Octopamine and cooperation: octopamine regulates the disappearance of cooperative behaviours between genetically unrelated founding queens in the ant

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We investigated whether octopamine (OA) is associated with the disappearance of cooperation in Polyrhachis moesta ant queens. Queens of P. moesta facultatively found the colony with genetically unrelated queens. The founding queens perform frequent food exchange with these non-related queens and partake in cooperative brood rearing, whereas single colony queens exclude non-related queens via aggressive behaviour. Thus, aggression is a factor that reduces cooperation. Given that aggression is generally associated with brain OA in insects, we hypothesized that OA controls the behavioural change in cooperation in the ant queen, via an increase in aggression. To test this hypothesis, we compared the amounts of OA and related substances in the brain between founding and colony queens, and observed the interaction of founding queens following oral OA administration. The brain OA levels in colony queens were significantly higher than those in founding queens. Oral administration of OA to founding queens caused significantly less trophallaxis and allogrooming behaviour than in the control founding queens, but with no significant increase in aggression. These results suggest that OA promotes the disappearance of cooperation in founding queens of P. moesta. This is the first study to reveal the neuroendocrine mechanism of cooperation in ant queens.

1. Introduction

Cooperation occurs under certain limiting conditions to ensure survival and reproduction between relatives and between non-relatives in a range of animal taxa. The former can be explained by kin selection theory, the latter by reciprocity, mutualism or manipulation [1]. Costs and benefits have a crucial role in the evolution and maintenance of cooperation. Thus, individuals can break their cooperative relation with other individuals when a change in conditions makes cooperation unbeneﬁcial.

The colony-founding queens in the ant Polyrhachis moesta (Emery) provide an ideal model to explore the transition of cooperation. Mated new queens facultatively aggregate in the cavities of dead twigs and found new colonies (generally, queen number ranged from 1 to 6) [2]. The multiple-founding queens are genetically unrelated and exchange food between one another and cooperatively lay eggs, care for the brood and produce initial workers to establish a colony [2–4]. Cooperation during colony founding results in lower mortality rates of founding queens, a higher production of initial broods, the
higher survival of the founding colonies and shorter periods of colony establishment [5,6]. However, when one or a few workers have emerged, the number of queens reduces and only a single queen is found in a mature colony [2,3]. The queens in the colonies are more aggressive towards other queens than founding queens are [2,4]. This indicates that *P. moesta* queens change their level of cooperation during their lifespan.

Cooperative behaviours may be regulated by biogenic amines, which function as neurotransmitters, neuromodulators or neurohormones, and regulate various behaviours in the nervous system in insects [7,8]. Among these amines, octopamine (OA) promotes the olfactory and visual systems, motor system and reproductive system in insects [7–9]. OA is also linked to aggressive behaviour in insects [9–12]. Given that aggression can reduce or diminish cooperation between individuals, we hypothesized that increased OA signalling reduces cooperation in *P. moesta* queens. In this study, we investigated the association between brain OA and behavioural changes in cooperation in *P. moesta* queens.

2. Material and methods

(a) Colony collection

Nests of *P. moesta* were collected in the Izu Peninsula in Central Japan. Given that queens of *P. moesta* mate between September and October, founding queens were sampled in October (n = 32 used for behavioural observations) and in December (n = 30 for measurements of brain biogenic amine levels). During the collections of the founding queens, queens in mature colonies were also collected (n = 9).

(b) Measurements of brain biogenic amine levels

Queens of *P. moesta* were euthanized using liquid nitrogen and stored until high-performance liquid chromatography with electrochemical detection (HPLC-ECD) analysis. Frozen brains including the suboesophageal ganglion were dissected in ice-cold bee saline [13,14]. Dissected brains were homogenized in ice-cold 0.1 M perchloric acid containing 100 ng ml⁻¹ 3,4-dihydroxyphenylacetic acid for 2 min. Each sample was then centrifuged at 14 000g for 30 min at 4°C. Supernatants were analysed by HPLC-ECD to quantify the biogenic amines present, following the procedures outlined in [13,14].

(c) Behavioural observations

A pair of founding queens were kept in a plastic case (5.5 x 7 x 2 cm). The queens were fed either 2 mg ml⁻¹ DL-octopamine hydrochloride dissolved in 10% sucrose solution (OA-treated queens) or 10% sucrose water (control queens). Eight pairs were selected for each group. They were kept at 25°C in dark conditions. After feeding the solutions, we recorded the queens’ behaviours for 24 h. Five types of behaviour were recorded; antennation, trophallaxis, allogrooming, avoidance after encounter, and attack (electronic supplementary material, table S1). After the observations, the brain biogenic amine levels of the queens were measured using the protocol described above.

(d) Statistical analysis

Mann–Whitney U-tests were performed to analyse the difference between the brain biogenic amine levels of founding and colony queens, between OA-treated and control queens, and the difference in the frequency, total duration and mean duration of behaviours performed by the OA-treated and the control queens.

3. Results

(a) Brain levels of biogenic amine in founding and colony queens

We quantified the brain levels of OA and related substances, including tyramine (TA, a precursor of OA) and N-acetyltyramine (NATA, a metabolite of TA) in the queens (figure 1a). The brain levels of OA in founding queens were significantly lower than those in colony queens (U = 64, p = 0.02, figure 1b), although the distribution of OA levels overlapped between founding and colony queens. No significant difference was detected in TA (U = 83, p = 0.083, figure 1c) and NATA (U = 128, p = 0.82, figure 1d).

(b) Behavioural analyses of octopamine-treated queens

During the 24 h observation period, OA-treated queens were less cooperative than were the control queens. The OA-treated queens performed trophallaxis significantly less frequently than did the control queens (U = 9, 5 = 0.02, figure 2a). Both the total and mean duration of trophallaxis were also significantly shorter in OA-treated queens than in control queens (total duration, OA-treated queen, 1009.1 ± 47.2 s, control queens, 1071.9 ± 319.2 s, mean ± s.e., U = 7.5, p = 0.009; mean duration, OA-treated queen, 9.0 ± 1.1 s, control queens, 21.1 ± 3.9 s, U = 9.5, p = 0.02).

The queens showed a similar trend in allogrooming as seen with trophallaxis. During the observation period, OA-treated queens performed allogrooming significantly less frequently than the control queens (U = 6, p = 0.005, figure 2b). Both the total and mean duration of allogrooming were also shorter in OA-treated queens than in control queens, although there was significant difference only in total duration (OA-treated queen, 1009.1 ± 607.6 s, control queens, 5684.1 ± 194.2 s, U = 9, p = 0.02), not in mean duration (OA-treated queen, 44.7 ± 11.8 s, control queen, 74.7 ± 12.2 s, U = 15, p = 0.083).

The queens showed no significant difference in performing antennation (U = 28, p = 0.721, figure 2c) and avoidance after encounter (U = 21, p = 0.272, figure 2d). During the observation period, attack was observed in one pair of OA-treated queens. It occurred five times and lasted 75 min in total. No attack was observed in control queens.

The brain levels of OA were significantly higher in OA-treated queens than in control queens (U = 66, p = 0.02, table 1), although these distributions overlapped. In TA, dopamine and serotonin, there were no significantly differences between OA-treated and control queens (table 1).

4. Discussion

The mechanisms underlying the disappearance of cooperation between unrelated individuals during colony founding in ant queens have not been clear. Therefore, we examined whether brain OA suppresses cooperative behaviours in *P. moesta* queens by measuring brain OA levels and evaluating various behaviours after oral administration of OA. We found higher
Figure 1. A synthetic and metabolic pathway of phenolamines (a) and the amount of OA (b), TA (c) and NATA (d) in the brain of founding (n = 30) and colony queens (n = 9). Box plots indicate first and third quartiles, median, minimum and maximum values.

Figure 2. Frequency of behaviours in OA-treated and control queens. (a) Trophallaxis, (b) allogrooming, (c) antennation and (d) avoidance. Box plots indicate first and third quartiles, median, minimum and maximum values. n = 8.
OA levels in the brains of colony queens showing aggressive behaviour compared with brains of founding queens showing cooperative behaviours. In addition, there was a significant reduction in cooperative behaviours associated with the increase in brain OA. However, no significant increase in aggression was detected in OA-administered queens. These results indicate that OA can suppress cooperation between founding queens under the behavioural states examined, without an increase in aggression.

Brain OA might be associated with the formation of a social partnership between genetically unrelated queens. The founding queens in *P. moesta* perform more food-begging followed by trophallaxis between unfamiliar individuals than occurs between nest-mate multiple-founding queens, even when the founding queens are supplied with a sufficient food reserve [4]. The queens performing more trophallaxis receive less aggression from nest-mates [15]. Thus, these interactions are suggested to promote social partnership [4]. Indeed, these interactions can result in the transfer of hydrocarbons between nest-mates in ant colonies [16], which is important in nest-mate recognition [3]. In this study, the increase in brain OA suppressed trophallaxis in founding *P. moesta* queens. Thus, the neuroendocrine function of OA is likely to be associated with impeding the development of sociality. Given that social cooperation needs to be continually reinforced by social bonding [16], the low brain OA levels in founding queens might be necessary to maintain the partnership among co-founding queens. The OA precursor, TA, is also a neuromodulator, with various suggested roles in insects [17,18]. We compared the brain TA levels between founding and colony queens, and the levels between OA-treated and control queens. In both results, the statistical values were marginal ($p = 0.08$). As TA has a role in the promotion of reproduction in insects [17], the colony queens with developed ovaries in *P. moesta* may have higher TA levels than founding queens. The higher TA levels in OA-treated queens may promote their reproduction as colony queens, resulting in the disappearance of cooperation.

OA modulates motor activities during flight in locusts (*Schistocerca gregaria*) [8,18] and flight and grooming in honeybee workers (*Apis mellifera*) [19]. In this study, administration of OA to founding queens reduced trophallaxis and allogrooming, but not antennation, avoidance after encounter or attack. Thus, OA can have selective influences on specific behaviours, rather than a general promoting effect on all types of motor activity. This is consistent with Boulay et al. [16], who found no effect of OA on locomotor activity in *Camponotus fellah* workers.

In conclusion, an increase in brain OA in founding *P. moesta* queens reduced cooperation, but not aggression, between genetically unrelated founding queens. Our study revealed, for the first time, the neuroendocrine mechanism underlying the disappearance of cooperation in ant queens. However, further study is needed to investigate whether brain OA regulates cooperation between a queen and workers within the colony and mediates generally the disappearance of cooperation in insect societies.

Data accessibility. Data used for all analyses are available as electronic supplementary material, tables S2–S4, and in Dryad repository (http://dx.doi.org/10.5061/dryad.sc14n).

Authors’ contributions. S.K., T.S. and K.S. designed the study. All authors collected the samples and S.M. and K.S. performed the experiments. All authors analysed the data and gave their ideas to make a draft of the manuscript. S.K. and K.S. wrote the manuscript following discussions with all authors.

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References


# Table 1. Brain levels of biogenic amines in OA-treated and control queens. OA, octopamine; TA, tyramine; DA, dopamine; 5HT: serotonin.

<table>
<thead>
<tr>
<th>OA-treated (pmol/brain)</th>
<th>control (pmol/brain)</th>
</tr>
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<tbody>
<tr>
<td>first quartile median third quartile mean ± s.e.</td>
<td>first quartile median third quartile mean ± s.e.</td>
</tr>
<tr>
<td>OA</td>
<td>0.46</td>
</tr>
<tr>
<td>TA</td>
<td>0.34</td>
</tr>
<tr>
<td>DA</td>
<td>7.02</td>
</tr>
<tr>
<td>5HT</td>
<td>0.74</td>
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</table>

OA = octopamine; TA = tyramine; DA = dopamine; 5HT = serotonin.


