Reproductive queue without overt conflict in the primitively eusocial wasp *Ropalidia marginata*

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Colonies of the primitively eusocial wasp *Ropalidia marginata* consist of a single egg layer (queen) and a number of non–egg-laying workers. Although the queen is a docile individual, not at the top of the behavioral dominance hierarchy of the colony, she maintains complete reproductive monopoly. If the queen is lost or removed, one and only one of the workers [potential queen (PQ)] becomes hyperaggressive and will become the next queen of the colony. The PQ is almost never challenged because she first becomes hyperaggressive and then gradually loses her aggression, develops her ovaries, and starts laying eggs. Although we are unable to identify the PQ when the queen is present, she appears to be a "cryptic heir designate." Here, we show that there is not just one heir designate but a long reproductive queue and that PQs take over the role of egg-laying, successively, without overt conflict, as the queen or previous PQs are removed. The dominance rank of an individual is not a significant predictor of its position in the succession hierarchy. The age of an individual is a significant predictor, but it is not a perfect predictor because PQs often bypass older individuals to become successors. We suggest that such a predesignated reproductive queue that is implemented without overt conflict is adaptive in the tropics, where conspecific usurpers from outside the colony, which can take advantage of the anarchy prevailing in a queenless colony and invade it, are likely to be present throughout the year.

reproductive conflict | reproductive succession | eusociality | cooperative breeding | insect societies

Reproductive division of labor is the hallmark of insect societies. This is achieved by the differentiation of colony members into reproductive (queen/king) and nonreproductive (worker) castes (1, 2). In insect societies such as those of ants, honeybees, swarm-founding wasps, and higher termites, which are traditionally referred to as highly eusocial, caste determination is achieved by preimaginal physiological processes that channel individuals into distinct reproductive or worker developmental pathways. Thus, caste of an individual is already determined at the time of eclosion and remains irreversible. If the reproductive dies, the colony has to rear a new one from the egg or early larval stage because the workers cannot change their caste in adulthood. In societies such as those of most social bees and wasps, which are traditionally referred to as primitively eusocial, all adult colony members are nearly totipotent and morphologically similar. The process of caste differentiation into reproductives and nonreproductives takes place largely in the adult stage and is based on social interactions among colony members (3–9). This makes the castes flexible and often reversible, so that workers can become queens upon the loss or death of the original queen. In addition to indirect fitness gained as workers, individuals, thus, also have a finite probability of direct reproduction as future queens. Similarly in wood-dwelling lower termites, workers that are still in their nymphal stages can be totipotent and can develop into future reproductives (10). This is also reminiscent of many cooperatively breeding birds and mammals in which adult helpers temporarily forego reproduction and assist breeders to rear and protect their offspring. Such helpers are also totipotent and can inherit breeding opportunities in the future (11, 12).

In most primitively eusocial species, colony members display aggressive dominance–subordinate interactions, on the basis of which they can be arranged in a linear dominance hierarchy. The queens typically occupy the top (alpha) position in this dominance hierarchy and may be sequentially replaced by individuals occupying successively lower positions in the dominance hierarchy. Thus, there is usually a reproductive queue based on the behavioral dominance hierarchy (13–19). In the primitively eusocial wasp *Ropalidia marginata*, on the other hand, queens are remarkably meek and docile individuals that seldom, if ever, occupy the alpha position in the aggression-based behavioral dominance hierarchy of their colonies, thus breaking the link between behavioral dominance and reproductive dominance (20–23). Lost queens are replaced by one of the workers, although this is not based on the position of the worker in the behavioral-dominance hierarchy. Thus, it appears that dominance–subordinate interactions are not used in this species to express or settle reproductive conflicts. Instead, these kinds of interactions appeared to have been coopted for use by intranidal workers to regulate foraging by extranidal workers (22, 24, 25).

Upon natural loss or experimental removal of the queen, one (and only one) of the workers becomes hyperaggressive, increasing her levels of dominance behavior several fold relative to her own levels in the presence of the queen. If the queen is not returned to the colony, this hyperaggressive individual will become the next queen, losing her dominance behavior by the time she begins to lay eggs in about a week. We, therefore, refer to her as the potential queen (PQ) (22, 23, 26, 27). The PQ cannot be identified by us in the presence of the original queen because she appears to be an unspecialized individual not unique in her behavioral profile, dominance rank, or ovarian development (26, 28). However, there is evidence that the rest of the wasps in the colony seem to "know" her identity, so that there appears to be a cryptic "heir designate," even though we cannot identify her beforehand (29).

Here, we ask whether there is just one such heir designate or whether there is indeed a longer reproductive queue with successive heirs, similarly designated, even in the presence of the original queen.

Results

Is There a Reproductive Queue in *R. marginata* Colonies? We already knew that upon removal of the queen, a PQ (PQ1) will reveal herself as a hyperaggressive individual. The question was what would happen if we similarly removed the PQ1 soon after her identity was revealed. To answer this question, we sequentially removed the queen and four successive PQs soon after they were evident (at intervals ranging from 30 to 90 min; modal value,

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30 min). These experiments, which were repeated on 19 different colonies, showed without exception that each time a PQ was removed, another PQ was evident, always as the one and only hyperaggressive individual. Thus, we were able to identify five successive PQs (PQ1 to PQ5) in each of the 19 colonies. The frequency per hour (freq/h) of dominance behavior shown by every PQ, in all colonies, was significantly greater than her own level of dominance behavior in the presence of the queen and also significantly greater than that of any other individual in her colony, during the period when she was the PQ (see Fig. 1).

Even more dramatic than the unfailing emergence of a single PQ after every removal of the queen or previous PQ was the fact that the PQs were almost never challenged by any member of their colonies. The frequency per hour of dominance behavior shown by the PQs (79.2 ± 56.3), in addition to being significantly greater was also 40–180-fold greater than the low levels of dominance behavior, if any, received by them (1.1 ± 2.4) (Fig. 2). Indeed, 68 out of 95 PQs observed did not receive a single act of dominance behavior from any colony member. The 95 PQs mentioned above were identified by their unique signature of being the sole hyperaggressive individuals in the colony. We have good reason to believe that such behaviorally identified PQs indeed go on to become the sole egg layers of their colonies. In a previous study, 13 PQ1s identified by their hyperaggression all went on to become the sole egg layers of their colonies in 3–11 d (5.62 ± 0.29 d) (30). In the present study, we found that 16 PQs (4 each of PQ2, PQ3, PQ4, and PQ5) identified by their hyperaggression, all laid their first egg in 3–8 d (5.25 ± 1.44 d) (Table S1) and went on to become the sole egg layers of their colonies. The reproductive monopoly of all these PQs (13 + 16 = 29) was also confirmed by the fact that no wasp other than these PQs in their respective colonies had any ovarian development.

Which Individuals Do the PQs Aggress? Our data on 95 PQs show that they do not appear to target any particular individual, not even the future PQs of their colonies. PQs directed their dominance behavior on average toward about half of their colony members (proportion of nestmates dominated, 0.50 ± 0.24). Considering the 76 PQs that could be studied (sample size here is 76 and not 95 because PQ5s could not be included in this analysis as PQ6s were not identified), the PQs did not target their immediate successors more than what is expected by chance alone (Wilcoxon matched-pairs, signed-ranks test; $P = 0.36$). Indeed, 35 of the 76 PQs did not show a single act of dominance toward their immediate successor PQs.

What Determines the Position of an Individual in the Reproductive Queue? In an attempt to understand the determinants of the position of an individual in the reproductive queue (i.e., succession rank), we used ordinal logistic regression models with dominance rank, frequencies per hour of dominance behavior, build cells, feed larva, proportion of time spent on the nest, indices of body size, ovarian development and productivity, and age, as potential predictor variables. Among these, age was the only significant predictor of the position of an individual in the

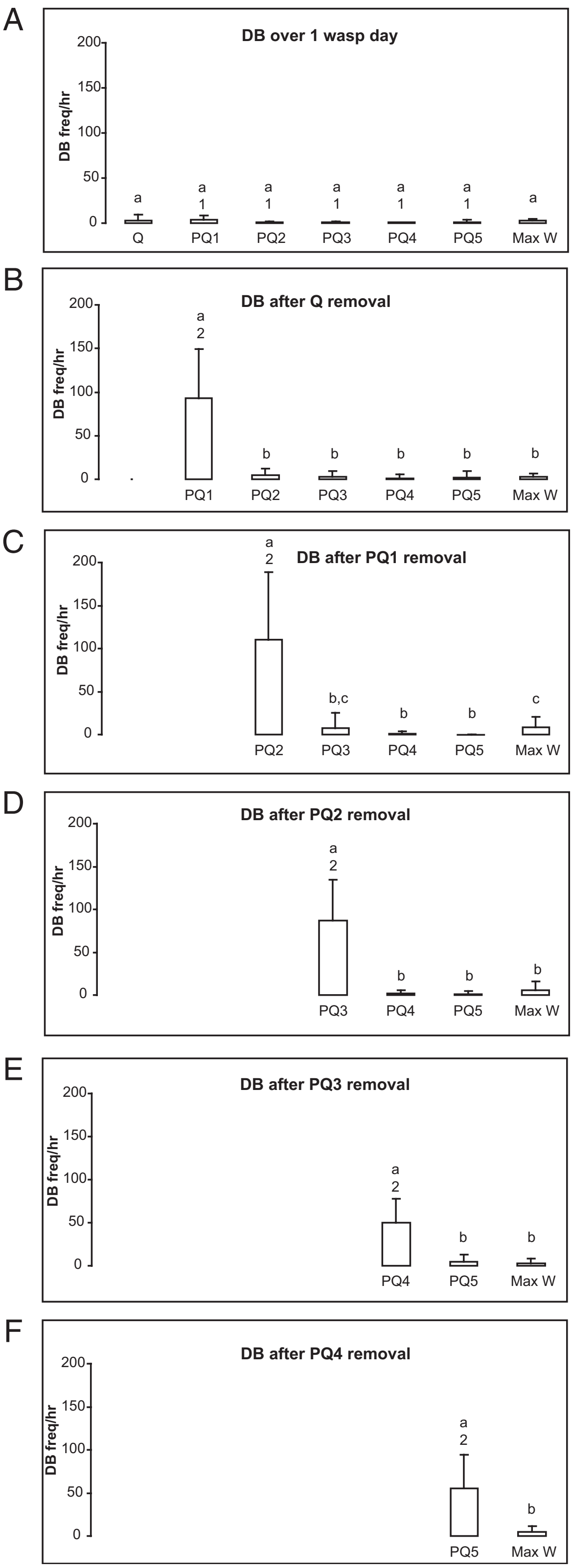

Fig. 1. Evidence for a reproductive queue in *R. marginata*. Means and SDs of frequencies per hour of behavioral dominance of the queen, five PQs, and the max worker (the individual that showed maximum behavioral dominance in the colony apart from the queen and the five PQs) in normal queenright colonies (*A*) and the PQs and the max workers in the absence of the queen and the preceding PQs (*B*–*F*) ($n = 19$ colonies). Bars that carry different letters are significantly different from each other within each panel (Wilcoxon matched-pairs, signed-ranks test; to achieve $P < 0.05$, α was set to 0.002, 0.003, 0.005, 0.008, 0.017, and 0.05 on account of Bonferroni correction, for *A*, *B*, *C*, *D*, *E*, and *F*, respectively). Bars that carry different numbers represent significant differences in freq/h of behavioral dominance of the focal PQ in normal queenright colony with her own value after removal of the queen or previous PQs (Wilcoxon matched-pairs, signed-ranks test; to achieve $P < 0.05$, α was set to 0.01 on account of Bonferroni correction). Note that each PQ showed significantly higher aggression after the queen and the previous PQs were removed than what she showed in the queenright colony and also compared with any other individual in the queenless colony. DB, behavioral dominance; Q, queen; PQ1 to PQ5, PQs 1–5; Max W, max worker.

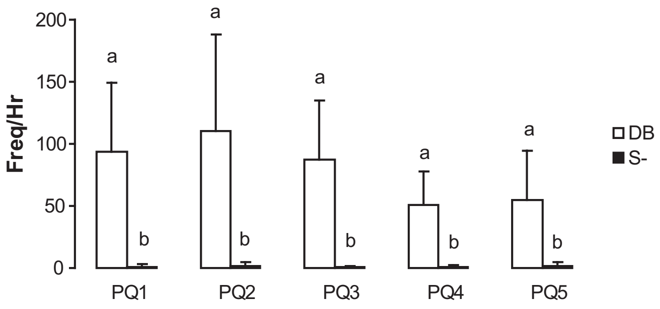

Fig. 2. Behavioral dominance shown and received (mean ± SD) by different PQs in the absence of the queen and previous PQs (Wilcoxon matched-pairs, signed-ranks test; to achieve $P < 0.05$, α was set to 0.01 on account of Bonferroni correction; $n = 19$ colonies). Note that each PQ showed significantly higher behavioral dominance than what she received. On an average, dominance shown by each PQ was ~40–180-fold higher than the dominance she received. DB, behavioral dominance shown; S-, behavioral dominance received; PQ1 to PQ5, PQs 1–5.

reproductive queue (Table 1). Unlike in other primitively eusocial species, dominance rank was not a significant predictor for succession rank. Indeed, PQs bypassed on average 6.73 ± 8.07 higher ranking individuals in becoming successors (Table S2). Although age is a statistically significant predictor of the succession rank of an individual, it is by no means a perfect predictor of which individual will become the next PQ. Only 10 out of 30 PQs (considering only those 6 colonies in which ages of all individuals were known) were the oldest members of their colonies at the time they became PQs. Three PQs bypassed one or more equal-aged individuals, and 17 PQs bypassed one or more older individuals in becoming PQs of their respective colonies. On average, PQs bypassed 1.72 ± 2.02 individuals that were of equal age or older. Considering only PQ1s, only one out of six PQs was the oldest member of the colony at the time of succession, whereas two PQs bypassed one or more equal-aged individuals and three PQs bypassed one or more older individuals at the time of succession (Fig. S1 and Table 2).

Discussion

The unfailing emergence of one and only one hyperaggressive individual (PQ) upon removal of the queen or previous PQ is the most remarkable result of this study. Coupled with the facts that no PQ encountered any challenge from her nestmates and that the PQs identified by their hyperaggression always went on to become the sole egg layers of their colonies, this suggests that there is a clear reproductive queue among the workers of *R. marginata* that is respected without exception and without overt conflict, upon the loss of the previous queen or PQ. Although we stopped the experiment after we found five successive PQs, there is no reason to believe that we would not have been able to identify even more successors, perhaps until the last wasp, had we persisted with the experiments. The presence of a reproductive queue with predesignated successors is likely to be adaptive in species with small colonies, in which the evolutionary persistence of the worker strategy often depends on future direct fitness, in addition to current indirect fitness (31–36). Information about their position in the succession hierarchy would help individuals make decisions about whether to stay on in their natal nests or leave to found new colonies.

Even though the PQs do not receive any appreciable amount of aggression from their nestmates at the time of succession, they themselves show very high levels of aggression toward their nestmates. At first, this seems rather surprising and raises the question of the function of such hyperaggression on the part of the PQs. On closer examination, we found that PQs distribute their aggression to as many as 50% of their nestmates and, thus, do not appear to target any particular individual, not even their immediate successors. Such a wide distribution of their aggression lends credence to an idea that we have suggested and supported previously, that the hyperaggression of the PQ may also be required for the rapid development of her own ovaries, so as to facilitate speedy establishment of herself as the sole new reproductive of the colony (30). These facts, taken together with other previous results showing that one and only one individual is acceptable to the colony as the successor (29), suggest that the hyperaggression of the PQ is not an expression of conflict over queen succession. Instead, the hyperaggression of the PQ is one-sided because she receives no challenge. Hence, our conclusion is that there is a reproductive queue without overt conflict in *R. marginata*.

An especially intriguing feature of the social biology of *R. marginata* is our continued inability to predict the identity of the PQ in the presence of the queen. Previous work has consistently shown that the PQ is an unspecialized intermediate among the workers, not unique in her behavioral profile, dominance rank, or level of ovarian development (26, 28). These previous efforts, however, considered only one successor per colony (PQ1) and did not adequately explore the potential role of age in the making of PQs. The present set of experiments, in which we studied several successive PQs and where we knew the ages of all

Table 1. Ordinal logistic regression analysis showing that age is the only significant predictor of the position of an individual in the succession hierarchy of the colony

Predictor variable	No. of nests	Regression coefficient	*P*
Dominance rank*	19	−0.026	0.397
Dominance behavior (freq/h)*	19	0.066	0.068
Build cell (freq/h)*	19	0.050	0.239
Feed larva (freq/h)*	19	−0.027	0.455
Proportion of time spent on nest*	19	−0.013	0.620
Index of body size[†]	7	0.101	0.090
Index of ovarian development[†]	7	−0.027	0.980
Index of productivity[‡]	6	−0.088	0.155
Age[§]	6	0.289	0.037

Similar superscript symbols (*, †, ‡, §) indicate that the predictor variables were measured in the same set of colonies and analyzed in the same model. Thus, four different ordinal logistic regression models were built. The validity of all models was confirmed by Pearson's goodness of fit test ($P > 0.05$). All individuals in a colony were given ranks based on a predictor variable, but only the five PQs from each colony were used in the regression models. All of the four regression models were also run, including nest identity as a potential predictor variable, but, in no case, was nest identity a significant predictor of the position of an individual in the reproductive queue.

Table 2. Numbers of older individuals bypassed by different PQs in different colonies

	No. of animals	PQ1	PQ2	PQ3	PQ4	PQ5	Mean no. of individuals bypassed in each colony, mean ± SD	Grand mean ± SD
Colony								
V1014	17	1	1.5	2	2.5	0	1.40 ± 0.96	
V1017	9	0	4.5	1	0	0	1.10 ± 1.95	
V1023	22	3	4	4.5	4	8.5	4.80 ± 2.14	
V1024	11	0.5	0	0	0	0	0.10 ± 0.22	
V1036	21	1	1.5	4	3.5	0	2.00 ± 1.70	
V1059	10	0.5	0.5	1.5	0	2	0.90 ± 0.82	
Mean no. of individuals bypassed by each PQ, mean ± SD		1.00 ± 1.05	2.00 ± 1.84	2.17 ± 1.75	1.67 ± 1.89	1.75 ± 3.40		1.72 ± 2.02

When a same-aged individual was bypassed, the number of individuals bypassed was considered to be 0.5.

of the wasps (in a subset of six colonies), therefore provides another opportunity to understand what makes a PQ and indeed what determines the position of an individual in the reproductive queue of her colony. Our attempts to identify the determinants of the position of an individual in the reproductive queue of the colony produced two notable results: (*i*) the rank of an individual in the behavioral dominance hierarchy of the colony has no effect and (*ii*) the age of an individual has a strong effect in determining her position in the reproductive queue. That dominance rank has no effect is not surprising considering what we already know about the determinants of PQ1 in *R. marginata* but is very surprising considering what we know about other primitively eusocial species (13–19). The failure of the dominance rank of an individual to predict her position in the reproductive queue is, however, consistent with another idea that we have suggested and supported previously, namely, that dominance–subordinate behavior is not involved in settling reproductive conflicts in *R. marginata* but, rather, has been coopted for the decentralized self-organization of work in the colony, by the workers themselves (27).

The emergence of age as a strong predictor of the position of an individual in the reproductive queue is rewarding because it brings us closer to predicting the identity of the PQ even in the presence of the queen or previous PQ. We suspect that it is not so much the absolute age of an individual but its age relative to other colony members that influences its ability to become a queen. This is reminiscent of the well-known role of relative age in work organization based on age polyethism in bees (37–39) and ants (40–42) and, indeed, even in *R. marginata* (43) and is supported by the fact that social insects appear to have mechanisms for assessing the relative ages of each other (43–46). However, it is also important to realize that age is not a perfect predictor of the position of an individual in the reproductive queue. At least one older individual appears to be bypassed by an average PQ when she takes over the colony as its next sole reproductive. The imperfection of age as a predictor may either be attributable to imperfections in assessing relative ages or, more likely, because age is only one of the factors that comes into play during queen succession and other factors may also play a role, especially in distinguishing between similar aged individuals. Elucidating such additional factors (including physiological and genetic factors) that may influence the position of an individual in the reproductive queue would, therefore, be the next major step in our studies of this fascinating species.

Age is known to be a strong determinant of the probability of an individual becoming a replacement queen in other species of primitively eusocial insects, but in all other cases, dominance behavior and/or dominance rank is an equally important confounding factor (47–49). What is unusual about *R. marginata* is that dominance behavior and dominance rank play no role in queen succession, hence making it a unique case of reproductive queue without overt conflict. Orderly queen succession without the incumbent being challenged after the loss of the queen or previous PQ is likely to be adaptive, especially in the tropics, where usurpers are present all year round to take advantage of a chaotic situation in a queenless colony. The threat of a conspecific foreign usurper invading the colony and taking over reproduction is indeed a major factor governing the lifecycle of *R. marginata* (50). However, there appears to be no overt conflict even in the presence of the previous queen (i.e., at the time the queue is formed in the first place). If age is a strong determinant and dominance behavior and/or dominance rank is of no importance, then we can indeed expect queen succession to be an orderly process without overt conflict both when the reproductive queue is being established and when it is being implemented. Because older individuals are higher up in the reproductive queue in *R. marginata*, a queue once formed can remain stable even if older individuals die, unlike some other species in which younger individuals become replacement queens (14, 51) and the reproductive queue has to be rearranged every time new individuals are born and enter the queue at the relative top.

However, why should age be such a strong determinant of the position of an individual in the reproductive queue? Because the whole colony appears to be accepting one of the oldest individuals without challenge, we may assume this is adaptive for the colony. Average age at which an *R. marginata* wasp maintained in isolation in the laboratory starts egg-laying is about 62 d (9). Given that these wasps live for an average age of about 30 d (52) in natural colonies, the oldest individual at the time of loss of the queen is likely to be that with the highest reproductive potential. Thus, long lifespans of the colonies, potentially long lifespans of the wasps, combined with their aseasonal, perennial, and indeterminate colony cycles (52, 53) and their tropical distribution make an age-based reproductive queue without overt conflict an ideal solution for *R. marginata*. It is true, of course, that age is not a perfect predictor of succession rank and that some older individuals are bypassed by PQs. Nevertheless, the advantage of age that *R. marginata* wasps seem to have is reminiscent of vertebrate (and human) societies in which age, experience, and wisdom contribute to the ability of an individual to inherit leadership roles (54, 55). This reinforces the frequent calls to social insect researchers to compare notes with those studying vertebrate societies, especially cooperatively breeding birds and mammals (56–59). We believe that it would be especially interesting to compare succession of queens, breeders, or leaders in vertebrate and invertebrate societies to examine the possibility that the patterns of succession are influenced more by ecological factors rather than taxonomic position of the species.

Materials and Methods

All experiments were carried out at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore (13° 00′ N, 77° 32′ E), India, between March 2006 and May 2009. Postemergence nests of *R. marginata* were collected from various sites in Bangalore and surrounding areas. *R. marginata* nests may contain between 1–300 individuals, although nests with more than 20–30 individuals are rather rare. Nests were brought to the vespiary (a room measuring 9.3 × 6 × 4.8 m and covered on all four sides by wire mesh with openings 0.75 × 0.75 cm, just large enough so that *R. marginata* individuals can fly in and out of the room unhindered but small enough to prevent the predatory wasps *Vespa tropica* and *Vespa affinis* from entering the room and attacking nests and brood of *R. marginata*) and transplanted in wood and wire-mesh cages measuring 45 × 45 × 45 cm (60). All individuals were uniquely marked with Testors enamel paints for individual identification.

We performed a series of experiments to determine whether a succession hierarchy exists, to ascertain that PQs identified by behavior indeed go on to become the sole egg layers, and to detect the possible determinants of the position of an individual in the succession hierarchies. Because more than one kind of information is obtained from a given experiment, exact sample sizes vary for different results (see *Results*).

Our experiments typically lasted 3 d each. Ten hours of observational data were obtained on normal, unmanipulated, queenright colonies in open cages where individuals were free to leave and enter the cage and the nest at will, over the first 2 d. On one of the 2 d, observations were made from 0800 to 1030 hours and 1300 to 1530 hours and on the other day from 1030 to 1300 hours and 1530 to 1800 hours (60). Behavioral observations consisted of equal numbers of randomly intermingled instantaneous scans to record a snapshot of the behavior of each individual at that moment, where frequent and/or long-duration behaviors were recorded (behaviors such as sitting, walking, grooming, etc.), and "all-occurrences" sessions to record every act of rare and short-duration behaviors of each individual at that moment (behaviors such as feeding larva, building the nest, different types of dominance behaviors, etc.) (61).

Each behavioral session (scan or all-occurrences) lasted for 5 min each, followed by a break of 1 min. Thus, by the end of day 2, each colony had been observed with 50 scans and 50 all-occurrences sessions. For a complete list of behaviors, see ref. 60. On the night of day 2, the cage windows and doors were closed to ensure that all individuals remained on or near the nest. On the morning of day 3 (at around 0830 hours), the queen was removed from the nest using a glass vial, and PQ1 was identified based on her behavioral profile. To do so, the colony was observed in blocks of 15 min with a 5-min break, for 30 min or until a PQ was evident (PQ was evident in 30–90 min; modal value, 30 min). An individual was designated as PQ if she showed a minimum of five acts of dominance and more than twice the number shown by anyone else in that colony during the same period. PQ1 is known to become hyperaggressive within minutes of queen removal and only gradually reduce her aggression in the course of the next week or so. We have also confirmed that such identification of PQ by her aggression in the first 30–90 min after queen removal is adequate for accurate identification (see below). Once identified, the PQ was removed, kept isolated in a glass vial, and observations of the nest were continued, to identify the next PQ, until five successive PQs were identified. All of the five PQs were identified on day 3 in a span of 4–5 h. At the end of day 3, the queen, five PQs, and rest of the workers were collected and preserved at −20 °C for later body measurements and dissection.

To investigate possible determinants of the position of an individual in the succession hierarchy, we measured the following nine predictor variables:

- *i*) Rank in behavioral dominance hierarchy, as calculated by the frequency-based dominance index (FDI). The index considers frequencies of direct and indirect dominance behaviors for each focal individual and devalues it by weighing against its direct and indirect subordinate behaviors. Direct dominance behavior (or subordinate behavior) is calculated as the sum of the frequencies with which the focal individual shows dominance behavior (or subordinate behavior) toward its colony members, and indirect dominance behavior (or subordinate behavior) is calculated as sum of the frequencies with which all individuals dominated by the subject (or all individuals to whom the subject showed subordinate behavior), in turn, show dominance behavior (or subordinate behavior) toward colony members. The index is described in more details in ref. 62.
- *ii*) Freq/h of behavioral dominance shown in the queenright colony.
- *iii*) Freq/h of build cell behavior in the queenright colony.
- *iv*) Freq/h of feed larva behavior in the queenright colony.
- *v*) Proportion of time spent on the nest in the queenright colony.
- *vi*) Index of body size, defined as the first principal component of the Principal Components Analysis (PCA) performed on 27 body-size parameters (12 head parameters, 6 wing parameters, 3 thoracic parameters, and 7 abdominal parameters) as described in ref. 60.
- *vii*) Index of ovarian development, defined as the first principal component of the PCA performed on six ovarian parameters (total number of oocytes; total numbers of mature, yolky, and resorbing oocytes; and average length and width of proximal oocyte) as described in ref. 60.
- *viii*) Index of productivity, determined by maintaining all individuals for 90 d in isolation in plastic boxes with ad libitum food and water. The index of productivity was computed as the weighted sum of the brood produced at the end of 90 d. The weightings used were as follows: egg, 1; small larva, 2; intermediate sized larva, 3; large larva, 4; pupa, 5; and imago, 6.
- *ix*) Age in days of all individuals eclosing after the nest was brought to the vespiary, ascertained from census data. The experiment was begun only after the individuals of unknown ages (eclosed before collecting the nests) had disappeared.

Not all predictor variables could be measured in the same colonies. More information on the numbers of colonies for each predictor variable is given in Table 1. Behavioral data (predictor variables *i* to *v*) were collected from observations on normal, unmanipulated, queenright colonies on the first 2 d. Data on body size and ovarian development (predictor variables *vi* and *vii*) were gathered from dissections performed on individuals after they were collected and preserved at the end of day 3. Data on index of productivity (predictor variable *viii*) were obtained from separate experiments in which individuals were not preserved at the end of day 3 but were isolated in plastic boxes with ad libitum food, water, and building material and kept in isolation for 90 d.

We used an additional 16 colonies (4 colonies each for PQ2, PQ3, PQ4, and PQ5) to ascertain that the individuals we termed as PQs based on their aggression in a span of 30–90 min indeed went on to become the sole egg layers of their colonies. On each of these nests, the queen was identified by egg-laying behavior, and the PQs were identified based on their aggression profiles as described above. For every focal PQ, the queen and the preceding PQs were removed. Nest-map records (indicating the contents of each cell, every day) were maintained, and the nest was tracked until a new egg was found in the nest map records. At this time, all individuals were collected, and their ovarian measurements were made to determine who had developed ovaries. The results of these experiments were pooled with previously published similar experiments on 13 colonies in which it was ascertained that the individual identified as PQ1 by her aggression became the sole egg layer of the colony (30), thus yielding results from a total of 29 colonies.

All experiments were done in accordance with the regulations for research involving animal subjects in India.

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