Brief Report

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Li-Or Lahmi , Ayelet Harari , Aviv Shaish , Ido Tsurim *

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The Carotenoid Composition of Larvae Feed Is Reflected in Adult House Fly (*Musca domestica*) Body

Li-Or Lahmi [1], Ayelet Harari [2], Aviv Shaish [3] and Ido Tsurim [4,*]

1 Achva Academic College, Beer-Tuvia Regional Council 7980400, Israel ; liorlahmi@gmail.com
2 The Bert W. Strassburger metabolic center, Sheba medical center, Tel-Hashomer 5265601, Israel; harari.ayelet1@gmail.com
3 The Bert W. Strassburger metabolic center, Sheba medical center, Tel-Hashomer 5265601, Israel. and Achva Academic College, Beer-Tuvia Regional Council 7980400, Israel; avivsha30@gmail.com
4 Katif Center for R&D Coastal Desert, Ministry of Innovation, Science and Technology, Sdot Negev Regional Council, P.O. Box 100, Netivot, 8771002, Israel. and Achva Academic College, Beer Tuvia Regional Council 7980400, Israel; tsurim@gmail.com
* Correspondence: tsurim@gmail.com; +972-52-6056461

Simple Summary: Carotenoids are organic compounds with various important functions in animals. Most animals can get these carotenoids only from their food. House flies are common pests with worldwide distribution. Their larvae feed on different kinds of organic materials, that can contain different types of carotenoids. We examined if the carotenoid content in the body of adult house flies would be similar to the ones they ate as larvae. We fed house fly larvae different diets with varying carotenoid content and then checked the carotenoid content of the adult flies. Our results showed that the carotenoids in adult house flies were influenced by what they ate as larvae, but were not exactly the same. Our findings suggest that we might be able to use carotenoid composition in adult flies to figure out where house fly infestations originate from. Also, we should carefully consider what we feed house fly larvae, used for animal feed.

Abstract: Carotenoids are common and diverse organic compounds with various functional roles in animals. Except for certain aphids, mites, and gall midges, all animals acquire necessary carotenoids only through their diet. The House fly (Musca domestica) is a cosmopolitan pest insect that populates diverse habitats. Its larvae feed on organic substrates that may vary in carotenoid composition according to their specific content. We hypothesized that the carotenoid composition in the adult House fly's body would reflect its composition in the larval feed. We reared house fly larvae on substrates that differed in carotenoid composition and characterized the carotenoid composition of the emerging adults. Our results indicate that the carotenoid composition of adult House flies is related to the carotenoid composition in its natal substrate, but does not directly reflect it. We suggest that these findings may be developed to identify sources of House fly infestations. We also recommend that care should be taken when considering the rearing substrates of House fly larvae used for animal feed.

Keywords: substrate; feed; larvae; insects; chemical composition; carotenoids; house fly

1. Introduction

Carotenoids are common and diverse organic compounds [1]. Some carotenoids are known to have essential functional roles in animals [2], such as in the visual and immune systems, diapause-related processes, protection against oxidative stress, ornament-based signaling, etc. [3–6]. However, the specific carotenoid composition in most animals and the function and metabolism of most carotenoids are largely unknown. Biosynthesis of carotenoids largely occurs in photosynthetic plants, algae, and some bacteria, archaea, and fungi [7], but is limited in Animalia to certain aphids, mites, and gall midges [8]. Most animals acquire necessary carotenoids from their diet and use them as gained from the feed or modified [9,10].

Both diet and genetic background likely affect carotenoid composition in insects' bodies [11,12]. Holometabolous insects acquire most building materials of the adult body from the larval feed, during the larval stages. Hence, adult carotenoid body composition and related functions may largely reflect the larvae's available feed resources [11–13]. However, knowledge of carotenoid pathways between larval feed and adult carotenoid composition and functionality is scant.

The House fly is a cosmopolitan pest insect that poses public health hazards [14], but also a growing economic importance in the animal feed industry [15]. The maggot develops in and feeds upon rotting organic substrates. The carotenoid composition of these substrates may differ substantially between different sources. [16] report that adult House flies contained the carotenoids Lutein and Zeaxanthin, but not β-carotene. However, our knowledge of this important issue is lacking beyond the scope of the visual system.

We hypothesized that the carotenoid composition in the adult House fly's body would reflect its composition in the larval feed (substrate). Hence, the carotenoid composition of adult flies is expected to vary according to differences in the carotenoid composition of the larval feed. Alternatively, if only specific carotenoids can be used for specific biological functions, then unsuitable carotenoids should be either modified into the specific suitable ones or excreted from the body. The carotenoid composition of adult flies is then expected to be homogenous, even if carotenoid composition differs among larval feeds.

2. Materials and Methods

To examine our hypothesis that the carotenoid composition of the adult fly will reflect the carotenoid composition in the maggot's substrate, we simultaneously reared four groups of House fly maggots, originating from the same laboratory colony, each group on a different substrate. Rearing substrates were cucumber, red tomato, yellow tomato, and control. The latter, contained bran and powdered rodent chaw, and lacked carotenoids. We placed, open petri dishes, 60 mm diameter, in a cage containing a colony of 5-6 days old House flies, for 24 hours, to allow oviposition in the dishes. Each petri dish was filled with one of the four substrates, two dishes of each substrate. We then transferred the substrate containing the eggs into a rearing cup (90 mm diameter×65 mm high), containing 300 ml of the same substrate, and placed the cups in a growing chamber (29±2°C, 70% humidity, 14:10 dark:light regime) for 10 days. We then sampled the aged substrates; 3 samples of 50 mg from each substrate type, for carotenoid composition analysis, and added a layer of dry coarse sawdust, to allow suitable environment for pupation. Pupa were then collected during the next five days and hatched in separate, empty containers. Substrate samples, and five hatched adult females, from each substrate type, were then analyzed using untargeted HPLC with photodiode array detector, as described in [17], to quantify the carotenoid composition in each sample.

3. Results

We found that the four different substrates differed substantially in their carotenoid composition (Table 1). The carotenoid composition analysis of the respective adult House flies indicates that adult House flies, bred on different larval substrate types, differed in their carotenoid composition. However, carotenoid composition in the adult flies was not identical to the carotenoid composition in the substrate. Adult flies only contained carotenoids that were present in the substrate, but not all carotenoids present in the substrate were found in the adults. Moreover, Lycopene and Prolycopene, the commonest carotenoids in the red and yellow tomato substrates, respectively, were completely absent from the adult flies (Table 1, Figure 1). Specifically,

<u>Control</u>: As expected, the Control substrate did not contain carotenoids. Accordingly, we did not detect carotenoids in the emerging adult flies.

<u>Cucumber</u>: In the Cucumber substrate we detected only chlorophylls, but not carotenoids. Here too, we did not detect carotenoids in the emerging adult flies.

<u>Red tomato</u>: In the red tomato substrate, we detected several carotenoids: Phytoene, two isomers of Phytofluene, β-carotene, Lutein, asymmetric ζ-carotene, 3 isomers of ζ-carotene, and as expected, several isomers of Lycopene, which was the dominant carotenoid. Surprisingly, however, the adult

House flies, emerging from this substrate, did not contain Lycopene at all. Phytoene, β-carotene and Lutein were also lacking in the adults. While so, adult flies did contain Phytofluene, asymmetric ζ-carotene and ζ-carotene, of similar isomers as in the substrate.

Yellow tomato: The yellow tomato substrate contained Phytoene, two isomers of Phytofluene, β-carotene, asymmetric ζ-carotene, 3 isomers of ζ-carotene and several isomers of Lycopene. Unlike the red tomato substrate, the primary carotenoids were Prolycopene and Neurosporene. Interestingly, the adult House flies, emerging from this substrate did not contain Prolycopene and Lycopene, but similarly to adult flies from the red tomato substrate, contained isomers of Phytofluene and ζ-carotene. In addition, adult flies also contained Neurosporene.

Table 1. Carotenoid content in the feed (3 replicates for each type; ng/g±1se) and in the adult House flies (5 flies from each substrate type; ng/fly±1se). Adult flies emerging from "Control" and "Cucumber" did not contain carotenoids, and hence not presented in the table. Different isomers of each carotenoid type are summed together.

Carotenoid	Tomato	Substrate (µg/g)	Adult Fly (ng/fly)
Phytoene	Red	12.8±2.7	0
	Yellow	33.5±3.3	0
Phytofluene	Red	3.1±0.7	323.0±248.9
	Yellow	7.8±1.1	88.1±43.3
Lutein	Red	0.13±0.03	0
	Yellow	0	0
asymmetric ζ-carotene	Red	0.04±0.01	13.8±6.0
	Yellow	0.6±0.1	22.2±11.0
ζ-carotene	Red	0.5±0.1	71.6±35.6
	Yellow	Detected	373.5±347.5
β-carotene	Red	4.1±0.5	0
	Yellow	1.4±0.6	0
Prolycopene	Red	0	0
	Yellow	9.2±1.8	0
Neurosporene	Red	0	0
	Yellow	1.8±0.3	16.4±17.9
Lycopene	Red	13.8±4.19	0
	Yellow	0.9±0.3	0

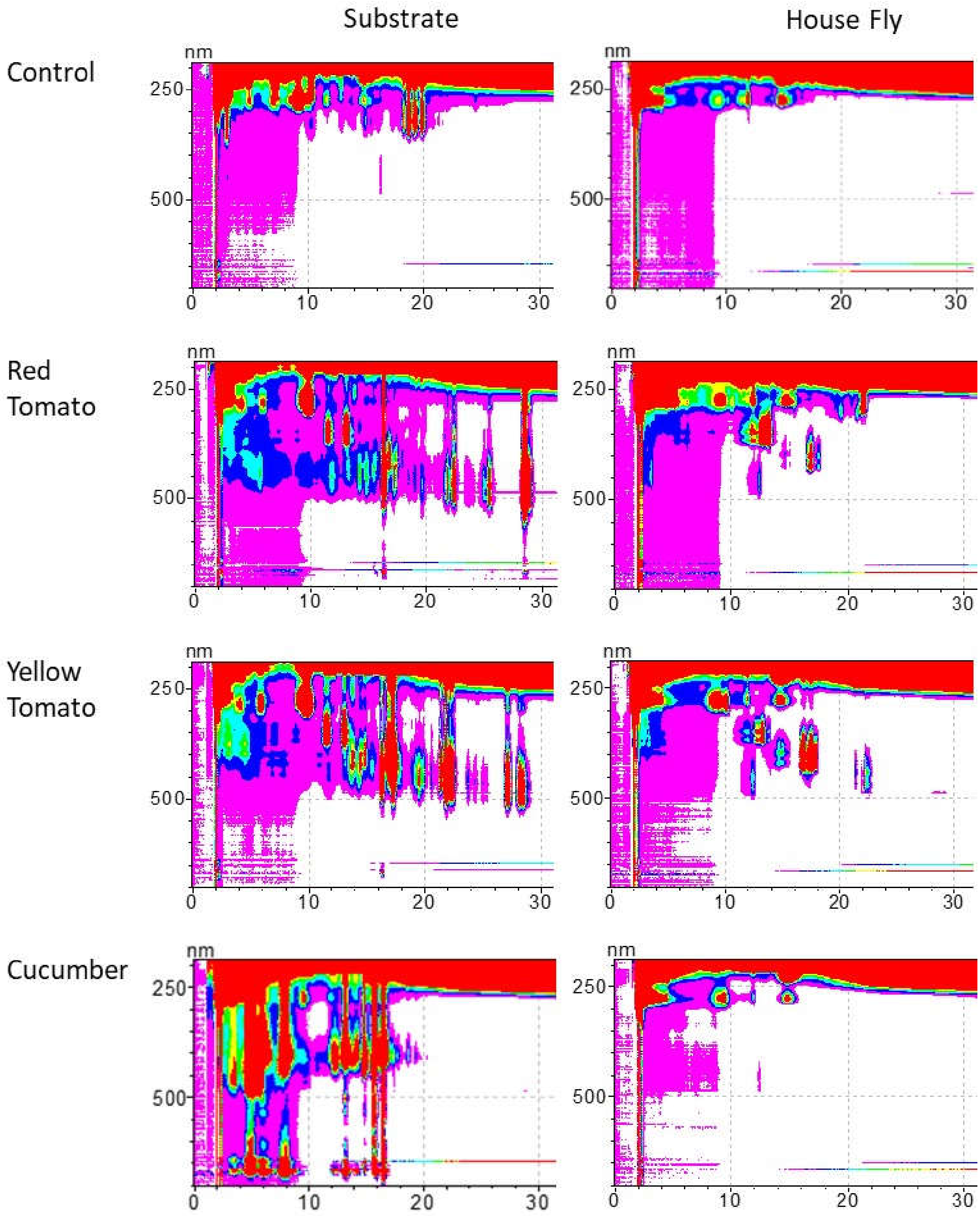

Figure 1. HPLC analysis of carotenoids of substrate and adult flies. The figure displays one representative contour plot from the different substrates and respective adult flies. Carotenoids were identified according to their characteristic retention time (Rt) and absorbance. Phytoene (Rt 5.8, 6.4, 9.9 min), Phytofluene (Rt 11.7, 12.4, 13.2 min), ζ-carotene (Rt 16.9, 17.1, 17.9 min), asymmetric ζ-carotene (Rt 14.4, 14.8 min), Neurosporene (Rt 21.4, 22.3 min), Lycopene (Rt 21.7, 22.3, 24.9, 25.2, 28.5 min), Prolycopene (Rt 17.3), Lutein (Rt 5.8).

4. Discussion

We used HPLC to characterize the carotenoid composition in the body of adult House flies and in the respective larval feed. We found that carotenoid composition in adult House flies can be diverse and that it is related to the carotenoid composition in the larval feed. These findings are in support of our primary hypothesis and in agreement with previous findings, e.g., [11] on silkworms and [12] on dragonflies. However, unlike our hypothesis, carotenoid composition in the adult flies did not directly reflect carotenoid identity in the respective substrate feed of the larvae. Interestingly, Phytoene, β-carotene, Lutein, Prolycopene, and Lycopene were absent from adult flies that originated from substrates that contained fair amounts of these carotenoids. As far as we know, we describe here for the first time the presence of the carotenoids Phytofluene, ζ-carotene, asymmetric ζ-carotene, and Neurosporene in House flies. These findings strongly suggest that House flies, and likely also other insects, can metabolize or selectively absorb and accumulate carotenoids from their feed [11].

In agreement with [16], we did not find β-carotene in the adult flies. However, [16] reports also Lutein and Zeaxanthin, which we did not find in our House flies, even though Lutein was present in the red tomato substrate. Indeed, Zeaxanthin was absent from all our substrates. [16] did not report the substrate carotenoid content, hence we can only speculate that β-carotene may have been missing from the substrates, and hence from the flies.

The specific function of the carotenoids we detected in the House flies and their transition and metabolic pathways are largely unknown. Further work is required to unravel the role of these carotenoids in the biology and ecology of House flies and insects in general, the consequences of habitat variability in carotenoid composition, and the biochemical pathways of carotenoid absorption and metabolism. Interestingly, our findings indicate that the carotenoid composition of adult House flies, and possibly other insects, reflect substrate-specific carotenoid fingerprints. This relation could potentially be used to relate between free-ranging adult House flies and their natal habitat, possibly aiding in identifying and locating House fly infestation sources. The use of insects, including House fly larvae, is increasingly promoted as a replacement for other animal feed sources, especially for fish and poultry [18,19]. Considering our findings, the specific composition of House fly feed should be considered not only with respect to protein and fat content but also to other essential compounds, such as vitamins and carotenoids.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, Aviv Shaish, Ido Tsurim. and Li-Or Lahmi.; methodology, Aviv Shaish, Ido Tsurim, Ayelet Harari and Li-Or Lahmi.; formal analysis, Ido Tsurim, Aviv Shaish and Ayelet Harari.; investigation, Li-Or Lahmi, Ido Tsurim.; resources, Aviv Shaish and Ido Tsurim; writing—original draft preparation, Aviv Shaish and Ido Tsurim.; writing—review and editing, Aviv Shaish, Ido Tsurim, Ayelet Harari and Li-Or Lahmi..; visualization, Aviv Shaish and Ayelet Harari.; supervision – Ido Tsurim and Aviv Shaish; project administration, Li-Or Lahmi and Ido Tsurim. All authors have read and agreed to the published version of the manuscript.

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