

Queens and Workers of the Primitively Eusocial Wasp *Ropalidia marginata* do not Differ in Their Dufour's Gland Morphology

by

Aniruddha Mitra¹ & Raghavendra Gadagkar^{1,2}

ABSTRACT

Ropalidia marginata is a primitively eusocial paper wasp found in peninsular India, where recent work suggests the role of the Dufour's gland hydrocarbons in queen signaling. It appears that the queen signals her presence to workers by rubbing the tip of her abdomen on the nest surface, thereby presumably applying her Dufour's gland secretion to the nest. Since the queen alone produces pheromone from the Dufour's gland and also applies it on the nest surface, the activity level of queen gland should be higher than that of worker gland, as the gland contents would have to get replenished periodically for queens but not for workers. The difference in activity level can be manifested in difference in Dufour's gland morphology, larger glands implying higher activity levels and smaller glands implying lower activity levels, as positive correlation between gland size and gland activity has been reported in exocrine glands of various taxa (including Hymenopteran insects). Hence we investigated whether there is any size difference between Dufour's glands of queens and workers in *R. marginata*. We found that there was no difference between queens and workers in their Dufour's gland size, implying that Dufour's gland activity and Dufour's gland size are likely to be uncorrelated in this species.

Keywords: Dufour's gland; morphometry; *Ropalidia marginata*; queens; workers

¹Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India.

²Evolutionary and Organismal Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore 560064, India.

*Corresponding author, email: mitra.aniruddha@gmail.com

INTRODUCTION

Monopolization of reproduction by the queen is an important distinguishing feature of eusocial insect colonies. *Ropalidia marginata* (Hymenoptera: Vespidae) is a primitively eusocial (queens and workers morphologically similar) paper wasp found in peninsular India. *R. marginata* queens are remarkably docile and thereby cannot be using dominance behavior to maintain reproductive monopoly (Gadagkar 2001). Therefore the queen must use other means, possibly pheromone, to suppress worker reproduction (Sumana & Gadagkar 2003). Recent work has shown the Dufour's gland, a small exocrine gland located near the tip of the abdomen, to be at least one source of the queen pheromone. In a bioassay it was found that a macerate of the queen's Dufour's gland can act as a proxy for the queen herself, thereby elucidating the role of this gland in queen signaling (Bhadra *et al.* 2010). Chemical analysis of Dufour's glands revealed long chain hydrocarbons that were found to be correlated with the state of ovarian activation of queens (Bhadra *et al.* 2010; Mitra *et al.* 2011; Mitra & Gadagkar 2011a, b), and it was also shown that the Dufour's gland hydrocarbon composition may act as an honest signal of fertility (Mitra & Gadagkar 2011a, b). These evidences unequivocally suggest the role of the Dufour's gland in queen signaling, and it appears that the queen signals her presence to workers by rubbing the tip of her abdomen on the nest surface, thereby presumably applying her Dufour's gland secretion to the nest (Bhadra *et al.* 2007). Workers appear to perceive the presence of their queen through her Dufour's gland compounds and react accordingly (Bhadra *et al.* 2010).

The activity of a gland is often correlated with the size of the gland. It has been found in both vertebrates as well as invertebrates that increase in activity of an exocrine gland can be reflected in increase of its size, and positive correlations between quantity of secretions and rates of biosynthesis with sizes of glands have been reported (Herrera 1992; Elmèr & Ohlin 1969; Ravi Ram & Ramesh 2002; Hassani *et al.* 2010; Tobe & Stay 1985; Roseler *et al.* 1980). With reference to the Dufour's gland, Ali *et al.* (1988) have found that gland size corresponds with amount of secretion in workers of the ant *Formica sanguinea*. Other studies have also suggested a connection between gland activity, gland size and reproductive status. Workers with well-developed

ovaries and egg-laying workers had larger Dufour's gland diameters than workers with poorly developed ovaries in the bumble bee *Bombus terrestris*, and gland size of queens also were found to increase with reproductive activity (Abdalla *et al.* 1999a, b).

In *R. marginata*, since the queen alone produces pheromone from the Dufour's gland and also applies it on the nest surface, possibly by rub abdomen behavior, the activity level of queen gland should be higher than that of worker gland, as the gland contents would have to get replenished periodically for queens but not for workers. The difference in activity level can be manifested in difference in gland size, larger gland size implying higher activity levels and smaller gland size implying lower activity levels. Hence the objective of our study was to see whether there is any size difference between Dufour's glands of queens and workers.

MATERIALS AND METHODS:

Six post emergence nests of *R. marginata* were used in this study. *R. marginata* forms aseasonal, perennial colonies and nests are founded and abandoned throughout the year. The study was conducted from September 2008 to September 2009. Nests were collected from various localities in Bangalore (13° 00' N and 77° 32' E), and transplanted to the vespiary at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore. The nests were maintained in closed cages made of wood and fine mesh, and provided with *ad libitum* food, water and building material. All wasps were uniquely color-coded with small spots of Testors® enamel paints (Gadagkar 2001) and, the queen was identified by egg-laying behaviour prior to beginning the study. The queen along with six randomly chosen workers were collected from each nest and the Dufour's gland of each wasp was dissected out along with the sting in Ringer's solution under a stereomicroscope (Wild M3Z). Each gland was photographed using a camera attached to a stereomicroscope (Leica S8AP0), using the software Leica IM 50, version 4.0.

The following measurements were made from the image of each gland using the same software (Fig 1):

total length of the gland (breaking up the length into several short straight-line segments and then summing them up) (μm)

maximum width of the gland (μm)

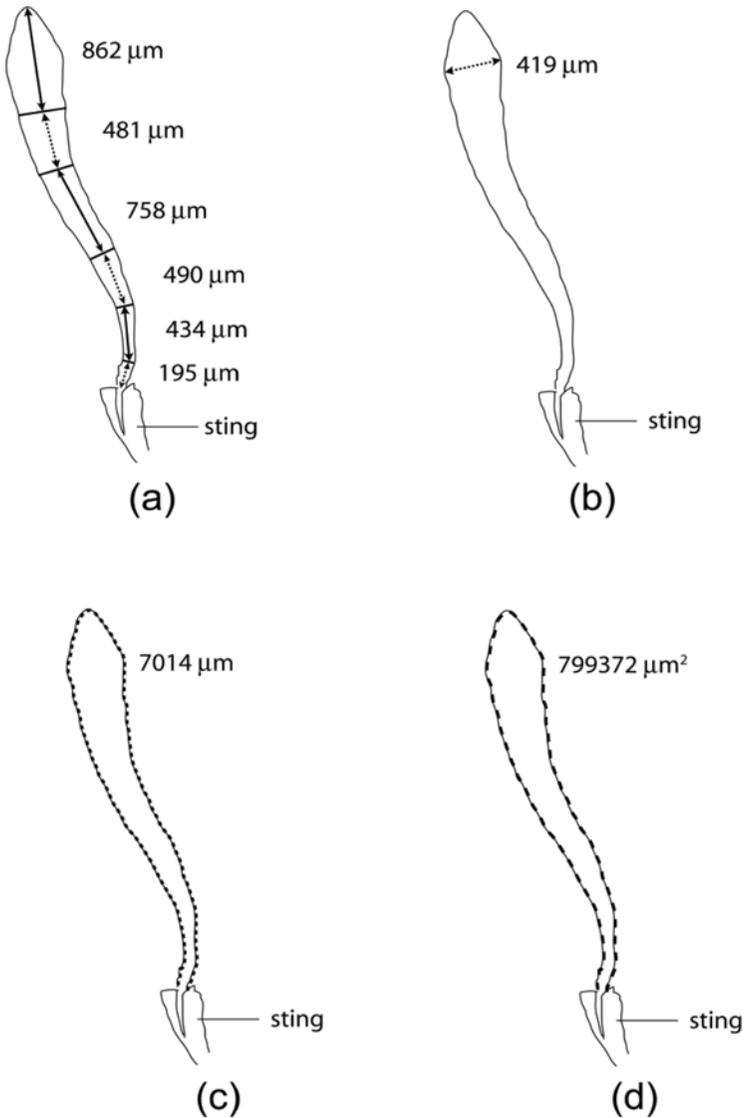


Fig 1: Diagrammatic representation of the measurements made from the image of Dufour's gland of *Ropalidia marginata*; (a): length of the gland (breaking up the length into several short straight-line segments and summing them up), (b): maximum width of the gland, (c): perimeter of the gland's image, and (d): area covered by the gland's image. (Diagram made by tracing from photograph, drawing: Aniruddha Mitra.)

perimeter of the gland's image (μm)
 area covered by the gland's image (μm^2)

Analysis of variance (ANOVA) was done to look at morphological difference between glands of queens and workers with respect to the four measurements made. Dufour's glands measurements were subjected to a principal components analysis and Dufour's gland size index calculated by taking scores of each individual on the first principal component (which accounted for 83.33% of total variance). Dufour's gland size indices of queens and workers were compared by a Mann Whitney U test. Following morphometry, the Dufour's glands were subjected to gas chromatography for another study (Mitra & Gadagkar 2011a).

Body measurements were done for each wasp by making 24 measurements (in mm) of different body parts (Table 1) using a stereomicroscope (Wild M3Z), and the measurements subjected to a principal components analysis. The score of each individual on the first principal component (which accounted for 55.6% of total variance) was used as an index of body size. Correlation between body size index and Dufour's gland size index was checked by Spearman rank correlation, and since a positive correlation was found, Dufour's gland size parameters were corrected by dividing with transformed body size index (made positive to get rid of negative values by linear transformation, done by adding 10 to all the values) and the resulting corrected values were subjected to ANOVA.

Statistical analyses were done using StatistiXL 1.7.

Table 1: List of 24 measurements made from different body parts of *Ropalidia marginata* for calculating body size index.

#	Measurement
1	Intra-ocellar distance
2	Ocello-optic distance (right)
3	Ocello-optic distance (left)
4	Head width
5	Head length
6	Mesoscutum width
7	Mesoscutum length
8	Alitrunk length
9	Wing length (left)
10	Length of 1st marginal cell (left wing)
11	Number of hamuli (left wing)
12	Length of 1st gastral segment
13	Width of 1st gastral segment
14	Height of 1st gastral segment
15	Length of 2nd gastral segment
16	Width of 2nd gastral segment
17	Height of 2nd gastral segment
18	Clypeus length
19	Clypeus width
20	Length of 1st antennal segment (right)
21	Length of 1st antennal segment (left)
22	Width of 1st antennal segment (right)
23	Width of 1st antennal segment (left)
24	Inter-antennal socket distance

RESULTS

Overall morphology of the Dufour's glands of queens and workers appeared to be similar. We did not find any difference between queens and workers with respect to any of the four parameters of their Dufour's gland size (Fig. 2).

Multivariate ANOVA: $F = 1.358$, $p = 0.267$

Univariate ANOVA:

Length of gland: $F = 0.007$, $p = 0.936$

Maximum width of gland: $F = 0.250$, $p = 0.620$

Perimeter of gland's image: $F = 0.001$, $p = 0.973$

Area covered by gland's image: $F = 0.795$, $p = 0.378$

In the Dufour's gland size index, it was found that workers had a greater range of gland size (having both undeveloped as well as large glands). Queens had a smaller range of gland size, but were not different from workers (Fig. 3) (Mann Whitney U test: $U = 124$, $p = 0.586$).

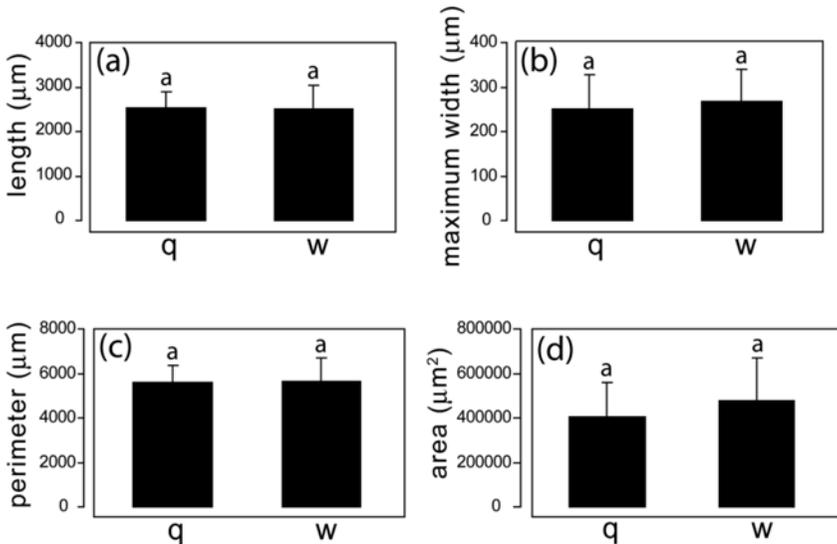


Fig 2: Mean and standard deviation of (a) length of the Dufour's gland, (b) maximum width of the gland, (c) perimeter of the gland's image and (d) area covered by the gland's image in queens (q) and workers (w) of *Ropalidia marginata*. Bars are carrying the same letter signifying that there is no difference between queens and workers for any of the four variables (ANOVA, $p > 0.05$, $n = 6$ queens and 36 workers).

Body size index and Dufour's gland size index were positively correlated implying that wasps with larger body size tend to have a larger Dufour's gland as well (Spearman rank correlation: $r_s = 0.311$, $p = 0.046$, $n = 84$).

Because body size was positively correlated with Dufour's gland size, all the Dufour's gland size parameters were corrected for body size and the resulting corrected values were subjected to ANOVA. There was no difference between queens and workers even after correcting for body size.

Multivariate ANOVA (corrected for body size): $F = 1.163$, $p = 0.344$

Univariate ANOVA (corrected for body size):

Length of gland: $F = 1.631$, $p = 0.209$

Maximum width of gland: $F = 1.165$, $p = 0.287$

Perimeter of gland's image: $F = 1.610$, $p = 0.212$

Area covered by gland's image: $F = 2.193$, $p = 0.147$

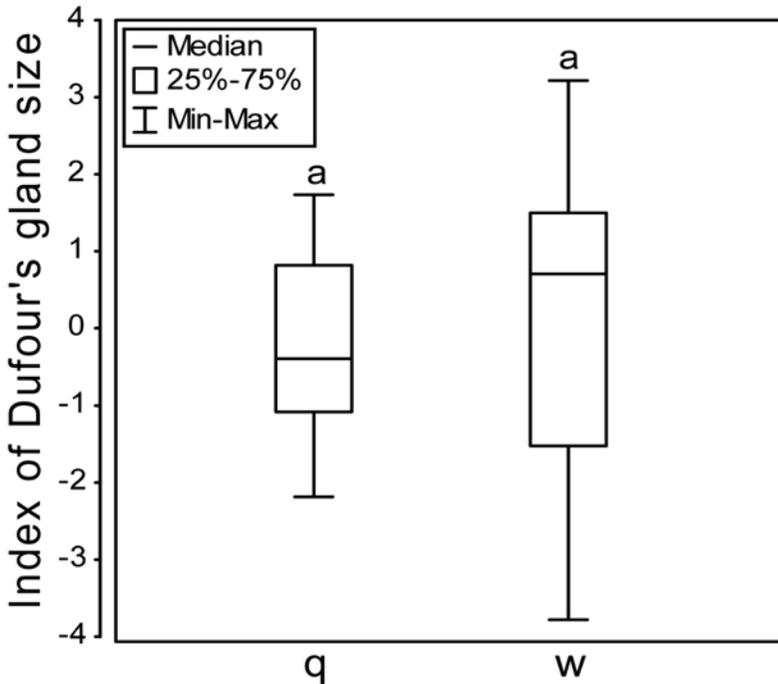


Fig 3: Dufour's gland size indices of queens (q) and workers (w) of *Ropalidia marginata*. Distributions carry the same alphabet indicating that they are not significantly different (Mann Whitney U test, $p > 0.05$, $n = 6$ queens and 36 workers).

DISCUSSION

We found that there was no difference between queens and workers in their Dufour's gland size. In Polistine wasps, Fratini *et al.* (1996) found in *Polistes dominulus* that Dufour's glands were larger in foundresses than in workers. In *R. marginata* however, the Dufour's gland of the queen is not different in size from that of the workers. Since the queen is the sole reproductive in a colony and she alone produces pheromone from the Dufour's gland, and also applies it on the nest surface, possibly by rub abdomen behaviour (Gadagkar 2001; Bhadra *et al.* 2007; Bhadra *et al.* 2010), we expected that the activity level of queen glands should be higher than worker glands, which can be reflected in size difference between Dufour's glands of queens and workers. Our results suggest that even if there is activity difference between Dufour's glands of queens and workers, this does not translate to size difference. Our results are reminiscent of what was found in *Polistes fuscatus*, where egg laying foundresses do not necessarily have larger Dufour's glands than subordinates (Downing & Jeanne 1983).

It may be possible that increase in secretory activity of a gland can be manifested in other ways like increase in abundance of cell organelles likely to be involved in secretion, rather than increase in gland size. Some studies suggest that actual synthesis of compounds in Hymenopteran exocrine glands might be low and such glands might be involved in uptake of compounds from the haemolymph and transporting them to the lumen of the gland, while the compounds themselves are synthesized elsewhere (Abdalla & Cruz-Landim 2004; Strohm *et al.* 2010). Since the Dufour's gland in *R. marginata* has been found to contain long chain linear and branched alkanes, which are typically found on the cuticle of insects (Bhadra *et al.* 2010; Mitra *et al.* 2011), it is possible that the Dufour's gland compounds are synthesized in oenocytes, which are usually involved in synthesis of insect hydrocarbons, and are then transported through the haemolymph to the Dufour's gland, and get sequestered there, as has been suggested for other Hymenopteran exocrine glands (Strohm *et al.* 2010). Hence difference in Dufour's gland activity need not necessarily translate to difference in gland size, as has been suggested in other endocrine and exocrine glands as well (Roseler *et al.* 1980; Zouboulis & Boschnakow 2001).

We conclude that Dufour's gland activity and Dufour's gland size are uncorrelated in *R. marginata*, and thereby any activity difference that exists between Dufour's glands of queens and workers does not translate to difference in Dufour's gland size.

ACKNOWLEDGMENTS

We thank the Department of Science and Technology, the Department of Biotechnology, the Council for Scientific and Industrial Research and the Ministry of Environment and Forests, Government of India for financial assistance. Dissections, morphometry and statistical analyses were done by AM. The paper was co-written by AM and RG, and RG supervised the overall work. All experiments reported here comply with the current laws of the country in which they were performed.

REFERENCES

- Abdalla, F.C. & C. da Cruz-Landim 2004. Occurrence, morphology and ultrastructure of the Dufour gland in *Melipona bicolor* Lepleiter (Hymenoptera, Meliponini). *Revista Brasileira de Entomologia* 48(1):9-19.
- Abdalla, F.C., H. Velthuis, M.J. Duchateau & C. da Cruz-Landim 1999. Secretory cycle of the Dufour's gland in workers of the bumble bee *Bombus terrestris* L. (Hymenoptera: Apidae, Bombini). *Netherlands Journal of Zoology* 49(3):139-156.
- Abdalla, F.C., H. Velthuis, C. da Cruz-Landim & M.J. Duchateau 1999. Changes in the morphology and ultrastructure of the Dufour's gland during the life cycle of the bumble bee queen, *Bombus terrestris* L. (Hymenoptera: Bombini). *Netherlands Journal of Zoology* 49(4):251-261.
- Ali, M.F., A.B. Attygalle, J.P.J. Billen, B.D. Jackson & E.D. Morgan 1988. Change of Dufour's gland contents with age of workers of *Formica sanguinea* (Hymenoptera: Formicidae). *Physiological Entomology* 13:249-255.
- Bhadra, A., P.L. Iyer, A. Sumana, S.A. Deshpande, S. Ghosh & R. Gadagkar R 2007. How do workers of the primitively eusocial wasp *Ropalidia marginata* detect the presence of their queens? *Journal of Theoretical Biology* 246:574-582.
- Bhadra, A., A. Mitra, S.A. Deshpande, K. Chandrasekhar, D.G. Naik, A. Hefetz & R. Gadagkar 2010. Regulation of reproduction in the primitively eusocial wasp *Ropalidia marginata*: on the trail of the queen pheromone. *Journal of Chemical Ecology* 36:424-431.
- Downing, H.A. & R.L. Jeanne 1983. Correlation of season and dominance status with activity of exocrine glands in *Polistes fuscatus* (Hymenoptera: Vespidae). *Journal of the Kansas Entomological Society* 56(3):387-397.
- Elmér, M. & P. Ohlin 1969. Compensatory hypertrophy of the rat's submaxillary gland. *Acta Physiologica Scandinavica* 76(4):396-398.

- Fratini, S., F.R. Dani & S. Turillazzi 1996. Preliminary study on size differences in Dufour's gland of *Polistes dominulus* (Christ) related to caste, season and type of founding (Hymenoptera, Vespidae). *Insect Social Life* 1:95-100.
- Gadagkar, R. 2001. The social biology of *Ropalidia marginata*: toward understanding the evolution of eusociality. Harvard University Press, Cambridge, Massachusetts.
- Hassani, S., R.F. Pour Abad, M.M. Fazel & D. Mohammadi 2010. Morphological differences in metathoracic glands of different populations of Sunn pest, *Eurygaster integriceps* Put. (Heteroptera: Scutelleridae). *Munis Entomology and Zoology* 5(1):266-269.
- Herrera, E.A. 1992. Size of testes and scent glands in capybaras, *Hydrochaeris hydrochaeris* (Rodentia: Caviomorpha). *Journal of Mammalogy* 73(4):871-875.
- Mitra, A. & R. Gadagkar 2011. Can Dufour's gland compounds honestly signal fertility in the primitively eusocial wasp *Ropalidia marginata*? *Naturwissenschaften* 98:157-161.
- Mitra, A. & R. Gadagkar 2011. Queen signal should be honest to be involved in maintenance of eusociality: chemical correlates of fertility in *Ropalidia marginata*. *Insectes Sociaux*. doi: 10.1007/s00040-011-0214-6
- Mitra, A., P. Saha, M.E. Chaoulideer, A. Bhadra & R. Gadagkar 2011. Chemical communication in *Ropalidia marginata*: Dufour's gland contains queen signal that is perceived across colonies and does not contain colony signal. *Journal of Insect Physiology* 57:280-284.
- Ravi Ram, K. & S.R. Ramesh 2002. Male accessory gland secretory proteins in *nasuta* subgroup of *Drosophila*: synthetic activity of Acp. *Zoological Science* 19:513-518.
- Roseler, P.-F., I. Roseler & A. Strambi 1980. The activity of corpora allata in dominant and subordinated females of the wasp *Polistes gallicus*. *Insectes Sociaux* 27(2):97-107.
- Strohm, E., M. Kaltenpoth & G. Herzner 2010. Is the postpharyngeal gland of a solitary digger wasp homologous to ants? Evidence from chemistry and physiology. *Insectes Sociaux* 57(3):285-291.
- Sumana, A. & R. Gadagkar 2003. *Ropalidia marginata* – a primitively eusocial wasp society headed by behaviourally non-dominant queens. *Current Science* 84:1464-1468.
- Tobe, S.S. & B. Stay 1985. Structure and regulation of the corpus allatum. *In*: Berridge, M.J. (ed.). *Advances in Insect Physiology*, Volume 18. London, Academic Press: 305-341.
- Zouboulis, C.C. & A. Boschnakow 2001. Chronological ageing and photoageing of the human sebaceous gland. *Clinical and Experimental Dermatology* 26:600-607.

