Title: *Polistes japonicus* (Hymenoptera: Vespidae) queens monopolize ovipositing but are not the most active aggressor in dominant–subordinate interactions

Running title: Dominant–subordinate relationship between the queen wasp and its workers

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Abstract In order to elucidate the dominant–subordinate relationship between the foundress and workers, five colonies of the paper wasp *Polistes japonicus* were observed in a netted and covered cage located outdoors. The number of workers in each colony ranged from four to eight. Workers were divided into first and second broods. Abdominal wagging and ovipositing were performed almost exclusively by the foundress throughout colony development. However, an analysis of aggressive encounters indicated that although the foundress hardly received dominance behaviors (aggression) from workers, it lacked either partially or completely the following characteristics of the queen that are usually seen in paper-wasp colonies with independent-founding queens (except in one colony that produced no second brood): the queen being socially dominant over any worker (The queen had more wins than losses in one-on-one dominance contests with any worker), exhibiting the highest frequency of dominance behaviors, and directing dominance behaviors primarily toward the socially most-dominant worker. In particular, during the mixed-brood period (when all first- and second-brood workers were present on the nest) the foundress hardly exhibited dominance behaviors toward socially dominant workers (mainly second brood) but frequently directed dominance behaviors toward socially subordinate workers (mainly first brood). The foundress disappeared in two colonies before the reproductives emerged; in these colonies the socially most-dominant worker inherited the colony and laid many eggs. The frequency of abdominal wagging by these two foundresses decreased during colony development, while it did not in the other colonies. This suggests that abdominal wagging provides information about the vigor of the performer. The superseder was socially dominant over all other workers, but spent little time wagging its abdomen and allowed some workers to lay eggs.

Introduction

Social insects that reside in colonies with a small number of workers, including *Polistes* species, are usually characterized by a linear dominance hierarchy among the individuals on the nest, including the queen (Pardi, 1948; Breed and Gamboa, 1977; Reeve, 1991; Monnin and Peeters, 1999; Jeanne, 2003). The dominance hierarchy is maintained by frequent aggressive encounters in which individuals exhibit dominance behaviors over others, such as rushing, mounting, or biting with the mandibles; this hierarchy is called a social dominance hierarchy, while a dominance hierarchy in reproduction is referred to as a reproductive dominance hierarchy hereafter (Röseler, 1991). The social dominance hierarchy usually functions as a mechanism for determining who lays eggs, and the queen is usually ranked highest (Röseler, 1991). When the queen disappears it is superseded by the highest-ranked worker in the social dominance hierarchy (Pardi, 1948; Jeanne, 1972; Litte, 1977; West-Eberhard, 1978, 1981; Yamane, 1986; Bridge and Field, 2007). Dominance behaviors are exhibited more often by more-dominant individuals (Wilson, 1974; Spradbery, 1991) and are directed primarily toward individuals ranked immediately below them (Downing and Jeanne, 1985; Miyano, 1986; Reeve and Gamboa, 1987; Hughes and Strassmann, 1988; Cant et al., 2006). In addition, the frequency of abdominal wagging is higher among more-dominant individuals in many species, but is limited to queens in others (e.g., Reeve, 1991; O'Donnell, 1998; Brillet et al., 1999; Cummings et al., 1999; Molina and O'Donnell, 2009). Either no or only certain workers oviposit in the

presence of the queen in species with a social dominance hierarchy (Reeve, 1991; Röseler, 1991), and eggs laid by workers are often eaten by the queen or by those workers who support the queen (Wenseleers and Ratnieks, 2006; Peeters and Liebig, 2009).

However, an exception to the above situation is found in *Ropalidia marginata*, where the queen is docile and is not ranked highest in the social dominance hierarchy, although it monopolizes ovipositing (Gadagkar, 1980; Chandrashekara and Gadagkar, 1991; Gadagkar, 2001, 2009). Pheromones may be used to maintain the reproductive dominance hierarchy (Bhadra et al., 2007; Gadagkar, 2009). This exception suggests that various mechanisms are used to establish the reproductive dominance hierarchy. Moreover, it is interesting to examine the mechanism that is used to establish the reproductive dominance hierarchy after the foundress has disappeared. In *R. marginata*, as soon as the foundress disappears, one worker (not the individual ranked highest in the social dominance hierarchy) becomes extremely aggressive in order to become the superseder (Premnath et al., 1996; Gadagkar, 2001; Sumana and Gadagkar, 2001). The extreme aggression disappears within 1 month, after which the relationship between the superseder and other workers becomes similar to that between the foundress and workers (Gadagkar, 2001).

Polistes japonicus Saussure (Hymenoptera: Vespidae), a primitively eusocial paper wasp, is a temperate species whose colonies usually have less than 10 workers (Matsuura, 1995). These wasps are rare in Japan. Our research group observed colonies reared in a netted cage during 2002–2004, and previously confirmed that there is a linear dominance hierarchy among workers (Ishikawa et al., 2010). However, we have not previously reported on the dominant–subordinate relationship between the queen and its workers.

By analyzing the data obtained from the above observations and preliminary observations made in 2000, the present study has elucidated whether the foundress establishes the highest rank in the social and reproductive dominance hierarchies, and which wasp supersedes the colony when the foundress disappears and how it does this.

Materials and methods

Colonies and rearing conditions

Five colonies [designated A–D and P (colony for preliminary observations)] were reared for observation in a field cage (3.2 × 3.4 × 1.9–3.7 m) with a roof on the Mie University campus in Tsu, Mie, Japan over 4 years: 2000 (colony P), 2002 (colonies A and B), 2003 (colony C), and 2004 (colony D). One wall of the cage was glazed and the remaining three walls were made of a vinyl chloride net with a 1 × 1 mm mesh, and the roof was constructed from corrugated clear-plastic sheets. The cage contained live trees (including *Neolitsea sericea*, *Prunus jamasakura*, and *Dendropanax trifidus*) that provided nesting sites and nest materials for the wasps. Foundresses were collected in April of each year in Tsu, reared for less than 2 weeks in a small cage (11 × 15 × 8 cm) containing a honey solution as food, and released into the field cage early in May. One exception was in 2000, when a natural nest with two workers that had just emerged was placed in a cage. A few foundresses were released at the same time, but all colonies were singly founded. Wasps were provided with flesh, honey, and water placed on dishes on a table in the cage that were renewed every day.

116 The flesh usually included lepidopteran larvae, adult cicadas (particularly their thoraxes),
117 and honeybee larvae or pupae, most of which were split in half.
118 Workers were divided into the first and second broods, each of which comprised
119 two to five individuals. The first brood comprised workers that emerged first or within 8
120 days of the first emerging day (Fig. 1). The second brood comprised workers that emerged
121 more than 8 days after the first emerging day of workers and before male reproductives had
122 emerged. The first brood was provisioned only by the foundress, while many individuals of
123 the second brood were provisioned by both the foundress and workers. Data were
124 analyzed separately for three periods (Fig. 1): (1) the first-brood period, when all first-brood
125 workers were present on the nest but second-brood workers were not, (2) the mixed-brood
126 period, when all individuals comprising the first and second broods were present on the
127 nest, and (3) the transition period between the first- and mixed-brood periods. Colony P
128 was not observed during the first-brood and transition periods, and colony B produced no
129 second brood. The foundress disappeared in colonies A and D just after the first-brood
130 period and during the mixed-brood period, respectively, but observations were continued to
131 determine who inherited the colony and the relationship between the superseder and the
132 other workers.
133
134
135 *Observing behaviors and determining the social dominance hierarchy*
136
137 Workers were marked by attaching small pieces of differently colored photographic paper
138 labeled with numbers to the mesonotum with clear nail polish. The paper was kept as

Fig 1

139 light as possible by removing its backing chartaceous part. The behaviors of individual
140 wasps on the nest were recorded by a video camera and recorder, except in 2000 when the
141 behaviors were observed directly by eye. Video recording usually started at noon and
142 finished at about 1800 hours; observations by eye were made from 1100 to 1400 hours.
143 The recordings and observations were made usually every few days before male emergence
144 in August. The recording/observation durations for the first-brood, transition, and
145 mixed-brood periods were 0, 0, and 15 h for colony P; 48, 66, and 24 h for colony A; 42, 0,
146 and 0 h for colony B; 12, 36, and 60 h for colony C; and 12, 6, and 72 h for colony D.
147 Dominance behavior was defined as aggressive contact of a dominant individual
148 with a subordinate individual, and was divided into two types: (1) an individual rushed at
149 another individual and pushed against it with its mandibles (called rushing hereafter) and
150 (2) an individual bit part of the body of another individual with its mandible (called biting
151 hereafter). A wasp approached another wasp more quickly when rushing than when
152 biting, and it did not bite after rushing. Episodes where an individual adopted a posture
153 for rushing but did not actually rush, or rushed but did not contact another wasp were not
154 counted as dominance behavior. The rank of the queen in the social dominance hierarchy
155 (this rank is called social rank hereafter) was determined based on the frequencies of
156 episodes of dominance behaviors for all possible pairs with workers (Ishikawa et al., 2010).
157 In each pair, the individual that directed dominance behaviors toward the other with a
158 higher frequency was considered to be ranked higher than the other of the pair. Since
159 rushing was rare (usually <3% for both the queen and workers), analysis results based on
160 the frequency of rushing and biting combined and those based on the frequency of biting
161 only were almost the same, and so only the former is presented here. The social ranks of

individual workers were obtained from the analysis of Ishikawa et al. (2010).

We also examined the frequencies and durations of abdominal wagging and lateral vibration of the abdomen (Reeve, 1991), and the frequency of ovipositing to confirm the reproductive dominance of the queen. Other well-known behavioral characteristics of the dominant individuals, such as buzzing its wings, stinging the subordinate, and rising on its legs above the subordinate (Wilson, 1974; Spradbery, 1991), were not observed. Neither queens nor workers struck cell walls with their antenna vigorously (antennal drumming) before, during, or after feeding food to larvae or checking cells (Jeanne, 2009).

Statistical analysis

All statistical analyses were performed using the software package NCSS 2007 (NCSS LLC, Kaysville, Utah). Regarding the characteristics of dominance behavior exhibited by the queen (see the Introduction), we statistically analyzed the following three points (hereafter called the three points for checking the function of dominance behavior): (1) whether the frequency of dominance behaviors was higher for those directed by the queen toward the highest-ranked worker than vice versa, (2) whether the frequency of dominance behaviors of the queen was higher for such behaviors directed toward the highest-ranked worker than for those directed toward the worker (usually the second ranked) who received dominance behaviors most frequently from the queen among all workers other than the highest-ranked worker, and (3) whether the total frequency of dominance behaviors was higher for the queen than for the worker (usually ranked the highest) who exhibited dominance behaviors most frequently among all workers.

186 Points 1 and 2 were determined by binomial tests. Frequencies for the individual
187 observation days during the target period were summed and used in binomial tests because
188 the trend was similar on different observation days. Point 3 was determined both by
189 comparing the hourly frequencies for individual observation days using paired *t*-tests and
190 by using a mixed model analysis for repeated measures. The mixed models were applied
191 to each of first- and mixed-brood periods, and included time as a covariate, colony as a
192 random factor, and the performer (queen or worker) as a fixed factor. Moreover, when the
193 highest-ranked worker never received dominance behaviors from the queen, we calculated
194 the probability that this situation would occur assuming that the queen directed all
195 dominance behaviors toward all workers at random.
196 The mean duration of one episode of abdominal wagging and its hourly frequency
197 were analyzed using the *t*-test (Aspin-Welch *t*-test when homogeneity of variance was not
198 satisfied), randomization test (for data including many zero frequencies), or ANOVA (with
199 Tukey-Kramer multiple comparison if necessary). The duration and frequency of
200 abdominal wagging by the queen were compared between different periods and between
201 different colonies. Differences in the hourly frequency between the queen and workers,
202 between the foundress and its superseder, and between before and after becoming a
203 superseder were also analyzed. In comparing between the queen and workers, many
204 workers never performed abdominal wagging during a given period, and such workers were
205 excluded from the analysis.
206 Regarding the hourly frequency of dominance behaviors and abdominal wagging
207 performed by the foundress, the effect of time (the number of days after the last worker of
208 the first brood had emerged) was determined by performing a mixed-model analysis for

repeated measures with time as a covariate and whether or not queen replacement occurred later as a fixed factor. When an effect of time was found, we calculated the correlation between the frequency of the event and the time for each foundress.

Results

Basic characteristics of the colonies

Totals of 49, 29, 79, 39, and 47 cells were constructed in colonies A, B, C, D, and P, respectively, with 3, 4, 4, 4, and 4 first-brood workers, and 4, 0, 3, 2, and 4 second-brood workers emerging. The fourth- and third-emerging workers in colonies A and P were missing just after emergence, resulting in seven and eight workers in those colonies, respectively. The foundress disappeared from colonies A and D on July 15 [this was incorrectly reported as July 9 in Ishikawa et al. (2010)] and August 12, respectively. The highest-ranked worker in the social dominance hierarchy inherited the colony in these two colonies (Table 1).

Table1

Ovipositing

Totals of 22, 4, 21, 9, and 1 ovipositions were observed in the presence of the foundress in colonies A, B, C, D, and P, respectively. All but one of these ovipositions were performed by

232 the foundress; the exception was performed by the third-ranked worker in colony C on
233 August 5, late during the mixed-brood period. This egg disappeared 3 days later, probably
234 due to it being eaten by the foundress or a worker. These observations indicate that the
235 foundress retained its highest rank in the reproductive dominance hierarchy and restricted
236 ovipositing by workers. After the foundress disappeared in colonies A and D, the
237 superseder did not monopolize ovipositing. Totals of 11 and 3 ovipositions were observed
238 in colonies A and D, respectively, of which 7 and 2 ovipositions were performed by the
239 superseder. In colony A, the second-, third-, and sixth-ranked workers laid two, one, and
240 one eggs, respectively, while in colony D the fourth-ranked worker laid one egg.
241
242
243 *Aggressive encounters*
244
245 During the first-brood period, the foundresses hardly received any dominance behaviors
246 from workers and directed dominance behaviors toward individual workers more
247 frequently than receiving dominance behaviors from them in colonies A–C (Table 2, Fig. 2),
248 suggesting that the foundress was ranked highest in the social dominance hierarchy.
249 However, the foundress in colony D evened the score against the highest-ranked worker
250 (one dominance behavior directed and one received), and had no aggressive encounters with
251 two other workers. In addition, the foundress in colony C directed dominance behavior
252 only twice toward the highest-ranked worker (Table 2), and the foundresses in colonies A, C,
253 and D did not direct dominance behavior significantly more often toward the
254 highest-ranked worker than toward any other worker (Table 2), and the hourly frequency of

Table 2
Fig 2

255 dominance behaviors exhibited by these foundresses was not the highest among individuals
256 on the nests (Table 2, Fig. 3). These results suggest that the foundress did not use
257 dominance behavior to retain its highest rank in the reproductive dominance hierarchy,
258 particularly in colonies C and D, even when it did actually retain it. One exception was
259 colony B, in which the three points for checking the function of dominance behavior were
260 satisfied (Table 2) — similar results were obtained even when colony B was analyzed after
261 division into early (early and mid-July) and late (late-July) stages (data not presented).
262 However, when mixed-model analysis for repeated measures was applied to the four
263 colonies combined, the hourly frequency of dominance behaviors was not significantly
264 higher for the foundresses than for any worker ($F_{1,6.2} = 0.002$, $P=0.966$).
265 The foundress hardly received dominance behaviors from any worker during the
266 transition and mixed-brood periods, as was the case during the first-brood period (Fig. 2;
267 values for the transition period are not presented because they were similar to those for the
268 mixed-brood period). However, the foundress hardly directed dominance behavior toward
269 dominant workers (Table 2, Fig. 2), which mainly composed the second brood; the
270 highest-ranked worker never received dominance behaviors from the foundress, and the
271 probability for this is <0.0001 assuming that the foundress directs dominance behaviors
272 toward workers at random. The foundress directed most of its dominance behaviors
273 toward subordinate workers, which mainly composed the first brood. Consequently, the
274 social rank of the queen was unclear. Moreover, the hourly frequency of dominance
275 behaviors directed toward other colony members was not significantly higher for the
276 foundress than for any worker (Fig. 3; mixed-model analysis for repeated measures: $F_{1, 3.7} =$
277 1.266, $P= 0.328$). Analysis of each colony also showed that this was true in colonies C and

Fig 3

D (Table 2), but not in colony P.

The hourly frequency of dominance behaviors that the foundress directed toward other colony members did not change with colony development, and was not influenced by whether or not queen replacement occurred later (mixed-model analysis for repeated measures: time*queen replacement, $F_{1,36.6} = 0.122$, $P=0.729$; time, $F_{1,32.2}= 0.835$, $P= 0.368$; queen replacement, $F_{1,1.5} = 4.599$, $P= 0.203$).

Fig 4

After the foundress disappeared, the dominant–subordinate relationship between the superseder and the other workers was typical of that for paper wasps (Figs. 4, 5): the three points for checking the function of dominance behavior were satisfied (Table 2), which suggests that the typical social dominance hierarchy was established.

Fig 5

Abdominal wagging and lateral vibration

The foundress often wagged its abdomen laterally (abdominal wagging) while walking from one cell to another when checking cells, and often simultaneously rubbed the ventral side of the posterior part of its abdomen on cell walls. The foundress always performed abdominal wagging while walking, and did not direct this toward any specific cells. It usually moved its abdomen with several strokes in a continuous episode, and during or after doing it the performer often expanded and contracted the abdomen from one to a few times. Abdominal wagging did not produce any sound or cause nest mates to change their behaviors, at least immediately after the wagging was performed. The duration of an episode of abdominal wagging did not vary between the first-brood, transition, and

mixed-brood periods except in colony C (Table 3), in which the duration increased during colony development, but it did vary significantly among different foundresses (ANOVA after log transformation for the first-brood period, $F_{3, 690} = 40.8$, $P < 0.0001$). The duration was significantly shorter for the superseder than for the original foundress in colonies A and D (Aspin-Welch t-test, $P = 0.001$ and $P < 0.001$, respectively). The duration of an episode of abdominal wagging for the highest-ranked worker on nests with the foundress present was 2 s ($n = 1$) for colony B, 14.7 ± 5.9 s (mean ± SE) ($n = 6$) for colony C (mixed-brood period), and 5.7 ± 2.8 s ($n = 7$) for colony D (mixed-brood period) (the highest-ranked worker did not wag its abdomen in colony A); it did not differ significantly from that for the foundresses in colonies C and D (Aspin-Welch t-test, $P = 0.564$ and $P = 0.055$, respectively).

Table3

The hourly frequency of abdominal wagging was far higher for the foundress than for the workers, with many workers never performing abdominal wagging during the first-brood period (Table 4; values for the transition period are not presented because they were similar to those for the first-brood period). During the early stage of colony development (for 7 days after emergence of the last worker of the first brood), the hourly frequency for the foundress was higher for colonies A and D than for colonies B and C (Fig. 6), although the difference was only significant for colony A (Tukey-Kramer multiple-comparison test, $P < 0.05$). This may be related to whether or not queen replacement occurs. The hourly frequency of abdominal wagging by the foundress was influenced by whether or not queen replacement occurred (mixed-model analysis for repeated measures: time*queen replacement, $F_{1,37.0} = 12.2$, $P = 0.001$). It decreased during colony development in colonies with queen replacement (colonies A and D) (Fig. 6; $r = -0.853$, $P < 0.001$ for colony A; $r = -0.865$, $P = 0.003$ for colony D), but not in colonies

Table4

Fig 6

without queen replacement (colonies B and C) (r = 0.742, P = 0.091 for colony B; r = 270, P = 0.350 for colony D). The hourly frequency for the highest-ranked worker (superseder) increased after the foundress disappeared [from 0/h (n = 6) to 0.47 ± 0.20/h (n = 6), randomization test, P = 0.018 for colony A; from 0.18 ± 0.10/h (n = 6) to 0.47 ± 0.08/h (n = 5), t-test, P = 0.062 for colony D], but it was far lower than that for the foundress that disappeared (compared with the value for the first-brood period: Aspin-Welch t-test, P < 0.001 for colony A; t-test, P < 0.0001 for colony D). After the foundress disappeared, the hourly frequency increased for all other workers in colony D, and consequently their hourly frequencies were similar to that of the superseder (Table 5). On the other hand, abdominal wagging was performed more often by the superseder than by other workers in colony A.

Table5

Lateral vibration was exhibited only by the foundresses in colonies B and C—once and three times, respectively, which corresponded to 0.024/h and 0.028/h for the entire observation period. When performing lateral vibration, the foundress remained steady on the lower portion of the outer walls of the comb with its head up and vibrated the abdomen laterally, rubbing the anterior to middle part of the ventral side on the walls so strongly that the nest shook and a sound was made. The foundress moved the abdomen faster in lateral vibration than in abdominal wagging. Nest mates did not change their behaviors during or immediately after lateral vibration. The duration of an episode of lateral vibration was 64 s for colony B (first-brood period), and 43, 84, and 66 s for colony C (the first duration corresponds to the first-brood period, the latter two to the transition period). During each episode, the foundress performed vibration continuously. Workers (including the superseder) never performed lateral vibration.

Discussion

The foundress retained the highest rank in the reproductive dominance hierarchy throughout colony development in *P. japonicus*. However, the foundress was not ranked highest in the social dominance hierarchy established through aggressive encounters during the transition and mixed-brood periods. To our best knowledge, this is the first report in the genus *Polistes* to indicate that the egg-layer is not the highest-ranked individual in the social dominance hierarchy, and only the second (the first being in *R. marginata*) in Polistinae with independent-founding queens (Röseler, 1991; Gadagkar, 2001; Jeanne, 2003). The foundress in one of the four colonies did not retain the highest rank, as was the case during the transition and mixed-brood periods, and the three points for checking the function of dominance behavior were often not satisfied during the first-brood period (except in colony B, which did not have a second brood), which suggests that the typical social dominance hierarchy was not perfectly established.

A candidate alternative mechanism for maintaining the reproductive dominance hierarchy is abdominal wagging. This is supported by the frequency of abdominal wagging decreasing during colony development in colonies A and D, in which the foundress disappeared. Abdominal wagging may function as an honest signal of the vigor of the performer (Keller and Nonacs, 1993; Sledge et al., 2001; Strauss et al., 2008, Peeters and Liebig, 2009). However, it should be noted that the frequency of abdominal wagging by the foundress in colony C— in which queen replacement did not occur—is similar to that

370 during the mixed-brood period for the foundress in colony D (Fig. 6), and that early during
371 the observation period the frequency of abdominal wagging by the foundress was higher in
372 colonies A and D than in colonies B and C. These observations suggest that the frequency
373 of wagging can be used as an indicator of relative temporal changes in the vigor of the
374 foundress, but not as an indicator of its absolute vigor. Alternatively, workers may be able
375 to detect the pheromones that may be released while the foundress is wagging its abdomen.
376 In *R. marginata* the foundress uses mechanisms other than aggressive encounters to retain
377 the highest rank in the reproductive dominance hierarchy; a promising candidate
378 mechanism is via pheromones rubbed on the cell wall (Bhadra et al., 2007; Gadagkar, 2009).
379 The same may be true in *P. japonicus* because we found that the foundress often rubbed its
380 abdominal tip on cell walls while wagging the abdomen. Jeanne and colleagues (Jeanne,
381 2009; Suryanarayanan et al., 2011) recently proposed that the frequency of vibrations
382 induced by antennal drumming determines whether larvae will become workers or
383 potential queens. Whether abdominal wagging of *P. japonicus* has such a function was not
384 determined in the present study.
385 The highest-ranked worker inherited the colony when the foundress disappeared,
386 which suggests that the social dominance hierarchy determines the reproduction sequence
387 among workers (Field and Cant, 2006; Field et al., 2006). As a result, socially dominant
388 workers are expected to be lazy in order to save their vigor for possible future reproduction
389 (Field and Cant, 2006; Field et al., 2006; Molina and O'Donnell, 2009); such laziness of
390 dominant workers has actually been found in *P. japonicus* (Ishikawa Y., unpublished).
391 Interestingly, the frequency of abdominal wagging by the superseder was far lower than
392 that by the original foundress even though the superseder increased its frequency of

393 abdominal wagging after it had inherited the colony. This may be associated with a
394 failure to constrain the ovipositing of other workers, which provides circumstantial
395 evidence that abdominal wagging functions as a mechanism for determining the egg-layer.
396 However, further investigation (including of the physiological status of the supersedes) is
397 required to determine why the frequency of abdomen wagging by the superseder was so
398 low.

399 The dominance hierarchy of *P. japonicus* resembles that of *R. marginata* in that
400 the foundress retains the highest rank in the reproductive dominance hierarchy using
401 mechanisms other than aggressive encounters, despite there being a small number of
402 workers. However, the two species differ in two important ways. First, the social
403 dominance hierarchy among workers functions as a mechanism for selecting the superseder
404 candidate in *P. japonicus* but not in *R. marginata* (Chandrashekara and Gadagkar, 1992;
405 Gadagkar, 2001); however, it should be noted that the social dominance hierarchy for *R.*
406 *marginata* performs such a function after the foundress has disappeared (see the
407 Introduction). Second, the foundress directs most of its dominance behaviors toward
408 subordinate workers in *P. japonicus*, which is not seen in *R. marginata* (Chandrashekara
409 and Gadagkar, 1991; Gadagkar, 2001).

410 A reasonable explanation for the first difference described above is that *R.*
411 *marginata* is a tropical species, and foundresses do not need to overwinter (Gadagkar,
412 2001). This would allow the foundress to retain its vigor for longer, meaning that workers
413 have to wait longer for the foundress to lose its vigor sufficiently for them to become
414 superseders. Moreover, a worker is most likely to become a new queen after the colony is
415 suddenly attacked and broken by their major enemy, the hornet *Vespa tropica* (Gadagkar,

2001). In this case the worker founds a new nest with a few individuals from the same nest, rather than superseding the colony. Under such conditions the dominant workers cannot predict when the foundress will disappear, and hence they will obtain more fitness returns by working harmoniously for a colony rather than by competing for a higher rank and being lazy in order to save their vigor for possible future reproduction. Therefore, dominance behavior is considered to promote effective foraging (Bruyndonckx et al., 2006; Lamba et al., 2008) rather than to determine the social rank.

In addition to the unique queen–worker relationship discovered in this study, *P. japonicus* has another unique characteristic—the dominance hierarchy among workers changes from older dominants to younger dominants during colony development (Ishikawa et al., 2010). The social structures of many eusocial insect species have recently been reported, but far more species remain to be investigated. Our investigations of *P. japonicus* suggest that there are greater variations in social structures than had been thought previously.

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552 **Table 1** Results of dominance contests between wasps in colonies A and D before the

553 foundress disappeared

Colony	Individual ID	Individual ID[a]								Score[b]	Social rank
		Foundress	w1	w2	w3	w4	w5	w6	w7		
A	Foundress	-	69-0	81-0	29-1[c]	-	0-3	1-0	0-0	4-1	2
	w1	0-69	-	13-25	15-7	-	0-4	0-0	0-0	1-3	5
	w2	0-81	25-13	-	121-1	-	0-3	0-1	0-0	2-3	4
	w3	1-29	7-15	1-121	-	-	0-6	1-4	0-0	0-5	7
	w5[d]	3-0	4-0	3-0	6-0	-	-	107-0	92-0	6-0	1
	w6	0-1	0-0	1-0	4-1	-	0-107	-	57-0	3-2	3
	w7	0-0	0-0	0-0	0-0	-	0-92	0-57	-	0-2	6
D	Foundress	-	3-1	18-0	8-0	5-0	1-0	0-0	-	5-0	1=
	w1	1-3	-	11-0	10-0	3-2	0-0	0-1	-	3-2	3
	w2	0-18	0-11	-	1-1	0-3	0-1	0-1	-	0-5	7
	w3	0-8	0-10	1-1	-	0-4	0-0	0-3	-	0-4	6
	w4	0-5	2-3	3-0	4-0	-	0-10	0-1	-	2-4	5
	w5	0-1	0-0	1-0	0-0	10-0	-	2-28	-	2-2	4
	w6[d]	0-0	1-0	1-0	3-0	1-0	28-2	-	-	5-0	1=

554 [a]w, worker, where the number following "w" indicates the emergence order

555 [b]The numbers of subordinate (left) and dominant (right) individuals for a target wasp.

556 The social rank was determined based on the difference between the two numbers

557 [c]The queen directed dominance behavior toward worker 3 and received it from worker 3

558 with frequencies of 29 and 1, respectively, before the foundress disappeared. In this case

559 the foundress was considered dominant over the worker 3

560 [d]Superseder

561 **Table 2** Characteristics of dominance behavior of the queen

Type of queen	Period	Colony	Queen vs highest-ranked worker[a]	Worker with highest frequency of dominance behaviors[b]	Total frequency[c]
Foundress	First brood	A	Pos. (<0.0001, 49)	Pos. (0.461, 90)	Neg. (0.248, 6)
		B	Pos. (<0.0001, 54)	Pos. (0.021, 84)	Pos. (<0.001, 6)
		C	Pos. (0.5, 2)	Neg. (0.013, 14)	Neg. (0.323, 2)
		D	No (1.0, 2)	Neg. (0.375, 5)	Neg. (0.234, 2)
	Transition	A	Neg. (0.25, 3)	Neg. (<0.0001, 32)	Neg. (0.215, 6)
		C	No (1.0, 2)	Neg. (<0.0001, 50)	Pos. (0.623, 4)
		D	— (—, 0)	Neg. (<0.001, 11)	Pos. (—, 1)
	Mixed brood	C	— (—, 0)	Neg. (<0.0001, 40)	Pos. (0.444, 8)
		D	— (—, 0)	Neg. (<0.001, 14)	Neg. (0.822, 6)
		P	— (—, 0)	Neg. (<0.0001, 34)	Pos. (0.003, 5)
Superseder	Transition	A	Pos. (<0.0001, 84)	Pos. (0.118, 148)	Pos. (0.049, 2)
	Mixed brood	A	Pos. (<0.0001, 179)	Pos. (<0.0001, 261)	Pos. (0.018, 4)
		D	Pos. (<0.0001, 42)	Pos. (<0.01, 62)	Pos. (0.045, 5)

562 [a] Whether the frequency of dominance behaviors was higher for those directed by the queen
563 toward the highest-ranked worker than vice versa
564 [b] Whether the queen directed dominance behaviors primarily toward the highest-ranked worker.
565 Comparison of the frequencies of dominance behaviors that the foundress directed toward
566 the highest-ranked worker and toward the worker (usually the second ranked) who

567 received dominance behaviors most frequently from the foundress among all workers other
568 than the highest-ranked worker
569 [c] Whether the queen exhibited the highest frequency of dominance behaviors. The total
570 frequency of dominance behaviors was compared between those exhibited by the queen and
571 those exhibited by the worker that exhibited dominance behaviors most frequently among
572 all workers
573 Values in parentheses indicate the *P* value for statistical tests (left) and the sample size (right).
574 Binomial tests were performed for columns 4 and 5, and paired *t*-tests for column 6. Pos.,
575 positive trend; Neg., negative trend; No, no trend. *P* values of <0.05 associated with "Pos." and
576 "Neg." indicate significant positive and negative trends, respectively
577
578

579 **Table 3** Time (mean ± SE values) spent on each episode of abdominal wagging

Colony	Type of queen	Period	Time[a] (s)	*n*	Hours observed
A	Foundress	First brood	5.7 ± 0.4 a	369	48
	Foundress	Transition	5.3 ± 0.7 a	110	54
	Superseder	Transition	3.9 ± 1.0	8	12
	Superseder	Mixed brood	3.1 ± 0.6	9	24
B	Foundress	First brood	4.1 ± 0.3	184	42
	Foundress	First half	3.8 ± 0.5 a	63	18
	Foundress	Last half	4.2 ± 0.4 a	121	24
C	Foundress	First brood	8.1 ± 0.8 a	53	12
	Foundress	Transition	9.2 ± 0.6 ab	119	36
	Foundress	Mixed brood	11.0 ± 0.8 b	241	60
D	Foundress	First brood	13.4 ± 1.2 a	88	12
	Foundress	Transition	11.7 ± 2.3 a	39	6
	Foundress	Mixed brood	12.5 ± 1.5 a	108	42
	Superseder	Mixed brood	5.4 ± 1.4	14	30

580 [a] Results of comparison between different periods for each foundress; values followed by
581 the same letters are not significantly different [Aspin-Welch *t*-test with sequential
582 Bonferroni correction, $P > 0.05$ (Rice, 1989)]

583

584 **Table 4** Hourly frequencies (mean ± SE values) of abdominal wagging performed by
585 individuals on nests with the foundress present

Period	Colony	Individual ID	Hourly frequency[a] (/h)	*n* (days observed)
First brood	A	F	7.79 ± 1.09 a	6
		w1	0	6
		w2	0.15 ± 0.08 b	6
		w3	0.03 ± 0.03 b	6
	B	F	4.31 ± 0.57 a	6
		w1	0.03 ± 0.03 b	6
		w2	0	6
		w3	0.06 ± 0.04 b	6
		w4	0.10 ± 0.05 b	6
	C	F	4.50 ± 0.17 a	2
		w1	0.17 ± 0.17 b	2
		w2-4	0	2
	D	F	7.33 ± 0.33	2
		w1-4	0	2
Mixed brood	C	F	4.03 ± 0.63 a	8
		w1	0.04 ± 0.04 b	8
		w2	0.04 ± 0.04 b	8
		w3	0.18 ± 0.06 c	8
		w4	0.07 ± 0.03 e	8
		w5	0.77 ± 0.14 d	8
		w6	0.13 ± 0.06 b	8
		w7	0.04 ± 0.04 b	8
	D	F	2.75 ± 0.54 a	6
		w1	0.18 ± 0.16 ab	6
		w2	0.31 ± 0.27 ab	6
		w3	0.06 ± 0.04 b	6
		w4	0.11 ± 0.08 ab	6
		w5	0.08 ± 0.06 ab	6
		w6	0.18 ± 0.10 b	6

586 [a] Values followed by the same letter are not significantly different (Tukey-Kramer
587 multiple-comparison test or *t*-test was used for performers of abdominal wagging in the
588 same colony for a specific period after log transformation)

589 **Table 5** Hourly frequencies (mean ± SE values) of abdominal wagging performed by

590 individuals on nests after the foundress disappeared

Period	Colony	Individual ID	Hourly frequency[a] (/h)	*n* (days observed)
Transition and mixed-brood	A	Superseder (w5)	0.47 ± 0.20	6
		w1-3	0	6
		w6	0.06 ± 0.06	6
		w7	0	6
		w8	0	4
Mixed-brood	D	Superseder (w6)	0.47 ± 0.08	5
		w1	0.23 ± 0.15	5
		w2	0.03 ± 0.03	5
		w3	0.17 ± 0.09	5
		w4	0.57 ± 0.33	5
		w5	0.37 ± 0.13	5

591 [a] There was a significant difference between the superseder and w6 (randomization test,

592 $P = 0.05$) in colony A, but not among colony members in colony D (Tukey-Kramer

593 multiple-comparison test after log transformation)

Figure captions

Fig. 1 Schema for discriminating the first-brood, mixed-brood, and transition periods. The numbers of workers belonging to the first and second broods vary in the different colonies. Each line represents the development of each offspring: E, egg; L, larva; P, pupa; A, adult.

Fig. 2 Hourly frequencies (mean and SE values) of dominance behaviors that the foundress directed toward (above) and received from (below) individual workers. (a) First-brood period, (b) mixed-brood period. F, first-brood worker; S, second-brood worker

Fig. 3 Hourly frequencies (mean and SE values) of dominance behaviors exhibited by individuals on the nest with the foundress. (a) First-brood period, (b) mixed-brood period

Fig. 4 Hourly frequencies (mean and SE values) of dominance behaviors that the superseder directed toward (above) and received from (below) individual workers after the foundress disappeared. Note that the superseder received dominance behavior only once during the transition period, and never during the mixed-brood period. (a) Transition period, (b) mixed-brood period. F, first-brood worker; S, second-brood worker

Fig. 5 Hourly frequencies (mean and SE values) of dominance behaviors exhibited by individuals on the nest after the foundress disappeared. (a) Transition period, (b) mixed-brood period

Fig. 6 Temporal changes in the frequencies of abdominal wagging performed by the foundress. (a) Colonies where the foundress was present throughout colony development, (b) colonies where the foundress disappeared. Open circles, first-brood period; solid circles, transition period; crosses, mixed-brood period. "D" indicates the disappearance of the foundress