Characterising the recolonisation of *Antechinus flavipes* following the restoration of a production landscape and its genetic implications in the Jarrah forest

Submitted by

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DVM

This thesis is presented for the degree of Master of Philosophy at Murdoch University.

2014

I dedicate this work to

Cinthya,

to whom half of this thesis belongs.

I declare that this thesis is my own account of my research and contains

work which has not previously been submitted for a degree

at any tertiary education institution.

Jose Luis Mijangos Araujo

“Indeed, every living organism can be viewed as an evolutionary success story”

Feder and Mitchell-Olds 2003

Abstract

Ecological restoration is emerging as a promising activity to contribute to biodiversity conservation. There is presently an increasing need to develop a stronger relationship between genetics and restoration. This is particularly necessary to investigate the effectiveness of restoration to maintain and conserve genetic diversity of recolonising faunal populations. This thesis investigated the links between genetics and restoration and how understanding the contribution of genetics can be used to further improve restoration outputs. A search of scientific literature identified 160 papers employing a genetic approach within a restoration context. Although genetic research in restoration is rapidly growing (59% of the identified articles were published during the last four years), I found that studies could make better use of the extensive toolbox developed by fields of applied genetics. 42% of reviewed studies used genetic information to evaluate or monitor restoration and 58% provided genetic information to guide pre-restoration decision-making processes. Reviewed studies suggest that restoration practitioners often overlook the importance of including genetic aspects within their restoration goals. Even though there is a genetic basis influencing the provision of ecosystem services, few studies inquired this relationship. I provide a view of research gaps, future directions and challenges in the genetics of restoration.

To evaluate how restoration affects the genetic diversity and dynamics of vertebrate species, this study uses a small marsupial (*Antechinus flavipes*) as a model. To this end, nine nuclear microsatellites and a 565-bp sequence of the mtDNA control region were used. *Antechinus flavipes* individuals were sampled in three locations with different disturbances (mining/restoration, dieback infected and dieback infected/mining) to investigate whether genetic bottlenecks, dispersal barriers, adverse environmental conditions or a skewed sex ratio affects genetic diversity and gene flow of this species. The findings showed:

1. A lack of evidence for the disruption of gene flow, suggesting that current restoration practices have been effective in maintaining adequate levels of landscape connectivity in this species.
2. There is a non-significant correlation between the distribution of individual heterozygosity and environmental conditions, suggesting that conditions in restored areas do not have a negative influence on genetic diversity.
3. Non-significant results from bottleneck tests probably indicate that restored areas provide enough resources to sustain several reproducing individuals and thus avoiding founder effects.
4. Parameters of neutral genetic diversity were high in both groups of individuals sampled in restored and in unmined sites and were not significantly different.
5. No detectable reduction of genetic diversity, despite a sampling effect that resulted in a skewed sex ratio.
6. The structure of a network of mtDNA suggests that historic gene flow occurred across the three locations.

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1 General introduction

1.1 Introduction

New scientific-based assessments and indicators of worldwide biodiversity and ecosystems status (e.g. Loh *et al*. 2005; Millennium Ecosystem Assessment 2005; Sanderson *et al*. 2002; Vié *et al*. 2009) provide compelling evidence that current rates of disturbance, exploitation and depletion of natural resources due to human activities are ecologically unsustainable. Specifically, the conversion of natural landscapes into production landscapes, where activities such as agriculture, grazing, logging and mining take place, is the main cause driving biodiversity loss (Vitousek *et al*. 1997). For instance, it has been estimated that croplands and pastures alone occupy approximately 40% of Earth's surface (Foley *et al*. 2005). The ecological restoration of these production landscapes, such as in cases of land abandonment (Cramer *et al*. 2008), land acquisition (Schultz & Crone 2005) or after mining of mineral ores (Nichols & Grant 2007), is emerging as a promising and effective strategy to support biodiversity conservation (Benayas *et al.* 2009; Bullock *et al.* 2011). Development of increasingly sophisticated restoration practices underpinned by the science of restoration, restoration ecology, has raised hopes that restoration contributes towards conservation efforts that aim to ameliorate biodiversity loss (Butchart *et al*. 2010) and also to help face the conservation challenges imposed by the emergence of novel ecosystems brought by climate change (Renton *et al*. 2012).

The main organisation supporting and promoting ecological restoration, the Society for Ecological Restoration International Science & Policy Working Group (*i.e.* SERI), defines ecological restoration as "the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed" (SERI; 2004).

According to SERI (2004), an ecosystem is ecologically restored when it contains sufficient biotic and abiotic resources to continue its development without further assistance, sustain itself structurally and functionally and demonstrate resilience and interactions with contiguous ecosystems. Restoration efforts to reach these goals comprise a wide array of objectives and activities: from simple management, such as fencing to prevent degrading actions caused by, for example, grazing, to complex activities that manipulate abiotic elements and biotic communities to reinstall complete ecosystems (Hobbs & Cramer 2008).

Restoration scientists and practitioners are becoming increasingly aware of the need to develop restoration protocols not only for the reconstruction of ecosystem biodiversity and demography of threatened populations, but also for the maintenance of their evolutionary potential (Hufford & Mazer 2003). It is clear that there is a gap in the restoration research agenda in developing a stronger relationship between genetics and restoration (see chapter 3). Particularly, the effectiveness of restoration efforts to maintain and conserve the genetic diversity of recolonising faunal populations is poorly understood. At present, faunal species richness and abundance are commonly used as measures of restoration success (Ruiz-Jaen & Aide 2005b), however, these parameters may not represent accurately the genetic trends occurring in restored landscapes. Therefore, whether restoration designed to avert biodiversity loss in production landscapes can also maintain genetic diversity in recolonising faunal populations, or whether it negatively affects their genetic diversity, remains unknown.

The work performed in this thesis comprises two components, one conceptual and one empirical. The conceptual component focuses on investigating the links between the field of genetics and the practice of ecological restoration and how the strengthening of these links could further improve restoration outputs.

In the empirical component, core concepts of the field of population genetics were employed to address the question of genetic diversity in recolonising faunal populations using a small, vagile, semelparous marsupial (*Antechinus flavipes leucogaster*) as the study species.

Please note chapters 3 and 4 are written in a different style to the rest of the chapters, since they were written as publications with stand alone introductions, therefore some repetition in describing the underlying concepts was necessary.

I begin the thesis by presenting a general introduction that places the genetic context on which the empirical component of the thesis is based (Chapter 1). This chapter provides a background of the Australian mining industry and its advances in developing restoration practices, and also includes the description of the study area, sampling sites and study species.

Next, I make a summary of the main genetic disciplines and how these are linked to the practice of ecological restoration (Chapter 2). I further discuss the suitability of molecular markers to be used as a routine tool within a restoration context. I then provide a comprehensive overview of the various ways that genetics has been used to inform ecological restoration (Chapter 3). Through a meta-analysis, I uncovered how genetic research topics have been aligned to different stages of restoration: from advances in theory to their implementation in decision-making, monitoring and evaluation processes. In the last section of the chapter research gaps, future directions and challenges in the genetics of restoration are pointed out.

The empirical component of the thesis used a genetic approach to assess the success of different aspects in an ecological restoration project (Chapter 4). Three sites located in ecosystems with different types (e.g. mining extraction and dieback) and levels of disturbances were used to investigate the effects of mining/restoration activities on various measures of genetic diversity and gene flow. This study, to my knowledge, is the first to apply a genetic approach to study faunal successional and recolonisation processes within a mining/restoration context.

The questions addressed in this chapter are:

1. Do restored mine sites provide functional landscape connectivity?
2. Are there genetic bottlenecks due to founder effects during the recolonisation process?

I finish with a general discussion of the main conclusions and management implications of the thesis (Chapter 5).

1.2 The genetic context

The use of genetics to guide ecological restoration is relatively recent. This interdisciplinary relationship is arguably quite broad, as the various genetics approaches, developed at the present, may have several applications on different restoration stages and processes. Particularly, the conservation of genetic diversity (i.e. conservation genetics) is a genetic approach that has been prominently applied on natural populations. The following paragraphs present basic genetic concepts about how genetics is related to restoration and the conservation of genetic diversity.

The importance of conserving genetic diversity in natural populations has been recognised by the International Union for Conservation of Nature (IUCN; McNeely *et al*. 1990), as it is the raw material upon which natural selection acts to bring about adaptive evolutionary change (Frankham *et al*., 2009). Its loss will reduce: (1) the ability of populations to respond and adapt to environmental change in the long and short terms (Burger & Lynch 1995); and (2) individual fitness due to the exposure and accumulation of deleterious mutations and loss of heterozygosity in overdominant loci (i.e.

inbreeding depression; Keller & Waller 2002). As a result, population persistence will be negatively affected (Frankham 2005; O'Grady et al. 2006).

Fragmentation, degradation and loss of habitat in production landscapes exacerbate the two main processes by which genetic diversity is lost at short time scales (i.e. 10s to 1000s of years). These are the disruption of landscape connectivity, which decreases gene flow, and the reduction of population size, which increases genetic drift. Ecological restoration has the potential to ameliorate these negative consequences by creating biological corridors to reconnect otherwise isolated patches of suitable habitat (Dixon *et al.* 2006), restoring and expanding natural habitats (Huxel & Hastings 1999) and, consequently, increasing effective population size. Counter-intuitively, the restoration process itself could potentially cause genetic side effects. For instance, genetic bottlenecks in recolonising individuals, or reintroduction of few genotypes into restored areas, have been reported as probable cause of reduction of population growth and individual fitness in the eelgrass *Zostera marina* (Williams 2001) and loss of genetic diversity in the terrestrial orchid *Dactylorhiza incarnate* (Vandepitte *et al.* 2012).

The term genetic drift refers to the random sampling of alleles being transmitted from generation to generation. As a result, within a population, rare alleles are prone to disappear and common alleles to become fixed. While the effect of genetic drift occurring in large populations might be minimal, in small populations it could have serious negative consequences. The effective population size (N_e) is a transcendental concept in population genetics. It is related to genetic drift and thus predicts rates of loss of neutral genetic variation, fixation of deleterious and favourable alleles and the increase in inbreeding experienced by a population (England *et al.* 2006). The effective population size is defined as the size of an idealised population that would have the same amount of inbreeding, or random genetic drift, as the population under consideration (Kimura & Crow 1963).

Overall, N_e in wildlife species is typically in the order of 14% of its census size (the total individuals in the population; Palstra & Ruzzante 2008), as not all individuals have the same efficiency in reproducing. For instance, some individuals will not have yet reached reproductive age or may die before reproducing. A population with skewed sex ratios, will also reduce N_e. Franklin's (1980) 50/500 rule, although criticised and questioned (Lande 1995; Reed & Bryant 2000), exemplifies the potential consequences of a low N_e. Populations with a N_e of 50 or less are at immediate risk of extinction because inbreeding in such small populations provokes the accumulation of deleterious mutations, reducing population fitness and facilitating an extinction vortex. Populations with a N_e of 500 or less are at long-term risk of extinction. The loss of genetic diversity is such that these populations may not be able to adapt to medium and long-term environmental changes.

Inferences of gene flow, or the exchange of genes, within a landscape are commonly based on the proportion of genetic differentiation existing between different populations. Therefore, populations and/or individuals separated by barriers that hinder gene flow (as occurs in fragmented or degraded habitats) will be more genetically differentiated than populations or individuals in landscapes lacking such barriers. It has been shown that the time necessary for a genetic signal to appear in order to detect a new barrier can be as short as 1 to 15 generations (Landguth *et al*. 2010), depending on the dispersal capabilities of the species, although the length of time lags can also be related to sampling scheme, effective population size and genetic substructure (Safner *et al*. 2011). Commonly, in empirical studies the level of gene flow is associated with landscape connectivity, defined as the degree to which the landscape facilitates or impedes movement between resource patches (Taylor *et al*. 1993).

It is important to differentiate structural from functional connectivity: while areas of suitable habitat within a landscape might be structurally connected (e.g. by corridors) they might not be functionally connected, as the species under study might not be able to disperse or immigrate between structurally connected habitat.

1.3 Australian mining industry and Alcoa of Australia

The empirical component of this thesis used a mining area under restoration as study area. The restoration of mining sites might be seen as an ideal study system to gain insight about how biological communities are assembled and structured or to investigate several ecosystem, successional and recolonisation processes. Although ecological restoration of mined sites is practiced as a post-disturbance measure to ameliorate potential adverse effects, and not as a mean of expanding or improving the ecosystem, it can be argued that the same principles by which restoration acts to maintain and conserve genetic diversity is likely to also occur in the ecological restoration of other types of production landscapes.

Restoration of mined sites has been a valuable source of experience to both the science and practice of ecological restoration around the world (Cooke & Johnson 2002). Particularly, Australia is one of the world's leading mining nations as regarding production of mineral ores and the environmental management of mining activities (Commonwealth of Australia 2006; Environment Australia 2002). Bauxite, the material from which alumina and aluminium are produced, is one of the main ores mined in Australia. In 2009, Australia was the world's largest producer, accounting for the 31.3% of world's production (Australian Bureau of Statistics 2012). The operations of the major Australian bauxite mining company, Alcoa of Australia, represent the world's largest integrated bauxite mining, alumina refining, aluminium smelting and rolling system (www.alcoa.com).

Alcoa commenced mining the northern jarrah forest in the south west of Western Australia in 1963 and up to 2006 Alcoa had restored about 13,000 ha of mined areas (Koch 2007). Alcoa has operated three mines within the jarrah forest: Jarrahdale, established in 1963 and closed in 1998, Huntly (world´s largest bauxite mine), established in 1976 and Willowdale, established in 1984, both still currently active (Figure 1.1).

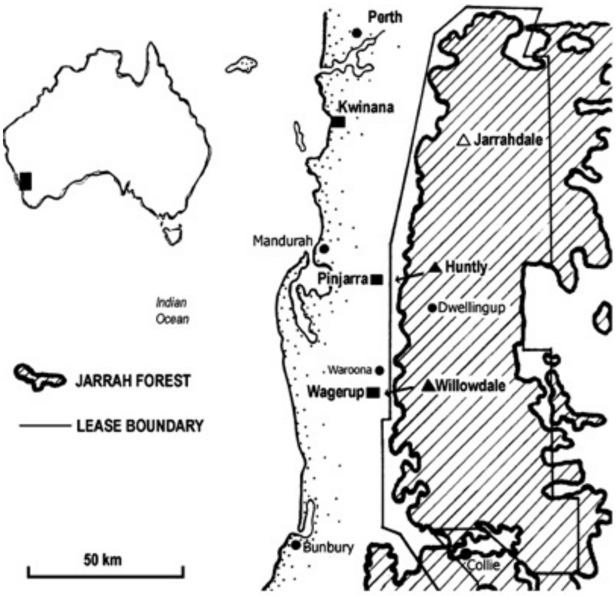

Figure 1.1 Location of Alcoa's mines, located in the northern jarrah forest in the southwest of Western Australia. Triangles represent Alcoa’s mine sites (Huntly and Willowdale in operation and Jarrahdale closed and rehabilitated), squares Alcoa’s refineries and dots main settlements (Source: Grant & Koch 2007).

Alcoa’s restoration practices have exceeded the rehabilitation targets set by Australian government agencies. For instance, Alcoa achieved 100% of plant species richness in their restored areas, compared to unmined reference sites, in recent times (Grant & Koch 2007). Additionally, Jarrahdale mine was the first mine in Western Australia to received a certificate of completion from the state government after fulfilling the rehabilitation criteria set by the Department of Environment and Conservation (now the Department of Parks and Wildlife; Government of Western Australia 2007). Alcoa's commitment to develop better restoration practices is further illustrated by its research partnerships with universities. Major studies have been undertaken on plant succession, vegetation patterns, tree growth, biomass accumulation, nutrient cycling, water use and timber quality (Alcoa of Australia 2010). Several fauna recolonisation and succession studies have also been completed (Craig *et al.* 2012; Craig *et al.* 2010; Nichols & Grant 2007; Nichols & Nichols 2003). However, no genetic studies on faunal species have been performed.

1.3.1 Mining process

Bauxite strip-mining is shallow (approx. 4–5 m) and takes place in pods of one to tens of hectares on the hillsides, but not in valley floors, swamps and streams, as they are alluvial and not bauxitic. As a consequence, when mining is complete, approximately 40–50% of the landscape has been mined and restored, leaving a mosaic of restored and unmined forest (Koch 2007). The mining process begins with a number of pre-mining surveys including aboriginal heritage, flora and fauna. Especially important is the mapping of the plant disease caused by *Phytophthora cinnamomi*, as this pathogen is responsible of killing approximately 40% of plant species in the jarrah forest, including jarrah trees which is the dominant tree species (for more information see section 1.4.2 Dieback, *Phytophthora cinnamomi*). The second step entails locating the bauxite ore by explorative drilling. Once the ore is located, the area is logged and cleared of remaining vegetation. In this step, logs, stumps and rocks are put aside and relocated to sites under restoration to provide shelter for faunal species. Afterwards, the topsoil (0-15 cm), which contains the majority of seeds, organic material, plant nutrients, and soil microbes, is stripped and directly returned to a nearby mined site under restoration (Koch 2007). The direct return avoids the degradation of the biological components of the topsoil by being stored in a stockpile.

The return of the topsoil has been identified as a critical step for a successful restoration (Cooke & Johnson 2002). Subsequently, the underlying soil layer comprising the following approximately 15-100 cm is stripped, stockpiled and returned after ore extraction. The final mining stage includes breaking the bauxite ore, either by blasting or by ripping, loading it into trucks and transporting it to a central crusher. Finally, the crushed ore is transported by a conveyor to refineries 20-25 km away.

1.3.2 Restoration process

Alcoa´s restoration process has been evolving continuously (Gardner & Bell 2007) with a major shift in 1988 from planting non-local eucalypt species to reseeding with the dominant jarrah forest canopy species (Grant & Koch 2007). Significant improvements to restoration practices have been mainly in the areas of landscaping after mining, soil return methods, deep ripping to relieve compaction, selection of appropriate plant species for restoration, plant propagation methods (e.g. tree nursery and seeding) and techniques to encourage return of fauna through the return of logs, rocks and woody debris as fauna habitat (Koch 2007). Further restoration management prescriptions include fertilising, thinning and burning, control of invasive species (e.g. red fox, *Vulpes vulpes*) and extensive monitoring programs (Grant & Koch 2007). On the other hand, it is clear that the ecosystem will never be identical to the pre-mining state but a modified jarrah forest ecosystem. There are downsides that still require improvement or temporal solutions, such as streamflow reductions due vigorous vegetation growth, imbalance of resprouter versus reseeder plant species and the lack of old trees, tree hollows and rotting wood (Nichols & Grant 2007). These components will probably take 100 years or more to become available (Whitford 2002), which could slow down the return of organisms that require this habitat. For example, 5% of birds and 13% of reptile species in the jarrah forest have not been recorded in restored sites yet (Nichols & Grant 2007). Also, mining operations have spread, although minimally (0.0006 ha for every hectare mined), the pathogen *P. cinnamomi* into uninfested forest (Colquhoun & Kerp 2007).

The experience gained by Alcoa during their history of mining and restoring the jarrah forest, has allowed them to set high restoration aims, such as achieving the best environmental and restoration performance of any mining company in the world (Gardner & Bell 2007). Alcoa's restoration process, which costs AU$34,000/ha, has already achieved high standards of environmental performance and is regarded as a very successful restoration operation in general ecological terms (Koch & Hobbs 2007).

1.4 Study area

1.4.1 Jarrah forest

The study area is the northern jarrah forest between Huntly and Willowdale mine sites in the south west of Western Australia. The jarrah forest is located in one of just five Mediterranean regions in the world that are characterised by dry summers and rainy winters. The average rainfall at Dwellingup, which lies approximately midway between Huntly and Willowdale mine sites, is 1240 mm/year (Bureau of Meteorology; www.bom.gov.au), with more than 75% falling between May and September. The overstory vegetation consists almost entirely of jarrah (*Eucalyptus marginata*) and marri (*Corymbia calophylla*). The jarrah forest forms part of a global biodiversity hotspot, where exceptional concentrations of endemic species are under threat from habitat loss (Myers *et al.* 2000). The remaining primary vegetation of this hotspot is only 10.8% of its original extent. Within this biodiversity hotspot live 5,469 plant and 456 vertebrates species of which 79.2% and 22% are endemic respectively (Myers *et al.* 2000). The main threats to this biodiversity hotspot have been agriculture and logging, invasive species and the plant pathogen *Phytophthora cinnamomi* (see below).

1.4.2 Dieback (*Phytophthora cinnamomi*)

Dieback *(Phytophthora cinnamomi)* is a soil-borne plant pathogen that kills many native plant species in the south-west of Western Australia (Shearer *et al*. 2004). The first evidence of the disease in the jarrah forest was noticed in 1921 (Podger 1972), however, it was not until 1965 that *P. cinnamomi* was identified as the causal agent (Podger *et al*. 1965). Approximately 40% of the recognised plant species in the south-west botanical province of Western Australia exhibit some susceptibility to *P. cinnamomi* (Shearer *et al*. 2004). It has been estimated that 14% of the area of the jarrah forest is affected by *P. cinnamomi* (Davison & Shearer 1989).

Phytophthora cinnamomi is mainly dispersed over large distances in infested soil moved by human activity and is naturally dispersed by propagules in water flowing in surface and near surface drainage systems and by growth through root systems (Shearer *et al*. 2007). Once an area becomes infected the subsequent decline in the vegetation is often dramatic and devastating, with the death of susceptible plants, foliage collapse and decomposition resulting in significant reductions in canopy cover, coarse woody debris and leaf litter (Shearer & Tippett 1989; Wardell-Johnson & Nichols 1991; Weste & Marks 1974). The most common primary symptom caused is root rot, while secondary symptoms resemble those of drought. Death time lag varies widely between species. For instance, *Banksia* species may die suddenly, whereas jarrah might take more than three years to die (Weste & Marks 1987). The expression of the disease may vary from total patch death to gradual crown decline and foliage dieback, depending on soil type, topography, hydrological cycles and presence of susceptible plant species (Davison 1994; Shea 1977; Wilson *et al.* 2003).

Plant pathogens, such as *P. cinnamomi,* have imposed several challenges to restoration in Australia and worldwide (Storer *et al.* 2001; Parke *et al*. 2003; Mitchell *et al.* 2011*)*. Specifically in the case of Alcoa's restoration process, *P. cinnamomi* has had major implications. Although, initially, Alcoa's mining operations (1960s) were performed mainly within *P. cinnamomi* infested areas, it was assumed that mining activities would spread the pathogen into uninfested areas (Colquhoun & Kerp 2007). According to this assumption, pine trees were used for restoration, since the pathogen would eventually kill jarrah trees. Later in the 1980s, it was realized that mining would not necessarily lead to the spread of *P. cinnamomi* and even more important its presence did not result in a complete death of jarrah trees (>80% survivorship; Colquhoun & Hardy 2000). Based on this new understanding, revegetation procedures could use entirely local tree and understory species with minimal risk of high mortality. Additionally, important procedures were developed to minimise *P. cinnamomi* infestations:

1. Elaboration of reliable, up-to-date maps and field demarcation of diseased sites.
2. Restricting vehicle movement from infested to uninfested areas
3. Prevent water draining from infested to uninfested areas
4. Cleaning vehicles before entering uninfested areas.
5. Preventing infested and uninfested soils mixing.
6. Training all field staff and planners.
7. Monitoring the spread of the disease attributable to mining and investigating the causes.

1.5 Sampling sites

Individuals of *A. flavipes* sampled for this study were captured in three locations with different types and levels of disturbance. Huntly, where extensive mining/restoration activities have been performed during more than 30 years; Dwellingup, an area largely affected by the occurrence of *P.*

cinnamomi, and; Willowdale, an area that has been highly disturbed by both *P. cinnamomi* and mining activities.

1.5.1 Huntly

The sampling area at Huntly mine site (32°36'S, 116°06'E) is relatively large (approximately 16,000 ha). Huntly is a heterogeneous landscape with intermingled patches of unmined forest and restored mine sites. Mined sites vary in size between 2 and 35 ha and comprise 33% of the study area (Fig. 1.2). Although there are some areas where *P. cinnamomi* has been detected, most sampling sites were free of this disease. Twenty-two trapping grids were randomly installed in unmined forest and seventeen in restored mine sites of different restoration ages and management prescriptions. The number of years that have passed since mined sites were restored ranged from 3 to 21 years. The mean distance between neighbouring grids (1483 m, SD=972 m, min=476 m, max=4558 m) was greater than both the home range size (a radius of approximately 56 m; Coates 1995) and average dispersal distance (approximately 350 m; Marchesan & Carthew 2008) of *A. flavipes*. Additionally, all grids were more than 70 m from other habitat types to maximise the probability of trapping individuals whose home ranges were entirely, or largely, in the sampled habitat. Precise density estimates have not been investigated at the study area, however, there are one or two individuals per hectare approximately (Michael Craig, personal communication). Further data suggests that *A. flavipes* recolonises restored areas relatively quickly (as soon as two years post-restoration; Nichols & Grant 2007), and its abundance in 12 and 17-year old restoration is the same or slightly higher than in unmined forest (Craig *et al*. 2012).

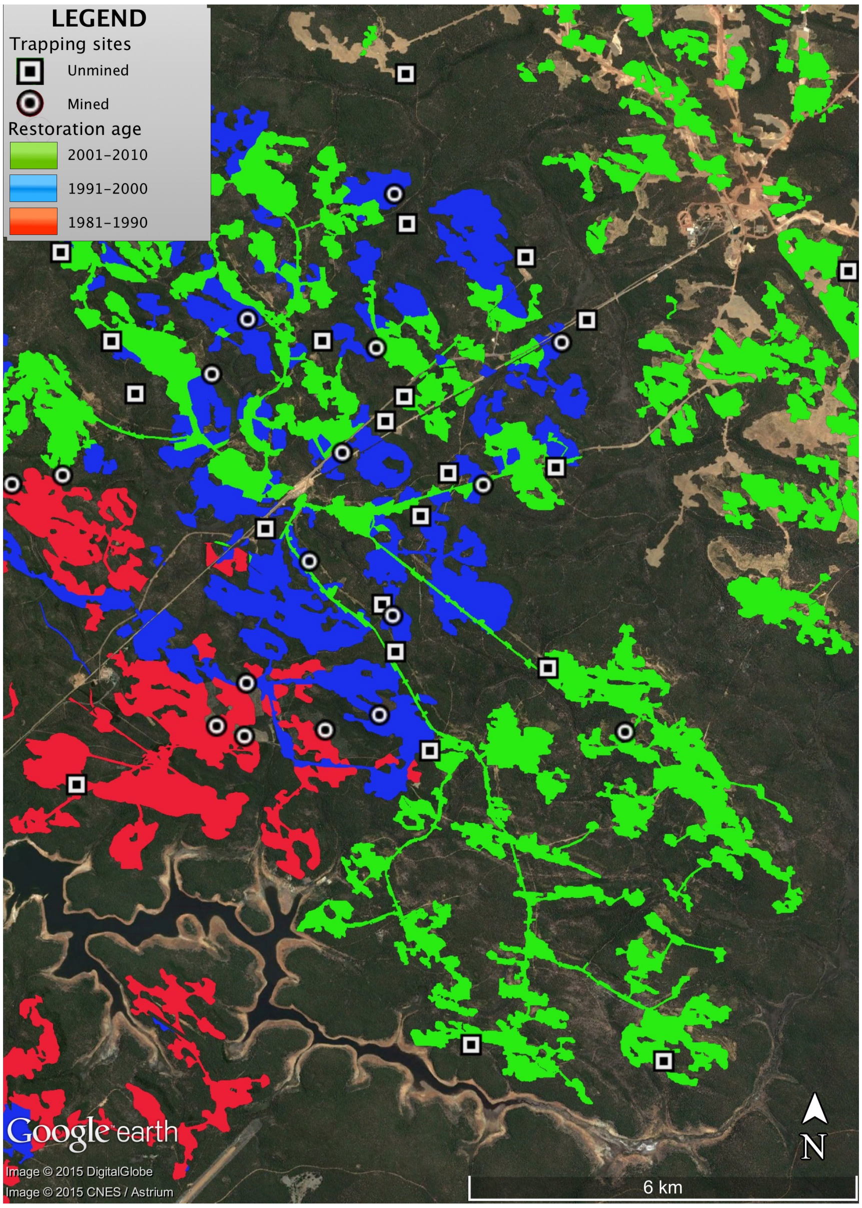

Figure 1.2 Location of trapping sites at Huntly (area=approximately 16,000 ha). Triangles represent traps installed in mined/restored areas and circles those installed in unmined forest. Coloured light green areas represent unmined forest, red colour represents areas that were restored between 1981-1990, bright green between 1991-2000 and purple between 2000-2010.

1.5.2 Dwellingup

Dwellingup trapping sites were located approximately 15 km south of Huntly mine site (Fig. 1.3).

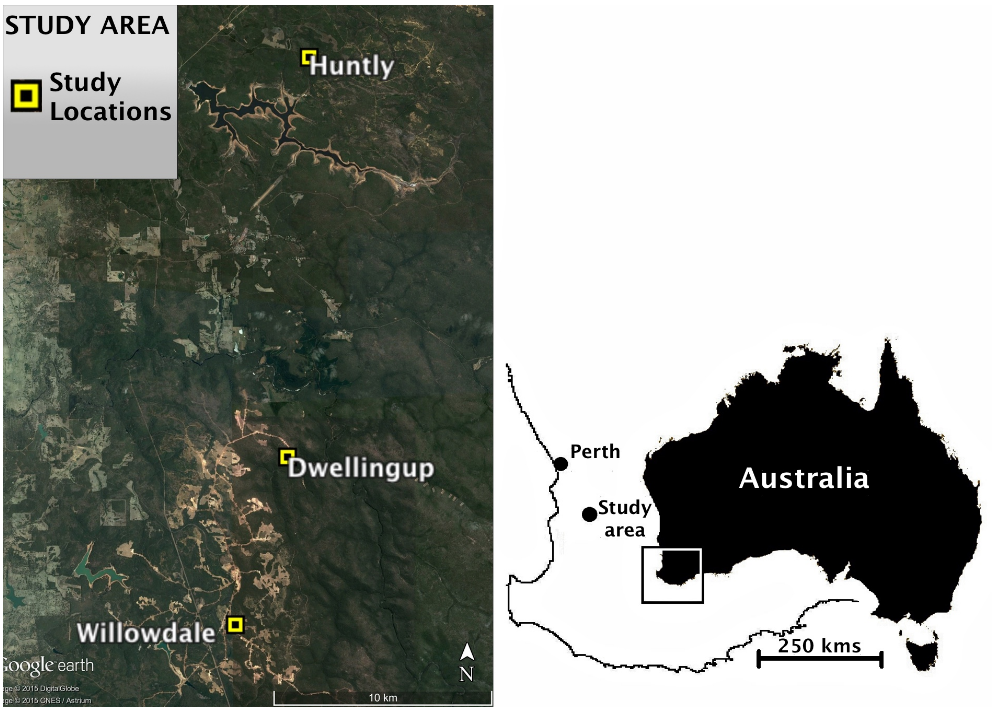

Figure 1.3 Location of studies areas within Australia.

Trapping sites at Huntly and Dwellingup were separated from each other by relatively important barriers such as human settlements and water bodies. Survey sites were selected to compare *P. cinnamomi* affected and unaffected areas of the northern jarrah forest (Table 1.1). Six 1-hectare survey sites were selected (Fig. 1.4). The mean distance between neighbouring grids was 675 m (SD=650 m, min=229 m, max=2081 m).

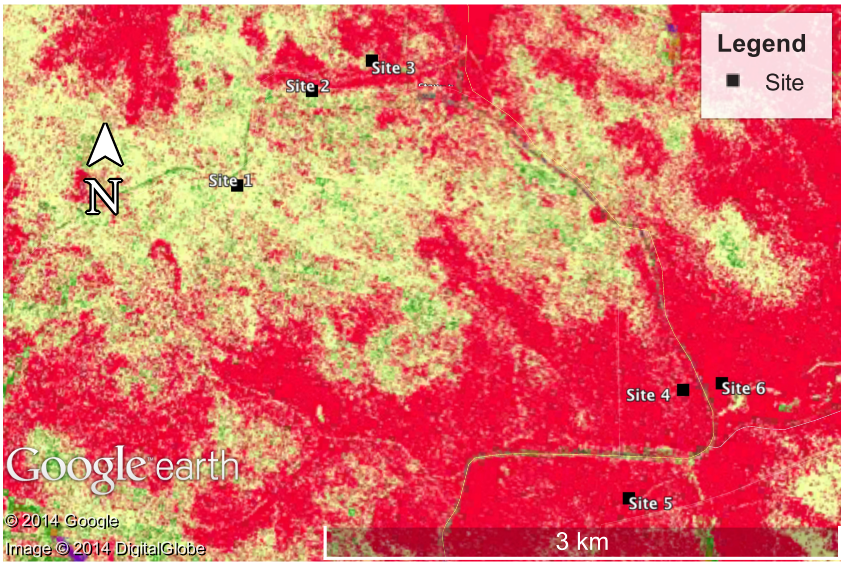

Figure 1.4 Location of trapping sites at Dwellingup (15 km south of Huntly) referred to in Table 1.1. Yellow and green colours represent levels of bare ground mainly due to a *P. cinnamomi* infection and red colour represents vegetation cover.

Table 1.1 Description of the presence and history of *P. cinnamomi* in the six sampling sites at Dwellingup, 15 km south of Huntly. See Figure 1.4.

	Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**
Approximate date of initial *P. cinnamomi* affection	Late 1950's to mid 1960's	Late 1950's to mid1960's	Late 1960's to mid 1970's	Late 1960's to mid 1970's	Uncertain	Not affected
Disease status	Post infestation. Old infestation, 50-60 years ago. Some colonisation by the susceptible species, *Banksia sessilis*	Post infestation. Old infestation, 50-60 years ago	Post infestation, 30-40 years ago. Some recent deaths suggest pathogen is still active	Post infestation, 30-40 years ago. Plants dying during survey period suggest pathogen is still active	Majority of site is disease free. Visual evidence of old infestation at 11 trap stations. Difficult to estimate date of initial infection	No disease expression, healthy. This site has not been disturbed for a long time
Trap stations affected by *P. cinnamomi*	25	25	22	25	11	0
Trap stations not affected by *P. cinnamomi*	0	0	3	0	14	25
Disturbance status	Severely affected	Severely affected	Moderately affected	Moderately affected	Healthy forest	Healthy forest

1.5.3 Willowdale

Willowdale trapping sites (Fig. 1.5) are located approximately 5 km south of Dwellingup sites and are relatively isolated from Dwellingup by extensive mined areas. Part of the site had been rehabilitated according to Department of Parks and Wildlife (DPaW) Dieback Forest Rehabilitation (DFR) procedures (Alcoa of Australia 2014). No accurate estimate could be made for the time of introduction of *P. cinnamomi* and its consequent spread. However, the site was probably infected by *P. cinnamomi* in 1980 according to Alcoa's Willowdale Area Dieback Distribution map. Confirmation of *P. cinnamomi* within the site was made in 1998 according to Alcoa's Willowdale hygiene mapping. The site was divided into 4 contiguous sub-sites (Table 1.2).

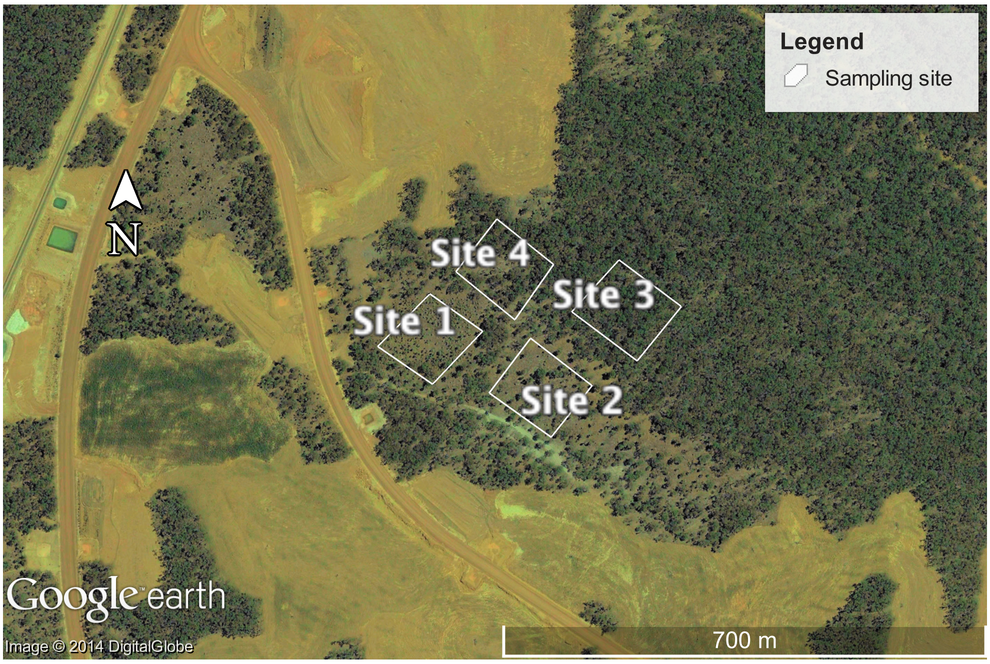

Figure 1.5 Location of trapping sites at Willowdale (approximately 5 km south of Dwellingup). The site was probably infected by *P. cinnamomi* in 1980.

Table 1.2 Description of the presence and history of *P. cinnamomi* in the four sampling sites at Willowdale, 5 km south of Dwellingup (see Figure 1.5).

	Site 1	Site 2	Site 3	Site 4
Disease status	Post infestation	Post infestation	No disease expression	Active
Disturbance status	Severely affected	Severely affected	Healthy forest	Moderately affected
Prescription	Dieback forest rehabilitation and plantation of dieback resistant jarrah (1998)	Dieback forest rehabilitation (1999)	None	None
Understorey	Little to none	None	Dense	Sparse
Leaf litter	Little to none	None	Dense	Sparse

1.6 The study species: the Yellow-footed Antechinus

The Yellow-footed Antechinus (*Antechinus flavipes*; Waterhouse, 1838) is a small carnivorous marsupial (21-80 g) member of the dasyurid family and is the most widely distributed *Antechinus* species in Australia (Menkhorst & Knight 2001). It is found continuously from South Australia to Queensland with isolated populations in north-eastern Queensland and south-western Western Australia (Fig. 1.6). The diet of *A. flavipes* consists mostly of insects, but may include anything from flowers and nectar to small birds and house mice (Van Dyck & Strahan 2008). The most notable trait of antechinuses, except for *A. swainsonii*, is their semelparity: a complete mortality of males occurring after a two-week mating season every year (Lee & Cockburn 1985), whereas approximately one third of females survive a second year to breed (Lada *et al.* 2008a). Antechinus *flavipes* are relatively vagile animals with some individuals able to disperse up to 720 m (mean=350 m; Marchesan & Carthew 2008). Their average home range has been estimated at 0.78 ha for females and 1.2 ha for males (Coates 1995). *Antechinus spp*. have developed a series of inbreeding avoidance mechanisms: the males disperse great distances after weaning (e.g. in *A. stuartii* up to 1230 m; Fisher 2005), whereas the females remain philopatric (Cockburn *et al*. 1985); individuals avoid sharing nests with opposite-sex relatives (Banks *et al.* 2005b); and multiple paternity within litters is common (Kraaijeveld-Smit *et al.* 2002b).

Figure 1.6 Range of *Antechinus flavipes* in Australia (yellow colour). It is found continuously from South Australia to Queensland with isolated populations in north-eastern Queensland and south-western Western Australia (Source: IUCN 2012).

Ecological genetics studies have been particularly useful in deciphering important ecological characteristics of various *Antechinus* species. For instance, Kraaijeveld-Smit *et al*. (2002a) in their study of *A. agilis* in South Australia, confirmed genetically the hypothesis of male-biased dispersal. Banks *et al*. (2005a) reported in *A. agilis* that, in a landscape dominated by a plantation of exotic *Pinus radiata* in south-eastern Australia, the pine plantation matrix did not pose a complete barrier to dispersal. Banks *et al*. (2005b) showed that *A. agilis* avoided sharing nests with opposite-sex relatives in large fragments but not in small ones, and Lada *et al*. (2007)

demonstrated that floods promote *A. flavipes* dispersal at the southern Murray–Darling Basin and that gene flow between populations is not restricted by rivers.

The local Western Australian subspecies of *A. flavipes*, the mardo (*A. flavipes leucogaster*; Fig. 1.7) has relatively specific habitat requirements, which have been related to the presence of grasstrees (*Xanthorrhoea preissii*; Swinburn *et al*. 2007). Studies carried out in the study area have recorded the presence of *A. flavipes* as soon as two years post-restoration (Nichols & Grant 2007), however its abundance remains lower than that recorded in unmined forest during the first eight years post-restoration (Craig *et al.* 2012). This is probably due to the absence of environmental elements that have been positively associated with the presence of *A. flavipes*, such as tree hollows, logs, stumps and leaf litter, and not by the availability of food itself (Nichols & Grant 2007).

Figure 1.7 Picture of *Antechinus flavipes leucogaster* (Yellow footed Antechinus) on the trunk of a *Banksia grandis* in the northern jarrah forest (Source: Armistead 2008). The snout:vent length of *A. flavipes* individuals is normally between 10 and 13 cm and their weight ranges from 21 to 80 g.

Antechinus flavipes may avoid areas affected by *P. cinnamomi* because of a lack of cover, food, nesting resources and a perceived increased risk of predation resulting from the decline or absence of vegetation structure and complexity, litter layer and *X. preissii* (Armistead 2008). The number of *A. flavipes* individuals, captures rates, detectability and patch occupancy rates are considerably lower in areas affected by *P. cinnamomi* compared to unaffected areas (Armistead 2008).

Antechinus flavipes was choose as study species because has a well-documented natural history that facilitates linking genetic findings to ecological characteristics, it is easily trapped, its short generation time minimises the time lag to detect genetic signals due to habitat fragmentation. Finally, although *A. flavipes* are not considered threatened, the studied subspecies (*A. f. leucogaster*) is confined to southwestern Australia with a large proportion of its distribution within the jarrah forest, which has been cleared and degraded to half of its original extent by extensive logging and clearing.

The landscape under study offers the suitable conditions to determine the potential of ecological restoration to contribute to the preservation of genetic diversity:

1. An ecological restoration project large enough to test landscape connectivity which allowed the sampling of a relatively large area (approximately 16,000 ha).
2. Restoration activities have been practiced long enough (since approximately 1976 at Huntly) which allows the detection of genetic changes. For instance, it has been shown that the time necessary for a genetic signal to appear in order to detect a new barrier can be as short as 1 to 15 generations (Landguth *et al*. 2010).
3. State of the art restoration methods. Alcoa's restoration process, which costs AU$34,000/ha, has achieved high standards of environmental performance and is regarded as a very successful restoration operation in general ecological terms (Koch & Hobbs 2007).
4. The lack of important natural landforms that could act as barriers, such as large water bodies or high elevations (range 250-350 mamsl).
5. A species with a well-documented natural history that facilitates linking genetic inferences to ecological characteristics. *Antechinus spp.* is among the most studied marsupials, due partly to its unusual semelparity.

2 Introduction to the genetics of restoration

2.1 Genetic disciplines to inform ecological restoration

The contributions of genetics to restoration originate from various genetic sub-disciplines, which use different research approaches. These sub-disciplines have emerged mainly from interdisciplinary research performed between the fields of genetics, ecology and conservation biology. Therefore, the field of genetics is able to contribute to restoration in several aspects, from theory to practice. This chapter comprises an overview about how the main genetic disciplines inform ecological restoration and how they are linked to its practice. For effects of practicality, I assigned specific areas of research to each discipline, however, there is no a defined line between them and their research interests frequently overlap.

2.1.1 Conservation genetics

Much research in conservation genetics has examined the negative consequences of the reduction and isolation of once large and connected populations (Frankham *et al.* 2009). Populations in these circumstances compromise their ability to respond and adapt to environmental stressors in the long and short-terms through the loss of genetic diversity from genetic drift and decreased gene flow. Consequently, both quantitative genetic variation and effectiveness of natural selection (if $Ne<500$; Franklin & Frankham 1998) are reduced and, ultimately, evolutionary potential is compromised. Additionally, the probability of mating between relatives in these populations increases, resulting in the potential exposure and accumulation of deleterious mutations and loss of heterozygosity (i.e. inbreeding depression). As a result, individual fitness and population persistence will be negatively affected (Frankham 2005; O'Grady *et al.* 2006).

In a restoration context, these genetic concepts highlight four genetic aspects that should be considered in restoration planning: 1) to choose genetically health donor populations, so translocated individuals perform better than otherwise inbred individuals (Kettle *et al*. 2008); 2) to capture enough genetic diversity from the donor population (ideally >95% of the standing genetic variation within the donor population; Weeks *et al*. 2011), so reinstalled populations retain evolutionary potential; 3) to monitor genetic diversity of recolonising populations to identify loss of genetic diversity; and 4) to maintain appropriate levels of gene flow in restored populations (one to ten migrants per generation; Mills & Allendorf 1996), either by reinforcement, or by reinstating ecological corridors with contiguous populations, to avoid inbreeding effects and promote evolutionary potential.

2.1.2 Restoration genetics

Specific genetic research on restoration has focused on the potentially negative consequences arising from the translocation of plants or animals that might be adapted to different environmental conditions than those occurring at the restoration site. The development of practical guidelines to avoid such negative consequences during restoration activities has been the primary outcome of research in restoration genetics (Broadhurst *et al*. 2008; Frankham *et al*. 2011; Rogers & Montalvo 2004; Weeks *et al*. 2011). Empirical evidence in plants, invertebrates and vertebrates, has shown that hybridisation between translocated individuals and individuals from established and/or surrounding populations, that are genetically divergent, reduces the fitness of subsequent generations due to disruptions of co-adapted gene complexes (i.e. outbreeding depression; Edmands 2007). However, the probability of outbreeding depression is elevated only when the populations have at least one of the following characteristics: are distinct species, have fixed chromosomal differences, exchanged no genes in the last 500 years, or inhabit different environments (Frankham *et al*. 2011). Additionally, translocated individuals might perform poorly if not adapted to local conditions (due to maladaptation). Therefore, a crucial research topic in restoration is to unravel the factors determining the strength of local adaptation and the geographic scale at which this occurs (McKay *et al*. 2005). To this end, seed transfer zones have received most attention from restoration geneticists. However, the latest research suggests the prevalence of outbreeding depression has been overestimated (Broadhurst *et al*. 2008; Frankham *et al*. 2011; Weeks *et al*. 2011) and represents a greater risk for plants (especially in selfing, rather than self-incompatible species) than for animals. Moreover, plant genetics studies suggest that, when outbreeding depression occurs, affected populations may recover in a few generations, after natural selection removes maladapted genes (Erickson & Fenster 2006). However, further research is needed to determine the generality of these conclusions.

2.1.3 Community genetics

Community genetics (the investigation of the role of genetic variation in influencing species interactions and determining community structure; Antonovics 1992) indicates that genetic diversity can influence evolution and ecological processes at community and ecosystem levels (Hughes *et al*. 2008). Major findings in this field suggest genetic diversity in foundation species may influence ecosystem processes and how communities are structured (Whitham *et al*. 2006). For instance, population genetic diversity in *Solidago altissima*, a dominant old-field plant species, determined arthropod diversity, community structure and increased ecosystem processes, such as aboveground net primary productivity (Crutsinger *et al.* 2006). In another example, recently developed community genetics models highlighted that, under conditions of high environmental heterogeneity, genetic diversity of foundation species can influence their capacity to exploit a wide range of niches (Gibson *et al.* 2012). Expanding these study to other species could provide important insights into how to improve restoration practices. Additionally, approaches that consider the genetics of multiple species could add useful insights into restoration in terms of community assemblages and ecosystem functioning, especially in the important early stages of restoration. Until now, the use of genetics for conservation and restoration purposes has been largely focused on single species. However, community genetics may provide one of the main research frameworks with which to expand theoretical concepts in restoration.

2.1.4 Landscape genetics

The emerging field of landscape genetics (Manel *et al*. 2003; Storfer *et al*. 2007; Storfer *et al*. 2010) combines population genetic theory with landscape ecology analyses to test the hypothesis that landscape features influence population dynamics, such as gene flow. New approaches to relate genetic data to landscape characteristics using Geographic Information System resources, such as resistance surfaces on raster images (Spear *et al*. 2010), circuit theory (McRae *et al*. 2008) and statistical procedures to correlate environmental factors with genetic data (Thomassen *et al*. 2010) have increased our ability to identifying landscape characteristics that influence the spatial distribution of genetic variation. This area of research can potentially inform restoration in many ways, such as identifying barriers to gene flow so they can be removed (Raeymaekers *et al*. 2008) and determining the suitability of different restoration management prescriptions to provide adequate levels of genetic connectivity (Spear *et al*. 2012). Landscape genetics has been also used to determine whether population differentiation is best explained as a function of environmental differences rather than geographical distances (Gao *et al*. 2012), ultimately allowing the identification of appropriate source populations. In a further example, using a regression of least cost paths and genetic differentiation, it was possible to determine the best management prescription for facilitating gene flow after a volcanic eruption (Spear *et al.* 2010; Spear *et al.* 2012). In the future landscape genetics applications could substantially contribute to the field of restoration ecology.

2.1.5 Molecular ecology

The field of molecular ecology, defined as the application of molecular genetic methods to gain insight into ecological features (Beebee & Rowe 2004), is one research area where the use of genetics has seen more practical applications. The collection of non-invasive genetic samples (e.g. hair or faeces; Beja-Pereira *et al*. 2009; Waits & Paetkau 2005) has facilitated the use of genetics in faunal conservation, with recent research developing and improving assignment and clustering models (Manel *et al*. 2005) to identify the population provenance of individuals. These models use multi-locus genotypic information with Bayesian inference methods (Beaumont & Rannala 2004), which main aim is to calculate the posterior distribution of the parameters (Excoffier & Heckel 2006), to infer dispersal, hybridisation, admixture proportions and delineate populations and genetic structure.

Efficient and powerful genetic methods to monitor census and effective population size (Luikart *et al*. 2010) have proven to be invaluable tools in the management of natural populations. Further research has developed methods to detect changes in population size (i.e. genetic bottlenecks), estimate kinship or infer migration rates (Sunnucks 2011), which can provide invaluable information to restoration projects in various ways. For example, connectivity estimations, past and present population trajectories (i.e. whether expanding or contracting; e.g. Beaumont 1999), migration rates, source and sink population identification – which inform whether the restored ecosystem is providing suitable conditions to sustain reproducing populations (Andreasen *et al.* 2012) – or the identification of the origin of individuals can readily assist restoration practitioners. With a different approach, Next Generation Sequencing technologies have considerable application in the survey of species richness (e.g. DNA metabarcoding and metagenomics; Taberlet *et al.* 2012; Williams *et al.* 2014), one of the most utilised parameter for pre-restoration baseline assessment and an important measure of restoration success with regard to faunal populations (Ruiz-Jaen & Aide

2005a). These new technologies will allow restoration practitioners to carry out faster and more affordable biodiversity assessments of ecosystems than current field-based techniques.

2.2 Genetic resources to be used in ecological restoration

The direct acquisition of genetic information of a target species is a powerful source of information to guide restoration efforts and a critical tool to apply the concepts mentioned in the above section. Methods to acquire this genetic information have different attributes, capabilities and limitations that require special consideration for their appropriate use in restoration. Genetic diversity may be measured indirectly by quantitative methods (i.e. expression of phenotypic traits) or directly by molecular methods through the actual DNA sequences. Unfortunately, determining the direct relationship between phenotypic traits and their corresponding DNA sequence (i.e. adaptive genetic diversity) is still a difficult endeavour as gene's expressions, interactions and inheritance are complex processes (i.e. polygenic traits, environmental effects, dominance, epistasis, pleiotropy and recombination). However, the genomics era promises to shed light on this restraint in the near future (Ekblom & Galindo 2011). As a result, genetic diversity measured by molecular markers (i.e. neutral genetic diversity) has been the main input of genetic information for ecological and conservation purposes in natural populations. Notable attributes of molecular markers are that they provide a measurement of genetic diversity that has a discrete distribution, which makes them amenable to model. Furthermore, they are relatively quick and affordable to amplify by PCR, are evenly distributed throughout the genome, and are mostly not under natural selection (Schlotterer 2004), allowing accurate inferences of genetic drift and gene flow. On the other hand, phenotypic traits allow the direct measure of genetic diversity of many ecologically important traits, which are mostly under natural selection (Storfer 1996). However, their continuous distribution makes them difficult to model, their measurement requires large sample sizes, long waiting periods and, in some cases, specialised equipment.

Although it has been shown that the ability of molecular markers to directly predict genetic diversity at loci under natural selection is limited (Reed & Frankham 2001), they are able to provide valuable information to predict patterns of local adaptation, as long as their limitations are taken into account and interpreted accordingly. It has been suggested that F_{ST} may be a better predictor of differentiation in genomic regions that underlie traits under natural selection than previously thought (Leinonen *et al*. 2013). From figure 2.1 it can be seen that when F_{ST} is close to 0, Q_{ST} can take any value. That is, evidence of low neutral genetic differentiation (e.g $F_{ST}<0.05$) is not to be taken as strong evidence to discard the possibility of local adaptation and, therefore, the risk of outbreeding depression. Conversely, high neutral genetic differentiation provides strong evidence for the opportunity for local adaptive differentiation to occur. That is, local adaptive differentiation is likely to occur between populations that have been isolated long enough to accumulate substantial neutral genetic differentiation (Allendorf *et al*. 2013, page 280).

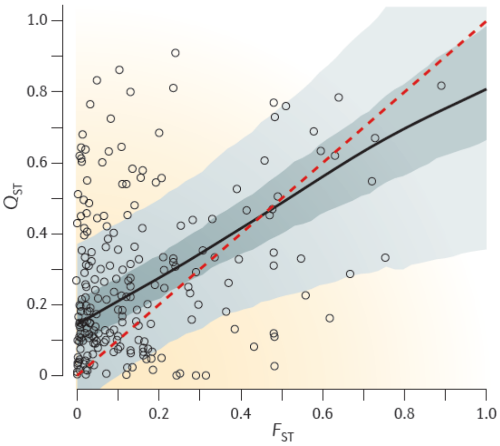

Figure 2.1 Comparison of mean Q_{ST} and F_{ST} across published studies. There is a significant non-parametric correlation (Spearman rank correlation coefficient = 0.24, n = 218, $P < 0.001$) between average Q_{ST} and F_{ST} estimates across all studies published up to 2013. Moreover, the fitted relationship between F_{ST} and the expected 'true' value of Q_{ST} does not significantly differ from a 1:1 relationship (dashed red line). The solid line denotes the posterior mode of predicted Q_{ST} estimated as a function of its relationship with F_{ST}, whereas the light grey and dark grey shaded areas denote the 50% and 95% posterior density intervals, respectively. Note that the 95% posterior density limits include the 1:1 line over the full range of possible F_{ST} values (taken from: Leinonen *et al.* 2013).

There is much work to be done before genetics can be routinely used to inform applied and basic research in restoration. Nevertheless, the genetics of restoration is already enhancing restoration science by providing novel research approaches and simultaneously broadening and improving research frameworks of both restoration ecology and conservation genetics.

3 Contribution of genetics to ecological restoration

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3.1 Abstract

Ecological restoration of degraded ecosystems has emerged as a critical tool in the fight to reverse and ameliorate the current loss of biodiversity and ecosystem services. Approaches derived from different genetic disciplines are extending the theoretical and applied frameworks on which ecological restoration is based. We performed a search of scientific articles and identified 160 articles that employed a genetic approach within a restoration context to shed light on the links between genetics and restoration. These articles were then classified on whether they examined association between genetics and fitness or the application of genetics in demographic studies, and on the way the studies informed restoration practice. Although genetic research in restoration is rapidly growing, we found that studies could make better use of the extensive toolbox developed by applied fields in genetics. Overall, 42% of reviewed studies used genetic information to evaluate or monitor restoration and 58% provided genetic information to guide pre-restoration decision-making processes. Reviewed studies suggest that restoration practitioners often overlook the importance of including genetic aspects within their restoration goals. Even though there is a genetic basis influencing the provision of ecosystem services, few studies explored this relationship. We provide a view of research gaps, future directions and challenges in the genetics of restoration.

Keywords: conservation genetics, meta-analysis, restoration, restoration ecology, restoration genetics, translocation.

3.2 Introduction

During the last four decades, conservation geneticists have developed countless concepts, methodologies and tools to inform the conservation of biodiversity and, together with other related fields, conservation genetics is experiencing a major innovation due to technological and analytical advances (see Allendorf *et al.* 2010 for examples, implications and limitations). Concurrently, ecological restoration is emerging as a promising and effective activity to return biodiversity and **ecosystem services** where they have been lost and/or reduced (see Box 1 for definition of terms; Benayas *et al.* 2009). Ecological restoration uses knowledge of an ecosystem's pre-existing structure, composition and functioning for "assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed" (SERI 2004), and has been increasingly taking advantage of conservation genetic applications to inform ecological restoration on a wide array of issues.

The links between genetics and restoration may span several aspects and genetics can provide information critical for the decision-making process and the monitoring of restoration projects (see Table 3.1 for a few examples). For instance, ecological restoration frequently involves the **translocation** of a range of different organisms and genetics tools may be highly informative to better plan and execute such exercises (e.g. identification of source populations, selection of founders, identifying adaptive variation, etc.). Genetics can facilitate the evaluation of a restoration project by, for example, quantifying gene flow or demographic changes in the targeted populations. The role of genetics is not only limited to indirectly evaluating population dynamics or ecosystem processes, however, as genetics can directly influence the success of restoration projects. Recent research has demonstrated the role of genetic diversity on individual fitness, population persistence and ecosystem processes, which are all elements of primary interest in restoration ecology. Furthermore, there is evidence of a direct relationship between population dynamics and genetic diversity (e.g. population size and dispersal; Beaumont 2008a; Sunnucks 2011), which require both aspects (demographic dynamics and genetics) to be taken into considerations concurrently. Specific research is enabling us to understand the principles and consequences of genetic disruption (i.e. loss of genetic diversity, inbreeding and **outbreeding depression**) resulting either directly or indirectly from restoration interventions (Hufford & Mazer 2003; Kramer & Havens 2009; McKay *et al.* 2005). These findings have served to develop guidelines and recommendations that have improved restoration practices and increased restoration success (Breed *et al.* 2013; Broadhurst *et al.* 2008; Frankham *et al.* 2011; Rogers & Montalvo 2004; Weeks *et al.* 2011). Furthermore, new advances with next generation sequencing tools are expected to make available molecular data for a wider spectrum of taxa, while being cheaper and faster than conventional methods. Applications of these techniques are also expected to provide insights into one of the most important current topics of genetic research in restoration: the identification of the strength of local adaptation and the geographic scale over which this local adaptation occurs (McKay *et al.* 2005).

Table 3.1 Applied studies exemplifying the broad range of restoration interventions and objectives in which genetics have been used.

Restoration intervention	**Target species**	**Objective**	**Method employed**	**Main conclusion or finding**	**Reference**
Reintroduction	Bighorn sheep (*Ovis canadensis*)	Avoid inbreeding	Bottleneck tests	Identification of genetic bottlenecks and small *Ne*	Ramey *et al.* 2000
Augmentation	Seagrass (*Zostera noltii*)	Improve evolutionary potential from an endangered species	Assignment tests	Successful location of the most suitable donor population	Diekmann *et al.* 2010
Seeding for river restoration	Common reed (*Phragmites australis*)	Delineation of seed sources zones to avoid maladaptation	Regression of allele occurrence and environmental variables	Environmental factors explained genetic structure	Gao *et al.* 2012
Eradication of invasive species	Brown rat (*Rattus norvegicus*)	Define eradication units	Migration rates and assignment tests	Eradication is feasible with low risk of recolonisation	Robertson & Gemmell 2004
Salvage logging and planting after disturbance	Coastal tailed frog (*Ascaphus truei*)	Evaluate management prescriptions	Regression of least cost paths and genetic differentiation	Natural regeneration maintain genetic diversity better than active management	Spear *et al.* 2012
Establishment of an ecological corridor	Australian rats (*Rattus fuscipes* and *Rattus leucopus*)	Monitor corridor efficiency to re-establish gene flow	Assignment and clustering tests	The use and occupation of the corridor differed between species	Paetkau *et al.* 2009
Removal of shrubs, mowing and grazing	Terrestrial orchid (*Dactylorhiza incarnata*)	Inference of colonisation patterns	Assignment and clustering tests and genetic parameters	Decrease in genetic diversity but not in population fitness	Vandepitte *et al.* 2012

Genetic research is expanding our understanding of the far-reaching influence of genetic diversity, not only at individual and population levels, but also at community and ecosystem levels (Benayas *et al.* 2009; Hughes *et al.* 2008). For example, studies in clonal plant species have shown that issues relevant for restoration, such as individual fitness, population growth, plant density, provision of ecosystem services, species richness and abundance are positively associated with genetic diversity (Reusch *et al.* 2005; Reynolds *et al.* 2012; Vandegehuchte *et al.* 2012; Williams 2001). However, further research is needed to determine how widely these results apply to other species, including fauna (Hughes *et al.* 2008).

Restoration ecologists need to appreciate that not all methods for measuring genetic diversity have the same attributes and their applicability to restoration will depend on the information being sought in any specific context, as well as financial and logistic limitations. Genetic diversity may be measured by quantitative methods, such as expression of phenotypic traits, or directly by molecular methods that quantify diversity at a genome level (e.g. DNA sequences). Unfortunately, determining the direct relationship between phenotypic traits and genetic diversity is not trivial because adaptive genetic diversity is confounded by complex processes such as gene expression, interactions and inheritance (e.g. Barrett & Hoekstra 2011; Stinchcombe & Hoekstra 2008). However, neutral molecular markers (e.g. microsatellite loci – tandem repeats of 2-4 nucleotides; Selkoe & Toonen 2006) possess some useful characteristics that make them generally suitable for applications of population genetic models including that they occur in a discrete distribution and they are generally highly discriminatory, quick, affordable and ubiquitous (Schlotterer 2004).

Despite all the above mentioned applications, how and where genetics may directly contribute to improving our ability to restore ecosystems is currently underappreciated and, as a consequence, restoration ecology underutilises genetic techniques (Brudvig 2011; Kettenring *et al.* 2014; Ruiz-Jaen & Aide 2005a; Wortley *et al.* 2013). Thus, the aim of this paper is to review how genetics has been utilised in restoration ecology to the present and to identify ways in which genetics could be better utilised to inform restoration ecology in the future. To achieve this, we first provide a comprehensive overview of the various ways that genetics has been used to inform ecological restoration. Then, through a meta-analysis, we cover how genetic research topics have been aligned to different stages of restoration, from advances in theory to their implementation in decision-making, monitoring and evaluation processes. We then utilise the finding from our meta-analysis to point out research gaps, future directions and challenges in the genetics of restoration.

Box 1. Glossary of terms in bold in the main text

Community genetics: the investigation of the role of genetic variation in influencing species interactions and determining community structure (Antonovics 1992).

Ecosystem services: benefits supplied by organisms and ecological processes at no cost to humankind, such as crop pollination, carbon sequestration and water purification.

Effective population size (N_e): the size of an idealised population that would have the same amount of inbreeding, or random genetic drift, as the population under consideration (Kimura & Crow 1963; Miller *et al.* 2011).

Foundation species: species with substantial effects on the structure of natural communities and modulation of ecological processes.

Outbreeding depression: reduction in mean population fitness resulting from hybridisation between genetically distinct individuals or populations of the same species (Hufford & Mazer 2003).

Seed dispersal: the movement or transport of seeds away from the parent plant.

Seed transfer zones: geographic areas within which plant materials can be moved freely with little disruption of genetic patterns or loss of local adaptation (Miller *et al.* 2011).

Translocation: human-mediated movement of living organisms from one area to another. The IUCN SSC Species Survival Commission (2012) considers four types of organism translocations:

- **Reinforcement/supplementation**: into an existing population of conspecifics;

- **Reintroduction**: inside its indigenous range from which it has disappeared;

- **Assisted colonisation**: outside its indigenous range to avoid extinction of populations of the focal species; and

- **Ecological replacement**: outside its indigenous range to perform a specific ecological function.

3.3 Meta-analysis of the use of genetics in ecological restoration

Using the “Web of Science” (www.isiknowledge.com) we searched up to the 31 December of 2013 for journal articles with the words restoration AND genetic* in the title or with the words “genetic*” and either “restoration ecology” OR ”ecological restoration” OR “restoration genetics" OR “revegetation” OR “rehabilitation” AND “min*” (to distinguish post-mining rehabilitation from medical rehabilitation) in the title, abstract or keywords. We recognise that using these keywords likely excluded articles from some active fields of research within conservation genetics, such as faunal translocations. These are valid ecological restoration activities, but they are not yet common practice in ecological restoration projects and are approached through the discipline of reintroduction biology (Seddon *et al.* 2007). Consequently, only studies that considered faunal translocations as being either ecological restoration or restoration ecology or restoration genetics were retained, whereas publications defined as genetic rescue or genetic restoration were not taken into account.

We retained those articles matching the following inclusion criteria: 1) acknowledging that its objectives were directly related to, or intended to be used in ecological restoration or restoration ecology; 2) employed genetics as their main approach to derive its conclusions, and; 3) used molecular markers. We acknowledge that phenotypic traits allow a measure of genetic diversity of many ecologically important traits and that there are authors who suggest that caution should be used when making management decisions using only neutral molecular variation (Kohn *et al.* 2006; McKay & Latta 2002; Stockwell *et al.* 2003). However, the continuous distribution of phenotypic traits makes them difficult to model, their measurement requires large sample sizes, long waiting periods and, in some cases, specialised infrastructure (Storfer 1996). Therefore, we decided to focus on neutral molecular methods as a measure of genetic diversity, as these marker-based methods are continually becoming quicker and more affordable (e.g. Abdelkrim *et al.* 2009) and are, in some cases, more cost-effective than comparable field-based methods (e.g. Johnson *et al.* 2013). This makes them easier to apply in a restoration context, where economic and time resources frequently limit restoration efforts. Furthermore, a recent review found a significant relationship between neutral genetic differentiation and natural selection of phenotypic traits (Leinonen *et al.* 2013).

To visualise general trends, we extracted the following information from each article: publication year, journal of publication, molecular marker used, the taxa of organism studied (plant, invertebrate, fish, bird, amphibian/reptile or mammal), ecosystem (aquatic or terrestrial) and continent where the study was conducted. Papers were classified based on whether they investigated changes in genetic diversity and its (possible) association with fitness (e.g. inbreeding or outbreeding depression); or application of molecular data to evaluate demographic parameters such as population size, dispersal or kinship. When studies used a combination of approaches the article was classified by the approach used to derive the main conclusion(s).

Additionally, we classified articles based on the way they informed restoration practice: 1) providing information to support decision-making processes, i.e. studies to develop restoration plans, which were carried out before the performance of any restoration intervention; or 2) providing information to monitor and evaluate restoration projects in on-going, or already finished, restoration projects. Finally, as one of the most important benefits of ecological restoration is the increase of ecosystem services (e.g. Nellemann & Corcoran 2010), we enquired how surveyed articles considered the relationship between restoration, genetics and ecosystem services.

3.3.1 General trends

Our search found 1347 articles, of which 160 satisfied the inclusion criteria given above. Genetic research in restoration is growing rapidly with 59% of articles published during the last four years (Fig. 3.1a). This trend likely reflects increasing interest in ecological restoration, as indicated by the number of published papers in the field during the last decade (Fig. 3.1b). However, unlike in conservation, the link between genetics and restoration still remains largely unexplored and untapped. A recent review of restoration research, (Brudvig 2011) found just one genetic study among 190 applied papers and, although the search conducted in this review would have likely underestimated the proportion of genetic studies, it is still indicative of the infrequent incorporation of genetics into most restoration projects. Furthermore, two other reviews of restoration success (Ruiz-Jaen & Aide 2005a; Wortley *et al.* 2013) failed to even consider genetic assessments of restoration success. The four journals most sought-after by authors were *Restoration Ecology* with 19 publications, *Conservation Genetics* with 15, *Biological Conservation* with 11, and *Molecular Ecology* with eight.

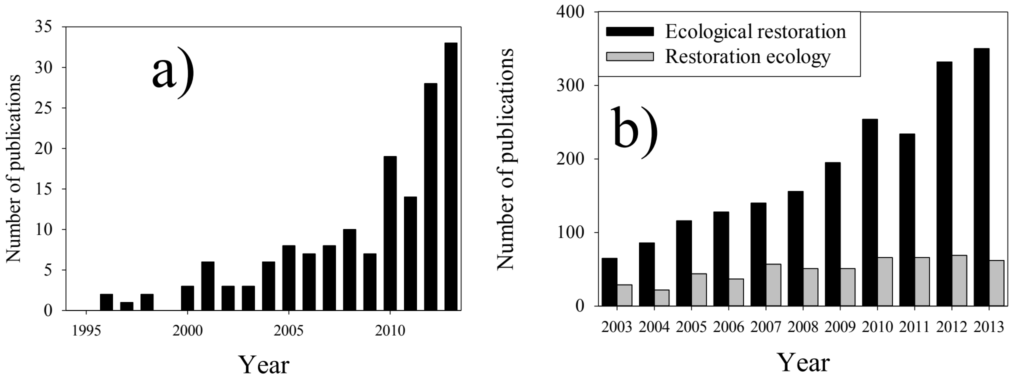

Figure 3.1 Trend in the number of published articles of restoration genetic studies (a; see text for inclusion criteria). Number of published articles (b) in scientific journals mentioning "ecological restoration" and "restoration ecology" in the title, abstract, or keywords, retrieved from a search within "The Web of Knowledge" (www. isiknowledge.com) from 2003 to 2013. Note that the number of publications in all three categories increased over time, with most publications in the last three years.

Continents where developed countries are located accounted for 85% of all studies (41% in North America, 23% in Australia and 21% in Europe), while continents where developing countries are located accounted for 15% (11% in Asia, 4% in Latin America and none in Africa; Fig. 3.2a). This mismatch between the overall number of scientific conservation publications relative to the world's conservation priority areas is ubiquitous in conservation science (Lawler *et al.* 2006). Economic constraints, language barriers or an affinity for publishing in regional journals, are typically the reasons explaining this publication bias (Lawler *et al.* 2006); however, the lack of infrastructure necessary for genetic studies in developing countries likely exacerbates this trend. Restoration in tropical terrestrial biomes, where many developing countries are located, shows a disproportionately higher response ratio in increasing both biodiversity and ecosystem services than is the case for temperate biomes (Benayas *et al.* 2009). This represents a window of opportunity for developing nations and an incentive for developed nations to invest in restoration practices in tropical ecosystems, as well as using genetics to improve the output of future restoration projects.

More research has been conducted on plants (81%) than on animals (19%; Fig. 3.2b) and more in terrestrial (69%) than in aquatic (31%) ecosystems. These percentages are similar to trends in overall restoration research (Brudvig 2011; Ruiz-Jaen & Aide 2005a; Wortley *et al.* 2013). At present, ecological restoration has largely focused on restoring floral, not faunal, communities. This focus could be partially explained by an assumption prevailing among restoration projects that "if you build it, they will come" (Palmer *et al.* 1997), which suggests that, if suitable environmental conditions exist, faunal recolonisation will occur passively. However, this assumption has been shown not to apply in all ecosystems as faunal species may have very specific habitat requirements (Pullin 1996) and take decades before recolonising restored areas (e.g. Craig *et al.* 2012; Kanowski *et al.* 2006). Their dispersal distance may be too short to recolonise within desirable timeframes (Jacquemyn *et al.* 2010; Kettle *et al.* 2012) or changing environmental conditions in restored ecosystems may represent filters that prevent their recolonisation (Craig *et al.* 2012). The genetics of fauna in a restoration context is understudied and future work in this area would help determine whether restoration is effective in helping retain the evolutionary potential of fauna populations. This would be particularly important to determine for species that are slow to recolonise restored areas.

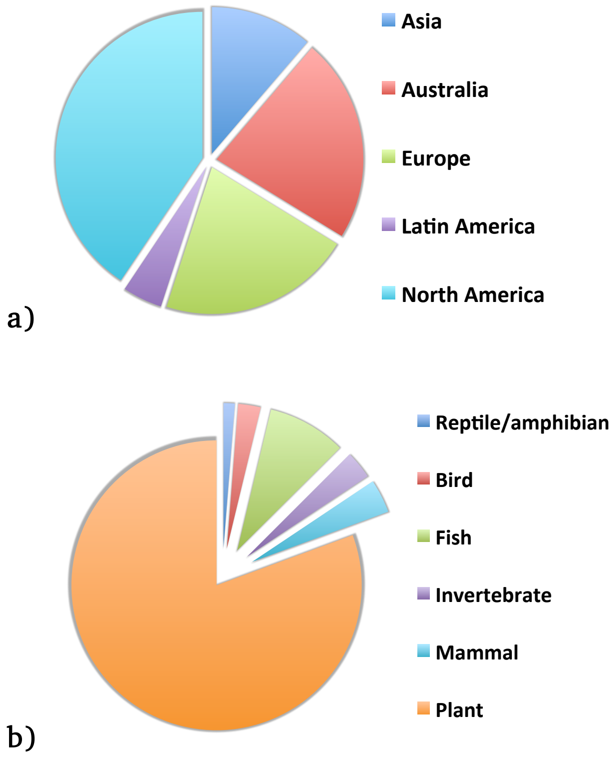

Figure 3.2 Proportion of empirical studies that were performed on (a) each continent and (b) classified by taxonomic group.

We documented 15 different types of molecular markers used in the articles sampled (Fig. 3.3). Nearly half the studies used microsatellites (47%) followed by amplified fragment length polymorphism (AFLPs; 25%) and random amplification of polymorphic DNA (RAPD; 8%). The strong bias towards microsatellite markers reflects their common use as they are highly polymorphic neutral loci, widely present in the genome, relatively cheap to study and provide resolution at the population level. Interestingly, only two studies employed DNA sequences and only a single study employed single-nucleotide polymorphism (SNPs) despite the increasing availability of new technologies such as next generation sequencing (NGS; Davey *et al.* 2011). However, the lack of use of

NGS is probably due to the time lag in publishing rather than an unwillingness to embrace this technology.

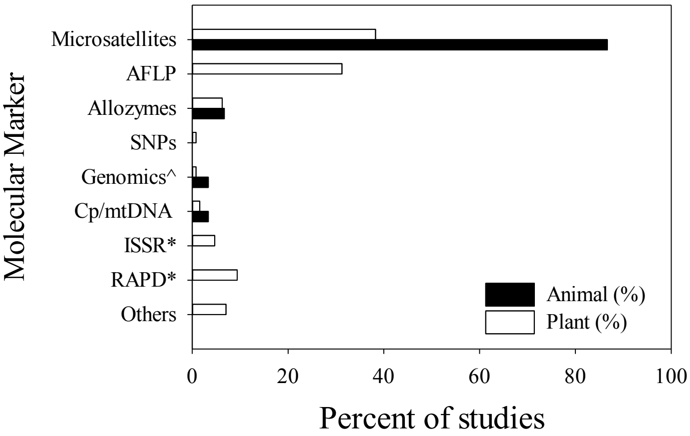

Figure 3.3 Percentage of empirical restoration genetics studies using different molecular markers in animals and plants. RAPD - random amplification of polymorphic DNA, ISSR – inter simple sequence repeat, Cp/mtDNA – chloroplast/mitochondrial DNA, AFLP - amplified fragment length polymorphism. ^ this term is used to refer to marker generation and the use of sequencing data from a large proportion of the genome for example generated by next-generation technologies. * The use of RAPD and ISSR markers has been questioned because of problems about reproducibility, dominance and homology and therefore their use is presently discouraged.

Inspection of genetic applications in restoration demonstrated that a relatively larger number of studies (58%) applied genetics to support decision-making processes rather than to evaluate the success of restoration projects (42%: Fig. 3.4). For examples, several studies used genetics to identify source populations and to delimit **seed transfer zones**. Secondly, the majority of studies explored changes in genetic diversity and the associations between genetics and fitness, while tools to assess demographic issues (such as gene flow, identification of migrants, effective population sizes or population trajectories) were less frequently used, suggesting that the latter approach may be underutilised in restoration genetic studies.

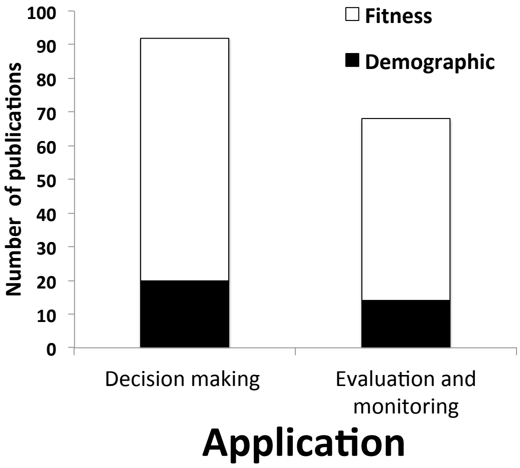

Figure 3.4 Graph representing the number of published studies summarising the application type of restoration genetics and the genetic approach used by these studies. Fitness refers to studies that examined the association between genetics and fitness (e.g. inbreeding, outbreeding and loss of genetic diversity) while demographic refers to papers that focused on demographic issues (e.g. population size, dispersal and kinship).

Experimental research in clonal species, such as sea grasses, indicates that genetic diversity plays an important role in the individual and population fitness of plants used for restoration, as well as in the provision of ecosystem services and faunal abundance (Procaccini & Piazzi 2001; Reynolds *et al.* 2012; Williams

2001), however further research is needed to generalise these conclusions. Recently developed **community genetics** models highlighted that, under conditions of high environmental heterogeneity, genetic diversity of **foundation species** can influence their capacity to exploit a wide range of niches, with broad implications for ecological restoration (Gibson *et al.* 2012). Conversely, some empirical studies have raised concerns about generalising theoretical genetic guidelines in restoration based solely on life history traits (i.e. mating system), since it is likely that species-specific characteristics limit the application of general criteria. For example, a common grassland herb (*Geranium pratense*) displayed low genetic diversity, high genetic differentiation among populations and a pronounced within-population spatial genetic structure, which was unexpected for an herbaceous, insect-pollinated and outcrossed species (Michalski & Durka 2012). In contrast, *Alexgeorgea nitens* (a dioecious, clonal, perennial species), displayed high levels of genetic diversity within populations, again unexpected for a clonal species with limited **seed dispersal** (Sinclair *et al.* 2010). These studies exemplify one of the advantages of using surveys of genetic diversity in restoration but also caution against following the generally accepted dogma as, if it had been followed in these cases, restoration outcomes may have been affected. In general, though, the relationship between restoration, genetics and ecosystem services remains understudied and is an important area for future restoration genetic studies.

3.3.2 Decision-making

Maximising restoration success requires a mindful decision-making process supported by reliable and accurate information. Genetic methods and tools can support the acquisition of this information as long as their capabilities, attributes and limitations are appreciated (see for example the discussion above about the limitations of using molecular markers *versus* quantitative genetics). The usefulness of genetic information in improving restoration outcomes was shown by Godefroid *et al*. (2011). These authors found that survival rates of reintroduced plant species were much higher when information about genetic diversity of the target species was included in the project design. Moreover, genetic methods and tools can provide important information that would be difficult to obtain through other methods, for example, estimating connectivity, past and present population trajectories (i.e. whether expanding or contracting), migration rates and identifying the origin of individuals.

Among the studies that guided pre-restoration decision-making processes, 78% focused on the relationship between genetics and fitness. The major focus of these studies was to inform the choice of the most suitable donor population(s) to avoid the risk of outbreeding depression when translocations of plants or animals were needed. To this end, genetic differentiation between potential donor populations across different spatial scales has been used as an *ad hoc* method to delineate seed transfer zones. Studies used a wide variety of methods to delineate seed transfer zones, including: analysis of molecular variance (Krauss & He 2006), principal component analysis (Lloyd *et al.* 2011), clustering methods (Broadhurst 2011), spatial autocorrelation (Krauss & Koch 2004), isolation by distance calculated through Mantel tests (Gonzalo-Turpin *et al.* 2010) and estimation of gene flow (Tanaka *et al.* 2011). Landscape genetics was used (although rarely) as an additional and informative approach to determine whether population differentiation is best explained as a function of environmental differences rather than geographical distances (Gao *et al*. 2012), ultimately allowing the identification of appropriate source populations.

The second objective in choosing a suitable donor population among studies was the identification of outbred populations with high neutral genetic diversity, under the assumption that outbred populations are less likely to suffer the effects of inbreeding depression (Kettle *et al.* 2008). Equally important was the capture of sufficient genetic diversity from the donor population, ideally >95% of the standing genetic variation within the donor population (Weeks *et al.* 2011), achieved through an adequate sampling strategy of the population(s) of organisms to be translocated (Blakesley *et al.* 2004; Sinclair & Hobbs 2009). By doing so, translocated populations might retain evolutionary potential, which is increasingly important to face the already on-going consequences of climate change (see also below in research gaps and future directions).

A different approach was used when **reinforcement** was the restoration aim. The objective, in these cases, was the genetic rescue of natural populations. Therefore, it was important to identify the population of origin, for example by using assignment tests (Diekmann *et al.* 2010), and the relative genetic differentiation between the established and potential donor populations. Prioritising and guiding stocking strategies of the lake trout (*Salvelinus namaycush*), by measuring the genetic contribution of different hatcheries was, for example, the primary aim in the management of a restoration project (Page *et al.* 2004). Lastly, genetic diversity information was used to decide whether populations require active management (e.g. Maloney *et al.* 2011), for example when low levels of genetic diversity or evidence of inbreeding, were found.

Genetic studies that examined gene flow, either directly (using individual genotypes) or indirectly (using allelic frequencies; Broquet & Petit 2009; Lowe & Allendorf 2010), and identified population structure provided information to a wide array of restoration activities. For example, information provided by gene flow studies have been used to define eradication units of invasive species with the aim of avoiding future recolonisations (Abdelkrim *et al.* 2010; Robertson & Gemmell 2004), to identify the principal barriers to gene flow to decide which sections of a river system should receive a higher restoration priority (Raeymaekers *et al.* 2008), or to determine levels of connectivity between streams to maximise resource outputs by using either a single stream or complete river system restoration approach (Cook *et al.* 2007). In another case, Balaguer *et al*. (2011) found no exclusive haplotypes or clear genetic structuring in their study of the tara tree (*Caesalpinia spinosa*), suggesting that this tree was introduced to a Peruvian archipelago by pre-Columbian cultures. This information gave crucial insights into the appropriate reference ecosystems to consider for ecosystem restoration. A further important resource offered by molecular-based genetics is the estimation of kinship. For example, parentage analyses have become crucial for inbreeding avoidance in the endangered Kootenai River white sturgeon (*Acipenser transmontanus*) aquaculture program (Schreier *et al.* 2012).

3.3.3 Evaluation and monitoring

Evaluation and monitoring are important sources of information for restoration management, each providing answers to different questions. Evaluation is often used at the end of projects and responds to questions like: did the project reach the set goals? Was the project successful? If not, what were the reasons? In turn, monitoring is often used to inform adaptive management strategies and is usually undertaken more frequently than evaluation. Monitoring responds to questions like: is the restoration on a desirable trajectory and within the expected timeframe? Are additional management interventions required?

Just under half the studies (42%) used genetic information to evaluate or monitor restoration interventions, of which 79% used neutral markers to explore possible associations between reduction in genetic diversity and fitness, although somewhat surprisingly none evaluated outbreeding depression, and 21% used genetics to evaluate demographic changes and gene flow. Arguably, the most appropriate method to evaluate outbreeding depression may be to compare hybrid fitness to that of the home parent through reciprocal transplantations, common garden experiments, or under controlled breeding designs (Edmands 2007). Although there is a need to perform more experimental work to evaluate outbreeding depression in other species besides plants (see Fraser *et al.* 2010), these experiments require long waiting periods and are of limited use for long-lived and endangered faunal species. On the other hand, molecular-based methods (Coulson *et al.* 1998) combined with fitness data potentially offer, in some circumstances, a cost and time effective alternative to evaluate outbreeding depression.

Typically, inbreeding depression and loss of genetic diversity in target species were concurrently determined. Several studies that monitored outputs of restoration projects recommended the need for additional management to maintain appropriate levels of gene flow (Mills & Allendorf 1996) and improve genetic diversity, using supplementation in plants (Sinclair *et al.* 2008) and animals (Ramey *et al.* 2000). For example, in a restoration project relying on spontaneous regeneration, it was found that a terrestrial orchid (*Dactylorhiza incarnata*) showed loss of neutral genetic diversity due to recurrent founding effects, although no relationship between neutral genetic diversity and individual fitness was found (Vandepitte *et al.* 2012). A decrease in neutral genetic diversity in restored populations, when compared to donor or reference populations, is a commonly reported finding. The reasons for these decreases encompassed inappropriate seed harvesting strategies (Burgarella *et al.* 2007), unreliable commercial seeds (Aavik *et al.* 2012; Fant *et al.* 2008; Gibbs *et al.* 2012), genetic bottlenecks in plant nurseries (Kettle *et al.* 2008) and founder effects due to recolonisation by few individuals (Hoban *et al.* 2012b; Vandepitte *et al.* 2012).

Efficient and unambiguous ways to measure restoration success are critical to improving ecological restoration outputs (Hobbs & Harris 2001). To this end, the availability of baseline data is essential to support the establishment of restoration targets. Especially important is the availability of baseline information to draw accurate conclusions from monitoring/evaluation programs, as demonstrated by a study on the recovery of bull trout (*Salvelinus confluentus*) populations following dam removal (DeHaan *et al.* 2011). Furthermore, several demographic studies used genetic methods to evaluate and monitor restoration, demonstrating the capabilities of genetic data to measure restoration success reliably. For example, long-term survival of out-planted abalone (*Haliotis kamtschatkana*; Read *et al.* 2012) and reproductive success in Pacific salmon (*Oncorhynchus* spp.; Baumsteiger *et al.* 2008) were assessed using parentage analyses and pedigree reconstruction (Blouin 2003; Jones *et al.* 2010). The use of assignment and clustering models (Manel *et al.* 2005) allowed an assessment of the function of an ecological corridor, revealing that corridor use and occupation differed between species and was neither symmetrical nor uniform (Paetkau *et al.* 2009). By using a landscape genetics approach, based on regression of least cost paths and genetic differentiation, it was possible to determine the best management prescription for facilitating gene flow after a volcanic eruption (Spear *et al.* 2010; Spear *et al.* 2012). Genetic data allowed these studies to draw well-founded conclusions based on quantifiable measures that would be otherwise difficult or impossible to obtain by traditional field methods.

3.3.4 Ecosystem services

While it may be appealing that genetics can aid in the enhancement of biodiversity (through restoration activities) while also increasing ecosystem services, the reality is that the relationship between biodiversity and ecosystem services is complex and not always positive (Benayas *et al.* 2009; Bullock *et al.* 2011). For example, efforts aimed to restore rare species may have smaller effects on ecosystem processes than those aimed on more common species (e.g.

Jain *et al.* 2014). Consequently, restoration projects may need to develop specific restoration objectives for biodiversity and ecosystem services separately.

Even though one of the most important benefits of ecological restoration is increasing ecosystem services (Nellemann & Corcoran 2010), and new insights indicate that there is a genetic basis influencing the provision of ecosystem services (Bailey 2011), we found relatively few studies that explored this relationship. While 12 studies mentioned the relationship between restoration and ecosystem services, only four suggested a relationship between genetics and ecosystem services and just two directly examined this relationship. In those two studies, Reynolds *et al*. (2012) found that a small increase in genetic diversity can improve restoration success, when measured by the provision of ecosystem services. Along the same lines, Ritchie & Krauss (2012) found genetic connectivity provided by pollinators maintained genetic diversity, seed germination and seedling performance of restored populations.

3.4 Research gaps, future directions and challenges

We argue that genetics should be considered a fundamental tool for planning, execution and monitoring of restoration projects, and research aimed to improve the applications of genetics to ecological restoration should be a priority. While we predict that the continuing advances and drop in prices of molecular techniques will further facilitate the use of genetics in this field, and identified several examples on how genetics informed the development of restoration management plans and supported the monitoring of their achievements (e.g. Aavik *et al.* 2012; Burgarella *et al.* 2007; Frankel 1974; Michalski & Durka 2012), we also argue that it is currently underutilised.

Furthermore, we noted that highly cited reviews on how a restoration project should be evaluated (Ruiz-Jaen & Aide 2005a, b; Wortley *et al.* 2013) did not consider any genetic aspects, suggesting that ecological restoration practitioners are overlooking the importance of incorporating genetics in their restoration goals. There are areas where more research is needed to better understand the role played by restoration genetics and how genetic data can be utilised to improve restoration outcomes. On the other hand, several genetic approaches and analytical techniques are already available to be applied in the field of restoration ecology, as demonstrated by the studies we found in our literature search. In some instances, genetic methods can provide important information that would be difficult to obtain otherwise. In others, they can be complementary to formal ecological methods. For example, connectivity estimations, past and present population trajectories (i.e. whether expanding or contracting; e.g. Beaumont 1999), migration rates, source and sink population identification – which inform whether the restored ecosystem is providing suitable conditions to sustain reproducing populations (Andreasen *et al.* 2012) – or the identification of the origin of individuals can readily assist restoration practitioners. We encourage managers and researchers to take full advantage of these techniques. With this in mind, we describe below the research directions and current genetic approaches that, in our opinion, should receive full attention in the near future.

Restoration ecologists are recognising the need to readjust restoration aims to face the challenges imposed by the emergence of novel ecosystems brought by climate change and other anthropogenic disturbances (Harris *et al.* 2006; Hobbs *et al.* 2009; Seastedt *et al.* 2008). It has been suggested that ecological restoration will be better suited for this challenge if management actions, in certain circumstances, focus on restoring ecosystem functioning and resilience, rather than on returning the ecosystem to a historic state (Heller & Hobbs 2014). Regardless of restoration aims, translocations will remain a fundamental tool for restoration ecologists, yet the genetic dynamics associated with translocation are only now being explored (e.g. Pacioni *et al.* 2013). The most recent research suggests the prevalence of outbreeding depression has been overestimated (Broadhurst *et al.* 2008; Frankham *et al.* 2011; Weeks *et al.* 2011) and local adaptation is less common in plants than generally assumed (Leimu & Fischer 2008). Other studies further suggest that, when outbreeding depression occurs, affected populations may recover in a few generations after natural selection removes maladapted genes (e.g. Erickson & Fenster 2006). However, further research is critical to determine and make clearer the generality and, arguably more importantly, the exceptions in applying genetic guidelines to different species, ecosystems and circumstances. We envisage that restoration genetics can play a key role in contributing to the development of better translocation guidelines. Research on minimum population sizes required to retain evolutionary potential (Willi *et al.* 2006), and linking these to restoration guidelines, will also be of critical importance. Although this review focused on neutral molecular markers, ideally, restoration genetic decisions should be based on a combination of neutral and quantitative genetic tools to decrease the risk of inbreeding and outbreeding depression.

Approaches that consider the genetics of multiple species could add useful insights into restoration in terms of community assemblages and ecosystem functioning, especially in the important early stages of restoration. Until now, the use of genetics for conservation and restoration purposes has been largely focused on single species. However, community genetics may provide one of the main research frameworks with which to expand theoretical concepts in restoration. A few community genetic studies suggest that genetic diversity in foundation species may influence ecosystem processes and how communities are structured (Whitham *et al.* 2006). For example, population genetic diversity in *Solidago altissima*, a dominant old-field plant species, determines arthropod diversity, community structure and ecosystem processes, such as aboveground net primary productivity (Crutsinger *et al.* 2006). Expanding this study to other species could provide important insights into how to improve restoration practices.

Simulation software in conservation genetics (Epperson *et al.* 2010; Hoban *et al.* 2012a) has been an important resource to test hypotheses and understand genetic responses under realistic conditions that would otherwise be difficult to infer empirically or experimentally. In turn, in restoration these programs remain underutilised, as does the development of specialised software for restoration purposes (but see McKenney *et al.* 1999). We encourage the use of these theoretical approaches because these can be highly informative as demonstrated by a recent study that used simulations to determine the best locations of restoration projects for maximising connectivity between patches (McRae *et al.* 2012). Computer simulations may also be useful for testing a number of hypotheses *in silico,* such as how the quality, size, spatial structure and configuration of restoration projects influence N_e, inbreeding and/or gene flow.

An overwhelming majority of restoration genetic studies were conducted in developed countries, highlighting the need for more work to be conducted in biodiverse developing countries, particularly those in the tropics. This could be achieved either by researchers from developed countries conducting their research in developing countries or by collaborating with colleagues based in these countries. One advantage of collaborations would be that they could contribute to develop professional expertise, provide funding opportunities and facilitate the upgrading of infrastructure in developing countries and reduce the current geographical bias of restoration genetic studies towards developed countries. This would have two further significant benefits. Firstly, the generalities of restoration genetic principles derived primarily from temperate zone ecosystems could be evaluated in tropical ecosystems. Secondly, as most biodiversity is contained within biodiverse tropical developing countries (Myers *et al.* 2000), conducting restoration genetic studies in those countries would improve overall restoration outcomes and increase the biodiversity benefits of restoration.

3.4.1 Application of new molecular techniques and analytical approaches

The use of molecular data to investigate past demographic fluctuations and connectivity, as well as to evaluate achievements of restoration projects, is an extremely useful, but currently underutilised, application of available genetic analytical methods. Especially when it is not possible to survey the ecosystem before anthropogenic alterations occur (possibly most of the time), these methods represent a suitable alternative to obtain baseline data. Additionally, the use of molecular markers with different mutation rates (that will accumulate genetic signals over different timeframes) and the use of ancient DNA techniques may complement this approach. In recent times there has been a dramatic improvement in the analytical approaches that are used to estimate the demography of a population and gene flow between populations. Amongst them, we argue that coalescent-based methods deserve special attention. Numerous statistical approaches and analytical packages are now available (e.g. Beaumont 1999; Cornuet *et al.* 2008; Drummond & Rambaut 2007; Kuhner 2006) that implement new models that allow analysis of multilocus and heterochronous data (e.g. Drummond *et al.* 2005; Heled & Drummond 2010), modelling of meta-population systems (Beaumont 2008b; Beerli & Felsenstein 1999, 2001) and offer a wide range of mutational models for fast mutating markers such microsatellites (Wu & Drummond 2011). Practitioners should note that the methods mentioned above estimate **effective population sizes** (N_e; Luikart *et al.* 2010; Schwartz *et al.* 2007) and it is important to consider that the ratio between N_e and actual population size (N) is highly variable among species and thus, N_e estimates should be treated as indicators rather than absolute numbers, and preferably compared within the same context and species. When the aim is the estimation of actual population size the collection of non-invasive genetic samples (e.g. hair or faeces; Beja-Pereira *et al.* 2009), in combination with capture-mark-recapture models, are possibly more efficient and economic than comparable field methods (Woods *et al.* 1999).

Landscape genetics (Storfer *et al*. 2007) is an approach that is highly informative in evaluating the effect of different environmental variables on population differentiation or genotype distribution, rather than the more simplistic, but more commonly used, geographical (linear) distance-based methods. We envisage that landscape applications could substantially contribute to the field of restoration ecology.

Among the various new molecular techniques, next-generation sequencing technologies (NGS) deserve particular attention. These technologies are solving some shortcomings of molecular applications in a number of ways. The faster and more affordable sequencing conducted using NGS is enabling the analysis of more samples and screening of a higher number of neutral loci (Abdelkrim *et al.* 2009; Williams *et al.* 2014), enabling concurrent research on larger numbers of species and increased coverage of the genome (e.g. Ekblom & Galindo 2011; Ouborg *et al.* 2010), as well as improvements in the quality of data from samples with low quantity and/or degraded DNA (i.e. invasive and ancient samples). The possibility of increasing the number of loci screened also has the secondary effect of facilitating the identification of those loci under selection (Vitalis *et al.* 2001; Williams *et al.* 2014), ultimately allowing the detection of local adaptation or lack thereof (Luikart *et al.* 2003). Moreover, NGS holds the potential to integrate the assessment of genetic diversity using neutral loci with the identification of adaptive and detrimental genes, and quantification of their genetic diversity, to help the decision making process. For instance, at the moment the use of neutral markers is the prevailing approach to delineate seed transfer zones, however, due to their neutrality, molecular markers may (Hufford *et al.* 2012) or may not (Sæther *et al.* 2007) reflect the same genetic patterns as traits under natural selection. The genomic era will shed light on the elusive endeavour of determining the actual mechanisms by which inbreeding and outbreeding depression influence fitness, and ultimately facilitate the prediction of their ecological and evolutionary consequences. In the meantime, NGS already has considerable application in the survey of species richness (e.g. DNA metabarcoding and metagenomics; Taberlet *et al.* 2012; Williams *et al.* 2014), one of the most utilised parameter for pre-restoration baseline assessment and an important measure of restoration success with regard to faunal populations (Ruiz-Jaen & Aide 2005a). This approach uses next-generation sequencing technologies to identify short DNA fragments present in environmental samples, such as soil and water (Williams *et al.* 2014), allowing restoration practitioners to carry out faster and more affordable biodiversity assessments of ecosystems than current field-based techniques. This approach has also allowed the identification of spatial patterns in response to environmental changes (e.g. ecotoxicology) and, more broadly, to investigate ecosystem-level processes (see Bohmann *et al.* 2014 for a review). Although significant methodological limitations and challenges remain with NGS, such as the high rate of incorrectly identified DNA bases in sequences and the challenge of processing and storing massive amount of sequence data (Williams *et al.* 2014), the many benefits of NGS, combined with continual reductions in the cost of NGS, will undoubtedly greatly increase the contribution that restoration genetics makes to ecological restoration.

3.5 Concluding remarks

We recommend that genetics is taken into consideration from the planning stage of restoration projects. Genetics can make an important contribution to obtaining baseline genetic data, which should improve the identification of restoration targets, and to evaluating restoration success, which is critical to improving ecological restoration outputs (Hobbs & Harris 2001). Currently, the genetics of restoration is contributing with novel approaches that are already broadening and improving research frameworks of both restoration ecology and conservation genetics. However, a further effort to direct, tailor and expand genetic concepts, tools and methods generated by conservation genetics and related research areas, to better inform and improve the practice of ecological restoration, will improve the efficiency of the effort made in this area.

The science and practice of ecological restoration, despite being a young field, has raised high expectations of our ability to reverse the loss of biodiversity and ecosystem services. It has even been argued that "our planet's future may depend on the maturation of the young discipline of ecological restoration" (Roberts *et al*. 2009). If ecological restoration is to meet these expectations, it must embrace a more holistic restoration approach: from plants to animals and from genes to ecosystems. Conceptual advances have been made in this regard, stating that restoration ecology and conservation biology are a subset of a broader enterprise: "intervention ecology" (Hobbs *et al*. 2011). Equally important for improving ecological restoration is the consolidation of the link between restoration and genetics. Realistically, decision making in restoration is based on incomplete knowledge (Rice & Emery 2003), as currently the implications of restoration on evolutionary processes remain poorly understood. Better understanding these implications, on which restored populations ultimately depend to adapt to current and future environmental variability, is perhaps the biggest challenge for restoration genetics.

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Author Contributions Box

All authors contributed equally to the writing of the paper.

Data accessibility

Results of the literature review conducted for this study are provided as Appendix 1.

4 Characterising the post-recolonisation of a small vertebrate and its genetic implications in a production forest landscape

Key words: *Antechinus flavipes*, ecological restoration, landscape connectivity, founder effect, mining.

Manuscript category: research paper.

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4.1 Abstract

Production landscapes, where activities such as timber harvesting, grazing and resource extraction take place, are taking up increasing parts of the Earth surface and have considerably reduced the extent of natural habitats. The ecological restoration of degraded parts of these production landscapes is, in many cases, the best remaining option to protect biodiversity. However, it is not clear whether dispersal, recolonisation and establishment patterns of faunal populations may diminish restoration genetic contributions. We employed core concepts in the field of population genetics to address questions of genetic diversity and gene flow in recolonising faunal populations using a small, vagile, semelparous marsupial (*Antechinus flavipes*) inhabiting a mined landscape under restoration, as a model. We did not detect a disruption of gene flow that led to genetic sub-structuring, suggesting adequate levels of gene flow across the landscape. Non-significant results from bottleneck tests probably indicate that restored areas provide enough resources to sustain several reproducing individuals. Parameters of neutral genetic diversity were high in both groups of individuals sampled in restored and in unmined sites and were not significantly different. Our results are encouraging as they indicate that ecological restoration of production landscapes has the potential to not just increase available habitat, but also to maintain genetic diversity in those landscapes. The integration of a genetic approach to restoration practices is helping to understand the full implications that these practices have on fauna inhabiting biodiversity-rich ecosystems.

4.2 Introduction

The conversion of natural landscapes into production landscapes, where activities such as agriculture, grazing, logging and mining take place, is the main cause driving biodiversity loss (Vitousek *et al.* 1997). For instance, it has been estimated that croplands and pastures alone occupy approximately 40% of Earth's surface (Foley *et al.* 2005). The ecological restoration of these production landscapes is emerging as a promising and effective activity to contribute to biodiversity conservation and the provision of ecosystem services (Benayas *et al.* 2009; Bullock *et al.* 2011). At the present, fauna restoration success is measured by estimating species richness and abundance (Ruiz-Jaen & Aide 2005b), however, these parameters may not represent accurately the genetic trends occurring in restored ecosystems. Therefore, whether restoration designed to avert biodiversity loss in restored ecosystems can also maintain and conserve genetic diversity in recolonising faunal populations is poorly understood.

Increasing attention has been put into the conservation of genetic diversity in natural populations, as it is the raw material upon which natural selection acts to bring about adaptive evolutionary change (Frankham *et al.* 2009). Its loss will: reduce the ability of populations to respond and adapt to long and short term environmental change (Burger & Lynch 1995); and reduce population fitness due to the exposure and accumulation of deleterious mutations and loss of heterozygosity in overdominant loci (i.e. inbreeding depression; Keller & Waller 2002).

Commonly, in empirical studies the level of gene flow is associated with landscape connectivity, defined as the degree to which the landscape facilitates or impedes movement between resource patches (Taylor *et al.* 1993). It is important to differentiate structural from functional connectivity: while areas of suitable habitat within a landscape might be structurally connected (e.g. by corridors) they might not be functionally connected, as the species under study might not be able to disperse or immigrate between structurally connected habitat. Due to genetic drift, the random sampling of alleles being transmitted from generation to generation, rare alleles are prone to disappear and common alleles to become fixed. While the effect of genetic drift occurring in large populations might be minimal, in small populations it could have serious negative consequences. Accordingly, ecological restoration may contribute in reducing loss of genetic diversity of faunal populations in two forms: 1) re-establishing landscape connectivity (Dixon *et al.* 2006) and therefore promoting gene flow; and 2) increasing the area of suitable habitat, with respect to what was left after the habitat was used for production, to sustain reproducing populations (Huxel & Hastings 1999) and therefore decreasing the negative effects of genetic drift. However, dispersal, recolonisation and establishment patterns, such as founder effects (e.g. Vandepitte *et al.* 2012), high-density blocking ("the process by which secondary dispersers arriving in an already colonised, densely occupied habitat fail to become established and reproduce" (Waters *et al.* 2013) or an unequal sex ratio (Allendorf *et al.* 2010) of recolonising individuals, might restrict these contributions.

To understand the implications of recolonisation patterns on the genetic diversity of faunal populations, we collected tissue samples of the small marsupial *Antechinus flavipes* from an ecosystem that has been extensively mined and subsequently restored. *Antechinus flavipes* (Yellow-footed Antechinus) is a small dasyurid marsupial (21-80 g) that displays semelparity, the complete mortality of males after mating every year, whereas approximately one third of females survive to breed a second year (Lada *et al.* 2008a). Their average home range has been estimated at 0.78 ha for females and 1.2 ha for males (Coates 1995) and they are relatively vagile, with individuals dispersing up to 720 m (mean=350 m; Marchesan & Carthew 2008). Additionally, *Antechinus* spp. have developed a series of inbreeding avoidance mechanisms whereby males disperse large distances after weaning, and females are philopatric (Lada *et al.* 2007), individuals avoid sharing nests with opposite-sex relatives (Banks *et al.* 2005b) and multiple paternity within litters is common (Kraaijeveld-Smit *et al.* 2002b).

Ideally, ecological restoration efforts should increase and improve landscape connectivity and natural habitat. However, we ask whether these ecological contributions may also entail genetic contributions; if so, individuals should be able to disperse or migrate between structurally connected habitat and several reproducing individuals should be able to successfully recolonise. Our study addressed the following questions:

a) Do restored mine sites provide functional landscape connectivity?
b) Are there genetic bottlenecks due to founder effects during the recolonisation process?

4.3 Methods

4.3.1 Fieldwork

Our study was conducted in the northern jarrah forest of south-western Australia, a multiple-use production landscape. The jarrah forest is a type of dry sclerophyll forest whose canopy consists almost entirely of jarrah (*Eucalyptus marginata*) and marri (*Corymbia calophylla*). The study area has a Mediterranean climate with hot, dry summers and cool, wet winters. Rainfall averages approximately 1240 mm/yr (Bureau of Meteorology; www.bom.gov.au), with more than 75% falling between May and September.

Antechinus flavipes individuals were trapped in three locations: Huntly, Dwellingup and Willowdale (Fig. 4.1). Huntly is a large bauxite mining site (32°36'S, 116°06'E), where mining/restoration activities have been performed since 1976. Since mining was undertaken from 1963 until 2006 of which 13,000 ha of mined areas have been restored (Koch 2007). Bauxite strip-mining is shallow (approx. 4–5 m) and takes place in pods of one to tens of hectares on the hillsides, but not in valley floors, swamps and streams, as they are alluvial and not bauxitic. As a consequence, when mining is complete, approximately 40–50% of the landscape has been mined and restored, leaving a mosaic of restored and unmined forest (Fig. 4.2; Koch 2007).

Restoration practices have evolved continuously, significant improvements have been in the areas of landscaping after mining, soil return methods, deep ripping to relieve compaction, selection of appropriate plant species for restoration, plant propagation methods (e.g. tree nursery and seeding with a mixture of between 78–113 trees) and techniques to encourage return of fauna through the return of logs, rocks and woody debris as fauna habitat (Koch 2007). Further restoration management prescriptions include fertilising, thinning and burning, control of invasive species (e.g. red fox, *Vulpes vulpes*) and extensive monitoring programs (Grant & Koch 2007). On the other hand, it is clear that the ecosystem will never be identical to the pre-mining state but a modified jarrah forest ecosystem. There are downsides that still require improvement or temporal solutions, such as streamflow reductions due vigorous vegetation growth, imbalance of resprouter versus reseeder plant species and the lack of old trees, tree hollows and rotting wood (Nichols & Grant 2007). These components will probably take 100 years or more to become available (Whitford 2002). For example, 5% of birds and 13% of reptile species in the jarrah forest have not been recorded in restored sites yet (Nichols & Grant 2007). These sophisticated restoration practices are regarded as a very successful restoration operation in general ecological terms (Koch & Hobbs 2007).

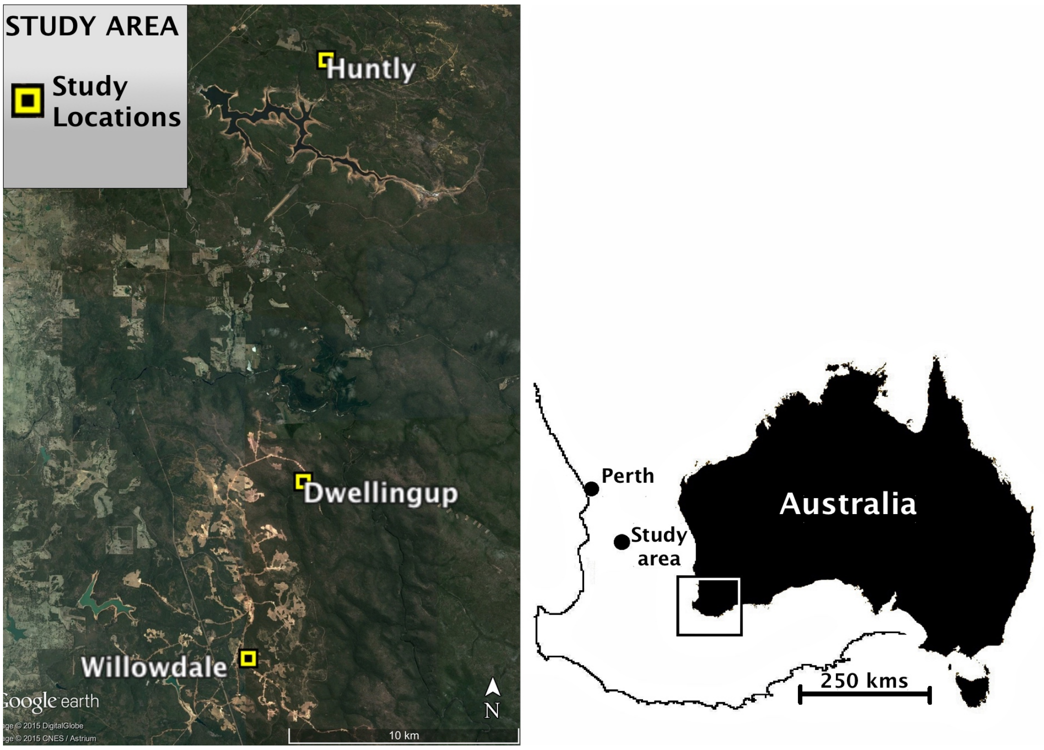


Figure 4.1 Location of studies areas (yellow squares) within Australia.

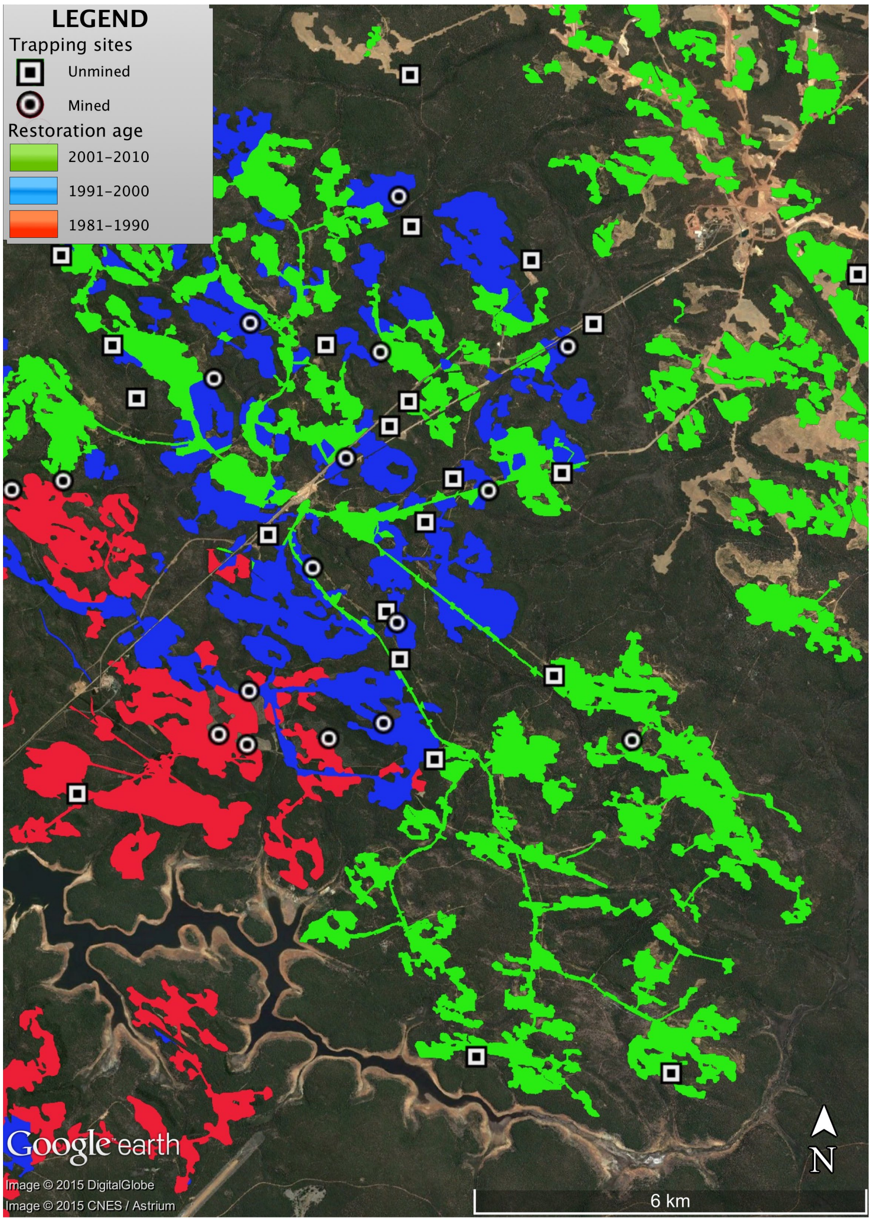


Figure 4.2 Location of trapping sites at Huntly (area= approximately 16,000 ha). Circles represent traps installed in mined/restored areas and squares those installed in unmined forest. Red colour represents areas that were restored between 1981-1990, blue between 1991-2000 and green between 2000-2010.

Due to a lack of a pristine and large enough area to serve as reference population, we set trapping grids at two surrounding disturbed locations (Dwellingup and Willowdale, approx. 15 and 20 km south of Huntly respectively), with the aim to compare our findings at Huntly to other common disturbances occurring in the jarrah forest. Trapping sites in these locations can be found in Fig. 4.3. Dwellingup is an area affected by the occurrence of *Phytophthora cinnamomi,* a soil-borne plant pathogen that kills many native plant species in the south-west of Western Australia (Shearer *et al.* 2004). Willowdale is surrounded by extensive mining (since 1984), and in addition it was infected by *P. cinnamomi* in 1980. From these locations we hypothesised that the more adverse environmental conditions at Dwellingup and Willowdale would derive into a lower genetic diversity of individuals inhabiting in these sites than individuals in Huntly.

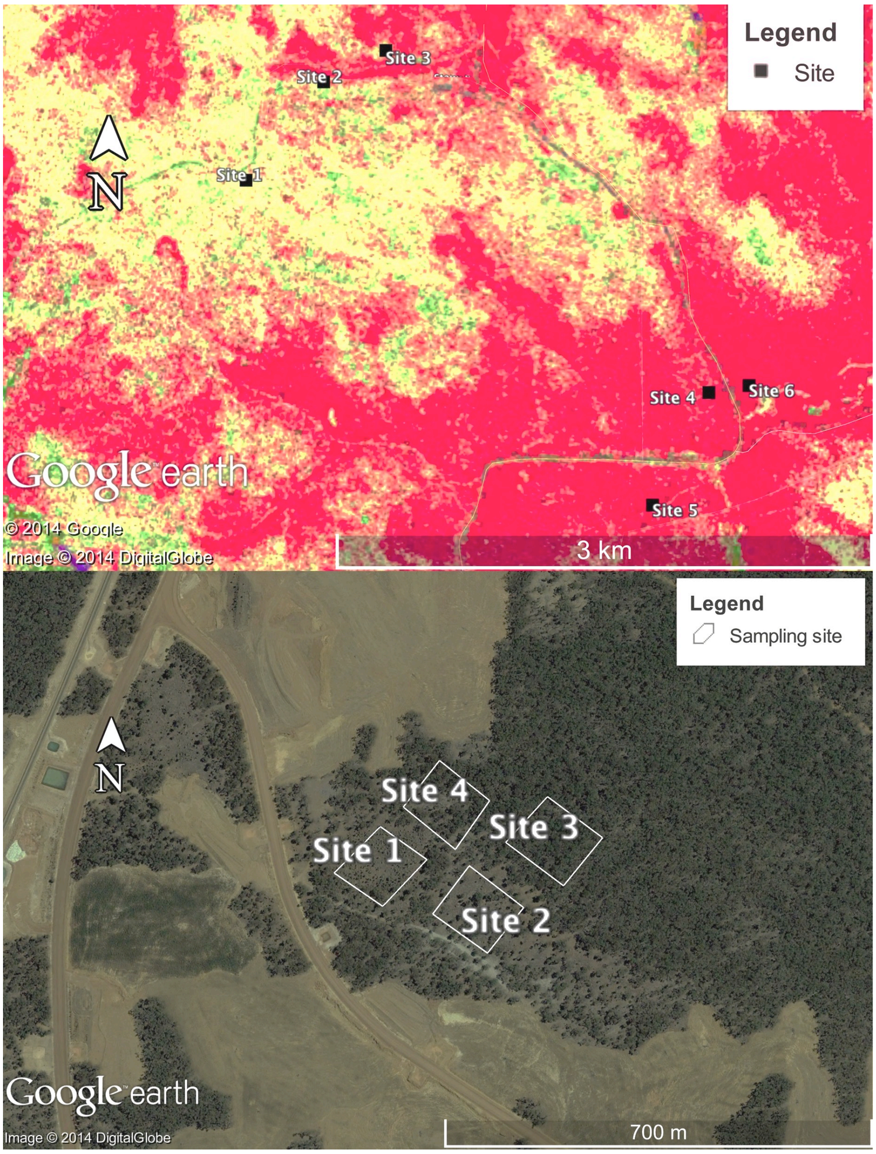


Figure 4.3 Location of trapping sites at Dwellingup and Willowdale. (Top) Dwellingup (15 km south of Huntly; yellow and green colours represent *P. cinnamomi* affected areas and red unaffected areas) and (Bottom) Willowdale (approximately 5 km south of Dwellingup; the site was suspected to be infected by *P. cinnamomi* in 1980).

At Huntly, trapping grids were randomly installed in unmined forest (n=22) and restored mined sites of different restoration ages and management prescriptions (n=17). The number of years that have passed since mined sites were restored ranged from 3 to 21 years. The mean distance between neighbouring trapping grids (1095 ± 134 m) was greater than both the home range size (a radius of approximately 56 m; Coates 1995) and average dispersal distance (approximately 350 m; Marchesan & Carthew 2008) of *A. flavipes*. All grids were more than 70 m from other habitat types to maximise the probability of trapping individuals whose home ranges were entirely, or largely, in the sampled habitat. Each grid consisted of pit, Elliott and cage traps following Craig *et al.* (2010; Fig. 4.4). Trapping sessions were performed from 2005 to 2012. Trapping grids were opened over four periods of two weeks each in spring, summer, autumn and winter, in every year except the winter in 2011 and 2012.

At Dwellingup trapping surveys were conducted from May 2002 to April 2004. Each survey was carried out over four consecutive nights, except during May-August 2002 (preliminary surveys), and December 2002 and November 2003, when only three nights were surveyed. Trapping surveys during October 2003 were conducted over two consecutive nights to reduce stress to adult females bearing pouch young. The trapping surveys scheduled for December 2003, February and March 2004 were cancelled due to inclement weather conditions. Trapping grids at Willowdale were installed and opened in 2002 from March to September. In both locations consisted of only Elliot traps. Ear tissue from trapped individuals was collected and placed into tubes containing salt-saturated 20% DMSO solution. The location of trapping grids was recorded using a GPS (Garmin International Inc., Olathe, Kansas, USA).

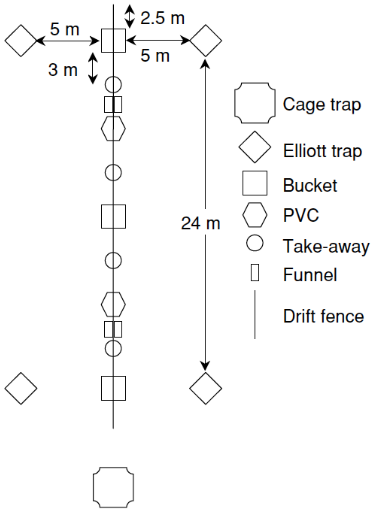


Figure 4.4 Schematic representation of trapping grids at Huntly. Reproduced from Craig *et al.* (2007).

4.3.2 Laboratory work

DNA was extracted with the DNeasy Blood & Tissue Kit (Qiagen). Sixteen microsatellites, initially developed for *A. agilis* as described in Banks *et al.* (2005a), were tested through the polymerase chain reaction (PCR), using a fluorescently M13 labelled 6-FAM primer (Schuelke 2000). Non-template and control samples were used in all PCR reactions. The PCR cycling conditions were as described in Banks *et al.* (2005a) with concentrations as follows: 100 ng of DNA template, 400 μM of each dNTP, 1.5-2 mM $MgCl_2$, 1X of reaction buffer, 5,000 ng of BSA (bovine serum albumin), 1-10 pmoles of forward and reverse unlabelled primers, 10 pmoles of 6-FAM-labelled primer, 0.25 U of *Taq* polymerase (Fisher Biotec) in a total reaction volume of 30 µL. For fragment analysis, 2 μL of the PCR products were combined with Hi-Di formamide (Applied Biosystems, Foster City, California) and 0.3 μL of Genescan LIZ-500 size standard (Applied Biosystems, Foster City, California) and separated by capillary electrophoresis on an ABI Prism 3737xl DNA Sequencer. Fragments were screened using the program GENEMARKER (v1.91, Soft Genetics LLC, State College, PA).

We also sequenced a 565-bp fragment of the mitochondrial control region (cytochrome *b* gene) from a total of 39 individuals from Huntly (n=13), Dwellingup (15) and Willowdale (11). Amplifications were performed using primers L15999M and H16498M (Fumagalli *et al.* 1997) using the conditions therein. PCR products were purified using QIAquick PCR purification kit (QIAGEN) as per manufacturer instructions. Sequences were aligned in Geneious v.6 (Biomatters, Auckland, New Zealand).

4.3.3. Data analyses

Firstly, to determine whether the difference in sex ratio between individuals (a factor reducing N_e) trapped at restored and unmined sites was significant we used a Pearson's chi-squared test contingency table using SPSS v21.

4.3.3.1 Genetic structure

We determined the number of populations occurring in the study area, using the whole dataset, by using a Bayesian clustering model implemented in STRUCTURE v2.3 (Hubisz *et al.* 2009). Bayesian clustering models assigns individuals to each simulated population, so that every subpopulation would be approximately at Hardy-Weinberg and linkage equilibriums between loci. We used the admixture model (Pritchard *et al.* 2000) and correlated allele frequencies (Falush *et al.* 2003) and set the number of populations (K) from one to eight, with twenty replications each and a burn-in period of 100,000 followed by 1,000,000 Markov Chain Monte Carlo (MCMC) iterations. To determine K, we used STRUCTURE HARVESTER (Earl & vonHoldt 2012) to inspect the mean loglikelihood averaged across the twenty replications and the second order statistic method described by Evanno *et al* (2005).

4.3.3.2 Genetic diversity

Deviations from Hardy-Weinberg Equilibrium (HWE), using all the samples, were verified through an exact test using GENEPOP 4.01. Tests of linkage disequilibrium were performed in FSTAT (Goudet 1995). The presence of genotyping errors was verified using Micro-checker (Van Oosterhout *et al.* 2004). Before pooling all samples collected at Huntly for genetic analyses, we first confirmed that each year cohort did not differ significantly from each other, by testing genic and genotypic differentiation for all pairs of cohorts in GENEPOP 4.01 (Rousset 2008) with 10,000 dememorisations, 1,000 batches and 5,000 iterations per batch.

To determine the levels of genetic diversity at the different trapping locations and between unmined and restored sites, we calculated various measures including, the mean number of alleles, fixation index (F), observed (H_o) and expected heterozygosity (H_e) using GENALEX 6 (Peakall & Smouse 2006). Private allelic richness and allelic richness were calculated with the software HP-RARE (Kalinowski 2005). Statistical differences between these genetic parameters were determined through Mann-Whitney U tests, and Wilcoxon tests between allelic richness, using SPSS v. 21. In addition, a number of genetic parameters were calculated at the individual level: proportion of heterozygous loci in an individual, standardised heterozygosity based on the mean observed and expected heterozygosity (Coltman *et al.* 1999), internal relatedness (Amos *et al.* 2001) and homozygosity by locus (Aparicio *et al.* 2006) with the aid of the R-function GENHET (Coulon 2010). We tested the correlation between these genetic parameters and three different categories according to the habitat type where individuals were sampled (Huntly: <15 and >15 years post-restoration and unmined forest; Dwellingup: severely affected, moderately affected and not affected) with a Spearman's Rho test using SPSS v. 21. We also calculated haplotype frequencies, haplotype diversity, and nucleotide diversity using ARLEQUIN 3.5 (Excoffier & Lischer 2010).

4.3.3.3 Bottleneck tests

When a population experiences a drastic reduction in its size, low frequency (rare) alleles are lost more rapidly than heterozygosity (*He*; Allendorf 1986). We investigated whether the effective population size had declined using three different methods. First, heterozygosity excess tests implemented in the program BOTTLENECK (Piry *et al.* 1999). It is assumed there will be a heterozygosity excess compared to the expected heterozygosity based on the number of alleles present in the population, under the assumption that the population is at mutation-drift equilibrium (H_{eq}; Cornuet & Luikart 1996). Statistical power of this test is dependent on the mutation model assumed and simulation settings (Williamson-Natesan 2005) and it is necessary to choose a trade-off between type I errors (detecting a bottleneck when there was none) and type II errors (not detecting a bottleneck when there was one; Williamson-Natesan 2005). Consequently, we used a range of different values (variance=12; proportion of step-wise mutation varying between 70-90%) that encompass realistic scenarios in vertebrates (Peery *et al.* 2012; Williamson-Natesan 2005). As a second approach we used the mean ratio (M-ratio) of the total number of alleles (k) to the overall range in allele size (r; M=k/r), which can be used to detect reduction in population sizes (when the ratio is below 0.68; Garza & Williamson 2001). We used the program M_P_Val to calculate M-ratios (Garza & Williamson 2001). Since the statistical power of the M-ratio method depends on the number of samples, mutation pattern and effective population size of the studied species, we used the program Critical M (Garza & Williamson 2001) to calculate a critical value (M-critical) that fits better to our dataset. As parameters of the simulations influence the statistical power of this test (similarly to the heterozygosity excess test, see above), we used the a range of different parameters: fraction of mutations that are larger than single steps=0.12, mean size for larger mutations=2.8 and a range of different pre-bottleneck values of Theta (1, 2, 4, 6, 8, and 10), based on a mutation rate (μ) of $5X10^{-4}$/locus/generation for a plausible range of N_e.

4.3.3.4 Landscape connectivity

To infer historic gene flow among the three sampling locations, we constructed a network of mtDNA sequence haplotypes using the median-joining algorithm (Bandelt *et al.* 1999) in Network 4·2 (Fluxus Technology Ltd). To examine the partitioning of genetic variation of mtDNA within and among the locations, we performed an AMOVA test (Excoffier *et al*., 1992), as implemented by ARLEQUIN 3.5 (Excoffier & Lischer 2010). Haplotype frequency and sequence divergence was used to calculate Φst (Excoffier *et al*., 1992)

To facilitate the interpretation of the effect of mining/restoration activities on landscape connectivity, we created a map to visualise the individual genetic distance between sampling sites. We first mapped the genetic distance between individuals using the genetic landscapes GIS Toolbox (Vandergast *et al.* 2011) in ARCGIS 10.1 (Environmental Systems Research Institute, Inc., Redlands, CA, USA). The GIS tool draws a network connecting all sampling locations to their nearest neighbours with non-overlapping edges. Next, genetic distance values (see below) are attached to the midpoints connecting each pair of connected locations. The genetic distances used were the residual values from a regression of geographic and genetic distances between sampling sites. We calculated the residuals using a reduced major axis regression, implemented in the software Isolation by Distance Web Service (Jensen *et al.* 2005). Residuals were used with the aim of removing the effects of simple distance on genetic divergence to reveal regions of unusually high or low divergence (Manni *et al.* 2004). The GIS tool then uses a spatial interpolation algorithm (inverse distance weighted interpolation; Watson & Philip 1985) to generate a continuous surface based on the mapped genetic distance values. Restored mine sites were categorised by decades (1981-89, 1990-99 and 2000-10). Subsequently, we visually examined this map in seek for a discernable pattern that could guide our hypotheses (Fig. 4.5).

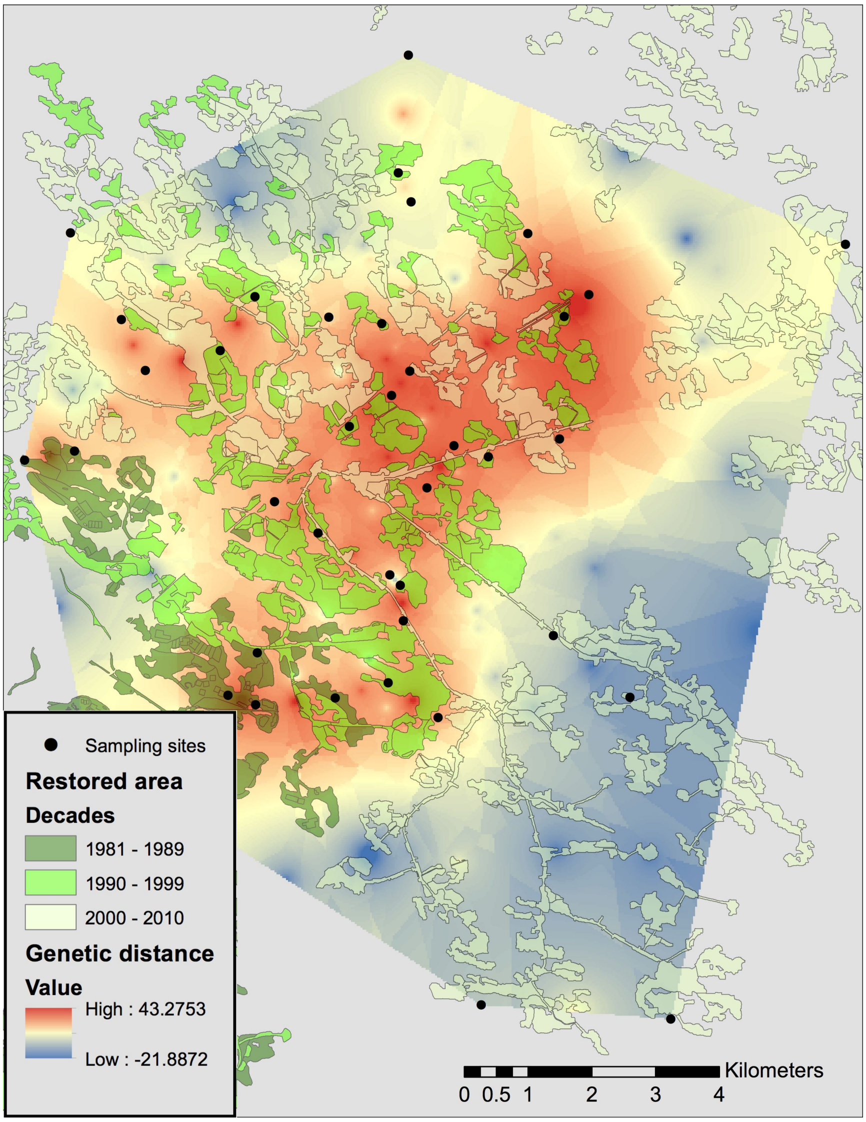


Figure 4.5 Interpolation of genetic distances between sampling sites. Genetic distances (residual values from a regression of geographic and genetic distances) between individuals were mapped. An inverse distance weighted interpolation was used to generate a surface from the mapped genetic distance values. Restored mine sites were categorised by decades (1981-89, 1990-99 and 2000-10).

We further hypothesised that if restored mine sites would act as barriers for gene flow we would find a significant correlation between the mean genetic distance between individuals and the proportion of surrounding area that has been mined/restored. We performed a Spearman's Rho test using SPSS v.21 to determine the correlation between the mean genetic distance between individuals (genetic distance/Euclidean distance) within a radius of 3 km for each sample and the proportion of mined area surrounding each sample in a radius of 1 km (Fig. 4.6). We used only samples with more than 4 comparisons.

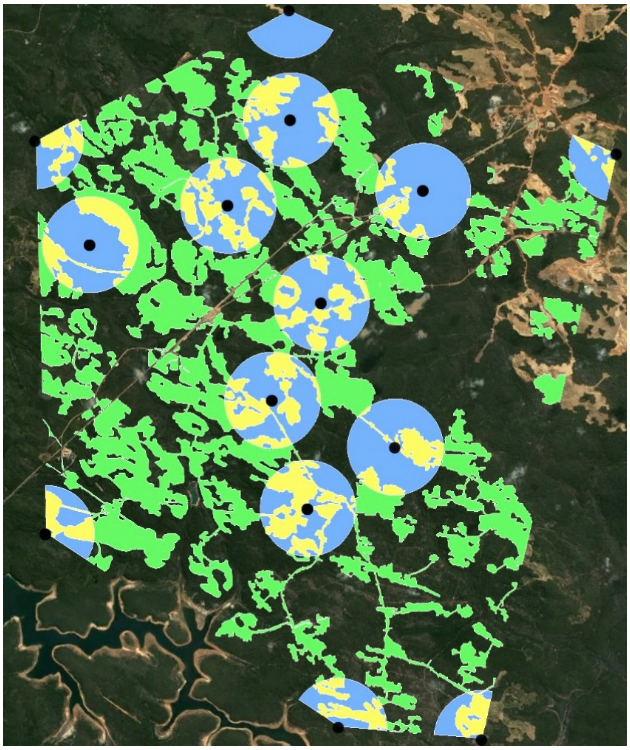


Figure 4.6 Representation of proportion of mined area surrounding trapping sites. Yellow colour represents the mined/restored area within a radio of 1 km from the trapping site.

4.4 Results

4.4.1 Fieldwork

We collected tissue samples from 122 individuals trapped between 2002 and 2012 in three locations. At Huntly, twenty-four individuals were sampled at restored sites (13 males and 11 females) and thirty-three at unmined sites (8 males and 25 females). In Huntly, the sex ratio was male biased at restored sites and female biased at unmined sites (P-value=0.0068, Table 4.1). At Dwellingup we sampled forty-two individuals and at Willowdale twenty-three individuals, there was no difference in sex ratio between affected and not affected *P. cinnamomi* areas at Dwellingup.

Table 4.1 Sex ratio of trapped individuals at Huntly mine site and Dwellingup, where the sites were categorised as mined and unmined and dieback affected and not affected, respectively. Number of trapped individuals differs from the number of individuals analysed because not all individuals were sampled.

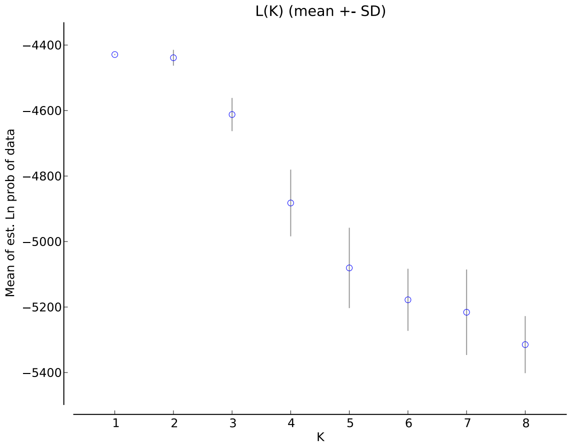
	Females	Males	Ratio (females:males)
Huntly			
Unmined	24	17	1.4:1
Restored	35	69	0.5:1
Dwellingup			
Severely affected[1]	4	6	0.7:1
Subtly affected[2]	8	14	0.6:1
Not affected	14	23	0.6:1
Willowdale	9	8	1.1:1

1. Subtly affected: infrequent and localised deaths of understory species, vegetation structure and canopy cover are moderately closed and leaf litter is thick but sparse.
2. Severely affected: plant species are unhealthy in all strata levels, vegetation structure and canopy cover are very open and leaf litter is very sparse.

4.4.2 Genetic structure

In STRUCTURE, K=1 and K=2 were almost equally likely (Fig. 4.7a) but the more parsimonious interpretation was K=1 (mean LnP(D)=-4429), while the second order statistic method indicated the existence of two populations (Fig. 4.7b). However, the latter does not allow us to evaluate if K=1 is more correct than K=2, so we visually examined estimated membership coefficients for each individual and in each run. We found that, when K=2, all individuals were symmetrically assigned among populations and concluded that K=1 was the most likely number of clusters.

a)



b)

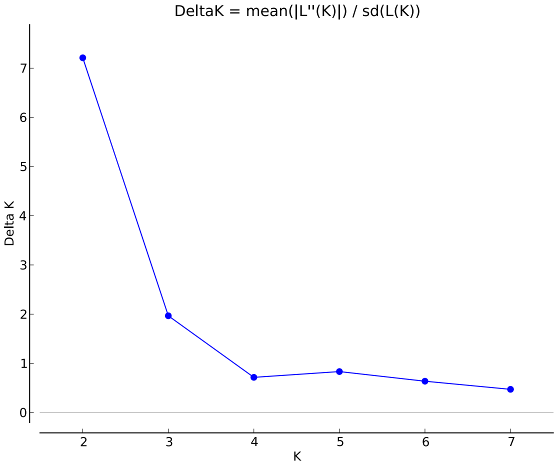


Figure 4.7 Graphics showing a) mean likelihoods across 20 replications indicating the presence of a single population; b) DeltaK values indicating the presence of two populations (K=2), however, all individuals were symmetrically assigned among populations suggesting K=1. Likelihood values were estimated with the program STRUCTURE (Pritchard *et al.* 2000) using the admixture model with correlated alleles frequencies and calculated following Evanno *et al*'s (2005) method.

4.4.3 Genetic diversity

We tested 16 microsatellite loci of which 11 successfully amplified a PCR product and were polymorphic. The loci 7H and 4A deviated from HWE (after sequential Bonferroni corrections) and showed null alleles, so they were removed from all analyses. At Huntly, the number of alleles per locus ranged from 6 to 16 with an average of 11 (±1.1 s.e.). The mean observed (0.848±0.022 s.e.) and expected (0.825±0.028 s.e.) heterozygosity over all the samples were relatively high (Table 4.2). None of the calculated parameters of gene diversity differed significantly between samples from unmined and restored sites ($P>0.05$, data not shown). None of the five individual genetic diversity parameters calculated was correlated with any habitat (Appendix 2), suggesting that environmental conditions are not an important factor influencing genetic diversity.

Eight mtDNA sequences were identified (Fig. 4.8, Tables 4.2 and 4.3). Haplotypes 5, 6, 7 and 8 were found just at Huntly, however three of these differ just by 1 bp and their frequency is small, suggesting that these haplotypes are recent mutations. Haplotype 4 was found just at Dwellingup. Haplotype 2 was shared between Dwellingup and Willowdale. Haplotypes 1 and 3, both with high frequencies, were found in all three locations and hence probably are the more ancestral. Overall, the network of mtDNA suggests that historic gene flow occurred across the three locations. Although, significant differences in the number of haplotypes, haplotype diversity and nucleotide diversity among the three locations (Table 4.2), these results are probably due to the different sizes of the area sampled at each location (i.e. Huntly 16,000 ha, Dwellingup 500 ha and Willowdale 10 ha). AMOVA test (Table 4.4) shows a high fixation index ($F_{st}=0.193$) and that partitioning of genetic variation is higher within locations (80.69%) than among locations (19.31%).

Table 4.2 Descriptive statistics of group of individuals sampled in the different trapping locations.

	All	**Huntly**	**Huntly unmined sites**	**Huntly restored sites**	**Dwellingup**	**Willowdale**
Microsatellites						
No. samples	122	57	33	24	42	23
Average no. alleles/locus	9.59 (0.637)	11.00 (1.106)	10.2 (0.9)	9.6 (1.0)	10.10 (1.148)	7.667 (0.816)
Effective no. alleles	6.11 (0.470)	6.730 (0.856)	6.3 (0.8)	6.1 (0.8)	6.491 (0.960)	5.108 (0.555)
Observed heterozygosity	0.813 (0.020)	0.848 (0.022)	0.837 (0.021)	0.822 (0.051)	0.778 (0.047)	0.814 (0.031)
Expected heterozygosity	0.807 (0.016)	0.832 (0.028)	0.829 (0.026)	0.817 (0.039)	0.820 (0.033)	0.803 (0.023)
Fixation index	-0.008 (0.017)	-0.032 (0.015)	-0.029 (0.022)	-0.025 (0.027)	0.046 (0.029)	-0.039 (0.035)
Private allelic richness	-	-	0.61	0.52	0.6	0.2
Allelic richness	-	-	9.14	9.11	8.66	7.35
mtDNA						
Sample size	39	13	10	3	15	11
Number of haplotypes	8	6	*	*	4	3
Haplotype diversity	0.772 (0.035)	0.821 (0.082)	*	*	0.667 (0.099)	0.473 (0.162)
Nucleotide diversity	0.015 (0.008)	0.019 (0.011)	*	*	0.015 (0.008)	0.007 (0.004)

Numbers in parentheses indicate standard error.

* MtDNA information for samples in unmined and restored sites was not calculated due to the difference in sample size.

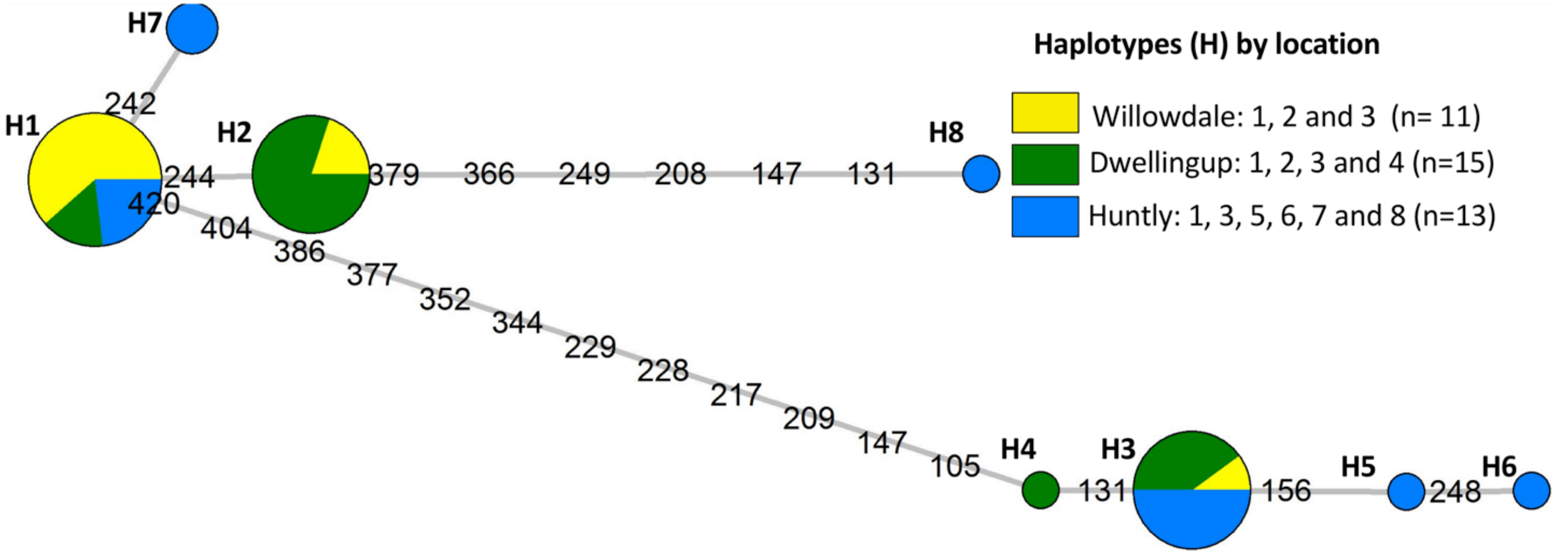


Figure 4.8 Network of mitochondrial DNA haplotypes of *A. flavipes* individuals trapped at Huntly, Dwellingup and Willowdale. Each pie represents one haplotype and its size is proportional to the number of individuals with that haplotype. Numbers along the links indicate the base pair positions where the mutation occurred.

Table 4.3 Distribution and frequency of the eight mtDNA Haplotypes found in three locations of *Antechinus flavipes*.

Haplotype	Huntly	Dwellingup	Willowdale
1	0.231	0.133	0.727
2	-	0.533	0.182
3	0.385	0.267	0.09
4	-	0.066	-
5	0.076	-	-
6	0.076	-	-
7	0.154	-	-
8	0.076	-	-

Table 4.4 Analysis of molecular variation showing the partitioning of genetic variation among and within locations (Huntly, Dwellingup and Willowdale).

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation
Among locations	2	2.713	0.07944	19.31
Within locations	36	11.953	0.33204	80.69
Total	38	14.667	0.41148	
Fixation index (FST)	0.193			

P-value<0.0001

4.4.4 Genetic bottlenecks

None of the heterozygosity excess tests were significant (data not shown). Similarly, all M-ratio and M-critical values were above 0.68 (a value below this number is an indication that a population has experienced a genetic bottleneck; Garza & Williamson 2001).

4.4.5 Correlation between mined area and genetic distance

The value of R was 0.20716 (P-value>0.05; Appendix 3) indicating that the association between mined/restored area and genetic distance is not statistically significant.

4.5 Discussion

The benefits and the potential limitations of restoration efforts to maintain genetic diversity of recolonising individuals have been rarely discussed in the scientific literature. Our results suggest that restoration practices have been effective in maintaining adequate levels of landscape connectivity. Likewise, they show that conditions in restored areas do not have a negative influence on neutral genetic diversity, probably because restored areas provide enough resources to sustain several reproducing individuals.

4.5.1 Sex ratio

A skewed sex ratio is a known factor reducing the effective population size (Allendorf *et al.* 2013). The sex ratio at restored sites was male biased (female:male ratio 0.5:1), whereas at unmined forest was female biased (female:male ratio 1.4:1). These results contradict a previous study, in the close related species *A. agilis*, where the sex ratio was male biased in unfragmented habitat and female biased in fragmented habitat (Banks *et al.* 2005a). In the latter study the male-biased sex ratio was believed to be an effect of a higher dispersal-associated mortality within the fragmented forest than in the unfragmented forest. In our case, the opposite trend we see in the sex ratio is probably because the males are responsible for most dispersal events and consequently constituting the majority of recolonisers migrating to the restored areas. Despite this sampling effect resulting in the skewed sex ratio described, we did not detect a reduction of genetic diversity.

4.5.2 Landscape connectivity

Patterns of dispersal, recolonisation and establishment in faunal populations are not random and highly complex. Dispersal may be influenced by several factors such as inbreeding risk, female abundance, patch size, patch quality and matrix permeability (e.g. Banks & Lindenmayer 2013). Specifically in the study area, *A. flavipes*, despite its relatively specific habitat requirements (Nichols & Grant 2007; Swinburn *et al.* 2007), has recolonised restored areas successfully (as soon as two years post-restoration; Nichols & Grant 2007), and its abundance in 12 and 17-year old restoration is the same or slightly higher than in unmined forest (Craig *et al.* 2012). Its relatively high vagility certainly plays an important role in *A. flavipes* recolonisation success. This trait has been documented by Lada *et al.* (2007), showing that gene flow between populations is not completely restricted by rivers. Nevertheless, other studies have shown that at finer scales, dispersal patterns can be influenced for example by roads (Burnett 1992) or by a plantation of a exotic species in a close related species (*A. agilis*; Banks *et al.* 2005a).

The availability of an undisturbed habitat would have been ideal to test landscape connectivity by directly comparing our data. Unfortunately, there are no pristine areas from where we could obtain *A. flavipes* samples. Therefore, we resolve to test the correlation between mined area and genetic distance in accordance with the general concept of the degree of isolation being proportional to genetic differentiation (Segelbacher *et al.* 2003); the failure of restoration goals can manifest itself with a fragmentation of suitable habitat throughout the study area, as a result, there would be isolated pockets of suitable habitat where small isolated populations reside. Under this scenario, we expected that individuals surrounded by a larger mined/restored area would be more genetically different from those individuals surrounded by a smaller mined/restored area. Our results did not support this hypothesis.

Likewise, the lack of genetic structure at the scale of our study supports the idea that even during the first years following restoration, restored areas do not represent dispersal barriers, at least at the short term. In contrast, AMOVA test using mtDNA haplotypes shows that partitioning of genetic variation is significantly higher within locations (80.69%) than among locations (19.31%). Although, mtDNA sequences may indicate the occurrence of barriers more back in time and detect genetic structure at a courser scale than microsatellites (Lada *et al*. 2008), our results do not support any of these scenarios. On the other hand, mtDNA is maternally inherited and is thus a better indicator of dispersal patterns in females than in males (Roffler *et al*. 2014), which is in accordance with the male-biased dispersal and female philopatry of the species. In this context, the major implication for restoration would be that the recolonisation and establishment process may be slower in this species as it requires that both sexes disperse (Arenas *et al*. 2014).

Taken together, these analyses suggest current restoration practices have been effective in maintaining gene flow across the landscape. Our data showed that landscape connectivity is not only at the structural, but also at the functional level.

4.5.3 Founder effect and genetic diversity

The occurrence of genetic bottlenecks following natural recolonisation within a restoration context has been documented in fungus (Aylward *et al.* 2015), plants (Hoban *et al.* 2012b; Vandepitte *et al.* 2012) and fish species (Marranca *et al.* 2015), but not yet in mammal species. An additional phenomenon that prolongs a genetic bottleneck may occur, if secondary recolonising individuals fail to establish and reproduce (i.e. high density blocking; Waters *et al.* 2013). A lack of a genetic bottleneck signal suggests that restored areas provide enough resources (e.g. carrying capacity) to sustain several reproducing individuals. Similarly, a non-significant correlation between the distribution of individual heterozygosity across different environmental conditions may suggest that conditions in restored areas do not have any negative influence on genetic diversity. However, we acknowledge that the sampling size might have reduced the statistical power of these tests.

We found high levels of neutral genetic diversity at Huntly, in agreement with previous studies using the same set of microsatellites in *Antechinus* spp. However, the gene diversity reported here is within the range of those populations inhabiting fragmented habitats (*He*=0.844; Banks *et al.* 2005a; and *He* =0.771-0.833; Lada *et al.* 2008b) and lower than those reported in continuous forests (*He*=0.860; Banks *et al.* 2005a; and *He*=0.886; Kraaijeveld-Smit *et al.* 2007). Lower genetic diversity could be also partially due to past contractions of the jarrah forest, which would have led to a significantly reduced population size. More specifically, we found that parameters of neutral genetic diversity were high in both groups of individuals sampled in restored and in unmined sites and were not significantly different. Although Dwellingup and Willowdale datasets were not as revealing as it was expected at the beginning of the study (*i.e.* they didn't show a lower diversity than Huntly and neither was possible to do the same tests done at Huntly, due to their low sample size and low number of trapping sites), these datasets serve to highlight the adaptability of *A. flavipes* to adverse conditions, probably mainly to its vagility and ecological traits to avoid inbreeding.

4.6 Conclusion

Our results are encouraging as they suggest that ecological restoration of production landscapes has the potential to not just increase available habitat, but also to maintain genetic diversity in those landscapes. However, restoration practitioners should bear in mind that incorporating genetic goals and success measures to the design of restoration projects is necessary to ensure their persistence in the long term. It is also important to recognise that further research is necessary to generalise the conclusions expressed here. We acknowledge that the small sample size of this study requires a cautious interpretation. We consider it is also necessary more studies to identify the genetic effects of restoration in recolonising individuals in other circumstances, such as different restoration conditions, at larger temporal scales and in other faunal and floral species.

This study exemplifies how restoration of mined sites, especially at great scale, may be used as a manipulative experiment to investigate and test genetic issues associated with restoration. Perform studies using larger sample sizes and studying species with other characteristics (e.g. specialists or slow-recolonising species) may provide additional insights about the genetic consequences of recolonisation within a restoration context. For example, recolonising individuals may not be a random subset of the individuals in source populations, but they may display differences in morphology, physiology, or behaviour (Hanski & Gaggiotti 2004), which would be under natural selection and represent other genetic implications. Similarly, other issues associated with recolonisation, such as genetic surfing (Excoffier & Ray 2008) and high-density blocking (Waters *et al.* 2013), deserve further research to understand the collateral genetic implications of restoration.

The use of genetic approaches in restoration science will help to achieve the ultimate goal of restoration ecology "to re-establish self-sustaining ecosystems that will resist future perturbation without additional human input" (Urbanska *et al.* 1997).

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5 General discussion

The ecological restoration of ecosystems that have been degraded, damaged, or destroyed, is one of the most transcendental activities that humankind may practice to reverse the current rate at which the three levels of biodiversity (i.e. genetic, species and ecosystem) are being depleted. Throughout this thesis, I argue that the building of a stronger bond between the field of genetics and the practice and science of restoration should be a high priority if restoration is going to accomplish its purpose. Restoration practitioners and scientists are becoming increasingly aware of the relevance of genetics in restoration. Nevertheless, it is still necessary to better understand how and where genetics may directly contribute to improving our ability to restore biodiversity. The aim of this thesis was directed to develop this understanding.

First, I discussed, from a population genetics perspective, the theoretical basis by which restoration may contribute to enhance genetic diversity of faunal populations (chapter 1). I then identified the main disciplines by which the field of genetics contributes to restoration and how these are linked to its practice. I also discussed the suitability of molecular markers, the main resource currently used in biodiversity conservation, to be used in a restoration context (chapter 2). In the next section, using a meta-analysis as an inference tool, I answered the following questions (chapter 3):

1. What is the relevance of using genetics in restoration?
2. How has genetics been used to inform ecological restoration?
3. What are the main trends in the genetics of restoration?
4. What are the current most important topics of genetic research in restoration?
5. What are the main research gaps, future directions and challenges?

If ecological restoration is going to be an effective response to the loss of biodiversity, not only must it recover ecosystems and species that have been lost, but also genetic diversity. However, there are no studies on the efficiency of restoration to restore or improve the genetic diversity of faunal species. In chapter 4, by using a small marsupial (*Antechinus flavipes*) as study species, I investigated whether ecological restoration is able to maintain adequate levels of gene flow by increasing landscape connectivity, and reducing genetic drift by increasing habitat to support reproducing populations. I also investigated the occurrence of founder effects and/or high-density blocking during the recolonisation of restored areas. I provided important insights to the following questions:

1. Do restored mine sites provide functional landscape connectivity?
2. Do genetic bottlenecks occur during the recolonisation process?

5.1 Major findings

Previous review articles in the genetics of restoration, have been plant focused and reviewed specific issues related to time (Rice & Emery 2003) and geographical (McKay *et al.* 2005) scales of local adaptation and the delineation of seed transfer zones (Hufford & Mazer 2003). In contrast, Chapter 3 offers an insightful and broad view about how genetics has contributed to restoration and how it has been used in restoration, in both plants and animals.

The use of genetics to inform ecological restoration is contributing to develop better restoration practices. By following genetic guidelines, restoration practitioners have been able to reduce negative genetic consequences derived from, for example, outbreeding and inbreeding depression. Genetics has also been useful to improve restoration in several issues: from monitoring and evaluating restoration projects to support pre-restoration decision-making processes.

We are far from completely understanding the real implications of restoration on micro evolutionary processes and, thus, decision-making in restoration is largely based on incomplete knowledge. Therefore, it is crucial to acknowledge how genetic factors may influence restoration outputs and understand the principles and consequences of genetic disruption resulting either directly or indirectly from restoration interventions.

The use of genetics in restoration is expanding, as 59% of the scientific papers found on this topic were published during the last three years. I found that these applications of genetics could be categorised in two main branches. Those focused on where genetic factors have a direct influence on population persistence and try to avoid/understand issues such as, outbreeding, inbreeding, maladaptation and loss of genetic diversity (i.e. conservation genetics). And secondly those focused on inferring population dynamics issues based on molecular markers (i.e. molecular ecology).

Overall, 42% of reviewed studies used genetic information to evaluate or monitor restoration and 58% provided genetic information to guide pre-restoration decision-making processes. Reviewed studies suggest that restoration practitioners often overlook the importance of including genetic aspects within their restoration goals. Even though there is a genetic basis influencing the provision of ecosystem services, few studies examined this relationship.

An important issue that has been reiterated in the restoration literature is the importance of evaluating restoration projects, and hence the need to set clear and measurable objectives, especially on some attributes that are difficult to measure, such as biotic flows and sustaining reproducing populations of species.

The use of genetic tools may have important contributions to monitoring and evaluating the efficacy of restoration and, ultimately, inform us on how to improve it.

The work performed in chapter three, will be useful to guide research in various topics. The meta-analysis detects important trends occurring in the genetics of restoration. The general trends I found may have several applications, for example: the analysis about how genetic research in restoration distributed across the world, might be useful to prioritise research efforts and economic resources; journal preference among restoration genetic studies, might help researchers to choose the best journal to publish their work. Further results may encourage restoration research to be less focused on plants and more on animals. I expect that this chapter will encourage both geneticists and ecologists to expand their research aims to the genetics of restoration.

One of the main goals of restoration is to restore biodiversity, which includes genetic diversity. However, there are no published studies investigating the efficiency of restoration to restore or improve the genetic diversity of faunal species. Chapter 4 is a first attempt to shed light on this matter, and raises the question that common measures of restoration success of faunal populations may not coincide with genetic goals.

The work done in this chapter illustrates and contributes to various issues expressed in chapter 3. By using a small marsupial as target species, this section contributes to restoration science by expanding research of faunal populations, a largely ignored component of restoration. In this chapter are also presented various ideas of how genetic methods may be used as measures of restoration success, monitoring and evaluation tools of faunal populations.

I did not find any indication of disruption of gene flow suggesting current restoration practices have not hindered the maintenance of adequate levels of gene flow across the landscape. I found high levels of neutral genetic diversity in both groups of individuals sampled in restored and in unmined sites and difference between them was not significant. These results suggest that ecological restoration of production landscapes has the potential to not just increase available habitat, but also to maintain genetic diversity in those landscapes. This study, to my knowledge, is the first to apply a genetic approach to study faunal successional and recolonisation processes within a mining/restoration context.

5.2 Future research

I predict in the near future the use of genetics in restoration will be as important tool as in conservation. At present, the most urgent topics of genetic research in restoration are the identification of the strength of local adaptation, the geographic scale over which this local adaptation occurs and predicting the restoration consequences on micro-evolutionary processes. Further to this, some potentially interesting research topics involving the use of genetics in restoration science should address:

1. Investigation of the genetic consequences of recolonisation (i.e. genetic surfing, founder effects, selection for dispersal behaviour).
2. Development of more specific genetic guidelines for restoration not just for different species (or other taxonomic ranks) but also for different ecosystems and circumstances.
3. Develop a closer link with community genetics based on evidence suggesting that genetic diversity in foundation species may influence ecosystem processes and how communities are structured.

4. Exploration of DNA metabarcoding to carry out faster and more affordable biodiversity assessments of complete restored ecosystems.
5. Explore the value of ecological restoration as a strategy to face the challenges brought by climate change, including its genetic implications.
6. How might adaptation be influenced by the new environmental conditions present in restored ecosystems?

5.3 Conclusion

Without a doubt the use of genetics in ecological restoration has developed its theoretical grounds, its practical principles and ultimately its final outputs. However, some questions such as, how and where genetics may directly contribute to improving our ability to restore ecosystems, have been not clearly approached. This thesis provides the basis to answer these important questions. A further contribution of this work is the presentation of insightful views about the main trends, research topics and gaps, future directions and challenges in the genetics of restoration, all of them key issues needed to further advance restoration efforts.

Ecological contributions of ecological restoration have been praised, however whether restoration practices are able also to contribute to restore genetic diversity of recolonising populations at short terms, has been overlooked. The results presented in this work indicate that restoration efforts have the potential to provide the suitable conditions to conserve genetic diversity (i.e. the restored habitat does not represent a dispersal barrier for faunal species and provide sufficient resources to support several reproducing individuals). However, it is important to acknowledge that improvement of genetic diversity of faunal or floral species through ecological restoration should not be taken for granted, but should be included in restoration goals.

If restored ecosystems are going to be resilient, self-sustaining and persist indefinitely, it will be crucial for restoration to better link its practice and science to genetic principles, but more importantly is the need to tailor and expand genetic concepts, tools and methods already developed by other fields, to answer the specific needs of restoration.

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Appendix 1

ıthor	Year	Species	Organism	Ecosystem	Country	Continent	Objective	Major finding/conclusion	Molecular marker	Application	Approach	Jo
ıpp	1996	*Elymus glaucus*	Plant/ herb	Terrestrial	USA	North America	Genetic structure to recommend transfer seed zones	High genetic differentiation. Seeds may need to be collected in close proximity to the proposed restoration site	Allozymes	Decision making	Genetic fitness	Res E
liams	1996	*Zostera marina*	Plant/ grass	Aquatic	USA	North America	Compare genetic diversity	Genetic diversity is less in transplanted beds	Allozymes	Evaluation/ monitoring	Genetic fitness	Es
enurm	1997	*Cordylanthus maritimus*	Plant/ flower	Aquatic	USA	North America	This study evaluated the genetic consequences of a reintroduction of the endangered annual plant	Rare allele frequency was lower than in the colonies from which seeds were collected at two of the three loci, and heterozygosity was reduced.	Enzyme	Evaluation/ monitoring	Genetic fitness	Res E
ıpp	1998	*Nassella pulchra*	Plant/ grass	Terrestrial	USA	North America	Isozyme and quantitative trait variation to recommend transfer seed zones	Quantitative traits are potentially better indicators of adaptation to regional and local environmental variation than isozymes for making recommendations about the spatial scales over which seed can be translocated	Isozyme quantitative traits	Decision making	Genetic fitness	Cons B
liams	1998	*Zostera marina*	Plant/ grass	Aquatic	USA	North America	Genetic diversity and differentiation	Transplanted plants were not reduced in genetic diversity. Natural populations exhibited very great genetic structure but transplanted beds not	Allozymes	Evaluation/ monitoring	Genetic fitness	Eco App
wn	2000	*Alosa sapidissima*	Animal/ fish	Aquatic	USA	North America	Assessment of genetic diversity	Significant genetic differentiation between donor and recipient populations	Microsatellites	Evaluation/ monitoring	Genetic fitness	Cons B
ney	2000	*Ovis canadensis*	Animal/ mammal	Terrestrial	USA	North America	Bottlenecks test and effective population size	Evidence of a severe bottleneck, small effective population size. Propose methods to mitigate deleterious long-term consequences. Discuss criteria for interventions	Allozymes and microsatellites	Evaluation/ monitoring	Genetic fitness	Res E
ulders	2000	*Cirsium dissectum* and *Succisa pratensis*	Plant/ flower	Terrestrial	Netherlands	Europe	To study genetic diversity to asses differentiation using neutral genetic markers.	Most of the genetic variation was found within populations. Small but significant genetic differences were found among populations	AFLP	Evaluation/ monitoring	Genetic fitness	F Eco Man
son	2001	*Nassella pulchra*	Plant/ grass	Terrestrial	United States	North America	Elucidate mode of reproduction and nucleotide variation among 11 natural populations and three selected natural germplasm.	Inbreeding, combined with founder effects and /or selection, has contributed to the differentiation of populations. Foundation seed populations of the selected natural germplasm releases were genetically well defined and most similar to natural seed collected near the corresponding source populations. Commercial germplasm sources will be made practically available and useful for conservation plantings within the intended areas of utilization.	AFLP	Decision making	Demographic study	Mo E
son	2001	*Poa secunda* and *Poa fendleriana*	Plant/ grass	Terrestrial	United States	North America	Analyze genetic variation within and among populations.	Successful characterization of naturally diverse germplasm sources that may be used for large-scale revegetation efforts.	AFLP	Decision making	Genetic fitness	Crop
caccini	2001	*Posidonia oceanica*	Plant/ seagrass	Aquatic	Italy	Europe	The role of genetic variability of donor beds in establishing transplantation criteria	Shoots growing and branching at the highest rate were those collected from populations with the highest heterozygosity values.	Microsatellites	Decision making	Genetic fitness	Res E
liams	2001	*Zostera marina*	Plant/ grass	Aquatic	USA	North America	Where and how genetic diversity is lost during eelgrass transplantation. Exploration of associations between genetic diversity and both vegetative propagation and sexual reproduction to evaluate the importance of genetic diversity for short-term population growth.	Genetic diversity contributes to eelgrass population viability even over the short term	Allozyme and quantitative traits	Decision making	Genetic fitness	Res E
ıng	2001	*Metrosideros excelsa*	Plant/ tree	Terrestrial	New Zealand	Australia	Assess genetic variation and interpopulation structure	Results suggest that revegetation programmes should source seed locally and avoid taking material from small populations in which	Allozime	Decision making	Genetic fitness	New Jou

								selfed seed may make up a significant portion of the seed crop.				B
vko	2001	*Leucopogon obtectus*	Plant	Terrestrial	Australia	Australia	Examine genetic diversity within and among all known populations for conservation.	The species exhibits high levels of genetic diversity despite small population sizes. Results suggest that this species comprises a single, genetically diverse group. Conservation and management should concentrate on maintaining the high levels of genetic variability through mixing genotypes and promoting outcrossing.	AFLP and RAPD	Decision making	Genetic fitness	Mo E
tafson	2002	*Dalea purpurea*	Plant/ leguminose	Terrestrial	USA	North America	Genetic diversity and performance between restored vs remnant	Our data indicate that using multiple local seed sources for restoration projects will maintain the local gene pool while enhancing the regional genetic diversity of this species	RAPD and allozyme	Decision making	Genetic fitness	Inter Jou Sc
ttner	2002	*Hemigenia exilis*	Plant/ schrub	Terrestrial	Australia	Australia	Delineate the origin and extent of seed collection for rehabilitation and level and partitioning of genetic variation and differentiation and to identify the impact of removing one population due to mining	Seed should be collected from a range of habitats of several populations, while keeping the two provenances separate. This strategy is likely to maintain high genetic diversity and locally adapted populations	RAPD	Decision making	Genetic fitness	Bi Con
vis	2002	*Spartina alterniflora*	Plant/ grass	Aquatic	USA	North America	Assessment of Genetic Diversity	Restored populations maintain levels of genetic diversity comparable to natural populations even higher. Suggesting significant rates of outcrossing. Small genetic population differentiation with IBD	AFLP	Evaluation/ monitoring	Genetic fitness	Ec App
pedes	2003	*Swietenia macrophylla*	Plant/ tree	Terrestrial	Costa Rica	Latin America	Levels of genetic diversity	There are high levels of gene flow and genetic diversity was not reduced, genetic differentiation between populations was low	Microsatellites	Evaluation/ monitoring	Genetic fitness	Mo E
oung	2003	*Odocoileus virginianus*	Animal/ mammal	Terrestrial	USA	North America	To assess genetic differentiation and diversity	There was significant genetic differentiation among all populations and the majority of genetic variation (≥ 93%) was contained within populations. No IBD. Evidence of bottlenecks. However genetic diversity was high. The recovery program was successful.	Microsatellites	Evaluation/ monitoring	Genetic fitness	Mo E
eeler	2003	*Eucalyptus marginata*	Plant/ tree	Terrestrial	Australia	Australia	Investigate genetic diversity	The lack of genetic structure and low population differentiation indicates that collection of germplasm for rehabilitation of forest sites following mining or *Phythophthora cinnamomi* infection, may be made at a regional scale.	RFLP	Decision making	Genetic fitness	S G
kesley	2004	*Castanopsis acuminatissima*	Plant/ tree	Terrestrial	Thailand	Asia	Genetic diversity and the selection of seed trees for forest restoration	Two algorithms were designed to select seed trees based on their individual genotype and to randomly select seed trees from a population of unknown genetic makeup. There is no differentiation between the populations	Microsatellites	Decision making	Genetic fitness	New
	2004	*Oryza rufipogon*	Plant/ leguminose	Terrestrial	China	Asia	The aim of this study was to elucidate the population genetic structure as an indicator of the species' evolutionary history and its future prognosis and thereby provide valuable data for the development of conservation management plans.	High degree of inbreeding in the species and severe subdivision within populations	Microsatellites	Decision making	Genetic fitness	Pl
tafson	2004	*Andropogon gerardii* and *Sorghastrum nutans*	Plant/ grass	Terrestrial	USA	North America	Genetic diversity and genetic relationships	Genetic diversity is much less of an issue in these perennial outcrossing autopolyploid grasses than genetic differences among local and non-local or cultivar seed sources and genetic differences and ecological performance among local and non-local seed sources are more of a concern than genetic diversity. Translocating non-local seed in order to increase diversity, or using cultivars, is likely to alter the genetic structure of remnant populations and potentially influence the associated community and affect ecosystem structure and function in unforeseen ways.	RAPD	Evaluation/ monitoring	Genetic fitness	Jo A E
uss	2004	*Lechenaultia biloba, Bossiaea*	Plant/ schrub	Terrestrial	Australia	Australia	Rapid delineation of genetic provenance by utilizing minimal sampling and a multivariate spatial autocorrelation	We demonstrate a new approach for the rapid delivery of genetic provenance delineation for native plant community restoration, and provide recommendations for seed collection zones for each of four	AFLP	Decision making	Genetic fitness	Jou N Con

		ornata, Lomandra hermaphrodita and *Dryandra lindleyana*					analysis	species. We discuss the limitations of the approach.				(
e	2004	*Salvelinus namaycush*	Animal/ fish	Aquatic	North America	North America	How genetic diversity is partitioned among remnant wild lake trout populations. We estimated measures of genetic diversity and the apportionment of genetic variance	Information on the relative contributions of different broodstocks to total gene diversity within the regional hatchery program can be used to prioritize the broodstocks to be retained and to guide future stocking strategies	Microsatellites	Decision making	Genetic fitness	Tra An Fi S
ertson	2004	*Rattus norvegicus*	Animal/ mammal	Terrestrial	South Georgia (UK)	Australia	Defining eradication units to control invasive pests. Identify gene flow between two isolated rat populations so populations could be eradicated with low risk of recolonization	The molecular definition of eradication units is a valuable approach to management	Microsatellites	Decision making	Demographic study	Jou A E
	2005	*Bromus ciliatus L.*	Plant/ grass	Terrestrial	Canada	North America	Assess the genetic diversity	The AFLP variability observed was significantly associated with the geographic origins of the fringed brome populations.	AFLP	Decision making	Genetic fitness	Ca Jou B
	2005	*Oryza rufipogon*	Plant/ leguminose	Terrestrial	China	Asia	(i) Assess the extent and patterns of microsatellite variability within and among the Chinese populations of *O. officinalis*; (ii) Determine evolutionary factors that may affect the observed population structure;	High genetic differentiation, limited gene flow, inbreeding	Microsatellites	Decision making	Genetic fitness	Mo E
and ang	2005	*Oryza officinalis*	Plant/ leguminose	Terrestrial	China	Asia	(1) Define the extent and pattern of microsatellite variability among Chinese populations of *O. rufipogon* and *O. officinalis*; (2) Determine evolutionary factors that may affect their differences in population genetic structure;	*O. officinalis* has slightly higher departures from Hardy–Weinberg expectations and heterozygosity deficits than *O. rufipogon*	Microsatellites	Decision making	Genetic fitness	Bio Con
vuer	2005	*Liatris scariosa*	Plant/ herbaceous/	Terrestrial	USA	North America	Population differentiation and genetic variation to inform translocation decisions	GST was relatively high. QST for leaf shape exceeded GST, indicating local adaptation, while QST for other traits fell within or below the GST range	Allozymes and quantitative traits	Decision making	Genetic fitness	Bi Con
ta	2005	*Miscanthus sinensis*	Plant/ grass	Terrestrial	Japan	Asia	Develop recommendations for the revegetation of devastated sites	The aspect ratios of leaf-blades varied significantly among populations, but both geographical proximity and morphological similarities did not precisely reflect genetic similarities	AFLP and cpDNA	Decision making	Genetic fitness	Ec R
o	2005	*Andropogon gerardii*	Plant/ grass	Terrestrial	USA	North America	Examine the potential for gene flow from large, recently introduced populations to small local populations	We found no significant differences in the genetic diversity or composition between the two groups (local vs. CRP). In summary, we found that local and introduced populations of Big bluestem have the potential to interbreed, based on their flowering periods,	RAPD	Decision making	Genetic fitness	Res E
ng	2005	*Haloxylon ammodendron*	Plant/ tree	Terrestrial	China	Asia	Genetic variation within and between nine populations	High levels of gene flow and The genetic structure also has important implications in ecological restoration practice. An overwhelming proportion of the variability is present within populations suggests that smaller numbers of populations will be required for effective conservation compared with island endemics with many, strongly isolated populations.	ISSR	Decision making	Genetic fitness	An B
bur	2005	*Argopecten irradians*	Animal/ invertebrate	Aquatic	USA	North America	Genetic monitoring program	Genetic evaluation of restoration efforts provides critical information about the efficacy of various strategies for supplementation and enhances our understanding of how we can best manage troubled fisheries	MtDNA sequence	Evaluation/ monitoring	Demographic study	Con G
lon	2006	*Salmo salar*	Animal/	Aquatic	USA	North	Genetic diversity	We found no genetic differences between the two populations. No	Microsatellites	Evaluation/	Genetic	Ices

			fish			America		genetic bottlenecks had occurred during the restoration program		monitoring	fitness	of S
adhurs	2006	*Acacia acinacea*	Plant/ schrub	Terrestrial	Australia	Australia	Investigate levels of genetic diversity and adaptively significant variation present across the species range	No one technique (morphology, fitness, ploidy level and AFLPs) was able to fully describe the variation present in A. acinacea and seed sourcing guidelines based on any single approach could have resulted in erroneous decisions being made.	AFLP	Decision making	Genetic fitness	Cons G
sel	2006	15 species	Plant/ various	Terrestrial	Australia	Australia	Assess genetic differentiation between potential seed source populations and the target population.	Species can be classified into three broad provenance classes – narrow, local and regional – to help guide decisions about appropriate seed-collection zones	AFLP	Decision making	Genetic fitness	*A* *E*
nsen	2006	*Salmo trutta*	Animal/ fish	Aquatic	Denmark	Europe	How populations could be restored after admixture has occurred. Identifying remaining no admixed individuals to be used for supportive breeding	This study demonstrates the feasibility of restoring populations that have been admixed with exogenous individuals, by identifying non-admixed individuals using genetic markers	Microsatellites	Decision making	Demographic study	Jo A E
ta	2006	*Alnus sieboldiana* and *Polygonum cuspidatum*	Plant/ grass	Terrestrial	Japan	Asia	Make preliminary recommendations for native species to use in the revegetation of a volcanically devastated area	Findings indicate the need to assess the genetic diversity empirically by using organelle and nuclear markers to avoid unintended consequences of genetic mixing associated with revegetation	AFLP and cpDNA	Decision making	Genetic fitness	Cons G
uss	2006	*Acacia rostellifera* and *A. cochlearis*	Plant/ leguminose	Terrestrial	Australia	Australia	Rapid genetic identification of local provenance seed collection zones for ecological restoration	Very high levels of genetic differentiation among populations. We suggest a narrow seed collection zone should be applied to these species for the conservation of genetic diversity	AFLP	Decision making	Genetic fitness	Jo A E
np	2006	*Lasthenia conjugens*	Plant/ flower	Terrestrial /aquatic	USA	North America	Genetic monitoring of both natural and restored populations can provide an assessment of restoration protocol success in establishing populations that maintain levels of genetic diversity similar to those in natural populations.	Genetic diversity remained constant across the three sampled generations.	ISSR	Evaluation/ monitoring	Genetic fitness	Cons G
garella	2007	*Quercus ilex*	Plant/ tree	Terrestrial	Italy	Europe	Empirically assess the effect of actual seed sampling strategy on genetic diversity	Significant reduction in genetic diversity and significant difference in genetic composition of the seedling lots compared to the seed origin stand were detected explained by an inappropriate forestry seed harvest strategy limited to a small number of spatially close trees.	Microsatellites	Evaluation/ monitoring	Genetic fitness	An F S
hman	2007	*Hedysarum boreale*	Plant/ legume	Terrestrial	United States	North America	Characterized the genetic relationships to maintain a broad genetic base within selected germplasm populations	Seventy percent of the total genetic variation was found within all 17 accessions, yet each accession showed significant isolation by distance. Two groups of metapopulations were identified	AFLP	Decision making	Genetic fitness	Crop
ok	2007	*Nannoperca australis*	Animal/ fish	Aquatic	Australia	Australia	Population connectivity (and isolation) and genetic diversity	Protecting and augmenting refugial habitat of extant populations and restoring corridor functions between refugia within streams will generate the greatest biological response at within-stream scales	MtDNA and microsatellitess	Decision making	Demographic study	Bio Cons
rson	2007	*Castanea dentata*	Plant/ tree	Terrestrial	USA	North America	Asses founder effect	No genetic differentiation between natural and restored populations. No loos genetic diversity	Minisatellites	Evaluation/ monitoring	Genetic fitness	Res E
n	2007	*Spartina alterniflora*	Plant/ grass	Aquatic	USA	North America	1) Identify multiple genotypes of *S. alterniflora* for use in restoration efforts and 2) Assess genetic variability of these genotypes at the molecular level.	Molecular marker analysis revealed that genetic diversity, essential for success in the restoration projects, was maintained in selected plants. These plants offer an enhanced germplasm base for current restoration efforts.	AFLP	Evaluation/ monitoring	Genetic fitness	Jo Aqua Man
tt	2007	*Rudbeckia fulgida*	Plant/ flower	Terrestrial	USA	North America	Assessment of genetic diversity	High levels of genetic diversity, little genetic differentiation between the cultivar and wild populations until more research these populations should be kept separate to prevent gene flow and maintain genetic diversity of wild populations	RAPD	Decision making	Genetic fitness	Tran of th Aca S
ugi	2007	*Nymphoides peltata*	Plant/ flower	Aquatic	Japan	Asia	Estimate the potential of soil seed banks for genetic restoration of the species,	Showed high inbreeding coefficients, suggesting that the seed banks were produced by inbreeding. Thus, although the seed banks had a certain potential to restore genetic diversity, the fitness reduction in	Microsatellites	Evaluation/ monitoring	Genetic fitness	Cons G

								seed banks caused by inbreeding could affect the success of restoration based on seed banks				
son	2007	*Sander vitreus*	Animal/ fish	Aquatic	USA	North America	Assess the success and relative contributions of past rehabilitation stocking and to investigate the relationship between historical and contemporary populations	Walleye restoration efforts in Lake Superior are working, and highlight the utility of and options for adaptive management approaches for restoring extirpated populations.	Microsatellites	Evaluation/ monitoring	Demographic study	Jou Gre Re
an	2008	*Baptisia leucantha, Asclepias incarnata, Coreopsis tripteris,* and Zizia aurea	Plant/ flower	Terrestrial	USA	North America	Test for loss of genetic variation in restored plant populations by comparing them with TNC's seed source nursery and with local remnant populations that were the source of nursery seed and of the first few restored sites.	Seed collection techniques used at Kankakee Sands captured the great majority of allozyme variation present in seed source remnant populations	Allozymes	Evaluation/ monitoring	Genetic fitness	Res E
eld	2008	*Epipactis palustris*	Plant/ flower	Terrestrial	Germany	Europe	Genetic structure and diversity	High within population variability and a moderate genetic differentiation among *E. palustris* populations. We found no differences between genetic diversity values of populations from former mining areas and those of natural habitats. No founder effects	RAPD and miRPF	Evaluation/ monitoring	Genetic fitness	Bio Con
t	2008	*Ammophila breviligulata*	Plant/ grass	Terrestrial	USA	North America	Assess genetic similarity between well-established and new native populations, restored populations, and restoration propagules obtained from two commercial suppliers.	Restorations derived from these nursery stocks were composed of genotypes that did not match local populations or had limited genetic diversity. In addition, native populations were more diverse than expected given the propensity of this species toward vegetative reproduction. Restoration projects should consider obtaining plants from multiple local suppliers or from neighboring *A. breviligulata* stands to include a diversity of local genotypes. This study points to the importance of obtaining baseline genetic surveys of remnant native populations and restoration propagules before restoration efforts are initiated, especially when the populations are threatened or endangered.	ISSR	Evaluation/ monitoring	Genetic fitness	Res E
ward	2008	*Phragmites australis*	Plant/ grass	Aquatic	USA	North America	Identification and incidence and growth of an introduced lineage	There is a high potential for the Eurasian *P. australis* lineage to displace the gulf coast lineage	AFLP and chloroplast DNA	Decision making	Demographic study	Bio Inv
tle	2008	*Araucaria nemorosa*	Plant/ tree	Terrestrial	New Caledonia, France	Australia	Evidence for genetic bottlenecks and elevated inbreeding	Extreme genetic bottleneck was detected in a nursery, the establishment of nursery stock for this endangered conifer is sampling established wild seedlings directly from the forest floor and rearing these on in nurseries.	Microsatellites	Evaluation/ monitoring	Genetic fitness	Bio Con
	2008	*Cyclobalanopsis myrsinaefolia*	Plant/ tree	Terrestrial	China	Asia	We compared genetic variation of the restored populations with the natural ones	Genetic variation were found to be lower, but not significantly, in the restored populations than they were in the natural population indicating a founder effect. High gene flow among populations. However, no deleterious consequences might be observed in restored populations due to high observed heterozygosity and high gene flow.	Microsatellites	Evaluation/ monitoring	Genetic fitness	Plan
ymaek :	2008	*Gasterosteus aculeatus*	Animal/ fish	Aquatic	Belgium	Europe	Detect the geographical determinants of genetic connectivity and population structure,	Anthropogenic structures came out as the strongest determinant of population structure. River restoration and conservation genetics may highly benefit from riverine landscape genetics, including model building, the detection of outlier populations, and a specific test for the geographical factors controlling the balance between gene flow and genetic drift	Microsatellites	Decision making	Demographic study	Evol App
clair	2008	*Conospermum triplinervium*	Plant/ tree	Terrestrial	Australia	Australia	Assess patterns of morphological and genetic variation	High level of population structure and low levels of genetic diversity in the smallest population and identification of the more desirable population for replanting and supplementation	AFLP	Evaluation/ monitoring	Genetic fitness	Au Jou B

ulders	2008	*Populus nigra*	Plant/ tree	Terrestrial	Europe	Europe	We analysed the structure of genetic diversity in relation to geography and river management.	Genetic variation was present within populations, no genetic differentiation along river but not between rivers. Gene flow and dispersal takes place across fairly large distances and between river catchments.	AFLP and microsatellites	Decision making	Demographic study	Plan
rnton	2008	*Jacquemontia reclinata*	Plant/ flower	Terrestrial	USA	North America	Assess the genetic structure and diversity. The primary goal of our project was to use molecular data to recommend management plans to contribute to the recovery of the species. We specifically aim to provide recommendations regarding appropriate propagation stock for use in reintroduction or augmentation activities.	High levels of polymorphic loci (94.7%) were found and larger populations had greater genetic diversity. One population was very differentiated and recommend the use of two management units.	RAPD	Decision making	Genetic fitness	Bic
ning	2009	*Purshia tridentata*	Plant/ schrub	Terrestrial	United States	North America	Analyze the range-wide genetic diversity to develop seed movement guidelines.	Moderate amount of genetic differentia- tion (FST=0.09, p < 0.001)	Microsatellite	Decision making	Genetic fitness	Jou Envi
a- brera	2009	*Lupinus elegans*	Plant/ leguminose	Terrestrial	Mexico	Latin America	Determined the genetic variation	Higher genetic variation within populations. Seed collection for restoration purposes should be done from the closest (geographical) population possible. Common garden experiment, suggests that populations might be highly adapted to local conditions	RAPD	Decision making	Genetic fitness	R Fit Me
tkau	2009	*Rattus fuscipes* and *Rattus leucopus*	Animal/ mammal	Terrestrial	Australia	Australia	Analysis to monitoring the re-establishment of connections between previously isolated populations.	The combination of movement and capture records allowed species-specific assessment of corridor function, revealing that the use and occupation of the corridor was higher for Bush Rat than for Cape York Rat and was neither symmetrical nor uniform.	Microsatellites	Evaluation/ monitoring	Demographic study	Ecc Man Res
ymaek	2009	*Gasterosteus aculeatus*	Animal/ fish	Aquatic	Belgium	Europe	We assessed the genetic structure of a barrier-sensitive species, to estimate the impact of fragmentation on a local to catchment scale	Dispersal was lower in above- than in below-mill populations, and water mills provoked an average loss of almost 4% of the genetic variation. This loss accumulated to 40% over the entire system. Simulations suggest that reassessment of stickleback genetic structure after a decade should reveal whether or not restoration actions have been effective.	Microsatellites	Evaluation/ monitoring	Demographic study	Jou A Ec
eman	2009	*Salvelinus namaycush*	Animal/ fish	Aquatic	USA	North America	This study examined the ancestry	Both pure strain and inter-strain hybrids were observed, and the majority of fish were classified as Seneca Lake strain or Seneca Lake hybrid. Stocked fish are successfully reproducing in Lake Huron indicating a level of restoration success.	Microsatellites	Evaluation/ monitoring	Demographic study	Jou Gre Re
arer	2009	*Acropora palmata, Montastraea cavernosa, Montastraea faveolata* and *Pocillopora damicornis*	Animal/ invertebrate	Aquatic	N/A	N/A	Assess the minimum number of donor colonies required to retain specific proportions of the genetic diversity of the population	Using 10 donor colonies randomly sampled from the original population would retain >50% of the allelic diversity, while 35 colonies would retain >90% of the original diversity. Scleractinian coral populations are genetically diverse and restoration methods utilizing few clonal genotypes to re-populate a reef will diminish the genetic integrity of the population.	Microsatellites	Decision making	Genetic fitness	Cor
clair	2009	*Daviesia divaricata*	Plant/ flower	Terrestrial	Australia	Australia	Sample size effects on estimates of population genetic structure	High levels of genetic diversity and genetic differentiation among populations. Recommend using a minimum of 30 plants for sourcing seed for restoration projects.	AFLP	Decision making	Genetic fitness	Aus Jou B
lelkrim	2010	*Rattus rattus*	Animal/ mammal	Terrestrial	New Zealand	Australia	Fine-scale genetic structure of mainland invasivepopulations to help conservation managers optimize control programs	Presence of a contiguous population with IBD pattern. Give management recommendations based on genetic data	Microsatellites	Decision making	Demographic study	Cons Ge

geletti	2010	*Aphanius fasciatus*	Animal/ fish	Aquatic	Italy	Europe	Effective population size and genetic diversity	Genetic variability of the killifish strongly reduced through time. Recommend translocation of fish from other populations to re-establish genetic variability.	Allozymes	Decision making	Genetic fitness	Ge
ttarai	2010	*Dalea ornata*	Plant/ legume	Terrestrial	United States	North America	Understanding the genetic and ecotypic variability for developing populations suitable for revegetation purposes.	Positive correlations were observed between phenotypic and genetic distance matrices (r 5 0.33, P 5 0.005), phenotypic and geographic distance matrices (r 5 0.35, P 5 0.002), and genetic and geographic distance matrices (r 5 0.31, P 5 0.009). It is recommended that two germplasm sources of western prairie clover be developed for use across the collection area.	AFLP	Decision making	Genetic fitness	Ran Eco Man
hman	2010	*Astragalus filipes*	Plant/ legume	Terrestrial	Canada/United States	North America	Determine which collections should be used to constitute a conservation unit or regional seed source.	Although there was significant correlation between genetic and linear geographic distance matrices, there was no correlation between genetic and phenotypic, elevation, temperature, or precipitation distance matrices.Results show that geographic distance contributes to genetic differentiation.	AFLP	Decision making	Genetic fitness	B
npanel	2010	*Zostera marina*	Plant/ grass	Aquatic	USA	North America	Determine whether the genetic stock origins of the plants used in management projects may affect restoration success. We assessed the genetic diversity and genetic structure	Populations are not genetically diverse, and there is also little divergence among populations. A low degree of outcrossing, evidence of historical bottlenecks. Two populations show high effective population size and therefore the more appropriate to use in restoration projects	Microsatellites	Decision making	Genetic fitness	Pop E
ard	2010	*Cervus elaphus*	Animal/ mammal	Terrestrial	USA	North America	We quantified genetic variability	Demographic factors maintaining positive population growth rates, increasing population size, and maintaining population stability, are strategies that will favor the retention of genetic variability in re-established populations. Continued monitoring of genetic variability in restored populations will elucidate the effectiveness of current management regimes on the genetic health of wildlife populations	Microsatellites	Evaluation/ monitoring	Genetic fitness	Res E
kmann	2010	*Zostera noltii*	Plant/ grass	Aquatic	Spain	Europe	An assignment test, using microsatellite data, was used to locate the most likely population of origin from a set of possible donor populations.	Allows locating the most closely related stands that may be the most successful donor populations for future restoration based on shoot or seed transplantation.	Microsatellites	Decision making	Demographic study	M B
zalo- rpin	2010	*Festuca eskia*	Plant/ grass	Terrestrial	Europe	Europe	We assessed population genetic differentiation and diversity patterns in the species including endophyte occurrence along altitudinal and longitudinal gradients	Molecular markers separated *F. eskia* into two East and West groups, and endophyte infection and genetic patterns were congruent with molecular data. Little evidence for genetic differentiation or difference in endophyte occurrence associated with altitude was detected. Little variation was found in within population diversity, regardless of provenance altitude and site, and/or endophyte infection frequency. The results of this study suggested the establishment of two distinct management units for *F. eskia* seed sourcing restoration	SSR and STS	Decision making	Genetic fitness	Cons Ge
quemy	2010	*Cirsium acaule*	Plant/ grass	Terrestrial	Belgium	Europe	Genetic diversity and structure	Both allelic richness and genetic diversity were significantly and positively related to population size. Estimation of observed and expected gene diversity provided evidence for population bottlenecks, low migration rates. Overall, our results indicate that in the absence of gene flow strong decreases in calcareous grassland area may have long lasting effects on genetic diversity of plant populations and may hamper the success of restoration projects that simply aim at restoring initial habitat conditions or enlarging population fragments, as indicated by the fact that none of the recently restored areas has been occupied by *C. acaule*	Microsatellites	Evaluation/ monitoring	Genetic fitness	Cons Ge
ias	2010	*Ostrea edulis*	Animal/ invertebrate	Aquatic	Europe	Europe	Genetic diversity, effective population size and pedigree reconstruction	Hatchery-produced populations from small numbers of broodstock showed a significant loss of genetic diversity relative to wild populations and small effective population size. Pond-cultured oysters were intermediate in genetic diversity and effective population size between hatchery and wild populations. Controlled hatchery	Microsatellites	Evaluation/ monitoring	Genetic fitness	Cons Ge

								production allows the development of bonamiosis-resistant strains, but at the expense of genetic diversity. Large scale pond culture on the other hand can provide a good level of genetic diversity. A mixture of these two approaches is required to ensure a healthy and sustainable restoration program for *O. edulis* in Europe.				
laval	2010	*Trifolium alpinum*, *Festuca eskia*, *Festuca gautieri* and *Rumex scutatus*	Plant/ various	Terrestrial	France	Europe	Definition of evolutionarily significant units	The delineation allowed integration of infraspecific plant variation in the management of natural resources for revegetation. Nevertheless, caution is needed for the establishment of seed pools in order to maximize genetic diversity in each of the pools during collection and production.	RAPD	Decision making	Genetic fitness	Ap Veg S
laval	2010	*Trifolium alpinum*, *Festuca eskia*, *Festuca gautieri* and *Rumex scutatus*	Plant/ herb	Terrestrial	France	Europe	Can genetic tools combined with phytogeography help to define local plants and how geographically close the source population should be to the restoration site?	Definition of two main evolutionarily significant units (esus) for the plants under study.	RAPD	Decision making	Genetic fitness	A Veg S
ry	2010	*Spartina alterniflora*	Plant/ grass	Aquatic	USA	North America	Genetic population structure	Small amount of genetic structure. It would be prudent to collect widely within the target marsh. We also recommend the practice of propagating plugs of *S. alterniflora* from wild seed, as opposed to using vegetative cuttings, when creating planting stock, in order to maximize genetic diversity in restored marshes.	Microsatellites	Decision making	Genetic fitness	W
ewell	2010	*Eucalyptus leucoxylon*	Plant/ tree	Terrestrial	Australia	Australia	Genetic structure and evaluate paddock trees as seed sources for revegetation	There was no genetic differentiation between paddock trees and remnant vegetation across our survey sites. These results suggest that paddock trees represent a potential source of seed to contribute to genetically diverse woodland restoration plantings,	Microsatellites	Decision making	Genetic fitness	Res E
hmach	2010	*Populus nigra*	Plant/ tree	Terrestrial	Germany	Europe	Genetic diversity and spatial genetic structure. Paternity analysis of seedlings as well as juveniles from a restricted area of natural regeneration was used to quantify pollen- and seed-mediated gene flow	Spatial genetic patterns of the adult tree population suggest small-scale isolation by distance due to short-distance gene flow, the major part of which (i.e. 70%) takes place within distances of less than 1 km.	Microsatellites	Decision making	Demographic study	Cons G
clair	2010	*Alexgeorgea nitens*	Plant/ flower	Terrestrial	Australia	Australia	Population genetic structure and variation	High levels of genetic diversity within populations, and surprisingly low levels of population differentiation. A best guess based in life traits of trees is not always the best option to choose source seed populations	AFLP	Decision making	Genetic fitness	Res E
vis	2010	*Spartina alterniflora*	Plant/ grass	Aquatic	USA	North America	Accurately predicting performance requires an understanding of a variety of underlying processes, particularly gene flow and selection, which can be measured, at least in part, using surrogates such as neutral marker genetic distances and simple latitudinal effects. Predicting performance for ecological restoration.	We suggest that dispersal distance and latitude should provide an adequate means of predicting performance in future *S. alterniflora* restorations and propose a maximum sampling distance of 300 km (holding latitude constant) to avoid the sampling of inappropriate ecotypes.	AFLP	Decision making	Genetic fitness	Res E
ng	2010	*Picea asperata*	Plant/ tree	Terrestrial	China	Asia	Compare genetic diversity	Mature cohorts in the intact stands exhibited the highest levels of genetic diversity. High genetic similarity and low genetic differentiation between progeny cohorts in the post-harvest stands and mature cohorts in the intact stands indicated a sufficient seed or	ISSR	Evaluation/ monitoring	Genetic fitness	Fo

							pollen migration from the surrounding intact stands into the regenerated area. These findings illustrate the important role of the mature cohorts in the intact stands as reservoirs of genetic diversity.				
	2010	*Stipa grandis*	Plant/ grass	Terrestrial	Mongolia/China	Asia	We wanted to know whether there were differences in genetic diversity among these populations and how genetic diversity varied among populations with different durations of fencing	Genetic diversity of the fenced populations was greater compared with the overgrazed population. There was a significant variation among the populations. Largest genetic diversity was in the moderately grazed population. The genetic diversity in the overgrazed population might be increased to some extent through fencing,	AFLP	Evaluation/ monitoring	Genetic fitness	Ra J
aguer	2011	*Caesalpinia spinosa*	Plant/ tree	Terrestrial	Peru	Latin America	Genetic diversity and genetic structure.	Nor clear geographical structuring of the genetic diversity. The importance of the present study lies in the demonstration, from a biological perspective, of the relevance of a holistic approach to ecological restoration in an oasis setting, but, obviously, deeper genetic and ecophysiological studies are needed for a further understanding of tara behaviour in the Peruvian loma fragments and in lomas undergoing experimental restoration.	Chloroplast sequence	Decision making	Demographic study	Pl
adhurs	2011	*Allocasuarina verticillata*	Plant/ tree	Terrestrial	Australia	Australia	Examine genetic diversity and population genetic structure to locate appropriate seed collection areas	Smaller populations produced seed crops with significantly fewer alleles, lower allelic richness and less gene diversity. No genetic differentiation.	Microsatellites	Decision making	Genetic fitness	Au Jou B
Haan	2011	*Salvelinus confluentus*	Animal/ fish	Aquatic	USA	North America	To inform restoration planning, we investigated levels of genetic variation, population assignments	There were greater levels of variation and gene flow in coastal watersheds compared to populations isolated by dams. Study population was highly differentiated. Despite long-term fragmentation, there was no significant genetic variation among Elwha bull trout separated by the dams. Baseline data from this study will be useful for monitoring bull trout recovery following dam removal.	Microsatellites	Evaluation/ monitoring	Genetic fitness	No S
ffiths	2011	*Salmo salar*	Animal/ fish	Aquatic	England	Europe	Assignment test	(I) previous causes of declines must be sufficiently ameliorated to allow new/translocated individuals to thrive, (ii) introduced individuals should originate from a stock that is closely related to the extirpated population, according to the principles of contemporary conservation biology, and (iii) dispersal and gene-flow from neighbouring populations may play a significant role in establishing new populations. This identifies the potential for natural recolonisation of rivers where salmon have become locally extirpated, whilst simultaneously highlighting the futility of long-term stocking without corresponding improvements in river navigability, habitat and water quality. In this sense, conservation strategies for salmon, as for most other organisms, should seek to restore ecosystem function and continuity, not apex species and their immediate habitat (Lawton et al., 2010), whereupon it seems likely that populations of salmon will re-establish naturally	Microsatellites	Decision making	Demographic study	Bio Cons
rgens	2011	*Rosa inodora Fries, Rosa sherardii Davies* and *Rosa subcollina*	Plant/ flower	Terrestrial	Germany	Europe	Genetic structure to delineate provenances for seed collection. UPGMA dendrograms and NMDS	Due to predominantly high values of genetic differentiation and spatial patterns of ordination, we suggest small provenance regions for endangered Rosa species for seed collection.	RAPD	Decision making	Genetic fitness	Plan
	2011	*Pinus monticola*	Plant/ tree	Terrestrial	USA	North America	To assess the genetic diversity and structure among populations	Genetic diversity and structure revealed by this study will aid recognition and selection of western white pine populations for species management and conservation programs.	AFLP	Decision making	Genetic fitness	Tree and
yd	2011	*Vallisneria americana*	Plant/ grass	Aquatic	USA	North America	We examined patterns of genetic diversity to look for seed zones	The majority of populations have high genotypic diversity and are not highly inbred. Substantial geographic structuring of genetic diversity suggests that caution should be used in moving propagules to locations distant from their source	Microsatellites	Decision making	Genetic fitness	Cons G

loney	2011	*Pinus lambertiana*	Plant/ tree	Terrestrial	USA	North America	Genetic diversity and genetic structure	Greater genetic drift, possibly due to a bottleneck caused by historical logging. Possible management strategies include restoring population numbers, deploying wpbrresistance, treating stands to promote natural sugar pine regeneration, and enhancing genetic diversity	SNPs	Decision making	Genetic fitness	F Eco Man
well	2011	*Acacia pinguifolia*	Plant/ schrub	Terrestrial	Australia	Australia	Investigate the effectiveness of prescribed burning to improve genetic diversity	Genetic diversity was marginally higher in the post-fire seedling cohort than the pre-fire adults	AFLP	Evaluation/ monitoring	Genetic fitness	Cons G
sons	2011	*Bottlebrush squirreltail*	Plant/ grass	Terrestrial	USA	North America	Genetic variation was assessed and correlations were calculated among phenotypic, genetic, environmental, and geographic distance matrices with the use of Mantel tests.	Environmental heterogeneity and isolation by distance are shaping ecotypic divergence driven by natural selection. Provide preliminary guidance to future research toward establishing seed-transfer guidelines.	AFLP	Decision making	Demographi c study	Ra Eco Man
sler	2011	*Cygnus buccinator*	Animal/ bird	Terrestrial	USA	North America	We assessed the consequences of this reintroduction program by comparing patterns of genetic variation at four restoration flocks (three wild-released, one captive) and their source populations.	Restorations from multiple versus single source populations may better preserve natural levels of genetic diversity	Microsatellites	Evaluation/ monitoring	Genetic fitness	Cons G
a	2011	*Juniperus brevifolia*	Plant/ tree	Terrestrial	Portugal	Europe	To evaluate genetic variability and differentiation	The majority of genetic variability was found within populations and among populations within islands. The genetic differentiation among populations suggests that provenance should be considered when formulating augmentation or reintroduction strategies	ISSR and RAPD	Decision making	Genetic fitness	Bio G
aka	2011	*Zostera marina*	Plant/ grass	Aquatic	Japan	Asia	This study examined population genetic structures and gene flow in eelgrass in Tokyo Bay to establish guidelines for conducting restoration.	Strong gene flow between the inner bay populations and higher diversity. We suggested that the restoration of eelgrass in the innermost part of Tokyo bay, where natural habitats have been lost, should be conducted using the inner bay beds.	Microsatellites	Decision making	Genetic fitness	M B
Looy	2011	*Origanum vulgare*	Plant/ herb	Terrestrial	Belgium	Europe	We aimed at relating neutral genetic variation of the populations with their status and sex ratio.	For *O. vulgare*, the gynodioecy proves a successful plastic strategy for expanding and new establishing populations to cope with pollen limitation and inbreeding depression.	AFLP	Evaluation/ monitoring	Genetic fitness	Plan Ev
ik	2012	*Lychnis floscuculi*	Plant/ flower	Terrestrial	Switzerland	Europe	Genetic diversity, inbreeding and genetic structure	Gene diversity and allelic richness were similar in natural and sown populations. In contrast, inbreeding coefficients were three times higher in sown than in natural populations. The sown populations were genetically distinct from the native populations. The use of commercially produced seeds may alter the genetic diversity and structure of plant populations. The observed higher inbreeding coefficients of sown populations could lead to reduced population viability. To restore genetically diverse populations, the seeds for further propagation should be collected from numerous individuals in large and non-isolated populations nearby restored sites.	Microsatellites	Evaluation/ monitoring	Genetic fitness	Bio Con
thier	2012	*Upupa epops*	Animal/ bird	Terrestrial	Switzerland	Europe	Assessment of artificial nest boxes	We found i) a low occurrence of extra-pair paternity, polygyny and conspecific brood parasitism, ii) a high level of neutral genetic diversity (mean number of alleles and expected heterozygosity per locus: 13.8 and 83%, respectively) and, iii) evidence for genetic connectivity through recent immigration of individuals from well differentiated populations.	Microsatellites	Evaluation/ monitoring	Demographi c study	Pl
trand- rcia	2012	*Uniola paniculata*	Plant/ grass	Terrestrial	USA	North America	(I) Identify improved sea oats lines; and (ii) Determine the genetic diversity of improved sea oats lines.	Genetically diverse sea oats lines were identified for restoration projects. These results provide useful genetic information that can be used by the sea oats breeding program to make crossing decisions	AFLP	Decision making	Genetic fitness	Eu
adhurs	2012	*Swainsona sericea*	Plant/ grass	Terrestrial	Australia	Australia	Reproductive output, progeny fitness and genetic diversity	Unexpectedly, four chromosome classes were observed. Population size influenced the number of alleles and percentage of polymorphic loci while ploidy influenced effective alleles and expected heterozygosity. Ploidy had a significant influence on height, shoot	AFLP	Decision making	Genetic fitness	Au Jo B

								weight, shoot to root ratio and days to germination. If ploidy differences are more common in herbaceous grassland species than is currently recognised, management strategies for endangered grassland communities, particularly with respect to the movement of seed, must explicitly take into account these associated complexities if the long-term negative effects of dysgenesis are to be avoided.				
ıklin	2012	*Spyridia filamentosa*	Plant/ seagrass	Aquatic	USA	North America	Understand the genetic diversity of this species in the Islands and determine if there are relationships between any molecular clades and anatomical characters	Presence of multiple clades. *Filamentosa* arrived in Hawai'i on at least six occasions.	MtDNA and gene sequence	Decision making	Demographi c study	Phy
ɔ	2012	*Phragmites australis*	Plant/ grass	Aquatic	China	Asia	Determination of spatial genetic structure	In conclusion, our findings not only provided insights into the population dynamics of common reed in changing environments, but also demonstrated the feasibility of using habitat patches in a mosaic landscape as test systems to identify appropriate genetic sources for ecological restoration. A strong association of genetic variation with habitat heterogeneity. Genetic distances among populations were best explained as a function of environmental difference, rather than geographical distance. Spatial structuring of genotypes in relation to patchy habitats.	Microsatellites	Decision making	Demographi c study	Mo E
bs	2012	*Lupinus perennis*	Plant/ flower	Terrestrial	USA	North America	We evaluated commercial and locally collected seed sources for potential use in a New York State-based, landscape-scale program for restoring blue lupine. (1) What is the genetic similarity between seed stock native to the targeted restoration area and that from commercial seed sources purporting to provide "native" seed stock? (2) Do differences in fitness-related traits at the restoration site correspond with degree of geneticsimilarity to known native stocks?	Seeds from some commercial suppliers were interspecific hybrids and therefore unreliable and from some other were genetically close to the aimed population. Performance of the latter was better. (1) commercial suppliers often but not always offer reliably characterized seed sources of sufficient genetic similarity to native populations to warrant their use in restoration projects and (2) genetic affinity of potential seed stock to native populations is positively related to its fitness in the environment targeted for restoration	Microsatellites	Evaluation/ monitoring	Genetic fitness	Res E
ɔan	2012	*Juglans cinerea*	Plant/ tree	Terrestrial	USA	North America	How mating patterns, dispersal and ecologically constrained regeneration influences genetic diversity and kinship in a naturally regenerating population of a threatened temperate forest tree	Natural regeneration at this site resulted in loss of allele richness due to a small number of parents, limited pollen and seed dispersal (<100 m)	Microsatellites	Evaluation/ monitoring	Genetic fitness	Cons G
ıston	2012	*Rhinanthus minor*	Plant/ flower	Terrestrial	England	Europe	Deciding on the optimal seed source for such restoration projects, e.g. Local genotypes or ecotypes.	We identified moderate levels of genetic differentiation between R. Minor populations within the UK.	Microsatellites and Chloroplast and nuclear rDNA ITS DNA sequencing	Decision making	Genetic fitness	Pers in E Ev Sys
ford	2012	*Stylidium hispidum*	Plant/ flower	Terrestrial	Australia	Australia	We compared intraspecific F1 hybrid performance and molecular marker differentiation among four populations	We found evidence of outbreeding depression in long-distance hybrids (111–124 km), and inbreeding depression among progeny of within-population crosses, relative to short-distance (3–10 km) hybrids.	AFLP	Decision making	Genetic fitness	Ecol Ev
sen	2012	*Elymus wawawaiensis*	Plant/ grass	Terrestrial	United States	North America	Estimate genetic relationships and underlying genetic components for seed and forage trait improvement between plant introductions and cultivars.	Through recurrent selection, populations of wheatgrass have been and can be developed to more effectively establish and compete on annual weed–infested rangelands.	AFLP	Decision making	Genetic fitness	Rar Eco Man
tle	2012	*Araucaria*	Plant/	Terrestrial	New	Australia	We indirectly estimate dispersal	Wright's genetic neighbourhood ranged from 22 to 876 trees, and	Microsatellites	Decision	Demographi	Dive

		nemorosa	tree		Caledonia		distances and project a maximal dispersal envelope around the extant populations	historic gene dispersal ranged from 10.8 to 82.4 m, indicating that most seed dispersal is <100 m. Our results also suggest that the majority of seed dispersal is too short to allow *A. nemorosa* to disperse to new more hospitable sites within an ecologically relevant time-scale.		making	c study	Dist
yd	2012	*Vallisneria americana*	Plant/ grass	Aquatic	USA	North America	We quantified the effect of restoration practices on genetic diversity	Restoration practices do not appear to negatively impact genetic diversity, and basic measures of genetic diversity within restored sites overlap with natural sites. However significant inbreeding was found in some sites. Corrections are proposed.	Microsatellites	Evaluation/ monitoring	Genetic fitness	Cons G
halski	2012	*Geranium pratense*	Plant/ grass	Terrestrial	Germany	Europe	We assess within- and among provenance differentiation patterns and aspects of gene flow by investigating the mating system, the genetic structure at regional and local scale, gene dispersal and potential selective mechanisms that may have contributed to differentiation patterns found.	Low genetic diversity, genetic differentiation between populations and spatial genetic structure. Limited dispersal ability, gene flow larger than 10 km is negligible. Our findings may question the approach of delineating provenances by general criteria, and highlight the importance of species specific studies on differentiation and adaptation patterns.	AFLP	Decision making	Genetic fitness	Cons G
lar	2012	*Acacia saligna*	Plant/ schrub	Terrestrial	Australia	Australia	Direct and indirect methods of paternity analysis were used to detect genetic contamination via inter-sub specific pollen dispersal from a planted stand of nonlocal species	Genetic contamination was detected and occurred over a distance of 1.6 km	Microsatellites	Evaluation/ monitoring	Genetic fitness	Res E
sner	2012	*Salix viminalis*	Plant/ tree	Terrestrial	Germany	Europe	We investigated clonal and genetic diversity patterns of *Salix viminalis* for 23 stands, taking into account young and old age classes and locations in front of the dykes in the active floodplain and behind the dykes in the inactive floodplain	One continuous population. Riparian softwood forest species have efficient dispersal strategies which allow the population to survive altered floodplain conditions from a genetic point of view	Microsatellites	Decision making	Genetic fitness	Bio Cons
	2012	*Zostera marina*	Plant/ seagrass	Aquatic	USA	North America	Assess population differentiation and to compare levels of within-population genetic diversity	The annual population had significantly higher clonal diversity than the others but showed no detectible differences in heterozygosity or allelic richness. Sufficient input of genetic variation through sexual reproduction or immigration prevent significant declines in the number and frequency of alleles. Genetic drift, sweepstakes recruitment, dispersal limitation, and possibly natural selection produce genetic differentiation over a spatial scale of 3–30 km	Microsatellites	Decision making	Genetic fitness	Jou H
kup	2012	*Rutidosis leptorrhynchoides*	Plant/ herbaceous	Terrestrial	Australia	Australia	We assessed the extent of local adaptation of fitness components and examined whether spatial scale, environmental distance, quantitative and neutral genetic differentiation, and size of the local and foreign populations could predict patterns of adaptive differentiation.	Local adaptation was observed for seedling survival, but not for biomass, while foreign genotype advantage was observed for reproduction (number of inflorescences). Among population pairs, local adaptation increased with Qst and local population size for biomass. Qst was associated with environmental distance, suggesting ecological selection for phenotypic divergence. However, low Fst and variation in population structure in small populations demonstrates the interaction of gene flow and drift in constraining local adaptation	Microsatellites and quantitative traits	Decision making	Genetic fitness	Evol App
d	2012	*Haliotis kamtschatkana*	Animal/ invertebrate	Aquatic	Canada	North America	Pedigree reconstruction approach to assess the survival of outplanted abalone	26% of the individuals sampled at the main outplanting site were from hatchery spawnings. Employing pedigree analyses to identify hatchery-released abalone in the wild may indeed be an effective way to quantify their long-term survival and impact on local population densities, especially if accurate spawning records are kept and DNA samples from all broodstock are obtained.	Microsatellites	Evaluation/ monitoring	Demographic study	Cons G
nolds, Glathe	2012	*Zostera marina*	Plant/ grass	Aquatic	USA	North America	Genetic diversity and quantitative traits	A small increase in genetic diversity enhanced ecosystem services (invertebrate habitat, increased primary productivity, and nutrient retention). Taken together, these results suggest that ecosystem	Microsatellites	Decision making	Genetic fitness	Pl

								restoration will significantly benefit from obtaining sources (transplants or seeds) with high genetic diversity and from restoration techniques that can maintain that genetic diversity.				
nolds, ycott	2012	*Zostera marina*	Plant/ grass	Aquatic	USA	North America	We examined the genetic diversity of restored *Zostera marina* meadows in a coastal bay system along the USA mid-Atlantic coast using microsatellite markers to compare donor and recipient meadows.	We show that donor meadows have high genetic diversity and that this diversity is maintained in meadows restored. No evidence of inbreeding depression. As long as donor sites are chosen carefully, issues that diminish fitness and survival such as heterosis or out-breeding depression can be avoided.	Microsatellites	Evaluation/ monitoring	Genetic fitness	M Ec Pr S
hie	2012	*Banksia attenuata*	Plant/ tree	Terrestrial	Australia	Australia	To assess the genetic variation of natural and restored populations, and their offspring,	Both natural and restored populations, and their offspring, displayed similarly high levels of heterozygosity. Very weak population divergence. Genetic structuring within the natural population was weak, but detectable at 10 m and more strongly genetically structured than the restored population. Results indicate the successful genetic management of *B. attenuata* in this restoration project,	Microsatellites	Evaluation/ monitoring	Genetic fitness	Res E
reier	2012	*Acipenser transmontanus*	Animal/ fish	Aquatic	North America	North America	Genetic monitoring may be used in the context of adaptive management to reduce deleterious genetic impacts of captive breeding in wild populations.	Very low levels of genetic diversity. We validate the power of parentage analysis to identify family relationships between individual. Parentage analysis will become crucial for inbreeding avoidance	Microsatellites	Decision making	Genetic fitness	End S Re
p	2012	*Limnanthes vinculans*	Plant/ flower	Terrestrial	USA	North America	We determined the temporal, and range-wide population genetic status	Most populations showed heterozygote deficiency indicating past bottlenecks, inbreeding or genetic drift. Genetic differentiation between populations. Seeds should only occur from genetically similar source sites that have been extensively genetically tested.	Microsatellites	Evaluation/ monitoring	Genetic fitness	Cons G
to	2012	*Austrocedrus chilensis*	Plant/ tree	Terrestrial	Argentina	Latin America	We analysed geographic patterns of genetic variation to examine its potential for restoration, determine the effects of historical factors affecting diversity, together with inbreeding and gene flow,	Genetic diversity decreased southwards, high genetic admixture in continuous western populations, higher inbreeding in northern populations, gene flow moderate.	Allozymes	Decision making	Genetic fitness	Res E
za	2012	*Croton floribundus* and *Peltophorum dubium*	Plant/ tree	Terrestrial	Brazil	Latin America	Analyse the genetic structure of different sampling points in order to establish strategies for collecting seeds	At least for these two species, seeds can be obtained from a few fragments, in one or two regions, as a strategy for optimizing the collection of seeds used in restoration, as well as part of genetic diversity lost during forest fragmentation.	RAPD	Decision making	Genetic fitness	R Bras Bio
ar	2012	*Ascaphus truei*	Animal/ amphibian	Aquatic/terrestrial	USA	North America	We investigated the genetic source of recolonization within the blast area and tested whether post-eruption salvage logging and subsequent tree planting influenced tailed frog movement patterns.	Widespread recolonization across the blast area from multiple sources, gene flow pathways within the blast area where salvage logging and replanting occurred post-eruption are strongly limited.	Microsatellites	Decision making	Demographic study	Ec App
depitt	2012	*Dactylorhiza incarnata*	Plant/ flower	Terrestrial	Europe	Europe	We related genetic variation and fitness to colonization history for all extant populations and assignment tests.	*D. incarnat*a suffered a severe bottleneck, recurrent founder effects, isolation due fragmentation provoke loss of genetic diversity but not of fitness	AFLP	Evaluation/ monitoring	Genetic fitness	Mc E
ik	2013	*Lychnis flos-cuculi*	Plant/ grass	Terrestrial	Switzerland	Europe	Evaluation of the functional connectivity of populations of a wet grassland plant in an intensively managed agricultural landscape	A few first-generation migrants were detected between sown and natural populations and among natural populations, suggesting that functional connectivity was now rather restricted. The connectivity of wet grasslands is likely to be best enhanced by establishing a relatively dense network of large habitat patches.	Microsatellites	Evaluation/ monitoring	Demographic study	Jou A E
lo	2013	*Columba janthina nitens*	Animal/ bird	Terrestrial	Japan	Asia	Performed a diet analysis using DNA barcoding and microhistological techniques	The DNA barcoding approach detected a much larger number of plants than the microhistological analysis. Barcoding analysis indicated the frequent consumption of introduced species, the rapid	Chloroplast trnL P6 loop sequences	Decision making	Demographic study	Ecol Ev

								eradication of specific introduced species may reduce the food resources available to this endangered bird.				
e	2013	*Grevillea sphacelata*	Plant/ schrub	Terrestrial	Australia	Australia	Test for evidence of genetic differentiation within and among populations	Movement of seed between populations separated by 4 km will not have detrimental consequences, despite significant differentiation at neutral genetic markers, and may be beneficial in maintaining genetic diversity and evolutionary potential	AFLP	Decision making	Genetic fitness	Bo Jou the S
vedo	2013	*Erythrina velutina Willd*	Plant/ tree	Terrestrial	Brazil	Latin America	Assess genetic diversity	The populations studied contain high genetic diversity suitable for seed collection for the restoration of degraded areas.	Isoenzimes	Decision making	Genetic fitness	R Aca Ci Ag Am
n	2013	*Micropterus dolomieu*	Animal/ fish	Aquatic	USA	North America	Survey of changes in introgression across the range of the studied species	No evidence that stocking of hatchery-reared individuals has led to severely depressed genetic variation.	Microsatellites	Evaluation/ monitoring	Demographic study	Tran An Fi S
z	2013	*Pulsatilla vernalis*	Plant/ flower	Terrestrial	Germany	Europe	Investigate the success of a population recovery programme and analyse whether potential bottlenecks during population reinforcement affected fitness and genetic variation	Increasing population sizes had no negative impact on fitness and genetic variation.	AFLP	Evaluation/ monitoring	Genetic fitness	Bio Con
dbury	2013	*Eucalyptus gomphocephala*	Plant/ tree	Terrestrial	Australia	Australia	Assess mating system variation	Genetic diversity was not consistently lower in offspring from fragmented, smaller and/or disturbed populations. The espcies mating system and the genetic diversity of offspring is robust to some habitat disturbance and/or fragmentation.	Microsatellites	Evaluation/ monitoring	Demographic study	Aus Jou B
adhurs	2013	*Eucalyptus melliodora*	Plant/ tree	Terrestrial	Australia	Australia	Determine whether plantings that will persist over time have been created	genetic diversity was significantly lower in restored trees. Significant genetic differentiation between restored and scattered trees indicated that seed was not necessarily sourced locally. The persistence of some of these Yellow Box sites may be constrained by inbreeding once scattered trees are lost.	Microsatellites	Evaluation/ monitoring	Genetic fitness	Bio Con
ni	2013	*Marsilea quadrifolia*	Plant/ fern	Aquatic	Various	Europe	Identify the most suitable genetic pool for plant reintroduction efforts	The populations suffered from low genetic variability. However, 4 populations were characterised by different genetic traits that are useful in defining a genetic pool for plant conservation.	AFLP	Decision making	Genetic fitness	A B
npanel	2013	*Zostera marina*	Plant/ grass	Aquatic	USA	North America	Identify which parental founding ecotypes survived the restoration process over multiple generations	The frequency of observed heterozygotes, although higher than in the natural populations, still indicates reduced levels of diversity and connectivity. The inbreeding frequency is high in the restored populations, but lower than what is seen in the native populations.	Microsatellites	Evaluation/ monitoring	Genetic fitness	Pop E
tes	2013	*Banksia cuneata*	Plant/ schrub	Terrestrial	Australia	Australia	Investigate spatial and temporal mating-system variations.	Temporal mating-system variation warrants increased consideration, not only in assessing the effects of habitat fragmentation on plant populations, but also in the design and establishment of successful translocation, augmentation and restoration programs.	Isoenzimes	Decision making	Genetic fitness	Aus Jou B
t	2013	*Cirsium pitcheri*	Plant/ thistle	Terrestrial	USA	North America	Measure the genetic diversity within reintroduced and native populations	Reintroduced populations have significantly higher genetic diversity than the native populations, however, they had significantly higher inbreeding coefficients.	Microsatellites	Evaluation/ monitoring	Demographic study	Bo Bo
sen	2013	*Origanum vulgare*	Plant/ herb	Terrestrial	Belgium	Europe	Investigate the genetic effects of recent colonization of restored semi-natural grassland patches	Spontaneous colonization after habitat restoration can lead to viable populations in a relatively short time, overcoming pronounced founder effects, when several source populations are nearby.	Microsatellites	Evaluation/ monitoring	Genetic fitness	Pl
ang	2013	*Caragana microphylla*	Plant/ schrub	Terrestrial	China	Asia	Evaluate levels of genetic variation within and among populations	Genetic variation in natural populations was more than that in plantation populations.	ISSR	Evaluation/ monitoring	Genetic fitness	Bioc Sys and
ry	2013	*Phragmites*	Plant/	Aquatic	Australia	Australia	Study genetic structure.	There was no genetic evidence of either salt tolerant or introduced	Microsatellites	Decision	Genetic	A

		australis	grass					variants.	and chloroplast DNA sequence	making	fitness	B
	2013	Various	Animal/ insects and birds	Terrestrial	Malaysia, China and the United Kingdom	Asia and Europe	Validate metabarcoding technology	Compared with standard biodiversity data sets, metabarcoded samples are taxonomically more comprehensive, many times quicker to produce, less reliant on taxonomic expertise and auditable by third parties, which is essential for dispute resolution	DNA sequences	Evaluation/ monitoring	Demographic study	Ec L
eph	2013	*Acicia spp.*	Plant/ tree	Terrestrial	Australia	Australia	Preliminary identification of salt tolerance in Acacia species	A phylogenetic tree was constructed to identify additional Acacia species closest to the four candidate salt tolerant species.	Nuclear ribosomal DNA markers (ITS and ETS)	Decision making	Genetic fitness	AF Pr
uss	2013	*Banksia menziesii*	Plant/ tree	Terrestrial	Australia	Australia	Derive seed sourcing guidelines from a novel range-wide assessment of spatial genetic structure	Population pairs separated by >60 km were, on average, twice as likely to be significantly genetically differentiated than population pairs separated by <60 km, suggesting that habitat-matched sites within a 30-km radius around a restoration site genetically defines a local provenance seed collection zone fot this species.	AFLP	Decision making	Genetic fitness	Ecol Ev
rsden	2013	*Vallisneria americana*	Plant/ grass	Aquatic	USA	North America	Observe population level differences in within-population and among-region crosses.	Levels of genetic relatedness among individuals, genetic diversity within populations, or differentiation across populations did not predict reproductive success.	Microsatellites	Decision making	Genetic fitness	Bio Con
wald	2013	*Crotaphytus collaris collaris*	Animal/ reptile	Terrestrial	USA	North America	Examine the genetic changes throughout all the stages of decline and recovery	Prior to prescribed burning, genetic diversity decreased within glades and differentiation increased among glades. With woodland burning, genetic diversity within glades first decreased during an expanding colonization phase, but then increased as a dynamically stable metapopulation was established.	Microsatellites	Evaluation/ monitoring	Genetic fitness	Mo E
ill	2013	*Acacia spp.*	Plant/ tree	Terrestrial	Australia	Australia	Test whether plant DNA barcoding regions are able to discriminate Acacia taxa of varying phylogenetic distances, and identify an ambiguously labelled seed collection.	Authors recommend using formal barcoding regions supplemented with data from other plastid regions, particularly rpl32-trnL, for barcoding in *Acacia*.	Plant DNA barcode regions	Decision making	Demographic study	Mo E Re
lot-naff	2013	*Berula erecta*	Plant/ herb	Aquatic	France	Europe	Analyse the population genetic diversity of an aquatic plant in wetlands restored by sediment dredging to assess the impact of ecological restoration on genetic diversity.	Benefits of restoration may depend strongly on the genetic diversity of established relict and dormant populations. Population diversity, in turn, may partly depend on the ecological characteristics of the site (i.e., connectivity of the water body to the surrounding wetlands, successional stage and site history).	Microsatellites	Evaluation/ monitoring	Genetic fitness	A B
ks	2013	*Juglans cinerea*	Plant/ tree	Terrestrial	USA	North America	Determine the frequency of hybridization	The presence of healthy trees and low rate of hybridization suggest that these trees may contribute to the development of disease-resistant genotypes for future restoration efforts.	N/A	Decision making	Demographic study	Natu J
nolds	2013	*Zostera marina*	Plant/ grass	Aquatic	USA	North America	Evaluate genetic structure and connectivity among restored and naturally recruited populations.	Genetic diversity achieved by active restoration in 10 years would have otherwise taken between 125 and 185 years to achieve through natural recruitment events.	Microsatellites	Evaluation/ monitoring	Genetic fitness	Jou E
malen rger	2013	Bacterial communities	Bacteria	Terrestrial	Ireland	Europe	Report the establishment of bacterial communities in bauxite residues with and without restoration amendments	While bacterial communities developed in the initially sterile bauxite residue, only the restoration treatments created diverse soil-like bacterial communities alongside diverse vegetation on the surface.	16S rRNA gene fragments	Evaluation/ monitoring	Demographic study	Envir l Sci Tec
clair	2013	*Posidonia australis*	Plant/ grass	Aquatic	Australia	Australia	Compare genetic diversity between transplant area and the original donor site.	Genetic diversity in the restored meadow was very high and comparable to the donor site, with no genetic differentiation detected.	Microsatellites	Evaluation/ monitoring	Genetic fitness	Ecc Man Res
nmervi	2013	*Wilsonia backhousei*	Plant/ schrub	Terrestrial	Australia	Australia	Investigate the implications of clonality for translocation; characterise samples	To maximise adaptive potential in translocated populations of *W. backhousei*, authors recommend sourcing propagules from multiple	Microsatellites	Decision making	Genetic fitness	We Eco

							and determine the size and distribution of genetically distinct individuals.	populations and planting representatives of the different populations in close proximity to facilitate sexual reproduction.				Man
gemor	2013	*Persoonia longifolia*	Plant/ schrub	Terrestrial	Australia	Australia	Assess genetic variation within, and differentiation among potential seed source populations.	Pairwise population genetic dissimilarity was correlated with both geographic distance and environmental distance derived from five climate variables. However, partial Mantel tests showed that the relationship between genetic and geographic distance was not independent of environmental distance, suggesting a non-neutral signature in these markers. Bayesian outlier analysis identified two markers, and spatial analysis method tests identified highly significant associations between these two markers and three environmental variables.	AFLP	Decision making	Genetic fitness	Rest E
etman	2013	*Schoenoplectus maritimus*	Plant/ herb	Aquatic	USA	North America	Investigate the patterns and structure of genetic diversity.	Each population should be treated as an independent management unit to preserve population structure and that seeds should be collected broadly within one or a few populations in close geographic proximity to a proposed restoration site	AFLP	Decision making	Genetic fitness	Ac B
azi	2013	*Copaifera langsdorffii*	Plant/ tree	Terrestrial	Brazil	Latin America	Investigate edge effects on the genetic diversity, mating system and pollen pool.	It is preferable that seed harvesting for conservation and environmental restoration strategies be conducted in the continuous savnnah woodland, where genetic diversity and variance effective size within progenies are higher.	Microsatellites	Decision making	Genetic fitness	He
der jnsbru e	2013	*Prunus spinosa*	Plant/ schrub	Terrestrial	Belgium	Europe	Investigate the genetic variation within and between populations and assessed their potential as seed source for gene conservation and ecological restoration.	The relatively high within-population diversities and moderate, although variable, between-population differentiation of the other Flemish populations point to a considerable amount of gene exchange and can justify extensive seed sourcing for the production of autochthonous planting stock	AFLP	Decision making	Genetic fitness	Plant Ev
ogawa	2013	*Polemonium kiushianum*	Plant/ herb	Terrestrial	Japan	Asia	Assess the genetic consequences of habitat degradation on the wild populations and the establishment of ex situ population	Genetic diversity in the ex situ populations was considerably lower than that of the wild populations. The low genetic diversity observed in the ex situ populations and different genetic composition between wild and ex situ populations may be due to genetic drift with few founders for the ex situ populations and the management strategy used for the ex situ populations	Microsatellites	Evaluation/ monitoring	Genetic fitness	Bio Cons
ng	2013	*Phragmites australis*	Plant/ grass	Aquatic	China	Asia	Assess bacterial diversity in the rhizosphere	Pyrosequencing of different *P. australis* ecotypes provided insight into the structural variation of the rhizosphere bacterial community.	DNA sequences	Decision making	Demographic study	Geom ogy

Appendix 2

R values resulting from a Spearman's Rho test to measure the correlation between a range of genetic parameters and groups of individuals trapped in different habitats. All correlations were not significant (P-value>0.05).

	r values	
Parameter	**Huntly**	**Dwellingup**
Proportion of heterozygous loci	0.148	0.078
Standardized heterozygosity based on the mean Ho	0.136	0.072
Standardized heterozygosity based on the mean He	0.139	0.075
Internal relatedness	-0.066	-0.079
Homozygosity by locus	0.112	0.058

Dwellingup categories: severely affected (n=9), moderately affected (7) and not affected (26).

Huntly categories: <15 years post-restoration (n=12), >15 years post-restoration (11), unmined (33).

Appendix 3

Correlation between mined area and genetic distance.

ID	Proportion of mined area (%)	Genetic distance	Number of comparisons
11226	24	9.0	5
11216	34	14.5	6
11230	34	8.0	6
11244	35	12.1	8
11135	35	15.5	12
11181	35	10.1	12
12018	37	13.4	6
12021	37	18.0	6
12029	38	20.2	5
12249	38	11.4	5
12022	40	16.7	9
12025	40	14.7	9
11157	40	16.2	11
11194	40	15.4	11
12037	42	16.4	11
12050	42	16.1	11
11282	43	15.1	10
12019	43	12.4	14
11265	45	17.0	4
12016	45	20.2	4
12017	48	15.8	11
11188	53	8.1	11
11189	53	7.0	11