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Development of the wing buds in *Megoura viciae*: a morphological study

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Abstract

This study describes the initial phases of wing morphogenesis of *Megoura viciae* Buckton (Homoptera Aphididae). We evaluated the development of wing buds in males (winged) and parthenogenetic females (presumed to become wingless) of *M. viciae* from birth to 4th instar. Wing bud epithelium in males is initially simple, becoming bistratified and then progressing to stratify. Mitotic activity is more intense in the proximal region of bud, and its behaviour seems to be synchronous. In parthenogenetic females, from birth to 1st instar, the buds resemble those of males at the same stages, but subsequently the epithelium returns to the simple state, and from the 3rd instar stage, it cannot be distinguished from the adjacent epithelium.

This study moreover defines distinctive wing bud features that can be used to identify these buds during development in other aphid species.

Key words: Aphids, dimorphism, development, wing buds.

Introduction

Winged-wingless dimorphism of aphids is among one of the most successful prerequisites allowing cosmopolitan diffusion and adaptation of these organisms. The determination of winged or wingless morphs is of special interest, due to the adaptive role of wingless females in the rapid colonization of host plants and that of winged females in species dispersal. On the other hand, photoperiod-dependent wing-wingless polymorphism allows sexuparae (mothers of sexual generation) to reach the primary host-plant, thus favouring outbreeding.

Wing dimorphism impacts on factors that go beyond mobility. Wingless individuals have a shorter developmental time and increased fecundity (Noda, 1959; Dixon, 1972), resulting in up to a 70% greater reproductive output than winged morphs (Müller *et al*., 2001).

Several studies address the environmental factors influencing this dimorphism. Poor nutritional quality aphid diet has also long been implicated in the production of winged morphs (Wadley, 1923; Bonnemaison, 1951; Mittler and Dadd, 1966; Dixon and Glen, 1971), but there is no clear relationship between nutrition and wing induction (Müller *et al*., 2001). The influence of temperature and photoperiod varies among species (Johnson and Birks, 1960; Lees, 1967; Bonvicini Pagliai *et al*., 1985; Vaz Nunes and Hardie, 1992). The role of overcrowding in inducing winged forms is discussed by Bonnemaison (1951), Johnson (1965) and Lees (1966). The major factor is contact stimulation among aphids under crowding conditions. This crowding stimulus during the adult period seems to indicate that the neurosecretory system is involved in wing formation (Kawada, 1987). A study carried out by Lees (1961) shows that wingless virginoparae of *Megoura viciae* Buckton (Homoptera Aphididae) reared in isolation from birth produce only wingless offspring and when reared in groups they produce winged forms. In this case, the wingless or winged character has been determined before birth and is controlled by a maternal regulatory mechanism.

Although evidence is still quite scarce, the induction of winged morphs could be due to interactions with natural enemies, such as parasitoids, predators or pathogens, and to mutualistic relationship with ants. In this case the interactions with organisms of a higher trophic level could stimulate or inhibit the induction of winged morphs either directly or indirectly via tactile stimuli (Muller *et al.,* 2001).

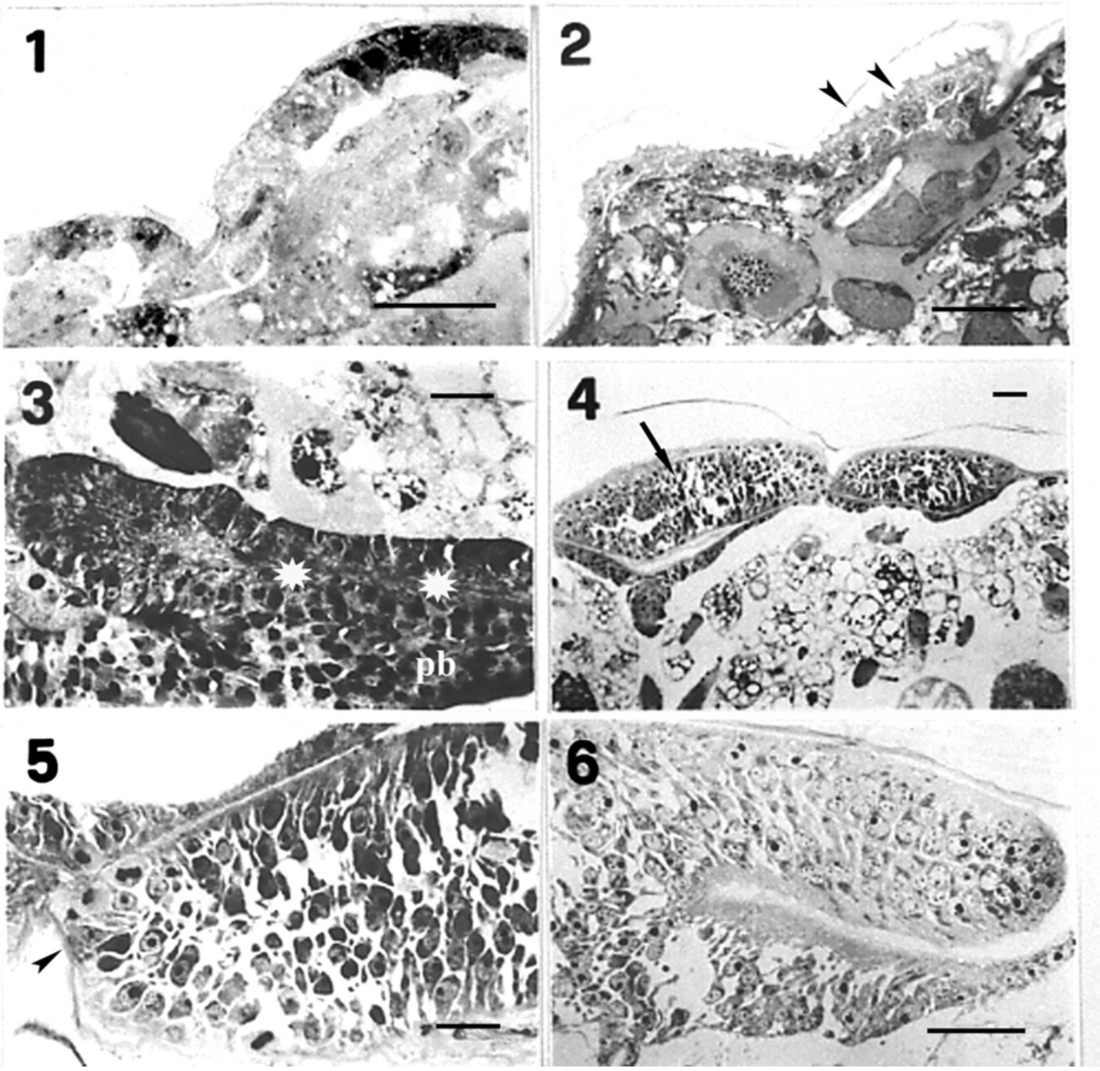
Winged virginoparae females usually generate only wingless nymphs, whereas wingless mothers can give birth to both winged and wingless nymphs (Lees, 1966). In *Macrosiphum euphorbiae* (Thomas) (Schull, 1938), *Aphis craccivora* Koch (Johnson, 1958) and *Acyrthosiphon pisum* (Harris) (Tsuji and Kawada, 1987) wing buds are present prior to birth in all individuals, irrespective of whether they are destined to develop into winged or wingless forms. Our previous investigations (Benedetti *et al*., 1991) demonstrated that wing buds in both males of *M. viciae* and winged parthenogenetic females of *A. pisum* are already present two days before birth. In the present study we followed the fate of these buds from birth (time 0) to the last instar (4th) in males (winged) of *M. viciae*, with the aim of defining the morphological features of this development. In order to recognize the period of post-embryonic life reactive to the wing development factors, parthenogenetic females (presumed wingless) at the same stages were also utilized. Under laboratory conditions in *M. viciae* species it is difficult to obtain winged females, thus we utilised males in this research.

Materials and methods

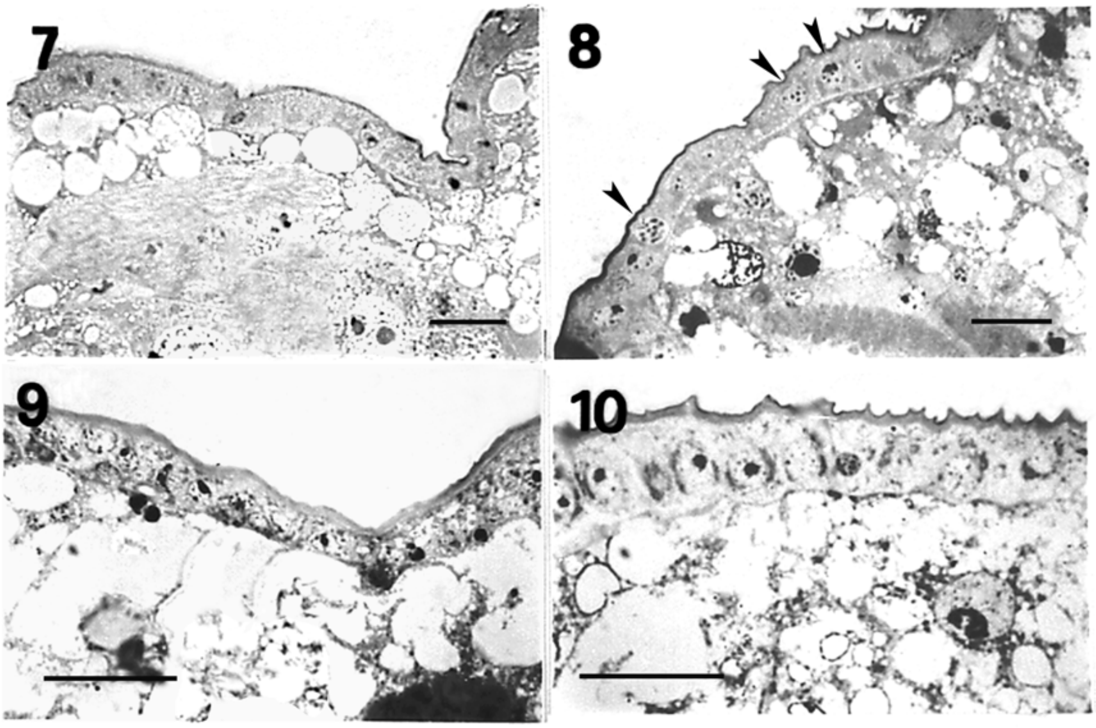
Specimens of *M. viciae* were reared in the laboratory under controlled environmental conditions (19 °C and 16L:8D photoperiod). Under these conditions reproduction occurs exclusively by parthenogenesis. Groups of 5 adult wingless females were transferred to a thermostatic cage and maintained at 19 °C and short photoperiod (8L:16D) to obtain sexual females and males (Bonvicini Pagliai and Crema, 1976). The adult parthenogenetic females were removed the following day, after birth of the offspring, born in a 20 h time slot. Ten days later, the adults were removed and the newborns, parthenogenetic sexuparae females, were maintained on plants under the same experimental environmental conditions. The male offspring thus obtained were collected at the day of birth (time 0) and at 1st, 2nd, 3rd, 4th instar nymphs.

In addition, groups of four adult wingless parthenogenetic females, taken from laboratory rearing, were separated and maintained under the same environmental conditions. The newborns (parthenogenetic presumed wingless females) were collected at time 0 and at 1st, 2nd, 3rd, 4th instar nymphs.

Ten specimens for each instar were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 6.8 at 4 °C for 10 min, in a microwave oven with 70-w power. The specimens were successively post-fixed with 1% osmium tetroxide in the same buffer for 2 min in the microwave oven at the same power. The specimens were dehydrated in acetone and embedded in Durcupan (Fluka). The semi-thin sections (1-2 µm) were stained with toluidine blue.



Figures 1-6. Frontal section of males of *Megoura viciae* with wing buds. Bar = 20 µm. Semi-thin sections (1-2 µm) stained with toluidine blue. (1) Simple epithelium at time 0. (2) The same simple epithelium with mitoses (arrowheads) at 1st instar. (3) The regularly bistratified epithelium with slot (white asterisks) at 2nd instar, pb=proximal buds. (4) Shows the stratified epithelium (arrow) at 3rd instar. (5) Enlargement of (4) with mitosis (arrowheads). (6) Stratified epithelium at 4th instar.



Figures 7-10. Frontal section of females of *Megoura viciae* with wing buds. Bar = 20 µm. Semi-thin sections (1-2 µm) stained with toluidine blue. (7) Simple epithelium at birth (time 0). (8) The same simple epithelium with mitoses (arrowheads) at 1st instar. (9) Epithelium not regularly bistratified at 2nd instar. (10) Monolayer epithelium at 3rd instar.

Results

Male at birth (time 0) - Wing buds were already evident on both sides of the meso/metathorax; each bud consisted of two small swellings. The monolayer epithelium consisted of cubical cells with large and round nuclei (figure 1).

Male 1st instar nymphs - The features were similar to those in males at birth, but spaces were observed among the cells, synchronous mitoses were frequent (figure. 2).

Male 2nd instar nymphs - The wing buds, larger than those of 1st instar, were greatly lengthened and leaned against the integument. They consisted of two epithelial layers, separated by a thin slot. The lengthened or polygonal cells had a round nucleus with a strongly basophilic nucleolus. Numerous intercellular spaces become very evident (figure 3). A large number of cells in mitosis were observed, particularly in the proximal bud.

Male 3rd instar nymphs - Wing buds butted up against the body wall and appeared to be constituted by a stratified epithelium. The slot was not evident because of the irregular disposition of cells and the numerous, large intercellular spaces. The cells were lengthened or irregular, with round nuclei and very evident nucleoli. Marked mitotic activity was also observed at this stage (figures 4 and 5).

Male 4th instar nymphs - The wing buds were similar to those of the 3rd instar, but the cells were more regularly arranged in two layers with again an evident slot between them. The epithelium near the buds was simple, like in the 3rd and 2nd instar (figure 6).

Female at birth (time 0) - The wing bud appeared slightly separated into two lobes. The simple epithelium was constituted by cubical cells (figure 7).

Female 1st instar nymphs - The bud features were similar to those of the previous instar; the epithelium had no intercellular spaces. A few mitoses were present (figure 8).

Female 2nd instar nymphs - The bud epithelium appeared irregularly bistratified. (figure 9).

Female 3rd instar nymphs - The bud epithelium was simple and indistinguishable from the surrounding epidermis (figure 10).

Female 4th instar nymphs - The buds were not recognisable.

Discussion and conclusions

The developmental stage sensitive to stimuli that influence wing induction varies among different species of aphids (reviewed by Dixon, 1998). Wing induction can be controlled either by the mother (pre-natal) or by the developing nymph (post-natal). In some species of aphid, both pre- and post- natal wing induction occurs. Most studies concentrated on the Aphidinae showed that results sometimes differed among clones of the same species [*Brevicoryne brassicae* (L.), *Myzus persicae* (Sulzer), *Sitobion avenae* (F.)], indicating large intraspecific variation for the timing of wing induction (Müller *et al*., 2001).

Our previous data (Benedetti *et al*., 1991) demonstrated that male and female (destined to become winged) embryos of *M. viciae* and *A. pisum* respectively, already have wing buds two days before birth.

Our present results show that in males at birth of *M. viciae*, the wing bud epithelium is initially simple, becoming bistratified and then progressing to stratify. Mitotic activity is more intense in the proximal region of bud, and the mitoses seem to be synchronous, following successive cycles. In parthenogenetic females (presumed wingless) from birth to 1st instar the buds resemble those of males at the same instar. At 2nd instar the epithelium is irregularly bistratified. Subsequently, the wing epithelium returns to be simple, and already in the 3rd instar, it cannot be distinguished from the adjacent epithelium.

Even in *A. craccivora* wing buds, distinguishable one day prior to birth, increase in size and differentiate intermittently at each moult in nymphs destined to become winged, whereas they disappear in nymphs destined to become wingless (Johnson, 1958). According to Johnson and Birks (1960) all virginoparae begin to develop as presumed winged forms, and environmental conditions or other stimuli can suppress this tendency, leading to the development of wingless forms. This behaviour has been termed diversion. Lees (1967) states that winged/wingless dimorphism is regulated by maternal humoral factors instead of extrinsic factors. The corpora allata is regarded as the most important factor for both maternal and nymphal regulation of wing determination (White, 1968). The development of winged and wingless morphs is under the control of this gland that produce juvenile hormone; in nymphs that develop into wingless adults the corpus allatum is more active than in nymphs that become winged (White, 1971). Hales (1976) has suggested that compounds with anti-juvenile hormones or corpus allatum blocking properties might provide experimental support that juvenile hormone not only controls metamorphosis but also wing dimorphism in aphids. However, the possible role of juvenile hormone is still controversial (Mittler *et al.,* 1976; Lees, 1977, 1980; Hardie, 1981), as is that of precocenes, substances with anti-juvenile hormone activity (Hales and Mittler, 1981; Kambhampati *et al*., 1984; Hardie, 1986, 1987). The effects of a number of precocene compounds have been examined on insects, mostly aphids, with regards to metamorphosis and wing dimorphism (*M. persicae,* Hales and Mittler, 1981; *M. euforbiae,* Delisle *et al*., 1983; *A. pisum*, Kambhampati *et al.,* 1984; Hardie *et al*., 1995, 1996; Gao and Hardie, 1996; *A. pisum*, *Aphis fabae* Scopoli and *M. viciae,* Hardie, 1986). Several studies failed to elucidate the role of juvenile hormone in wing development, since the mode of precocene action on aphids has been contradictory, evoking either wing induction or inhibition, depending on the precocene analogue. Precocene II has been found to induce wing formation in *A. pisum* and *M. euphorbiae* but appears to induce precocious adult development only in the latter species (Mackauer *et al*., 1976; Delisle *et al*., 1983). Precocene III induces both precocious metamorphosis and wing formation in *A. pisum* (Kambhampati *et al*., 1984).

Interaction with natural enemies and mutualists may also influence wing induction even further (Müller *et al.,* 2001). Aphid parasitoids can interfere with the wing development of their host (Johnson, 1959; Bonnemaison, 1970; Liu and Hughes, 1984; Christiansen-Weniger and Hardie, 1998). In some individuals of *A. pisum* wing development was completely inhibited after a parasitoid attack during the early second nymphal stage of presumptive winged virginoparae and males. It seems likely that factors from the adult parasitoid, released during oviposition, or from the parasitoid egg are responsible for the inhibition of wing development (Christiansen-Weniger and Hardie, 2000).

The specimens we used were obtained from controlled laboratory rearing, and thus we can rule out the influence of extrinsic factors that may have interfered with wing development. Our study demonstrates that in *M. viciae* the intrinsic factor (or factors) stopping wing development are active between 1st and 2nd instar stages. Thus, in absence of extrinsic factors, this short period is the only moment of post-embryonic life during which the aphid is reactive to the stimulus. Our observations, describe for the first time the initial phases of wing morphogenesis and define the characteristic morphology of the wing bud epithelium, which can be useful for identifying these buds during development even in other aphid species. Moreover, this morphologic study lays the groundwork for future research to elucidate the mechanisms behind control of winged/wingless dimorphism.

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