Towards an understanding of variation in the Mulga complex (*Acacia aneura* and relatives)

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SUMMARY

Acacia aneura and its close relatives form a highly variable species complex commonly known as Mulga. They are small trees that dominate the vegetation of arid regions, in all occupying around 20% of Australia. This paper discusses and illustrates some of the more important types of variation found in Mulga, especially growth form and phyllode and pod morphology. This variation occurs both between and within populations and often results in a very complex mosaic of mixed Mulga populations. The underlying genetic and biological factors responsible for this variation are explored. While hybridisation is probably one cause of the variation, our use of microsatellite markers has not been able to provide direct evidence of this; however, the sampling done to date has been very small. Genetic developmental mechanisms such as polyploidy, apomixis and neoteny are maintaining this diversity. The Mulga complex contains multiple ploidy levels, including triploids, tetraploids and pentaploids, and polyembryony is a common feature in all these polyploids. Microsatellite data have identified fixed heterozygozity in populations with some genetic differences among morphotypes. Progeny arrays of 24 morphotypes indicated that over 95% of the plants have the same genotype as the mother plant. This accumulating evidence indicates that the Mulga complex is reproducing through facultative apomixis. Additionally the retention of juvenile characteristics (neoteny) is seen in many populations and also increases the variability. Given the importance of Mulga to the ecology, management and sustainable utilisation of arid zone ecosystems, it is important that the classification of the group adequately reflect the biological reality that exists in nature, if indeed this is achievable. The work reported here, and in related molecular and population studies, provides a basis for testing new classifications of Mulga. It also provides new information that can contribute to an improved classification of the group.

INTRODUCTION

The name Mulga is most commonly applied to the large, woody, perennial, arid zone species *A. aneura* F.Muell. ex Benth. and its close relatives. The name also denotes the vegetation type that is dominated by these species and which covers about 20% of the land surface of Australia (Everist 1949). According to Maiden (1907) the name Mulga alludes to a long, narrow shield made by Aborigines from *Acacia* wood.

Mulga is a very common, significant component of Australian arid zone vegetation and as such is important to the ecology and environmental management of these ecosystems. Furthermore, Mulga is the most economically significant acacia of the arid zone, primarily because it is an important source of fodder, especially during times of drought.

Species of the Mulga group, especially *A. aneura* itself, are notoriously variable, and identification of these taxa, both in the field and from herbarium material, is often very difficult. Understanding this variation and its causal factors, and being able to identify the taxa reliably, are critically important to the effective management, conservation and utilisation of this valuable resource. Two recent revisions of Mulga (Randell 1992; Pedley 2001) have helped clarify some of the taxonomic complexity within the group and have provided aids to identification (for naming species see also Maslin 2001). Very little is known, however, about the genetic basis of this variation, the origin of the different forms of Mulga, or the ecological factors that allow them to coexist in often bewilderingly complex mixed populations. Previous studies have suggested that these patterns of variation are due to a complex mix of sympatry and putative hybridisation in the major species (Davies 1976), geographic variation within the species, and sympatric variation within *A. aneura* itself (Pedley 1973; Lamont and Fox 1981; Fox 1986; Cody 1989). In the light of our own field studies it appears likely that while hybridisation is a major factor in creating diversity, there are other mechanisms maintaining this diversity. These mechanisms could include polyploidy and apomixis such as are found in other legume taxa of the arid zone, e.g. the *Senna artemisioides* group (Randell 1970), as well as neoteny.

This overview paper introduces some of the more important patterns of variation in Mulga and some of the genetic and biological mechanisms responsible for the great diversity displayed by species of this group. In so doing this will lead to a better understanding of the evolutionary history of these plants and how they relate to their environment, and quite possibly provide the basis for an improved classification of Mulga. The fundamental genetic question is whether plants of the same morphotype[1] share immediate common ancestry, irrespective of whether they grow side-by-side or are widely distributed. Furthermore, it is important to understand the genetic relationships among morphotypes, in particular whether morphotypes arise independently within populations through *in situ* hybridisation events, or whether the morphotype has a more ancient origin from a single area and has spread independently through arid Australia.

The data presented here have been acquired from four field excursions we conducted over the last few years to Mulga-diverse areas in the Mt Magnet and Pilbara regions of W.A., southern N.T., S.A. and north-western N.S.W. Based on this field experience and subsequent glasshouse and preliminary laboratory studies, hypotheses have been developed concerning variation in Mulga and a preliminary account of these ideas is presented below.

Distribution and ecology

Communities with Mulga species as the dominant or co-dominant occupy 15 million square kilometres or about 20% of the Australian continent (Everist 1971, cited in Johnson and Burrows 1981). They occur in a discontinuous belt between c. 20° and 35°S latitude and 113° and150°E longitude in the central and southern parts of the arid zone from near the west coast of W.A. eastwards through N.T. and S.A. to central Qld and N.S.W., and far north-western Vic. The distribution of the 'core group' of Mulga, namely, *A. aneura* (10 varieties), *A. ayersiana, A. minyura* and *A. paraneura*, is shown in Figure 1. Mulga is adapted to environments where the soil water regime is almost always limiting for growth, but where there is a possibility of recharge at all seasons (Johnson and Burrows 1981). As discussed by Midgley and Gunn (1985), Mulga communities predominate where the mean maximum temperature of the hottest month is 36-40°C and the mean annual rainfall is mainly 200-250 mm (although in the easternmost areas it rises to 500 mm); they are conspicuously absent from the semi-arid regions with a regular summer or winter drought (Nix and Austin 1973). In the driest areas direct precipitation figures may give a misleading impression of the amount of water the trees get as Mulga often receives 'run off' water from surrounding areas (it is in these areas that the densest stands occur). Mulga communities grow on a range of soil types but the densest stands are usually on red earth and sand or red clayey sand; rarely on alkaline soil and almost never on black cracking clay.

As discussed by Johnson and Burrows (1981), *A. aneura* is very well adapted for survival in harsh, arid conditions. Its phyllodes are held vertically rather than horizontally, thereby aiding water redistribution and minimising heat absorption. The sclerophyllous phyllodes are hairy, have a resinous covering and are drought resistant by means of dormancy. The plants 'aestivate' when drought occurs and resume growth within four days after water again becomes available (Slatyer 1965). They also have the capacity to channel water down their phyllodes and stems so that rainfall is concentrated at the base of the trunk (Slatyer 1965; Pressland 1973). This stem flow increases significantly the amount of water available to the roots, for example, 25 mm of rainfall can be concentrated into 140 mm within the root zone of the tree, making small showers more effective (Kerle 1995).

Utilisation

Mulga is not only a very important component of arid zone natural ecosystems but also provides many commodities for man. In traditional Aboriginal cultures *A. aneura* is an important source of food; not only are its seeds consumed (first being roasted and ground into a paste) but Mulga plants provide habitats for honey ants, lerps (which produce 'honey dew') and wasps (which produce 'mulga apples'). Mulga is also a source of wood for weapons, digging sticks and sacred objects; *A. minyura* provides copious resin, called 'kiti', which was traditionally used by men in the production of tools and spear throwers. For further information on Aboriginal usage of Mulga see Aboriginal Communities of the Northern Territory (1993), Kerle (1995), Latz (1999) and Cowley (2001).

As summarised by Doran and Turnbull (1997), Mulga forms a significant part of the dry-range diet of sheep in Australia, but without supplementary high quality feed it is barely sufficient for subsistence. Further information on the fodder value of Mulga is given in Everist (1949, 1969), Chippendale and Jephcott (1963), Askew and Mitchell (1978), Cunningham *et al.* (1981), Goodchild and McMeniman (1987) and Mitchell and Wilcox (1994).

TAXONOMY

The taxonomic imperative

It is somewhat surprising that a species-group like Mulga that is so widespread, common and important to the structure of arid zone ecosystems, and which is so important to the pastoral industry, has received so little taxonomic attention. In terms of species numbers the Mulga group is not especially large, but in terms of variation and taxonomic complexity few Australian plant groups would rival Mulga. Over the past 100 years or so

[1] We use the term morphotype to define a group of plants that share similar-looking morphological features, e.g. growth form, phyllode shape, size and/or colour, pod form, etc.

an extensive body of literature has been published on Mulga, dealing primarily with its ecology, biology, Aboriginal utilisation and relevance to the pastoral industry (for reviews see Everist 1969, Johnson and Burrows 1981 and Doran and Turnbull 1997). Although some authors did recognise that different 'forms' of Mulga exist and discussed them as separate entities (e.g. Everist 1969; Beard 1976: 43; Pedley 1973), many treated Mulga simply as a single species, namely, *A. aneura*. As discussed below, the current classification of Mulga recognises four distinct species (comprising 13 separate taxonomic entities) as the 'core' of the group, and future studies may well show that more taxa should be recognised. Therefore, caution should be exercised when interpreting previous studies because it may not always be clear which of the taxa as defined in Pedley's classification (2001) is meant. Furthermore, as noted by Randell (1992), as the taxonomic concepts of Mulga become increasingly refined there may well be a need to re-evaluate earlier concepts. Extending this notion to the future it is imperative that the classification of Mulga reflect the biological reality that exists in nature, if indeed this is achievable. The importance of defining and naming meaningful biological entities is discussed by Maslin (this proceedings) and what is said there applies particularly to Mulga.

In 1971 Preece considered that, because of the amount of variation in *A. aneura*, it was dangerous for ecologists to apply their results too far beyond the population being studied. While this was sound advice at the time the situation today has improved through the taxonomic work of Randell (1992) and Pedley (2001). There now exists a taxonomic framework that helps users to generalise the results of their studies beyond the population level. As discussed below, however, it is quite possible that future studies will further refine the classification of Mulga and produce a scheme that accommodates even better the complex patterns of variation in many populations.

Definition of Mulga

As used here the term 'Mulga group' refers to 10 species (Table 1), comprising a core group of four species, *A. aneura* F.Muell. ex Benth. (Common Mulga, containing 10 varieties), *A. ayersiana* Maconochie (Uluru Mulga), *A. minyura* Randell (Shrubby Desert Mulga) and *A. paraneura* Randell (Weeping Mulga), together with six close relatives, namely, *A. atopa* Pedley, *A. brachystachya* Benth. (Turpentine Mulga or Umbrella Mulga), *A. clelandii* Pedley (Cleland's Mulga), *A. craspedocarpa* F.Muell. (Hop Mulga), *A. ramulosa* W.Fitzg. (Bowgada or Horse Mulga, containing two varieties) and *A. subtessaragona* Tindale and Maslin. Other species not far removed from the Mulga group include *A. coolgardiensis* Maiden (Sugar Brother) and perhaps *A. catenulata* C.T.White (Bendee).

Although field workers can often recognise plants as belonging to the Mulga group through characters such as growth form, foliage colour and their place in the landscape, such identifications are not necessarily reliable. To identify Mulga accurately one must resort to cryptic characters (i.e. attributes that are often visible only with the aid of magnification). However, there is no single character that uniquely defines the entire Mulga group and, indeed, the group is so variable that even a combination of characters produces a definition that is almost meaningless, at least in terms of gross morphological features. The four species of the core group of Mulga, however, can be uniquely defined by a reasonably small suite of characters. This combination of attributes (listed below) is found in *A. aneura*, *A. ayersiana*, *A. minyura* and *A. paraneura* but not in any other species.

- Trees or (usually tall) shrubs with hard, grey or blackish bark.
- Branchlets (at least when young) invested with short, straight, silvery white, appressed hairs.
- Phyllodes not spiny tipped, flat or terete (never angular in cross-section), striate by numerous, fine, closely-spaced, longitudinal nerves, the nerves commonly resinous (at least on young phyllodes) and with short, straight, silvery white, appressed hairs between them (hairs sometimes cover the entire surface of the phyllode).
- Inflorescences simple (i.e. not racemose or paniculate) with the flowers arranged in cylindrical spikes or sometimes in obloid (never globular) heads.
- Sepals free or very shortly united at their base.
- Pods flat, thin-textured (often papery, never woody), commonly reticulately veined and with or without a narrow marginal 'wing' (see Randell 1992: 108 for discussion of the 'wing').
- Seeds transversely or obliquely aligned in the pod, the aril rather small and white or very pale yellow.
- Distribution largely confined to the arid zone (Figure 1).

The six close relatives of the core group possess many but not all of the above attributes; each relative has one or more characters that are at variance. For example, *A. craspedocarpa* has reticulately veined phyllodes, *A. ramulosa* and *A. brachystachya* have non-resinous phyllodes and usually terete pods, *A. atopa* and *A. subtessaragona* have quadrangular pods and *A. clelandii* has ± longitudinal seeds. Some of these taxa, especially *A. craspedocarpa*, hybridise with various members of the core group of Mulga.

VARIATION IN MULGA

Mulga species show a great deal of variation in morphological features, especially growth form, phyllode shape, size and colour, and pod morphology. This variation is evident both between different populations of the one taxon and between different taxa within the one population. People with field experience of Mulga commonly encounter mixed-populations containing few

TABLE 1
The Mulga core group and related taxa.

Mulga core group	*A. aneura* F.Muell. ex Benth. (Common Mulga) var. *aneura* var. *argentea* Pedley var. *conifera* Randell var. *fuliginea* Pedley var. *intermedia* Pedley var. *macrocarpa* Randell var. *major* Pedley var. *microcarpa* Pedley var. *pilbarana* Pedley var. *tenuis* Pedley *A. ayersiana* Maconochie (Uluru Mulga) *A. minyura* Randell (Shrubby Desert Mulga) *A. paraneura* Randell (Weeping Mulga)
Closely related to Mulga core group	*A. atopa* Pedley *A. brachystachya* Benth. (Umbrella Mulga,Turpentine Mulga) *A. clelandii* Pedley (Cleland's Mulga) *A. craspedocarpa* F.Muell. (Hop Mulga) *A. ramulosa* W.Fitzg. (Bowgada, Horse Mulga) var. *ramulosa* var. *linophylla* (W.Fitzg.) Pedley *A. subtessaragona* Tindale & Maslin
More distantly related to Mulga core group	*A. coolgardiensis* Maiden (Sugar Brother) ?*A. catenulata* C.T.White (Bendee)

Figure 1. Distribution of the 'core group' of Mulga: A. aneura *(10 varieties)*, A. ayersiana, A. minyura *and* A. paraneura. *Data derived from a compilation of the maps presented in Pedley (2001). Cartography by John Maslin.*

to many different morphotypes[2]. Applying names to the morphotypes has in the past been extremely difficult, not only because very few formal names were available, but also because it is difficult to know with certainty which morphotypes represent the same taxonomic entity. The studies of Randell (1992) and Pedley (2001) have helped clarify some of this taxonomic complexity within the group and have provided aids to identifying the taxa, but the genetic basis of the variation is little understood.

Our preliminary field studies have provided us with an excellent, albeit somewhat limited, opportunity to examine variation in a number of Mulga populations. This work has led to a better understanding of population variation and the distribution of morphotypes, which in turn has allowed the definition of hypotheses that can be tested by molecular methods (see below).

The difficulty in understanding Mulga and applying names to the myriad of forms, lies in the extreme variability in plant habit, foliage and pods. The distribution of morphotypes within a population can provide an indication of the underlying genetic mechanisms responsible for this variation. The following discussion attempts to describe the morphological variation in the core group of Mulga. In a later section we discuss the possible genetic determiners of this variation.

Habit variation

Mulga plants often have distinctive field facies and the main growth attributes that produce these are the following:

- height (shrubs or trees about 3-15 m high);
- number of main stems arising from ground level (one to many);
- angle of divergence of lateral branches from the main stems (horizontal to erect);
- posture of the main branches and branchlets (erect to pendulous);
- overall shape of the plants (rounded, obconic, diffuse, etc.);
- colour and density of the crown foliage.

Figures 2–4 illustrate some of the morphotypes found in members of the 'core group' Mulga. These images show that not only is Mulga very variable in growth form, there is no such thing as a 'typical' Mulga habit. Nevertheless, many taxa have characteristic features and these aid their identification in the field, provided that one knows what characters to look for and that hybridisation has not obscured them. Although many Mulga plants are obconic trees (Figure 2A, 2B, *A. aneura* var. *aneura*) some are shrubs, for example, *A. minyura* (Figure 3A) which is characteristically multi-stemmed with a dense, rounded crown, and *A. aneura* var. *macrocarpa* (Figure 3B) which has a more open, spreading crown. The number of stems arising from ground level can vary, not only between taxa but also within the one taxon; for example, *A. ayersiana* may be either single-stemmed (Figure 4B) or multi-stemmed (Figure 4C). The angle of divergence of the lateral branches from the main stems commonly imparts a distinctive aspect to the plants. For example, *A. aneura* var. *tenuis* (Figure 3E) typically has a distinctly erect branching pattern, whereas in *A. aneura* var. *conifera* (Figures 2E-G) the lateral branches diverge more or less at right angles from the main stem. A very distinctive growth form is that of *A. paraneura* (Figure 4A) which has a whispy open crown with pendulous branchlets. Crown colour in Mulga is most often a greyish green (e.g. *A. aneura* var. *aneura*), but it is silvery grey or blue-grey in taxa such as *A. ayersiana* and *A. minyura* and typically pale green in others such as *A. aneura* var. *tenuis*. Crown colour may be dominated by the colour of the new shoots and can therefore vary seasonally; in taxa such as *A. aneura* var. *fuliginea* the plants assume an overall brownish hue when making new growth.

Field observations suggest that, while some morphotypes maintain a single habit throughout their life, others change as they mature. In the latter case the adolescent plants are often more bushy and rounded than the adults (Figures 4B-D, *A. ayersiana*; Figures 3C-D, *A. aneura* var. *pilbarana*). Juvenile plants also appear to have distinctive facies and there would be a practical advantage (to ecologists for example) in being able to ascribe names to these confidently; further study of juveniles is needed.

It seems likely that in some taxa, at least, growth can be arrested at particular stages of development (e.g. the adolescent phase), and therefore some of the variation observed in Mulga populations may be due to this phenomenon. In other words, plants may look superficially very different (in both growth habit and phyllode morphology) but may in fact represent the same genetic entity. The very distinctive 'pine tree' habit of *A. aneura* var. *conifera* may possibly be an example of arrested development, although this is yet to be confirmed through detailed field study and genetic investigation. Typical var. *conifera* plants grow to tall trees (reaching c. 10 m high) with one (or sometimes two) straight, erect trunks and numerous, short lateral branches which diverge almost at right angles from the main trunk (Figures 2E-G). These plants have a scattered distribution (Pedley 2001: 476) and appear not to be especially common. An intriguing question is whether the so-called 'Christmas tree' form of Mulga that is encountered in some populations of at least *A. aneura* var. *aneura*, var. *pilbarana* and var. *tenuis* is the same entity as *A. aneura* var. *conifera*. Pedley (2001) thinks not, and he may well be correct. However, our field observations at a few sites have provided insights into the origins of 'Christmas tree' Mulga and, even if these morphotypes are taxonomically different from var. *conifera*, they may provide clues as to the origin of this variety. Figure 2D shows a typical 'Christmas tree' morphotype that occurred (at a very low frequency) within a mixed Mulga population; this population, about 30 km north of Meekatharra, W.A., contained plants of *A. aneura*

[2] The term morphotype is used here to denote a group of plants that share similar-looking morphological features, e.g. growth form, phyllode shape, size and/or colour, pod form, etc.

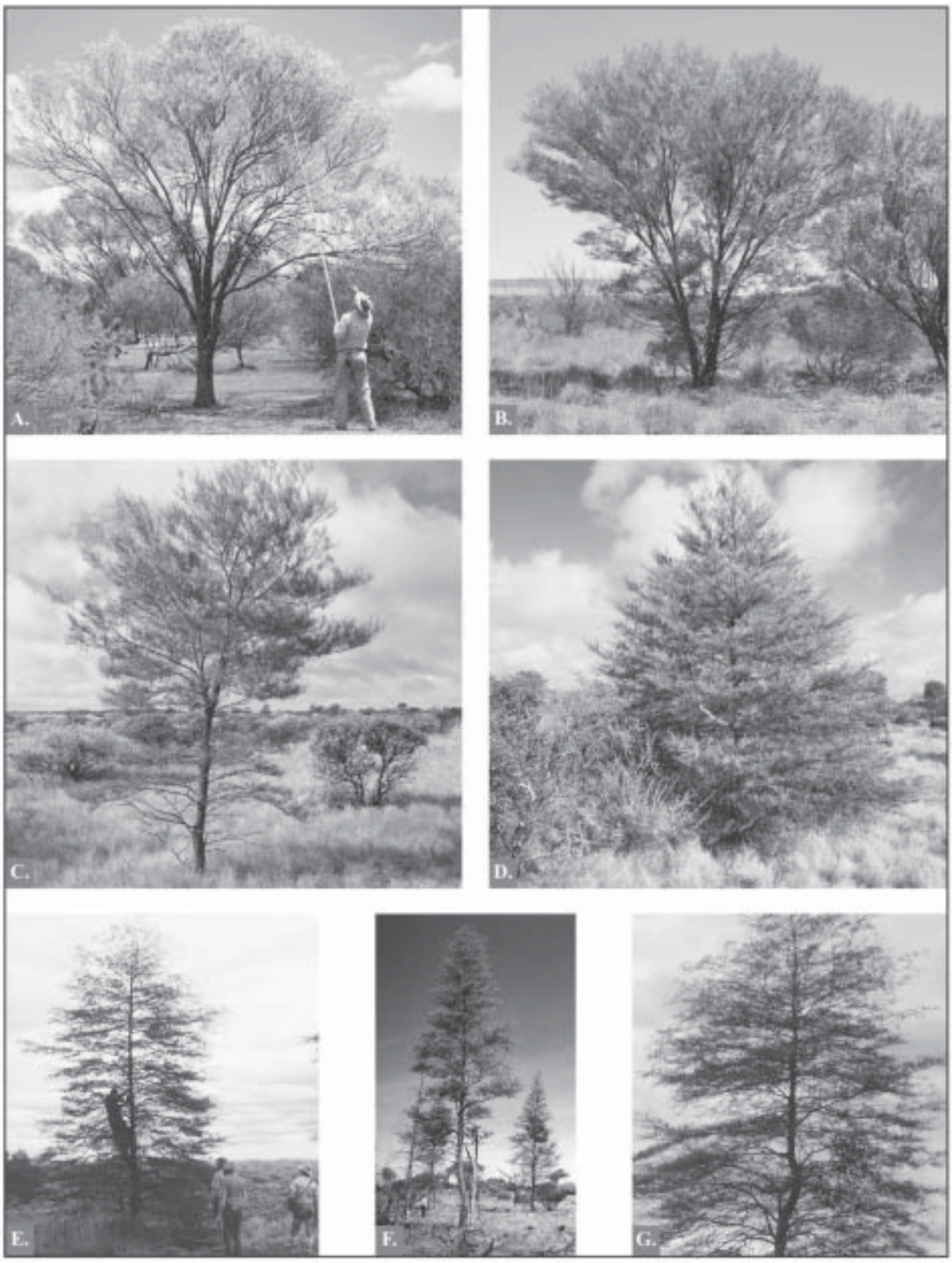

Figure 2. Habit variation in A. aneura. ***A*** – A. aneura var. aneura *(single-stemmed);* ***B*** – A. aneura var. aneura *(multi-stemmed);* ***C*** – A. aneura var. aneura *(note wide-spreading branches below fork and ascending branches above fork);* ***D*** – A. aneura var. aneura *('Christmas tree' morphotype);* ***E-G*** – A. aneura var. conifera.
All photographs by B.R.Maslin except 2F by H.Hewitson.

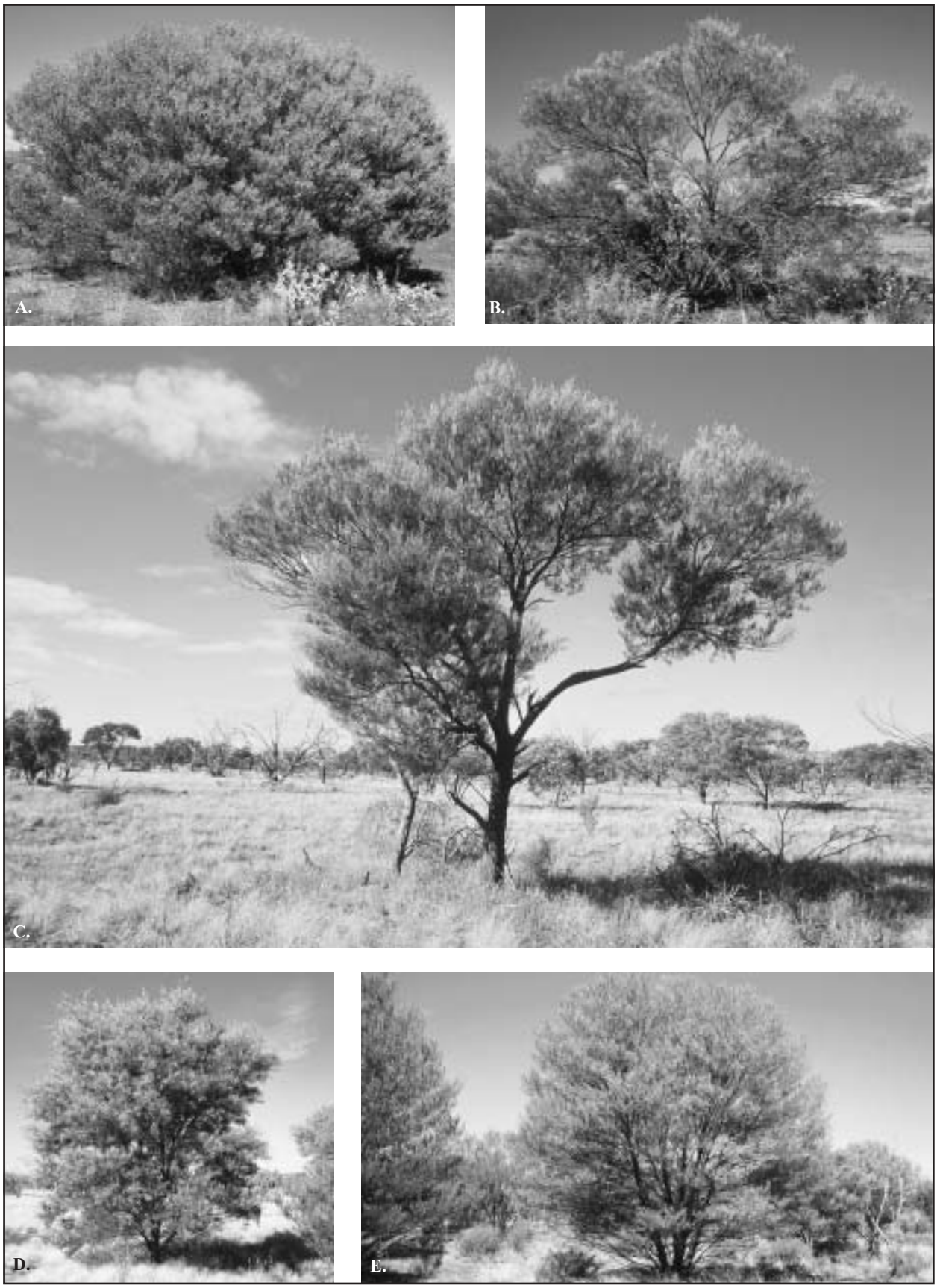

Figure 3. Habit variation in A. aneura *and* A. minyura. ***A*** – A. minyura*;* ***B*** – A. aneura var. macrocarpa*;* ***C*** – A. aneura var. pilbarana *(mature plant);* ***D*** – A. aneura var. pilbarana *(juvenile plant);* ***E*** – A. aneura var. tenuis.
All photographs by B.R.Maslin.

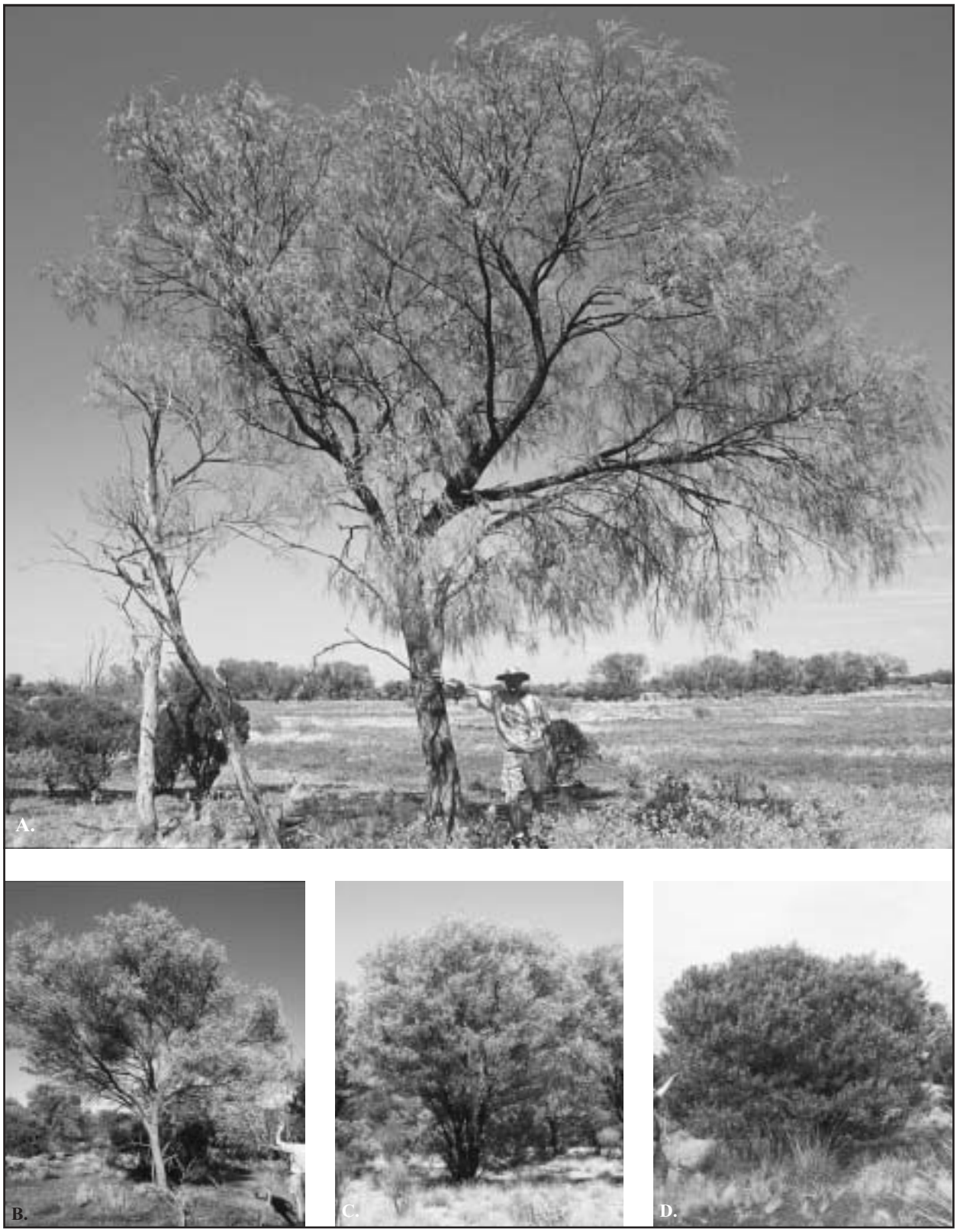

Figure 4. Habit variation in A. paraneura *and* A. ayersiana. ***A*** – A. paraneura *(note pendulous branchlets);* ***B-D*** – A. ayersiana *(**B** – mature, single-stemmed plant; **C** – mature, multi-stemmed plant; **D** – juvenile plant).*
All photographs by B.R. Maslin.

var. *aneura* and other Mulga taxa. Here, the 'Christmas tree' morphotype possessed a single, straight, erect main trunk and widely spreading lateral branches, as in var. *conifer*. Adjacent to this plant, and occurring at a higher frequency within the population, were morphotypes that can best be described as having a 'pseudo-conifer' growth habit (Figure 2C). These plants had a single trunk for about 1-2 m above ground level, at which point it divided into two main stems. The lateral branches below the divide were quite short and diverged almost at right angles, whereas above the divide the lateral branches were longer and had an ascending to erect aspect. It is not hard to imagine that once the lower branches were lost and the plant continued to grow, it would attain a mature growth form similar (or identical) to that which is commonly seen in var. *aneura* (Figure 2A). Similarly, it is easy to imagine a 'Christmas tree' morphotype resulting, had the trunk of the 'pseudo-conifer' plant not divided and had all the lateral branches retained their horizontal aspect. Whether or not these 'Christmas tree' morphotypes would ultimately mature into 'typical' var. *conifera* is not known.

In several isolated populations *A. aneura* var. *conifera* maintains its characteristic growth habit but the phyllodes vary significantly (terete and green vs. flat and greyish) which suggests that plants attributed to this variety may not all share a common ancestry. More field studies are needed to understand these developmental phenomena better, but this is type of problem can also be addressed by DNA methods.

Phyllode variation

Perhaps the most striking variation among Mulga plants is in phyllode shape and size, although colour also varies and can be a significant factor contributing to the overall 'look' of the plants (see above under *Habit variation*). Phyllode variation can be expressed between individuals of the one taxon over its geographic range, between individuals of different taxa within the one population, or at different stages of growth within an individual plant. It is intra-population variation that is normally the most striking (and the most confusing for field workers), and this is often compounded by genetic factors (discussed below) such as hybridity between morphotypes. Figure 5 shows a range of phyllodes from one mixed Mulga population encountered during the course of our study. This demonstrates the great complexity that can exist within populations.

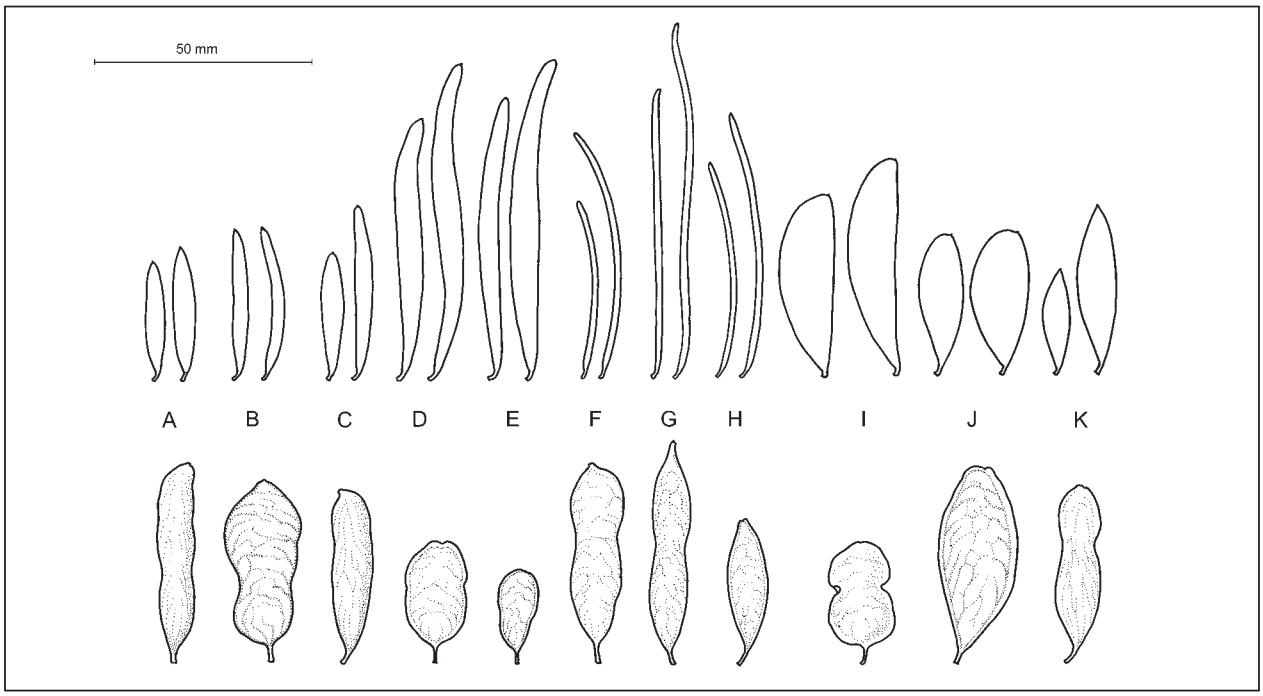

Figure 5. Phyllode and pod variation sampled randomly (and not thoroughly) from a mixed Mulga population near Mt Magnet, W.A. ***A and C*** *–* A. minyura x A. ramulosa var. ramulosa *(B.R.Maslin & J.T.Miller 7924C & G respectively);* ***B*** *–* A. aneura var. macrocarpa x A. minyura *(B.R.Maslin & J.T.Miller 7924B);* ***D and E*** *–* A. aneura var. major *(B.R.Maslin & J.T.Miller 7924F & H respectively);* ***F*** *–* A. aneura var. aneura *(B.R.Maslin & J.T.Miller 7924J);* ***G*** *–* A. aneura var. microcarpa x A. ramulosa var. ramulosa *(B.R.Maslin & J.T.Miller 7924);* ***H*** *–* A. aneura *var.* microcarpa *(B.R.Maslin & J.T.Miller 7924A);* ***I*** *–* A. aneura var. argentea *(B.R.Maslin & J.T.Miller 7924I);* ***J*** *–* A. craspedocarpa *(B.R. Maslin & J.T.Miller 7924D);* ***K*** *–* A. craspedocarpa x A. minyura *(B.R.Maslin & J.T.Miller 7924E)*
Determinations by L.Pedley (BRI) from herbarium material, except 7924F & H which were determined by B.R.Maslin. Voucher specimens lodged at the W.A. Herbarium (PERTH).

Within the 'core group' of Mulga the phyllodes vary from terete to flat, straight to variously curved, short to long and linear to narrowly elliptic. The smallest phyllodes occur on *A. minyura* (0.5-2.5 cm long and 2-4 mm wide), the widest on *A. ayersiana* and *A. aneura* var. *major* (reaching 10 mm and 8(-13) mm wide respectively) and the longest on *A. paraneura* (7-20 cm). Although phyllode size is an easy character to observe and measure, it is variable and therefore is, in itself, generally not a reliable means of identifying taxa.

A striking example of phyllode variation on a single plant was observed in two populations of a probable new taxon (referred to herein as Holey Mulga) that we inspected near Alice Springs, Northern Territory. Plants of this species show three distinct phyllodes forms (Figure 6):

- juvenile phyllodes are oblong, elliptic or oblanceolate and are short and broad (about 15-25 x 5-10 mm);
- adult phyllodes are narrowly linear and 40-70 x 1.5-2 mm;
- adolescent phyllodes are intermediate in both shape and size, i.e. narrowly oblong-elliptic and about 25-40 x 3-5 mm.

Some plants contain all three phyllodes types, juveniles on the lower branches, adults on the upper branches and the adolescents in between. However, plants bearing only juvenile, adolescent or adult phyllodes also occur. From observations to date it is probable that in this taxon both the juvenile and adolescent foliage may persist on fully grown plants, but it is not known if these plants ever become biologically mature, i.e. capable of producing flowers and fruits (to date only fully grown plants bearing adult foliage have been collected in fruit). Thus, in Holey Mulga one may encounter plants bearing any one of three very different phyllode types and this can cause problems with identification, particularly when using herbarium material. In the field, however, plants of this taxon can usually be reliably identified by their fluted trunks (similar to those found in *A. coolgardiensis* from W.A. and *A. catenulata* from Qld and north-eastern N.T.) and by the fact that they always grow on rocky ridges. Also, when the plants die from old age, drought or fire they show very distinctive holes in their trunks where lateral branches once emerged, thus the common name Holey Mulga.

It is likely that at least some of the variation observed in Mulga populations can be accounted for by neoteny and heteroblasty, as just described for Holey Mulga. To what extent this occurs will be determined only by further field study.

Pod variation

The most important variable in the fruit of the core group of Mulga is the position of the vein that lies at or near the margin of the pod. As discussed by Randell (1992), this vein supplies the placenta of the developing seed via the funicle. In the past the area between the vein and the margin of the pod has been incorrectly termed a 'wing', and the width of this wing can be useful in distinguishing certain taxa. In *A. aneura* var. *aneura*, var. *macrocarpa* and var. *pilbarana*, for example, the wing is absent or extremely narrow, whereas in taxa such as *A. ayersiana*, *A. minyura* and *A. aneura* var. *intermedia* and var. *major* it is 1-2 mm wide (Pedley 2001). Sometimes the wing is not easy to discern because the submarginal vein may not be overly prominent when viewed on the outer surface of the pod. In these cases it is best to split the pod open and inspect the vein on the inner surface of an individual pod valve. Other carpological features useful in identifying taxa include pod (and seed) size, pod colour and to a less extent the prominence of surface reticulations and density of the indumentum. Acacia *aneura* var. *tenuis*, for example, has distinctively shiny, pale brown pods, while *A. aneura* var. *macrocarpa* and var. *microcarpa* have large and small pods respectively (Pedley 2000).

There is some evidence that pod and phyllode size and shape covary. Both Cody (1989) and Andrew *et al.* (in review) found that phyllode and pod variation are congruent. This suggests that, at least in the plants studied by these authors, pod and phyllode traits are either linked or the same genes may affect the traits.

Variation within populations

Perhaps the most perplexing aspect of the Mulga complex is that the above-mentioned variation in plant habit, phyllodes and pods often occurs in a single population. Such populations usually contain from a few to more than a dozen morphotypes. It is not uncommon for some of these morphotypes to re-occur in adjacent or more distant populations, often in association with different morphotypes. This results in a very complex mosaic of morphotypes in mixed populations across arid landscapes. Single morphotype populations are apparently rare but have been seen, for example, in *A. aneura* var. *pilbarana*.

An important approach to understanding what controls variation in Mulga is to know the way that characters vary within populations. If the variation for a particular attribute (e.g. phyllode size) patterns into discrete classes then this indicates that there may be a barrier to gene flow between the morphotypes. However, if the variation is continuous it may indicate that hybridisation occurs among the morphotypes. A few studies have examined phyllode length variation within Mulga populations and the results of these indicate, not unexpectedly, that different patterns exist. Cody (1989), for example, found a continuous distribution of phyllode dimensions at Yelma in Western Australia. On the other hand, Andrew *et al.* (in review) found a disjunct distribution of phyllodes characters in a population near Mt Connor in the Northern Territory. As discussed below, developmental, genetic or environmental factors (or a combination of these) may be responsible for the observed patterns of character variation within populations.

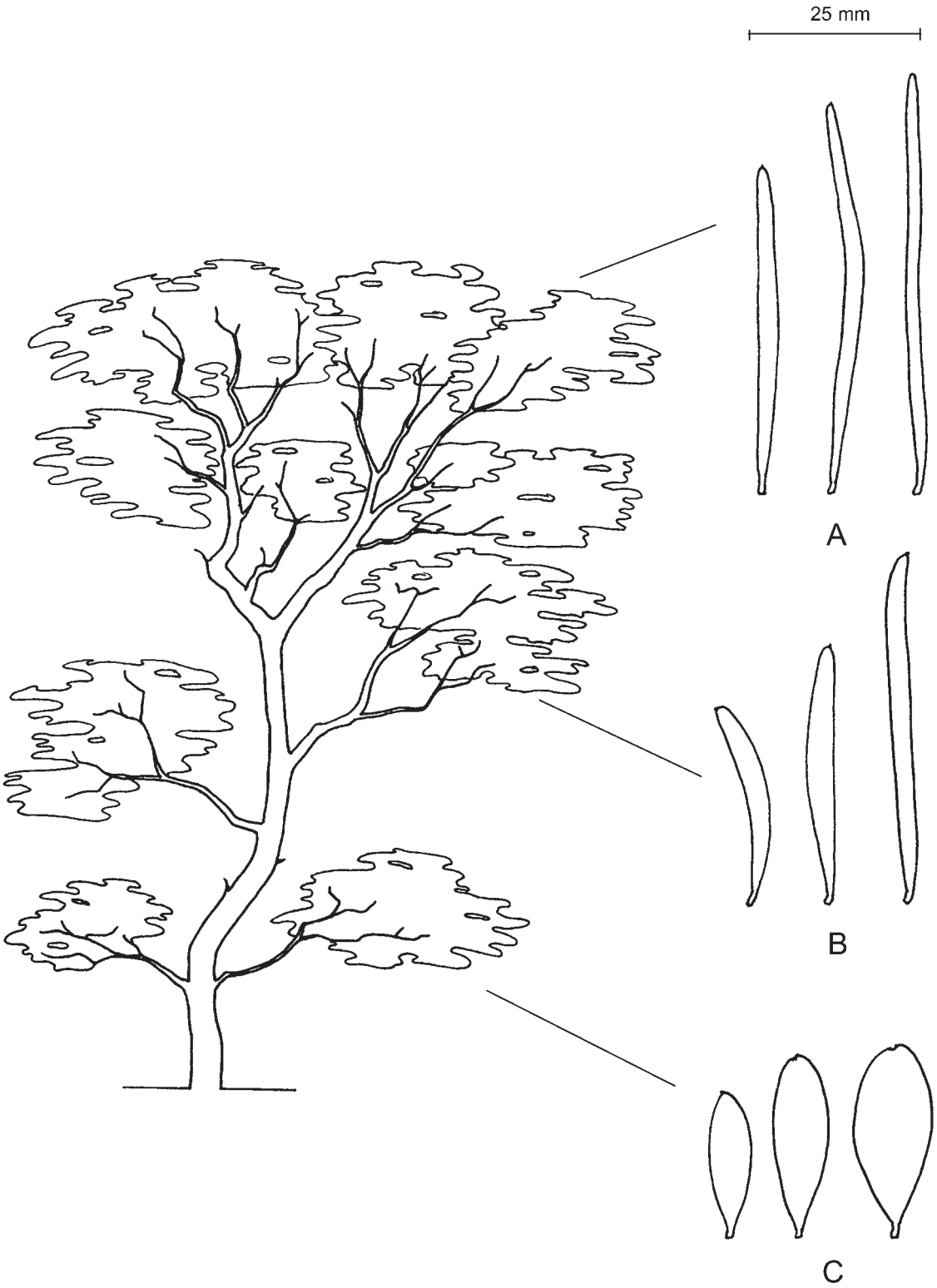

Figure 6. Acacia *sp. nov. (Holey Mulga) showing heteroblastic foliage on a single plant. A – mature phyllodes found on upper branches; B – intermediate phyllodes; C – juvenile phyllodes found on lower branches.*
A from B.R.Maslin 8149(iii); B from B.R.Maslin 8149(ii); C from B.R.Maslin 8149(i). Voucher specimens lodged at the W.A. Herbarium (PERTH). Collection from near Alice Springs, N.T.

Variation between populations

As mentioned above, both discrete classes and continuous variation in phyllode size have been found in Mulga populations. Figure 7 presents data from 18 populations in the Mt Magnet area of W.A. where plants were collected to represent the range of variation. The figure depicts the length versus width of phyllodes and shows the variation in phyllode size from obviously small in *A. minyura* to large in *A. ayersiana*. Other than the extremes of the size range it is not possible to detect distinct size classes in this particular study. These results confirm field observations that populations consist of morphotypes varying in phyllode size and that a single morphotype can have a wide distribution. This sort of quantitative approach should be applied also to other structures such as pods and seeds, and extended to populations across the entire geographic range of Mulga.

Another important key to understanding the complexity in Mulga is to determine whether the similar-looking morphotypes in different populations actually represent the same genetic entity. For example, a plant from Central Australia may have the same habit and phyllode morphology as one from W.A. The critical question is whether these two plants have a common ancestor (and occur in their respective geographic areas by natural distribution processes) or whether they have arisen independently in each area by local processes (such as hybridisation), resulting in similar-looking forms. If these plants are shown to be of the same genetic type it would indicate a common origin of the morphotype. This would suggest that the morphotype is maintaining its genetic integrity and could be considered worthy of formal taxonomic recognition. If, however, the two morphotypes had different genetic signatures it would indicate that they may be of local origin, and that the similar morphology may be due to parallel evolution or separate hybridisation events. It is this type of question that may be addressed with molecular marker techniques.

FACTORS CONTRIBUTING TO VARIATION

Genetic techniques used to study variation

The past decade has seen an explosion in tools available to study plant genetics and, in particular, the relatively new DNA technique Simple Sequence Repeats (SSRs or microsatellites). These methods allow the study of genetic relationships among plants both within and between populations. In particular, these markers can identify the parents and offspring of hybridisation events. Other important genetic techniques, and ones that we have employed in our study of Mulga, are cytology and embryology. Data derived from observations of chromosomes can identify barriers to reproduction, since plants with different numbers of chromosomes are generally incompatible.

While field study of Mulga has contributed significantly to our understanding of variation, the use of glasshouse grown plants has been critical to developing an understanding of the underlying genetics. Growing plants to study development, cytogenetics and molecular genetics is helping to unveil some of the mysteries surrounding the morphological variability in Mulga.

Hybridisation

Hybridisation among Mulga morphotypes, leading to the creation of new morphotypes, has been considered a major factor in Mulga diversification (Davies 1976; Randell 1992; Pedley 2001). Our own field observations and morphometric studies (Figures 7 and 8) show that, in many Mulga populations, morphotypes occur that seem intermediate in form (e.g. in shape and size of phyllodes) between two or more other morphotypes. This suggests that the intermediate form may be a hybrid derived from the local parental plants. However, hybrids do not always express the intermediate character states of the parents, as some contain novel or transgressive morphological characters (McDade 1990; Riesberg and Ellstrand 1993).

Additionally, two morphotypes may hybridise to create unique progeny but these may be infertile. In these cases the hybrids may increase the amount of variation observed within a population but, being infertile, do not contribute to future gene pools.

Locally derived hybrids appear to dominate some populations. For example, in the Mt Magnet area of W.A. many populations contain *A. aneura*, *A. craspedocarpa* and putative hybrids between the two. *Acacia craspedocarpa* has relatively short, broad phyllodes with a distinctive reticulate nervature (due to anastomosing minor nerves) and large pods (Figure 8D); *A. aneura*, on the other hand, has long, narrow phyllodes with parallel (not reticulate) nerves, and smaller pods (Figure 8A). The putative hybrids can be readily recognised by the phyllodes which are intermediate between the putative parents not only in size but also in their nervature (i.e. they have relatively few anastomoses, therefore the reticulum is not pronounced) (Figures 8B, 8C). Populations can be dominated by the hybrids (often also including F2 back crosses to one or other of the putative parents) with various *A. aneura* and *A. craspedocarpa* morphotypes interspersed, creating a complex mixture of plants. It is not known whether these hybrids persist and are reproductively successful although mature seeds have been collected from many of them. If this same type of complex hybridisation occurs between similar-looking morphotypes then it is more difficult than in the *A. aneura/craspedocarpa* example to distinguish between the parents and hybrids. If these hybrids are interfertile then the complex genetic mixture gets even more complicated, and the differences between the morphotypes may become even less evident.

Molecular markers have been employed to investigate the role of hybridisation in one population of *A. craspedocarpa* containing one morphotype of *A. aneura* and several putative hybrids. This population was located

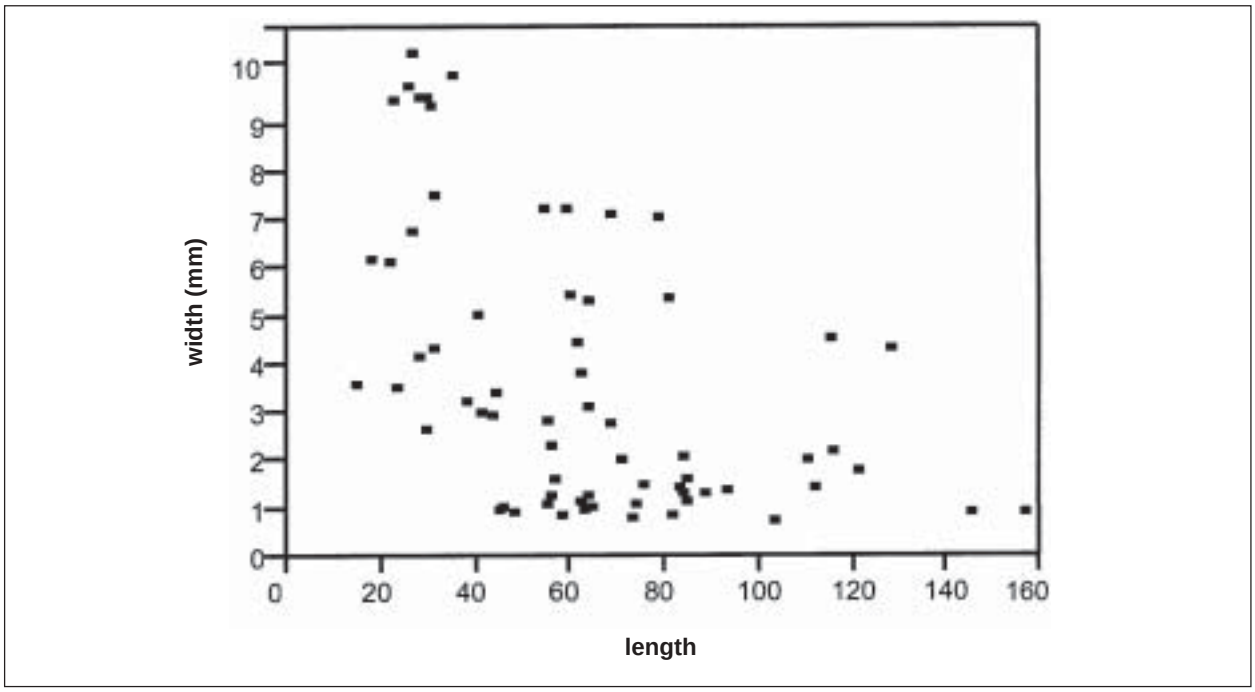

Figure 7. Plot of the length (x-axis) and width (y axis) of various mulga phyllodes collected in the general Mt. Magnet/Meekatherra area of Western Australia (B.R.Maslin, J.Miller, L.Sweedman & B.Cole BRM 7892-7924). Each point is the average from the measurement of three phyllodes from a single tree.

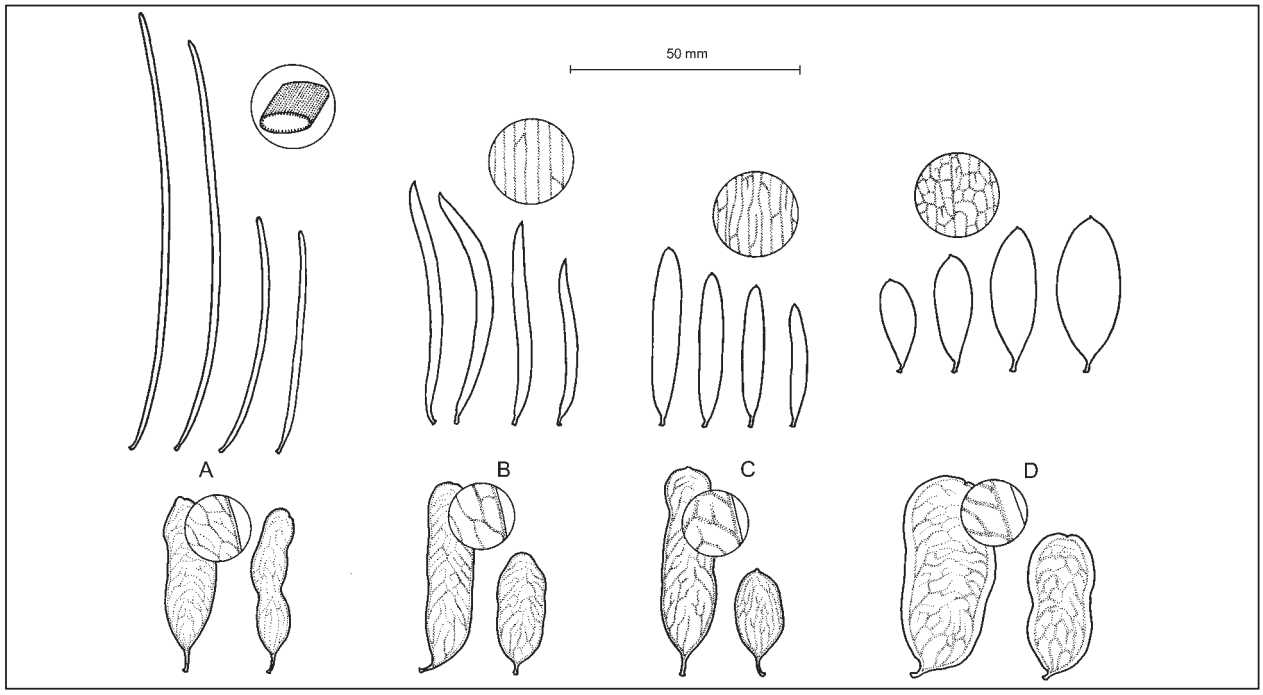

Figure 8. Acacia aneura var. aneura x A. craspedocarpa *hybrid complex near Mt Magnet, W.A.* ***A*** – A. aneura var. aneura *(pods not winged; phyllodes linear with insert showing finely striate, parallel nervation);* ***B*** *and* ***C*** – A. aneura var. aneura x A. craspedocarpa *hybrids showing intermediate pods and phyllodes (note inserts showing few anastomosing minor nerves, i.e. phyllodes not conspicuously reticulate);* ***D*** – A. craspedocarpa *(pods winged; phyllodes short and broad, with insert showing distinctive reticulate nervature).*
A from B.R.Maslin, J.Miller, L.Sweedman & B.Cole (BRM 7892); B from B.R.Maslin, J. Miller, L.Sweedman & B.Cole (BRM 7892E); C from B.R. Maslin, J.Miller, L.Sweedman & B. Cole (BRM 7892D); D from B.R.Maslin, J.Miller, L.Sweedman & B.Cole (BRM 7892C). Voucher specimens lodged at the W.A. Herbarium (PERTH).

23.5 km west of Mt Magnet and the plants referred to below were vouchered under *B.R. Maslin et al.* 7892A-E. Hybrid plants will receive half of their alleles from one parent and the other half from the second parent. If each parent has specific alleles not found in other plants these alleles will be added together in the hybrid progeny. However, microsatellites in the Mt Magnet population did not show this additive profile of alleles of the putative parents in the putative *A. aneura/craspedocarpa* hybrids (Miller *et al.* in prep). The sample size in this particular study, however, was small and not all possible parental plants were sampled. This population appeared to have only one *A. aneura* morphotype which suggests that there may be less genetic variation within this morphotype. Chloroplast DNA from the *psb*A-*trn*H intergenic spacer was also sequenced for these plants. *Acacia craspedocarpa* differed from *A. aneura* by two deletions and the putative hybrid also had these two deletions. Since chloroplast DNA in *Acacia* is maternally inherited this suggests that *A. craspedocarpa* is the maternal parent of the putative hybrid. More detailed sampling of this population is necessary to test whether there is sufficient genetic variation within the *A. aneura* morphotype to suggest its parentage in the hybrid. Alternatively, the parent of this hybrid may have died out in the population. If not, the putative hybrid may fall within the natural range of genetic variation of *A. craspedocarpa*. If this is indeed the case, then it will require a major redefinition of *A. craspedocarpa* because phyllode nervature and shape in putative hybrid plants is very different from that found in typical *A. craspedocarpa* (compare Figures 8B and 8C with Figure 8D).

Microsatellite sampling was more detailed in a population near Mt Connor in the N.T., studied by Andrew *et al.* with the same molecular DNA markers. This population contained five non-overlapping morphotypes including a putative *A. aneura* x *A. minyura* hybrid. The microsatellite markers were consistent with the morphology in recognising the same genotypes and found little or no genetic variation within morphotypes. Analysis of the hybrid using SSR did not show alleles in common with the samples of the two parental species. A multivariate analysis did not place the putative hybrids as intermediate to the two parents. While the sampling in this study was more detailed than in the *A. craspedocarpa* example, the putative parents at Mt Connor may not have been sampled. One possible explanation for this might be that the hybridisation events occurred elsewhere and that the seed was transported to Mt Connor by water; it is common in these arid environments for large volumes of water to flood the landscape when heavy rains occur.

The lack of direct proof for hybridisation to date should not be regarded as evidence against hybridisation occurring in Mulga. Another possible explanation for the lack of additive alleles in the putative hybrid may be backcrossing of the hybrids to parental species and with other hybrids containing genetic variation, thus obscuring the ability to identify additivity of alleles. Also, if hybridisation events occurred sufficiently in the past, the hyper-variable microsatellite markers may have changed their alleles through mutation and the evidence for hybrid additivity lost. At present it is not clear which explanation is most likely to account for the data found to date.

Polyploidy

The Mulga complex has been considered to be polyploid at least in part. Tetraploids have been reported by Buhkari (1997) and Maslin (unpublished). The recent work by Andrew *et al.* at Mt Connor led to the discovery of triploids, tetraploids and pentaploids. During meiosis the number of copies of chromosomes is halved as the gametes form. Since the halving of triploids and pentaploids does not lead to complete sets of chromosomes in the gametes these plants usually are not fertile. Chromosomes were counted from seedling root tips from seeds collected off trees with heavy seed set. Some of these seedlings were triploids and others pentaploids, suggesting that the mother plant had the same ploidy level and had higher fertility than would be expected by sexual reproduction alone.

Diploid Mulga has been reported only from material cultivated outside Australia (Tixier 1965). A diploid plant of *A. ayersiana* var. *latifolia* (= *A. ayersiana*, *fide* Pedley 2001: 322) was found by flow cytometry but has not been confirmed by the more accurate method of chromosome counting (Miller unpublished). This specimen was a seedbank accession from the Charleville area of south-western Queensland. This area has not been extensively collected by us and may hold a key piece to the puzzle. Diploid members of the complex could point to an ancestral types that have given rise to new morphotypes via auto or allopolyploidisation events. The use of cytogenetics to study chromosome pairing during meiosis within a polyploid genome may give a better understanding of the origin of the polyploid event. A fruitful future study in Mulga would be to undertake a concerted search for naturally occurring diploid plants.

Polyembryony

During field trips seeds were collected whenever possible. When these seeds were germinated in glasshouses, many aberrant plants with more than two cotyledons or two multiple meristems were noted. Fox (1979) also noted twin meristems in *A. aneura* but found that these plants soon died, but this mortality was not seen in our glasshouse experiments. In our case further germinations were undertaken in petrie dishes to examine the aberrant seedlings more closely. Twenty or more seeds harvested from each of over 24 plants throughout the range of Mulga in Central and Western Australia were germinated. This survey represented all available morphotypes for which we have been able to collect in seed. A proportion of aberrant seedlings were identified in all populations, the most common having three cotyledons. Further dissection of these plants led to the discovery of two sets of true first leaves. Two complete plants, with two distinct pairs of

cotyledons, two hypocotyls and roots, were also commonly seen. The relative placement of these twin plants varied. At times the two plants could be easily separated and could be grown on individually in pots. After re-establishment in glasshouse pots the plants grew normally. Other twins were intertwined and could not be separated without damaging the plants. Between these extremes there was a range of twins including forms with two apical meristems and a single root. A cross-section of the hypocotyl indicated that the plants had two vascular systems. It appears that usually only the strongest of the two plants survives the germination process, with a low level of survival for multiple plants.

The presence of more than one embryo in a seed is called polyembryony. It can have multiple causes and has been found in sexual (Asker and Jerling 1992) and asexual species. Two or multiple embryos may be due to apomixis, the adventitious growth of an asexual embryo from maternal tissue. Cell division of the maternally derived nucellus tissue, which surrounds the embryo sac, gives rise to multiple asexual embryos in *Senna* series *Subverrucosae*. In this species the sexually derived embryo may or may not persist and develop, leading to multiple embryos of both sexual and asexual origin (Randell 1970).

Apomixis

The arrays of seedlings used in testing seedling development and polyembryony were surveyed with SSR markers for variation among the siblings and to the mother plant (Miller *et al.* in prep). Over 95% of the progeny were identical to the mother plant and the siblings, suggesting that they are of asexual origin (Miller *et al.* in prep). Most loci studied were heterozygous, such as a tetraploid plant with three alleles at a single locus. If the plant was self-pollinating (most *Acacia* are outcrossers) the expected segregation of alleles in the self-pollinated progeny did not occur. It is also unlikely that outcrossing would have produced the identical genotype of the maternal parent in the progeny.

The progeny data and the microsatellite data from Andrew *et al.* (in review) indicate that Mulga can produce a clonal population genetic structure via apomixis. Andrew *et al.* found more genetic differences among morphotypes than within morphotypes and some morphotypes, including triploids and pentaploids, displayed identical genotypes within the dozen plants sampled, providing evidence of fixed heterozygozity that can not be well-explained by sexual reproduction.

These multiple lines of evidence indicate that apomixis plays an important role in Mulga populations. The observations of polyploidy, microsatellite marker patterns and especially polyembryony suggest that these plants are apomictic. Apomixis is asexual reproduction through a seed. There are several forms of apomixis depending on the origination of the asexual embryo.

Based on data and observations outlined above the following is a working hypothesis explaining part of the Mulga variation. Ancestral morphotypes of diploid Mulga, either through hybridisation or autopolyploidy, gave rise to polyploid morphotypes. The polyploid morphotypes are facultative apomicts. Sporadic hybridisation occurs both within morphotypes and among plants differing in morphological characteristics. These plants are maintained in the populations due to apomictic reproduction. The polyploidy and apomixis act as barriers to gene flow and allow the maintenance of these hybrids. Recurring hybridisation among morphotypes and their subsequent maintenance by apomictic reproduction creates the highly variable populations.

Neotony

Neoteny, the retention of juvenile features in the adult growth phase, as described above for Holey Mulga (see *Phyllode variation*). Understanding ontogenetic developmental sequences in foliage, from the juvenile through adolescent to adult, is likely to be more important in understanding variation within Mulga than has hitherto been appreciated. As mentioned above in discussing phyllode and habit variation, a working field hypothesis to explain the variation in Mulga was neoteny. We consider this a retention of juvenile characters when the juvenile form is reproductively mature.

This was shown to be a major factor in Holey Mulga in which we observed three classes of phyllodes. Some trees bore one class, others all three. Holey Mulga grows only on rocky ridges in central Australia where other Mulga morphotypes tend not to grow, hence the three classes of phyllodes could be identified easily. This is important because some trees with juvenile and intermediate-sized phyllodes are as large as trees with mature phyllodes.

Other Mulga morphotypes may have a similar range of phyllode diversity relating to developmental stages. Putative developmental stages have been seen in other populations which, however, all consisted of multiple morphotypes, hence these predictions will need to be tested with molecular markers.

Neoteny also appears to be a possible causal factor in producing some growth forms in the Mulga group. As discussed above under *Habit variation*, there appears to be a relationship among the habits of *A. aneura* var. *conifera*, the 'Christmas tree' form as well as the pseudo-conifer habit. As mentioned above these same DNA markers can play an important role in identifying juvenile plants and associating these to morphotypes of the corresponding adults.

In this case the plants of varying maturity for phyllode and habit will be tested with DNA markers. These markers will identify closest relatives regardless of maturity. If *A. aneura* var. *conifera* originated multiple times from different Mulga morphotypes the markers will identify that each *A. aneura* var. *conifera* population is more closely related to a different local morphotype than it is to other plants of *A. aneura* var. *conifera*. This information will be invaluable in reassessing the classification of this taxon.

Additionally, patterns may be discernible among morphotypes in their maturation process from juvenile to adulthood, thereby giving us a better understanding of Mulga development as a whole.

FUTURE WORK

Field, glasshouse and laboratory work has begun to shed some light on the genetic reasons for the variation in Mulga. In order to form a useful nomenclatural system for Mulga more work must be done. Firstly, more collections should be made in areas so far not visited. These collections will amplify the knowledge of variation and may uncover diploid members of the complex that could be putative progenitors. DNA markers should be used at the population level to address the issues of hybridisation and neoteny. These markers also should be used to test the relationships of morphotypes both within and among populations. Even with this detailed knowledge the construction of a nomenclature for Mulga will most probably prove difficult due to the extreme genetic complications within this intriguing group of plants.

ACKNOWLEDGMENTS

We acknowledge help from David Albrecht (Northern Territory Herbarium, Alice Springs) for assistance with field work in the Alice Springs area; Peter Latz (Alice Springs, N.T.) for information about Holey Mulga and *A. minyura*; Trevor McKenzie (Mt Magnet, W.A.) for field assistance, provision of information about Mulga and accommodation assistance in the Mt Magnet area; Steve van Leeuwen (CALM, Karratha, W.A.) in the Pilbara; and Dioni Walsh (Department of Agriculture, Meekatharra, W.A.) for field assistance and collection of pods from tagged plants. We thank Penny Butcher (CSIRO Forestry and Forest Products, Canberra), Randy Bayer and Neil Bagnell (CSIRO Plant Industry, Canberra) for their assistance with field and lab work. H.Hewitson took the photograph of *A. aneura* var. *conifera* featured in Figure 2F. A portion of the field work was funded by a grant to JM from the Australian Biological Resources Study.

REFERENCES

Aboriginal Communities of the Northern Territory (1993). *Traditional Aboriginal Medicines in the Northern Territory of Australia*. Conservation Commission of the Northern Territory of Australia: Darwin.

Andrew, R.L, Miller, J.T., Peakall, R., Crisp, M.D. and Bayer R.J. (in review). Genetic, cytogenetic and morphological variation in a mixed population of *Acacia aneura* (Leguminosae: Mimosoideae) species complex members. In *Advances in Legume Systematics*, vol. 11, (M.D.Crisp, ed.). CSIRO Publishing, Melbourne.

Asker, S.E. and Jerling, L. (1992). *Apomixis in Plants*. CRC Press, Boca Raton.

Askew, K. and Mitchell, A.S. (1978). *The Fodder Trees and Shrubs of the Northern Territory*. Division of Primary Industry Extension Bulletin No. **16**, 1-84. Division of Primary Industry, Alice Springs.

Bukhari, M.Y. (1997). Nuclear DNA amounts in *Acacia* and *Prosopis* (Mimosaceae) and their evolutionary implications. *Hereditas* **126**, 45-51.

Beard, J.S. (1976). *Vegetation Survey of Western Australia: Murchison*. University of Western Australia Press, Nedlands.

Carr D.J. and Burdon, J.J. (1975). Temperature and leaf shape in seedlings of *Acacia aneura*. *Biochemie und Physiologie der Pflanzen* **168**, 307-318.

Chippendale, G.M. and Jephcott, B.R. (1963). *Topfeed: the Fodder Trees and Shrubs of Central Australia*. Extension Article no. 5, Animal Industry Branch, Northern Territory Administration, Alice Springs.

Cody, M.L. (1989). Morphological variation in Mulga, I, Variation within and among *Acacia aneura* populations. *Israel Journal of Botany* **38**, 241-257

Cowley, T. (2001). The Green & Gold. *Australian Geographic* **61**, 68-85.

Cunningham, G.H., Mulham, W.E., Milthorpe, P.L. and Leigh, J.H. (1981). *Plants of Western New South Wales*. Government Printer, Sydney.

Davies, S.J. (1976). Studies of the flowering season and fruit production of some arid zone shrubs and trees in Western Australia. *The Journal of Ecology* **64**, 655-687.

Doran, J. and Turnbull, J. (1997). *Australian Trees and Shrubs: Species for Land Rehabilitation and Farm Planting in the Tropics*. ACIAR Monograph No. **24**. Australian Centre for International Agricultural Research, Canberra.

Everist, S.L. (1949). Mulga (*Acacia aneura* F.Muell.) in Queensland. *Queensland Journal of Agricultural Science* **6**, 87-131.

Everist, S.L. (1969). *Use of Fodder Trees and Shrubs*. Queensland Department of Primary Industries, Division of Plant Industry Advisory Leaflet no. 1024.

Everist, S.L. (1971). Continental aspects of shrub distribution, utilisation and potentials: Australia. In *Wildland Shrubs: Their Biology and Utilisation*, pp. 16-25. US Forest Service General Technical Report INT-1.

Fox, J.E.D. (1979). Observations on the germination and early growth of *Acacia aneura*. Mulga Research Centre, Annual Report No. 3: 45-51.

Fox, J.E.D. (1986). Vegetation: diversity of the mulga species. In *The Mulga Lands* (P.S.Sattler, ed.), pp. 27-32. Royal Society of Queensland, Brisbane.

Goodchild, A.V. and McMeniman, N.P. (1987). Nutritive value of *Acacia* foliage and pods for animal feeding. In *Australian Acacias in Developing Countries* (J.W.Turnbull, ed.), pp. 118-122. ACIAR Proceedings no. 16. Australian Centre for International Agricultural Research, Canberra.

Johnson, R.W. and Burrows, W.H. (1981). *Acacia* open-forests, woodlands and shrublands. In *Australian Vegetation* (R.H.Groves, ed.), pp. 198-226. Cambridge University Press, Cambridge.

Kerle, J.A. (1995). Mulga – Wanari. In *Uluru, Kata-Tjuta & Watarrka* (Ayers Rock, the Olgas & Kings Canyon, Northern Territory) (T.Lee, ed.), pp. 74-76. University of New South Wales Press, Sydney.

Latz, P.K. (1999). *Pocket Bushtucker: a Guide to the Plants of Central Australia and their Traditional Uses.* IAD Press, Alice Springs.

Lamont, B.B. and Fox, J.E.D. (1981). Spatial pattern of six sympatric leaf variants and two size classes of *Acacia aneura* in a semi-arid region of Western Australia. *Oikos* **37**, 73-79.

Maiden, J.H. (1907). *Acacia aneura*, F. v. M. The Mulga. *The Forest Flora of New South Wales* **3(6)**, 106-110, pl. 99 & photographs. Government Printer, Sydney.

Maslin, B.R. (coordinator) (2001). *WATTLE: Acacias of Australia*. CD-ROM. Australian Biological Resources Study, Canberra, and Dept. of Conservation and Land Management, Perth.

McDade, L. (1991). Hybrids and phylogenetic systematics I. Patterns of character expression and their implications for cladistic analysis. *Evolution* **44**, 1685-1700.

Midgley, S.J. and Gunn, B.V. (1985). *Acacia aneura* seed collections for international provenance trials. *Forest Genetic Resources Information* **13**, 21-29. FAO, Rome.

Miller, J. T., Butcher, P., Moran, G. and Bayer, R.J. (in prep.). Transfer of SSR markers within the Australian Acacias and their applicability in population genetic studies.

Mitchell, A.A. and Wilcox, D.G. (1994). *Arid Shrubland Plants of Western Australia*, 2nd edn. University of Western Australia, Nedlands, and Department of Agriculture, Perth.

Nix, H.A. and Austin, M.P. (1973). Mulga: a bioclimatic analysis. *Tropical Grasslands* 7, 9-22.

Pedley, L. (1973). Taxonomy of the *Acacia aneura* complex. *Tropical Grasslands* 7, 3-8.

Pedley, L. (2001). *Acacia aneura* and relatives. *Flora of Australia* **11B**, *Mimosaceae, Acacia part 1* (A.E.Orchard and A.J.G.Wilson, eds), pp. 309-328. Australian Biological Resources Study, Canberra, and CSIRO Publishing, Melbourne.

Preece, P.B. (1971). Contributions to the biology of mulga, I, Flowering. *Australian Journal of Botany* **19**, 21-38.

Pressland, A.J. (1973). Rainfall partitioning by an arid woodland (*Acacia aneura* F.Muell.) in south western Queensland. *Australian Journal of Botany* **21**, 235-245.

Randell, B.R. (1970). Adaptations in the genetic system of Australian arid zone *Cassia* species (Leguminosae, Caesalpinioideae). *Australian Journal of Botany* **18**, 77-97.

Randell, B.R. (1992). Mulga, a revision of the major species. *Journal of the Adelaide Botanic Garden* **14**, 105-132.

Riesberg, L.H. and Ellstrand, N.C. (1993). What can molecular and morphological markers tell us about plant hybridizations? *Critical Reviews in Plant Sciences* **12**, 213-241.

Slatyer, R.O. (1965). Measurements of precipitation interception by an arid plant community (*Acacia aneura* F.Muell.). *Arid Zone Research* **25**, 181-192.

Tixier, P. (1965). Données cytologiques sur quelques Legumineuses cultivées ou spontanées du Vietnam et du Laos. *Revue de Cytologie et de Biologie Vegetales* **28**, 133-173.