

Pollination ecology of *Tetratheca juncea* (Tremandraceae): finding the pollinators

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Abstract: *Tetratheca juncea* Smith (family Tremandraceae) is a terrestrial herbaceous plant now mainly found in the Lake Macquarie area of coastal NSW and listed as Vulnerable under Schedule 2 of the NSW *Threatened Species Conservation Act* 1995. This study carried out from July 2001 to June 2002 records the observation and identification of two species of native bee buzz-pollinating its flowers and describes a direct relationship between the first appearance of a pollinator and the commencement of seed set. Findings from this study with respect to the pollination ecology of *Tetratheca juncea* are:

- There is a strong flowering period from September to January, though a number of flowers can be found on some plants across the geographic range of the plant in all months of the year;
- Two species of native bee *Lasioglossum convexum* and *Exoneura* sp, were confirmed collecting pollen from the flowers by way of buzz pollination;
- Fruiting only occurred in coincidence with flower pollination by these bees;
- Flowering, seed set and seed release was a concurrent process while ever the bees were active;
- The bees are polylectic and the sexual reproductive process in *Tetratheca juncea* appears to be pollinator-limited.

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Introduction

Tetratheca juncea Smith (Tremandraceae) is a terrestrial herbaceous plant endemic to NSW. It is listed as Vulnerable under Schedule 2 of the NSW *Threatened Species Conservation Act* 1995, as Vulnerable in the *Commonwealth Environment Protection and Biodiversity Conservation Act* 1999 and has a ROTAP coding of 3VCa (Briggs & Leigh 1995). *Tetratheca juncea* is now known only from Wyong to Bulahdelah and inland to the edge of the main ranges with the greatest concentration of records being from the Wyong and Lake Macquarie local government areas (Payne 2000). There species was recorded in what are now suburbs of Sydney in the late 19th Century, from Port Jackson and suburbs to the south (Thompson 1976) but is now regarded as extinct there.

In the Munmorah area *Tetratheca juncea* grows in low open forest in association with *Angophora costata*, *Eucalyptus capitellata*, *Eucalyptus haemastoma* and with a mixed shrub and grass ground cover (Payne 1993). Payne (2000) concluded that the preference for the plant was that of Munmorah Conglomerate geology with the Awaba Soil Landscape Unit and that the plant showed a preference for dry ridges and shade. Table 1 shows the number of records for 400 records of *Tetratheca juncea* sites compiled from published and unpublished surveys (Payne 2000, Bartier et al. 2001, and S. Bell & C. Driscoll unpublished) in vegetation communities occurring within the Lower Hunter and Central Coast (LHCCREMS) (NPWS 2000, Eco Logical Pty Ltd 2002).

Table 1. Distribution of reports of *Tetratheca juncea* across vegetation map units in the Lower Hunter and Central Coast.

Description	Map Unit	Percentage
Coastal Plains Smoothbarked Apple Woodland	MU 30	62%
Coastal Plains Scribbly Gum Woodland	MU 31	14%
Coastal Foothills Spotted Gum-Ironbark Forest	MU 15	10%
Alluvial Tall Moist Forest	MU 5	2%
Heath	MU 55	2%
Lower Hunter Spotted Gum-Ironbark Forest	MU 17	1%
Wyong Paperbark Swamp Forest	MU 43	1%
Coastal Sheltered Apple-Peppermint Forest	MU 11	1%
Coastal Sand Wallum Woodland-Heath	MU 34	1%
Swamp Mahogany-Paperbark Forest	MU 37	1%
Riparian Melaleuca Swamp Woodland	MU 42	1%
Coastal Clay Heath	MU 48	1%
Coastal Wet Sand Cyperoid Heath	MU 44	<1%
Coastal Wet Gully Forest	MU 1	0.5%
Coastal Sand Apple-Blackbutt Forest	MU 33	0.5%
Hunter Valley Moist Forest	MU 12	<0.5%

GIS analysis of regional distribution of records against 1:250 000 geological maps (Environment Australia) shows that *Tetratheca juncea* plants have been found growing on following underlying geologies: Quaternary Sands; Triassic Sandstones; Triassic Shales; Permian Coal Measures and Carboniferous Volcanics.

Tetratheca juncea is distinguished from other members of the *Tetratheca* genus by having generally leafless stems that have a distinctly angular, winged structure (Thompson 1976). The flowers however share the four-petalled, pink form that is characteristic of the genus (although, during this study, flowers with 5, 6, and 7 petals proportionately arranged were recorded). The flowering period is generally reported as being from mid to late winter through to late summer (Gardner

& Murray 1992). The flowers grow from nodes on the mostly leafless stem and are commonly solitary, but occasionally in pairs with each flower facing downward, suspended on a peduncle of about 10 mm length. The four petals range in colour from mauve through pink to almost white (Thompson 1976). There are eight dark mauve poricidal anthers surrounding the carpel with the stigma protruding beyond their length (Bartier et al. 2001). Payne (2000) describes the anthers as containing the pollen in a basal sac with Bartier et al. (2001) also noting that the pollen is contained basally in tapetal fluid.

Reproduction in *Tetratheca juncea* is through both asexual rhizomal spread and sexual pollination, seed development and germination (Payne 2000). Bartier et al. (2001), using hand pollination, showed that while the plant is capable of autogamy, the quantity of seed development was far greater with outcrossing. The potential for autogamous seed set was considered to be low because of the pendant presentation of the flowers. Recruitment by seed appears to be limited by a dispersal mechanism involving ants collecting the seed, for the lipid-rich elaiosome (Brew et al. 1989, Boeswinkel 1999).

Pollen vectors of *Tetratheca juncea* have not been described to date despite considerable effort. Bartier et al. (2001) spent almost 100 observation hours in set periods of 15–25 minutes, but did not observe any insects collecting pollen from the flowers. Payne (2000) proposed that a Carpenter Bee could extract pollen from the flowers by way of vibration, or that pollen might simply dry in the anthers and fall out with some grains lodging on the stigma. Hingston (1999) in southern Tasmania, collected *Lasioglossum* species and *Homalictus niveifrons/megastigmus* from the flowers of *Tetratheca glandulosa*. Bartier et al. (2001) proposed that a buzz-pollinator for *T. juncea* could be members of the *Homalictus* genus and possibly *Homalictus megastigmus*. The aim of this investigation was to determine the identity and behaviour of pollen vectors involved in the sexual reproductive process for *Tetratheca juncea*.

Methods

Stamen and anther detail

In order to better understand the mechanism for pollen removal, details of the stamens and their arrangement in the developing flower were examined using dissecting, compound and scanning electron microscopes (SEM).

Field observation for pollinators

Fieldwork was carried out at Wakefield, near Toronto (Figure 1) where there were large populations of *Tetratheca juncea* extending over a wide area. Two populations were selected: Site 1 had about 200 plants growing within an area of approximately one hectare and Site 2, about one kilometre southeast of Site 1, had around 50 plants within an area of approximately 50 × 25 metres. The habitat of the *Tetratheca juncea* here had a low grassy ground cover with a sparse shrub cover that facilitated observer movement around the

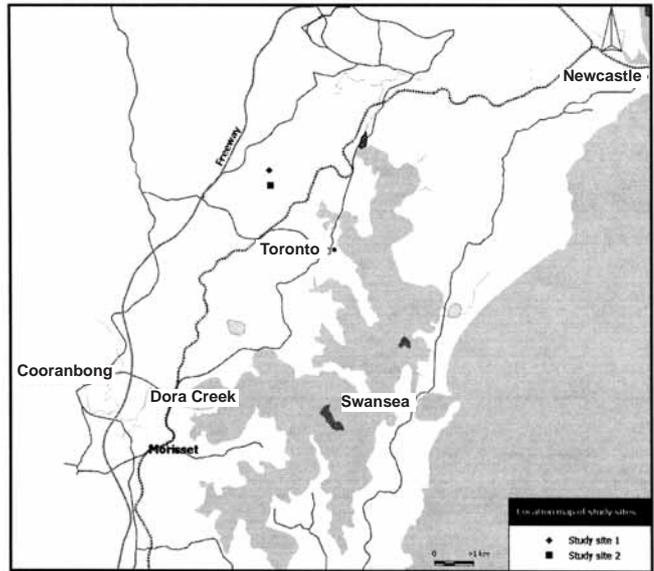


Fig. 1. Location of the study sites

populations, and allowed observation of a wide area from any one position. Each site contained some large patches of *Tetratheca juncea* plants in dense flower and the sites were surrounded by many hectares of relatively undisturbed forest. Plant names follow Harden (1992–2002)

A basic premise at the start of this study was that the amount of pollen available from *Tetratheca juncea* flowers would be so small as to only provide a satisfactory reward-for-effort to a small insect and that the search for a pollinator would require very close observation. Within both sites, areas with the greatest density of flowers were given the closest attention, commencing at the start of flowering in July 2001. Initially, because nothing was known about the behaviour of, or type of pollinators of *Tetratheca juncea*, each site was continuously walked (with arbitrary changes of direction) over a period of two to three hours at a time. Effort was limited to days where the weather was fine and with no more than a light breeze. The start time each day varied and was the time by which any dew on the flowers had finally evaporated. These conditions allowed for up to three observation periods per week. This was done from August 2001 to November 2001 with more general monitoring continuing after that period.

Once a pollinator had been observed at either site, attention was directed towards dense patches of flowers and these were watched for up to two hours continuously and bees were captured for identification. The vegetation surrounding the *Tetratheca juncea* flowers made capture of the bees with nets impractical. Instead bees were captured by clamping a flat lid over the flower with the bee attached, on to a container about 75 mm wide, so capturing bee and flower together. The bees, still in the containers in which they were captured, were placed briefly in a deep freeze and then kept in a refrigerator. Dr Michael Batley of Macquarie University Division of Earth and Life Sciences and who is also associated with the Australian Museum Entomology Department provided the identification of the bees and the pollen that they carried.

Because the bees are out of sight under the flower when extracting pollen, an Anabat II bat-call detector (Titley Electronics, Murwillumbah) set on Division Ratio 4 (not an application the makers would have envisaged) was used to 'listen' for the characteristic buzz of the bees extracting pollen. A major advantage of the Anabat device over straight audio recording was the elimination of the majority of background noise. Using the detector, the buzz was detectable from around half a metre; at this distance it was inaudible to the un-aided ear.

Table 2. Flowering plant species occurring at study Sites 1 and 2.

(Nomenclature follows Harden 1992, 1993, 2000, 2002)

Species	Site 1	Site 2
<i>Acacia ulicifolia</i> (Mimosoideae)	+	+
<i>Billardiera scandens</i> (Pittosporaceae)	+	+
<i>Bossiaea obcordata</i> (Faboideae)	+	+
<i>Comesperma ericinum</i> (Polygalaceae)	+	+
<i>Cryptostylis subulata</i> (Orchidaceae)	+	
<i>Dampiera stricta</i> (Goodeniaceae)	+	
<i>Daviesia squarrosa</i> (Faboideae)	+	+
<i>Dianella caerulea</i> var. <i>assera</i> (Phormiaceae)	+	+
<i>Dillwynia retorta</i> (Faboideae)	+	
<i>Epacris pulchella</i> (Epacridaceae)		+
<i>Gompholobium glabratum</i> (Faboideae)		+
<i>Gompholobium minus</i> (Faboideae)		+
<i>Goodenia heterophylla</i> (Goodeniaceae)	+	
<i>Hardenbergia violacea</i> (Faboideae)	+	+
<i>Hibbertia empetrifolia</i> (Dilleniaceae)	+	+
<i>Hibbertia linearis</i> (Dilleniaceae)		+
<i>Hovea linearis</i> (Faboideae)	+	
<i>Leptospermum polygalifolium</i> (Myrtaceae)	+	
<i>Leptospermum trinervium</i> (Myrtaceae)	+	+
<i>Leucopogon juniperinus</i> (Epacridaceae)	+	+
<i>Lomandra glauca</i> (Lomandraceae)	+	
<i>Patersonia glabrata</i> (Iridaceae)	+	
<i>Persoonia levis</i> (Proteaceae)	+	+
<i>Persoonia linearis</i> (Proteaceae)	+	+
<i>Pimelia linifolia</i> (Thymelaeaceae)	+	+
<i>Podolobium ilicifolium</i> (Faboideae)	+	+
<i>Pultenea daphnoides</i> (Faboideae)		+
<i>Tetradthea thymifolia</i> (Tremandraceae)	+	

Results

Habitat associated with Tetradthea juncea

Both study sites were located in an area that was designated as MU 30, Coastal Plains Smoothbarked Apple Woodland (Eco Logical Pty Ltd 2002), and the overstorey tree species present were generally consistent with this designation. The trees present were *Angophora costata* and *Corymbia gummifera* as dominant with *Eucalyptus umbra* and *Eucalyptus capitellata* as co-dominant along with the inclusion of *Eucalyptus piperita*. Table 2 contains a list of all native flowering herb or shrub species that were growing along with the *Tetradthea juncea* at both sites. By far the dominant ground cover were the grasses *Themeda australis* and *Austroanthonia fulva*; these made up around 90% of the ground cover. All of these species flowered for a time during the flowering of *Tetradthea juncea*. During the observation period the flowering of the plants listed in

Table 2 was spasmodic and did not appear to relate to a conventionally described flowering season. The detailed monitoring of the flowering periods of these plants in relation to the flowering of the *Tetradthea juncea* and the pollinator activity is part of a continuing study.

The presence of *Tetradthea thymifolia* was of interest as Payne (2000) comments that at the western extent of its range, *Tetradthea juncea* is replaced by *Tetradthea thymifolia*. In one instance both species were growing together with their stems only a few centimetres apart.

Tetradthea juncea flower and anther structure

Inspection of the stamens shows that they are arranged around the gynoeceum in four pairs and an examination of the developing flower reveals the reason for this. Each of the four petals is separately folded around two stamens for their length and the outer end of the petal is tightly folded beyond the ends of the anthers so that they are completely enclosed, and they remain this way until anthesis. This arrangement in *Tetradthea juncea* is similar to that described by Thompson (1976) for *Tetradthea hirsuta*.

The stamens themselves consist of a stout filament that merges into the anther which ultimately dehisces through a rostral apical pore (Thompson 1976, Buchmann 1983). The scanning electron micrograph (Figure 2) shows the macroscopic structure and proportions of a single stamen. A cross section (Figure 3) reveals that the internal structure of the anther is made up of four parallel locules with the gametophytic cells that produce the pollen grains lining the walls. Thompson (1976) describes this locular arrangement as generally applying to the *Tetradthea* genus and also notes that the walls between the loci can disintegrate as the anther matures. This disintegration may be the explanation for observations (Payne 2000, Bartier et al. 2001) that the pollen is held in a sac at the base of the anther. During the current study, the integrity of the locular structure of the *Tetradthea juncea* anthers was still apparent in mature flowers. Examination of the anther under a dissecting microscope reveals that it contains pollen in a syrupy tapetal fluid for the full length. This locular structure is similar to that of most angiosperm anthers (Buchmann 1983) during the early stages of development. However, at anthesis most anthers develop parallel or confluent slits for pollen dispersal whereas in *Tetradthea*, pollen release is through the terminal pore. The folding of the petal around the stamens and over the terminal pore appears to serve the purpose of protecting the developing pollen prior to anthesis.

Pollinator observations

As with the Bartier et al. (2001) investigation, this work involved over 100 hours of monitoring; the main difference was the length of continuous time that each monitoring event involved (2–3 hours in contrast to 15–25 minutes by Bartier et al.). Figure 4 shows timing of flowering intensity, bee observations and visits, seed capsule development and seed release during this study.

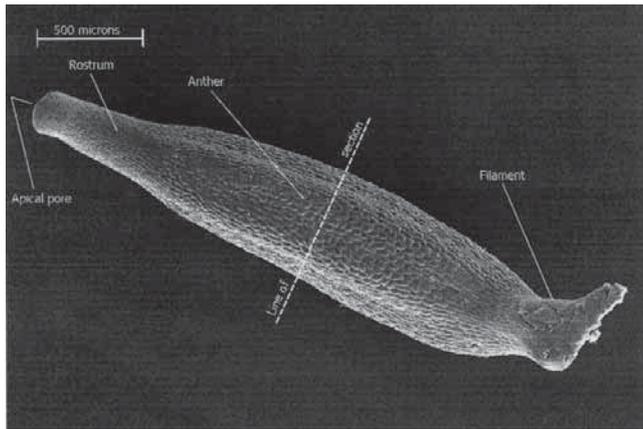


Fig. 2. SEM of one stamen.

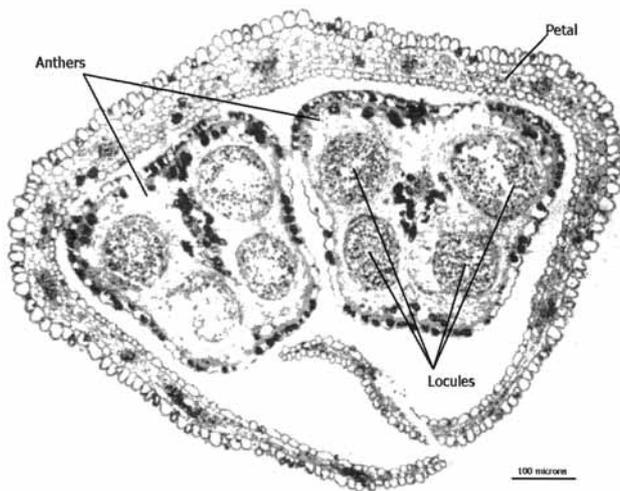


Fig. 3. Mid-section (refer Fig. 2) of a pair of stamens folded in a petal in the unopened *Tetratheca juncea* flower.

A number of insect taxa were attracted to the *Tetratheca juncea* flowers although none did more than hover near the flowers or land on the back of the flower for a brief period. These were Butterfly, *Hesperiidae* (Skipper); Butterfly, *Nymphalidae* (Dingy Ring); Hover Fly, *Syrphidae*; introduced Honey-bee, *Apis mellifera*; Blue-banded Bee, *Amegilla* sp.

On 2 September 2001 at Site 1 a small native bee about 6 mm long was observed to be methodically 'working' through a patch of the *Tetratheca juncea* flowers. The bee would land on the back of a flower and then move to the underside for a few seconds and then fly to another flower. Pollen clumps could be seen on the legs of this bee indicating that it was a female. After attending about six flowers the bee landed nearby and combed the pollen that had been collected. Pollen-combing behaviour was seen often throughout this study. On 24 September 2001 at Site 2 a voucher sample of this bee, identified as *Lasioglossum* (*Chilalictus*) *convexum* Walker, was captured on flowers of *Dianella caerulea* var. *assera* flowering among the *Tetratheca juncea* plants. This bee was carrying *Dianella* and *Myrtaceae* pollen but no *Tetratheca juncea* pollen. On 29 September 2001 at Site 2 a different species of native bee, identified as a member of the *Exoneura* genus, and smaller than *Lasioglossum convexum*,

was collected while underneath a *Tetratheca juncea* flower. This bee was carrying about 30% *Tetratheca juncea* pollen.

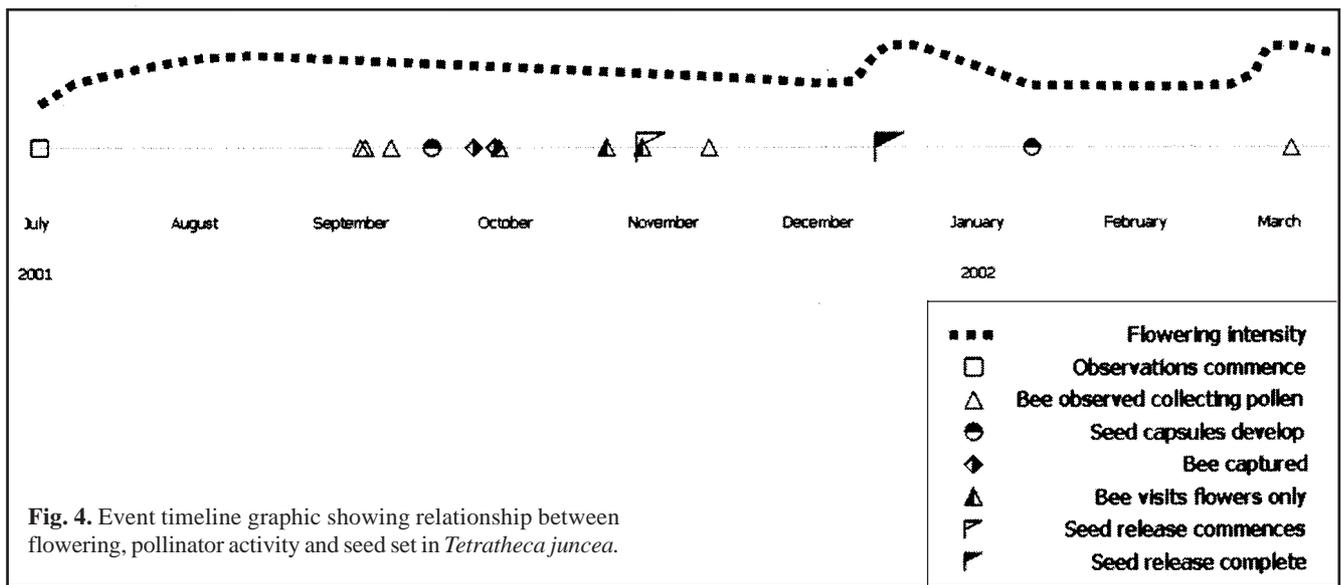
Flowering of *Tetratheca juncea* in the Wakefield population in 2001 commenced at the beginning of July but up until 16 September no seed capsules had developed. The first observation of a native bee collecting pollen was on 2 September and by 16 September the first seed capsules were developing on plants through both observation sites. In one instance a seed capsule developed from a flower from which a *Lasioglossum convexum* bee had been seen collecting pollen. It is interesting to note that no seed development was seen on any of the *Tetratheca thymifolia* plants that were flowering in the immediate area; perhaps the same pollinator limitations apply to both species. At other locations where *Tetratheca thymifolia* was the only *Tetratheca* species present, seed capsules were evident.

Up to April 2002 there were three distinct flowering/seed-development/seed-release cycles over the ten-month period of this study with the third flowering continuing at that time. The flowering of plants at a location near Beresfield, about 30 kilometres further north, commenced about two weeks later than those at Wakefield, and this difference in time was maintained for all subsequent cycles. Flowering during early April 2002 was also observed in the Karuah Nature Reserve (S. Bell pers. com.).

The number of approaches to the *Tetratheca juncea* flowers by the bees was limited to no more than one visit in two hours by either species of bee to a patch of flowers. More often than not, an entire morning would pass without any bees being observed. All sightings were up until around the middle of the day and this is consistent with other studies where it has been shown that the time of maximum pollen availability is before midday (Goodwin 1986, King & Lengoc 1993) or that bee activity is in the morning (Bernhardt 1995, Larson & Barrett 1999). Buchmann (1983) notes that floral anthesis generally occurs around sunrise resulting in maximum pollen availability in the morning.

It is possible that in the absence of an observer, the bees could visit more frequently; there were a number of occasions when a bee would fly to a patch of flowers and then clearly notice the observer, fly erratically just in front of the observer for quite some time and then leave. Rayment (1935) describes this behaviour well, '... tedious, memorising flights, backward and forward, this way, that way, until one's patience is almost exhausted.' When the bee observes a change in the environment around the flowers that it has previously visited it goes through a process, 'memorising flight' to imprint that change into its memory.

The least obtrusive method of static observation was being seated about 2 metres from the flowers, although the method of randomly moving around a large area of plants proved more successful for finding bees. If they were approached after they had settled down to 'work' on a patch of flowers, the bees were found to be less prone to disturbance. On one occasion it was possible to get a few centimetres from a bee and watch it through a magnifier.



The bees appeared to go through a process of assessment as to the suitability of the flowers for pollen removal, and several times were seen to make very brief inspections of two or three flowers in a group, and then leave. Buchmann and Cane (1989) have shown that *Bombus* bees make a similar quick assessment of the pollen return available from *Solanum* flowers. This process of assessment of potential reward should be expected and has been shown to occur in birds, for example the Glossy Black-Cockatoo (*Calyptorhynchus lathami*) that makes a quick assessment of the nutrient content of the seeds from the Forest Oak (*Allocasuarina* sp.) fruit before it expends further energy (Crowley & Garnett 2001).

Using the Anabat II detector it was possible to 'listen' for the buzz of the pollen extraction process, but the only opportunity came when a female *Lasioglossum convexum* was seen and landed first on a flower of *Comesperma* form A at Site 2. The bee burrowed into the central tube of the flower that is formed by a keel and lateral sepals, but made no detectable sound. This observation was probably of the bee collecting nectar since dissection of *Comesperma ericinum* flowers revealed them to be nectariferous. These flowers have a structure that suggests that pollination would be incidental to nectar harvesting by bees. The anthers open by broad apical slits and are positioned where pollen would fall on to the bee as it pushed into the tube for nectar. The stigma is flexed downward and would come into contact with the dorsal surface of the bee.

Following the visit to the *Comesperma*, the bee then landed on a flower of a nearby *Dianella caerulea* var. *assera* and immediately bursts of buzzing were recorded. Figure 5 shows a sound spectrograph of that recording. *Dianella* flowers have a solanoid structure and are known to require buzz-pollination (Bernhardt 1995).

The total duration of this event was 5.47 seconds with the longest burst being 0.58 seconds. This method of observation has potential in detecting the presence of a very

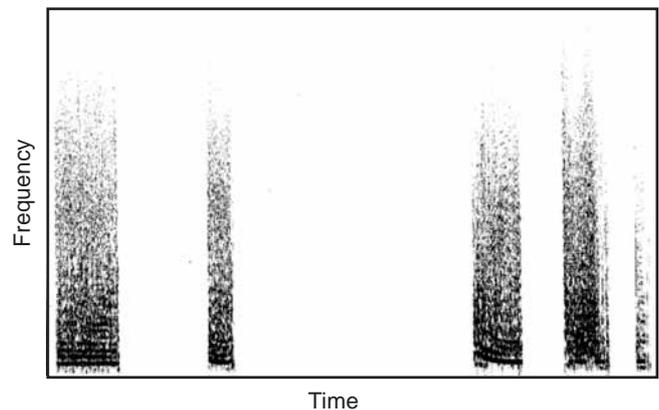


Fig. 5. Sound spectrograph of Anabat II detector recording of *Lasioglossum convexum* buzz-pollinating a *Dianella caerulea* flower. (Spectrograph created using Syrinx, Burt 2001)

tiny and fast moving bee and for accurately measuring the time elements of a flower visit. The Anabat II alters the sound structure and might not be informative about frequencies. Research into the application of this monitoring method is ongoing.

Discussion

Tetralochea juncea pollination

The flowers of *Tetralochea juncea*, as with other members of the genus *Tetralochea* produce no nectar that could serve as a pollinator attractor, and it would appear that pollen is the sole reward available to an insect such as a bee. Payne (2000) observes that under ultra violet light there are apparent pathways evident in the petals of *Tetralochea juncea* that could serve as nectar guides for potential pollinators. It is beyond the scope of this study to determine the significance of this except to note that the presence of these areas *per se* does not necessarily mean that they are used by pollinating insects. A number of factors such as colour contrast, flower symmetry and the visual capability of an insect in ranges of frequency

are involved in the function of nectar pathways (Penny 1983, Daphne & Kevan 1996). It may well be that these apparent nectar pathways are a part of a floral cue to the buzz pollinating bees in that the bees were all seen to land on the back of the flower before crawling between the petals to get to the pollen. The transient interest in the flowers that was shown by other insects may be a result of a misleading cue for nectar seeking insects.

The *Tetralochea* flower structure resembles what has been termed a *Solanum*-type (Vogel 1978), and later Solanoid (Faegri 1986), because of the dominance of these structural characteristics in *Solanum* (Solanaceae). Pollen extraction and thereby pollination of flowers with poricidal/porose anthers generally occurs through the process of buzz-pollination (also termed vibratory-pollination or sonication), achieved by bees grasping the androecium with the anthers against the venter and transferring vibrations through their body by means of rapid contraction of their indirect flight muscles (Buchmann 1983, Buchmann & Cane 1989, Harder & Barclay 1994). (Not by the buzzing of their wings as is often thought — the pollen extracted would be all blown away!). The audible component of the vibratory process is a useful detectable artefact to the energetics being applied to the flower parts by the bee in order to extract the pollen (Buchmann 1983). The poricidal anthers of *Tetralochea juncea* contain the pollen within locules in tapetal fluid. In common with other buzz-pollinated plants (King & Lengoc 1993, King & Buchmann 1996) the *Tetralochea juncea* anthers appear to dispense pollen, probably through the localised drying of the tapetal fluid. Dissection of a number of *Tetralochea juncea* anthers showed that at any time only a fraction of the total pollen content was dry, just inside the disseminating pore.

While it has been demonstrated that *Tetralochea juncea* is capable of self pollination (Bartier et al. 2001) the gynoecium architecture is not consistent with that of wind-pollinated flowers (Proctor et al. 1996) with the stigma surface being minute. Also the downward presentation of the flowers further limits the opportunities for air-borne pollen to land on the stigma surface. Bartier et al. (2001) determined that rates of autogamy were about 1 in 50 flowers in an undisturbed population and concluded that the majority of seed set found during their investigation would have been as a result of autogamy given the total absence of observed pollinators. This is not consistent with the observations in this study where the abundant flowers of both study sites produced no seed capsules for almost two months from July and that seed set occurred within two weeks of the first pollinator being observed (see timeline, Figure 4). Furthermore, in contrast to observations by Payne (2000) that seed capsule development and subsequent seed release only occur following the flowering period, during this study there was a concurrent process of flowering, seed capsule development and seed release that followed the appearance of the first pollinators and this continued into 2002 until bees were no longer active in colder weather. A low level of flowering continued through the colder months (with some flowers present for each of the 12 months) with no seed

capsule development and this was taken as an indication that the bees were no longer active.

Conservation importance of pollinators

For some plants, pollinators are readily available and these can simply be the wind or a wide variety of both invertebrate and vertebrate fauna. For other plants pollination is a specialised process with limited pollinators available or with limited pollinating opportunities being made available by the plant. Without a pollinator most plant species, even those able to vegetatively propagate, would become extinct and a decline in pollinators has broad implications for biodiversity (Allen-Wardell et al. 1998, Kremin & Ricketts 2000).

Members of the two genera of bee observed collecting pollen from *Tetralochea juncea* in this study, *Lasioglossum* (*Chilalictus*) sp. and *Exoneura* sp., have been recorded collecting pollen from a number of other plant species throughout Australia such as *Dianella caerulea* var. *assera*, specifically by *Lasioglossum convexum* (Bernhardt 1995) and *Acacia* sp., (Bernhardt 1987). In southern Tasmania, bees of the *Lasioglossum* genus (*Austrevylaeus* and *Chilalictus*) were recorded at nectarless plants of *Dianella*, *Acacia*, *Hibbertia*, *Tetralochea glandulosa* and nectariferous plants of Fabaceae, Epacridaceae and Myrtaceae (Hingston 1999). During the current study, plant species other than *Tetralochea juncea* from which *Lasioglossum convexum* was observed collecting pollen or nectar were *Comesperma ericinum* form A (Polygalaceae), *Dianella caerulea* var. *assera* (Phormiaceae), *Dillwynia retorta* (Faboideae), *Hibbertia serpyllifolia* (Dilleniaceae), and *Persoonia levis* (Proteaceae). *Exoneura* sp. was seen collecting pollen from *Tetralochea juncea* at a Beresfield population about 30 kilometres north of the two study sites but was not seen collecting from other species. Bernhardt (1987) notes that bees which collect from nectarless flowers are generally members of polylectic genera (i.e. they collect pollen and nectar from a number of plant genera across several families).

Both bee species are loosely social with different ecology. *Lasioglossum convexum* nests in tunnels in the ground with side brood chambers; an environmental requirement would be suitably textured soil that the bee can tunnel into. *Exoneura* sp., often referred to as 'reed bees', nest in hollow stems and rely on either the centre of a deteriorating dead stem such as in fern stems or *Lantana*, or hollows formed by the larvae of wood-boring beetles (Rayment 1935, Michener 2000). Bees of both genera remain dormant for winter and emerge as the weather warms (Dollin et al. 2000) though this has not been specifically demonstrated for *Lasioglossum convexum*.

The pattern of bee visitation as observed directly, and as inferred by the distribution of seed set among the plants, has implications for the genetic diversity of the plant populations. The bees exhibited flower constancy (Goulson 1994) over the short period that the flowers were visited and it became apparent that only small patches (generally much less than 1 m²) were being pollinated. Given that, as a consequence of rhizomal spread (Payne 2000, Driscoll & Bell unpub.) the

plants in that small area would most likely all be ramets from the same genetic source, pollination was of the kind that has been described as 'zoophilous autogamy' (Bernhardt 1976). The possibility also exists for facilitated self pollination where pollen being extracted attaches to the stigma of the same flower (Larson & Barrett 1999). In one instance at site 1, a single plant with five stems developed 15 seed capsules from 20 flowers in a location that was surrounded by other plants in plentiful flower but with few to no seed capsules. During January 2002, a group of plants in about 1 m² that had not produced any seed capsules since July 2001 developed a number of seed capsules. This conclusion is consistent with early work reviewed by Levin (1978) showing that pollinator foraging, based on the efficient use of distance and time, will have an effect on the genetic composition within and between plant populations. Considerable investigation into the genetic structure within these populations will be needed before the proportion of autogamy to outcrossing in *Tetralochea juncea* can be determined with confidence.

The very low seed set reported in *Tetralochea juncea* populations (Payne 2000, Bartier et al. 2001) is a likely indication of a pollinator-limited reproductive system. The concept of pollinator-limitation is built around the premise that seed set in a flowering plant population is below a level that would be achieved were more pollinators available (Calvo & Horvitz 1990). Bartier et al. (2001)'s work on hand pollination provides supporting evidence for *Tetralochea juncea* being pollinator-limited with fruit-set percentages being significantly higher in hand pollinated flowers than in unmanipulated flowers. The combination of pollinator limitation and fragmented and disjunct populations of *Tetralochea juncea* has implications for the future prospects of the plant. Agren (1996) demonstrated that for a pollinator limited species, there was a positive correlation between population size and seed production indicating inadequate pollen transfer in small populations. Bartier et al. (2001) found for *Tetralochea juncea*, that there was a positive correlation between fruit production and population size as measured by plant biomass. The question arises as to whether the pollinators of *Tetralochea juncea* have always been a limiting factor in the reproductive process of the plant or whether there has been a decline in pollinator numbers over time.

The two bee species collecting pollen from *Tetralochea juncea* are polylectic and so it would appear that the bees are less dependant on *Tetralochea juncea* than it is on the bees. This might provide a lead as to why *Tetralochea juncea* has such a long and profuse flowering with continued spasmodic flowering throughout a year and could also suggest that the reproductive process has always been pollinator-limited.

If it is the case that the flowering pattern of *Tetralochea juncea* is an adaptation to a pollinator-limited breeding system then knowledge of the ecology of pollinators in the ecosystem is of significance in relation to the long-term viability of the species. Hingston (1999) has shown an affinity between plants and their bee pollinators in Tasmania in such a way that a variety of species and genera with overlapping flowering

periods encourage bee activity, and that nectariferous plants flowering in the same area as nectarless plants facilitates pollinator visits to the latter. In an earlier study, again in Tasmania, Hingston (1998) showed that the composition of native bee fauna varied within vegetative communities throughout the year. It is possible that habitat degradation through various anthropogenic activities, and maybe in particular frequent fire, has resulted in a simplification of floristic composition and vegetative structure that has led to a decline in pollinator numbers.

Conclusion

Findings from this study with respect to the pollination ecology of *Tetralochea juncea* are:

- There is a strong flowering period from September to January, though a number of flowers can be found on some plants across the geographic range of the plant in all months of the year;
- Two species of native bee *Lasioglossum convexum* and *Exoneura* sp, were confirmed collecting pollen from the flowers by way of buzz-pollination;
- Fruiting only occurred in coincidence with flower pollination by these bees;
- Flowering, seed set and seed release was a concurrent process while ever the bees were active;
- The bees are polylectic and the sexual reproductive process in *Tetralochea juncea* appears to be pollinator-limited.

This study, along with the work of others (Bartier et al. 2001), has shown that refreshment of the genetic content within populations and between very disjunct populations is poor. It could well be that the current populations of *Tetralochea juncea* are long established, predominantly clonal colonies each containing little genetic variation and with local adaptive genotype differences between the disjunct occurrences of the plant. If this were the case and pollinator numbers have significantly declined there could be a loss of mutualism between the bees and their floristic habitat and *Tetralochea juncea*, being nectarless and a pollen source of last resort, could be undergoing a process of insidious delayed extinction (Johnson & Steiner 2000). It would be presumptuous to decide that this was not a possibility and further research is needed into the dynamics of the ecology of this rare plant and its pollinators.

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References

- Agren, J. (1996) Population size, pollinator limitation and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology* 77(6): 1779–1790.
- Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S.L., Cane, J.H., Cox, P.A., Dalton, V., Feinsinger, P., Ingram, M., Inouye, D., Jones, C.E., Kennedy, K., Kevan, P., Koopowitz, H., Medellin, R., Medellin-Morales, S. & Nabham, G.P. (1998) The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology* 12(1): 8–17.
- Anderson, G.J. & Symon, D. (1998) Insect foragers on *Solanum* flowers in Australia *Annals of the Missouri Botanical Gardens* 75: 842–852.
- Bartier, F.V., Gross, C.L., Mulligan, D.R., Bellairs, S.M., & Bowen, D. (2001) Understanding the biology and ecology of vulnerable plant species: A case study with *Tetralochea juncea* occurring over coal leases, ACARP Project C8012. A report prepared for Australian Coal Research, June 2001.
- Bernhardt, P. (1976) The pollination ecology of *Hepatica acutiloba* DC. (Ranunculaceae) *Bulletin of the Torrey Botanical Club* 103(6): 255–258.
- Bernhardt, P. (1987) A comparison of the diversity, density and foraging behaviour of bees and wasps on Australian *Acacia*. *Annals of the Missouri Botanical Garden* 74: 42–50.
- Bernhardt, P. (1995) The floral ecology of *Dianella caerulea* var. *assera* (Phormiaceae) *Cunninghamia* 4(1): 9–20.
- Boeswinkel, F.D. (1999) Ovules and seeds of Tremandraceae. *Australian Journal of Botany* 47: 769–781.
- Brew, C.R., O'Dowd, D.J. & Rae, I.D. (1989) Seed dispersal by ants: behaviour-releasing compounds in elaiosomes. *Oecologia* 80: 490–497.
- Briggs, J.D. & Leigh, J.H. (1995) *Rare or threatened Australian plants* (CSIRO).
- Buchmann, S.L. (1983) Buzz pollination in angiosperms. In: (eds Jones C.E. & Little R.J.) *Handbook of experimental pollination biology*. 73–113 Van Nostrand Reinhold, New York, NY.
- Buchmann, S.L. & Cane, J.H. (1989) Bees assess pollen returns while sonicating *Solanum* flowers. *Oecologia* 81: 289–294.
- Burt, J. (2001) Syrinx Version 2.2L, www.syrinxpc.com.
- Calvo, N.C. & Horvitz, C.C. (1990) Pollinator limitation, cost of reproduction, and fitness in plants: a transition matrix demographic approach. *The American Naturalist* 136(4): 499–516.
- Crowley, G.M. & Garnett, S.T. (2001) Food value and tree selection by Glossy Black-Cockatoos (*Calyptorhynchus lathami*). *Austral Ecology* 26: 116–126.
- Daphne, A. & Kevan, P.G. (1996) Floral symmetry and nectar guides: ontogenetic constraints from floral development, color pattern rules and functional significance. *Botanical Journal of the Linnean Society* 120: 371–377.
- Dollin, A., Batley, M., Robinson, M. & Faulkner, B. (2000) *Native bees of the Sydney region. A field guide* (Australian Native Bee Research Centre: Sydney).
- Eco Logical Pty Ltd (2002) *Vegetation survey, classification and mapping Lower Hunter and Central Coast region*. Version 1.2. A project undertaken for The Lower Hunter and Central Coast Regional Environment Management Strategy CRA Unit Sydney Zone National Parks and Wildlife Service.
- Faegri, K. (1986) The solanoid flower. *Transactions of the Botanical Society of Edinburgh, 150th Anniversary Supplement* (eds I.J. Alexander & N.M. Gregory) 51–59. Botanical Society of Edinburgh, Edinburgh.
- Gardner, C. and Murray, L. (1992) Tremandraceae. In: *Flora of New South Wales Vol 3* (ed. G.J. Harden) pp. 74–78 (UNSW Press).
- Goodwin, R.M. (1986) Kiwifruit flowers: anther dehiscence and daily collection of pollen by honey bees. *N. Z. Journal of Experimental Agriculture* 14: 449–452.
- Goulson, D. (1994) A model to predict the influence of insect flower constancy on interspecific competition between insect pollinated plants. *Journal of Theoretical Biology* 168: 309–314.
- Harden, G.J. (ed.) (1992) *Flora of New South Wales Vol 3* (UNSW Press: Sydney).
- Harden, G.J. (ed.) (1993) *Flora of New South Wales Vol 4* (UNSW Press: Sydney).
- Harden, G.J. (ed.) (2000) *Flora of New South Wales Vol 1* (UNSW Press: Sydney).
- Harden, G.J. (ed.) (2002) *Flora of New South Wales Vol 2* (UNSW Press: Sydney).
- Harder, L.D. & Barclay, R.M.R. (1994) The functional significance of poricidal anthers and buzz pollination: controlled pollen removal from *Dodecatheon*. *Functional Ecology* 8: 509–517.
- Hingston, A.B. (1998) Temporal and spatial variation in abundances of native bee species on an altitudinal gradient in southern Tasmania. *Australian Journal of Zoology* 46: 497–507.
- Hingston, A.B. (1999) Affinities between southern Tasmanian plants in native bee visitor profiles. *Australian Journal of Zoology* 47: 361–384.
- Johnson, S.D. & Steiner K.E. (2000) Generalisation versus specialisation in plant pollination systems. *Tree* 15(4): 140–143.
- King, M.J. & Buchmann, S.L. (1996) Sonication dispensing of pollen from *Solanum laciniatum* flowers. *Functional Ecology* 10: 449–456.
- King, M.J. & Lengoc, L. (1993) Vibratory pollen collection dynamics. *Transactions of the American Society of Agricultural Engineers* 36(1): 135–140.
- Kremen, C. and Ricketts, T. (2000) Global perspectives on pollination disruptions *Conservation Biology* 14(5): 1226–1228.
- Larson, B.M.H. & Barrett, C.H. (1999) The pollination ecology of buzz-pollinated *Rhexia virginica* (Melastomataceae) *American Journal of Botany* 86(4): 502–511.
- Levin, D.A. (1978) Pollinator behaviour and the breeding structure of plant populations, In: *The pollination of flowers by insects* (ed. A.J. Richards) pp.133–150. Academic Press.
- Lundberg, S. and Ingvarsson, P.K. (1998) Population dynamics of resource limited plants and their pollinators. *Theoretical Population Biology* 54: 44–49.
- Michener, C.D. (2000) *The bees of the world*. Johns Hopkins University Press.
- NPWS (2000) *Vegetation survey, classification and mapping Lower Hunter and Central Coast region*. Version 1.2. A project undertaken for The Lower Hunter and Central Coast Regional Environment Management Strategy CRA Unit Sydney Zone National Parks and Wildlife Service.
- Payne, R.J. (2000) Lake Macquarie *Tetralochea juncea* conservation management plan final report November 2000. A report prepared for Lake Macquarie City Council, NSW National Parks and Wildlife Service and BHP Pty Ltd.
- Penny, J.H.J. (1983) Nectar guide and color contrast: a possible relationship with pollination strategy. *New Phytologist* 95: 707–721.
- Proctor, M., Yeo, P. & Lack, A., (1996) *The natural history of pollination* (Timber Press, Oregon).
- Raymont, T. (1935) *A cluster of bees* (Endeavour Press: Sydney).
- Thompson, J. (1976) A revision of the genus *Tetralochea* (Tremandraceae). *Telopea* 1(3): 139–215.
- Vogel, S. (1978) Evolutionary shifts from reward to deception in pollen flowers. In *The pollination of flowers by insects, Linnean Society Symposium Series Number 6*, (ed. A. Richards) pp. 89–96. *The Linnean Society of London* (Academic Press: London).