

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 important 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

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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 fluorochromic 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

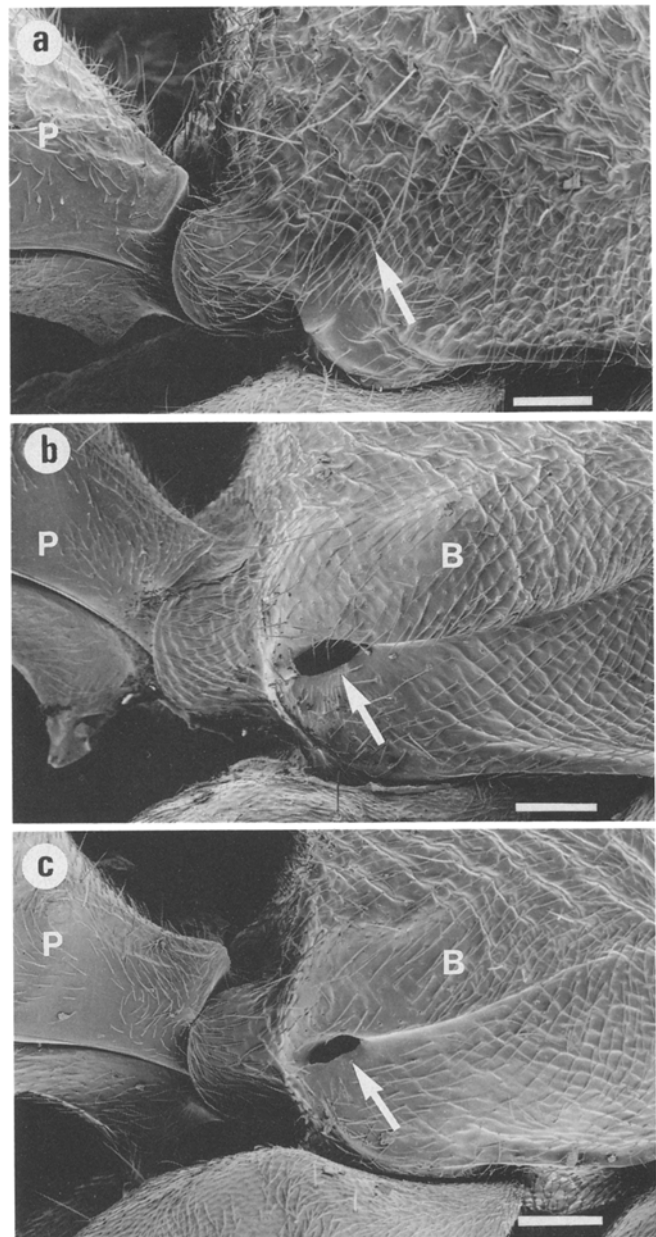


Fig. 1 a-c. SEM of the posterior lateral corners of the propodeum from the 3 castes of *Myrmecia urens*. **a** Male; **b** Female; **c** Worker. **B** bulla, **P** petiole. Arrows indicate absence of the metapleural gland in the male and the opening of the gland in female and worker. Scale bar = 100 μ m

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.

Table 1. Changes in the pollen germinability of *Brassica campestris* and results of *G*-tests (Sokal and Rohlf, 1981) for differences between control pollen and pollen treated by direct contact with male, female and worker castes of *Myrmecia urens*

Ant caste	Median	Range	Probability of					<i>n</i>
			-ve effect (+ve effect)					
			NS	0.05	0.01	0.001		
Pollen-direct contact								
Males	-43.9	-55.2/+6.4	1	0	1	8	10	
Males in captivity 5 days	-41.6	-44.4/-31.5	0	0	0	4	4	
Females	-30.8	-30.1/-21.4	0	0	1	2	3	
Workers	-29.30	-45.9/-17.5	0	1	3	6	10	

Table 2. Changes in the pollen quality (percentage of pollen grains fluorescing with FCR) of *Leporella fimbriata* and results of *G*-tests (Sokal and Rohlf 1981) for differences between control pollen and pollen treated by direct and indirect contact with male and worker castes of *Myrmecia urens*. ¹ These males had been isolated in captivity for 7, 12 and 16 days (refer to text for details)

Ant caste	Median	Range	Probability of					<i>n</i>
			-ve effect (+ve effect)					
			NS	0.05	0.01	0.001		
Pollen-direct contact								
Males ¹	-44.8	-51.0/-44.8	0	0	0	3	3	
Workers	-32.9	-52.9/-7.1	2	1	1	6	10	
Pollen-indirect contact								
Workers	-3.41	-18.2/+7.2	1	(1)	0	1	3	

Effects of males and workers on fruit and seed set

There were no significant differences in mature fruit diameter, seed weight, embryo size or the percentage of seeds with normal embryos between self-pollinated and male-pollinated fruits (Table 3) and between self-pollinated and worker-pollinated fruits (Table 4).

Discussion

The data in Tables 1 and 2 are very similar to previous data from other ant species and pollen types demonstrating that the ant surface is harmful to pollen (Beattie et al. 1984, 1985; Hull and Beattie 1988). However, they also show that absence of the metapleural gland does not reduce the harmful effects as we had previously predicted (Peakall et al. 1987). These findings raise at least two questions:

1) How does *Leporella* pollen avoid the harmful effects of the ant integument? Table 2 showed that pollen loose on the integument quickly loses viability. However, Table 4 showed that pollinia attached to the integument by stigmatic secretions functions normally. Therefore, it appears that the stigmatic secretions insulate the pollen from harmful secretions by preventing direct contact with the ant surface. While this result is clear it does

Table 3. Comparisons of fruit diameter, seed weight, embryo size and percentage of seed with normal embryos for self and male ant pollinated fruits of *Leporella fimbriata*

Mean \pm SD	Self pollinated mean \pm SD	Male ant pollinated mean \pm SD	<i>t</i>	<i>df</i>	<i>P</i>
Fruit diameter (mm)	3.77 \pm 0.44	3.9 \pm 0.44	0.66	19	NS
Seed weight (mg)	1.6 \pm 0.7	1.5 \pm 0.01	-0.29	19	NS
Mean embryo width ^a	1.46 \pm 0.06	1.40 \pm 0.09	-1.79	19	NS
Mean embryo length ^a	2.07 \pm 0.07	2.03 \pm 0.09	-1.22	19	NS
%Normal embryos	91.8 \pm 3.4	80.4 \pm 21.1	-1.59	19	NS
<i>n</i>	9	12			

^a Based on measurements of 30 embryos per fruit, 1 unit = 25 μ m

Table 4. Comparisons of fruit diameter, seed weight, embryo size and percentage of seed with normal embryos for self and artificial worker ant pollinated fruits of *Leporella fimbriata*

Mean \pm SD	Self pollinated mean \pm SD	Worker ant pollinated mean \pm SD	<i>t</i>	<i>df</i>	<i>P</i>
Fruit diameter (mm)	3.81 \pm 0.62	3.86 \pm 0.46	-0.25	20	NS
Seed weight (mg)	1.1 \pm 0.6	1.1 \pm 0.6	0.00	20	NS
Mean embryo width ^a	1.33 \pm 0.12	1.34 \pm 0.12	-0.17	20	NS
Mean embryo length ^a	1.98 \pm 0.14	2.04 \pm 0.11	0.75	20	NS
%Normal embryos	62.7 \pm 11.3	57.8 \pm 17.9	0.76	20	NS
<i>n</i>	11	11			

^a Based on measurements of 30 embryos per fruit, 1 unit = 25 μ m

not rule out the possibility that there is also a dilution effect so that even if some pollen is harmed, enough of the approximately 100000 grains remain functional for full seed set.

2) Where do the harmful effect on males come from? Previous work with *Camponotus*, a genus that does not bear metapleural glands, has shown that pollen is nevertheless damaged following contact with the ant integument (Beattie et al. 1985; Hull and Beattie 1988). It has also been found that mandibular glands secrete antibiotic substances in *Calomyrmex* sp. and *Polyrachis* sp. (Brough 1983; Bellas and Holldobler 1985). It is possible that males of *Myrmecia urens* secrete substances either from other glands, or from the integument itself. Another possibility is the transfer of metapleural secretions from one caste to another. This has been observed by Maschwitz et al. (1970) in the genus *Myrmica*.

Leporella fimbriata is obligately dependent upon ants for pollination. It exhibits highly specialised mechanisms

for the attraction and manipulation of ants (Peakall et al. 1987; Peakall 1989). As the ants nevertheless harm pollen, the avoidance of these harmful effects appears to be primarily associated with plants traits.

In contrast, both ant and plant traits may be important in the avoidance of harmful effects on pollen in the worker ant pollination of the orchid *Microtis parviflora* (Peakall and Beattie 1989). Neither orchid or *Brassica* pollen was harmed by contact with the principal pollinator, *Iridomyrmex gracilis* suggesting that in this species the metapleural secretions are less potent or that the metapleural glands are not active during this critical period. Furthermore, the pollinium in this orchid species is connected by a short stalk (stipe) to the viscidium which attaches to the head of the pollen vector and holds the pollen above the ant surface.

The plant traits associated with the avoidance of harmful effects on pollen seen in *Leporella* and *Microtis* are widespread throughout the orchid family. These traits are unlikely to be adaptations to ant pollination but may have been essential preadaptations to the evolution of ant pollination. Similarly, the absence of metapleural glands in male *Myrmecia urens* can be ruled out as an adaptation for ant pollination because the ant receives no benefit. It is most likely associated with the male's relatively brief sojourn in the ant colony which means that they have little contact with nest mates and fewer opportunities to spread or contact infection (Baroni Urbani 1989). It will be of interest to elucidate the mechanisms associated with the protection of pollen in proven cases of ant pollination of non-orchid species.

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References

- Baroni Urbani C (1989) Phylogeny and behavioural evolution in ants, with a discussion of the role of behaviour in evolutionary processes. *Ethol Ecol Evol* 1:137–168
- Beattie AJ, Turnbull CL, Hough T, Knox B (1986) Antibiotic production: a possible function for the metapleural gland of ants (Hymenoptera: Formicidae). *Ann Entomol Soc Am* 79:448–450
- Beattie AJ, Turnbull CL, Hough T, Jobson S, Knox B (1985) The vulnerability of pollen and fungal spores to ant secretions: evidence and some evolutionary implications. *Am J Bot* 72:606–614
- Beattie AJ, Turnbull CL, Knox RB, Williams E (1984) Ant inhibition of pollen function: A possible reason why ant pollination is rare. *Am J Bot* 71:421–426
- Bellas T, Holldobler B (1985) Constituents of mandibular and Dufour's glands of an Australian *Polyrachis* weaver ant. *J Chem Ecol* 11:525–537
- Brewbaker JL, Kwack BH (1963) The essential role of calcium ion in pollen germination and pollen tube growth. *Am J Bot* 50:859–865
- Brough EJ (1983) The antimicrobial activity of the mandibular gland of a formicine ant *Calomyrmex* sp. *J Invert Pathol* 42:306–311
- Heslop-Harrison J, Heslop-Harrison Y (1970) Evaluation of pollen viability by enzymatically induced fluorescence: intracellular hydrolysis of fluorescein diacetate procedure. *Stain Technol* 45:115–120
- Hull DA, Beattie AJ (1988) Adverse effects on pollen exposed to *Atta texana* and other North American ants: implications for ant pollination. *Oecologia* 75:153–155
- Maschwitz U, Koob K, Schildknecht H (1970) Ein Beitrag zur Funktion der Metathoracaldrüse der Ameisen. *J Insect Physiol* 16:387–404
- Peakall R (1989) The unique pollination of *Leporella fimbriata* (Orchidaceae): Pollination by pseudocopulating male ants (*Myrmecia urens*, Formicidae). *Plant Syst Evol* 167:137–148
- Peakall R, Beattie AJ (1989) Pollination of the orchid *Microtis parviflora* R. Br. by flightless worker ants. *Funct Ecol* 3:515–522
- Peakall R, James SH (1989) Outcrossing in an ant pollinated clonal orchid. *Heredity* 62:161–167
- Peakall R, Beattie AJ, James SH (1987) Pseudocopulation of an orchid by male ants: a test of two hypotheses accounting for the rarity of ant pollination. *Oecologia* 73:522–524
- Sokal RR, Rohlf FJ (1981) *Biometry*, 2nd Ed. Freeman, New York