Journal of Insect Physiology 59 (2013) 552–559

Contents lists available at SciVerse ScienceDirect

Journal of Insect Physiology

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

Water balance characteristics of pupae developing in different size maggot masses from six species of forensically important flies

David B. Rivers [a,*], Jay A. Yoder [b], Andrew J. Jajack [b], Andrew E. Rosselot [b]

[a] Department of Biology, Loyola University Maryland, Baltimore, MD 21210, USA
[b] Department of Biology, Wittenberg University, Springfield, OH 45501, USA

ARTICLE INFO

Article history:
Received 27 November 2012
Received in revised form 5 March 2013
Accepted 6 March 2013
Available online 18 March 2013

Keywords:
Heat stress
Dehydration tolerance
Blow fly
Scuttle fly
Flesh fly

ABSTRACT

The impact of maggot mass size on body water content, net transpiration rate, and dehydration tolerance of fly pupae was examined in six species of necrophagous flies. Species that spent more time on food as larvae, produced pupae with high body water contents. Dehydration tolerance limits of pupae were modest, matching the moisture-rich conditions of decaying carrion for larvae. *Protophormia terraenovae* pupariates on food as it dries, and this was reflected by pupae having the highest body water content and lowest net transpiration rate. *Megaselia scalaris* featured the lowest body water content and highest dehydration tolerance, implying that this species is arid-suited, which matches its ability to feed and colonize on post-decay carrion. *Lucilia illustris* was the most sensitive to larval overcrowding, resulting in a dramatic decrease in pupal size, early dispersal from food, fed less and had fast net transpiration rates. By contrast, *Lucilia sericata* was the most resistant, by showing no pupal size decrease and no change in net transpiration rate. Other species were between these extremes, requiring larger maggot mass sizes to produce the effect of decreasing pupal size and increasing net transpiration rate. We conclude: (1) the pupa's response to overcrowding and water balance profile are species-specific, varying according to pupal size and net transpiration rate as independent characteristics; (2) water balance profile of the pupae reflects the behavior and microhabitat of the larva; and (3) danger of lethal desiccation to smaller-sized pupae is circumvented by a faster developmental rate rather than enhanced water conservation.

1. Introduction

During infestation of carrion tissue by necrophagous fly larvae, overcrowding and heat production in large masses of feeding larvae (e.g., maggot masses) can lead to physiological stress, including food shortage and heat stress (Villet et al., 2010; Rivers et al., 2011). Such stressors commonly have the profound effect of producing smaller-sized pupae (Kamal, 1958; Rivers et al., 2010). The reduced-sized pupae yield truncated adults, and at least in the case of calliphorids, when this occurs due to development in large larval aggregations, leads to an increase in developmental rate, i.e., shorter duration in puparia than adults arising from smaller larval assemblages. Interestingly, this same trend of reduced length of puparial development does not occur with species of sarcophagids that have been tested under similar conditions (Byrd and Butler, 1998; Rivers et al., 2010). Besides a reduction in pupal size, another consequence of stress from maggot mass size is that smaller pupae from larger maggot masses have a more 'fragile' puparium, the tanned cuticle of the old third instar larva that encases the pupa, in that smaller puparia do not appear to be as thick and generally are more susceptible to damage when handled by comparison to larger puparia of the same flies (Rivers, pers. obs.). The capacity by these smaller-sized puparia to yield fully functional adults, albeit with reduced fecundity (Kamal, 1958; Rivers et al., 2010), necessarily implies that despite the stress imposed from maggot mass size, the pupa maintains adequate levels of body water (i.e., water balance; Wharton, 1985; Hadley, 1994), remains viable and hydrated to develop properly, and permit adult eclosion. However, the heightened evaporative water losses due to increased surface area to volume ratio for a smaller-sized pupa (Hadley, 1994) and seemingly less resiliency in the physical properties of the puparium that protects against dehydration (Yoder and Moreau, 1994) from being raised in a large maggot mass represent extreme water balance problems, especially for fly pupae and pharate adults that cannot replenish water stores by drinking or feeding, or through water vapor absorption (Yoder and Denlinger, 1991a,b; Yoder and Moreau, 1994).

Does heat stress or food shortage from maggot masses lead to alterations in the resultant pupa to maintain water balance? To answer this question, we conducted a water balance study on pupae

* Corresponding author. Address: Department of Biology, Loyola University Maryland, 4501 North Charles Street, Baltimore, MD 21210, USA. Tel.: +1 410 617 2057; fax: +1 410 617 5682.

E-mail addresses: drivers@loyola.edu (D.B. Rivers), jyoder@wittenberg.edu (J.A. Yoder), s14.ajajack@wittenberg.edu (A.J. Jajack), s14.arosselot@wittenberg.edu (A.E. Rosselot).

0022-1910/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jinsphys.2013.03.002

of six forensically relevant species: the calliphorids, *Lucilia illustris*, *Lucilia sericata*, *Phormia regina*, *Protophormia terraenovae*; the sarcophagid *Sarcophaga bullata*; and the phorid *Megaselia scalaris*. Some of these species also have relevance for maggot debridement therapy, particularly *L. sericata*, and medical-veterinary significance for inducing various forms of myiasis (Mullen and Durden, 2009). The flies selected for this study are necrophagous yet differ in aspects that potentially impact their water balance characteristics. For example, each species typically develops in large feeding aggregations, but the size necessary that convey group-feeding benefits, how tightly formed is the mass, and whether homogeneous versus heterogeneous composition is common for the masses are not the same for each species (Rivers et al., 2011). *Megaselia scalaris* develops in the most loosely structured aggregations of all the species, and it has not yet been determined whether cooperative feeding occurs or is even necessary for this fly. The flies also utilize carrion at different stages of decomposition, as the calliphorids are typically initial colonizers, sarcophagids are more commonly associated with the tail end of the first wave and into the second wave of insect colonization of animal remains, while phorids prefer later stages of decay characterized by 'drier' soft tissues (Goff, 2010).

Water balance characteristics of pupae, not adults, have been shown to be ecologically defining toward predicting habitat suitability for a fly species, in particular looking at the pupa's net transpiration rate, percentage body water content, and dehydration tolerance (Yoder and Denlinger, 1991a,b; Kleynhans and Terblanche, 2009, 2011). Our study comparing the water balance characteristics of pupae of the six different species also seeks to make a contribution to this area, with the added effect of examining whether maggot mass size influences habitat preferences if the water balance profile of pupae becomes altered. Net transpiration rate is viewed as the best indicator of moisture requirements in relation to habitat preference for a species, and this is based on the correlation that arthropods that thrive in moisture-rich habitats have high water loss rates and those that reside in drier habitats typically have low water loss rates that enhances water conservation (Hadley, 1994). Percentage body water content and dehydration tolerance limit are secondary characteristics, with the more arid-inhabiting species having low body water contents as well as high tolerance for dehydration that allows them to sustain large body water losses and remain viable (Hadley, 1994). All of the fly species that we examined co-occur and were field-collected from the same geographic area, but it is likely that their water balance characteristics differ because these physiological features tend to be species-specific (Hadley, 1994). Here, percentage body water content, net transpiration rate, and dehydration tolerance limit were determined for pupae from fly species that were raised in maggot masses of different sizes, with the purpose of constructing a water balance profile for each species. Our hypothesis is that water balance characteristics of pupae may shift to more enhanced water conservation as maggot mass size, and intensity of the stress from overcrowding larvae, increases. A second hypothesis is that some species may be more capable of making this shift toward water conservation enhancement than others, because of the species-specific nature of how water balance is achieved.

2. Materials and methods

2.1. Collection and rearing of flies

Colonies of *L. illustris* and *L. sericata* originated from larvae collected from raccoon (*Procyon lotor*) carrion placed in an urban location on the campus of Loyola University Maryland in Baltimore, MD. Pupae of *P. regina* were provided by Dr. John Stoffalono Jr. (University of Massachusetts–Amherst) and used to establish a laboratory colony.*Protophormia terraenovae* and *S. bullata* have been maintained in colony for more than 2 years. The flies were reared in the laboratory under conditions that prevent diapause (25 °C, 15-h:9-h L:D, 70–75% RH) as described (Denlinger, 1972; Rivers et al., 2010). Larvae of *M. scalaris* were isolated from infested beef liver previously exposed to adults of *L. sericata* within the Fly Laboratory at Loyola University Maryland. A colony was not established for this species; rather, larvae were 'field' collected for each experiment and raised on fresh beef liver as with the other fly species.

2.2. Instrumentation and measurements

Handling and transferring of pupae were done with an aspirator that was made by affixing a mesh-covered plastic pipette tip to a piece of Tygon tubing (Fisher Scientific, Pittsburgh, PA). An electrobalance (CAHN; SD ± 0.2 μg precision and ±6 μg accuracy at 1 mg; Ventron Co., Cerritos, CA) was used to weigh the pupae; the pupae were weighed and monitored individually, in less than one minute, and without the use of anesthesia or enclosures. Relative humidity (SD ± 3% RH) was measured using a hygrometer (SD ± 0.5% RH; Thomas Scientific, Philadelphia, PA) and was maintained in 5000-cc (l × w × h) sealed glass desiccators by placing saturated salt solutions (Winston and Bates, 1960) in the base of the desiccator. A relative humidity of 0% was provided by calcium sulfate, $CaSO_4$ (0% RH; 1.5×10^{-2}% RH, Toolson, 1978; W.A. Hammond Drierite Co., Xenia, OH). Pupae were placed, with one fly per well, into 12 depression hole porcelain spot plates (115-mm l × 90-mm w × 12-mm h; Fisher). The porcelain plate was placed inside the desiccator to expose them to a specific relative humidity. Desiccators were then placed at 25 °C, the basic experimental temperature, in programmable environmental cabinets (SD ± 1.0 °C; Fisher). A drying oven (Blue M Electric Co., Chicago, IL) set at 90 ± 2.0 °C containing Drierite was used to dry the flies to constant mass as described by Hadley (1994).

2.3. Determination of water balance characteristics

Maggot masses of 100, 500 and 1000 individuals per mass were started as first instar larvae or eggs put on 150 g of fresh beef liver: the group size of 100 is the control and represents typical rearing group size and an uncrowded condition, 1000 represents a crowded condition, and 500 falls in between these extremes (Rivers et al., 2010). None of the maggots ran out of food in any of the treatments. It is important to note that separate cohorts of these flies from each of the experimental maggot mass sizes eclosed completely with greater than 90% survival. Before beginning any water balance characteristic determination experiments, flies were desiccated by 3–5% of their body mass at 33% RH, as a standard pretreatment so that differences in mass measurements represent changes in body water levels of the pupae (Arlian and Eckstrand, 1975). Water balance characteristics were determined based on Wharton's (1985) equations, updated by Benoit et al. (2005). Flies were weighed (defined as the initial mass or fresh mass, f), placed at a specific relative humidity, and re-weighed at various intervals of time. At the end of the experiment, the flies were placed in the drying oven and dried for several days until their mass became constant, and this was defined as the dry mass, d (Wharton, 1985; Hadley, 1994).

The dry mass, d, was used to calculate the water mass, m, from the initial mass, f ($m = f - d$) for determination of the percentage body water content: percentage $m = 100 (f - d)/f$. The net transpiration rate was determined by weighing the flies that were placed at 0% RH (only relative humidity where water loss is exponential; Wharton, 1985) at different intervals of time enough to obtain five