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Author(s)	Ishikawa, Asano; Miura, Toru
Citation	Sociobiology, 50(3), 881-893
Issue Date	2007
Doc URL	https://hdl.handle.net/2115/32888
Type	journal article
File Information	Miura-Ishikawa-Sociobaiol.pdf

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Morphological differences between wing morphs of two Macrosiphini aphid species *Acyrthosiphon pisum* and *Megoura crassicauda* (Hemiptera, Aphididae)

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ABSTRACT

In addition to caste polyphenism in social insects, aphids display one of the widest ranges of polyphenism, which produces several distinct phenotypes in response to environmental stimuli, e.g., daylength and density conditions. However, the mechanisms underlying aphid polyphenism remain poorly understood. This study was designed to evaluate the developmental impact of density conditions. We compared the morphologies of winged and wingless parthenogenetic females in the aphids *Acyrthosiphon pisum* and *Megoura crassicauda*, based on body-part measurements and observations by scanning electron microscopy. The earliest and the most remarkable differences between winged and wingless morphs appeared in the mesothoracic parts from the third instar, where wings are formed in the winged morphs. Winged adults also have narrower heads, well-developed compound eyes and well-sclerotized thoracic cuticles, compared with wingless adults. Although differences in hind-tibia length were identified in both species, the relationships between the two morphs were reversed in the two species, probably because the ecological significances of the winged/wingless morphs are different between the two species.

KEY WORDS: aphid, density condition, morphological difference, wing polyphenism.

INTRODUCTION

Caste differentiation of social insects is a distinctive type of polyphenism, in which discrete multiple phenotypes are produced depending on extrinsic factors such as social interactions among colony members (Nijhout 1999, 2003). Other than social insects, polyphenism is also observed in various insects such as seasonal polyphenism of butterflies and phase polyphenism of locusts. Especially, aphids (superfamily Aphididae, order Hemiptera) display various morphs in their complex life cycles (Fig. 1). Among them, some soldier-producing species have evolved in parallel (Aoki 1977, Stern and Foster 1996). Behind this background, it can be considered that Aphididae exhibits extreme polyphenic development to cope with environmental uncertainty and possess potential to produce multiple phenotypes.

In general, aphids display two main types of polyphenisms in their life cycles: reproductive polyphenism and wing polyphenism (Fig. 1). Reproductive polyphenism involves cyclical switching between asexual and sexual reproduction in their annual life cycle (Kawada 1987). In spring, parthenogenetic females (fundatrices) hatch from diapausing eggs, and their progeny produce vast numbers of clonal offspring through parthenogenetic viviparous reproduction. By late summer, the shorter photoperiod and lower temperature triggers the production of sexupari, which are parthenogenetic females that produce sexual morphs. Then, male and sexual female (ovipara) mate in autumn and produce diapausing eggs (Tagu et al. 2005). These morphs differ in a range of morphological features, especially reproductive organs and flight apparatuses (Tsuji and Kawada 1987).

Wing polyphenism occurs primarily among parthenogenetic females in summer and involves switching between the wingless sedentary morph and the winged morph capable of dispersal. In this case, increased aphid density mainly triggers wing formation in many species. In addition, host plant quality and interactions with predators, pathogens and plant viruses are known as environmental factors that also affect wing polyphenism (Lees 1966, Sutherland 1969, Müller et al. 2001, Braendle et al. 2006).

Density effect (tactile stimulation) can be regarded as the simplest social interaction. The density-dependent switching system in aphid life cycle will be helpful to our understanding of the impact of social interactions on ontogenesis and production of various morphs such as castes. In this regard, two aphid species, *Acyrthosiphon pisum* and *Megoura crassicauda*, have been studied extensively (Lees 1966, Sutherland 1969, Müller et al. 2001, Braendle et al. 2005) and are major candidates for the model insects to analyze the developmental mechanism underlying wing polyphenism. There are several studies on the morphological differences between winged and wingless morphs. Winged morph have well-developed flight apparatus and sense organs (Kalmus 1945, Kring 1977, Miyazaki 1987).

In the present study, we evaluated the developmental impact of social interaction among individuals (i.e. density), by carefully examining the morphological differences between wing morphs during postembryonic development in two species of aphids.

MATERIALS AND METHODS

Insects

We used the pea aphid *Acyrthosiphon pisum* and the vetch aphid *Megoura crassicauda* (Hemiptera, Aphididae, Macrosiphini) as the focal aphid species. Parthenogenetic aphid cultures were maintained in tubes (diameter: 2.5 cm, height: 10 cm), in which a vetch seedling (*Vicia faba L.*) was placed on wet vermiculite, under long day-length condition (16L: 8D, 20°C) over generations (Wilkinson and Ishikawa 2000). Since the population density (tacticle stimulation) is known as an environmental cue to induce the winged morphs in these aphid species (Lees 1966, Sutherland 1969), we manipulated the density condition to induce wing types. Thus, to induce the winged morphs, more than 30 adult aphids were reared on a single vetch seedling of about 3 cm high. The induction ratio of winged aphids under high-density (HD) condition was 60-80%. To induce wingless aphids under low-density (LD) condition, only one adult individual was kept on a 3 cm vetch seedling. Under the latter conditions, the rate of wingless aphids was 100%. In *A. pisum*, since the effect of rearing conditions lasts over 2 or 3 generations (MacKay and Wellington 1977), we maintained stock insects over three generations under LD condition before the rearing experiments under both LD and HD conditions.

Morphometric analysis

In these aphid species, there are four nymphal instars and the fifth instar is the adult stage. To determine the nymphal-instar stage, we measured the hind-tibia length using

an image analysis system HIM-1N (HOGA, Osaka, Japan) and aligned the measurements in ascending order for each species. From the third instar, we were able to distinguish winged lines from wingless lines based on the external morphology of the thoracic part, i.e. wing buds. To determine the developmental differences in body parts between the two morphs, we measured the mesothoracic length (wing bud/wing length) and head width, in addition to hind tibia length, of the third- to fifth- instars in both winged and wingless lines.

Scanning electron microscopy

To describe the ultrastructural morphological features of the head, compound eyes and thoracic parts, aphid samples were fixed in FAA solution (formalin: ethanol: acetic acid = 6: 16: 1), then transferred into increasing concentrations of ethanol, and then into t-butanol. Subsequently, they were freeze-dried using Freeze Dryer ES-2030 (Hitachi, Tokyo), and coated with gold ion with Ion Sputter E-1010 (Hitachi). The head and thoracic structures were examined under a scanning electron microscopy JSM-5510LV (JEOL, Tokyo) (cf. Miura et al. 2004)

RESULTS

Determination of nymphal instars

Based on the bar diagrams of hind-tibia length measurements in ascending order for each species, we can detect four nymphal instars and the fifth adult instar. A careful examination of the graphs shows points at which the values increased steeply. These points presumably reflect the points of molting (Fig. 2). On average, the hindtibia becomes approximately 1.3 to 1.5 times longer through each molt in *A. pisum* (Fig. 2a). The hind-tibia length in each instar is as follows (mean±SD, ranges are shown in parentheses); 1st instar: 0.57±0.05 mm (0.46-0.67 mm, n=40), 2nd instar: 0.88±0.05 mm (0.77-1.01 mm, n=84), 3rd instar: 1.30±0.08 mm (1.16-1.46 mm, n=76), 4th instar: 1.76±0.09 mm (1.59-1.91 mm, n=46), and 5th instar: 2.42±0.15 mm (2.15-2.65 mm, n=11). In *M. crassicauda*, the hind-tibia length increased 1.4 to 1.5 times through each molt (Fig. 2b), and the hind-tibia lengths were as follows; 1st instar: 0.53±0.04 mm (0.41-0.59 mm, n=26), 2nd instar: 0.78±0.04 mm (0.67-0.88 mm, n=32), 3rd instar: 1.11±0.06 mm (0.95-1.20 mm, n=50), 4th instar: 1.62±0.12 mm (1.34-1.82 mm, n=56), and 5th instar: 2.38±0.20 mm (2.01-2.71 mm, n=43) (Fig. 2b). There was no overlap between the ranges of different instars. Therefore, discrimination of the instars in this study was based on the hindtibia length (Fig. 2).

Morphometric analysis

To examine morphological differences between two morphs, we compared the mesothoracic length, hind-tibia length and head width of the third to fifth instar aphids for winged and wingless lines.

In *A. pisum*, mesothoracic lengths of aphids from the winged line were longer than those from the wingless line (Fig. 3a, left), and the differences were statistically significant from the third instar (student's t-test: $p<0.01$), and especially the fourth ($p<0.0001$) and fifth instars ($p<0.0001$). Similarly in *M. crassicauda*, the mesothoraces of the winged line were longer (Fig. 3a, right). The differences in mesothoracic lengths between the winged and wingless lines were significant in all three instars (3rd instar: $p<0.0001$, 4th instar: $p<0.0001$, 5th instar: $p<0.0001$).

In *A. pisum*, the hind-tibia length of the fourth nymph in the wingless line was significantly longer than that in winged lines ($p<0.01$), and the lengths of the third and fifth instars showed similar tendencies but the differences were not significant (Fig. 3b, left) (3rd instar: $p>0.05$, 5th instar: $p>0.05$). In contrast, in *M. crassicauda*, the hind-tibia length in the winged line was longer by the fourth instar (Fig. 3b, right, $p<0.01$) and was even significantly longer for the fifth instar ($p<0.0001$), compared with the wingless line. These results indicate that the difference in hindtibia length between morphs is smaller than that between instars.

Differences in head width between the two wing types followed similar tendencies in the two species. In *A. pisum*, head widths of the aphids in the wingless line were larger than those in the winged line in fourth and fifth instars (Fig. 3c, left) (3rd instar: $p>0.05$, 4th instar: $p<0.01$, 5th instar: $p<0.01$). In *M. crassicauda*, the heads of

the wingless morphs were significantly wider than those of the winged morphs at the fifth instar (Fig. 3c, right) (3rd instar: $p>0.05$, 4th instar: $p>0.05$, 5th instar: $p<0.01$).

Scanning electron microscopic findings

Observations with a scanning electron microscope (SEM) showed that the larger head width of the wingless adults was due to the distance between eyes in both species (Fig. 4). In addition, the thoracic parts of the winged adults were massive and covered with well-sclerotized cuticle with smooth surfaces, in both *A. pisum* and *M. crassicauda* (see the ventral sides of both species, Fig. 4, arrowheads). Consistently, histological examination of cross-sections also indicated that the presence of thicker thoracic cuticles in the winged adults (date not shown).

SEM also revealed that the compound eyes of the winged morph were significantly more convex in both *A. pisum* and *M. crassicauda* (Fig. 5). In both species, the ratio of height to width of the compound eyes was approximately 1.2 times larger in the winged morph than the wingless morph; *A. pisum*: 0.74±0.07(WD), 0.61±0.04(WL), n=4; *M. crassicauda*: 0.69±0.04(WD), 0.57±0.06(WL), n=4.

DISCUSSION

Wing primordia are not only recognizable, but the mesothoracic parts where the wing primordia exist become longer from the third instar of the winged line in both examined species. Although we did not examine the metathoraces, this part would also elongate in the winged line. The flight apparatus contain complex structures such as large amount of flight muscles, which require abundant energy for development. In the winged line, therefore, it takes some time from the third instar to develop the wing primordia and to invest energy into the flight apparatus.

In addition, the winged adults have thick cuticles in their thoraces. Structurally, the dorso-ventral flight muscles attach to these thick cuticles, and thus any muscle contraction for flight would require such sclerotized cuticles (Fig. 4) (Chapman 1998, Ishikawa et al. unpublished data). Moreover, since lipids deposited in the epicuticle are engaged in desiccation resistance (Hadley 1982), the cuticles of winged morphs may contain more lipid to manage starvation and desiccation during flight.

With regard to the cephalic structures, the winged adults have narrower head and well-developed compound eyes in comparison with the wingless adults. This finding is consistent with a previous report showing that the number of ommatidia increased in the winged morphs (Kring 1977). In this report, there was no difference in the compound-eye diameter between the morphs. Thus, the increase in the number of ommatidia should make the compound eyes swollen, which is consistent with our results here. Presumably, the winged adults require well-developed eyes to find new host plants at the time of dispersal flight. Some studies on insect development have

shown that production of a morphological structure often comes at a cost to the development of other morphological traits because of competition between traits for limited resources (Emlen 2001, Nijhout and Emlen 1998). Therefore, it is possible that production of the more developed compound eyes in the winged morph results in a narrower head width. Furthermore, the small head with well-developed ‘goggling eyes’ may provide the morph with a panoramic view (cf. Parker 2003).

With regard to the hind-tibia length, while there were differences between wing morphs, opposite relationships were seen from species to species. Although both *A. pisum* and *M. crassicauda* belong to Macrosiphini and their host plants are similar legume plants, each species should have different habits and therefore the leg length varies accordingly. In *A. pisum*, the wingless morphs might require longer legs, as they have no wing so that migration depends only on their legs. On the other hand, in *M. crassicauda*, the winged morphs might need long legs, as they must walk indefinitely in search of host plants after flight.

In conclusion, our study described the morphological differences between aphid phenotypes caused by interactions among individuals. The mechanisms underlying wing polyphenism are probably utilized or co-opted in the case of soldier-producing aphid species. As a circumstantial evidence, for example, soldiers with long legs and thick cuticle are produced in response to density condition (Shibao et al. 2004). Future comprehensive developmental studies of various aphid taxa should provide the underlying mechanisms of social interactions and developmental regulations in aphids.

ACKNOWLEDGEMENTS

We thank S. Koshikawa and S. Hongo for the valuable comments on this study. Thanks are also due to M. Imai, H. Ishigaki and S. Miyazaki for their help in the maintenance of aphid stocks. This work was supported by Grant-in-Aid for Scientific Research (B) (No. 18370007).

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FIGURE LEGENDS

Fig. 1. Schematic diagram showing the annual life cycle of *Acyrthosiphon pisum* and *Megoura crassicauda.*

Fig. 2. Bar graph showing hindtibia lengths of all instars in ascending order. (a) *Acyrthosiphon pisum*, (b) *Megoura crassicauda*. Vertical lines indicate the point of molting events.

Fig. 3. Comparisons of polyphenic traits between wing types in *Acyrthosiphon pisum* (left) and *Megoura crassicauda* (right); (a) mesothoracic length, (b) hindtibia length, (c) head width.

Fig. 4. Scanning electron micrographs of the ventral view of the cephalic and thoracic parts, showing morphological differences between the two morphs; (a) winged and (b) wingless morphs of *Acyrthosiphon pisum*; (c) winged and (d) wingless morphs of *Megoura crassicauda*.

Fig. 5. Scanning electron micrographs of the compound eyes; (a) winged and (b) wingless morphs of *Acyrthosiphon pisum*; (c) winged and (d) wingless morphs of *Megoura crassicauda*.

Fig. 1.

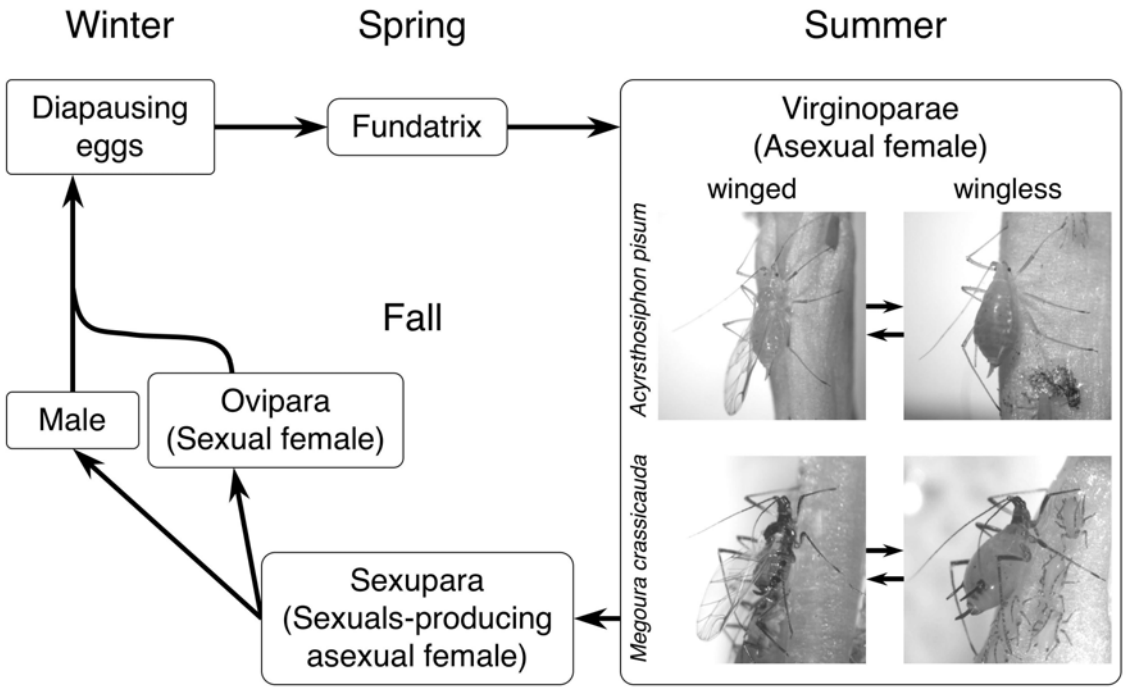

Winter
Spring
Summer
Diapausing eggs
Fundatrix
Virginoparae
(Asexual female)
winged
wingless
Acyrsthosiphon pisum
Megoura crassicauda
Fall
Male
Ovipara
(Sexual female)
Sexupara
(Sexuals-producing
asexual female)

Fig. 2.

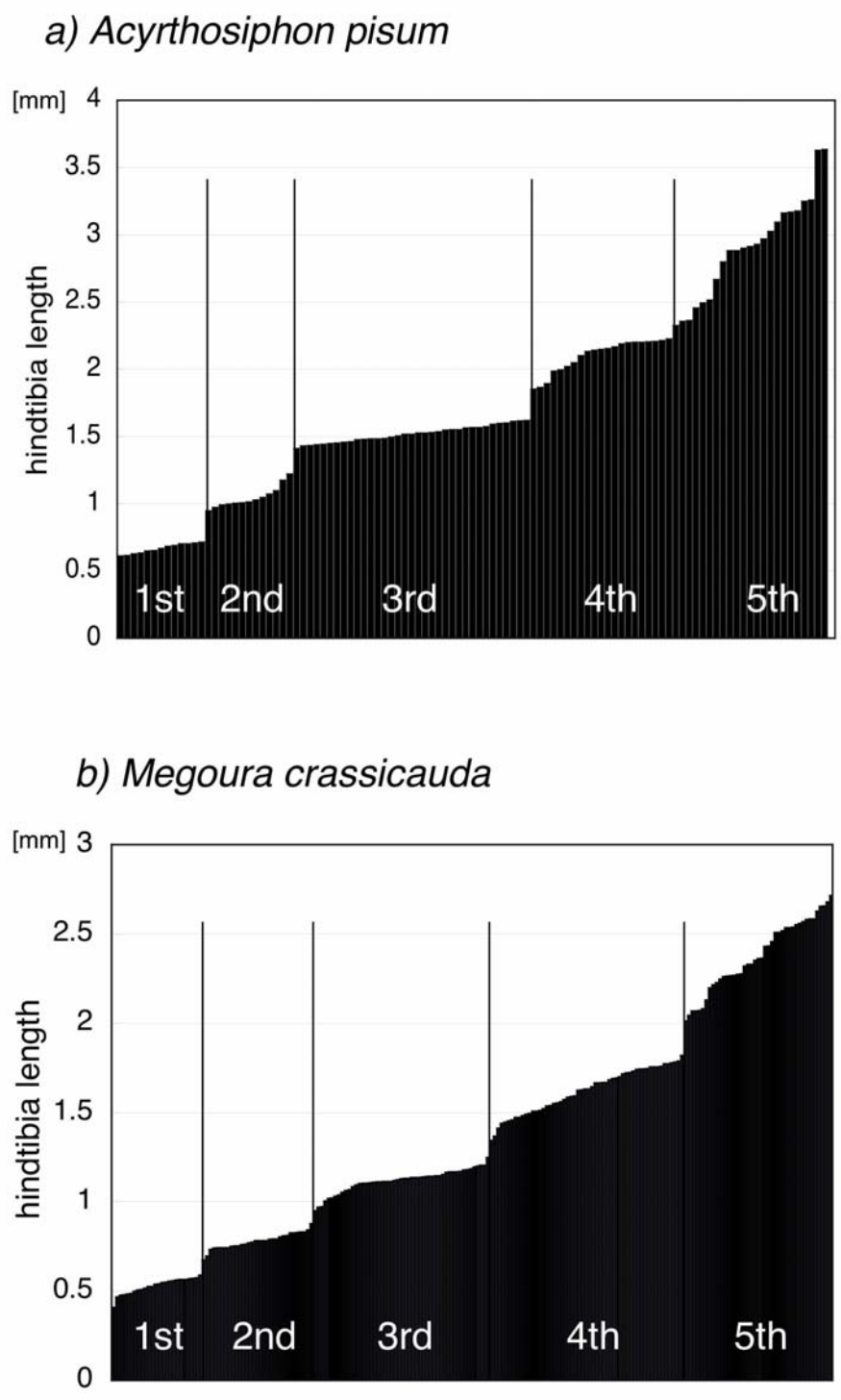
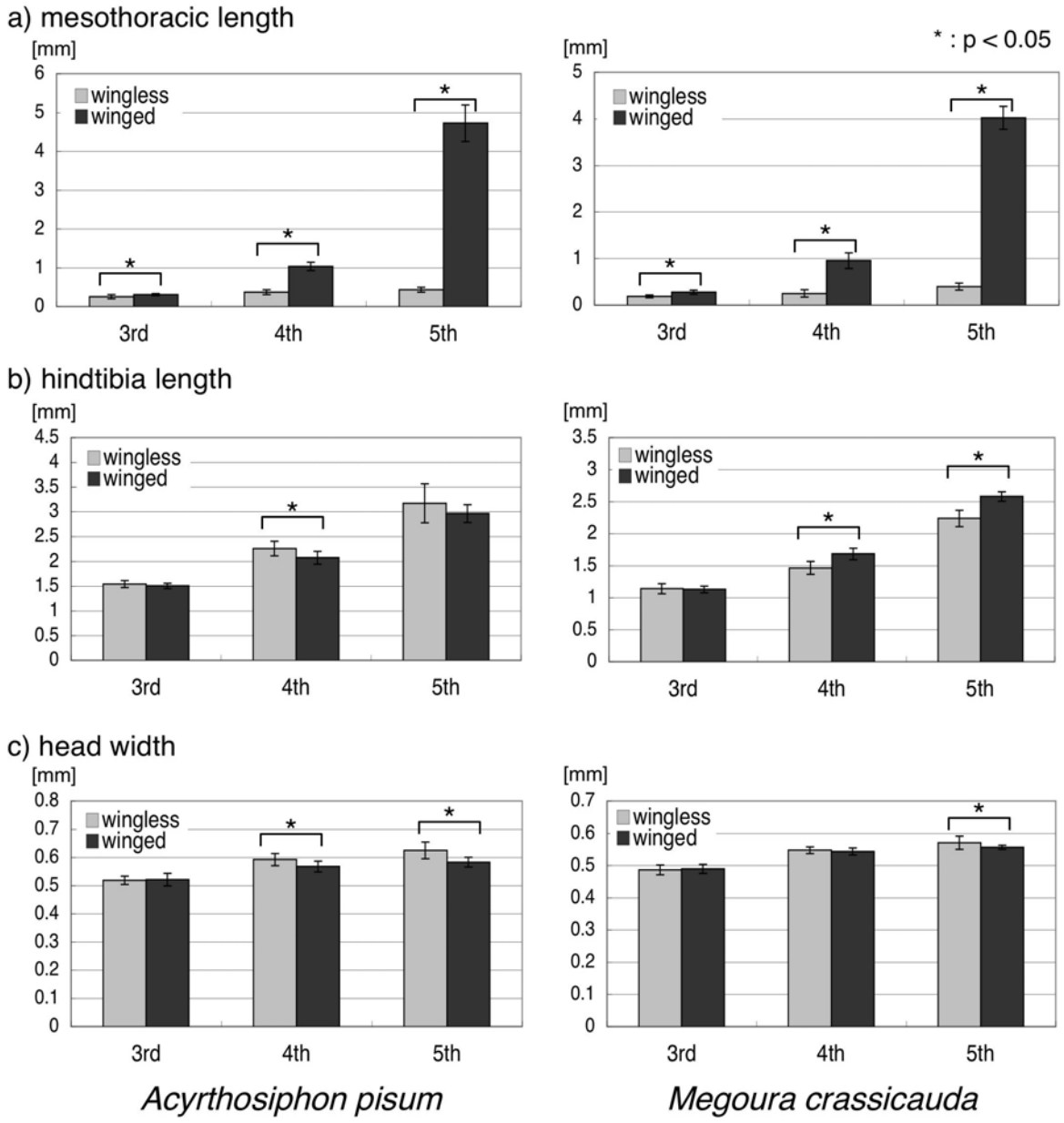

a) Acyrthosiphon pisum
[mm]
4
3.5
3
2.5
2
1.5
1
0.5
0
hindtibia length
1st
2nd
3rd
4th
5th
b) Megoura crassicauda
[mm]
3
2.5
2
1.5
1
0.5
0
hindtibia length
1st
2nd
3rd
4th
5th

Fig. 3.


a) mesothoracic length
b) hindtibia length
c) head width
[mm]
wingless
winged
* : p < 0.05
3rd
4th
5th
Acyrthosiphon pisum
Megoura crassicauda

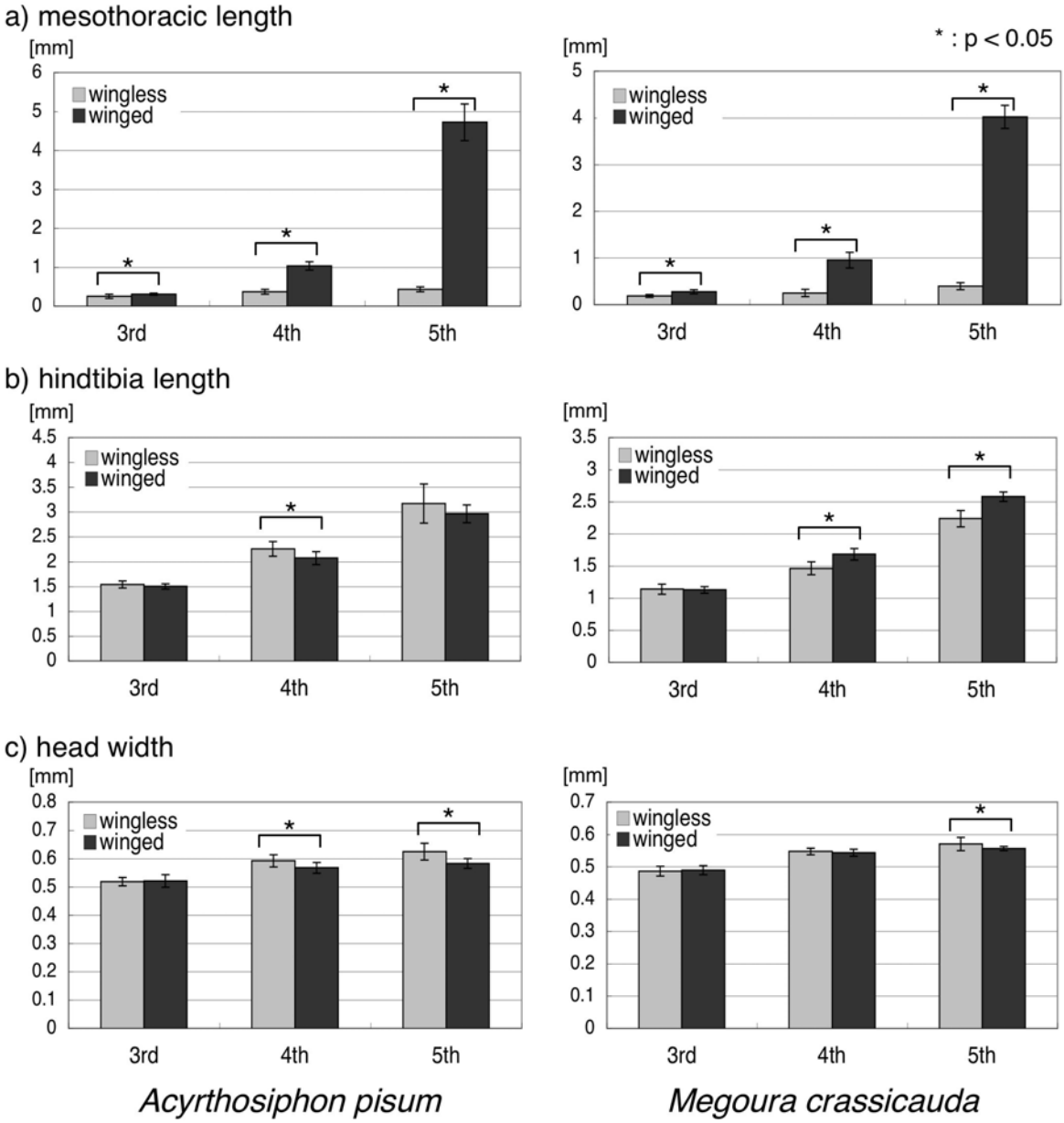

a) mesothoracic length
[mm]
wingless
winged
3rd
4th
5th
* : p < 0.05
b) hindtibia length
c) head width
Acyrthosiphon pisum
Megoura crassicauda

Fig. 4.

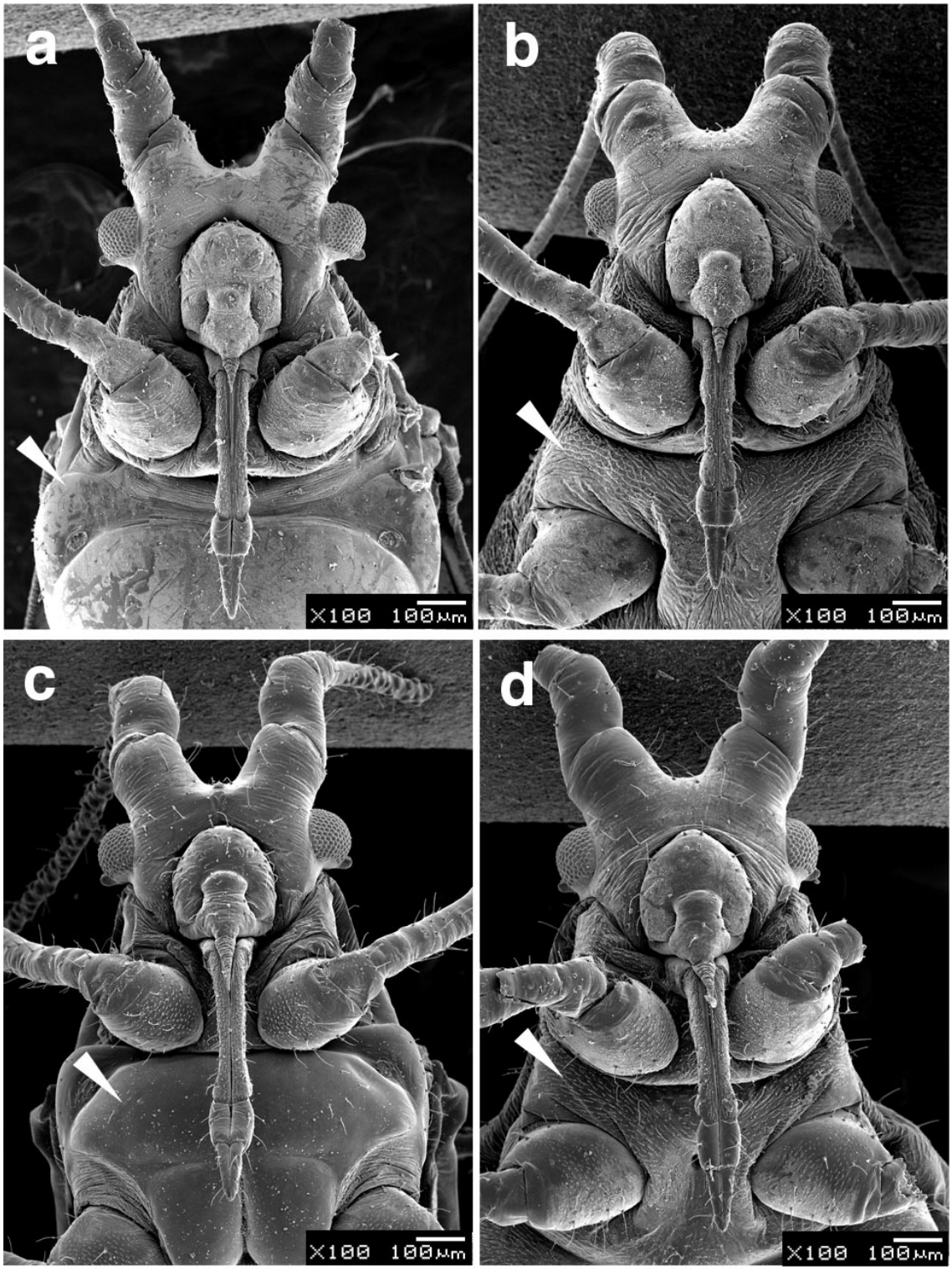
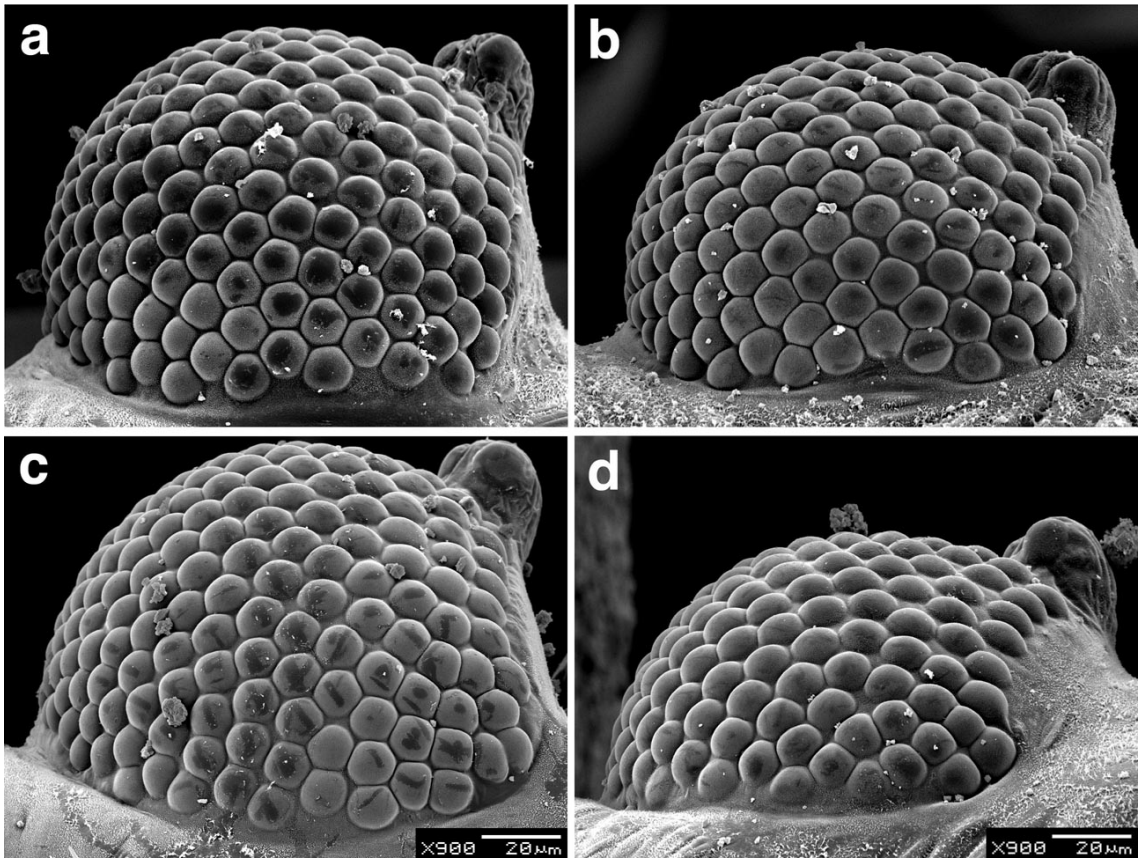



Fig. 5.