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RESEARCH ARTICLE - WASPS

The Queen of the Paper Wasp *Polistes jokahamae* (Hymenoptera: Polistinae) Is Not Aggressive but Maintains Her Reproductive Priority

H Yoshimura, J Yamada, YY Yamada

Insect Ecology Laboratory, Graduate School of Bioresources, Mie University, Tsu, Japan

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Corresponding author
Yoshihiro Y. Yamada,
Insect Ecology Laboratory
Graduate School of Bioresources
Mie University
1577 Kurimamachiya-cho
Tsu, Mie 514-8507, Japan.
E-Mail: yamada-y@bio.mie-u.ac.jp

Abstract

The behaviors performed on the nest by the foundress queen and workers of the paper wasp *Polistes jokahamae* were observed in three colonies in the field and one colony in a cage set in the field. Each queen was rarely ranked top in the dominance hierarchy determined by the pairwise dominance-subordinate interactions and did not display more frequent direct aggression toward the top-ranked worker than toward other workers. Furthermore, the queen exhibited aggression less frequently than did the most aggressive workers in all four colonies. The dominance order among the workers was positively correlated with the emergence order, with older workers being more dominant. The queen laid eggs in a dominant or monopolized way; some dominant workers laid eggs in three colonies. These observations suggest that the queen maintained her queen status, including her reproductive priority, using signals rather than aggression. Lateral vibrations (rapidly laterally vibrating the abdomen) and abdominal rubbing (rubbing the abdomen onto the comb) appeared to be candidate signals of the fertility or reproductive potential of the performer. Lateral vibrations were performed only by the queen, and their frequency was positively correlated with the frequency of ovipositing. The queen and some dominant workers performed abdominal rubbing; the frequency was higher for the queen than for any of the dominant workers early in the colony's development, but not later. Although performers of abdominal rubbing were more likely to lay eggs than nonperformers, the frequency of abdominal rubbing was not a predictor of the frequency of ovipositing.

Introduction

Eusocial insects are divided into two groups according to their caste characteristics: primitively and advanced eusocial groups (Wilson, 1971; Jeanne, 2003). The latter include hornets and honeybees and are characterized mainly by morphologically discernible differences between the queen and workers. In contrast, primitively eusocial groups, which include *Polistes* (paper wasps) and bumble bees, are characterized by the absence of critical morphological differences between the queen and workers. The queen of an advanced eusocial group maintains her queen status (including reproductive priority) using the queen pheromone (Wyatt, 2014), while the queen of a primitively eusocial group has been considered to achieve this using physical aggression (van Doorn & Heringa, 1986; Monnin & Peeters, 1999; Jandt et al., 2014). The physical aggression usually includes biting with mandibles, rushing, chasing, and mounting on the backs of subordinate individuals. In primitively eusocial insects, aggression is also used to establish and maintain the dominance hierarchy among colony members, including the queen: higher-ranked individuals are more aggressive in that they perform aggressive behaviors more frequently (Reeve, 1991; Monnin & Peeters, 1999). The queen (or replacement queen) in primitively eusocial insects is the most aggressive colony member, is ranked the highest in the dominance hierarchy and monopolizes reproduction (e.g., Pardi, 1948; Strassmann & Meyer, 1983; Reeve, 1991; Ishikawa et al., 2011).

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Moreover, the aggression is directed primarily toward individuals ranked immediately below the performers of the aggression in the dominance hierarchy (Reeve, 1991; Cant et al., 2006; Ishikawa et al., 2010). The chemicals (cuticular hydrocarbons) on the surface and/or the face pattern function as signals of the bearer's agonistic ability in some species, and the costs associated with fighting over rank are reduced (Tibbetts & Lindsay, 2008; Dapporto et al., 2010a, 2010b; Tibbetts et al., 2010). However, aggression still plays a primary role: the top-ranked wasp in the dominance hierarchy determined by aggression monopolizes oviposition. Dapporto et al. (2010a) refer to a mild type of aggression as dominance behavior (probably examination of the chemical profile of cuticular hydrocarbons) and a harsh type of aggression as attack or aggression, but here, we use the term aggression for both actions.

However, the rank in a dominance hierarchy determined by aggression may differ from that in a dominance hierarchy in reproduction (Röseler, 1991); the former and latter hierarchies are often called social and reproductive dominance hierarchies, respectively. Such a difference between reproductive and social dominance is found in two primitively eusocial species of the Polistinae: *Ropalidia marginata* and *P. japonicas* (Chandrashekara & Gadagkar, 1991; Sumana & Gadagkar, 2003; Ishikawa et al., 2011). The queens of these two species are peaceful and not top-ranked in the social dominance hierarchy, but they do monopolize ovipositing. These species are considered to maintain the queen's status by signaling her fertility, that is, by using an honest signal, as does the queen of advanced eusocial groups (Keller & Nonacs, 1993; Peeters & Liebig, 2009). The *R. marginata* queen uses a pheromone to maintain her queen status (Mitra, 2014), which is considered an honest signal of her fertility. It is likely that the *P. japonicas* queen also uses a pheromone to maintain her status in the colony, since only the queen performs abdominal wagging (Ishikawa et al., 2011), as does the *R. marginata* queen while releasing a pheromone.

It is important to realize that the social dominance hierarchy of paper wasps has mostly been investigated for a foundress association established before the emergence of workers or for workers after the disappearance of the queen. To the best of our knowledge, among the species with colonies composed of one queen and her daughters (henceforth called queen-daughter colonies), the dominance ranks of individual colony members (including the foundress) have been revealed only in the following four species: *Polistes chinensis antennalis* (Morimoto, 1961), *Mischocyttarus cassununga* (Murakami & Shima, 2010), and the above two species, *R. marginata* and *P. japonicus*. Colonies of *R. marginata* may include several foundresses because a colony was founded by one or several foundresses (Shakarad & Gadagkar, 1995); observations are usually made of nests for which the number of foundresses is unknown. The foundress queen of *P. chinensis* and *M. cassununga* is ranked top in the social dominance hierarchy.

The relatedness between the current and future colony members is higher in a queen-daughter colony than in a foundress association or an orphan colony if the foundress queen is mated once - such monandry is common in paper wasps (Strassmann, 2001). Moreover, the queen in queen-daughter colonies is mated, while her daughters are unmated (at least at the early stage) and cannot produce females. As a result, as long as the queen is sufficiently fertile, unmated daughters benefit from working for the colony (Bourke & Franks, 1995). These characteristics of the queen-daughter colonies make the conflict between the queen and daughters more likely to be resolved using an honest signal of fertility rather than physical aggression.

It would be interesting to determine how frequently a peaceful queen is found in queen-daughter colonies and what determines the peacefulness or aggressiveness of the queen in these colonies. These questions could be answered by characterizing the dominance hierarchies in queen-daughter colonies of many species. Such information would also provide a deeper understanding of the evolution of the mechanisms underlying the establishment and maintenance of the queen's status in the Polistinae (Jandt et al., 2014).

We, therefore, investigated the mechanisms used to maintain the queen's status in queen-daughter colonies of *P.* (*Megapolistes*) *jokahamae* Radoszkowski, 1887 (nee *P. jadwigae*) (Vespidae: Polistinae). *Polistes jokahamae* queens usually found a nest solitarily and independently of conspecifics (Kasuya, 1981). First, we determined whether the *P. jokahamae* queen maintains her status using aggression by addressing the following three questions: (1) whether the queen is the most aggressive and top-ranked individual in the social dominance hierarchy determined by aggression; (2) whether the queen directs aggression primarily toward the top-ranked worker in the social dominance hierarchy (the top-ranked worker becomes the primary egg-layer after the disappearance of the queen [Miyano, 1991]); and (3) whether the queen is the exclusive (or primary) egg layer. We also wanted to identify any interactions between the emergence order and dominance rank in the dominance hierarchy since older workers are usually more dominant in temperate paper wasps (Tsuji & Tsuji, 2005). Our present results showed that the queen was peaceful but still the exclusive or the primary egg layer (see "Results"). We then explored the possibility that a behavior functions as an honest signal of the queen's fertility.

Materials and Methods

Biology of P. jokahamae

Polistes jokahamae is an independent, solitary founding paper wasp (Kasuya, 1981) and is common on the islands of Japan except for Hokkaido. Overwintered queens emerge from diapause in late March and begin to found a nest in mid-April to early May (in Mie, Japan). Workers emerge in late May to July, followed by the emergence of males and gynes (potential queens of the next generation) (Yoshimura & Yamada, 2018).

Colonies and videoing

Four colonies of *P. jokahamae* were observed over five years on the Mie University campus in Tsu, Mie, Japan: 2010 (colony 3), 2011 (colony 5), 2013 (colony 6), and 2014 (colony 9) (Table 1): observed colonies were numbered each year according to the order of discovery of them, but many colonies were lost before the appearance of reproductives. The observations began around the emergence of the first worker (Table 1). We could easily distinguish between the queen and early-emerging workers from the differences in body size (Yoshimura & Yamada, 2018), the level of wing damage, and the size of the yellow patches on the abdomen; the queen has larger yellow patches than early-emerging workers (H. Yoshimura & Y.Y. Yamada, unpublished). Colonies 3 and 9 were reared in a tent (pyramidal with a curved ceiling, 2.0 m × 1.5 m × 1.3 m) with mesh sheets on two sides facing each other. The tent windows were closed in colony 3, and sufficient wax moth larvae, honey, water, and dried trees for nest materials were provided for the wasps to collect ad libitum. The tent windows for colony 9 were opened to enable the wasps to forage outside the tent. Colonies 5 and 6 were founded naturally on a lintel in greenhouses whose windows were kept open. Honey and water were placed near the nest for these three colonies. The wasps were marked by attaching small pieces of differently colored photographic paper, which were labeled with numbers, to the mesonotum using instant glue (Aron Alpha® jerry type, Toagosei, Osaka, Japan). The label paper was kept as light as possible by removing its chartaceous backing.

The behavior of individual wasps on the nest was recorded by a digital video camera (HDC-TM 35 or V700M, Panasonic, Osaka, Japan) every day from 6 a.m. to 6 p.m. for a period from the day of first worker emergence or colony discovery (Table 1) until colony disappearance or August 20. Video sessions were canceled when there was a strong wind or heavy rain. Video analysis was performed for the worker phase (Reeve, 1991) when the colony comprises the queen and workers. We analyzed several periods during which the colony comprised almost the same colony members (Table 1). Each analysis period typically lasted four to seven days, during which we analyzed the behavior of individual wasps for two to five days (usually every other day) (Table 1). The behavior was analyzed for six consecutive hours (from 11 a.m. to 5 p.m.) on the days selected, although the video time did not reach this stablished time on some days due to bad weather or difficulties encountered while videoing (Table 1).

Analysis of behavior and determination of rank in the dominance hierarchy

Aggression, ovipositing, and foraging behaviors were recorded by watching the videos, and their hourly frequencies were calculated. The aggressive behaviors included biting, rushing, and chasing: biting part (usually the head) of the opponent with the mandibles; rushing at the opponent, which sometimes resulted in the head touching part of the opponent; and chasing the opponent, which often occurred when the opponent was trying to escape the rushing. Rushing and chasing were classified in the same category because these two behaviors were often confused. Mounting a foreign wasp was sometimes observed, but not against other nestmates. Individuals who exhibited or received each aggressive behavior were identified and recorded. When ovipositing, the female wasp first inserts her abdomen into a cell and then lays an egg on the cell wall. We observed that female wasps often inserted their abdomens into cells without laying an egg. We observed 172 cases in which it was possible to determine whether or not a focal wasp laid an egg after inserting her abdomen into the cell. The female laid an egg in 38 of the 41 cases in which

Table 1. Information on colony development and analysis periods.

Colony	Date of first worker emergence or colony discovery	Date of first male emergence	Date of queen disappearance	Analysis period	Total analysis time (h)	Observation dates	Number of workers on each observation day
3	June 22 (2)[a]	August 4	August 3	1	17.0	July 4, 6, 7, 9, 16	5
				2	30.0	July 18, 20, 22, 24, 25	6 or 7
				3	29.0	July 26, 27, 28, 29, 30	7 or 8
5	July 3 (4)[a]	-[b]	August 16	1	12.0	July 17, 18	4
				2	11.0	July 28, 30	7 or 8
				3	12.0	August 12, 14	12
6	June 24 (4)[a]	August 11	July 21	1	7.7	July 3, 5	3
				2	15.1	July 7, 9, 11	3
				3	5.2	July 15, 17	3
				4	8.5	July 18, 19, 20	3
9	June 11	October 9	July 18	1	24.0	June 16, 17, 19, 21	8 or 10
				2	24.0	June 23, 25, 27, 29	12
				3	20.0	July 1, 4, 5, 6	12
				4	17.5	July 15, 16, 17	9

[a] Some workers had already emerged when the colony was discovered. Numbers in parentheses indicate the numbers of workers when colonies 3, 5, and 6 were discovered.

[b] The colony collapsed due to an ant attack on August 16, just before the reproductives were assumed to emerge.

she was observed to keep her abdomen in the cell for ≥120 s, while no egg was laid in the 131 cases in which her abdomen remained in the cell for <120 s. Thus, when we could not verify whether or not an egg was actually laid after the abdomen was inserted into a cell, we assumed that oviposition occurred only when the female kept her abdomen in a cell for ≥120s.

Lateral vibrations, rushing with flapping, and abdominal rubbing were recorded as candidate behaviors for a fertility signal, and the relationship between their frequencies and the frequency of ovipositing was analyzed. Lateral vibrations refer to rapidly vibrating the abdomen laterally for a period of approximately 1 s. Rushing with flapping refers to a focal wasp rushing at several wasps one after another on the nest while flapping ($M = 22.8$, $SD = 39.9$ s, max = 362 s, min = 9 s, $N = 549$). Abdominal rubbing refers to rubbing the abdominal tip onto one or several cells one after another ($M = 3.8$ s, $SD = 2.7$ s, max = 33 s, min = 1 s, $N = 249$).

The hourly frequency of each kind of behavior was obtained by dividing the total frequency observed during the daily analysis period (usually six hours) by the time during which the focal wasp stayed on the nest, that is, the daily analysis period minus the total foraging time, with each foraging time corresponding to the time span from leaving the nest to returning to it.

The dominance rank was determined using two methods: (1) calculating the dominance index (DI; Premnath et al., 1990) and (2) calculating standings, corresponding to the recorded wins and losses in agonistic contests (i.e., pairwise dominance-subordinate interactions) for all possible pairs of wasps on the nest (Ishikawa et al., 2010). These two methods were used because they might show different dominance ranks; however, as reported in the Results section, this difference was quite small. Episodes of all kinds of aggression were counted without weightings. When using standings, we assumed that for a pair of wasps, the wasp displaying aggression over the other with a greater frequency was dominant over the other. A wasp was then ranked according to the number of subordinate individuals for that wasp minus the number of dominant individuals over it.

Statistical analysis

The hourly frequencies of each of five kinds of behavior (aggression, ovipositing, rushing with flapping, lateral vibrations, and abdominal rubbing) performed by the queen in a day were compared with those performed by the worker with the highest frequency of the corresponding behavior in individual colonies. A stratified Wilcoxon signed-rank test (an exact test for paired samples) was used, with the colony incorporated in a stratum variable (Cytel, 2012) because the data did not conform to a normal distribution. To analyze the possible effect of time, the observation days were dichotomized into the early and late stages of the worker phase, which were defined as periods before and after the time point of two weeks before disappearance of the queen. The observed frequencies of a focal behavior were then compared between the early and late stages using the exact permutation test for two independent samples with strata (Cytel, 2012). Moreover, we used a binomial test in each analysis period to determine whether the queen directed aggression more intensively toward the highest-ranked worker or the worker with the highest frequency of aggression (these were often actually the same individual). In other words, we compared the observed frequency with the frequencies obtained if she randomly directed aggression toward other colony members.

The relationship between the dominance rank and emergence order was also analyzed for each colony using the generalized Cochran-Mantel-Haenszel test with the analysis period incorporated as a stratum variable (Cytel, 2012), with the p values calculated using the exact method. This analysis revealed whether a relationship was present throughout all the analysis periods for each colony. Some workers emerged on the same day and some workers had already emerged on the nest-discovery day (Table 1); in such cases, they were assigned the same number (the mean number of a range of emergence orders for them).

The above tests were implemented using StatXact 10 software (Cytel, Cambridge, MA, USA), except the binomial test, which was conducted using NCSS 11 (NCSS Statistical Software, Kaysville, UT, USA). Statistical significance was assessed using the sequential Bonferroni multiple-comparison method (Holm, 1979) where necessary. When the relationship between the dominance rank and emergence order was analyzed, the relative ranks or orders among all colony members present on the nest were used to control for differences in the total number of colony members on the nest. Relative ranks or orders were calculated using the expression (rank or order of a given worker - 1)/(number of workers - 1), which produced values of 0 and 1 for the first- and last-ranked or emerging wasps, respectively.

We also examined the relationships between the hourly frequencies of ovipositing and those of lateral vibrations, abdominal rubbing, and rushing with flapping. This analysis was applied to (1) the queen of each colony and (2) all colony members; the latter procedures were not applied to lateral vibrations because only the queen performed them. The analysis was performed using a linear mixed model. The frequency of the focal behavior was incorporated into the model as a fixed factor, and the colonies and individuals (nesting in the colonies) were incorporated as random factors. Regarding random factors, random intercepts or both random slopes against the frequency of the focal behavior and random intercepts were considered. The decision of which to choose was based on the AIC values; consequently, the former was chosen except when analyzing rushing with flapping for the queen. The linear mixed model analysis was performed using the lme4 package in R version 3.4.1 (R Foundation, 2017).

Unless we detected a significant relationship between the frequencies of oviposition and focal behavior, we explored whether performers of the focal behavior were more likely to oviposit than nonperformers. We compared the proportion (r)

of ovipositing days among the days on which a focal wasp performed the focal behavior with the proportion (r') of ovipositing days among the days on which the focal wasp did not. This analysis was performed both for the queens and for all colony members. Unfortunately, this analysis did not detect a significant difference in any cases (not presented in the Results); i.e., if a female performs the focal behavior on a given day, it does not necessarily indicate that she is likely to oviposit on that day. Then, we compared the proportion (s) of those females who oviposited at least once among those females who performed the focal behavior at least once during four designated days with the proportion (s') of the ovipositing females among those females who never performed the focal behavior during the four days. This analysis was applied to the last four observing days for adult females who stayed on the nest for at least four observing days. If a difference is detected, it indicates that focal behavior performers are more likely to oviposit than nonperformers, although they do not necessarily oviposit on the days when they perform it.

The significance of the difference for the above two analyses was assessed by calculating exact probability values (Cytel, 2012) after verifying the homogeneity of odds ratios $r/(1 - r)$: $r'/(1 - r')$ or $s/(1 - s)$: $s'/(1 - s')$ among the individuals or colonies; i.e., after verifying that different individuals or individuals of different colonies are similarly likely to oviposit when they perform the focal behavior. The individuals or colonies were incorporated as a stratum factor. The SatXact® software was used for the calculation.

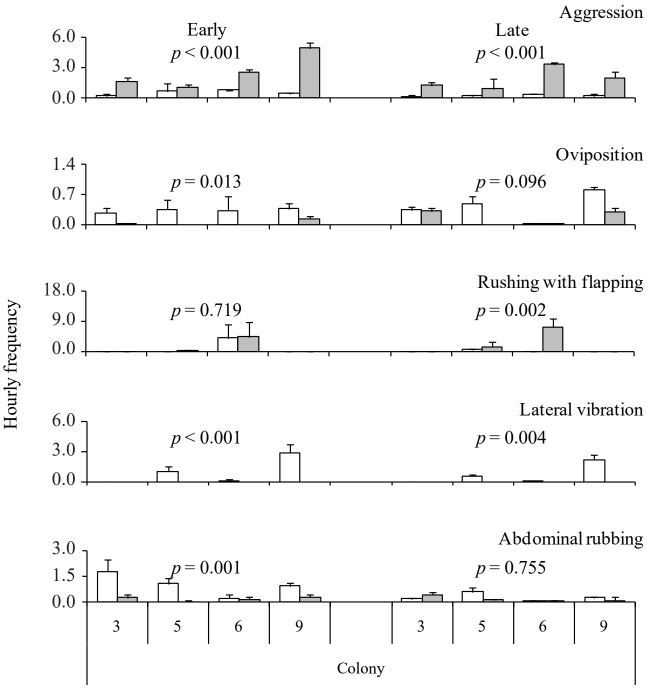

Fig 1. Comparison of the frequencies of five kinds of behavior between the queen (open) and the worker (gray) that performed the focal behavior most frequently. Comparisons were made separately for the early and late stages of the worker phase. The p values were obtained using the stratified Wilcoxon signed-rank test, and those for comparisons between the early and late stages for each behavior performed by the queen (q) and the worker (w) were as follows: aggression, 0.057 (q) and 0.063 (w); ovipositing, 0.114 (q) and 0.004(w); rushing with flapping, 0.170 (q) and 0.504 (w); lateral vibration, 0.418 (q); and abdominal rubbing, <0.001 (q) and 0.972 (w) (exact permutation test).

Results

Colony characteristics

The total numbers of cells constructed and workers emerging in individual colonies ranged from 36 to 48 and 11 to 22, respectively (Table 2). One or several reproductives (males and gynes, i.e., females emerging after the emergence of the first males) emerged in each colony except colony 5, which produced no reproductives because the colony collapsed due to being attacked by ants just before the reproductives were assumed to emerge. In colony 3, the queen disappeared after the male emergence, while the queens of colonies 5, 6, and 9 disappeared before this (Table 1); the queen of colony 5 disappeared when the colony collapsed.

Ovipositing

Individual queens performed 0-35 ovipositions during each analysis period (Table 2). Meanwhile, one to several workers began to oviposit in the individual colonies as the colony developed, except colony 5. Individual workers performed up to 11 ovipositions during each analysis period, but their number was smaller than the ovipositions by the queen with the exception of analysis period 3 in colony 3. The hourly frequency of ovipositing was significantly higher for the queen than for the workers ovipositing most frequently among the workers early in the colony's development, but not later (Fig 1). Workers that oviposited usually had a high ranking, but ovipositing was performed most frequently by

Table 2. Colony size and eggs laid.

Colony	Total number		Analysis period	Frequency of oviposition	
	Cells	Reproductives[a]		Queen	Workers[b]
3	42	5, 0	1	7	2 (1–1, 4–1)
			2	14	8 (1–5, 4–3)
			3	10	17 (1–5, 4–11, 6–1)
5	36	–	1	0	0
			2	8	0
			3	6	0
6	38	5, 4	1	2	0
			2	2	1 (3–1)
			3	0	0
			4	0	0
9	48	1, 0	1	7	0
			2	11	9 (2–5, 3–1, 6–1, 7–2)
			3	14	16 (2–9, 7–3, 9–4)
			4	35	3 (2–1, 9–1, 10–1)

[a] Left, males; right, females. Colony 5 collapsed just before the reproductives were assumed to emerge.

[b] Numbers in parentheses are the number of eggs for each ovipositing worker. The numbers before and after each en dash indicate the emergence order of the workers and the frequency of oviposition, respectively.

the second- or third-ranked worker rather than the top-ranked one (Appendix S1).

Dominance hierarchy

The queens were rarely ranked top in the dominance hierarchy during the individual analysis periods in the four colonies irrespective of whe ther the dominance rank was based on the DI or standings for agonistic contests (Fig 2). Older workers were the more dominant workers during most of the analysis periods for the two kinds of dominance ranks based on the DI and standings (generalized Cochran-Mantel-Haenszel test: colonies 3, 5, and 9, $p < 0.001$ [DI], $p < 0.001$[standings]; colony 6, $p = 0.056$ [DI], $p = 0.037$ [standings]). However, the oldest worker often failed to achieve the highest rank. The queens were never the most frequent aggressors among the colony members during any analysis period (Appendix S2); the hourly aggression frequency of the queen was significantly lower than that of the most aggressive worker (Fig 1). Higher-ranked workers often - but not always - performed aggressive behaviors more frequently (Appendix S2). However, the most dominant worker often did not exhibit the highest frequency of aggression in any colony.

The queen rarely displayed intensive aggression toward the top-ranked worker during any analysis period in the four colonies (Fig 3). The frequency of aggression did not differ significantly from the assumption that she was randomly aggressive toward all workers ($p > 0.05$, binomial test; specific statistical results not presented), irrespective of whether the dominance rank was based on the DI or standings. One exception was period 4 in colony 9 for the dominance rank based on standings ($p < 0.001$), during which the queen directed all aggressive behaviors toward the top-ranked worker; this individual was not top-ranked using the DI. The queen also did not display intensive aggression toward the worker exhibiting the highest frequency of aggressive behaviors ($p > 0.05$, binomial test; specific statistical results not presented). A particularly interesting finding was that the queen often received aggression from many workers irrespective of their dominance ranks (Fig 3).

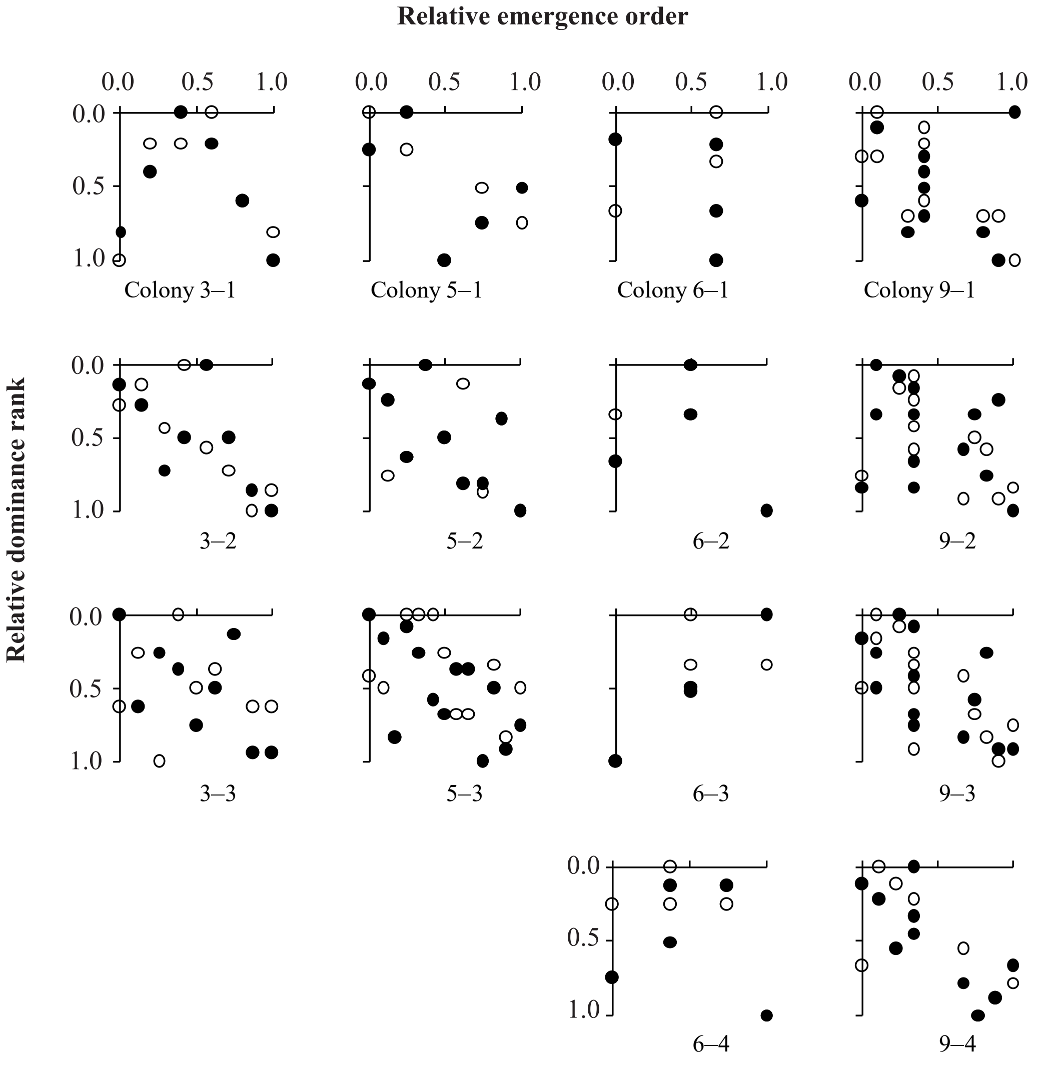

Fig 2. Relationship between the dominance rank and the emergence order. The dominance rank was based on the dominance index (DI, solid) and standings (open). The dominance rank and emergence order are expressed according to the relative rank or order: the first and last ones are indicated by 0 and 1, respectively. The queen was designated by a relative emergence order of 0.

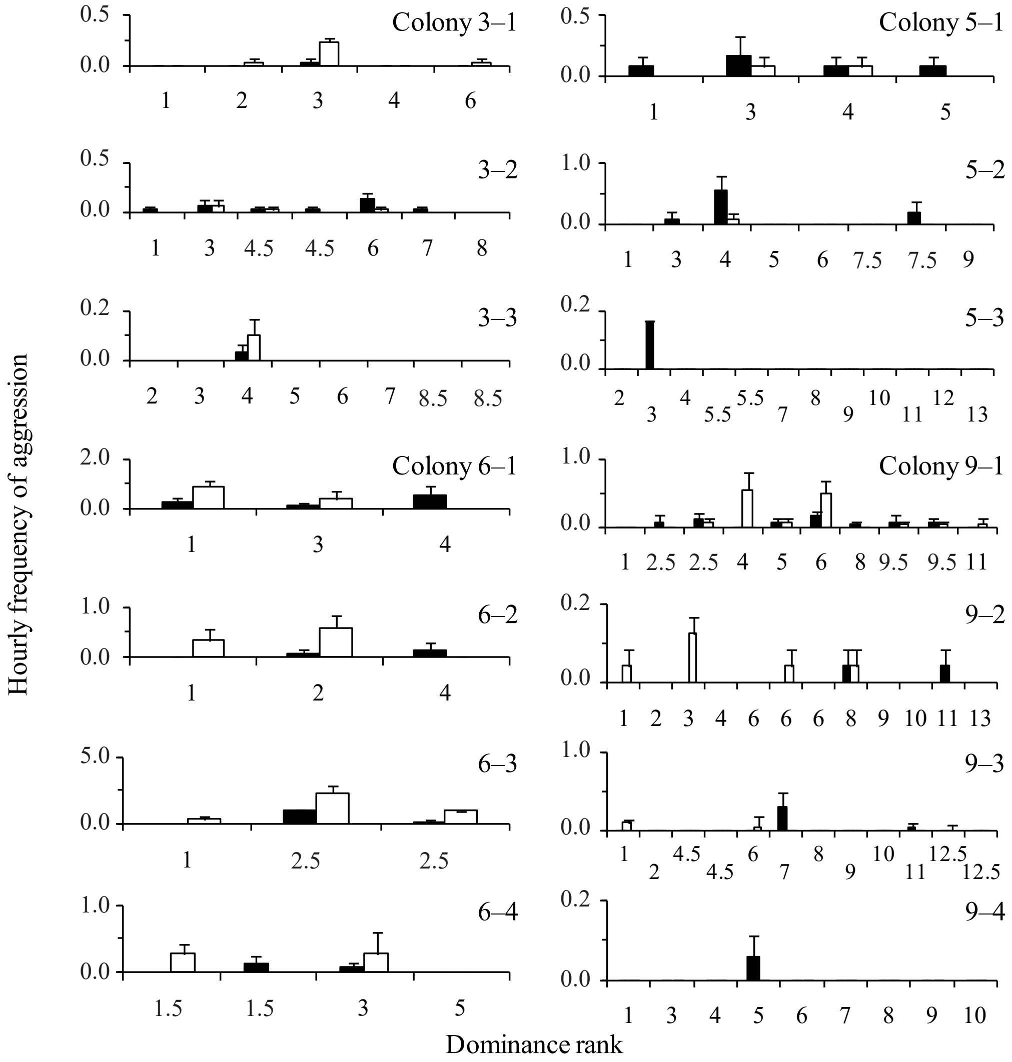

Fig 3. Hourly frequencies (means and SE values) of the aggressive behaviors that the queen exhibited toward (solid) and received from (open) individual workers, who are ordered according to their dominance ranks based on the DI. The missing dominance rank for each analysis period indicates the queen's rank.

Lateral vibrations, abdominal rubbing, and rushing with flapping

Lateral vibrations were performed only by the queen, never by workers (Fig 1). The hourly frequency of ovipositions performed by the queen was significantly related to that of the lateral vibrations ($\chi^2 = 6.6$, $df = 1$, $p = 0.010$; Fig 4).

Some high-ranked workers, as well as the queen, performed abdominal rubbing (Appendix S1). The queen exhibited this behavior most frequently among the colony members early but not late in the colony's development (Fig 1). The frequency of the queen performing abdominal rubbing did not reflect her frequency of ovipositing ($\chi^2 = 0.4$, $df = 1$, $p = 0.554$; figures not presented). The same was true for the frequency of all nest members ($\chi^2 = 0.0$, $df = 1$, $p = 0.851$; figures not presented). However, females who performed abdominal rubbing at least once during the four days were more likely to oviposit than nonperformers during that period (Table 3).

The frequency of rushing with flapping differed markedly between the colonies (Fig 1, Appendix S2), with no and very few events seen in colonies 9 and 3, respectively.

Table 3. Comparison of proportions of ovipositing females among ones performing abdominal rubbing and those among nonperformers.

Colony	Abdominal rubbing	
	Performers	Nonperformers
3	3/3	3/4
5	1/1	1/7
6	0/2	0/2
9	2/2	2/11
p values[a]	0.011 (1.0)	

[a] Exact test for comparison between the odds for performers and nonperformers; figure in parentheses indicates the *p* value for the exact test for homogeneity of odds ratios among different colonies.

Rushing with flapping was mainly performed by dominant workers and the queen (Appendix S2). However, the top-ranked worker did not always do this with the highest frequency among the workers, and many workers (including low-ranked ones) performed rushing with flapping during period 3 in colony 5. The hourly frequency for the worker performing rushing with flapping most often among the workers was similar to that for the queen early in the colony's development, but later, the former was higher than the latter (Fig 1).

None of the analyses detected a relationship between oviposition and rushing with flapping. It is unlikely that colony members can estimate who is likely to oviposit or how many eggs the ovipositor islikely to lay based on rushing with flapping.

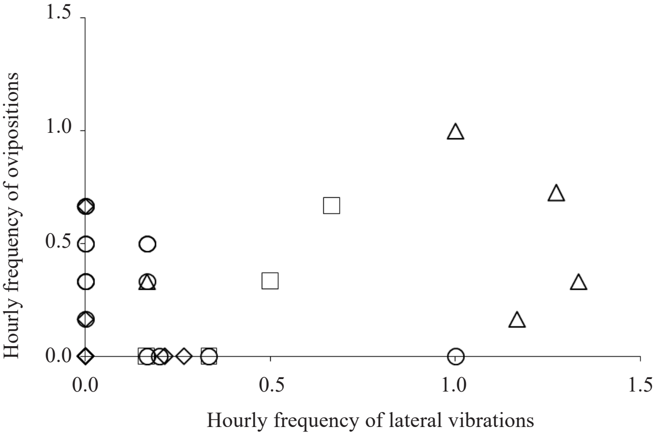

Fig 4. Relationship between hourly frequencies of ovipositing and lateral vibrations performed by the queen during one day. Circles, squares, diamonds, and triangles indicate colonies 3, 5, 6, and 9, respectively. Note that the queen of colony 6 performed neither ovipositing nor lateral vibration during one day on six occasions.

Discussion

The *P. jokahamae* queen was not aggressive or ranked top in the dominance hierarchy determined by aggression, but she oviposited either monopolistically or dominantly. Moreover, the queen did not direct aggression more frequently toward the most aggressive or top-ranked worker (a potential successor) in the social dominance hierarchy compared with the frequency obtained if she randomly directed aggression toward the other workers. These observations strongly suggest that the queen maintains her queen status without using aggression. Instead, the queen is considered to maintain her status by demonstrating her fertility or reproductive potential via some signal. The most likely candidate signal is lateral vibration because the frequency of lateral vibrationis positively related to that of ovipositing. In addition, abdominal rubbing also appears to function as a signal. However, this signal only predicted who is likely to oviposit but not how many eggs the ovipositor is likely to lay. Rushing with flapping is not considered to be involved in maintaining the queen's status.

Miyano (1991) observed that the queen was not aggressive in a queen-daughter colony of *P. jokahamae*. However, the observation period in that study was only four hours, during which time the queen did not oviposit, and the workers originated from colonies different from the queen's. Therefore, our present study have disclosed for the first time that the *P. jokahamae* queen is not aggressive but she oviposits either monopolistically or dominantly.

Lateral vibrations are exhibited by many *Polistes* paper wasps, including *P. dominulus* (Brillet et al., 1999), *Polistes fuscatus* (Savoyard et al., 1998), and *Polistes instabilis* (Molina & O'Donnell, 2009). The queen usually performs lateral vibrations with the highest frequency in the colonies of these species and is the only individual to perform them in the *P. jokahamae* colony. Lateral vibrations have been assumed to have two main functions: (1) as a signal for communicating between larvae and adults; and (2) as a form of aggression in the dominance hierarchy (Jeanne, 2009) since lateral vibrations are usually performed while feeding larvae or encountering other colony members. The *P. jokahamae* foundresses did not perform lateral vibrations under these situations, so lateral vibrations performed by the *P. jokahamae* foundresses do not appear to have such a function. Moreover, the lateral vibration signal is mechanical, but it might also be chemical if odor release accompanies the vibrations. Experiments are required to further clarify the characteristics and function of the lateral vibrations.

Performers of abdominal rubbing were more likely to oviposit than nonperformers, although the daily frequency of ovipositing was not related to that of abdominal rubbing. One plausible explanation is that the frequency of abdominal rubbing is related to the level of egg maturation. Workers are thought not to be allowed to lay eggs easily in the presence of the queen, and even the queen does not oviposit unless cells suitable for oviposition, that is, empty cells or cells containing pupae or last-instar larvae, are available. As a result, the frequency of abdominal rubbing might not be related to that of ovipositing.

Abdominal rubbing appears to correspond to the abdominal wagging observed in *P. japonicus* (Ishikawa et al., 2011) and the abdominal rubbing of the ventral surface of the abdominal tip on the nest observed in *R. marginata* (originally referred to as "rub abdomen" in *R. marginata*; Bhadra et al., 2007, 2010; Mitra & Gadagkar, 2011). These behaviors appear to accompany the release and/or smearing of a chemical or queen pheromone on the cells of the nest, since it has been recently discovered that *R. marginata* queens maintain their queen status with the queen pheromone that is released during abdominal rubbing (see Mitra, 2014 for a review). However, it should be noted that the similar behavior of abdominal stroking found in some other paper wasps has been suggested to have a different function (e.g., Cervo & Lorenzi, 1996; van Hooser et al., 2002; Lorenzi et al., 2011) - the performer demonstrating her own status (e.g., queen or high rank in the dominance hierarchy) to the immatures. Future further experiments are needed to clarify the function of the abdominal rubbing observed in *P. jokahamae*.

Lateral vibration is considered to be an honest signal of the queen's fertility. In general, to keep a signal honest, displaying a signal should incur some cost (Bradbury & Vehrencamp, 2011). Lateral vibration may be physically demanding. Alternatively, the cost of lateral vibration may be ignorable, but workers may check the queen's fertility by directly examining the numbers of eggs laid by the queen, as do *P. dominulus* workers (Liebig et al., 2005), and/or by indirectly checking the profile of chemicals that may be released while performing abdominal rubbing or that of cuticular hydrocarbons (note that the queen often received aggression). In other words, workers do not absolutely rely on the signal, and then, they check the queen's fertility in alternative ways. If workers find the signal dishonest, workers may cease being workers and start to prepare for ovipositing. Whether workers check the queen's fertility directly as well as receiving the signal related to her fertility is an interesting topic for future studies (see Tibbetts & Izzo, 2010).

Some dominant workers started ovipositing late in the colony's development, but the queen did not hinder these ovipositing behaviors by using aggression. However, some eggs laid by workers were eaten by the queen (queen policing) and workers (worker policing), and some eggs laid by the queen were also eaten by workers. Such behaviors have been analyzed in some paper wasps (Saigo & Tsuchida, 2004; Liebig et al., 2005; Dapporto et al., 2010a), but unfortunately, in the present study, it was not possible to identify all such events by observing the video or to perform a quantitative analysis.

Our research team has disclosed that the queen in queen-daughter colonies of two *Polistes* species (*P. japonicus* and *P. jokahamae*) is peaceful and does not use a social dominance hierarchy to maintain her queen status. A particularly interesting finding is that *Polistes snelleni* queens are also peaceful (Yamasaki & Tsuchida, 2014), which suggests that queen's signals control the colony, although the dominance hierarchy needs to be investigated in detail. It is of great importance to determine whether aggression is involved in maintaining the queen's status in queen-daughter colonies of other paper wasp species and to determine which signal is involved if aggression is not involved.

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Authors' Contributions

YY Yamada conceived and designed the experiment. J Yamada and H Yoshimura carried out the fieldwork and assembled the data. YY Yamada and H Yoshimura performed the data analyses and wrote the manuscript.

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Appendix

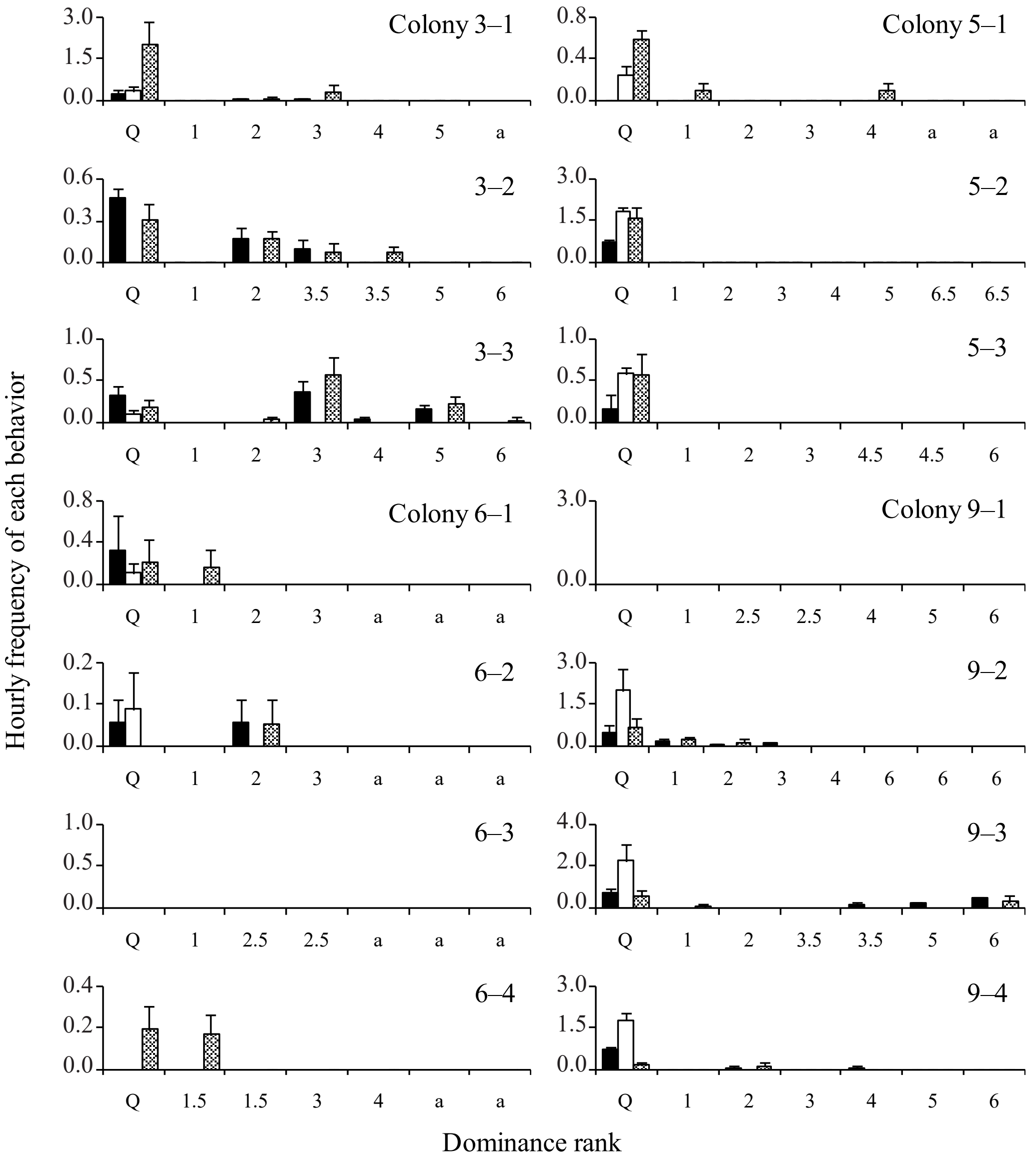

Fig S1. Hourly frequencies (means and SE values) of ovipositing (solid), lateral vibrations (open), and abdominal rubbing (checked) performed by the queen (Q) and the first- to sixth-ranked workers. Dominance ranks were based on the dominance index (DI). No workers performed lateral vibrations. The number following the colony number indicates the analysis period. The letter “a” on the x-axis indicates no corresponding worker. Seventh- and lower-ranked workers occasionally performed ovipositing and abdominal rubbing: ovipositing, 0.06 ± 0.06 (mean ± SE) for the eighth-ranked worker during period 4 in colony 9; abdominal rubbing, 0.17 ± 0.00 for the eighth-ranked worker during period 3 in colony 5, and 0.08 ± 0.00 for the eighth-ranked worker during period 1 in colony 9.

Appendix

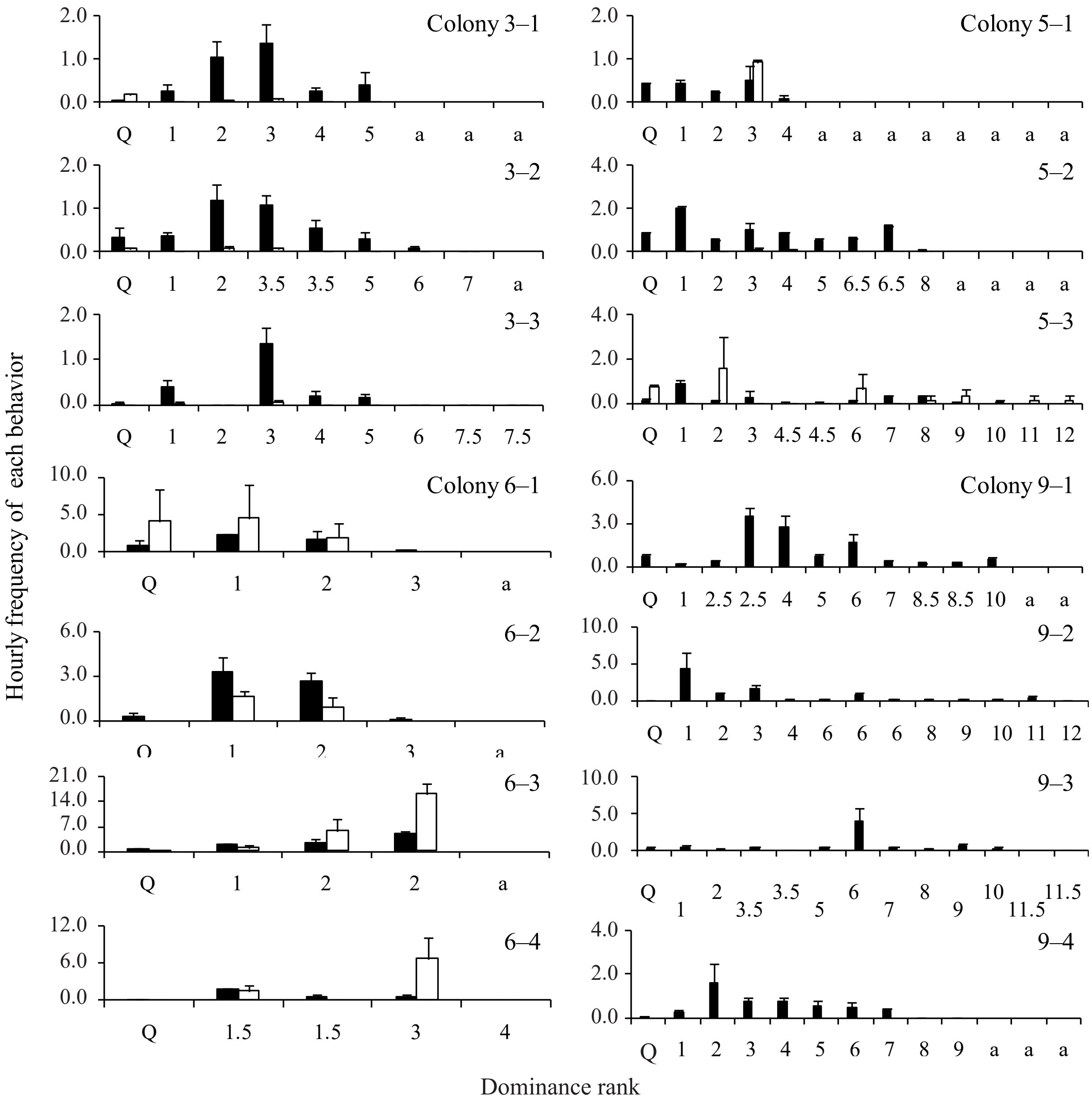

Fig S2. Hourly frequencies (means and SE values) of aggressive behaviors (solid) and rushing with flapping (open) performed by the queen (Q) and workers, who are ordered according to their dominance ranks based on the DI.