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Population size, plant size and reproductive output in key populations of the endangered terrestrial orchid *Genoplesium baueri* in eastern New South Wales

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Abstract: *Genoplesium baueri* R.Br. (family Orchidaceae), an endangered terrestrial orchid species endemic to the Sydney Basin, New South Wales, Australia, is known from 13 populations but little is known about its population demographics. To assess the emergent population size, plant size and reproductive output of two key northern Sydney populations, at Ku-ring-gai Chase National Park (KCNP) and Ku-ring-gai Wildflower Garden (KWG), and how these fluctuate through time, intensive field surveys of these populations were carried out over a ten-year period (2009-18).

Plants emerged at both sites in every year with a total of 1,249 plant counts made over the 10-year period. From this, we estimate that a total of approximately 500 perennial individuals may exist across both populations, more than previously thought. Although emergent population size did not differ between populations, we found significant differences between the populations in the other measures. The KWG population had taller plants that produced more flowers per plant than the KCNP population, while the plants in the KCNP population had more pollinated flowers and fruit per plant. All of these measures significantly fluctuated through time. This 10-year study has shown that the resilience of key *Genoplesium baueri* populations is greater than previously thought. However, it has also shown the boom-and-bust nature of emergence, flowering and fruit set in this species, which has highlighted the need for multi-year intensive studies when assessing likely persistence of endangered terrestrial orchid species.

Keywords: demographics; effective fruit-set; multi-year survey; plant height

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Introduction

Orchidaceae is a widely distributed non-woody plant family with Antarctica being the only continent currently bereft of orchids (Jones 1988; Givnish *et al*. 2016). A global fascination with orchid flowers and the use of orchid tubers as a food source and ingredient in herbal preparations has led to their extensive collection from the wild (Low 1991; Bernhardt *et al.* 2017). However, in Australia, changing land use represents the most significant threat to orchids and has contributed to the decline of many native species (Swarts & Dixon 2009). In fact, Jones (2006) considers it is likely that hundreds of native orchid species have become extinct in Australia in the past 200 years, with many more becoming threatened with extinction.

One such threatened orchid is *Genoplesium baueri* R.Br. (Jones 1988), Brittle Midge Orchid or Bauer's Midge Orchid, a rare terrestrial orchid species, listed as Endangered under both the New South Wales (NSW) Biodiversity Conservation Act (2016) and the Commonwealth Environment Protection and Biodiversity Conservation Act (1999). Robert Brown and Ferdinand Bauer first collected the species in 1805 (Watts *et al.* 1997), and Brown later described it from a drawing by Bauer, of the material they collected (Brown 1810). *Genoplesium baueri* is endemic to the Sydney Basin in NSW Australia, with most populations being recorded between northern Sydney and Ulladulla (NSW Scientific Committee 2012). However, little is known about the dynamics of these *Genoplesium baueri* populations, making conservation management challenging, and resolving knowledge gaps is important as urbanisation has drastically reduced *Genoplesium baueri* habitat putting it under continuing pressure.

Assessing *Genoplesium baueri* population dynamics is challenging as individual plants do not emerge on a regular basis but rather, emerge from underground tubers at an unknown frequency (W. Grimm, pers. obs.). Furthermore, *Genoplesium baueri* plants do not exhibit a vegetative (leaf-only) period of growth (Grimm *et al*. 2020), unlike several more intensively investigated Australian terrestrial orchid genera such as *Prasophyllum*, *Caladenia* and *Drakaea* (Coates *et al.* 2006, Coates & Duncan 2009; Brundrett 2016). This means that consistent monitoring of *Genoplesium baueri* populations over multiple years is required to assess their size and number. In addition, *Genoplesium baueri* plants emerge singularly or in tight clumps of up to 14 plants (Grimm *et al*. 2020) making tracking individual plants in longitudinal studies problematic.

In 2010 the total number of *Genoplesium baueri* plants across the 13 known populations was estimated to be fewer than 250 plants (Stephenson 2010*;* 2011), an estimate likely to be conservative given the difficulties associated with monitoring the species. Of the 13 populations, two northern Sydney populations, in Ku-ring-gai Chase National Park (KCNP) and Ku-ring-gai Wildflower Garden (KWG) and two South Coast populations, with the largest estimated population sizes have been identified as key populations by the 'Saving Our Species' conservation program (http://www.environment.nsw.gov.au/savingourspecies). These have the greatest chance of long-term persistence in the wild over the next century with appropriate site management. The objectives of this study were to assess the emergent population size, plant size and reproductive output of the two northern Sydney populations and how they fluctuate through time through an intensive ten-year field study. The desired outcome of this research was to expand the data available for *Genoplesium baueri* and provide information that will help secure its future survival in the wild. The two key *Genoplesium baueri* South Coast populations are being monitored in an ongoing longitudinal study managed by staff of the NSW Department of Planning, Industry and Environment, based in Wollongong.

Methods

The two *Genoplesium baueri* populations located at Ku-ring-gai Chase National Park (KCNP) and Ku-ring-gai Wildflower Garden (KWG) were surveyed during all flowering seasons from 2009-2018. For the KCNP population, plants were distributed along both verges of an unpaved fire trail and occupied an area of 100 x 10 m. For the KWG population, plants were distributed along both verges of a paved fire trail and occupied an area of 80 x 12 m, and an adjacent area 30 x 30 m bounded by the same paved fire trail on one side, by a rubble fire trail on an adjacent side and by a perennial and an ephemeral creek on the two remaining sides. KCNP and KWG were classified as separate populations because they are separated by 5 km, which means it is unlikely that genetic exchange would occur between them. Both population sites were located on the Hornsby Plateau (Carolin & Tindale 1994), in areas characterised by low woodland and heathy vegetation that grow on soils of very low fertility derived from Hawkesbury Sandstone (Thomas & Benson 1985, Chapman & Murphy 1989, Martyn 2018).

From late December each year, the population sites were systematically searched on a weekly basis for *Genoplesium baueri* plants. When a plant was found it was allocated a unique sequential code, photographed, plotted onto a site map and GPS location recorded. A fine bamboo skewer marker, 20-30 cm long and bearing codes for year and plant number, was inserted a short depth into the substrate at a distance of 10 cm in a predetermined direction from the base of each inflorescence. The plant marker was positioned 10 cm from the base to avoid damaging the tuber. The shoot of the plant occasionally travelled sideways within the surface detritus before emerging above ground and so was likely to be displaced from a position directly above the tuber. Consistent relative spacing of markers was not possible where plants occurred in very close proximity to each other (Grimm *et al*. 2020) and the use of robust permanent markers was considered to be a threat to the safety of the endangered plant population. Plants were also photographed at each subsequent survey as flower and fruit development progressed. These data were used to track the plants through time in that growing season. In addition to the location data, the following were recorded for each plant at each survey: plant height (to top of inflorescence), flower count, pollinated flower count and dehisced fruit count. Plant heights were measured to nearest 0.5 cm and were not recorded in the 2011-12 flowering season at both sites. To determine the pollinated flower count, a flower was considered pollinated when swollen ovaries were observed (Bower *et al*. 2015). Monitoring was generally terminated by June, at the conclusion of fruit dehiscence.

Data analysis

Differences in emergent population size between sites and across years were tested using one-way ANOVAs. When significant differences existed, Tukey post-hoc analyses were used to determine where they existed. Differences in plant height, flower production per plant, flowers pollinated per plant and fruit production per plant between sites and across years were tested using Kruskal-Wallis non-parametric analyses because the data for these traits were not normally distributed even after transformation. All statistical analyses were performed using IBM SPSS statistical software, Version 21.0.0 (www.spss.com) with the significance level set at 0.05. Emergent population size data were $\log_{10}$ transformed to satisfy the normality requirement of ANOVA.

Results

Emergent population size

Plants emerged at both sites in every year with a total of 1,249 plant counts made over the 10 year period (Fig. 1). There were no significant differences in plant number between the two populations ($F_{1,18}$=2.66, p=0.120, Fig. 1) and across years ($F_{9,10}$=2.44, p=0.094, Fig. 1).

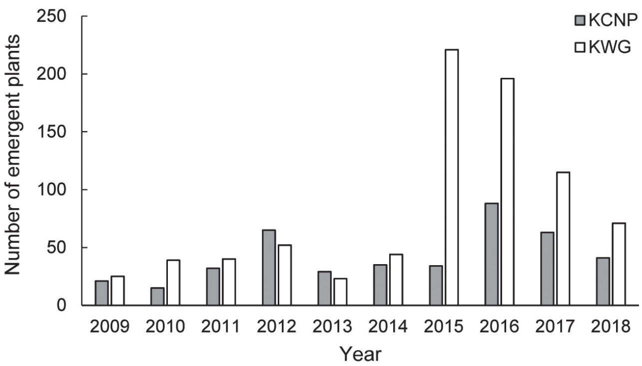

Fig. 1. The number of emergent plants recorded for the *Genoplesium baueri* populations at Ku-ring-gai Chase National Park (KCNP) and Ku-ring-gai Wildflower Garden (KWG) surveyed between 2009-18.

Plant height

Plant height was measured for 1,041 plants. Overall, the mean plant height was 13.3 cm (±0.173 SE). There was a significant difference in plant height between populations (H_2=308.68, p<0.001) with the KWG population having significantly taller plants than the KCNP population. There were also significant fluctuations in plant height across years (H_8=78.20, p<0.001).

Flower count and per plant

Plants flowered in both populations every year with 5,238 flowers being counted on 1,249 plants (Fig. 2a). Overall, the mean number of flowers produced per plant was 4.19 (±0.05 SE). There was a significant difference in the number of flowers produced per plant between populations with the KWG population producing more flowers per plant than the KCNP population (H_1=60.42, p<0.001, Fig. 2b). There were also significant fluctuations in flowers produced per plant across years (H_9=25.03, p=0.003, Fig. 2b).

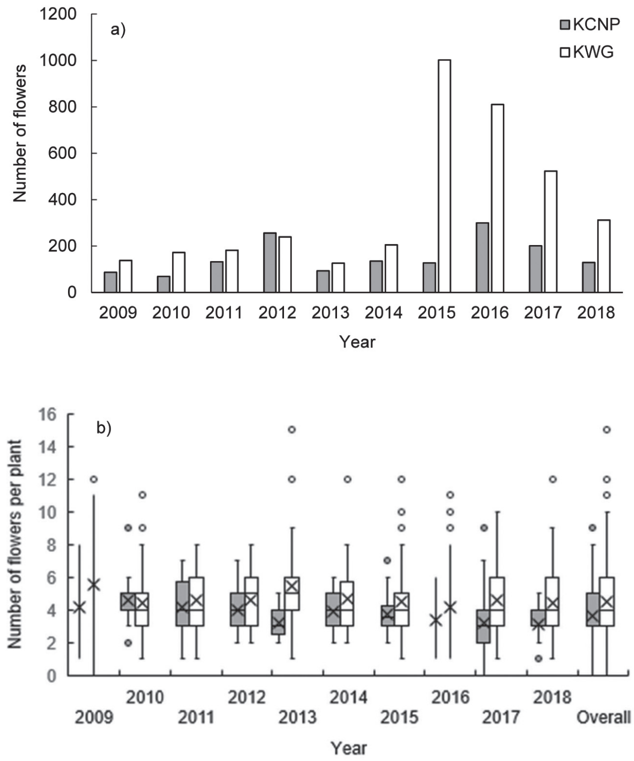

Fig. 2. *Genoplesium baueri* a) Total number of flowers and b) number of flowers produced per plant for each population (KCNP, KWG) across years (2009-18). Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box the horizontal bar represents the median value and the X represents the mean value. Vertical bars extend to 1.5 IQR, while outliers beyond 1.5 IQR are indicated by dots.

Pollinated flower count and per plant

Pollination occurred in both populations every year with 1,081 out of 5,238 flowers being pollinated. Overall, the mean number of flowers pollinated per plant was 0.865 (±0.043 SE). There was a significant difference in the number of flowers pollinated per plant between populations with the KCNP population having more flowers pollinated per plant than the KWG population (H_1=7.68, p=0.006). There were also significant fluctuations in flowers pollinated per plant across years (H_9=144.29, p<0.001).

Fruit count and per plant and effective fruit set

Fruit production occurred in all years but did not occur at both sites in every year with 427 fruit produced from the 1,249 plants (Fig. 3a). Overall, the mean number of fruit produced per plant was 0.342 (±0.029 SE). There was a significant difference in the fruit produced per plant between populations (H_1=6.15, p=0.013, Fig. 3b) with the KCNP population producing more fruit per plant than the KWG population. There were also significant fluctuations in fruit produced per plant across years (H_9=254.33, p<0.001, Fig. 3b).

The total effective fruit set (the percentage of flowers that produced mature fruit) was 8.2% (427 fruit formed from 5,238 flowers; Fig. 3c) with a higher effective fruit set for the KCNP population (11.2%) than the KWG population (6.9%).

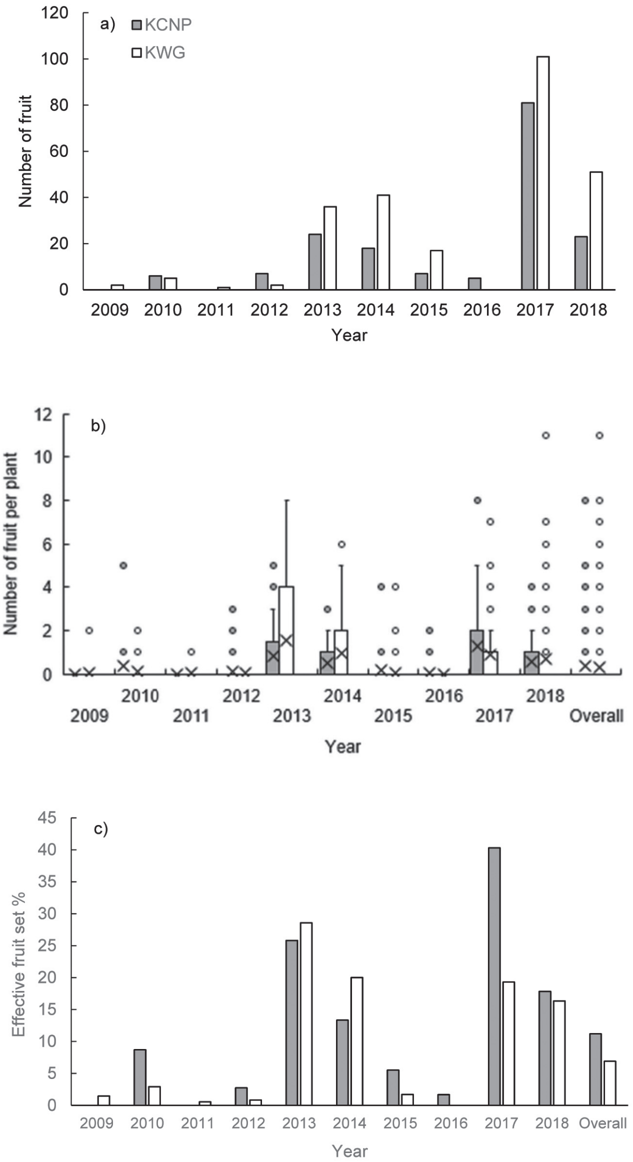

Fig. 3. *Genoplesium baueri* a) Total number of fruit, b) number of fruit per plant and c) effective fruit set (% fruit per flower) for each population (KCNP, KWG) across years (2009-18). Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box the horizontal bar represents the median value and the X represents the mean value. Vertical bars extend to 1.5 IQR, while outliers beyond 1.5 IQR are indicated by dots.

Discussion

Population size

In 2012 the NSW Scientific Committee accepted the number of mature *Genoplesium baueri* plants across its entire distribution to be low (<2,500) and to have an area of occupancy of 168 km^2. These criteria, together with the severely fragmented nature of the populations and other documented threats, qualified *Genoplesium baueri* for listing as Endangered under the NSW Threatened Species Conservation Act (1995). However, there was a high level of uncertainty in the population estimates and the first objective of this study was to determine the size of the key northern Sydney *Genoplesium baueri* populations. In the first two-years of our study, the number of emergent plants we reported for the combined northern Sydney populations (n=46, 2009, n=54, 2010), made up approximately a quarter of the <250 individuals estimated across the species entire distribution (Stephenson 2010). From the observations made during the remainder of our 10-year study, we suggest that these estimates were conservative, with the maximum number of emergent plants at each site found to be greater (n=88, KCNP; n=221, KWG). It should be acknowledged that the proportion of tubers that remain dormant each year is unknown, so the true size (i.e. number of emergent and non-emergent plants) of the two northern Sydney *Genoplesium baueri* populations is still unknown. However, we estimate there to be about 500 individuals spread across the two populations based on an annual record of the positions of emergent plants in linear sections and the number of emergent plants. These size estimates show that the KCNP and KWG populations are likely to fit the Brundrett (2016, p. 52) definition of 'core habitat' as being 'the most essential area(s) for survival of the taxon' by virtue of having 'the highest densities of and/or the majority of currently known individuals' in the northern part of its range. Therefore, it is vitally important that these populations be protected. Currently, KCNP population is protected within Ku-ring-gai Chase National Park boundaries; however, the KWG population at Ku-ring-gai Wildflower Garden is on Crown Land and is thus less secure from land re-zoning and development.

An important consideration in assessing the viability of populations is determining the variability in their size through time. For both populations, we found that their size did not fluctuate significantly through time. However, they did exhibit an increasing trend for the seven years from 2009, which peaked at 284 emergent plants in 2016, and then dropped by 30-40% each of the following couple of years to 112 plants in 2018. This last count was close to the ten-year mean of 125 plants. The fluctuations in emergent population size through the duration of the study were consistent across both populations and was most likely due to natural variation rather than loss of habitat or decline in habitat suitability. This is contrary to the reports of other temperate Australian terrestrial orchid species, where declines in population sizes have been related to reduced habitat suitability with longer fire intervals (>3 years), allowing increased competition from shrubs and herbs (Coates *et al.* 2006; Coates & Duncan 2007, 2009; see Brundrett 2016 for exceptions).

Plant size

Plants in the KCNP population were significantly shorter than the plants in the KWG population. These population-level differences may be a result of dissimilar abiotic or soil conditions present. For example, although the same rainfall record was common to both population sites, KCNP is noticeably drier and the area where the orchid occurs supports a sparser, more open forest vegetation than is present at KWG.

Flower production and pollination and effective fruit set

Genoplesium baueri was not previously considered to flower annually (Jones 1988; Fairley 2004) although it has been reported to flower at one site in three consecutive years (Benson & McDougall 2005). We observed that the two northern Sydney populations flowered annually over the 10-year study period but the mean number of flowers produced per plant did show a weak downward trend through time at both sites. Of the flowers produced, only 20% were pollinated; the rest were either not pollinated, lost to herbivory or sustained other damage. This pollination rate varied significantly through time and was particularly low at both sites in 2016. The proportion of pollinated to non-pollinated flowers is much lower than we would expect from an obligately self-pollinating species and consistent with our observations of pollinarium removal by chloropid flies (Grimm *et al*. 2020). Although the plants in the KCNP population produced fewer flowers per plant than those in the KWG population, they had significantly more flowers pollinated per plant.

Effective fruit set is likely to be the most important determinant of the reproductive success of a species, enabling population persistence if seeds are able to transition successfully to seedlings and mature plants, or, in terms of the distinct life cycle stages of *Genoplesium baueri*, to protocorms, tubers and emergent plants. Of the pollinated flowers recorded, 40% produced fruit that reached seed release resulting in an effective fruit set of 8.2% across both populations, lower than the median fruit set reported for non-autogamous temperate orchid species (34.6±2.3%; n=123, Tremblay *et al.* 2005). For the Critically Endangered, fly-pollinated rewarding orchid, *Genoplesium (Corunastylis) littorale,* Bower *et al*. (2015) found that 45.8% of plants remained viable 6 weeks after flowering, with 42.6% of flowers produced by those plants setting fruit. This equates to an effective fruit set of roughly 20% in a single season. However, the effective fruit set of our two *Genoplesium baueri* populations in the same season (2013) as Bower conducted his study, were 25.8% (KCNP) and 28.6% (KWG), and much higher than the 8.2% found across both populations over the 10-year duration of the study (2009-18). This highlights the significant variation in population size and plant size through time and the need for future orchid surveys to evaluate populations across multiple years, once the initial snapshot survey has been reported. This in turn will yield better quality data that can be utilised as a reliable tool in guiding conservation management of threatened orchid species.

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