

1 **Queen succession conflict in the paper wasp *Polistes dominula* is mitigated by age-**  
2 **based convention**

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20 **Abstract**

21

22 Reproduction in cooperative animal groups is often dominated by one or a few individuals,  
23 with the remaining group members relegated to non-reproductive helping roles. This  
24 reproductive skew can evolve if helpers receive fitness benefits such as potential future  
25 inheritance of the breeding position, but the mechanisms by which inheritance is  
26 determined are not well resolved. *Polistes* paper wasps form highly reproductively skewed  
27 groups and inheritance of the breeding position is likely to play a key role in the  
28 maintenance of this social structure, making them excellent models for the processes by  
29 which simple societies are maintained. Reproductive succession is thought to be  
30 determined via an age-based convention in some *Polistes* species, but there is also  
31 evidence for contest-based succession systems in which the replacement queen uses  
32 physical aggression to overpower and thereby subordinate her nestmates. Here we  
33 provide evidence that queen succession in colonies of the European paper wasp *Polistes*  
34 *dominula* is determined via convention rather than contest, with little disruption to the  
35 colony's social functioning. We use queen removal experiments and fine-scale behavioral  
36 analyses to confirm that age is a strong predictor of succession, and that behavioral  
37 responses to queen removal are restricted to the oldest individuals rather than being  
38 experienced equally across the group. We provide the most comprehensive and detailed  
39 experimental analysis on the dynamics of breeder succession in a cooperatively breeding  
40 invertebrate to date, thereby shedding light on the mechanisms by which animal societies  
41 are able to maintain cohesion in the face of within-group conflict.

42

43 Key words: convention; inheritance; *Polistes*; queen replacement; reproductive conflict.

44

45

46 **Introduction**

47

48 Group living offers substantial benefits in terms of efficiency of energy acquisition and  
49 protection against predators and parasites (Krause and Ruxton 2002; Olson et al. 2015).  
50 However, conflicts of interest between group members can threaten the cohesion of the  
51 group as a whole (Beekman et al. 2003; Ratnieks et al. 2006). Intragroup conflicts are  
52 likely to be major drivers in social evolution, and mechanisms that facilitate their resolution  
53 are predicted to be favored by selection (Rubenstein 2012; Cant and Young 2013). The  
54 mechanisms regulating the resolution of conflict in social groups have major impacts on  
55 the costs and benefits of group living, so determining these mechanisms is fundamental to  
56 achieving an understanding of the evolution of sociality. However, investigating the nature  
57 of social conflict mediation mechanisms requires detailed data regarding individuals'  
58 behavioral and physiological responses to social disruption, which are difficult to obtain  
59 when dealing with complex societies.

60

61 Reproductive conflict is most intense in societies in which reproductive opportunities are  
62 partitioned unequally, as is the case in the cooperatively breeding groups of many birds,  
63 mammals, and social insects. A common feature of such societies is the presence of  
64 strong reproductive skew, with a relatively small number of high-ranking individuals  
65 monopolizing reproductive opportunities (Cant and English 2006; Nonacs and Hager  
66 2011). Much research into the evolution of cooperative breeding has focused on the  
67 benefits of this social system for subordinate individuals who would appear to be  
68 disadvantaged relative to the reproducing dominant individuals. These benefits could  
69 either be direct, e.g. 'queuing' for a chance to occupy the dominant reproductive role; or  
70 indirect, e.g. by boosting the fitness of dominant individuals to whom they are closely  
71 related (Cant and English 2006; Field and Cant 2009; Nonacs and Hager 2011). However,  
72 even when the benefits of helping are well-established, it remains the case that

73 subordinate individuals typically gain significantly lower fitness returns than dominant  
74 individuals, resulting in potentially high conflict over access to dominant reproductive  
75 role(s). A complete understanding of the evolution of cooperative breeding therefore  
76 requires that we understand not just the benefits of helping, but also the mechanisms by  
77 which conflict between individuals is resolved.

78

79 The loss of a reproductive dominant opens up opportunities for conflict within otherwise  
80 stable societies. Subordinate individuals may compete for the newly vacant reproductive  
81 role, but this competition is likely to come at a cost to the group as a whole (Gobin et al.  
82 2003; Strassmann et al. 2004). It is thus possible that selection will favor the evolution of  
83 mechanisms that facilitate the replacement of lost dominant individuals without outright  
84 conflict (Aureli and de Waal 2000; Aureli et al. 2002). Subordinate individuals may instead  
85 compete for a vacant reproductive role via 'conventional' traits that serve to differentiate  
86 between candidates reproductives without direct conflict. For example, while dominance  
87 and reproductive succession are determined by aggressive interactions in many vertebrate  
88 societies (e.g. Creel et al. 1992; Clarke and Faulkes 1997; Nichols et al. 2012), in other  
89 societies wholly or partially conventional phenotypic traits such as song complexity, age or  
90 length of tenure may serve as cues to dictate dominance without the need for outright  
91 conflict (e.g. East and Hofer 1991; Spencer et al. 2004; Duncan et al. 2018). Likewise,  
92 evidence exists for both conflict-based and convention-based reproductive succession  
93 mechanisms in invertebrate societies. Injurious fights over queen succession are  
94 particularly likely to occur when candidate reproductives are unrelated, as in the case of  
95 pleometrosis in ants (e.g. Bernasconi and Strassmann 1999) or multiple-foundress nesting  
96 in social wasps (e.g. West-Eberhard 1969). Reproductive succession in invertebrate  
97 groups with higher relatedness appears to more often involve traits such as age (e.g.  
98 Bridge and Field 2007). Evidence for the role of age in predicting dominance is particularly

99 strong in *Polistes* paper wasps (Pardi 1948; Strassmann and Meyer 1983; Miyano 1986;  
100 Hughes and Strassmann 1988; Tsuji and Tsuji 2005) but it remains unclear whether the  
101 role of this trait in fact reflects differences in e.g. fighting ability or ovarian development, or  
102 whether it represents a genuinely arbitrary convention.

103

104 In-depth analysis of the social dynamics of reproductive replacement in cooperatively  
105 breeding societies requires the collection of detailed behavioral and physiological data that  
106 are not readily available for most vertebrates. By contrast, cooperatively breeding  
107 invertebrate societies such as those of *Polistes* paper wasps are relatively small, short-  
108 lived and easily-manipulable, making them excellent systems with which to analyze the  
109 dynamics of reproductive conflict resolution (Starks and Turillazzi 2006; Jandt et al. 2014).

110

111 While several studies have involved observations of *Polistes* colonies from which queens  
112 have either been experimentally removed or naturally lost, few of these have paid close  
113 attention to the social dynamics that give rise to replacement reproductives. In particular,  
114 only a very small number of studies have investigated the mechanisms of queen  
115 succession in nests following the eclosion of workers (**Table 1**). Reproductive conflicts  
116 during this worker phase may be radically different from those that occur during the  
117 founding phase because workers are usually closely related to one another and to the  
118 queen, which subordinate foundresses may not be (Zanette and Field 2008; Leadbeater et  
119 al. 2011).

120

121 The close relatedness among siblings within post-eclosion nests means that fitness  
122 interests of individuals in such groups are more strongly aligned than in foundress groups,  
123 where relatedness can be low (Zanette and Field 2008; Leadbeater et al. 2011); this may  
124 have favored the evolution of more robust conflict-resolution mechanisms in post-eclosion

125 nests. Unlike clonal social organisms (e.g. social aphids Uematsu et al. 2013), however,  
126 groups of *Polistes* workers are not genetically identical and so some degree of selfish  
127 behavior may persist. The intermediate level of relatedness observed among group  
128 members in post-eclosion paper wasp colonies thus contributes to their value as a model  
129 for the tradeoff between cooperation and conflict.

130

131 The European paper wasp *P. dominula* is the most intensely-studied species within its  
132 genus (Starks and Turillazzi 2006; Jandt et al. 2014), and partial evidence exists for both  
133 convention-based and contest-based resolution mechanisms following queen succession  
134 in this species (**Table 1**). We thus chose *P. dominula* as a focal system with which to  
135 perform an in-depth analysis of the queen replacement process in *Polistes*. We focused  
136 our analyses upon colonies following the emergence of the first adult workers, because we  
137 expected the higher within-colony relatedness during this phase to favor the expression of  
138 mechanisms that mitigate group-level costs during queen replacement.

139

140 In this paper we conduct a detailed analysis of the social dynamics of queen succession in  
141 *P. dominula*. By removing queens from monogynous colonies, we generate detailed data  
142 on behavioral and ovarian development with which we test several hypotheses pertaining  
143 to the social mechanisms of queen replacement in this species. First, we determine the  
144 individual-level traits that dictate queen succession. We predict that queen succession is  
145 explained either by age, which is known to influence dominance in many *Polistes* (Pardi  
146 1948; Strassmann and Meyer 1983; Miyano 1986; Hughes and Strassmann 1988; Tsuji  
147 and Tsuji 2005), suggesting convention; or alternatively by size, a trait thought to influence  
148 fighting ability in *Polistes* (Turillazzi and Pardi 1977; Cervo et al. 2008; Tibbetts and  
149 Shorter 2009; but see Reeve et al 2000; Cant et al. 2006), suggesting contest  
150 (**Hypothesis 1**). Additionally, we test the hypothesis that queen succession reflects pre-

151 existing physiological constraints on ovarian development (**Hypothesis 2**). Second, we  
152 examine group-level responses to queen removal to contextualize the succession process.  
153 We predict that queen removal will be followed by significant social disruption if succession  
154 is dictated by conflict but not if it is dictated by convention (**Hypothesis 3**). We investigate  
155 the distribution of behavioral responses to queen removal: these should be evenly  
156 distributed among multiple individuals within each colony under a contest-based  
157 succession mechanism, whereas in the case of convention-based succession only one or  
158 a few individuals should undergo significant behavioral changes (**Hypothesis 4**). We also  
159 test the hypothesis that group-level responses to queen removal will be minimized when  
160 there is reduced ambiguity regarding the identity of the replacement queen (**Hypothesis**  
161 **5**). Hypotheses 1, 3 and 4 address whether the process of queen replacement is dictated  
162 by contest or convention (**Table 2**), while Hypotheses 2 and 5 investigate the proximate  
163 mechanisms of this succession process.

164

165

## 166 **Methods**

167

### 168 ***Colony collection and ageing of wasps***

169 *Polistes dominula* colonies ( $N = 76$ ) were collected from rural areas near Florence, Italy,  
170 and transferred to a laboratory in the University of Florence during mid-May 2017, before  
171 the emergence of the first brood. Only nests with at least 20 cells and at least one capped  
172 cell were collected. *P. dominula* wasps typically do not initiate flight when the ambient  
173 temperature is below  $\sim 22^{\circ}\text{C}$  (Weiner et al. 2011) and colonies were therefore collected in  
174 the morning when temperatures were still low ( $< 20^{\circ}\text{C}$ ) to ensure that all nestmates were  
175 collected together. Colonies were maintained in glass boxes (15 cm x 15 cm x 15 cm)  
176 under natural light conditions with ad libitum access to water, sugar, dipteran larvae and

177 nest materials (cardboard and paper). On cool days (midday temperatures <25 °C)  
178 heaters were used to maintain high daytime temperatures within the laboratory; otherwise,  
179 temperatures were ambient. Immediately after colony collection, individuals were given  
180 unique markings by applying spots of colored paint (Testor Corporation) to the thorax  
181 and/or to the tips or upper portions of the wings.

182

183 Both single foundress (monogynous) and multi-foundress (polygynous) colonies were  
184 collected (mean 1.9 foundresses/colony; range 1-5 foundresses/colony). In order to ensure  
185 that all adult offspring emerged into an equivalent social environment on each colony, all  
186 subordinate foundresses were removed from the multiple-foundress colonies. This loss of  
187 subordinate foundresses is not an unnatural state for *Polistes* colonies, which experience  
188 high rates of foundress loss in the wild (Strassmann 1981; Miyano 1986; Strassmann et al.  
189 2004). Colonies were observed for five minutes hourly for two days following relocation to  
190 the lab in order to identify the dominant foundress. Dominant foundresses were identified  
191 based on two criteria: firstly, the dominant spends the large majority of her time on the nest  
192 and occupies the central portion of the nest carton, whilst her subordinate co-foundresses  
193 forage (Baracchi 2017); secondly, dominant individuals could be observed antennating  
194 subordinate individuals (see Methods; Pardi 1948; Jandt et al. 2014), while subordinates  
195 were never observed antennating dominants. In all cases, the dominant foundress of a  
196 colony was identified within two days of collection and before the eclosion of adult workers.  
197 Once the dominant foundress of a colony had been identified, all subordinates were  
198 removed from the nest box. Colonies were then checked daily and any newly emerged  
199 individuals were given unique identification markings. This allowed us to assign ages to  
200 each emerging individual in each colony, to an accuracy of 24 hours.

201

202 ***Queen removal experiments***



203 Mature colonies were randomly allocated either to a queen removal (QR), or to a control  
204 (C) treatment. Control colonies were subjected to a sham removal in which an individual  
205 was removed from and then immediately placed back onto the nest. To capture the  
206 process of queen succession, we further randomly separated colonies into those in which  
207 we sampled wasps and assessed their reproductive dominance early during the  
208 succession period and those where this was done late in succession. A previous queen  
209 removal study in *P. dominula* (Strassmann et al. 2004) found that the process of queen  
210 replacement is ongoing at three days following queen loss but largely complete after  
211 twelve days. In line with this, we chose three and twelve days following queen removal as  
212 focal timepoints for our analyses. The four treatments are henceforth referred to as QR3  
213 ( $n=20$ ), QR12 ( $n=20$ ), C3 ( $n=20$ ) and C12 ( $n=15$ ).

214

215 A colony was considered 'mature' once it had produced a minimum of four adult offspring  
216 (Dapporto et al. 2005). Each colony was manipulated according to its treatment group on  
217 the morning of the fourth day following maturity. All manipulations were performed between  
218 07:00 and 08:00, during which time period the temperatures in the lab were still well below  
219 22°C. For colonies belonging to QR3 or QR12 treatments, manipulation consisted of  
220 removing the queen and immediately preserving her body in a 1.5 mL Eppendorf tube  
221 containing 80% EtOH, which was stored at -20°C for later dissection. For C3 and C12  
222 treatments, manipulation consisted of removal of a randomly pre-selected non-foundress  
223 individual. Following this manipulation, each colony was maintained under standard lab  
224 conditions for an additional period of time dictated by treatment group: three days for QR3  
225 and C3 colonies, and twelve days for QR12 and C12 colonies.

226

## 227 **Data collection**

228 *Ovarian and body size measurements*

229 All individuals were dissected after completion of the experiment. For each individual, an  
230 index of ovarian development was obtained by removing and photographing the ovarioles,  
231 and subsequently measuring the mean length of the six largest oocytes/ova present. This  
232 is a standard measure of ovarian development and has been shown to strongly correlate  
233 with more complex ovarian indices in *P. dominula* (Cini et al. 2013). Individuals with  
234 extremely underdeveloped oocytes (all oocytes <15  $\mu\text{m}$  in length) were assigned an  
235 ovarian index of 15  $\mu\text{m}$  as measurements were imprecise below this value.

236

237 We obtained measures of body size by removing the left forewing of each individual and  
238 measuring the maximum distance from the tip of the wing to the thoracic wing joint, which  
239 is a reliable proxy for body size in *Polistes* wasps (Haggard and Gamboa 1980; Cant et al  
240 2006). Wing length was used in place of head width because head tissues had been  
241 consumed in a separate analysis. All measurements were performed using *Fiji*, a  
242 distribution package of the image processing program *ImageJ* 1.52 (Schindelin et al. 2012;  
243 Schneider et al. 2012). All measurements were performed by a single observer, blind with  
244 respect to each individual's identity and treatment group.

245

#### 246 *Behavioral recording of nestmate interactions*

247 To obtain data regarding the individual- and group-level mechanisms of queen  
248 replacement, we recorded the individuals' behavior before and after manipulation.  
249 Colonies were filmed before and after experimental manipulation in order to assess  
250 individual-level and group-level effects of queen loss. Recordings were made for 30  
251 minutes daily for three days before and three days after experimental manipulation, using  
252 Sony HDR-CX405 HD video cameras mounted on tripods. Additionally, colonies in the  
253 QR12 and C12 treatments were filmed for 30 minutes daily on days 10 through 12  
254 following experimental manipulation. All filming occurred between the hours of 10:30 and

255 16:30 when *P. dominula* activity levels are at their highest (Cini et al. 2013). The time of  
256 filming for each colony and day was randomized. Between 07:00 and 08:00 on the  
257 morning following the end of the treatment-specific time period, the entire colony was  
258 terminated by removing all individuals and preserving their bodies in alcohol as described  
259 above.

260

261 Digitally recorded behaviors for each colony were annotated using BORIS observation  
262 software (Friard and Gamba 2016). To permit efficient analysis of the >200 hours of videos  
263 recorded, behaviors not directly related to dominance interactions (e.g. nest building) were  
264 not recorded. An observer recorded the time and duration of each instance of dominance  
265 behavior that occurred within each video, and also tracked the proportion of time spent on  
266 and off the nest carton by each individual within each 30-minute video. In line with  
267 previous behavioral observations of *P. dominula* colonies, mounting followed by  
268 antennation was by far the most common behavior observed at all treatment stages (Pardi  
269 1948; Tibbetts and Huang 2010; Jandt et al. 2014). More aggressive dominance  
270 interactions, such as wing chewing, were observed extremely rarely (<20 times across the  
271 entire experiment) and so all subsequent analyses focus solely on mounting followed by  
272 antennation (hereafter referred to simply as 'antennation').

273

274 To reduce the likelihood of false positives, antennation bouts were only recorded if they  
275 were longer than 1 second in duration. Antennation bouts that did not occur in conjunction  
276 with an unambiguous instance of mounting were not recorded, as these may simply reflect  
277 affiliative or communicative functions rather than dominance behavior. In each instance of  
278 antennation, the dominant and subordinate individuals' identities were recorded. Dominant  
279 and subordinate actors in a bout of antennation are usually easily identified: the dominant  
280 strikes the subordinate with her antennae while the subordinate remains still and lowers

281 her head and body to the nest surface (Pardi 1948; Jandt et al. 2014). In a small  
282 proportion (~5%) of antennation bouts, antennation was resisted by the targeted individual,  
283 resulting in an inconclusive struggle between the two individuals without either establishing  
284 a clear dominant role. For the purposes of downstream social network analyses, these  
285 bouts were recorded as draws.

286

287 In order to test whether queen removal results in a group-level change in dominance  
288 behavior, we calculated the mean and variance of antennation rate within each colony for  
289 each three-day time period. We additionally calculated the average proportion of time  
290 individuals spent off the nest carton (a proxy measure of participation in off-nest activities  
291 such as foraging or internidal drifting) for each time period, to test whether queen removal  
292 exacts a cost in terms of the group's ability to continue normal colony functions. As  
293 interactions were only recorded when they occurred on the nest carton itself, antennation  
294 rate was measured relative to the amount of time that an individual was present on the  
295 nest.

296

### 297 ***Analyzing the Process of Queen Succession***

#### 298 *Assignment of Elo ratings to estimate within-group dominance*

299 We used the Elo rating system (Elo 1978; Albers and De Vries 2001; Neumann et al. 2011)  
300 to determine a dominance score for each individual within each observation period. The  
301 Elo rating system assigns each individual an equal, arbitrary starting value and then uses  
302 the results of sequential pairwise interactions, each with a winner and loser, to adjust the  
303 individuals' values based on the discrepancy between the actual outcome of the interaction  
304 and that expected from previous interactions. Thus, an individual experiences a large gain  
305 in Elo rating if she wins unexpectedly (i.e. if she defeats an individual of higher prior Elo  
306 value than herself), but only a small gain if she was already expected to win the contest

307 (i.e. if she already has a higher Elo value than the individual she defeats). Elo ratings are  
308 well-suited to our data, as they can accommodate repeated interactions between specific  
309 pairs of individuals.

310

311 We collated all observed behaviors for a given colony within each of three set time  
312 periods: the three days prior to manipulation, the three days immediately following  
313 manipulation (for all colonies), and days 10 through 12 following manipulation (for QR12  
314 and C12 colonies only). We then generated Elo rankings for each individual during each  
315 period using the *AniDom* package in R (Farine & Sanchez-Tojar 2018) with an initial Elo  
316 value of 1000 and scaling constant  $K = 100$ . Elo rankings are sensitive to the order of  
317 interaction, but each video represents only a small portion of the total number of  
318 interactions that may have occurred within a 3-day period and the order of in which  
319 interactions were observed may not have been meaningful. To remove potential bias, we  
320 therefore randomly re-ordered the list of contests collated within each 3-day period 1000  
321 times and obtained an Elo ranking for each individual for each permutation; final Elo  
322 scores were then calculated as the mean score across all permutations for the given time  
323 period. Each time period (three days pre-removal, and days 1-3 and 10-12 post-removal)  
324 was treated as independent. In addition, we discarded dominance values for any individual  
325 that was observed on the nest carton for fewer than 30 minutes within the focal three-day  
326 time period rather than arbitrarily assign 'neutral' Elo scores to individuals that were under-  
327 observed.

328

### 329 *Social network analyses*

330 As a complement to individual-level measures of dominance, we used social network  
331 analyses to capture group-level consequences of queen removal. Network characteristics  
332 were calculated using the behavioral interactions for each colony for each three-day

333 period, again treating each period as independent. Collated lists of behavioral interactions  
334 were converted to social networks in R using the *igraph* package (Csardi and Nepusz  
335 2006) with each individual representing a node and each interaction representing an edge  
336 (connection) between nodes. For each network, we then generated two measures of social  
337 network structure. The *transitivity coefficient* (or *clustering coefficient*) of a network  
338 measures the global density of closed node triads, i.e. the proportion of instances in which,  
339 when an individual A has interacted with two other individuals B and C, those two  
340 individuals have also interacted with one another. Transitivity may be interpreted as a  
341 measure of the cohesiveness of the group (Croft et al. 2008). *Degree centrality* is the  
342 extent to which a particular node (individual) occupies a central location within a network  
343 (Croft et al. 2008). Variance in degree centrality indicates the level of social monopoly  
344 within a group, and high degree centrality variance indicates that one or a few individuals  
345 dominate the network relative to a larger number of poorly-connected individuals. If new  
346 queens establish themselves by directing frequent dominance behavior towards their  
347 nestmates, then we should expect to observe an increase in the variance of degree  
348 centrality in colonies following queen removal.

#### 349 350 *Estimation of individual-level transition from worker to queen roles*

351 In order to robustly assign queen identity, we fit a Bayesian logistic regression model with  
352 the R package *arm* (Gelman & Su 2018) using ovarian development indices and Elo  
353 ratings from queens and workers from queenright control colonies as the independent  
354 variables and caste as a binary response variable. This allowed us to subsequently  
355 estimate the degree to which individuals on post-removal colonies phenotypically  
356 resembled workers or queens. Workers were coded with a value of 0 and queens were  
357 coded with a value of 1. We applied a flat Bayesian prior to the model output to account for  
358 the fact that phenotypic scores for workers and queens exhibit perfect separation on

359 queenright colonies (Gelman et al. 2008), whereas our expectation is that workers  
360 transitioning to queens must necessarily pass through some intermediate stage in which  
361 their phenotype is wholly or partially intermediate between that of a 'normal' queen and  
362 'normal' worker. For each QR3 and QR12 colony, we then estimated the 'queenness' of  
363 each individual by supplying the model with ovarian scores and dominance scores  
364 pertaining to the three days prior to colony termination. Using this method, each individual  
365 from each QR3 and QR12 colony was assigned a value from 0 to 1 indicating the degree  
366 of phenotypic identity to workers or queens from queenright colonies (0 = 100% similarity  
367 to worker phenotype; 1 = 100% similarity to queen phenotype), except where Elo ratings  
368 could not be established due to insufficient observational data.

369

### 370 ***Testing the hypotheses***

371 General linear mixed models (GLMMs) except those with queenness as the response  
372 were constructed in R using the *lmer* function, part of the package *lme4* (Bates et al 2015).  
373 For individual-level analyses, response variables were queenness (Section 2.4.3), Elo  
374 rating, or ovarian development. Models with queenness as the response were constructed  
375 using the *metafor* R package (Viechtbauer 2010), which allowed us to weight individuals'  
376 queenness estimates by the inverse of the standard error of those estimates. For all  
377 individual-level models, fixed effects were wing length, age and pre-manipulation Elo rating  
378 and their pairwise interactions. The possibility that caste-biased traits such as ovarian  
379 development might be negatively correlated within nests (if, for example, replacement  
380 queens suppress their nestmates' phenotypic plasticity) led us to question the validity of  
381 including colony as a random effect in the individual-level models. To account for this, we  
382 ran each individual-level model both with and without the random effect. We report the  
383 results of the latter fixed-effect models alongside the mixed-effect models in the  
384 supplementary materials.

385

386 For group-level analyses, response variables were within-group mean antennation rate,  
387 antennation rate variance, network transitivity, degree centrality or mean time off-nest.  
388 Fixed effects were treatment group (Control vs Queen removal), and experimental stage  
389 (Pre-manipulation vs Post-manipulation days 1-3 vs Post-manipulation days 10-12),  
390 number of individuals in the colony, and the interaction between treatment and time period.  
391 Colony was included as a random factor in each model, to account for repeated  
392 measurements of the same colonies, which were assumed to be independent. For group-  
393 level analyses, response variables were transformed using Tukey's ladder of powers  
394 transformation ( $\lambda = 0.450, 0.175, 0.925, 0.950$  and  $0.775$  respectively for mean, variance,  
395 transitivity, centrality and time off-nest transformations), and were thereafter confirmed to  
396 adhere adequately to the assumption of normality using the package *rcompanion*  
397 (Mangiafico 2018). In all cases, continuous variables were centered and scaled to facilitate  
398 model comparison. Full details and results for each model are given in supplementary  
399 tables **S3-S12**.

400

401

## 402 **Results**

403

404 Of 76 colonies collected, eight were excluded due to colony failure: six did not produce the  
405 minimum required number of four adult workers within 21 days of collection, and in a  
406 further three the queen died before the emergence of the first brood. Additionally, we  
407 excluded 12 colonies that were heavily styloped by strepsipteran flies (*Xenos vesparum*),  
408 defined as displaying >50% stylopedisation among all offspring produced across the course  
409 of the experiment. Following these exclusions, final colony sample sizes for the experiment



410 were  $n=55$  colonies, with  $n=16$  colonies each for treatments QR3, QR12 and QC3, and  
411  $n=7$  colonies for treatment QC12.

412

413 Approximately half of the remaining 55 colonies produced at least one styloped individual  
414 (mean $\pm$ SE  $1.56\pm 0.28$  styloped wasps/colony across all 55 colonies, representing  
415  $13.6\pm 2.1\%$  of workers in each colony). Styloped individuals become asocial, fail to  
416 perform typical foraging and feeding behaviors, and eventually disperse from the nest  
417 (Hughes et al. 2004; Dapporto et al. 2007; Kathirithamby 2009; Beani et al. 2011; Geffre et  
418 al. 2017). Styloped individuals do not typically engage in or respond to dominance  
419 interactions, and disperse within a few days of emergence (Hughes et al 2004). Here, they  
420 comprised 12% (86/701) of experimental individuals but took part in a total of just 0.3%  
421 (56/1633) observed dominance interactions. Accordingly, we excluded styloped  
422 individuals from further analysis.

423

424 A very small number of males emerged during the experiment (3/701 observed  
425 individuals). Since male *P. dominula* very rarely engage in dominance behavior (5/1633  
426 observed dominance interactions in this experiment) and are known to disperse relatively  
427 quickly following eclosion in nature (Reeve 1991), we excluded males from all analyses.

428

429 Following these exclusions, behavioral and ovarian data for 55 queens and 557 workers  
430 from 55 colonies remained (mean $\pm$ SE  $12.2\pm 0.66$  wasps/colony; **Tables S1 & S2**).

431

### 432 ***Hypothesis 1: Predictors of queen succession***

433 Age was the sole significant predictive variable in each of the three complete models of  
434 individual phenotypes following queen removal, suggesting that age is a reliable predictor  
435 of both post-removal ovarian development (Est = 0.43, SE = 0.12,  $p = 0.0023$ ) and post-

436 removal dominance (Est. = 0.28, SE = 0.10,  $p = 0.008$ ), and thus of post-removal  
437 queenness (Est. = 0.17, SE = 0.044,  $p < 0.001$ ; **Table S3**). The slope of queenness on age  
438 was similar when models were constructed using data from QR3 colonies only (Est. =  
439 0.18, SE = 0.15,  $p = 0.22$ ) or from QR12 colonies only (Est. = 0.14, SE = 0.11,  $p = 0.21$ ;  
440 **Table S4**).

441

442 Excluding two colonies which failed to produce any candidate queen replacements (see  
443 **Hypothesis 3** below), in 19/30 (63%) queen removal colonies the individual with the  
444 highest queenness was also the oldest individual (**Figure 1**). In a further 8/30 (27%)  
445 colonies this individual was the second oldest, leaving three colonies in which neither of  
446 the two oldest individuals was the most queenlike. These proportions did not differ  
447 between QR3 and QR12 treatments ( $\chi = 0.89$ ,  $p = 0.64$ ; **Table S5**). Thus, while age  
448 appears to act a strong predictor of succession, it does not perfectly explain variation  
449 between individuals' caste identities.

450

451 Neither body size (measured by wing length) nor pre-manipulation Elo rating emerged as  
452 significant terms in any of the models, indicating that queen succession in *P. dominula* is  
453 unlikely to be strongly dictated by physical strength. A possible exception appeared in the  
454 model with post-manipulation Elo rating as the response variable, in which there was a  
455 near-significant negative interaction between body size and age (Est. = -0.18, SE = 0.10,  $p$   
456 = 0.074; **Table S3**). This might plausibly indicate that for particularly large individuals, age  
457 is a less important determinant of dominance following queen removal.

458

### 459 ***Hypothesis 2: Role of ovarian development in queen succession***

460 Contrary to the hypothesis that post-eclosion queen succession in *Polistes* reflects  
461 inherent physiological constraints upon ovarian development in workers, when we included

462 control colonies in our ovarian model, we found no evidence for a relationship between  
463 age and ovarian development on queenright colonies (Est. = 0.060, SE = 0.096,  $p = 0.53$ ;  
464 **Figure 2 & Table S6**). Only following queen loss did older individuals begin to display  
465 greater ovarian development than their younger sisters (Est. = 0.52, SE = 0.12,  $p < 0.001$ ).

466

### 467 ***Hypothesis 3: Group-level resilience to queen loss***

468 We found little evidence that queen removal significantly altered the group-level social  
469 dynamics within colonies. Neither mean (Est. = 0.24, SE = 0.27,  $p = 0.37$ ) nor variance  
470 (Est. = 0.12, SE = 0.11,  $p = 0.28$ ) of antennation rates were significantly increased  
471 following queen removal, indicating that loss of the dominant does not result in a  
472 meaningful increase in the rate and variance of dominance behavior (**Table S7, Figure 3**).

473

474 Both social network centrality (Est. = 0.49, SE = 0.28,  $p = 0.089$ ) and clustering coefficient  
475 (Est. = 0.63, SE = 0.37,  $p = 0.091$ ) exhibited borderline-significant increases in the three  
476 days following queen removal, but we otherwise found no evidence of group-level  
477 responses to queen loss (**Table S8**). By contrast, control colonies exhibited significantly  
478 decreased centrality (Est. = -0.76, SE = 0.33,  $p = 0.022$ ) and increased mean time off-nest  
479 (Est. = 1.1, SE = 0.41,  $p = 0.0096$ ) at days 10-12 following manipulation (**Table S8**).

480

481 Network centrality (Est. = 0.20, SE = 0.028,  $p = <0.001$ ) and mean (Est. = 0.16, SE =  
482 0.034,  $p = <0.001$ ) and variance (Est. = 0.065, SE = 0.014,  $p < 0.001$ ) of antennation rate  
483 were all strongly positively correlated with colony size.

484

485 One notable manner in which queen loss may have altered nests' social structures was in  
486 the production of multiple replacement queens. A large majority of individuals on post-  
487 removal colonies were assigned queenness values very close to zero, as expected if most  
488 individuals remain as un-reproductive, low-dominance workers. We thus considered any

489 individual with a queenness estimate greater than 0.1 to be exhibiting significantly  
490 divergent caste expression relative to the normal worker profile; any such individual might  
491 represent a potential replacement queen. While the majority of nests produced either one  
492 (15/32 nests) or two (11/32) potential replacement queens, a small number (4/32)  
493 produced three or more replacements, and a further 2/32 failed entirely to produce a  
494 potential replacement queen. These numbers did not differ significantly between QR3 and  
495 QR12 conditions ( $\chi = 0.16$ ,  $p = 0.98$ ; **Table S9**).

496

#### 497 ***Hypothesis 4: Within-group distribution of behavioral responses to queen removal***

498 In accordance with the predictions of a convention-based succession model, the change in  
499 individuals' antennation rates between the three days preceding and the three days  
500 following queen removal was strongly predicted by age (Est. = 1.2, SE = 0.22,  $p < 0.001$ ),  
501 although there was also a strongly significant negative interaction between the effects of  
502 body size and age (Est. = -0.69, SE = 0.22,  $p = 0.0018$ ; **Table S10**). This negative  
503 interaction term indicates that age was a weaker predictor of antennation rate for larger  
504 individuals.

505

506 The mean increase in dominance rates was close to zero for the youngest (latest-eclosing)  
507 individuals on each colony: an increase of 0.09 antennations/hour for individuals that were  
508 fifth or below in the order of eclosion vs an increase of 2.42 antennations/hour for  
509 individuals that eclosed first or second on their respective colonies, and an increase of  
510 0.71 for those that eclosed third or fourth.

511

#### 512 ***Hypothesis 5: Efficacy of gerontocracy in the presence of low age rank resolution***

513 Age-based convention appears to act as an effective means of conflict mitigation during  
514 queen succession in *P. dominula*. Despite this, we found no evidence that a lack of  
515 resolution within a colony's age hierarchy results in the failure of this conflict-resolution

516 mechanism, i.e. a reversion to a contest-based system. The age gap between the two  
517 oldest workers in a colony was not a significant predictor of the increase in antennation  
518 rate in that colony in the three days immediately following queen removal (Est. = 0.50, SE  
519 = 0.40,  $p = 0.22$ ; **Table S11**), suggesting that colonies with multiple oldest individuals of  
520 similar ages are nonetheless able to transition to a successor queen without a significant  
521 increase in intra-group conflict. We also found no correlation between the difference in  
522 antennation rates of the two most dominant workers on a colony prior to queen removal  
523 and increases in colony-wide antennation rate on that same colony following queen  
524 removal (Est. = -0.22, SE = 0.43,  $p = 0.62$ ; **Table S12**).

525

526

## 527 **Discussion**

528

529 The potential future benefit of inheriting the reproductive role is thought to be an important  
530 factor ensuring the ongoing cooperation of helpers in societies with high reproductive  
531 skew. Despite this, the mechanisms by which replacement reproductives arise in  
532 cooperative societies are not well understood. Here we have presented the results of the  
533 most in-depth analysis of the mechanisms of reproductive succession in an invertebrate  
534 society to date. In addition to confirming that queen succession among workers in *P.*  
535 *dominula* is dictated by age, we show that this gerontocratic system is not underlain by  
536 age-dependent constraints upon size or reproductive development. We additionally  
537 provide evidence that group-level characteristics, such as the rate and network structure of  
538 dominance interactions between individuals, are not strongly perturbed by queen loss.  
539 Behavioral responses to queen removal were concentrated among the oldest individuals,  
540 suggesting that the gerontocratic convention effectively mitigates potentially costly conflict  
541 over reproductive succession in post-eclosion colonies of this species.

542

543 Age acted as a strong predictor for queen succession, both strongly predicting individuals'  
544 chances of inheriting the queen position and seemingly moderating social disruption  
545 following queen loss. Despite this, we did not find evidence that groups were less able to  
546 mitigate within-colony conflict when the strength of this cue was relatively weak. Groups  
547 with a poorly-resolved age ranking did not experience greater social disruption than ones  
548 in which the age gap between the oldest individuals was large. Moreover, the most  
549 queenlike individual on a given nest was not always the oldest, although in 90% of nests  
550 she was one of the two oldest individuals. Colonies were only maintained for a relatively  
551 short period of time in order to minimize any behavioral effects of the laboratory  
552 environment (Jandt et al. 2015), but as a result the age difference between individuals on  
553 any given colony was small. The influence of the gerontocratic convention identified here  
554 might be stronger on colonies that have undergone a larger number of brood cycles, with a  
555 larger range of ages therefore represented.

556

557 The very low level of group-level perturbation we observed following queen removal  
558 contrasts with results from Strassmann et al. (2014), who found significant increases in  
559 within-group conflict immediately following queen loss in mature nests outside the  
560 laboratory context. We consider three possible explanations for the discrepancy between  
561 these results and our own. First, colony size is known to predict conflict between dominant  
562 and subordinate individuals in *Polistes* (Cant et al 2006), and thus it may be that the early-  
563 stage nests we observed were too small to merit conflict over succession. Second, it is  
564 possible that some aspect of the lab context, such as *ad libitum* access to food, reduced  
565 the propensity of individuals to engage in conflict. Finally, the fact that we enforced a  
566 single-foundress context upon our colonies in question may have been a factor. From the  
567 perspective of an early-emerging worker, the absence of subordinate foundresses on the

568 nest may act as an indicator of particularly high within-nest relatedness, since in a  
569 monogynous colony the workers are guaranteed to be fully matrilineally related.

570

571 Intriguingly, we identified a strongly negative interaction between age and size in predicting  
572 the change in individuals' antennation rate following queen removal. Age was a weaker  
573 predictor of antennation rate increase for larger individuals, which might indicate that larger  
574 individuals were attempting to compete for the dominant position even while young.  
575 Despite this there was no meaningful influence of size upon ovarian development or Elo  
576 rating. This suggests that the gerontocratic convention operates effectively even in the  
577 face of physically large competitors, at least for the early-season colonies described here.  
578 One possibility is that such 'queue jumpers' might become more aggressive in their efforts  
579 later in the season when the indirect fitness benefits of cooperation have declined, as  
580 occurs in certain other reproductively plastic social insects such as bumble bees (Rottler-  
581 Hoermann et al. 2016).

582

583 Our results are consistent with the established notion that social hierarchies on queenright  
584 *P. dominula* colonies are age-based, with the oldest individuals being the most dominant  
585 (Pardi 1948; Theraulaz et al. 1990). The physiological bases of this age-based system  
586 have remained elusive, however. One established hypothesis is that gerontocracy in *P.*  
587 *dominula* reflects a physiological constraint, i.e. that younger individuals might have  
588 underdeveloped ovaries and so as a result are poorly positioned to transition to a  
589 reproductive role (Pardi 1948). Contrary to this, our data show that the positive relationship  
590 between workers' age and ovarian development is present only following queen removal.  
591 This is what we would expect to observe if gerontocracy is antecedent to, rather than a  
592 consequence of, variation in ovarian development. Moreover, while *P. dominula* expresses  
593 a positive relationship between age and reproductive dominance, this is not the case in all

594 *Polistes* species: several species are thought to express the reverse relationship, with  
595 younger individuals more likely to inherit the queen role, possibly due to ecological  
596 variables that affect future fitness payoffs (Tsuji and Tsuji 2005). The existence of age-  
597 based conventions acting in opposite directions in different *Polistes* species seems  
598 incongruent with the idea that ovarian development is limited by age in this genus, but is  
599 unsurprising if age acts as a predominantly arbitrary signifier of dominance.

600

601 The speed with which colonies appear to have generated replacement queens and the  
602 low-conflict nature of this transition indicate a remarkably robust and efficient conflict  
603 resolution mechanism operating within *P. dominula* colonies. Such robust mechanisms for  
604 the mitigation and resolution of intragroup conflict are essential components in the long-  
605 term maintenance of animal social groups (Aureli and de Waal 2000). Despite this, the  
606 nature of these mechanisms has been difficult to elucidate, especially outside of the non-  
607 human primates (Aureli et al. 2002). It has proven particularly challenging to separate  
608 different aspects of the phenotype in order to identify the specific cues that matter for  
609 reproductive succession in complex vertebrate societies within which measuring behavior  
610 and physiology is difficult and time consuming. Our ability to generate detailed phenotypic  
611 data for a large number of individuals over a short period of time was key in revealing the  
612 capacity for gerontocracy to act as a robust conflict-resolution system in a society with very  
613 high reproductive skew. Though labor-intensive to produce even in an invertebrate system,  
614 such in-depth data will be necessary to advance our understanding of social conflict  
615 resolution.

616

617 Understanding how social cohesion is maintained in the face of within-group conflicts is a  
618 key project of social evolutionary biology, and social insects are among our best models  
619 for testing hypotheses in this field. The results presented here represent the most in-depth



620 analysis of reproductive succession in a cooperatively breeding social insect to date. Our  
621 results confirm that the process of queen succession can occur with minimal social  
622 disruption, and we provide strong evidence in favor of the hypothesis that reproductive  
623 succession can be successfully dictated by a convention rather than by violent and costly  
624 within-group conflict. Our findings provide novel insights into the mechanisms by which  
625 social groups can mitigate potential sources of internal conflict.

626

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628

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631

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633

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638

639 Data accessibility: Analyses reported in this article can be reproduced using the data  
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799

800 **Tables and figures**

801

802 **Table 1.** Review of published literature examining the effects of queen loss in post-  
803 emergence *Polistes* colonies. *Fighting*: evidence of fighting between potential successors.  
804 *Colony disruption*: evidence of significant disruption to colony functioning or growth  
805 following queen loss. *Conventional cues*: evidence that traits other than size or fighting  
806 ability predict queen succession. *Lack of colony disruption*: evidence that colony  
807 functioning or growth are unaffected by queen loss. Parentheses indicate partial evidence.  
808 Fuller details are provided in supplementary table **S13**.

| Paper                   | Species                               | Data type: Behavioural (B)/<br>Genetic (G)/ Ovarian (O)/<br>Chemical (C) | Subordinate foundresses<br>present? | Evidence for conflict |                      | Evidence for convention |                              |
|-------------------------|---------------------------------------|--|-------------------------------------|-----------------------|----------------------|-------------------------|------------------------------|
|                         |                                       |  |                                     | Fighting              | Colony<br>disruption | Conventional<br>cues    | Lack of colony<br>disruption |
| Hughes et al 1987       | <i>Polistes annularis</i>             | B  | Both considered                     | Y                     |                      |                         | Y                            |
| West-Eberhard 1969      | <i>Polistes canadensis</i>            | O  | N                                   |                       | Y                    |                         |                              |
| Miyano 1986             | <i>Polistes chinensis antennalis</i>  | B  | N                                   | Y                     | Y                    | Y                       |                              |
| Dapporto et al 2005     | <i>Polistes dominula</i> <sup>1</sup> | CO   |                                     |                       |                      |                         |                              |
| Strassman et al 2004    | <i>Polistes dominula</i> <sup>1</sup> | BO   | N                                   | (Y)                   | Y                    |                         |                              |
| Monnin et al 2009       | <i>Polistes dominula</i> <sup>1</sup> | BO   | Y                                   |                       |                      |                         | Y                            |
| Tibbetts & Huang 2010   | <i>Polistes dominula</i> <sup>1</sup> | BC   | N                                   | (Y)                   |                      |                         |                              |
| Strassman & Meyer 1983  | <i>Polistes exclamans</i>             | B  | N                                   | (Y)                   |                      | Y                       |                              |
| West-Eberhard 1969      | <i>Polistes fuscatus</i>              | O  | N                                   |                       | Y                    |                         |                              |
| Reeve & Gamboa 1983     | <i>Polistes fuscatus</i>              | B  | N                                   |                       | (Y)                  |                         |                              |
| Page et al 2002         | <i>Polistes fuscatus</i>              | BO   | Not reported                        |                       |                      |                         |                              |
| Paroli 1948             | <i>Polistes gallicus</i> <sup>2</sup> | BO   | Both considered                     |                       |                      | Y                       |                              |
| Hughes & Strassman 1988 | <i>Polistes instabilis</i>            | BO   | N                                   |                       |                      | Y                       |                              |
| Miyano 1991             | <i>Polistes jadvigae</i>              | BO   | N                                   | Y                     | Y                    | Y                       | Y                            |
| Metcalf & Whitt 1977    | <i>Polistes metricus</i>              | G  | Both considered                     |                       |                      |                         | (Y)                          |
| Metcalf 1980            | <i>Polistes metricus</i>              | BG   | N                                   |                       |                      |                         |                              |
| Hagiwara & Kojima 2002  | <i>Polistes nipponensis</i>           | B  | N                                   |                       |                      |                         |                              |
| Metcalf 1980            | <i>Polistes variatus</i>              | BG   | N                                   |                       |                      |                         |                              |

<sup>1</sup>*P. dominula* is frequently referred to as *P. dominulus*

<sup>2</sup>*P. gallicus* and *P. dominula* are difficult to distinguish and were not widely recognised as separate until ~1980; it is thus possible that Paroli's (1948) data actually derive from *P. dominula*

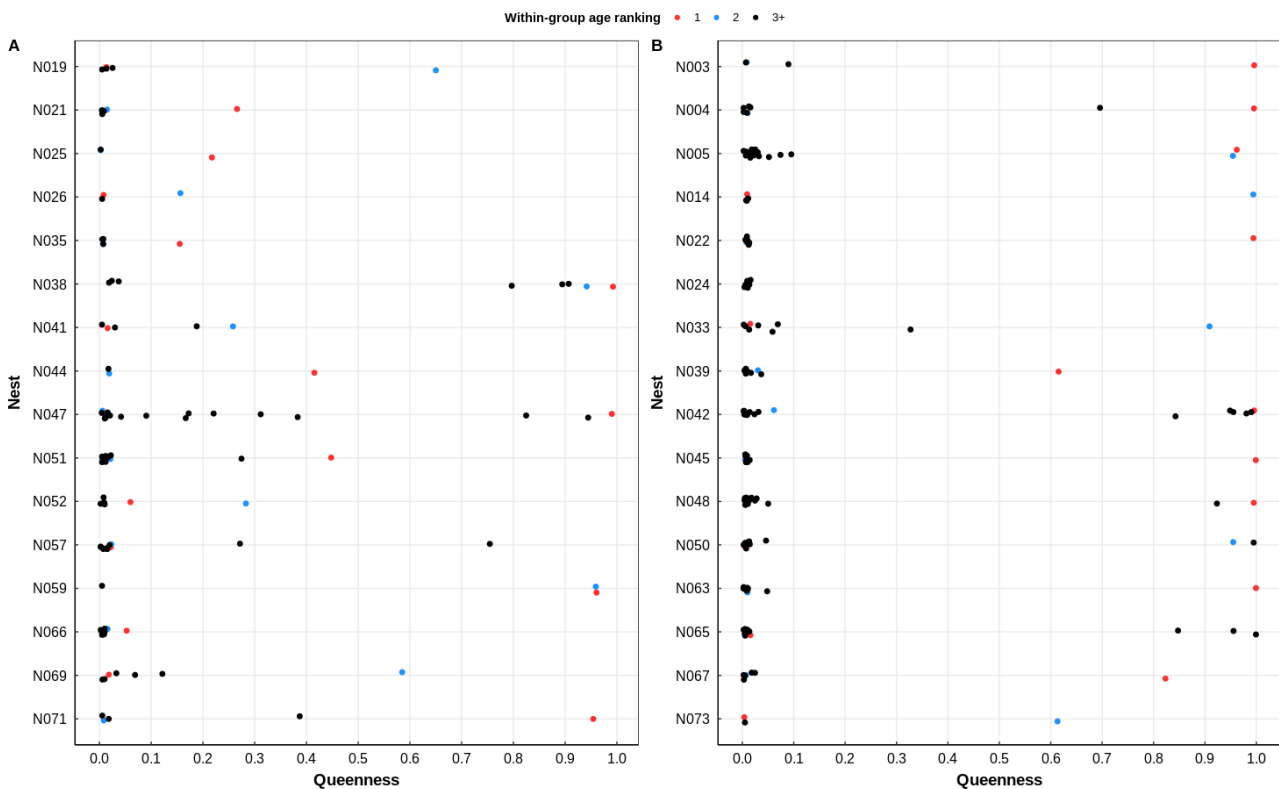
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814 **Table 2.** Summary of predictions for Hypotheses 1, 3 & 4.

|   | Convention  | Conflict  |
|---|---|---|
| <b>H1: Cues predicting queen succession</b>                       | Cues not directly related to competitive ability (e.g. age) | Correlates of fighting ability or resource holding potential (e.g. body size, aggression) |
| <b>H3: Extent of social disruption following queen removal</b>    | Minimal   | Substantial   |
| <b>H4: Distribution of behavioural responses to queen removal</b> | Restricted to one or a few individuals                      | More evenly distributed among multiple competing individuals                              |

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817 **Figure 1.** Queenness estimates for individuals from (A) QR3 and (B) QR12 nests. Colored

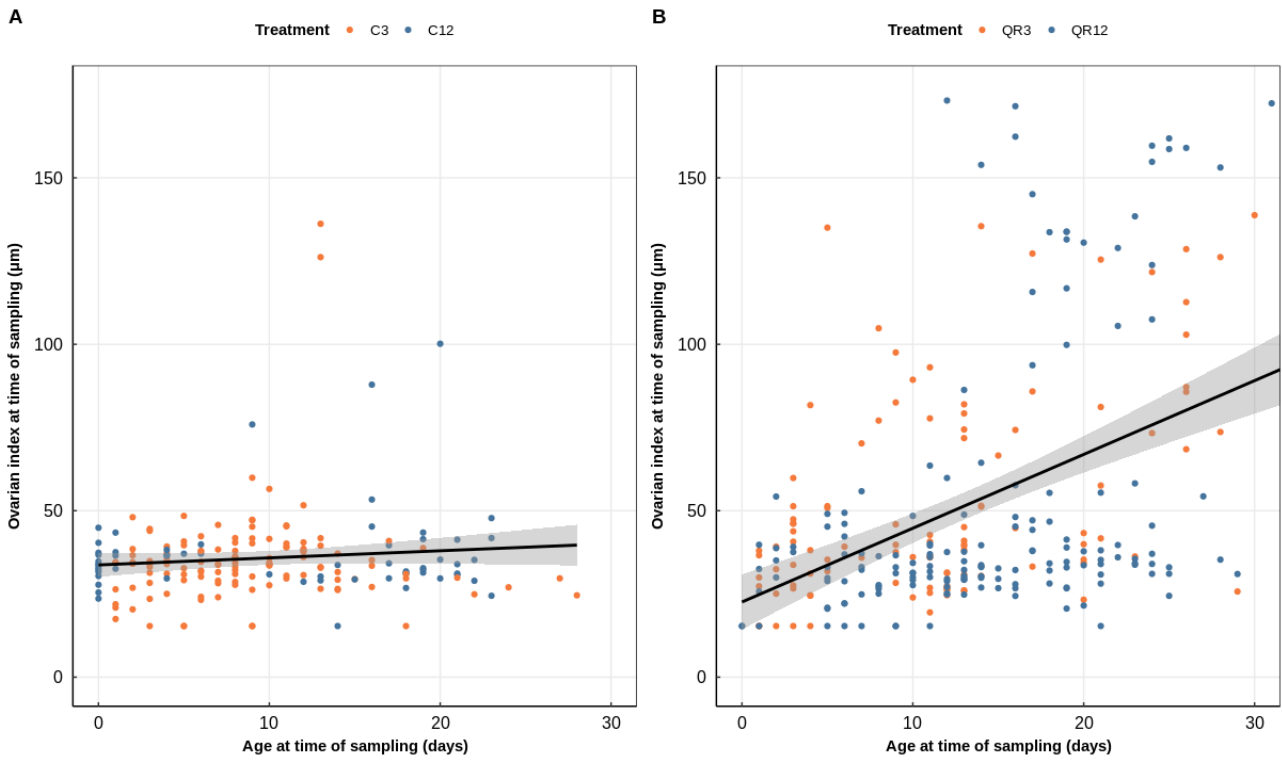


818 points represent the oldest (red) and second-oldest (blue) individuals from each nest.

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824 **Figure 2.** Ovarian index plotted against age for workers from (A) control and (B) queen



825 removal colonies. Trend lines with 95% confidence intervals are shown in black.

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