

Reproductive success and rarity of the variable midge orchid *Genoplesium insigne*

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Abstract: *Genoplesium insigne*, the variable midge orchid, is a rare terrestrial orchid with a highly restricted distribution and low total population size. There is limited published data on its ecology and life history, and ongoing management has been dependent on ecological knowledge of other *Genoplesium* species.

Observations and results from four years of monitoring *Genoplesium insigne* across multiple sub-populations is presented, including counts of the number of flowering stems, phenological data, pollinator observations and reproductive success. Increased survey effort during spring 2020 demonstrated that the total population size of the species greatly exceeded previous estimates of less than 20 individuals, however the total population size remains low.

Observations from open flowers, including droplets on column auricles and removal of entire pollinia by small flies in the family Chloropidae, suggest the pollination mechanism for *Genoplesium insigne* is xenogamy, or geitonogamy, mediated by small Diptera. The pollination success of *Genoplesium insigne* in each of the four years of monitoring was lower than all published rates for other *Genoplesium* species and comparatively low for nectar producing orchids globally. Pollination success did not vary between populations or seasons despite differences in population size, habitat fragmentation or weather conditions. The low pollination success in *Genoplesium insigne* may be due to limitations in the attractiveness of its nectar to pollinators, or result from timing of flowering. Its comparatively early flowering period in spring, compared to other *Genoplesium* species in summer, may represent a decoupling of the flowering period and the life cycle of its primary pollinator, or may increase competition for pollinators.

Priorities for further research include investigating the causes of the comparatively low reproductive success and how this may contribute to the rarity of the species.

Cunninghamia (2022) 22: 045–052

doi: 10.7751/cunninghamia.2022.004

Introduction

The Orchidaceae family is the most diverse of all angiosperm families, with estimates of approximately 25,000 species (Cribb et al. 2003). The pollination mechanisms of the Orchidaceae have been the subject of research dating back to Darwin (1862). Across the Orchidaceae a variety of pollination mechanisms have been identified, with different rates of pollination or reproductive success associated with different mechanisms (Neiland & Wilcock 1998). Breeding systems based on autogamy and/or agamospermy are reproductively very successful with high fruit set levels recorded in these orchids (Neiland & Wilcock 1998). Among the xenogamous orchid species, those species which offer a reward to their pollinators (most commonly nectar), have higher pollination rates compared to those species which are deceptive and offer no rewards (Neiland and Wilcock 1998; Brundrett 2019). Within these broader trends, the pollination success of individuals and populations can be influenced by additional factors including habitat fragmentation, plant density and pollinator type (Tremblay et al. 2005; Aguilar et al. 2006; Parra-Tabla et al. 2011). Reduced reproductive success has been recorded among isolated and highly dispersed orchid populations and species pollinated by flies (Tremblay et al. 2008). While the causes of rarity are diverse, low reproductive success has been associated with rarity within members of the Orchidaceae (Neiland and Wilcock 1998).

The genus *Genoplesium* R. Br. or midge orchids, comprises over 50 species (Jones 2006; Jones & Clements 2018) distributed within Australia, New Zealand and New Caledonia (Jones 2001). *Genoplesium* was reinstated by Jones and Clements (1989) for the distinctive group of species which were then placed in *Prasophyllum* sect. *Genoplesium*. Jones et al. (2002), proposed Fitzgerald's genus *Corunastylis* also be reinstated, leaving *Genoplesium* monotypic. This latter proposal has not been accepted by the Council of Heads of Australasian Herbaria and reflected in the Australian Plant Census (APC). Nomenclature used throughout this paper follows that of the National Herbarium of NSW and Jones & Clements (1989) with *Corunastylis* treated as a synonym. This genus of geophytic herbs is a member of the Prasophyllinae subtribe within the Orchidaceae and is distinguished by a hinged and mobile labellum, a cushion like callus on the labellum and deeply notched column wings (Jones 2001). Other characteristics of the genus include short plants with small, often dark coloured flowers, which can make detection of individuals in the field difficult. *Genoplesium* species reproduce solely from seed and grow as individuals or widely spaced colonies (Jones 2001).

The dominant pollination mechanism in the genus *Genoplesium* is considered to be xenogamy or geitonogamy mediated by small Diptera (Bower 2001a; Bower et al. 2015). With the exception of those species which have been identified as being autogamous (*Genoplesium nudum*, *Genoplesium pumilum* and south-west Tasmanian *Genoplesium archeri*) or apomitic (*Genoplesium apostasioides*), most *Genoplesium* species are thought to reward fly pollinators with nectar produced either in the column appendages or

the labellum callus (Garnet 1940; Jones 2001; Bower 2001; Bower et al. 2015; Ren et al. 2020). Attraction of pollinators to flowers of *Genoplesium* species appears to be by odour (Bower 2001) with various reports of *Genoplesium* species producing aromas that are detectable to humans including lemon scents and sour milk (Jones 2001; Bower 2001; Bower et al. 2015; Ren et al. 2020). Consistent with global and regional trends, studies of reproductive success in nectar rewarding *Genoplesium* species have identified high levels of reproductive success (Bower et al. 2015; Ren et al. 2020a). In contrast with trends identified for British orchids, many species of nectar-rewarding *Genoplesium* are naturally rare with restricted distributions or disjunct occurrences (Bishop 2000; Jones 2006) including nine species of *Genoplesium* (including synonymous *Corunastylis* species) listed as endangered or vulnerable under the NSW *Biodiversity Conservation Act 2016*.

Genoplesium insigne is a somewhat atypical member of the *Genoplesium* genus, as peak flowering of the species typically occurs in the spring months (September to November), while almost all other members of the genus flower in the summer and autumn months. The species is endemic to the northern portions of the Central Coast Local Government Area (LGA) and the southern portions of the Lake Macquarie LGA, and is listed as critically endangered under the NSW *Biodiversity Conservation Act 2016* because of its highly restricted distribution and very low total population size (NSW Scientific Committee 2015).

In 2015 the extent of occurrence (EOO) measured using a minimum convex polygon (as per IUCN [2011]) was 40 km² when considering the three known extant locations or 100 km² when all previously known locations were included (NSW Scientific Committee 2015). In 2015 the NSW Scientific Committee (2015) estimated the area of occupancy (AOO) to be between 12–20 km² based on three to five 2 × 2 km grid cells, the scale recommended for assessing AOO by IUCN (2011). Over the period of this study additional sub-populations of the species were detected and the EOO was increased to approximately 210 km² with the AOO increased to 576 km² as per IUCN (2011; 18 grid cells [2 × 2 km]) or 400 km² as per Bland et al. (2016; four grid cells [10 × 10 km²]).

Habitat for *Genoplesium insigne* has been lost, fragmented and disturbed by a variety of land-uses, and extant populations vary in terms of population size, remnant size and degree of fragmentation. There is little published data on the life history of *Genoplesium insigne* and many aspects of the ecology of the species have been assumed to follow that of other *Genoplesium* species (Commonwealth Threatened Species Scientific Committee 2014).

This paper presents the findings of four years of monitoring the reproductive success of *Genoplesium insigne*. Data on population size, phenology, pollination strategies and reproductive success are presented, including analysis of whether population size or patch size effect reproductive success. The study aims to increase understanding of the ecology of this very rare species to assist in ongoing management and conservation planning.

Methods

All populations of *Genoplesium insigne* surveyed as part of this study were located within the Central Coast and Lake Macquarie LGAs (Figure 1) and within the area bound by the localities of Wyong, Chain Valley Bay and Freemans Waterhole. To protect extant populations from poaching, specific locations are not included within this paper, following the Sensitive Species Data Policy in New South Wales (Andrews 2009). All observations and results as part of this study were made on in-situ individuals of *Genoplesium insigne* with no collections of the species made due to an ongoing germination trial including seed collection.

Surveys were undertaken during flowering seasons commencing in late winter or early spring from 2017 to 2020 and included four flowering seasons. Hereafter individual flowering seasons are referred to by the year in which the flowering season commenced. The survey methods involved visits approximately every 2-3 weeks (minimum of four surveys for each sub-population) across each flowering season to four known sub-populations of *Genoplesium insigne* in 2017 and five sub-populations from 2018 to 2020. During each visit the entire known extent of each sub-population was traversed with survey effort broadly consistent as the extent of each sub-population was well defined and time spent traversing individual sub-populations was consistent between seasons.

Floral presentation and phenology

The total number of flowering individuals, unopened flower buds, open flowers, closed unfertilised flowers and fertilised flowers were recorded during each visit. Plant locations were recorded using handheld Global Positioning Systems and were marked in the field using wooden sticks to avoid drawing attention to the plants which were frequently located in publicly accessible locations. Where large populations were detected (>20 individuals), counts of the entire populations of flowering stems were undertaken, although only a subset of the total population (comprising a minimum of 10 randomly selected individuals) was surveyed repeatedly to record the conversion of flowers to fruits.

Population size

Surveys involving counts of the number of flowering individuals within all known and accessible sub-populations of *Genoplesium insigne* were undertaken in each season from 2017 to 2020. The number of sub-populations surveyed as part of the project increased over time as new sub-populations were discovered. A peak of 14 sub-populations were surveyed in 2020.

Pollination

All flowers of individual plants were examined closely for the presence of pollinators and whether they were carrying the distinctive *Genoplesium* pollinarium. No collections of observed pollinators were attempted due to the rarity of the subject species and the seed collection occurring from these

populations as part of a separate ongoing germination trial. Prospective pollinators and insects wearing pollinaria were photographed and identified by Dr. Dan Bickel (Australian Museum).

Measurement of pollination success followed that of Bower et al. (2015) and numerous other studies in the Orchidaceae. Measurements were taken post anthesis by calculating the percentage of flowers setting fruit on each inflorescence (Neiland and Wilcock 1998, Tremblay et al. 2005). In *Genoplesium* there is a marked difference between the distension of fertilised versus unfertilised ovaries. Accordingly, it was not considered necessary to assess seed viability as a measure of reproductive success. Additionally, seed viability assessment would ultimately destroy tested embryos which could no longer be used in propagation trials (see above).

Statistical analysis

To assess changes in pollination rates between populations (sites) and flowering seasons (years), two quasi-binomial generalised linear models were fit to pollination data using site and year as explanatory factors (Wakefield 2013). These were compared to a third model of the same type with no explanatory factors, referred to as the Null Model. Due to the small number of samples at two of the sites (CRBG [$n = 2$] and MTRD [$n = 2$]), these have been combined into a single group, 'OTHER' [$n = 4$].

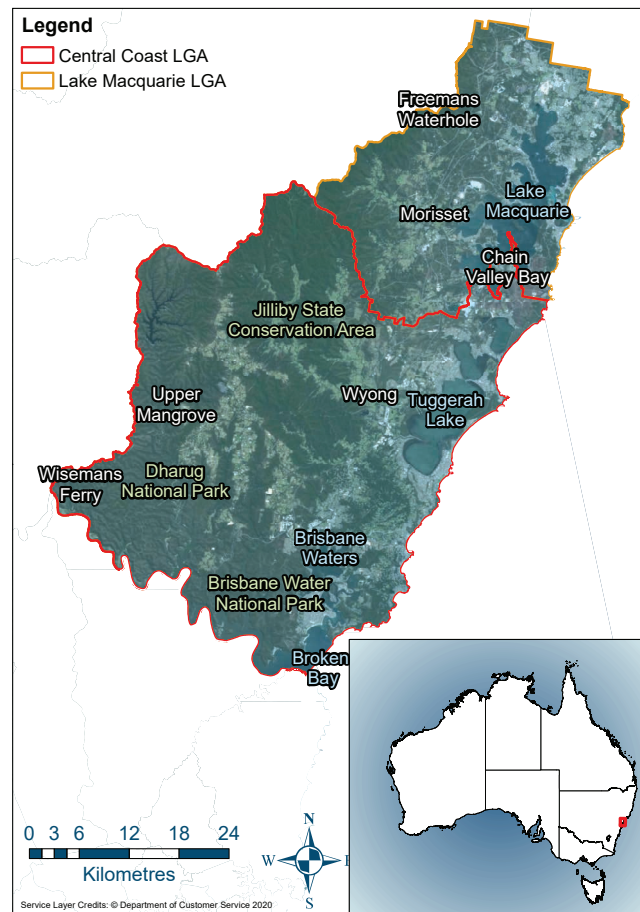


Figure 1: The Central Coast and Lake Macquarie Local Government Areas

Results

Floral presentation and phenology

Flowering of *Genoplesium insigne* has been confirmed in October and November in each flowering season (Table 1). While open flowers were observed as early as 29 July (in 2020), this observation was made of a solitary individual which already had seven open flowers suggesting flowering commenced up to one week earlier. The latest-open flowers of *Genoplesium insigne* in a flowering season were observed on the 29 February 2020, representing the end of a season which commenced the previous September. Across the extended flowering periods of *Genoplesium insigne* in 2019 and 2020 flowering of the population was asynchronous with some scapes in bud while other had dehiscent capsules.

Flowering of *Genoplesium insigne* was acropetal, with lower flowers on the raceme opening first. The duration of an individual scape retaining open flowers varied greatly and correlated with factors including pollination success,

the number of flowers present within an inflorescence and damage by florivorous invertebrates. The most detailed account of the floral duration on a single scape was recorded in spring 2020, when an individual with nine flowers on the raceme, none of which were successfully pollinated, retained at least one open flower across a 21-day period (August 24 – 15 September 2020). The mean number of flowers recorded across all seasons was 7.8 flowers per inflorescence (95% Confidence Interval [CI] from 7.3 to 8.3) with the mean number of flowers ranging from 6.8 to 8.3 in individual seasons (Table 2). The maximum number of flowers observed on a single raceme was 16.

While no detailed monitoring of the presence or absence of leaves throughout the annual cycle was undertaken, individual leaves were observed to persist for the full annual cycle. For some individuals the flaccid leaf from the previous season could be observed at the same time as the erect fused leaf and peduncle of the current flowering season.

Table 1: Months in which flowering of *Genoplesium insigne* was observed from 2017 to 2020. Highlighted months are those in which open flowers were observed

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2017												
2018												
2019												
2020												

Table 2: Mean number of flowers per inflorescence, fruits and percentage pollination success from 2017 to 2020

Year	2017	2018	2019	2020	Total
Number of inflorescences	10	26	26	53	115
Number of flowers	71	216	176	431	894
Mean number of flowers per inflorescence	7.1	8.3	6.8	8.1	7.8
Number of fruits	7	58	25	65	161
Pollination success (%)	9.4	26.1	11.3	14.1	15.7

Population size

The total known population of flowering stems of *Genoplesium insigne* monitored as part of this study has increased from 27 in 2017 to 432 in 2020 (Figure 2). This increase resulted from the ongoing discovery of new populations from only five populations in 2017 to 14 populations in 2020 (some populations surveyed in 2018 were not included in 2019 counts due to access restrictions on private land). For the four populations where flowering stems were counted from 2017 to 2020 the number of plants has remained relatively constant, although some annual fluctuation has occurred (Figure 2). The number of flowering stems detected within individual sub-populations varied greatly, ranging from a single individual to more than 100.

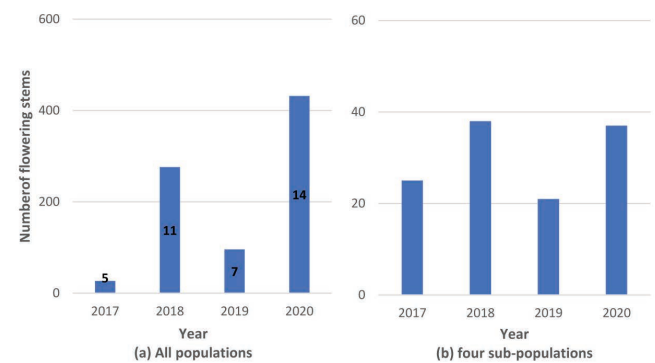


Figure 2: Total population of flowering stems of *Genoplesium insigne* from: (a) 2017 to 2020 across all surveyed sub-populations [labels indicate the number of sub-populations included within each count]; (b) the four original sub-populations.

Pollination

Inspections of open flowers of *Genoplesium insigne* identified droplets on the lobes of the column wings (Figure 3), similar to those observed for *Genoplesium fimbriatum* and *Genoplesium filiforme* (Ren et al. 2020a). Inspections of the labellum did not detect any droplets in the groove of the labellum callus such as those observed in *Genoplesium littorale* (Bower et al. 2015).

Observations of floral visitors on *Genoplesium insigne* in 2019 and 2020 were limited to three observations of flies moving between flowers on a single raceme. During one of these observations a single small Chloropidae fly, (identified by D. Bickel, 2020), was observed removing a pollinarium from an open flower of *Genoplesium insigne* and then visiting other open flowers on the same raceme (Figure 3). As no evidence of pollen deposition on stigmas was recorded we cannot ‘confirm’ this insect was a pollinator as per Adams and Lawson (1993). The remaining two observations were of up to two Chloropid flies on a single raceme, moving between and entering open flowers via individual labella (Figure 3). Based upon the yellow lunate marking on the distal frons, antenna colour and apparent yellow/dark banding on tibia II, the Chloropid observed and photographed removing pollinia from *Genoplesium insigne* (Figure 3) belonged to the same morphotype identified as the primary pollinator of *G. fimbriatum*, *G. filiforme* and *G. ruppilii* (D. Bickel pers. comm. 2020; Ren et al. 2020b).



Figure 3: *Genoplesium insigne* entire inflorescence (top-left), droplets observed near the column (top-right), Chloropid with pollinia attached (bottom-left) and two Chloropids on a single flower (bottom-right). Photos: B. Towle.

Fruit set

Across the various sub-populations in 2017 to 2020, the combined mean proportion of 115 inflorescences of *Genoplesium insigne* flowers developing into capsules was 15.7% (Table 3). Flowers setting fruit ranged from 9.4% in 2017 to 26.1% in 2020. The GLMs for pollination rate were assessed using analysis of deviance (Wakefield 2013) using a 5% level of significance. The analysis found no significant increase in explained variance by accounting for site ($p = 0.94$), or year ($p = 0.12$), when compared to the Null model. Analysis of deviance using a 5% level of significance found no significant increase in explained variance by accounting for site ($p = 0.94$), or year ($p = 0.12$), when compared to the Null Model. Estimates of pollination rates from each model are presented in (Figure 4).

Table 3: Reproductive success, patch size and maximum population size across four populations of *Genoplesium insigne*.

Sub population	Max number of flowering individuals in a single season	Mean reproductive success (%)	Patch size estimate*
BW	22	16.1	1.5 ha
CRBG	9	35.3	> 100 ha
CVB	19	14.5	>100 ha
MRST	158	16.5	> 100 ha

* Patch size was estimated from aerial photographic interpretation and includes the area of contiguous native-dominated vegetation.

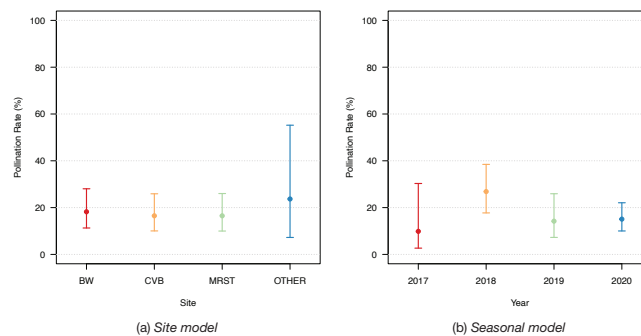


Figure 4: Estimated mean pollination rates (%) and associated 95% CIs

Discussion

At the time of its description in 2001, fewer than 20 *Genoplesium insigne* plants from three localities were known (Jones 2001; NSW Scientific Committee 2001). In 2013 only 13 inflorescences of the species were recorded across all the then-known sites (Commonwealth Threatened Species Scientific Committee 2014). In 2020 the total known population of flowering stems was 432 individuals resulting from a targeted monitoring program under the NSW Saving our Species program. As a result of these additional individuals and sub-populations the EOO was increased to 210 km² and the AOO was 576 km² as per IUCN (2011) or 400 km² as per Bland et al. (2016).

The initial low estimates of population size (Jones 2001) were most likely influenced by difficulties in detecting and then identifying such small plants to species. In 2020 the total number of flowering individuals within one well defined fenced population occupying approximately 100 m² was not observed until five counts were completed over a period of 100 days (Figure 5). A similar survey effort has been demonstrated as being necessary to achieve counts of the total population size of *Diuris praecox* (Yare et al. 2020). The multiple surveys required to detect the total sub-population size in surveys of known populations of *Genoplesium insigne* should be primarily attributed to asynchronous flowering. However, the very cryptic nature of the species' size and floral pigmentation can also result in reduced detection during surveys based only on a single count.

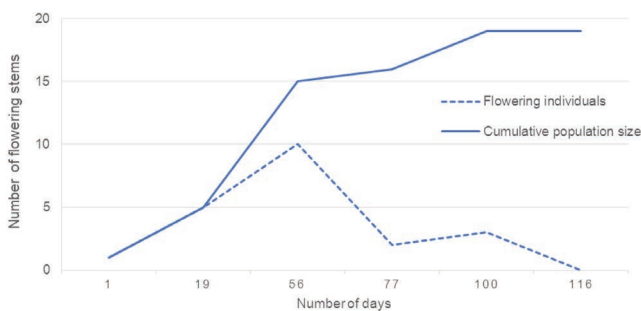


Figure 5: Cumulative total of *Genoplesium insigne* observed at a single population across six visits

Consistent with other *Genoplesium* species (Bower et al 2015; Ren et al. 2020), *Genoplesium insigne* is most likely to be dependent on small flies (family Chloropidae) for pollination. The observations of a Chloropid removing an entire pollinarium from an open flower, and the low pollination rate suggest a lack of apomixis and/or mechanical self-pollination (autogamy). Bagging experiments (as per Ren et al. 2020) would confirm this. *Genoplesium insigne* appears to offer a liquid reward to pollinators – as confirmed for other *Genoplesium* species (Ren et al. 2020); additional microscopy of excised flowers could confirm this.

Pollination rates observed for *Genoplesium insigne* across four flowering seasons are lower than reported for other *Genoplesium* species. Three common *Genoplesium* species (*Genoplesium ruppilii*, *Genoplesium fimbriatum* and *Genoplesium filiforme*) which overlap with the physical distribution of *Genoplesium insigne*, have all recorded higher rates of reproductive success. Ren et al. (In prep) reported conversion rates of flowers to fruits for these species with *Genoplesium ruppilii* scoring 35% (n = 696, SD = 29), *Genoplesium fimbriatum* scoring 38% (n = 1085, SD = 33) and *Genoplesium filiforme* achieving 43% (n = 718, SD = 31). Bower et al. (2015) recorded the conversion rates for the rare *Genoplesium littorale* to be an average of 42.6 % (n = 695). All four species are higher than *Genoplesium insigne* which achieved an average pollination rate of 15.7%.

Canackle et al. (2020) report that all flowering plants of the rare *Genoplesium superbum*, which flowered in 2020, had some capsule development and “very high” pollination

rates, although reproductive success was not quantified. Rates of successful fruit set in *Genoplesium insigne* in this study are low compared to regional and global comparisons with nectariferous and outbreeding orchid species (Neiland and Wilcock 1998; Brundrett 2019). The comparatively low pollination rates of *Genoplesium insigne* have been accompanied by our very infrequent observations of potential pollinators. No quantifications of pollinator observations are reported by Bower et al. (2015) or Ren et al. (2020), however these studies, which involved generally similar methods, included much more frequent pollinator detections than our observations for *Genoplesium insigne*.

A possible explanation for the low reproductive success of *Genoplesium insigne* is that the small and widely dispersed populations are less attractive to pollinators compared to larger populations of more common *Genoplesium* species (i.e. the Allee effect). Small and widely dispersed populations may also cause low reproductive success due to low genetic diversity within populations and inbreeding depression (Frankham 2015). However, the absence of any increase in reproductive success within the larger populations of *Genoplesium insigne* compared to smaller populations does not support this theory. Alternatively, the low reproductive success of *Genoplesium insigne* may be attributed to resource limitation within its habitat. However, the increased reproductive success of other summer/autumn flowering *Genoplesium* species within the same habitat does not support this explanation. It is hypothesized that the low reproductive success of *Genoplesium insigne* is due to pollinator limitations, which may be due to a decoupling of the flowering period and the activity period of its pollinators. Alternatively, there may be increased competition for pollinators in spring when numerous other flora species are flowering. The common observations of shared pollinators on summer and autumn flowering *Genoplesium* species (Figure 6) supports these theories.

The low reproductive success of *Genoplesium insigne* may be contributing to the rarity of the species. Severely limited seed production and recruitment can increase probabilities of local extinctions by producing population structures comprised of old adults with few recruits (Jacquemyn et al. 2005). Calvo (1990;1993) found that low reproductive success in orchid species is not always linked to low population growth, or rarity, if reproductive success is not correlated with recruitment. Where low fruit set is sufficient to saturate potential habitat for germination, low reproductive success may not be linked to population growth (Calvo 1990; 1993). While specific features of *Genoplesium insigne* habitat are not fully understood, the species is associated with relatively common habitats suggesting that the reported fruit production is unlikely to be saturating potential germination sites, at least in comparison to the more common *Genoplesium* species that occupy the same habitat. Therefore, the low reproductive success of *Genoplesium insigne* is likely to represent one of the factors contributing to the rarity of the species. It is noted that in a review of orchid extinctions in a European context, Jacquemyn et al. (2005) concluded that habitat loss and deterministic threats associated with habitat deterioration are probably more important than reproductive

success in determining population viability and extinction rates of orchid species.

While low reproductive success in *Genoplesium insigne* may only be one factor, and even a minor factor, contributing to the rarity of the species, it is likely to influence the rate at which the species is able to respond to conservation efforts including habitat protection (Jersakova et al. 2002). Encouragingly, Ackerman et al. (1996) demonstrated that when fruit and seed production of the deceptive orchid *Tolumnia variegata* were experimentally increased, seedling recruitment and population growth rates increased. Therefore, in addition to current conservation efforts which are focused on habitat protection and management, conservation efforts for this species should consider actions such as hand pollination, to increase reproductive success. Only through long-term monitoring of the species, including recruitment will the results of any experimental manipulation of reproductive success be detected.

Reproductive success of *Genoplesium insigne* did not vary significantly between populations, or between seasons, despite large differences between the size of each population and their degree of fragmentation. While the low reproductive success may be contributing to its rarity, the broadly similar levels of reproductive success in small and fragmented habitat patches with small populations, highlight that all populations continue to produce seed, and remain important to the conservation of the species.



Figure 6: *Genoplesium acuminata* (left) and *Genoplesium fimbriatum* (right) with multiple Chloropid flies (as indicated by red arrows) within habitat which supports monitored populations of *Genoplesium insigne*. Photos: B. Towle.

Conclusions

Targeted surveys and monitoring of reproductive success of *Genoplesium insigne* have provided insights into the population dynamics and ecology of this very rare species. The population now greatly exceeds previous very low estimates, but the total known population still remains low.

Fruit set rates in *Genoplesium insigne* are low, compared to rates for other *Genoplesium* species, and nectariferous orchids in general; further research should investigate causes of low fruit set and whether it contributes to ongoing rarity. Low population numbers and low fruit set rates combined with *Genoplesium insigne* inhabiting land under constant development pressure, means continued long-term monitoring and appropriate management of known populations are critical for the survival of this species.

Acknowledgements

This project has been funded by the New South Wales Government Saving our Species Program. Organisational support and land access has also been provided by Central Coast Council, Lake Macquarie City Council, Delta Electricity, NSW Crown Lands, Busways Pty Ltd and Darkinjung Local Aboriginal Land Council. We would also like to thank and acknowledge the assistance and work provided by Antony von Chrismar, Nigel Hunter, Garon Staines, Boris Branwhite and Katie Elsley during earlier stages of the monitoring project. We thank Ian Shannon who reviewed the statistical analysis. We also thank Dr. D. Bickel, of the Australian Museum (Sydney), for his expertise in identifying pollinators and Professor Peter Bernhardt for providing ongoing discussion and insightful comments on an early manuscript. We also thank an anonymous reviewer who provided insightful comments on the draft manuscript.

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