

**THE REPRODUCTIVE ECOLOGY OF TWO
TERRESTRIAL ORCHIDS, *CALADENIA RIGIDA* AND
*CALADENIA TENTACULATA***

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Philosophy

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DECLARATION

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Farrington L, Macgillivray P, Faast R, Austin AD (2009) Evaluating molecular tools for *Caladenia* (Orchidaceae) species identification. Australian Journal of Botany 57:276-286.

Phillips RD, Faast R, Bower CC, Brown GR, Peakall R (2009) Implications of pollination by food and sexual deception for pollinator specificity, fruit set, population genetics and conservation of *Caladenia* (Orchidaceae). Australian Journal of Botany 57:287-306.

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Cover photos : *Caladenia rigida* (left) and *Caladenia tentaculata* (right). Photos by author.

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THESIS SUMMARY

The reproductive outcome of plants is often determined by a multitude of interacting factors operating at both the plant level and the population level. For many plants, fruit production and the subsequent release of seeds are paramount for the persistence of the species. Understanding the processes that influence variation within and among populations is therefore crucial for the successful long-term management of threatened plants. While abiotic factors such as resource availability and environmental conditions can influence seed production directly through their effects on plant growth, biological interactions such as those between plants and pollinators or herbivores can be equally important. The relative intensity and direction of such interactions are often determined by the nature of the plants themselves, or by characteristics of the plant population or the habitat in which it occurs.

This thesis examines the processes that influence spatio-temporal variation in the reproductive success of two terrestrial orchids, *Caladenia rigida* and *Caladenia tentaculata*. The study was carried out over three years (2005 – 2007), in several populations located in the Mount Lofty region of South Australia. A detailed investigation of the pollination strategy employed by *C. rigida* revealed that this species is a generalist, being pollinated by a suite of food-seeking insects, possibly attracted by the presence of small amounts of nectar. Successful pollination and seed release for *C. rigida* was highly variable across space and time. Furthermore, both measures were consistently higher than for the sexually deceptive species, *C. tentaculata*, leading to the suggestion that the highly specialised pollination syndrome of the latter species may place it at a reproductive disadvantage. Pollination success of *C. rigida* was influenced by the height of flowers, but not by the local density of conspecifics. Small populations of *C. rigida* did not produce capsules when environmental conditions were stressful, suggesting that resource availability may indirectly restrict reproductive success by limiting the availability of pollinators. Poor seed quality in some populations may also be attributed to reduced population size.

Both orchid species were subject to intense levels of vertebrate florivory and capsule predation, leading to significant reductions in seed output. A herbivore exclusion experiment was carried out to help elucidate the size and type of herbivores, and video-surveillance identified birds as a predominant florivore in some populations. The intensity of florivory varied within and among populations, as well as among years, in response to several factors including flower height, the local density of conspecifics, concealment amongst neighbourhood vegetation and proximity to the habitat edge. Spatio-temporal variation in seed release was thus the net outcome of processes acting on both mutualistic and antagonistic interactions.

This work provides valuable baseline data of factors that influence the reproductive ecology and, hence, population dynamics of *Caladenia* species. Implications for the conservation and management of threatened populations are discussed, with respect to both short-term and long-term goals. The thesis is presented as a series of five manuscripts. Two of these have been published, and the remaining three have been prepared for submission as publications.

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