REVIEW

Assessing genetic risk in revegetation

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Summary

1. Revegetation can provide major environmental benefits in degraded landscapes, but there is also potential for negative impacts from genetic change in local native populations. Broad areas of revegetation may provide a large source of foreign genes in landscapes where small remnant native populations act as a sink. Genetic change from hybridisation can threaten population persistence and contribute to species extinction through genetic assimilation or demographic swamping.

2. Implementation of revegetation within a risk management framework allows identification of risk factors, analysis and evaluation of risk to inform decision-making and management to minimise and mitigate the risk. Informed analysis and evaluation of genetic risk is important in revegetation because it will be difficult to control or reverse the impacts in natural ecosystems and they are often not expressed until the second generation or later.

3. A risk assessment protocol is presented based on evaluation of factors that influence the likelihood and consequences of adverse genetic change from revegetation arising through pollen dispersal.

4. The assessment is applicable to a broad range of revegetation activities and contributes to the development of informed decision-making processes in implementation of revegetation systems and land use practices that protect and enhance biodiversity in degraded landscapes.

5. *Synthesis and applications.* Implementation of revegetation programmes within a risk management framework will help to ensure that significant environmental benefits are captured with minimal concomitant negative impacts on the surrounding biodiversity. A genetic risk protocol provides a tool for evaluation of potential adverse genetic impacts on native populations from revegetation and can be implemented in conjunction with weed risk assessment. Risk assessment as an integral part of evaluation of environmental impact for large-scale revegetation programmes will contribute to the development of informed decision-making processes in the implementation of revegetation systems, and ultimately, it will aid in the development of land uses that protect and enhance biodiversity in degraded landscapes.

Key-words: gene flow, genetic change, hybridisation, outbreeding depression, pollen dispersal, risk assessment

Introduction

Revegetation is a major activity undertaken to restore degraded and fragmented landscapes, particularly in agricultural regions where extensive land clearing has led to considerable environmental problems such as dryland salinity, soil erosion, contamination of waterways and reduction in potable water supplies. Linking of protected areas through vegetation corridors is being undertaken as a means of facilitating adaptation to climate change in many countries. Whilst the environmental benefits of revegetation are usually clear, although not often explicitly quantified, the potential for negative impacts is not generally considered, except in relation to the source of seed for the planting itself (Lessica & Allendorf 1999; Broadhurst *et al.* 2008), and occasionally as a source of weed incursions.

One negative impact of revegetation that has generally not been considered is the potential for genetic change in surrounding native populations (Hufford & Mazer 2003; Laikre *et al.* 2010). Many revegetation initiatives involve planting over large areas, particularly where they are used to restore major

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environmental processes or for carbon sequestration (Laikre *et al.* 2010). These broad areas of revegetation potentially provide a large source of foreign genes, particularly in degraded landscapes where small remnant native populations act as a sink. The potential for genetic change is receiving increasing attention with a strong call for monitoring of genetic diversity in large-scale releases (Schwartz, Luikart & Waples 2007; Laikre *et al.* 2010). Whilst monitoring documents the effects on genetic diversity, risk assessment as a tool for minimising the effects of large-scale releases has received little attention.

Scientific research on gene flow and hybridisation has increased knowledge on the likelihood of adverse genetic change, and this knowledge can contribute to policy- and decision-making in determination of acceptable levels of risk associated with revegetation. However, this scientific knowledge is not usually incorporated into practical assessment tools that are readily available. Here, we review the factors influencing genetic impact of revegetation programmes and present a genetic risk assessment protocol that is suitable for use by land managers and practitioners responsible for implementation of revegetation programmes. We restrict our focus to the impacts of revegetation on surrounding biodiversity and do not discuss issues associated with source of seed for revegetation as this is focussed on the planting itself and has been adequately covered in recent publications (Broadhurst *et al.* 2008; Millar, Byrne & Coates 2008; Sgrò, Lowe & Hoffmann 2011).

This risk assessment is based on well-established genetic principles and is presented as a decision tree that enables evaluation of risk using the information and knowledge available, and identification of cases requiring further knowledge for more rigorous evaluation or implementation of management practices to minimise the risk. We have focussed on the concept of genetic dispersal of whole genomes and do not include aspects of risk associated with specific genes such as introgression of transgenes from genetically modified organisms (Chandler & Dunwell 2008). In this context, we focus on the risk of genetic effects arising from the pollen dispersal component of gene flow in particular, as this risk has not generally been recognised in revegetation programmes. Seed dispersal introduces new genetic material into ecosystems and can be extensive in water- and wind-mediated systems, but does not contribute directly to genetic change in the receiving population until pollen dispersal into the next generation. Thus, the risks associated with ongoing genetic contribution to the native population following seed dispersal are similar to those from the original planted individuals. Seed dispersal is a major component of invasiveness, and negative impacts are effectively evaluated through weed risk assessments (Pheloung 2001; Stone, Byrne & Virtue 2008; Setterfield *et al.* 2010; Virtue 2010). We recommend that weed risk assessment is also routinely implemented in revegetation programmes in conjunction with genetic risk assessment.

Genetic risk

Genetic risk refers to the potential for negative impacts from the movement of foreign genes from domesticated or other non-local populations into native populations via pollen dispersal (Arnold 1992). It includes hybridisation both between species and between differentiated taxa, cultivars or populations within species. Hybridisation threatens population persistence and contributes to species extinction through either genetic assimilation or demographic swamping or the synergistic action of both processes over multiple generations (Levin, Francisco-Ortega & Jansen 1996; Wolf, Takebayashi & Rieseberg 2001; Hufford & Mazer 2003). The potential for genetic risk may be high in modified landscapes because pollen dispersal can be extensive (White, Boshier & Powell 2002; Ward *et al.* 2005), particularly in ecosystems with large or fecund planted populations and small populations of remnant vegetation (Sampson & Byrne 2008; Millar *et al.* 2011). Gene flow through seed and pollen dispersal is an important factor in natural population and ecosystem processes and is usually considered to be beneficial to plant conservation (Ellstrand & Elam 1993). It shapes gene pools and the population genetic structure of species, acting as a force to maintain genetic continuity between populations and preventing the loss of genetic diversity through inbreeding. However, gene flow may have negative impacts when there is substantial genetic divergence between gene pools of source and sink populations (Ellstrand & Elam 1993; Ellstrand, Prentice & Hancock 1999; Vila, Weber & Antonio 2000; Ellstrand 2003). In revegetation, genetic effects arise both from use of native species where there are related species with compatible mating systems or where there is genetic divergence within the species, and from use of exotics where there is potential for hybridisation with native relatives (Vila, Weber & Antonio 2000; Laikre *et al.* 2010).

Hybridisation is defined here as interbreeding of individuals from genetically distinct populations regardless of their taxonomic status. Hybridisation can result in additivity, where the hybrid progeny show equivalent fitness to the parental populations, or the hybrids can deviate from the parental fitness through expression of either heterosis or outbreeding depression (Ellstrand & Elam 1993; Arnold *et al.* 1999). Expression of heterosis (enhanced hybrid fitness relative to the parental population) leads to establishment of hybrids in natural populations and associated introgression, the transfer of genes from one population to another by the repeated backcrossing of hybrids to the parental population. If the hybrids maintain increased fitness through successive generations, especially with ongoing pollen dispersal into the population from revegetation, then introgression will lead to loss of genetic identity, and potential extinction, through assimilation of the foreign genome (Ellstrand, Prentice & Hancock 1999). Modelling by Haygood, Ives & Andow (2003) found that genetic assimilation can be rapid even for non-advantageous alleles, particularly where demographic swamping occurs.

An alternative outcome arising from hybridisation is outbreeding depression, where reduced fitness means that most hybrids do not become established in the native population or that they do not reproduce because of sterility, although some hybrid individuals may still exhibit hybrid vigour (Arnold *et al.* 1999). Outbreeding depression can still pose a threat to small populations as it reduces the reproductive capacity of the

population through demographic swamping, thus increasing the probability of population extinction (Ellstrand 1992; Ellstrand & Elam 1993; Levin, Francisco-Ortega & Jansen 1996; Rhymer & Simberloff 1996). This effect will be greatest for small populations when pollen swamping is acute, seed production is low, or inbreeding is already reducing population fitness (Lopez, Potts & Tilyard 2000). Outbreeding depression has been detected after crossing among geographically isolated populations of a range of plant species (reviewed by Hufford & Mazer 2003), and a review by Edmands (2007) found that the effects of outbreeding depression were similar in magnitude to those of inbreeding depression.

Risk management framework

Operation of any endeavour within a risk management framework allows identification of factors contributing to potential risks, analysis and evaluation of the risk in specific cases to inform decision-making, and management of the activities to minimise and mitigate the risk (Raybould 2007). Implementation of revegetation programmes within a risk management framework will help to ensure that the significant environmental benefits are captured without any concomitant negative impacts on the surrounding biodiversity that may cause further environmental degradation in the future. Informed analysis and evaluation of genetic risk is important in revegetation because it will be difficult to control or reverse the impacts in natural ecosystems (Brunner *et al.* 2007). Furthermore, the impacts may not become apparent for many years because of the long life cycles of woody plants and because they may not be expressed until the second or later generation (Hails & Morley 2005; Edmands 2007).

Risk is a function of the likelihood of the hazard occurring and the consequences of the hazard should it occur (Wilkinson, Sweet & Poppy 2003; Andow & Zwahlen 2006). In relation to genetic risk, the hazard is an adverse change in the genetic constitution of native gene pools, resulting in genetic or demographic extinction. The likelihood of adverse genetic change relates to presence of a source of foreign genes and pollen dispersal that facilitates transmission of these genes into a native gene pool. The consequences of movement of foreign genes into native gene pools depend on the scale and cause of the divergence between the gene pools as these influence the level of fitness expressed in the context of any selection pressures.

Risk assessment is a tool used to quantify the likelihood and consequences of risk to inform decision-making according to the level of risk deemed acceptable, evaluation of other benefits and the values of society (Andow & Zwahlen 2006; Raybould 2007). Risk assessment requires clear identification of the hazard so that exposure can be determined (Wilkinson, Sweet & Poppy 2003). Assessment and mitigation of the likelihood of adverse genetic change is feasible, whereas the consequences are controlled by fitness not migration. Consequences are difficult to evaluate or predict as genetic and ecological processes are complex, local adaptation is hard to measure, and impacts may not be evident until the second generation following

hybridisation (Hails & Morley 2005; Edmands 2007; Farnum, Lucier & Meilan 2007). Therefore, current frameworks for risk assessments of genetic impact have focussed on factors influencing the likelihood of genetic exchange between populations that are sufficiently divergent that some negative impact is possible, rather than the consequences (e.g. Potts *et al.* 2003; Barbour *et al.* 2008a,b).

Factors contributing to genetic risk

LIKELIHOOD OF GENETIC IMMIGRATION

Factors that have a major influence on the likelihood of genetic immigration are those that facilitate the movement of foreign genes into native gene pools, including the presence of taxonomic or genetic divergence, compatibility of mating systems and the distance and level of pollen dispersal (Chapman & Burke 2006; Chandler & Dunwell 2008). The presence of genetic divergence between the source and sink gene pools provides the source of foreign genes. Use of exotic or non-local species or genotypes in revegetation will result in divergent gene pools when there are related species or genotypes present in natural vegetation in the landscape. Where local species are used, the presence of divergent gene pools may arise through use of species with taxonomic structure such as subspecies or variants, chromosomal differences such as polyploidy or use of germplasm from widespread species with divergent lineages and/or local adaptation. The expression of outbreeding depression is related to the degree of genetic divergence and historical isolation (Frankham *et al.* 2011). Identification and characterisation of germplasm resources used in revegetation programmes is an essential first step in effective assessment of taxonomic and genetic divergence.

Revegetation as a source of foreign genes in the landscape presents a risk when there is compatibility of mating systems between the plantings and the native populations allowing successful seed production following hybridisation (Vila, Weber & Antonio 2000; Potts *et al.* 2003). Barriers to successful seed production include both pre-zygotic and post-zygotic reproductive factors (Potts *et al.* 2003). At the species level, both pre-zygotic and post-zygotic systems may operate to prevent hybridisation, whilst within species, flowering phenology is the most likely pre-zygotic barrier to hybridisation. Knowledge of the flowering phenology of revegetation plantings and native populations is crucial information as this is the first barrier to hybridisation. Flowering phenology is readily assessed, although flowering time is influenced by environment and can vary markedly across different environments (Potts *et al.* 2003).

Pollen dispersal provides a mechanism for the transfer of foreign genes into native gene pools. Pollen dispersal in plants is nearly always described by a leptokurtic distribution, with the majority of pollen travelling a short distance from the source and a tail of long distance dispersal that greatly influences the rate of spread of genes (Chapman & Burke 2006; Smouse, Robledo-Arnuncio & Gonzalez-Martinez 2007). Many studies have revealed fat-tailed dispersal curves in trees

(e.g. Dick, Etchelecu & Austerlitz 2003; Austerlitz *et al.* 2004; Ward *et al.* 2005; Byrne *et al.* 2008; Mimura *et al.* 2009) confirming the potential for substantial long distance dispersal.

The extent of pollen dispersal is directly related to the method of pollination and pollinator behaviour (Chandler & Dunwell 2008). In general, wind-pollinated species show greater pollen dispersal distances than animal- or insect-pollinated species, although recent studies have shown that animal and insect pollination can lead to extensive pollen dispersal in trees and shrubs even in fragmented landscapes (White, Boshier & Powell 2002; Dick, Etchelecu & Austerlitz 2003; Ward *et al.* 2005; Bacles, Lowe & Ennos 2006; Byrne *et al.* 2008; Sampson & Byrne 2008; Millar *et al.* 2011). Regardless of pollination system, dispersal is highly idiosyncratic and can vary greatly in different environments at different times, and even for different individual plants in the same environment (Ellstrand 1992; Ellstrand & Elam 1993; Byrne *et al.* 2007, 2008; Chandler & Dunwell 2008; Mimura *et al.* 2009).

CONSEQUENCE OF GENETIC IMMIGRATION

The consequences of adverse genetic change in native gene pools are influenced by factors that contribute to the fitness of individuals and populations (Hails & Morley 2005; Andow & Zwahlen 2006; Chandler & Dunwell 2008). In a sensitivity analysis, the fitness of advanced hybrids had the greatest impact on persistence of gene in populations (Hooftman *et al.* 2008). The scale of taxonomic and genetic divergence between revegetation and native populations and the nature of the evolutionary process generating the divergence influence the fitness of hybrid progeny at the individual level. Genetic divergence will occur when non-local species are used. Divergence can also occur with use of local species because native species used for revegetation are generally widespread species that occur across broad environmental gradients, as these are characteristics that increase their utility. In addition, they may have taxonomic structure, such as subspecies or variants, that indicates greater levels of genetic divergence that may or may not be fully resolved. Selective breeding may also be a factor leading to differentiation of planted populations.

Expression of outbreeding depression following crossing between differentiated populations is dependent on the nature of the genetic processes underlying the divergence between source and sink populations. If population fitness has occurred through selection for locally adapted genotypes, then hybridisation will result in the dilution of these genotypes, as hybrids show heterozygosity and underdominance at loci formerly fixed for adapted alleles (Templeton 1986). If population fitness occurs through the development of co-adapted gene complexes (distinct combinations of epistatically interacting loci), then hybridisation may lead to disruption of complexes through recombination. The effects of outbreeding depression through disruption of co-adapted gene complexes are often not expressed until the F_2 generation and are seen as advanced generation breakdown (Templeton 1986; Fenster & Dudash 1994; Hufford & Mazer 2003; Potts *et al.* 2003).

The size of the pollen source and sink populations will determine whether hybridisation has an impact at the population level, and the functionality of the ecosystem will determine whether interaction with other processes will dispose the populations to greater impacts. The risk of genetic immigration from planted populations depends greatly on the relative size and distribution of native and planted populations (Ellstrand 1992; Ellstrand & Elam 1993; Bacles *et al.* 2005; Chapman & Burke 2006; Farnum, Lucier & Meilan 2007). In disturbed landscapes, broadscale revegetation to restore environmental processes may generate large amounts of contaminant pollen (source) in comparison with small natural pollen pools (sink). As native population sizes decrease, the relative proportion of immigrant pollen increases (Ellstrand & Elam 1993) and the impacts of genetic change are greater. The effective size of the population is also affected by flowering capacity produced not just numbers of plants. A small introduced population in the vicinity of a larger native population may still lead to significant pollen swamping if the introduced population produces a larger number of flowers (Anttila *et al.* 1998; Millar *et al.* 2011).

Most revegetation projects occur in disturbed and/or fragmented landscapes where ecosystem processes have been highly disrupted. These landscapes are typically composed of small remnant populations where gene flow is especially important in shaping genetic structure. In small, genetically or geographically isolated populations, low effective population sizes may lead to a significant decrease in reproductive performance through inbreeding depression (Ellstrand & Elam 1993). This is especially relevant to predominantly outbreeding species where significant increases in inbreeding can lead to reduced genetic variation within populations and increased differentiation among populations. Low genetic variation reduces opportunities for small populations to respond to selection pressures caused by changing environments (Stockwell, Hendry & Kinnison 2003) and ultimately decreases the probability of population persistence. Therefore, further reduction in effective mating in small populations in disturbed ecosystems increases the risks of population extinction (Levin, Francisco-Ortega & Jansen 1996). In functional ecosystems with large native populations, the relative impact of pollen dispersal between planted and natural populations would be less because these populations have greater genetic diversity and greater population fitness.

The level of disturbance in the landscape is also a factor influencing the consequence of genetic immigration because ecological disturbance creates heterogeneous habitats that provide greater opportunity for hybrid establishment (Vila, Weber & Antonio 2000; Wolf, Takebayashi & Rieseberg 2001). Establishment of hybrid individuals may also lead to increased herbivore and pathogen pressure in native vegetation. Hybrids may be more vulnerable to pests and may support considerable and diverse pest assemblages (Whitham *et al.* 1999; Dungey & Potts 2001), thus acting as sources of pest incursion into native populations, particularly if the hybrid individuals provide sufficient habitat to support viable pest populations.

To assess the risks posed to native populations, it is also important to have an understanding of the conservation context of the landscape, both at the species level in relation to the occurrence, size and distribution of populations of rare species as well as their genetic diversity, and at the landscape scale in relation to ecological processes, such as fragmentation, disease threats and hydrological balance. By placing a conservation value upon individual populations, it becomes possible to evaluate acceptable levels of genetic risk. If a population has low conservation value (e.g. it is a widespread species, or its genetic diversity is captured in several other populations that occur in conservation reserves), then the benefits from revegetation may be considered greater than the risk of genetic immigration.

Risk assessment protocol

Risk assessment is a well-established tool for identification of risk associated with particular activities although risk assessment methodologies are continuing to evolve (Andow & Zwahlen 2006). Genetic risk assessment is implemented in regulation of planting of genetically modified organisms. These assessments are quite complex and involve both risk of escape of the transgene into natural populations or traditionally bred cultivars, and the potential impact of the transgene on non-target organisms and ecological processes (Raybould & Wilkinson 2005; Andow & Zwahlen 2006; Chandler & Dunwell 2008; Gressel & Rotteveel 2000). Similarly, genetic risk assessment can be implemented in revegetation programmes to identify and manage potential risk to native biodiversity. Risk assessment for revegetation will be less complex and more targeted than that required for genetically modified organisms, as it focusses on escape of whole genomes rather than ongoing impacts of specific transgenes.

Assessment of the likelihood of genetic change involves an evaluation of the presence of genetic divergence among the planted and native gene pools, and the degree of pollen dispersal from revegetation plantings to native plants of the same or related species (Chandler & Dunwell 2008). Although the consequences of the genetic change are not directly evaluated in the risk assessment, some of the factors that influence the consequences can be considered, and both are assessed within the geographical context of the revegetation programme. Future climate change predictions may need to be considered in evaluation of the geographical criterion.

The assessment protocol presented here (Fig. 1) is in the form of a decision tree or exposure pathway that is based on three criteria – taxonomy, biology and geography – with a series of questions relating to each criterion. The protocol is similar to other protocols for assessment of risk from genetically modified
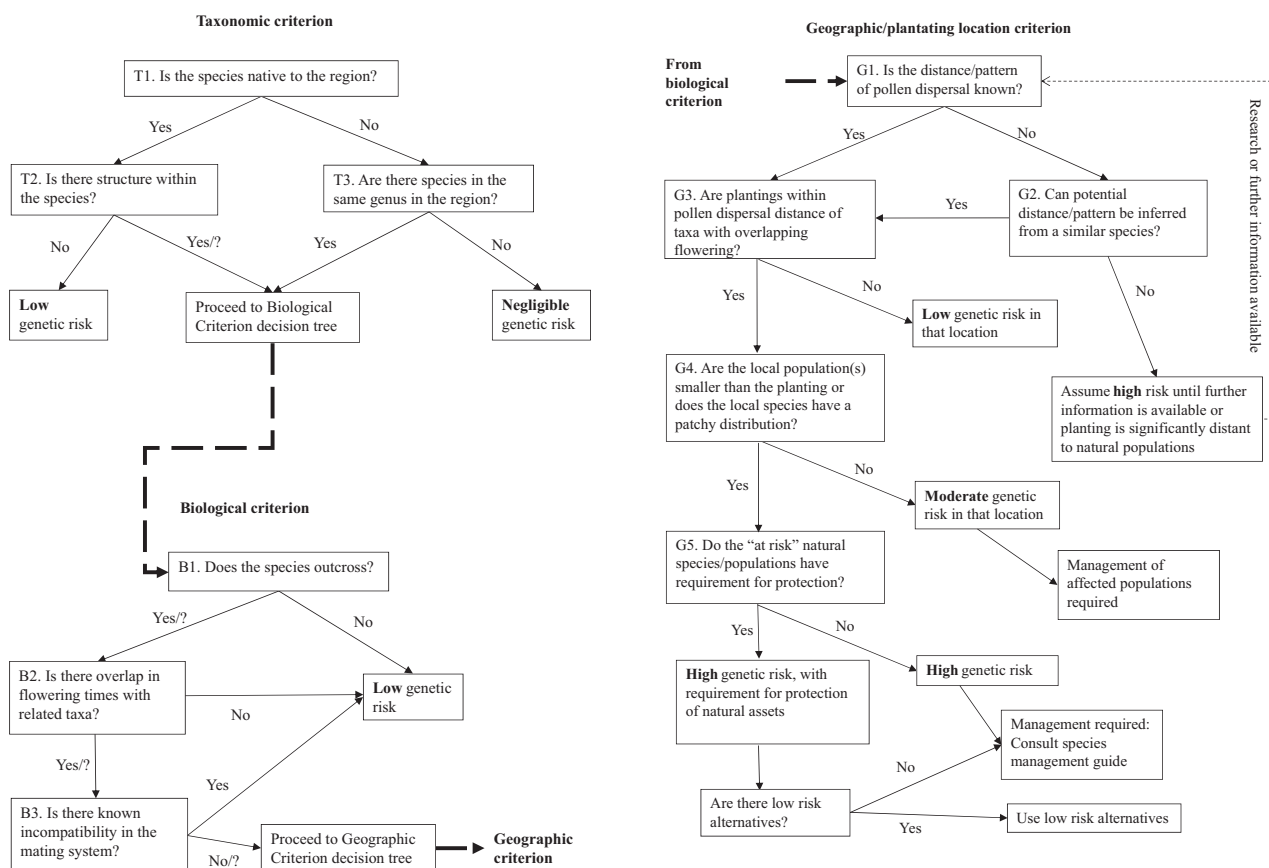

Fig. 1. Assessment protocol for analysis and evaluation of risk in native populations of adverse genetic change from revegetation.

organisms (Gressel & Rotteveel 2000; EFSA 2010) but is focussed in more detail on the specific aspects of gene escape relevant to revegetation. Uncertainty is an inherent aspect of risk assessment, and the level of detail available is usually highly variable. Thus, risk assessments need to be flexible in relation to the level of complexity, so that high likelihood of exposure leads to rigorous evaluation, but identification of limited exposure early in the assessment means that risks can be discounted and the assessment concluded (Wilkinson, Sweet & Poppy 2003; Andow & Zwahlen 2006). A tiered approach that allows increase in complexity within the assessment provides greatest scope to accommodate variability in risk, availability of information and the context of the decision (Wilkinson, Sweet & Poppy 2003; Andow & Zwahlen 2006). The assessment developed here is tiered such that further evaluation is undertaken as greater likelihood of risk is identified throughout the process. Identification of negligible or low risk can be determined at several points in the process without the need to complete the whole assessment. The assessment is designed to be specific in encompassing a range of factors influencing the likelihood and consequences of hybridisation but generic enough to be applicable to a broad range of revegetation activities. There are five categories of genetic risk described in the decision tree: negligible risk, low risk, moderate risk, high risk and high risk with requirement for protection (Table 1).

Table 1. Factors contributing to risk categories identified in the risk assessment protocol, the factors influencing risk are cumulative and increase with increasing risk category

Risk category	Risk factors	Consequence
Negligible	No compatible species present	
Low	No taxonomic or genetic divergence	
	No physical proximity	
Moderate	Taxonomic or genetic divergence	Hybridisation
	Physical proximity	
	Compatible mating systems	
High	Taxonomic or genetic divergence	Hybridisation with demographic swamping and/or genetic assimilation
	Physical proximity	
	Compatible mating systems	
	Large source/sink ratio	
	Patchy distribution	
High with requirement for protection	Taxonomic or genetic divergence	Hybridisation with demographic swamping and/or genetic assimilation
	Physical proximity to populations under protection	Population/species extinction
	Compatible mating systems	
	Large source/sink ratio	
	Patchy distribution and requirement for protection	

TAXONOMIC CRITERION

Knowledge about taxonomy and genetic variation within and between natural populations and planted stands is required to evaluate whether the revegetation planting will constitute a source of foreign genes in the landscape. The questions addressing this criterion (T1–T3, Fig. 1) pertain to the taxonomic and genetic relationships of the planted species to related natural populations. Information on whether the species is native or has native relatives (T1, T3) can be obtained from herbarium data bases. Genetic divergence within species (T2) can occur through taxonomic structure such as subspecies or variants, which indicates genetic divergence, through chromosomal differences such as polyploidy or through the non-random distribution of alleles or genotypes. Information to answer this question may require a literature search, or undertaking a study of genetic diversity for the species. The presence of genetic divergence may also be inferred from the distribution of the species, because patchy or disjunct distributions may indicate the possibility of genetic divergence among geographically distinct populations because of isolation and drift, and occurrence in different habitats may indicate genetic divergence because of selection.

There are three possible outcomes of assessment of the taxonomic criterion. Negligible genetic risk is identified if the revegetation species is not native and there are no other species in the same genus. Identification of low genetic risk occurs if there is no taxonomic or genetic structure within the species. Further evaluation of the biological criterion is required for all other cases.

BIOLOGICAL CRITERION

The questions addressing the biological criterion (B1–B3, Fig. 1) relate to the reproductive biology of the revegetation species and its relatives. Ability to outcross, presence of suitable pollinators and overlap in flowering phenology between planted stands and related taxa or local populations of the revegetation species will all contribute to the genetic risk (Potts *et al.* 2003; Chandler & Dunwell 2008). Species with outcrossed mating systems (B1) that have wind pollinated or have abundant generalist pollinators will have a greater level of pollen dispersal than those that have self-pollinated mating systems. However, predominantly self-pollinating species generally have some level of outcrossing (van de Wiel 2007), and even low levels of outcrossing can result in the persistence of genes in wild populations (Hooftman *et al.* 2008). For species with outcrossing, information on flowering phenology and mating compatibility (B2, B3) is required to determine whether genetic immigration is possible because of overlapping flowering with related taxa. The plasticity of flowering phenology and the influence of environment also need to be considered in evaluation of this component.

There are three possible outcomes of assessment of the biological criterion. Identification of low genetic risk occurs if the species is self-pollinating or predominantly self-pollinating with occasional or very low outcrossing (< 5%). Identification of high genetic risk occurs if there is overlap in flowering times

with related taxa. Further evaluation of the geographical criterion is required for all other cases.

Plantings of non-local or genetically distinct populations can be separated from native vegetation by distance to minimise risk of genetic immigration in either direction. Knowledge of the pattern of pollen flow is important to make the best decisions for site selection and management. The questions in the geographical criterion (G1–G5, Fig. 1) give an indication of the potential for movement of genes through pollen dispersal. The questions identify knowledge of pollen dispersal distance in the species or whether it can be inferred from information available in related species (G1–G3). Species planted within the pollen dispersal distance of taxa with overlapping flowering time will have a high risk of genetic immigration. The questions also require information on remnant vegetation in the landscape (G4, G5) because attributes of native populations will affect the potential for genetic immigration from foreign gene pools (Lessica & Allendorf 1999; Potts *et al.* 2003; Chandler & Dunwell 2008). Small patchy populations of native species will have a higher risk than a continuous or large population because of the effects of pollen swamping from a large planted stand (Wolf, Takebayashi & Rieseberg 2001; Farnum, Lucier & Meilan 2007; Sampson & Byrne 2008; Robledo-Arnuncio *et al.* 2009; Millar *et al.* 2011). Small populations of species that are threatened or identified as requiring protection under conservation legislation will require particular attention because interspecific mating between an endangered species and a planted stand may, in the extreme case, lead to extinction via hybridisation (Farnum, Lucier & Meilan 2007). Information on species requiring special protection will be available from government departments responsible for biodiversity conservation and protection.

There are four possible outcomes of assessment of the geographical criterion. Identification of low genetic risk at a particular location occurs if the distance and pattern of pollen dispersal are known and the plantings are not within the pollen dispersal distance of taxa with overlapping flowering. Identification of moderate risk occurs if the distance and pattern of pollen dispersal are known and the plantings are within the pollen dispersal distance of populations of taxa with overlapping flowering. If the 'at-risk' populations are small or occur in a patchy distribution, the risk is high. This high risk is increased if the species or populations have a requirement for protection.

Genetic risk management

The first steps in risk management are to identify the hazard and evaluate the risks through use of an assessment protocol such as that presented here. A major advantage to the use of risk assessment protocols is that many revegetation programmes that represent low risk can be readily identified and undertaken with confidence. For higher-risk activities, decisions can then be made in regard to the level of risk that is acceptable in association with the level of management that is feasible (Wolf, Takebayashi & Rieseberg 2001; Andow & Zwahlen 2006). It is very difficult to eliminate risk, but it can be minimised by reducing the hazard and the exposure, and operation in an adaptive management framework will improve both risk assessment and the biodiversity outcomes of revegetation. Where there is a high feasibility of managing risk, the information from the risk assessment can be used to develop strategies for minimising the likelihood of genetic immigration, and these strategies can be integrated into design and implementation of revegetation programmes.

The most effective management of high-risk species is to select species or germplasm sources that will provide the same benefits but have a lower risk. This can be achieved in two ways: (i) by selecting germplasm that is not genetically divergent so that movement of genes through pollen dispersal does not lead to introgression or outbreeding depression or (ii) by selecting species that are not compatible with vegetation in the local neighbourhood such that pollen dispersal does not lead to availability of a foreign pollen source during mating. Evaluation of potential revegetation species through the genetic risk assessment will quickly determine those species that do not present any risk as these factors will lead to identification of low risk in the taxonomic and biological sections of the decision tree. Selection of non-compatible species or non-divergent germplasm of compatible species is most feasible for localised areas of revegetation. However, these plantings should still maintain a focus on use of native species to prevent any adverse ecological impacts from the use of exotic species.

For revegetation programmes that are designed to achieve specific environmental outcomes or produce specific marketable products, such as agroforestry, the options for the selection of species and germplasm may be more limited. In these cases, management of genetic risk through cultural management practices has been suggested, such as selection for different flowering times, planting so that flowering times do not coincide or harvesting before flowering. However, these practices are virtually impossible to implement effectively with the long life cycles of woody perennials. The most reliable practices to limit gene flow from agroforestry crop include keeping the crop isolated from native populations by distance or by the use of barriers (Andow & Zwahlen 2006).

Use of isolation distances has been promoted as a containment strategy (Wilkinson, Sweet & Poppy 2003; Andow & Zwahlen 2006; Chandler & Dunwell 2008) and requires knowledge of the shape of the pollen dispersal curve (Smouse, Robledo-Arnuncio & Gonzalez-Martinez 2007). Pollen dispersal curves in woody perennials have confirmed some guidelines that isolation distances should be greater for wind- and bird-pollinated species than for insect-pollinated species and that isolation distances should be greater for a patchy as opposed to a spatially continuous flowering resource (Potts *et al.* 2003). However, these are general guidelines that will be imprecise because of the sensitivity of pollen dispersal to environmental conditions (Andow & Zwahlen 2006; Chandler & Dunwell 2008) and the spatial distribution of source and sink populations.

A management strategy used in crop management is the use of barrier rows, or guard rows, of different non-interbreeding species or populations of the same species that act to intercept a portion of pollen entering or leaving the crop. Models have predicted that buffer zones will substantially reduce pollen leakage from crops (Manasse & Kareiva 1991). Empirical studies have also shown that barrier rows are effective in the management of crop species, and Barbour *et al.* (2008b) found reduced flowering in inside rows compared to edge rows in eucalypt plantations. However, revegetation is undertaken over long time frames to realise significant environmental benefits; therefore, physical containment through barrier rows may be less effective because a low level of escape occurring over many years has a cumulative effect.

When revegetation confers high environmental benefits and the risks to compatible species in the natural vegetation are moderate, then management of potentially affected populations may be appropriate. This may include monitoring the population for hybrid recruitment, for example, after disturbance events such as fire or flood, and culling of hybrids if required (Barbour *et al.* 2010). It would also involve declaration that the population not be used as a source of seed for other revegetation projects, and this may be controlled through licensing of seed collection permits.

Conclusions

There are genetic risks associated with the use of native species for large-scale revegetation, even within their natural range. Many genetic and ecological factors interact to make the consequences of hybridisation difficult to determine without major research over multiple generations. However, the likelihood of pollen dispersal as the means by which foreign genes may infiltrate native gene pools and the presence of genetic divergence as an indicator of potential for outbreeding depression are easier to identify and can be determined through a genetic risk assessment. Attributes of native populations that influence the impact of genetic exchange can also be identified and incorporated into risk assessments.

The risk assessment presented here is based on well-established genetic principles and enables identification of factors contributing to risk, assessment of whether there is sufficient information available to assess the risk and evaluation of management options. Risk assessment provides a means of identifying specific areas where further scientific data can improve evaluation through reducing uncertainty, and thus guiding research priorities to areas with the greatest implications for decision-making (Raybould & Wilkinson 2005; Raybould 2007). Implementation of revegetation programmes within a risk management framework will help to ensure that the significant environmental benefits are captured with minimal concomitant negative impacts on the surrounding biodiversity. Greater understanding of the dynamics of pollen dispersal for dominant species in fragmented landscapes will increase the capacity for rigorous assessment of the risks involved in the use of these taxa in large-scale revegetation programmes. It is important to note that risk assessment is only one of the factors used to reach a decision and information from a range of factors relating to the costs and benefits of an particular action are evaluated in any decision-making process. Further scientific discussion leading to improved risk frameworks in revegetation is encouraged to reinforce risk assessment as an integral part of evaluation of environmental impact for large-scale revegetation programmes. Development of informed decision-making processes for implementation of revegetation systems will ultimately enable the development of land uses that protect and enhance biodiversity in degraded landscapes.

Acknowledgements

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