate dependent variable in each treatment.

Data analysis

We used factorial analysis of variance (factorial-ANOVA) to test the individual and combined effect of both larval densities and diet on dependent variables, followed by post-hoc Tukey test, after calculating the ANOVA premises and inclusion criteria of our data. Pearson correlations were carried out to evaluate the possible covariation between larval parameters for each species in each treatment. All analyses were carried out on Statistica ® 7.0 [25] ($\alpha < 0.05$).

RESULTS

In general, the pork-based diet positively affected species fitness, with both *Dermestes* species larvae being ca. 1.1 (*D. caninus*) and 1.7 (*D. maculatus*) times heavier on this substrate, than on dog food (Figure 1). Diet type also impacted the development time for both beetle species. *Dermestes maculatus* fed dried pork needed 21.2 ± 4.5 d to develop to the adult stage, which was 0.5 d less (on average) than in dog food ($F_{2;18}$ = 29.998; $P < 0.0001$ - Table 1). Unfortunately, comparisons could not be made with *D. caninus* since their larvae did not survive until the adult stage on dog food treatments. Curiously, larval density did not affect any dependent variables for both *Dermestes* species (Table 1). The larval life-history traits for both species were not affected by the interaction of larval densities and type of diet (Table 1).

Correlation matrices were conducted for each species with consideration of the influence of food type on the larval parameters monitored. As no *D. caninus* larvae completed development when fed dog food, only a simple Pearson correlation (using larval parameters) was carried out for this species in this type of food. *Dermestes caninus* showed a strong and significant correlation between length and weight on the dog food diet (r = 0.94; $P < 0.05$). *Dermestes maculatus* presented only significant correlation between larval length and weight (r = 0.92; $P < 0.05$) when fed dog food; however, no correlation was determined with development time or larval fitness. Larval length and weight were correlated for members of both species on the pork diet (*D. caninus*: r = 0.90; P < 0.05; *D. maculatus*: r = 0.82; P < 0.05). Finally, there were no differences in development time of *D. maculatus* between the colonies with and without handling, irrespective of diet ($F_{1;36}$ = 0.877, $P = 0.355$). All *D. caninus* specimens on the dog food diet died prior to adulthood, which did not allow us to make a comparison in relation to contact.

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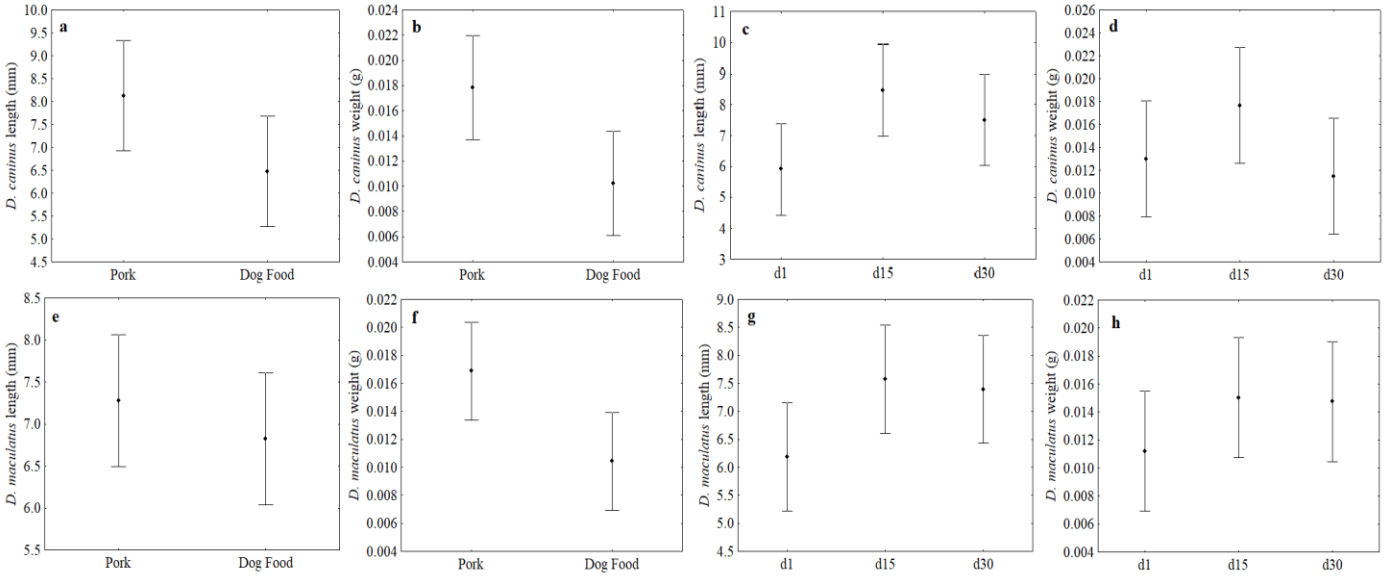

Figure 1 Mean and standard deviation from life-history trait parameters (larval length and weight) for *Dermestes caninus* in different type of diet (a, b) and larval densities (c, d); and for *Dermestes maculatus* in different type of diet (e, f) and larval densities (g, h).

Table 1 Univariate results of factorial ANOVA for both *Dermestes caninus* and *Dermestes maculatus* according to type of food and larval density. Significant results are in bold; NA (not applicable)

D. caninus	Length		Weight		Development Time	
	F	*P*	F	*P*	F	*P*
Diet	**4.134**	**0.057**	**7.502**	**< 0.001**	**29.593**	**NA**
Density	3.320	0.059	1.786	0.196	0.646	0.536
Diet*Density	1.150	0.339	1.032	0.377	0.646	0.536
D. maculatus	-	-	-	-	-	-
Diet	**0.738**	**0.402**	**7.541**	**< 0.001**	**29.998**	**< 0.001**
Density	2.726	0.092	1.083	0.360	0.852	0.443
Diet*Density	1.954	0.171	1.298	0.297	0.713	0.504

DISCUSSION

Results from the current study demonstrate diet type impacts life-history traits of *D. maculatus* and *D. caninus*, affecting both bionomic factors (larval parameters) and survival. The pork-based diet resulted in accelerated growth and final length and weight when compared to the dog food. However, larval aggregation had no influence on life-history traits of either species. Interestingly, *D. caninus* did not survive when fed dog food, irrespective of other treatment conditions. This study is, to the best of our knowledge, the first to test the effect of larval aggregation and type of diet on *D. caninus*.

Dermestes species are known as a pest of stored products and are colonizers of carrion in the late stages of decomposition [8]. Nonetheless, generalizations about diet and aggregation on associated development cannot be drawn for the genus, based on results from the current study. Both species had faster development and higher growth (both length and weight) when fed with a dry pork diet, and only *D. maculatus* completed development on dog food [26]. Osuji [27] argues that low protein content within food substrates may delay development time from larvae to adults as well as reduce survival on *D. maculatus*. However, protein levels in both diets were similar (e.g., 6.5 g of protein into a 30 g of food). Thus, other factors such as fat and moisture, may have affected the development time of both species. For example, Cambron *et. al.* [28] observed that *Manduca sexta* (Linnaeus, 1763)

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(Lepidoptera: Sphingidae) caterpillars fed high fat diets had higher mortality rates and lower body masses. Similarly, feeding blow flies with high fat diets increased mortality rates and suppressed development [29]. In contrast, blow flies fed diets with low levels of fat had enhanced longevity, higher body sizes and lifespan [30].

The absence of a proper pupation site has an influence on the survival rates or time of development of pupae since cannibalism of larvae and pupae by other larvae is possible [19]. According to Fontenot *et al.* [26] the amount of refugia is as important as the material of it, and it is necessary to avoid cannibalism. In the current study, pupation behavior was observed when larvae burrowed into pieces of pork and, sometimes, dog food. Difficulties in locating pupation sites in the dog food treatment, possibly due the small size of the grains, could have been an accessory factor delaying the development times and increasing mortality rates.

Among carrion fauna, dipteran larvae aggregate as a mean to increase temperature, accelerating their development and allowing survival in lower environmental temperatures [18]. Blow fly aggregation has also been demonstrated to influence larval mortality rates and pupal weight [18, 31]. However, this behavior is not common in Coleoptera. Even though some level of aggregation can be observed in the field (corpses and carcasses), this behavior had no significant effect on larval development in the current experiment. However, a recent study involving *D. maculatus* showed that when placed in containers at a high larval density (60 individuals), mortality can reach 30% [32]. It is reasonable to think that the larval density increases intraspecific competition, leading to decrease of larval parameters and inflating mortality. Considering the outcomes of the present study and those found by Zanetti *et al.* [32], *D. maculatus* seems to have a threshold for the triggering of competitive behavior.

Although *D. caninus* is a species of forensic importance, information on its dietary habits and immature behavior is scarce. Data generated through the current study could serve as a foundation for potential application of this species as an indicator of time of colonization in relations to a minPMI. However, validation is still needed to determine the accuracy and precision of these calculations.

CONCLUSIONS

Artificial diets are not ideal for conducting studies to determine development time of forensically relevant arthropods when the aim is to apply these data in investigations. For this reason, even though *D. maculatus* can survive on both diets (pork and dog food), it is highly recommended to use pork when rearing for case validation. Although a *D. caninus* could be maintained in a colony with dog food as a diet, mortality can be high. This study represents the first publication with biological data for *D. caninus*. Future research should focus on possible competition effects between *D. maculatus* and *D. caninus*, as the presence of both species on the same corpse was not observed under field conditions (personal observation). Other future directions for investigation include the influence of macronutrients on both species' development, and food preference experiments. More sophisticated knowledge of factors that have influence on those *Dermestes* species may be of great importance for carrion ecology studies or PMI estimates.

Conflict of Interest

Authors declare none

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Authors' Contribution

Rodrigo C. Corrêa: Initial drafting and critical editing of manuscript; study concept and design; data collection; interpretation of results; manuscript writing.
Rodrigo R.F. Carmo: Study concept and design; statistical analysis and interpretation of results; manuscript writing.
Ann R. George: Data collection; manuscript writing.
Jeffery K. Tomberlin: Study concept and design; interpretation of results; manuscript writing.

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