Understanding Mulga

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PROGRESS REPORT: YEARS 1-3 (2006-2009)

EXECUTIVE SUMMARY

This report summarizes progress with the *Understanding Mulga* project for the three year period, October 2006 to November 2009.

A comprehensive field program was undertaken resulting in the collection of 1266 herbarium samples, 1682 genetic samples and 200 seed samples which form the basis of the study. Most of the important Mulga areas of the State were sampled; the main areas omitted were the eastern deserts but previously-collected specimens from these areas were available for study therefore this omission has not seriously constrained the project. Field studies have proved especially important because, apart from providing material for laboratory examination, they have facilitated a better understanding of the extremely complex and often cryptic patterns of variation within Mulga. Further field work in the Pilbara in 2010 would be informative if pods are produced this year. The drought conditions that have persisted for the past three years have posed particular and serious problems for the field program. As a result we were often not able to collect pod material which is so important to resolving the taxonomy of Mulga; this constraint was ameliorated by the use of previously collected herbarium material.

A major achievement of the project has been the elucidation of variation patterns within the Western Australian Mulga flora. Mulga in this state is now viewed as comprising about 15 species that are arranged in three major alliances (the Blue, Green and Grey-green alliance). Attaining this taxonomic resolution is very significant because the alliances represent the conceptual framework for understanding Mulga and the species form the taxonomic 'backbone' for the study. Work on refining this classification is currently in progress and is expected to be completed by the middle of 2010. Species descriptions having been commenced; 10 of the 15 species will be described as new to science.

Detailed study of characters has revealed that the following are the most useful in both naming species and in defining species and alliances: branchlet resin (translucent vs opaque); new shoot colour, resinosity and indumentum; phyllode shape, size, colour, curvature and cross-section shape; flower sepal fusion and size; pod shape, size and the nature of the marginal 'wing'. The importance of the branchlet resin and new shoot characters was hitherto unknown.

On a broad scale the chloroplast DNA data correlates well with the morphological data with respect to definition of species and alliances within Mulga. Genetic results also show that most Mulgas are polyploids and that hybridity, apomixis and neoteny occur within at least some communities and contribute to the variation and complexities within these communities.

An electronic key for the identification of Western Australian Mulga entities (i.e. species, variants and some hybrids) has been commenced and the first draft will be distributed in June 2010 for testing. This key will prove an invaluable tool for anyone wishing to quickly and easily name Mulga entities (even when plants are without flowers or fruits), and

to view descriptions and images (photographs and line drawings) of them. The key will be distributed on CD and via the web.

A number of partnerships have been established and these include: Dr. Shao Fang Wang (Chemistry Centre of Western Australia) – study of gum chemistry; Gerald Page (University of Western Australia) – ecological study of West Angelas Mulga populations; Dr Rolf Rutishauser (University of Zurich) – study of pod anatomy; and Curtin University of Technology – cultivation of Mulga seedlings.

Publicity for the Understanding Mulga project has been undertaken through two actively-maintained websites, http://www.worldwidewattle.com/infogallery/projects/mulga.php and http://www.cpbr.gov.au/jmiller/mulga.php, and a Department of Environment and Conservation Information Sheet (which is included with this report). An article in the popular DEC magazine, *Landscope*, will be produced towards the end of the project.

A workshop designed to inform sponsors about the project and to provide them with the opportunity to guide future activities was conducted in August 2008. A workshop on the use of the identification key can be delivered after June 2010 should the sponsors require it.

The project will be completed by June 2011 and a schedule of activities for the next 18 months is presented. The principal outputs that will be completed within this time-frame will include: (1) a comprehensive taxonomic revision of Western Australian Mulgas; (2) a genetic overview of Mulga that will include a focus on factors responsible creating and maintaining variation within the group; (3) a 'user friendly' Mulga Manual that will comprehensively describe, discuss and illustrate W.A. Mulga species; and (4) an electronic key to facilitate the identification of Mulgas (to be produced on CD and via the web). Miscellaneous small scientific papers will also be produced, the first of which is currently in preparation (pods of Mulga).

Notwithstanding some constraints encountered over the past three years (most notably the prolonged drought that has prevented collection of flowers and pods) we consider that the outputs of this project will represent a major step forward in the understanding of Mulga in Western Australia, and will establish a sound foundation for further studies of this, large, complex and important arid zone group of plants.

INTRODUCTION

The following Report summarizes progress with the *Understanding Mulga* project for the three year period, October 2006 to November 2009. In previous years we provided annual reports of activities (copies of these two reports are attached as Appendix 2 and 3 for your information), however, the present concatenated report is deemed more appropriate this year as it provides a clearer overview of progress to date. This is particularly relevant since a request has been made to extend the project by one year.

FIELD STUDY AND SPECIMEN PROCESSING

A comprehensive field program has been undertaken over the past three years. Details of individual trips are provided in Appendix 1 where it is seen that a total of 1266 herbarium collections, 1682 genetic samples and 200 seed samples have been gathered. All collections have been processed (i.e. mounted and data based) and are now lodged at the W.A. Herbarium; genetic material has been extracted for DNA which is with Miller in Canberra; most seed has been germinated. Additional to our own gatherings we have used

the collections at the W.A. Herbarium and a selection of specimens sent to us on loan from the Adelaide, Alice Springs, Brisbane and Melbourne herbaria.

Most of the important Mulga areas of the State have been visited over the course of this study, but as would be expected, our sampling of populations has been selective within the regions that we visited. Nevertheless, we suspect that most the significant Mulga taxa have been encountered in our field studies.

The only Mulga areas in W.A. not yet visited include much of the eastern deserts and in particular, the central ranges to the east of Warburton. Apart from being logistically challenging to collect from these regions given their remoteness and the relatively short duration of the project, we considered the likelihood of getting useful (pods/flowers) material from these regions to be extremely low because of the prolonged drought that has affected the area. We therefore assessed that it was not a good use of resource (either time or money) to mount trips to these regions. We do not consider, however, that this omission from the field program has compromised the project because we have been able to examine preserved specimens (with pods and flowers) from the region at the Perth, Adelaide and Alice Springs herbaria.

Understanding Mulga morphotypes in the field has been especially important to this study. Being able to study intra- and inter-population variation provided a critical context for our subsequent examination of collections. In particular it enabled us to identify possible hybrids (hybridity is seemingly very common in this group) and to gain insights into the extremely complex and very often cryptic patterns of variation that exist both within and between Mulga morphotypes. Compared with other *Acacia* species the variation that has been observed in Mulga is extreme. Although we have analysed and documented much of this variation it is not at all likely that we have identified all the variants, forms, etc. that occurs within the group. We do believe, however, that the main entities have been recognized and elucidated (see **MAJOR TAXONOMIC GROUPINGS WITHIN MULGA** below).

A very serious problem associated with our field studies has been the drought conditions that have persisted for the three year period over the entire project area. As a result there are many instances where we have not been able to collect pod material which is so important to resolving the taxonomy of Mulga. An additional problem occurred in 2009 when health problems prevented the chief taxonomic investigator (Maslin) from participating in the comprehensive Pilbara field studies that had been planned for October of that year. Although a scaled-down trip was undertaken by Reid (accompanied by visiting Swiss anatomist Dr R. Rutishauser), much of what was hoped to be achieved in the Pilbara in 2009 has had to be deferred/cancelled.

For the remainder of this project the Pilbara region remains our main focus of attention insofar as field studies are concerned. To this end we retain regular contact with workers in the region in order that we may plan trips to coincide with flowering and, more particularly, fruiting events.

HERBARIUM STUDY & MORPHOLOGICAL ANALYSES OF VARIATION

A sort and scrutiny of the almost 3000 dried herbarium specimens of Mulga housed at the Western Australian Herbarium has been undertaken. Primary focus was given to the Maslin/Miller material collected in 1999 and 2000, the Maslin *et al.* material collected in connection with our 2006-2009 field program (see above), and a West Angelas collection by PhD student Gerald Page (see **PARTNERSHIPS** below). These primary-focus collections represent the most taxonomically informative material available to the project because the specimens are well-documented, comprehensive in their scope at the population level, and most are complemented by material gathered for genetic scrutiny. Additional to the above we have studied selected critical material from interstate herbaria in Alice Springs, Adelaide and Melbourne.

Morphological analysis of variation was undertaken using conventional taxonomic methodology. This study has revealed that most of the variation in Mulga in Western Australia can be accommodated by about 15 species (see below under **MAJOR TAXONOMIC GROUPINGS WITHIN MULGA**). Morphological characters that have proved most useful in defining Mulga taxa and groups of Mulga taxa include the following: branchlet resin; new shoot colour, resinosity and indumentum; phyllode shape, size, colour, curvature and cross-section shape; flower sepal fusion and size; pod shape, size and the nature of the marginal 'wing'. The study of branchlet resin revealed the existence of an important and hitherto unknown taxonomic character, namely, the resin comprised two morphologically distinct types, a red-brown translucent resin and an opaque milky white resin. This character has proved very useful in helping name specimens, especially when pods and flowers are lacking. Also, when resin type is used in combination with the pod 'wing' character we are able to establish what is considered a broad conceptual framework for understanding variation within Mulga. This framework comprised the recognition of three major groupings of Mulga which are called the Green, Grey-green and Blue alliances, for which there is genetic support (see under **GENETIC STUDIES** below). These alliances now provide a useful framework for discussing the broad patterns of variation within the Mulga group.

GENETIC STUDIES

The goals of the genetic work are two-fold. Firstly, in concert with the morphological investigation, to determine species boundaries and evolutionary relationships of the Mulga entities. Secondly, to investigate the underlying processes that generate and are maintain the complicated diversity found in Mulga.

The genetic component of the study uses two major sources of data, namely, DNA sequences from the maternally inherited chloroplast and secondly, DNA fingerprinting with biparentally inherited microsatellite markers. Additionally we explored two other types of data, DNA ploidy analysis and the new technology of Next Gen DNA sequencing.

A. DNA SEQUENCES OF THE MATERNALLY INHERITED CHLOROPLAST

Chloroplast DNA sequence provides a conservative evolutionary signal. The goal is to determine broad patterns of diversity within mulga. A secondary goal is to find deviations from this broad pattern that

indicate evolutionary processes that are acting upon the plants. Five regions of the chloroplast DNA were determined in the first part of the Understanding Mulga project to contain DNA polymorphisms. DNA sequences have been generated for over 500 collections of Mulga. These samples represent an even sampling of the diversity of Mulga in Western Australia and include a small sampling of other material (mostly N.T. taxa) needed to develop an evolutionary context. The sampling strategy was driven by recommendations based on the morphological analyses.

On a broad scale the chloroplast DNA data correlates well with the morphological data with a few exceptions. In consultation with the morphological analyses the genetic data recognize a set of collections define certain Mulga entities (Figure 1A). The DNA sequences of this subset was analyzed separately and is shown in Figure 1B). In these figures each branch off the core black stem represents an evolutionary lineage. The lineages are colored based on the morphological groupings (see **MAJOR TAXONOMIC GROUPINGS WITHIN MULGA** below). For example, the grouping marked with blue branches are most members of the Blue Alliance, on the left marked in green are most members of the Green Alliance and near the bottom marked in grey are most members of the Grey-green Alliance.

Several major trends are evident. Firstly, the chloroplast DNA results suggest that the Blue Alliance is more closely related (i.e. shares more evolutionary history) to the Green Alliance than to the Grey-green Alliance. Secondly the *A. aneura* collections from the type locality in the Flinders Range in South Australia are not part of any of the three major alliances. They are, however, most closely related to the Grey-green Alliance (Figure 1, marked in purple).

There are some taxa and also some individual plants in which the genetic data does not correlate directly with the morphological data. For example *A. minyura* (Figure 1, marked in red) is an independent lineage genetically distinct from the rest of the Blue Alliance. The genetic data suggests that while it maintains similarity with the Blue Alliance in some morphological traits such as opaque branchlet resin and winged pods, it is a distinctive evolutionary lineage. It could have originated by either of two phenomena. Either *A. minyura* resulted from a hybridzation event between a an ancestral member of the Blue Alliance and another Mulga entity, most likely a ancestor or the Grey-green Alliance or *A. minyura* has been an independent lineage and some of its morphological characters are randomly similar to the Blue Alliance (lineage sorting). Our data can not discriminate between these alternatives. In either case the taxonomic status of *A. minyura* is not affected by its evolutionary origin. A similar scenario is hypothesized for *A. ayersiana*.

A surprising result is the grouping in the chloroplast DNA data of is the grouping of two entities of the Green Alliance (*A. aneura* var. *macrocarpa* and *A.* sp. Mulga Paraburdoo (B.R. Maslin et. al. BRM 9201);

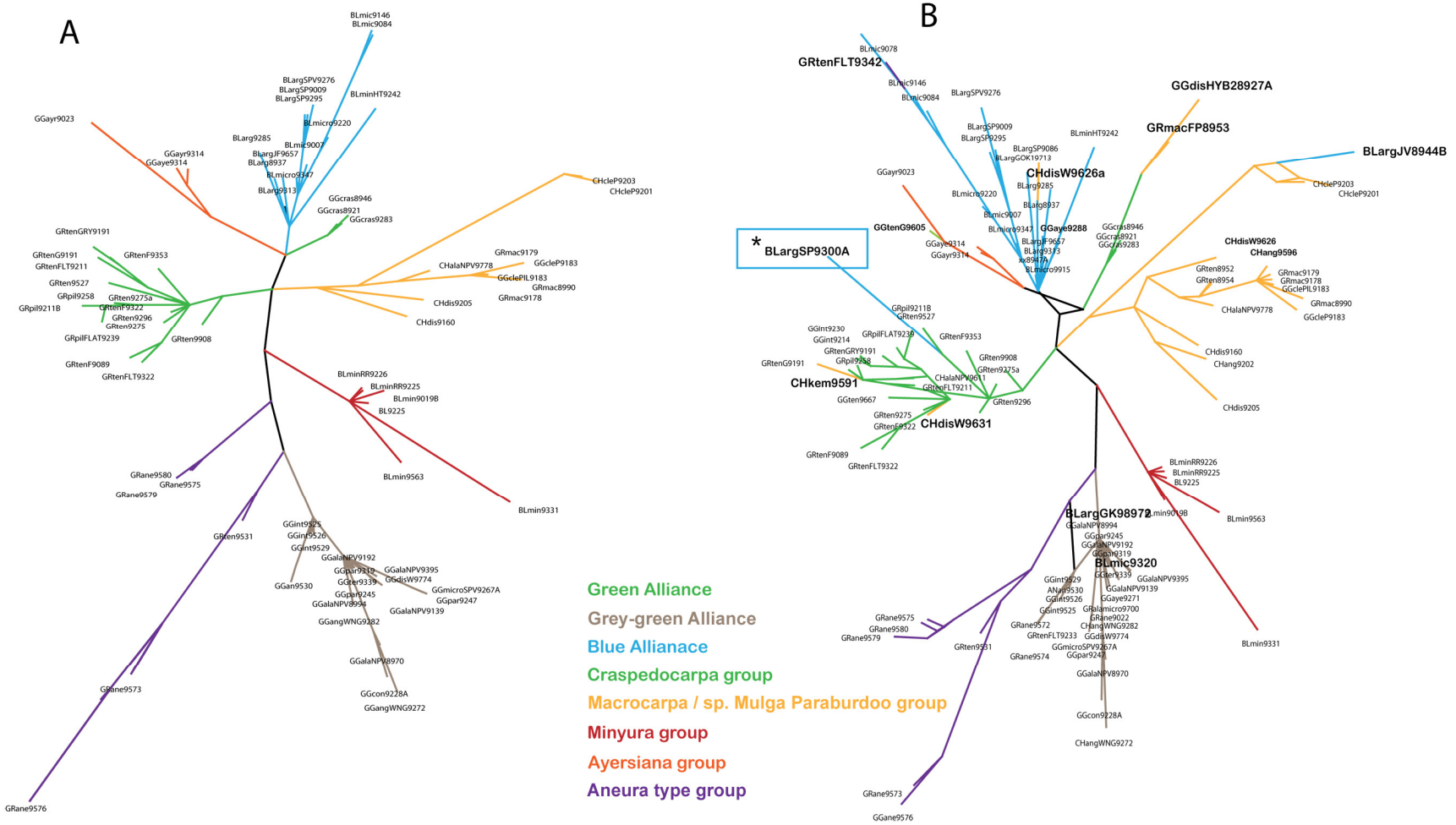


Figure 1. Phylogenetic tree based on plastid DNA sequence showing the relationship of individual Mulga plants. A. The plants used in this tree are confirmed members of the alliance or group based on morphological analysis. Branches are colour coded to each group. B. DNA sequences of other Mulga plants were added to the analysis of A. Several plants such as BLargSP9300A, boxed, have the morphological characteristics of one group, Blue, but the DNA sequence of another group, Green; this suggests that this plant is a hybrid.

marked in yellow in Figure 1) on a separate branch from the rest of the Green Alliance. The Green Alliance and the macrocarpa/sp. Mulga Paraburdoo group branch off the main backbone at the same location indicating that they are closely related but that there is a deep evolutionary divergence between these groups.

Within the three major alliances distinctive species can be recognized by morphological characters. For example, the two most common forms of the Blue Alliance are *A. aneura* var. *argentea* and *A. aneura* var. *microcarpa* can be distinguished based on phyllode characters. However, the chloroplast DNA is not variable enough and it can not distinguish the two entities. A similar situation occurs in the Green Alliance among var. *tenuis*, var. *pilbarana* and var. *angusta* (Mt Augustus). The lack of genetic resolution among these entities could be due to their evolutionary history has not been old enough to accumulate changes, or because there is gene flow among the entities, or most likely that both phenomena are occurring.

The data above describes the interrelationships of the major lineages and suggest that hybridization and lineage sorting are factors affecting the evolution of Mulga. Many plants sampled in the study do not easily correlate to the entities described below. To investigate these species their chloroplast DNA was sequenced and added to the previously discussed dataset. Several examples are indicated on Figure 1B. For example, BLargSP9300A can be classified morphologically as a member of the Blue Alliance, however, its chloroplast DNA is that of a member of the Green Alliance, indicating recent hybridization between the two alliances. Other examples are indicated by the non-correlating branch colours. This interactive morphological-genetic analysis can help identify collections that do not easily fit in morphological categories or can partially identify collections that are sterile, that is they do not have pods which are necessary to determine the defining pod 'wing' character state.

B. DNA FINGERPRINTING WITH BIPARENTALLY INHERITED MICROSATELLITE MARKERS

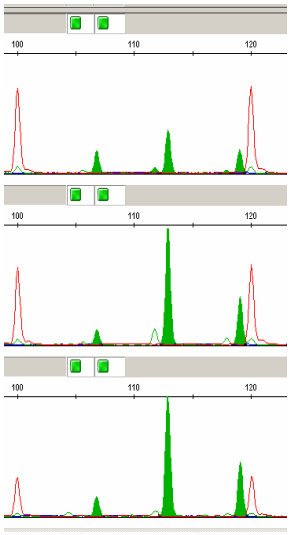
The goal of the DNA fingerprinting was to use this more variable marker system to learn more about the relationships among entities within alliances. Early in the project a microsatellite DNA library was generated with over a dozen microsatellite loci developed. Eight microsatellite regions proved reliable for DNA amplification and scoring of alleles. Genotypes were generated for over 500 collections of Mulga. These samples represent an even sampling of the diversity of Mulga in Western Australia. The sampling strategy was driven by recommendations based on the morphological and chloroplast DNA analyses. Many of the same samples were used for both the Chloroplast and microsatellite studies.

The microsatellite markers were not able to resolve relationships any better or differently than the chloroplast DNA sequence data. There are two reasons for this result, one biological and one experimental.

As we have seen within the chloroplast DNA there is extensive hybridization both within and among morphological entities of Mulga. The hybridization has the effect of homogenizing the genetic signal and this is what was seen in the results.

Previous chromosome counts and flow cytometry (see below) have indicated that Mulga contains multiple ploidy levels. That is two plants may not have the same number of chromosomes and therefore we would expect different number of markers from plants of different ploidy levels. It was not within the scope of this project to measure the ploidy level of the plants because we lacked access to the appropriate equipment. This incomplete nature of the data has been more important to the data than originally expected. The analysis methodology of the data does not work optimally without the knowledge of ploidy level. I suspect that some of the lack of resolution is due to this phenomenon. Refer below for more information on ploidy and flow cytometry.

Microsatellites were used to test the role of apomixes (asexual reproduction by seed) in Mulga. Twelve seed lots were germinated and DNA extracted and analyzed by microsatellites and compared to the microsatellite profile of the mother tree. In 10 of 12 cases, most of the seedlings were derived from apomictic reproduction. That is, the seedlings were genetically identical to the mother plant (see Figure 2).



Mother plant

Seedling 1

Seedling 2

In this case the three alleles of the mother plant (green peaks) are found in both seedlings. This phenomenon was found in several microsatellite loci. This indicates that it is highly unlikely that sexual reproduction, either outcrossing or self fertilization, produced this pattern.

Figure 2. Microsatellite analysis to test apomixes.

Unfortunately the inability to collect mature seed-containing pods has restricted our ability to test the how widespread this phenomenon is and if it correlates to specific entities. Future work to address this issue will be undertaken when more fruiting material is available.

C. GOALS NOT DIRECTLY PART OF THE MULGA RESEARCH PLAN

I. PLOIDY LEVELS

As noted above we have prior evidence of differing ploidy levels in Mulga. Measurement of ploidy levels was not in the scope of this project but we have been able to generate some preliminary data. This data indicates that all samples studied of the Mulga complex are polyploid ranging from triploid, tetraploid,

pentaploid to hexaploid (see Figure 3). This wide variation has a large affect on the morphological and molecular variation seen in Mulga.

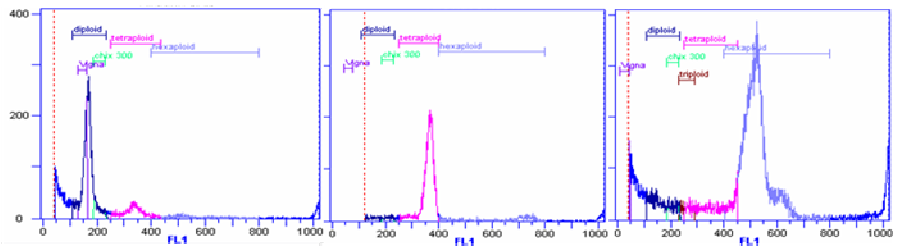


Figure 3. Differing ploidy levels in Mulga.

Recently, Miller has purchased a flow cytometer which is used to measure ploidy level. While it will be difficult to measure ploidy of previously collect material, the machine will be used in the future to measure ploidy. These measurements will be applied to field collected seed.

II. NEW METHODS IN DNA MARKERS

The use of chloroplast DNA is informative for answering Mulga genetic questions. However the application of microsatellite DNA markers was less helpful. Miller is investigating new DNA tools that may provide better resolution to the genetic relationships and clarify the parental types in putative hybrid plants and species. These new markers will be investigated, in conjunction with the new data from flow cytometry, during the coming year. The generation of these data will not require additional funds.

ANCILLARY STUDIES

Pod anatomy. The principal aim of this study is to better understand the taxonomically important pod 'wing' character of Mulga. In 2008 a selection of pod samples (representing the Green, Grey-green and Blue alliances) were sent to Rutishauser in Zurich for anatomical examination and in late 2009 Rutishauser visited Western Australia to undertake field work and herbarium studies. Rutishauser has now completed his anatomical investigations and together with Reid and Maslin is currently preparing a scientific paper for publication.

Seedling ontogeny. The principle aim of this study is to test for neoteny (i.e. the retention of juvenile features in the adult growth stage) within different Mulga morphotypes by observing seedling leaf development. Over the course of the project approximately 200 viable seed samples have been collected, the seed germinated and seedling leaf development documented (i.e. characters measured, plants at different stages of development photographed). For further study judiciously selected subsets of these seedlings were destructively sampled and preserved at the W.A. Herbarium or planted out in a dedicated plot at a Field Trial Area of the Department of Environmental Biology at Curtin University of Technology. It is unlikely that these plantings will be maintained beyond 2010. Preliminary collation and analysis of the

seedling data suggests that neoteny is not present within members of the Green and Grey-green Mulga Alliances, however, further work on the Blue Mulga Alliance is required to assess this group.

Branchlet resin. The aim of this study is to better understand the taxonomically important branchlet resin character (i.e. opaque vs translucent). Ten resin samples have been sent to Dr Shao Fang Wang from the Chemistry Centre of Western Australia for analysis. However, during 2009 there were major disruptions at the Centre due to their premises being relocated to the Curtin University Bentley campus. We therefore have not yet received any results from this analysis.

MAJOR TAXONOMIC GROUPINGS WITHIN MULGA

Based on the above studies we now consider that the majority of variation within Mulga in Western Australian can be accommodated in about 15 species (listed below). These species are arranged under the three major Mulga alliances (called Blue, Green and Grey-green) that have been recognized by this study. Reaching these taxonomic decisions were significant milestones because the alliances represent the conceptual framework for understanding Mulga and because the species will now form the taxonomic 'backbone' for the remainder of the project. They will form the basis of our scientific reviews, the electronic identification key and the Mulga Manual. Each of these species is morphologically variable and Maslin and Reid are currently documenting this variation; they are also precisely characterizing the morphological boundaries of the species to help ensure that each will be effectively identified in the electronic key. This is a slow, accretive process and is the activity that is occupying most of their time at present. This work may possibly result in some additional taxa having to be recognized. Draft descriptions of each of the species has been commenced and are being built up and refined as the detailed taxonomic work progresses. Illustrations of these species will be commenced soon.

At present an interim naming convention has been applied to the 15 species which are listed below. Some already have validly published species names which will persist into the future, namely, *A. ayersiana*, *craspedocarpa*, *A. minyura* and *A. paraneura*. Some have validly published variety names that will need to be raised to species rank in the future, namely, *A. aneura* var. *argentea*, var. *macrocarpa*, var. *microcarpa* and var. *tenuis*. The remaining taxa do not have proper names and have been provided with temporary phrase names (which will later be changed to conventional species names).

Some taxonomic issues remain unresolved and will need to be sorted out prior to publication, namely:

1. The status of *A. aneura* var. *pilbarana* is still uncertain, however, it most likely will turn out to be the same taxon as *A. aneura* var. *tenuis*. Also within the Pilbara considerable uncertainty remains concerning 'upland populations' listed below as *A. aneura* var. *angusta* (Mt Augustus): this entity is currently under review and may eventually be shown to be an ecotype of *A. aneura* var. *tenuis.* This work on the Pilbara plants has been significantly compromised by the dearth of fruiting material and because we had to limit our field work in the area in 2009 (see under **FIELD STUDY AND SPECIMEN**

PROCESSING above). Resolution of this matter is the highest priority and is the most challenging of the outstanding taxonomic issues.

2. The taxonomic status of the entity listed below as *Acacia* sp. Mulga narrow wings (B.R. Maslin *et al.* BRM 9149) needs confirmation. This entity appears is a 'good' taxon and is reasonably common in W.A.; however, we still need to make final decisions concerning its rank. It is not anticipated that it will take much time to resolve this matter.

3. The taxonomic status of typical *A. aneura* (i.e. var. *aneura*) requires further work. This species was originally described from specimens collected from the southern Flinders Range in South Australia in 1851 by Ferdinand von Mueller. We collected material for morphological and genetic analysis from this same population in 2008. The genetic results showed the species to be somewhat discrete but close to other members of the Grey-green alliance while the morphological results could not confidently separate the material from what was subsequently described as *A. aneura* var. *intermedia* (also in the Grey-green alliance). *Pro tem* we have combined the two varieties under the name var. *aneura* (see list below) but more detailed study of the pods is needed to determine if this is a sound decision. Resolution of this matter is not an especially high priority issue of Western Australia but it might have implications regarding how we name some of our plants (i.e. are they var. *aneura* or var. *intermedia,* or do both varieties occur in this state).

4. A probable new Mulga species has been identified by the Understanding Mulga project for Northern Territory; it is called '*A. aneura* var. Blue Mulga (*C.R. Dunlop* 1951)'. It grows east of the Docker River but as yet we have no confirmed occurrences from Western Australia. Botanists from Alice Springs Herbarium are keeping an eye on these plants and if they come into fruit this year (2010) they will try and arrange for us to join them on a quick trip to the region to see if we can better define its geographical distribution. This is not a particularly high priority matter for the present project.

5. Finally, it appears that there are discordant elements within what is currently called *A. aneura* var. *conifera*; those entities which occur within Western Australia will need to be resolved ahead of publication. Current indications are that plants with a conifer habit are simply juvenile forms and that these occur in a number of different Mulga species. Resolution of this matter should not be troublesome.

Our studies to date have confirmed the extremely diverse and complex nature of the variation within Mulga. Perhaps surprisingly none of the species we recognize is strongly discriminated from its presumed closest relative(s). This can probably be taken as an indication that this is a recently derived and actively evolving species complex. The presence of numerous hybrids further complicates the picture. Although morphological characters such as phyllode nervature and pod length: width ratios are useful in identifying hybrids work on this subject is in progress.

Considering the above matters, and bearing in mind the somewhat selective nature of our field sampling, it is not expected that in the future every entity encountered within the vast Mulga lands of Western Australia will able to be satisfactorily named or accommodated by our classification. Nevertheless, we subjectively assess that our study will have captured and documented the vast proportion of the variation within Mulga. Certainly it will be a major taxonomic step forward and will provide a robust framework for future study of this complex group of species.

The following list summarizes the provisional Mulga species that we currently recognize for Western Australia, arranged according to which Mulga alliance each occurs in.

1. Green Alliance (branchlet resin translucent; pods not winged)
    - *Acacia aneura* var. *macrocarpa*
    - *Acacia aneura* var. *tenuis*
    - *Acacia* sp. Mulga Paraburdoo (B.R. Maslin et al. BRM 9201) [Formerly we applied the informal manuscript name *Acacia* aff. *clelandii* to this entity]
    - *Acacia aneura* var. *pilbarana* [This entity is probably same as var. *tenuis*; it is currently under review]
    - *Acacia aneura* var. *angusta* (Mt Augustus) [This entity may be a ecotype of *A. aneura* var. *tenuis* and is currently under review; it therefore is not given a formal phrase name]
2. Grey-green Alliance (branchlet resin translucent; pods winged)
    - *Acacia aneura* [This entity now includes plants previously called *A. aneura* var. *intermedia*]
    - *Acacia ayersiana* [Including a narrow phyllode variant]
    - *Acacia craspedocarpa*
    - *Acacia paraneura*
    - *Acacia* sp. Mulga broad wings (B.R. Maslin 9058) [Formerly we applied the manuscript name *Acacia aneura* var. *alata* (narrow phyllode variant) to this entity]
    - *Acacia* sp. Mulga narrow wings (B.R. Maslin et al. BRM 9149) [Formerly we applied the manuscript name *Acacia aneura* var. *angusta* (winged) to this entity; we need yet to formally decide on the appropriate rank/status of this entity]
    - *Acacia* sp. Mulga dark shoots (B.R. Maslin & J.E. Reid BRM 9754) [Formerly we applied the manuscript name *Acacia aneura* var. *discolor* to this entity]
    - *Acacia aneura* var. Blue Mulga (C.R. Dunlop 1951) [A Northern Territory taxon that may possibly occur in Western Australia]

3. Blue Alliance (branchlet resin opaque; pods winged)

    - *Acacia* var. *argentea* [Including a variant with narrow, recurved phyllodes]
    - *Acacia aneura* var. *microcarpa*
    - *Acacia minyura*
    - *Acacia sp.* Mulga short phyllodes (B.R. Maslin et al. BRM 9276) [This entity was formerly called *A. minyura* (Hilltop variant) or *A*. sp. Hamersley Range hilltops (S. van Leeuwen 3552) or *A. aneura* var. *argentea* (short phyllode variant)]

ELECTRONIC IDENTIFICATION KEY

The electronic identification key that will enable Mulga taxa to be identified is currently in preparation. This key contains all the main Mulga species that we recognize for Western Australia (see list above); it also contains the most common hybrid taxa (it would be impractical to include every known Mulga hybrid) and the more significant of the informal variants that are discussed in our taxonomic treatments. Because so many Mulga specimens are submitted for identification in a sterile state (i.e. without flowers or fruits) we have devoted considerable time in trying to establish effective vegetative characters for use in the key. We have been reasonably successful in this endeavour: users will often be left with just two or three species when using only vegetative characters to conduct an identification.

The production timeline of this key has been divided into three phases; Phases 1 and 2 involve the construction, assemblage, testing and reviewing of all content, while Phase 3 focuses on the production and distribution of the final product. Specific activities referable to each Phase are outlined below.

Phase 1 (in progress; expected completion date is end of June 2010):

- Select morphological characters considered useful for identification purposes (define and illustrate these characters).
- Establish the taxon content of the key (i.e. determine what species/hybrids/variants will be included).
- Create key content (i.e. code each taxon for its morphological characters).
- Attach photographs having diagnostic value to each taxon.
- Distribute draft version of key for testing by users.

Phase 2:

- Review user feedback and implement any necessary/recommended modifications.
- Add formal descriptions, diagnostic line drawings, distribution maps and additional images to each taxon.

- Distribute key (draft version) on CR-ROM and WorldWideWattle website for further user testing.

Phase 3:

- Review user feedback and implement any necessary/recommended modifications.
- Create user guide booklet (to accompany CD-ROM).
- Produce final version of key and distribute as appropriate (i.e. CR-ROM accompanying the Mulga Manual and on WorldWideWattle website).

Phase 1 is currently in progress and is expected to be completed in April 2010.

PARTNERSHIPS

Chemistry Centre of Western Australia. Mulga branchlet resin has been shown to have taxonomic significance (see above); therefore a collaboration to investigate these compounds was established with Dr Shao Fang Wang, a biochemist from the Chemistry Centre of Western Australia. Ten resin samples representing the three major Mulga Alliances have been collected and submitted to Dr Wang for analysis during 2009. However, because of disruptions caused by the Centre being relocated to the Curtin University campus we have not yet received any results.

University of Western Australia. A collaboration with Gerald Page, a PhD student at the University of Western Australia was established to investigate Mulga populations in the West Angelas area (Pilbara) which form part of Page's study (*Mulga ecology and applications to site restoration at West Angelas, Pilbara*). During the 2007 field program participants of the *Understand Mulga* project visited a number of Page's study sites in order to examine the range of variation and number of Mulga morphotypes present. Herbarium and genetic material collected during this time as well as additional material provided by Page have since been processed and analyzed. Page has been provided with names for his morphotypes so that his studies will tie-in with the taxonomic classification that will be produced from the *Understanding Mulga* project. The genetic results from Page's sample set have been included within the *Understanding Mulga* genetic dataset; the referable genetic data is currently being extracted and sent to Page for inclusion in his studies. Examination, sampling and subsequent analysis of Page's study sites and associated collections have yielded much valuable information concerning the variation in Pilbara Mulga entities as well as increasing the robustness of the Pilbara Mulga dataset.

University of Zurich. Collaborative studies with Dr R. Rutishauser from Zurich have been established to investigate pod anatomical characteristics. Material has been sent to Rutishauser for study and in 2009 he visited Western Australia (with funds provided by the *Understanding Mulga* project) to participate in field studies aimed at familiarizing him with the taxonomic issues in Mulga and providing him with the opportunity to collect and study a greater range of pod variation than he had hitherto been able to do. Rutishauser has now completed his anatomical investigations and together with Reid and Maslin is

currently preparing a scientific paper for publication. Further collaborative work with Rutishauser is possible.

Curtin University of Technology. The Department of Environmental Biology has provided a facility on their Bentley campus which has enabled us to grow-on seedlings in order to investigate morphological changes associated with increasing age of Mulga plants (see under **ANCILLARY STUDIES** above).

PUBLICITY

Websites relating to the *Understanding Mulga* project have been constructed by Maslin and Reid (see http://www.worldwidewattle.com/infogallery/projects/mulga.php) and Miller (see http://www.cpbr.gov.au/jmiller/mulga.php). These sites are current, operational, provide information on the project, and among other things acknowledge the support of the three sponsors of this project.

An Information Sheet aimed at communicating information and current findings regarding the *Understanding Mulga* project has been written by Maslin and Reid. This Information Sheet is available for download from the *Understanding Mulga* website constructed by Maslin & Reid (see http://www.worldwidewattle.com/infogallery/projects/related-articles.php) and from the Department of Environment and Conservation's Science Division Publications and resources webpage (see http://www.dec.wa.gov.au/index.php?option=com_content&Itemid=2103&id=3569&lang=en&task=view). A copy of this Information Sheet is attached with this Report.

An article for the DEC magazine *Landscope* was supposed to have been prepared in the first year of the project. However, it has become clear now that it is too early to publish such an article because the Mulga taxa are not yet formally circumscribed and documented. It is more appropriate to publish the *Landscope* article towards the end of the project (at the same time as publication of the *Mulga Manual* and key to species).

WORKSHOPS

On 4 August 2008 we conducted a Mulga Workshop for the *Understanding Mulga* sponsors and selected guests. This Workshop was conducted at Technology Park Function Centre in Bentley. The primary aim of the Workshop was to inform sponsors first-hand about the project rationale and progress, and providing them with the opportunity to guide future activities. A PowerPoint presentation the Workshop proceeding has been distributed to sponsors; a copy of this is attached with the present Report.

Rio Tinto has requested that we conduct a Mulga identification workshop during 2010. This is a good idea and it would be appropriate that it happen after Phase 1 of the identification key has been completed (in June). Logistically the best place to conduct such a workshop would be in Perth, especially if the workshop is to be open to a wide audience (we suspect that there would a number of people interested in participating). However, sponsors may wish to have 'in house' workshops conducted for their

staff/contractors, in which case a Pilbara venue might be more appropriate. Using the Karijini Visitor Centre to conduct the workshop is also a possibility. These matters require further discussion.

OUTPUTS

Now that the taxonomic framework has been established for Mulga we are in a position to systematically assemble the information to produce the outputs required of the project. Work has commenced on assembling these deliverables and it is expected to take about one year to complete them.

Scientific publications. These will include both taxonomic and genetic papers, and will include a revision of Mulga in Western Australia with descriptions of new species and revised descriptions for existing species. These descriptions have been commenced and are at varying stages of development. An artist has been found who will prepare line drawings of all the taxa. A paper (with Rutishauser in Zurich) on Mulga pods is currently in preparation and a paper (with Sao Fang Wang) on Mulga gum chemistry is expected to be produced (see **PARTNERSHIPS** above).

Electronic key. First draft expected to be completed in April 2009 (see **ELECTRONIC IDENTIFICATION KEY** above).

Mulga Manual. Work specifically on the Mulga Manual has not yet started. However, the species descriptions that are currently being prepared will be adapted for use in the Manual. Also, we have assembled a large collection of photographic images for the Manual. The artist who will illustrate taxa for the taxonomic paper will also do drawings for the Manual.

Landscope article. As noted below under **PUBLICITY** a popular article on Mulga will be published in *Landscope* to coincide with the release of the Mulga Manual.

PROJECT EXTENSION

Following the August 2008 workshop a request to extend the *Understanding Mulga* project was made. One of the main reasons for needing this extension related to the excessively dry seasons that have occurred throughout the project, resulting in fewer than expected pods having been collected. A copy of our formal request for an extension is attached to this report (see Appendix 4).

Sponsors responded positively to our request for a year extension of the project and to date we have received the supplementary funding from two of the sponsors.

COMPLETION OF MULGA PROJECT

The *Understanding Mulga* project will be completed by June 2011. Initially the project was scheduled for completion at the end of 2009 but for reasons noted above a one year extension was requested of the sponsors. Subsequent to making that request an opportunity has occurred for Maslin and Miller to participate in a significant, new national program involving *Acacia.* This new activity is funded by the

Atlas of Living Australia program and is time-critical because funds must be expended by the middle of 2012. The new project involves, among other things, a revision of the WATTLE identification key which is a widely used identification tool for naming Australian species of *Acacia*, particularly by consultants doing environmental work associated with the mining industry. It is therefore considered as advantageous to the Mulga sponsors that we take advantage of this new opportunity. Our participation in this activity will necessitate moving the completion date of *Understanding Mulga* a further 6 months, but will not involve any additional funding from the Mulga sponsors. The new WATTLE project is expected to commence in July 2010 and for the period until June 2011 our time will be divided equally between this activity and the Mulga project.

With the above in mind the following schedule for completion of the *Understanding Mulga* project is presented:

<u>January – June 2010</u>

- Complete taxonomic discrimination of all W.A. Mulga taxa and commence descriptions (Maslin & Reid)
- Prepare draft electronic Mulga identification key and distribute for testing (Maslin & Reid)
- Complete genetic scrutiny of relevant WA. Mulga collections (Miller)
- Prepare draft of Mulga pod paper (Rutishauser, Reid & Maslin)
- Analyse seedling ontogeny data (Reid & Maslin)
- Maintain Mulga websites (Reid, Maslin & Miller)

<u>June – December 2010</u>

- Commence draft of taxonomic revision of W.A. Mulga species (Maslin & Reid)
- Commence draft of Mulga genetic overview paper (Miller, Byrne, Sampson, Reid & Maslin)
- Commence draft of Mulga Manual (Maslin, Miller, Reid & van Leeuwen)
- Distribute Mulga identification key on web and elsewhere for use and further testing (Maslin & Reid)
- Publish Mulga pod paper (Rutishauser, Reid & Maslin)

- Undertake field study (for about 1 week) in Pilbara, primarily to examine *Acacia aneura* var. *angusta* (Mt Augustus), subject to pods being set (Maslin & Reid)

- Undertake Mulga identification Workshop if requested to do so

- Maintain Mulga websites (Reid, Maslin & Miller)

January – June 2011

- Complete taxonomic revision of W.A. Mulga species (Maslin & Reid)

- Complete Mulga genetic overview paper (Miller, Byrne, Sampson, Reid & Maslin)

- Complete Mulga Manual, incorporating electronic identification key (Maslin, Reid, Miller & van Leeuwen)

- Prepare *Landscope* article regrading *Understanding Mulga* project; publication to coincide with launch of Mulga Manual (Maslin, Reid, van Leeuwen & Miller)

- Maintain Mulga websites (Reid, Maslin & Miller)

Post June 2011

- Formally launch Mulga Manual

- Publish taxonomic revision and genetic over view papers

- Where appropriate prepare and publish miscellaneous 'spin off' papers relevant to the Mulga project (e.g. seedling ontogeny, Mulga gum, genetic studies, etc.)

CONCLUDING REMARKS

Our taxonomic judgements concerning Mulga have, quite naturally, been made on the basis of available evidence. We are now in the process of producing a reasonable classification and understanding of Western Australia Mulga, but these plants are not as elucidated as thoroughly as we would have liked. The reasons for this are many but the sheer size and variability of the group and the complex genetic systems that operate within it are contributing factors. Another important factor is, as already noted, because the project has been seriously compromised by the prolonged drought across most of rangeland Western Australia. These unexpected weather conditions have meant in many cases we have not been able to collect flowers and, more importantly, pods. This lack of critical material has been particularly regrettable with respect to our attempts to understand the patterns of variation in the var. tenuis/pilbarana complex in

the Pilbara. Notwithstanding these constraints we believe that what we will produce will represent a major step forward in the understanding of Mulga in Western Australia, and will establish a sound foundation for further studies of this amazing and important arid zone group.

APPENDIX 1: Field studies undertaken in connection with Understanding Mulga project, 2006-2009.

The following field trips were undertaken during Year 1 (2007):

Date:	31 August – 13 September, 2006
Destination:	Paynes Find – Yalgoo – Mt Magnet – Sandstone – Leinster – Wiluna – Meekatharra
Trip participants:	B.R. Maslin, J. Miller and R. Fairman.
Trip details:	A total of 214 herbarium collections were made, plus 380 phyllode samples for genetic analysis. There had been some rains in the more southerly regions and pod crops were good but immature, therefore, two subsequent trips had to be undertaken (see below). This trip yielded much valuable information concerning the variation in Mulga, and a good quantity of material to work with throughout 2007 and subsequent years. Western Nickel provided valuable logistic support for the period that we were in the Leinster area.

Date:	October and November, 2006
Destination:	Area traversed as above.
Trip participants:	R. Fairman.
Trip details:	A total of 137 herbarium collections were made, plus over 120 seed collections, many from plants tagged from the previous field trip (see above). These trips yielded a good quantity of mature seed from a wide range of Mulga morphotypes for future ontogenetic studies.

The following field trips were undertaken during Year 2:

Date:	2-31 October 2007
Destination:	Mullewa – Mt Augustus – Pilbara – Meekatharra – Lorna Glen, east of Wiluna – Laverton – Mt Magnet
Trip participants:	B.R. Maslin, J. Miller, J.E. Reid and DEC volunteers, G. Marsh and D. Edinger.
Trip details:	A total of 328 herbarium collections were made, plus 841 phyllode samples for genetic analysis and 20 seed collections for ontogeny studies. In the Pilbara we examined sites at West Angelas that were under study by UWA PhD student, Gerald Page; material for herbarium and genetic study was collected. Unfortunately most of the areas we visited were excessively dry on account of the prolonged drought. Nevertheless, this trip yielded much valuable information concerning the variation in Mulga, and a good quantity of material to work with throughout 2008 and subsequent years.

Date:	21-27 September, 2008.

Destination: Northern Territory (Napperby Station, north of Alice Springs)

Trip participants: B.R. Maslin, J. Miller, J.E. Reid, and N.T. botanists David Albrecht and Peter Latz.

Trip details: The primary aims of this short trip to the Northern Territory were to examine relevant Mulga collections at the Alice Springs Herbarium and to collect material of *Acacia aneura* var. *intermedia* from its type locality on Napperby Station, north of Alice Springs. A total of 78 herbarium collections were made, plus many phyllode samples for genetic analysis were collected. An examination, albeit brief, of Northern Territory Mulga is important in order to contextualize the W.A. species. An examination of the material we collected has enabled us to accurately apply the name var. *intermedia* and to determine whether or not there is a significant distinction between W.A. Mulgas and those in the east. Also, the material we loaned from the Alice Springs Herbarium has helped to provide insights into the Mulga morphotypes that are found in the central range areas to the east of Warburton.

Date: 28 September - 2 October, 2008

Destination: South Australia (Flinders Range)

Trip participants: B.R. Maslin, J. Miller, J.E. Reid, and S.A. botanist Martin O'Leary

Trip details: The primary aim of this very short trip to South Australia was to revisit the Type locality of *Acacia aneura* (i.e. Cudnaka) in the southern Flinders Range, just south of Hawker. A total of 20 herbarium collections were made, plus phyllode samples for genetic analysis. Having knowledge of the Mulga morphotype that was collected in 1851 by Baron von Mueller from the Type locality is absolutely critical to this study. This information will enable us to accurately apply the name *Acacia aneura*, and this in turn is the essential starting-point when it comes to reporting the scientific results of our study. It was disappointing that because of the prolonged drought the Cudnaka Mulga plants did not possess any pods. Nevertheless, the herbarium and genetic material we collected, together with specimens that Mr O'Leary has sent us on loan from the South Australian State Herbarium (Adelaide), will enable us to achieve this goal. A most interesting discovery was that two different Mulga morphotypes exist in the Cudnaka populations visited by Muller.

Date: 8-27 October, 2008.

Destination: Mt Augustus – Meekatharra – Cue – Mt Magnet – Laverton/Leonora – Menzies – Paynes Find.

Trip participants: B.R. Maslin, J.E. Reid and DEC staff/volunteers M. Falconer, S. Carroll, G. Marsh and D. Edinger

Trip details: A total of 239 herbarium collections were made, plus 240 phyllode samples for genetic analysis and 39 seed collections for ontogeny studies. However, in areas north of Cue conditions were disappointingly dry because of the prolonged drought and therefore the numbers of good collections made were fewer than we had wanted. Also, because of the dry conditions we were obliged to eliminate sites north of Meekatharra – Wiluna. There had been good rains in more southerly regions and pod crops in many areas were good; however, because some pods were not quite mature a subsequent trip had to be undertaken (see below). Despite the conditions this trip yielded much valuable information concerning the variation in Mulga, and a good quantity of material to work with and supplement previous collections.

Date: 10-13 November 2008.

Destination: Paynes Find – Sandstone – Mt Magnet.

Trip participant: B.R. Maslin

Trip details: A total of 57 herbarium collections were made, plus 32 phyllode samples for genetic analysis and 21 seed collections for ontogeny studies. Most plants in this region were with pods/seeds. This area is of particular relevance to this study because the Mulga populations here are diverse, complex and often contain many different morphotypes. This trip yielded much valuable information concerning the variation in Mulga and a good quantity of mature seed from a wide range of Mulga morphotypes for future ontogenetic studies.

<u>The following field trips were undertaken during Year 3:</u>

Date: 20-30 April, 2009

Destination: Paynes Find – Mt Magnet – Cue – Meekatharra – Mt Augustus – Wiluna – Leonora – Menzies

Trip participants: B.R. Maslin and J.E. Reid.

Trip details: A total of 76 herbarium collections were made. The primary aim of this trip was to collect flowering specimens of Mulga, however, most of the areas we visited were excessively dry on account of the prolonged drought and therefore the numbers of good collections made were fewer than we had wanted. Nevertheless, this trip yielded much valuable information concerning the variation in Mulga, its patterning across the landscape and a reasonable quantity of material to work with and supplement previous collections.

Date: 21-27 May, 2009

Destination: Pilbara region

Trip participants: B.R. Maslin and J.E. Reid.

Trip details: A total of 61 herbarium collections were made. We worked to obtain a more detailed overview of Mulga variation and patterning within the Pilbara region, as well as to fill in specific knowledge gaps. However, most of the areas we visited were excessively dry on account of the prolonged drought and therefore the numbers of good collections made were fewer than we had wanted. Nevertheless, this trip yielded much valuable information concerning the variation in Mulga, its patterning across the Pilbara landscape and a reasonable quantity of material to work with and supplement previous collections. Rio Tinto provided valuable logistic support for the period 20-21 May.

Date: 26-31 October, 2009

Destination: Pilbara region

Trip participants: J.E. Reid and Swiss anatomist Rolf Rutishauser.

Trip details: A total of 41 herbarium collections were made, plus 117 phyllode samples for genetic analysis and samples for anatomical studies. Collection sites to cover the range of Mulga variation in the Pilbara region were visited. A number collection sites from previous Pilbara trips (see above) were re-examined and genetically sampled. A number of the areas we visited were excessively dry on account of the prolonged drought and therefore the numbers of good collections made were fewer than we had wanted. However, a judicious selection of samples for anatomical study were obtained. This trip yielded valuable information (fill in specific knowledge gaps) and a sufficient quantity of material to work with and supplement previous collections. Rio Tinto provided valuable logistic support for the period 29-30 October.

Date: 8-10 November, 2009

Destination: Paynes Find – Mt Magnet

Trip participants: B.R. Maslin and Swiss anatomist Rolf Rutishauser.

Trip details: A total of 15 herbarium collections were made, plus samples for anatomical studies. This area is of particular relevance to this study because the Mulga populations here are diverse, complex and often contain many different morphotypes. This trip yielded a judicious selection and quantity of samples from a wide range of Mulga morphotypes for anatomical study.

APPENDIX 2: Understanding Mulga Progress Report: Year 1 (2006-2007).

Understanding Mulga

By B.R. Maslin, J. Miller, M. Byrne and S. van Leeuwen

PROGRESS REPORT: YEAR 1 (2006-2007)

The following Report is aligned against the activities that are outlined in the *Understanding Mulga* prospectus under Project Schedule, Year 1.

Herbarium study

A preliminary sort of the almost 1 500 dried herbarium specimens of Mulga housed at the Western Australian Herbarium (PERTH) has been undertaken. Primary focus was given to the Maslin/Miller material collected in 1999, 2000 and 2006, because this is the most taxonomically informative material available (i.e. it is well documented, comprehensive in its scope at the population level, and is mostly complemented by material gathered for genetic scrutiny). This material has now been sorted into what is provisional groups that have been assigned temporary names; as we gain a better understanding of these groups the names will be converted to formal names which will then comprise the taxa of the Mulga Manual. All the PERTH specimens have been databased.

The necessary type and other historically important specimens lodged at the National Herbarium of Victoria (Melbourne) have been sent on loan to the Western Herbarium. This material will prove critical at the stage of assigning formal names to our groups.

Field survey

A number of targeted field studies were conducted during 2006. The purpose of this work was to gain a better understanding of the Mulga entities in the field, to understand the nature of the variation at both the intra- and inter-population levels and to collect material for subsequent taxonomic, genetic and ontogenetic analyses. Both pure and mixed populations of Mulga were targeted, however, it is becoming clear that pure (i.e. populations containing only one morphotype) are not common. The following field trips were undertaken:

1. 31 August – 13 September (Paynes Find – Yalgoo – Mt Magnet – Sandstone – Leinster – Wiluna – Meekatharra). Trip participants: B.R. Maslin, J. Miller, R. Fairman. Total number of collections made: 205. Western Nickel provided valuable logistic support for the period that we were in the Leinster area.
2. October and November (area traversed as above). Trip participant: R. Fairman. Total number of collections made: about 100. The purpose of these two follow-up trips was to collect mature seed from plants that we with immature pod in August/September.

All collections have been processed (i.e. mounted and databased) and examined and are now lodged at the W.A. Herbarium. See also under DNA extractions Ontogenetic studies below, and Herbarium study above.

These trips and the results obtained from analysis of the material collected has formed the basis for the 2007-2008 field program.

Collection processing

All 300+ collections made during the 2006 field survey are now lodged at the W.A. Herbarium.

Morphological analyses of collections

Preliminary analysis of all Maslin/Miller specimens collected in 1999, 2000 and 2006 collections have now been done. This material will be re-analysed in conjunction with material collected in 2007-2008 in order to better understand/circumscribe the taxonomic entities.

Microsatellite library

Because of the extreme complexity of the variation in the Mulga complex the genetic information will be a critical adjunct to the morphological information that is derived from the taxonomic scrutiny of the material collected. Additional to microsatellite information, Dr Miller is presenting genetic information derived from chloroplast DNA analyses, and from flow cytometry (this latter activity, which enables chromosome numbers to be determined) did not form part of the initial proposal, but is being carried by Dr Miller because of the importance of the information to developing an understanding of the variation). A microsatellite library specific to Mulga was developed by a Canadian company specializing in this work. This library and the markers created earlier by Dr Byrne have been tested in Iowa using a DNA analyzer. A group of eight markers has been selected to generate data to this point.

DNA extractions

DNA has been extracted from approximately 700 samples. These samples included the following:

1. 400 extracted in W.A. from material collected during the 2006 field study (this DNA has been sent to Miller at University of Iowa). Several problems were encountered during this DNA extraction process, resulting in a few samples with low (but mostly useable) DNA yields.
2. 300 (including both mature phyllodes and seedlings), extracted in Iowa based on material collected during the 1999 and 2000 field surveys. This material was old but a majority of the samples provided useable DNA. Additionally, multiple seeds were germinated from several mother plants. DNA was extracted from the seeds and was used to test the microsatellite library and to generate data when DNA was not extractable from old collections.

Genetic fingerprinting

To date microsatellite markers have been run on more than 600 samples, including the 300 samples collected in the field in 2006 and the 300 samples previously collected and stored by Miller in Iowa. These

data indicate that the markers have varying levels of divergence, indicating that both closely and more distantly related taxa will be able to be defined. These data are now being analyzed to test the relationships of plants within populations and to assess hybridization among the morphotypes. In addition, more than 150 samples from both new and older collections have been genotyped for several chloroplast polymorphisms. These makers are less conserved than the microsatellites and are also maternally inherited. This analysis defined three major groups of plants and analyses are presently in progress to test the correlation of these data with the morphological groups. As noted a flow cytometer has been acquired by Miller and protocols are being developed to quickly analyze ploidy level of future collections.

Ontogenetic study

Around 120 viable seed samples were collected from plants sampled in 2006. This seed has now been germinated and the seedling leaf development documented (i.e. characters measured, plants at different stages of development photographed). Furthermore, genetic scrutiny of seedling material will be undertaken by Dr Miller in the second year of the project; the methodology whereby this may happen is currently being developed. The information (morphological and taxonomic) will be valuable in our attempts to elucidate the variation patterns in Mulga.

Publicity

Web publicity. A website relating to the *Understanding Mulga* project has been constructed by Miller (see http://www.biology.uiowa.edu/newccg/jmiller/mulga.php). Among other things this site acknowledges the support of the three funders of the project).

A complementary web site has also been constructed by Maslin. However, because of unavoidable technical problems this information to the WorldWideWattle website (http://www.worldwidewattle.com/) this information is not yet posted on the web. However, it will be posted in the next month or so (although the 2007 field program may possibly prevent this happening until early December).

An article for the DEC magazine *Landscope* was supposed to have been prepared in the first year of the project. However, it has become clear now that it is too early to publish such an article because the Mulga taxa are not yet formally circumscribed and documented. If appropriate we will publish the Landscope article in 2008.

Summary

Progress in the first year of the Understanding Mulga was very satisfactory and almost all of the First Year targets were met. We made significant collections of 'critical' material, developed a much better understanding of the patterns of variation and the taxa involved, were not constrained by adverse weather conditions (which could have resulted in our not being able to collect pod material), we able to make significant progress with the taxonomic analysis of the collections (this work was greatly facilitated by the appointment of Ms Jordan Reid to the project), were able to extract DNA from most of the material collected and have made significant inroads into the genetic scrutiny of the taxa. Furthermore, we have

established working relations with Prof. Pauline Grierson at the University of Western Australia (who has a PhD student working on Mulga in the West Angelas area); this liaison will be further progressed during Year 2 of the project.

APPENDIX 3: Understanding Mulga Progress Report: Year 2 (2007-2008).

Understanding Mulga

B.R. Maslin, J. Miller, M. Byrne and S. van Leeuwen

PROGRESS REPORT: YEAR 2 (2007-2008)

EXECUTIVE SUMMARY

Progress in this second year of the Understanding Mulga *project has been gratifying with the following major achievements attained:*

- *Significant numbers of herbarium specimens (567) and genetic samples (1,185) have been collected, producing one of the most comprehensive datasets ever assembled for a single group of plants in Australia.*
- *A broad conceptual framework for understanding Mulga has been established through the recognition of Blue, Green and Grey-green Alliances within the group.*
- *There is broad congruence between the genetic and morphological datasets supporting the above groupings, and this can be taken as a measure of the robustness of the groups.*
- *The recognition of more than 20 taxa within W.A. Mulga and the preparation of draft descriptions of these taxa.*
- *An electronic key for the identification of W.A. Mulga taxa has commenced.*
- *Correct application of two Mulga names (*A. aneura *var.* aneura *and var.* intermedia*) have been established through visits to relevant type localities in S.A. and N.T., respectively.*
- *Genetic evidence has determined that most collections of WA Mulga are tetraploid.*
- *Pod anatomical studies have commenced in Zurich by Rutishauser.*
- *Collaborative partnerships (involving resin chemistry and Pilbara ecology) have been established to help better understand variation in Mulga.*
- *Seedling studies are ongoing and somewhat ambiguous but results suggest that neoteny seems not to be prevalent in the Green and Grey-green Alliances.*
- *A workshop was conducted to inform sponsors and selected guests of progress with the* Understanding Mulga *project.*
- *Two Mulga websites, one hosted in Perth and the other in Canberra, have been established and maintained.*

Notwithstanding the above, work during 2007-08 was constrained to some extent for two main reasons; firstly, relatively low numbers of critically important pods were collected on account of the

prolonged drought throughout much of the study area, and secondly, Miller's move from Iowa to Canberra disrupted the genetic study so some extent.

INTRODUCTION

The following Report covers the period from October 2007 to November 2008 and is aligned against the activities outlined in the *Understanding Mulga* prospectus under Project Schedule, Year 2.

FIELD SURVEY AND SPECIMEN PROCESSING

A very comprehensive field program was undertaken during the 2007-08 period. We not only recollected critical material identified as a result of the 2006-07 herbarium/genetic study, but targeted many new areas in an attempt to encompass as much of the diversity of Mulga in Western Australia as possible. For reasons stated below we also undertook short field excursions in the Northern Territory and South Australia. Insofar as Western Australia is concerned the only major region not yet visited is the central ranges area to the east of Warburton. Apart from being logistically challenging to visit such a remote region we considered that the likelihood of collecting useful (pod/seed) material from this area was not high on account of the prolonged drought. Furthermore, our visit to the Northern Territory herbarium may have obviated the need to visit the central ranges, but this will need to be confirmed following our examination of the material we collected in the Northern Territory and loaned from the Alice Springs Herbarium.

In 2009 the primary focus of our field studies will be the Pilbara region and also the complex populations between Mt Magnet and Menzies.

The following field trips were undertaken during 2007-08:

Date:	2-31 October 2007
Destination:	Mullewa – Mt Augustus - Pilbara – Meekatharra – Lorna Glen, east of Wiluna – Laverton – Mt Magnet
Trip participants:	B.R. Maslin, J. Miller, J.E. Reid and DEC volunteers, G. Marsh and D. Edinger.
Trip details:	A total of 272 herbarium collections were made, plus many phyllode samples for genetic analysis and some seed for ontogeny studies. In the Pilbara we examined sites at West Angelas that were under study by UWA PhD student, Gerald Page; material for herbarium and genetic study was collected. Unfortunately most of the areas we visited were excessively dry on account of the prolonged drought. Nevertheless, this

trip yielded much valuable information concerning the variation in Mulga, and a good quantity of material to work with in 2008.

Date: 21-27 September, 2008.

Destination: Northern Territory (Napperby Station, north of Alice Springs)

Trip participants: B.R. Maslin, J. Miller, J.E. Reid, and N.T. botanists David Albrecht and Peter Latz.

Trip details: The primary aims of this short trip to the Northern Territory were to examine relevant Mulga collections at the Alice Springs Herbarium and to collect material of *Acacia aneura* var. *intermedia* from its type locality on Napperby Station, north of Alice Springs. A total of 74 herbarium collections were made, plus many phyllode samples for genetic analysis were collected. An examination, albeit brief, of Northern Territory Mulga is important in order to contextualize the W.A. species. An examination (in 2009) of the material we collected will enable us to accurately apply the name var. *intermedia* and to determine whether or not there is a significant distinction between W.A. Mulgas and those in the east. Also, the material we loaned from the Alice Springs Herbarium will help provide insights into the Mulga morphotypes that are found in the central range areas to the east of Warburton.

Date: 28 September - 2 October, 2008

Destination: South Australia (Flinders Range)

Trip participants: B.R. Maslin, J. Miller, J.E. Reid, and S.A. botanist Martin O'Leary

Trip details: The primary aim of this very short trip to South Australia was to revisit the Type locality of *Acacia aneura* (i.e. Cudnaka) in the southern Flinders Range, just south of Hawker. A total of 11 herbarium collections were made, plus phyllode samples for genetic analysis. Having knowledge of the Mulga morphotype that was collected in 1851 by Baron von Mueller from the Type locality is absolutely critical to this study. This information will enable us to accurately apply the name *Acacia aneura*, and this in turn is the essential starting-point when it comes to reporting the scientific results of our study. It was disappointing that because of the prolonged drought the Cudnaka Mulga plants did not possess any pods. Nevertheless, the herbarium and genetic material we collected, together with specimens that Mr O'Leary will send us on loan from the South Australian State Herbarium (Adelaide), will enable us to achieve this goal. A most interesting discovery was that possibly two different Mulga morphotypes exist in the Cudnaka populations visited by Muller. Our 2009 examination of the material collected will determine if indeed this is the case.

Date: 8-27 October, 2008.

Destination: Mt Augustus – Meekatharra – Cue – Mt Magnet – Laverton/Leonora – Menzies – Paynes Find.

Trip participants: B.R. Maslin, J.E. Reid and DEC staff/volunteers M. Falcolner, S. Carroll, G. Marsh and D. Edinger

Trip details: A total of 210 herbarium collections were made, plus many phyllode samples for genetic analysis and some seed for ontogeny studies. However, in areas north of Cue conditions were disappointingly dry because of the prolonged drought and therefore the numbers of good collections made were fewer than we had wanted. Also, because of the dry conditions we were obliged to eliminate sites north of Meekatharra – Wiluna. There had been good rains in more southerly regions and pod crops in many areas were good; however, because some pods were not quite mature a subsequent trip had to be undertaken (see below). Despite the conditions this trip yielded much valuable information concerning the variation in Mulga, and a good quantity of material to work with in 2009.

Date: 10-13 November 2008.

Destination: Paynes Find – Sandstone – Mt Magnet.

Trip participant: B.R. Maslin

Trip details: A total of 48 herbarium collections were made, plus phyllode samples for genetic analysis and seed for ontogeny studies. Most plants in this region were with pods/seeds. This area is of particular relevance to this study because the Mulga populations here are diverse, complex and often contain many different morphotypes.

All 2007 collections have now been processed (i.e. mounted and databased) and examined and are now lodged at the W.A. Herbarium. The 2008 collections are currently being processed.

Additional to our own gatherings we have used the collections at the W.A. Herbarium and a selection of specimens sent to us on loan from the Adelaide, Alice Springs, Brisbane and Melbourne herbaria.

HERBARIUM STUDY & MORPHOLOGICAL ANALYSES OF COLLECTIONS

Morphological analysis of variation of herbarium material was undertaken using conventional taxonomic methodology. This study revealed the existence of an important and hitherto unknown taxonomic character, namely, the branchlet resin comprises two seemingly distinct types, a red-brown translucent resin and an opaque milky white resin. Somewhat surprisingly we were able to establish a broad conceptual framework for understanding variation in W.A. Mulga by using just this resin character in combination

with the character of the pod 'wing'. Consequently three major Mulga Alliances have now been recognized to accommodate the more than 20 Mulga taxa that we identify, namely:

4. Green Alliance (branchlet resin translucent; pods not winged). Included taxa: *Acacia aneura* var. *macrocarpa,* var. *macrocarpa* (flat phyllode variant), var. *pilbarana*, var. *tenuis* and var. *tenuis* (flat phyllode variant);

5. Grey-green Alliance (branchlet resin translucent; pods winged). Included taxa: *Acacia aneura* var. *fuliginea*, var. *fuliginea* (narrow phyllode variant), var. *intermedia*, var. *intermedia* (linear phyllode variant), var. *intermedia* (resinous variant), var. *alata* ms name, var. *alata* (narrow phyllode variant), var. *alata* (narrow pod variant), var. *alata* (pseduo-winged variant), *Acacia craspedocarpa* and *Acacia paraneura.*

6. Blue Alliance (branchlet resin opaque; pods winged). Included taxa: *Acacia* var. *argentea*, var. *argentea* (narrow phyllode variant), var. *argentea* (short phyllode variant), var. *microcarpa*, var. *microcarpa* (thick resin variant), var. *microcarpa* (broad incurved phyllode variant), var. *microcarpa* (broad recurved phyllode variant), *Acacia ayersiana*, *Acacia minyura* and *Acacia* aff. *minyura* (Hilltop variant).

This classification has been shown to be broadly congruent with results from the genetic analyses.

During 2009 we will test the robustness of this classification, including whether or not additional alliances need be recognized. Furthermore, it will be necessary to determine which of the above-listed provisional entities represent 'good' taxa, and whether or not additional ones need to be recognized. Apart from the two characters already noted the following ones have proved useful in defining taxa:

- branchlet resin thickness;
- new shoot colour, resinosity and indumentums;
- phyllode shape, size, colour, curvature and cross-section; and
- pod shape and size.

A detailed examination of Mulga flowers and seeds has not yet been undertaken. Critical examination of new collections will enable verification and better definition of the provisional taxa that are currently recognized. Once the taxa are clearly defined we can continue with preparing descriptions and encoding them for use in an electronic identification key.

The existence of numerous hybrids within the Mulga group has proved a complicating factor. Although characters such as phyllode nervature and pod length: width ratios are useful in identifying hybrids further work on this subject is required.

GENETIC STUDIES

During 2007-08 Miller moved from the University of Iowa, U.S.A., to a new position at the CSIRO Center for Plant Biodiversity Research in Canberra. This move caused some interruption to the genetic program. Nevertheless, the part-time appointment of Dr J. Sampson to help analyse microsatellite results, and the appointment in 2009 of a laboratory technician to assist Miller will greatly facilitate the generation of genetic information in the coming year (both these positions used *Understanding Mulga* funds).

DNA EXTRACTIONS

During the 2007-08 a total of 1,185 phyllode samples were collected (1,113 from W.A. taxa and 72 from N.T./S.A. taxa). By any measure this is a large number of samples and once analysed will contribute towards an enormous genetic dataset for Mulga. DNA has been extracted from the 841 samples that were collected during the 2007 field study. This material has been sent to Miller at the Centre of Plant Biodiversity, Canberra for analysis. Improved DNA extraction protocols as a result of new equipment (not obtained using *Understanding Mulga* funds) have eliminated extraction problems encountered during year one of this project. Of the344 genetic samples collected in 2008, 272 will be extracted in Perth and the DNA send to Miller; the remaining 72 samples have been extracted by Miller.

MICROSATELLITES

While in Iowa during the first half of the second year of the project 120 plants, specifically chosen to cover the entire range of morphological variation within W.A. Mulga, were analyzed with five microsatellite markers. These selections were based on the preliminary data generated in year 1 of the project. These data were presented at the Sponsor's Workshop held in Perth in July. In addition to the eight microsatellite markers selected last year, an additional four markers have been identified from the earlier constructed libraries. These markers were chosen due to their ability to genetically differentiate individual plants or to differentiate species. Currently data is being generated using these 12 microsatellite markers. The plants used are targeted to the samples identified in the morphological study as being critical to identification of taxa with Mulga.

CHLOROPLAST DNA SEQUENCING

The chloroplast DNA sequence data has been expanded to include five chloroplast genes. These data have been generated in over 150 plants chosen to cover the entire range of morphological variation within Mulga. The plants studied in this dataset are essentially the same that those used in the microsatellite dataset. Specific DNA differences are now known and can be used to assign a plant to a taxonomic grouping.

As mentioned above in the Herbarium studies, three alliances have been identified. The major result from the genetic work is congruence with the morphological data. The genetic results, both microsatellite and chloroplast sequence data also identify three major alliances. These alliances correlate well with those uncovered in the morphological analyses. This congruence in morphological and molecular data indicates that the results are robust. The goals of the third year are to further dissect relationships among taxa and to test morphological hypotheses by increasing sampling of different Mulga types and DNA markers. The use of threes types of data sets permits rapid identification of hybridization among the Mulga species.

DNA PLOIDY LEVEL

Flow cytometry analysis was undertaken in Iowa on seedlings from previous field collections. This analysis determined that most Mulga collections, including *A. craspedocarpa*, are tetraploid, with a small fraction being hexaploid. All outgroups are diploid. With these data we have adjusted our data analysis to account for this important information. Presently we do not have access to a flow cytometer. Further research in this area will depend on the successful application of a CSIRO equipment grant proposal to be submitted by Miller in December 2008.

ANATOMICAL STUDY

A limited number of pods have been sent to Rutishauser in Zurich for examination. These pods include representatives from the three main Mulga alliances, Green, Grey-green and Blue. The aim of this study is to examine the underlying anatomical structure of the so-called pod 'wing' and to determine whether or not there is information of taxonomic value therein. Unfortunately, because of staff/resource shortages in Zurich, we have had to significantly reduce the amount of anatomical investigation that will be undertaken by Rutishauser.

ONTOGENETIC STUDY

Continued monitoring of seedlings germinated from 2006 seed collections has occurred throughout the year with further seedling leaf development documented. Subsets of seedlings have been destructively sampled for herbarium vouchers (lodged at the W.A. Herbarium) or planted out in a dedicated plot at the Field Trial Area of the Department of Environmental Biology at Curtin University of Technology. We thank Professor Jonathon Majer, Head of Department of Environmental Biology at Curtin University of Technology, for making this facility available to the project. Seeds collected during the 2007 and 2008 field program have been stored and will be prepared and germinated as required during 2009.

Preliminary analysis of seedling data suggests that neoteny is most likely not present within members of the Green and Grey-green Mulga alliances. Because of excessive seedling mortality it is not yet possible to assess neoteny within the Blue alliance of Mulga. Therefore, during 2009 further study of the Blue alliance will be undertaken. A more detailed discussion of the ontogenetic study is provided in the CD from the *Understanding Mulga* sponsors workshop presented on 4 August 2008.

PARTNERSHIPS

Because Mulga branchlet resin was shown to have taxonomic significance (see above) a collaboration to investigate these compounds was established with Dr Shao Fang Wang, a biochemist from the Chemistry Centre of Western Australia. Ten resin samples representing the three major Mulga alliances have been collected and submitted to Dr Wang for analysis during 2009.

As already noted we collected material of Mulga from sites at the West Angelas that formed part of a study (*Mulga ecology and applications to site restoration at West Angelas, Pilbara*) by UWA PhD student, Gerald Page. Following analysis of the material collected we will provide Page with names for his entities so that his study may tie-in with the taxonomic classification that will be produced from the *Understanding Mulga* project.

Pod anatomical studies by Dr R. Rutishauser in Zurich are noted above.

PUBLICITY

Websites relating to the *Understanding Mulga* project have been constructed by Maslin and Reid (see http://www.worldwidewattle.com/infogallery/projects/mulga.php) and Miller (see http://www.cpbr.gov.au/jmiller/mulga.php). These sites are current, operational, provide information on the project, and among other things acknowledge the support of the three sponsors of this project.

An article for the DEC magazine *Landscope* was supposed to have been prepared in the first year of the project. However, it has become clear now that it is too early to publish such an article because the Mulga taxa are not yet formally circumscribed and documented. If appropriate we will publish the *Landscope* article in 2009.

SPONSORS WORKSHOP

On 4 August 2008 we conducted a Mulga Workshop for the *Understanding Mulga* sponsors and selected guests. The Workshop was conducted at Technology Park Function Centre in Bentley. The primary aim of this Workshop was to inform sponsors first-hand about the project rationale and progress, and providing them with the opportunity to guide future activities. Workshop subject titles included the following:

- Introduction and background (by Stephen van Leeuwen)
- A new taxonomic evaluation of Mulga (by Bruce Maslin)
- An ontogenetic (seedling) study of Mulga (by Jordan Reid)
- Genetic Approaches to Assist in the Taxonomic Evaluation of Mulga (by Joe Miller)
- Wrap up & Way Forward (by Steve van Leeuwen)

A CD containing the above presentation has been prepared and presented to Sponsors.

Apart from providing sponsors with relevant technical information concerning the *Understanding Mulga* project the Workshop highlighted problems encountered primarily because of the excessively dry seasons during the first 18 months of the project. As a consequence fewer than expected pods have been collected. This in turn has constrained the project to some extent (i.e. less critically important pod material available for study) and has caused a greater reliance upon genetic information in order to make taxonomic decisions. Because of these and other problems that were discussed in the wrap up session, we indicated a desire/need to prolong the project by one extra year. Therefore, a request for additional funding has been submitted with this Report.

APPENDIX 4: Copy of letter to BHP Billiton requesting support to extend the *Understanding Mulga* project for one extra year. A similar letter was sent to the other two Mulga sponsors, Rio Tinto and Fortescue Metals Group.

Your Ref:		Phone:	+61 8 9405 5169
Our Ref:		Fax:	+61 8 9306 1641
Enquiries:	Dr Stephen van Leeuwen	Email:	stephen.vanleeuwen@dec.wa.gov.au

Gavin Price
Manager, Environment & Sustainable Development
BHP Billiton Iron Ore
PO Box 7122
Cloisters Square PERTH WA 6850

Dear Gavin

Re: ***Understanding Mulga***
Progress Report - Year 2 & Works Program – Year 2

Attached is a brief progress report on activities undertaken over the past 12 months on the project.

As indicated at the August sponsors workshop we seek your support to extend the project for an additional 12 months. Detailed below is our justification for this request.

Taxonomic studies:

It is extremely difficult to precisely determine how long any taxonomic project will take because until detailed research commences one does not know with certainty what taxonomic problems will be encountered, or how many species are involved. In the case of Mulga this problem is compounded because the group is large and is distributed over a very wide geographic area. Other complicating factors in Mulga relate to the fact that most of the plants are polyploids, many are hybrids and their in situ mode of speciation is unique within *Acacia*. These factors make Mulga a very challenging, and time-consuming group of species to study and elucidate.

Apart from the above we have encountered a particular problem that has compounded our taxonomic difficulties. To confidently name Mulga plants it is necessary, at this stage of the study, to inspect pods of the specimens that we collect. However, because of prolonged drought-like conditions over much of the W.A. study area many of the plants encountered on field trips were without pods. In an attempt

to ameliorate this problem we have resorted to collecting additional material for genetic typing of these podless entities. However, this in turn has caused an impost on the genetic program and has slowed taxonomic progress.

Genetic studies:

During this second year of the project the leader of the genetics program, Dr Joe Miller, relocated his operation from Iowa in the U.S.A. to CSIRO in Canberra. This move has caused some disruption to the genetic work. The situation has been compounded because in Canberra Miller does not have ready access to a flow cytometer which was available to him in Iowa. This piece of equipment is important for generating information that is used for interpreting genetic information. An application through CSIRO will be made by Miller in December 2008 to purchase this equipment (not involving *Understanding Mulga* funds). Another problem for the genetic study is that it is proving harder than expected to find pure populations of each of the Mulga morphotypes; these populations are needed to calibrate the molecular data.

Logistics:

Mulga has an extremely wide geographic distribution and it is therefore very time-consuming, expensive and logistically challenging to undertake a meaningful collection of all the morphotypes. During the first two years of the project (2006-2008) most critical areas have been sampled but we have not had time to undertake a detailed field study of Mulga in the Pilbara. This is regrettable because the Pilbara is of particular significance to the sponsors of the project. It is therefore our desire to focus on the Pilbara in 2009 with work done over the past two years providing the necessary context from which to accurately assess Mulga in this region. This Pilbara can only be adequately done if we are afforded sufficient time (one year) and funds. Obviously, seasonal conditions will influence the success of the Pilbara sampling program.

Seedling studies.

A seed germination program was commenced in the first year of the project and seedlings are currently being grown-on in plots at Curtin University of Technology. Although it would be useful to germinate more seed (especially of “Blue alliance” taxa that failed in year 1) we have not done so because unless the project can run for an extra year the plants will not be mature enough to produce useful taxonomic information.

We are confident that with your ongoing support this project will deliver all outputs as articulated in the prospects. We are also extremely confident that the project will deliver a significant advancement in our knowledge of Mulga, the conservation status of entities within the Mulga group and above all a user friendly identification tool that is based on a stable and functional taxonomy.

To extend the project for this additional year an extra $150,000 is required. This funding, which equates to $50,000 from each of the three partners, will primarily be used to cover salary costs for morphological and molecular studies, although some will be allocated to cover field costs.

If you have any questions about this invoice, progress on the research project or require clarification regarding the request for a 12 month extension and the budget associated with this extension please do not hesitate to call. If you would like a personal face to face briefing on progress or the request for an extension please do not hesitate to ask.

Yours sincerely

DR STEPHEN VAN LEEUWEN
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