

The effect of population structure, plant size, herbivory and reproductive potential on effective population size in the temperate epiphytic orchid, *Sarcochilus australis*

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Abstract: Distribution of plant size and reproductive success is investigated in the temperate epiphytic orchid *Sarcochilus australis* (Lindl.) Rchb. f. at Kinglake National Park, Victoria, in south-eastern Australia, and applied to estimating the effective population size. Plant size distribution (leaf number, length of longest leaf and number of flowers) was not normally distributed. Most individuals were vegetative and it is estimated that more than half of all individuals are too small to flower, however exceptionally large individuals even though rare are able to have more than one active inflorescence. Flowering probability is plant size dependent and follows a sigmoid curve. The minimum observed leaf size of a flowering individual was 26 mm, however these small individuals have a low probability of flowering (<30%), while large plants (> 80 mm) have a much higher probability of flowering (90%). The effective population size (N_e) of the Kinglake population of *Sarcochilus australis* was estimated from the distribution of flower production, and shown to be small ($N_e = 10\text{--}19\%$) and comparatively similar to some of the other published estimates of effective populations size in orchids. From this basic survey of size distribution in *Sarcochilus australis* it is predicted that genetic diversity is low.

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Introduction

Plant population structure such as the distribution of plant size, and distribution of reproductive effort and success has important evolutionary and conservation implications. For example, plant size distribution has frequently been shown to effect survivorship and reproduction (Harper 1977, Wesselingh et al. 1997) and can be related to inter- and intra- specific competition (Harper 1977, Jack, 1971). In many plant species a minimum size is required to produce flowers while size and growth directly influence the size of the floral display (Pino et al., 2002; Metcalf et al., 2003). The size of floral display is frequently positively correlated with reproductive success either through increase male success (pollen removal) or female success (fruit production; Wesselingh et al., 1997; Pino et al., 2002; Metcalf et al., 2003). Since plant size affects reproductive output by influencing flower production (Harper 1977) and variation in plant size influences variation in reproductive success, reproductive effort ultimately influences effective population size through fruit set and progeny recruitment (Tremblay & Ackerman 2001).

The assumption of size influencing reproductive output is well documented, for example in the tree *Lindera erythrocarpa* seed production was highest in the largest individuals (> 28 cm dbh), which produced twice as many seeds as smaller trees (= 20 cm dbh; 7.7×10^4 and 4.2×10^4 seeds, respectively, Hirayama et al., 2004). This pattern is found

in many species of angiosperms (Herrera 1991) including gymnosperms (Enright & Ogden 1979). Knowledge of plant size distribution and its relationship with reproductive potential can allow estimates of effective population size (Kimura & Crow 1963, Heywood 1986, Tremblay & Ackerman 2001) which is directly related to the likelihood of evolution by natural selection and genetic drift.

The relationship between genetic drift (random changes in genetic or morphological variation among generations) and selection, and their relative role in evolution depends on the effective population size (N_e), the number of effective breeding adults in a generation (Wright 1931, 1939, 1968). The smaller the N_e , the higher the probability that loss or fixation of alleles in the population will be caused by genetic drift and the higher the selection coefficients need be for natural selection to be important. When effective population sizes are below 50, drift is expected to be strong (Wright 1968; Franklin 1980).

Consequently, the relative contribution of progeny to the next generation has evolutionary and conservation implications. Do most individuals leave an equal amount of progeny or are some individuals more effective at leaving progeny? It is common that later stage individuals usually have a higher survival probability and reproductive success (Øien & Moen 2002, Pino et al., 2002) and consequently the distribution of size will influence the effective population size.

In this study five questions are evaluated for the temperate orchid *Sarcochilus australis* (Lindl) Rchb.f. 1) What is the plant size distribution as measured by the number of leaves, the length of the longest leaf and the number of flowers and are these variables normally distributed? 2) Which of the two size variable (leaf length or leaf number) a better predictor of flower production? 3) What is the probability of plants flowering based on leaf length? 4) How common and what impact is the effect of herbivores on floral production? 5) Ultimately, considering the variation in flower display what is the most likely estimate of effective population size?

Methods

Model species and sampling strategy

Sarcochilus australis is a twig epiphyte on a variety of host trees in wetter, cooler parts of eastern Australia, from Tasmania to Queensland (Backhouse & Jeanes 1995; Bishop 1996). I sampled all observed plants along a more-or-less random walk on the slopes of a small creek dominated by ferns in the understorey at one site at Kinglake National Park, Victoria. The survey included only plants that were reachable, though few observed plants were observed beyond reach.

For each plant the number of leaves was counted, the length of the longest leaf was measured (mm), and number of inflorescences and flowers per inflorescence were counted. The presence of herbivory on flowers was noted when the tip of the inflorescence was damaged. The total number of flowers produced by a plant was calculated as the sum of flowers from all inflorescences.

Statistical analysis and plant size distribution

Initial data exploration involved graphing the frequency distribution of plant size (number of leaves and length of longest leaf) and number of flowers per plant to determine the pattern and evaluate if the variables studied follow a normal distribution. Test of normal distribution was performed with the “Shapiro-Wilk W test for normality” (Shapiro & Wilk 1965). The relationship between leaf length and leaf number with flower production was evaluated with multivariate linear regression analysis. The variable that explained most of the variation was then used exclusively to evaluate the likelihood of plants flowering. The likelihood of flowering was evaluated using logistic regression, the length of the longest leaf as the predictive variable and the presence (or absence) of an inflorescence as the response variable. All statistics (excluding effective population size (N_e) calculations) were performed with the statistical package JMP 5.1.2 (SAS Institute Inc., Cary, N.C., USA).

In all cases statistical analyses were performed on the square root of the number of leaves and number of flowers and the log of the length of the longest leaf to reduce heteroscedasticity.

Tables and figures are shown with non-transformed data for ease of interpretation.

Estimating effective population size from variation in flower production.

The model “variance as a function of size distribution” (Kimura & Crow 1963) describes the effective population size as a function of the progeny distribution among individuals. The model assumes that size of an individual is directly related to the number of progeny produced and this is largely supported with empirical and theoretical analyses (Metcalf et al., 2003). Flower production is usually highly correlated with fruit set and recruitment (Herrera 2004). Heywood (1986) reformulated the function considering the variance in terms of reproductive success or potential. Here I use the number and variance in flower production as a measure of reproductive potential. Because this model is most appropriate for species with non-overlapping generations or species with small variance in reproductive lifespan, it serves as a comparison for models developed for overlapping generations and also provides a general estimate when populations have a short lifespan with little or no overlapping generations, consequently a upper range of the effective population size if variance in reproductive success is high (Tremblay & Ackerman 2001).

The effective population size is estimated as ,

$$N_e = \frac{1}{(1 + F)\left(\frac{s_b^2}{\bar{x}_b^2}\right) + 1} * N$$

where b is the variable of interest (number of flowers), s^2 and \bar{x} are the variance and the mean of the variable, F is the genetic estimate of inbreeding (F_{is} ; ranging from 0 to 1), and N is the number of individuals sampled (Husband & Barrett 1992).

Results

Leaf number

The number of leaves per individual varied from one to eight and the population size distribution was skewed towards plants with fewer leaves, 2 to 5 leaves being the most common plant size (Fig. 1). The mean number of leaves per plant was 3.5 ± 0.12 (s.e.; $n=154$) and the median was 3. Leaf number was not normally distributed rather the distribution was skewed with more plants having fewer leaves (Shapiro-Wilk W Test for normality, $W = 0.9249$ $p < 0.0001$).

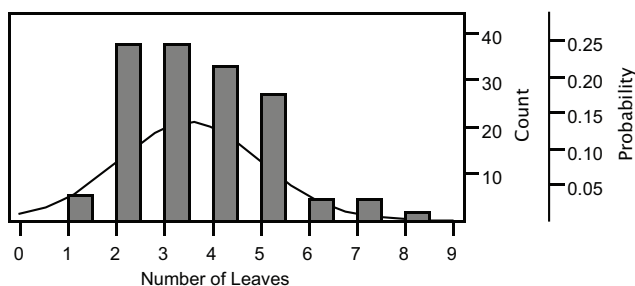


Fig. 1. Frequency and probability distribution of number of leaves per individual in 154 individuals of the temperate epiphyte *Sarcophilus australis* at Kinglake, Victoria.

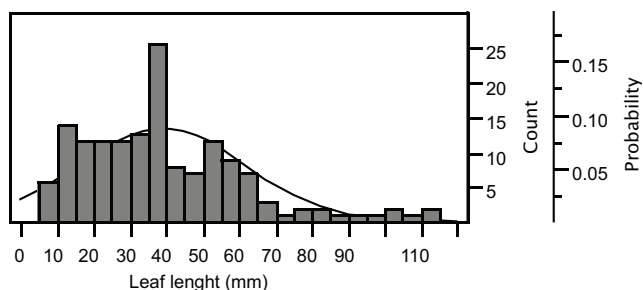


Fig. 2. Frequency and probability distribution of the length of the longest leaves of 154 individuals in the temperate epiphyte *Sarcophilus australis* at Kinglake, Victoria.

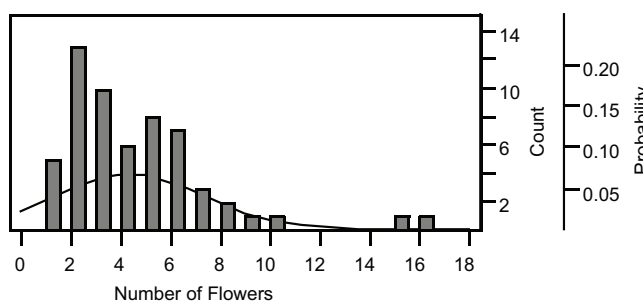


Fig. 3. Frequency and probability distribution of number of flowers per individual in 58 flowering individuals of the temperate epiphyte *Sarcophilus australis* at Kinglake, Victoria.

Leaf length.

The length of the longest leaf varied from 6 to 112 mm, with a mean of 38.9 mm (se = 1.8; median 35, n = 153), with 50% of the individuals lying between 22 and 53 mm (Fig. 2). The distribution of length of the longest leaf was significantly skewed (Shapiro-Wilk W Test for normality, $W = 0.9215$ $p < 0.0001$) with an excess of individuals with very long leaves.

Flower production

A total of 58 of the 154 plants had active inflorescences. The distribution of number of flowers per plant ranged from two to 16, the mean number of flowers was 4.4 for a total of 254 flowers (se = 0.40, n = 58, Fig 3). The distribution of flower production was significantly skewed (Shapiro-Wilk W Test for normality, $W = 0.8268$ $p < 0.0001$) towards fewer flowers, and few individuals had many flowers.

Plant size and flower production.

A multiple stepwise regression analysis of the total model including the length and number of leaves shows that flower production was positively correlated with the measures of plant size (multiple regression $F_{2,57} = 24.68$, $p < 0.0001$, $R^2_{adj} = 0.45$). However, leaf length explained more of the variation (log of leaf length, $F_{1,57} = 44.20$, $p < 0.0001$, $R^2_{adj} = 0.43$; Fig.4) compared to the number of leaves (sqrt of leaf number, $F_{1,57} = 20.7$, $p < 0.0001$, $R^2_{adj} = 0.26$). Leaf length and leaf number were correlated ($\text{Sqrt}(N. \text{leaves}) = 0.4075274 + 0.9462978 \text{Log}(\text{leaf})$, $F_{1,153} = 117.06$, $p < 0.0001$, $R^2_{adj} = 0.43$).

Minimal plant size for flowering.

The minimum leaf length of a flowering individual was 26 mm. Fifty individuals were smaller than 26 mm. Logistic regression of inflorescence presence and length of longest leaf suggested that small individuals had a low probability of producing inflorescences (logistic regression (log leaf length), n = 154, Chi Square = 68.97, $p < 0.0001$, $R^2_{adj} (U) = 0.34$). The probability of flowering was plant size dependent and followed a sigmoid curve. Plants with a 20 mm leaf length had approximately 10% probability of flowering, at 46 mm leaf length plants had a 50% probability of flowering, while a 90% probability of flowering was attained only when the longest leaf is larger then 73 mm (Fig. 5).

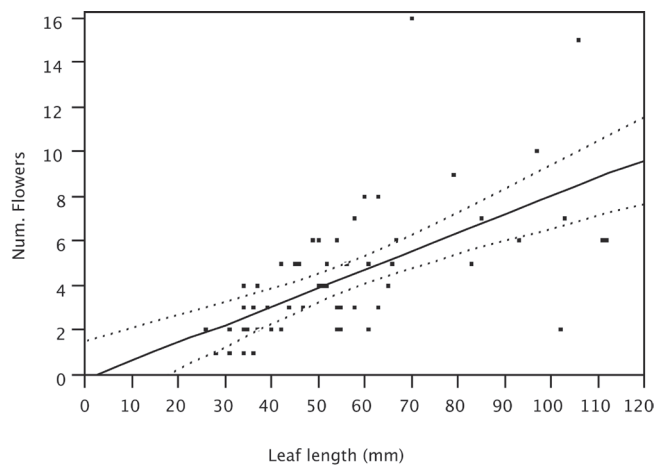


Fig. 4. Linear relationship (\pm standard error) between length of the longest leaf and the number of flowers born by *Sarcophilus australis* at Kinglake, Victoria. $\text{Sqrt}(\text{flowers}) = -2.571755 + 2.6573918 \text{Log}(\text{leaf})$.

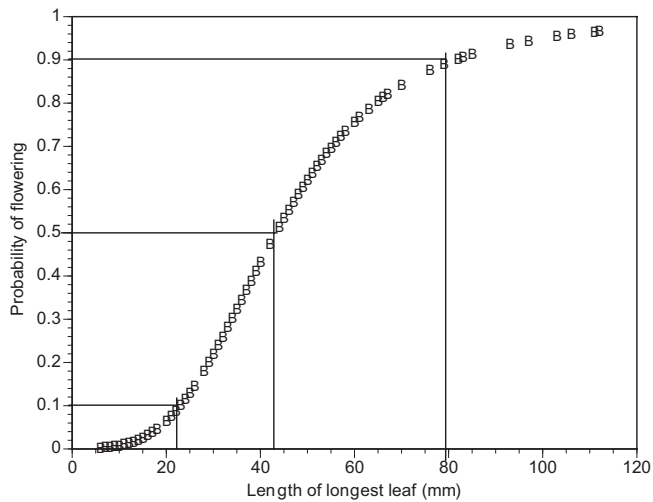


Fig. 5. Logistic regression of probability of flowering as influenced by the length of the longest leaf of *Sarcochilus australis*. The length of the longest leaf for a 10%, 50% and 90% flowering probability is shown.

Herbivory.

Evidence of herbivory on floral production was noted in 19 of 58 inflorescences. In none of the observed cases were all of the flowers on the inflorescence damaged; at least two flowers were present. The real effect of herbivory on these plants can be evaluated by comparing damaged to non-damaged flowering plants. Plants with evidence of herbivory had marginally fewer flowers (mean and s.e., 3.68 ± 0.44) than plants with no evidence of herbivory (mean and s.e., 4.72 ± 0.54). However, the effect of herbivory on plants was not significant (Sqrt of number of flowers, t-test, assuming unequal variance $t_{48.2} = 1.22$, $p = 0.23$).

Estimates of effective population size.

The variance in the number of flower per individual was 9.117 and the mean was 4.393. Consequently the range of N_e is from 10.3% of the standing reproductive individuals for a population that is completely inbred ($F_{is} = 1$) and up to 18.8% for a population that has little inbreeding ($F_{is} = 0$). Thus for *Sarcochilus australis*, the 58 flowering individuals are likely behaving as if 6 to 11 individuals.

Discussion

Size distribution

Leaf number, leaf length and flower production all exhibited skewed distributions with a high number of individuals in the smaller categories. Skewed distributions of vegetative characters are common in many plants (Harper, 1977). Plants size can be affected by plant density (Harper, 1977). *Sarcochilus australis* plants surveyed at the Kinglake site

were most frequently alone or in groups of two or three, only on a couple of trees were a large number of plants present (>9), and on these hosts most of the plants were small. Were these plants recent recruits or are they competing for resources that ultimately affected their size? In general we know little about the effect of competition in orchids and population structure (Kull, 2002, Rivera-Gómez, et al., 2006). If density effects are present in orchids then this suggests that space and resources are limited (Kery et al., 2000). However, there is little evidence to suggest that space is the limiting factor to population dynamics of epiphytic orchids (Calvo & Horvitz 1990, Ackerman, et al., 1996), whereas some evidence of resources limitation on reproduction and growth patterns is known in field studies (Fernández et al., 2003). Most of the known effects of resource limitation have been observed as a consequence of the cost of reproduction among reproductive bouts (for an extensive review see Tremblay et al, 2005).

A predictor of flowering size

Leaf length is a better predictor of flower production than number of leaves and explains 43% of the variation. While adding leaf length and leaf number increases the resolution it only improves the resolution slightly ($R^2_{adj} = 0.45$), consequently, measuring the length of the longest leaf is sufficient for a quick survey of the reproductive potential in a population.

One third of individuals were smaller than the smallest individuals (leaf length of 26 mm) with an inflorescence. Considering plants equal and larger than 26 mm, 50 of the 108 (48.3%) did not flower. The likelihood of small individuals flowering with leaves below 30 mm is only about 20%, individuals with leaves over 40 mm have a 50% probability of flowering. All plants with leaves over 83 mm produced flowers ($n=9$). Production of multiple inflorescences was only noted three times in the survey, two of the largest individuals present in the survey produced two inflorescences (106 mm, 7 leaves; 112mm, 3 leaves) and one with three inflorescences (70 mm, 5 leaves). The conditions for a plant to produce multiple inflorescences is likely dependent on multiple variables including size and number of leaves, resource availability as well as choice location for maximising photosynthesis.

Plant size as a measure of reproductive potential and output is well known (Pino et al., 2002; Metcalf et al., 2003). In some species the relationship between plant size and fecundity measures can reach r^2 as high as 92% (for a review see Metcalf et al., 2003).

Resource limitations and growth.

In general the interaction of epiphytes and their physical and biological environment is still unknown. However, it has been documented that moss can dramatically affect water (Mägdefrau & Wutz 1951 in Proctor 1982, Pócs 1980) and nutrient availability (Coxson et al. 1992) but its interaction

with epiphytic flora is unknown. The presence of moss cover on trees in the Kinglake site was common and should be evaluated in relation to orchid presence and growth rates. Moss diversity does not appear to relate to epiphytic distribution (Tremblay et al. 1998). Otero et al. (2002) found that different fungi dramatically affect the germination and growth rates of seedlings of some orchid species. Different species of trees have diverse microfloras (Tremblay et al. 1998) and it is probable that this could influence population dynamics including recruitment (Bayman et al. 2002; Willems et al. 2001), and consequently the presence, growth rate and density of orchids.

Herbivory

Herbivory could affect size distribution if the amount consumed is preferentially more on one of the stages than the others. In general, when herbivory has been noted in orchids the effect on survivorship appears to be minimal (Whigham & O'Neill, 1988; Primack & Hall 1990, Calvo 1990). Mammalian herbivory of young leaves of many terrestrial orchids is known in the Australian orchid flora, but little is known of its short and long-term effects (Coates, et al., 2006). Very little herbivory (insect damage) appears to be present in orchids in general. When herbivory was noted in *Sarcochilus australis* it was rare and limited to the tip of the inflorescence. In general the number of flowers that were damaged does not appear to be significant. Herbivory appears to be occurring prior to anthesis with all the bud being consumed. None of the open flowers had partial damage.

Effective population size.

How many individuals there are in a population and how they distribute the reproductive success among them has conservation and evolutionary consequences. It is generally held that, in theory, effective population sizes smaller than 50 are highly likely to go extinct and that genetic drift (loss of genetic and morphological diversity), is highly probable (Wright 1968, 1977). If by luck all *Sarcochilus australis* plants were sampled at Kinglake, this population has a N_e of 6–11 and is likely to experience genetic drift, which could lead to inbreeding depression and/or cladogenesis. However, the total population is unlikely to have been measured, as it is likely that many plants were missed in areas that were not surveyed. Unnoticed plants are likely higher up in the canopy and branch falls with orchids were found on the ground or hanging on to other living trees in the understorey, supporting the hypothesis that plants grow higher up in the canopy, as well as in the area surveyed. What is the true population size of *Sarcochilus australis* at Kinglake? For effective populations to be considered large and viable on a long-term basis, N_e larger than 100 is often considered sufficient. In this instance, considering the estimated effective population, over 1000 reproductive plants are needed.

The limitations to the N_e estimates have to be recognized, the formula used to calculate the effective population size in *Sarcochilus australis* is most appropriate for annual plants. Because *Sarcochilus* is a perennial and the estimate is likely a higher bound for N_e , as the estimated variance in reproductive success among individuals will likely increase among years resulting in smaller N_e . A larger N_e would be possible if and only if individuals that reproduced reduced their reproductive success in future time periods, and those that were non-reproductive become reproductive in following reproductive episodes, thus reducing the variance in reproductive potential among individuals (flower production). Note that the larger the variance among individuals in reproductive success, the smaller the effective population size. Moreover, if a more appropriate estimate of N_e , such as the lifetime reproductive success (Barrowclough & Rockwell, 1993), which considers individual survivorship, long-term reproductive success (multi-year survey) and variation in survivorship, had been included for this long-lived species, the estimate of effective population size would likely be even lower (Tremblay & Ackerman 2001).

Estimates of effective population size including variance in lifetime reproductive success in orchids have rarely been calculated (Tremblay & Ackerman 2001, Chung, et al., 2004). Tremblay & Ackerman (2001) found that using the same formula here for total reproductive potential (pollinaria removal and fruit set) that N_e in three epiphytic orchids of the Caribbean were variable among species (*Lepanthes rupestris*, $F_{is} = 0$, $N_e = 0.25$, $F_{is} = 1$, $N_e = 0.39$; *Lepanthes rubripetala*, $F_{is} = 0$, $N_e = 0.15$, $F_{is} = 1$, $N_e = 0.25$; *Lepanthes eltoroensis*, $F_{is} = 0$, $N_e = 0.10$, $F_{is} = 1$, $N_e = 0.18$) and that variance in survivorship and reproductive success (fruit set) was high, suggesting that genetic drift is highly likely in isolated populations (Tremblay & Ackerman, 2003, Tremblay et al, 2005). When they applied a more complex estimation of N_e , using lifetime reproductive success (Barrowclough & Rockwell 1993), the estimates of N_e were frequently smaller, in the range of 5% to 20% of the standing population. The only other survey of N_e in orchids is in the terrestrial *Cremastra appendiculata* in Korea (Chung et al., 2004) where, based on the variance in clone size, N_e as a proportion of standing individuals was estimated as 64% (mean, with a range of 0.64–0.97 in six populations). Chung et al. used a different formula to estimate N_e , but the results are equivalent to the formula used here. Their estimate of variation is a single time period and does not evaluate variance in lifetime reproductive success. Because they chose a character with very small variance (clone size, range 1–12 ramets per genet, with a skewed distribution to one) which may not represent the size of the variance in reproductive success from which to measure N_e , their estimate of N_e is much larger than *Sarcochilus australis* or the *Lepanthes* species; however if they had chosen inflorescence size (which varies from 20–50) they may have found a different result as it is likely that the variation in reproductive potential would be larger, resulting in smaller N_e .

This preliminary survey of *Sarcochilus australis* population structure suggests that a minimum size must be attained for flowering, and that only very large individuals have the possibility of having multiple inflorescences. The variation in floral display in orchids is likely to be a consequence of age, genetics and phenotypic plasticity (Lacey 1986). Effective population size estimates are low and suggest that genetic drift could be occurring at the Kinglake site if *Sarcochilus* population sizes are not more than of 500 reproductive individuals (for an N_e of 50 individuals, approximately 10% of the standing population). Consequently it is likely that high inbreeding coefficients (F_{is}) would be found if genetic variation were surveyed, unless gene flow among populations of *Sarcochilus australis* is high. This is unlikely to be the case as estimates of gene flow in orchids are commonly low (Tremblay & Ackerman 2003, Tremblay et al., 2005). Moreover, the closest extant populations of *Sarcochilus australis* are in Bunyip State Park c. 60 km away. It is possible that in the past there were other closer populations, and that gene flow among local populations occurred, but, as a result of forest clearing and drying of the gullies, no other surviving populations at Kinglake are known.

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