A question of origin: Where and how to collect seed for ecological restoration

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Abstract

Native plant species are routinely planted or sown in ecological restoration projects, but successful establishment and survival depend on where and how seeds are collected. Research suggests that it is important to use locally adapted seeds. Local populations often show a home-site advantage and non-local genotypes may be maladapted to local environmental conditions. Furthermore, intraspecific hybridisation of local and non-local genotypes may have a negative impact on the genetic structure of local populations via mechanisms such as outbreeding depression. Many species show a strong small-scale genetic differentiation between different habitats so that matching habitats of the restoration and donor site can be more important than minimizing geographical separation. It is a challenge to identify appropriate seed sources because strong small-scale population differentiation makes it difficult to delineate geographically defined seed zones to which seed exchange should be limited. Moreover, it is important to consider the genetic diversity of introduced material because it may be crucial to avoid genetic bottlenecks, inbreeding depression and poor establishment of plant populations. Repeated propagation in stock, which is often required to obtain a sufficient amount of seeds, can further reduce genetic diversity and may select for particular genotypes. Negative impacts of improper seed choice for nursery planting stock may become detectable only after many years, especially in long-lived and slow growing plants. Although scientific information on many species remains limited, the increasing demand for translocation of seed means that mandatory regulations are necessary. Guidelines should prescribe a specification of seed provenance, a record of genetic diversity of wild collections and rules for subsequent processing such as direct transfer and propagation of stock or seed orchards. We use a literature review to evaluate current legislation and to develop recommendations for herbaceous and woody species.

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Zusammenfassung

Es ist heute gängige Praxis, heimische Pflanzenarten zu Renaturierungszwecken auszubringen. Die erfolgreiche Etablierung hängt jedoch davon ab, wie und wo das Saat- oder Pflanzgut gewonnen wurde. Forschungsergebnisse legen nahe, dass nur lokal angepasste Herkünfte eingesetzt werden sollten. Es ist vielfach festgestellt worden, dass sie eine höhere Fitness als fremde Herkünfte besitzen, und letztere sind daher schlecht an lokale Umweltbedingungen angepasst. Eine intraspezifische Hybridisierung mit gebietsfremden Populationen kann weiterhin einen negativen Einfluss auf die genetische Struktur von lokalen Populationen haben wie z.B. outbreeding depression. Einige Arten zeigen eine starke kleinräumige genetische Differenzierung zwischen verschiedenen Habitaten, so dass die Übereinstimmung von Spender- und Renaturierungsflächen wichtiger sein kann als eine minimale Entfernung. Diese kleinräumige Differenzierung macht es schwierig, geographische Regionen festzulegen, innerhalb derer ein Austausch von Pflanzenmaterial unbedenklich ist. Darüber hinaus muss die genetische Diversität des gesammelten und eingeführten Materials berücksichtigt werden, um genetische Flaschenhälse, Inzuchtdepression und eine geringe Etablierungsrate zu vermeiden. Eine wiederholte Kultivierung und Vermehrung von Pflanzenarten, die oft notwendig ist, um eine ausreichende Samenmenge zu produzieren, kann zu einer weiteren Reduktion der genetischen Diversität beitragen oder zu einer Selektion bestimmter Genotypen führen. Negative Auswirkungen einer ungeeigneten Auswahl von Herkünften werden insbesondere bei langlebigen und langsam wachsenden Arten häufig erst nach vielen Jahren feststellbar. Angesichts des wachsenden Bedarfs an Wildpflanzensaatgut sind verbindliche Regelungen notwendig, auch wenn die Datenlage für viele Arten immer noch unzureichend ist. Diese Richtlinien sollten Vorgaben zur Herkunft und Diversität des Saatgutes bei der Sammlung am natürlichen Standort machen und die nachfolgende Prozesskette von der Saatgutreinigung und Lagerung über die Vermehrung bis hin zur Ausbringung regeln. Unter Verwendung von Literaturdaten bewerten wir die gegenwärtige Gesetzgebung und entwickeln Empfehlungen sowohl für krautige Pflanzen als auch für Gehölze.

Introduction

In the last two centuries the human impact on the world's ecosystems has increased and natural habitats have been degraded. During recent decades, interest has grown among conservationists, restoration practitioners, forest managers and landscape designers, in restoring altered landscapes, woodlands and forests back to more 'natural' ecosystems, which are rich in native species. Currently, native herbaceous plant species are routinely sown in ecological restoration projects and, the use of indigenous woody species instead of exotics is standard in European forestry. A major challenge for restoration practitioners is to consider genetic variation and diversity within native species (Walker, Hodder, Bullock, & Pywell 2004).

Population differentiation within plant species is well documented. This differentiation is partly driven by local adaptation resulting in a home-site advantage for the offspring. For example, provenance trials of trees and shrubs showed that stock of British origin is better adapted to British conditions than continental stock (e.g. Jones, Hayes, & Sackville Hamilton 2001; Worrell 1992). Here we use the term 'local' as a synonym for autochthonous, i.e. to mean that the populations originate where found, and by extension, are adapted to local environmental conditions. Local adaptation in herbaceous species can occur rapidly, e.g. on soils contaminated with heavy metals (Antonovics 2006). For long-lived species such as trees, adaptation to variable environmental conditions such as climate, 'local' is assumed to imply a continuous existence at a specific site since post-glacial immigration (Kleinschmit, Kownatzki, & Gregorius 2004). However, while it is often assumed (using the rationale we outlined above) that local populations are superior to non-local populations, there have been several studies demonstrating that non-local populations can have a higher fitness (e.g. Bischoff, Vonlanthen, Steinger, & Müller-Schärer 2006b; Crespi 2000; Saltonstall 2002).

Four mechanisms have been identified through which the introduction of non-local genotypes may have negative consequences (e.g. Edmands 2007; Krauss, Zawko, Bussell, Taylor, & Hood 2005; McKay, Christian, Harrison, & Rice 2005).

- Non-local genotypes may suffer from maladaptation to the local environment resulting in a lower fitness. For long-lived perennials problems may only be evident many years after seemingly successful establishment.
- Intraspecific hybridisation of local and introduced genotypes may result in outbreeding depression, i.e. reduced fitness in subsequent generations due to the

introgression of maladaptated genes or due to hybrid breakdown. Hybrid breakdown describes the disruption of positive epistatic interactions among co-adapted gene complexes, and its negative effects on fitness are often delayed until the F2 or later generations (Edmands 2007; Hufford & Mazer 2003).

- Occasionally, introduced genotypes are superior to local ones and become invasive. Such a spread of alien genotypes is called cryptic invasion because it is much more difficult to detect than the spread of alien species (Hufford & Mazer 2003; Saltonstall 2002).
- The introduction of non-local material may have negative effects on associated plant and animal species. Imported hawthorn has been shown to flower up to 5 weeks earlier than native hawthorn, potentially threatening the insects and birds whose reproductive cycles are synchronized with this event (Hubert & Cottrell 2007). The implications of using non-local seed for resident invertebrate herbivores are uncertain, but there is some evidence that invertebrates can distinguish plants of different origin (Keller, Kollman, & Edwards 1999) and that the development of Lepidoptera larvae can be influenced by food-plant origin (Smith 2007).

Although small and inbred populations may benefit from genetic reinforcement by crossing with non-local populations (Edmands 2007; Petit et al. 2001), the translocation of plant material to other sites always involves at least one of the risks mentioned above and there is an ongoing debate on their importance (Sackville Hamilton 2001; Wilkinson 2001). The aim of this paper is to discuss where and how seeds should be collected, what to take into account when growing plants in stock, and which methods are most appropriate for transferring seeds.

We focus on the practical implications and seek to integrate work on the importance of local adaptation (McKay et al. 2005; Sackville Hamilton 2001) and genetic diversity (Edmands 2007). We also aim to integrate the approaches that have been outlined for forestry and grassland management, which are normally separated in the literature. In particular, attention is paid to the importance of considering small-scale differentiation, a subject which has been only briefly referred to in other reviews. Throughout the paper we refer to restoration practice in three European countries (Flanders (Belgium), Switzerland and Great Britain).
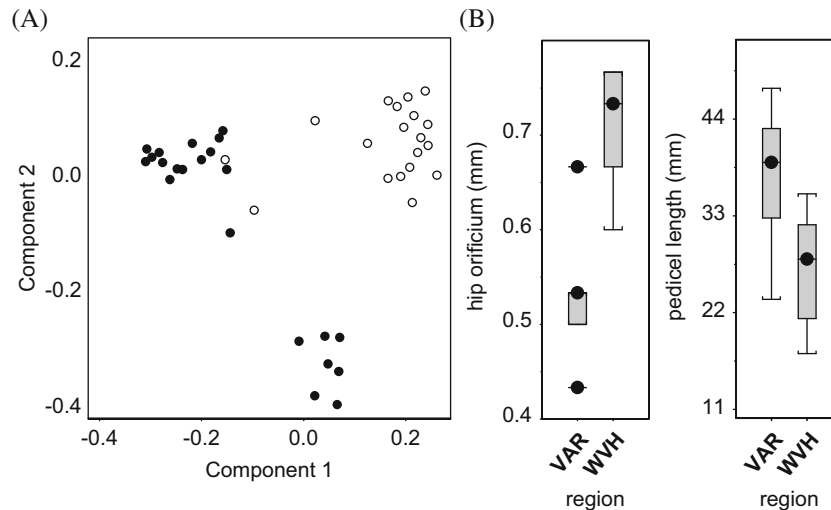
Seed origin: where to collect seed

Large-scale differentiation: delineation of seed zones.

Adaptive genetic differentiation between populations has been found to increase with geographical distance reflecting a correlation between distance and differences in environmental conditions to which populations are adapted (e.g. Becker, Colling, Dostal, Jakobsson, & Matthies 2006; Etterson 2004; Joshi et al. 2001). Climate has been identified as the main driver of selection at such large scales but other environmental factors that show large-scale patterns may also act as diverging selective forces (Etterson 2004; Joshi et al. 2001; Macel et al. 2007; Santamaria et al. 2003). In addition, the probability of gene flow and genetic exchange between populations decreases with distance enhancing genetic differentiation even if environmental conditions and selective forces are very similar (Epperson 2003). As gene flow in plants is usually restricted to a few hundred metres, genetic drift may contribute to population differentiation although this differentiation is not adaptive (Galloway & Fenster 2000; Linhart & Grant 1996; Savolainen, Pyhäjärvi, & Knürr 2007).

To reduce potential risks, restricting plant translocation to certain regions or "seed zones" is a useful approach. Seed zones represent an area in which natural genetic exchange occurs, so that within the zones, transfer of plant material should have no negative impact. One approach to delineate seed zones is to test seeds of different origin in common garden experiments resulting in species-specific models, which relate genetic variation to geographic and/or climatic variation (e.g. Campbell 1991; Lindgren & Ying 2000; Rehfeldt 1995). However, for many species, such data are lacking and the distance over which gene flow takes place is unknown. A simplified approach is the delineation of seed zones using environmental criteria such as geographical distance, climate and geomorphology. Seed zones are administratively delimited by latitude, longitude and altitude which serve as surrogates for environmental descriptors (Ying & Yanchuk 2006). Seed zones have been developed in particular for forest trees whereas few examples exist for herbaceous species (Mortlock 2000; Waters 2001). In Flanders four main seed zones are delineated for woody species, based on variation in climate and soil, and on the distribution ranges of different plant species (Vander Mijnsbrugge, Cox, & Van Slycken 2004). In the UK, 24 local seed zones are defined based on major geological and climatic regions modified by altitude (Herbert, Samuel, & Patterson 1999). In Switzerland, guidelines include herbaceous species and recommend seed exchange within four macroregions delineated by a combination of geomorphology and climate. For rare and genetically variable species eleven regions are distinguished (SKEW 2002). Frequently, the borders of environmentally based seed zones are arbitrary and it is unlikely that they reflect the real genotypic differentiation of a plant species. Molecular markers such as AFLP (amplified fragment length polymorphisms), microsatellites or chloroplast DNA provide a relatively new, powerful

Fig. 1. Population differentiation for *Rosa arvensis* in Flanders, sampled in two regions 50 km apart, both belonging to the same official seed zone Brabants District West. (A) Biplot of a Principal Components Analysis of AFLP results for individuals sampled in both regions. Full circles: West-Vlaams Heuvelland; open circles: Vlaamse Ardennen. (B) Box plots showing the morphological differentiation of the diameter of the hip orificium (opening where styles emerge, left panel) and the length of the pedicel (hip stalk, right panel). WVH: West-Vlaams Heuvelland; VAR: Vlaamse Ardennen (based on unpublished data).

tool, which may help to delineate seed zones (e.g. Krauss & He 2006; Krauss & Koch 2004). For example, for *Rosa arvensis* a Flemish seed zone is divided, as both a morphological and a molecular marker analysis demonstrated population differentiation in two regions within the seed zone (De Cock 2008; Fig. 1). The delineation of seed zones for *Quercus petraea* in Flanders aims to maintain the structure of the evolutionary migration lineages of oak, as revealed by cpDNA analysis (Vander Mijnsbrugge, Coart, Beeckman, & Van Slycken 2003), because these reflect the historic biogeography (Petit et al. 2002). In Great Britain, seed zones for *Pinus sylvestris* have been developed on the basis of biochemical and molecular data (Hubert & Cottrell 2007).
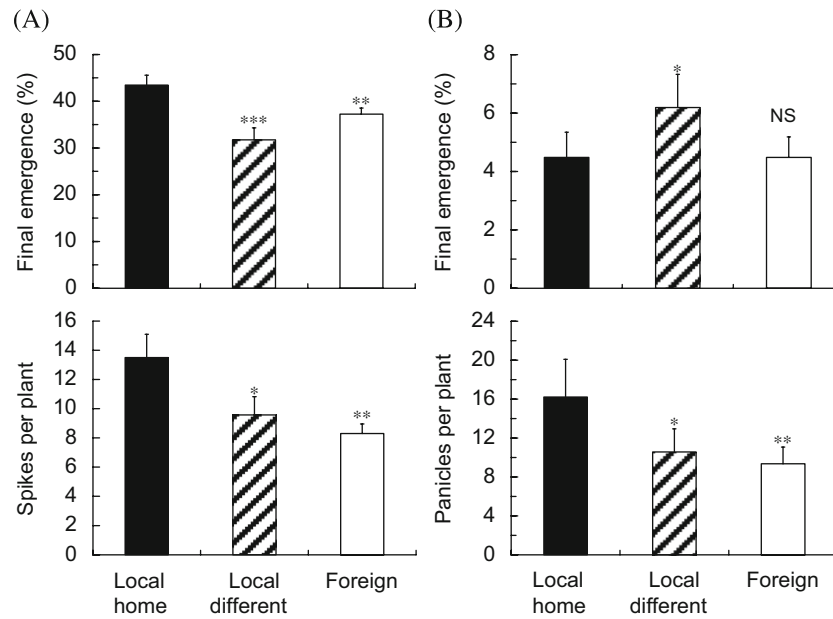
However, molecular markers are neutral and do not respond to selection as adaptive traits do (e.g. McKay & Latta 2002). They may overestimate differentiation between distant populations of similar habitats because isolation and random genetic drift increases neutral variation, whereas similar selective forces result in small differentiation of quantitative traits (Petit et al. 2001). Advanced molecular methods such as QTL mapping, which relate genetic analyses to the phenotype, or a combined analysis of neutral molecular markers and quantitative traits can help to overcome these limitations (Petit et al. 2001; Savolainen et al. 2007).

Small-scale differentiation and "naturalness" of source populations

The delineation of seed zones has two potential constraints: small-scale differentiation and the long tradition of translocation by humans (agro-ecosystems, forestry). The relationship between the geographical distance over which a population is translocated and the fitness of translocated plants may be weak (Montalvo & Ellstrand 2000; Smith, Diaz, Winder, & Daniels 2005), and studies comparing populations at different scales sometimes reveal larger within than among region differentiation (Berg, Becker, & Matthies 2005; Santamaria et al. 2003).

Adaptation to different local habitat types has been reported at small scales of 500 m or less (Waser & Price 1985). In particular, genetic differentiation has been found to be strong along humidity and heavy metal gradients (Bradshaw 1984; Linhart & Grant 1996; van Tienderen 1992). In *Hydrocotyle bonariensis* and *Ranunculus reptans,* local adaptation occurred even within populations among plants of higher and lower elevations of a sand dune ecosystem (Knight & Miller 2004; Lenssen, van Kleunen, Fischer, & de Kroon 2004). Thus, genetic differentiation has been maintained in spite of the homogenizing effect of gene flow. Small- and large-scale adaptive differentiation was directly compared in a study on *Holcus lanatus* and *Plantago lanceolata* (Bischoff et al. 2006a). Differences in fitness-related traits were nearly as large between contrasting local habitats (distance 0–30 km) as among European regions (distance 600–1100 km; Fig. 2). The results suggest that small-scale differentiation can be a serious constraint in the delineation of geographically determined seed zones. Populations that are further away but from similar habitats may be better adapted than neighbouring populations from a contrasting habitat. In such cases the concept of seed zone would be only valid for populations of the same habitat type, i.e. the zones

Fig. 2. Seedling emergence (upper figures) and reproduction (lower figures) of two populations from contrasting local habitats ("local home" and "local different") and from distant foreign provenances; significant linear contrasts "local home" vs. "local different" and "local home" vs. "foreign" are indicated; ns: not significant, *$P<0.05$, **$P<0.01$, ***$P<0.001$; $\pm$SE; modified from Bischoff et al. (2006a). (A) *Plantago lanceolata* and (B) *Holcus lanatus*.

have to be defined on the basis of environmental distance, which is only weakly correlated with geographical distance (Montalvo & Ellstrand 2000; Smith et al. 2005; Wilkinson 2001). This complicates the collection of appropriate plant material because environmental conditions which determine habitat differentiation may not always be obvious. Molecular genetic analysis provides valuable additional information on spatial scales of differentiation but again results based on neutral markers have to be interpreted with care. Gene flow reduces neutral variation at small scales but differentiation in traits that are under strong selection may be maintained (Steinger, Haldimann, Leiss, & Müller-Schärer 2002).

The second constraint in defining seed zones are 'non-natural populations' resulting from a former introduction of non-local plant material. This is of particular importance for woody plant species as transportation of seeds and planting stock over long distances is a much older and more widespread tradition than in wild herbaceous plants. However, some herbaceous crop plant and forage grass genotypes have been translocated for centuries. The risk of selecting inappropriate seed sources is twofold. First, an introduced population may be mistaken for a local one and maladapted seed may be sourced. Second, a local population may be collected, that is growing close to introduced plants, which may influence the offspring of the local population through natural cross-fertilisation. Hence, the reliability of seed zone delineation depends on the degree of former plant translocations and on the possibility of distinguishing

local from non-local populations. One way to tackle this problem is the establishment of authorised registers to identify remaining local populations of woody plants (Flanders: Vander Mijnsbrugge, Cox, & Van Slycken 2005; Great Britain: Wilson & Samuel 2004; Germany: Kowarik & Seitz 2001). Established registers are restricted to woody species and there are no similar initiatives for herbaceous species.

Genetic diversity of the sampled material: how to collect seeds

General considerations

If seeds are collected from limited sources, i.e. individuals or populations, insufficient genetic diversity may result in low establishment rates and a low fitness of the restored population (Falk & Holsinger 1991; Hufford & Mazer 2003). Inbreeding depression and genetic drift are potential causes for such founder effects. Inbreeding depression may occur because the probability of subsequent mating between closely related offspring increases if introduced seeds are only collected from few individuals. The negative effect of inbreeding on plant population fitness has been demonstrated for many species (Luijten, Kéry, Oostermeijer, & den Nijs 2002; Montalvo 1994; Vergeer, Sonderen, & Ouborg 2004). Population fitness is usually correlated with the degree of heterozygosity which is low in inbred

populations (Oostermeijer, van Fijek, & den Nijs 1994; Williams 2001; Vergeer, Rengelink, Copal, & Ouborg 2003). Procaccini and Piazzi (2001) found that degree of heterozygosity might be more important than plant provenance. A low degree of heterozygosity could also be the result of random genetic drift which is an additional risk if populations are small and isolated (Ellstrand & Ellam 1993; Oostermeijer et al. 1994; Vergeer et al. 2003). If seeds are collected from small inbred populations even a relatively high number of source plants may not be sufficient to avoid negative founder effects. Montalvo et al. (1997) propose the 'effective population size' (N_e) to evaluate genetic diversity in source and restored populations by considering percentage of reproductive individuals, sex ratio and fluctuations in population density. Typically N_e is smaller than the census population size (N_c) but there are situations in which N_e and N_c are similar (Gomory, Longauer, Paule, & Bruchanik 2008). However, N_e is difficult to estimate for plants, particularly if vegetative reproduction occurs, and for practical reasons census population size is used as an estimate of genetic diversity in ecological restoration (Montalvo et al. 1997).

More recently, positive effects of high genetic diversity on population fitness have been demonstrated independent of genetic drift and inbreeding depression (Bischoff, Steinger, & Müller-Schärer in press; Johnson, Lajeunesse, & Agrawal 2006). Extending the idea of the species diversity–productivity function, there may also be a positive relationship between genetic diversity and population biomass (Crutsinger et al. 2006). A high genetic diversity may be a biological insurance against fluctuations in ecosystem processes and it increases the stability of restoration measures (Hughes & Stachowicz 2004; Reusch, Ehlers, Hämmerli, & Worm 2005).

Number of individuals to be sampled

Research on the relationship between genetic diversity and population fitness has demonstrated its importance in ecological restoration but the question of how much diversity is needed remains. Falk and Holsinger (1991) and Brown and Briggs (1991) recommended sampling 10–50 individuals per population, a suggestion that has been adopted in other guidelines and publications (Roberts & Bishir, 1997; SKEW 2002). However, their aim was to establish *ex-situ* cultures of very rare species, which should be sampled with care in order to minimize the impact on source populations. Meanwhile, many studies have shown a clear positive relationship between population size and plant performance (Fischer & Matthies 1998; Vergeer et al. 2003) suggesting that a sample of 10 or 20 individuals would be far too small to avoid founder effects. The shape of the population size-performance curve appears to be species specific and

depends largely on the breeding system (Leimu, Mutikainen, Koricheva, & Fischer 2006). The fitness of self-incompatible species is much more dependent on genetic diversity and population size than that of self-compatible or purely selfing species. There is still a need for further research in order to identify the minimum numbers of individuals to be sampled.

Collecting several populations to increase genetic diversity?

If source populations are small and/or inbred, sampling several populations could be an alternative to maximizing plant number per population. Falk and Holsinger (1991) advocate the collection of 3–5 populations for the re-introduction of endangered plants if the species are not extremely rare. Vergeer et al. (2004) recommend a mix of non-local populations when local populations of sufficient diversity are not available. However, the risk of out-breeding depression increases with genetic differences between parents, and genetic differences are usually correlated with spatial distance. Fischer and Matthies (1997) and Waser and Shaw (2000) found the highest fitness in progeny from crosses between parents that were 10 m apart. Both lower (inbreeding depression) and higher distances (outbreeding depression) including interpopulation crosses, resulted in lower progeny fitness. So far, there is more and stronger experimental evidence for the negative effects of inbreeding (and other negative effects associated with low genetic diversity) than for outbreeding depression (Keller & Waller 2002). Yet experiments and manipulative studies do not sufficiently consider mechanisms for avoiding inbreeding in natural populations (Edmands 2007). Furthermore, outbreeding depression may be underestimated because it can be superimposed by simultaneous inbreeding depression for several generations. We suggest that different populations should only be mixed when there is hard evidence for inbreeding depression (Edmands 2007) and/or when source populations are very small. This also applies to genetic reinforcement approaches, i.e. the introduction of individuals from other populations to increase genetic diversity of small and inbred populations (Becker, Reinhold, & Matthies 2006; Luijten et al. 2002; Petit et al. 2001). The importance of outbreeding depression in natural plant populations is still poorly understood and this aspect of conservation genetics requires further research.

Seed transfer to restoration sites

Direct transfer techniques

The efficacy of methods for transferring local grass-land species from source to restoration sites have

recently been investigated both in Germany (Kiehl, Thormann, & Pfadenhauer 2006; Kiehl & Wagner 2006) and the UK (Edwards et al. 2007; Mortimer et al. 2007). The principal methods are hay strewing (known as 'green hay' in the UK), and sowing brush harvested seed. Hay strewing involves the immediate transfer of vegetative material cut at an appropriate time. It transfers a wide range of species and enhances the successful establishment of invertebrate communities (Edwards et al. 2007; Kiehl & Wagner 2006). But, as hay cutting removes the majority of seeds, it has a relatively high impact on the donor site which matters at least for monocarpic species. In contrast, brush harvesting requires specialist machinery and captures only a proportion of the species present, however, it does not deprive the donor site of seeds, as each harvest collects between 10% and 40% of the seed available (D. MacIntyre, Emorsgate Seeds (UK) 2007, personal communication). On balance, the hay strewing method is more accessible to contractors and farmers as it uses readily available machinery and samples a wider phenological window, which reduces the need for multiple collections, although it is not possible to store collected material (Mortimer et al. 2007). Brush harvesting is a specialist activity but it allows the practitioner to target the collection of species and material can be cleaned and stored providing an opportunity to remove 'weed' species. Both methods have been shown to be effective (Edwards et al. 2007) but one harvest may not suffice to collect the full spectrum of species (Kiehl et al. 2006).

Propagation in stock or seed orchards

The alternative to direct transfer techniques is to collect seeds and propagate them as a crop. Recently, seedsmen have taken up the challenge and tailored herbaceous seed mixtures have been produced using hand collected, propagated seeds. These mixtures are known to be well targeted and effective. In contrast to direct transfer methods, a sampling strategy must be devised and seed cleaning is required to remove undesired species. First, to guarantee successful germination and a high offspring fitness, seed companies tend to select for larger seeds during seed cleaning. Such a selection may reduce genetic diversity; the production of seeds of variable size may be part of an adaptive bet hedging strategy. Seed size is known to affect long-lasting differences in fitness among offspring (Halpern 2005) and smaller seeds have been shown to have advantages in some conditions (Hendrix, Nielsen, Nielsen, & Schutt 1991). Second, it is known that variation in seed production per plant can lead to a reduction in effective population size (N_e), which, as we have observed above, is a factor leading to genetic drift.

If seed collection is conducted carefully, the relationship between N_e and the census population size (N_c) can be optimized. Johnson, Bradley, and Evans (2004) showed that sampling a constant number of inflorescences per plant across a number of plants will increase N_e and that 3 inflorescences per grass is sufficient to maximise N_e/N_c. Third, seeds of short-lived species are often grown over several generations in stock and repeated selection processes (i.e. harvest date and technique, and seed cleaning) can lead to genetic changes or even impoverishment. There are three possible deleterious effects of keeping stock ex-situ and regenerating seed over several cycles: (1) natural populations will track environmental changes and adapt accordingly but populations held ex-situ will not (Lynch & Lande 1993), (2) concomitantly, the plants raised in a garden may adapt to the cultural conditions they are exposed to (Schoen & Brown 2001) and (3) loss of variation by genetic drift will increase with each cycle so that a threefold increase in sample size is a minimum for the maintenance of allelic diversity (Schoen & Brown 2001). Therefore, in cases of repeated propagation from a narrow genetic base, seeds should be harvested for a low number of regeneration cycles (Schoen & Brown 2001 suggest that six is a reasonable number) and fresh collections should be made at regular intervals. Overall, propagation is expensive, requiring substantial investment in land, equipment and time; it is less likely to be used to raise seeds from discrete locations on a commercial scale, but is likely to be adopted by nature conservation groups on best practice sites. There are a number of initiatives across Europe to provide appropriately sourced seeds for small-scale projects (see www.floralocale.org in the UK). Although commercial scale production of 'local' seeds is rare in Europe, in America the USDA supports and manages projects raising seed from discrete areas on a commercial scale (http://www.fs.fed.us/wildflowers/nativeplant-materials accessed 08/07/2008) and similar work is carried out in Australia (www.florabank.org.au accessed 08/07/2008) although typically projects receive financial backing from governments or NGOs.

For trees and shrubs seed orchards or officially approved seed stands are common methods of propagating economically important species. The challenge is to implement similar methods for local populations. In Flanders, local populations from an authorised register are approved as official seed sources. Also, local individuals are vegetatively propagated and planted in orchards (Vander Mijnsbrugge et al. 2005). The limitations of this approach are predominately due to the inevitable selection process undertaken when collecting individuals for the orchard. In addition, the consumer demand for uniform planting stock leads to further loss of variability in transferred material. This problem has already been identified in the course of current tree breeding programs (El-Kassaby 2000). For ecological

restoration purposes, maintenance of the genetic diversity is a priority and it is better to avoid any type of selection during seed collection, seed processing and in the nursery practices.

Legislation

Legislation controlling the use of seeds is limited and much of what exists relates to the use of native species at the national scale. World-wide, the most developed area of legislation is that which controls forestry. Within Europe, national laws of member states regulating the procurement and sale of forest reproductive material have been streamlined through a European directive (Council directive 1999/105/EU amending 66/404/EEC and 71/161/EEC within the Forest Reproductive Materials (FRM) Regulations). The Member States are required to establish regions of provenance and national registers of approved material so that reproductive material which is intended for marketing, or which is placed on the market, is properly identified from collection to delivery to the end user. However, the EU directive does not control the use of FRM. This is effected by national subsidies and guidelines which are frequently voluntary. The attention to non-commercial species is relatively recent and will take time to filter into policy. There is poor legislation across Europe for herbaceous 'wildflower' and grass species sown in restoration schemes (no EU directive). Guidelines are issued piecemeal under national legislation and vary in detail and utility. While the marketing of herbaceous seed is partly regulated, the use of seed is unregulated in all member states, relying on uptake of voluntary initiatives and adoption of guidelines. Guidance on seed collection is patchy and there is no legislation and little advice covering use.

Legislation requires a broad consensus of scientists and affected users. Results of scientific research fail to influence policy, but this is partly due to unsuccessful communication (Rayner 2004). The inevitable ambiguity of scientific research may be used to avoid difficult decisions (Lawton 2007) and is a serious threat to the development of effective environmental law (Carden 2006). Furthermore, policymakers and managers may be influenced by a number of pressure groups and are constrained by commercial concerns (Brunner & Clark 1997). Two approaches may go some way to addressing these issues. First, the scientific community has to express the results in a non-scientific language that can easily be understood by practitioners, something which scientists in the field of restoration ecology have been accused of failing to do (Weiher 2007). Second, and crucially, stakeholders must be engaged, as the barriers to change are at least partly social in nature (Szaro et al. 1998).

Recommendations

When natural colonisation is unlikely, practitioners need to make informed decisions on seed sourcing. In addition to the general rules that apply in all cases, we identify two specific approaches (Table 1). The first is a conservative approach, focussed on the re-introduction of rare or locally extinct species/plant communities in areas of high conservation value. The second is a coarser approach, relevant in areas of lower conservation value, which aims to increase the species richness of degenerate plant communities or establish vegetation on bare ground in less sensitive areas. The latter approach may also be appropriate for sites requiring large amounts of seeds such as forest plantations and (re)vegetation schemes along motor- and waterways, on ex-arable land or in mining areas. In the first approach (Table 1), direct methods such as manual transfer or the application of hay cuttings are preferable, using the closest source population from the same habitat type. Ideally, the degree of heterozygosity within the source populations would be assessed in order to identify inbreeding depression and the need to sample more than one population. Location and size of the source population, the number of mother plants collected and a potential mixing of different populations should be documented. It is also important to consider whether populations at the recipient site are threatened by high levels of inbreeding and need to be 'rescued' by the introduction of unrelated stock (Kaye 2001). Here, conservation priorities justify the potential higher costs of small-scale seed collection. The process should be overseen by nature conservation authorities. In the second approach (Table 1), direct transfer methods are less feasible as the large amount of seed requires propagation in stock. Here, seed zone delineation, based on environmental criteria rather than on geographical distance, would be a valuable aid for commercial seed collection. Criteria for seed zone delineation should include large-scale environmental factors such as climate, geology and geomorphology and as long as data on spatial scales of population differentiation are unavailable, the transfer within a seed zone should be restricted to the same habitat type. Regulations should stipulate that the habitat of the collection site is well documented and that populations are kept separate. Zones may be further refined by considering species-specific life history traits such as breeding system, pollination vectors and dispersal regimes (e.g. Krauss & He 2006). A genetic analysis of source populations may be too expensive but the size of sampled populations and the number of plants from which seeds are collected should be documented. Seeds should be sampled from at least 50 mother plants to guarantee sufficient genetic diversity. If individual source populations are too small, mixing

Table 1. Summarised recommendations for seed collection and use.

	Where to collect	How to collect	Transfer method and propagation
General rules	• Collect locally • Match habitat as closely as possible	• Sample at least 50 individuals per source population, record the number and location • Collect preferably one population, mix different source populations if they are too small or inbred	• Choose an appropriate transfer method considering conservation value and amount of seeds required
First approach: areas of high conservation value, reintroduction of rare plants	• Collect from closest source populations • Collect from same habitat type	• Record size of the source population and/or determine degree of heterozygosity to identify need for population mixing	• Transfer directly without propagation, e.g. hay strewing, brush harvesting or manual transfer
Second approach: areas of low conservation value, restoration of industrial/agricultural sites, grasslands, reforestation, requiring large amounts of seed	• Collect from populations of the same seed zone, based on climate and geomorphology • If possible, refine seed zones by considering life history traits and genetic structure of source populations	• Collection of several source populations often not avoidable to obtain sufficient amounts of seed but keep them separate and respect general rules (see above)	• Propagate collected seeds and replenish stock at regular intervals to prevent genetic drift • Avoid seed or seedling selection during propagation and transfer

of different populations within the same seed zone and the same habitat type is possible but should be considered with care, as little is known about genetic architecture and spatial scales of population differentiation. In general, it is preferable to source from the closest population of sufficient size than to have mixtures from many populations within a seed zone.

In both approaches, there should be no selection of seed-based criteria such as size or production date, especially during propagation in stock. To do so may lead to reduced genetic diversity. If seed is known to be polymorphic and different types of seed are produced in different periods then repeated sampling is recommended. Seed cleaning should be employed to remove undesired species but not smaller seeds, as seed size affects dormancy and selection may change the genetic architecture of the seed banks and restored populations. Stocks or orchards of perennial species may be kept for a long time but stocks of short-lived species should be regularly replaced (maximum 6 years) with fresh collections from the wild unless selection effects and inbreeding are controlled by genetic analyses. Inevitably, there remains a need to continue research. Currently, much research is novel, small scale and short term (Robertson & Hull 2001). The few large-scale studies (Becker, Colling, Dostal, Jakobsson, & Matthies 2006; Becker, Reinhold, & Matthies 2006; Bischoff et al.

2006a; Joshi et al. 2001) are also time limited. In particular, further information on the loss of diversity during propagation in stock is required.

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