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Ensuring biodiversity offset success

The right kind of seed for a rare daisy (*Rutidosis lanata*)

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Executive summary

Development of gas fields in south-eastern Queensland by Australia Pacific LNG involves impacts on populations of the endemic daisy *Rutidosis lanata*. Ecological offset work is required, including the restoration or creation of genetically healthy, long-term viable populations. Observations of low seed set in the field and under nursery conditions and a lack of knowledge about the biology of the species prompted the present project. Its aims were to study the genetics and reproductive ecology of *R. lanata* to develop recommendations for offset work, in particular on how seeds or plants should be sourced to create self-sustaining, healthy populations.

We found *R. lanata* to represent a polyploid complex, meaning that individuals with different numbers of genome copies co-exist in the same species. The geographic distribution of these individuals shows a clear north-south pattern in the study area, with northern populations predominantly having six genome copies, and southern populations predominantly having four.

The results of the crossing experiments confirmed *R. lanata* to be self-incompatible. Mate availability varied from c. 55% to c. 90% across populations. Most populations showed mate availability of c. 60-80%, suggesting that mate limitation resulting from lack of local genetic diversity may cause or at least contribute to reduced seed set. Crossing between populations resulted in significantly higher reproductive success for all populations except the two least mate-limited, suggesting the possibility of 'genetic rescue' through population mixing.

However, the crossing experiments also showed that hybrids arising from crosses between individuals with different numbers of genome copies suffer from a severely reduced ability to pollinate other plants. Any additional hybrids as would, for example, be created by mixing populations with different genome copy numbers during seed sourcing, would consequently exacerbate mate limitation and thus reduce population viability.

Taken together, the results of cytological surveys and crossing experiments indicate that seed set and thus population viability can be maximised by mixing populations with the same number of genome copies, but that populations with different numbers should be kept spatially separated.

1 Introduction

Rutidosis lanata (Asteraceae: Gnaphalieae) is a perennial herbaceous daisy endemic to southern Queensland (Holland 1994) (Figure 1). Its range is restricted to an area of approximately 160 x 190 km from around Yuleba and Barakula State Forest in the north to around Westmar and Moonie in the south, and populations have been fragmented by land use changes (Figure 1). The species is listed as vulnerable under the Queensland Nature Conservation Act 1992.

Construction of the Australia Pacific LNG project in the Surat and Bowen Basins of south-western and central Queensland has some impacts on populations of *Rutidosis lanata*. In accordance with Australia Pacific LNG's Environmental Offset Strategy and the Queensland Biodiversity Offset Policy, ecological offset work is required.

To ensure the establishment of long-term viable offset populations and to guide efficient management practices, knowledge of the biology of a species is indispensable. Very little, however, was known about the reproductive ecology and genetics of *Rutidosis lanata*, prompting the development of the present research project to inform conservation management.

Information available for better studied relatives of *Rutidosis lanata* suggested the possibility of the existence of two traits that have the potential to complicate the establishment of genetically healthy populations: polyploidy and self-incompatibility.

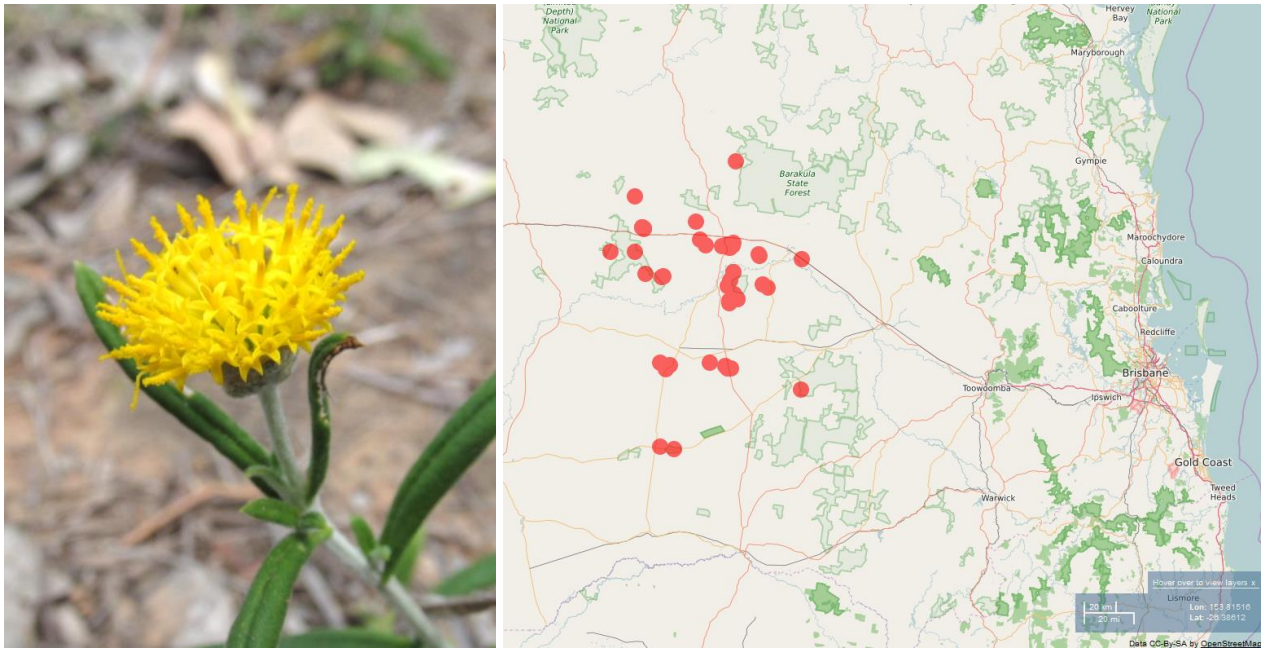

Figure 1 Left, flowering head of *Rutidosis lanata*. Right, approximate area of distribution indicated by records in the Atlas of Living Australia (ala.org.au). Additional populations have been discovered by ECOAUS staff

1.1 Polyploidy

Polyploidy is the phenomenon of genome duplication. Most animals and vascular plants have two genome copies, one inherited from each parent, a situation that is called diploid. In many plant groups, however, polyploids can arise spontaneously, leading to the existence of populations or species with higher numbers of genome copies (Leitch and Bennett 1997).

From the perspective of conservation management, the most relevant issue is hybridisation between populations with different numbers of genome copies. If, for example, a diploid with two copies and a tetraploid with four copies are crossed, their offspring will have three copies (one from one parent, two from the other). Individuals with uneven numbers of genome copies are often partially or fully sterile because the genetic material cannot be evenly distributed during the cell divisions that produce egg or sperm cells (Ramsey *et al.* 2011).

Consequently, the formation of additional hybrids between plants with different numbers of genome copies should be avoided to ensure that populations are genetically healthy and reproductively successful. If populations with such different numbers exist, they or their seeds should be kept geographically separate and not be mixed.

Only one chromosome count of *R. lanata* has been published (Kokubugata and Holland 2002). At 72 chromosomes, comparison with the chromosome numbers of diploid relatives suggests hexaploidy, i.e. six genome copies. It remains unknown whether there is variation between populations, but close relatives of *R. lanata* are known to constitute such polyploid complexes (Murray and Young 2001).

1.2 Self-incompatibility

Many flowering plants exhibit self-incompatibility, a mechanism by which flowers reject the pollen grains of potential fathers that exhibit the same genetic marker (S-allele) as that possessed by the mother (Hiscock and Tabah 2003). The purpose of this mechanism is to avoid inbreeding and the resultant risk of genetic disorders arising in offspring.

In large and well-connected populations, large numbers of S-alleles are present and guarantee that most plants will have genetically compatible mates available. In small and isolated populations, however, little diversity in S-alleles can be maintained, until most plants are surrounded by incompatible mates with which they share at least one S-allele. This situation, which is characterised by low seed set and consequently low population viability, is called mate limitation (Young and Pickup 2010).

Mate limitation can be managed by adding individuals or seeds from other, genetically distinct populations, in other words by mixing populations (Pickup and Young 2007). This injects new S-alleles into a genetically impoverished population, making new mates available and restoring seed set.

Close relatives of *Rutidosis lanata* are known to be self-incompatible (Young *et al.* 2000). Anecdotal observations suggested that the species may suffer from reduced seed set in the field and in the nursery (Brad Dreis, pers. comm.). It is possible that mate limitation due to a scarcity of S-alleles in the self-incompatibility system is at least partly responsible, but the mating system of *R. lanata* remains unknown.

1.3 Aims of the study

The goals of the present study were to generate basic information about the genetic makeup and reproductive biology of *Rutidosis lanata* that could be used to guide offset activities, so as to maximise long-term population viability. Specific aims were to:

1. Confirm whether *Rutidosis lanata* is a polyploid complex, i.e. whether there is variation in the number of genome copies between selected populations.

   1.1. If so, provide an understanding of the geographic distribution of different genome copy numbers;

2. Confirm whether the species is self-incompatible;

   2.1. If so, assess if there is evidence for mate-limitation in selected populations;

   2.2. If so, test if mate availability can be increased by crossing between populations;

3. Develop management recommendations, in particular with regard to seed sourcing and whether populations should be mixed during ecological offset work.

2 Methods

2.1 Study plants

153 plants were collected by Brad Dreis, Liz Fisher, and Chays Ogston from ten sites southwest (Gilmore) and southeast (Chaplin 1 & 2, Little 1 & 2) of Miles and southeast of Condamine (Campbell 1-5) (Table 1).

The plants were transferred to research greenhouses in Canberra, ACT, in autumn 2015. Initially they experienced some mortality, reducing the number of study plants to c. 106 at seven to twelve individuals per population. The surviving plants, however, subsequently proved to be very vigorous.

Table 1 Provenance of study plants

Site ID	Collection Date	Lot plan	Latitude	Longitude
Campbell 1	19/01/2015	62RG44	-26.99188	150.237821
Campbell 2	19/01/2015	59RG356	-27.019334	150.260247
Campbell 3	19/01/2015	59RG356	-27.022234	150.268114
Campbell 4	11/03/2015	60RG44	-27.007933	150.246333
Campbell 5	11/03/2015	60RG45	-27.008933	150.254492
Chaplin 1	11/03/2015	51RP896380	-26.708953	150.231738
Chaplin 2	22/01/2015	51RP896380	-26.709605	150.239118
Gilmore	12/03/2015	25BWR215	-26.72405	150.116542
Little 1	23/01/2015	53BWR143	-26.715191	150.215878
Little 2	11/03/2015	2RP90330	-26.701363	150.21888

2.2 Cytology

2.2.1 GENOME SIZES

Genome sizes were measured in all study plants using flow cytometry (Doležel *et al.* 2007). Fresh leaf material was collected from living specimens and kept on ice. A single triploid clone of *Bellis perennis* L. (2C = 3.45 pg) was used as a standard. Approximately 50 mg of *Rutidosis lanata* and 10 mg of standard leaf material were each placed in 1,200 µL of ice cold Modified Galbraith's buffer (Galbraith et al. 1983; Price 2010) [4.58 g MgCl2, 2.1 g MOPSO, 4.44 g Citric Acid, 15.0 g PVP-10, 0.5 ml Triton X-100, 2.5 ml Tween 20, made up to 500 ml with distilled water. The pH was adjusted to 7.0 – 7.1 with 10 M NaOH and filtered through a 0.22 µm filter, transferred to 15 ml tubes, and stored at −20°C] in a Petri dish and were manually chopped with a razor blade for approximately 30 s each to slice the leaf apart in parallel lines perpendicular to the main leaf vein. The chopped leaf material and buffer were gently mixed to release intact nuclei. The liquid was filtered through a 40 µm cell strainer and then centrifuged at 2,000 rpm for 1 min. Supernatant was removed until approximately 400 µL were left, and cells were gently resuspended. The sample was mixed with 20 µL of 1 mg/ml propidium iodide, then loaded into a Beckman Coulter Cell Lab Quanta MPL flow cytometer equipped with a 488 nm laser at 22 mW and run at 22 µL/min. Histogram data was

collected for both the internal standard and sample using the FL2 detector, and data analysis was performed with Beckman Coulter Cell Lab Quanta SC MPL analysis Software.

2.2.2 CHROMOSOME COUNTS

Several actively growing root tips were collected from selected study plants and were immediately placed in vials containing 0.5% colchicine solution. After refrigeration for approximately 4 h the root tips were transferred into freshly made fixative (3 parts 95% ethanol to 1 part glacial acetic acid [GAA]). They were refrigerated overnight, rinsed with water and transferred into 70% ethanol for long term storage at -20°C. To prepare squashes, two or three root tips were transferred to a new vial, rinsed to remove dirt, and softened for 10 min at 60°C in 1M hydrochloric acid. Subsequently, the root tips were rinsed with water and transferred into 45% GAA.

One or two root tips were placed on a glass slide, and the first c. 1 mm separated out while discarding the rest. Excess liquid was removed and replaced with a drop of FLP Orcein stain. Softened root tips were spread out by tapping with a brass rod for 30 s to 2 min. A cover slip was added and the sample heated over an open flame alcohol burner for 2-4 s. It was then squashed firmly under soft tissue paper.

Approximate chromosome counts were made from digital photographs from a Zeiss Axioimager compound microscope at the 63x water immersion level.

2.3 Crossing experiments

Flower-heads were bagged with custom-made bridal tulle mesh bags at the bud stage to exclude uncontrolled pollination by insects entering the greenhouses. An individual crossing experiment started when the two flower-heads selected for crossing began to bloom. The relevant pots were placed next to each other, mesh bags were removed, and the two flower-heads were gently brushed across each other and then re-bagged. Watchmaker's tags were attached to the stalks of flowering heads noting the crossing partner and the day the experiment started. Cross-pollination was repeated three times over the course of five to six days for each experiment to cover all individual, successively blooming flowers of both flower-heads.

All plants were crossed with themselves by reciprocally pollinating two flower-heads of the same plant, to test for self-incompatibility. All plants of a population were crossed with each other, except when individual plants produced an insufficient number of flowers, to estimate mate availability and number of S-alleles within populations. Finally, a representative number of inter-population crosses were conducted to explore the improvement of mate availability through population mixing.

Approximately three to five weeks after pollination, when a seed-head was shedding either ripe or abortive seeds, the entire seed-head was collected and placed in a paper envelope labelled with the identifiers of maternal and paternal parent. Fertile seed (recognised by their large size and dark colour) were counted.

As even self-pollinated flowers may sometimes set very few fertile seeds (generally 0-2), either because of pollen contamination or because of a malfunction of the self-incompatibility mechanism, seed-heads were scored as successfully pollinated only if the number of fertile seeds was larger than $M + 2 * \frac{S}{\sqrt{N}}$ rounded up, where M is the mean of fertile seeds from all self-crosses, S is the standard deviation of fertile seeds from all self-crosses, and N is the number of self-crosses. For our study the relevant criterion was three or more fertile seeds.

Mate availability in a given group of plants was calculated by dividing the number of successfully pollinated seed heads by the number of all seed heads harvested from that group, excluding self-pollinations. R 3.2.0 was used for statistical evaluation. S-allele numbers were inferred from the crossing diallels using a custom-written Python script, assuming the existence of dominance effects in the female function and polyploidy of the S-locus.

3 Results

3.1 Polyploidy

Study plants showed three clearly distinct genome size classes of c. 15.2-17.3 pg, 19.4-21.4 pg, and 23.0-25.9 pg (Figure 2). The averages of the size classes showed a ratio of 1.00 : 1.24 : 1.49, suggesting the existence of four, five and six genome copies (tetraploidy, pentaploidy and hexaploidy).

This observation was confirmed by mitotic chromosome counts of c. 48 for representative plants in the smallest genome size class (#72, Little 1, #94, Campbell 4), c. 60 for plants in the intermediate class (#74, Campbell 5; #80, Campbell 4), and c. 72 for plants in the largest class (#16, Chaplin 2; #33, Little 1).

Six of the ten study populations were pure, and four were mixed (Table 2). Of the latter, three contained at least one pentaploid. In one (Campbell 5), pentaploids were in the majority among the sampled plants.

The distribution of ploidy levels in *Rutidosis lanata* showed a clear geographic structure across the sampled area, with hexaploid populations predominantly in the north, and tetraploid populations predominantly in the south (Figure 3).

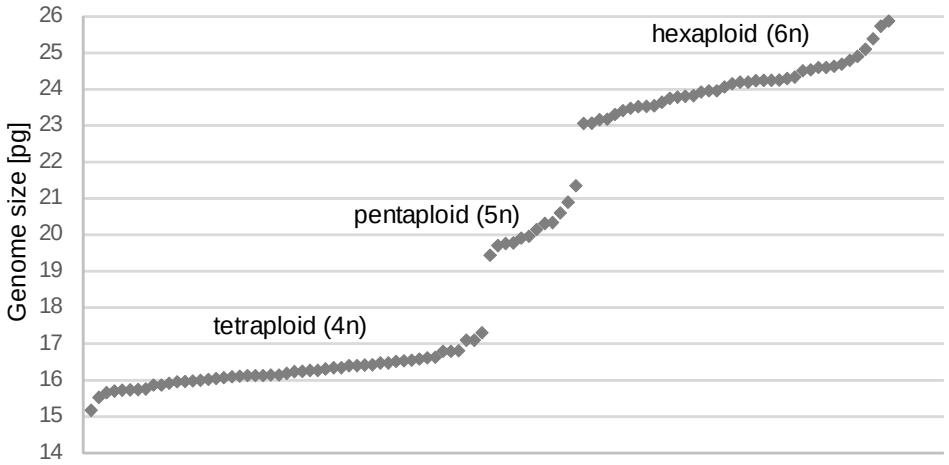

Figure 2 Genome size measurements formed three clearly distinct size classes

Table 2 Summary of ploidy levels observed in the study populations

Population	Tetraploid (4 sets)	Pentaploid (5 sets)	Hexaploid (6 sets)	Summary
Campbell 1	7	0	0	All tetraploid
Campbell 2	10	0	0	All tetraploid
Campbell 3	12	0	0	All tetraploid
Campbell 4	9	4	1	Mixed but mostly tetraploid
Campbell 5	2	7	1	Mixed, with many hybrids
Chaplin 1	0	0	9	All hexaploid
Chaplin 2	0	0	9	All hexaploid
Gilmore	7	1	4	Mixed
Little 1	4	0	5	Mixed

Little 2	0	0	10	All hexaploid

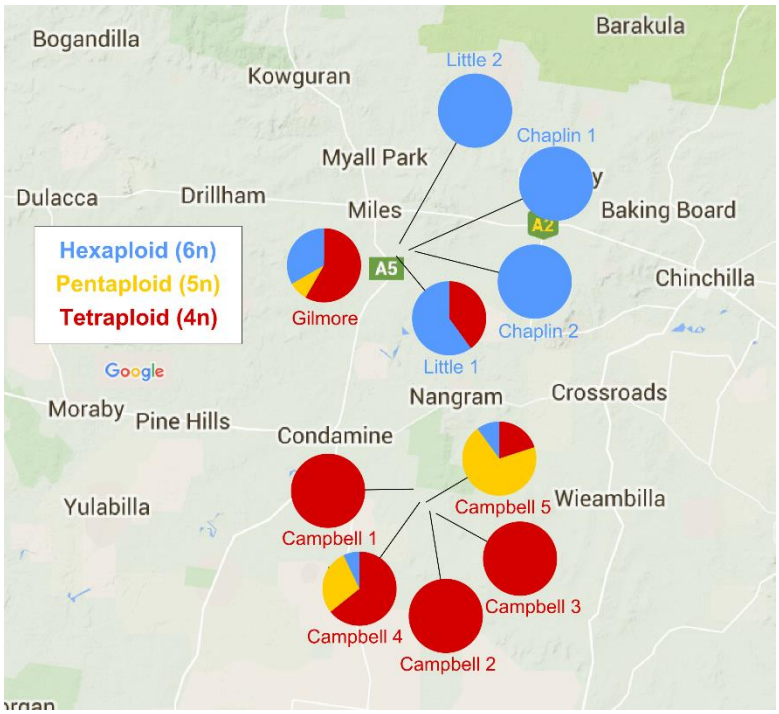

Figure 3 Geographic distribution of ploidy levels in the study area

3.2 Crossing experiments

All plants except one (#105, Gilmore) were unable to set any significant amount of seed after being self-pollinated, confirming the existence of a self-incompatibility mechanism in *Rutidosis lanata*. Tables summarising pair-wise crossing success were asymmetric (not shown), suggesting that the self-incompatibility mechanism is sporophytic, as in many other Asteraceae.

Mate availability varied across populations with a maximum of 90.0% in Chaplin 1 and a minimum of 56.9% among the tetraploids of Campbell 4 (Table 3). Most populations showed values in the range of 60-80%, suggesting that mate limitation may be a frequent phenomenon in wild populations of *Rutidosis lanata*.

Crossing plants at the same ploidy level between populations improved mate availability significantly for most populations (Table 3). Only the two least mate limited populations, Campbell 1 and Chaplin 1, did not show any improvement. Conversely, the benefits of cross-population pollination were strongest for the most mate limited populations (Figure 4). Populations showing a mate availability of less than c. 75% saw a benefit, those starting out with a higher mate availability did not.

The availability of twelve naturally occurring pentaploids among our study plants allowed us to explore their reproductive fitness by including them in the crossing experiments. Pentaploids did not suffer in their role as pollen recipients (mothers), experiencing a similar pollination success and producing similar numbers of seeds in successfully pollinated seed-heads as tetraploids and hexaploids. In their role as pollen donors (fathers), however, they were able to pollinate only c. half as many flower-heads as the even-numbered ploidy levels, and even the heads counted as successfully pollinated by pentaploids set only a third to half as many fertile seeds as those pollinated by other plants (Figure 5).

Table 3 Results of within- and between-population crossing experiments at the same ploidy level. Asterisks indicate mixed populations where the rarer ploidy levels were excluded from mate availability inference because they accounted for less than five plants each. Population Campbell 5 was excluded because was is dominated by pentaploid hybrids

Population (and ploidy level)	S-allele estimates	Mate availability in population	Mate availability when crossed with other pops.	Improvement
Campbell 1 (tetraploid), 7 plants	17-19	78.9%	77.4% (n=31)	None
Campbell 2 (tetraploid), 11 plants	19-22	63.4%	78.9% (n=19)	15.5%
Campbell 3 (tetraploid), 12 plants	21-23	67.7%	78.3% (n=23)	10.6%
Campbell 4 (tetraploid), 9 plants*	15-17	56.9%	90.0% (n=30)	33.1%
Gilmore (tetraploid), 7 plants*	11-13	67.0%	75.0% (n=16)	8.0%
Chaplin 1 (hexaploid), 9 plants	30-32	90.0%	89.7% (n=39)	None
Chaplin 2 (hexaploid), 9 plants	27-29	71.0%	86.4% (n=22)	15.4%
Little 1 (hexaploid), 5 plants*	23-26	75.0%	79.4% (n=34)	4.4%
Little 2 (hexaploid), 11 plants	33-36	65.7%	74.3% (n=35)	8.6%

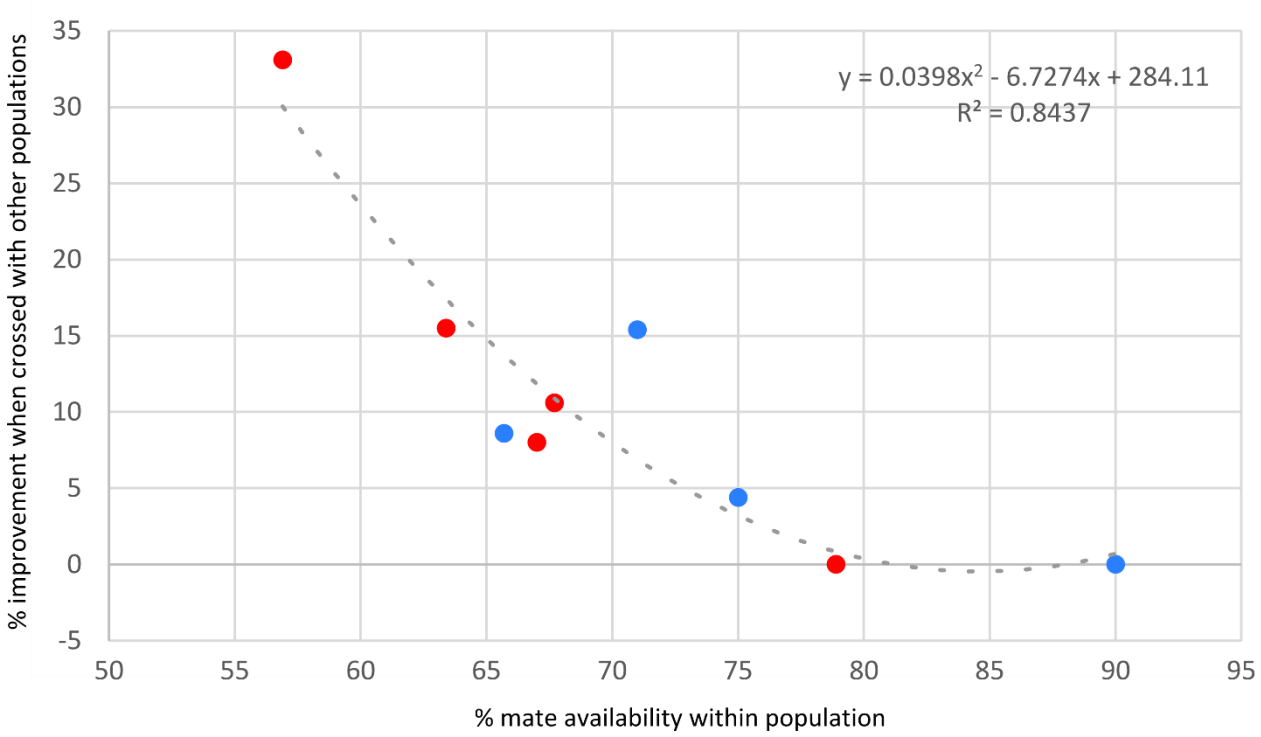

Figure 4 The beneficial effect of inter-population crosses on mate availability was stronger the more mate limited a population was

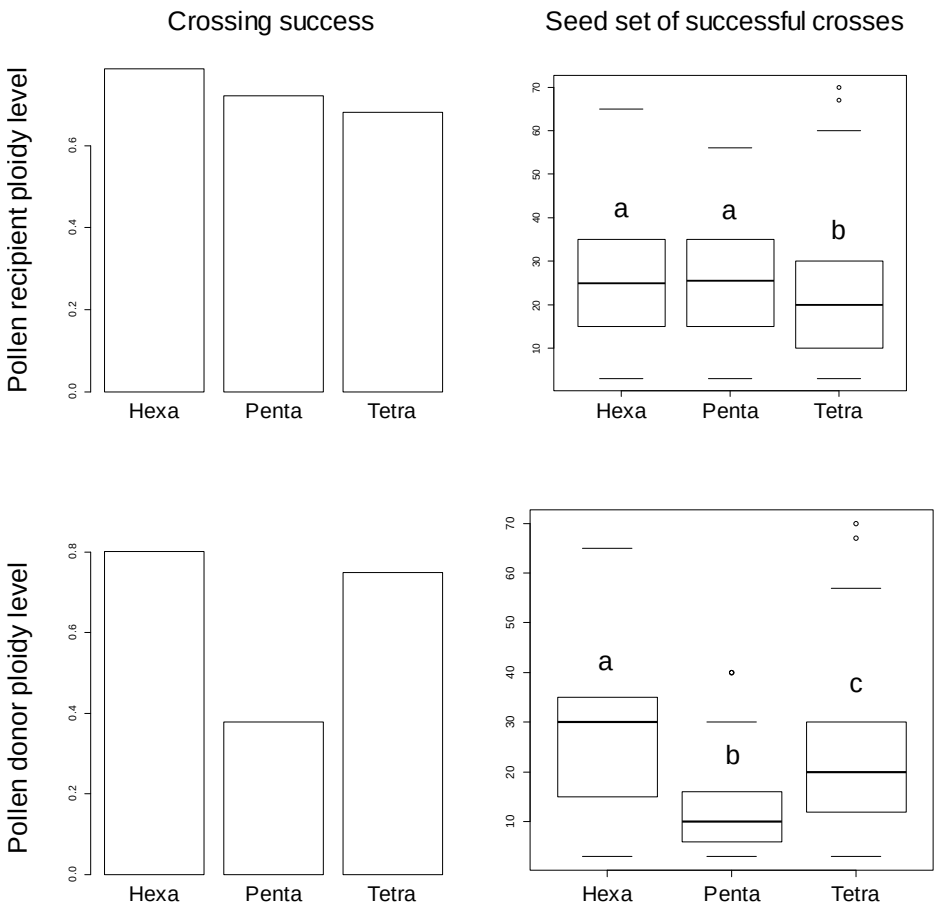

Figure 5 Reproductive fitness of different ploidy levels. Top row shows results of plants acting as pollen recipients (mothers), bottom row shows them acting as pollen donors (fathers). Left column shows proportion of crossing successes, right column shows seed set of successfully pollinated flower-heads. Different lowercase letters indicate significant difference in Welch Two Sample t-tests. Note poor performance of pentaploid hybrids as fathers

4 Discussion

4.1 Breeding system

4.1.1 SELF-INCOMPATIBILITY

Our results indicate that *Rutidosis lanata* has a strong sporophytic self-incompatibility system. Plants cannot self-pollinate and in addition reject pollen of closely related plants. This means that populations have to be genetically diverse if long-term reproductive success is to be ensured (Young and Pickup 2010). Because even purely stochastic processes will lead to a loss of diversity in incompatibility markers (S-alleles) in small and fragmented populations, populations need to be large enough to maintain the necessary diversity to be sustainable.

4.1.2 MATE AVAILABILITY IN EXISTING POPULATIONS

Several populations for which mate availability could be estimated showed evidence of significant mate limitation, i.e. individual plants would reject a large percentage of the pollen they receive from other members of their population. This suggests that a lack of S-allele diversity may explain or at least contribute towards low set seed in natural populations and harvested plants.

4.1.3 MATE AVAILABILITY IN INTER-POPULATION CROSSES

Crosses between plants from different populations were generally more successful than within-population crosses, with only the two least mate limited populations not showing an improvement. This suggests that mixing plants from different sources (but with the same chromosome number, see 4.2.2) should improve seed set and thus the long-term viability of translocated or restored populations.

4.2 Polyploidy

4.2.1 CO-EXISTENCE OF DIFFERENT PLOIDY LEVELS IN THE SPECIES

Our study has demonstrated that *Rutidosis lanata* forms a polyploid complex characterised by individuals with different numbers of genome copies or chromosome sets. Tetraploid, pentaploid and hexaploid individuals (four, five and six genome copies, respectively) co-exist within the species. It can be assumed that the rare and intermediate pentaploids are hybrids of the more frequent tetraploids and hexaploids.

Six of the ten study populations appeared to be either purely tetraploid or purely hexaploid, while four populations showed some naturally occurring admixture, with three of them containing at least one pentaploid hybrid.

Despite this the distribution of cytotypes showed a clear geographic structure, with predominantly hexaploid populations in the north of the study area and predominantly tetraploid populations in the south. This suggests that mixing of populations or seeds during relocation or offset work has the potential to produce even more pentaploid intermediates than exist naturally, especially when managing populations whose genome copy numbers are unknown.

No diploids (two genome copies) were observed in the study, but they must have existed at least historically, as the hexaploid cytotype cannot arise from genome duplication alone; it would have come about through hybridisation either of tetraploids with octoploids or of diploids with tetraploids followed by

genome duplication. Study populations covered only part of the entire range of *Rutidosis lanata*, and it is consequently possible that more undiscovered diversity in cytotypes exists in other areas.

4.2.2 EFFECT OF CROSSING BETWEEN PLOIDY LEVELS

Results of the crossing experiment demonstrated a significantly reduced reproductive fitness in the male function of pentaploid hybrids compared to the parental, even-numbered ploidy levels. They pollinated successfully at approximately half the normal rate, and even pollination events that were counted as successful produced only half as many seeds as when the father was tetraploid and two thirds less seeds than when the father was hexaploid.

As crossing success and seed set are subsequent hurdles to be overcome, their effects are multiplicative. This means that any additionally produced pentaploid in a mixed population would on average represent an at least 75% loss to reproductive success compared to a non-pentaploid plant. Existence of pentaploids thus can be expected to compound the effect of mate limitation through reduced genetic diversity, further contributing to reduced seed set and lower population viability.

Interestingly, there was no significant effect of pentaploidy on the female function, a result that was unexpected because pentaploids in other plant species have been observed to be sterile (Bringhurst and Khan 1963).

4.3 Management recommendations

Several of the study populations benefited from inter-population crossing leading to higher mate availability and thus higher seed set. On the other hand, crosses between populations with different genome copy numbers should be avoided because the resulting intermediates are at least partly male-sterile and thus exacerbate the problems of mate limitation and lower seed set. Given the relatively small geographic range of the species, it is not expected that population mixing would produce adverse effects due to outbreeding depression resulting from local adaptation.

Accordingly, to maximise seed set and population viability, existing populations could be augmented with plants from other, especially larger populations with the same number of genome copies. When creating offset populations, seed provenances with the same number of genome copies should be mixed, but populations with different numbers of genome copies should be kept spatially separated.

4.4 Suggestions for future studies

Our knowledge of the geographic distribution of different ploidy levels is still comparatively limited, mostly to the north-east of the range of *Rutidosis lanata*. It would be desirable to obtain a more complete picture, e.g. of the genetics of the populations around Jackson, Dulacca, and Glenmorgan in the west of its range, and near Moonie and Westmar in the south. Of particular interest would be any populations that have to be managed for conservation purposes.

The present study has examined mate availability and inferred approximate S-allele numbers for nine study populations. Ecological models are available that allow predictions to be made about the number of individuals and the number of S-alleles needed to establish a sustainable population of self-incompatible plants (often defined as genetically viable for at least 100 years even in the absence of immigration) (Thrall *et al.* 2014). However, even the most appropriate models were developed for diploid species, not for polyploids such as *Rutidosis lanata*, and most commonly used models do not consider genetics at all. Polyploids are faced with a trade-off: due to the higher number of genome copies per plant they can maintain higher numbers of S-alleles in a population of the same size than diploids, but on the other hand they also need higher numbers because a pollen recipient will test pollen for a larger number of S-alleles, making it less likely that pollen is accepted. It is unclear which effect will be stronger, and if polyploids need larger or smaller population sizes than diploids to achieve long-term viability.

Expanding the relevant population models to apply to polyploid species would be beneficial beyond the present study species, as polyploidy is common across the land plants. Once developed, these models could inform management decisions for many different species given knowledge of their ploidy level and breeding system.

Another potential factor in population viability are pollinator services. Small and fragmented plant populations may attract less pollinators, different species of pollinators, or experience changed pollinator behaviour compared with large and continuous populations, and these effects can compound that of mate limitation. Very little, however, is currently known about pollination in *Rutidosis lanata* beyond anecdotal observations during field work or in the greenhouse that tend to suggest that it may be reliant on generalist insects, in particular native bees, hoverflies, small beetles and butterflies.

5 Client workshop

Part of the project plan was a client workshop to communicate and discuss the results as well as the impact of genetics on the conservation management of plant species more generally. It took place on 15 September 2016 in the CSIRO EcoSciences Precinct at 41 Boggo Road, Dutton Park, Brisbane, under the title "Viable or Vulnerable? Ecological and genetic considerations for restoration plantings" (Table 4).

Table 4 Schedule of the client workshop

10:00-10:30	Introduction, morning tea. Welcome by **Graeme Bartrim**.
10:30-10:50	**Andrew Young**, Small population effects: inbreeding, founder effects, drift, fitness.
10:50-11:10	**Linda Broadhurst**, Genetic structure: local adaptation, polyploidy.
11:10-11:30	**Alexander Schmidt-Lebuhn**, Case study: mate availability and polyploidy in a perennial daisy managed by Origin Energy.
11:30-12:00	**Francisco Encinas-Viso**, Population Viability Analysis.
12:00-1:00	Lunch break.
1:00-2:00	**Alexander Schmidt-Lebuhn**, Introduction to group work. Work in groups on participant-supplied case studies.
2:00-3:00	Group presentations, discussion and feedback.

In addition to the four speakers and GISERA Communications Advisor Tsuey Cham, the Workshop was attended by 13 participants representing industry, government and academia: Leanne Stevens (Arrow Energy), Brad Dreis (ECOAUS), Graeme Bartrim (Origin Energy), Laura McCallion and Cameron Playsted (QGC), Daryl Robinson (Sibelco), Karalyn Herse, Ahlia Karam, Teva Kohring, Frank Mills, and Gordon Murrell (Department of the Environment and Heritage Protection), Jennifer Silcock (University of Queensland), and Sarah Mathews (CSIRO).

Talks in the morning provided an introduction to the importance of genetics for the viability of populations and the long-term survival of rare species, with the present study used as a practical example. For the afternoon, participants were divided into three groups, each of which was provided with basic information on two participant-nominated species of current or potential management interest. These were *Acacia curranii* (Curly-bark Wattle), *Philotheca sporadica* (Kogan Waxflower), *Eucalyptus curtisii* (Plunkett Mallee), *Homopholis belsonii* (Belson's Panic), *Cadellia pentastylis* (Ooline), and *Calytrix gurulmundensis*.

Taking into account the presentations and discussions of the morning, the groups considered what observations or traits were important for population viability in each of the species, what additional knowledge might be needed most and how to obtain or infer it, and how these considerations might affect the management of the relevant species (Figure 6). Each group presented its results in a general discussion.

Figure 6 Group work on participant-nominated species during the client workshop

Glossary

Mate availability

The probability of an individual being able to mate with a randomly chosen individual of the same population. It is calculated by dividing the number of successfully pollinated seed heads by the number of all seed heads harvested in the experiment, excluding self-pollinations.

Polyploidy

The state of having more than two genome copies or sets of chromosomes. Ploidy levels are described using Greek prefixes: diploid (2 copies), triploid (3), tetraploid (4), pentaploid (5), hexaploid (6), etc.

S-alleles

The alleles of the gene locus controlling self-incompatibility in plants. They consist of two tightly linked genes one of which codes for a marker protein expressed on the pollen surface, while the second codes for the matching receptor protein expressed on the stigma of a flower. If a receptor recognises its own marker protein on a pollen surface, the stigma does not allow that pollen grain to fertilise the flower.

Self-incompatibility

A mechanism used by many plant species to recognise and reject their own and closely related pollen. It serves to avoid inbreeding, but accordingly requires the availability of distantly related individuals to produce seed and avoid reproductive failure.

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