



1 **Sensory plasticity in a socially plastic bee**



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11 **Abstract**

12 The social Hymenoptera have contributed much to our understanding of the 
13 evolution of sensory systems. Attention has focussed chiefly on how sociality and
14 sensory systems have evolved together. In the Hymenoptera, the antennal sensilla
15 are important for optimising the perception of olfactory social information. Social
16 species have denser antennal sensilla than solitary species, which is thought to
17 enhance social cohesion through nest-mate recognition. In the current study, we test
18 whether sensilla numbers vary between regional populations  the socially plastic
19 sweat bee *Halictus rubicundus*. We found region level differences in both olfactory
20 and hygro/thermoreceptive sensilla numbers. We also found evidence that olfactory
21 sensilla density is developmentally plastic: when we transplanted bees from Scotland 
22 to the south-east of England, their offspring (which developed in the south) had more
23 olfactory hairs than the transplanted individuals themselves (which developed in
24 Scotland). The transplanted bees displayed a mix of social (a queen plus workers)
25 and solitary nesting, but neither individual nor nest phenotype was related to sensilla

26 density. We suggest that this general, rather than caste-specific sensory plasticity
27 provides a flexible means to optimise sensory perception according to the most
28 pressing demands of the environment, and may support social plasticity in *H.*
29 *rubicundus*. 

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35 **Introduction** 

36

37 Natural selection on sensory abilities has resulted in enormous diversity in the
38 mechanisms that organisms use to perceive their environment. For example, species
39 of Lake Tanganyika cichlids that have colonised more complex, rocky habitats have
40 greater visual acuity than sand-dwelling species (Dobberfuhl et al. 2005). These 
41 mechanisms can also feedback into selective regimes, facilitating micro- and
42 macroevolutionary change through sensory drive. Sensory drive occurs when local
43 adaptation results in populations with diverging modes of communication and
44 perception. This has been proposed to lead to assortative mating between partners
45 with complementary signalling and perception systems, reducing geneflow and
46 promoting speciation (Boughman 2002).


47

48 The Hymenoptera have emerged as a model system to understand both the
49 proximate and ultimate factors that underlie sensory evolution. These insects have
50 antennal receptors with diverse functions and distinct forms that can easily be

51 characterised within and between species. This work has revealed that both the
52 density and form of antennal sense organs (sensilla) correlate with diet (Polidori et
53 al. 2012; 2020), sex (Babu 2019; Do Carmo Queiroz Fialho et al. 2014) and a
54 cleptoparasitic lifestyle (Wcislo 1995; Galvani et al. 2017). Attention has focussed
55 particularly on how eusociality has imposed selection to optimise antennal
56 perception. Eusociality involves reproductive division of labour with a queen/s
57 producing eggs and workers provisioning the brood. However, sociality takes diverse
58 forms across the bees and wasps and group sizes vary enormously, providing rich
59 opportunities for comparative study. Many obligately social species show worker
60 polyphenism, where some workers specialise on brood care and others on foraging
61 or guarding the nest (Andersson 1984). Compared with solitary species, social
62 species require more finely tuned communication systems to distinguish intruders
63 from kin and gain information from nestmates (Wcislo 1997; Renner and Nieh 2008).
64 Across the Hymenoptera, social species tend to have greater densities of antennal
65 sensilla that are involved in the perception of olfactory cues than solitary species
66 (Elgar et al. 2018). This is thought to enhance social cohesion and performance by
67 facilitating nest-mate recognition (Ozaki et al. 2005; Gill et al. 2013; Couto et al.
68 2017) and supporting caste polyphenisms (Spaethe et al. 2007; Riveros and
69 Gronenberg 2010; Gill et al. 2013; Grueter et al. 2017; Elgar et al. 2018).
70
71 The olfactory antennal sensilla have been proposed as a pre-adaptation that acts
72 alongside haplodiploidy to predispose the Hymenoptera to the evolution of sociality
73 (Couto et al. 2017). A conserved, ancestral olfactory subsystem involving the
74 basiconic antennal sensilla has been suggested to facilitate kin selection allowing
75 nestmate recognition from cuticular hydrocarbon (CHCs) profiles (Ozaki et al. 2005;

76 McKenzie et al. 2016; Couto et al. 2017; Pask et al. 2017). While empirical evidence
77 for this subsystem exists for only two species (an ant and a hornet), the halictid bees
78 have also provided support for the possibility that sensory pre-adaptations might
79 contribute to the evolution of sociality. The Halictidae or sweat bees exhibit a range
80 of social structures, from solitary nesting through to obligate sociality, including
81 within-species social polymorphism and social plasticity (Gibbs et al. 2012). A
82 comparative study by Wittwer et al (2017) has shown that ancestrally solitary species
83 and their social sister species have equivalent densities of hair-like sensilla
84 (basiconic and trichoid sensilla) that detect olfactory cues. Species that have
85 reverted to a solitary existence from sociality on the other hand, have reduced
86 densities of these sensilla. This suggests that in the Halictidae, sensitivity to olfactory
87 cues precedes the evolution of sociality rather than evolving as a consequence of it.

88
89 In addition to caste polyphenisms, where individuals always form social groups but
90 vary in the tasks that they perform, a number of species are facultatively social. In
91 these species, individuals vary in their propensity to form social groups or nest as
92 solitary individuals (Gibbs et al. 2012). This social plasticity is thought to be key to
93 the evolutionary diversification of the Hymenoptera as it provides a genetic base
94 from which obligate eusociality and caste polyphenism can evolve repeatedly (West-
95 Eberhard 2003; Jones et al. 2017). It also provides an opportunity to test for
96 correlates of social evolution without the complication of interspecific differences. In
97 this study we characterise the antennal sensilla of a facultatively social species,
98 *Halictus rubicundus*, for the first time. We measure the density of different antennal
99 sensilla types between regional populations of this socially plastic sweat bee and test
100 whether sensory plasticity mirrors social plasticity in a transplant experiment. Our


101 results represent an important step in disentangling the sequence of events that led
102 to the evolution of eusociality and reproductive division of labour in the
103 Hymenoptera. 

104

105 **Materials and methods**

106

107 *Study system*


108 *Halictus rubicundus* is a ground-nesting bee that can be found throughout Europe
109 and North America in areas with sandy-loamy soil that receive sufficient sunlight. In
110 Great Britain and Ireland, *H. rubicundus* demonstrates social variation according to
111 latitude but retains social plasticity throughout its range (Field et al. 2010; 2012). In
112 the north bees are typically solitary as the short growing season and cooler climate
113 precludes the bivoltinism that is required for social living. In a typical year in the
114 north, foundresses produce a single brood of offspring per year, the females of which
115 enter hibernation and emerge to become foundresses the following spring (figure
116 1a). In the south, growing seasons tend to be longer and the weather is warmer, so
117 bivoltinism and sociality can occur more frequently. After overwintering underground,
118 females produce a first brood (the B1) which become workers and provision a
119 second brood (B2) which are offspring of either the original foundress or a B1
120 replacement queen. B1 females are also observed to provision their nests alone 
121 (without workers) in the same year that they emerge. The B2 hibernate and emerge

122 the following year to found their own nests (figure 1b; Field et al. 2010; 2012).

123

124 Sociality is extremely plastic in *H. rubicundus* in Great Britain, and when solitary


125 northern bees are transplanted to the south, the B1 brood will often provision as

126 workers or solitary foundresses in the same year (Field et al. 2010; 2012; this study). 

127 Here we test for fixed and plastic differences in antennal sensilla density in *H.*
128 *rubicundus* across and within populations. We first test whether olfactory sensilla
129 densities differ between bees from southern populations, where the climate allows
130 some individuals to form social nests most years, and northern populations with
131 limited opportunity to form social nests. Second, we test whether antennal sensilla
132 numbers are developmentally plastic by transplanting bees from the north to the
133 south. Do the offspring of northern bees that develop in the warmer south have
134 different numbers of antennal sensilla than their parental generation which
135 developed in the north? We also test for differences in sensilla number between
136 individual and nest-level phenotypes: do social individuals/nests exhibit greater
137 densities of olfactory sensilla to aid in nestmate recognition?

138

139 *Collection*

140 *Halictus rubicundus* females were collected from 4 sites in the UK in 2018, 2019 and
141 2020 (see fig 1c). Two sites in the south-west (SW): Boscastle (Cornwall: N 50° 41'
142 24" W 4° 41' 24"; N = 12; 2018) and Bodmin (Cornwall, N 50° 30' 36" W 4° 33' 36"; N
143 = 4; 2019); a site at a mid-latitude (MID): Belfast (Northern Ireland, N 54° 32' 24" W
144 5° 58' 48"; N = 19; 2020); and a site in the far north of Scotland (SCO): Migdale
145 (Scotland, N 57° 53' 24" W 4° 15' 0"; N = 15; 2018 and 2020). Previous studies and
146 our own observations indicate that while a proportion of nests are social in SW (this
147 study), nests are always non-social in Belfast (MID; Field et al. 2010) and Scotland
148 (SCO; this study). However, bees from Belfast (MID; Field et al. 2010) and Scotland
149 (SCO; this study) are socially plastic and may have social nests when moved to
150 more southerly sites.  Bees sampled were expected to be foundresses based on

151 collection date. We collected females by hand-netting in in late June in SCO, and in
152 May and early June in the SW, when all bees had fairly worn wings indicative of
153 several weeks of provisioning. In Belfast (MID) we excavated overwintering
154 foundresses in February.

155

156 *Transplant*

157 In addition to sampling from native populations, we collected bees that had been
158 transplanted from Scotland (SCO) to a site in the south-east (SE), the Knepp estate
159 in West Sussex (N 50° 53' 60" W 0° 21' 36"; N = 47; see fig 1c). Buckets of soil were
160 embedded within a nest aggregation at the Scottish site during winter 2018-19, and
161 native foundresses subsequently nested in them during 2019. Their B1 offspring
162 then emerged in late summer 2019 and hibernated in the buckets. The buckets
163 containing hibernating Scottish bees were taken from Scotland to the south-east in
164 the spring of 2020, where they were re-embedded in the ground. SCO foundresses
165 that emerged were marked with a dot of enamel paint when they provisioned the B1
166 brood in the south-east. When the B1 emerged, they were marked with a different
167 colour of enamel paint. Each nest was marked with a numbered nail at the entrance.
168 This allowed us to distinguish fresh B1 females from the original foundresses and to
169 determine whether nests were solitary (with a single B1 female or original foundress
170 provisioning alone) or social (with multiple B1 females provisioning the nest). At the
171 end of the B1 provisioning phase we collected SCO foundresses (which had
172 emerged in Scotland and been transplanted to the SE) and their B1 offspring (which
173 had developed and emerged in the SE).

174

175 *Specimen storage and preparation*

176 All specimens were stored in 95% Ethanol until they were prepared for imaging. One
177 antenna was removed from each bee (whether it was the left or right was recorded)
178 and mounted on a JEOL aluminium stub (10mm dia x 10mm high) using a PELCO
179 carbon conductive sticky tab (10mm dia). Specimens were mounted dorsal side up.
180 Specimens were carbon coated using spectrographically pure carbon.

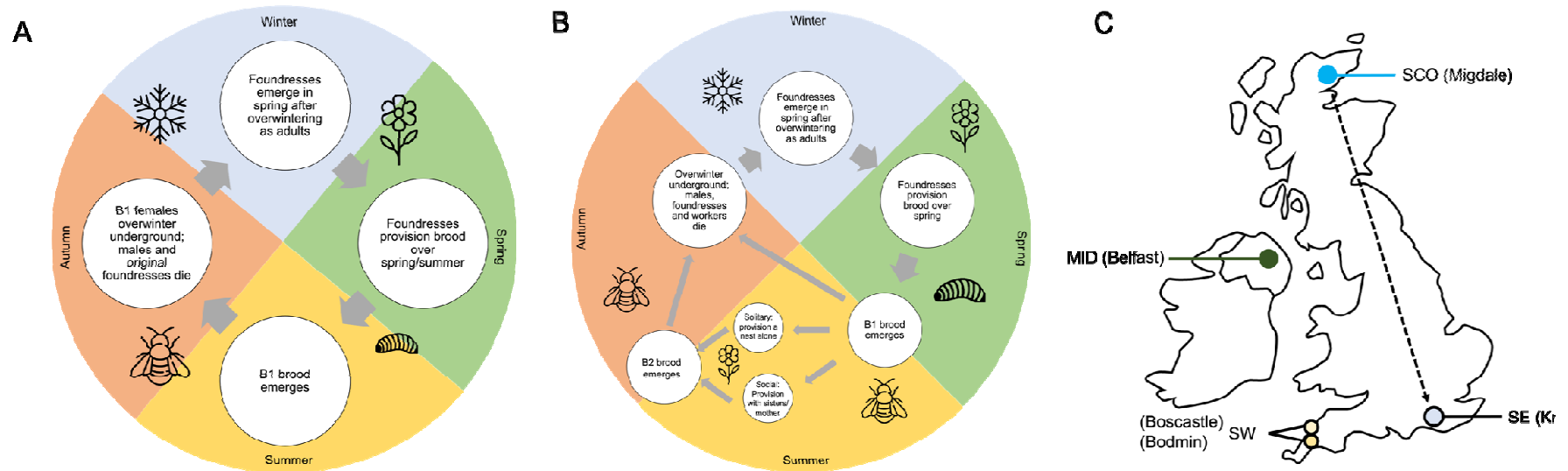
181

182 *Imaging*



183 Mounted and carbon coated specimens were imaged using a TESCAN Vega SEM in
184 High Vacuum mode at 20.0kV. The scan mode was set to resolution and the
185 magnification was 485x. Only the two distal segments (11 and 12), which have the
186 highest density of sensilla, were imaged. The length and area of each antennal
187 segment was measured during imaging and images of each antennal segment from
188 the SEM were saved in TIF format.

189

190



191

192 **Figure 1**  univoltine lifecycle of *Halictus rubicundus* which prohibits social nesting (B) bivoltine lifecycle of *Halictus rubicundus*
193 where sociality is possible as the 1st brood (B1) can provision a second brood (B2). Note that in (B) the B1 females can either (i) 
194 overwinter and provision the following year as a solitary foundress (ii) provision a nest alone as a solitary foundress in the year that
195 they emerge or (iii) provision the nest socially with their B1 sisters with a queen (their mother or a sister) in the year that they
196 emerge. (C) Map depicting the sites where *H. rubicundus* females were studied. Dashed arrow represents the direction of the
197 transplant experiment (SCO to SE).

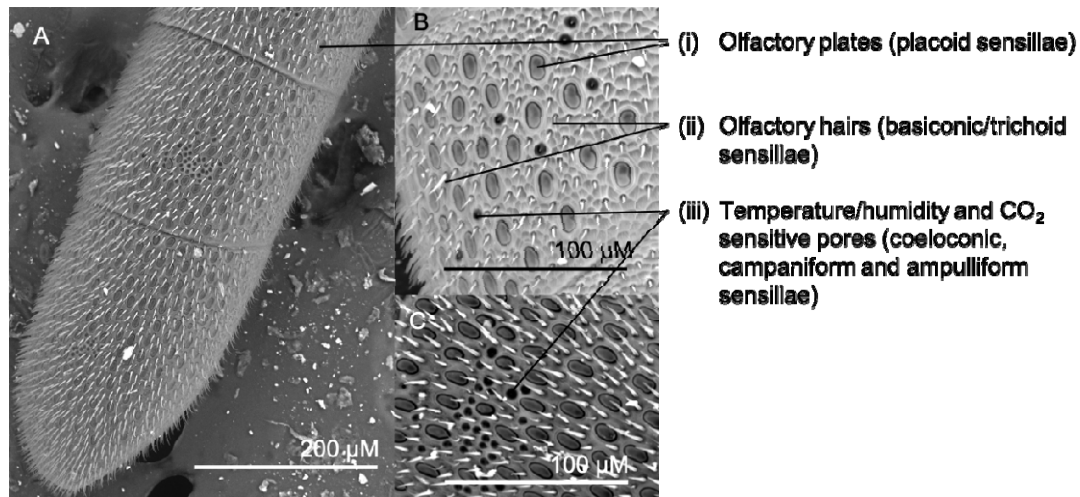
198 *Image scoring*

199 Eight distinct types of sensilla with overlapping functions have been characterised in
200 the Hymenoptera (Do Carmo Queiroz Fialho et al. 2014). Placoid sensilla are plate-
201 like structures that are involved in olfaction and chemoreception. Tricoid and
202 basiconic sensilla are hair-like projections with multiple sub-types that are used in
203 olfaction involving contact. Coeloconic, campaniform and ampulliform sensilla are
204 pore-like and are involved in sensing temperature, humidity and CO₂ concentration
205 (Do Carmo Queiroz Fialho et al. 2014). For this study we grouped all sensilla types
206 into three structural/functional groups that allowed for repeatability in scoring: (i)
207 olfactory plate sensilla (sensilla placodea, fig 2), (ii) olfactory hair-type sensilla
208 (sensilla trichodea and basiconica, fig 2) and (iii) temperature/humidity/CO₂ pore
209 sensilla (coeloconic/campaniform/ampulliform sensilla; fig 2).

210

211 Images of antennal segments 11 and 12 were scored using ImageJ software. For
212 each segment, three 50µM x 50µM 'quadrats' were selected, the X and Y
213 coordinates of which were derived by generating a random number using the excel
214 function RANDBETWEEN. Each quadrat was saved as a TIF image and the number
215 of sensilla types i and ii were counted. We counted sensilla type iii across the entire
216 segment as they are distributed unevenly on the surface of the antenna. In some
217 cases, bare patches with no sensilla were present on the surface of the antenna.
218 Any quadrats which fell on the bare areas were discarded and another area was
219 selected. We note that the scorer was not blind to the population of origin as the
220 same person was required to mount, image and score the antennae.

221



222

223 **Figure 2** SEM image showing a *Halictus rubicundus* antenna tip. (A) shows the
224 distal 2 antennal segments (11 and 12) that were imaged, (B) and (C) show the
225 types (i, ii, and iii) of antennal sensilla that were counted. Scale is given in
226 μM.

227 *Analysis*


228 *Repeatability*

229 Thirty-three of the 130 sampled antennae were scored a second time (by the same
230 person) for all sensilla types. The repeatability of image scoring was assessed using
231 the intra-class correlation coefficient, function *ICC* in the R package *irr* (Gamer et al,
232 2019).

233

234 *Sensilla numbers*

235 We used linear mixed models in the R package *lme4* (Bates et al. 2019) to test for
236 differences in the numbers of sensilla types (i-iii). For all models outlined below, the
237 response variable was total sensilla counted. For i) olfactory plates, total across 3
238 quadrats for each segment per individual; ii) olfactory hairs, total across 3 quadrats
239 for each segment per individual; or iii) temperature/humidity pores, total across each

240 segment per individual. The package DHARMA was used to determine the most
241 appropriate error structure for all models (Gaussian) and to interpret residual plots
242 for lack of fit (Hartig, 2020). 


243

244

245 *Region, development site and social phenotype*

246 To test whether bees from different regions have different numbers of sensilla we
247 included region as a fixed factor with 3 levels in LMMs: SW (Boscastle and Bodmin
248 combined; N = 16 individuals), MID (Belfast, Northern Ireland; N = 19 individuals)
249 and SCO (Migdale, Scotland, far north; N = 15 individuals).

250

251 We ran additional models to test whether sensilla density varies depending on where 
252 bees complete their development (which may suggest developmental plasticity). To
253 do this we compared bees that developed in Scotland (SCO) with their offspring that
254 developed in the south-east after transplantation. LMMs included a fixed effect of
255 development site, with 2 levels: SCO (bees that emerged in Scotland and were
256 either collected there (N = 15 individuals, same individuals that were used in the
257 region models; or were moved to the south-east as adults, N = 4) and SE (bees that
258 emerged as adults after immature development in the south-east, the B1 offspring of
259 the bees that were transplanted from Scotland to the south-east; N = 27). Note that
260 *H. rubicundus* females mate before overwintering and so both the mother and father
261 of the B1 bees collected in the south-east originated in Scotland.

262

263 Additional LMMs were used to test whether B1 offspring collected in the SE (using
264 the same individuals used in the *development site model* for which nest phenotypes
265 were known, N = 14) had higher counts of sensilla if they were workers from social

266 nests of transplanted females compared to those which left and started their own
267 solitary nests. Social nests had a queen and one or more workers, while solitary
268 nests had a single B1 foundress that produced offspring in the same year. For these
269 models we included nest phenotype as a fixed effect with 2 levels (social, N = 6 or
270 solitary, N = 8). We did not sample multiple individuals from the same nest, so did
271 not include nest as a random effect. We did not recover any queens from social
272 nests and so could not test for an effect of bee phenotype within social nests. We
273 also ran a model to test for effects of individual, rather than nest-level, phenotype
274 across social and solitary nests, using the same bees. For this analysis bees were
275 characterised as (1) a future reproductive (a B1 individual that had emerged in 2020
276 and did not provision that year; N = 13); (2) a worker (a B1 individual that
277 provisioned a nest containing a queen; N = 7); (3) a solitary foundress from 2019 (a
278 foundress that emerged in SCO in 2019 and laid eggs/provisioned a nest alone
279 without workers in the south-east); (4) a solitary foundress from 2020 (a foundress
280 that emerged in the B1 generation in 2020 in the SE and provisioned a nest alone; N
281 = 4).

282

283 Finally, we ran a model using the bees collected in the SE to test whether age-
284 related wear and tear reduces density of all 3 sensilla types. For these analyses age
285 was binary: bees were scored as fresh (newly emerged and had not provisioned a
286 nest; N = 12) or old (had provisioned a nest for several weeks N = 16).

287

288 All LMMs included antenna segment number (11 or 12) as a fixed factor. The
289 interaction effect between region/development type/social phenotype and segment
290 number was also fitted. We included a random effect of individual as two segments

291 were imaged for each bee. Models were run using the function *lmer* in the R
292 package lme4 (Bates et al, 2015).

293

294 **Results**

295

296 *Repeatability and effects of age*

297 Counts of all sensilla types were highly repeatable (Koo and Li 2016; ICC greater
298 than 0.85 for all; for a detailed summary see supplementary material table S1).


299

300 We also found no evidence that age-related wear and tear influences the variation in
301 sensilla density; there was no effect of age on density of any of the three types of
302 sensilla (type i: $X^2 = 0.25$, $df = 1$, $p = 0.62$; type ii: $X^2 = 0.01$, $df = 1$, $p = 0.92$; type iii:
303 $X^2 = 0.09$, $df = 1$, $p = 0.76$).

304

305

306 *Differences between antennal segments*

307 Olfactory plates (type i) and olfactory hairs (type ii) were found in greater numbers on
308 the most distal antennal segment, segment 12, while hygro/thermoreceptors (type iii)
309 were found in higher numbers on segment 11. There were no interaction effects
310 between segment number and region (table 1) 

311

312 *Region*


313 There was a significant effect of region on the counts of type ii (olfactory hairs) and iii
314 (thermo/hygroreceptors) sensilla, but not type i (olfactory plate receptors; see table
315 1, figure 3a-c). Pairwise tests show that bees from mid-latitude (MID; Belfast; N = 19)

316 had significantly more olfactory hairs (type ii) than bees collected from the far north
317 (Scotland; N=30; $p = 0.03$; figure 3b), whereas bees from the south-west (N = 16) did
318 not differ from MID (N = 32; $p = 0.06$) or Scottish (N = 15; $p = 0.93$) bees. Bees from
319 SCO and MID had more thermo/hygro (type iii) receptors than SW bees ($p < 0.05$;
320 figure 3c). Bees from the south-west had the most olfactory plate sensilla (type i) but
321 this was not statistically significant (figure 3a).

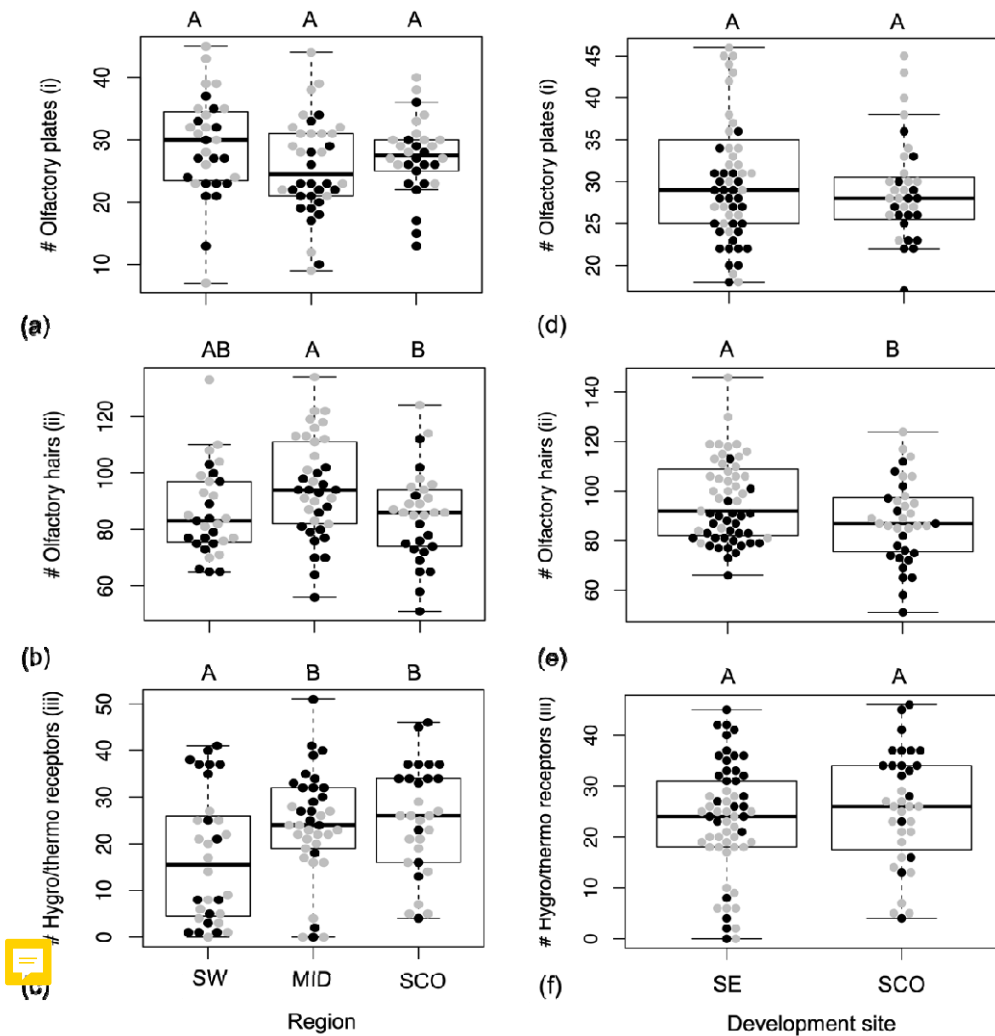
322

323 **Table 1** LMM results showing effect of region on antennal sensilla counts across
324 segments using type II sums of squares (p-values for main effects are calculated
325 independent from interaction effects which in all cases are non-significant).

326

Sensilla type 	Effect	X ²	df	P
Type i) olfactory plates	Region	2.31	2	0.31
	Segment	19.48	1	1.01×10^{-5}
	Region*Segment	0.45	2	0.80
Type ii) olfactory hairs	Region	7.81	2	0.02
	Segment	41.74	1	1.04×10^{-10}
	Region*Segment	2.70	2	0.26
Type iii) thermo/hygro-receptive	Region	9.14	2	0.01
	Segment	19.17	1	1.20×10^{-5}
	Region*Segment	0.35	2	0.84



327



328

329 **Figure 3** Numbers of (a) and (d) olfactory plates, (b) and (e) olfactory hairs, ii; and
 330 (c) and (f) hygro/thermoreceptive pores, iii, for *Halictus rubicundus* across: a-c three
 331 sampled regions: SW (South-west; mostly social), MID (Belfast; solitary) and SCO
 332 (Scotland; solitary); d-e for *Halictus rubicundus* originating from Scotland and
 333 collected after either developing as larvae in Scotland (SCO) or after developing as
 334 larvae at the transplanted site in the south-east (SE). Different uppercase letters over
 335 bars indicate significantly different counts of sensilla. Black points represent sensilla
 336 counts on segment 11 and grey points on segment 12. Box plots show median and
 337 quartiles across segments 11 and 12.

338 *Development site*

339 The density of olfactory hairs (type ii) varied according to where bees originating
340 from Scotland spent their immature development (table 2; figure 3d-e).  spring of
341 transplanted bees which developed in the south-east (N=27) had more olfactory
342 hairs (type ii, $p = 0.01$) than conspecifics that developed in their natal Scottish site
343 (N=19; fig 3e). A similar trend was seen in olfactory plates (I; fig 3d) but this was not
344 statistically significant ($p = 0.06$). There was no effect of origin 
345 thermo/hygroreceptor numbers (iii; fig 3f).

346

347

348 **Table 2** LMM results showing effect of origin of emergence on antennal sensilla counts across segments (p-values for main effects
 349 are calculated independent from interaction effects which in all cases are non-significant).

350

Sensilla type	Effect	X ²	df	P
Type i) olfactory plates	Development site	3.44	1	0.06
	Segment	34.82	1	3.6x10 ⁻⁹
	Development site *Segment	0.61	1	0.43
Type ii) olfactory hairs	Development site	6.41	1	0.01
	Segment	61.35	1	4.79x10 ⁻¹⁵
	Development site *Segment	1.57	1	0.21
Type iii) thermo/hygroreceptive	Development site	0.74	1	0.39
	Segment	33.25	1	8.12x10 ⁻⁹
	Development site *Segment	0.15	1	0.69

351

352

353 *Sociality*

354 B1 workers from social nests (N= 6) and solitary B1 females that founded new nests
355 alone (N= 8) had equivalent numbers of all 3 sensilla types (i-iii, see table 3; fig 4a-c)
356 and there were no interactions between nest phenotype and antenna segment
357 number. There was some suggestion that solitary bees had higher numbers of
358 olfactory plates (type i), but this was not statistically significant ($p = 0.07$). Similarly
359 there were no differences in numbers of any sensilla types across different individual
360 phenotypes (B1 future reproductive emerged in 2020 in SE and did not provision that
361 year, will overwinter, N = 13; B1 worker emerged in 2020 in the SE, N = 7; B1
362 solitary foundress that emerged 2019 in SCO and provisioned in the SE in 2020, N =
363 4; and B1 solitary foundress emerged and provisioned in 2020 in SE, N = 4; see
364 table 4; figure 4d-e). These results were the same if B1 solitary foundresses from
365 2019 (that developed and emerged in SCO) were excluded (see archived code and
366 data; <https://github.com/DrBecky-B/Bee.Antennae>).

367

368 **Table 3** LMM results showing effect of nest phenotype (social/solitary) on antennal
369 sensilla counts across segments (p-values for main effects are calculated
370 independent from interaction effects which in all cases are non-significant).

371

Sensilla type	Effect	X ²	df	P
Type i) olfactory plates	Nest phenotype	2.91	1	0.08
	Segment	11.80	1	0.0005
	Nest phenotype*Segment	1.08	1	0.30
Type ii) olfactory hairs	Nest phenotype	0.08	1	0.78

	Segment	42.03	1	8.98x10 ⁻¹¹
	Nest phenotype*Segment	1.29	1	0.26
Type iii) thermo/hygroreceptive	Nest phenotype	0.29	1	0.59
	Segment	33.08	1	8.86x10 ⁻⁹
	Nest phenotype*Segment	0.10	1	0.75

372

373 **Table 4** LMM results showing effect of bee phenotype (future
 374 reproductive/worker/solitary foundress 2019 and solitary foundress 2020) on
 375 antennal sensilla counts across segments (p-values for main effects are calculated
 376 independent from interaction effects which in all cases are non-significant).

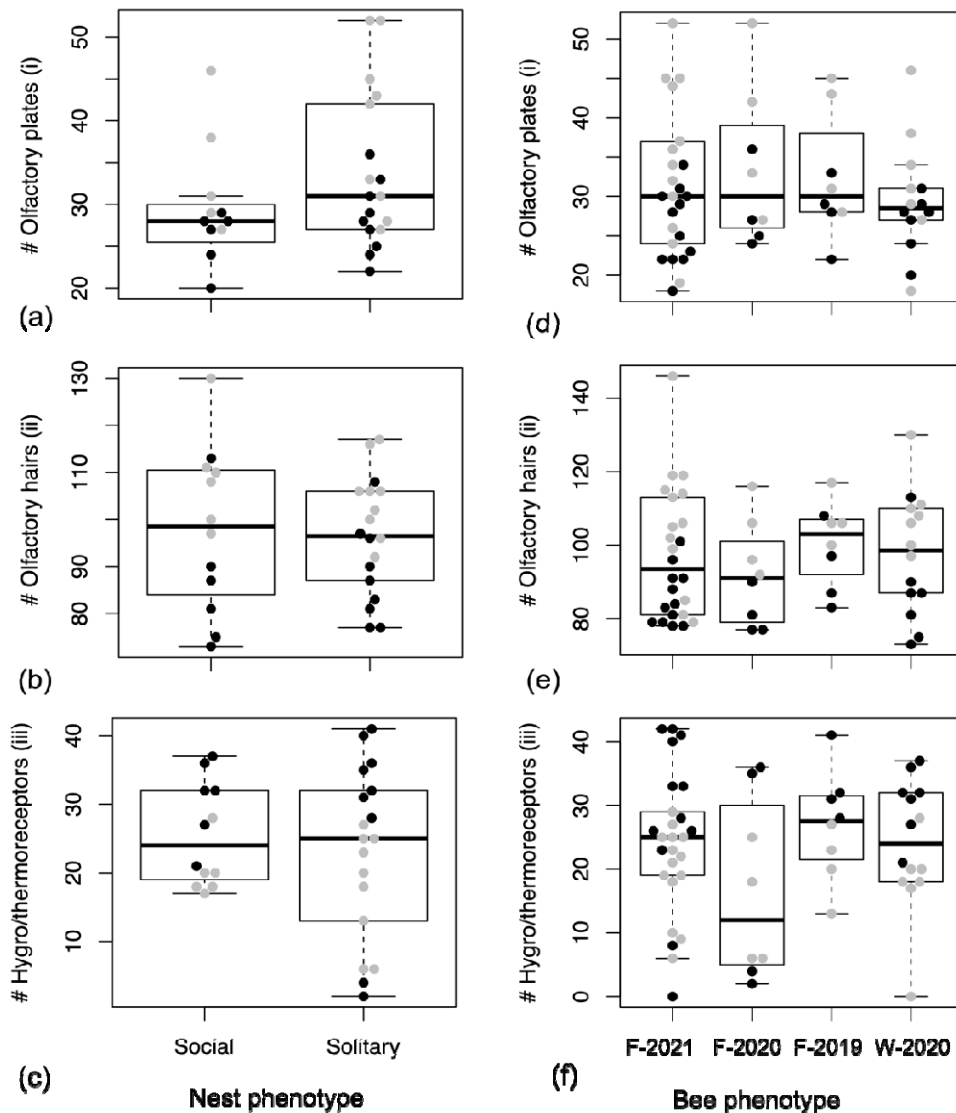
377

Sensilla type	Effect	X ²	df	P
Type i) olfactory plates	Bee phenotype	1.51	3	0.67
	Segment	22.34	1	2.29x10 ⁻⁶
	Bee phenotype*Segment	1.81	3	0.61
Type ii) olfactory hairs	Bee phenotype	1.24	3	0.74
	Segment	42.03	1	8.33x10 ⁻¹¹
	Bee phenotype*Segment	0.85	3	0.84
Type iii) thermo/hygroreceptive	Bee phenotype	3.39	3	0.34
	Segment	30.18	1	3.95x10 ⁻⁸
	Bee phenotype*Segment	2.26	3	0.52

378

379

380



381




382 **Figure 4** Numbers of (a) and (d) olfactory plates, i; (b) and (e) olfactory hairs, ii; and
383 (c) and (f) hygro/thermoreceptive pores, iii; for *Halictus rubicundus* from Scotland
384 collected from (a-c) social and solitary nests and according to individual phenotype
385 (d-f) after being transplanted to the south-east in 2020. For figures 4d-4f: F-2021
386 female that emerged in the SE in 2020 but did not provision that year; F-2020 female
387 that emerged in the SE in 2020 and provisioned a nest as a solitary foundress; F-
388 2019 female that emerged in SCO in 2019 and provisioned a nest as a solitary

389 foundress in 2020; W-2020 female that emerged in the SE in 2020 and provisioned a
390 social nest in the same year as a worker. Black points represent sensilla counts on
391 segment 11 and grey points on segment 12. Box plots show median and quartiles
392 across segments 11 and 12. There were no significant differences in counts of any
393 sensilla type across the groups.

394

395 Discussion

396

397 The social Hymenoptera have contributed much to our understanding of the
398 proximate and ultimate factors that underlie the evolution of sensory systems. 
399 Attention has focussed chiefly on how sociality has imposed selection to optimise 
400 olfactory communication, enhancing social cohesion through nest-mate recognition
401 and supporting caste polyphenisms (Wcislo 1997; Ozaki et al. 2005; Spaethe et al.
402 2007; Renner and Nieh 2008; Riveros and Gronenberg 2010; Gill et al. 2013; Couto
403 et al. 2017; Grunter et al. 2017; Elgar et al. 2018). In the current study we measured
404 sensilla density in the socially plastic sweat bee *Halictus rubicundus*. We found that
405 when bees were transplanted from Scotland, where they are solitary, to the south
406 where they can be social, their offspring had higher densities of olfactory hairs.
407 These results suggest that the density of olfactory hairs may, like sociality, be
408 phenotypically plastic in *H. rubicundus*. We also found evidence for between-region
409 differences in the density of basiconic/trichoid olfactory hairs (and
410 hygro/thermoreceptive sensilla) for untransplanted bees, with mid-latitude bees
411 having higher densities than more northerly Scottish bees. Differences were not 
412 perfectly correlated with the expected degree of sociality, however, because bees
413 from the south-west did not differ from mid-latitude or Scottish bees.

414



415 The results from our transplant experiment suggest that olfactory hair density may be
416 phenotypically plastic, and may vary depending on the conditions that bees
417 experience during development. When Scottish bees were transplanted to the south-
418 east, their offspring that developed there (where air and soil temperatures in June
419 and July are 2-5°C warmer than their native site; table S2) had greater densities of
420 trichoid/basiconic hairs. In this scenario, warmer temperatures during development,
421 rather than social phenotype, lead to greater densities of olfactory hairs. A similar
422 pattern is seen in the parasitoid wasp *Trichogramma*; when males develop under
423 colder temperatures they have reduced numbers of basiconic hairs (Pinto et al.
424 1989). A direct response to temperature might explain why untransplanted
425 foundresses from the south-west, where bees are often social, but temperatures are
426 not as high as in the south-east, did not have higher basiconic hair densities than
427 non-social populations (Fig. 3).

428


429 In addition to plasticity within a population, we also found evidence that olfactory hair
430 density (type ii, basiconic/trichoid sensilla) varies across regional populations in *H.*
431 *rubicundus*. In contrast we saw no differences in the densities of placoid (plate-like,
432 type i) olfactory sensilla across regions. This may relate to the function of these
433 receptor types. Basiconic/trichoid hairs respond to contact with the CHCs of other
434 individuals, and so are likely to be required for nest-mate recognition and
435 communication, while placoid plates are thought to be involved in longer range
436 olfaction to detect food and hosts (Ozaki et al. 2005; McKenzie et al. 2016; Couto et
437 al. 2017; Pask et al. 2017). We know of no clinal variation in diet breadth in this


438 species (although this remains to be rigorously studied), which fits with the pattern
439 we see here.

440

441 Bees collected from Belfast at a mid-latitude had the highest density of olfactory hair-
442 like sensilla (type ii),  higher than bees from Scotland (450km north of Belfast) but
443 statistically equivalent to bees collected in the south-west. We predicted that *H.*
444 *rubicundus* collected further south would have the greatest densities of olfactory
445 sensilla to support social communication between nestmates (Wittwer et al. 2017;
446 Elgar et al. 2018). In the south, summers are typically warmer and longer and a
447 higher proportion of the population is expected to exhibit social behaviour more
448 regularly. While we did see regional differences in olfactory sensilla density, bees
449 from the south-west did not have the highest densities as we would have predicted
450 based on clines in sociality. The regional differences we saw may be related to 
451 developmental plasticity, which could limit the scope for fixed population level
452 differences to evolve in the predicted direction. Longitudinal studies of these same
453 populations and additional, reciprocal transplant experiments (from south to north)
454 would help to elucidate the extent to which genetically fixed differences in olfactory
455 hair density and plastic expression contribute to the population level differences we
456 saw in this trait.

457

458 We also found that the density of sensilla (type iii) involved in the perception of 
459 humidity, temperature and CO₂ varies across regions, but unlike olfactory hairs this
460 does not appear to be plastic. Bees from a mid-latitude and the north of Scotland
461 (Belfast and Scotland) have more thermo/hygro receptive sensilla than bees from the
462 south-west (400 km south of Belfast and 850 km south of the Scottish site). The

463 density of these sensilla does not appear to be plastic as it was not influenced by
464 where Scottish bees developed or their social phenotype. This pattern may be a
465 result of a more extreme, variable climate in the north, which leads to consistent
466 selection for higher densities of thermo/hygro receptors. Sweat bees including *H.*
467 *rubicundus* are highly sensitive to temperature and rain. Flight activity is constrained
468 by low ambient temperatures, lack of sunlight and rainfall (Potts and Wilmer 1997).
469 In the north, bees experience colder ambient temperatures and greater rainfall. Bees
470 with more of these receptors may be more sensitive to current and oncoming
471 weather conditions, so that they have reduced mortality and improved foraging
472 based on the climate. The potentially severe fitness consequences of incorrect
473 perception of climatic cues may explain the lack of plasticity in the number of
474 thermo/hygro receptors if selection has a strong and canalising effect on this trait.
475
476 Our results add a new dimension to the growing body of evidence that
477 developmental temperature may contribute to adaptations which support sociality in
478 the Hymenoptera. While previous studies suggest that temperatures experienced by
479 developing larvae contribute to individual differences in social phenotype (i.e. caste
480 polyphenism: Czekońska and Tofilski 2020; Becher et al. 2009; the development of
481 status badges: Green et al. 2012; memory formation: Jones et al. 2005 and olfactory
482 learning: Anton and Rossler 2021), sensilla density in the socially plastic *H.*
483 *rubicundus* may be a more general response to the environment that is not directly
484 related to the social phenotype of the nest or individual (i.e. a worker, solitary
485 foundress or queen). Scottish bees that developed in the south-east had more
486 olfactory hairs than their conspecifics that emerged in Scotland irrespective of their
487 caste or nest phenotype. 

488

489 In the Halictidae, Wittwer et al (2017) found that halictid bee species that had
490 reverted back to solitary nesting from a state of sociality had reduced olfactory hair
491 density compared to social species and ancestrally solitary species. They suggest
492 that this is because dense olfactory sensilla are a pre-adaptation that facilitates the
493 evolution of sociality and may contribute to the repeated evolutionary transitions to
494 sociality seen in the halictid bees. Our results expand on this, suggesting that
495 olfactory hair density may also be phenotypically plastic. More broadly, plasticity in
496 traits such as this might contribute to the evolutionary lability of sociality in the
497 Halictidae and in the Hymenoptera, acting alongside social plasticity in the
498 hymenopteran ancestor to provide the 'flexible stem' which allowed for repeated
499 evolutionary transitions to obligate sociality across the order (West-Eberhard 2003;
500 Wittwer et al. 2017).

501

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507

508 **Conflict of interest**

509 The authors have no conflict of interests to report.

510

511 **Ethics**

512 This research adheres to ethical codes of practice at the University of Exeter. No
513 vertebrates were used in this research. Bee antenna samples were taken from living
514 and dead stored specimens. Handling during antenna removal of live specimens was
515 done as quickly as possible to minimize stress to individuals. Bees frequently lose
516 parts of and whole antennae in nature and we did not see any negative effects on
517 the survival or provisioning behaviour of live sampled individuals compared to intact
518 bees. The sample sizes reported were considered appropriate to maximize statistical
519 power while reducing the number of individuals involved in the experiments.
520 Individuals were sampled from large populations > 500 individuals and numbers
521 taken did not pose a threat to the persistence of these populations.

522

523

524 **Data accessibility**

525 All code and data are archived on Github and can be accessed here:

526 <https://github.com/DrBecky-B/Bee.Antennae>

527

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