

1 **Differential investment in visual and olfactory brain regions mirrors the sensory needs of**
2 **a paper wasp social parasite and its host**

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22 **ABSTRACT**

23 Obligate social parasites evolve traits to effectively locate and then exploit their hosts, whereas
24 hosts have complex social behavioral repertoires, which include sensory recognition to reject
25 potential conspecific intruders and heterospecific parasites. While social parasite and host
26 behaviors have been studied extensively, less is known about how their sensory systems function
27 to meet their specific selective pressures. Here, we compare investment in visual and olfactory
28 brain regions in the paper wasp *Polistes dominula*, and its obligate social parasite *P. sulcifer*, to
29 explore the link between sensory systems and brain plasticity. Our results show opposite and
30 significant differences, consistent with their very different life-histories, in the sensory
31 investments between these two closely-related species. Social parasites initially invest in the
32 optic lobes to likely locate their hosts. After host colony usurpation, the parasite increases its
33 brain volume, with specific investment in antennal lobes, which mirrors the behavioral switch
34 from a usurping parasite to an integrated parasitic queen of the host colony. Contrastingly, hosts
35 initially invest in the antennal lobes and sensory processing compared to social parasites, as
36 predicted by their need to maintain social cohesion, allocate colony tasks, and recognize con- and
37 heterospecific intruders. Host queens show a trend of higher investment in all sensory brain
38 regions compared to workers, paralleling differences in task allocations. Our work provides
39 novel insights into how intraspecific brain plasticity can facilitate the unique sensory adaptations
40 needed to perform specific tasks by the host or to transition from searching to successful host
41 exploitation by the social parasite.

42

43 **Keywords:** brain plasticity, coevolution, host exploitation, sensory systems, social parasite,

44 *Polistes dominula*, *Polistes sulcifer*

45 INTRODUCTION

46 Brood parasitism, in which a parasitic individual takes advantage of the parental care of a host,
47 is a reproductive strategy that has evolved independently in diverse lineages, and is well-
48 represented in birds and social insects (Antonson et al., 2020; Buschinger, 2009; Cini et al.,
49 2019; Kilner & Langmore, 2011). The significant costs of parasitism to host species result in a
50 coevolutionary arms race, where the parasite must locate and successfully exploit parental care
51 by the host, while the host, in turn, must recognize and reject potential parasites (Feeney et al.,
52 2012; Hauber et al., 2006; Lenoir et al., 2001). In particular, deceiving hosts is critical to
53 obligate social parasites, a type of insect brood parasite that has lost the worker caste and
54 solely depends on exploiting their social host for brood care (Rabeling, 2020). Although the
55 adaptive behaviors of host and obligate social parasites have been studied extensively (Cervo,
56 2006; Lhomme & Hines, 2019; Loope et al., 2017; Nehring et al., 2015), the evolution and
57 function of sensory systems to facilitate their behavioral interactions and arms-race remain
58 poorly understood (Aidala et al., 2012; Stevens, 2013). Hosts have large sensory repertoires
59 that facilitate general foraging decisions, social interactions, task allocation and the
60 recognition of intruders. In contrast, the main selective pressure on obligate social parasites is
61 to find and then deceive their hosts. Therefore, fine-tuned sensory systems are critical to
62 mediate both the enemy-recognition by the host, and the successful deception and exploitation
63 by the social parasite (Stoddard & Hauber, 2017).

64 However, developing brain tissue needed to process sensory stimuli is energetically
65 expensive (Kotrschal et al., 2013; Niven & Laughlin, 2008; O'Donnell et al., 2011). Therefore,
66 variation in the demands of specific sensory stimuli drives differential investment in specific
67 sensory brain regions (Arganda et al., 2020; Barton et al., 1995; Keesey et al., 2020). For

68 example, nocturnal lineages of mammals, birds, and insects have larger olfactory over visual
69 brain structures compared to diurnal lineages (Barton et al., 1995; Corfield et al., 2008;
70 O'Donnell et al., 2015; Sheehan et al., 2019; Stöckl et al., 2016). In social insects, specifically,
71 differential investment in brain regions is also associated with colony size, caste, social
72 interactions, and the need for distinguishing colony members from intruders (Arganda et al.,
73 2020; Ehmer et al., 2001; O'Donnell et al., 2007; Seid et al., 2008; Seid & Junge, 2016).
74 Therefore, in systems where brood parasites attack social insects, hosts use their specialized
75 sensory systems to recognize and reject intruders (Cervo et al., 1996; Cini, Bruschini,
76 Signorotti, et al., 2011; Lenoir et al., 2001). In turn, parasites would need to use their own
77 sensory systems to first locate potential hosts (Cervo et al., 1996; Cervo & Turillazzi, 1996),
78 and then identify the correct host species, replace the host queen, and exploit the host workers
79 for brood care (Cini, Bruschini, Poggi, et al., 2011; Nehring et al., 2015; Ortolani et al., 2010).

80 To explore adaptive investment in specific brain regions by hosts and obligate brood
81 parasites, we here take advantage of a unique system composed of two closely-related paper
82 wasp species (Choudhary et al., 1994). *Polistes sulcifer* is the obligate social parasite of
83 *Polistes dominula* (Cervo & Turillazzi, 1996; Cini, Bruschini, Poggi, et al., 2011). Paper
84 wasps use cuticular hydrocarbons (CHCs) as odor signals that indicate fertility and dominance
85 (Dapporto et al., 2007), and to distinguish nestmates from intruders (Bruschini et al., 2011;
86 Dani, 2006; Dani et al., 2001; Lorenzi et al., 1997; Mora-Kepfer, 2014). Therefore, precise
87 and swift identification of potential parasites by CHCs recognition and visual inspection of
88 facial markings is crucial to the host *P. dominula* (Cervo et al., 2015; Cini, Ortolani, et al.,
89 2015; Ortolani et al., 2010; Sledge et al., 2001; Turillazzi et al., 2000). Paper wasp hosts
90 queens and workers also have distinct sensory needs according to division of labor. Workers

91 forage to collect nest material and prey, while queens remain on the nest and interact with
92 these incoming subordinate workers (Queller et al., 2000).

93 In contrast, the social parasite *P. sulcifer* must overcome a different and complex
94 challenge that requires a switch both in behavior and sensory modalities. A *P. sulcifer* female
95 emerges from its overwintering site at the top of high mountains and migrates to lower
96 elevations to locate the nests of *P. dominula* (Cervo, 2006), requiring navigational and visual
97 acuity. After finding a host colony, the parasite usurps and functionally replaces the host
98 queen to take reproductive control (Cervo, 2006), and acquires the CHCs of the colony
99 (Bagnères & Lorenzi, 2010; Dapporto et al., 2004; Sledge et al., 2001; Turillazzi et al., 2000).
100 However, host workers may eventually detect the new parasite queen, perhaps due to an
101 imperfect chemical and/or behavioral integration into the host colony (Cini et al., 2020; Cini,
102 Bruschini, Poggi, et al., 2011; Sledge et al., 2001). Nonetheless, when successful, the parasite
103 queen becomes the sole egg layer (Cervo, 2006; Turillazzi et al., 2000). After the adult female
104 and male parasite brood emerge from the host nest, they migrate to the top of mountains to
105 mate, and fertilized females overwinter to start the search and usurpation cycle the following
106 spring (Cervo, 2006). Therefore, *P. sulcifer*'s sensory needs should switch from an initial
107 investment in vision needed to first migrate to mate and overwinter and migrate to locate a
108 host colony the following spring, to a subsequent investment in olfaction to chemically
109 deceive the host workers.

110 We hypothesize that relative proportions of select brain regions reflect the differential
111 investment by hosts and parasites to meet their specific sensory needs. In particular, here we
112 focus on insect brain regions with known functions. First, the antennal lobes (AL) receive
113 olfactory stimuli from the antennae (Anton & Homberg, 1999). Second, the optic lobes (OL) are

114 known to process visual input from the eyes and are associated to visual ecology needs
115 (Gronenberg & Hölldobler, 1999). The OL are divided into the lamina (LA), medulla (ME) and
116 lobula (LO), which provide contrast enhancement, color vision and motion detection, and shape
117 discrimination, respectively (Arganda et al., 2020; Strausfeld, 1989; Yang et al., 2004; Yilmaz et
118 al., 2019). Third, the mushroom bodies (MB) are the neuropils associated to learning and
119 memory (Ehmer & Hoy, 2000; Strausfeld et al., 1998). In the calyx (CA) of the MB, the lip (LI)
120 and collar (CO) primarily processes olfactory visual information, respectively (Ehmer & Hoy,
121 2000; Strausfeld et al., 1998). Finally, the Central complex (CX), the navigation center of the
122 brain, may be implicated in long distance-migrations (Honkanen et al., 2019).

123 Given the known functions of these specific brain regions and the broad knowledge
124 about the natural history of this social parasite-host system, we first predicted that *P. sulcifer*
125 would show greater investment in the OL and CX compared to its host due to their need to
126 migrate to overwinter and then find new hosts in the spring. Second, we explored if brain
127 plasticity mirrors the drastic change from finding a host to integrating as a parasite queen. We
128 predicted that parasites initially invest in brain structures necessary to navigate and locate a
129 host and then switch to an investment in sensory structures after successfully integrating into a
130 host nest. Third, we expected that hosts would show higher AL investment compared to its
131 parasite, due to the different and complex olfactory stimuli they encounter and must assess
132 (Dani, 2006). Finally, because social interactions increase the CA volume in *Polistes* wasps
133 (Ehmer et al., 2001; Molina & O'Donnell, 2007), queen and worker hosts would show higher
134 investment in the LI and CO, compared to the social parasite before it integrates into the host
135 colony.

136

137 **METHODS**

138 **Field collection and usurpation experiments**

139 In Spring 2016 and 2017, we collected host colonies from unparasitized populations in the
140 surroundings of Florence (Tuscany, Italy). Colonies had 2-4 foundresses and brood, but no adult
141 workers. We fixed each nest to the ceiling of a glass cage (15 cm x 15 cm x 15 cm) and
142 maintained it under controlled laboratory conditions with ad libitum sugar, water, fly maggots
143 as larvae food, and paper for nest building. We individually marked colony members with
144 enamel paint dots (Testor ©) on the thorax and wings. We also collected *P. sulcifer* females
145 emerging from their overwintering sites at 2050 m altitude on Monti Sibillini (Umbria-Marche,
146 Italy). We kept them in the same type of glass cages, inside a fridge at 4°C with ad libitum
147 water and sugar, until it was time to end diapause. We simulated spring conditions by exposing
148 the parasites to direct natural and artificial sunlight, and low-elevation natural temperature
149 according to the established protocol (Cini et al., 2020; Cini, Bruschini, Poggi, et al., 2011;
150 Ortolani et al., 2008)

151 We ran experimental trials during the last week of May, when usurpation usually occurs
152 in the field (Cervo et al., 1996; Ortolani et al., 2008; Turillazzi et al., 1990). We focused on four
153 experimental categories: usurping parasites, post-usurpation parasites, and host and workers in
154 unparasitized colonies. For usurpation trials, we randomly chose host colonies and introduced a
155 single social parasite female inside the glass cage of a putative host nest (Cini, Bruschini, Poggi,
156 et al., 2011). We only chose parasites that showed usurpation behavior, confirmed by the clear
157 attempts to land on the colony and the attacks toward the host foundresses. First, we confirmed
158 that a parasite was trying to usurp a colony, and instead of allowing to integrate into the host
159 colony, it was collected and categorized as a usurping parasite (N =4). Second, we allowed a

160 subset of landing parasites to remain on the host nest. One week after usurpation, we confirmed
161 that parasites showed typical behaviors of integrated parasite queens, such as occupying the
162 central position of the nest, lack of foraging, stroking its abdomen on the nest cell rims, and
163 dominating host individuals (Turillazzi et al., 1990). We categorized them as post-usurpation
164 parasites (N =6). Finally, we reared unparasitized host colonies under the same conditions for
165 one week after the emergence of workers. Each worker was marked with paint individually. We
166 then collected the behaviorally dominant female and categorized it as host queen (N =7). From
167 the same colony, we also collected a host worker, after confirming it was active and exhibiting
168 typical worker behavior (N =4). To check that these behavioral categories matched the
169 predicted reproductive physiology, we dissected the abdomens and assessed ovary development
170 of each specimen using the established method for *Polistes* (Barth et al., 1975; Cini et al., 2013;
171 Pardi, 1948; Walton et al., 2020). Therefore, we confirmed that host queens and parasite queens
172 had developed ovaries, while host workers and usurping parasites had undeveloped ovaries.

173

174 **Histology and quantification of brain structures**

175 We employed an established histological protocol for *Polistes* brains to test for
176 differences in investment in brain structures that receive and process sensory information
177 (Ehmer & Hoy, 2000; Molina & O'Donnell, 2008; O'Donnell et al., 2011; O'Donnell et al.,
178 2007; O'Donnell et al., 2019). Each specimen was imbedded in epoxy resin to preserve the
179 dimensions of the head capsule and the brain regions to avoid confounding measurements due
180 to changes in brain size (Ocampo et al., 2020). To facilitate subsequent quantification of each
181 brain region, heads were sectioned into 17 μm -thick coronal axis sections and stained with the
182 NISSL stain toluidine blue. We then photographed each consecutive brain section per

183 specimen by using a Canon camera (EOS 5D Mark III) mounted on a Leica DM IL LED
184 microscope at 4x magnification and a 1000-micron scale. We used the image software
185 AxioVision Software (version 4.8; Zeiss) to trace the AL, and the three substructures of the
186 OL that receive visual information (LA, ME and LO). We also traced two CA substructures:
187 LI and CO. Finally, we traced the CX, and the central brain (CB). We traced every other
188 section in each specimen, as established by the reported accuracy of less than 3.5% error for
189 34 micro meter thick sections (Ehmer et al., 2001). Furthermore, we traced one hemisphere of
190 the brain for each specimen, quantified area and calculated volume of each brain region
191 (Molina & O'Donnell, 2008; Molina & O'Donnell, 2007; O'Donnell et al., 2011). Tracing and
192 quantification of brain structures were performed blindly to the 2 species and 4 categories.
193 Each image was standardized using a 100 μ m scale, and head width was measured as a proxy
194 for body size. Finally, we used the software RECONSTRUCT to generate the 3D brain
195 reconstructions for these two species (Fiala, 2005).

196

197 **Statistical analyses**

198 Observed differences in proportional investment in different brain regions can arise from
199 changes in allometric scaling through grade shifts (e.g, change in elevation) and/or changes in
200 the slope of the covariance between brain regions (Eberhard & Wcislo, 2011; O'Donnell et al.,
201 2013; Ott & Rogers, 2010; Seid et al., 2011; Sheehan et al., 2019; Stöckl et al., 2016). Thus, we
202 here explored 1) if hosts and parasites differed in the relative size of specific brain regions, and
203 2) if changes in grade shift and/or slope explained the investment in specific sensory regions
204 compared to non-sensory regions. To test for differences in visual and olfactory investment
205 between host and social parasites, we first quantified absolute volume for each brain region and

206 for the WB. For the specimens used in this study, the OL contribute on average to 42% of the
207 total brain in *P. dominula* and 46% in *P. sulcifer*, which can influence the scaling of relative
208 brain regions. Therefore, we normalized individual brain regions by CB volume to control for
209 the effect of the OL in relative neuropil scaling, and avoid distortions of brain volume and size
210 per species (Ott & Rogers, 2010; Sheehan et al., 2019; Stöckl et al., 2016).

211 To determine the relationship in the investment between sensory brain regions and central
212 brain, we implemented Standardized Major (SMA) regression analyses, using the SMATR v.3
213 package for R (Warton et al., 2012; Warton et al., 2006). We utilized the scaling relationship
214 between brain regions x and y , using the allometric equation $y = a \cdot x^\beta$ (Dubois, 1897; Huxley &
215 Teissier, 1936). We then used the linear equation $\log(y) = \beta \log(x) + \log(a)$, where $\log(a) = \alpha$, as
216 this logarithmic transformation estimates β from the slope and α from intercept of a regression
217 (Huxley & Teissier, 1936), used in previous studies that calculated investment in brain regions
218 (Ott & Rogers, 2010; Sheehan et al., 2019; Stöckl et al., 2016).

219 First, we tested for a Common Slope between host and social parasite ($H^0 = \beta_{host} =$
220 $\beta_{parasite}$) by using a log-likelihood test. Specifically, we ran the following comparisons in neural
221 tissue investment: 1) WB and body size, 2) CB and WB, 3) pooled sensory regions and CB, and
222 4) each sensory region normalized by the CB. Second, if the host and parasite shared a Common
223 Slope, we then tested for a Slope Index (SI), a Common Shift and a Grade Shift Index (GSI) for
224 the four comparisons described above. The Slope Index ($SI = \beta_{host\&par}$) tested if investment in a
225 brain region was isometric, calculated by a log-likelihood test. Therefore, if $\beta \neq 1$, the
226 proportion of sensory brain region (brain region y) and central brain (x) is allometric, meaning
227 that x/y would change with size. The Common Shift ($H^0 =$ equal axis between host and parasite)
228 indicated any shift along the major axis, calculated by a Wald Test. The Grade Shift Index (GSI)

229 quantified how much larger a sensory brain region (region y) is for a given size of central brain
230 (region x) for hosts compared to parasites ($H^0 = \alpha_{host} = \alpha_{parasite}$). GSI represents changes in
231 elevation (intercept α) with no changes in the slope (β), reflecting volumetric differences
232 between the two species as $e^{\alpha_{host} - \alpha_{parasite}}$, calculated by a Wald test. If the GSI > 1, hosts had a
233 larger brain region than parasites, and if GSI < 1, parasites had a larger brain region than hosts.
234 Log-likelihood tests and Wald tests were implemented in the SMATR package. Finally, we
235 compared investment between the host and social parasite in specific sensory regions by
236 normalizing each brain region by the CB, and running Mann-Whitney U tests. We ran these
237 same comparisons among queen hosts, worker hosts, usurping parasites and parasite queens by
238 using Kruskal-Wallis tests with posthoc pairwise comparisons, adjusted with Bonferroni
239 corrections.

240

241

242 **RESULTS**

243 **Contribution of allometry to host and social parasite differences**

244 Hosts and parasites did not share a common allometric slope when we compared investment
245 between absolute WB and body size ($P = 0.026$, Fig. 1A, Suppl. Table 1). Hosts showed an
246 isometric pattern with workers having small WB and body size while host queens showed a large
247 WB and body size. In contrast, the social parasites showed a hyperallometric relationship in
248 brain investment in the transition from usurpation to post-usurpation. Specifically, usurping
249 parasites had small WB, but parasite queens increased their WB volume after successful
250 integration into host colonies (Fig. 1A). Both the relationship between WB and CB (Fig 1. B),
251 and between CB and pooled sensory brain regions (Fig 1.C) (See Suppl. Table 1) shared a

252 common slope, respectively. Hosts showed a grade shift, with increased CB volume compared
253 to the usurping social parasites, as indicated by significant differences in elevation ($GSI > 1$,
254 $P=0.014$). Parasites also showed higher investment in volume of pooled sensory brain regions
255 compared to hosts, with the OL representing $> 65\%$ of these structures ($GSI < 1$, $P=0.016$).
256 Notably, there is overlap in investment of pooled sensory regions between host queens and
257 parasite queens (Fig. 1C). We found no effect of body size on brain region volume (SI) in the CB
258 or pooled sensory regions ($P=0.25$ and $P=0.9$, respectively, Suppl. Table 1).

259 Next, hosts and social parasites shared a common slope in the relationship between each
260 sensory brain region and CB, but showed unique differences in the GSI and SI depending on the
261 specific region (Suppl. Table 1, Suppl. Figure 2). The LA that provides visual contrast
262 enhancement, was significantly larger in parasites compared to hosts ($GSI < 1$, $P < 0.001$) and
263 allometric, as indicated by significant differences in the SI ($P < 0.001$). The ME and LO, that
264 facilitate color vision, motion detection and shape discrimination, also had increased volume in
265 parasites compared to hosts ($GSI < 1$, $P < 0.001$ and $P=0.01$, respectively), but were isometric.
266 However, the AL showed an opposite and allometric increase in the hosts compared to the
267 parasites. Both the CO and LI, that process visual and olfactory stimuli respectively, also showed
268 this allometric pattern. The LI also had a significant grade shift ($GSI > 1$, $P < 0.001$) in the hosts
269 compared to parasites. Finally, the CX, the navigation center of the brain, had significantly
270 increased volume in parasites compared to hosts ($GSI < 1$, $P=0.045$).

271

272 **Differential relative investment and tradeoffs between sensory brain regions**

273 When comparing the relative investment in sensory regions normalized by the CB, we
274 found significant and opposite differences between hosts and social parasites. Hosts had

275 increased AL volume ($U=19.00$, $df=1$, $P=0.011$, Fig 2A,) and a larger CA than social parasites
276 ($U=17.00$, $df=1$, $P=0.007$, Suppl Fig. 1) Both CA substructures, LI and CO, were significantly
277 larger in the host than in the parasite ($U=8.00$, $df=1$, $P=0.001$ and $U=9.76$, $df=1$, $P=0.03$
278 respectively, Fig. 2B). In contrast, OL volume was larger in social parasites compared to hosts
279 ($U=103.00$, $df=1$, $P=0.001$, Suppl. Fig 1A). Specifically, social parasites invested highly in the
280 LA ($U=105.00$, $df=1$, $P<0.001$, Fig. 2D), ME ($U=95.00$, $df=1$, $P=0.005$, Fig. 2D) and LO
281 ($U=91.00$, $df=1$, $P=0.011$, Fig. 2D). Social parasites also showed higher volume in the CX,
282 compared to the hosts ($U=79.00$, $df=1$, $P=0.025$, Fig. 2E).

283 We then tested for differential investment in sensory brain regions among the four
284 categories: 1) host queens and 2) host workers from the same nest, 3) usurping parasites, 4)
285 post-usurpation (parasite queens). Both usurping parasites and parasite queens had significantly
286 larger OL volume, followed by the host queen, while the host workers had the smallest OL
287 ($\chi^2=12.36$, $df=3$, $P=0.006$, Suppl. Fig. 1). We found the same pattern for investment among
288 categories in the LA ($\chi^2=12.79$, $df=3$, $P=0.005$, Fig. 3), ME ($\chi^2=8.89$, $df=3$, $P=0.031$) and LO
289 ($\chi^2=10.14$, $df=3$, $P=0.017$, respectively, Fig. 3C). Contrastingly, host queen had the highest
290 investment in AL while usurping parasites had the lowest investment. However, parasite
291 queens had a similar AL volume to host workers, which reflects an increase in investment after
292 usurpation ($\chi^2=11.11$, $df=3$, $P=0.01$, Fig 3A). Usurping parasites and parasite queens had
293 significantly smaller CA compared to host queens. However, parasite queens had similar CA
294 volume to host workers, showing a trend towards increased investment in this brain region after
295 usurpation ($\chi^2=12.14$, $df=3$, $P=0.007$). Host queens also had a significantly larger LI volume,
296 followed by an intermediate volume in host workers, and small LI in usurping parasites and
297 parasite queens. ($\chi^2=13.12$, $df=3$, $P=0.004$, Fig. 3B). Host queens and workers had the largest

298 CO, while usurping parasites had relatively small CO. However, parasite queens showed a
299 significant increase in CO volume, compared to ($\chi^2=9.76$, $df=3$, $P=0.021$, Fig. 3B).

300

301 **DISCUSSION**

302 Social parasites have evolved sensory and behavioral strategies to effectively locate host nests
303 and integrate as the new parasitic queen, while wasp hosts have evolved complex behavioral
304 repertoires needed to recognize nestmates and intruders (Bagnères & Lorenzi, 2010; Brandt et
305 al., 2005; Cervo, 2006; Cini, Patalano, et al., 2015; Cini et al., 2019). In this study, hosts and
306 parasites showed an inverse relationship in the investment towards visual and olfactory brain
307 regions, which supports the hypothesis that expensive brain tissue is allocated according to
308 specific sensory needs (Keeseey et al., 2020). For instance, the most important selective pressure
309 in the social parasite is to first find a host nest, then functionally replace the host queen and
310 deceive the host workers (Cini, Patalano, et al., 2015; Cini et al., 2019; Grüter et al., 2018),
311 which requires complex changes in sensory processing. We find several lines of evidence
312 supporting the hypothesis that brain plasticity facilitates these crucial behavioral and sensory
313 transitions in the social parasite. First, *P. sulcifer* shows high investment in the optic lobes,
314 which coincides with the selective and energetic pressures for migration, host detection and
315 invasion, as the visual system is expensive to maintain (Niven & Laughlin, 2008). Second,
316 during the usurpation period, there is less investment in the antennal lobes and both visual and
317 olfactory processing by the calyx. Third, our results suggest that brain plasticity is associated
318 with the switch from a searching solitary parasite to invading a host colony and disguising itself
319 as a parasite queen. The social parasite significantly increases whole brain volume as it
320 transitions from usurping to post-usurpation, reflected in the significant increase in antennal

321 lobes and a trend in increase of the calyx. In sum, our results suggest that brain plasticity is
322 advantageous, allowing parasites to adaptively respond to changes in sensory needs (Beani et al.,
323 2014; Molina & O'Donnell, 2008; Molina & O'Donnell, 2007).

324 Notably, energetic allocation towards the optic lobes mirrors the initial visual needs of
325 this obligate social parasite. Investment in the lamina, medulla and lobula may enhance contrast,
326 motion detection, color vision and discrimination (Arganda et al., 2020; Gronenberg et al., 2008;
327 Strausfeld, 1989; Yang et al., 2004; Yilmaz et al., 2019), which may be critical during migration
328 to first overwinter in its mountain site, and then migrate again to find suitable host nests to
329 invade at lower elevations (Cervo, 2006). In addition, the parasite uses visual cues to localize
330 nests, and then olfactory cues to distinguish *P. dominula* from sympatric *P. gallicus* or *P.*
331 *nimpha*, as the parasite's large size can hinder effective usurpation in the latter two social species
332 (Cervo, 2006; Cervo & Turillazzi, 1996; Cini, Bruschini, Signorotti, et al., 2011). The high
333 investment in vision and low investment in sensory processing (e.g., calyx) also reflects the
334 initial needs of the parasite, which is remarkably similar to neural tissue allocation in the
335 obligate parasitic ant *Polyergus mexicanus* (Sulger et al., 2014). In addition, the central complex,
336 the navigation center of the brain, is significantly larger in the social parasite compared to the
337 host (Honkanen et al., 2019).

338 After successfully finding a host nest then replacing the host queen, the parasite
339 experiences a drastic change in sensory needs from its usurping phase. We find that the change is
340 reflected by an increase in whole brain volume, which is explained by reallocation of investment
341 in the antennal lobe and a trend towards increasing sensory processing. This pattern suggests the
342 need to adopt the CHCs of the colony to become reproductively dominant (Turillazzi et al.,
343 2000). If the parasite succeeds, host workers will treat the parasite as if it were the host queen

344 (Cervo, 2006; Cini, Patalano, et al., 2015). However, recent experimental works suggests that
345 host workers eventually recognize the parasite queen as an intruder, so the time to produce and
346 rear successful parasite offspring is limited (Cini et al., 2020; Cini et al., 2014). Behavioral and
347 neurogenomic responses by host workers (Cini et al., 2020) may elicit the investment by the
348 parasite queen to increase the antennal lobes to receive olfactory information, and both in the lip
349 and collar, which process olfactory and visual information respectively. Once the parasite
350 integrates itself as parasite queen, this increase in the calyx is similar to the investment by host
351 workers, which is associated to social interactions in *Polistes* wasps (Ehmer et al., 2001; Molina
352 & O'Donnell, 2008; Molina & O'Donnell, 2007). However, calyx investment in parasite queens
353 is less than in host queens, which may be explained by differences in their behavioral repertoires
354 (Cini, Patalano, et al., 2015), with host queens having more frequent social interactions.

355 In contrast, parasite attacks do not represent the strongest selective pressure in the
356 sensory system of hosts, as incidence of obligate parasitism is almost null in this host population
357 (RC & AC, pers. comm.). Our results of high investment in olfaction and sensory processing are
358 consistent with other studies that show preferential brain investment in brain region associated
359 needed for effective communication, maintenance of division of labor, learning and memory
360 (Farris et al., 2001; Gronenberg et al., 1996; Jaumann et al., 2019; O'Donnell et al., 2015; Rehan
361 et al., 2015; Seid et al., 2005; Smith et al., 2010). Host queens had significantly higher antennal
362 lobes, as they spend most of their time on the nest and communicate with incoming subordinate
363 workers, who spend more time foraging off the nest (Molina & O'Donnell, 2008). Host queens
364 also use olfaction to assess fertility and policing attempts by subordinate workers, and to
365 maintain their dominance in the nest hierarchy (Dapporto et al., 2010). As predicted, host
366 queens also had more developed antennal lobes than usurping parasites and parasite queens. The

367 antennal lobes in *P. dominula* may facilitate identifying non-nestmate conspecifics or social
368 parasites, as recent work in paper wasps shows both an expansion and rapid evolution in the 9-
369 exon odorant receptors, responsible for detecting CHCs (Legan et al., 2020). Because *P. sulcifer*
370 must adopt the odor of the host colony to become chemically integrated, it is critical for the host
371 queen to detect any intruders approaching the nest, including social parasites (Cervo, 2006; Cini
372 et al., 2020; Turillazzi et al., 2000). Consistent with the critical task of recognition, we found that
373 both host queens and workers invested more in brain regions for olfactory processing compared
374 to obligate parasites. Hosts also invested more in visual processing compared to parasites, as
375 indicated by higher collar volume. These findings suggest that differential brain investment may
376 be a response to the important role of olfaction and vision in discriminating nest intruders, which
377 has been shown experimentally in this host-parasite system (Cini, Ortolani, et al., 2015).
378 Another possibility is that high investment in the calyx by hosts is due to the visual and olfactory
379 demands of foraging. Established parasitic queens, in contrast, will not forage and instead remain
380 on the host nest (Cervo, 2006). This pattern is consistent with the observed higher calyx
381 investment for the host ant *Formica fusta* compared to workers of its obligate social parasite *P.*
382 *mexicanus*, which rarely forage (Sulger et al., 2014). These two hypotheses may not be mutually
383 exclusive, and further experiments that include molecular, cellular and circuitry approaches
384 (Godfrey & Gronenberg, 2019) would elucidate the effect of these two selective pressures
385 towards sensory investment.

386

387 **Conclusions**

388 Our study provides novel insights into how intraspecific brain plasticity facilitates the
389 preferential investment of specific neural structures to meet an individual's sensory needs. In

390 the obligate parasite, our results support the hypothesis that preferential investment in specific
391 brain regions reflects the changing sensory needs of an individual, as it switches from searching
392 for a host colony to invading and disguising itself as a parasite queen. Specifically, the social
393 parasite may first invest in the optic lobes to facilitate migration and locating host nests, and
394 then transition to an increase in the antennal lobes to successfully integrate into the host colony.
395 Given that this social parasite evolved from a eusocial ancestor, it is likely retaining traits that
396 facilitate this cognitive switch from a solitary parasite to a social parasite queen in the host
397 colony. Previous work suggest that social insects share genetic toolkit genes (Berens et al.,
398 2014; Toth et al., 2010), so the social parasites likely respond to changes in sensory needs
399 similarly to their eusocial relatives (Cini, Patalano, et al., 2015; Cini et al., 2019). In the
400 potential host, our data suggests that queens and workers take advantage of their existing, and
401 well-developed sensory system, and particularly the antennal lobes, for intruder detection to
402 increase social parasite rejection. A trend towards differential investment in sensory structures
403 is also mirrored in the differential task allocation between host queens and workers. Finally,
404 future work comparing host populations highly attacked or not attacked by *P. sulcifer* will
405 effectively test the role of these social parasites as a selective pressure on the differential
406 investment in specific brain sensory regions in their hosts.

407

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418

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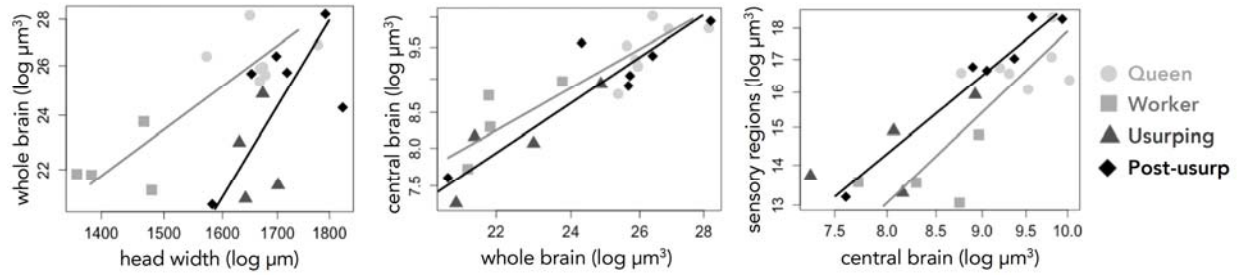
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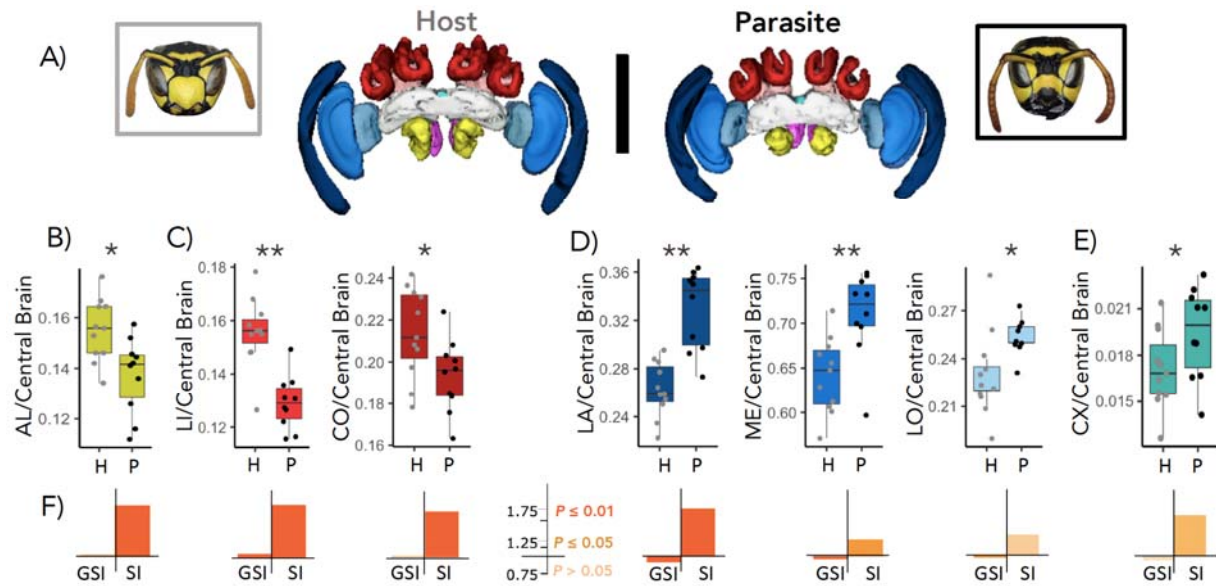
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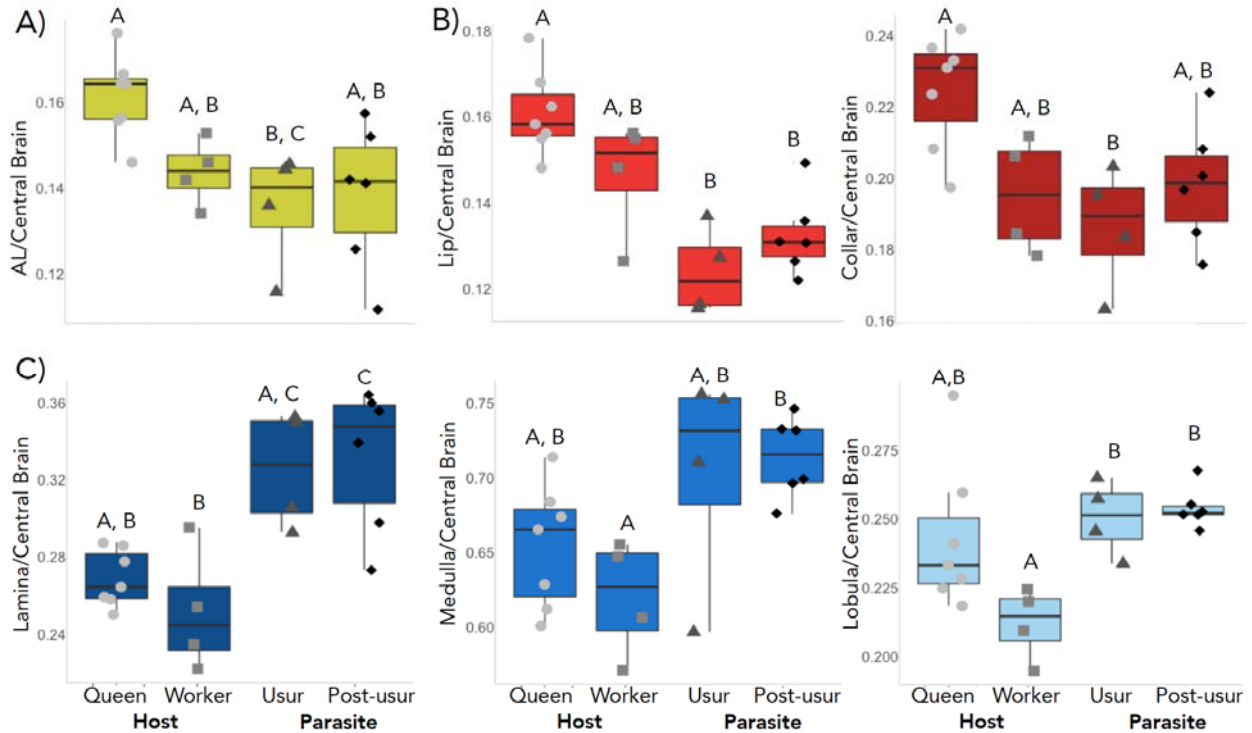
682 **Fig. 1. Role of allometry in investment of whole brain (WB), central brain (CB) and**
683 **combined sensory brain regions between host and social parasite.** Relationship between
684 A) head width (as a proxy for body size) and WB, B) WB and CB and C) CB and combined
685 sensory regions. Species and categories per species are depicted as: host queens (light grey
686 circles) and workers (medium grey squares), usurping parasites (dark grey triangles) and post-
687 usurpation parasite queens (black diamonds). Standardized major axis fits are log transformed
688 per species with the lines based on intercepts and slopes (grey for hosts and black for social
689 parasites). For full statistical tests, see Supplementary Table 1.

690



691

692 Figure 2. **Visual and olfactory brain regions in *Polistes dominula* (host) and *P. sulcifer***
 693 **(social parasite).** A) Frontal view of 3D reconstructed brain regions for the host (grey and to
 694 the left) and the parasite (black and to the right). Black scale bar = 1 mm. B) Antennal lobes
 695 (AL). C) Substructures of the calyx (CA): lip (LI) and collar (Co). D) Substructures of the
 696 OL: lamina (LA), medulla (ME) and lobula (LO) in sequence. E) Central complex (CX). All
 697 brain regions are normalized by the central brain shown in light grey. The subesophageal zone
 698 (ZEZ) is shown in dark pink and the peduncle (PED) in light pink. Each dot represents an
 699 individual and is grey for hosts (H) and black for parasites (P). Each box plot shows the
 700 median, 25th and 75th percentiles and the whiskers show the 5th and 95th percentiles. E) The
 701 Grade Shift Index (GSI) was calculated by scaling differences in normalized sensory brain
 702 regions between species. If GSI > 1, hosts had higher investment than parasites, and vice
 703 versa for GSI < 1. The Slope Index (SI) is represented by the deviation of the estimated
 704 common allometric slope β from 1. Statistical results based on Mann-Whitney U tests (* $P <$
 705 0.05, ** $P < 0.01$). Full statistical tests can be found in the Results sections and
 706 Supplementary Table 1. See Suppl. Fig 1. for OL and CA results.



707

708 Figure 3. **Visual and olfactory brain regions in host queens (light grey circles) and workers**

709 **(medium grey squares) and usurping parasites (dark grey triangles) and post-usurpation**

710 **parasite queens (black diamonds). A) Antennal lobes (AL), B) Substructures of the calyx**

711 **(CA): lip (LI) and collar (CO), C) Substructures of the optic lobes (OL): lamina (LA), medulla**

712 **(ME) and lobula (LO) in order from dark to light blue. Each dot represents an individual and**

713 **each box plot shows the median, 25th and 75th percentiles and the whiskers show the 5th and 95th**

714 **percentiles. Results reported by Mann-Whitney U tests, with different letters stating significant**

715 **differences between categories. Full statistical tests can be found in the results section.**