

# 1 **Specialization and adaptation in pollen sterol use by wild bees**

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## 11 **Abstract**

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14 Sterols are stabilizing components of membranes and hormone precursors in eukaryotes. Honeybees  
15 incorporate a subset of pollen-derived sterols into their tissues, rather than converting phytosterols to cholesterol  
16 as occurs in most insect herbivores. To establish whether this approach to sterol acquisition was typical  
17 of all bees, we measured the sterolome of 56 bee species representing all major bee families to  
18 identify and quantify which pollen sterols they used. The  $\Delta 5$  sterols, 24-methylenecholesterol,  
19 isofucoesterol and  $\beta$ -sitosterol, which are common components of pollen sterolomes were also the  
20 main sterols in most bee species, but this was not so in a minority of species suggesting they had  
21 different sterol requirements. Generalists contained more 24-methylenecholesterol than their specialist  
22 congeners, suggesting an adaptation to use pollen sterols that occur widely in plants whereas  
23 Asteraceae specialists use pollen sterols that are not used by generalists, which may explain the  
24 Asteraceae paradox. Overall, our data suggests an ecological rather than phylogenetic driver of bee  
25 sterol composition.

26  
27 **Keywords** Bee sterolome, wild bee nutrition, pollen sterols, 24-methylenecholesterol, isofucoesterol,  
28 campesterol.

## 29 30 31 **1 | Introduction**

32 The co-evolution of bees and flowers has led to an inter-dependency whereby many flowers are  
33 reliant on animal pollination and the flower visiting bees depend on floral nectar for carbohydrate and  
34 pollen for their protein and lipid requirements (Cappellari et al., 2016). However, the chemical

35 composition of pollen with respect to nutrient ratios, amino acid profiles, defence compounds and  
36 lipids including sterols is highly variable, potentially influencing the suitability of the pollen for  
37 palynivores (Arnold et al., 2014; Wright et al., 2018; Palmer-Young et al., 2019; ; Zu et al., 2021; Lau  
38 et al., 2022; Furse et al., 2023a; Baker et al., 2025).

39 Sterols are essential micronutrients serving key functions as membrane components and steroid  
40 hormone precursors in animals and plants. Sterols vary significantly in pollen with most variation  
41 explained by the position of a double bond in the steroid B ring and substitution at C-24 (Baker et al.,  
42 2025). For example, six pollen sterols are required to optimise brood production in honeybees and  
43 these are characterised by a double bond at C-5 ( $\Delta^5$  sterols) (Moore et al., 2025). Since pollen sterols  
44 vary widely across taxa and most pollen contains only a few major sterols some pollen may not be a  
45 suitable food source for all bees (Baker et al., 2025). Many of these pollen sterols such as 24-  
46 methylenecholesterol, isofucosterol, and desmosterol are not found in animal tissues and are scarce in  
47 terrestrial plant vegetative tissues (Villette et al., 2014; Zu et al., 2021, Baker et al., 2025). Honeybees  
48 have lost the capacity to synthesise cholesterol, and unlike other insects cannot dealkylate  
49 phytosterols at C-24 to produce cholesterol (Furse et al., 2023b). Instead, honeybees rely on  
50 phytosterols derived from pollen for tissue sterol composition, in place of cholesterol (Herbert *et al.*,  
51 1980; Moore et al., 2025). Moore et al., (2025) showed that 6 pollen sterols including 24-  
52 methylenecholesterol, isofucosterol and desmosterol supported substantial increases in brood  
53 production while Bogaert et al. (2025) also reported that 24-methylenecholesterol and isofucosterol  
54 were required by honeybees to produce offspring. This contrasts with many insect herbivores that can  
55 dealkylate dietary phytosterols to cholesterol, although sterol metabolic capacity varies substantially  
56 across taxa and some herbivores retain appreciable phytosterols in their tissues (Jing and Behmer,  
57 2020)..

58 Whether or not all bee species share the sterol metabolism of honeybees is unknown, though the few  
59 studies that have reported sterols from the tissues of other bees indicate that they have several of these  
60 sterols in common (e.g., 24-methylenecholesterol) (Feldlaufer *et al.*, 1993; Jing and Behmer, 2020;  
61 Vanderplanck, Zerck, *et al.*, 2020). Given the diversity of sterols in pollen (Baker et al, 2025), it is  
62 possible that other bee species show similarities between the sterolome of their food and the sterols  
63 they use in their tissues. The tissues of solitary bees contain phytosterols in common with honeybees  
64 but in different proportions. For example, *Diadasia rinconis* is dominated by 24-  
65 methylenecholesterol, while isofucosterol is three times higher in *Megachile rotundata* than  
66 honeybees, and  $\beta$ -sitosterol accounted for over 50% of total sterol content in *Colletes cunicularius*  
67 (Svoboda and Lusby, 1986; Feldlaufer *et al.*, 1993; Vanderplanck, Zerck, *et al.*, 2020). This suggests  
68 that they either ingest a different sterol content to honeybees and/or that these bee species have  
69 specific sterol transporters in the gut that favour particular sterols in food (Vought *et al.*, 2007).

70 Wild bees provide an important pollination service for many wildflower species as well as  
71 contributing to pollination of many crops (Hutchinson *et al.*, 2021). Some wild species also exhibit  
72 dietary specialisation in pollen foraging. Monolectic bee species for example forage from a single  
73 plant species for pollen, although this degree of specialisation is not common (Cane, 2021; Falk and  
74 Lewington, 2015) while oligolectic species collect pollen from species belonging to a single genus or  
75 family (Muller and Kuhlmann, 2008). Specialisation may limit distribution and phenology of wild  
76 bees. For instance, *Melitta dimidiata* (sainfoin bee) is confined to Salisbury plain in the south of  
77 England due to its reliance on *Onobrychis viciifolia* (Falk and Lewington, 2015). Asteraceae pollen is  
78 associated with oligolectic bee species and is rarely gathered by polylectic species such as honeybees,  
79 a phenomenon referred to as the ‘Asteraceae paradox’ (Müller and Kuhlmann, 2008; Praz, Müller and  
80 Dorn, 2008; Vanderplanck, Gilles, *et al.*, 2020). Polylectic bee species collect pollen from multiple  
81 families and are often referred to as generalist foragers. In the UK where the present study was  
82 undertaken all bumblebees and some solitary bee species are polylectic (Falk and Lewington, 2015).  
83 The UK also hosts approximately 70 kleptoparasitic bee species, including six cuckoo bumblebee  
84 species which do not have any morphological adaptations for collecting pollen on their bodies and  
85 rely on host bee species to provision their young (Falk and Lewington, 2015) and so present an  
86 interesting target for nutritional studies.

87 There are over 270 bee species native to the UK, which range in their distribution as a result of the  
88 distribution and phenology of different flowering plants, nesting requirements and climate (Goulson *et al.*,  
89 2005; National Biodiversity Data Centre, 2022). Florally homogenous landscapes may be  
90 nutritionally incapable of supporting a diverse bee community with a wide range of nutritional  
91 requirements, notably specialist species. In nutritionally homogenous arable landscapes, seed mixes  
92 designed to support bee populations are often targeted at common, generalist taxa such as  
93 bumblebees, however the nutritional value of these flowers for other bee species is currently  
94 unknown. Bumblebees are often target taxa of seed mixes designed to increase floral resources in  
95 amenity and agricultural settings (Edwards and Jenner, 2018). However, plant selection for these  
96 mixes is not based on an understanding of the nutritional value of specific flowers to a range of  
97 different bee species (Williams and Lonsdorf, 2018). Since sterols vary across plant taxa (Baker *et al.*,  
98 2025) specific sterol compounds may not be available in pollen from plants in all habitats.  
99 Therefore, we sought to determine for the first time how sterols varied in wild bees and how this  
100 related to pollen sterols.

101 Specifically the study addressed the following questions: 1) Which sterols are found in wild bee  
102 species and do they differ across taxa and in different floral landscapes?; 2) How variable is the  
103 sterolome of related generalist and specialist solitary bees and do kleptoparasites have similar  
104 sterolomes to their hosts?; 3) How variable are the sterolomes of bumble bees?; 4) Do bees regulate or  
105 bias their sterol incorporation?; 5) Can bees adapt to the pollen sterols of different plants? In

106 addressing these questions, we present sterol data from taxonomically diverse solitary bee species and  
107 bumblebees representing all bee families worldwide except for the Stenotritidae which is restricted to  
108 Australia. We examined the data for differences based on lecty and on phylogenetic relatedness and  
109 present a comprehensive overview of the patterns in sterol use across these species. In doing so we  
110 also reveal how the sterolome in bees is related to their pollen food (e.g., in monoleptic species and  
111 Asteraceae specialists); and how the sterols vary between bee tagmata and between sexes of the same  
112 bee species.

113

## 114 **3 | RESULTS**

### 115 **3.1 | The sterol profile of bees is largely defined by the three pollen sterols**

116 Total sterol content and sterol profile (sterolome) varied both within and between bee species (Figures  
117 1 and 2). Nineteen sterols were detected across 56 wild bee species of which 13 sterols were assigned  
118 using standards. The dataset included sterol analysis for seven eusocial *Bombus* species and 49  
119 solitary bee species and included 21.

120 The sterolomes of bees analysed here contained isofucosterol,  $\beta$ -sitosterol and 24-  
121 methylenecholesterol at a median percentage of >10%. The relative abundance of these three sterols  
122 varied as a function of bee species (Figure 1, Table S1). For example, the relative quantities of 24-  
123 methylenecholesterol within the genus *Lasioglossum* ranged from 3% in the shaggy furrow bee (*L.*  
124 *villosulum*, species mean) to 72% in Smeathman's furrow bee *L. smeathmanellum*.

125 The relative amount of  $\beta$ -sitosterol was never lower than 2% and it was recorded < 10% for only six  
126 species. In addition, 24-methylenecholesterol and  $\beta$ -sitosterol were the only sterols that were recorded  
127 at >70% of the sterolome of any given species (e.g.,  $\beta$ -sitosterol: *Hylaeus signatus*, 24-  
128 methylenecholesterol: *Halictus rubicundus*, *Lasioglossum smeathmanellum*). Fourteen of the nineteen  
129 sterols recorded accounted for less than 2% of the total sterols on average (Table S1). In Asteraceae  
130 specialists, cycloartanol was an important sterol (>15%: *Andrena denticulata*, *A. humilis*, *A. fulvago*,  
131 *Colletes daviesanus*, *Heriades truncorum*, *Osmia leaiana*, *Dasygoda hirtipes*) (Figure 1). The fungal  
132 sterol, ergosterol was recorded in 39 bee species and was likely recorded as a result of fungal  
133 contamination of pollen as it is recorded widely on plant pollen (Baker et al., 2025) (Table S1).

134 Most bee sterolomes contained at least 17 out of the 19 sterols we recorded (Table S3). Only three  
135 species contained fewer than 10 sterols: *Halictus rubicundus* (9), *Ceratina cyanea* (8), *Nomada*  
136 *flavopicta* (8). Five species contained no measurable cholesterol: *Andrena wilkella*, *Nomada*  
137 *flavopicta*, *Halictus rubicundus*, *Lasioglossum smeathmanellum* and *Coelioxys inermis*. Furthermore,  
138 campesterol was not detected in either *Halictus rubicundus* nor *Ceratina cyanea*. The occurrence of  
139 campesterol and cholesterol in relatively small quantities almost universally in bees was expected

140 since they are precursors to key hormones, ecdysone and makisterone (Furse et al., 2023) thus their  
141 absence from two species was surprising.

142 Only the proportions of three out of 19 sterols in the bee sterolome showed consistently phylogenetic  
143 signal (Figure 1): desmosterol ( $K=0.327$ ,  $p<0.050$ ,  $\lambda=0.790$ ,  $p<0.005$ ), campesterol ( $K=0.525$ ,  
144  $p=0.001$ ,  $\lambda=1.012$ ,  $p=0.001$ ) and ST(28:1)B ( $K=0.479$ ,  $p<0.050$ ,  $\lambda=0.758$ ,  $p=0.001$ ) (Figure S1). Four  
145 additional sterols showed significant phylogenetic signal (i.e.  $p < 0.05$ ) for at least one metric  
146 (Blomberg's  $K$  or Pagel's  $\lambda$ ): ST(28:1)A, 24-methylenecholesterol, sitostanol and salisterol. We also  
147 grouped the sterols by carbon chain and B-ring saturation and found that the 28 carbon chain sterols  
148 group and  $\Delta 0$  group showed significant phylogenetic signals for a single metric. In all except  
149 sitostanol, salisterol and the  $\Delta 0$  group, this significance was in Blomberg's  $K$  (Table S4).

150 We analysed separately the relationship between sterolome and phylogeny in the genus *Bombus*  
151 (Figure 2). None of the sterols, carbon number/B-ring substitution or total sterol ( $\mu\text{g/g}$ ) were  
152 associated with a significant phylogenetic signal in this genus. Across the bumblebee dataset (18  
153 species), whole-body tissues were dominated by 24-methylenecholesterol, isofucoesterol and  $\beta$ -  
154 sitosterol (Supplementary Table 2), reflecting a sterolome like that of honeybees (Moore *et al.*, 2025).  
155 Cholesterol was present in very low proportions across all species ( $< 0.5\%$  median) compared to  
156 campesterol (median 6.6%). Ergosterol was detected in all species at a median proportion of 2.1%.

157 Total sterol quantities in the bees varied more than the sterol profile. For example, the shaggy furrow  
158 bee, *Lasioglossum villosulum*, showed the widest range with a maximum of 1143  $\mu\text{g/g}$  and a  
159 minimum of 87  $\mu\text{g/g}$ . The species with the highest mean values (*Nomada rufipes* = 551  $\mu\text{g/g}$  and  
160 *Lasioglossum villosulum* = 489  $\mu\text{g/g}$ ) were both driven by outliers. For *Lasioglossum villosulum*, the  
161 two highest total sterol samples also had elevated quantities of the unassigned sterol, ST(28:1)B; these  
162 samples were collected from a different site than the other four samples. The red-thighed epeolus,  
163 *Epeolus cruciger*, had a less variable but higher total sterol content (293  $\mu\text{g/g}$ ) and was also better  
164 sampled.

165

### 166 **3.2 | The sterolomes of solitary bees and bumblebees differ.**

167 In the nine most sampled bee species in our dataset, the sterolome differed significantly among  
168 species except for one of the kleptoparasites and its host (Figure 3A, PERMANOVA,  $F_{8,118}=25.306$ ,  
169  $p=0.001$ , NMDS stress value = 0.151, pairwise comparisons,  $p < 0.05$ ). In this case, the ashy mining  
170 bee, *Andrena cineraria*, and its kleptoparasite, *Nomada fucata*, had a distinct sterolome from other  
171 bee species but the two species were not significantly different from each other ( $p>0.100$ ). It is  
172 notable that the sterolome of the heather colletes, *Colletes succinctus*, and its kleptoparasite, *Epeolus*

173 *cruciger*, shared similar sterolomes, presumably due to their parasite-host relationship but  
174 nevertheless still differed significantly.

175 Species identity explained over 50% of the variation in the data ( $R^2=0.632$ ). However, species groups  
176 also displayed significantly different variances (Fig 3,  $F_{8,118}=6.825$ ,  $p<0.001$ ). All species were  
177 associated with a difference in at least one sterol with the exception of *Epeolus cruciger* (Figure 3B),  
178 a parasite of *Colletes* and which had a sterolome that was similar to *Colletes hederiae* (Figure 3B). The  
179 pantaloon bee, *Dasypoda hirtipes*, an Asteraceae specialist, showed the most distinct sterolome which  
180 included strong associations with cycloartanol and cholesterol (Figure 3B).

181 In comparison to the larger dataset, the sterolomes of bumblebees exhibited only small differences in  
182 the proportions of sterols (Figure S2, PERMANOVA:  $F_{5,145}=2.938$ ,  $p=0.001$ , dispersion:  $F_{5,145}=2.095$ ,  
183  $p>0.050$ , NMDS stress value = 0.128). Species identity explained a low proportion of the variation in  
184 the data ( $R^2=0.092$ ). Pairwise comparisons showed the garden/ruderal bumblebee, *Bombus*  
185 *hortorum/ruderatus* and the brown-banded carder bee, *B. humilis*, are species with characteristically  
186 long tongues and thus typically distinctive floral preferences had the most distinct sterolomes. Both  
187 differed significantly from *B. pascuorum* ( $p<0.050$ ) and *B. terrestris/lucorum* ( $p<0.050$ ) as well as  
188 each other ( $p<0.050$ ). In addition, *B. humilis* differed significantly from the red-tailed bumblebee, *B.*  
189 *lapidarius* ( $p<0.050$ ).

190 Comparing all bumblebee species, the red-shanked carder bee, *B. ruderarius*, was more associated  
191 with campesterol (IV=0.32,  $p<0.050$ ). It is worth noting the indicator value for this association is very  
192 low (<0.4) suggesting there is little difference between species that can be attributed to individual  
193 sterols. Furthermore, *B. ruderarius* was represented only by a single sample.

194

### 195 **3.3 | The sterolomes of generalist and specialist bees**

196 While all bumblebees (*Bombus*) are generalist foragers, other genera display a wider variety of  
197 foraging types. For instance, the genus *Andrena* includes polylectic, oligolectic and monolectic  
198 species. Generalist (polylectic) *Andrena* species showed a significantly different sterol profile from  
199 specialist *Andrena* (oligo/monolectic) species (Figure 4, NMDS: stress = 0.077, PERMANOVA:  
200  $F_{1,11}=3.417$ ,  $p<0.050$ , dispersion:  $F_{1,11}=1.105$ ,  $p>0.100$ ). Generalist bees in the genus *Andrena* were  
201 strongly and significantly associated with 24-methylenecholesterol (IV= 0.85,  $p=0.010$ ) and the  
202 unassigned sterol ST(28:1)B (IV=0.86,  $p<0.015$ ). In contrast, specialist species were associated with  
203 cholesterol (IV=0.924,  $p<0.015$ ) and the unidentified sterol ST(30:1)A (IV=0.76,  $p=0.025$ ). However,  
204 foraging strategy only explained a small proportion of the variation in the data ( $R^2=0.237$ ).

205 In addition to differences between generalist and specialist bees, it should also be expected that  
206 specialist bees displayed distinct sterol profiles as a result of their host plant pollen. Two specialist

207 bees in the genus *Colletes* illustrate this relationship well; *C. succinctus* which targets heather  
208 (especially *Calluna vulgaris*) pollen, and *C. hederæ*, the ivy bee, which is a specialist of ivy pollen  
209 (*Hedera helix*) (Figure 5, stress= 0.105). These bees display significantly different sterolomes despite  
210 being closely related (Figure 5A, PERMANOVA,  $F_{1,31}=37.418$ ,  $p=0.001$ , dispersion:  $F_{1,31}=27.761$ ,  
211  $p<0.001$ ), with species identity accounting for over 50% of the variation in the data ( $R^2=0.547$ ).

212 *Colletes hederæ*, an ivy flower specialist, was most strongly associated with cycloartenol (IV=0.946,  
213  $p=0.005$ ) and avenasterol (IV= 0.856,  $p=0.005$ ) which are both prominent sterols in ivy pollen (Baker  
214 et al., 2025). In contrast, the profile of the heather specialist, *Colletes succinctus*, was strongly  
215 associated with  $\beta$ -sitosterol (IV= 0.814,  $p=0.005$ ) and isofucosterol (IV= 0.788,  $p=0.005$ ) (full list of  
216 associations not shown). These associations reflect sterols which are at higher proportions in their  
217 respective pollens, indicating that diet strongly influences bee sterolome (Figure 5C).

218

### 219 **3.4 | Bumblebees maintain a consistent sterol profile**

220 Bumblebees are generalist foragers and have been reported collecting pollen from a wide range of  
221 plants (Falk and Lewington, 2015). We compared the sterolome of bumblebee corbicular pollen to the  
222 mean sterolome of eighteen bumblebee species and to the sterolome of 295 different UK pollens  
223 (Baker et al. 2025). The sterol profile of corbicular pollen was more similar to bumblebees than the  
224 hand collected pollens suggesting bumblebees target pollen specifically. Corbicular pollen and  
225 bumblebees showed higher 24-methylenecholesterol than hand collected pollen which had higher  
226 cycloartenol and which is either absent or a minor sterol in two bumblebees (Figure S3A, NMDS  
227 stress value= 0.179, PERMANOVA:  $F_{2,350}=13.030$ ,  $p=0.001$ ). There was also a significant difference  
228 in the variance of the groups ( $F_{2,350}=28.015$ ,  $p <0.001$ ; Note: as the pollen sample types covered a  
229 much wider taxonomic breadth than the bumblebees, this difference in variation was expected). Hand  
230 collected pollen was strongly associated with sterols containing a cyclopropane ring, cyclolaudenol  
231 and cycloartenol, whereas corbicular pollen was strongly associated with the  $\Delta 5$  sterols, desmosterol  
232 and cholesterol (Figure S3 B).

233 To understand whether bumblebees maintained the same sterol profile in their tissues under different  
234 floral landscapes, a set of widely distributed and common *Bombus* species was sampled from at least  
235 three floristically distinct sites, each in a different UK county. Intraspecies variation in sterolomes  
236 between sites could indicate that bees are flexible in the sterols they can use. Only the common carder  
237 bee, *Bombus pascuorum*, exhibited a significantly different sterol profile at different collection sites  
238 (NMDS stress = 0.177, PERMANOVA:  $F_{3, 22}=1.818$ ,  $p<0.050$ , dispersion:  $F_{3,22}=0.504$ ,  $p>0.500$ ).

239 However, this was not highly significant ( $p=0.046$ ), and the variance explained by collection site was  
240 low ( $R^2=0.199$ ). Both the red-tailed bumblebee, *B. lapidarius*, and the buff-tailed bumblebee, *B.*  
241 *terrestris/lucorum*, showed no significant difference in sterolome between sites (*B. lapidarius*: NMDS

242 stress = 0.123, PERMANOVA:  $F_{2,13}=1.540$ ,  $p>0.100$ , dispersion:  $F_{2,13}=1.103$ ,  $p>0.100$ . *B.*  
243 *terrestris/lucorum*: NMDS stress = 0.172, PERMANOVA:  $F_{3,35}=1.162$ ,  $p>0.100$ , dispersion:  
244  $F_{3,35}=0.609$ ,  $p>0.500$ ). All NMDS biplots are shown in Figure S4. This suggests that bumblebee  
245 foraging can fulfil sterol needs in landscapes where floral resources differ.

246 The sterol use by bumblebees was consistent among species and across different habitats. In addition,  
247 the sterol requirements of bumblebees appeared also consistent between sexes and body tagmata.  
248 Analysis of individual head, thorax and abdomens of bumblebees showed that the sterol profile of the  
249 head differed significantly from the abdomen ( $p<0.050$ ) and thorax ( $p<0.010$ ). However, the variance  
250 explained by both was very low and differences varied by species (Figure S5, NMDS: stress=0.150,  
251 PERMANOVA: Tagmata:  $F_{2,181}=4.581$ ,  $p=0.001$ , Species:  $F_{5,181}=2.382$ ,  $p=0.001$ , Variance explained:  
252 Tagmata:  $R^2=0.045$ , Species:  $R^2=0.059$ ). *Bombus vestalis* had the highest comparable number of  
253 male/female specimens and there was no difference in sterolome between these groups (NMDS  
254 stress= 0.121, PERMANOVA:  $F_{1,17}= 2.06$ ,  $p=0.100$ , dispersion:  $F_{1,17}=0.339$ ,  $p>0.500$ ). When all  
255 eight species with male and female specimens were compared there was also no difference between  
256 sexes. NMDS biplots are shown in Figure S6.

### 257 **3.5 | Bee sterolomes diverge from pollen sterol profiles**

258 The sterolomes of specialist bees analysed in this dataset do not directly resemble those in their  
259 pollens indicating, as in honeybees (Moore et al., 2025), that bee sterolomes do not directly mirror  
260 pollen sterol composition.

261 For example, the sterolomes of the monoleptic bees *Melitta dimidiata*, *Macropis europaea*, *Melitta*  
262 *tricincta*, *Andrena florea* and two other species heavily dependent on a single pollen source (*Colletes*  
263 *hederae*, *C. halophilus*) differed significantly from their pollen ( $F_{1,56}=53.733$ ,  $R^2=0.231$ ,  $p=0.001$ ).  
264 There were also significant differences between bee-pollen paired groups ( $F_{5,56}=18.635$ ,  $R^2=0.401$ ,  
265  $p=0.001$ ). The interaction between these effects was also significant ( $F_{5,56}=5.854$ ,  $R^2=0.126$ ,  
266  $p=0.001$ ), indicating that the relative difference between bee and its host pollen depended on the  
267 pairing of specialist and host (Figure S7 NMDS: stress = 0.162).

268 Furthermore, some of the specialist bee species contained sterols which were not detected in their  
269 pollen. For example, the bryony mining bee, *Andrena florea*, and the sainfoin bee, *Melitta dimidiata*,  
270 both contained ergosterol (0.706% and 0.012% of total sterols respectively) while their pollen did not.  
271 The yellow-loosestrife bee, *Macropis europaea*, and the ivy bee, *Colletes hederae*, both contained  
272 cholesterol, which did not occur in their pollen (0.265% and 0.926% respectively) while desmosterol  
273 was absent from ivy (*Hedera helix*) but present in the ivy bee, *Colletes hederae* (1.299%). One  
274 interpretation of this is that these bees convert pollen sterols to cholesterol. However, the relative  
275 amounts were very low and bees likely obtain these minor sterols elsewhere. *M. europea*, for  
276 example, is an oil collecting species so may obtain cholesterol from floral oils or from microbial

277 activity in the larval provisions. Only the red bartsia bee, *Melitta tricincta*, contained sterols that were  
278 totally represented in its host species' pollen, *Odontites vernus*.

279

### 280 **3.6 | Bees can adapt to the pollen sterols of different plants**

281 The adaptation to using phytosterols in pollen may have benefits to bees from reducing the metabolic  
282 cost of sterol production. However, since honeybees are limited in the phytosterols they can use and  
283 restricted to acquiring these from pollen (Moore et al., 2025; Bogaert et al., 2025), this may also be  
284 the case for other species of bee. Plants could produce pollens that are 'undesirable' so to deter  
285 palynivory by producing higher quantities of unusable sterols. It would, therefore, be beneficial to  
286 bees if they could tolerate some of these 'undesirable' phytosterols in their tissues. Asteraceae was  
287 highlighted in Baker et al. (2025) as producing lower proportions of  $\Delta 5$  sterols and higher proportions  
288 of sterols with a B-ring cyclopropane substitution but still has specialist bee species associated with its  
289 pollen. To determine whether these bees have adapted to these sterols, we compared the sterolomes of  
290 generalist bee species, Asteraceae specialist bee species, and non-Asteraceae specialists to the pollen  
291 sterols of all three Asteraceae tribes which differ greatly in their pollen sterolomes (Cichorioideae,  
292 Asteroideae, and Carduoideae) (Figure 6A). While bee and pollen sterolomes were distinct, those of  
293 the Asteraceae specialist bees were most similar to Asteraceae pollen, specifically Cichorioideae  
294 (PERMANOVA, stress=0.151;  $F_{5,96}=16.041$ ,  $p=0.001$ ). Generalist bee species were significantly  
295 associated with 24-methylenecholesterol (ISA, Figure 6C). Non-Asteraceae specialist bees were  
296 significantly associated with  $\beta$ -sitosterol and Asteraceae specialists with cholesterol. Different  
297 Asteraceae tribe pollens were associated with at least one sterol with a B-ring cyclopropyl substitution  
298 (CPR) each: Asteroideae with cycloartenol (IV=0.564,  $p=0.005$ ), Carduoideae with cyclolaudenol  
299 (IV=0.568,  $p=0.005$ ) and Cichorioideae with cycloartanol (IV=0.576,  $p=0.010$ ). Carduoideae was  
300 associated with the most individual sterols (Figure 6C).

301 When comparing all 56 bee species, Asteraceae specialists showed significantly lower proportions of  
302  $\Delta 5$  sterols and higher proportions of sterols with a B-ring cyclopropane substitution (CPR) than non-  
303 Asteraceae specialist, generalist and parasitic bee species (CPR:  $H(3)=19.077$ ,  $p<0.005$ ,  $\Delta 5$ :  
304  $H(3)=12.743$ ,  $p<0.050$ , *post-hoc* Dunn tests: CPR ( $p<0.005$ ),  $\Delta 5$  ( $p<0.050$ )) (Figure 6B).

305

## 306 **4 | DISCUSSION**

307 This study represents the largest survey of wild bee sterolomes to date. The whole-body tissues of all  
308 56 bee species analysed were dominated by phytosterols rather than cholesterol, indicating that in  
309 general, bees do not convert dietary sterols into cholesterol. Instead, they use a range of phytosterols

310 that they acquire from pollen. Overall, bee sterolomes were characterized by the presence of 24-  
311 methylenecholesterol,  $\beta$ -sitosterol and isofucoesterol, which is consistent with previous work (Svoboda  
312 and Lusby, 1986; Vanderplanck, Zerck, *et al.*, 2020). These three sterols were the major sterolome  
313 components in *Bombus* spp. which was similar to that in honeybees (Svoboda *et al.*, 1980; Moore *et*  
314 *al.*, 2025). These sterols occur in high proportions across a wide taxonomic range of floral pollen  
315 (Baker *et al.*, 2025; Zu *et al.*, 2021) indicating that many flowering species may provide suitable  
316 sterols for many bees. Our data implies that these three sterols are therefore best suited to bee  
317 physiological requirements, perhaps in part due to their  $\Delta^5$  B-ring substitution. However, some sterols  
318 including cycloartenol, cycloeucalenol and obtusifoliol that occur in the pollen of plants commonly  
319 visited by bees were not recorded as being used by bees, suggesting that sterol incorporation is  
320 regulated rather than passive. The lack of phylogenetic signal in most sterols across the dataset  
321 indicates the bee sterolome is driven by other forces such as foraging choice and life history, creating  
322 significant differences between even closely related bees.

323 Sterols are a stabilizing compound in cell membranes which impact membrane permeability and  
324 fluidity (Reviewed in Dufourc, 2008). The phytosterols stigmasterol and  $\beta$ -sitosterol can form rafts  
325 and maintain a stable membrane fluidity across a wider temperature range than cholesterol (Beck *et*  
326 *al.*, 2007). Abundance of these specific sterols in bees could therefore be an adaptation to colder  
327 temperature conditions, as observed in *Drosophila*. Flies are flexible in their uptake of specific sterols;  
328 when they are reared in cold conditions (7.1°C), they took up proportionally more  $\beta$ -sitosterol and  
329 stigmasterol than cholesterol from food than flies reared at 28.6°C (Knittelfelder *et al.*, 2020).  
330 Although not as widely abundant as more common sterols, campesterol, cycloartenol and avenasterol  
331 were present in proportions (>30%) in some bee species suggesting their incorporation into  
332 membranes. Cycloartenol for instance has been shown to substitute for cholesterol when maintaining  
333 membrane dynamics in model tissues (Dufourc, 2008).

334 Insects also require sterols for the synthesis of steroid hormones: in most species, cholesterol is  
335 converted into moulting hormones (Jing and Behmer, 2020). Honeybees need cholesterol and  
336 campesterol, respectively, to synthesize the hormones 20-hydroxyecdysone and makisterone A  
337 (Feldlaufer *et al.*, 1986). Other dietary sterols can also be essential to successful development in bees,  
338 as seen with the reliance of a stingless bee on ergosterol to pupate (Paludo *et al.*, 2018). The few bee  
339 species that have been studied for their steroid hormone precursors show that bees may share the trait  
340 of using campesterol as the basis for production of the alternative moulting hormone, makisterone A  
341 (Feldlaufer *et al.*, 1993; Paludo *et al.*, 2018). In our data, the phylogenetic trend seen in campesterol,  
342 where closely related species showed similar proportions, may therefore be due to its importance in  
343 hormone production. Further, the absence of cholesterol or campesterol from some species (*Andrena*  
344 *wilkella*, *Nomada flavopicta*, *Halictus rubicundus*, *Lasioglossum smeathmanellum*, *Coelioxys inermis*  
345 and *Ceratina cyanea*) could indicate that these species use other moulting hormone synthesis

346 pathways (Feldlaufer *et al.*, 1986; Furse, Koch, *et al.*, 2023). It is however important to note that these  
347 species were all represented by a single sample. Therefore, it is possible cholesterol and campesterol  
348 were present in concentrations too low to be detectable in these samples.

349 It was shown from the analysis of bees at different collection sites that locations with different plant  
350 communities did not affect sterolomes in two of three bumblebee species analysed. This suggests that  
351 bumblebees maintain a relatively consistent sterolome even when foraging on different pollen  
352 sources, implying regulated use of dietary sterols even in generalist species. The seemingly stable  
353 ratio between sites may therefore represent a compromise between readily available dietary sterols  
354 and a physiological optimum (Knittelfelder *et al.*, 2020). This strategy may contribute to the  
355 ecological success of bumblebees, allowing them to forage in an extremely wide range of habitats,  
356 using pollen from many different plant species. Within the bumblebee dataset, the interspecies  
357 differences and species-sterol associations were the result of small variations in proportions of  
358 different sterols, likely a result of their similar foraging and life history strategies. As such, the  
359 ecological relevance of these differences may be limited.

360 Corbicular pollen collected from bumblebees showed a profile distinct from hand-collected pollens,  
361 correlating with higher 24-methylenecholesterol. It is therefore possible that bees are targeting certain  
362 sterols in pollen, though there is currently no evidence to suggest they can detect sterols directly.  
363 There are also other potential causes of variation between hand and bee collected pollens; bumblebees  
364 can change their pollen foraging target during a single trip (Martínez-Bauer *et al.*, 2021) and they add  
365 saliva to collected pollen to aid compaction. Further, the bees analysed in this study will likely have  
366 collected different pollen to those they were raised on.

367 Despite some similarities, the sterolomes of congeneric generalist and specialist bees were distinct.  
368 For instance, polylectic *Andrena* species had higher proportions of 24-methylenecholesterol than  
369 oligo/monolectic species in this genus. The sterolome of honeybees is comprised of the sterols they  
370 require including 24-methylenecholesterol along with isofucoesterol and sitosterol (Moore *et al.*, 2025;  
371 Bogaert *et al.*, 2025) and therefore the sterols we find in other bee species may also be the sterols they  
372 need. Thus, where generalists forage across a range of flowers to ensure access to desirable sterols  
373 such as 24-methylenecholesterol and isofucoesterol, they may do so at a cost of competition for  
374 resources. Specialist foraging strategies may be driven by several factors and specialist bees may  
375 benefit from adapting their sterolome to make best use of their pollen host, as seen in *Colletes*. That  
376 said, there is no evidence that specialists experience less competition than generalists (Wcislo & Cane  
377 1996) although specialisation could have benefits that outweigh its cost (e.g., greater foraging  
378 efficiency). Our data, however, do suggest there is flexibility in sterol use in some specialist bees that  
379 have developed specific sterol needs and concurs with the concept of sterol nutritional niches  
380 (Behmer and Joern, 2006). This is illustrated in highly specialised bees, which exhibit sterolomes that  
381 are distinct from those of their host pollen, consistent with regulated incorporation of dietary sterols,

382 as recently shown for honeybees (Moore et al., 2025). Some bee species contained sterols that were  
383 absent from their pollen, indicating that the specialist plant-bee relationship is flexible and bees are  
384 using pollen from additional plant species or other sources such as plant oils and fungal contaminants.  
385 It could alternatively be explained by the fact that these sterols were not detected in our samples. In  
386 addition, the sterol requirements of specialist bees could be fulfilled by plants other than their  
387 preferred species; for this reason, we expect that the sterols are not what are driving specialization in  
388 host-pollinator relationships. Given that relying on a single pollen host may mean a bee is more  
389 vulnerable to changes in flowering time and distribution, other nutritional or ecological factors must  
390 be driving this relationship.

391 We were able to identify potential specialist sterol adaptation most distinctly in Asteraceae specialist  
392 bees due to their frequency in the dataset. The pollen sterolomes of Asteraceae flowers display lower  
393 levels of  $\Delta 5$  sterols such as 24-methylenecholesterol,  $\beta$ -sitosterol and isofucosterol which are found  
394 commonly in generalist bees and higher levels of sterols with  $\Delta 8$  B-ring bonds and cyclopropane rings  
395 (Baker et al., 2025). This may be a potential reason for the infrequent use by generalist bee species of  
396 sterols without a  $\Delta 5$  sterols (Müller and Kuhlmann, 2008; Vanderplanck, Gilles, *et al.*, 2020) as these  
397 sterols do not appear suitable for the majority of bee species and may prevent successful growth and  
398 development (Jing, Grebenok and Behmer, 2012; Behmer, 2017). Our results showed that this trend  
399 was reflected in the sterolomes of Asteraceae specialist bees which often had higher proportions of  
400 cycloartanol and cholesterol and lower proportions of  $\Delta 5$  sterols. The ability to incorporate much  
401 higher levels of cycloartanol in their tissues than most other bee species suggests that these species  
402 have adapted to Asteraceae pollen. However, these bees still maintained a dominance of  $\Delta 5$  sterol,  
403 indicating there are limits to sterolome adaptation in bees. The ability to overcome the nutritional  
404 deficiencies of Asteraceae pollen may have allowed these bees to benefit from the widespread and  
405 abundant availability of these flowers.

406 Cichorioideae flowers produced some of the highest cholesterol proportions reported in Baker et al.  
407 (2025) (*Helminthotheca echioides*: 60.05%, *Crepis capillaris*: 44.21%, *Leontodon saxatilis*: 31.33%,  
408 *Crepis vesicaria*: 27.77%). The use of cholesterol as a dietary sterol has been proposed as facilitating  
409 the transition from carnivory to palynivory in Apoidea evolution (Santerre, 2023). As bees evolved  
410 from carnivorous ancestors to pollinivorous diets, cholesterol rich pollens may have provided a  
411 suitable source of useable sterols before bees adapted to use a wider range of phytosterols, as  
412 suggested by Dötterl and Vereecken (2010). *Dasypoda* species, which occupy a basal position in bee  
413 evolution, are also ancestrally oligolectic on Asteraceae flowers (Michez *et al.*, 2008) and their  
414 sterolome had strong associations with cholesterol.

415 Our data does not reveal a singular clear trend in sterol profiles of wild bees. There is no evidence for  
416 a strong phylogenetic signal in most sterols, therefore, the drivers of sterol profile in bees are likely to  
417 be ecologically, rather than phylogenetically, constrained but with some plasticity. However, there are

418 multiple instances of sterol profiles differing from those expected by ecological drivers, for instance  
419 where monoleptic bee species sterol profiles do not match the sterol proportions of their pollen host.  
420 Proportional differences between pollen and bee sterol profiles could arise from several non-mutually  
421 exclusive processes, including regulated incorporation or retention of sterols, as well as other  
422 nutritional or ecological factors that influence pollen foraging choices. Future work can build on these  
423 data by experimentally manipulating the diets of bees to determine if they are fixed to a profile of  
424 specific sterols or if they demonstrate greater flexibility. The ecological and economic importance of  
425 wild bees makes a comprehensive knowledge of their nutritional requirements an important step in  
426 understanding the impact of floral community change on bee populations.

427

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447

#### 448 **AUTHOR CONTRIBUTION**

449 EB, GAW, PCS and WEK designed the study, performed the analyses, and drafted the first version of  
450 the manuscript. EB, EL and KB undertook sampling and prepared samples for analysis. All authors  
451 contributed to analyses, interpretation of the results, and the final form of the manuscript.

## 452 2 | MATERIALS AND METHODS

453 Two datasets were collected and analysed for this paper. The first was a set of bumblebee specimens,  
454 covering 18 species, comprising whole bodies, tagmata and corbicular pollen loads. The second was a  
455 set of 56 wild bee species, comprising only whole-body samples. Processing, sample analysis and data  
456 analysis for both sets of samples is detailed below.

457

### 458 2.1 | Sample collection

#### 459 2.1.1 | Bumblebees

460 Female workers of all UK bumblebee species were targeted for collection. A minimum collection  
461 target of 5 individuals per species was set and specimens collected with landowner permission. Rare  
462 species (*Bombus distinguendus* and *B. sylvarum*) were collected only with prior arrangement with the  
463 Bumblebee Conservation Trust. Queens from abundant and common species ('The big 7': *Bombus*  
464 *hortorum*, *B. hypnorum*, *B. lapidarius*, *B. lucorum*, *B. pascuorum*, *B. pratorum* and *B. terrestris*) were  
465 collected to assess tagma composition. Female worker *Bombus lapidarius*, *B. terrestris* and *B.*  
466 *pascuorum* were collected from at least three different sites to facilitate intra-species analysis. For  
467 kleptoparasitic bumblebee species, which do not have workers, queens were collected, with males  
468 collected when this was not possible. For *Bombus ruderarius* and *B. campestris*, no female workers  
469 could be collected and so males were collected instead. To ensure there was not a difference between  
470 the sterols of male and females of the same species, males were opportunistically collected from well  
471 sampled common species (*Bombus hortorum*, *B. lapidarius*, *B. lucorum*, *B. ruderatus*, *B. rupestris*, *B.*  
472 *vestalis*) to enable statistical comparison.

473 Bees were captured using a net and euthanized in a -20°C freezer. They were then stored at -20°C and  
474 moved to -80°C storage in batches. After sample collection, bees were defrosted for dissection and the  
475 full gut, crop, sting and venom sacs removed from the abdominal cavity. This was to prevent  
476 misinterpretation of sterols detected from gut contents with those incorporated into body tissues. Any  
477 visible parasites were also removed. Ovaries, where present, were retained. A hind leg was also  
478 removed from all samples for future barcoding of specimens with indeterminate characters. For any  
479 male specimens, genitalia were retained, and guts removed. A set of queen bumblebees were also  
480 separated into body tagmata (head, thorax, abdomen). Any loose pollen in corbiculae or on the body  
481 was removed by brushing and full corbicular pollen loads collected into 1.5ml glass vials. Dissected  
482 specimens were then refrozen at -80°C.

483 Species with indeterminate characters were barcoded to confirm identification as follows. Hind legs  
484 were stored at -80°C in a 1.5ml Eppendorf. DNA was extracted from legs using a Qiagen DNeasy  
485 Blood & Tissue Kit following the supplementary protocol 'Purification of total DNA from insects

486 using the DNeasy® Blood & Tissue Kit'. Legs were homogenised using four 2.3mm ceramic beads in  
487 an MP FastPrep-24™ 5G bead beating grinder and lysis system in five cycles of 35s at 6 m/second  
488 followed by 180s rest. The genomic DNA was then sent to SureScreen Scientifics for PCR, barcoding  
489 and species identification.

490

### 491 **2.1.2 | Wild solitary bees**

492 Species were chosen to cover a wide phylogenetic range and included all foraging strategies. These  
493 included monolecty a foraging strategy whereby bees feed from a single species (Cane, 2021),  
494 oligolecty, polylecty and kleptoparasitism. Close relatives that had different foraging strategies were  
495 prioritised to understand the effects of phylogeny and ecology. A sample size of three individuals for  
496 each species was targeted initially but this was not possible for some species. Very common bee  
497 species were collected at least three times, and from different locations at the collection site to best  
498 capture intra-species variation. In total, 56 bee species were collected, with ten of these represented by  
499 a single sample. All samples were female except for *Melecta albifrons* which was represented by two  
500 male specimens. Collection was primarily focused in the South of England, from Royal Botanic  
501 Gardens (RBG), Kew and Wakehurst by HK. Other species procured from specific sites were done so  
502 with the permission of landowners. Bees were collected with a net then euthanised and stored at -  
503 20°C. Pollen was brushed from the outside of the body and removed from any pollen-collecting  
504 structures such as corbiculae or scopae. Guts were retained within specimens as removal would have  
505 likely damaged other internal tissues for the smallest specimens and so processing was kept  
506 consistent.

507

## 508 **2.2 | Sample preparation**

### 509 **2.2.1 | Bumblebees**

510 All whole-body and tagma samples were covered in 5ml or 3ml of deionised water respectively and  
511 re-frozen at -80°C before being freeze dried. Samples were then transferred to plastic bead-beating  
512 vials containing four 2.3mm steel beads and homogenised at 30rpm in a Qiagen tissue raptor for 3  
513 minutes in two 1.5 minute intervals. Homogenised tissue was then transferred back to their glass vial  
514 in a 1:50 (mg:µl) dilution of GCTU (Guanidine (6 M guanidinium chloride) and thiourea (1.5 M)  
515 dissolved in deionised H<sub>2</sub>O together and stored at room temperature out of direct sunlight) and re-  
516 frozen at -80°C. For each sample, 60µl of homogenate was transferred to a 96-well plate for sterol  
517 extraction. GCTU was used as a buffer as previously reported (Furse et al., 2024) because it  
518 suppresses lipase activity, is antimicrobial, aids in the break-up of cellular structure without damaging  
519 target lipid metabolites and provides a pipettable solution.

520

521 Paired corbicular pollen loads weighing >20mg were separated and those weighing 10-20mg were  
522 combined for sterol analysis. As previously described (Furse et al., 2023a), pollens were suspended in  
523 200µl KOH and vortexed to disperse. They were then boiled at 75°C for two hours. Twelve which  
524 had dried out due to methanol evaporation during that time were re-suspended in 150 µl methanol. All  
525 saponified material was then transferred to a 96-well plate for sterol extraction.

526

### 527 **2.2.2 | Wild bees**

528 Specimens were covered in deionised water, frozen at -80°C and freeze dried until all water had been  
529 removed. Samples were then stored at -80°C before being homogenised. Specimens were  
530 homogenised in GCTU using a BioSpec Tissue Tearor with a 14mm head attachment.

531

### 532 **2.3 | Sample data collection**

533 All plates included controls to monitor instrument functioning: Blanks (60µl GCTU) were used to  
534 detect and remove background noise and at the start of each plate. A stock solution of reference  
535 materials verified by multi-nuclear NMR (QA) was used to calculate coefficient of variance.  
536 Reference materials were available for: 24-methylenecholesterol, 24-methylenecycloartenol,  
537 anthelsterol (a sterol found in *Baccharoides anthelmintica*, ST(28:3), that is active under 330 nm UV  
538 but whose structure has not been determined formally using NMR) (Furse et al., 2023a), avenasterol,  
539 β-sitosterol, brassicasterol, campesterol, cholesterol, cycloartenol, cycloeucaleanol, cyclolaudenol,  
540 desmosterol, episterol, ergosterol, isofucoesterol, schottenol, sitostanol, spinasterol and stigmasterol.  
541 The  $m/z$  and retention time for each sterol is shown in Table S5. Aliquots of 40 µl were used in the  
542 run. A QC solution of homogenised commercially available honeybee collected pollens (*Helianthus*  
543 *annuus*, *Nymphaea* sp., *Fagopyrum esculentum*) and bumblebee adults, larvae and pupae in GCTU (6M  
544 guanidine + 1.5M thiourea to make 1 L dissolved in deionised water) was run at a series of  
545 concentrations to test the range of linearity of the instrument. Three concentrations of QC were used:  
546 100%, 50%, 25%, corresponding to 40 µl, 20 µl and 10 µl aliquots of the QC stock solution. These  
547 were analysed in the same way as the samples to calculate the correlation between analyte  
548 concentration and signal size. Variables whose ratio was <0.75 were discarded.

549 All samples were then extracted as 96-well plates by SF (96-well plate, Esslab Plate+™, 2.4 ml/well,  
550 glass-coated) using a 96-channel pipette (liquid-handling robot, Integra Viaflow 96/384 channel  
551 pipette) as follows. First 150µl of internal standard ( $d_7$ -cholesterol) was added to all samples.  
552 Concentration differed between plates as detailed above.

553 Then 500µl DMT (250µl ×2) was added to all samples. DMT comprised dichloromethane (DCM) (3  
554 parts), methanol (1 part) and triethylammonium chloride (0.0005 parts, i.e. 500 mg/l), mixed and

555 stored at room temperature out of direct sunlight. Then 500 µl of deionised water was added before  
556 agitating using the multi-channel pipette. Layers were separated by spinning for 2 min (methanol +  
557 water, Solid, DMT + sterol) and 50 µl of extract (DMT & sterol) transferred to a 384-well plate and  
558 left for 30 min for the DMT to evaporate. Once the 384-well plate was full, 150 µl of LCMS quality  
559 methanol was added and the plate sealed with foil.

### 560 **2.3.1 | Bumblebees**

561 Samples were run across two 384-well plates comprising eight individual 96-well plates. Sample  
562 order was randomised within each 96-well plate.

563 For plate A, whole-body bumblebee samples, the concentration of internal standard (*d*<sub>7</sub>-cholesterol)  
564 was 5 mg/l (1.99098e<sup>-9</sup> Moles/sample). For plate C, body tagmata and pollen samples, it was 1 mg/l  
565 (3.98196e<sup>-10</sup> Moles/sample).

### 566 **2.3.2 | Wild bees**

567 For each sample, 60µl of homogenate was transferred to a 96-well plate for sterol extraction. Samples  
568 were run across two 384-well plates comprising eight individual 96-well plates. Sample order was  
569 randomised within each 96-well plate. The concentration of *d*<sub>7</sub>-cholesterol for all plates in this run  
570 was 5 mg/l (1.99098e<sup>-9</sup> Moles/sample).

571

## 572 **2.4 | Sample analysis and data processing**

573 The liquid chromatography- mass spectrometry analysis of samples was completed following Furse *et*  
574 *al.* (2023a) and Baker *et al.* (2025). Instrument output was also processed as established in Furse *et al.*  
575 (2023a). Relative abundance was calculated by dividing the signal area for each metabolite by the  
576 signal of internal standard (*d*<sub>7</sub>-cholesterol).

577

578 These values were used for the majority of data analysis. For total sterol analysis, instrument output  
579 (*m/z*) was converted to µg/g (dry weight of the sample) first by dividing the signal area for each  
580 metabolite by the signal of internal standard (*d*<sub>7</sub>-cholesterol) then using the following formula:

$$581 \frac{\text{Sterol } (\mu\text{g})}{\text{Tissue } (g)} = \frac{\text{sterol molecular mass } \left(\frac{g}{\text{mol}}\right) \times (\text{signal} \times \text{mol of } d_7\text{chol.}) \times 1,000,000}{\text{Starting weight } (mg)/1,000}$$

582

583 The amount of mol of *d*<sub>7</sub>-cholesterol was:

- 584 • Bumblebee plate (whole bodies): 1.99098e<sup>-9</sup> Moles/sample
- 585 • Bumblebee plate (tagmata and pollen): 3.98196e<sup>-10</sup> Moles/sample

- 586 • Wild bee plates (whole bodies):  $1.99098e^{-9}$  Moles/sample

587

#### 588 **2.4.1 | Bumblebees**

589 Due to the impact of the COVID-19 pandemic a second analysis run was needed to analyse samples  
590 of *Bombus distinguendus* and *B. jonellus* which were collected a year later than intended. Ten samples  
591 which had also shown much higher proportions of un-named sterols than the rest of the samples in the  
592 first run were also re-analysed as part of this run. The second analysis detected a different selection of  
593 low-abundance sterols compared to the previous run. As the primary aim of this work was to enable  
594 comparison between species and sites, any sterols which were not detected in both runs were therefore  
595 removed from data analysis to enable the most accurate comparisons. This led to the removal of two  
596 sterols from the second run (stigmasterol, ST(28:2)C, ST(31:2)A, ST(30:3)) and ten from the first (24-  
597 MCA, sitostanol, ST(27:2)C, ST(28:2)C, ST(29:0)A, ST(29:0)B, ST(29:2)A, ST(29:2)B, ST(29:2)C,  
598 ST(30:2)A, ST(30:2)C and ST(31:2)A). Proportions were re-calculated using only sterols detected in  
599 both runs (24-methylenecholesterol,  $\beta$ -sitosterol, campesterol, cholesterol, cycloartenol,  
600 cyclolaudenol, desmosterol, ergosterol and isofucosterol). Total sterol values for this dataset are  
601 therefore lower than if all detected sterols were included. Inclusion of this second data set did not alter  
602 the significance of any findings regarding phylogenetic and interspecies analysis.

603

#### 604 **2.4.2 | Phylogenetic analysis**

605 Phylogenetic signal was calculated separately for each dataset following Zu *et al.* (2021). Phylo4d  
606 objects were created using phylo4d() from PHYLOBASE (R Hackathon *et al.*, 2024).

607 Phylosignal(reps=999) from PHYLOSIGNAL (Keck *et al.*, 2016) was used to calculate Pagel's  $\lambda$  and  
608 Blomberg's K. These measures of phylogenetic signal were calculated for the individual proportions  
609 of all 19 sterols, total sterol ( $\mu\text{g/g}$ ) and proportion of sterols belonging to different carbon chain  
610 lengths (27, 28, 29, 30, 31) and double bond saturation categories (cyclopropane ring(CPR), 0, 5, 7,  
611 NA). Ergosterol was classed as NA for double bond position as it has double bonds at both 5 and 7.  
612 All unidentified sterols were also classed as NA.

613 The phylogenetic trees were plotted by creating a phy-data object using treedata() from GEIGER  
614 v.2.0.11. (Pennell *et al.*, 2014). Family and sub-genus clades were identified using getMRCA() from  
615 APE v.5.8.(Paradis and Schliep, 2019). Phylogeny was plotted with ggtree() from GGTREE  
616 v.3.12.0.(Yu *et al.*, 2017, 2018; Yu, 2020). Heatmap and bar graph associated with phylogeny were  
617 plotted using ggplot() from TIDYVERSE v.2.0.0.(Wickham *et al.*, 2019). The composite graph was  
618 then plotted using APLOT v.0.2.2.(Yu, 2023).

#### 619 **2.4.3 | Bumblebees**

620 The most comprehensive phylogeny of the *Bombus* genus to date was used with permission from  
621 Hines (2008). Phylogeny was trimmed using `keep.tip()` from APE v.5.8.(Paradis and Schliep, 2019) to  
622 only include sampled species. *Bombus sylvestris* was not available in the phylogeny and so not  
623 included in these analyses. Subgenera were defined following Williams *et al.* (2008). Individuals of  
624 *Bombus terrestris* and the *B. lucorum* complex were combined into *Bombus terrestris/lucorum* for  
625 analysis as many samples had indeterminate characters. Similarly, *Bombus hortorum* and *B. ruderatus*  
626 were combined into *B. hortorum/ruderatus*. To plot these species groups on the phylogeny branch,  
627 tips for *Bombus terrestris* and *B. hortorum* were used.

628

#### 629 **2.4.4 | Wild bees**

630 The supermatrix phylogeny produced by Henríquez-Piskulich, Hugall and Stuart-Fox (2024) was used  
631 to assess phylogenetic signal in the sterol dataset for the wild bee dataset. The 4,586 bee species dated  
632 chronogram with outgroups removed was selected for analysis. The tree was downloaded from Dryad,  
633 imported into R and pruned to 56 species using APE v.5.8.(Paradis and Schliep, 2019). Our dataset  
634 contained six out of the seven currently recognised families within the clade of Anthophila (bees):  
635 Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae and Melittidae. Supplementary taxonomic  
636 data from Henríquez-Piskulich, Hugall and Stuart-Fox (2024) for all 56 species is available in the  
637 supplementary resources (Table S6).

638

#### 639 **2.5 | Data analysis**

640 All data analysis was done in R version 4.4.0 (2024-04-24 ucrt) (R Core Team, 2024) and R studio  
641 (2024.9.1.394) (Posit team, 2024). For each data type (whole-body, individual tagma, corbicular  
642 pollen), a Bray-Curtis dissimilarity matrix was used to identify outliers using `disana()` from the  
643 LABDSV package v.2.1-0 (Roberts, 2023). Any data point with a minimum dissimilarity over 0.5  
644 compared to all other points for that group was identified as an outlier, as per author guidance, and so  
645 was removed. No samples exceeded the outlier threshold in the bumblebee data, so all data was  
646 included in the analysis. For the wild bee dataset, each species with at least three replicates was tested  
647 for outliers. This led to the removal of five samples in total: One *Andrena flavipes*, two *Andrena*  
648 *florea*, one *Anthophora plumipes* and one *Colletes hederæ*. Seven specimens were removed from  
649 total sterol analysis as their post-freeze drying weight suggested an error, meaning their signals data  
650 could not be converted into  $\mu\text{g/g}$  units. This was likely due to incorrectly calibrated weighing  
651 equipment.

652 Summary statistics were calculated from species means using the STATS package from R (R Core  
653 Team, 2024). To carry out statistical comparisons of sterol profile between groups, proportion data  
654 (0-1) was used, where zero denotes a sterol is absent from a sample, and one denotes it is completely

655 dominant. Groupings used in individual analyses are specified below. Data were arcsine square root  
656 transformed and used to calculate a Bray-Curtis dissimilarity matrix using `vegdist(method="bray")`.  
657 The matrix was used to plot an NMDS using `metaMDS(autotransform=FALSE)`. The lowest number  
658 of axes was selected where stress was  $\leq 0.2$  (established cut off, (Bakker, 2024)). The stress value  
659 reflects how well the ordination represents the original data. `Adonis2()` was used to carry out a  
660 PERMANOVA testing for significant differences between groups. Differences in variance between  
661 groups which could invalidate comparisons were tested using `anova()` and `betadisper()`. Pairwise  
662 comparisons between groups were carried out following Bakker (2024), using `adonis2()` with a  
663 Benjamini-Hochberg correction. All functions were from VEGAN (Oksanen *et al.*, 2024) except  
664 `anova()` from the STATS package (R Core Team, 2023). A 2D biplot was created from the NMDS  
665 with a stress value of  $< 0.2$ . Strong ( $> 0.7$  absolute value) and significant ( $p < 0.0100$ ) sterol associations  
666 were plotted over the datapoints using `ggplot()` and `ggrepel()` from GGLOT2 (Wickham, 2016) and  
667 GGREPEL (Slowikowski, 2024).

668 The INDICESPECIES package (De Cáceres and Legendre, 2009) was used to carry out Indicator  
669 Species Analysis (ISA) using `multiplot()` to determine significant associations between and individual  
670 sterols. ISA was done at the level of individual groups only and not for combinations of groups.  
671 Hereafter IV refers to indicator value in the results section.

## 672 **2.5.1 | Bumblebees**

### 673 *2.5.1.1 | Differences in sterol profile among UK bumblebee species*

674 The six species with the highest number of samples, including male and female specimens, were used  
675 in an NMDS (`trymax=200`, `k=3`) to compare sterol profile between species. The first two dimensions  
676 of the NMDS are plotted in Figure S2. All available whole-body samples were used in an ISA to test  
677 for associations between individual sterols and species.

### 678 *2.5.1.2 | Comparison between bumblebee collected pollen and whole-body tissue sterol profiles*

679 A total of 40 large pollen baskets were collected from females to analyse for their individual sterol  
680 content. An NMDS was calculated using this data ( $n=40$ ), means of all bumblebee species ( $n=18$ ) and  
681 all 295 pollen species detailed in Baker *et al.*, (2025) ( $n=295$ ) (`trymax = 200`, `k=2`). ISA was then  
682 carried out to test for associations between sterols and each of these groups. As the pollen analysis  
683 had produced a wider range of sterols, only those present in both analyses were used to calculate total  
684 sterols and then proportions for the pollen data. These sterols were 24MC,  $\beta$ -Sitosterol, campesterol,  
685 cholesterol, cycloartenol, cyclolaudenol, desmosterol, ergosterol and isofucosterol.

### 686 *2.5.1.3 | Intra-species sterol profile across different foraging environments*

687 To determine whether there were significant intra-species differences driven by habitat, female  
688 workers of a set of species that are common in a range of habitats were collected from an urban

689 garden environment (Royal Botanic Gardens, Kew), a semi-natural, dry, calcareous grassland site  
690 (sites near Rollestone Camp and Bulford Camp, Salisbury Plain Training Area, Wiltshire)(Natural  
691 England, 2015), and a semi-natural grass moor and heather moorland site (Harthope Valley, Cheviot  
692 hills, Northumberland National Park, Northumberland) (Natural England, 2013). These species were  
693 *Bombus terrestris/lucorum*, *B. pascuorum*, and *B. lapidarius*. Between site comparisons were plotted  
694 for *Bombus pascuorum*, *B. lapidarius* and *B. terrestris* individually using NMDS (trymax = 200, k =  
695 2) and sites were compared statistically using PERMANOVA.

#### 696 2.5.1.4 / *Bumblebee body tagmata (head, thorax, abdomen) sterol profiles*

697 Tagmata were analysed by NMDS (trymax = 200, k=2) and PERMANOVA to test for significant  
698 differences in sterol profile between head, thorax and abdomen. The strata argument was used to  
699 control for the effect of tagma being from the same individual. Species identity was also included as a  
700 fixed effect in the model. Additionally, ISA was carried out on the data to test for associations  
701 between tagmata and individual sterols.

#### 702 2.5.1.5 / *Sex differences in sterol profile*

703 Where collection of females was not possible, male bumblebees were collected instead. For each  
704 species which had at least one male and female specimen (*B. barbutellus*, *B. hortorum/ruderatus*, *B.*  
705 *lapidarius*, *B. monticola*, *B. terrestris/lucorum*, *B. rupestris*, *B. sylvestris*, *B. vestalis*) an NMDS and  
706 PERMANOVA was calculated using means for each species-sex combination to prevent the often-  
707 higher female sample sizes affecting the results (trymax=200, k=2). Sex and species identity were  
708 included as fixed effects. *Bombus vestalis* had highest number of male specimens (11) and a  
709 comparable number of female specimens (8) and was therefore used for a separate NMDS and  
710 PERMANOVA (trymax=200, k=2).

711

### 712 2.5.2 / *Wild bees*

#### 713 2.5.2.1 / *Differences in sterol profile across bee species*

714 Species which were represented by at least 10 samples in the dataset were used for an NMDS  
715 comparison of inter-species differences. This included nine species from seven genera. An NMDS  
716 was calculated from proportion data using all available samples for these species (trymax=200, k=2).

#### 717 2.5.2.2 / *Differences in sterol profile between generalists and specialists*

718 *Andrena* was the most speciose genus in the dataset and contained a similar number of  
719 oligolectic/monolectic (6) and polylectic (7) species which were not in monophyletic groups. *Andrena*  
720 species were therefore used in a NMDS (trymax=200, k=2) to calculate if the two lecty groups had  
721 significantly different sterol profiles.

722 2.5.2.3 | Differences in sterol profile between specialists

723 *Colletes hederæ* and *C. succinctus* were both sampled from >3 sites with at least 10 replicates and  
724 have different foraging preferences. They were therefore used in an NMDS (trymax=200, k=2) to  
725 compare sterolome between these two closely related species that had different pollen hosts.

726 2.5.2.4 | Sterol profiles of monolectic and narrowly oligolectic bee species compared with their  
727 pollens

728 The dataset contained four monolectic bee species and two species which are narrowly specialised in  
729 the UK (*Colletes halophilus* and *Colletes hederæ*). Comparisons were made between the bee and  
730 pollen samples and between the bee-pollen groups via NMDS (trymax=200, k=2) and PERMANOVA  
731 to determine whether specialist bees grouped closer with their pollen host or other bee species.

732 2.5.2.5 | Asteraceae specialist bees

733 An NMDS (trymax=200, k=2) was calculated to compare the sterol profiles of solitary bees  
734 specialising on Asteraceae, non-Asteraceae specialists, polylectic bees and pollen from three tribes of  
735 Asteraceae (Carduoideae, Cichorioideae, Asteroideae). When comparing pollen and solitary bee sterol  
736 profiles, only sterols that had been detected in both datasets were used and proportions re-calculated  
737 using the remaining sterols. This led to a total of 17 sterols with ST(30:1)A and ST(30:1)B being  
738 removed from the bee dataset. Aligning data in this way will have altered the proportions of the  
739 remaining sterols in both datasets. However, to retain them would have made it impossible to fairly  
740 compare in an analysis.

741

742 Sterol data were then also grouped by B-ring double bond saturation (cyclopropane ring (CPR),  $\Delta 0$ ,  
743  $\Delta 5$ ,  $\Delta 7$ ,  $\Delta 8$ , NA) with ergosterol and all unidentified sterols classed as NA (ergosterol is a  $\Delta 5$  and  $\Delta 7$   
744 sterol) after Baker et al., (2025). In order to compare the proportions of different B-ring double bond  
745 groups between Asteraceae and non-Asteraceae specialist bees a Kruskal-Wallis rank sum test was  
746 carried out using `kruskal.test()` followed by pairwise Wilcoxon tests using `pairwise.wilcox.test()`, both  
747 from the STATS package (R Core Team, 2024). Both analyses used a Benjamini-Hochberg correction  
748 for multiple testing.

749

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## Figure legends

### 889 **Figure 1.**

890 *Phylogeny of 56 bee species subset from the supermatrix phylogeny produced by Henríquez-Piskulich,*  
 891 *Hugall and Stuart-Fox (2024), showing family clades and foraging status. Species mean sterol*  
 892 *percentages for seven key sterols are plotted along with total sterols ( $\mu\text{g/g}$ ) for all samples (species*  
 893 *means are shown by grey bars, error bars show  $\pm$  standard error). 24-methylenecholesterol,*  
 894 *isofucosterol and  $\beta$ -sitosterol account for the majority of sterols in most bee species. However, their*  
 895 *proportions are highly variable between species. Bee families do not appear to show conserved*  
 896 *sterolomes and only 3/19 sterols tested showed significant phylogenetic signal across the tree. Higher*  
 897 *levels of cycloartanol appear across the tree and are often associated with species which are*  
 898 *specialist (oligolectic) on Asteraceae flowers.*

### 899 **Figure 2.**

900 *Bombus species phylogeny subset from Hines (2008) with subgenera highlighted. Heatmap shows*  
 901 *mean species proportion for the most commonly dominant five sterols plus cholesterol. Bar graph*  
 902 *shows mean species total sterol production  $\pm$ SEM. Points show individual samples of each species*  
 903 *with different colours for each county the species was sampled from. The kleptoparasitic Bombus*  
 904 *species do not show a sterolome distinct from other Bombus species. All bumblebee species analysed*  
 905 *were dominated by 24-methylenecholesterol, isofucosterol and  $\beta$ -sitosterol, with no phylogenetic*  
 906 *signal in any of the nine sterols analysed. Bombus sylvestris was omitted from this tree and so not*  
 907 *included in phylogenetic analyses. Ruder = ruderalis & Hort = hortorum.*

### 908 **Figure 3.**

909 *A) Interspecies comparison of sterol profile among species with  $\geq 10$  samples demonstrating*  
 910 *significant interspecies differences in sterolome (PERMANOVA,  $F_{8,118}=25.306$ ,  $p=0.001$ ). Grey lines*  
 911 *indicate significant ( $p<0.010$ ) and strong (absolute value  $>0.7$ ) correlations. Outlined points*  
 912 *represent means for each group and ellipses show 95% confidence interval. There are clear*  
 913 *similarities in the sterol profile of ecologically linked species such as Epeolus cruciger with Colletes*  
 914 *succinctus and Nomada fucata with Andrena cineraria, both of which are kleptoparasite-host pairs.*  
 915 *All pairwise comparisons were significantly different ( $p < 0.05$ ) except Nomada fucata and Andrena*  
 916 *cineraria. There were significant differences in variance between species with Colletes succinctus*  
 917 *displaying the most conserved sterolome and Nomada fucata the most variable. NMDS stress value =*  
 918 *0.151.*

919 *B) Indicator Species Analysis of species with  $\geq 10$  samples as shown in Figure 3A. Of the 19 sterols*  
 920 *tested, 15 were indicators of individual species groups. Indicator values (IV) and p-values are shown*  
 921 *for significant sterols. Dasypoda hirtipes was associated with the most sterols and shows a distinct*  
 922 *grouping in Figure 3A. Epeolus cruciger was not associated with any individual sterols, potentially as*  
 923 *a result of its high similarity in sterolome with Colletes succinctus as well as other bee species.*

### 924 **Figure 4.**

925 *A) NMDS of generalist (polylectic) and specialist (oligolectic/monolectic) Andrena species (stress*  
 926 *value =0.077) which showed a significant difference between groups (PERMANOVA:  $F_{1,11}=3.417$ ,*  
 927  *$p<0.050$ ). Grey lines indicate significant ( $p<0.010$ ) and strong correlations (absolute value  $>0.7$ ).*  
 928 *Outlined points represent means for each group and ellipses show 95% confidence interval.*  
 929 *Generalist species were correlated with higher 24-methylenecholesterol and specialist species are*  
 930 *associated with higher cholesterol.*

931 **B)** Proportions of dominant sterols across all *Andrena* species included in Figure 4A. All remaining  
932 sterols are collapsed into a single group. Generalist species show higher proportions of 24-  
933 methylenecholesterol and specialist species that target Asteraceae species (*A. denticulata*, *A. fulvago*  
934 and *A. humilis*) show higher proportions of cycloartanol. *Andrena florea*, which specialises on  
935 *Bryonia cretica*, contains very high proportions of un-named sterols suggesting this bee's sterolome  
936 requires further characterisation. *Andrena fuscipes* and *A. hattorfiana* specialise on *Ericaceae* and  
937 *scabious* flowers (*Knautia* and *Scabiosa* spp.) respectively.

938 **Figure 5.**

939 **A)** Sterol composition of all replicates of *Colletes hederæ* and *C. succinctus* (*Hedera helix* and  
940 heather species respectively). Only major sterols are named with the remainder collapsed into single  
941 group. *Colletes succinctus* demonstrated much higher proportions of  $\beta$ -sitosterol whereas *C. hederæ*  
942 contains higher proportions of avenasterol. Both species show a sterolome that is largely consistent  
943 across samples.

944 **B)** NMDS of *Colletes hederæ* and *C. succinctus* (stress= 0.105). Species show distinct sterolomes  
945 (PERMANOVA,  $F_{1,31}=37.418$ ,  $p=0.001$ ) despite their relatedness. *C. hederæ* is associated with  
946 higher avenasterol and cycloartenol whereas *C. succinctus* is associated with higher  $\beta$ -sitosterol.  
947 *Colletes hederæ* shows a larger variation in sterolome, potentially due to its broader foraging habit.  
948 Grey lines indicate significant ( $p<0.010$ ) and strong correlations (absolute value  $>0.7$ ). Outlined  
949 points represent means for each group and ellipses show 95% confidence interval.

950 **C)** Sterol composition of the main pollen hosts of *Colletes hederæ* and *C. succinctus* (*Hedera helix*  
951 and heather species). Only major sterols are named with the remainder collapsed into single group.  
952 The three *Ericaceae* heather species show a similar sterol profiles, dominated by isofucosterol and  
953  $\beta$ -sitosterol. *Hedera helix* sterol profile differs more from that of *C. hederæ* than pollen heather  
954 sterols do from *C. succinctus*.

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956 **Figure 6.**

957 **A)** NMDS comparing the sterol profile of bees which specialise on Asteraceae pollen to those which  
958 do not (stress = 0.151). Bees and pollen species appear distinct in NMDS space. Most Asteraceae  
959 specialists target Cichorioideae and can be seen to cluster closer to these pollen than other  
960 Asteraceae. Groups were significantly different (PERMANOVA, stress=0.151;  $F_{5,96}=16.041$ ,  
961  $p=0.001$ ) and group identity explained nearly 50% of the variation in the dataset ( $R^2=0.455$ ). All  
962 pairwise comparisons between groups were significant ( $p<0.050$ ) except Asteroideae versus  
963 *Carduoideae* pollen ( $p>0.100$ ). Grey lines indicate significant ( $p<0.010$ ) and strong correlations  
964 (absolute value  $>0.7$ ). Outlined points represent means for each group and ellipses show 95%  
965 confidence interval.

966 **B)** Proportion of sterols belonging to different double bond position groups across solitary bee  
967 species. Asteraceae specialists show a lower proportion of  $\Delta 5$  sterols and higher proportion of sterols  
968 with a B-ring cyclopropane substitution (CPR) than the rest of the dataset. (CPR:  $H(3)=19.077$ ,  
969  $p<0.005$ ,  $\Delta 5$ :  $H(3)=12.743$ ,  $p<0.050$ , post-hoc Dunn tests: CPR ( $p<0.005$ ),  $\Delta 5$  ( $p<0.050$ )). No other  
970 comparisons were significantly different.

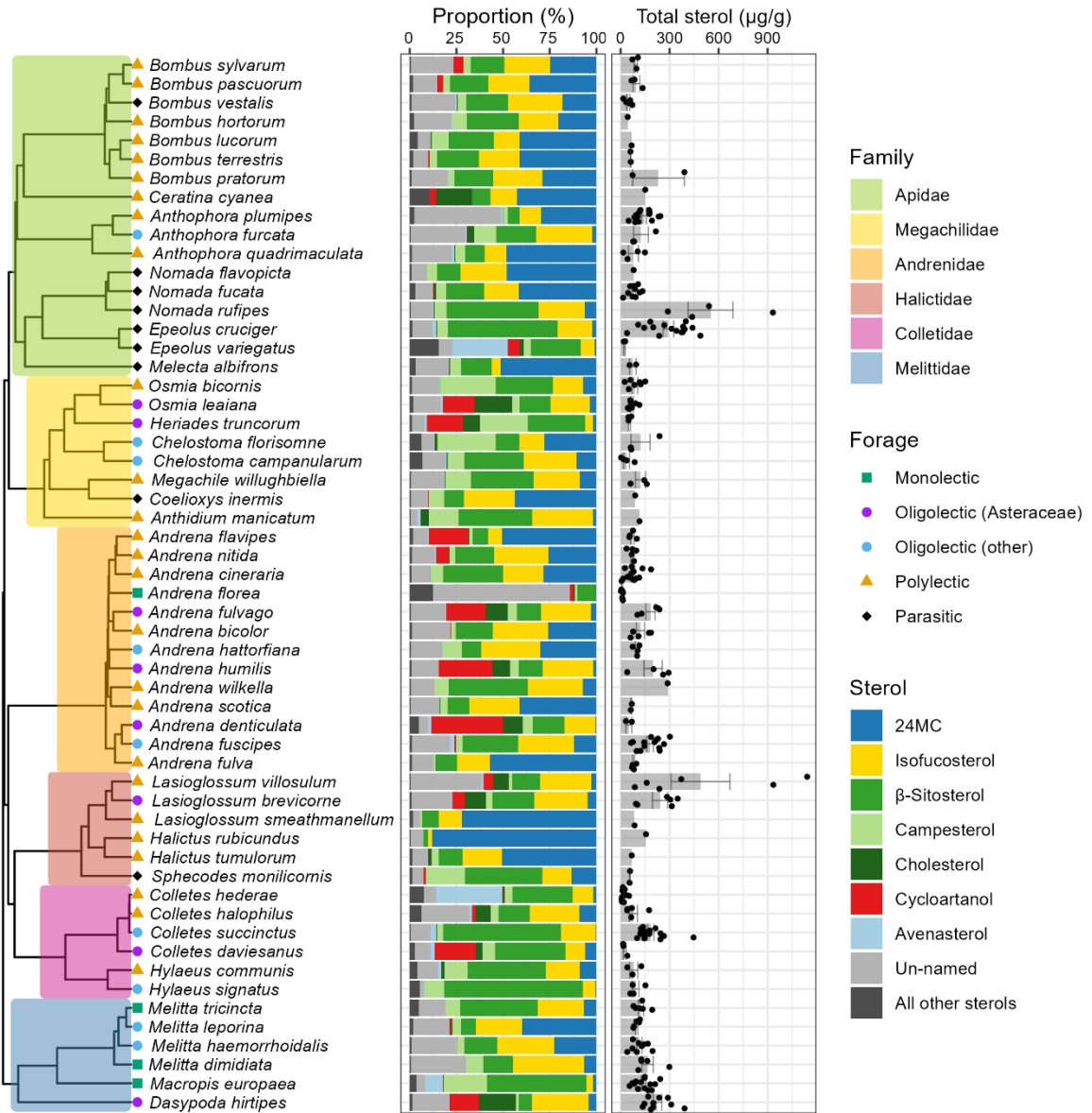
971 **C)** Summary of Indicator Species Analysis results showing associations between groups in Figure 6A  
972 and individual sterols. All bee groups were associated with  $\Delta 5$  sterol whereas each pollen group was  
973 associated with a sterol containing a cyclopropane ring.

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977 **Figure 1.**



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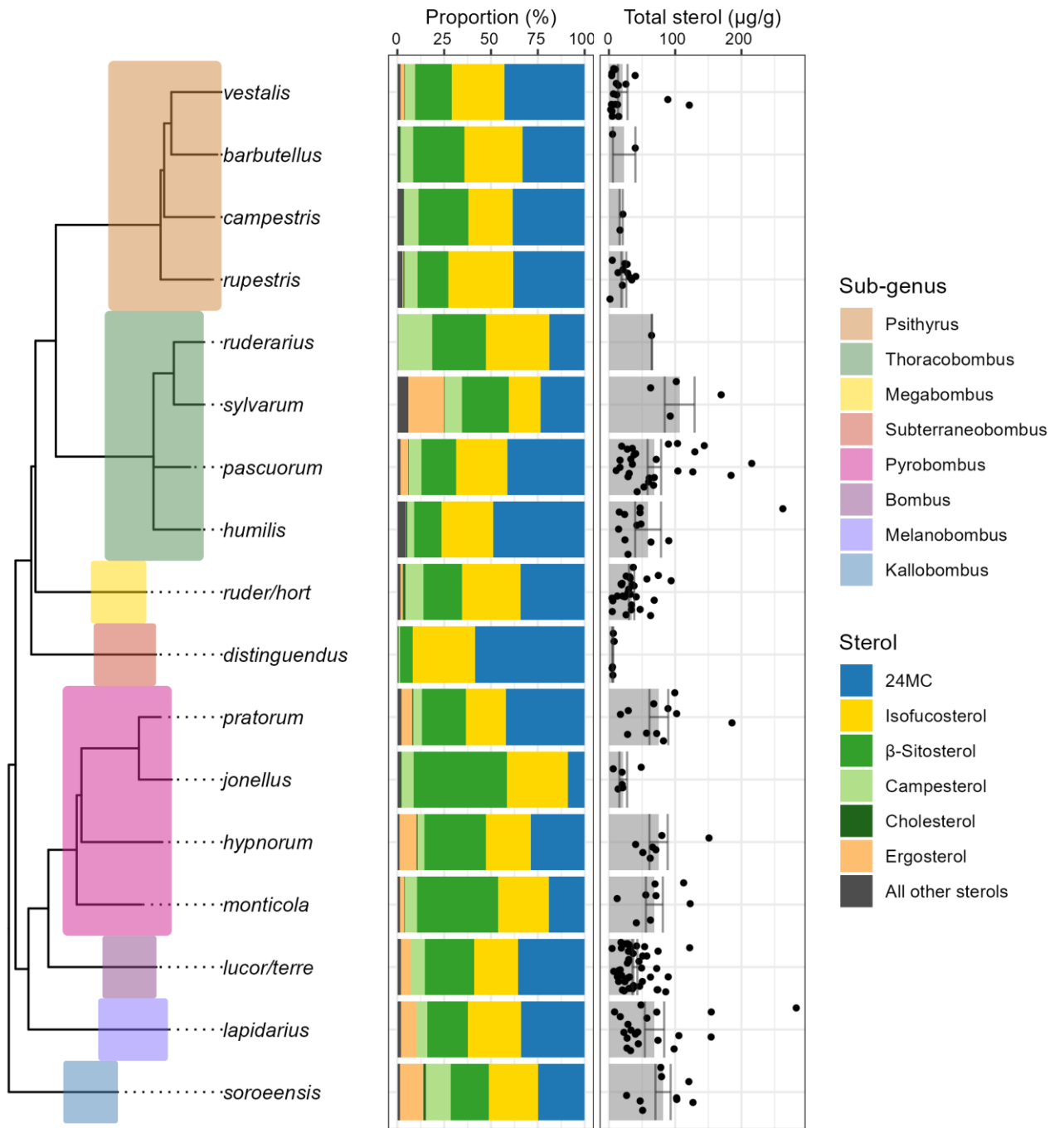
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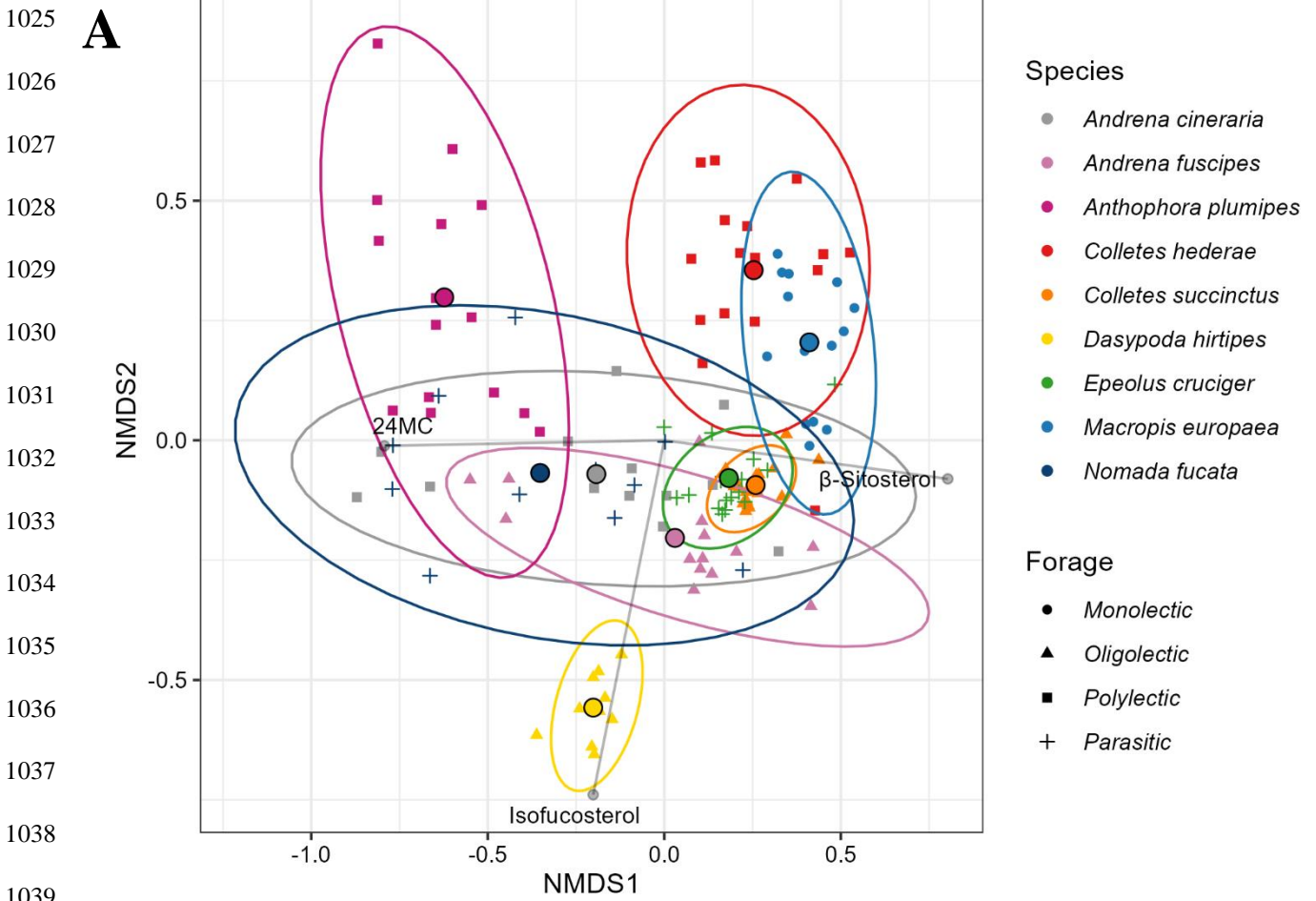
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1024 **Figure 3.**



**B**

| Sterol                     | IV    | P-value | Sterol                     | IV    | P-value |
|----------------------------|-------|---------|----------------------------|-------|---------|
| <i>Andrena cineraria</i>   |       |         | <i>Dasygaster hirtipes</i> |       |         |
| ST(28:1)A                  | 0.523 | 0.005   | Cycloartanol               | 0.803 | 0.005   |
| <i>Andrena fuscipes</i>    |       |         | Cholesterol                | 0.744 | 0.005   |
| ST(29:1)A                  | 0.419 | 0.020   | Ergosterol                 | 0.589 | 0.005   |
| <i>Anthophora plumipes</i> |       |         | ST(29:2)A                  | 0.416 | 0.020   |
| ST(28:1)B                  | 0.830 | 0.005   | Isofucosterol              | 0.394 | 0.005   |
| Sitostanol                 | 0.477 | 0.005   | <i>Macropis europæa</i>    |       |         |
| <i>Colletes hederæ</i>     |       |         | Campesterol                | 0.497 | 0.005   |
| Avenasterol                | 0.611 | 0.005   | <i>Nomada fucata</i>       |       |         |
| Cycloartenol               | 0.581 | 0.005   | 24MC                       | 0.515 | 0.005   |
| ST(30:1)B                  | 0.477 | 0.005   |                            |       |         |
| <i>Colletes succinctus</i> |       |         |                            |       |         |
| β-Sitosterol               | 0.416 | 0.005   |                            |       |         |

1041 **Figure 4.**

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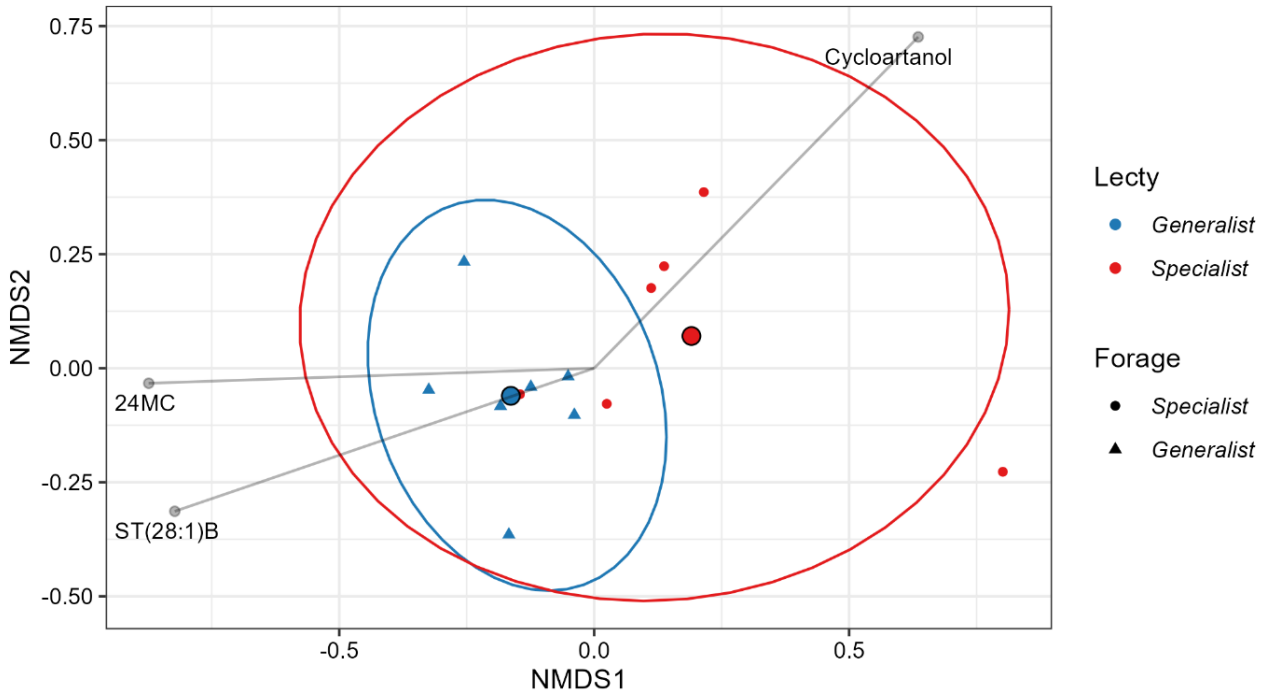
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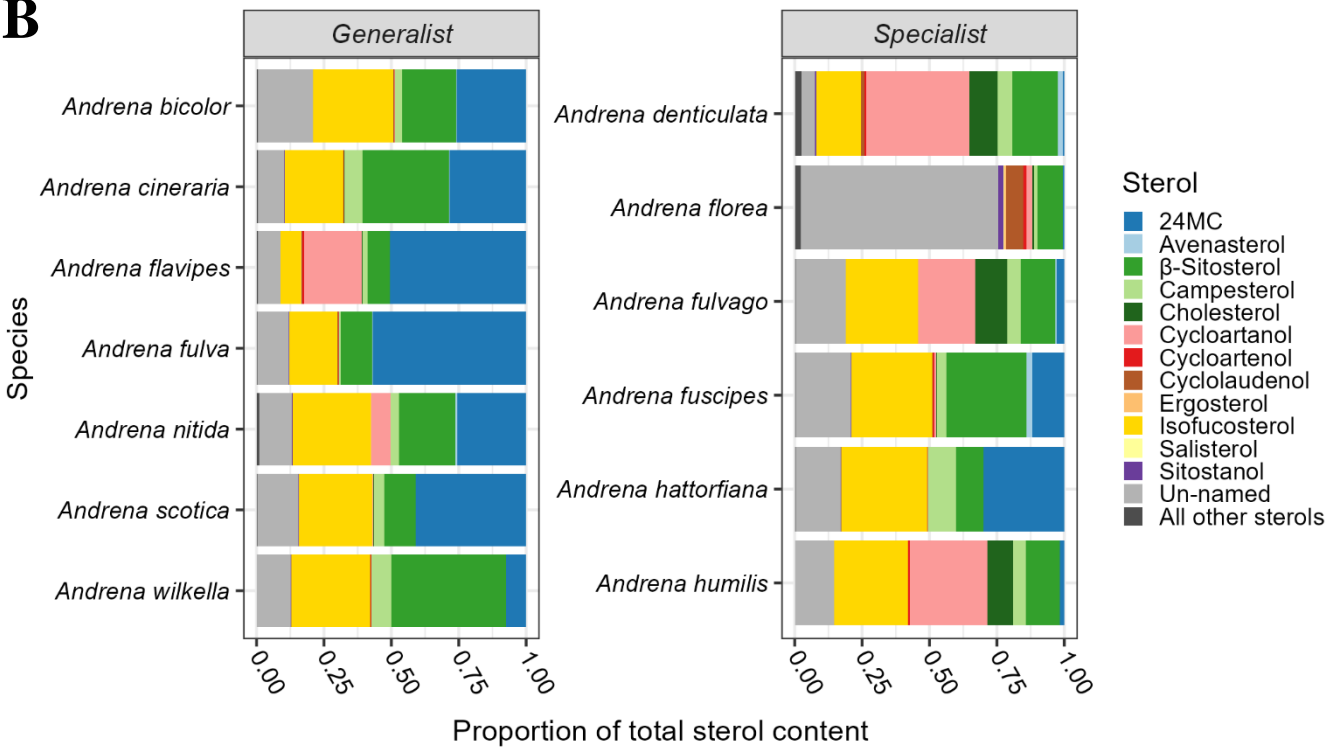
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**A**



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**B**

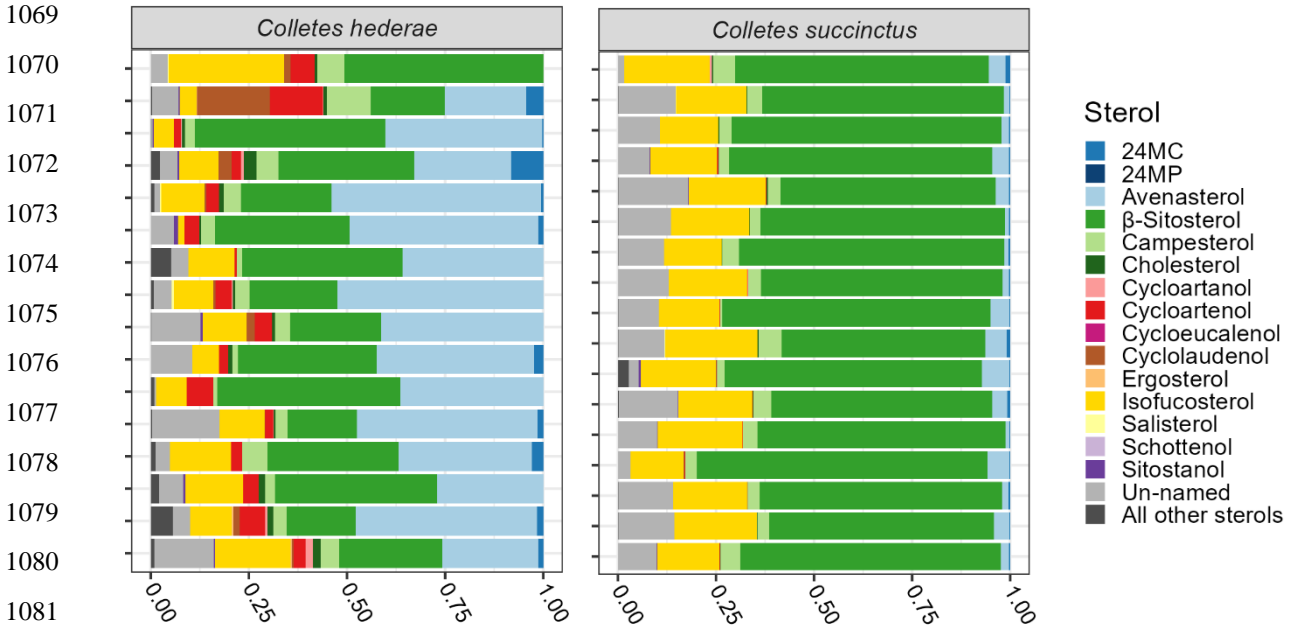


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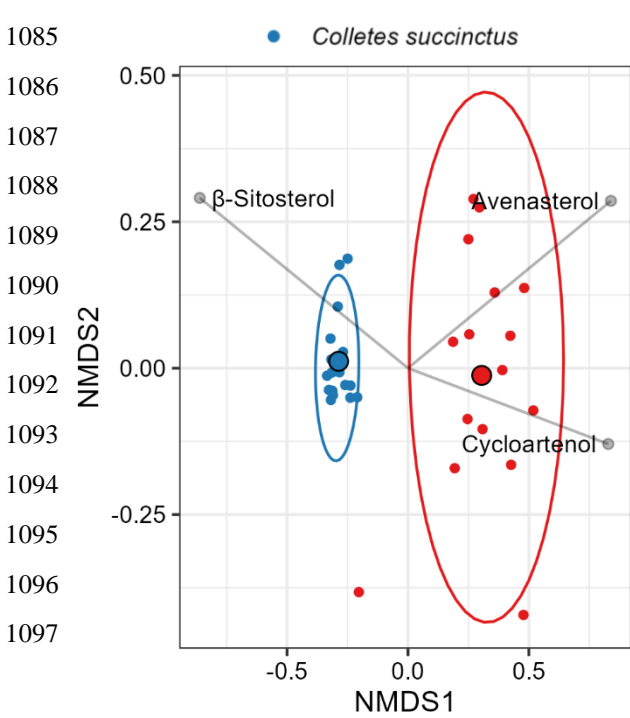
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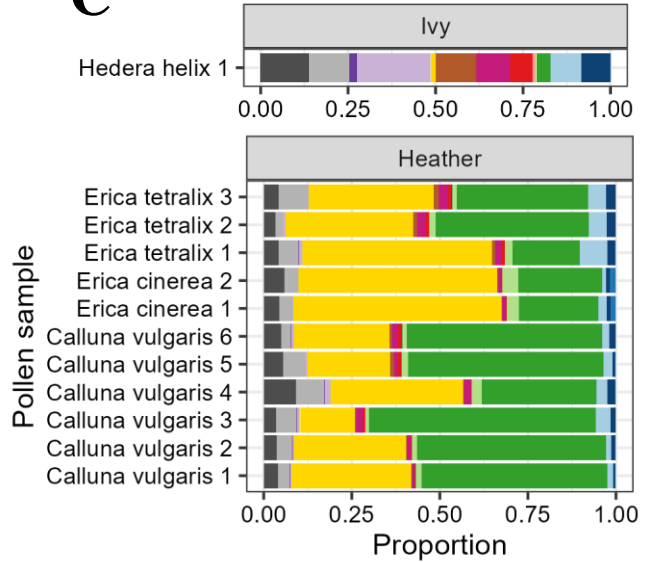
1068 **A**



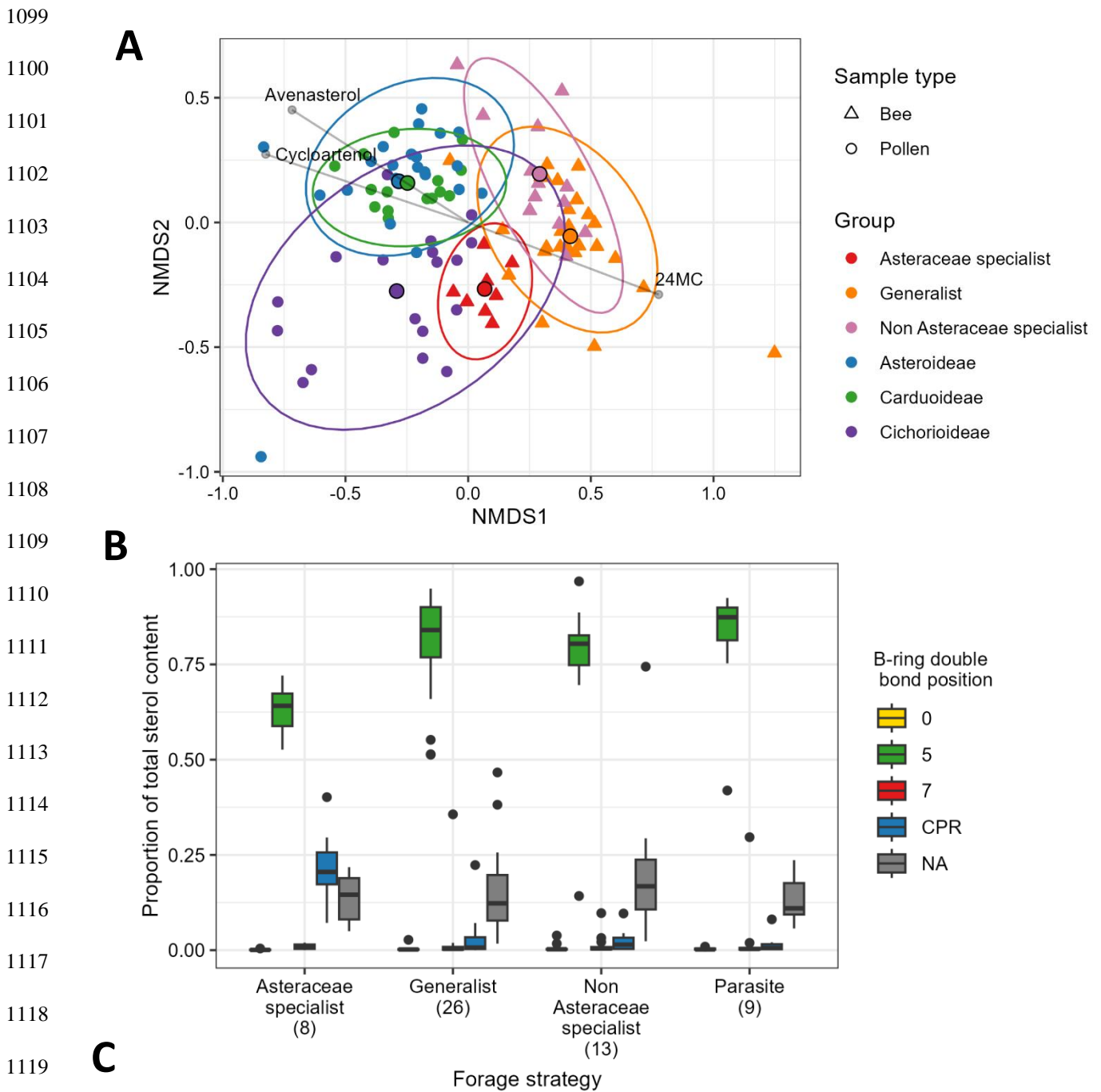
1083 **B**



1083 **C**



1098 **Figure 6.**



| Sterol                    | IV    | P-value | Sterol        | IV    | P-value | Sterol        | IV    | P-value |
|---------------------------|-------|---------|---------------|-------|---------|---------------|-------|---------|
| Asteraceae specialist     |       |         | Asteroideae   |       |         | Carduoideae   |       |         |
| Cholesterol               | 0.614 | 0.005   | Cycloartenol  | 0.564 | 0.005   | Avenasterol   | 0.609 | 0.005   |
| Non-Asteraceae specialist |       |         | Cichorioideae |       |         | Parasite      |       |         |
| $\beta$ -sitosterol       | 0.477 | 0.005   | Cycloartenol  | 0.576 | 0.010   | Salisterol    | 0.590 | 0.005   |
| Generalist                |       |         |               |       |         | Cyclolaudenol |       |         |
| 24MC                      | 0.632 | 0.005   |               |       |         | Sitostanol    |       |         |
|                           |       |         |               |       |         | 0.555         |       |         |
|                           |       |         |               |       |         | 0.010         |       |         |

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