biology letters
Animal behaviour

Biol. Lett. (2008) **4**, 634–637
doi:10.1098/rsbl.2008.0455
Published online 16 September 2008

We know that the wasps 'know': cryptic successors to the queen in *Ropalidia marginata*

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Unlike other primitively eusocial wasps, *Ropalidia marginata* colonies are usually headed by remarkably docile and behaviourally non-dominant queens who are nevertheless completely successful in maintaining reproductive monopoly. As in other species, loss of the queen results in one of the workers taking over as the next queen. But unlike in other species, here, the queen's successor cannot be predicted on the basis of dominance rank, other behaviours, age, body size or even ovarian development, in the presence of the former queen. But the swiftness with which one and only one individual becomes evident as the potential queen led us to suspect that there might be a designated successor to the queen known to the wasps, even though we cannot identify her in the queen's presence. Here, we present the results of experiments that support such a 'cryptic successor' hypothesis, and thereby lend credence to the idea that queen (and potential queen) pheromones act as honest signals of their fertility, in *R. marginata*.

Keywords: *Ropalidia marginata*; primitively eusocial; cryptic successor; potential queen; queen succession; honest signal

1. INTRODUCTION

Primitively eusocial wasp societies (i.e. those that lack queen–worker dimorphism) are known to have active, behaviourally dominant queens who occupy the alpha position in the dominance hierarchies of their colonies. When the queen is lost or removed, the beta individual in the dominance hierarchy takes over as the new queen. Thus, the queen's successor can be identified even in the presence of the original queen (Pardi 1948; West-Eberhard 1969; Röseler *et al.* 1986; Turillazzi 1991; Deshpande *et al.* 2006; Cronin & Field 2007).

By contrast, *Ropalidia marginata* (although a primitively eusocial species) has strikingly docile and behaviourally non-dominant queens. In spite of her docility, however, an *R. marginata* queen is able to maintain complete reproductive monopoly in her colony. When the queen is lost, one of the workers becomes extremely aggressive within minutes. If the queen is not replaced, this individual develops her ovaries and starts laying eggs within a few days. She also gradually decreases her levels of aggression, and eventually becomes a typically docile queen. We have designated this individual as the potential queen (Premnath *et al.* 1996; Gadagkar 2001; Sumana & Gadagkar 2001; Kardile & Gadagkar 2002). Unlike other species, potential queens do not occupy any unique rank in the dominance hierarchies of their colonies and they cannot be predicted on the basis of their body size, age and even their ovarian development, in the queen's presence (Chandrashekara & Gadagkar 1992; Deshpande *et al.* 2006).

We undertook a specific comparative study of the more typical primitively eusocial species *Ropalidia cyathiformis* and *R. marginata* to predict the queen's successors in the two species. While we could predict the identity of the potential queen in all colonies of *R. cyathiformis*, the potential queens of *R. marginata* colonies were completely indistinguishable from other workers (Deshpande *et al.* 2006). Nevertheless, the facts that (i) only one individual steps up her aggression after queen removal, (ii) the swiftness with which she does so and (iii) she alone is unanimously accepted by the rest of the workers led us to suspect that, just as in other primitively eusocial species, there may also be a designated successor to the queen in *R. marginata*, who, however, is 'cryptic' to us in the presence of the queen. In this paper, we present the results of experiments that support such a 'cryptic successor hypothesis'.

2. MATERIAL AND METHODS

Each experiment lasted for two days. On the first day, behavioural observations were carried out on the unmanipulated colony. The queen was identified by her egg-laying behaviour, prior to the experiment. On the second day, the nest was split into two fragments that were fixed on either side of a wire mesh partition in a closed cage. The adults were randomly distributed between the two sides such that one fragment had the queen and half the set of workers (queenright (QR) fragment) and the other had only half the set of workers (queenless (QL) fragment). This experimental design has previously proved successful in demonstrating that the workers cannot sense their queen across the mesh partition (Sumana *et al.* 2008), and it permits a single observer to observe both nest fragments simultaneously.

Behavioural observations on day 2 were made in three sessions. After fixing the nest and releasing the adults, observations were carried out for 2 hours. A potential queen always became evident due to her elevated levels of aggression on the QL fragment during this session. This individual was designated as the potential queen 1 (PQ1), because she was observed to establish herself as the potential queen in session 1. At the end of session 1, the queen and PQ1 were removed from their respective sides and released on the opposite sides of the mesh. This was termed as Q–PQ exchange. Thus, the previous QL side now became the QR side and vice versa. Two hours of behavioural observations were then carried out as before. In some experiments, PQ1 continued as the potential queen on the new QL side. In other experiments, a different individual became the potential queen in the new QL side, and we designated her as PQ2. At the end of session 2, another Q–PQ (PQ1 or PQ2, as the case may be) exchange was carried out, and again observations were made for two hours in session 3.

If *R. marginata* colonies have cryptic successors to their queens, we make the following two predictions in the outcome of the experiment described above.

(i) Since the workers are randomly distributed between the two sides, the cryptic successor has a 50 per cent chance of being on either the QR or the QL fragment. In those experiments where the cryptic successor happened to be in the QL side, she would become PQ1 and, being the true successor, she should be acceptable to the workers on both sides. Hence, she should remain as the potential queen even when she is moved to the opposite side and there should be no PQ2 in session 2. In those experiments where the cryptic successor happened to be on

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2008.0455 or via http://journals.royalsociety.org.

Received 13 August 2008
Accepted 28 August 2008

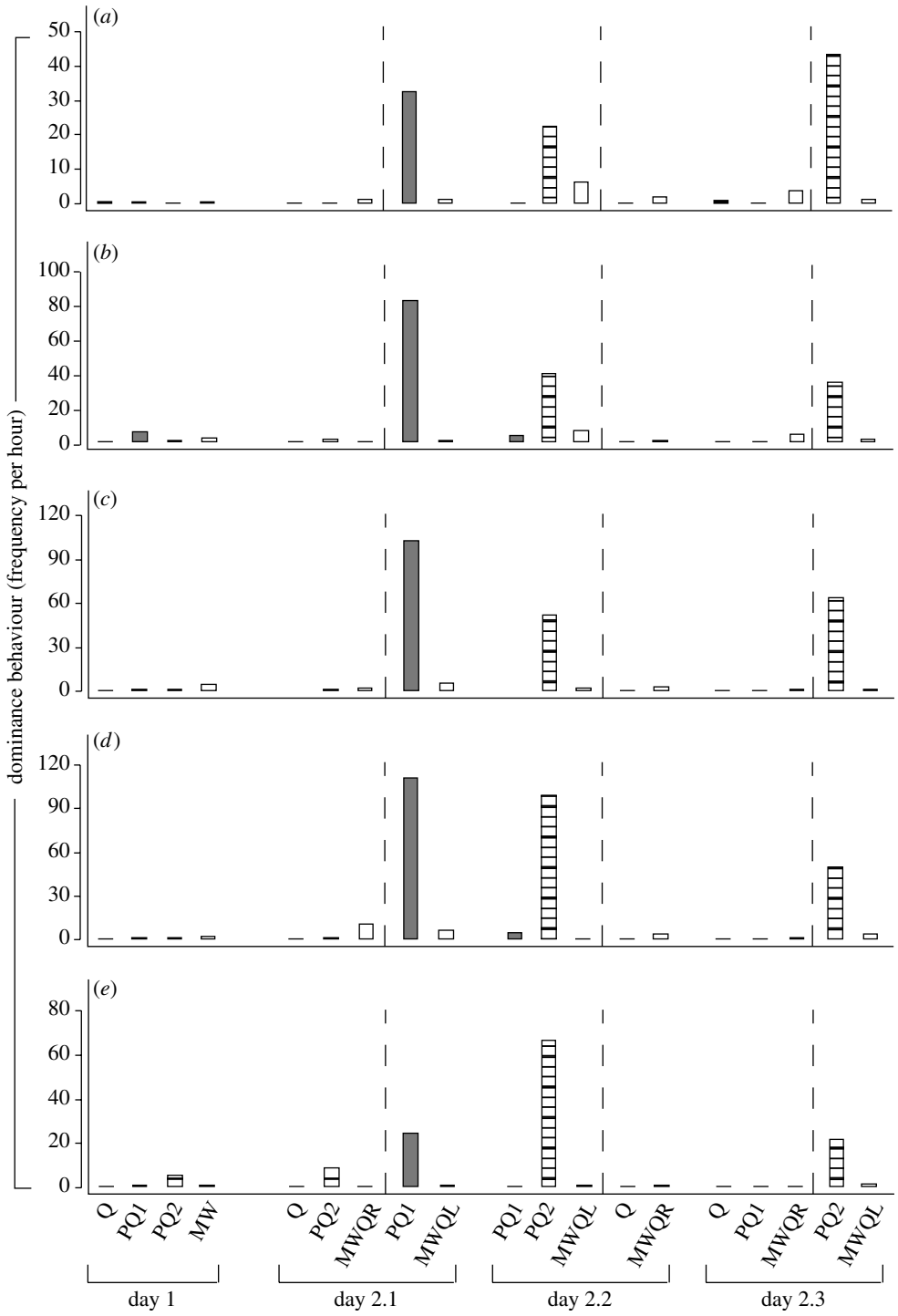

Figure 1. The frequency per hour of dominance behaviour exhibited by the queen, PQ1, PQ2 and max worker (MW) on day 1 in the normal colony, and on the QR and QL fragments in the three sessions on day 2 in the five colonies ((*a*) V532, (*b*) V586, (*c*) V621, (*d*) V638 and (*e*) V657) in which the PQ2 was the cryptic successor.

the QR side, a different individual should become the PQ1 on the QL side, due to the absence of the legitimate successor on this side. Hence, she should lose to the real cryptic successor in the opposite side and there should therefore be a PQ2 in session 2. Thus, PQ1 should lose her status upon exchange and there should be a PQ2 in about half the experiments.

(ii) Since the cryptic successor has to be either PQ1 or PQ2, there should be no third individual who can lay claim to the status of a potential queen. Hence, there should be no PQ3 in session 3, in any of the experiments.

3. RESULTS

All eight colonies used in the experiments had an egg-laying queen on day 1. In session 1, on day 2, a potential queen, whom we designated as PQ1, was observed in the QL side in all the colonies. When the PQ1 was exchanged with the queen in session 2, a new individual, designated as PQ2, was seen to establish herself as the potential queen on the new QL side, in five of the eight colonies (figure 1). In the remaining three colonies, the PQ1s held their position as the potential queen even in the new QL side, and no other individual was seen to step up her rate of aggression (figure 2). Since a PQ2 was observed to establish herself in about half (five out of the eight) colonies, the first prediction of the cryptic successor hypothesis is upheld.

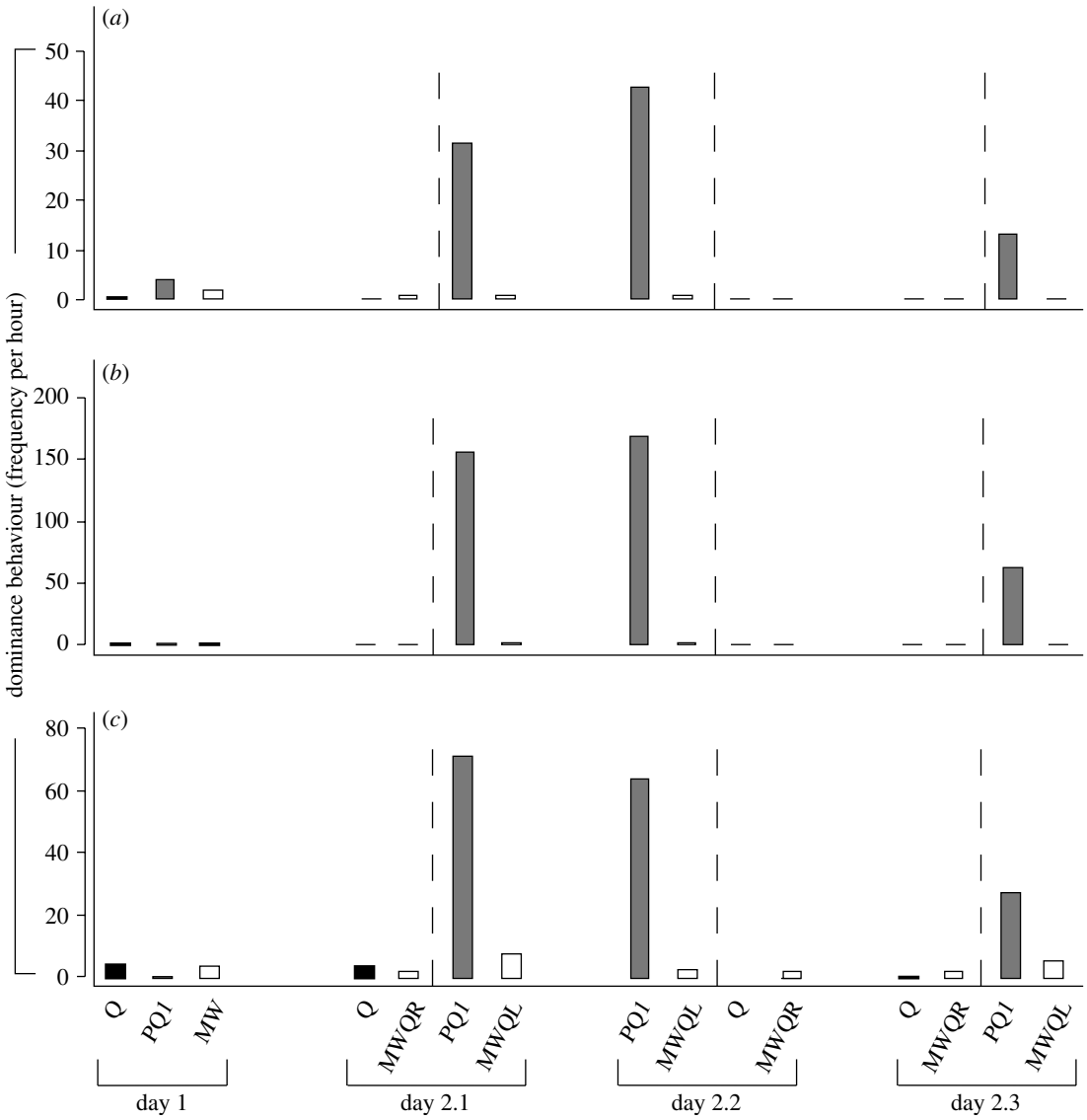

Figure 2. The frequency per hour of dominance behaviour exhibited by the queen, PQ1 and MW on day 1 in the normal colony, and on the QR and QL fragments in the three sessions on day 2 in the three experiments ((*a*) V519, (*b*) V614 and (*c*) V800) in which the PQ1 was the cryptic successor.

In the three colonies where the PQ1s held their position in session 2, they continued to do so in session 3. Similarly, in the five colonies where there was a PQ2 in session 2, they (the PQ2s) continued to hold their position in session 3. Thus, no PQ3 was evident in any colony, thereby upholding the second prediction of the cryptic successor hypothesis (figures 1 and 2).

4. DISCUSSION

Because both the predictions of the cryptic successor hypothesis are upheld, we claim that even in *R. marginata*, there is a designated successor to the queen, as in other species. But we refer to her as a cryptic successor because, in this species, we cannot identify her in the presence of the queen by the same criteria that are adequate to identify the potential queen in a typical primitively eusocial species such as *R. cyathiformis*.

There is another important aspect of our results that we wish to highlight. In all colonies and in all sessions, the individuals we designated as the potential queens did not receive a single act of aggression from any individual, although they themselves showed high levels of aggression. More interestingly, the PQ1, on being released on the new QL fragment in session 2, did not show any aggression at all towards the PQ2, even though the PQ2 took several minutes to initiate aggression after the exchange and the PQ2 did not usually direct any aggression towards PQ1. In fact, the PQ1 showed very little or no aggression at all on exchange in all the colonies where a PQ2 was seen (figure 1). Thus, the presence of the PQ2 was obvious to PQ1 even before the PQ2 had begun to show aggression. Hence, our claim that the cryptic successor is 'known' to the wasps.

In having a designated successor who alone is acceptable to all the wasps, *R. marginata* appears to be similar to other primitively eusocial species, the only difference being that the successor in *R. marginata* cannot be identified in the presence of the queen. However, *R. marginata* may be unique in the fact that the successor receives no challenge from any other

wasp, including the PQ1 who was behaving as a successor in her fragment only a few minutes ago. If *R. marginata* is indeed unique in this regard, our claim that the wasps 'know' is stronger in the context of our species than it would be for species in which the designated successor is subjected to some challenge before being accepted.

But why is *R. marginata* so different from other primitively eusocial species in having cryptic rather than obvious successors to the queen? We have speculated that *R. marginata* queens may use pheromones to regulate, rather than use physical aggression to inhibit, worker reproduction (Premnath *et al.* 1996; Sumana & Gadagkar 2003; Bhadra *et al.* 2007; Sumana *et al.* 2008). If this speculation is correct, it may well be that the successor is known to the wasps in a colony, on account of her unique smell, rather than her unique behaviour, in the presence of the queen. This of course would make her cryptic to us.

And why is *R. marginata* so different from other primitively eusocial species in its absence of challenge to the queen's successors? If our speculation that *R. marginata* queens use pheromones to regulate worker reproduction is true, then such a difference is to be expected. It has been convincingly argued that while suppression of worker reproduction against their inclusive fitness interests is possible when queens use physical aggression, such suppression by pheromones cannot be expected to be evolutionarily stable. Queen pheromones may thus be more correctly said to provide an honest signal of queen fertility to the workers who in turn would be selected to suppress their own reproduction for optimum inclusive fitness gains (Keller & Nonacs 1993). It is therefore not surprising that in typical primitively eusocial species where aggression is used to suppress reproduction, potential queens face a challenge from other contenders. On the other hand, since *R. marginata* queens employ pheromones for regulation of reproduction, it is not surprising that potential queens are not challenged, as their fertility must also be honestly signalled by their smell. This of course raises the question of why the potential queens themselves are so aggressive. We are gathering evidence that the aggression of potential queens helps them to develop their ovaries rapidly (Lamba *et al.* 2007).

Our experiments comply with regulations for animal care in India.

This work was supported by grants from the Department of Science and Technology, Department of Biotechnology and the Ministry of Environment and Forests, Government of India (to R.G.), and a CSIR Junior Research fellowship to A.B. A.B. and R.G. designed the study with inputs from Dr Sujata Deshpande. Behavioural observations and data analysis were carried out by A.B. A.B. and R.G. co-wrote the paper and R.G. supervised the work. We are grateful to Keith Jensen, John Wenzel and Jeremy Field for their helpful comments on an earlier version of this manuscript.

Bhadra, A., Iyer, P. L., Sumana, A., Deshpande, S. A., Ghosh, S. & Gadagkar, R. 2007 How do workers of the primitively eusocial wasp *Ropalidia marginata* detect the presence of their queens? *J. Theor. Biol.* **246**, 574–582. (doi:10.1016/j.jtbi.2007.01.007)

Chandrashekara, K. & Gadagkar, R. 1992 Queen succession in the primitively eusocial tropical wasp *Ropalidia marginata* (Lep.) (Hymenoptera: Vespidae). *J. Insect Behav.* **5**, 193–209. (doi:10.1007/BF01049289)

Cronin, A. J. & Field, J. 2007 Social aggression in an age-dependent dominance hierarchy. *Behaviour* **144**, 753–765. (doi:10.1163/156853907781476436)

Deshpande, S. A., Sumana, A., Surbeck, M. & Gadagkar, R. 2006 Wasp who would be queen: a comparative study of two primitively eusocial species. *Curr. Sci.* **91**, 332–336.

Gadagkar, R. 2001 *The social biology of* Ropalidia marginata: *toward understanding the evolution of eusociality*. Cambridge, MA: Harvard University Press.

Kardile, S. P. & Gadagkar, R. 2002 Docile sitters and active fighters in paper wasps: a tale of two queens. *Naturwissenschaften* **89**, 302–304. (doi:10.1007/s00114-002-0306-2)

Keller, L. & Nonacs, P. 1993 The role of queen pheromones in social insects: queen control or queen signal? *Anim. Behav.* **45**, 787–794. (doi:10.1006/anbe.1993.1092)

Lamba, S., Kazi, Y. C., Deshpande, S., Natesh, M., Bhadra, A. & Gadagkar, R. 2007 A possible novel function of dominance behaviour in queen-less colonies of the primitively eusocial wasp *Ropalidia marginata*. *Behav. Process.* **74**, 351–356. (doi:10.1016/j.beproc.2006.12.003)

Pardi, L. 1948 Dominance order in *Polistes* wasps. *Physiol. Zool.* **21**, 1–13.

Premnath, S., Sinha, A. & Gadagkar, R. 1996 Dominance relationship in the establishment of reproductive division of labour in a primitively eusocial wasp (*Ropalidia marginata*). *Behav. Ecol. Sociobiol.* **39**, 125–132. (doi:10.1007/s002650050274)

Röseler, P.-F., Röseler, I. & Strambi, A. 1986 Studies of the dominance hierarchy in the paper wasp, *Polistes gallicus* (L.). *Monitore Zool. Ital.* (*N.S.*) **20**, 283–290.

Sumana, A. & Gadagkar, R. 2001 The structure of dominance hierarchies in the primitively eusocial wasp *Ropalidia marginata*. *Ethol. Ecol. Evol.* **13**, 273–281.

Sumana, A. & Gadagkar, R. 2003 *Ropalidia marginata*: a primitively eusocial wasp society headed by behaviourally non-dominant queens. *Curr. Sci.* **84**, 1464–1468.

Sumana, A., Deshpande, S. A., Bhadra, A. & Gadagkar, R. 2008 Workers of the primitively eusocial wasp *Ropalidia marginata* do not perceive their queen across a wire mesh partition. *J. Ethol.* **26**, 207–212. (doi:10.1007/s10164-007-0049-9)

Turillazzi, S. 1991 The Stenogastrinae. In *The social biology of wasps* (eds K. G. Ross & R. W. Matthews), pp. 74–98. Ithaca, NY: Comstock.

West-Eberhard, M. J. 1969 The social biology of polistine wasps. *Misc. Publ. Mus. Zool. Univ. Mich.* **140**, 1–101.