The significance of ant and plant traits for ant pollination in *Leporella fimbriata*

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**Summary.** Ant metapleural glands secrete surface antibiotics that affect pollen as well as bacteria and fungi. This may be one reason why ant pollination is rare. It is predicted that pollination by ants is possible only in the presence of certain ant and/or plant traits. Two traits are investigated; first, absence of the metapleural glands, and second, the presence of stigmatic secretions that insulate pollen from the ant integument. The pollinator of the orchid *Leporella fimbriata* is the ant *Myrmecia urens*. Only one caste is involved, the winged males, and they differ significantly from the queen and worker castes in that they do not possess metapleural glands. This paper reports experiments which test for differential effects on pollen between the males and other castes and evaluates the importance of stigmatic secretions. The results show that the absence of metapleural glands makes no difference as all three castes have strong disruptive effect on pollen artificially applied to the integument. However, during pollination the orchid secures the pollen mass to the ant surface by stigmatic secretions and normal pollen function, fruit production and seed set occur. It appears that both ant and plant traits are pre-adaptive having evolved for functions other than ant pollination.

**Key words:** Ant – Pollination – Pollen – Metapleural gland – Antibiotic

The paired, thoracic metapleural glands of ants produce anti-bacterial and anti-fungal secretions (Maschwitz et al. 1970; Beattie et al. 1986). The presence of these secretions on the integument of ants also disrupts the normal function of pollen grains and the development of pollen tubes (Beattie et al. 1984, 1985). It has been suggested that this is one reason why ant pollination is rare and leads to the prediction that ant pollination systems require ant and/or plant traits that result in the avoidance of the harmful integumental effects (Hull and Beattie 1988; Peakall et al. 1987; Peakall and Beattie 1989).

An opportunity to examine the relative importance of ant and plant traits has been provided by the recent discovery of a unique, obligate ant pollination mechanism in the orchid *Leporella fimbriata* (Lindl.) George. Pollination is effected by winged males of the ant *Myrmecia urens* Lowne that attempt copulation with the labellum (Peakall et al. 1987; Peakall 1989). While the queen and worker castes of this species possess metapleural glands, the pollinating males do not. We have predicted that this trait may be import for effective ant pollination in *Leporella* (Peakall et al. 1987). The majority of ants possess metapleural glands and they are virtually diagnostic of the family Formicidae (Baroni Urbani 1989). If the absence of these glands is critical, ant pollinators should be restricted to the small subset that does not possess them. However, in this orchid pollen is secured to the ant surface by stigmatic secretions and has no direct contact with the ant integument. This plant trait may effectively protect pollen from harmful secretions.

In this paper we examine whether the absence of the metapleural glands is crucial for the pollination of *Leporella fimbriata* by looking at the effects of the three castes on pollen function, fruit production and seed set. We then evaluate the importance of stigmatic secretions to the pollination process.

**Materials and methods**

**Scanning electron microscopy**

SEM examination of the posterior end of the thorax where the metapleural glands open to the outside was undertaken for worker, queen and male castes.

**Effects of the different castes on pollen**

Two kinds of pollen were used: *Brassica campestris* and *Leporella fimbriata*. *Brassica* was used because it has been shown to be highly susceptible to the surface secretions of other ant species (Beattie...
et al. 1985). *Leporella* secures the pollinium to the ant with stigmatic secretions which are smeared on the thorax during pseudocopulation. Pollen is never in direct contact with the ant surface and much of the pollen is held above the ant body. Pollen grains are loosely bound within the pollinium and the stigma normally receives portions of the pollinium rather than the entire mass (Peakall 1989).

Individual males, queens and workers of *Myrmecia urens* were each introduced into a small conical tube and gently secured with a bung of cotton wool. Fresh pollen was applied to the thorax through a window cut into the side of the tube and left for 30 min. Fourteen males, 10 workers and 3 queens were tested using *Brassica* pollen, while 3 males and 10 workers were tested using *Leporella* pollen detached from the pollinium. In an additional 3 workers pollinia were secured to the thorax by means of the stigmatic secretions that normally attach the pollinia to the insect. In these trials the pollen was not in direct contact with the ant surface. Control pollen was taken from the same anther or pollinium and exposed to the air for the same length of time.

Effects on *Brassica* pollen were assayed by transferring it to a germination medium (20 ml 0.1 TAPS ph 8.0, 10 ml 10X Brewbaker and Kwack (1963) medium, 20 g sucrose, 70 ml distilled water) for 15 h and then determining percentage germination. Treatments and controls were counted “blind”. *Leporella* pollen is very difficult to germinate in vitro and so effects of ants were assessed using the fluorochromatic procedure of Heslop-Harrison and Heslop-Harrison (1970). This procedure tests for the integrity of cell membranes. Viable pollen grains absorb the substrate fluorescein diacetate which is hydrolysed by esterases to fluorescein and retained within the cell. Pollen quality is assessed by scoring the percentage of fluorescent pollen grains.

### Effects of males on fruit and seed set

Twelve flowers were bagged immediately following pollination by male ants. As *Leporella fimbriata* is fully self-compatible (Peakall and James 1989) a second flower on the same inflorescence was then pollinated by hand. Mature fruit size, desiccated seed weight, mean embryo size and the percentage of seeds with embryos were compared for ant pollinated and selfed fruits.

### Effects of castes with metapleural glands on fruit and seed set

In an attempt to simulate the natural deposition of pollinia on a caste with metapleural glands, pollinia were attached to the thorax of 11 workers using natural stigmatic secretions as before. Each pollinium was firmly attached to the ant surface but not in direct contact with it. After 30 min pollen was removed and used to pollinate a fresh flower. Control pollen was obtained from the second pollinium of each flower used for ant treatment but, instead of being exposed to the ant integument, it was transferred directly to a second flower on the same inflorescence as the treatment.

Thirty minutes is a realistic time interval between pollinium removal and deposition on the stigma in the field although pollen loads can be carried for several hours and even over night (Peakall 1989). Mature fruit size, desiccated total seed weight, mean embryo size and the percentage of seeds with embryos were compared for treatments and controls.

### Results

**Scanning electron microscopy**

Scanning electron microscopy confirmed that the male caste has no external openings for metapleural glands while the queens and workers do, Fig. 1. Further work in progress shows that queens and workers also have internal secretory cells while the males have none. This is the subject of a future paper.

**Effects of the different castes on pollen**

The data are summarised in Tables 1 and 2. *Brassica* pollen germination and *Leporella* pollen fluorescence was significantly reduced by all castes, the exceptions being a single male for *Brassica* and a single worker with *Leporella*. The effects persisted in males that had been isolated for several days.