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## **Contribution of genetics to ecological restoration**

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## 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 127 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, 41% of reviewed studies used genetic information to evaluate or monitor restoration and 59% 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.

## 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 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

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on individual fitness, population persistence and ecosystem processes (e.g. Reynolds *et al.* 2012; Williams 2001), which are all elements of primary interest in restoration ecology. Furthermore, there is evidence of a direct relationship between population dynamics and genetic diversity (Beaumont 2008a; Sunnucks 2011), which require both to be taken into consideration concurrently. Research is enabling us to understand the principles and consequences of genetic stochasticity (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 becoming 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).

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 – repeats of 2-4 nucleotides; Selkoe & Toonen 2006) have been shown to 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 (Schlötterer 2004). 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 (e.g. Diekmann *et al.* 2010).

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-Jaén & 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 use of 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.

### **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



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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 whereby the former is a suitable proxy for estimating complex polygenic 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.

### *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. 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. 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-Jaén & 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 and *Biological Conservation* with 11, and *Molecular Ecology* with eight.

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. 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. 2b) and more in terrestrial (69%) than in aquatic (31%) ecosystems. These percentages are similar to trends in overall restoration research (Brudvig 2011; Ruiz-Jaén & 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.

We documented 15 different types of molecular markers used in the articles sampled (Fig. 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.

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. 4). For example, 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.

The meta-analysis also suggested that few studies investigate the link between restoration, genetics and ecosystem services. Experimental research in clonal species, such as seagrasses, 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 & Piazzini 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

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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 to establish appropriate genetic targets for focal taxa. They also caution against following the generally accepted dogma as, if it had been followed in these cases, inappropriate restoration targets may have been formulated. In general, though, the relationship between restoration, genetics and ecosystem services remains understudied and is an important area for future restoration genetic studies.

#### *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.

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 to increase genetic diversity while maintaining local adaptations 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 where to eradicate invasive species with the aim of avoiding future recolonisations and increasing restoration success (Abdelkrim *et al.* 2010; Robertson & Gemmill 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).

#### *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

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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



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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.

### *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.

### **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

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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-Jaén & 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.

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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). These reviews suggest that the importance of local provenance to restoration success should be questioned and the inclusion of non-local provenances considered in some instances (Frankham *et al.* 2011; Weeks *et al.* 2011), particularly where predictions are that future climate change may lead to the emergence of novel ecosystems (Hobbs *et al.* 2009). 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 by better understanding the role of foundation species in ecosystems.

Simulation software in conservation genetics (Epperson *et al.* 2010; Hoban *et al.* 2012a) is 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 and ultimately how they maintain genetic diversity.

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.

#### *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 on effective population size and gene flow, which can potentially inform restoration by setting desirable targets for restoration success (e.g. Bourke *et al.* 2010). 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

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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 as 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 (see Palstra & Fraser 2012 for caveats on using  $N_e/N$  ratios). 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 (Luikart *et al.* 2010; Woods *et al.* 1999).

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-Jaén & 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 to assess restoration success (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.

### **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 S1 (Supporting information).

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**Table 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 ( <i>Ovis canadensis</i> )	Avoid inbreeding	Bottleneck tests	Identification of genetic bottlenecks and small $N_e$	Ramey <i>et al.</i> 2000
Augmentation	Seagrass ( <i>Zostera noltii</i> )	Improve evolutionary potential from an endangered species	Assignment tests	Successful location of the most suitable donor population	Diekmann <i>et al.</i> 2010
Seeding for river restoration	Common reed ( <i>Phragmites australis</i> )	Delineation of seed sources zones to avoid maladaptation	Regression of allele occurrence and environmental variables	Environmental factors explained genetic structure	Gao <i>et al.</i> 2012
Eradication of invasive species	Brown rat ( <i>Rattus norvegicus</i> )	Define eradication units	Migration rates and assignment tests	Eradication is feasible with low risk of recolonisation	Robertson & Gemmell 2004
Salvage logging and	Coastal tailed frog	Evaluate management	Regression of least cost	Natural regeneration maintain	Spear <i>et al.</i> 2012

Recovery after disturbance	( <i>Ascaphus truei</i> )	prescriptions	paths and genetic differentiation	genetic diversity better than active management	
Establishment of an ecological corridor	Australian rats ( <i>Rattus fuscipes</i> and <i>Rattus leucopus</i> )	Monitor corridor efficiency to re-establish gene flow	Assignment and clustering tests	The use and occupation of the corridor differed between species	Paetkau <i>et al.</i> 2009
Removal of shrubs, mowing and grazing	Terrestrial orchid ( <i>Dactylorhiza incarnata</i> )	Inference of colonisation patterns	Assignment and clustering tests and genetic parameters	Decrease in genetic diversity but not in population fitness	Vandepitte <i>et al.</i> 2012

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## Figures

**Figure 1.** Trend in the number of published articles of restoration genetic studies per year (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.

**Figure 2.** Proportion of empirical studies that were performed on (a) each continent and (b) classified by taxonomic group.

**Figure 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.

**Figure 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).

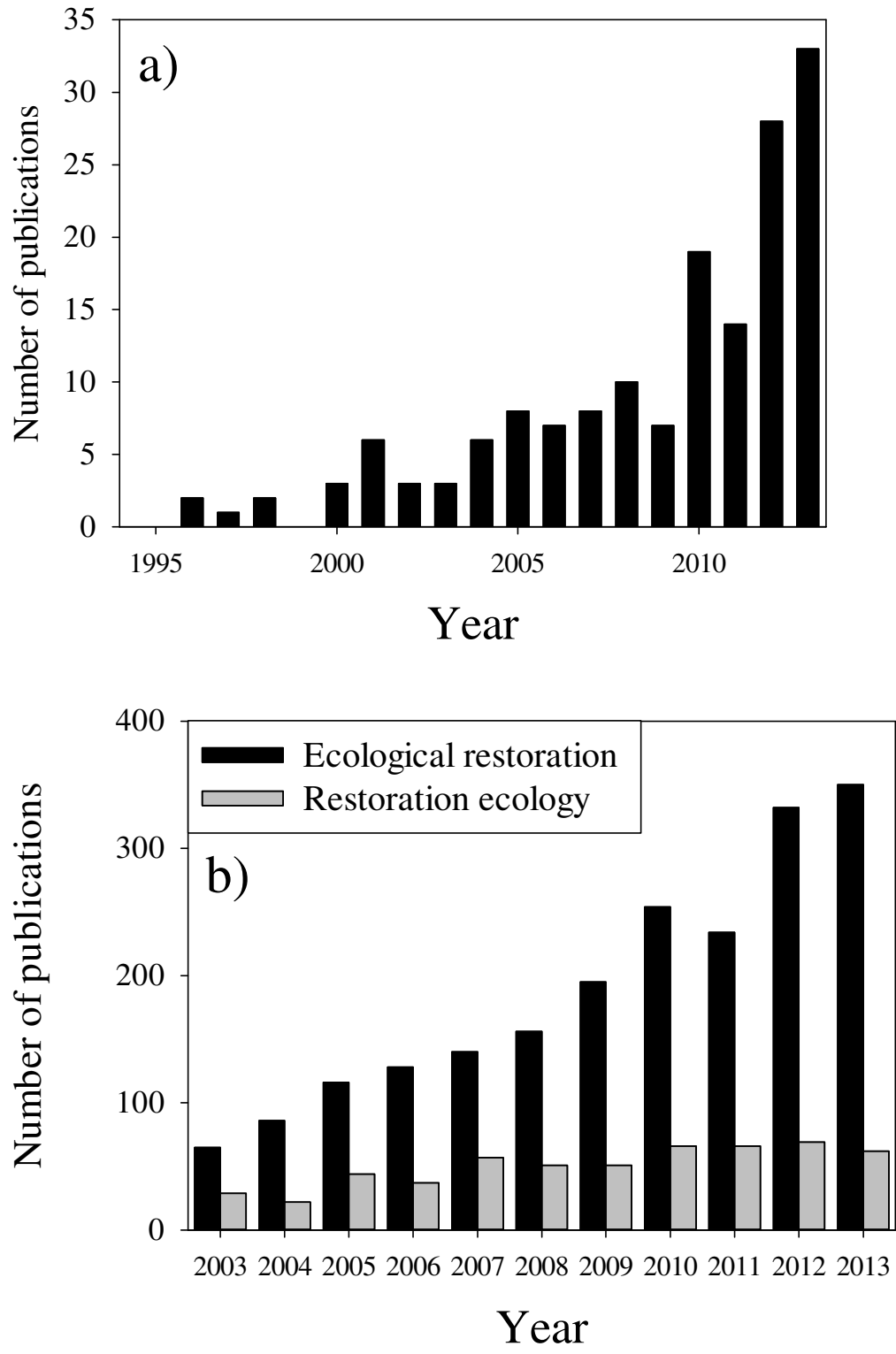


Fig. 1

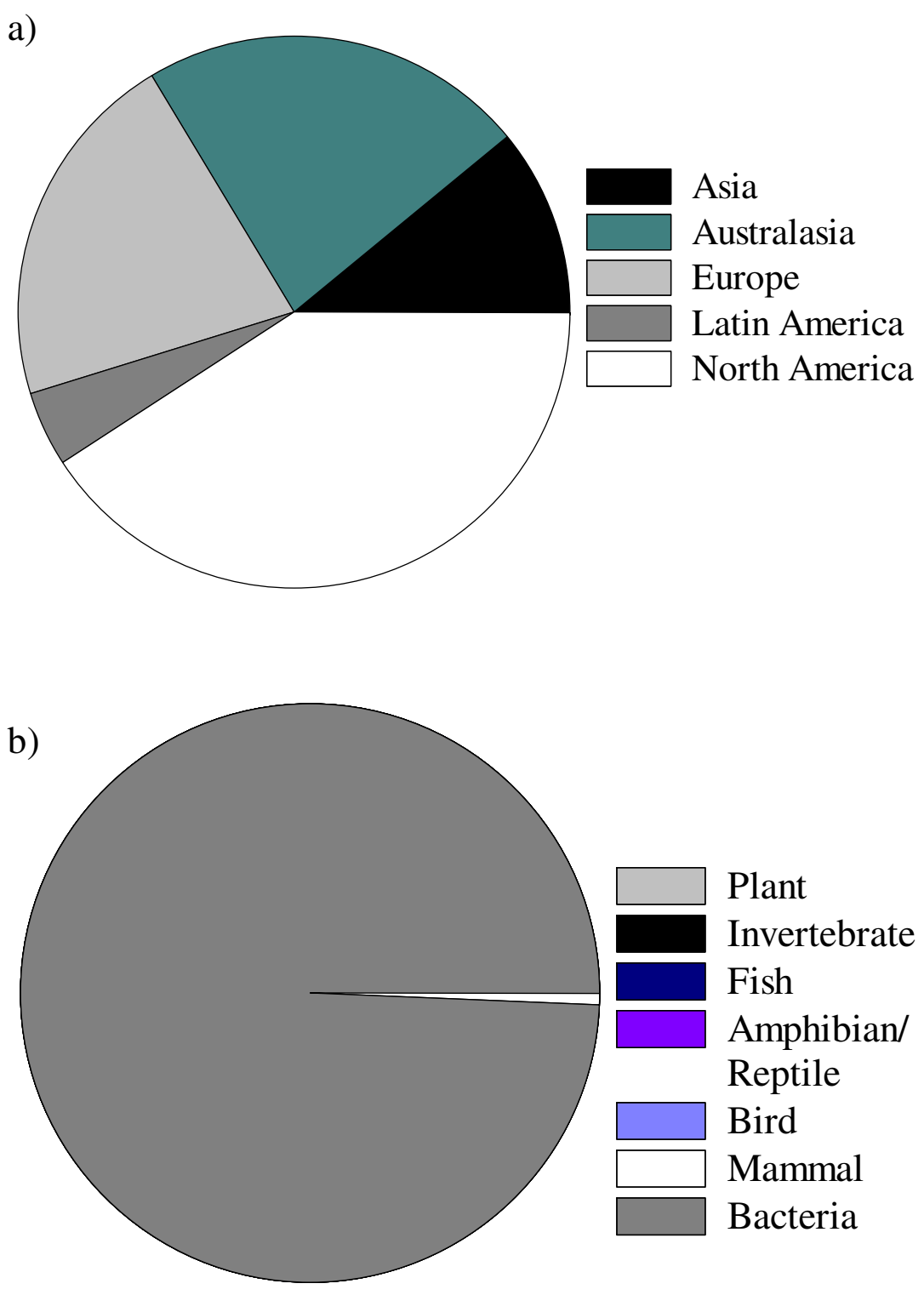


Fig. 2

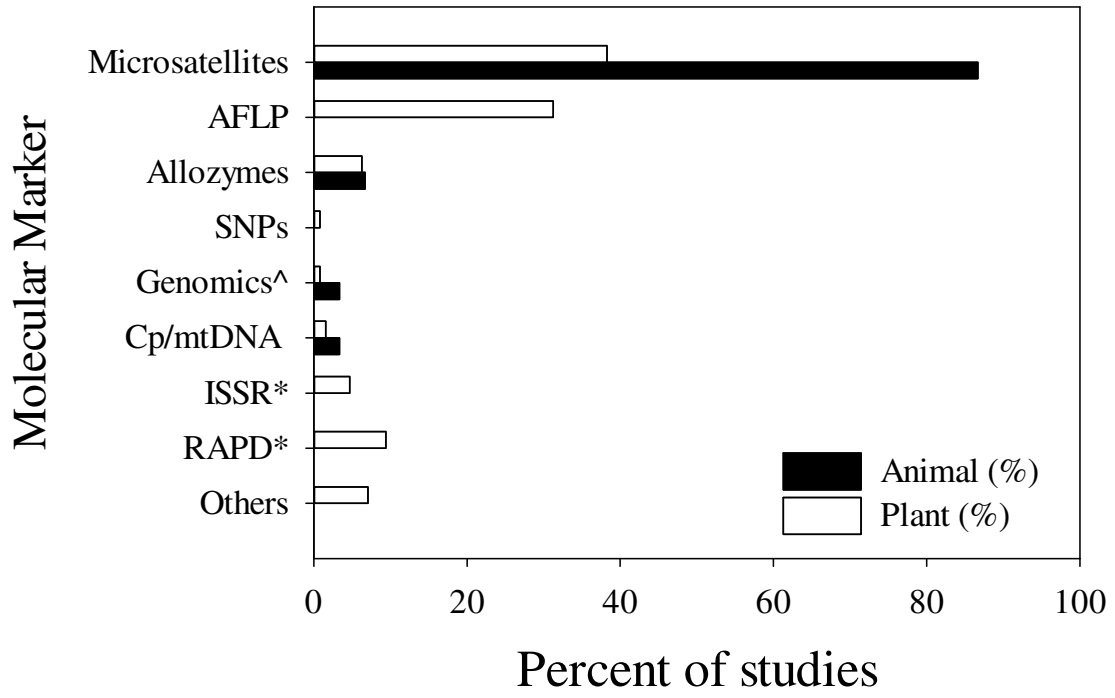


Fig. 3

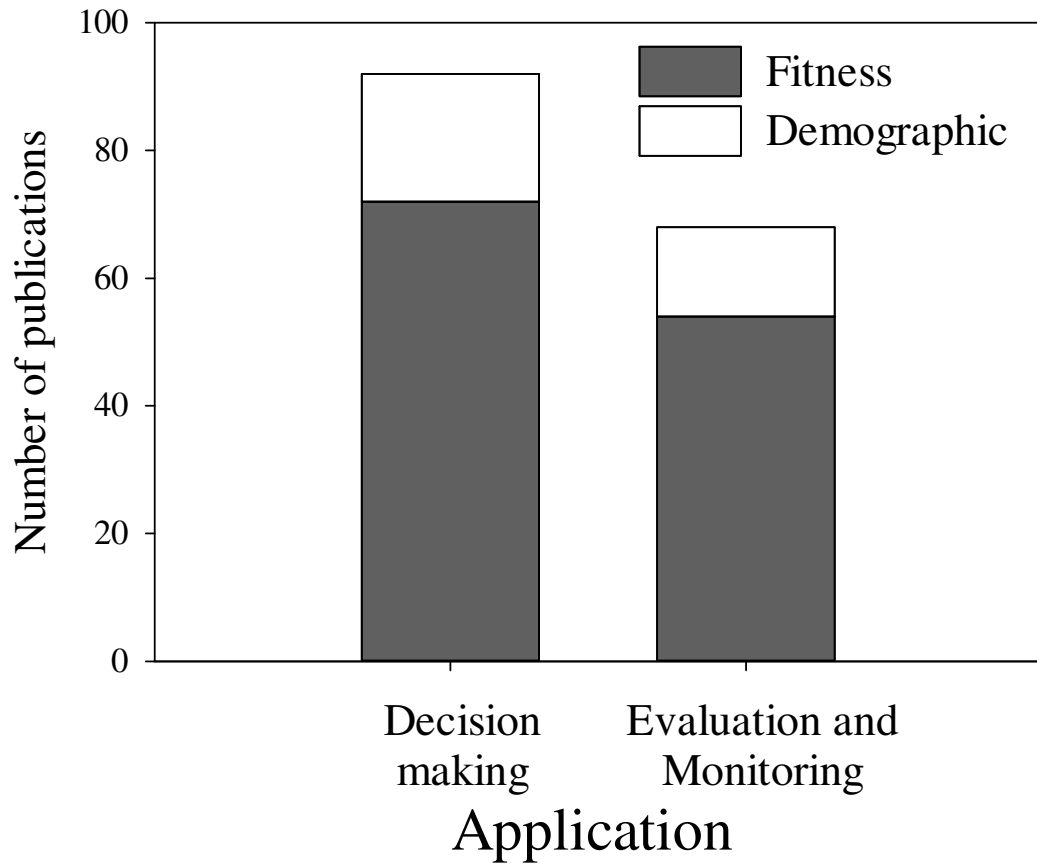


Fig. 4