

Pollination ecology of the Australian cycad *Lepidozamia peroffskyana* (Zamiaceae)

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Abstract. Experiments carried out to investigate the reproductive ecology of the Australian cycad *Lepidozamia peroffskyana* (Regal, *Bull. Soc. Imp. Nat. Mosc.* 1857, 1: 184) revealed that this species is pollinated exclusively by host-specific *Tranes* weevils (Pascoe 1875). The weevils carry out their life cycle within the tissues of the male cones but also visit the female cones in large numbers. Female cones from which insects (but not wind) were excluded had a pollination rate that was essentially zero. In contrast, female cones from which wind (but not insects) were excluded had a pollination rate comparable with naturally pollinated cones. Assessment of *Tranes* weevil pollen load indicated that they are effective pollen-carriers. No other potential insect pollinators were observed on cones of *L. peroffskyana*. Sampling of airborne loads of cycad pollen indicated that wind-dispersed grains were not consistently recorded beyond a 2-m radius surrounding pollen-shedding male cones. The airborne load of cycad pollen in the vicinity of pollination-receptive female cones was minimal, and the spatial distribution of the coning population indicated that receptive female cones did not usually occur close enough to pollen-shedding male cones for airborne transfer of pollen to explain observed natural rates of seed set. These multiple lines of evidence suggest that wind—once considered the only pollination vector for cycads and other gymnosperms—plays only a minimal role in the pollination of *L. peroffskyana*, if any at all. The global diversity of insects associated with cycads suggests that some lineages of pollinating beetles may have been associated with cycad cones since Mesozoic times.

Introduction

Cycads comprise an ancient group of gymnosperms with a fossil record extending back to the Permian (Gao and Thomas 1989). Their diversity was greatest during the Jurassic and subsequently declined during the Cretaceous transition from gymnosperm to angiosperm-dominated floras (Biswas and Johri 1997). Some 200 cycad species in 11 genera persist in Central and South America, Southern Africa, South-East Asia and Australia (Jones 2002). Cycads are dioecious, and both sexes bear reproductive structures that are relatively massive in comparison with those of conifers. For example, mature seed cones of *Lepidozamia peroffskyana* weigh up to 38 kg and are reputedly the largest of any gymnosperm, living or extinct (Ornduff 1989). Pollination of cycads has traditionally been attributed to wind, in a process comparable to that of conifers (Chamberlain 1919, 1935; Brough and Taylor 1940; Dyer 1965; Giddy 1974). However, this claim has been based largely on the untested assumption that wind pollination was

‘characteristic of the whole group of gymnosperms’ (Chamberlain 1935, p. 127). More recently, experimental studies have shown all cycads so far examined to be primarily insect-pollinated (Table 1). Pollination is typically by various species of host-specific beetle, which are associated with cycad cones on every continent where cycads occur (Vovides 1991; Forster *et al.* 1994; Donaldson 1995, 1997; Tang *et al.* 1999). Perhaps because their life cycle has an obligate dependence on the seasonal availability of cones (Norstog and Fawcett 1989) the cycad-pollinating beetles are characteristically specific to cycads, and often host-specific to a particular genus or species (Oberprieler 1995a, 1995b).

Studies of cycad reproduction have fostered a new awareness that insect pollination has evolved more than once in the history of the plant kingdom (Norstog 1987; Farrell 1998; Pellmyr 2002). The ancient origins of cycads and their Pangean biogeography naturally suggest a unique insight into the early evolution of insect pollination. However, there

Table 1. Published experimental studies of cycad pollination

Species	Experiment locality	Insect pollinator(s)	Author(s)
<i>Zamia furfuracea</i>	Central America	<i>Rhopalotria</i> weevil (beetle)	Norstog <i>et al.</i> (1986)
<i>Zamia pumila</i>	Central America	<i>Rhopalotria</i> weevil (beetle)	
		<i>Pharaxonotha</i> (languriid beetle)	Tang (1987a)
<i>Encephalartos cycadifolius</i>	South Africa	<i>Metacucujus</i> (boganiid beetle)	Donaldson (1995)
		Undescribed languriid beetle	
<i>Encephalartos villosus</i>	South Africa	<i>Porthetes</i> weevil (beetle)	Donaldson (1997)
		Undescribed languriid beetle	
<i>Macrozamia macdonnellii</i>	Central Australia	<i>Cycadothrips</i> (thrips)	Mound and Terry (2001)
<i>Macrozamia communis</i>	Eastern Australia	<i>Tranes</i> weevil (beetle)	Terry (2001)
		<i>Cycadothrips</i> (thrips)	
<i>Bowenia spectabilis</i>	Northern Queensland	<i>Miltotranes</i> weevil (beetle)	Wilson (2002)

is perhaps a danger that the old ‘conventional wisdom’ of wind pollination may simply be replaced by a new assumption that ‘cycads are basically insect pollinated’ (Jones 2002, p. 55). While this assessment may well prove correct, it must be remembered that detailed pollination studies currently exist for only four of the world’s 11 cycad genera. In this account of *Lepidozamia peroffskyana* pollination, we present a fifth. The aims of our study were as follows:

- (i) to investigate the hypothesis that *L. peroffskyana* is pollinated by the beetle associated with its cones—in this case, a weevil in the *Tranes* genus;
- (ii) to determine whether wind plays any subsidiary role in cycad pollination, a question that has remained somewhat ambiguous in previous studies (see Norstog *et al.* 1986; Tang 1987a; Donaldson 1995, 1997); and
- (iii) to record *L. peroffskyana* coning phenology, aspects of *Tranes* weevil life history, and any symbiotic connections that exist between the two taxa.

Methods

Study site and organisms

Lepidozamia peroffskyana is a large cycad endemic to wet sclerophyll forests and rainforest margins of eastern Australia between Gympie in south-eastern Queensland and Taree in north-east New South Wales (Johnson 1961; Jones 2002). The study was undertaken in Mooball National Park in the Burringbar Range of northern New South Wales (28°22'S, 153°27'E) on 1 ha of steep hillside. The vegetation consisted of wet sclerophyll forest with a *Eucalyptus* canopy and an understorey dominated by *L. peroffskyana* and *Xanthorrhoea* sp. grass trees. The duration of the study was from early January (late summer 2001), when the first pollen-shedding male cones were observed, until April of the same year, by which time all male cones had been spent and pollen-shedding activity had ceased. Voucher specimens of *L. peroffskyana* from the study site (collected by P. Machin) have been lodged with the Queensland herbarium.

Cones of the Australian cycads *Macrozamia*, *Bowenia* and *Lepidozamia* are associated with a complex of related weevil species informally known as the ‘*Tranes* group’ (Oberprieler 1995a). ‘*Tranes* group’ weevils have been identified as the pollination vector of *Macrozamia communis* (Terry 2001) and *Bowenia serrulata* (Wilson 2002). A ‘*Tranes* group’ weevil that is presently undescribed (Rolf Oberprieler, pers. comm.) is associated with *L. peroffskyana* cones in

large numbers (Fig. 1) and several authors have speculated about its probable role in pollination (Ornduff 1989; Kennedy 1991; Forster *et al.* 1994). None of these authors found any other insect species consistently associated with both male and female cones of



Fig. 1. Mass gathering of *Tranes* weevils on male cone of *Lepidozamia peroffskyana* in the early stages of pollen-shed. The characteristic spirally opening fissure allows weevils access to the cone interior. Scale-bar interval = 5 cm.

L. peroffskyana, a finding confirmed by our own experience in the field. Voucher specimens of the *Tranes* sp. from cones of *L. peroffskyana* at the study site (collected by J. Hall) have been lodged with the University of Queensland insect collection.

Demography and cone production

A demographic sample of *L. peroffskyana* was collected at the study site to determine the proportion of reproductive plants and the relationship between size class and cone production. Measurements were recorded for all cycads present within three randomly selected circular plots of 10 m radius. The relative position of each cycad was recorded, in addition to the following attributes: number of fronds; length of the longest frond; height and girth of the trunk (if present); and sex and developmental stage of the cones (if present). To record the cone development sequence of *L. peroffskyana*, a representative young cone of each sex was selected and monitored over the duration of the pollination season.

Pollinator-exclusion experiments

The relative effectiveness of wind and insects as pollinators of *L. peroffskyana* was compared by establishing experimental treatments, detailed below, that selectively excluded either wind or *Tranes* weevils from receptive female cones. Uncovered controls were also monitored. Treatments were established on immature female cones before they had become receptive to pollination. The experimental design was based on previous studies of cycad pollination by Norstog *et al.* (1986), Tang (1987a), Donaldson (1995, 1997), Terry (2001) and Wilson (2002).

The insect-exclusion treatment ($n = 8$) created a barrier to *Tranes* weevils while still allowing potentially pollen-bearing wind movements to reach the female cone. A mesh bag was placed over the cone and sealed at the top of the trunk with Selleys brand space-filling foam (Selleys Australia, Sydney). *Tranes* weevils were so persistent in their attempts to penetrate this exclusion that a single layer of mesh was an insufficient barrier. Therefore, all fronds were removed and a second mesh bag was placed over the first and sealed lower down the trunk with wire and a layer of engine grease. This removal of fronds was not expected to retard cone development for two reasons: in pollination tests of the cycad *Macrozamia lucida*, open control cones with and without leaves were not statistically different in seed set (95.5 v. 97.6%—Irene Terry, pers. comm.), and completely denuded *L. peroffskyana* have been observed presenting cones after bushfire (Paul Kennedy, pers. comm.). The mesh diameter of the insect-exclusion bags was 2 mm. In handbooks of pollination biology, mesh diameters down to 0.25×0.25 mm, and the use of double layers of netting are both recommended as acceptable insect-exclusion measures, still allowing a free movement of airborne pollen (Dafni 1992; Kearns and Inouye 1993).

The wind-exclusion treatment ($n = 9$) was intended to create a baffle to wind currents while still allowing *Tranes* weevils access to the female cone. A black cloth bag was wired in place over the cone. Weevils could enter by crawling up from the base. A control treatment ($n = 8$) consisted of female cones left to pollinate in their natural state.

Cones were collected for dissection in the first week of April. Pollinated and non-pollinated ovules could be distinguished because in *Lepidozamia* non-pollinated ovules fail to develop as the pollinated ovules mature (John Donaldson, pers. comm.). The pollinated ovules increase in size, and develop a hard sclerotesta and external red coloration. The non-pollinated ovules remain small and yellow and the hard sclerotesta does not develop.

Airborne load of cycad pollen

A series of pollen traps was used to assess the airborne load of *L. peroffskyana* pollen at the study site, and test whether this airborne pollen load was comparable with that of wind-pollinated conifers.

Microscope slides were smeared with a thin layer of Carberla's solution adhesive and mounted on stakes at a height of 1.5 m (a typical height at which female *L. peroffskyana* bear cones). The traps were fitted into a small open-sided box to shelter the adhesive surface from rain. Trap lines were established around three developing male cones that were nearing pollen-shed. Each cone was surrounded by three equidistantly spaced and outwardly radiating trap lines, with the adhesive surface facing back towards the pollen source. Pollen traps were placed at 0.5, 2, 4 and 8 m along the trap line and left in the field for 1 week to cover the entire period of pollen shedding. A second series of traps was established around three female cones approaching pollination receptivity. Each was surrounded by three equidistantly spaced traps (placed immediately adjacent to the cone) with the adhesive surface facing outwards. These traps were left in the field for 2 weeks to cover the entire period of receptivity to pollination. Pollen on the slides was counted by three microscope eyepiece graticule transects over the coverslip area at $\times 10$ magnification, resulting in a total pollen count for 54 mm^2 (16.8% of the coverslip area). This transect count was extrapolated to estimate total pollen captured by the slide.

Pollen load of *Tranes* weevils

Tranes weevils were collected from pollen-shedding male cones to test their ability to carry pollen on their bodies. Specimens were examined with a scanning electron microscope to determine which external surfaces were associated with pollen transport. Total pollen load was estimated by the centrifuge method of MacGillivray (1987), as adapted by Donaldson (1995, 1997) for South African cycad beetles. Weevils ($n = 10$) were shaken individually in centrifuge tubes containing xylene and 400 mL of glycerol gelatine. The weevil was removed and the tube was centrifuged at 15000 rpm for 1 min, embedding the xylene-suspended pollen into the glycerol gelatine. The glycerol gelatine pellet was then melted onto a microscope slide for examination of the pollen load. Pollen on the slides was counted by three microscope eyepiece graticule transects over the coverslip area at $\times 10$ magnification, resulting in a total pollen count for 54 mm^2 (16.8% of the coverslip area). This transect count was extrapolated to estimate the total pollen load.

The cycad–weevil relationship

Additional details of the cycad–weevil relationship were elucidated by field observation of weevil behaviour, counts of weevil numbers associated with opportunistically collected male and female cones, and dissections of the weevil digestive system.

Results

Demography and cone production

Coning plants represented 12.2% of the sampled population (Table 2) and their sex ratio was 62.5% male. Coning plants typically possessed a trunk, although a small proportion did

Table 2. Demography of *Lepidozamia peroffskyana* coning at the study site

'Seedling' size class	78
'Juvenile' size class	96
'Mature' size class	88
Total plants	262
Total plants with trunk	59
Total plants with cone ^A	32
Total male cones	20
Total female cones	12

^AConing *L. peroffskyana* at the study site always produced one cone per plant.

not (15.6%; $n = 32$), indicating that *L. peroffskyana* can reach sexual maturity before the development of an arborescent trunk. Hence, using total plants with trunk as a conservative estimate of the sexually mature population, participation in the coning episode was limited to (at most) only slightly more than half of the sexually mature individuals present (see also maps in Fig. 2). Such restricted participation in the annual coning event is a typical feature of cycad populations, possibly owing to the high resource cost associated with such massive reproductive structures (Clark and Clark 1987; Ornduff 1989, 1993; Vovides 1990).

The observed *L. peroffskyana* coning episode (i.e. the period of time when male and female cones in the pollination phase were present in the population) lasted 3 months. For individual plants, the period from cone emergence to the pollination phase was approximately 6 weeks for cones of both sexes. The developmental sequence of representative male and female cones is presented in Fig. 2.

The sample population was categorised into size classes of 'seedling', 'juvenile' and 'mature' plants (Table 2) according to a size index defined by leaf number \times length of the longest leaf. This combined index provided a better indication of overall plant size; for example, it distinguished

between small leaved 'seedlings' and larger leaved 'juveniles' with a similar number of fronds. However, it should be stressed that there are no accurate techniques for quantifying the age of a cycad. The size classes were intended only to provide an estimate of reproductive maturity and do not represent a precise division of the population age structure. The index values used to define the size classes were as follows: 'seedling' = 1–99; 'juvenile' = 100–999; 'mature' = 1000–6000. This subjective classification was judged to be successful, since only plants in the 'mature' size class were characterised by trunk development and/or the production of cones. The coning status recorded for these 'mature' plants is illustrated in maps of the three sample plots (Fig. 3). These maps emphasise that the development of cones was not precisely synchronised between individuals, and that there were relatively small numbers of cones in the pollination phase at any one time. These maps also indicate the estimated limit of effective airborne pollen transport (see *Airborne load of cycad pollen* below).

Pollinator-exclusion experiments

Pollination success varied among control treatments. The average percentage of pollinated ovules across cones was high

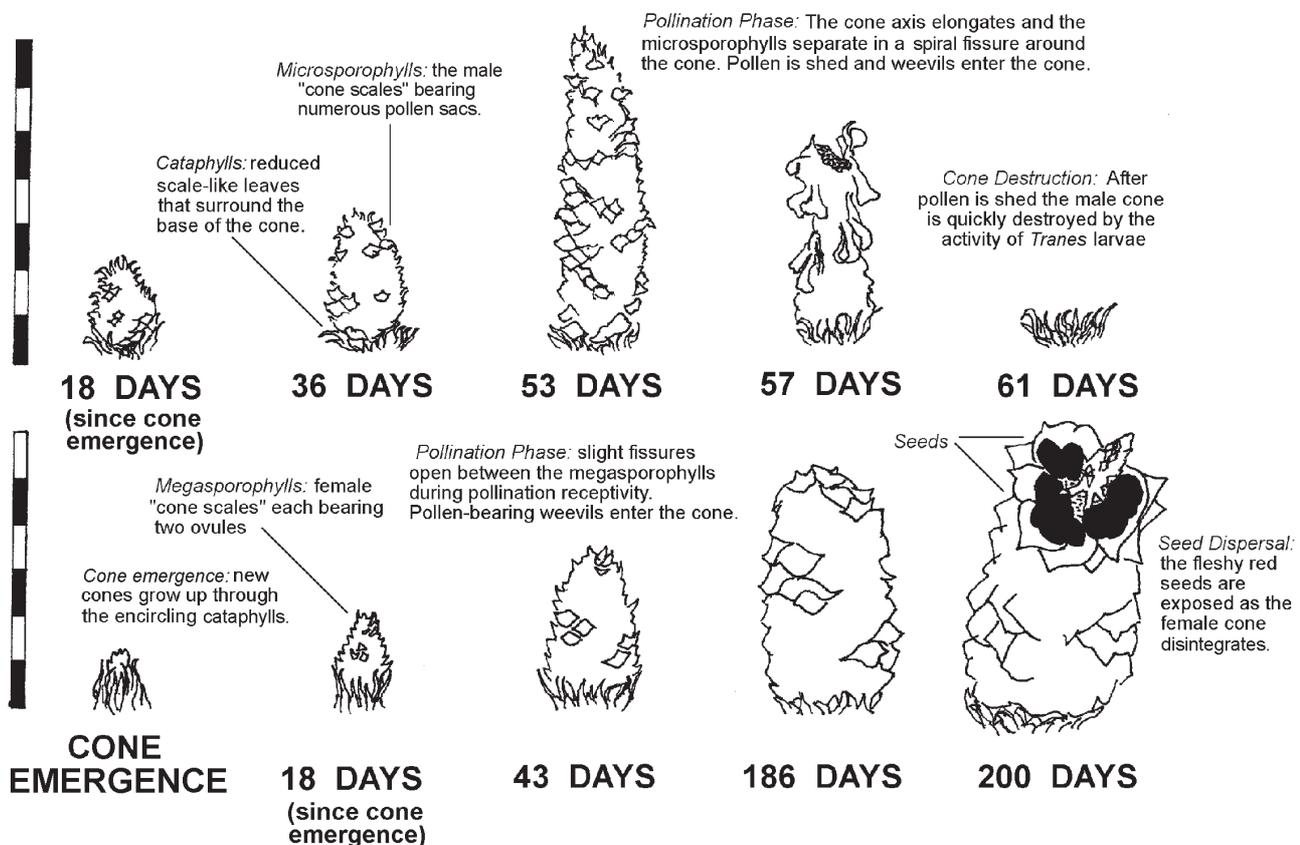


Fig. 2. Sequence and duration of male and female cone development in *Lepidozamia peroffskyana*. Male cones are completely destroyed in the course of the weevil life cycle, mainly through larval feeding. Scale-bar interval = 10 cm.

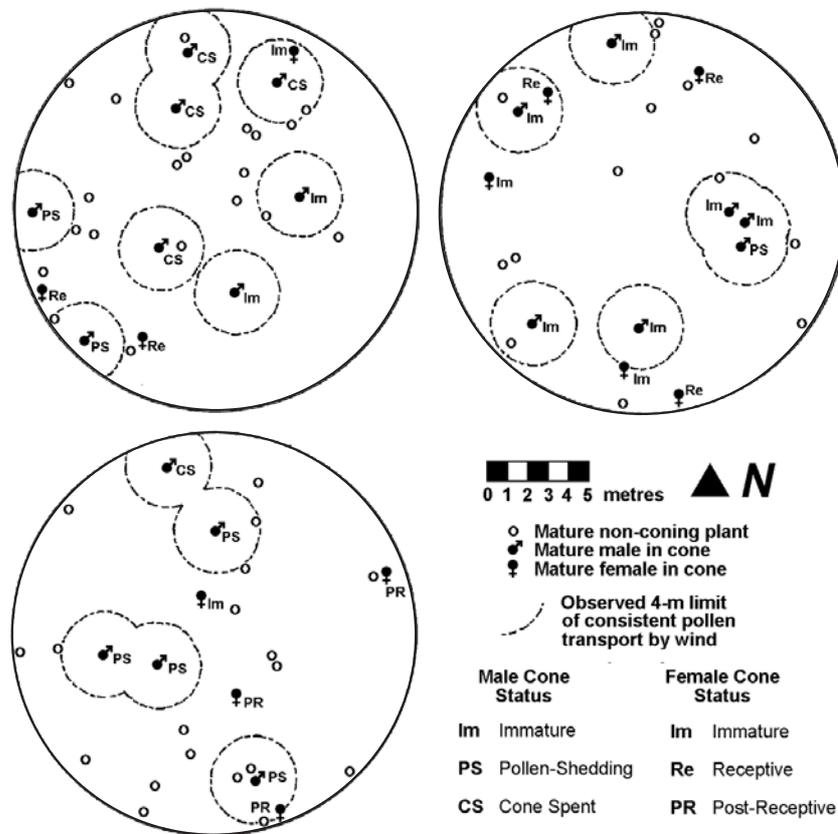


Fig. 3. Maps of *Lepidozamia peroffskyana* coning activity, Mooball National Park, northern New South Wales, February 2001. The sample consisted of three circular plots of 10-m radius.

at 84% (Table 3). Cones from which wind was excluded but to which weevils had access had an average 70% of ovules pollinated. In most of the insect-exclusion treatments, pollination was reduced to zero when *Tranes* weevils were successfully excluded. Of the 1729 ovules present in the eight insect exclusion cones, only two (0.1%) were pollinated.

Airborne load of cycad pollen

The airborne load of cycad pollen trapped in the vicinity of male cones was seldom abundant, often minimal, and always sporadic (Table 4). Pollen was most abundant on traps within 50 cm of the male cone, but even at this short distance its quantity varied greatly among trap lines (Table 4). In each

replication the highest pollen load at 50 cm was between 250° and 290°, presumably the direction of the prevailing wind. At a distance of only 2 m from the male cone, pollen was no longer always recorded on traps; and the trapping of pollen at 4 and 8 m was sporadic and exceptional. Dashed circles around male cones mapped in Fig. 2 indicate this 4-m limit of consistent pollen transport by wind. No receptive female cones were recorded within 4 m of pollen-shedding males.

The quantity of airborne cycad pollen in the immediate vicinity of receptive female cones was minimal, the estimated pollen captured by the average trap for each of the three female cones being 4, 6 and 0 grains, respectively.

Table 3. Pollination of *Lepidozamia peroffskyana* female cones naturally and after exclusion of wind and insect vectors

Treatment	<i>n</i>	Mean seed number ^A (range in parentheses)	Mean percentage pollinated (range in parentheses)
Natural pollination	8	270 (163–355)	83.9 (68.5–95.2)
Wind exclusion	9	230 (170–304)	70.3 (31.5–94.6)
Insect exclusion	8	216 (114–240)	0.1 (0.0–0.4)

^ANumber of seeds per cone.

Table 4. Changes in airborne load of cycad pollen trapped at increasing distances from *Lepidozamia peroffskyana* male cones

Male	One			Two			Three		
Trap line	1	2	3	1	2	3	1	2	3
Degree bearing	270°	0°	95°	290°	50°	150°	250°	50°	100°
Distance (m)									
0.5	446	65	24	1190	28	298	280	28	214
2	298	24	0	0	0	12	0	0	0
4	0	6	0	59	0	0	0	6	0
8	0	0	0	0	0	113	0	0	6

L. peroffskyana pollen captured by airborne traps adjacent to female cones was typically exceeded by pollen from other species (although this was not quantified).

Pollen load of *Tranes weevils*

Cycad pollen adhering to *Tranes* weevils collected from *L. peroffskyana* male cones was conspicuous under the electron microscope. Significant amounts of pollen were consistently carried on the legs and tarsi, whose numerous setae favoured the retention of pollen (Fig. 4). The estimated average pollen load for *Tranes* weevils collected from male cones was 1745.4 grains per weevil ($n = 10$, max. = 4230, min. = 588). In the course of the study, more than 500 *Tranes* weevils were observed to visit a single receptive female cone during the course of one week. Thus, even allowing for half the pollen load to be lost during movement from male to female cones, *Tranes* weevils could collectively deliver approximately 435 000 pollen grains to a receptive female cone, or approximately 1800 grains for every ovule present in the average female cone.

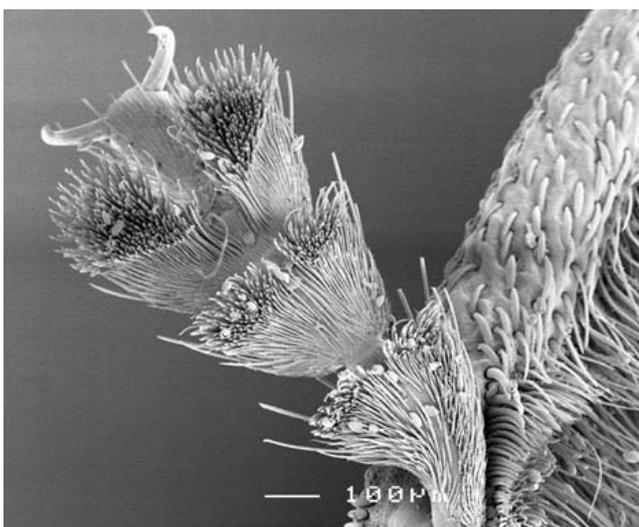


Fig. 4. Transport of *Lepidozamia peroffskyana* pollen on *Tranes* weevil tarsus. Cycad pollen (resembling grains of rice) is visible among the setae that cover the underside of the tarsus (foot segment). Scale bar = 100 μm .

Tranes weevil behaviour on *Lepidozamia peroffskyana*

Tranes weevils congregate on male cones of *L. peroffskyana* in spectacular numbers approximately 24 h before the cones distend in the final phase of their development. Thus, large numbers of weevils are present and ready to enter the cone as soon as the first microsporophylls separate. A male cone collected for dissection at first microsporophyll separation yielded more than 700 weevils. Rather than all the sporophylls coming apart at once, a subset of the sporophylls separate to produce a characteristic fissure that spirals around the male cone as it extends (Fig. 1). Consequently, most of the sporophylls shed their pollen over internal spaces of the male cone rather than into the outside air. *Tranes* weevils enter the male cone to feed upon this dehisced pollen. Weevils are also active on the external surfaces of male cones throughout the daylight hours. Copulation and oviposition activity was frequently observed, in addition to aggressive interactions between males in which rivals briefly grappled with each other by locking rostrums over each other's abdomen. Male *Tranes* weevils can be distinguished by the exaggerated brush of setae they bear along the foreleg tibia.

Female *Tranes* use their rostrum to excavate oviposition holes into the microsporophylls. Some 24–48 h after initiation of pollen shedding, the surfaces of the male cone are extensively marked by the resulting oviposition scars. By this stage in cone development most of the microsporangia have dehisced and the adult weevils depart. The larvae bore internally within the microsporophylls, hollowing them out completely before tunnelling into the central cone axis, which they completely reduce to frass. After less than a week of such larval activity, the structure of the male cone is entirely destroyed (Fig. 2). Late-instar *Tranes* larvae burrow into the soil to pupate. Pupae were collected in the first 10 cm of topsoil beneath male plants that had borne cones in the previous weeks. *Tranes* pupae possibly diapause in the soil during the months when *Lepidozamia* cones are unavailable—as has been reported for *Miltotrane*s weevils on *Bowenia* (Wilson 2002)—but as yet we have been unable to confirm this.

Pollination of *L. peroffskyana* depends upon movement of pollen-bearing weevils from male to female cones, and the

female cones clearly have a strong attraction for *Tranes* weevils. One of the insect-exclusion treatments was penetrated by *Tranes* and when collected for dissection a week later it contained more than 500 weevils. Weevil behaviour on the external surfaces of receptive female cones differed from that observed on pollen-shedding male cones. Weevils were not numerous on the exterior of the female cones (typically <12) and the mating, male–male aggression and oviposition behaviours so prominent on male cones were absent. Oviposition scars were not conspicuous and *Tranes* larvae were never observed within the megasporophylls of the female cone. Rather than congregating on the external surfaces of the female cone, weevils moved into the cone interior soon after arrival.

Diet of *Tranes* weevils

Dissection of the weevil digestive system indicated that adult *Tranes* feed on cycad pollen. The ingested pollen is passed through the proventriculus, which in *Tranes* is a bell-shaped structure bearing longitudinal and transverse rows of cuticular plates (Fig. 5A). These seem to fracture the exine of

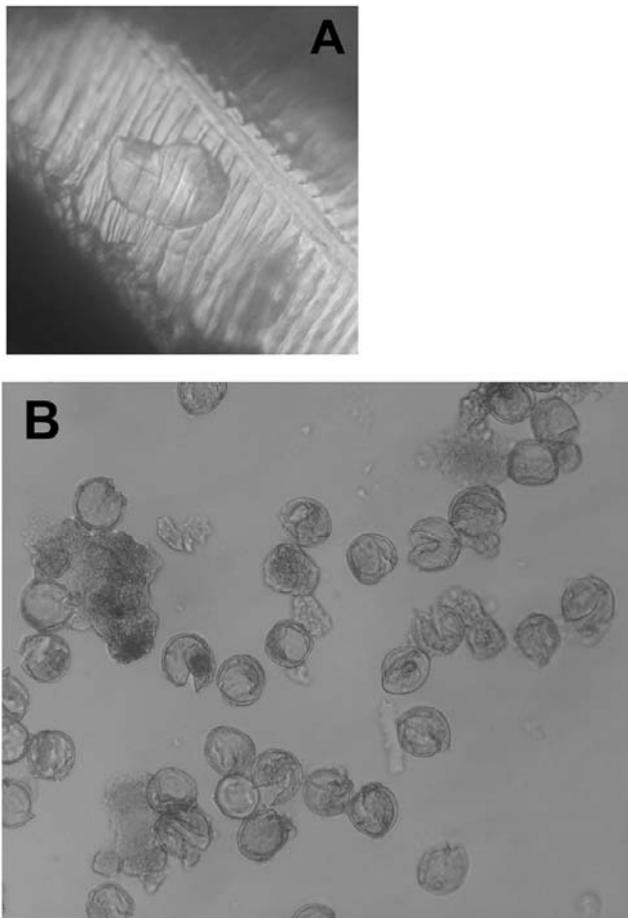


Fig. 5. (A) Fractured grain of cycad pollen associated with cuticular plates of *Tranes* weevil proventriculus. (B) *Tranes* weevil stomach contents, showing cycad pollen with fractured exine.

pollen grains. *Tranes* stomachs contained large amounts of cycad pollen that had undergone some form of mastication or crushing (Fig. 5B). The exine of the pollen had been fractured and the internal contents apparently digested. Pollen grains with this same damage to the exine were also associated with the proventricular structure (Fig. 5A).

Discussion

Insect pollination of *Lepidozamia peroffskyana*

The cycad *L. peroffskyana* is pollinated exclusively by a species of *Tranes* weevil that carries out its life cycle in association with the male cones but that also visits the female cones in large numbers. No other insect visitor was consistently recorded from both male and female cones. Previous studies of cycad pollination (Norstog *et al.* 1986; Donaldson 1995, 1997) have recorded limited pollination of cones from which insects had been excluded, but were unable to distinguish between subsidiary pollination by wind or contamination of the treatments by insects as the cause of this fertility. The results of our study demonstrate that wind does not play any role in the pollination of *L. peroffskyana*, a conclusion that is supported by multiple lines of evidence, as follows:

- (i) The pollinator-exclusion experiment (Table 3) demonstrated that pollination was virtually eliminated in the absence of *Tranes* weevils, whereas the exclusion of wind had a comparatively negligible effect, since weevils still located the cone and pollinated the microsporophylls.
- (ii) The presence of airborne cycad pollen was not consistently recorded beyond a 2 m radius surrounding pollen-shedding male cones (Table 4). In contrast, the pollen range of wind-pollinated conifers is routinely measured in dozens of kilometres (Faegri and Van der Pijl 1979). In the case of *Lepidozamia* cycads, the pollen is not 'light and dry and easily blown by the wind' as claimed by Chamberlain (1935, p. 127). *Lepidozamia* pollen on both weevil specimens and airborne traps had a tendency to clump, whereas pollen grains of wind-pollinated species are typically non-sticky and disperse singly (Proctor *et al.* 1996). The architecture and development of the *L. peroffskyana* male cone is such that most of the pollen is shed over enclosed internal spaces rather than directly into the surrounding air.
- (iii) The airborne load of cycad pollen was minimal in the immediate vicinity of receptive female cones. By extrapolation, the maximum airborne pollen load recorded in the vicinity of a female cone was approximately 20000 grains per square metre. In contrast, Proctor *et al.* (1996, p. 265) suggest that 'every square metre of the [wind pollinated] plant's habitat must receive around a million pollen grains to make pollination reasonably certain'. In the case of cycads,

this theoretical figure would be even higher, since the micropyles are not exposed to the open air but are sheltered behind a barrier of interlocking sporophylls.

- (iv) The onset of pollination receptivity was not precisely synchronised within the coning population (Table 2), and coning individuals were scattered beyond the distance at which airborne pollen transport was effective (Fig. 3). In contrast, genuinely wind-pollinated plants are characterised by mechanisms to ensure that the pollination phase is closely synchronised between individuals and that the pollination event is initiated when meteorological conditions for airborne pollen transport are optimal (Faegri and Van der Pijl 1979; Proctor *et al.* 1996).

In summary, the reproductive ecology of *L. peroffskyana* is completely unlike that expected for a wind-pollinated plant.

The relationship between *L. peroffskyana* and its associated *Tranes* weevils appears to be a host-specific mutualism in which the long-term persistence of both participants is dependent upon the survival of the other. Since wind pollination is ineffective and no other potential insect pollinator species are present, populations of *L. peroffskyana* would fail to reproduce in the absence of *Tranes* weevils. A similar scenario appears to have eventuated in Cuba, where the endangered cycad *Microcycas calocoma* has failed to regenerate naturally for many decades following the probable extinction of its insect pollinator (Vovides *et al.* 1997). The *Tranes* weevils at the Mooball study site were dependent upon the male cones of *L. peroffskyana* as a food source for both adults and larvae. They were never observed feeding on any other tissues of *L. peroffskyana*, or in association with any other host plant (see also Ornduff 1989; Forster *et al.* 1994; Oberprieler 1995a). The *Tranes* sp. on *L. peroffskyana* is a different species from *T. insignipes* from the cones of *L. hopei*, the only other cycad in the *Lepidozamia* genus (Wilson and Rowles 1997). The taxonomist Rolf Oberprieler (pers. commun.) considers the *Tranes* sp. on *L. peroffskyana* to be distinct from *T. lyterioides*, the pollinator of *Macrozamia communis* (Chadwick 1993), and also from the *Tranes* sp. on *M. machinii*, taxonomically the closest relative of the *Tranes* sp. we observed on *L. peroffskyana*. For these reasons, it seems reasonable to suppose that the *Tranes* sp. on *L. peroffskyana* is host specific, but further taxonomic work would be required to confirm this absolutely.

The pollination symbiosis between *L. peroffskyana* and *Tranes* depends upon the movement of pollen-bearing weevils from male to female cones. However, the stimulus for such behaviour remains unclear, since female cones do not 'reward' pollinating weevils with resources for either pollen-feeding or reproduction. A similar situation exists in other cycad-insect pollination systems that have been studied (Norstog *et al.* 1986; Tang 1987a; Donaldson 1995,

1997). At present the prevailing hypothesis is that non-rewarding female cones attract pollinating insects by mimicking the volatile odours insects use to identify the truly rewarding male cones (Tang 1987a, 1987b; Pellmyr *et al.* 1991). We noted that receptive *L. peroffskyana* female cones on which weevils were present emitted a pungent fruity odour similar to the scent released by pollen-shedding male cones.

Lepidozamia peroffskyana and other cycad pollination systems

Studies of insect pollination now exist for all of the Australian cycad genera in the families Zamiaceae and Stangeriaceae, allowing the pollination system of *Lepidozamia* to be compared with that of *Macrozamia* (Chadwick 1993; Mound and Terry 2001; Terry 2001) and *Bowenia* (Wilson 2002). All these plants are pollinated by weevils in the 'Tranes group' species complex, and the basic elements of their pollination systems are similar. The male cones are the centres of weevil activity, providing resources for feeding and larval development. Intensive feeding by adults and larvae severely damage (if not destroy) male cones, but not before they have dehisced their pollen. Weevils transport pollen on their bodies as they move between cones. *Tranes* weevils visit female cones in sufficient numbers to effect pollination, even though the 'unrewarding' female cones do not provide resources for feeding or completion of the life cycle. Hence the hypothesis that pollinating insects are 'temporarily duped' into visiting female cones, perhaps because they mimic the sensory cues associated with males (Terry 2001). In general outline, the pollination system between 'Tranes group' weevils and Australian cycads is similar to beetle pollination of *Zamia* cycads in the Americas and *Encephalartos* cycads in Africa (Table 1). However, the pollinating beetle taxa on these continents have no close relationship with 'Tranes group' weevils, and the similarities in pollination system are a result of convergent evolution to a specialised niche (Oberprieler 1995a, 1995b). The inability of pollinating insects to feed on, or mature larvae in, the tissues of the female cone has been attributed to toxicity mechanisms that protect female cones from insect attack during the lengthy maturation of the seed (Norstog and Fawcett 1989; Vovides *et al.* 1993).

Although the 'Tranes group' weevil pollination systems of *Lepidozamia*, *Macrozamia* and *Bowenia* are broadly similar, details of weevil behaviour are divergent between the genera, lending ecological support to taxonomic hypotheses of host specificity. As reported by Chadwick (1993) and Terry (2001), *Tranes* on *Macrozamia* are nocturnal, their activity peaking between dusk and early evening. During the day they are inconspicuous, remaining within the cone interior. The average number of *Tranes* collected from individual male cones of *Macrozamia* was about 150. In contrast, *Tranes* sp. on *L. peroffskyana* are diurnal and active

on cone surfaces throughout the day, feeding, mating and ovipositing. Average numbers of *Tranes* collected from male cones of *L. peroffskyana*, at about 700, was far greater than that typical of *Macrozamia*.

Another significant difference between *Lepidozamia* and *Macrozamia* pollination systems is the absence of thrips pollination in *Lepidozamia*. Thrips of the genus *Cycadotherips* are associated with *Macrozamia* throughout Australia (Mound 1991; Chadwick 1993; Forster *et al.* 1994; Mound and Terry 2001; Terry 2001). Like 'Tranes group' weevils, they carry out their life cycle in the cones, and Terry (2001) demonstrated that *Cycadotherips* are effective pollinators of *Macrozamia* cycads. In some *Macrozamia* species, *Cycadotherips* and *Tranes* weevils occur together as 'dual, specialist pollinators' (Terry 2001, p. 1293); but in other populations either *Cycadotherips* or *Tranes* are the only insect pollinator present. Using thrips-trapping techniques of Mound and Terry (2001), we found no evidence of *Cycadotherips* associated with *L. peroffskyana* at our study site. The absence of *Cycadotherips* from *Lepidozamia* is intriguing, given that *Macrozamia* exclusively pollinated by *Cycadotherips* grow sympatrically with *Tranes*-pollinated *L. peroffskyana* in south-east Queensland (Forster *et al.* 1994). *Cycadotherips* failure to cross between genera could be interpreted as further evidence of the tendency towards host specificity in cycad-insect pollination systems.

Antiquity of cycad pollination by insects

The association of both weevils and thrips with *Macrozamia* pollination (Terry 2001) demonstrates that cycad relationships with pollinating insects have developed more than once in the history of these plants. The evolutionary antiquity of cycads (Gao and Thomas 1989) prompts the question of how long these pollination relationships have been in place. The *Lepidozamia*-*Tranes* pollination system is unlikely to be older than the final separation of South America and Australia during the middle Tertiary, since *Tranes* weevils are not associated with cycads on any other continent (Oberprieler 1995a). Although weevil genera in the same family as *Tranes* (Curculionidae) are associated with cycads in southern Africa and South-East Asia (Oberprieler 1995a; Tang *et al.* 1999) they are not closely related to each other and therefore their relationships with cycads evolved independently (Oberprieler 1995a, 1995b). Because the Curculionidae as a group did not evolve until the early Cretaceous, genera such as *Tranes* presumably underwent a host shift onto cycads from original angiosperm hosts (Oberprieler 1995a, 1995b).

The biogeography of the beetle family Languriidae provides more substantial evidence for the antiquity of cycad pollination by insects. The languriid subfamily Xenoscelinae contains cycad-associated genera on every continent where cycads occur: Southern Africa (Donaldson 1995, 1997), Central America (Vovides 1991), South-East Asia (Tang

et al. 1999) and Australia (Ornduff 1993; Forster *et al.* 1994). Exclusion experiments have confirmed that languriid beetles can pollinate their cycad hosts (Donaldson 1995, 1997) and for some cycad species, languriids are the only pollinating insects present (Vovides 1991; Donaldson 1995). Because of the global distribution of languriids, it seems probable they developed their relationships with cycads before late-Mesozoic continental drift. Investigation of the phylogenetic relationship among the global diversity of cycad-associated languriid beetles could clarify this point. An ancient relationship between cycads and pollinating beetles is also indicated by the distribution of the beetle family Boganiidae, whose most primitive subfamily, the Paracucujinae, is exclusively associated with cycads in Southern Africa and Western Australia (Crowson 1981; Goode 1989; Ornduff 1993; Lawrence and Britton 1994; Donaldson 1997). The Jurassic biogeography of languriid and boganiid cycad beetles provides the best evidence at this time that cycad-insect pollination systems were already in place before the Cretaceous evolution of the angiosperms.

If the Mesozoic ancestors of Australian *Lepidozamia*, *Macrozamia* and *Bowenia* cycads were insect pollinated, the Mesozoic beetle that originally pollinated these genera must have subsequently become extinct, to be replaced by the 'Tranes group' weevils some time during the Tertiary. In the case of *Macrozamia*, there is tangible entomological evidence for such an 'older' beetle pollinator. *Tranes* weevils are now the only pollinating beetles associated with *Macrozamia* cycads in eastern Australia (Chadwick 1993; Forster *et al.* 1994), but in south-west Western Australia there are relict *Macrozamia* species associated with boganiid and languriid beetles as well as *Tranes* (Ornduff 1993). The distribution of *Macrozamia* was once continuous across Australia, since a relict species still persists in the central Australian ranges (Jones 2002). The relict *Macrozamia* of central and western Australia were isolated by increasing desertification of central Australia during the late Tertiary (White 1994). Hence, the western Australian boganiids and languriids probably also once had a continent-wide distribution. They represent the most likely beetle pollinators of the ancestral *Lepidozamia*, *Macrozamia* and *Bowenia* cycads before the current relationships with 'Tranes Group' weevils evolved during the Tertiary. That these cycads appear to have survived the extinction of their original insect pollinators is testament to the remarkable evolutionary persistence of these plants.

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