BULLETIN

OF THE

WESTERN AUSTRALIAN NATIVE ORCHID STUDY AND CONSERVATION GROUP (INC)

JULY 1991

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OBJECTIVES OF THE GROUP

a. To promote interest in and preserve Western Australian indigenous Orchids.

b. To learn the best means of cultivation and do all things possible for the conservation of native orchids in their environments.

c. To learn their habitats and keep records.

d. To have field days and learn to recognize the different genera and species.

e. To hold meetings for the exchange of knowledge and furthering of interest in Western Australian orchids.

f. To affiliate with kindred organisations.

g. To make rules for the governing of the Group's domestic affairs.

h. To do all such other lawful things as are incidental to or conductive to the attainment of the above objectives.

NOTE: The opinions expressed by contributors to this bulletin are not specifically endorsed by the group.

POSTAL ADDRESS OF GROUP
PO Box 323
Victoria Park 6100

NEXT COMMITTEE MEETING - Wednesday 17th July 1991 at 7.00pm, Kings Park Board Administration Centre.

NEXT GENERAL MEETING - Wednesday 17th July 1991 at 8.00pm, Kings Park Board Administration Centre.

Topic for General Meeting:-

Mrs. Margo O'Bryne will be speaking about system 6. Mrs. O'Bryne is the community involvement coordinator.

BULLETIN CONTRIBUTIONS

Contributions are needed for every edition of the Bulletin. Articles should be sent to Marie French [redacted]

The article submission deadline for the next issue of the Bulletin is 1st August, 1991.

ANNUAL GROUP MEMBERSHIP FEES -- RED SPOTS

Does your bulletin have a red spot? If so, you are currently an unfinancial member of WANOSCG. 1991 Membership Fees are now due!

The group membership fees remain unchanged for 1991 as follows:

Single Membership	$15.00 pa
Family Membership	$15.00 pa
Junior Membership	$ 2.00 pa

NEW MEMBERS

The committee and members would like to welcome the following people to the club, Mr. & Mrs. Gary & Suzanne Clark of [redacted], Joanne Armstrong and Frank Kelly of [redacted], and Mr. E (Jim) Smith of [redacted]. We hope you all have a rewarding association with our group.

RIP

The committee and members wish to extend to Mrs. Thelma Spice and her family our sincerest condolences on the sad passing of her husband George.

GET WELL

Paul Schaber will soon be having an eye operation. We hope it is successful and you feel well soon Paul.

FORTHCOMING FIELD TRIPS

John Forrest Sunday 21/7/91

We meet at the main entry on the left going up Greenmount Hill at 10.30am. There should be Pterostylis vittata, P. barbata and P. recurva in flower. On the other side of the road has been a summer burn and an early inspection has revealed quite a lot of leaves although I think we will be too early to see very much in flower.

Wave Rock - 17th & 18th August

Bookings have been made at the Wave Rock Caravan Park for all those who requested them, Bill Jackson will be leading the trip and has asked that everyone is booked in and ready to leave the caravan park at 10.00am sharp! If anyone else wants a booking they must contact me soon, as there is very little left in the way of on-site accommodation, no problems with caravans and tents.

Leeman - 24th & 25th August

No bookings have been made at this stage, but this trip is straight after the Wave Rock weekend so all those wanting accommodation please advise me so that I can make bookings, we also require a trip-leader please.

Nye Evans

PAPER AS A CULTURAL MEDIUM FOR TERRESTRIAL ORCHIDS

This article appeared in the May 1991 "The Orchidophile" courtesy of The Australian Native Orchid Society - Sydney Group Inc.

The use of tissue and towel paper has surprisingly proven to be a promising medium for the cultivation of a few terrestrial orchid genera. I have not yet proven it to be a reliable medium for flowering orchids but it has shown exceptional qualities for the propagation of a number of species and in particular for recovering sick or ailing terrestrial orchids.

It provides a clean cellulose environment, disease resistant, easy to wet, retaining a beautifully balanced level of moisture, and is as pliable a medium as one could wish. It is impossible to over water and terrestrial orchid tubers formed in it are healthy and covered with white filaments.

The paper medium also aids in the recovery of plants such as Pterostylis species effected by tuber rot.

The idea dawned when I wanted to send a friend some bare root Genoplesium species by mail. I found that I had run out of sphagnum moss, and thought of using tissue paper as a substitute. The tissue paper was wrapped around the tuber in a furled or quarter folded style and put then in a coffee mug to await finding a suitable box and the energy to go to the post office. Well they grew so well next to a window ledge in the study over the intervening weeks on nothing but tap water that I decided not to send them but to continue the experiment. They have now been in the mug for two years and half of them flowered this summer. During this time they have only had one dilute application of fertilizer.

Following up the notion that there may be promise in this method of cultivation I re-potted about 14 lots of species that were looking sick or unhappy during April to July last year. I tried a range of paper products including paper towels and toilet paper. I used newspaper and cardboard in larger pots for dividing the pot volume into sections to make the placement of the tissue wrapped terrestrials easier to manage. I filled in any spaces with a combination of casurina needles and buzzer chips or small pebbles.

The initial results were again promising with a lot of root growth and new tuber formation showing at the bottom of the pots containing Pterostylis species. The only thing that concerned me was how they would fare over the summer dormancy. Would they desiccate or would the paper covering maintain the tubers?

Well good results to report - all the pots are showing healthy and thriving plants although they seem to be later in their emergence with new season growth. Some difficult species such as P. parviflora and P. acuminate are multiplying. There seems to be an indication the Pterostylis species may require some added nutriments as the Pterostylis plants are vegetatively smaller in size than their relatives in pots with soil medium. However, Thelymitra and Caladenia species seem to be enjoying the paper medium most as they look larger and healthier than soil medium grown plants. I have yet to try it with Diuris species but I can perceive that it should prove an excellent medium for enticing additional tuber growths in Diuris plants from which the new tuber has been removed.

I am also trialling this season the use of the paper medium as a more reliable way in inducing symbiotic growth of terrestrial seedlings from seed sown into mother pots.

A WARNING - use of paper as a medium is still at an experimental stage so I wouldn't advocate anyone changing over to paper until it has been more extensively trialled. The question of which fertilizers to use and how long the paper retains its structure before composting and becoming a problem needs further study.

I would however suggest that if you have a few ailing plants that you might consider using this technique to recover them. As a medium it might also prove effective in the deflasking of terrestrial seedlings. Time will tell.

Jim Lykos

"PIONEER BOTANISTS OF W.A" - by G.G. Smith

The following is a continuation of the article we ran in the June Issue and it is part of our groups' tribute to the 200th anniversary of WA Native Orchid collection and research.

"Visiting Botanists and Voyages of Discovery".

The earliest botanical observations of the vegetation of Western Australia appear to be those of William Dampier on his two investigations of the coast of New Holland. On his first voyage in 1688 Dampier, the Buccaneer, made a few notes on the soil and vegetation in the region of Cygnet Bay as well as reporting unfavourably on the natives.

Eleven years later Captain Dampier, under the instructions of the British Government again visited New Holland, landing at Shark Bay, which he named in August, 1699. On this occasion he reported more fully on the vegetation, remarking on the aridity of the soil, the variety of leaf forms of the plants, and the colour and scents of the flowers. Dampier was particularly impressed by the presence of blue flowers in the flora of the area and very likely he observed one of the blue flowering species of the Leschenaultia family - probably one of the Dampieras - a genus named in his honour. The Sturt Pea and the Lucky Bean were also mentioned in his report. The coastal shrub Olearia axillaris reminded him of rosemary and led him to name Rosemary Island in the Dampier Archipelago after the profusion of this shrub on some of these islands.

Dampier collected and dried some forty native plants and so made the first collection of West Australian plants to reach European herbaria. A dozen of these pressed specimens are at present in the Herbarium of the Oxford University.

Nearly a century passed before the next collection of native plants was made by Archibald Menzies, the naturalist of the Vancouver Expedition. When in 1791 Vancouver discovered and named King George Third Sound, Menzies made large collections about the Sound and these were described and named some years later by the illustrious botanist of Kew, Robert Brown.

Following Vancouver the French dominated the exploration of the coast of New Holland for a time, and they also contributed to the botanical knowledge of this newly discovered land. D'Entrecasteaux's Expedition visited the southern coast of W.A. towards the end of 1792 in the corvettes, "La Recherche" and "L'Esperance". Their first landing was made on one of the islands in Esperance Bay and later on the mainland, Jacques Julien H. de Labillardiere, one of the naturalists of the expedition collected plants extensively in the Esperance region during their brief stay of a few weeks. We know from his excellent publication on the natural history of the Esperance area that Labilladiere collected Banksias, Myrtaceous plants and numerous other small plants as well as observing most accurately the geological relationships of the limestone and granite and their respective soils.

Labillardiere was followed by two naturalists, Leschenault de la Tour and M. Guichenot, who accompanied the ships "Geographe" and "Naturaliste" under Baudin and Peron. This expedition landed in Geographe Bay in May 1801 and the two naturalists eagerly collected the flowering plants of the vicinity of Cape Naturaliste. Later the "Geographe" sailed north, where Leschenault collected and made notes on the flora of Bernier Island and Shark Bay, while the" Naturalist" explored the Swan River District and Rottnest Island. The expedition then explored the Southern and Eastern shores of Australia, returning two years later to Western Australia. On the return voyage, King George's Sound was visited where Guichenot and Leschenault made large collections of the plant life of the Sound. Leschenault's collections were deposited in the Museum of Natural History in Paris but were never thoroughly worked on or described by British or European botanists. Leschenault's only publication on his and his colleagues' labours is his appendix to Freycinets chronicle of the Expedition under the title "On the Vegetation of New Holland and Van Diemen's Land". However, both men were commemorated by the two attractive genera of plants Leschenaultia and Guichenotia.

Yet another french expedition was to explore the western coast of Australia between 1817 and 1820 under the command of Freycinet in the ships "L'Uraine" and "La Physicienne". The botanist of this expedition was C. Gaudichaud. His collections made chiefly at Shark Bay, were taken back to Paris where he described a few of the species in the published account of that voyage.

The part played by the French in this period is clearly marked by place names about the western coast of Australia and is again reflected in the names of several wild flowers, namely Leschenaultia, Guichenotia and Billardiera.

The apparent interest of the French in New Holland spurred the British to take more interest in the west coast and eventually it was claimed for the Crown. Likewise the interest of British scientists was also extended to the west coast and the development of its natural history was largely instigated by the scientists of Kew and the British Natural History Museum.

While Baudin and Peron were still exploring the western shores of Australia, Captain Flinder's expedition arrived at King George's Sound in December of 1801. The botanist of this expedition was the illustrious Robert Brown, of whom it has been said "as keeper of the Botanical Department of the British Museum - for over forty years, sat spider-like at the centre of the botanical web". Brown's whole life was given to botanical research and his many excellent publications on a wide field of botanical research earned him a very high place in the history of botany.

In the three weeks of the Investigator's stay at the Sound, Brown made a very thorough collection of the plants flowering at the time. These pressed specimens and others from the Recherche Archipelago and Cape La Grande became the foundation of his great Australian collection which was to prove so valuable in the preparation of Bentham's "Flora Australiensis". With Brown was the botanical artist Ferdinand Bauer, who illustrated many of Brown's descriptions of species with fine line drawings, accurate in minutest botanical detail.

The earliest period of the botany of New Holland came to a close with the visit of Alan Cunningham in 1818 when he accompanied Captain King's survey voyage. Cunningham, a botanical collector of Kew Gardens, studied the flora of the coastal regions, including King George's Sound, Swan River and very briefly, Dirk Hartog's Island.His accurate and detailed observations and his notes on the species collected made a valuable contribution to the growing knowledge of the flora of this new

country. A general account of the vegetation was written by Cunningham in an appendix to King's - "Narrative...of Australia".

While Robert Brown was at King George's Sound he collected the drum-stick blackboy, or black gin, a species then new to botanical science. Twenty-four years later Brown named and described this handsome plant as Kingia australis when further knowledge of its habit had been obtained for him by Cunningham and by Captain King and Mr. William Baxter, who collected the flowers and ripe fruits necessary for a complete description of the plant. Brown honoured Captain King in naming this blackboy; to put it in his own words - "To this new genus I have given the name of my friend. Captain King, who, during his important surveys of the coasts of New Holland, formed valuable collections in several departments of Natural History, and on all occasions gave every assistance in his power to Mr. Cunningham, the indefatigable botanist who accompanied him. The name is also intended as a mark of respect to the memory of the late Captain Phillip Gidley King, who, as Governor of New South Wales, materially forwarded the objects of Captain Flinders' voyage; and to whose friendship Mr. Ferdinand Bauer and myself were indebted for important assistance in our pursuits while he remained in that colony".

SNAKES AND SNAKE BITE - a summary of last month's general meeting talk by Derek Mead-Hunter.

SNAKE BITES AND AVOIDING BEING BITTEN

Most reported snake-bite cases in Australia could be avoided if the person had used a little common sense. By far the most bites occur when people try to catch, handle or kill snakes. I don't think anybody should try to catch snakes unless they are an expert.

In the bush you can avoid snake bite by:-

1. Leaving them alone and they will leave you alone on most occasions. Therefore do not pick up logs, pieces of corrugated iron or similar things that snakes may be under. If you must shift things on the ground, do it with your foot first.

2. Don't put your hand down hollow logs - if you have to get something out of a log - look first.

3. If you are in long grass or thick vegetation tread fairly heavily or drag your feet. The snake will pick up the vibrations off the ground and realize that something big and heavy is coming through and it will move off pretty quickly.

4. If you come across a big log or rock in the bush, never step straight over it. Always step on the object first and look straight down. If the snake is sunning itself, particularly on a windy day, it will hide behind something that will break the wind. They have to sun themselves to get their body temperature up to their right operating temperature and find these sheltered spots ideal. A lot of people have been bitten by just stepping over objects in the bush - the snake won't like being trodden on and will usually strike.

5. Do not wear thongs, sandals or similar open shoes. Wear a stout pair of walking shoes or boots, ankle protection is preferable as most snakes tend to strike low down on the legs - wear jeans as well for added protection. Most Australian snakes have very short fangs compared to other countries' snakes. The short fangs may get caught in your jeans - rather than your leg.

IF YOU ARE BITTEN

If you get bitten by a snake a few rules should be followed:-

1. Don't' panic - <u>if possible!</u> Suppress panic as best you can as Blood Pressure goes up and venom spreads through the body very quickly.

2. Don't use a tourniquet or any other old fashioned remedy - such as slashing with a razor blade - sucking out the venom, cutting off the leg etc.

3. Don't give alcohol or any other stimulant.

4. Do not wash the area around the snake bite. The venom around the bite area is not going to do any harm to the victim. Leaving the venom on the victim's skin, will only enhance the Medical experts ability to identify the snake, therefore making it possible for them to administer the correct anti-venene.

   If somebody can actually safely kill the snake for proper identification then all the better. (Remember colour is not a good guide to snake identification).

If the hospital does not have Mono-valent anti-venene, they can administer a poly-valent anti-venene. Mono-valent anti-venene is administered to a victim when proper identification of the snake has been made, whereas Poly-valent anti-venene is used when the identification of the snake is not known. Poly-valent is given through a drip into the blood stream and a larger quantity of Poly-valent must be used to neutralise the wound compared to the Mono-valent anti-venene.

5. DO apply a broad pressure bandage around the wound and preferably right up the limb. IMMOBILIZE the limb using splints, and if the bite is on the arm, use a sling. Bandage as if you were bandaging a bad sprain - you don't want to cut off the blood flow - you just want to suppress the lymph travel rate rather the actual blood flow. Venom is actually carried through the lymph. The victim should be helped or carried as quickly and calmly as possible to a hospital where qualified people can then take over.

GENERAL INFORMATION

If there are a lot of bites around the wound it doesn't necessarily mean a non-poisonous python is the snake that has bitten you, a poisonous snake using it's two main fangs at the front may actually have one to four fangs in biting position - so always bandage any snake bite and get to a hospital even if you suspect that the snake is not poisonous.

If you do get bitten by a known non-venomous snake, wash the wound very well as snakes do carry a lot of bacteria on their teeth. You may need a course of antibiotics if you get an infection.

Any day that is fairly warm and overcast, snakes will usually be active. They don't like it too hot and also they will move about a lot at dawn and dusk.

Baby Dugites have a black head but when they get older they lose the black markings. This black marking doesn't necessarily help you identify the Dugite - as quite a few snakes have a black head. So leave any snake alone - unless you know what you are doing because head-shape and colour won't help you identify them.

Very few people actually die of snake bite in Australia. The deadliest snake in the world is an Australian Snake, the Inland Python. There have been four recorded bites and everybody has survived - it is just a matter of getting the victim to a hospital.

One man was bitten on the Birdsville Track and still made it! Some people of course do die (some people also die of bee-stings) but they may be allergic to the venom - so it will react a lot more quickly in their bodies. If you are bitten on the femoral artery, you probably won't last long either! The average healthy adult snake bite victim, with correct bandage and immobilization, will last a couple of hours at least. Australian snakes have a neuro- toxic venom - so it stops you breathing (which of course results in death). If the victim begins to gasp for air, then mouth-to-mouth resuscitation should be applied, this will keep the victim alive until medical experts can take over.

Did you know, African and American vipers have myo-toxic venom, which destroys all your muscle cells and you get massive ulcers? To cure you, large sections of muscle have to be removed and you have to be rebuilt by a plastic-surgeon! So get bitten by an Australian snake if you want to be bitten!

Most snakes have an anti-venene - even sea snakes. The main time to be wary of snakes is spring (i.e. Orchid time). They have just finished "Hibernation" and their venom is more toxic because it is more concentrated (venom is toxic saliva) - their first urge it to get something to eat and their second urge is to mate! September to December and February to March are the bad times.They quieten in the real heat of December and January.

KILLING SNAKES

There are a lot of misconceptions about killing snakes - one popular belief is that to kill a snake a person has to cut its head off, but if you actually miss the snake your hands are so close to its head that they are within striking range. There is a documented case of a person actually cutting the head off a snake and the head flying up and a fang lodging the in person's forehead!

Of course all snakes are protected and they really shouldn't be killed unless they are realistically endangering someone's life. I have heard a lot of people saying in National Parks - "We killed this snake, it could have bitten someone" - this is not a valid reason for killing the snake. 1) It is in a National Park and 2) it was probably minding its own business.

I also have a lot of enquiries about "back-yard snakes" every year at the University. On most occasions the snake can be left alone and it will move off on its own accord, but occasionally they are a danger to lives and have to be killed.

The safest way to kill a snake is to break its back. Use the back of a shovel or a rake or a green stick - i.e., one that will bend - a dry stick will break on impact. Whatever you do, be humane and kill the snake as quickly as possible, with a hit to the head.

The reason for breaking the snake's back is that it reduces the snake's striking range. A snake can actually strike to at least 2/3rd's to 100% of its body's length. So if you are confronted by a six foot snake it has a striking range of almost four feet! If you break its back 1) it is in agony and it is more interested in writhing around and 2) if it actually strikes at you it will have a shortened strike radius.

FOR SALE

ORCHIDS OF WESTERN AUSTRALIA..2ND EDITION.

WANOSCG's second publication of the cultivation and natural history of WA native orchids is now available with 14 pages of COLOUR PHOTOGRAPHS.

THE BOOK IS PRICED AT...
$ 9.00 (plus $2.00 postage) for WANOSCG members. OR
$12.00 (plus $2.00 postage) for non-members.

THELYMITRA APICULATA BADGES

The badge features Thelymitra apiculata, a beautiful orchid, the status of which is not declared rare and endangered, but is under the scrutiny of CALM.

The badges cost $4.00 (plus $1.00 postage and packaging).

ANOS BADGES

Remember the Diuris purdiei conservation badge?

The second conservation badge, featuring Dendrobium bigibbum, is available for $4.50. All profits from the sale of this badge will be used for orchid conservation.

Also available for $3.00 is the badge for the 1st Australian Native Orchid Conference. This badge features Pterostylis gibbosa which has been chosen as the logo for this conference.

Please add $1.00 postage and packaging to each order.

ORCHID SPOONS

The Victorian Group of ANOS has announced it intends producing a third series of three spoons featuring Australian Native Orchids.
This series will feature:-
Dendrobium kingianum
Calochilus richae
Sarcochilus ceciliae

The spoons will be released in July/August of this year and will be priced at $5.95 (less 25% for order of 12 or more).

W.A.N.O.S.C.G. GENERAL MEETING
19th JUNE, 1991

PRESENT and APOLOGIES as per attendance book.
Visitors present and welcomed were Amanda Walker, and Rob Ward who is from New Zealand.

MINUTES.
The minutes of 15th May were published in the Bulletin.
Moved Burdinat/Burton "that the minutes be accepted as read."
Carried.

BUSINESS ARISING.
There is much to be done in preparation for the Orchid Conference and display to be held in W.A. in September.

CORRESPONDENCE.
Inward As tabled.
Outward As tabled.
Moved Clarke/Greeve "that Inward Correspondence be accepted and Outward Correspondence be ratified."
Carried.

TREASURER'S REPORT.
As circulated.
Moved Parker/Swarts "that the Treasurer's Report be accepted.
Carried.

FIELD TRIPS.
As Bulletin.
A trip planned to The Gnangarra Pine Plantation on Saturday 29th June. A barbeque will be held if the weather is suitable.
Chris French will try to organise a rescue dig at Australind.

PLANT TABLE.
Noel Clarke talked about the orchids brought in by W. Burton and I. Greeve.

GENERAL BUSINESS.
There is a very interesting and possibly helpful article in the Orchidophile on the use of paper for cultivating some species of orchid.

Guest speaker Derek Mead-Hunter gave a most interesting talk on snakes and snakebite.

Supper was provided by A. Evans, and L.George provided the raffle.

The next meeting is to be held on July 17th, 1991.

Meeting closed 9.38.PM.

Oecologia (1990) 84:457–460

Oecologia

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

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Received January 3, 1990 / Accepted in revised form May 18, 1990

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

* This is contribution number 98 to the Research Unit for Biodiversity and Bioresources, Macquarie University

Offprint requests to: R. Peakall

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 germinaton 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, dessicated 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.

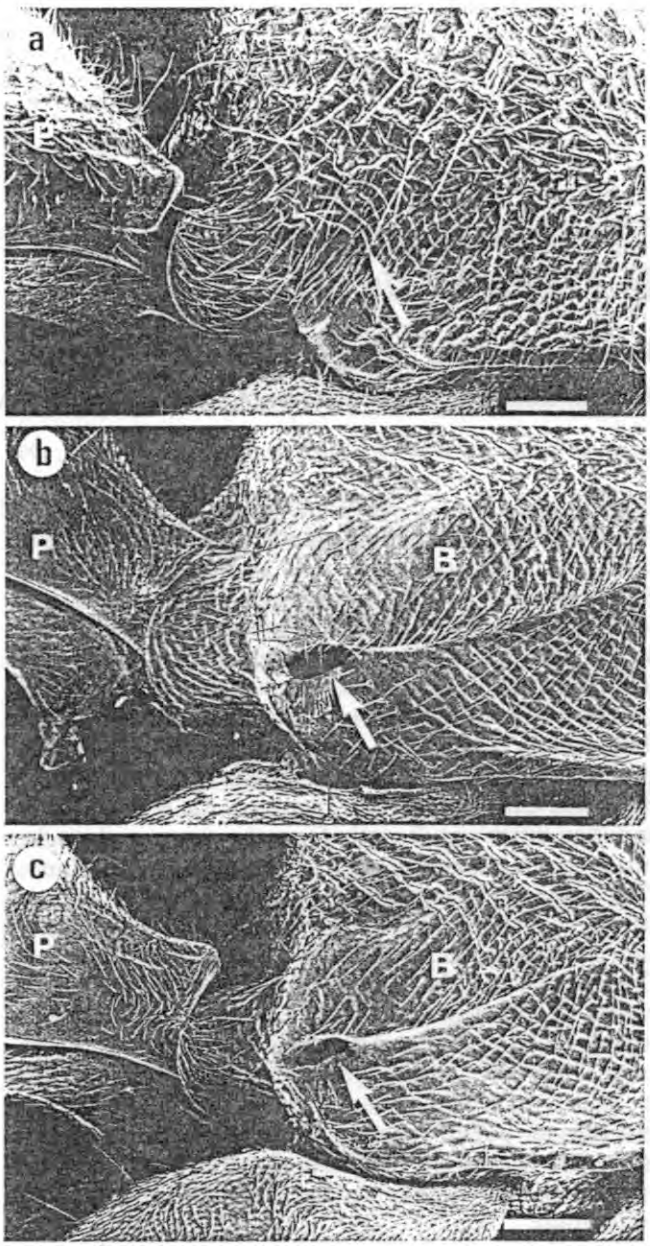

Fig. 1 a–c. SEM of the posterior lateral corners of the propodeum from the 3 castes of *Myrmecia urens*. a Male; b Female; c Worker. *B* bulla, *P* petiole. Arrows indicate absence of the metapleural gland in the male and the opening of the gland in female and worker. Scale bar = 100 μm

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 −ve effect (+ve effect)				
			NS	0.05	0.01	0.001	*n*
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 −ve effect (+ve effect)				
			NS	0.05	0.01	0.001	*n*
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).

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

[a] Based on measurements of 30 embryos per fruit, 1 unit = 25 μm

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 ± SD	Self pollinated mean ± SD	Worker ant pollinated mean ± SD	*t*	*df*	*P*
Fruit diameter (mm)	3.81 ± 0.62	3.86 ± 0.46	−0.25	20	NS
Seed weight (mg)	1.1 ± 0.6	1.1 ± 0.6	0.00	20	NS
Mean embryo width[a]	1.33 ± 0.12	1.34 ± 0.12	−0.17	20	NS
Mean embryo length[a]	1.98 ± 0.14	2.04 ± 0.11	0.75	20	NS
%Normal embryos	62.7 ± 11.3	57.8 ± 17.9	0.76	20	NS
n	11	11			

[a] Based on measurements of 30 embryos per fruit, 1 unit = 25 μm

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 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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(4) Number of mature plants held on behalf of the Group.

(5) Estimated number of tuberoids avail. for sale in current season.

(6) Estimated number of tuberoids required in current season.

NAME	3	4	5	6	7	8	NAME	3	4	5	6	7	8
e.g. DIURIS longifolia													
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