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Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2016.06.002

Comparison of sucrose intake and production of elimination spots among adult *Musca domestica*, *Musca autumnalis*, *Phormia regina* and *Protophormia terraenovae*

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ARTICLE INFO

Article history:
Received 17 Feb 2016
Received in revised form 22 Mar, 2nd revised form 30 Mar 2016
Accepted 10 May 2016
Available online 11 Jun 2016

Keywords:
Regurgitation
Defecation
Non-hematophagous Diptera
Pathogen transmission
Food safety

ABSTRACT

Objective: To compare the differences in intake and excretion between *Musca domestica* and other three species from families Muscidae and Calliphoridae which may help explaining the significance of house fly in the transmission of pathogens.

Methods: The four adult species were supplied with two concentrations of sucrose via modified capillary feeder assay system. The two sucrose concentrations were applied to one adult male/each experiment and the elimination spots were counted. Using 0.25 mol/L sucrose + 0.25% bromophenol blue, one active non-starved male/cup was observed carefully for 1 h to record its behavior. As a growing medium used in bacterial transmission experiments, undiluted trypticase soy broth was used to feed 3-day-old females and males of *Musca domestica* following two different diets upon emergence and the frequency of elimination spots was estimated.

Results: The two *Musca* species have half the weight of the two *Phormia* species. Comparing the volume of intake per hour, house fly took as much as the other species, all of which were larger. House fly produced twice, or more, the number of elimination spots/h than the other three species. Feeding the flies a sugar liquid diet resulted in producing more fecal spots than regurgitation spots. The male house flies produced less elimination spots/h when fed with trypticase soy broth than with the two sucrose solutions.

Conclusions: House flies eliminated more than the other examined fly species and most of these elimination events were defecation which implicates the fecal route for pathogen transmission by this important vector.

1. Introduction

Musca domestica L. (*M. domestica*) often regurgitates and defecates. Due to these elimination events, it is an extremely important vector for both veterinary and medical entomological research because it is able to transmit important pathogens to humans and their domestic animals. The topics of fly regurgitation and defecation are being intensively investigated, and both can result in pathogen transfer. These two elimination events were shown to be significant for transmission of various pathogens [1,2]. Regurgitation behavior by non-hematophagous Diptera has diverse functions depending on the species [3]. In addition to its role in removing excessive water from the crop, regurgitation can be involved in pathogen dissemination [4]. However, defecation is also involved in pathogen transmission [5]. In addition to its importance, as stated above, regurgitation and defecation have also recently taken the forefront in forensic entomology [6,7].

Since we proposed that the difference between regurgitation and defecation rates could help to explain the significance of adult *M. domestica* in either the oral transmission pathway or the fecal pathway for certain pathogens [8], it was essential to investigate whether this applies to other flies. This study was conducted to examine these differences amongst four different

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Foundation Project: Supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, the Massachusetts Agricultural Experiment Station and the Stockbridge School of Agriculture at the University of Massachusetts, Amherst under project number of MAS00448.

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

species of adult flies and to compare the intake per hour of each species as related to fly size. Also, we wanted to study the house fly elimination rate after feeding trypticase soy broth (TSB), a growing medium which is used in bacterial transmission experiments and to follow the origin of the small spatters observed after feeding TSB [1].

2. Materials and methods

2.1. Insect colonies

M. domestica, *Musca autumnalis* De Geer (*M. autumnalis*), *Phormia regina* Meigen (*P. regina*) and *Protophormia terraenovae* Robineau-Desvoidy (*P. terraenovae*) were reared in a 16:8 h light/dark cycle at (28 ± 2) °C and 50% relative humidity [9,10]. All experiments were conducted under these same laboratory conditions. Males of all species were separated at emergence with their wings clipped for ease of handling, and then placed in metal screened cages where they were given only granulated sucrose and water for two days. For most experiments, only males were used in order to avoid the compounding effects of ovarian development [1]. If females were used or diets changed, it will be noted. During all experiments, one to two flies/replicate died and have not been counted in the results.

2.2. Feeding procedure for intake determinations

When 3-day-old, all species were weighed, transferred to 90 mm plastic Petri dishes (one male/Petri dish), and offered with various diets using a modified capillary feeder (CAFE) assay system [11]. Glass microcapillary tubes (Drummond Scientific®, Broomall, PA) were filled with 25 μL of 0.25 mol/L sucrose + 0.25% of the non-toxic, pH-sensitive bromophenol blue sodium salt dye (Sigma®B5525, St. Louis, MO) using a Hamilton® microsyringe. The 0.25% dye solution was made using the 0.25 mol/L sucrose solution as the stock solution and, when mentioned elsewhere in the paper, this is how it was made. Descent of the liquid diet in the microcapillary tubes was clearly visible every 3 h, allowing continuous and unambiguous measurement of consumption and permitting refilling to the 25 μL level; thus determination of consumption over a 12 h period was performed. The experiment was repeated three times with 10 flies/replicate for each species. Two Petri dishes, without flies, were used as evaporation controls to calculate the net intake value. The same procedure was repeated using 0.5 mol/L sucrose + 0.25% bromophenol blue (using the 0.5 mol/L sucrose as the stock solution to make the dye solution). The two concentrations of sucrose were used to detect any concentration effect.

2.3. Estimation of the frequency of elimination spots using sucrose solutions

Non-starved, 3-day-old males of each species were placed individually into a test plastic cup. Initially, each cup contained 0.25% bromophenol blue solution added to a 0.25 mol/L sucrose solution soaked in cotton. The soaked cotton was placed into a small bottle cap on top of the Whatman No. 5 filter paper (Sigma®, St. Louis, MO), which was then placed into a 500 mL transparent plastic test cup with a transparent lid. After 24 h, flies were moved to a new test cup and spots on the original cup were counted on the filter paper, the sides and lid of the container. The experiment was repeated with 10 flies for three consecutive days and, the whole experiment was repeated three times/each species. The same procedure was repeated using 0.5 mol/L sucrose with bromophenol blue as well.

2.4. Behavioral experiment

The procedure was similar to the previous experiment using 0.25 mol/L sucrose + 0.25% bromophenol blue. After 24 h from the beginning of the experiment, one active fly/cup was carefully observed for 1 h to record its behavior and to determine the percentages between regurgitation and defecation spots. Behavioral observations were made with 15–25 flies/each species in three replicates.

2.5. Estimation of frequency of elimination spots by *M. domestica* using TSB

The test containers were prepared and the solutions used were identical to those in the above experiment, but this time the larger container was supplied with one female or one male *M. domestica* (non-starved and 3-day-old). The experiment consisted of two groups of *M. domestica* given access to one of the following diets upon emergence: one group received granulated sucrose + water, while the other group received granulated sucrose + water + powdered milk. On Day 3, flies were placed into the larger test containers. The experiment was similar to the previous procedure using undiluted TSB (Sigma®, St. Louis, MO) instead of sucrose, supplemented with 0.25% bromophenol blue. After removal of the flies, colored spots on the filter paper, sides and lid were counted and the number of elimination spots produced/fly recorded. The spatters or very small spots were not counted but recorded as tiny spots. The experiment was replicated three times with 10 flies for each sex given each of the two accessible diets.

2.6. Statistical analysis

One-way ANOVA was performed by using Statistical Package for the Social Sciences version 13.0. Means were compared using student Newman–Keuls multiple comparison tests where significant level was set at $P < 0.05$.

3. Results

3.1. Determination of sucrose intake relative to body weight

The intakes of sucrose solution showed that all species consumed significantly less concentrated solution per hour than the diluted one (Table 1). *M. domestica* consumed significantly more 0.25 mol/L sucrose than *M. autumnalis* and *P. terraenovae*, and non-significantly less than *P. regina* ($F = 6.092$; $P < 0.01$ for the four species). At the 0.5 mol/L concentration of sucrose, *M. domestica* consumed non-significantly less sucrose than *P. terraenovae* and *P. regina*, but significantly more than *M. autumnalis* ($F = 1.279$; $P = 0.301$ for the four species).

Table 1
The intake of different concentrations of sucrose by 3-day-old males of *M. domestica*, *M. autumnalis*, *P. regina* and *P. terraenovae*.

Species	Weight of fly (mg)	Intake of sucrose (range) (μL/h)	
		0.25 mol/L	0.5 mol/L
M. domestica	12.45 ± 0.03	1.50^{a} ± 0.12 (0.56–1.66)	$0.53^{*,a}$ ± 0.11 (0.22–0.86)
M. autumnalis	20.40 ± 0.01	1.14^{b} ± 0.14 (0.64–1.82)	$0.30^{*,b}$ ± 0.08 (0.06–0.72)
P. regina	42.08 ± 0.02	1.82^{a} ± 0.19 (0.62–2.32)	$0.63^{*,a}$ ± 0.18 (0.11–1.53)
P. terraenovae	46.90 ± 0.03	1.04^{b} ± 0.14 (0.55–1.49)	$0.54^{*,a}$ ± 0.13 (0.08–0.87)

Values were expressed as mean ± SE. *: Significant at $P < 0.05$ within the same species. Means with different letters are significantly different from each other between different species ($P < 0.05$).

Results showed that the weight of *M. domestica* was about 25% less than that of the two larger species, and it was about 60% the weight of *M. autumnalis*; it had a significantly higher consumption rate/mg body weight than the other three species at both sucrose concentrations (Figure 1).

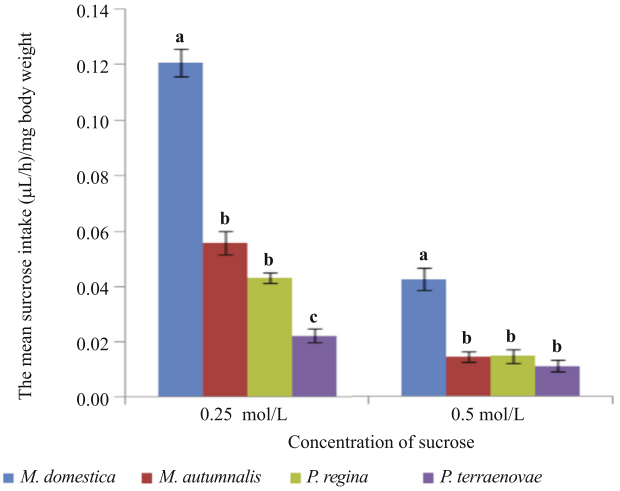

Figure 1. Mean sucrose intake/mean body weight of 3-day-old males of *M. domestica*, *M. autumnalis*, *P. regina* and *P. terraenovae* fed with different concentrations of sucrose.
Values were expressed as mean ± SE, $n = 10$ with three replicates. Means with different letters are significantly different from each other ($P < 0.05$) at the same sucrose concentration.

3.2. Estimation, location and size of the elimination spots of flies fed with sucrose solutions

The term elimination spots or just spots refers to both regurgitation and defecation spots added together. *M. domestica*, being the smallest, produced significantly more elimination spots compared to the other species, especially for *P. terraenovae* that *M. domestica* produced more than three times the number of spots when fed with both sucrose concentrations (Table 2) ($F = 21.934$; $P < 0.0001$ for the four species fed with 0.25 mol/L sucrose and $F = 67.647$; $P < 0.0001$ for the four species fed with 0.5 mol/L sucrose).

The mean number of elimination spots produced was significantly higher within the same species when using diluted sucrose solution (0.25 mol/L) compared to concentrated one (0.5 mol/L). In the two *Musca* species, the number of spots on the sides and lids of the cup constituted about a third and fifth of the total number of spots for both sucrose concentrations, respectively. The two blow fly species preferred to deposit spots on the filter paper rather than on the sides or lids (*i.e.*, their spot production on sides and lids together was 6.01% or less of the total number of spots produced). By measuring spot diameter in the same species, a slight non-significant increase was found at 0.5 mol/L sucrose concentration (Table 2). As the weights of the blow flies were heavier than those of *Musca* species, the diameters of their elimination spots were significantly larger ($F = 174.136$; $P < 0.0001$ for the four species fed with 0.25 mol/L sucrose and $F = 59.071$; $P < 0.0001$ for the four species fed with 0.5 mol/L sucrose).

3.3. Regurgitation versus defecation

The percentage of defecation spots to elimination spots was 93.76% for *M. domestica* and more than 97% for the other three species (calculated from Table 2) (*i.e.*, 12–20 fecal spots/h for muscid flies and 8–14 fecal spots/h for calliphorid flies). The defecation spots were round or pear-shaped with a string-like filament coming from the anus; they had a violet color and were considerably bigger than regurgitation spots, which were round-shaped with a navy blue color. Tear- or pear-shaped defecation spots were produced only when the fly moved during defecation. When the cotton piece was too saturated with sucrose dye solution, the labellum, tarsi and, less often, the anus of the flies were seen to touch the filter paper immediately after food intake, leaving minute spatter spots around the small cup of food. After 24 h, these spatters exceeded one hundred in number/observation dish. Spatters on filter paper were never noticed in the CAFE system feeding experiments. Spatters could be easily separated from regurgitation or defecation spots due to their small size. Long time touches of the labellum to the food (0.2–1.0 min) usually immediately led to one defecation spot. However, short time touches to the food source were noticed and they were exceedingly fast. Sometimes, the fly lingered around the cup for 10–15 min or bubbled for several minutes and defecated without touching the food. During all the observations and for all species, flies never came near their spots (regurgitation or defecation) to re-ingest them whether they were still in liquid form on the sides and lid of the container or whether they were dry on the filter paper. In other words, flies never re-ingested their own liquid regurgitation or fecal material. The blue color of bromophenol blue dye began to appear in bubbles immediately after ingesting food and in defecation spots after 18–22 min from the beginning of the experiment.

3.4. Feeding related behavioral observations on all species

All flies bubbled (*i.e.*, really a droplet and not a hollow bubble) by consistently standing at one place without moving and produced 1–10 bubbles per hour. Flies spent more time with larger bubbles and vice versa. Flies usually re-ingested the bubble even if they stood still with the bubble for 9 min (range of 4 s to 9 min). Some flies didn't bubble at all under the same conditions and for the same species (1–2 flies/replicate for each species failed to bubble). Crop volumes of flies were not determined with respect to bubbling or non-bubbling. The effect of fly density on bubble production was not studied.

Table 2
The number, percentage and size of elimination spots for 3-day-old males of *M. domestica*, *M. autumnalis*, *P. regina* and *P. terraenovae* fed with different concentrations of sucrose.

Species	No. of spots/h (range)		% of spots on sides and lid of the cup to total no. of elimination spots		% of regurgitation spots to total no. of elimination spots/h	Diameter of elimination spot (range) (cm)	
	0.25 mol/L	0.5 mol/L	0.25 mol/L	0.5 mol/L	0.25 mol/L	0.25 mol/L	0.5 mol/L
M. domestica	7.15^{a} ± 0.85 (4.88–9.29)	$5.89^{*,a}$ ± 0.38 (3.13–7.50)	33.41	19.43	6.24	0.10^{a} ± 0.01 (0.02–0.19)	0.12^{a} ± 0.01 (0.02–0.23)
M. autumnalis	3.18^{b} ± 0.25 (1.38–4.21)	$2.05^{*,b}$ ± 0.22 (0.54–3.46)	34.46	22.28	2.98	0.14^{b} ± 0.01 (0.04–0.27)	0.15^{a} ± 0.03 (0.05–0.26)
P. regina	4.88^{b} ± 0.34 (3.04–6.00)	$2.62^{*,b}$ ± 0.20 (1.25–4.05)	5.44	6.01	2.33	0.27^{c} ± 0.01 (0.08–0.54)	0.28^{b} ± 0.02 (0.09–0.71)
P. terraenovae	1.77^{c} ± 0.28 (0.33–2.83)	$0.82^{*,c}$ ± 0.21 (0.17–1.77)	4.93	2.67	2.04	0.34^{d} ± 0.04 (0.10–0.75)	0.37^{c} ± 0.02 (0.11–0.95)

Values for no. of spots/h and diameter of elimination spots were expressed as mean ± SE. *: Significant at $P < 0.05$ within the same species. Means with different letters are significantly different between different species ($P < 0.05$). $n = 10$ and replicated three times.

3.5. Estimation of the frequency of elimination spots using TSB for *M. domestica*

Males fed on both diets prior to feeding on TSB produced significantly more elimination spots than females of the same group ($F = 14.850$; $P < 0.001$ for males and females fed with sucrose + water and $F = 25.587$; $P < 0.0001$ for males and females fed with sucrose + water + milk) (Figure 2).

The male house flies fed on TSB produced less elimination spots compared to those fed on the two sucrose solutions. Males fed on water + sucrose prior to TSB produced a total of 4.33 spots/h while those fed on water + sugar + milk produced a total of 3.86 spots/h (Figure 2).

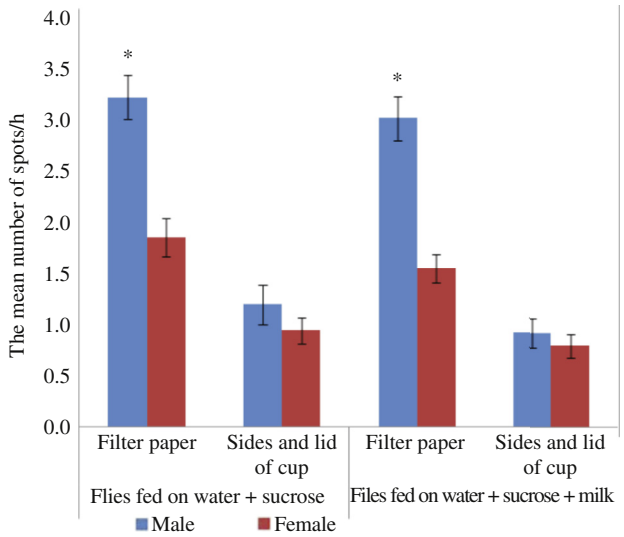

Figure 2. Difference in the number of elimination spots produced by male and female *M. domestica* fed on TSB following two days feeding on two different diets.
Values were expressed as mean ± SE. *: Significant at $P < 0.05$ compared with female. $n = 10$ and three replicates.

4. Discussion

4.1. Intake and elimination spots

All species took more of the diluted sucrose solution and this agrees with the previous results for *P. regina* [12]. The surprising result was that *M. domestica* imbibed significantly more than *M. autumnalis*, but there was little difference when compared to *P. regina* and *P. terraenovae*. This difference in intake only makes sense when one looks at the greater number of elimination spots for *M. domestica*. *M. domestica* produced significantly more elimination spots than the other three species, thus leaving more room in their digestive system to explain for a greater consumption.

4.2. Regurgitation and defecation

This study showed that house flies eliminated more than the other examined fly species and most of these elimination events were defecation. Our statements in this study and what we conclude or suggest are based on flies only fed on a liquid sucrose diet. On other diets, the situation might be different as the rate of fly elimination seemed to vary with the kind of food and the temperature [13]. Also, movement, feeding, regurgitation and defecation between *Calliphora vicina* Robineau-Desvoidy and *Lucilia sericata* Meigen were different and depended on the state of the diet (*i.e.*, liquid or dried) [7].

In the current study, the defecation spots were bigger and easily differentiated from regurgitation spots. In previously published studies, most authors failed to describe how they differentiated the spots, which spots were from regurgitation, and which from the anus. They usually mentioned spots for both regurgitation and fecal spots combined [1,14], or sometimes they were identified as regurgitation spots only [15]. Few reports vaguely stated that the two different spots were easily distinguishable [16]. In our study, both spot types varied from 0.10 to 0.12 cm (1.0–1.2 mm) in diameter, which falls within the range reported by others [7,14]. On the contrary, regurgitation spots based on scanning electron microscope varied from 200 to 500 μm in diameter [17]. In this study, we specified the type of the filter paper, while most researchers just stated filter paper or card stock. This can be misleading because each filter paper number or card stock will produce a different size for the same measured drop. Most studies on defecation of flies focused on post-feeding diuresis. A new technique using *Drosophila melanogaster* provided both a novel/model way of doing this [18]. The most extensive study to date on adult fly defecation was on adult *D. melanogaster* [19]. The researchers examined, using various genetic probes, the neural and hormonal mechanisms involved in gut function and focused on the impact of fluid intake and ionic balance on defecation spots.

4.3. Behavior

Both *Musca* species spent significantly more time on the sides and lids of the cups than the two blowfly species. The cause of this disparity in activity between the two groups needs further investigation. A previous study found that *P. regina* exhibited preferences for particular areas of the experimental containers and, also that preference patterns changed with fly density [20]. We noted that a few flies of each species failed to bubble and bubbling flies did not move. Previous studies assumed that a fly would bubble if a specific crop volume was reached and the essential bubbling/crop volume somehow caused the flies to remain still [21]. In the current study, *M. autumnalis*, *P. regina* and *P. terraenovae* seldom dropped their bubble, but *M. domestica* did. The fact that house flies often dropped their bubbles while the other species did not is another factor that makes house fly a good vector. We did not study the effect of fly density on flies dropping their bubbles, but this external disturbance from other flies might cause flies to drop their bubbles in nature.

We found that the minute spatters around a container filled with TSB were derived from frequent contacts by house fly contaminated mouthparts, tarsi and anus. Also, we recorded that flies sometimes contacted the food source for only a few seconds. Graham-Smith noted that flies can obtain within few seconds enough nutrients to keep them alive for days [13]. This short contact with a diet is significant, as the flies can pick up pathogens on the labellum and tarsi in a short time to make them major pathogen transmitters [22,23].

In our study, when flies were fed with a liquid sucrose diet, we never observed a single fly attempting to re-ingest its own regurgitation or feces. This is in contrast with another report which found that flies fed on blood re-ingest their own artifact (*i.e.*, a blood spot they previously created but now was dry) [7]. Differences between the above study and ours may be due to blood having an odor while sucrose has none. Also, flies in their study might have been in a different reproductive state (*i.e.*, protein starved).

This study was essential for our current work on *Vibrio cholerae* because it directs one's attention to the possibility that the route of pathogen transmission in *M. domestica* might be through feces rather than through the oral route. Future comparative studies using different foods are essential to prove our current findings on the best transmission route of pathogens. Diet and the pathogen species involved may also influence which route of infection is important.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Thanks to Alan Thomson for re-reading the manuscript following the initial review process. Thanks to Tyler Godek and Bhavi Patel for assisting in data collection and rearing the flies. Roger Moon was essential by providing pupae of *M. autumnalis*. This study is based upon research supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, the Massachusetts Agricultural Experiment Station and the Stockbridge School of Agriculture at the University of Massachusetts, Amherst, under project number MAS00448 to Professor Stoffolano. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the U.S. Department of Agriculture or National Institute of Food and Agriculture. Appreciation to the Egyptian Cultural Affairs and Missions Sector, Ministry of Higher Education and Faculty of Science, Cairo University, Egypt for their support to Dr. El-Bassiony for her research in the Stoffolano laboratory.

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