



# HOKKAIDO UNIVERSITY

Title	Morphological differences among three female castes: Worker, queen and intermorphic queen in the ant <i>Myrmecina nipponica</i> (Formicidae : Myrmicinae)
Author(s)	Miyazaki, Satoshi; Murakami, Takahiro; Azuma, Noriko et al.
Citation	Sociobiology, 46(2), 363-374
Issue Date	2005
Doc URL	<a href="https://hdl.handle.net/2115/44108">https://hdl.handle.net/2115/44108</a>
Type	journal article
File Information	socioB46-2_363-374.pdf



**Morphological differences among three female castes: worker, queen and intermorphic queen in the ant *Myrmecina nipponica* (Formicidae: Myrmicinae)**

Satoshi Miyazaki<sup>1</sup>, Takahiro Murakami<sup>2</sup>, Noriko Azuma<sup>1, 3</sup>,

Seigo Higashi<sup>1</sup> and Toru Miura<sup>1</sup>

<sup>1</sup> Graduate School of Environmental Earth Science, Hokkaido University,  
Sapporo 060-0810, JAPAN

<sup>2</sup> Center for Developmental Biology, RIKEN, Kobe 650-0047, JAPAN

<sup>3</sup> Present address: Graduate School of Fisheries Sciences, Hokkaido University,  
Hakodate 041-8611, JAPAN

*Correspondence:*

Prof. Toru Miura,

Graduate School of Environmental Earth Science,

Hokkaido University,

Sapporo 060-0810, JAPAN

## ABSTRACT

In social insects, caste morphologies are different depending on their tasks allocated in their colonies. Although many ant species generally possess two female castes: winged queen and wingless worker, in some *Myrmecina* species, some colonies produce a wingless reproductive caste instead of alate queens. This wingless reproductive caste is termed ‘intermorphic’ queen, and its morphological features are intermediate between winged queen and worker. In the present study, we analyzed the morphological features of the three female castes to explore the adaptive meaning of the intermorphics in terms of reproductive strategy, in addition to the developmental and evolutionary origin in *Myrmecina nipponica*. We first performed the principal component analysis based on the morphometric data of the three female castes of adults (alate queen, intermorphic queen and worker). The results of analysis showed that allometry of the intermorphic queen differ from those of alate queen and worker. In intermorphics, compound eyes and gasters were more developed in comparison with heads and thoraxes. We also examined several body parts in detail by scanning electron microscopy in the three female castes. The morphological features of intermorphics varied widely compared with the other two castes. Intriguingly, some parts of intermorphics were queen-like, while others were worker-like. Our findings suggest that the morphological features of intermorphic queens have specialized reproductive strategy involving budding.

## INTRODUCTION

In ant colonies of most species, the reproductive division of labor between queen and worker castes is generally established. Accordingly, the morphological features of adult individuals are specialized according to the caste roles. For example, in many ant species, the queens have well-developed thorax and two pairs of deciduous wings that are necessary for nuptial flight. On the other hand, workers have a relatively simpler thorax and are wingless (Hölldobler and Wilson 1990). In some species, however, in addition to these two distinctive castes, there is another caste, whose morphological traits are intermediate between queen and worker. In ants, such morphologically intermediate castes are thought to have evolved secondarily, as there have been arguments on the origin of those castes, particularly soldier caste (Hölldobler and Wilson 1990; Urbani and Passera 1996; Ward 1997; Urbani 1998). Moreover, with regard to the morphologically intermediate reproductive castes, their evolutionary significance and genetic factors that cause complex caste differentiation have been so far studied in only some species (Heinze and Buschinger 1987, 1989; Peeters 1991; Heinze 1998; Winter and Buschinger 1986).

*Myrmecina nipponica* (= *Myrmecina glaminicola nipponica*) is one of those species, which produce intermediate reproductive castes, termed 'intermorphic queen' (Ohkawara et al. 1993; Murakami et al. 2000, 2002; Kikuchi et al. 2002). The intermorphic queens (here we call them 'intermorphics') in *Myrmecina nipponica* are wingless, and their body size was reported as intermediate between alate/dealate queens (here we call them just 'queens') and workers, although the morphological

traits vary widely and continuously (Ohkawara et al. 1993). Although the reproductive abilities of the queens and intermorphics are not significantly different, which was inferred by the ovariole number (Ohkawara et al. 1993; Murakami et al. 2002), each type of reproductives shows different reproductive strategies (Murakami et al. 2000). The intermorphics are considered to adapt to an environment with low food resources and/or uneven distribution of nest sites (Ohkawara et al. 1993; Murakami et al. 2002). In this species, colonies can be categorized into two types: 'queenright colonies' which are headed by queens, or 'queenless colonies' headed by intermorphics (Ohkawara et al. 1993). Queens are exclusively produced in queenright colonies, whereas intermorphics are produced only in queenless colonies. Therefore, both types of reproductives rarely coexist in the same colony (Ohkawara et al. 1993). In Indonesia and Europe, morphologically intermediate reproductives were reported in *Myrmecina* species (Tsuji et al. 2001; Buschinger et al. 2002; Buschinger 2003). Although a wealth of ecological information is currently available on intermorphics, there is little information on caste differentiation system and developmental origin.

Detailed examination of the specific morphological features of intermorphics, would allow the elucidation of their adaptive significance. Furthermore, examination of the developmental processes of such specific features could allow elucidation of their developmental origin. To our knowledge, detailed examination of the morphological features of various body parts is scarce. In the present study, we analyzed the morphological features of three female castes in detail, and discussed the adaptive significance of intermorphics.

## MATERIALS AND METHODS

### Ants

*Myrmecina nipponica* Wheeler is widespread in Japan (Murakami et al. 1997), inhabiting mosses, fern roots and decayed logs on forest floor. The body length is 3 – 4 mm, and the colony size is approximately 40 individuals (Ohkawara et al. 1993; Murakami et al. 2002). In this study, colonies were sampled in Tomakomai Experiment Forest of Hokkaido University and natural forests near Chitose City from May to September 2004. Adult females were fixed in 70% ethanol later in the laboratory, and used for morphological analyses. Although queens can be readily identified by their external structures of the mesothorax and wing stumps, differentiation of intermorphics from workers is difficult since intermorphics had simplified thoraces that resemble those of workers.

### Morphometric studies

Since allometric differences among castes show the caste tasks in many ant species (Hölldobler and Wilson 1990), we performed morphometric analyses to compare the allometry among the three female castes. Seven body parts, i.e., head length, head width, compound eye width, alitrunk length, pronotal width, gastral length and gastral width, were photographed with a CCD camera (HIM – 1, HOGA, Kyoto, Japan) and measured using an image analysis system. This was followed by principal component analysis of these body parts using the statistical program SSSMCTH2 (ESUMI, Tokyo, Japan).

### **Detailed morphological examination by scanning electron microscopy**

In order to compare the morphological features of the three female castes in more detail, we focused on the following features: compound eye, ocellus, thorax in dorsal view and lateral view, examined by a scanning electron microscope (JOEL, JSM-5510, Tokyo, Japan). These parts showed large variation among the three female castes, and also within intermorphics in *Myrmecina nipponica* (Ohkawara et al. 1993; Murakami et al. 2002).

## RESULTS

### Morphometric studies

In queenright colonies, the compound eye width and alitrunk length showed dimorphic frequency distributions (Fig. 1a). Queens had larger compound eyes and alitrunks than workers. In queenless colonies, on the other hand, although the compound eye width showed a continuous dimorphic distribution, that of alitrunk length was monomorphic (Fig. 1b). According to previous studies on *Myrmecina* species (Buschinger et al. 2002, Murakami et al. 2002), wingless individuals with larger compound eyes were intermorphics, but their alitrunk length is similar to workers.

The results of principal component analysis indicated that the three female castes could be distinguished by analysis of seven body parts (Fig. 2a). The principal component 1 accounted for 80.3% of the total variance and positively correlated with all measurements (Table 1). Therefore, the principal component 1 was considered to show the general body size. The principal component 2 accounted for 7.5% of the total variance, positively correlated with compound eye width, gastral length, and gastral width, and negatively correlated with head length, head width, alitrunk length, and pronotal width (Table 1). These results suggest that this principal component expresses the allometric differences, primarily reflecting the relative size of compound eyes and gaster. The principal component 1 was the highest in queens ( $4.93 \pm 0.78$ ,  $n = 10$ ), followed by intermorphics ( $1.87 \pm 1.08$ ,  $n = 22$ ) and workers ( $-0.90 \pm 1.76$ ,  $n = 101$ ), while the principal component 2 was the highest in intermorphics ( $1.12 \pm 0.40$ ,  $n$

= 22), followed by workers ( $-0.19 \pm 0.54$ ,  $n = 101$ ), and queens ( $-0.51 \pm 0.45$ ,  $n = 10$ ). Thus, the intermorphics had larger compound eyes and gaster relative to the intermediate sizes of head and thorax. The three female castes were well separated in two dimensions of alitrunk length vs. compound eye width (Fig. 2).

### **Detailed morphological examination by SEM**

#### *Compound eyes*

Whereas the mean widths of compound eyes and ommatidia were not notably different between the two reproductives, the number of ommatidia was smaller in intermorphics than in queens (Table 2). Thus, the compound eye of the queen (Fig. 3a) was larger and contained more ommatidia on its surface than that of the intermorphics (Fig. 3b). Workers had the smallest number of ommatidia but each ommatidium was the larger than those of the others (Fig. 3c and Table 2).

#### *Ocelli*

Each queen had three ocelli (Fig. 4a) each measuring 0.5 - 0.6 mm in diameter (Fig. 4d). Many intermorphics had only one ocellus at the position of the anterior ocellus in queens; the diameter of their ocelli was 0.03 mm in maximum (Fig. 4b) and occasionally smaller than 0.01 mm (Fig. 4e), which makes it invisible under a stereomicroscope. A few intermorphics were completely devoid of ocelli (Fig. 4f). Many workers were also devoid of ocelli (Fig. 4h), although a few workers had only one small ocellus measuring 0.01 - 0.03 mm in diameter (Fig. 4c) or even  $< 0.01$  mm (Fig. 4g) in diameter.

*Dorsal view of the thorax*

Queens had well-developed mesonotum, which covered most part of the pronotum (Fig. 5a). Intermorphics showed a large and continuous variation of the mesonotum bulge, in which a relatively developed mesonotum occasionally contained a pair of wing buds (Fig. 5d), while other intermorphics had no wing buds on their relatively bulgy (Fig. 5e) or less bulgy thoraces (Fig. 5f). Workers showed a variation of mesonotum bulge: in Fig. 5, b is relatively bulgy but c is less bulgy. The variability of the mesonotum bulge was smaller in workers than in intermorphics, with a small overlap between the two castes.

*Wing regions*

Queens had pairs of fore- and hindwings on meso- and metapleuron, respectively (Fig. 6a, d). Out of 11 intermorphics in which the lateral view was examined (Fig. 6b), only one individual possessed a pair of forewing buds (Fig. 6e, the same individual as Fig. 5d); two individuals had a small hole at the forewing position, in which there was a small wing bud (Fig. 6b); six individuals had a wing process at the thoracic position for forewing (Fig. 6f); and two individuals had neither wing buds nor processes. All of the 11 intermorphics had neither wing buds nor processes at the hindwing position. Workers were completely apterous with no vestiges of fore- and hindwings (Fig. 6c, g).

*Thoracic spiracles*

All three female castes had a pair of metapleural spiracles (MS in Fig. 6h). In queens, the openings of metapleural spiracles were located in a deep groove between the mesopleuron and metapleuron (Fig. 6i). Intermorphics had a small opening in an invaginated round punctum (Fig. 6j) located at the mesopleuron – metapleuron boundary that was not as conspicuous as in queens. In some intermorphics, the openings were plugged by fragments of unknown substances, probably secretions. In workers, the spiracle openings located at the inconspicuous boundary between the two pleurons were open in some individuals (Fig. 6k), but covered with cuticle in the others (Fig. 6l). Propodeal spiracles and metapleural glands (PS and MG, respectively, Fig. 6h) were morphologically similar in the three castes.

## DISCUSSION

Based on the results of morphometric analyses of the three female castes of *M. nipponica* (Fig. 2), it is clear that intermorphics possess specific morphological features, which are different from those of queens and workers. However, certain body parts of intermorphics resemble those of queens, while others resemble those of workers. For example, compound eyes, gasters and metapleural spiracles are queen-like, while ocellus and thorax are worker-like (Figs. 3-6). It is suggested that these specific-morphological features of intermorphics are related to their reproductive roles, which involve dispersal by budding (Murakami et al. 2000). Namely, intermorphics probably lose their flight abilities and instead invest in reproduction.

The most remarkable differences between the intermorphics and queens are the structures or organs involved in flight. First, the thoracic structures of intermorphics lack functional wings, and are worker-like (Fig. 5). Second, the ocellus does not develop in intermorphics like in queens (Fig. 4). In general, the ocellus of insects is known to be sensitive to light and functions in stabilization of flight (Simmons 2002).

However, intermorphics possess queen-like features such as compound eyes, gasters and metapleural spiracles (Fig. 3 and 6). These features are thought to be important in reproduction. First, intermorphics possess developed reproductive organs and function as reproductives similar to queens (Ohkawara et al. 1993; Murakami et al. 2002). Moreover, intermorphics were suggested to possess poison glands, which release sex pheromone to attract males to their gasters (Buschinger 2003), although no

such glands were found in our SEM studies. Thus, the gastral parts of intermorphics are relatively well developed like queens, probably because the gasters contain important reproductive organs.

Compound eyes are known to resolve visual images (Land 1997).

Intermorphics develop compound eyes as well as queens because they probably need to resolve visual images when mating and budding. In addition, insects that are active under darkness tend to have larger ommatidial facet (Land 1997). The reason why intermorphics, in addition to queens, have smaller ommatidia than workers is probably that they are devoted to reproduction and do not actively move in their colony.

Morphological differences of metapleural spiracles among the three castes (Fig. 6i – l) are also considered to be involved in their caste roles. Spiracles play an important role in gas exchange, when water loss from spiracles must be minimized (Chapman 1998). The metapleural spiracle openings of queens (Fig. 6i) and intermorphics (Fig. 6j) are invaginated from the cuticle surface, suggesting that this structure may function in the control of water loss. Because both types of reproductives come out from their natal nests and disperse by nuptial flight or budding, they must minimize water loss from the spiracles at the time of dispersal. In comparison, workers mainly remain in their underground nests. On the other hand, the metapleural glands, which secrete a mixture of antiseptic substances (Hölldobler and Wilson 1990), were not morphologically different among castes, suggesting that these glands probably serve a similar function in the three castes.

Typically, intermediate castes between queen and worker castes are thought to have evolved secondarily from either type of castes (Wilson 1975). Although the

evolutionary and developmental origin of the intermorphics remains unsolved, the caste developmental pathway of queens or workers would have been modified under given conditions, resulting in the appearance of intermorphics. Based on the accumulated information on the intermorphics in this species, we suggest that the intermorphics possess similar reproductive ability like queens, but are specialized in the dispersal strategy by budding. Studies on caste differentiation of intermediate castes could provide further information that enhances our understanding of the evolution of the caste systems in Formicidae.

## ACKNOWLEDGMENTS

We thank T. Kikuchi, T. Toita, M. Kidokoro and T. Yamada for helping us in field sampling and maintenance of ant colonies. Thanks are also due to F. G. Issa for their comments on the manuscript. This work was supported partly by a Grant-in-Aid for Scientific Research (Nos. 15687001, 17207003).

## REFERENCES

- Buschinger, A., and M. Schreiber 2002. Queen polymorphism and queen-morph related facultative polygyny in the ant, *Myrmecina graminicola* (Hymenoptera, Formicidae). *Insectes Sociaux* 49: 344-353.
- Buschinger, A. 2003. Mating behavior in the ant, *Myrmecina graminicola* (Myrmicinae). *Insectes Sociaux* 50: 295-296.
- Chapman, R. F. 1998. *The Insects: structure and function* (4<sup>th</sup> edition). Cambridge: Cambridge University Press.
- Heinze, J. 1998. Intercastes, intermorphs, and ergatoid queens: who is who in ant reproduction? *Insectes Sociaux* 45: 113-124.
- Heinze, J. and A. Buschinger 1987. Queen polymorphism in a non-parasitic *Leptothorax* species (Hymenoptera, Formicidae). *Insectes Sociaux* 34: 28-43.
- Heinze, J. and A. Buschinger 1989. Queen polymorphism in *Leptothorax* spec. A: its genetic and ecological background (Hymenoptera: Formicidae). *Insectes Sociaux* 36: 139-155.
- Hölldobler, B. and E. O. Wilson 1990. *The ants*. Belknap Press of Harvard University Press, Cambridge, MA.
- Kikuchi, T., R. Yoshioka and S. Higashi 2002. Effects of worker manipulation on sex ratio of a Japanese ant species, *Myrmecina nipponica*. *Eco. Res.* 17: 717-720.
- Land, M. F. 1997. Visual acuity in insects. *Annu. Rev. Entomol.* 42: 147-177.

- Murakami, T., K. Ohkawara, and S. Higashi 2002. Morphology and development plasticity of reproductive females in *Myrmecina nipponica* (Hymenoptera: Formicidae). *Entomol. Soc. Am.* 95: 577-582.
- Murakami, T., L. Wang and S. Higashi 2000. Mating frequency, genetic structure, and sex ratio in the intermorphous female producing ant species *Myrmecina nipponica*. *Eco. Entomol.* 25: 341-347.
- Murakami, T., Y. Ishibashi, S. Abe, M. C. Yoshida and S. Higashi 1997. CAP-PCR useful for analyzing the kin relatedness of a small ant species. *Naturwissenschaften* 84: 32-34.
- Ohkawara, K., F. Ito, and S. Higashi 1993. Production and reproductive function of intercastes in *Myrmecina graminicola nipponica* colonies (Hymenoptera: Formicidae). *Insectes Sociaux* 40: 1-10.
- Peeters, C. P. 1991. Ergatoid queens and intercastes in ants: two distinct adult forms which look morphologically intermediate between workers and winged queens. *Insectes Sociaux* 38: 1-15.
- Simmons, P. J. 2002. Signal processing in a simple visual system: The locust ocellar system and its synapses. *Microsc. Res. Tech.* 56: 270-280.
- Tsuji, K., K. Ohkawara, and F. Ito 2001. Inter-nest relationship in the Indonesian ant, *Myrmecina* sp. A with some considerations for the evolution of unicoloniality in ants. *Tropics* 10: 409-420.
- Urbani, C. B. 1998. The number of castes in ants, where major is smaller than minor and queens wear the shield of the soldiers. *Insectes Sociaux* 45: 315-333.
- Urbani, C. B. and L. Passera 1996. Origin of ant soldiers. *Nature* 383: 223-224.

- Ward, P. S. 1997. Ant soldiers are not modified queens. *Nature* 385: 494-495.
- Wilson, E. O. 1975. *Sociobiology*. Belknap Press of Harvard University Press, Cambridge, MA.
- Winter, U. and A. Buschiger 1986. Genetically mediated polymorphism and caste determination in the slave-making ant, *Harpagoxenus sublaevis* (Hymenoptera: Formicidae). *Entomol. Gener.* 11: 125-137.

Table 1. The eigenvectors of body part parameters in the first and second principal components. Principal components 1 and 2 accounted for 87.8% of total variance.

	Principal component 1	Principal component 2
Head length	0.37	-0.25
Head width	0.41	-0.14
Compound eye width	0.34	0.66
Alitrunk length	0.38	-0.39
Pronotal width	0.38	-0.33
Gastral length	0.36	0.46
Gastral width	0.40	0.09

Table 2. Measurements of compound eyes and ommatidia of the three female castes.

Ommatidium width was measured in 28, 31 and 47 ommatidia from 5 queens, 5 intermorphics and 10 workers, respectively.

	queen	intermorphics	worker
Compound eye diameter (mm)	0.14±0.01 (n=20)	0.14±0.01 (n=22)	0.09±0.01 (n=101)
Number of ommatidia/ compound eye	92.4±1.4 (n=5)	78.8±10.3 (n=5)	24.6±6.2 (n=10)
Ommatidium diameter (µm)	20.2±1.1 (n=28)	20.2±1.0 (n=31)	21.2±1.4 (n=47)

Data are mean ± SD

## FIGURES

Fig. 1

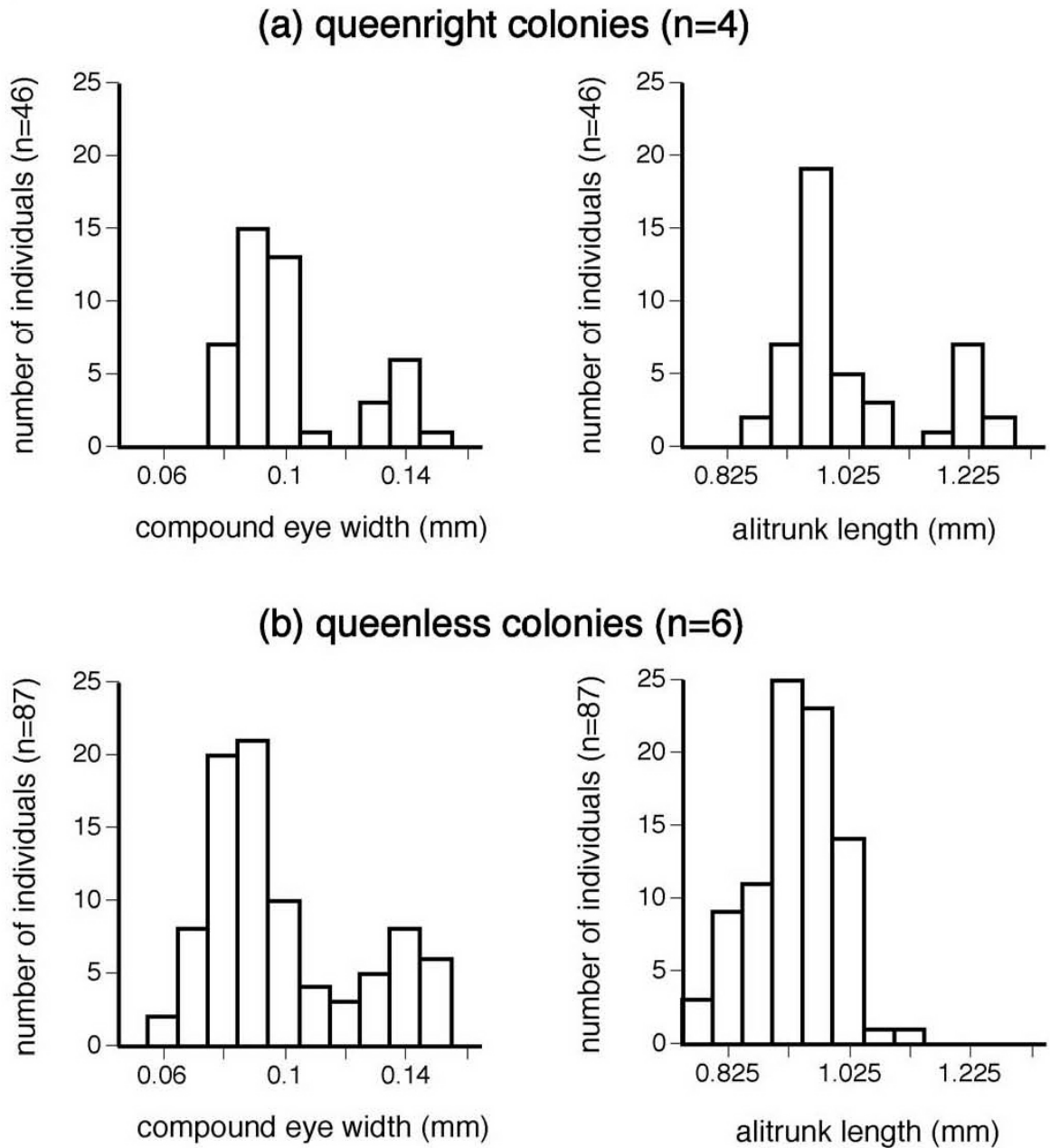


Fig. 1. Comparison of compound eye width and alitrunk length between queenright (a) and queenless colonies (b). The queenright and queenless colonies respectively produce queens and intermorphics as reproductives, and both types of reproductives never coexist in the same colony.

Fig. 2

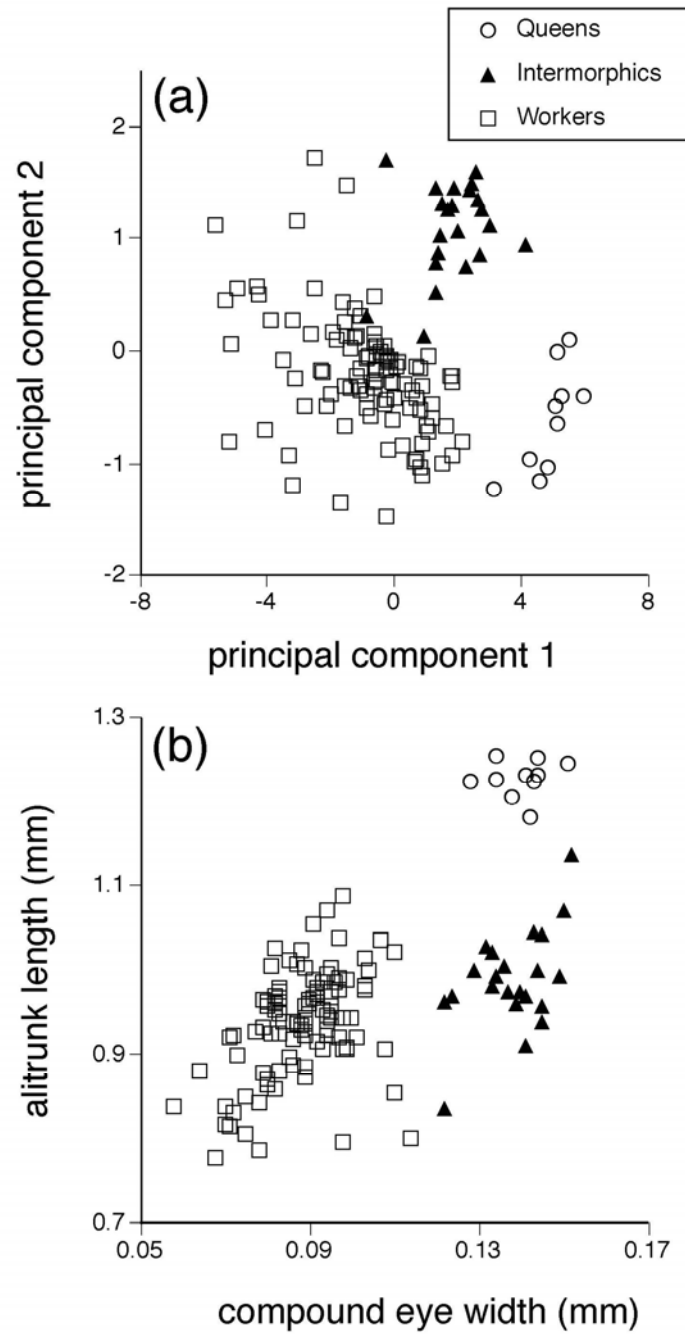


Fig. 2. Morphometric analysis of the three female castes. (a) Plot obtained by the principal component analysis with the first and second component axes, accounting for 87.8% of the total variance. (b) Plot of alitrunk length vs. compound eye width, showing that these two parameters well explain the morphological differentiation among the three castes.

Fig. 3

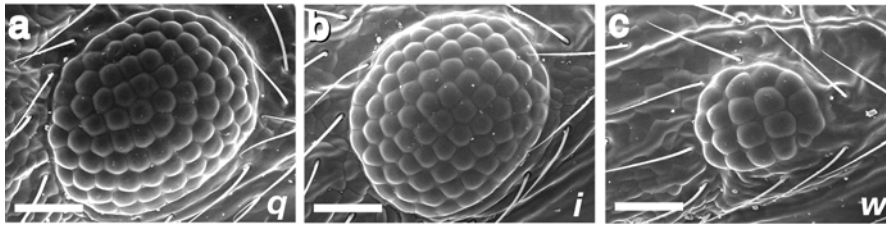


Fig. 3. Scanning electron micrographs of compound eyes of representative queen (a), intermorphic (b) and worker (c). The compound eyes of queens and intermorphics contain more ommatidia than those of workers. Bars = 50  $\mu$ m.

Fig. 4

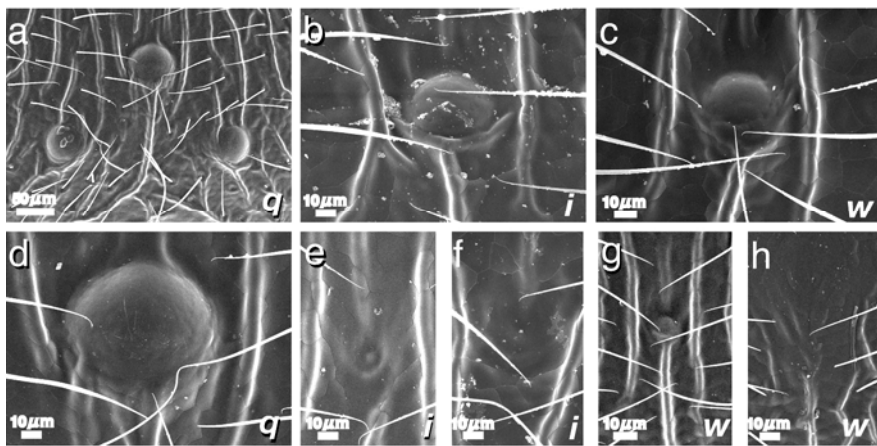


Fig. 4. Scanning electron micrographs of ocelli of three representative female castes. A queen has three larger ocelli (a, d), whereas an intermorphic and a worker have at most one small ocellus (b-c, e-h). *q*: queen, *i*: intermorphic, *w*: worker.

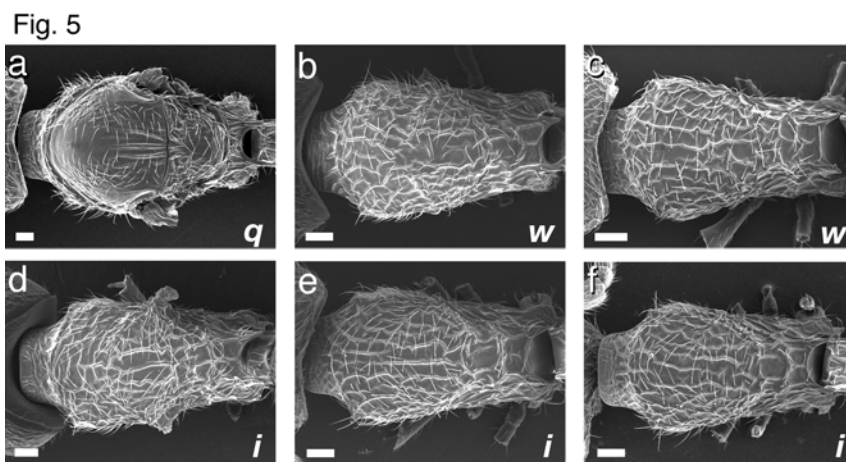


Fig. 5. Scanning electron micrographs of thoraces of three representative female castes (dorsal view). Queens have well-developed thoraces with little morphological variability (a), whereas intermorphics and workers have more simplified thoraces than queens (b-f). In particular, the thoraces of intermorphics show wide variability ranging from a well-developed thorax with a pair of forewing buds (d) to a simple one like those of workers (f). *q*: queen, *i*: intermorphic, *w*: worker. Bars = 50  $\mu$ m.

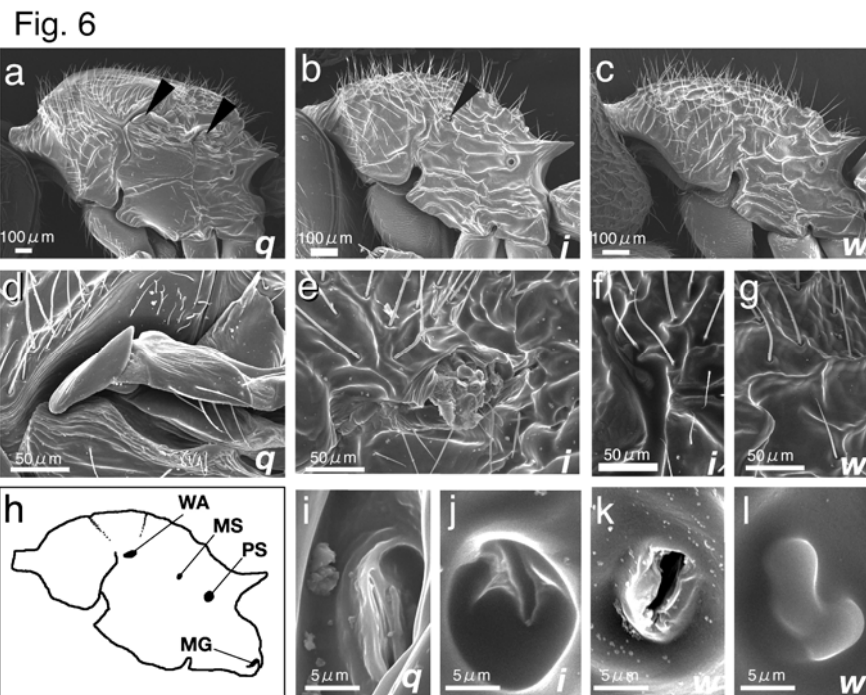


Fig. 6. Scanning electron micrographs of thoraces (lateral view) (a-c), wing articulations (d-g) and spiracles (i-l) in three representative female castes. Queens have two pairs of wings: fore- and hindwings (a) with robust articulation (d). Only a few intermorphics possess relatively well-developed wing buds (e), while most of them have a pair of small wing processes (f). Workers do not show any structures of wing rudiments (c, g). Metapleural spiracles also show structural differences among the three female castes (h-l). In queens, metapleural spiracles are hidden between the mesopleuron and metapleuron (i). In intermorphics, the cuticle around the openings of metapleural spiracles is invaginated and shows some hollow structures (j). Some workers have open metapleural spiracles (k), while others possess closed ones (l). *q*: queen, *i*: intermorphic, *w*: worker. WA: wing articulation, MS: metapleural spiracle, PS: propodial spiracle, MG: metapleural gland.