Effect of Crop Volume on Contraction Rate in Adult House Fly

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ABSTRACT The functional aspects of the adult house fly crop have not been studied even though various human and domestic animal pathogens have been discovered within the crop lumen. The average volume consumed (midgut and crop) by flies starved for 24 h was 3.88 μ l by feeding both sexes on a sucrose phosphate glutamate buffer. In addition, various volumes of a solution (0.125 M sucrose plus Amaranth dye) were fed to 3-d-old adult female house flies to quantify the crop contraction rate as affected by crop volume. As crop volume increased, the contraction rate increased until it reached a peak at 2 μ l, after which it declined. It is hypothesized that the high contraction rate of the crop, which in house fly is almost twice the rate of three other fly species, is one of the factors that makes house fly an excellent vector. The mechanism for such a high contraction rate needs to be investigated.

KEY WORDS dipteran diverticulated crop, pathogen transmission, crop volume–contraction rate, trachoma, *Chlamydia trachomatis*

Adult *Musca domestica* L., the common house fly, is an established vector of many disease-causing agents (Greenberg 1973). Recent studies showed that adults can vector *Escherichia coli*, the pathogen is found in the crop, and probably disseminated by regurgitation of crop contents (Sasaki et al. 2000, Wasala et al. 2013). House flies were implicated as vectors of *Chlamydia trachomatis* (Forsey and Darougar 1981, Emerson et al. 1999), and no studies have been conducted since these reports have implicated or focused on the house fly crop organ as a potential site for storage of this pathogen.

The dipteran crop is vital to the fly because it not only acts as a storage organ for nutrients, but its contents are also used to moisten dry food through a process called regurgitation (Graham-Smith 1914, Stoffolano and Haselton 2013). Recently, however, Wasala et al. (2013) suggest that *E. coli* can multiply in house fly regurgitant. Over the years, extensive research has been done on the crop in *Phormia regina* Meigen (Dethier 1976, Solari et al. 2013); however, this fly is not as medically or veterinarily important as the house fly. Our study focused on the house fly because it is expected that *M. domestica* would provide an excellent model system for investigators without access to *Musca sorbens* Wiedemann to study *Chlamydia trachomatis* (Busacca 1935) transmission.

The crop is a diverticulated foregut organ whose duct enters the foregut just before food entering the

cardia or proventriculus, which then leads into the midgut. The transport of food in and out of the crop is facilitated by coordinated contractions of visceral muscles that surround the crop cuticle in the form of general peristalsis or contractions involving both the crop lobes–pumps and the opening and closing of valve-like sphincters in the crop duct (Thomson and Holling 1975a). “Proper crop function depends on muscle relaxation during food ingestion and rhythmic muscle contractions to push food back into the midgut for digestion and absorption” (Haselton et al. 2004), which is why the number of contractions in the crop is so important. In addition, both crop contractions and volume are essential for the fly to perform “bubbling” behavior, which is when the crop contracts pushing the crop contents out onto the proboscis tip in the form of a droplet (i.e., not a true bubble; Stoffolano et al. 2008). Unlike blood-feeding Diptera, which get rid of excess water in the bloodmeal via the anus, nonblood-feeding flies get rid of excess water in their meal through “bubbling” behavior (Hendrich et al. 1992). Most flies reingest this droplet and some share it with the female as a nuptial gift (Stoffolano and Haselton 2013), but some like *Bactrocera tyroni* (Froggatt) (Coronado-Gonzalez et al. 2008) and house fly generally deposit the droplet on the substrate, which in the house fly literature (Greenberg 1973) is recorded as a vomit spot (i.e., really regurgitant as it only comes from the crop and not the midgut).

This study was conducted to examine the effects of crop lobe volume on crop lobe contraction rates and to determine the amount of sucrose phosphate glutamate buffer (i.e., used to culture *Chlamydia tracho-*

Dedication: This article is dedicated to Dr. Bernard Greenberg who pioneered the interaction between flies and the pathogens that cause disease. His two volume series, *Flies and Disease*, will remain a major source of information for future researchers.

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matis; Brunham et al. 1985) a fly starved for 24 h will consume.

Materials and Methods

Maintaining Flies. A colony was started from the Florida, U.S. Department of Agriculture (USDA) house fly colony maintained by Dr. Geden. Housing and rearing procedures followed those described by Hogsette (1992).

Feeding Procedure Before Bioassay. Only females were fed 0.125 M sucrose solution for 2 d, and then on the third day they were starved for a period of 18 h with only access to water. This time period showed by dissection and examination of the crop that after the 18-h starvation period, the crop was completely empty. After the starvation period, flies were cold-immobilized and each fly was waxed down by covering the wings with Tackiwax (Fisher Scientific, Pittsburg, PA). All legs were then removed using No. 5 forceps. Using a microcapillary pipette, each fly was then fed 1 ml of 0.125 M sucrose containing 0.02 M Amaranth solution. This was repeated using different volumes of 0.125 M sucrose with 0.02 M Amaranth. Ten females were tested for each volume. Immediately after feeding, flies were dissected.

Dissections and Bioassay. Females were restrained as described above in the Feeding Procedures Before Bioassay section. The abdomen was opened using No. 5 forceps by tearing open the abdomen at all the ventral intersegmental membranes until the crop was exposed. With the crop exposed, 200 μ l of a physiological saline (Haselton et al. 2004) was added using a pipette, which prevented the specimen from drying out. The fly was left for 1 min to adjust to the surgery. Then, using a dissecting microscope, a spot was visually picked on the crop lobes and counts always focused on that same spot area for each fly. The number of the muscle contractions of the crop lobes was counted for 1 min. This same procedure was repeated until 10 flies for each treatment volume were tested.

Sucrose Phosphate Glutamate Buffer (SPG) Feeding Studies. Because *Chlamydia* is maintained in a SPG buffer, it was necessary for future vector competence studies to determine how much of the SPG adults would ingest. To ensure that the flies would consume the SPG, they were starved for 24 h before the experiment. Unlike the previous experiment, 25 flies of both sexes were used. Each fly was individually cold-anesthetized (2–4 min) and placed into its own cup and numbered. The SPG contained 7.5 g of sucrose, 0.052 g of KH_2PO_4 , 0.122 g of Na_2HPO_4 , and 0.072 g of glutamic acid dissolved with 100 ml of distilled water. Flies were cold-anesthetized (2–4 min) and placed on ice before weighing. After each fly was initially weighed and individually placed into a separate cup, a small piece of the SPG-soaked cotton wick was placed into the cup. Each fly was allowed to feed for exactly 1 h and then reweighed. This permitted the determination of the volume (μ l) of SPG each fly ingested as a consequence of its initial body weight mass (g). Once determined, we compared, using re-

gression analysis, fly mass (g) with microliter of SPG consumed.

Statistics Used. SAS v. 9.3 was used for all analyses (SAS Institute 2009). Analysis of variance (PROC ANOVA) was used to determine the effect of crop volume on the crop contraction rate. Differences among the means were determined by Tukey's post hoc test (SAS Institute 2009). The relationship between initial fly weight and the volume of SPG buffer consumed was determined using correlation analyses (PROC CORR).

Results

The crop contraction rates differed significantly among the different volumes tested ($F = 199.86$; $df = 5, 54$; $P < 0.0001$). The average contraction rate per minute and the SE for the different concentrations of solution were determined (Fig. 1). When graphed, the data showed that as the crop volume increased, the number of contractions per minute increased and reached a peak at 2 μ l and then declined to a contraction rate at 4.5 μ l, which was not significantly different than at 1 μ l (Fig. 1).

The average weight of the flies was 0.012 g with a SE of 0.00048 and the average volume of SPG ingested was 3.88 μ l with a SE of ± 0.51 . Furthermore, there was a positive, but nonsignificant relationship between the initial fly weight and volume of SPG ingested ($P > 0.05$; Fig. 2).

Discussion

Volumes of SPG Consumed. Adult house flies starved for 24 h will consume SPG sufficient to fill the crop. The consumption volume information produced in this study is essential for future studies aimed at determining the crop involvement in house fly possibly serving either as a vector of *C. trachomatis* or being used as a model system in those areas where *M. sorbens* is not available. Even though Miller et al. (2004) showed that *M. sorbens* is a vector of the pathogen, no studies have identified the fly crop as a source for pathogen storage and involvement or tested whether house fly is also a suitable vector. The ingested volume of SPG in adult *M. domestica* reported in this study is about the same volume used to infect house flies with other pathogens (McGaughey and Nayduch 2009). Our study also suggests that the volume of SPG ingested may be dependent on the size of the fly, and the variance in Fig. 2 may also be because of sex difference, which was not considered in this study. However, future studies with a larger sample size and sorting by sex may provide a better estimate of the relationship between size and of individual flies and the volume of SPG ingested. Root (1921) showed that house fly starved for 17 h had an average intake of 3.9 μ l, while those starved for 21 h consumed 6.6 μ l (i.e., both values are within the range of values determined for flies in this study).

Fig. 1. Crop contractions per minute at varying microliter volumes of a 1 M sugar and Amaranth solution (mean $\pm$ SE). Bars with the same letters above them are not statistically different ($\alpha = 0.05$).

Crop Contraction Rate and Its Effect on Pathogen Transmission. The effect of volume on crop muscle contraction rate for house fly (Fig. 1) follows a similar pattern as that shown by Thomson (1975) for *P. regina*, but with house fly having a considerably smaller crop capacity, and twice the contraction rate. A comparison of crop contraction rates (ccr) per minute for four species of flies (*Protophormia terraenovae* (Robineau-Desvoidy): 57–62 ccr, Stoffolano laboratory (J.G.S., unpublished); *Drosophila melanogaster* Meigen: 47 ccr, Kaminski et al. 2002; and *P. regina*: 49–51 ccr, Thomson 1975, Liscia et al. 2012) determined at the maximum crop volume and giving the highest contraction rate reveals that house fly has almost twice the

adult crop contraction rate (89–92 ccr, Haselton et al. 2004 and this study) as the other flies. Thomson and Holling (1975b) demonstrated that crop contraction rate was positively correlated with crop emptying (i.e., in this case regurgitation or “bubbling”). House fly is reported to produce many more regurgitant spots compared with fecal spots (Graham-Smith, 1914, p. 84) and this suggests that regurgitation rates are significantly higher than fecal deposition rates. Coast (2004) reported that only 3% of the water loss in house fly adults is due to excretion. In personal correspondence with him (2014), Coast stated “What I referred to as losses via salivation may correspond to regurgitation of the crop contents, but this had only a minor

Fig. 2. First order regression line (with 95% CI) showing nonsignificant, but positive relationship between initial house fly mass and the volume of SPG consumed.

role to play in total water loss, which was mainly via the cuticle and spiracles. Noninjected control flies very rarely void any urine or 'saliva,' and evaporative losses via the cuticle and respiratory system account for virtually all water loss. Control flies were given free access to water and sugar." Based on his results, one can conclude that house fly adults excrete (i.e., which does not include regurgitant) considerably less than one would think (i.e., 3% total for excretion based on his article). We propose that this difference between regurgitation and fecal rates could help explain the significance of adult house flies in the oral transmission pathway versus the fecal pathway for certain pathogens. Whether this applies to other flies needs to be investigated.

Studies have shown that the microbiome of adult house flies is large (Gupta et al. 2011) and that various pathogens may be stored in the crop (Sasaki et al. 2000, McGaughey and Nayduch 2009), while others have implicated the crop as a major site for the pathogen storage and transmission process (Sasaki et al. 2000, Calibeo-Hayes et al. 2003). It also has been demonstrated as a site where horizontal transmission of antimicrobial resistance takes place (Petridis et al. 2006, Macovei and Zurek 2006).

In conclusion, the crop of *M. domestica* is an extremely important organ that ultimately influences the fly's locomotor activity, rate of feeding, and regurgitation propensity. The high contraction rate of the house fly crop muscles may account for its being such an important vector.

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