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Comparing reproductive success in the rare sedge *Gahnia insignis* and the common *Gahnia clarkei* in north-east New South Wales.

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Abstract: Species may become vulnerable because of a reduction of habitat, leading to reduction of population sizes and an increase in geographic isolation between populations, leading to genetic drift that may result in reduced reproductive fitness. The restricted sedge *Gahnia insignis* S.T. Blake (family Cyperaceae), occurring in isolated pockets in north-east New South Wales and Queensland, was compared to a closely related, sympatric, common and widespread *Gahnia clarkei* Benl for flowering phenology and reproductive success. Flowering patterns, examination of pollen, fertilisation and embryo development and seed-ovule ratios show *Gahnia clarkei* has every indication of successful sexual reproduction, but that *Gahnia insignis* appears to reproduce mostly by vegetative means, with an occasional sexual event. This was due to the rarity of pollination opportunities, and to poor pollen viability and pollen quantity, resulting in a much lower seed-ovule ratio than *Gahnia clarkei.* The additional high level of vegetative reproduction in *Gahnia insignis* suggests it may be largely clonal in Nightcap National Park. A genetic study of the whole distribution would add knowledge of the species genetic diversity and differentiation between populations.

Key words: Reproductive success, phenology, disjunct, sympatric

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Introduction

The population size of many Australian plant species may be reduced through loss of habitat due to clearing, geomorphological processes and climate change. The occurrence of vegetative reproduction or self-fertilisation in a plant species, along with reduced population sizes, can lead to a loss of genetic diversity through an increase in homozygosity (Schemske & Lande 1985) or genetic drift, both resulting in a loss of genetic variation and fitness (Luitjen et al., 1996). This may affect their ability to adapt to changing environments, decrease their levels of reproductive performance and increase their susceptibility to predators (Watson et al., 1994). Small populations may experience poor seed set due to poor pollen quantity and quality (Byers, 1995), and this may contribute to the occurrence of local extinctions.

Measures of reproductive success such as flower morphology and phenology, insect predation, root structure, seed/ovule ratios, seed set, and pollination success at microgametogenesis and megagametogenesis were determined, observed and compared in the rare sedge species *Gahnia insignis* (family Cyperaceae) and the sympatric, more common species *Gahnia clarkei* in 1995. The dioecious flowers of *Gahnia* produce the stigmata first, followed by the stamens approximately one week later. *Gahnia insignis* was found to have little opportunity for pollination, with low viable pollen production and synchronous temporal separation of male and female flowers., resulting in a low fruit set.

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Materials and methods

Gahnia insignis S.T. Blake (Schoeneae: Cyperaceae) is federally listed as rare in Australia (ROTAP code = 3RcaQN, Briggs and Leigh, 1996). It occurs in isolated pockets in Nightcap National Park in northeast NSW, Australia (152^{o} 20'E 28^{o} 35'S). Other disjunct populations include Mt Jerusalem National Park, Comboyne Plateau and Copmanhurst in north-east New South Wales, and in Moreton and North Kennedy districts in Queensland (Blake 1957; NSW Herbarium records). *Gahnia clarkei* Benl also occurs in Nightcap National Park but is a much more common species in eastern Australia, occurring in coastal districts in moist habitat in NSW, Queensland, Victoria, Tasmania and South Australia.

Two populations of each species were observed. *Gahnia clarkei* has a clumping habit for each plant with individuals up to one metre wide and approximately 1.5 metres high; numbers of plants in each population ranged from 100–300 plants. In contrast the root structure in *Gahnia insignis* consists of many widely spreading horizontal roots, any of which may produce upright shoots. It was difficult to determine what comprised an individual plant of *Gahnia insignis,* however one population covered the forest floor for an area of 500 sq. m. while the other colonised the edge of an old logging track for 100 m. *Gahnia insignis* was examined for root structure, with a clump being considered here as an 'individual' plant.

Inflorescences were examined and the number of florets calculated by counting the number of florets per spikelet, spikelets per panicle, panicles per plant, and plants per population for both species. The flowering period, flowering strategies, breeding systems and the seed/ovule ratios (Wiens 1984) were observed. The seed/ovule ratio is the percentage of ovules that develop into seeds/fertile fruits, considered to be approximately 50% in perennials (Wiens 1984). Opportunistic observations on possible insect predators, insects which inhabit the plants, potential pollinators and possible fruit dispersal agents were also included. Inflorescence morphology, seed set (through dissecting fruits), pollen viability, and root structure of the two species were compared.

Destructive sampling for *Gahnia insignis* was used to determine seed/ovule ratios (Wiens 1984), seed set and embryo viability, since there was a high predation rate of spikelets from bud to young fruiting stages, and the pollination rates were low. Plants were tagged at anthesis, then one panicle from three 'plants' from each population was collected every three weeks and fixed in Formalin-Propionic-Alcohol (FPA), and then stored in 70% ethanol before being dissected. The numbers of fruit (and their viability) and the numbers of spikelets from each panicle collected were recorded. *Gahnia insignis* was collected and fixed at three stages of the reproductive process, from one month preceding anthesis to one month following anthesis.

Gahnia clarkei had a much shorter flowering period with much higher production levels and larger panicles, so *in-situ* counts were made on the panicles that were tagged at anthesis. *Gahnia clarkei* was collected from two months preceding anthesis.

Material from both species was dehydrated in a series of tertiary butyl alcohol dilutions embedded in paraffin wax, sectioned at 11–12 µm, and stained with safranin and fast green.

Results

Floral morphology was similar for both species. *Gahnia insignis* had one or two florets per spikelet, while *Gahnia clarkei* always had two. All florets had six anthers and a trifid stigma. However the number of panicles per population was much greater for *Gahnia clarkei* (3812–7769) compared with *Gahnia insignis* (22.5–27.8) (Table 1). The dioecious flowers of *Gahnia* produce the stigmata first, followed by the stamens approximately one week later.

In Nightcap National Park in 1995 flowering in *Gahnia insignis* continued intermittently from early April to late September. Flowering was synchronous within and often between populations, with the presentation of stigma and stamens being temporally separated throughout the whole

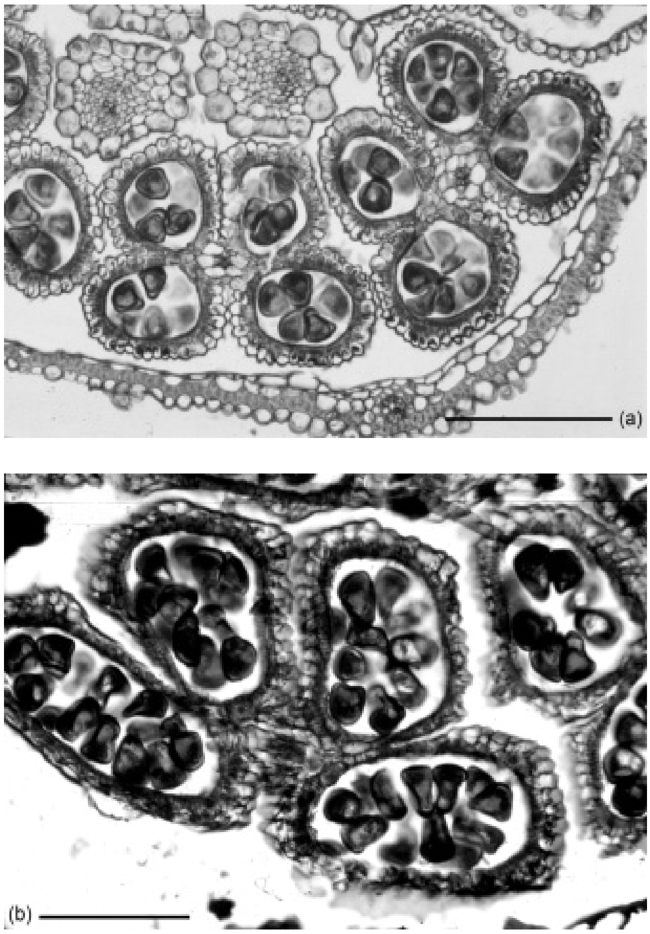

Fig. 1. Transverse sections of mature anthers of (a) *Gahnia insignis* and (b) *Gahnia clarkei.* Scale = 25µm.

forest, i.e. in the whole forest you may see only stigmata, or another day you may see only stamens. Thus pollination was impossible most of the time. Plants of *Gahnia insignis* always produced stamens one week after the stigmata appeared, (with the exception of one occasion, presumably very briefly when stamens and stigma were present simultaneously at both sites), suggesting that there is little opportunity for pollination. The stigmata generally last two or three days, and stamens probably similar. Pollen was released but there were only very rarely stigmata present to accept it. Panicles were visible for approximately three weeks prior to anthesis.

Flowering in *Gahnia clarkei* lasted two weeks, with each plant producing flowers simultaneously, but not synchronously with other plants. Pollen was produced in large amounts. Panicles were visible for more than eight months prior to anthesis.

Two stages of microsporogenesis were observed in *Gahnia clarkei*, and four stages in *Gahnia insignis.* The epidermis, middle layer and tapetum are evident in both species, and the endothecium can be seen developing in *Gahnia insignis*. Figure 1 shows transverse sections of anthers with mature pollen grains in *Gahnia clarkei* and almost mature in *Gahnia insignis*, with the spiral endothecium visible. The pollen of *Gahnia insignis* has more unstained pollen grains than *Gahnia clarkei,* which has more grains per anther, larger grains, and more grains which have taken up stain. The unstained pollen grains indicate the presence of cytoplasm and cellulose, and an absence of chromosomes, indicating a lack of viability. Lack of maturity in the *Gahnia insignis* pollen is most unlikely due to the collection regime and the large numbers of samples processed and viewed.

Three stages of megasporogenesis were observed in *Gahnia clarkei,* and four stages in *Gahnia insignis* and appeared to be normal in both species. A tetrad was observed in *Gahnia insignis,* but no embryos were observed in young fruits, suggesting that they may develop late, the fruit was unfertilised or the embryo was aborted. *Gahnia clarkei* showed normal development, with plenty of evidence of fertilisation and normal embryo development, with numerous embryos sighted. This may explain the Seed-ovule ratios results (Table 1) showing *Gahnia insignis* (0.02 and 0.03), has roughly only one tenth of the seed/ovule ratio of *Gahnia clarkei* (0.25 and 0.36).

No insects were observed on *Gahnia clarkei*, but the effects of insect predation on *Gahnia insignis* were obvious even from casual visits. 28% of developing spikelets were attacked by insects, but only 5% of the remaining fruit. Grasshoppers and lepidopteran larvae were observed feeding on *Gahnia insignis.*

Discussion

The widespread sedge species *Gahnia clarkei* has good outcrossing opportunities, reflected by the evidence of abundant sexual reproduction, and the differences shown in plants within and between populations. Plants showed variation in appearance, the large numbers of flowers produced with abundant pollen produced at varying times, and the large numbers of fruits produced on each plant. Compared to *Gahnia clarkei,* the geographically restricted (within Nightcap National Park) *Gahnia insignis* was found to have little opportunity for pollination due to the temporal separation of the male and female flowering phases throughout the forest and synchronous temporal separation of male and female flowers, reflected in the low seed/ovule ratios (Table 1) and low pollen viability (Figure 1), resulting in a low fruit set.

Gahnia clarkei has a clumping habit for each individual plant but the root structure in *Gahnia insignis* consists of many widely spreading horizontal roots, any of which may produce upright shoots. It appears to be reproducing mostly by vegetative means, or occasionally pollinating/fertilising with a closely related individual, resulting in populations that may be largely clonal.

Other largely clonal plant species have also been observed to have low reproductive success, such as some members of the Orchidaceae and Rosaceae families (Gill 1989, Aspinwall & Christian 1992, and Sydes and Calder 1993). While it is possible that *Gahnia insignis* is a relatively new species, the disjunct distribution suggests that the distribution of *Gahnia insignis* was much wider in the past. This hypothesis supported by the presence of a similar species, *Gahnia sclerioides,* growing only in south-west Western Australia (Wilson, 1997). *Gahnia sclerioides* has a similar appearance and habit, grows in a similar habitat, and also has a restricted distribution and abundance, but greater fruit fertility (Wheeler 1996).

Table 1. Seed/ovule ratios for *Gahnia insignis* and *Gahnia clarkei* populations.

Each spikelet of *Gahnia insignis* were assumed to contain 1.5 florets. † = Number of viable fruits per panicle/ Number of florets per panicle.

Species	Site	Number of spikelets per panicle (+/-s.e.)	Number of resultant fruits containing embryos (+/-s.e.)	Mean % embryo viability	Seed/ovule ratio †
Gahnia insignis#	1	27.8 (3.00)	2.3 (0.58)	61	0.03
	2	22.5 (2.37)	1.1 (0.04)	61	0.02
Gahnia. clarkei	1	3812 (392)	2131 (326)	90	0.25
	2	7769 (1590)	6221 (1109)	90	0.36

A low level of viable pollen production and low resistance to insect predation may indicate low genetic variability in *Gahnia insignis* in Nightcap National Park. Testing the genetic variability between populations throughout the whole distribution of the species would provide valuable information for its future conservation, and may point to the possibility of cross-pollinating two or more populations with the aim of increasing genetic diversity and improving the species long-term outlook.

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