Project Order, Variations and Research Progress

Project Title: Ensuring biodiversity offset success: Chromosomal and breeding system analysis of Rutidosis Lanata to determine optimal seed sourcing strategies for reproductive success and ecological viability of translocated populations

This document contains three sections. Click on the relevant section for more information.

1 Original Project Order

Project Order

Proforma 2014

1. Short Project Title (less than 15 words)

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

Long Project Title	Ensuring biodiversity offset success: Chromosomal and breeding system analysis of *Rutidosis lanata* to determine optimal seed sourcing strategies for reproductive success and ecological viability of translocated populations.
GISERA Project Number	B4
Proposed Start Date	1 August 2014
Proposed End Date	30 June 2015
Project Leader	Prof. Andrew Young, CSIRO

2. GISERA Research Program

☒ Biodiversity Research	☐ Marine Research	☐ Land Research
☐ Water Research	☐ Social & Economic Research	☐ GHG Research

3. Research Leader, Title and Organisation

Prof. Andrew Young Director, Centre for Australian National Biodiversity Research Research Director, National Research Collectors CSIRO Time commitment: 7%

4. Summary (less than 300 words)

The perennial herb *Rutidosis lanata* is currently the subject of a large-scale translocation operation by Origin Energy as part of its biodiversity offsets program. Relocation of in excess of 100,000 plants may be necessary in order to offset impacts from construction of the Australia Pacific LNG project. Successful establishment of a self-sustaining (demographically viable) population relies on knowledge of basic reproductive ecology and genetic diversity (chromosomal variation) for the species. This project will provide data on these issues and use this information to generate seed sourcing and deployment strategies that will maximise the viability of translocated *R. lanata* populations. This information will form the basis for development of a biologically based translocation plan. Lessons from this research will be communicated to partners through a workshop that will outline both the results obtained for this species and also provide general information regarding genetic and reproductive considerations that are relevant to plant tranlocations aimed at providing successful biological offsets.

5. Budget Summary (From Excel Budget Pack worksheet "Project Plan Summary")

Expenditure	2011/12 Year 1	2012/13 Year 2	2013/14 Year 3	2014/15 Year 4	2015/16 Year 5	Total
Labour				173,055		173,055
Operating				25,000		25,000
Total Costs				**198,055**		**198,055**
CSIRO				198,055		198,055
Total Expenditure				**198,055**		**198,055**

Expenditure per Task	2011/12 Year 1	2012/13 Year 2	2013/14 Year 3	2014/15 Year 4	2015/16 Year 5	Total
Task 1				19,805		19,805
Task 2				19,805		19,805
Task 3				59,419		59,419
Task 4				59,416		59,416
Task 5				19,805		19,805
Task 6				19,805		19,805
Total Expenditure				**198,055**		**198,055**

Cash Funds to Project Partners	2011/12 Year 1	2012/13 Year 2	2013/14 Year 3	2014/15 Year 4	2015/16 Year 5	Total
CSIRO				118,833		118,833
Sub Total				118,833		118,833
Total Cash to Partners				**118,833**		**118,833**

Source of Cash Contributions	2011/12 Year 1	2012/13 Year 2	2013/14 Year 3	2014/15 Year 4	2015/16 Year 5	Total
GISERA				118,833		118,833
Total Cash Contributions				**118,833**		**118,833**

In-Kind Contribution from Partners	2011/12 Year 1	2012/13 Year 2	2013/14 Year 3	2014/15 Year 4	2015/16 Year 5	Total
CSIRO				79,222		79,222
Total In-Kind Contribution from Partners				**79,222**		**79,222**

	Total funding over all years	Percentage of Total Budget
GISERA Investment	118,833	60%
CSIRO Investment	79,222	40%
Total Other Investment	0	0
TOTAL	**198,055**	

Task	Milestone Number	Milestone Description	Funded by	Participant Recipient	Start Date (mm-yy)	Delivery Date (mm-yy)	Fiscal Year	Fiscal Quarter	Payment $
Task 1	1.1	On signing of contract	GISERA	CSIRO	Aug 14	Aug 14	14-15	1	11,883
Task 2	2.1	Specimen delivery	APLNG	CSIRO	Sept 14	Oct 14	14-15	2	11,883
Task 3	3.1	Completion of flow cytometry	GISERA	CSIRO	Oct 14	Apr 15	14-15	2	35,651
Task 4	4.1	Completion of Pollination experiment	GISERA	CSIRO	Oct 14	Apr 15	14-15	2	35,650
Task 5	5.1	Client Workshop	GISERA	CSIRO	Apr 15	May 15	14-15	4	11,883
Task 6	6.1	Delivery of report	GISERA	CSIRO	May 15	Jun 15	14-15	4	11,883

6. Other Researchers (include organisations)

(State time commitment to project by each Researcher listed)

Researcher	Time Commitment (project as a whole)	Principle area of expertise	Years of experience	Organisation
Andrew Young	0.07	Experimental design, crossing studies and data analysis	25 plant reproductive ecology and genetics	CSIRO
David Marshall	0.95	Flow cytometry	25 ecology, 1 in flow cytometry	CSIRO

7. GISERA Objectives Addressed

Carrying out of research and improving and extending knowledge of social and environmental impacts and opportunities of CSG-LNG projects for the benefit of the CSG-LNG industry, the relevant community and the broader public.

8. Program Outcomes Achieved

Details are provided in *Section 13. Project Objectives and Outputs*.

9. Program Outputs Achieved

Details are provided in *Section 13. Project Objectives and Outputs*.

10. What is the knowledge gap that these research outputs will address?

Two key knowledge gaps will be addressed by this project. The first will be the identification of any chromosome races that exist within R. lanata to advise sourcing and deployment of plants for translocation. Unintentional mixing of plants with different chromosome numbers will severely compromise restoration success through generation of sterile plants. Second, current observations of seed set in R. lanata indicate reproductive failure (very low seed set) in nursery populations. This may well be because populations have low numbers of genetic mating types (S-alleles). Controlled pollination experiments aim to confirm the species' self-incompatibility system and identify how mixing populations can increase reproductive success by combining genotypes with different mating types.

11. How will these Research outputs and outcomes be used in State Government and other water managers to achieve Adaptive Management of Water Resources?

NA

12. Project Development (1 page max.)

The project was developed through consultation between Origin Energy and the CSIRO Biodiversity Portfolio (Young) regarding genetic and ecological factors that were likely to limit the long-term success (viability) of restoration plantings.

The value of the project is in its application of scientific data about genetic structure and breeding systems gathered from focused experiments and observations to directly improving the efficiency and effectiveness of replanting that are required under Australia Pacific LNG's various environmental approvals.

As per the Coordinator-General's report on the EIS, Australia Pacific LNG may only clear plants protected under the NC Act in accordance with a clearing permit (or exemption) and must provide offsets for the permanent loss of EVNT plants (Appendix 1, Part 1, Condition 7(a)[i&ii]). The condition stipulates that offsets must be provided in accordance with the "Queensland Government Environmental Offsets Policy 2008" (QGEOP) and generally in accordance with the "Queensland Government Policy for Biodiversity Offsets (Consultation Draft)". This policy was finalised in October 2011.

Australia Pacific LNG has obtained multiple permits for the clearing of R. lanata which typically require "an offset to be provided in accordance with the 'Australia Pacific LNG Environmental Offset Strategy' and generally in accordance with the Queensland Biodiversity Offset Policy 2011". Under this policy, offsets for Endangered plants must be provided at a ratio of 1:5. These permits also require contribution to the enhancement of knowledge of the species.

This work builds on a growing body of outcome-focused restoration science that integrates genetic and ecological analyses to understand and overcome limits to plant population viability. Similar work has been conducted successfully by this CSIRO research team on restoration of several other grassland species in particular the daisy *Rutidosis leptorrhynchoides* and the pea Swainsona recta.

13. Project Objectives and Outputs

The objective of this project is to provide guidelines as to how best to undertake *R. lanata* translocations to minimise biological limits to reproductive success and maximise population viability. The outputs may also inform requirements for future *R. lanata* offset requirements. Benefits of the project will include; 1) Development of clear guidelines for *R. lanata* offset plantings with regard to seed sourcing and deployment; 2) improved likelihood of success of *R. lanata* translocations through establishment of genetically and reproductively viable populations; 3) Communication of general information regarding the genetic and reproductive constraints to be considered when undertaking plant translocations for biodiversity offset purposes – these will be outlined in the partners workshop.

This project will examine two specific biological issues that may limit the success of the proposed translocations of *Rutidosis lanata* and provide data-based translocation guidelines to minimize their effects on reproductive performance and population viability. Specifically these are:

1. **Chromosome number and inter-population cytogenetic structure.** Assessment of variation in chromosome number among *R. lanata* populations. Cytogenetic races have been identified in the con-generic species *Rutidosis leptorrhynchoides* which has diploid (2n=2x=22), tetraploid (2n=4x=44) and hexaploid (2n=6x=66) plants. Mixing of individuals with different base chromosome numbers is likely to result in the generation of dysgenic progeny (seed) with uneven chromosome numbers that will be infertile. Such chromosome mixing events cannot be reversed and are likely to represent a long-term threat to the viability of any re-established population. Therefore identification of any chromosome races that exist within *R. lanata* is very important in terms of sourcing and deployment of plants for translocation. **Research:** Screen 10-15 populations for differences in genome size using flow cytometry (5-10 individuals per population) and confirm chromosome numbers of each ploidy group using chromosome counts from root squashes of representative individuals (if available).

2. **Reproductive constraints due to low numbers of genetic mating types.** Current observations of seed set in *R. lanata* indicate reproductive failure (very low seed set) in nursery populations. Two other species in the genus *Rutidosis* (*R. leptorrhynchoides* and *R. leiolepis*) are both known to have genetic self-incompatibility systems. These systems prevent selfing and mating between relatives and in populations that have low genetic variation at the self-incompatibility locus (S-locus) this will severely constrain seed set. Current observations of low seed set in *Rutidosis lanata* may well be because populations have low numbers of genetic mate types (S-alleles). If this is the case translocation planning should aim to maximize genetic diversity at the self-incompatibility locus by mixing genotypes from different populations to restore mate availability and seed set. This has proved a successful strategy for *R. leptorrhynchoides*. **Research:** Conduct controlled-pollination experiments to: a) demonstrate whether *R. lanata* has a genetic self-incompatibility system; b) Measure genetic variation at the incompatibility locus in five populations to determine if low seed set is due to low numbers of genetically compatible mates; c) Conduct inter-population crosses to determine whether mixing plants from different populations can restore S-locus genetic diversity, mate availability and significantly increase seed set.

14. Project Plan

All experimental design, cytogenetic analysis and crossing studies as well as data analysis and interpretation and writing of the final report will be undertaken by CSIRO staff. Origin Energy will be responsible for sourcing and transporting live plants to CSIRO Plant Industry in Canberra to be used for both cytogenetic (10-15 populations of 5-10 plants each) and experimental crossing work (5 populations of 10-15 plants each, can be five of the same populations as use for cytogenetic work). This includes obtaining all required permits and permissions.

14.1 Project Schedule

ID	Task Title	Task Leader	Scheduled Start	Scheduled Finish	Predecessor
1	On signing of contract	Andrew Young	Aug 14	Aug 14	
2	Specimen delivery	Andrew Young	Sept 14	Oct 14	
3	Completion of flow cytometry	Andrew Young	Oct 14	Apr 15	
4	Completion of Pollination experiment	Andrew Young	Oct 14	Apr 15	
5	Client Workshop	Andrew Young	Apr 15	May 15	
6	Delivery of report	Andrew Young	May 15	Jun 15	

Task 1.

TASK NAME: Sign contract.

TASK LEADER: Andrew Young

OVERALL TIMEFRAME: August 2014

BACKGROUND: Contract needs to be signed to allow project to proceed.

TASK OBJECTIVE: To sign the contract.

TASK OUTPUTS & SPECIFIC DELIVERABLES: Signed contract.

Task 2.

TASK NAME: Specimen Delivery.

TASK LEADER: Andrew Young

OVERALL TIMEFRAME: Sept 2014 – Oct 2014

BACKGROUND: Plants must be transferred from the nursery in Brisbane to CSIRO Canberra where they can be grown. Department of Environment and Heritage Protection Guidelines must be followed to undertake this process.

TASK OBJECTIVE: Live plants to be transferred from the nursery in Brisbane to CSIRO Canberra.

TASK OUTPUTS & SPECIFIC DELIVERABLES: Delivery of live plants to CSIRO Canberra.

Task 3.

TASK NAME: Completion of flow cytometry

TASK LEADER: Andrew Young

OVERALL TIMEFRAME: Oct 2014 to Apr 2015

BACKGROUND: Cytogenetic races have been identified in the con-generic species *Rutidosis leptorrhnychoides* which has diploid (2n=2x=22), tetraploid (2n=4x=44) and hexaploid (2n=6x=66) plants. Mixing of individuals with different base chromosome numbers is likely to result in the generation of dysgenic progeny (seed) with uneven chromosome numbers that will be infertile. Such chromosome mixing events cannot be reversed and are likely to represent a long-term threat to the viability any re-established population. Therefore identification of any chromosome races that exist within *R. lanata* is very important in terms of sourcing and deployment of plants for translocation.

TASK OBJECTIVE: Screen 10-15 populations for differences in genome size using flow cytometry (5-10 individuals per population) and confirm chromosome numbers of each ploidy group using chromosome counts from root squashes of representative individuals (if available).

TASK OUTPUT: Geographical mapping of cytogenetic variation in *Rutidosis lanata* and guidelines identifying common cytogenetic population groups that can be mixed and those that can't due to the likelihood of generating dysgenic plants.

SPECIFIC DELIVERABLE: Guidelines for translocation and population mixing that explicitly take cytogenetic variation into account.

Task 4.

TASK NAME: Completion of Pollination experiment

TASK LEADER: Andrew Young

OVERALL TIMEFRAME: Oct 2014 to Apr 2015

BACKGROUND: Current observations of seed set in *R. lanata* indicate reproductive failure (very low seed set) in nursery populations. Two other species in the genus *Rutidosis* (*R. leptorrhynchoides* and *R. leiolepis*) are both known to have genetic self-incompatibility systems. These systems prevent selfing and mating between relatives and in populations that have low genetic variation at the self-incompatibility locus (S-locus) this will severely constrain seed set. This suggests that low seed set in *Rutidosis lanata* may well be because populations have low numbers of genetic mate types (S-alleles). If this is the case translocation planning should aim to maximize genetic diversity at the self-incompatibility locus by mixing genotypes from different populations to restore mate availability and seed set. This has proved a successful strategy for *R. leptorrhynchoides.*

TASK OBJECTIVE: Conduct controlled-pollination experiments to: a) demonstrate whether *R. lanata* has a genetic self-incompatibility system; b) Measure genetic variation at the incompatibility locus in five populations and determine if low seed set is due to low numbers of genetically compatible mates; c) Conduct inter-population crosses to determine whether mixing plants from different populations (but of the same cytogenic race) can restore S-locus genetic diversity, mate availability and significantly increase seed set.

TASK OUTPUT: Definitive information about the breeding system of *Rutidosis lanata* and whether or not it is self-incompatible. Determination of whether a low S-allele number is likely to be responsible for observed reproductive failure and if so whether inter-population transfer of plants can increase genetic mate availability and restore seed set.

SPECIFIC DELIVERABLE: Guidelines regarding how moving plants among populations can be used to increase local genetic variation and restore mate availability and reproductive success (seed set).

Task 5.

TASK NAME: Client workshop

TASK LEADER: Andrew Young

OVERALL TIMEFRAME: Apr 2015 – May 2015

TASK DESCRIPTION: A workshop will be held with the client.

TASK OBJECTIVE: Review project results and implications for translocation as well as presenting generic information regarding biological factors affecting restoration targets and success.

TASK OUTPUTS & SPECIFIC DELIVERABLES: Client workshop explaining results and implications for translocation, as well as presenting generic information regarding biological factors affecting restoration targets and success.

Task 6.

TASK NAME: Delivery of Report

TASK LEADER: Andrew Young

OVERALL TIMEFRAME: May 2015 – June 2015

BACKGROUND: Project reporting is a key deliverable.

TASK OBJECTIVE: To produce a final report.

TASK OUTPUTS & SPECIFIC DELIVERABLES: Final report.

15. Budget Justification

The project leader Prof. Andrew Young (CSIRO) has 25 years of experience in plant conservation biology research specifically in the areas of genetic and ecological work that form the basis of this project. He has a strong international publication record and has been awarded an Australian Academy of Science medal for his work in plant conservation. He will take direct responsibility for completion of activities in Canberra.

Mr David Marshall is an experienced plant ecology technician who has recently undertaken training in plant cytogenetics.

The budget is primarily for the salaries of Young (5%) and Marshall (65%) who will undertake the bulk of the technical work associated with the project.

Additional budget items are for laboratory and glasshouse costs for the chromosome analysis and crossing studies.

16. Project Governance

Project reporting will be as per standard GISERA project reporting procedures. Interim reporting schedule will be:

Task	Milestone description	Due date
3	Report on flow cytometry	Apr 15
4	Report on pollination experiment	Apr 15
5	Client workshop	May 15
6	Final report	Jun 15

CSIRO will provide Graeme Bartrim and/or Laura Hahn with draft copies of scientific outputs/papers four weeks prior to submission for publication.

17. Communications Plan

Communications activities or this project will be undertaken in line with the GISERA Communications Plan.

18. Risks

The key project risk is the availability of sufficient flowering plants for the crossing studies and the ability to source fresh leaf material and roots tips for the chromosomal analyses. To minimise this risk live plants will be transferred from the nursery in Brisbane to the CSIRO Canberra where they can be grown. Department of Environment and Heritage Protection Guidelines must be followed to undertake this process. Note that CSIRO has suitable quarantine facilities but should it be necessary to use these rather than standard glasshouses additional costs are likely

Laboratory and glasshouse procedures in Canberra will all be compliant with CSIRO OHS regulations.

The project will not generate commercially valuable IP.

Capacity to deliver: The CSIRO science team is experienced in both chromosomal analysis and the crossing experiments involved in the plant breeding system work. If plants cannot be successfully transferred to Canberra then Origin Energy or Greening Australia staff will have to be trained to undertake crossing experiments and harvest roots tips and leaf samples from plants in Brisbane. This should be relatively straight forward to achieve.

Project Management: The project will be managed by Prof. Andrew Young (CSIRO) who has 25 years of experience in plant conservation biology research. He will take direct responsibility for completion of activities in Canberra and will work to assist with planning and training of staff to undertake activities that may be required in Brisbane should this be necessary.

19. Intellectual Property and Confidentiality

Background IP (clause 10.1, 10.2)	**Party**	**Description of Background IP**	**Restrictions on use (if any)**	**Value**
	CSIRO	None		$
				$
Ownership of Non-Derivative IP (clause 11.3)	NA			
Confidentiality of Project Results (clause 15.6)	Project results are not confidential.			
Additional Commercialisation requirements (clause 12.1)	NA			
Distribution of Commercialisation Income (clause 1.1)	NA			
Commercialisation Interest (clause 1.1)	**Party**		**Commercialisation Interest – N/A**	
	APLNG			
	CSIRO			
	QGC			

GISERA Gas Industry Social & Environmental Research Alliance

20. Approval from Project Parties

In signing this document you are committing your organisation to provide the specified funds, personnel and the required in-kind contributions.

Australia Pacific LNG

SIGNED for and on behalf of Australia Pacific LNG, exercising authority delegated by the GISERA Management Committee

.....Dan O'Sullivan.....

by
in the presence of

.....
Signature of witness

KRISTEN FORBES
.....
Name of witness

21/8/14.
.....
Date

QGC Pty Ltd

SIGNED for and on behalf of QGC Pty Ltd, exercising authority delegated by the GISERA Management Committee

.....
Brett Smith

by
in the presence of

.....
Signature of witness

TAWANCHAI TIRRIS
.....
Name of witness

25/08/14
.....
Date

GISERA Gas Industry Social & Environmental Research Alliance

CSIRO

SIGNED for and on behalf of CSIRO, exercising authority delegated by the GISERA Management Committee

.....

by
in the presence of

.....
Signature of witness

EUGENIA TAN
.....
Name of witness

28 August 2014
.....
Date

2 Variations to Project Order

Changes to research Project Orders are approved by the GISERA Director, acting with authority provided by the GISERA National Research Management Committee, in accordance with the National GISERA Alliance Agreement.

The table below details variations to research Project Order.

Register of changes to Research Project Order

Date	Issue	Action	Authorisation
09/12/14	Recent extreme weather conditions in south Queensland has resulted in natural populations of Rutidosis Lanata suffering from severe stress. Unfortunately harvesting individuals in these dry, hot conditions will not likely provide any live plants that are in good condition. Assuming that forecast rain occurs in December/January, Origin staff plan to survey mid-January, providing CSIRO with live plants in February.	Milestones 2, 3, 4, 5 and 6 will be pushed back by 5 months.	
11/11/15	Work is running late due to original delays in getting plants and also in plants acclimatising to Canberra glasshouse conditions. This has meant that we are only now seeing large amounts of flowering. Given this we will need to change the delivery dates for remaining milestones.	Milestones 4, 5 and 6 will be pushed back.	
14/06/16	The workshop date has been pushed back to ensure maximum participation. This will impact delivery date of final report.	Milestone 5 will be pushed back to July 2016 and milestone 6 will be pushed back to September 2016.	

3 Progress against project milestones

Progress against milestones are approved by the GISERA Director, acting with authority provided by the GISERA National Research Management Committee, in accordance with the National GISERA Alliance Agreement.

Progress against project milestones/tasks is indicated by two methods: Traffic Light Reports and descriptive Project Schedule Reports.

1. Traffic light reports in the Project Schedule Table below show progress using a simple colour code:
   - Green:
     - Milestone fully met according to schedule.
     - Project is expected to continue to deliver according to plan.
     - Milestone payment is approved.
   - Amber:
     - Milestone largely met according to schedule.
     - Project has experienced delays or difficulties that will be overcome by next milestone, enabling project to return to delivery according to plan by next milestone.
     - Milestone payment approved for one amber light.
     - Milestone payment withheld for second of two successive amber lights; project review initiated and undertaken by GISERA Director.
   - Red:
     - Milestone not met according to schedule.
     - Problems in meeting milestone are likely to impact subsequent project delivery, such that revisions to project timing, scope or budget must be considered.
     - Milestone payment is withheld.
     - Project review initiated and undertaken by GISERA Research Advisory Committee.

2. Progress Schedule Reports outline task objectives and outputs and describe, in the 'progress report' section, the means and extent to which progress towards tasks has been made.

Project Schedule Table

ID	Task Title	Task Leader	Scheduled Start	Scheduled Finish
Task 1	On signing of contract	Andrew Young	Aug 14	Aug 14
Task 2	Specimen delivery	Andrew Young	Sept 14	Mar 15
Task 3	Completion of flow cytometry	Andrew Young	Oct 14	Sep 15
Task 4	Completion of Pollination experiment	Andrew Young	Oct 14	Mar 16
Task 5	Client Workshop	Andrew Young	Apr 15	Jul 16
Task 6	Delivery of report	Andrew Young	May 15	Sep 16

Project Schedule Report

Task 1.

TASK NAME: Sign contract.

TASK LEADER: Andrew Young

OVERALL TIMEFRAME: August 2014

BACKGROUND: Contract needs to be signed to allow project to proceed.

TASK OBJECTIVE: To sign the contract.

TASK OUTPUTS & SPECIFIC DELIVERABLES: Signed contract.

PROGRESS REPORT:

The contract has now been signed, and the project released for research to commence.

Task 2.

TASK NAME: Specimen Delivery.

TASK LEADER: Andrew Young

OVERALL TIMEFRAME: Sept 2014 – Oct 2014

BACKGROUND: Plants must be transferred from the nursery in Brisbane to CSIRO Canberra where they can be grown. Department of Environment and Heritage Protection Guidelines must be followed to undertake this process.

TASK OBJECTIVE: Live plants to be transferred from the nursery in Brisbane to CSIRO Canberra.

TASK OUTPUTS & SPECIFIC DELIVERABLES: Delivery of live plants to CSIRO Canberra.

PROGRESS REPORT:

Plants have now been received in Canberra from a range of populations. They are now housed in CSIRO glasshouses and are acclimatizing to the new conditions. Many plants appear to have been dug straight from the ground and potted up including associated vegetation. Mortality from transplant shock is ongoing and may reach 25%. Depending on this some re-sampling may be required.

Task 3.

TASK NAME: Completion of flow cytometry

TASK LEADER: Andrew Young

OVERALL TIMEFRAME: Oct 2014 to Apr 2015

BACKGROUND: Cytogenetic races have been identified in the con-generic species Rutidosis leptorrhynchoides which has diploid (2n=2x=22), tetraploid (2n=4x=44) and hexaploid (2n=6x=66) plants. Mixing of individuals with different base chromosome numbers is likely to result in the generation of dysgenic progeny (seed) with uneven chromosome numbers that will be infertile. Such chromosome mixing events cannot be reversed and are likely to represent a long-term threat to the viability any re-established population. Therefore identification of any chromosome races

that exist within R. lanata is very important in terms of sourcing and deployment of plants for translocation.

TASK OBJECTIVE: Screen 10-15 populations for differences in genome size using flow cytometry (5-10 individuals per population) and confirm chromosome numbers of each ploidy group using chromosome counts from root squashes of representative individuals (if available).

TASK OUTPUT: Geographical mapping of cytogenetic variation in Rutidosis lanata and guidelines identifying common cytogenetic population groups that can be mixed and those that can't due to the likelihood of generating dysgenic plants.

SPECIFIC DELIVERABLE: Guidelines for translocation and population mixing that explicitly take cytogenetic variation into account.

PROGRESS REPORT:

120 plants from the 10 populations supplied have been screened for genome size using flow cytometry. Results indicate significant inter-population variation in genome size exists with tetraploid (2n=48), pentaploid (2n=60) and hexaploid (2n=72) plants observed. These genome sizes have been confirmed with chromosome counts of plants in each genome size group. There appears to be some general north to south structure to this variation, but analysis of a wider geographical range of populations will be required to confirm this.

Population specific crossing guidelines will be delivered as part of final report once information on fertilization rates from inter-population crosses (Task 4) is available.

Status – complete.

Task 4.

TASK NAME: Completion of Pollination experiment

TASK LEADER: Andrew Young

OVERALL TIMEFRAME: Oct 2014 to Apr 2015

BACKGROUND: Current observations of seed set in *R. lanata* indicate reproductive failure (very low seed set) in nursery populations. Two other species in the genus *Rutidosis* (*R. leptorrhynchoides* and *R. leiolepis*) are both known to have genetic self-incompatibility systems. These systems prevent selfing and mating between relatives and in populations that have low genetic variation at the self-incompatibility locus (S-locus) this will severely constrain seed set. This suggests that low seed set in *Rutidosis lanata* may well be because populations have low numbers of genetic mate types (S-alleles). If this is the case translocation planning should aim to maximize genetic diversity at the self-incompatibility locus by mixing genotypes from different populations to restore mate availability and seed set. This has proved a successful strategy for *R. leptorrhynchoides.*

TASK OBJECTIVE: Conduct controlled-pollination experiments to: a) demonstrate whether *R. lanata* has a genetic self-incompatibility system; b) Measure genetic variation at the incompatibility locus in five populations and determine if low seed set is due to low numbers of genetically compatible mates; c) Conduct inter-population crosses to determine whether mixing plants from different populations (but of the same cytogenic race) can restore S-locus genetic diversity, mate availability and significantly increase seed set.

TASK OUTPUT: Definitive information about the breeding system of *Rutidosis lanata* and whether or not it is self-incompatible. Determination of whether a low S-allele number is likely to be

responsible for observed reproductive failure and if so whether inter-population transfer of plants can increase genetic mate availability and restore seed set.

SPECIFIC DELIVERABLE: Guidelines regarding how moving plants among populations can be used to increase local genetic variation and restore mate availability and reproductive success (seed set).

PROGRESS REPORT:

Within each of the ten experimental populations provided crosses were conducted with the aim of filling in a complete diallel of every plant against every plant of the same population, including self-crosses. Self-crosses were used to confirm the existence of self-incompatibility in *R. lanata* and to calculate a statistical cut-off for the differentiation of successful and unsuccessful crosses. The full diallels were used to estimate the number of S-alleles in each population. In addition, numerous crosses were conducted between populations to test if mate availability could be increased by outcrossing. Each cross included bagging of the flower heads before fertility to exclude uncontrolled pollination, three controlled pollinations 2-3 days apart, harvesting of ripe seed-heads c. 2-4 weeks later, and counting of fully developed seeds. As of 18 March 2016, more than 900 individual crosses have been conducted, resulting in 1,848 counted seed-heads. Mate availability has been inferred, and S-allele numbers have been inferred with a custom-written computer program. **Status – complete.**

Results indicate that:

a) *Rutidosis lanata* has a strong self-incompatibility system. Only one experimental plant in the Gilmore population was consistently able to self-pollinate.

b) Low genetic variation for S alleles does mean that mating success is limited in some populations, and this could be contributing to low observed seed set in populations in the field.

c) Crosses between plants from different populations are generally more successful than within-population crosses suggesting that mixing plants from different sources (but with the same chromosome number, see task 3) should maximize translocation success.

Mate availability

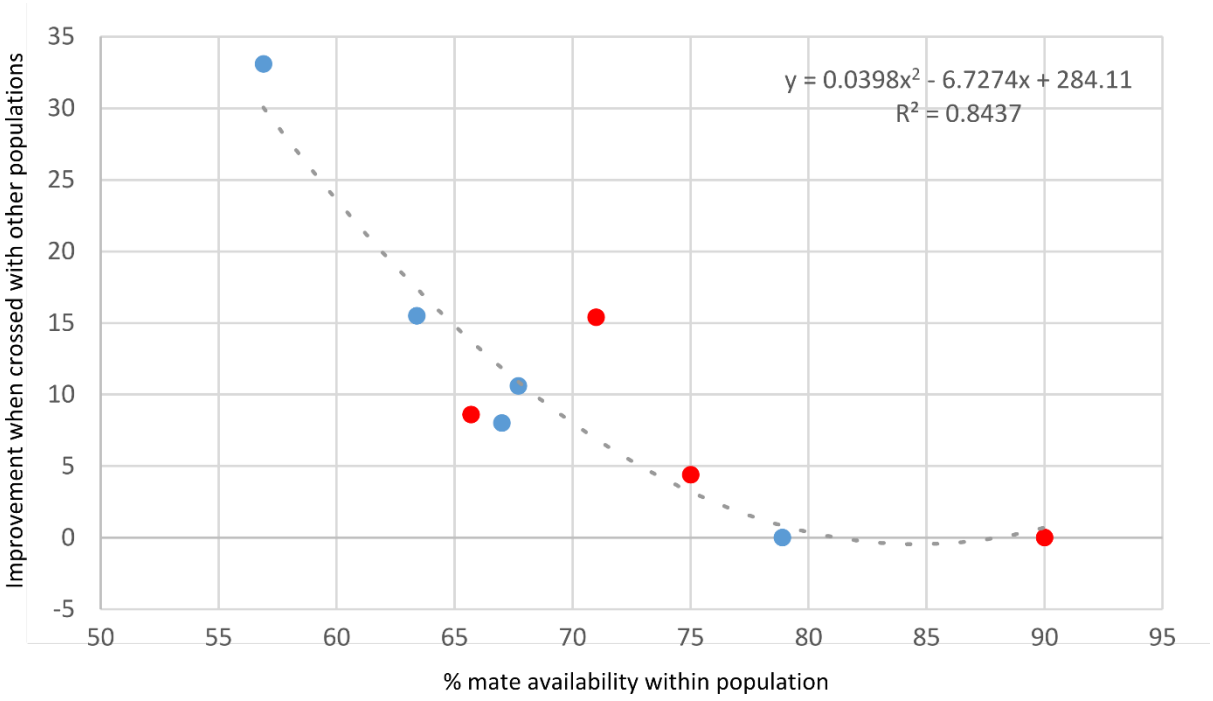
The following table shows mate availability (MA) in each population, separate by ploidy level, and improvement when crossed against plants at same ploidy level outside of the population. No statistics were generated for minor cytotypes in populations marked with asterisks as they represented only four or less experimental plants. No mate availability was calculated for pentaploid plants because in their case male sterility as opposed to genetic compatibility has the greatest impact on the results. (Note that Campbell 5 consequently had to be excluded as it had too few tetraploids and hexaploids.) S-allele estimates under the assumption of polyploidy of the S-locus and codominance are included for comparison.

Population (and ploidy level)	S-allele estimates	MA in pop.	MA when crossed with other pops	Improve-ment
Campbell 1 (tetraploid), 7 plants	18-24	78.9%	77.4% (n=31)	None
Campbell 2 (tetraploid), 11 plants	23-31	63.4%	78.9% (n=19)	15.5%
Campbell 3 (tetraploid), 12 plants	27-36	67.7%	78.3% (n=23)	10.6%
Campbell 4 (tetraploid), 9 plants*	16-21	56.9%	90.0% (n=30)	33.1%
Gilmore (tetraploid), 7 plants*	14-18	67.0%	75.0% (n=16)	8.0%
Chaplin 1 (hexaploid), 9 plants	42-51	90.0%	89.7% (n=39)	None
Chaplin 2 (hexaploid), 9 plants	32-45	71.0%	86.4% (n=22)	15.4%
Little 1 (hexaploid), 5 plants*	24-27	75.0%	79.4% (n=34)	4.4%
Little 2 (hexaploid), 11 plants	39-50	65.7%	74.3% (n=35)	8.6%

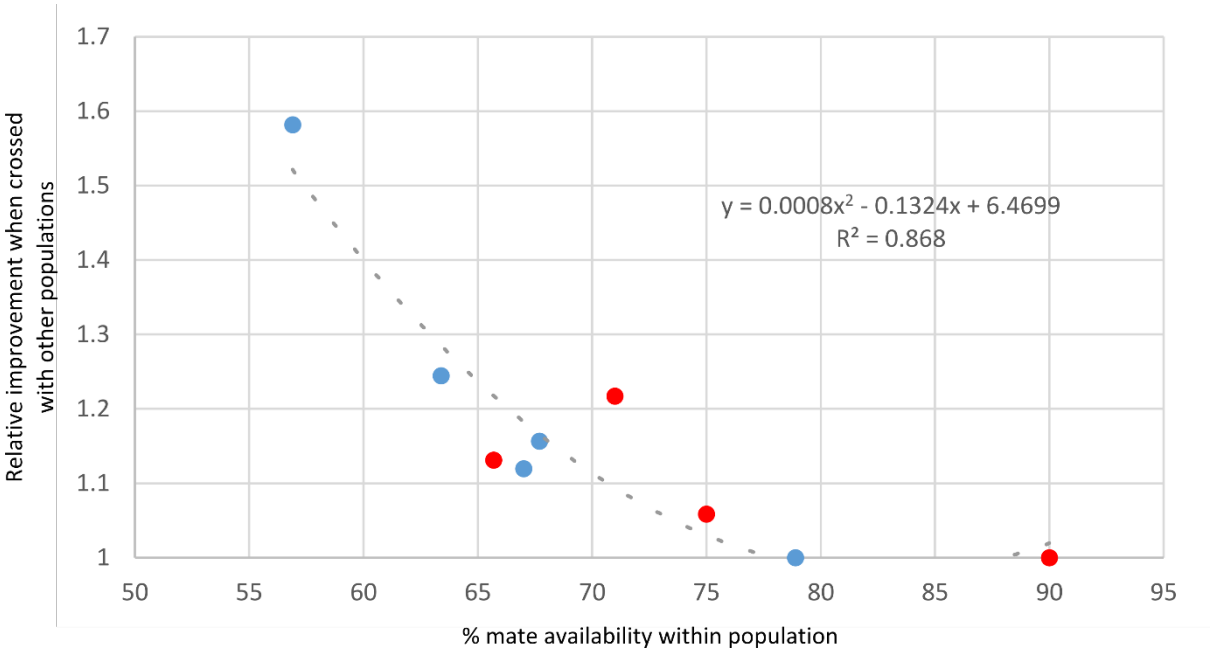
Improvement of mate availability through crossing between populations

Mate availability can be increased by introducing plants from other populations at the same ploidy level. Populations with more than c. 75% mate availability do not appear to see a benefit, but genetically impoverished populations may see very significant improvements in seed set.

Graph showing absolute increases in MA (e.g. 15% if it rises from 60% to 75%):



Graph showing relative increases in MA (e.g. 1.25 if it rises from 60% to 75%):



Tetraploid populations are blue, hexaploid ones red.

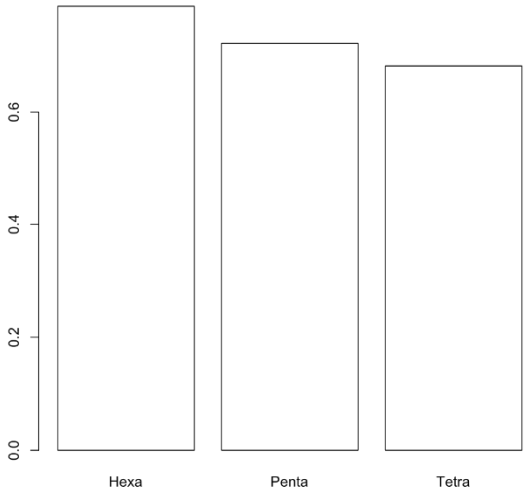
Pentaploid plants suffer major reproductive fitness loss in their male function

Results indicate that mixing of cytological races (different ploidy levels) should be avoided. Four of the experimental populations are naturally mixed, and in at least three of them crosses between tetraploid and hexaploid plants have produced pentaploid hybrids. The crossing experiments demonstrated that pentaploid plants suffer from a significant loss of male fertility, which will likely have a negative impact on population viability.

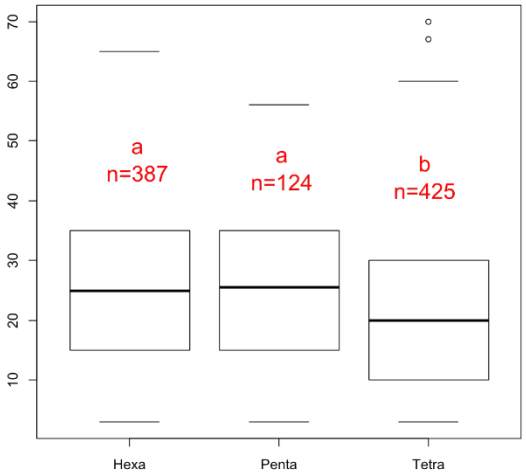
In the following figure, the left plots show crossing success, the right plots show seed set of successful crosses. The top row shows how different ploidy races perform as mothers (producing seeds themselves), the bottom row shows how they perform as fathers (fertilizing seeds of other plants).

Pentaploids can set seed normally. As fathers, however, they produce only ca. half as many successful crosses as the even ploidy levels, and even in their successful crosses they produce very few seeds.

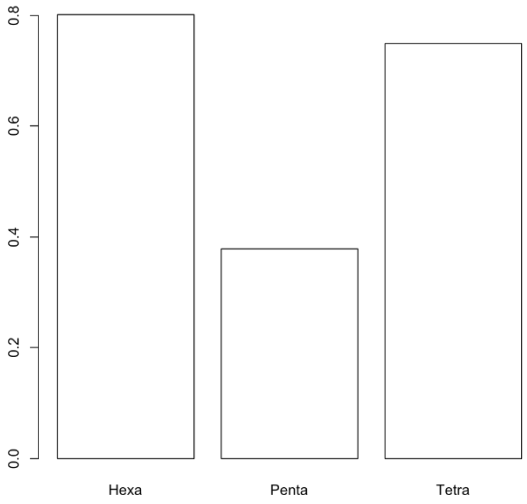
Percentage of successful crosses for mothers of different ploidy levels (within and between populations)



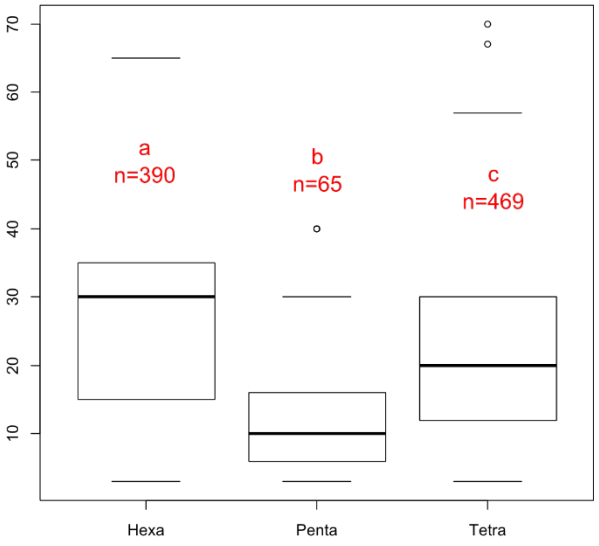
Seed set of successful crosses for mothers of different ploidy levels (within and between populations)



Percentage of successful crosses for fathers of different ploidy levels (within and between populations)



Seed set of successful crosses for fathers of different ploidy levels (within and between populations)



Task 5.

TASK NAME: Client workshop

TASK LEADER: Andrew Young

OVERALL TIMEFRAME: Apr 2015 – May 2015

TASK DESCRIPTION: A workshop will be held with the client.

TASK OBJECTIVE: Review project results and implications for translocation as well as presenting generic information regarding biological factors affecting restoration targets and success.

TASK OUTPUTS & SPECIFIC DELIVERABLES: Client workshop explaining results and implications for translocation, as well as presenting generic information regarding biological factors affecting restoration targets and success.

PROGRESS REPORT:

The delays in Tasks 3-5 led to the workshop being postponed. However, in the interim, both a poster and a project information sheet describing the results so far and the implications for *R. lanata* translocation planning have been developed so that they can be used as communications resources. They were presented at the GISERA meeting 27-28 Oct 2015 in Brisbane. We discussed our results with industry stakeholders (Origin Energy, ECOAUS) in a phone meeting on 17 March 2016. The results were again communicated in a c. 20 min talk presented at the GISERA Knowledge Transfer Session for the biodiversity projects on 27 May 2016 in Brisbane.

The client workshop 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". It was attended by c. twenty participants representing a good diversity of industry stakeholders, policy makers and academics: Leanne Stevens (Arrow Energy), Brad Dreis (ECOAUS), Graeme Bartrim (Origin Energy), Laura McCallion and Cameron Playsted (QGC), Ben Barker and 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), Tsuey Cham (GISERA communications advisor), and Linda Broadhurst, Francisco Encinas-Viso, Sarah Mathews, Alexander Schmidt-Lebuhn and Andrew Young (CSIRO).

Four talks in the morning provided an introduction into the importance of genetics for long-term successful and efficient ecological offset work, one of them presenting the results of the present project. The afternoon was dedicated to group work on participant-nominated species of current or future management concern for the CSG industry, applying the information discussed in the morning.

Task 6.

TASK NAME: Delivery of Report

TASK LEADER: Andrew Young

OVERALL TIMEFRAME: May 2015 – June 2015

BACKGROUND: Project reporting is a key deliverable.

TASK OBJECTIVE: To produce a final report.

TASK OUTPUTS & SPECIFIC DELIVERABLES: Final report.

PROGRESS REPORT:

The delays in Tasks 3-5 led to the report being postponed. It was submitted to GISERA on 19 Sept 2016.