Comparative Biochemistry and Physiology, Part A 218 (2018) 70–79

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Fatty acid composition in native bees: Associations with thermal and feeding ecology

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ARTICLE INFO

Keywords:
Thermal ecology
Diet consumption
GC-FID
Transesterification
Proboscis-length
Linear discriminant analysis
Cluster analysis

ABSTRACT

Fatty acid (FA) composition of lipids plays a crucial role in the functioning of lipid-containing structures in organisms and may be affected by the temperature an organism experiences, as well as its diet. We compared FA composition among four bee genera: *Andrena, Bombus, Megachile,* and *Osmia* which differ in their thermal ecology and diet. Fatty acid methyl esters (FAME) were prepared by direct transesterification with KOH and analyzed using gas-liquid chromatography with a flame ionization detector. Sixteen total FAs ranging in chain length from eight to 22 carbon atoms were identified. Linear discriminant analysis separated the bees based on their FA composition. *Andrena* was characterized by relatively high concentrations of polyunsaturated FAs, *Bombus* by high monounsaturated FAs and Megachilids (*Megachile* and *Osmia*) by relatively high amounts of saturated FAs. These differences in FA composition may in part be explained by variation in the diets of these bees. Because tongue (proboscis) length may be used as a proxy for the types of flowers bees may visit for nectar and pollen, we compared FA composition among *Bombus* that differed in proboscis length (but have similar thermal ecology). A clear separation in FA composition within *Bombus* with varying proboscis lengths was found using linear discriminant analysis. Further, comparing the relationship between each genus by cluster analysis revealed aggregations by genus that were not completely separated, suggesting potential overlap in dietary acquisition of FAs.

1. Introduction

Eukaryotic cells devote substantial resources (~5% of their genes) to producing and maintaining lipid assemblages that provide key evolutionary advantages (Sud et al., 2007; van Meer et al., 2008; Vance and Vance, 1985). Lipids serve as key energy reserves (Arrese and Soulages, 2010; Hahn and Denlinger, 2007), are fundamental components of cell membranes (Hochachka and Somero, 2002), and play key roles in neural signaling processes (reviewed in van Meer et al., 2008). Variation in the functional demands and diet availability of lipids can lead to consistent differentiation of lipid profiles among organisms and habitats (Castell et al., 1972; Hanson et al., 1985). As such, lipid profiles can be used to differentiate among certain taxa (Arts and Wainmann, 2012; Brett and Müller-Navarra, 1997; Torres-Ruiz et al., 2007).

Temperature and diet are the two primary factors that can drive differentiation of lipid profiles among organisms (Hanson et al., 1985; Hazel, 1995; Hochachka and Somero, 2002) through effects on physicochemical properties of lipid constituents. The functioning of lipids, and hence lipid-containing structures, depends strongly on the physicochemical properties of lipid constituents (Hochachka and Somero, 2002), particularly fluidity. Because lipids are key components of biological membranes, changes in lipid composition can alter membrane fluidity, which can, among other things, disrupt ion balance and the function of lipid-derived second messengers (reviewed in Hazel, 1995). Additionally, given that lipids can only be metabolized when fluid (Frank, 1992; Holmstrup et al., 2007; Kostal and Simek, 1998; Ohtsu et al., 1993; Ruf and Arnold, 2008), changes in lipid fluidity can also alter metabolism and energy production.

Fluidity of lipids is determined by various factors including the ratio of unsaturated to saturated fatty acids (UFA:SFA) that constitute the lipid (Hochachka and Somero, 2002). The ratio of UFA to SFA is primarily determined by the temperatures an organism experiences (Hazel, 1995; e.g. Marr and Ingraham, 1962) and by its diet (Barlow, 1966; Frank, 1992). Whereas UFAs provide less energy when metabolized but maintain fluidity at low temperatures, SFAs are less fluid at low temperatures but provide relatively more energy when metabolized. Therefore, based on the homeoviscous adaptation (HVA) hypothesis, organisms experiencing colder temperatures should have

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https://doi.org/10.1016/j.cbpa.2018.01.013
Received 12 November 2017; Received in revised form 19 January 2018; Accepted 22 January 2018
Available online 01 February 2018
1095-6433/

higher UFA:SFA (to maintain fluidity and functionality of lipid containing structures), whereas organisms experiencing warmer conditions should favor SFAs for their increased energy yield (although the quantity may vary with tissue under study). The basic predictions of the HVA hypothesis (Sinensky, 1974) have been tested and appear to hold for diverse organisms (Anderson et al., 1981; Carey and Hazel, 1989; Fudge et al., 1998; reviewed in Hazel, 1995; Holmstrup et al., 2007; Raynard and Cossins, 1991) including insects (Atapour et al., 2007; Barlow, 1964; Fast, 1966; van Dooremalen and Ellers, 2010). Insects often show strong shifts in fatty acid (FA) composition in response to temperature (e.g. Hahn and Denlinger, 2011; see Hazel, 1995; Kostal and Simek, 1998), likely in part because, as small ectotherms they have limited scope for decoupling body temperature from environmental temperature and therefore must cope physiologically with environmental temperature fluctuations.

Changes in FA composition that facilitate tolerance of diverse environmental temperatures arise from three main processes: de novo synthesis of FAs, assimilation of FAs from the diet, and modification of existing FAs. Although insects, like other organisms, can synthesize many FAs de novo, polyunsaturated fatty acids (PUFAs) must be obtained from the diet (reviewed in Stanley-Samuelson et al., 1988). Consequently, the amount of these essential FAs in the diet will influence FA distribution in organism tissues (Barlow, 1966; Frank, 1992; Frank et al., 2008). Furthermore, the composition and concentrations of FAs in the diet that are precursors for the synthesis of other FAs may also alter the concentrations of FAs that are synthesized de novo, ultimately affecting insect FA composition. Thermal and feeding ecology therefore both independently and interactively, may be strong determinants of and provide useful information on FA biochemistry in insects and the mechanistic basis for their adaptations to predicted changes in environmental conditions (Hartmann et al., 2013).

Native bees vary widely in body size (Michener et al., 1994) and associated thermal ecology (Bishop and Armbruster, 1999; Stone and Willmer, 1989), as well as in diet (Michener et al., 1994; Willmer, 2011), providing an excellent group for comparative studies of FA composition. Body temperatures of small bees likely track changes in environmental temperatures, whereas larger bees and those that live in social aggregations can regulate body temperatures physiologically (via regulation of loss of metabolic heat; Heinrich, 1974a, 1974b), and behaviorally (Heinrich, 1975; Heinrich and Esch, 1994; Kronenberg and Heller, 1982). For example, eusocial bees nesting together can maintain relatively constant and warm hive temperatures (Engels et al., 1995; Heinrich, 1975; Kronenberg and Heller, 1982), unlike solitary bees (see Heinrich, 1993). Bee morphology and behavior also influence the types of flowers bees visit and their ability to collect pollen and nectar (Brian, 1947; Michener et al., 1994; Willmer, 2011) with possible effects on dietary FAs and hence, on tissue FA composition.

We compared FA composition among four bee genera (*Andrena*, *Bombus*, *Megachile* and *Osmia*) that differ in their thermal ecology and diet. The smallest bees in this study, *Andrena* (mean wet mass = 0.06 g) and *Osmia* (mean wet mass = 0.05 g), may be less likely to retain metabolic heat compared to *Bombus* (mean wet mass = 0.25 g) and *Megachile* (mean wet mass = 0.12 g) given their greater surface area to volume ratio (Stone and Willmer, 1989) and given the higher pilosity of *Bombus* (and to some extent, *Megachile*). We, therefore, expected higher UFA: SFA in *Andrena* and *Osmia* relative to *Bombus* and *Megachile* collected in similar environments. *Bombus* are heterothermic and eusocial and hence benefit from elevated and constant body temperatures when foraging and return to nests with regulated temperatures (Heinrich, 1993, 1975). Lipid profiles in *Bombus* may therefore be less influenced by environmental temperatures compared to the other three genera.

The four bee genera in this study also vary in their feeding ecology. Differences in proboscis length and body size in part determine from which flowers bees can successfully extract nectar and pollen (Willmer, 2011). Variation among plants in lipid composition of pollen (and to a lesser extent nectar, Willmer, 2011) could result in differences in lipid composition of bee diets. This variation in lipids in the diet could subsequently lead to differences in lipid profiles of bee tissues.

We describe a protocol adapted for FA composition analysis in bees using gas-liquid chromatography with a flame ionization detector (GC-FID). We then measure FA composition of 73 bees from four bee genera: *Andrena, Bombus, Megachile* and *Osmia*. We describe clear differences in FA composition among genera, likely related to thermal ecology and diet. We further show that FA profiles differ with proboscis length in bumble bees. Using cluster analysis, we also show that genus aggregations are not completely separated, suggesting potential overlap in dietary acquisition of FAs.

2. Methods

2.1. Field collection of samples

We collected bees near Phelps Lake in Grand Teton National Park (N 43° 30.220′, W 110° 48.327′, 2060 m asl; June to August 2012) in an open meadow with abundant flowers supporting a diverse bee community. Bees were euthanized in cyanide within 30 s of capture and subsequently transported on ice to the University of Wyoming (UW)-National Park Service (NPS) Research Station. Bee body mass was measured in the field within 24 h of capture (± 1 mg, Acculab PP2060D) and then re-measured within eight days of capture when samples were transported to the lab (± 0.1 mg, Acculab ALC-210.4, NY, USA). We identified bees to genus and bumble bees to species (all workers, Koch et al., 2012; Michener et al., 1994). All analyses were performed on female bees for *Andrena*, *Bombus* and *Megachile*. However, given that none of the analyses showed variation among sexes, male and female data were combined for analyses in *Osmia*. We then cleaned off any pollen particles present on the body, and then stored the bees at −20 °C until performing FA composition analyses.

2.2. Extraction and methylation of fatty acids

All reagents were analytical grade and glassware and utensils were washed with Liquinox (Alconox, Inc.) followed by hexane to eliminate organic residue prior to usage. Using lab-reared *Bombus impatiens*, we first compared three approaches to fatty acid methyl ester (FAME) preparation for FA analysis in bees using gas-liquid chromatography (GLC): i. methylation of directly saponified FAs, ii. direct transesterification using methanolic HCl, iii. direct transesterification using methanolic KOH (KOH approach). Triacylglycerol (tritridecanoate, C 13:0, 1 mg) was used as an internal standard (IS) for all methods. Fourteen worker *B. impatiens* collected from a lab-reared hive were lyophilized (Freezone 4.5, Labconco, Kansas City, USA) and homogenized (Tekmar, Telex no: 21-4221, Vernon, BC, Canada) together and subsamples from this homogenate were used for FA analysis to compare the three methods.

For methylation of directly saponified tissue, we followed procedures as described by Lake et al. (2006). Briefly, in the homogenized sample placed in 16 × 125 mm glass tubes with teflon-lined screw caps, we added 4 mL of ethanol and 1 mL of 33% (wt/vol) KOH in DI water followed by heating at 80 °C for 60 min, vortexing every 5 to 10 min. We next added 1 mL of 12 M HCl and 3 mL high purity hexane, vortexed and centrifuged at 2600 rpm for 3 min (the same conditions apply hereafter unless otherwise mentioned; Beckman TJ-6/TJ-6R centrifuge, MN, USA). The supernatant layer containing hexane and FAs was transferred to a clean tube with IS. We then dried the samples (evaporation of hexane) using N_2 gas at 50 °C, added 4 mL 0.545 M methanolic HCl, and incubated at 80 °C for 45 min, vortexing every 5 min. We next added 2 mL of DI water and 1 mL high purity hexane, centrifuged for 3 min and transferred the supernatant, the hexane layer containing FAs, to gas liquid chromatography (GLC) auto-sampler vials (2 mL, 13 × 32 mm, Agilent Technologies, USA) containing a bed of Na_2SO_4, and stored at −20 °C until run in the GC.