

1 **Diversity in membrane composition is associated with variation in thermoregulatory**
2 **capacity in hymenopterans.**

3
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10 Running head: Membrane composition diversity in heterothermic insects

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23

24 **Abstract**

25

26 Thermoregulatory capacity varies widely among bees and wasps, but the cellular physiology
27 required to support such thermogenic ability remains unclear. Studies conducted on ectothermic
28 species living in varying temperature show that cellular membrane composition is adjusted to
29 remain functional, a process named homeoviscous adaptation. We show that the fatty acid
30 composition of flight muscle membranes varies with thermogenic capacity in species of bees and
31 wasps. The relative abundance of palmitate (16:0) and linoleate (18:2) decreased, while oleate
32 (18:1) increased with increasing thoracic temperature. Species selected for the study varied over
33 ten-fold in body mass, which in turn affected species thoracic temperature and their fatty acids
34 profile. Nevertheless, all analyses conducted show that thoracic temperature is the main driver of
35 flight muscle membrane composition in hymenopterans with diverse thermoregulatory capacity.
36 These findings are in line with the predictions based on the homeoviscous adaptation hypothesis
37 and further show that thermogenic strategy used by insect species impacts cellular membrane
38 composition.

39

40 Key words: Membrane, fatty acids, thorax, temperature, thermoregulation, homeoviscous
41 adaptation, body mass, hymenopterans

42

43 **Introduction**

44

45 Insects are widely distributed around the world, inhabit almost every environment, and also
46 show remarkable diversity in thermal properties. Many are ectotherms poikilotherms, as their
47 internal temperature varies, but they are unable to generate and maintain enough heat to increase
48 their body temperature above that of the environment. Therefore, environmental temperature
49 constrains their daily activities. Other insect species are heterotherms, thus switching periodically
50 between ectothermy and endothermy. Thermoregulation is strongly linked to the evolution of high
51 metabolic rate associated with insect flight (Heinrich, 1981, 1995), and bee and wasp species of
52 the Hymenoptera order display important interspecific and intraspecific variation in endothermy
53 and thermoregulatory capacity (Stone, 1994; Stone and Willmer, 1989; Willmer and Stone, 2004).
54 Within a species, high-altitude hymenopteran populations can be more endothermic than
55 populations at sea level and are consequently able to forage at lower ambient temperatures, while
56 temperate populations with poor thermoregulatory capacities rely on behavioral strategies, such as
57 long periods of basking, to warm up (Herrera, 1995; Stone, 1993). Interspecific differences in
58 endothermic heat generation are also found in honeybees or wasps and are thought to be linked to
59 differences in foraging and nesting behaviour, but also in morphological and physiological features
60 of workers (Dyer and Seeley, 1987; Kovac et al., 2009).

61 Hymenopterans warm up for flight through different mechanisms, from basking to
62 shivering thermogenesis, or a mix of both. During the latter, muscles do not operate myogenically
63 as during flight. The opposite sets of muscles are declutched from the wings and contract
64 simultaneously in a tetanus (Esch and Goller, 1991). These tetanic contractions of the dorsoventral
65 and dorsal longitudinal indirect flight muscles generate sufficient heat during pre-flight warm-up,

66 elevating thoracic temperature above the environmental temperature (Willmer and Stone, 2004).
67 Overheating is avoided via enhanced convective heat loss (shunting of the haemolymph to the
68 abdomen) or behaviorally by searching for shade, restraining activity, or by dissipating excess heat
69 through evaporation (Heinrich, 1995). Hymenopterans thus differ in their ability to
70 thermoregulate, but the implications of such variation on cellular properties associated with
71 variable thermal regime remains unexplored.

72 Cellular membranes are sensitive to temperature, and poikilothermic organisms ranging
73 from bacteria to plants and animals can adjust their composition to maintain homeostasis in a
74 process named homeoviscous adaptation (HVA) (Hazel and Williams, 1990; Sinensky, 1974). The
75 cold-induced ordering of the membrane is commonly countered by an increase in the relative
76 abundance of unsaturated fatty acids, causing disorder to maintain membrane fluidity (Logue et
77 al., 2000). The two fatty acid chains constitute the hydrophobic part of phospholipids which are,
78 along with other lipid classes and various proteins, the backbone of cellular membranes. Fatty
79 acids (FAs) are carboxylic acids with a long hydrocarbon tail, and can be either saturated or
80 unsaturated. Saturated FAs (SFAs) only possess single bonds and allow rotation around the
81 carbon-carbon bond, while unsaturated FAs can have one (monounsaturated, MUFAs) or two to
82 six double bonds (polyunsaturated, PUFAs). Double bonds constrain the rotation around the
83 consecutive carbons, and provide a kink in the chain that can affect the surrounding lipids in the
84 bilayer (Hulbert and Else, 1999). This variability in FA composition affects the local membrane
85 fluidity and the function of various membrane-associated structures and processes such as various
86 oxidative enzymes (Guo et al., 2005; Miyasaka et al., 1996; Power and Newsholme, 1997),
87 ATPases (Swanson et al., 1989; Turner et al., 2005), hormone receptors (Corcoran et al., 2007)
88 and ion channels (Leaf et al., 2005).

89 Membrane FA composition has been linked with habitat-specific temperatures, as species
90 of vertebrates living in cold climates generally possess more unsaturated membranes than species
91 found in warmer environments (Logue et al., 2000). Moreover, this remodeling of cellular
92 membranes with temperature acclimation has been established in a wide variety of organisms
93 (Ayala-Del-Río et al., 2010; Uemura et al., 1995). In teleost fish, a heat stress can affect the
94 regulation of genes linked to FA metabolism (Buckley, 2006), and membrane saturation varies
95 with season (Guderley, 2004; Kraffe et al., 2007). In reptiles, membranes are also subject to
96 changes in polyunsaturation such as in cold-acclimated crocodiles (Seebacher et al., 2009). Fruit
97 flies (*Drosophila melanogaster*) subjected to rapid cold hardening, were found to respond by
98 reductions in membrane SFAs and MUFAs, and increases in PUFAs (Overgaard et al., 2005). An
99 appropriate phospholipid FA composition of membranes appears to be crucial to survival at
100 freezing temperatures in many organisms such as the freeze-tolerant earthworm *Dendrobaena*
101 *octaedra* (Bindesbol et al., 2009). Globally, it appears that the cellular thermal environment greatly
102 influences membrane composition in a variety of organisms.

103 This study investigates how the diversity in thermoregulatory capacity found in
104 hymenopterans affects the membrane phospholipid composition of flight muscle tissue. We tested
105 relationships between thoracic temperature and the thoracic FA composition in temperate bee and
106 wasp species. We expected to find higher proportions of PUFAs at lower thoracic temperature, in
107 line with the predictions from the HVA hypothesis, while muscles operating at higher temperature
108 should contain more MUFAs and SFAs.

109 **Materials and Methods**

110

111 *Sampling of hymenopterans and temperature measurements*

112 Individuals from various species of hymenopterans were sampled during the spring and
113 summer at various locations in the Ottawa area. Two superfamilies were targeted, Apoidea-
114 Anthophila and Vespoidea. A total of 109 individuals were sampled, pertaining to 21 species and
115 two different superfamilies of the Hymenoptera order. For one species, *Bombus bimaculatus*, both
116 workers and queens were sampled and treated as separate data point; all analyses were also
117 performed without the queens and results were essentially the same. Body masses of the collected
118 specimens ranged from 27.5 to 550mg. Immediately after capture individuals were transferred to
119 a restraining device (50mL syringe with netting at the open end) to immobilize them, and their
120 thoracic surface temperature (T_{th}) was measured in under a minute. Thermograms were taken in
121 the shade using an infrared camera (EX300, FLIR systems). Individuals were then transported in
122 50mL transparent tubes, placed on ice in a cooler until arrival at the lab where they were stored in
123 the freezer at -20°C . Within 24 to 48h, specimens were identified to the species level (when not
124 possible, to the subgenus level) using various keys (Buck et al., 2008; Michener, 2007); their body
125 parts separated, weighted and stored in 1.5 mL CryoVials at -80°C . Thermographic data was stored
126 on a computer before further analysis and extraction of the T_{th} . An Enviro-meter (Fisher scientific)
127 was used to measure the ambient air temperature (T_a). The thorax temperature excess, the
128 difference between thoracic surface and ambient air temperature ($T_{th}-T_a$) was also used to assess
129 the endothermic capacity (Kovac and Stabentheiner, 2012). For all analyses, results for thoracic
130 temperature excess and T_{th} were essentially the same, thus only results for T_{th} are reported.

131

132 *Fatty acid composition of membrane phospholipids*

133 Total lipids from flight muscle were extracted by homogenizing the thorax (Polytron,
134 Kinematica, Luzern, Switzerland) in 2:1 chloroform:methanol (v/v) (Folch et al., 1957) and

135 completing 3 cycles of shaking, centrifugation (10 min, 2000 g), and filtration. Before the last
136 cycle, 0.25% KCl was added to help removing aqueous contaminants. The aqueous phase was
137 discarded, and the organic phase was dried on a rotating evaporator (Büchi Rotavapor, Flawil,
138 Switzerland). Phospholipids were separated by resuspending total lipids in chloroform before
139 loading on solid-phase extraction columns (Supelclean 1 ml, 100 mg LC-NH₂; Sigma-Aldrich; St.
140 Louis, USA). Neutral lipids, non-esterified fatty acids, and phospholipids were separated by
141 sequential elution using solvents of increasing polarity: isopropyl ether:acetic acid (98:2 v/v),
142 chloroform:isopropanol (3:2 v/v), and methanol (Maillet and Weber, 2006). The FA composition
143 of membrane phospholipids was measured after acid transesterification in 1M acetyl chloride and
144 methanol (90°C for 2 h). FA methyl esters were analyzed on an Agilent Technologies 6890N gas
145 chromatograph (Mississauga, Ontario, Canada) equipped with a flame-ionization detector and a
146 fused silica capillary column (Supelco DB-23, 60 m, 0.25 mm i.d., 0.25µm film thickness; Sigma-
147 Aldrich) using published procedures (Magnoni and Weber, 2007). Individual FAs were identified
148 by determining exact retention time with authentic standards (Supelco, Bellefonte, PA, USA).
149 Only the fatty acids accounting for >1% of total FAs in membrane phospholipids are reported.

150

151 *Statistical analysis*

152 All statistical analyses were performed using the Systat 13 software. All values are
153 presented as mean ± SE. Dependent and independent variables were first tested for normality using
154 the Shapiro-Wilk test. Relationships between body mass and FA relative abundance in birds and
155 mammals follow power functions (Hulbert et al., 2007). Therefore, variables were log-transformed
156 to linearize the data. Nevertheless, we also tested several non-linear regression models, but the
157 data fit was marginally improved in only a few cases and subsequent results were essentially the

158 same. Normality of residuals was also verified using the Shapiro-Wilk test, and homoscedasticity
159 was assessed using Levene's test.

160 We first tested the effect of body mass on T_{th} , taking superfamily as a covariate using an
161 analysis of covariance (ANCOVA). For each FA, we first tested the relationships with each
162 independent variable: body mass or thoracic temperature (T_{th}), taking superfamily into account
163 using ANCOVAs. We further tested the effect of body mass, thoracic temperature and superfamily
164 combined. For all analyses, interactions were tested and only reported when significant. Finally,
165 we present correlations between thoracic temperature and membrane composition independent of
166 body mass, by performing analyses on the residuals obtained from significant regressions with
167 body mass.

168

169 **Results**

170 *Thoracic temperature variation with body mass*

171 Thoracic temperature (T_{th}) increased with increasing body mass of the Hymenopteran
172 species studied ($r^2=0.54$, $P<0.001$; Fig. 1). An ANCOVA showed no significant differences
173 between superfamilies Apoidea and Vespoidea (Mass: $F_{(1,19)}=23.755$, $P<0.001$; Superfamily:
174 $F_{(1,19)}=2.057$, $P=0.168$).

175

176 *FAs in the thorax membrane phospholipids of local hymenopterans*

177 The thoracic membranes of the 21 species were composed of five major FAs, where 16:0
178 and 18:0 were the main SFAs, 18:1 ($n=9$) was the main MUFA, 18:2 ($n=6$) and 18:3 ($n=3$) were the
179 main PUFAs. Other FAs including 14:0, 16:1 ($n=7$), 20:0, 20:1, 21:0, 22:0, 20:5, 22:1, 22:6 and
180 24:0 were found in relatively low abundances (<1%) and their presence varied among individuals
181 of a species.

182 The relative abundance of two FAs in the phospholipid fraction changed significantly with
183 T_{th} and with body mass. Figure 2 illustrates the influence of these two variables on FA
184 composition. SFA 16:0 decreased significantly with body mass ($r^2=0.56$, Mass: $F_{(1,19)}=26.726$,
185 $P<0.001$; superfamily: $F_{(1,19)}=2.965$, $P=0.101$; Fig. 2A) and with T_{th} ($r^2=0.73$, T_{th} : $F_{(1,19)}=46.682$,
186 $P<0.001$; superfamily: $F_{(1,19)}=0.383$, $P=0.543$; Fig. 2B). MUFA 18:1 increased significantly with
187 body mass ($r^2=0.30$, Mass: $F_{(1,19)}=9.241$, $P=0.007$; superfamily: $F_{(1,20)}=1.769$, $P=0.199$; Fig. 2C)
188 and with T_{th} ($r^2=0.43$, T_{th} : $F_{(1,18)}=14.693$, $P=0.001$; superfamily: $F_{(1,18)}=7.225$, $P=0.015$;
189 superfamily* T_{th} : $F_{(1,18)}=6.757$, $P=0.018$; Fig. 2D). The significant interaction terms reflect that
190 18:1 is significantly related to T_{th} in Apoidea (n=14), but not Vespoidea (n=7), possibly owing to
191 the smaller sample size. A similar result was found for 18:2, where there was a significant
192 interaction between mass and superfamily ($r^2=0.52$, Mass: $F_{(1,18)}=0.178$, $P=0.678$; superfamily:
193 $F_{(1,18)}=0.853$, $P=0.368$; superfamily*mass: $F_{(1,18)}=5.932$, $P=0.025$), where only Apoidea showed a
194 significant relationship. A similar relationship was found when looking at thoracic temperature,
195 where the interaction between T_{th} and superfamily was almost significant ($r^2=0.60$, T_{th} :
196 $F_{(1,18)}=1.353$, $P=0.260$; superfamily: $F_{(1,18)}=4.512$, $P=0.048$; superfamily* T_{th} : $F_{(1,18)}=3.939$,
197 $P=0.063$).

198 The effect of body mass and thoracic temperature on membrane phospholipid composition
199 was analyzed with variables combined and final models are presented in Table 1. The variation in
200 content of 16:0, 18:1 and 18:2 is explained by T_{th} . In the case of the latter FA, results from the
201 ANCOVA showed a significant interaction between superfamily, mass and thoracic temperature.
202 This again suggests differences in relationships between T_{th} , mass and %18:2 between
203 superfamilies, although T_{th} appears to drive most of the variation. When analyzed separately, there

204 was a significant effect of T_{th} on 18:2 abundance in Apoidea ($r^2=0.56$, Mass: $t_{(12)}=0.630$, $P=0.540$;
205 T_{th} : $t_{(12)}=-2.782$, $P=0.017$), but not in Vespoidea.

206

207 *Residual analysis to account for the effects of mass on membrane composition and thoracic*
208 *temperature*

209 To represent the relationships between FA composition and thoracic temperature, we
210 further analyzed data using residuals obtained from the regressions with body mass, and
211 superfamily when applicable. The decrease in 16:0 with T_{th} remained significant when correcting
212 for the effect of body mass on both variables ($r=-0.67$, $P=0.001$; Fig. 3A), as did the increase in
213 18:1 with T_{th} ($r=0.45$, $P=0.035$; Fig. 3B). Residuals of the relationship between relative 18:2
214 abundance and superfamily were found to decrease nearly significantly with residual thoracic
215 temperature ($r=-0.42$, $P=0.051$; Fig. 3C).

216

217 **Discussion**

218 This study shows that the FA composition of flight muscle membranes varies with
219 thermogenic capacity in hymenopterans. Specifically, the relative abundance of palmitate (16:0),
220 oleate (18:1) and linoleate (18:2) is associated with species differences in thoracic temperature.
221 These findings are in line with the prediction based on the HVA hypothesis (Hazel and Williams,
222 1990) developed in ectothermic organisms, and further show that thermogenic strategy used by
223 insect species impacts cellular membrane composition. Species selected for the study vary over
224 ten-fold in body mass, which in turn affects species thoracic temperature and their FA profile.
225 Nevertheless, all analyses conducted show that thoracic temperature is the main driver of flight
226 muscle membrane composition in hymenopterans with diverse thermoregulatory capacities. The

227 apparent correlated evolution between muscle membrane composition and thermoregulatory
228 capacity in this group of animals should help reveal the causes and functional consequences of
229 such association.

230 Membrane phospholipid composition of the species sampled consist of essentially five
231 major fatty acids, where the relative abundances of several of these FAs are associated with species
232 thoracic temperature (see results and Fig. 2 B, D). Species membrane composition, however, also
233 changes systematically with body mass in hymenopterans. The significant increase in 18:1 (Fig
234 2C), the decrease in a SFA (16:0) (Fig. 2A) and the lack of significant variation in 18:2 with body
235 mass resemble what we previously found among tropical Euglossine bees (Rodríguez et al., 2015).
236 Despite similar relationships, the group of Euglossine bees was distinct, as it varied very little in
237 thoracic temperature and variation in muscle membrane composition was associated with species
238 difference in flight metabolic rate according to the “membrane pacemaker” theory of metabolism
239 (Hulbert, 2003). The current findings further show how thoracic temperature variation is another
240 main driver explaining membrane composition diversity among species.

241 The thermogenic capacity of Apoidea and Vespoidea is largely influenced by species body
242 mass: smaller species have cooler thoraxes than bigger ones (Fig. 1). Such an association has been
243 documented in hymenopterans, between groups such as superfamily (Apoidea and Vespoidea)
244 (Heinrich, 1995; Kovac and Stabentheiner, 2012; Willmer and Stone, 2004), but also species
245 within a genus (*Anthophora*) (Stone, 1994) and populations within a species (*Anthophora*
246 *plumipes*) (Stone, 1993). We report that not only thorax temperature varies with body mass, but
247 membrane composition also changes systematically with body mass in hymenopterans. When
248 looking at the association between membrane composition, body mass and thorax temperature
249 simultaneously, we find thoracic temperature as the main driver of membrane composition

250 variation. This is further emphasized when representing body mass-independent variation where
251 residual variations in 16:0, 18:1 and 18:2 are related to residual variation in thoracic temperature.

252 The relationships found between species FA content and thoracic temperature (Table 1;
253 Fig. 3), are in the directions predicted by the HVA hypothesis (Hazel, 1995; Sinensky, 1974). We
254 find that thoraxes functioning at lower temperatures appear to contain more PUFA 18:2 than those
255 of highly endothermic species when controlling for the effects of body mass, perhaps conferring
256 more fluidity to the membrane at low temperatures (Fig. 3C). Compensating for this decrease in
257 18:2 with temperature is a significant increase in the MUFA 18:1, also independent of body mass
258 (Fig. 3B). Contrary to our initial predictions, the abundance of SFA 16:0 decreases with thoracic
259 temperature, independently of body mass (Fig. 3A). Our study thus suggests that for heterothermic
260 insects with varying levels of endothermy to ectothermy, FA composition of the membrane is
261 adjusted to provide or maintain muscle functionality.

262 Membrane FA composition adjustments in ectothermic vertebrates involve increases in
263 PUFAs with cold temperatures while MUFAs remain unchanged (Guderley, 2004; Logue et al.,
264 2000; Seebacher et al., 2009). In cold-acclimated ectothermic invertebrates (such as most insects),
265 increases in PUFAs are often accompanied by decreases in MUFAs and SFAs (Bindesbol et al.,
266 2009; Cuculescu et al., 1995; Overgaard et al., 2005). However, modifications of the FA chain
267 seem to differ as a function of the directionality of the temperature change, tissue, and class or
268 subclass of phospholipids (Kraffe et al., 2007). The decrease in 16:0 with thoracic temperature in
269 our study could be understood from the point of view of chain length rather than saturation, as
270 exposure to cold temperature often elicits reduction in the average length of the FA chain (Hazel
271 and Williams, 1990). 16:0 is the shortest FA found amongst the five important FAs in the thorax
272 membrane, nearly doubling the abundance of the other SFA, 18:0. Its higher abundance in cooler

273 hymenopterans is reminiscent of the results found in the cold-acclimated flesh fly *Sarcophaga*
274 *similis* (Goto and Katagiri, 2011) or in the firebug *Pyrrhocoris apterus* (Tomcala et al., 2006).
275 Therefore, 16:0 abundance could also be part of the strategy to maintain the function of the
276 membrane in hymenopterans with different thermoregulatory capacities, because of the
277 importance of chain length in maintaining fluidity at lower temperatures (Lewis et al., 1989).

278 Changes in membrane composition associated with thermal acclimation has been a central
279 topic in thermal physiology, but diversity among species living in different thermal environments
280 is far less documented. Crockett (2008) reviewed the importance of lipid restructuring during cold
281 acclimation in ectotherms and cites the work of Logue et al. (2000) reporting species differences
282 associated with thermal environment in vertebrates. It is noteworthy that this study mostly focused
283 on differences among fish species, but included comparisons with endothermic vertebrates, a rat
284 and a turkey. Work testing the validity of the HVA hypothesis has more recently been extended to
285 natural populations of *Drosophila melanogaster*, where populations living along latitudinal
286 gradient show varying levels of plasticity as function of the variability of their thermal
287 environments (Cooper et al., 2014). We further show that species from the same geographical
288 location and therefore thermal environment, but with diverse thermogenic capacity influencing
289 their cellular environment, also exhibit a phospholipid profile according to the HVA hypothesis.
290 These findings help bridge the gap between thermal acclimation and adaptation in ectothermic and
291 endothermic species.

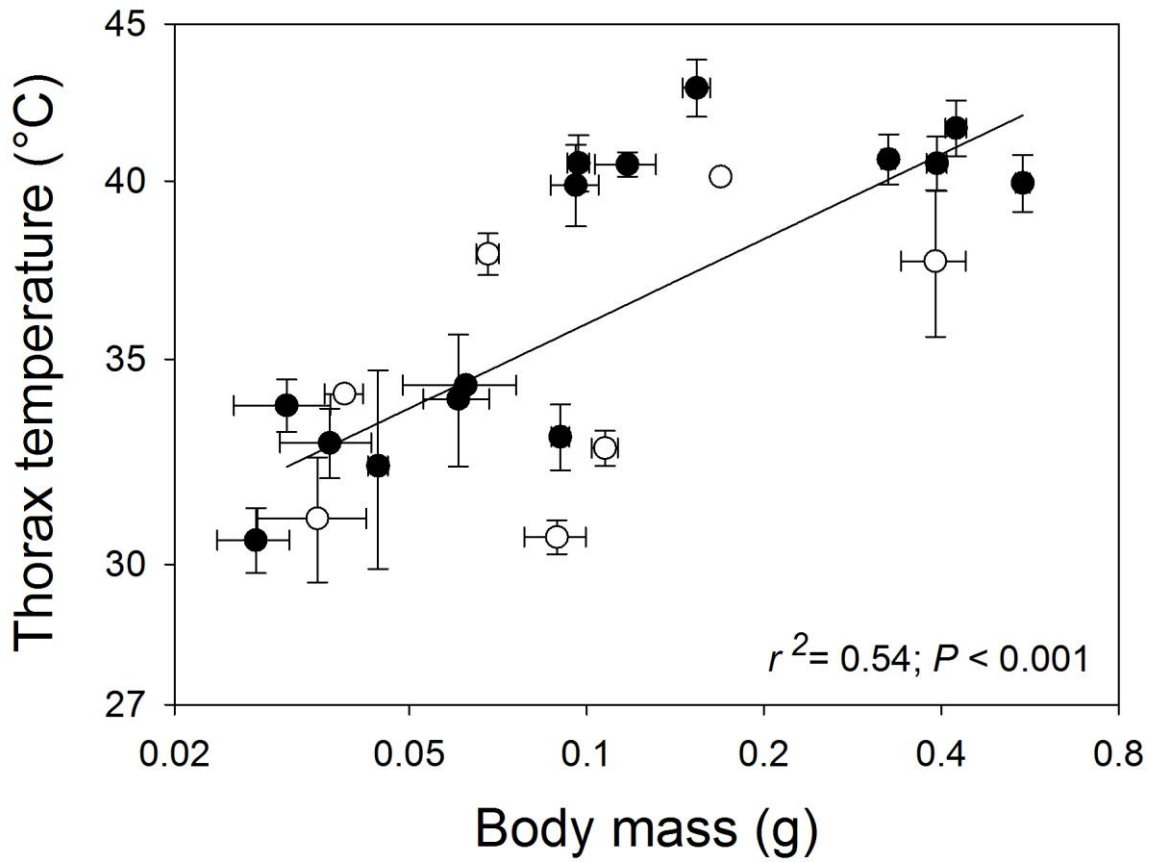
292 Together, this work shows that membrane composition varies across species with diverse
293 thermoregulatory capacities and according to the HVA hypothesis. We know that morphological
294 traits such as body size, pile length and wing size are linked with thermoregulatory capacity in
295 insects such as bees and wasps (Osorio-Canadas et al., 2016; Willmer and Stone, 2004).

296 Nevertheless, physiological and cellular properties of endothermic species are potentially also
297 distinct (Stone and Willmer, 1989), and membrane composition may be a key functional trait
298 evolving with thermoregulatory capacity.

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303 memory of Peter Hochachka, a mentor and friend with continuing influence.

304



306

307 **Figure 1.** Relationship between body mass and thoracic temperature in 21
308 species of Hymenoptera, pertaining to two superfamilies: Apoidea (filled circles)
309 and Vespoidea (open circles). Values are means (\pm S.E.M.).

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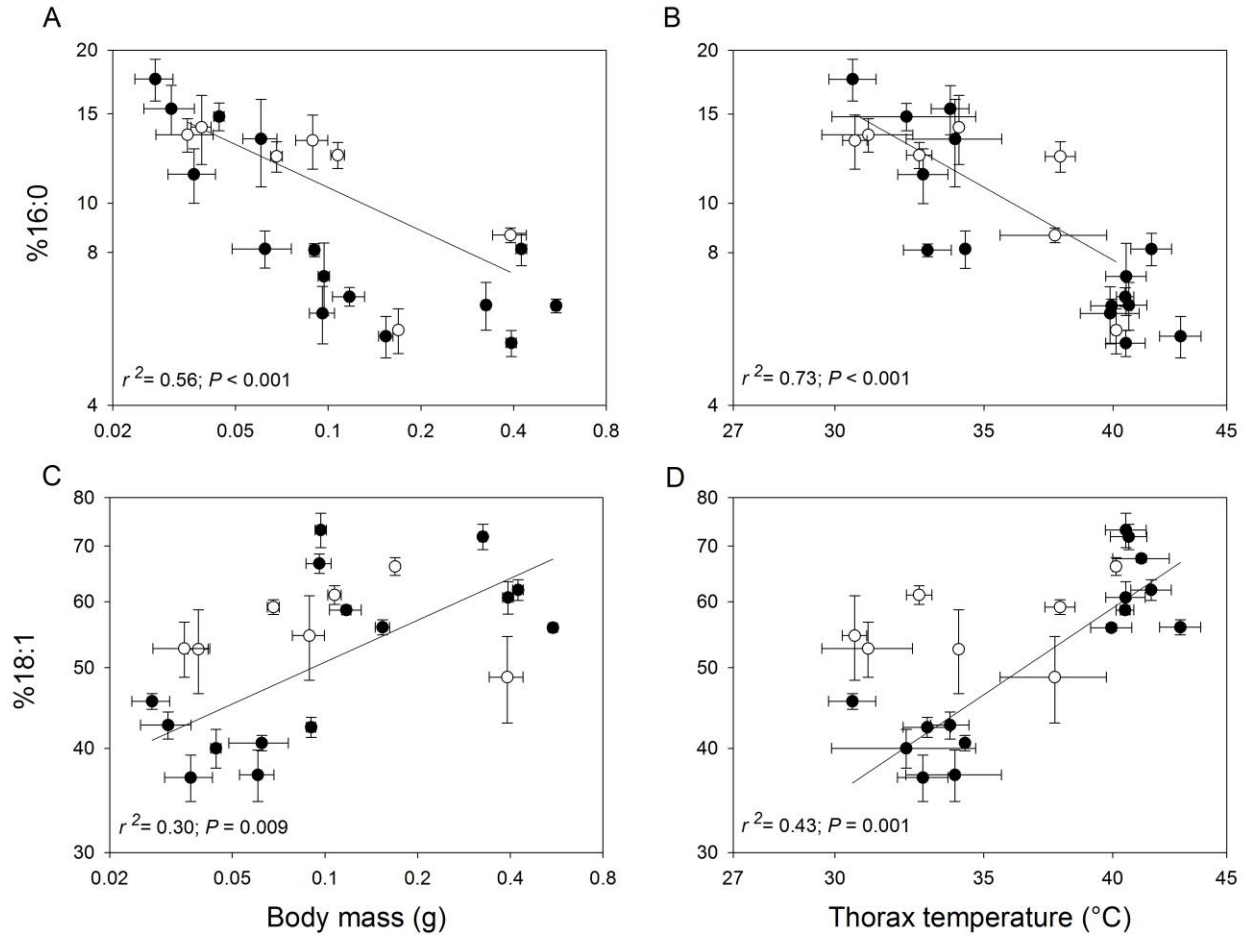
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322 **Figure 2.** Relationship between (A, C) body mass or (B, D) thoracic
 323 temperature and the relative abundance of 16:0 and 18:1 in 21 species of
 324 Hymenoptera, pertaining to two superfamilies: Apoidea (filled circles) and
 325 Vespoidea (open circles). Values are means (\pm S.E.M.) and results from the
 326 ANCOVA including superfamily as a covariate are presented.

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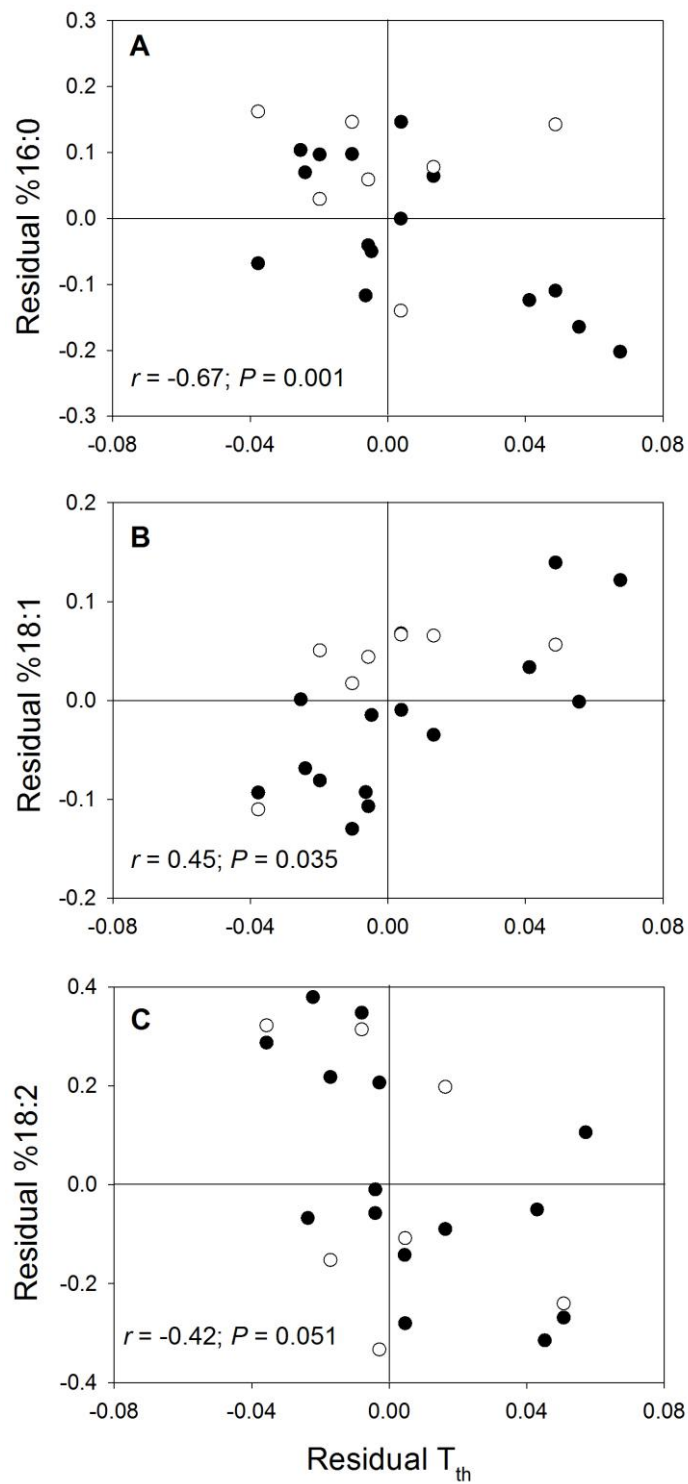
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Table 1. Sources of variation of Apoidea and Vespoidea species
FA composition traits. All variables were log₁₀-transformed. For
all models presented df: 1,18, except for 18:2 where df: 1,17.

	Variable	<i>F</i>	<i>P</i>
	16:0 Superfamily	0.946	0.344
	LogMass	3.057	0.097
	LogT _{th}	11.995	0.003
	18:0 Superfamily	0.223	0.642
	LogMass	0.186	0.672
	LogT _{th}	0.004	0.950
	18:1 Superfamily	6.363	0.021
	LogMass	0.011	0.919
	LogT _{th}	10.001	0.005
	18:2 Superfamily	0.227	0.640
	LogMass	2.985	0.102
	LogT _{th}	4.647	0.046
	Superfamily*LogMass*LogT _{th}	5.086	0.038
	18:3 Superfamily	0.020	0.890
	LogMass	0.463	0.505
	LogT _{th}	0.688	0.418



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Figure 3. Residual variation of (A) %16:0, (B) %18:1 and (C) %18:2 as a function of residual T_{th} in 21 species of Hymenoptera, pertaining to two superfamilies: Apoidea (filled circles) and Vespoidea (open circles).

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Table S1. Number and detailed classification of sampled Hymenopteran specimens (total $n=109$). Some specimens could only be identified to the Subgenus level. Queens (q) and workers (w) are indicated.

Superfamily (Series)	Family	Subfamily	Genus	Species	<i>n</i>		
Apoidea (Anthophila)	Andrenidae	Andreninae	<i>Andrena</i> (<i>Leucandrena</i>)	Sp.	4		
			<i>Andrena</i> (<i>Melandrena</i>)	Sp.	8		
			<i>Andrena</i> (<i>Scapteropsis</i>)	Sp.	2		
			<i>Andrena</i> (<i>Trachandrena</i>)	Sp.	6		
	Apidae	Apinae	<i>Apis</i>	<i>mellifera</i>	4		
	Halictidae	Halictinae	<i>Augochlora</i>	<i>pura</i>	5		
	Apidae	Apinae		<i>Bombus</i>	<i>affinis</i> (q)	3	
				<i>Bombus</i>	<i>bimaculatus</i> (q)	10	
				<i>Bombus</i>	<i>bimaculatus</i> (w)	5	
				<i>Bombus</i>	<i>griseocollis</i> (w)	2	
				<i>Bombus</i>	<i>impatiens</i> (q)	14	
				<i>Bombus</i>	<i>rufocinctus</i> (w)	5	
				<i>Bombus</i>	<i>ternarius</i> (q)	5	
				<i>Nomada</i>	<i>ruficornis</i> group	2	
				Apidae	Nomadinae	<i>Nomada</i>	<i>ruficornis</i> group
Megachilidae				Megachilinae	<i>Osmia</i>	<i>lignaria</i>	2
Vespoidea	Vespidae	Eumeninae	<i>Ancistrocerus</i>	<i>catskill</i>	2		
		Vespinae	<i>Dolichovespula</i>	<i>maculata</i>	2		
		Eumeninae	<i>Eumenes</i>	<i>fraternus</i>	3		
		Polistinae	<i>Polistes</i>	<i>dominula</i>	5		
			<i>Polistes</i>	<i>fuscatus</i>	13		
		Vespinae	<i>Vespula</i>	<i>flavopilosa</i>	4		
Vespinae	<i>Vespula</i>	<i>maculifrons</i>	3				

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