

Ensuring biodiversity offset success: the right kind of seed for a rare daisy (*Rutidosia lanata*)

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The woolly wrinklewort *Rutidosia lanata* (Asteraceae) is a rare and protected daisy from southern Queensland.

As part of the biodiversity offset program for the Australia Pacific LNG project, more than 100,000 plants of this species will be translocated. Successful establishment of a self-sustaining population relies on knowledge of basic reproductive ecology and genetic diversity for the species. The Gas Industry Social & Environmental Research Alliance (GISERA) have initiated a research project on the woolly wrinklewort to better inform the conservation of this rare species.

What do we need to know to make functioning populations?

Seed production is critical for plants to maintain functioning populations. Many factors can influence how much seed a plant produces. These include both reproductive biology and genetic diversity. It is therefore important to understand these factors in the woolly wrinklewort to ensure that the translocation project is successful.

Reproductive biology – Self-incompatibility

Many plants are self-incompatible, using genetic mechanisms to recognise their own or closely related pollen and preventing it from fertilising seed. This avoids inbreeding and ensures the next generation is genetically diverse. However, in small populations plants are often closely related, making it difficult for self-incompatible plants to find a suitable mate. This leads to very low seed set and endangers the long-term survival of populations and species.



A *Rutidosia lanata* flower bagged ready for pollination

Before the start of this project, the breeding system of the woolly wrinklewort was unknown and it was also unclear to what degree the existing populations are already suffering from inbreeding. Knowing this information is important to the success of the project. If the species is self-incompatible then the translocated populations need to have a suitable number of pollen partners, which could be achieved by mixing populations during the project.

Genetic diversity – Polyploidy

Polyploidy means that the genetic code (genome) of a plant is duplicated. It occurs frequently in plants and plays an important role in speciation and evolution. Some species have populations with two copies of the genome in the same way as humans do, while other plants can have more.

Before the start of the project, it was unclear if polyploidy occurs in this species but it does occur for its close relative, the button wrinklewort. If polyploidy does occur then mixing populations with different numbers of copies of the genome should be avoided during translocation. This is because such mixing produces sterile offspring.

What research is being done?

120 plants from ten wild populations were collected and are now being grown in greenhouses at CSIRO's Black Mountain Laboratories.

Crossing experiments

Flowering heads are bagged before opening to prevent uncontrolled pollination. In five populations, all plants are crossed with each other as well as with themselves to check for how much self-pollination can occur. To do this the flower heads are rubbed together and harvested after four weeks. Evaluation of successful and failed crosses in a population allows an estimate of whether it is suffering from a shortage of suitable pollen partners.

Flow cytometry

To measure genetic variation, fresh leaves are chopped in a buffer solution to release the plant's cell nuclei, which is where the genome copies (DNA) are stored. These nuclei are then passed through a flow cytometer which uses a laser to count them and measure their size. This gives a good estimate of how many genome copies the woolly wrinklewort has. In addition, the chromosome numbers of selected plants are being counted to confirm the results of flow cytometry.

Preliminary results

Preliminary data indicate that the woolly wrinklewort is self-incompatible. This suggests that it will be beneficial to mix populations during translocation. However, given that there is a lot of genetic variation between populations it will be important to select which populations to mix to avoid producing sterile seed.



The controlled pollination process



Studying genetic variation using flow cytometry

Next steps

The next steps will include the completion of assessments of genetic variation and the mapping of populations with similar and different genetic make-up. Finally, population mixing guidelines will need to be developed to ensure the maximisation of seed production.