

**Restoration of wooded landscapes –  
Informing assumptions with microsatellite technology**

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NB. Whilst this review aims to have been as extensive as possible in addressing the commissioning brief, it cannot guaranteed to be exhaustive.

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## Executive summary

Microsatellites are a very powerful molecular marker tool. They can and have been used extensively to understand the contemporary population dynamics and connectivity of individuals within different landscape settings, and are arguably the best molecular marker currently available for such purposes. The literature on conservation genetics has ballooned since the introduction of these markers. Numerous studies now exist that examine the dynamics of genetic diversity across changing landscapes, including those subject to fragmentation. Using specialized statistical methods and variation within nuclear and organelle genomes, microsatellites also offer insights into historical colonization processes and population evolutionary history, which are informative for making predictions of contemporary and future colonisation dynamics and range shifts, e.g. due to landscape changes and climate change.

One of the strengths of a PCR-based marker like microsatellites is that it can be used with very small quantities of living and/or dead tissue of the study organism. Thus it is possible to use historically collected material (e.g. from herbaria or museums), and has added a temporal context to several studies. Whilst not a universal marker (one which can be applied to any organism), microsatellites can be developed for almost any species with even modest research budgets. Microsatellites have been isolated for a range of species, including, plants, mammals, birds, insects and fungi. Whilst microsatellites do not give the researcher direct access to community or habitat assessments of species richness, by applying a range of recently developed statistical methods, they inform on the processes of propagule, individual and population movement and dynamics for specific species.

Microsatellites have been applied to an increasing number of studies of European flora and fauna. This review documents a representative sample of this research to examine; historical colonization, contemporary gene flow dynamics and the impacts and restoration of fragmented landscapes. This review finds that microsatellites are suitable for assessing each of these parameters and give detailed information on an individual species response. A key concern of many surveyed studies is that genetic diversity and connectivity processes are impacted by fragmentation. This is evident across taxonomic groups. Strategies to improve habitat connectivity, such as corridors and stepping stones, are demonstrated to improve patch connectivity and increase genetic diversity. However the literature also indicated that connected remnant networks of this type were not as effective at maintaining diversity as large blocks of continuous habitat.

Three key and related issues are prioritized for future research development:

- Undertake genetic studies for a range of species (flora and fauna with a range of life history characteristics) within heterogeneous fragmented landscapes containing different patch sizes, isolations and histories. Studies should either be done within a single location or aim to integrate studies that control for scale and habitat quality.
- Improved evaluation of natural dispersal/recruitment processes and corridors/stepping stones as methods to maintain diversity and rehabilitate habitats.
- Improved understanding of genetic diversity and differentiation of individuals/populations to be used for reintroduction and rehabilitation. Knowledge is required on the number, proximity and habitat/environmental context of reintroduced individuals to maximize diversity and adaptive potential whilst minimizing genetic erosion.

## Detailed summary

This review aims to provide a detailed survey of the existing literature on the application of hypervariable molecular markers, such as microsatellites, to studies of species genetic diversity and dynamics (dispersal and colonization) within wooded landscapes, and their potential application to inform restoration programmes.

Within species diversity, assessed using neutral genetic marker systems such as microsatellites, is largely dictated by the number and spatial scale of individuals that are in reproductive contact (connectivity). Thus, studies of species dynamics within past and contemporary wooded landscapes using such molecular marker systems tend to focus on the dispersal and colonization dynamics of individuals, propagules and populations of a species. These processes are generally grouped under the label, gene flow. However, it is important to differentiate gene dispersal - the total dispersal capacity of individuals and populations in terms of genes and migrants; and gene flow - successful dispersal events (i.e. leading to successful migration of an individual or establishment of reproductive propagule). Gene flow and dispersal can be split into gamete (e.g. pollen), propagule (e.g. seed) and adult (e.g. migrant) components, the latter two of which can be described as colonization. This terminology will be applied throughout this review.

The review is divided into four sections, as follows;

1. **Introduction to genetic diversity and gene flow:** a background on the main factors (biological and environmental) that influence genetic diversity and gene flow. Includes review of the main approaches used to study genetic variation and dynamics within populations, and the temporal and spatial scales at which such methods can be applied.
2. **Review of genetic marker systems:** a background to all the potential molecular marker methods that are used to quantify genetic diversity and gene flow including their scope and disadvantages, with a more detailed justification of microsatellites and the effort involved to develop them. This section also includes a discussion of guidelines for best sourcing and preserving practices of material for genetic analysis, and a list of British woodland species (plants, animals, insects) for which SSRs are currently available.
3. **Statistical methods for analyzing gene flow:** a background and brief review of the main statistical methods used to describe genetic diversity and gene flow.
4. **Review of studies of genetic diversity and gene flow within wooded systems:** a review of British and European studies based on the available literature.  
This section is ordered under three main subheadings:
  - a. Historical colonization of woodland species in Britain
  - b. Contemporary gene dynamics within woodland systems
  - c. Impacts of woodland clearance and potential for restoration.

By way of summary, the following specific questions, asked in the commissioning brief, are answered succinctly based on the more extensive content of the review.

**1. *Does the development of hypervariable molecular markers, such as microsatellites, offer a novel tool for studying whether new woodlands reverse the effects of fragmentation? For example, is it possible to identify unique markers for populations (present in source woodlands) and therefore identify the source of individuals present in more recent woods?***

Microsatellite markers have now been in use for more than a decade and they have proved to be an extremely useful molecular marker system. They are very suitable for assessing the dynamics of genetic diversity, differentiation and gene flow of populations over time due to natural and human-induced habitat and environmental changes. Nuclear encoded microsatellites are codominantly expressed and can be used with a broad range of statistical analyses to provide insights into population histories and dynamics. Although other universal marker systems are available, these are generally inferior to microsatellites due to the lower levels of polymorphism highlighted (isozymes) or the dominant nature of markers generated (AFLP, RAPD, ISSR). Another set of molecular markers is derived from the organelle genomes (chloroplasts and mitochondria), which are haploid and lack recombination. Microsatellite loci can also be found within the organelle genomes and are particularly common in the chloroplast of plants (cpSSRs). Whilst superior to nuclear microsatellites for identifying population/species evolutionary history (due to the phylogenetic interpretability of genetic variation), organelle molecular variation (including cpSSRs) are generally not biparentally inherited and thus only give the evolutionary history of the maternal or paternal lines (depending on inheritance pattern of the genome). Such organelle markers are however often used in consort with nuclear microsatellite studies, where they provide complementary information on male gametic or propagule only gene flow and colonization.

The ability to uncover high levels of genetic variation is an essential prerequisite to the calculation of many genetic diversity and gene flow parameters. Being highly polymorphic (although this depends on the type and number of loci screened), microsatellites are probably the best marker system for identifying private alleles (unique to populations or individuals). The identification of such alleles (in both nuclear and organelle SSR loci) is a great asset in assessing the dynamics of gene flow within and between populations, and can be appropriately used to determine the source population of newly established individuals/populations (e.g. woodland species).

**2. *Do limits exist for the temporal scale at which conclusions can be drawn regarding species dispersal?***

Depending on the type of sampling procedure, molecular marker system and statistical analysis applied, a range of temporal scales can be assessed using genetic technologies.

Contemporary scale – gene flow dynamics can be assessed over the course of a single year, season or day and can include male gamete-mediated gene flow (e.g. pollen), propagule/progeny dispersal (e.g. seeds or young individuals) or migration. Following genotyping of potential source individuals/populations a sample of new gametes,

propagules, progeny or individuals can be assigned to a most probable source using statistical matching techniques which utilize the exclusion probabilities generated by hypervariable markers (e.g. SSR, AFLP and if enough loci are used, isozymes and RAPD/ISSR). Assessing organelle markers can also provide complementary insights into gamete or propagule-only dynamics.

Recent generation scale – individual and population level mating system/gene flow dynamics, and population connectivity/colonization/contraction/expansion can be inferred by assessing the genetic structure of a sample of mature individuals. The ‘genetic footprint’ of past events can last for a few generations (approximately 1 to 4), particularly if populations of a species are characterized by overlapping generations of long lived individuals (e.g. trees). However, for species with a short generation time (e.g. insects and annual plants) historical processes (e.g. colonization bottlenecks) will be very difficult to differentiate from contemporary population drivers. Codominantly expressed nuclear markers are the best systems for such analyses (SSR or isozymes), although dominant markers can also be used (AFLP, RAPD, ISSR), recognizing that the latter type of marker have more limited analytical power. The assessment of organelle markers (including cpSSRs) can provide complementary insights into gamete or propagule-only dynamics.

Evolutionary history – the historical colonization dynamics of species (processes occurring over many generations, from 4 to hundreds, and dating back thousands/millions of years) can be followed by assessing the geographical distribution of phylogenetically interpretable markers. The source and sink of populations and whether they are in expansion or contraction phases can be inferred from such markers. Time scales include colonization dynamics over a few hundred years, range shifts since the last ice age (last 10,000 years), and the ancient expansion of species into new regions following major geological/environmental changes (processes generally of millions of years). Successful analysis of historical dynamics requires that contemporary populations have accumulated a proportion of genetic variation over the time period of interest and that populations in historical source and sink regions still exist. Organelle markers are most suitable for such phylo geographic studies, although some nuclear regions (applying sequencing information) can also be used. Microsatellites have also been used for such studies, when a stepwise mutation model that assumes an evolutionary relationship between alleles according to size differences between them, is applied. The results of applying microsatellites to such investigations is generally not as powerful as more phylogenetically robust marker systems (e.g. from the organelle genomes) due to homoplasy (surveying multiple loci limits this problem). A maximum time limit may operate due to limitation on the number of tandem repeat size differences that can accumulate at microsatellite loci.

- 3. For which taxa is this analysis possible? Ideally I would like to consider a selection of plants, invertebrate and mammals, either listed or with SAPs. Are microsatellites the most appropriate markers and if so, in which British woodland species have microsatellites been developed? Are there any particular species for which it is a priority to develop such markers?***

Unlike other molecular techniques (i.e. isozymes, RAPD, AFLP, ISSRs, organelle markers), microsatellite loci have to be isolated, developed and optimized for each species to which they are applied. There are no 'universal primers' available for SSR loci, unlike the above mentioned techniques which can be considered off-the-shelf. Whilst some microsatellites can be transferred amongst species within the same genus and even within some families, there are no short cut methods to ensure success. Even if transferred loci are polymorphic, there is always the risk of null alleles (PCR product is not amplified due to mutations in the primer binding site).

To date many microsatellites have been isolated for a range of species (including many British woodland species, See Appendix 1). However, the cost of microsatellite development is fairly substantial, particularly if a multi-species approach is required. A microsatellite enriched clone library (50-90%) for a single species can be generated by a number of biotech companies for around US\$5-10,000, although it could be done much cheaper using in-house facilities (<US\$1500) or within a negotiated large-scale commercial contract. In order to obtain 10 polymorphic microsatellite loci, it is usually necessary to sequence an average of 72 clones from a highly enriched library. On average, this would be followed by the design and testing of 24 primer pairs (a contingency of 10-20% should be included for difficult species). These further steps add further cost but are difficult to quantify as they tend to be laboratory specific.

It is now appreciated that microsatellites also exist in coding regions of the genome. Based on a total of 561,932 EST sequences, large numbers of microsatellites have been found in the coding regions of spruce (n=188), poplar (n=1523), loblolly pine (n=426), *Arabidopsis* (n=3626) and rice (n=5138). From tests across 23 spruce species, 57% of EST-SSRs were widely applicable, compared to only 18% of traditionally developed markers. Thus a new generation of microsatellites, which have greater utility across species, may be on the horizon.

Despite restrictions and costs, microsatellites can be isolated for a whole range of species (i.e. plants, animals, birds, insects and fungi), and due to their utility, millions of loci have now been developed for hundreds, if not thousands of species. There is even a new journal, *Molecular Ecology Notes*, published quarterly, which is primarily dedicated to reporting the development of new microsatellite loci. A list is provided within this review of representative British woodland species (including plants, animals and insects) for which SSR loci are currently available.

**4. What are the practical limitations to these techniques? For example, must samples be living tissue or is hair etc suitable? (Important as this will determine species choice) and what are the minimum quantities of tissue required for analysis?**

As for all PCR-based methods, only small amounts of preserved or fresh material are required for analysis of microsatellites (grams or milligrams of material). The exponential amplification basis of the polymerase chain reaction (PCR), means that if the chemistry is correctly optimized, contamination minimized and sufficiently sensitive screening methods are used (e.g. capillary sequencer), only a few copies of the target locus need to be present in a sample to obtain a genetic profile. This principle has allowed DNA to be

analysed from a variety of miniscule samples (single hairs) and ancient sources (herbarium, museum and macrofossil remains). The combination of contemporary and historical sources has added a further dimension to many population genetic studies. However it should be noted that for many animal samples, Home Office licenses are required which may be difficult to obtain.

**5. *What criteria make a species, or a range of species, suitable for analysis?***

All species should, in theory, be suitable for microsatellite analysis. However, recently evolved polyploid taxa may have more than one copy of a locus (there are 2 copies in heterozygous diploid individuals), and make calculation of population genetic parameters problematic/impossible. This problem would also apply to other marker systems. Other problematic species are those with high levels of asexuality or autogamy, or those that have been through a recent and very severe bottleneck, as this will limit the amount of genetic variation between individuals within populations to calculate statistical parameters. Again other marker systems would not be any better than microsatellites when applied to such species.

**6. *What limitations exist for such studies? For example, do we have the statistical methods for dealing with gene flow data on a landscape scale?***

The statistical methods to analyze gene flow within and between populations and individuals has seen something of a recent renaissance and, in particular, historical and spatial components of individual and population dynamics have been developed adding to an already mature and well tested set of statistical procedures. This review summarizes the main procedures that can be applied to analyze historical and contemporary dispersal and the colonization of individuals, populations and species.

# 1. Introduction – Genetic diversity and gene flow

Genetic diversity within species is largely dictated by the number and spatial scale of individuals within genetic connectivity. Where low numbers of individuals are in contact (e.g. small populations or self fertilizing individuals) genetic diversity will be low, but where large numbers of individuals are in genetic contact high levels of genetic diversity will be maintained within populations. The spatial scale of such genetic neighbourhoods is determined by the dispersal capabilities of the propagules and individuals of a species. Even for non-vagile species (e.g. trees) their gametic (pollen) and propagule (seed) dispersal mechanisms can be extremely effective in establishing genetic neighbourhoods of several 10s to 100s of kilometers. Thus, studies of species dynamics within past and contemporary landscapes tend to focus on dispersal and colonization dynamics of individuals, propagules and populations of a species. Under contemporary and future scenarios of habitat clearance, abandonment and climate change it is also these processes that will be most critical in dictating species' responses (Young *et al.*, 1996; Williams *et al.* 2003; Davies *et al.* 2004).

Term definition: Dispersal describes the movement of individuals and/or their gametes/propagules within or between populations. Gene flow describes the proportion of newly immigrant genes moving into a given population (Endler 1977), and implies successful dispersal of genes. In contrast, not all dispersal events will result in successful gene dispersal, thus these two terms will be used explicitly to differentiate these two processes throughout this review.

Gene flow is an essential aspect of the proliferation and spread of species, and migration of genes may be mediated by adult or juvenile individuals and/or their reproductive propagules in the form of sexual/asexual gametes, zygotes, and/or clones. The nature and extent of gene flow is largely dependent on the intrinsic biological characteristics of the organism: mode of reproduction (asexual versus sexual; hermaphroditism versus biparental outcrossing); mobility of individuals (vagility) and their propagules (gametic and zygotic dispersal mechanisms and behaviour); timing of reproduction (can result in temporal isolation of populations and affect potential gene flow patterns); individual density (can affect the size of breeding neighborhoods, Franceschinelli and Bawa 2000); behaviour of animals or plant pollinators and seed dispersers; progeny survival and establishment (dependent on adaptation to microsite conditions, Nathan and Muller-Landau 2000).

In addition to these intrinsic characteristics, it is becoming increasingly clear that the magnitude and rate of gene flow is strongly affected by the environment that an organism inhabits. Topographic and hydrological features of an organism's environment strongly influence the scale and direction of genetic connectivity between individuals and populations of a species. Furthermore, changes in land use and habitat fragmentation can also strongly affect gene flow dynamics (Young *et al.*, 1996). It is only once we understand how organisms disperse their genes and the ecological requirements for propagule establishment, that we will be able to understand and predict the likely effects of contemporary habitat or environmental change on genetic diversity and gene flow.

A suite of methods and models has been developed to study gene flow, and can be classified into two approaches; indirect and direct methodologies.

Indirect methods use the distribution of genetic variation within contemporary populations to infer the magnitude of gene flow between them. Using indirect methods, only successful gene flow events are considered, that is, those that have led to the establishment of a successful migrant/propagule. The problem with using indirect approaches is that genetic structure is also influenced by other factors. For example, current patterns of gene flow that have only recently been contributing to the genetic structure of a population may be masked by the influence of historical gene flow or colonization processes that have little relevance within a contemporary landscape. Conversely, some indirect methods may give insights into the past gene flow and colonization dynamics of populations and species. For example, the expected proportion of heterozygotes and relative abundance of rare alleles can be used to indicate whether a population has been through a genetic bottleneck, associated with recent colonization, within the last few generations. In addition, the evolutionary relationship between genotypes (phylogenetic information content) can be used to infer historical connectivity relationships between populations containing those genotypes. Methods applying coalescent and phylogenetic measures of gene flow can be usefully applied in such circumstances. Such inference may also be usefully applied to a spatial context. The field of phylogeography examines the geographic distribution of evolutionary lineages and has been used to work out historical colonization routes, patterns and dynamics (most documenting processes since the last ice age), for a range of woodland species. These techniques are documented in more detail in Section 3 and example case studies are given in Section 4.

Direct methods use genetic variation exhibited by progeny or juveniles to directly identify parental contribution or variability and calculate dispersal parameters of gametes or propagules. Direct measures offer a very accurate way of assessing gene dispersal. However, an unknown proportion of propagules/gametes will be successful and therefore it is unclear whether this gene dispersal parameter is a good approximation of successful gene flow. Gene movement studies can easily be integrated into survival and ecological studies to provide information on successful establishment/migration. Whilst the study of contemporary patterns of gene flow has been critically under investigated, important observations of fundamental importance to ecological and conservation genetic studies can be revealed (Adams 1992, Ellstrand and Elam 1993).

For both methods, the presence of genetic variation is an essential component of calculating gene flow parameters. It is also preferential to have private alleles (those only exhibited by single individuals or population) as this aids the identification of a gene flow source.

### ***Factors influencing gene flow***

Both intrinsic (i.e., biological, e.g., reproductive system, vagility and dispersal behaviors) and extrinsic (i.e., environmental, e.g., physical barriers and selection gradients) factors influence gene flow.

### **Mating system**

The movement of individuals between populations constitutes gene flow (migrants). Migrations may, however, have a range of outcomes from no influence if the individual moves back to its source population to reproduce, to a long-lasting influence if a group of individuals with high longevity permanently relocate to a new population (immigration) where they breed successfully. Most plant species and some lower animals do not move of their own accord once established, but vegetative propagation and asexual modes of egg/seed production may allow an exact genetic copy (clone) of the individual to be dispersed extensively. Asexual methods of propagation may result in many free-living and separate individuals (ramets) that constitute a single genetic individual (genet).

Self-fertilization (selfing) or autogamy is a reproductive system that produces a zygote following the fusion of two gametes derived from the same individual, i.e. possess both male and female reproductive organs (hermaphrodites). Self-fertilization is common in many plant and lower animal groups. Whilst selfing allows the fixation of preferential gene combinations and may advantage species establishing on particularly stable environments, a lack of recombination accords slower species' adaptation rates to environmental change and can lead to an accumulation of deleterious mutations (inbreeding depression). Species that self also tend to harbour low genetic diversity within populations but have high differentiation between populations.

Mating systems where individuals of a species have separate sexes, ensure that zygotes are the product of gametes that originate from two different individuals (outcrossing). Outcrossing is the normal mode of reproduction for most higher animals and some plants (dioecious are obligate outcrossers), but can occur in hermaphrodite species if mechanisms exist to limit the fusion of gametes derived from the same individual (e.g., self-incompatibility). Populations of outcrossing species tend to have high diversity within populations but low differentiation between populations. One consequence of having truly random outcrossing is that alleles should be distributed amongst individuals of a population according to mathematical principles known as the Hardy–Weinberg equilibrium.

The Hardy–Weinberg equilibrium derives the expected proportion of homozygous and heterozygous genotypes in a randomly mating population of diploid organisms according to the relative frequency of alleles in the population (see Lowe et al. 2004 for a further explanation). In its basic form and for a diallelic single locus system, the frequency of three genotypes AA, Aa and aa is expected to be equal to  $p^2$ ,  $2pq$  and  $q^2$  respectively, where  $p$  and  $q$  are the allelic frequencies of A and a in the population, and  $p^2 + 2pq + q^2 = 1$ . Deviation from predicted allele frequencies can be used to test whether a population is randomly mating or not. If not then further investigation of potential inbreeding, assortative mating, or reproductive cliques/clusters is required.

### **Vagility and dispersal**

In addition to mating system, which determines the composition of transmitted genetic material, the vagility of individuals and dispersal of propagules (including gametes and zygotes) and behaviour of progeny are important factors when considering the extent of gene flow between individuals and populations of a species.

Some animals may range over extremely large distances and therefore have reproductive neighborhoods spread over very large areas. Whereas animals with low vagility and most plants, may not move very far at all and therefore it is their gametes and/or zygotes, which must be dispersed to allow sexual reproduction, genetic connectivity, and colonization to occur. Male gamete dispersion can be extensive (e.g., wind pollinated trees), or limited (e.g., most terrestrial animals need direct contact to fertilize females). Female gametes are usually retained within the female individual/reproductive structure, and therefore only dispersed as a fertilized zygote. Zygote or progeny dispersal can be similarly asynchronous (e.g., free floating pelagic larvae compared to heavy gravity dispersed seeds).

Differences in gene dispersal (both gametic and zygotic) have a profound impact on the magnitude of gene flow and structuring of genetic diversity within and between populations. Such dynamics should be known to help interpret gene flow parameters accurately. For species with low vagility, the effect of restricted dispersal will produce genetic structuring within populations. Advances in the analysis of fine-scale genetic structure have resulted from the availability of highly polymorphic markers and concurrent development of statistical analyses. The availability of these methods has helped infer gene dispersal dynamics at field sites (Sork et al 1999). For example, studies of spatial genetic structure within populations of two sympatric oak species have found that pedunculate oak (*Quercus robur*), which has acorns which protrude from leaf whorls that facilitates seed dispersal by birds (e.g. jays), was found to exhibit no spatial genetic structuring within native populations sampled in Britain and France (Streiff *et al* 1998; Cottrell *et al* 2003). In contrast sessile oak, *Quercus petraea*, which has no specialized seed dispersal adaptations, was found to exhibit strong spatial genetic structuring within populations. On average, patches of genetically similar individuals covered a diameter of 100m, which coincided with the maximum expected distance of most gravity dispersed acorns of this species.

Gender bias in animal vagility may be strongly determined by size or strength of individuals, resulting in different neighborhood sizes for the two sexes, which will affect the distribution of genes on the sex chromosomes (e.g., Y chromosome) or uniparentally inherited genomes (e.g., mtDNA). The relative dispersal potentials of gametes and progeny can also have strong influences on the genetic connectivity of uniparentally inherited genomes relative to biparentally inherited genomes (nuclear DNA), and is an important consideration when choosing markers to examine gene flow parameters. For example, in plants differences in dispersal between male gametes (pollen) and maternally produced propagules (seed) may establish differential spatial patterns for the different cellular genomes (Berg and Hamrick 1995).

High vagility of individuals may offer the potential for gene flow across the range of a species, however, if individuals return to their birth site to reproduce, then gene flow will be severely reduced and a strong differentiation between populations based on natal site is expected. For a range of highly mobile animals (e.g., whales, Baker *et al.* 1990; salmon, Bermingham *et al.* 1991; and birds, Avise and Nelson 1989), significant genetic differentiation between populations or species ranges has been demonstrated due to philpatric effects.

Mating between closely related individuals (assortative mating) may also increase spatial structure for species with low vagility, particularly if closely related individuals have synchronous reproductive timings (Bacilieri *et al.* 1994). However, studies of temperate forest trees have found little correlation between phenological groups and genetic similarity (Gram and Sork 2001). Such observations indicate that spatial clumping of phenological groups may be due to common environmental conditions or that phenological differences are not great enough to isolate groups reproductively (Gram and Sork 2001).

### **Historical dimension**

Many studies of gene flow focus on summary statistics inferred from population genetic structure. These estimates reflect gene flow that is averaged over time and space. Conclusions from these estimates therefore reflect historical as well as contemporary events. Thus, indirect estimates of gene flow may not accurately reflect contemporary processes, as the population genetic structure may carry a predominant signal from a drastic but historical event.

Contemporary gene flow parameters can be measured within the current landscape by using direct estimates of gene flow. For example, one may wish to examine gene flow between populations that have been recently fragmented. Gene flow parameters that have established the genetic structure within the mature element of contemporary populations can be inferred by indirect gene flow estimations and compared to parameters inferred from direct contemporary measures of gamete/propagule dispersal to assess change in gene flow/genetic structure due to the fragmentation process. For example, White *et al.* (1999) investigated the level and distribution of genetic diversity within a highly fragmented population of *Swietenia humilis* in a tropical dry forest in Honduras, which had experienced extensive deforestation in the 1960's. Loss of low-frequency alleles was observed in fragmented blocks compared to a continuous stand, with the percentage of this allelic loss increasing with a decrease in fragment size (White *et al.*, 1999). Despite the high level of fragmentation and small size of remnant subpopulations, indirect and direct estimates of gene flow revealed an extensive network of gene exchange over the spatial scale of the study ( $R_{ST} = 0.032$ ;  $Nm = 8.9$ ), and high levels of genetic variation across all microsatellite loci remaining within all fragments (mean  $H_E = 0.5458$ ; White *et al.*, 2002). This contemporary gene flow appears to be facilitated by native pollinators, which move between spatially isolated trees, and may be assisted by increased wind movement following canopy opening. The increased pollen flow among fragmented populations enables the remnant stands to persist as metapopulations, but could also

maintain, or even augment levels of genetic variation in *S. humilis* (although see Aldrich and Hamrick 1998).

Signatures of allelic diversity and combination can also be used to infer past population processes (e.g. bottlenecks during colonization) if the process has occurred within the recent past. For more ancient processes (up to several million years ago), it is also possible to use phylogenetically informative markers within a population genetic context to infer historical events. Such studies apply coalescent theory to estimate the historical size, connectivity, and relative colonization history of populations, and are discussed in more detail in Section 3. Case studies applying these techniques are presented in Section 4.

### **Physical landscape**

Physical barriers (e.g., mountains, large bodies of water, predominant oceanic currents) have a tremendous effect on the genetic connectivity of individuals occurring on either side of such barriers. Thus, gene flow between populations of a species is a complex interaction between the innate vagility/dispersal ability of a species and its physical environment. The physical landscape needs to be considered when interpreting the relative gene flow exchange distances, and landscape approaches, which consider physical features, are recommended (Sork *et al.* 1999; Lowe *et al.*, 2004).

An example of a study that examines the extent of influence of a physical barrier is the work of Cavers *et al.* (2003a and b) on the wide spread neotropical tree, Spanish cedar, *Cedrela odorata* (Meliaceae). Spanish cedar is monoecious, predominantly outcrossing, is pollinated by insects, has wind-dispersed seed, and is highly valued for its timber (being as it is a close relative of mahogany), which has resulted in significant overexploitation for more than two centuries. Variation at 145 AFLP fragments was used to describe genetic variation within 6 populations of a wet ecotype form in Costa Rica, which is found occurring on either side of the central mountain (up to 4000 m high) which divides the country longitudinally. No significant spatial correlation was found using Euclidean distances between populations (that is the linear distance between populations separated by the mountain range). However a Mantel's test demonstrated that pairwise genetic distance between populations fitted an isolation-by-distance model when the distance between populations that considered the mountain range (i.e. used the distance to the point on the mountain range where Spanish cedar can grow, Cavers *et al.* 2003a). These data indicate that whilst historical colonization probably played its part in establishing genetic structure, contemporary barriers to gene flow (i.e., the mountain range) are maintaining genetic divergence within this species.

### **Heterogeneous environments and changing landscapes**

The local environmental conditions can be a strong determinant of the survival of a particular species, and therefore limit its distribution. Change in habitat may be as strong as the physical barriers, and in certain circumstances is part of the same effect (the high altitudinal effect of mountains causes significant environmental changes, in addition to increasing the distance between populations by adding a vertical dimension). Strong

selection gradients can cause sharp clines across which adaptive genes are structured. However, adaptive clines may limit the overall level of gene dispersal across them and cause disruptions in neutral gene connectivity, thus there may be a strong discrepancy between potential and successful gene dispersal events.

A shift to a new environment may also change the reproductive associations of a population. For example, in plants, changes in environment are commonly associated with changes in flowering time. Such an effect increases differentiation between populations distributed across different environments and drift and adaptation effects, associated with the environmental influx. Selection may also act to disrupt or enforce positive spatial structure within populations of low vagility species (Epperson and Allard 1989). Much of the spatial genetic correlation caused by familial clumping of juveniles may subsequently be removed by intensive competition, leaving only a few adults to survive to reproductive maturity (Dow and Ashley 1996).

A change in habitat or land use can dramatically affect population genetic diversity and structure, at least in theory, by changing the dynamics of genetic connectivity and gene flow between individuals and populations. Fragmenting blocks of habitat can reduce gene flow between populations, particularly if the distance between fragments is greater than the maximum dispersal distance of species inhabiting those fragments (isolation). The quality of the environment between fragments may further serve to reduce gene flow if it is detrimental to individual migration or dispersal mechanism. Over time, the size, quality and even shape of remaining isolated fragments will directly influence the species and genetic diversity harboured in remnant patches (Young *et al.* 1996; Newton *et al.* 1999; Sork *et al.* 1999; Young and Clarke 2000). Gene flow dynamics can change depending on environment, and in some situations may mitigate against genetic diversity loss even in chronically fragmented landscapes. The replanting and expansion of forest blocks, construction of corridors and maintenance of stepping stone fragments may also serve to maintain species and genetic diversity through encouraging genetic connectivity. (Sork *et al.* 1999; White *et al.* 1999; 2002; Dick 2001)

Past climatic change has affected the distribution of habitats, in some cases, dramatically. Most temperate communities (wooded or not) have undergone extreme range shifts since the last ice age (approx 10,000 years; Hewitt 1996; 1999). Future predicted global climate and human-mediated landscape change would exert further pressures on our native biotic communities (Williams *et al.* 2003). Summarising the evidence for some of the assumptions of how species and gene flow will respond under differing landscape conditions is the purpose of this review and a detailed section at the end of this report describes what is known about past and contemporary gene dynamics for species inhabiting natural and degraded woodland ecosystems.

### ***Indirect versus direct methods of estimating gene flow***

Gene flow estimates can be made using genetic markers in two fundamentally different ways. Indirect methods derive gene flow parameters from analyzing the genetic structure of adult populations whereas direct methods analyze gametes or progeny arrays and utilize identified gene dispersal events to calculate gene flow parameters.

The basis, assumptions and drawbacks of the two methods of analysis are described briefly below, and outlined in much more detail in Section 3 following a detailed description of the genetic marker systems that can be applied to genetic diversity and gene flow studies (Section 2).

### **Indirect methods**

Indirect estimates of gene flow can be obtained mathematically from the relative proportion of population differentiation and inbreeding within and between populations (Wright 1951). Whilst estimates are essentially a cumulative combination of gene flow contributions across generations, it is also important to note that only successful gene flow events are represented. Indirect estimates of gene flow are however most useful for assessing the relative, rather than absolute, contribution of historical gametic and zygotic dispersal (Hu and Ennos 1997). For example, Ennos (1994) used existing data, describing the differences in partitioning of variance between the maternally inherited chloroplast genome and the biparentally inherited nuclear genome, to estimate the asymmetry of gene flow due to seed and pollen in European oaks (a ratio of 1:196, respectively).

Estimates of gene flow can also be made using assignment tests. Populations are characterized according to their component genetic variation (whatever the marker used), and sample individuals are assigned statistically to a population with which they share most genetic similarity. Such tests are very robust, and can be used with caution for populations that do not fit Hardy-Weinburg assumptions (see above). The amount of allelic variation and proportion of heterozygotes identified by a particular marker system can also be used to determine if populations have experienced a severe bottleneck within the recent past (a few generations) due to theoretical predictions of the rate of allelic diversity and heterozygosity recovery following such processes.

An alternative to using population genetic differentiation to make indirect estimates of gene flow is to use genealogy reconstruction methods. Genealogical methods can also use a coalescent approach to calculate the level of sharing of common ancestors between populations, and therefore act as a measure of historical gene flow (Neigel 1997). The spatial distribution of evolutionary phylogenetic lineages within species (phylogeography) can also be traced to gain an understanding of large scale colonization processes (for example post glacial migration across central and northern Europe).

Indirect gene flow methods can be used to derive effective estimates of historical genetic connectivity between populations, or for assessing the lifetime contribution of gene flow to the genetic structure across a range of populations. However, there are several serious problems that should be considered before such techniques are applied. Data should only be used to give estimates of the relative contribution of gene flow between different gametes or between zygotes and gametes. There are often cases where absolute distances and frequencies of gene flow are required (e.g., for ecological modeling of the impact of gene escape, or for devising genetic resource management strategies that consider genetic erosion by gene flow). Indirect estimations of gene flow require several assumptions regarding genetic structure of the study population, which are often not met in reality. For

example, genetic equilibrium between gene flow and drift, and a randomly mating metapopulation of equal population sizes that are linked by constant gene flow (Ennos 1994, Sork *et al.* 1999). The analysis of indirect gene flow parameters is actually a complex parameter that also includes selection. Therefore, it is impossible to separate the difference in genomic diversity that is attributable to actual dispersal from other factors such as post-dispersal selection. Indirect gene flow estimates are also easily influenced by historical factors that perturb the theoretical population genetic equilibria (Cottrell *et al.* 2003, Gram and Sork 2001, Sork *et al.* 1999). However the cumulative nature of genetic partitioning that these methods consider, has been used as justification for using such data to assess changes in historical land use (Sork *et al.* 1999).

### **Direct methods**

For assessing contemporary gene flow dynamics it is recommended that a direct method be used (Cain, Milligan, and Strand 2000, Schnabel 1998). Genetic identification of the parents of gametes and/or progeny provides a direct estimate of realized short-term gene dispersal at the individual or population level (Schnabel 1998), and requires few assumptions.

Direct methods also have limitations, whilst not influenced by historical events, gene dispersal is measured for a given period of time only. Due to the limited temporal dimension of most gene dispersal estimates, they are vulnerable to variability between recording periods. The effort required to sample and analyze the genotypes of many parents and gametes/offspring is significant and if only small numbers of progeny arrays are considered then there may be considerable inter-individual variation. Ideally, an estimate of direct gamete/progeny dispersal would be obtained by repeated samplings over time for many individuals, but resource limitation clearly restricts the scope of such replication. Direct estimates of gene flow may only actually be estimating dispersal if the survival or success of a gamete/progeny is not assessed. Whilst not suffering to the same extent as observational methods, the magnitude of the problem depends on the stage at which parental contribution of propagules was assessed. If measured directly on pollen or sperms then there is no estimation of success and this is purely a dispersal measure. However, if juvenile individuals are genotyped and the migration or seed dispersal parameters estimated, by comparing the distance to the individual's parents, then the reproductive contribution to the next generation can be assessed which is a good indication of successful gene flow. Selection pressures can, of course, be assessed for each life stage (gametes, propagules, young and old juveniles, young and old adults), if time and resources permit. However, a combination of indirect and direct approaches may also provide similar resolution of dispersal, stochastic, and selection impacts on population genetic structure.

The recent proliferation of highly variable markers for a range of species (which allows calculation of the high exclusion probabilities), alongside statistical advances have seen a sharp increase in the number of studies of contemporary gene flow (Carvalho 1998), which can produce truly landscape-wide assessments of gene dynamics.

## **2. Review of genetic markers and state of knowledge**

Depending on the approach to be considered a variety of molecular markers can, and perhaps should, be applied to study gene flow and population genetics.

### **Isozymes**

Isozyme markers have been the mainstay of indirect estimates of gene flow until very recently. Indeed, many models based on population differentiation were developed with this marker in mind (Latter 1973, Slatkin 1985). Whilst isozymes do not exhibit particularly high levels of variation, their codominant nature, known genomic origin (usually nuclear), and the fact that they can be applied to almost any species, means that they are very suitable for use with indirect measures of gene flow, and if enough polymorphic loci are used (approximately 10), sufficiently high exclusion probabilities can be achieved to also calculate direct estimates of gene flow. The relationship between alleles cannot however be phylogenetically interpreted, i.e. they are unordered).

### **Dominant markers**

The class of marker represented by AFLPs, RAPDs, and ISSRs are dominantly expressed multilocus variation, which represents unordered alleles derived from all cellular genomes. The application of dominant markers to differentiation-based indirect estimates of gene flow is problematic due to the unknown proportion of heterozygotes in populations. However, several estimates of differentiation have been proposed for use with dominant data, and their careful application can allow some inference of indirect gene flow parameters (Lynch and Milligan 1994). Despite the development of these statistical procedures, codominant data are still clearly superior to dominant data for such estimates. Dominant multilocus markers are, however, an excellent source of private alleles, and can be used without problem in direct estimates of gene flow. Comparisons between AFLP and microsatellite markers suggest that although the latter have higher exclusion probabilities, AFLP analysis can be used successfully for parentage analysis (Gerber *et al.* 2000). The authors note that low-frequency AFLP fragments produce higher exclusion probabilities, but are unlikely to be found consistently in natural populations. Therefore, a large number of fragments (more than 200) may have to be screened to derive enough markers for calculation of satisfactory exclusion probabilities.

### **MtDNA – animals**

Variation in the animal mitochondrial genome is high and can be phylogenetically interpretable (i.e. it is ordered). It is an excellent marker to apply to indirect estimates of gene flow, including differentiation and private allele models. However, the main advantage of this marker comes with applying phylogenetic and coalescent models to gene flow estimation. Being predominantly maternally inherited in animals it can only be used to assess female-mediated or propagule specific gene flow. In addition, high intrapopulation mtDNA variation should allow this marker to be used to assist maternity analysis, although exclusive identification is not usual unless a unique haplotype is identified.

### **Organelle markers – plants (cpDNA and mtDNA)**

Chloroplast genomes tend to harbor low levels of variation, although mutations are phylogenetically interpretable. Indirect estimates of gene flow (differentiation, private allele and phylogenetic) are possible using this marker as long as enough variation can be found. Chloroplast genomes are predominantly inherited maternally in angiosperms and paternally in conifers and can thus only be used for uniparental estimates of gene flow. Within populations, cpDNA diversity tends to be low, but some species harbor population-level polymorphism, which can be used to assist in parental identification. Microsatellite loci exist in the chloroplast genome and some are highly polymorphic (Provan *et al.* 1999; 2001), and have been used to make direct estimates of gene flow (Ziegenhagen *et al.* 1998). In this review the term ‘microsatellite’ used on its own will refer to nuclear loci, microsatellites from an organelle genome will be specifically referenced as such (e.g. cpSSRs). Plant mitochondrial DNA has low levels of unordered variation and is maternally inherited in angiosperms and gymnosperms. Variation in this genome may help provide markers to assess female-mediated gene flow. However, the low level of variation limits application of this genomic marker, particularly within populations (Wolfe and Liston 1998).

### **nDNA variation**

Variation at neutral loci (point of indel mutations) within the nuclear genome offers great potential for gene flow studies. Nuclear encoded variation is codominant and in many cases can be phylogenetically interpreted. These properties make nuclear loci suitable for estimating a range of indirect gene flow parameters using differentiation, private allele or phylogenetic models. If loci harbor high levels of polymorphism then they can be used to calculate direct estimates of gene flow. The utility of nuclear markers (other than SSRs) is presently limited due to relatively slow screening procedures, which are not particularly suitable for interpreting codominant expression of alleles (i.e., RFLP and sequencing). However, the screening of single nucleotide polymorphisms (SNPs) at nuclear loci offers the potential for developing a highly effective and rapid technique for screening codominant, polymorphic nuclear loci. The fact that it is possible to find SNPs in coding regions means that there is a high possibility that such markers will be orthologous and transferable across species. If this is indeed the case then such markers will become very useful, as they will be easier to develop than is the case for microsatellites at the moment.

### **Microsatellites (simple sequence repeats - SSRs)**

Microsatellite markers are highly polymorphic, codominant loci and are abundant in the nuclear genomes of most species. To date many microsatellites have been isolated for a range of species (including many British woodland species, See Appendix 1). However, this type of marker needs to be specifically developed for the focal species of the study, requiring additional time and resources (see below). The high number of alleles per locus, particularly low frequency alleles, allows calculation of high exclusion probabilities, and offers much potential for identifying private alleles (Estoup and Angers 1998, Schnabel 1998). A stepwise mutation model can also be applied to repeat number variation to infer evolutionary relationships between alleles, although such estimates suffer from homoplasy problems (Schlötterer 1998) and a large number of loci (more than 10) should

be applied if such estimates are to be used. These properties make SSRs highly suitable for almost all gene flow estimates, and are often the marker of choice (Parker *et al.* 1998). However, one potential problem with this marker type can be the high mutation rate, which leads to inconsistencies between parental and offspring genotypes. The mutation rate of highly polymorphic loci may have to be estimated and progeny arrays carefully screened for evidence of *de novo* mutation.

### ***How easy are SSRs to isolate?***

The benefits of hyper-variable co-dominant markers are well documented, but unlike the other molecular techniques listed above (isozymes, RAPD, AFLP, ISSRs, organelle markers, but not including other nuclear loci and SNPs), microsatellite loci have to be isolated, developed and optimized before they can be applied to a new species. There are no 'universal primers' available for SSR loci, unlike the abovementioned techniques which can be considered off-the-shelf. Whilst some microsatellites can be transferred amongst closely related species within the same genus and even family (Lowe *et al.* 2002), there are no short cut methods that ensure rapid success. Even if transferred loci appear polymorphic, there is always the risk of null alleles (no allele is present due to mutation in the primer binding site causing a failure of PCR). Microsatellites have already been developed for a range of species groups (see Appendix 1 for details of loci developed for a range of British woodland species), however developing new loci is not trivial and not without risk.

Reviews by Zane *et al.* (2002) and Squirrell *et al.* (2003) suggest that microsatellites should be isolatable for the whole range of species (i.e. plants, animals and insects). But microsatellite development comes at a cost that is not insubstantial particularly if a multi-species approach is required. A microsatellite enriched clone library (50-90%) for a single species can be generated by a number of new biotech companies for US\$5-10,000 (Zane *et al.* 2002), although it could be done much cheaper using inhouse facilities (<US\$1500) or within a negotiated large-scale commercial contract. Squirrell *et al.* (2002) estimate that to generate ten polymorphic 'well-behaved' microsatellites from such a library, approximately 72 clones will need to be sequenced, primers designed for approximately 24 of those, which will then be assessed for polymorphism and presence of null alleles, leading to the remaining attrition. Whilst the report of Squirrell *et al.* (2002) only surveyed the available literature for development of microsatellites in plants, results are likely to be similar for animal groups. However, the authors suspect that there may be an underreporting of SSR failure rate in the literature and they advise a further 10-20% contingency plan be included for unforeseen failures/difficult taxa. Sequencing costs are reducing rapidly, but the design and screening of SSRs still require good technical competence and can be time consuming. From start (library construction) to finish (polymorphic loci) the process can take 3-6 months, although considerable savings in time and consumables would be achieved by concurrent isolation of a large number of SSR loci for multiple species. It is now being appreciated that microsatellites also exist in coding regions of the genome and EST-SSR databases exist for spruce (n=188), poplar (n=1523), loblolly pine (n=426), Arabidopsis (n=3626) and rice (n=5138) based on a total of 561,932 EST sequences. Of the SSRs tested across the 23 spruce species, 57% of EST-SSRs were widely applicable, compared to only 18% of the previously developed

markers. This suggests that this new generation of microsatellites may show greater utility across species than those isolated by conventional means (Rungis *et al.*, 2004).

### ***Source material for genetic analysis***

The type of material collected for sampling depends on the marker chosen. Freshly collected material is generally necessary for isozyme studies (this material needs to be stored on ice and processed within 24 hours). However, for all PCR-based methods (including microsatellites) only small amounts of preserved material are required (grams or milligrams of material), but fresh material can also be used if available. The exponential amplification basis of the polymerase chain reaction (PCR), means that if the chemistry is correctly optimized and sufficiently sensitive screening methods are used (e.g. capillary sequencers), only a few copies of the target locus need be present in a sample to obtain a genetic profile. This principle has allowed DNA to be analysed from a variety of ancient sources including Egyptian mummies, the Tirolian Ice Man, prehistoric bone fragments (Scholz *et al.* 2000), fossil bones of penguins (Lambert *et al.* 2002) and thousand-year-old wood samples from peat bogs (Dumolin-Lapègue *et al.* 1999). DNA has also purported to have been extracted from million year old insects encased in amber and leaf specimens laid down in sedimentary rocks. However, possible contamination from contemporary sources cannot be ruled out in such cases. In this respect, microsatellites also have another advantage over the off-the-shelf DNA profiling methods (e.g. AFLPs) in that the primers used are species specific. Even if sample material is contaminated with fungi or bacteria, SSR analysis would still only amplify DNA from the intended source. In contrast, AFLPs amplify DNA from all sources, potentially producing erroneous signals in contaminated samples. Organelle markers (mtDNA and cpDNA) are somewhere between microsatellites and AFLPs in their specificity and tend to be group specific (i.e. plant, mammal, bird, insect, fungal). So pathological contamination can be avoided but not laboratory introduced contamination of other group sources.

There are several texts advising the best source of material for DNA analysis (e.g. Weising *et al.* 1996). For plants, dried leaf tissue (Chase and Hills, 1991), dried cambium plugs (where leaves are inaccessible, e.g. tall trees), dormant buds and seed material are all suitable sources for extracting large quantities of good quality DNA. For insects, only destructive sampling is really possible and recently killed or desiccated specimens can be used. DNA concentrations are highest from head and thorax sections compared to abdomen or peripheral parts (i.e. legs, antennae). For animals there are a range of non-descriptive sampling methods that can be used, including, skin plugs/scrapings, blood samples, hair traps and scat samples (e.g., Taberlet and Waits, 1998). However for many mammals (e.g. red squirrel) Home Office licenses are required for sampling, which may be difficult to obtain. Numerous methods for collection of genetic material and DNA isolation from animals and plants have been developed (e.g., Laulier *et al.* 1995; Doyle and Doyle 1987, Lookerman and Jansen 1995). In addition to sampling in the field, alternative sources of material for genetic studies include *ex situ* conservation collections (e.g., seed banks, zoological gardens, botanical gardens, sperm and egg banks) and museum collections (e.g., herbaria, skeleton collections, study skin collections, spirit collections) can be used to supplement collections or to provide a historical dimension to the analysis of population genetic changes (e.g. Hale *et al.* 2001).

### 3. Statistical methods for analyzing genetic diversity and gene flow

#### *Measuring gene flow - indirect estimates*

Indirect methods of assessing gene flow use the observed genetic structure within adult populations (not propagules) to compare with models of expected genetic structure. Some of these tests can be very powerful but several have restrictive conditions of population composition and relations before they can be correctly applied.

#### **Clonality, inbreeding, family structure and bottlenecks**

##### *Clones*

The distribution of a clonal individual (genet) in terms of number of ramets and size of area covered can be assessed easily using genetic fingerprinting approaches. The problem is at what level is genetic identity accepted as indicating two ramets of a clone, rather than two individuals which are genetically very similar but indistinguishable using the chosen marker system? Unless the entire genomes of both individuals are sequenced this cannot be truly proven. However, in practice, it is possible to apply a reasonable number (usually defined by available resources) of highly polymorphic markers (e.g., RAPD, AFLP, SSRs, ISSRs, RFLPs), to demonstrate that individuals are identical and show the distribution of a single clone. The confidence of correct clonal identity can be shown by examining the variation present within individuals of non-clonal origin (if they exist) and by calculating an exclusion probability. Gerber *et al.* (2000) define an exclusion probability as “the average capability of any marker system to exclude any given relationship” and it is conditional on the genotypes of the reported relatives, frequency of alleles at loci, and the number of independent marker systems (loci) tested (Jamieson and Taylor 1997). An exclusion probability basically allows a probability to be assigned to the possibility of identifying two non-identical individuals as identical-by-accident. As long as the exclusion probability is close to 1 then identity of clones can be made fairly confidently.

##### *Inbreeding*

Under the Hardy–Weinberg principle (see above), differences in the proportion of heterozygotes observed and expected within a population, can be used to estimate the proportion of inbreeding present, and an inbreeding coefficient  $F_{IS}$  can be calculated. If one assumes that all the deficit in heterozygosity is due to selfing then a direct relationship between selfing and the inbreeding coefficient is calculable (Wright 1965). However, there are two reasons why  $F_{IS}$  may not be a good indicator of selfing. First, matings between close relatives may be mistaken for selfing as it causes an increase in homozygosity using this parameter. Second, homozygotes resulting from selfing are likely to suffer from inbreeding depression, and may be under selective pressure and removed from a population, causing an upward bias in the proportion of observed heterozygosity. Overall,  $F_{IS}$  remains a reasonable indicator of the level of inbreeding within a population or species, and should be estimated before applying estimates of gene flow based on population genetic structure if any level of autogamy or family clustering

is suspected. This statistic can only be calculated using nuclear codominant markers (e.g. SSRs).

#### *Coefficients of relatedness and family relationships*

Genetic relatedness can be expressed as a single measure,  $r$ , between individuals (Avisé 1994). For full sibs and parent–offspring pairs  $r = 1/2$ , for half-sibs and between aunts/uncles and nieces/nephews or between grandparents and grandchildren  $r = 1/4$ , for unrelated individuals  $r = 0$ . These principles can be used to calculate relatedness where the pedigree of relationship between individuals is known (Michod and Anderson 1979). However, where the pedigree is unknown, which is the usual case in natural populations, a mean coefficient of relatedness can be calculated among group/population members. These methods rely on comparing the observed and expected heterozygosity levels within family groups to a wider population (Queller and Goodnight 1989; Pamilo and Crozier 1982).

#### *Detecting population bottlenecks*

Populations that have experienced bottlenecks suffer a reduction in both allelic richness and heterozygosity. However allelic richness declines faster than heterozygosity (Cornuet and Luikart, 1996), and is the basis of the sign test for heterozygote excess. Following the bottleneck, recovery of heterozygote proportions, which are in equilibrium ratio, will be quicker than the recovery of allelic richness. The increase in rare allele numbers will take time to achieve in a population due to the accumulation of new mutation events (and gene flow), whereas the alleles remaining following the sampling effect will still contribute to overall heterozygosity in future generations. More recently other approaches have emerged that identify bottlenecks using phylogenetic and coalescent approaches and are outlined below.

#### **Using population genetic structure**

Gene flow can be inferred by examining the partitioning of genetic variation between populations and calculating expectations of genetic structure according to theoretical models. The population genetic statistic for subdivision,  $F_{ST}$ , was originally described by Wright (1951) to partition departures from random mating into components due to non-random mating within and between populations. Whilst codominant, nuclear markers (e.g. SSRs) provide the best loci for such estimates, several other population subdivision statistics can also be used depending on the marker technique used, including, gene diversity ( $G_{ST}$ , Nei 1973), organelle partitioning ( $G_{ST}$  for haploid genomes, Pons and Petit 1995, Takahata and Palumbi 1985), nucleotide diversity ( $N_{ST}$ , Lynch and Crease 1990), and dominant variation (a PhiST derived from an analysis of molecular variation; Excoffier, Smouse, and Quattro 1992; and a corrected  $F_{ST}$  using mid-frequency markers; Lynch and Milligan 1994).

Besides gene flow, drift can also change allelic frequencies within populations and its effect is increased with decreasing effective population size. As these two processes are difficult to discern, population genetic approaches only allow the inference of the number of individuals exchanged between populations per generation,  $Nm$  (Wright 1951). The theoretical relationship between  $Nm$  and drift is such that, on average, the movement of

one individual per generation between populations ( $Nm = 1$ ) is sufficient to prevent genetic differentiation by genetic drift alone (Allendorf 1983). This  $Nm$  value equates to a mean  $F_{ST}$  of 0.20 and is used as a criterion to classify populations or species as having high (above this value) or low (below this value) gene flow. Several animal groups with high vagility also have low population differentiation and consequently high  $Nm$  values. Where genetic differentiation estimates are available for markers that have different inheritance characteristics (e.g. maternal vs. biparental) it is possible to estimate the relative ratio of gene flow occurring due to male gametes and maternally produced propagules. Although most commonly used to derive pollen vs. seed gene flow ratios for plants, this measure can also be used for animal cases (Ennos 1994).

#### *Private alleles*

Alleles that are only found in a single population can be classified as private. The logarithm of the average frequency of private alleles [ $p(1)$ ] has an approximate linear relationship with  $Nm$  over a range of population parameters and thus can be used as an alternative measure of gene flow (Slatkin 1985). This relationship is expected to occur because private alleles will only become prevalent when gene flow (as measured by  $Nm$ ) is low. When tested with sufficient genetic information, private allele and  $F_{ST}$  methods yield similar estimates of gene flow, although a correction is recommended for  $Nm$  when very different population sizes are sampled (Barton and Slatkin 1986, Slatkin and Barton 1989).

#### *Assignment tests*

Estimates of gene flow can also be made using assignment tests. Populations are characterized according to their component genetic variation (whatever the marker system used), and individuals from the entire sample are then assigned statistically to the population average with which they share most genetic similarity. Such tests are fairly robust, and can be used with caution for populations that do not fit Hardy-Weinberg assumptions (e.g. Cornuet *et al.* 1999; Paetkau *et al.* 2004).

### **Using phylogenetic information – adding the historical dimension**

Tests that use phylogenetic information are generally only applied to those loci or genomes for which robust phylogenies can be reconstructed (e.g. the non-recombining organelle genomes; animal mtDNA and plant cpDNA; and sequence information from some nuclear loci). Under certain circumstances and restrictions, SSR loci could be substituted for such markers if a stepwise mutation model is used, which assumes that the difference in allelic size reflects evolutionary divergence. Whilst results from such studies have been ambiguous, and the method not fully proven, Schlotterer (1998) recommends using a large number of loci (10-20) if such statistics are to be applied to microsatellites.

#### *Phylogeography*

The spatial distribution of evolutionary phylogenetic lineages within species (phylogeography) can be traced to gain an understanding of large scale colonization processes. The basis of the assumption is that remnant populations are isolated and will have diverged genetically (which can be traced using phylogenetic reconstruction), over

time from other such remnant populations. If range expansion occurs out of a remnant then the newly colonized populations will only take a subset of the remnant variation with them due to the bottlenecking effects of colonization. Thus, remnants can be identified by the high genetic diversity of phylogenetically related variation, and newly colonized sites should only have a subset of the remnant variation or may have a mixture of variation if colonized by several different source remnants. These principles have been formalized into a powerful statistical test by Templeton (1998), the Nested Clade Analysis that classifies populations, depending on the level and phylogenetic relationship of genetic variation they contain, into remnant, admixture or recently expanded or contracted regions.

#### *Cladistic measures of gene flow*

DNA sequence data are often available for more limited numbers of individuals than unordered allelic state information (e.g., isozyme data) and thus discarding the known gene tree and using such data in a simple  $F_{ST}$  analysis represents a loss of information and compromised analysis (Neigel 1997). DNA sequence data are better employed to calculate gene flow using a cladistic approach (Slatkin and Maddison 1989). The sampled sequence data, which should represent the distribution of allelic clades within geographic populations, is used as multistate unordered characters to construct a gene tree. Then parsimony is used to determine the minimum number of character state transitions (i.e., migration events) required to construct a robust phylogenetic tree of sequence relationships. The distribution of this minimum number of migration events was shown through simulation studies to be a simple function of  $Nm$  (Slatkin and Maddison 1989) and that there is a linear relationship between  $Nm$  and distance between samples (Slatkin and Maddison 1990). Thus, a powerful indirect estimator of gene flow can be obtained using the genealogical information of sequence data.

Unfortunately, this method cannot be applied evenly to all genomic regions for which phylogenetic are available. Additional simulations by Hudson, Slatkin, and Madison (1990) indicate that the best correlation between  $F_{ST}$  and the cladistic method is obtained for loci with low recombination and moderate to high migration, and thus this method is probably best applied to animal mtDNA variation which is not subject to recombination (unlike nDNA) and can provide fully resolved genealogies (unlike cpDNA).

#### *Timing dispersal distance*

An evolutionary clock for molecular divergence can be used to calculate the expected distribution, due to dispersal, of various aged, gene tree lineages by applying a multigeneration “random-walk” process from the specified center of origin for each clade (Neigel, Ball, and Avise 1991). This approach is most useful when considering low-dispersal species and using a rapidly mutating, non-recombining genetic marker, such as animal mtDNA. In these circumstances, mutations that delineate new descendent lineages may be dispersed at rates sufficiently low to prevent the attainment of equilibrium between genetic drift and gene flow that many earlier models assume.

### *A coalescent approach*

As phylogenetically interpretable data have been introduced to population genetic analyses, genealogical approaches have been developed for testing evolutionary hypotheses and population genetics parameters (Felsenstein 1992). Genealogical approaches exploit two properties of gene trees, and can be used to make inference about gene flow (Neigel 1997). First, branch lengths that connect sequences correspond to a time of coalescence to the most recent common ancestor. Second, the relative order of branches in a tree corresponds to the order of coalescence events and therefore constitutes a cladistic relationship amongst the sequences.

Slatkin (1991) demonstrated a relationship between coalescence time and  $F_{ST}$  for loci where mutation rate is too low to affect  $F_{ST}$ . This  $F_{ST}$  estimator can be used in the  $Nm$  calculation and allows the demographic processes of migration and genetic drift to be separated from mutation (Neigel 1997). The power of this analysis means that further advances in calculating gene flow parameters utilizing coalescent approaches are expected, and allow insights into ancient gene flow and colonization events.

### **Incorporating the spatial context**

Gene flow in organisms with limited vagility may be restricted by distance alone, and is known as isolation-by-distance (Wright 1943, 1946). When using an indirect approach to calculate gene flow, the isolation-by-distance concept has been used to infer the extent of genetic connectivity within and/or between populations. In a simple form, pairwise  $F_{ST}$  estimates can be calculated between populations. These pairwise differentiation estimates can be plotted as a dendrogram or used to examine an isolation-by-distance effect using a spatial correlation test, and have been used to examine range-wide and fine-scale genetic structure and infer gene flow dynamics.

More formal spatial autocorrelation analysis can also be undertaken to allow a comparison between the correlation in allelic or genotypic state of portions of a population grouped into defined spatial distance classes and that which is expected under random spatial distribution of variation (Ennos 2001). Positive autocorrelation indicates that genetically similar individuals cluster together spatially, for which one explanation is limited gene flow. Several types of analysis can be conducted to show spatial genetic structure. Perhaps the most commonly applied method is Moran's autocorrelation statistic,  $I$  (Moran 1950), which measures the correlation in gene frequencies between samples of genotypes within specific distance classes. Analysis can be conducted separately for individual loci or data combined over loci and the combined  $I$  value depicted for the range of distance classes as a single graph (e.g. Smouse and Peakall 1999).

If each sample is composed of a single individual then the frequency of alleles within an individual can be used. Under these conditions, there is a direct relationship between  $I$  and Wright's coefficient of relatedness  $r_{(x)}$  (Pamilo and Crozier 1982, above; Queller and Goodnight 1989) and so a direct link between spatial autocorrelation and population genetic analysis can be made (Ennos 2001). If populations are at equilibrium and genetic structure has been solely generated by isolation-by-distance, then quantitative estimates

of gene flow can be made (Hardy and Vekemans 1999). However, the assumptions under which such analysis may be conducted are easily violated in natural populations, and greatly reduce the power of interpretation of such a test. These spatial tests of genetic structure are most often applied to plant systems, although in theory they should and have been applicable to sedentary or highly territorial animal systems (Peakall *et al.* 2003).

### ***Measuring gene flow – direct estimates***

Direct measures of gene flow can be distinguished from indirect measures by the fact that propagules, and not adult individuals, are used, and that genotypes are determined directly following gene movement and used to retrace gene flow parameters directly relating to dispersal events. A number of direct gene flow methods are available.

### **Using progeny from a non-exhaustively sampled population**

By screening codominant (and sometimes dominant) marker variation within progeny of known maternal origin, the predominant breeding system of the mother/population/species can be inferred. Clonality (apomixis) can be identified if all the progeny are the same as the mother. Selfing is assumed if only the maternal alleles are present in the progeny arrays, but they should segregate according to Mendelian principles. Outcrossing is assumed if each progeny has only a single maternal allele and a second “foreign” allele, presumably from the father.

### ***Mating system parameters***

Tests of mating system parameters have been developed into formal models by Ritland and colleagues (Ritland 1986, 2002, Ritland and Jain 1981). Genetic variation from progeny arrays (where both the maternal genotype is known and unknown) at codominant or dominant loci can be used to calculate the proportion of inbreeding to outcrossing. Usually two parameters are estimated. A single locus estimate of outcrossing, where information from individual loci are used and the averaged, and is based on the number of non-maternal alleles, the total number of alleles, and their segregation ratios. Similarly a multilocus estimate of outcrossing is made, where multiple or all loci are used to calculate this parameter.

Differences in these two statistics are also applied to calculate two further parameters. The first is the proportion of biparental inbreeding (mating between relatives, which causes increased homozygosity), which is calculated as the difference between the single and the multilocus estimates. For truly outcrossed progeny arrays, the single locus and multilocus estimates will be similar/identical, whereas for matings involving closely related individuals, the single locus outcrossing estimate will be significantly lower than the multilocus outcrossing rate. The second parameter is correlated matings, the degree to which siblings share the same male parent, which is calculated as the relationship between the probability that two progeny taken from a single array are outcrossed full sibs (known as the “correlation of paternity”) or selfed full sibs (known as the “correlation of selfing”). This estimate is made once an overall estimate of selfing rate has been made across families and variances are normalized. These statistics can be made spatially explicit if the location of the mother is known, although in general they are used as a population average.

This suite of measures provides a very powerful test of the mating system of a species and gives an indication of the number of males in a population contributing to the family of a particular female.

#### *Number of males and effective reproductive neighbourhood size*

For organisms with low vagility (mostly plants, although the technique can also be used with sedentary or highly territorial animals), differences in genetic differentiation between the parent and progeny generation can be used to great effect to infer gene flow parameters, and with further development, these methods offer the potential to dissociate historical gene flow and contemporary dispersal events. Smouse *et al.* (2001) proposed this new measure of male dispersal, which only needs genotype information from a non-exhaustive sample of females and their progeny (Austerlitz and Smouse 2001). The method defines an  $F_{ST}$  equivalent for male gametes in a population, which allows a quantification of the heterogeneity of the male gamete pool sampled by females scattered across a landscape, and is used to estimate mean male gamete dispersal distance and effective neighborhood size. The method appears to be sensitive to marker systems with low exclusion probabilities, but the model can be confidently applied using a relatively small number of regularly segregating, highly polymorphic SSR markers (e.g., four loci).

Similar principles have been applied by Burczyk *et al.* (2002), who have adapted earlier work by Adams and Birks (1991) to develop a model that can be applied to angiosperms and gymnosperm plants (although it should also in theory be applicable to sedentary or highly territorial animals). The model apportions offspring to three categories: selfed; outcrossing with males in a circumscribed area around the mother (neighborhood); and outcrossing to males outside the neighborhood. The model allows the derivation of average and specific mating system, number of males, neighborhood size for outcrossed events, and the proportion of matings within and outside this area.

#### **Assignment of parental contribution within an exhaustively sampled area**

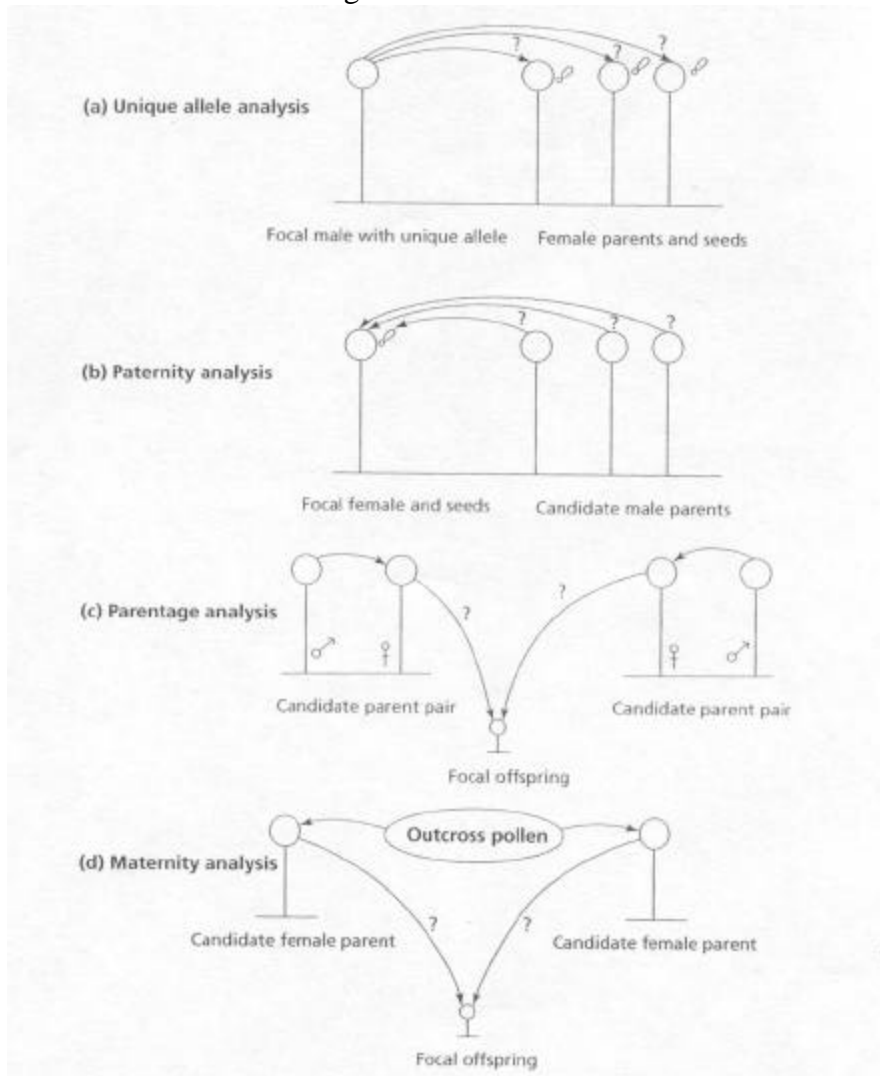
Surveying the genetic variation of progeny or juveniles of a population also offers the potential to directly identify the parents of these progeny. However, to undertake such analyses with any hope of obtaining a meaningful result requires two conditions. First, the parent population must be sampled sufficiently that potential parents are included in the sample (usually an exhaustive sample within a particular area is chosen when not working with small, closed systems). Second, a suitable molecular marker system (preferably codominant and highly polymorphic, e.g. SSRs) must be chosen so that exclusion probabilities are sufficiently high to allow confident assignment of parental identity.

Successful identification of one or both parents of progeny within or between populations may allow calculation of several parameters, including:

- Fertility or mating success of an individual or population (Roeder *et al.* 1989; Schnabel *et al.* 1998)
- Propagule dispersal frequency between populations or amongst regions/family groups within a population.

- For low-vagility species, the distance and frequency of propagule dispersal or a dispersal distance/frequency curve can be calculated.

Calculation of such parameters for a range of propagules (gamete, dispersed progeny, established progeny, juvenile, recently matured adult) will also provide an insight into selection pressures acting at the different life stages and the total proportion of dispersal that translates as effective gene flow.



**Figure 1.** Schematic representation of different types of direct analysis of dispersal analysis in plant populations (from Ennos, 2001): a) using unique alleles to measure pollen dispersal; b) using paternity analysis to measure pollen dispersal; c) using parentage analysis to measure pollen and seed dispersal; d) using maternity analysis to measure seed dispersal.

#### *Paternity, maternity, and parentage analysis*

It is possible to work out three types of parent/offspring relationship using genetic exclusion methods (Fig. 1).

- Paternity analysis: For progeny where the mother (and her genotype) are known (e.g., seeds on trees, eggs in a nest, newly born mammals), the identity of the father can be

established by comparing the genotype of the progeny with potential males in the population, once the maternal genotype has been subtracted. This process can involve screening progeny for a unique allele carried by only one male individual in the population, and provides a good estimate of the mating success of this individual. An alternative method is to use information from all available loci to exclude males, due to incompatible genotypes. Such analyses may be used to provide evidence for extra-pair matings in supposedly monogamous animals. The possibility of autogametic or apomictic progeny does not exclude the female parent from also being the male parent in some species (e.g., hermaphrodites and apomicts).

- **Parentage analysis:** For free-living or established progeny where the identity of both parents is required (e.g., animal offspring which have left the maternal home or plant seedlings), the problem is more difficult. In such cases it is necessary to show that two potential parent individuals have compatible genotypes with the progeny (i.e., have not been excluded by the available marker variation). However, it is also necessary to show that the contribution of both individuals (or only a single individual for selfed progeny) is required to establish the full genotype of the progeny (Schnabel 1998). For dioecious species, this pair must clearly comprise different sexes. However, for hermaphrodite individuals, molecular markers exclusively inherited through different gametes (e.g. organelle markers are often only maternally inherited) could be used to differentiate the nature of the sexual contribution of parents, or specific maternal tissue still associated with the propagule can be genotyped (e.g. pericarp or megagametophyte tissue of plant seeds), or an assumption could be made based on the proximity of progeny and parents. For example, Dow and Ashley (1996) assumed that the closest identified parent to oak seedlings must be the female due to the restricted nature of acorn dispersal. This assumption appears to have been valid for their system, where direct tracking observations found that 99% of acorns fell within 50 m of the maternal tree canopy. Another principle of parentage analysis, when applied to non-vagile species, is that the distance between the two parents corresponds to the dispersal distance of the male gamete.
- **Maternity analysis:** For progeny where the mother is unknown, she can be deduced using two principles. For dioecious species, the correct mother can be identified by excluding potential mothers with incompatible genotypes. Such analysis could be used to identify whether a female bird is actually the mother of all the eggs in her brood or whether there is evidence for intrabrood parasitism. If offspring are fertilized by random mating events involving a homogeneous outcross male gamete pool (e.g., free-swimming sperm of marine species or wind-dispersed pollen) then the most likely mother can be identified using genotype information of the offspring, the outcross male gamete pool and potential female parents.

#### *Problems with parental identification*

Parental analysis by genetic exclusion across loci results in one of three outcomes:

- A single parent (or parent pair for parentage cases) may be found, in which case parental contribution is documented.
- All parents may be excluded leaving no potential parent(s). There are several explanations for this. First, both parents may be outside the sampled area. Second, there may have been a mutation between the parent and progeny generation, consequently the true parent has been excluded by incompatibility (depending on the marker this may be a significant factor, although is rarely assessed; Dow and Ashley 1996). Third, one or both parents may have died, the probability of which increases if an individual's life span is short or if the progeny analyzed are no longer young juveniles.
- Several potential parents within a plot may be identified. Although highly polymorphic molecular markers (e.g., microsatellites) exhibit high exclusion probabilities (Gerber *et al.* 2000), unique assignment is rarely possible for all offspring (Chase *et al.* 1996, Schnabel 1998). Therefore to select one individual as the correct parent from several possibilities, a model for exclusion has to be adopted (e.g. simple exclusion, maximum likelihood or fractional parentage methods). Exclusion methods are most accurate when the numbers of loci and alleles per locus scored are high (e.g. SSRs, although AFLPs can be used Gerber *et al.* 2000), and when the number of potential parents is low. Several assumptions are made using the different exclusion models, however, simulation approaches allow an estimation of the confidence of assignment. The use of exclusion models usually increases the proportion of offspring for which parents can be allocated. This advantage is important. Parental assignment is usually a means to an end, such as investigating pollinator ecology or gene flow changes. Discarding data in the form of offspring, because no parent can be assigned wastes effort and time in the data sampling, and leads to a reduction in the explanatory power of results (Snow and Lewis 1993), which in extreme cases may lead to incorrect conclusions.

Another problem with exclusive assignment of parents is the possibility of assigning false positives. This problem increases significantly as the exclusion probability decreases below 1, and can significantly skew gene dispersal estimates. For example, if an individual within a plot is identified as a progeny's parent but the real parent is actually located outside the plot, then the amount of gene flow inferred by this misidentification is underestimated (i.e., the real level of gene flow is much higher than the analysis indicates). This phenomenon is referred to as "cryptic gene flow" because the real gene dispersal event has been missed. Fortunately the magnitude and variance of cryptic gene flow has a close relationship with the exclusion probability and can be estimated statistically.

## 4. Review of dispersal, colonization and gene flow studies in woodland systems

This review aims to provide a detailed survey of the British and European literature on the application of hypervariable molecular markers, such as microsatellites, to studies of species genetic diversity and dynamics (dispersal and colonization) within wooded landscapes, and their potential application to inform restoration programmes.

This section is broken down into three parts: Historical colonization of woodland species in Britain; Contemporary gene dynamics within woodland systems; and Impacts of woodland clearance and potential for restoration.

### *Historical colonization of woodland species in Britain*

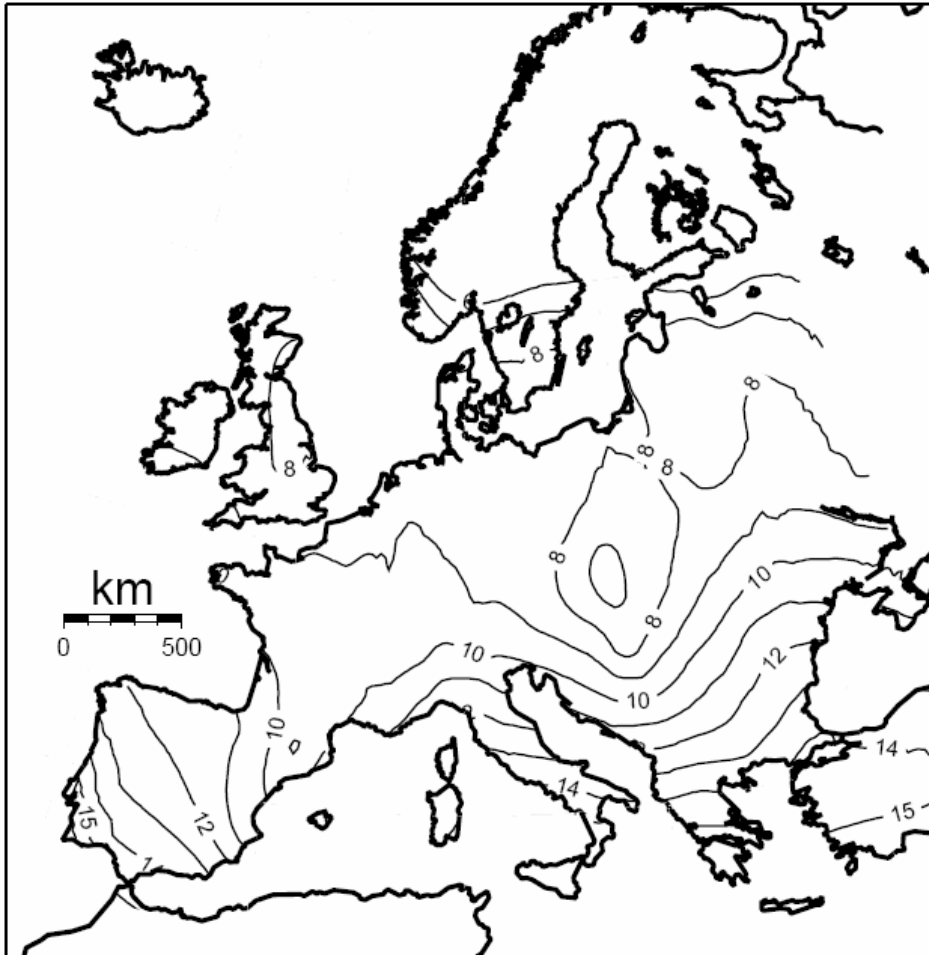
Past climatic change has dramatically affected the distribution of species and ecosystems. For most European species, range shifts have been especially extreme since the last ice age (approx 10,000 years), and future predicted global climate and human-mediated landscape change will exert further pressures on our native biotic communities. There are now a number of studies that integrate fossil evidence and molecular phylogeographic data to examine the distribution of species during the last glacial maximum and map out their routes and dynamics of post glacial colonization (e.g. Hewitt 2004; Lascoux *et al.* 2004). Such studies can also be used to infer the relative rate of migration and dynamism of species under future global climate change scenarios.



**Figure 2.** Distribution of ice sheets (hatched area), tundra, permafrost and sea ice during last glacial maximum (approx 20,000 year BP). Fossil evidence suggests that at this time temperate European floral and faunal communities survived south of the permafrost line (from Hewitt 1999).

### Fossil evidence of species history

Macro (e.g. bones, teeth, carapaces and wood) and micro (e.g. pollen) fossil records offer the possibility to determine the historical geographic distribution of species, and they can be of tremendous use to verify/test phylogeographic conclusions. The fossil record for the European flora and fauna is particularly good, and has allowed detailed description of species dynamics since the last glacial maximum (LGM, 18 000 BP).



**Figure 3.** Pollen core record indicating the date that oak pollen was first recorded at sustained levels between 13 000 and 6000 BP (from Brewer *et al.* 2002). During the last glacial maximum (>13 000 BP) the Iberian, Italian and Balkan Peninsulas are clearly identified as refugia, and following post glacial climatic warming oaks recolonized central and northern European areas from these refugia. Whilst the pollen core record indicates the timing of events it is not a good indicator of the route the particular oaks took during recolonization (apart distinguish overall south/north range change and to indicate a leading western edge of colonization).

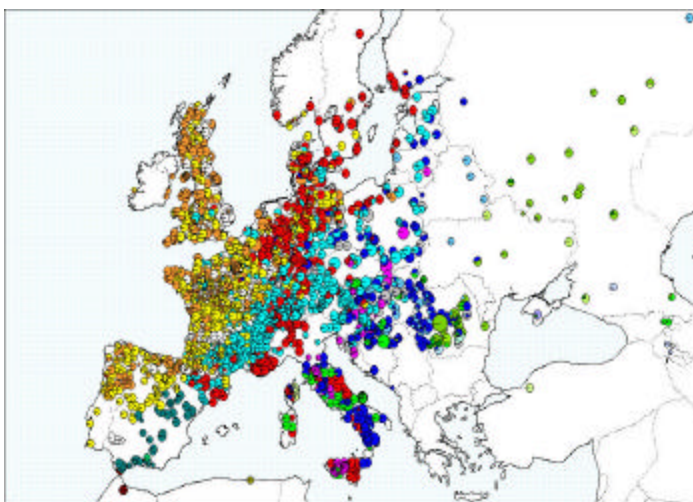
Much of the northern part of the continent (including most of the British Isles and Scandinavia) was covered in ice sheets (Fig. 2), and fossil data suggests that areas south of the ice fields were treeless with abundant arctic-alpine and steppe vegetation (Godwin and Deacon, 1974). Based on the fossil data, three main Pleistocene refugia that harboured most of the contemporary temperate species assemblages are speculated in southern Europe, in Iberia (Spain and Portugal), Italy and the Balkans (Huntley and

Birks, 1983; Taberlet *et al.*, 1998). Many species were able to emerge from these refuge areas as the ice caps began to retreat about 12 000 BP, and were able to colonize as far north as southern Britain and Scandinavia by approximately 10 000 BP (Godwin, 1975; Birks, 1989). However, the direction of colonization and the influence that the different refugial areas have had on the contemporary species range is not discernable from fossil data, and requires combination with phylogeographic studies.

### Case study of post glacial colonization - oaks

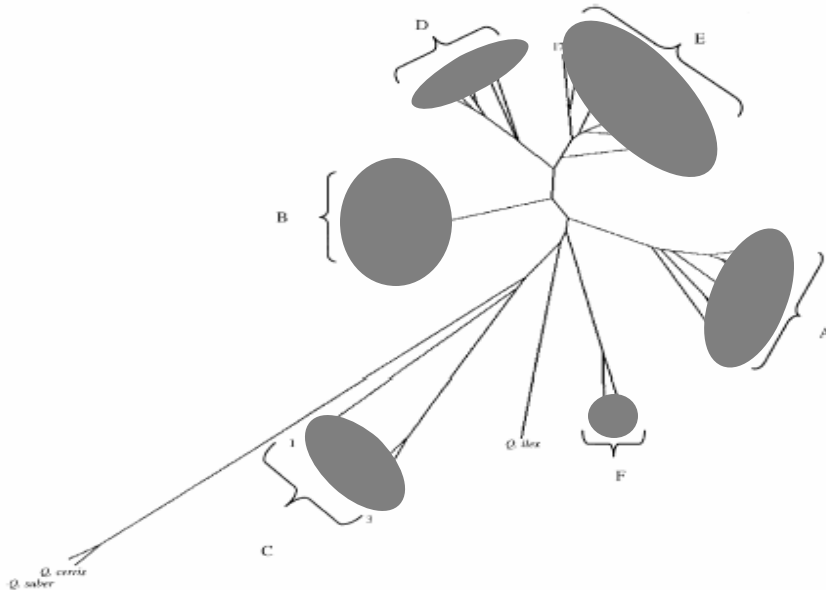
Pollen core analysis of 600 profiles across Europe (Brewer *et al.* 2002), recently confirmed earlier findings of Huntley and Birks (1983), that during the glacial maximum oaks were restricted to the southern peninsulas of Iberia, Italy and the Balkans (Fig. 3).

The analysis by Brewer *et al.* (2002) also highlighted some particularly interesting insights into temporal and geographic variation in oak colonization. The initial stages of colonization took place in the late-glacial interstadial (13-11 000 BP) when oaks spread into central Europe from primary refugia of the peninsulas. It appears that this range expansion was rapid and extensive, and oaks could even have reached areas of northern France and Germany at the end of this period (Fig. 3). This expansion was halted by a more recent cold period, between 8 000 and 10 000 BP (known as the Younger Dryas), which appears to have caused extinction of oak populations in northern areas. During this time oaks survived in mountainous regions of central Europe (e.g. Alps and Carpathian mountains) identified as secondary refugia. Oak spread into central and northern European regions, from primary and secondary interstadial refugia, following climatic stabilization in the Holocene (since 8 000 BP). Range expansion was noted to be faster in western compared to central regions, probably due to more rapid warming in coastal areas and physical barriers (e.g. mountain ranges) in central areas. Shifts in climatic temperature and competition with other species are both thought to play a role in the colonization dynamics of oak during this time. However, by 6 000 BP, oak had reached its maximum European extension (i.e. northern Britain and Scandinavia).



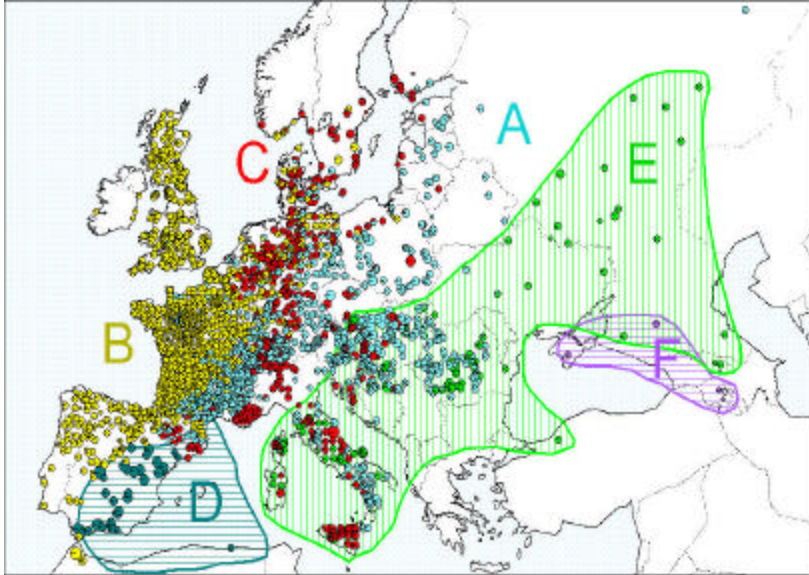
**Figure 4.** Distribution of chloroplast DNA variation within a total of 2,593 oak populations (11,828 individual trees from eight white oak species) sampled and genotyped from 38 countries in western and central Europe (from Petit *et al.* 2002b). A total of 45 chloroplast variants were detected and their individual distribution (numbered in key) is presented.

Onto this detailed temporal picture of colonization have been superimposed the results of a combined analysis of chloroplast DNA from 16 European laboratories. The phylogeographic analysis (Figs. 4 and 5) is based on a sample of 2,613 populations (12,214 individuals trees and eight oak species) comes from 37 countries, analysed for four cpDNA loci (highlighting 45 variants) in eight white oak species (Petit *et al.* 2002a, 2002b).

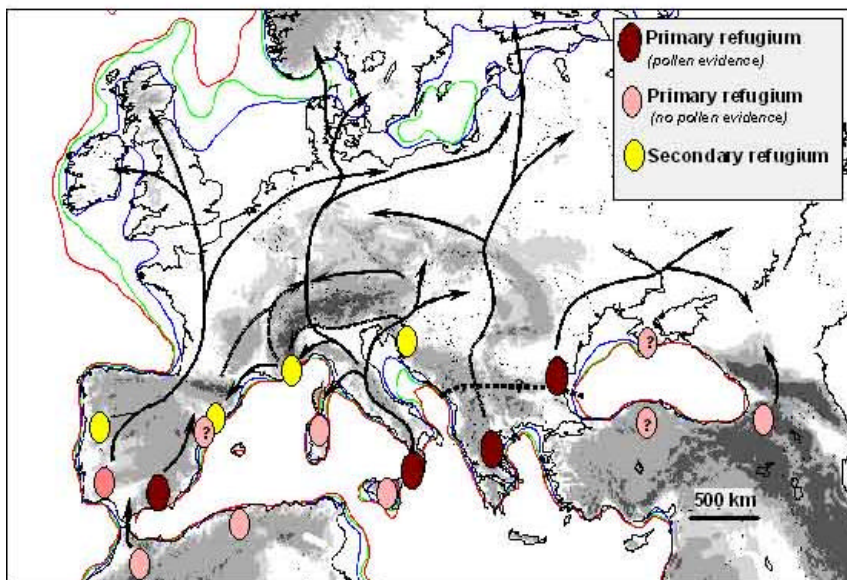


**Figure 5.** Phylogeny (based on Fitch-Margoliash method) and six main lineages identified for cpDNA variants found during screening of 11,828 individuals from 2,593 oak populations (from Petit *et al.* 2002b). The lineages are labelled A to F and the distribution of the individually numbered cpDNA variants can be seen in Fig. 6.

A strong phylogeographic structure was present within the data set (Fig 6), and allowed five important inferences: First, nearly all the haplotypes found in northern Europe were also present in southern populations, although the reverse was not true. This result suggests that most mutations were generated prior to postglacial expansion. Second, a total of six cpDNA lineages were identified, including one in Italy (C), two in Spain (B and D, the later which is also shared with northern Africa), and those from the Balkan region resolved into three lineages (A, E and F). These findings shed more light on the colonization dynamics in this important and previously understudied refugial area (Fig 7; Dumolin-Lapègue *et al.* 1997). Third, whilst postglacial colonization appears to have been predominantly from the primary refugia, the role of the secondary refugia, identified by the pollen core analysis (Brewer *et al.* 2002), also appears to have been significant (Fig 7). In particular, secondary refugia, which existed in the Alps and Carpathian mountains during the Younger Dryas, have been implicated in the unusual colonization pathway of one of the Balkan lineages, which spread into southern France and northeastern Spain (Petit *et al.* 2002b).



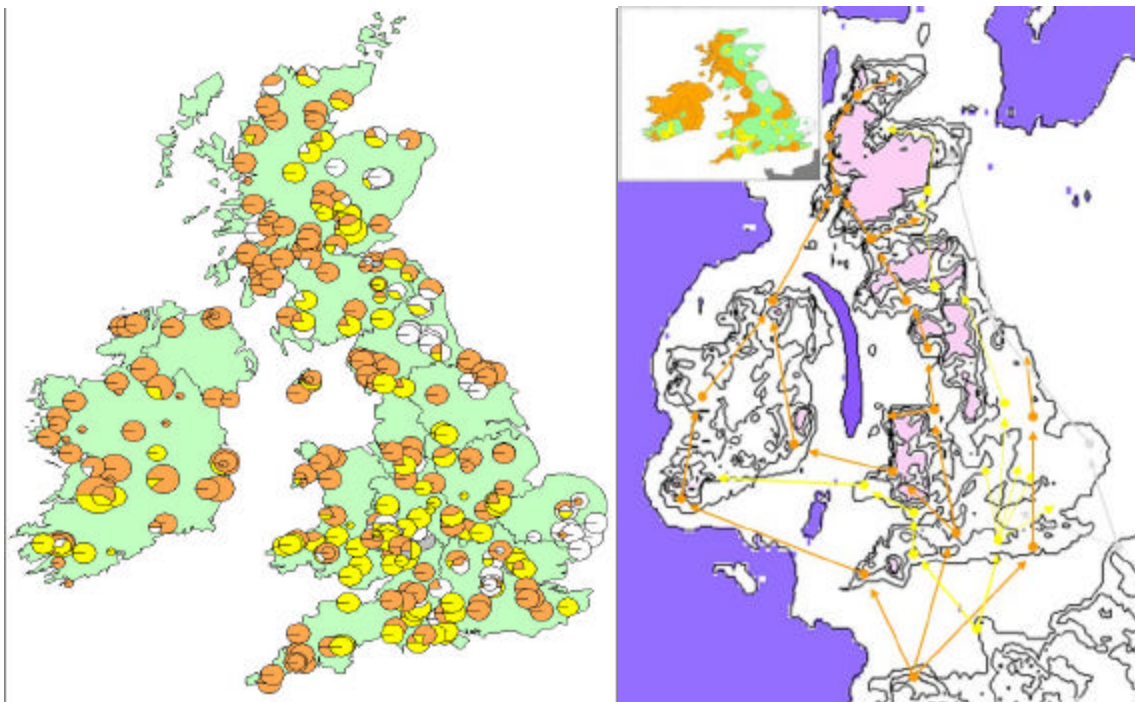
**Figure 6.** Superimposition of six identified phylogenetic clades (Fig. 5) onto sample locations (Fig. 4), indicating the distribution of refugial lineages (from Petit *et al.* 2002a). Lineage A is of Balkan origin and trees possessing these haplotypes have colonized central/eastern European areas and the Baltic states to reach the south of Finland. Lineage B is of Iberian origin and has colonized much of France, the British Isles and extends along the southern edge of the North Sea into Denmark. Lineage C is of Italian origin and trees possessing these haplotypes have colonized central European areas and southern Scandinavia. Lineage D is of Iberian origin and appears to have been restricted to Iberian and North African regions. Lineage E is of Balkan origin and is spread across much of the Balkans, further east in Ukraine and Russia, but is also found in Italy. Lineage F was found along the north coast of the Black Sea but sampling intensity is too low in this region to fully determine its pattern of distribution/postglacial spread.



**Figure 7.** It is possible to identify the main primary and secondary refugia and routes of colonization for oaks from each of the main cpDNA lineages (Fig. 6; from Petit *et al.* 2002a). The area of the southern Balkans remains unresolved due to poor sampling for molecular and pollen core analysis.

Fourth, chloroplast haplotypes tended to be shared amongst the eight oak species where they occurred in sympatry, but partitioning of variation in populations appeared to be

linked to a species' life history and regeneration ecology. Indeed, the ability of certain cpDNA lineages to spread out of refugia may be linked to the colonization ability of the species possessing those haplotypes. A fifth and final important observation concerned the age of chloroplast variation, which arose from analysis of the cpDNA phylogeny. Although a clock for the rate of mutation accumulation in the chloroplast DNA of plants is notoriously difficult to calibrate (Wolfe, Li and Sharp 1987), it appears that it was not possible for all the observed variation to have accumulated whilst regions were separated during the last glacial maximum (approximately 100 000 BP). Some of the observed lineage splits are so deep that they must have been isolated for millions rather than tens of thousands of years, and are expected to be the product of isolation during multiple glacial cycles. Vegetation changes before the onset of glacial periods are predicted to be very rapid and lead mainly to the extinction of northern populations rather than invasion of saturated, climax communities already present in southern refugial locations (Hewitt 1996). Under such dynamics, there is expected to be little mixing of different cpDNA lineages during inter-glacial periods.



**Figure 8.** Distribution of three main native cpDNA types in the British Isles (left), and the likely colonization pathways that these three types followed (right), calculated by interpolation (inset top) and topographical considerations. Sea levels deeper than 100m are shaded purple and are unlikely to have been land during the last ice age, and land higher than 300m is shaded pink and is the upper limit of growth of oaks in the British Isles (from Cottrell *et al* 2002).

The European wide pattern of cpDNA variation has been accompanied by a suite of papers detailing patterns of cpDNA at the country or local scales (e.g. France, Petit *et al.*, 2002c; Italy, Fineschi *et al.*, 2002; Scandinavia, Jensen *et al.*, 2002; Iberia, Olalde *et al.*, 2002). Within the British Isles 98% of the autochthonous oaks sampled possess one of three Iberian haplotypes (Cottrell *et al* 2002; Fig 8) that also occur in western France (Petit *et al.* 2002c). Two populations possess haplotypes from an Italian source but due to

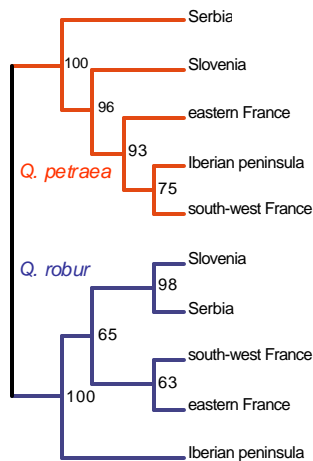
the polymorphic nature of these populations this material is considered to be recently introduced by man rather than naturally colonising. A fifth haplotype from the Balkan region is also found in some populations, particularly those from the Forest of Dean and Welsh Marches region. A further analysis of ancient woodlands (Cottrell *et al.* 2004) and seed source forests in the region (Lowe *et al.* 2004) indicates that this material is most likely to have been introduced by natural means. The three most commonly occurring haplotypes were strongly spatially structured across Britain, with one predominantly occurring in western areas, one in central areas (although predominantly in south England), and one in eastern areas (Fig 8). In addition to this longitudinal structuring, large areas of forest were fixed for a single chloroplast haplotype (some over several hundred square kilometres), and is a pattern also noted in several country-scale studies of oaks in Europe (e.g. France, Petit *et al.* 1997).

A series of simulation studies modelling dispersal strategies by Hewitt (1996), Ibrahim, Nichols and Hewitt (1996), Le Corre *et al.* (1997), Petit *et al.* (2001) and Davies *et al.* (2004), describe the results of a mode of dispersal called leptokurtic, where most seed dispersal is local but with occasional very long distance events of between 10 and 100 km. After several hundred generations, large patches of the recolonized landscape are dominated by a single genotype mimicking empirical observed patterns. In addition, haplotype diversity was found to reduce only slowly across the colonized landscape and this helps to explain why relatively high cpDNA diversity is maintained across northern Europe. Such a model of seed dispersal is realistic for oaks that have seeds that are predominantly gravity dispersed but for which additional dispersal can occur over very long distances by birds (e.g. Jays). Thus, once long distance dispersal events have established a small patch of trees possessing a single haplotype ahead of an advancing wave of colonization, local dispersal would increase the size and density of the patch. This would occur to such an extent that by the time the advancing wave had reached the single haplotype patch it would be almost impenetrable to the influence of external seed influx (although not pollen influx). The establishment and maintenance of this patchy distribution of cpDNA variation thus appears to be a consequence of the peculiar combination of rare long distance dispersal together with heavy local dispersal and the long generation time of oaks (Le Corre *et al.* 1997).

Such estimations of very long distance dispersal have only been so far been possible using simulation modelling approaches. For direct measures in the field one of the problems is that an exhaustive sample of all individuals over large spatial scales is required, which may include thousands if not millions of individuals. Where the spatial scale of sampling has allowed longer distance dispersal to be estimated, seed flow distances of > 1km and >5 km have been recorded for oak and ash respectively (S. Gerber, unpublished data; Bacles 2004). In both cases this was a minimum estimated dispersal distance and was the distance from the established seedling to the edge of the intensively sampled plot. As the actual dispersing tree was not identified, the real dispersal distance could be just outside the sampled plot or indeed from many kms or 10s of kms further away. In addition to the sampling effort required, Clark (1998) notes that the very long dispersal distances inferred from required post glacial colonization dynamics may not be found today because of changes in environment and ecosystem,

where propagules are invading climax communities rather than bare land which makes establishment much less likely.

In contrast to surveys of organelle DNA, range-wide analysis of nuclear-encoded variation indicates that oaks maintain high levels of diversity but that most variation is partitioned within populations (91% in a survey of 15 allozyme loci in 32 French populations by Kremer *et al.* 1991; 74% in survey of six allozyme loci in 26 Danish populations by Siegismund and Jensen, 2001). Indirect estimates of gene flow are consequently extremely high, presumably due to the highly efficient wind-pollinated reproductive mode of the species. Despite the high proportion of variation partitioned within populations, a small but significant component is partitioned between populations and appears to have been influenced by post glacial colonization dynamics. Allozyme analysis of 81 *Q. petraea* populations across Europe indicated that populations from the central part of the natural range exhibited fewer alleles but higher heterozygosities than populations from the margins of distribution (Zanetto and Kremer 1995), and the authors speculated that these patterns were due to Pleistocene colonization dynamics. Population differentiation across the range of the sample (Iberia to the Balkans) was very low ( $F_{ST} = 0.025$ ), but fitted an isolation by distance model (Zanetto and Kremer, 1995), as did a RAPD analysis across a similar range of samples (Le Corre, Dumolin-Lapègue and Kremer, 1997).



**Figure 9.** UPGMA dendrogram of 10 European *Q. robur* and *Q. petraea* populations (162 individuals) based on the proportion of shared alleles at 20 microsatellite loci (Muir, Fleming, Schlötterer, 2000). One population from each species was sampled at five locations. Numbers are bootstrap support values. In accordance with the assumption that *Q. robur* and *Q. petraea* are separate taxonomic units, all populations of the same species group together. The separation between the two species is low but supported by high bootstrap values (100%). Similarly,  $F$ -statistics also indicated a significant differentiation between the two species ( $P < 0.01$ ), but was mainly influenced by allele frequency differences at only 3 of the 20 loci surveyed. There was also a significant component of variation due to the geographic origin of the population ( $P < 0.01$ ), and distribution partially corresponds to glacial refugial regions.

Further analysis of allozyme and RAPD variation found that there was a correlation with chloroplast variation (Le Corre, Dumolin-Lapègue and Kremer 1997; Kremer *et al.* 2002a), and when geostatistical tools were applied to the data, gene frequency variance reflected the postglacial pathways of colonization (Le Corre *et al.* 1998). These

observations suggest that the nuclear genome must have been differentiated between populations inhabiting different Pleistocene refugia. Thus glacial refugia populations were differentiated for nuclear although probably to a lesser extent than organelle genomes. However, once postglacial colonization began, extensive gene flow would have led to contact between refugia areas and caused extensive mixing of nuclear genomes (Kremer *et al.* 2002a). Indeed, no difference in diversity has been observed for nuclear microsatellite loci sampled from northern (British and Irish) vs. southern (French, Spanish and Italian) oak populations (Mariette *et al.* 2002; Muir *et al.* 2004). Despite this mixing, there is still a refugial imprint of nuclear differentiation, which remains in contemporary populations and can be identified using microsatellites (Fig. 9; Muir, Flemming and Schlotterer 2000; Muir *et al.* 2004).

An imprint of postglacial colonization is, however, not evident for morphological or adaptive variation, which display no correlation with cpDNA variation (Kremer *et al.* 2002a) and exhibit very high population differentiation (Kremer, Zanetto and Ducusso, 1997). For such quantitative characters, local selection pressures are probably responsible for the contemporary distribution of genetic variation.

### **Other phylogeographic studies of forest species colonization**

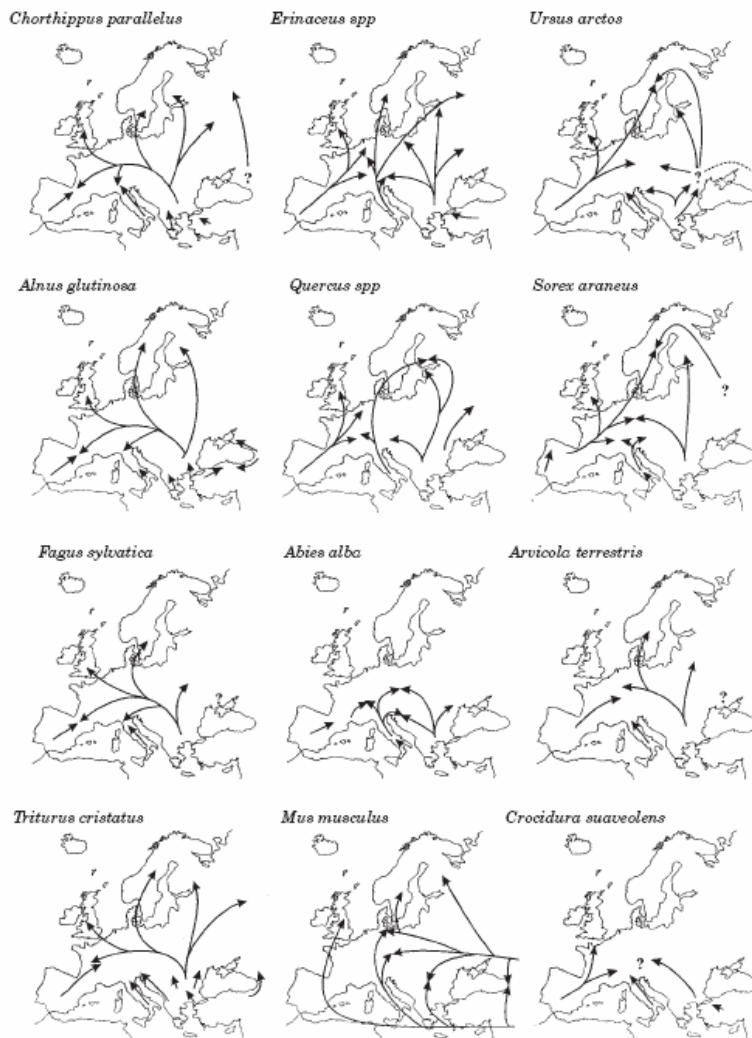
Many other forest dwelling plants and animals have been studied to reconstruct post glacial colonization routes and dynamics. Whilst some have combined molecular, palynological and computer simulation studies most have relied totally on phylogeographic structure with an organelle genome.



**Figure 10.** Main zones of contact or suture between different refugial colonization lineages across a range of species (Taberlet *et al.* 1998).

Examples of phylogeographic studies of other plant and animal species across Europe are: alder (*Alnus glutinosa*; King and Ferris 1998), beech (*Fagus sylvatica*; Demesure, Comps and Petit 1996), ivy (*Hedera spp.*; Grivet and Petit 2002), hornbeam (*Carpinus betulus*; Grivet and Petit 2003), buckthorn (*Frangula alnus*; Hampe *et al.* 2003), common ash (*Fraxinus excelsior*; Heuertz *et al.* In Press), silver fir (*Abies alba*; Liepelt *et al.*

2002), *Prunus spinosa* (Mohanty *et al.* 2003), wild cherry (*Prunus avium*; Mohanty *et al.* 2001), silver birch (*Betula pendula*; Palmé 2003a), downy birch (and dwarf birch (*B. pubescens* and *B. nana*; Palmé *et al.* 2004), hazel (*Corylus avellana*; Palmé and Vendramin, 2002); willow (*Salix caprea*; Palmé *et al.* 2003b), Scots pine (*Pinus sylvestris*; Sinclair *et al.* 1999; Soranzo *et al.* 2000), Norway spruce (*Picea abies*; Taberlet *et al.* 1998), English holly (*Ilex aquifolium*; Rendell and Ennos 2003), heather (*Calluna vulgaris*; Rendell and Ennos 2002), a woodland sedge (*Carex digitata*; Tyler 2002), red squirrel (*Sciurus vulgaris*; Hale *et al.* 2004), European hedgehog (*Erinaceus europaeus*; Hewitt 1999; Seddon *et al.* 2001), brown bear (*Ursus arctos*; Hewitt, 1999), shrew (*Sorex araneus*; Hewitt, 1999), lesser white toothed shrew (*Crocidura suaveolens*; Taberlet *et al.* 1998), red deer (*Cervus elaphus*; Hewitt, 1999) and woodmouse (*Apodemus sylvaticus*; Hewitt, 1999).



**Figure 11.** Proposed post-glacial expansion routes deduced from molecular data and fossil evidence. Three broad patterns emerge – the grasshopper, *Chorthippus parallelus*, the hedgehog, *Erinaceus europaeus* & *concolor*, and the bear, *Ursus arctos*. Species with similar patterns are placed below each of these (from Hewitt 1999).

Despite the range of taxa, there have been several attempts to identify common colonization pathways. Taberlet *et al.* (1998) was able to identify some common patterns of areas where glacial refugial lineages are likely to meet (called suture zones, Fig 10). However, no common pathways of post glacial colonization are obvious across the range of studies. Indeed, depending on the species, the British Isles has been colonized by populations from Iberian, Italian and Balkan origin (Fig. 11; Hewitt 1999). It appears that the routes of colonization followed, and the pattern of genetic variation established during migration, are particular to the life history of individual species (Taberlet *et al.* 1998; Comps *et al.* 2001). Interestingly a meta-analysis of chloroplast phylogeographic variation for 22 European trees, including populations sampled in Britain, found that maximal genetic diversity is not harboured in the refugial areas of the Iberia, Italy and Balkans but in middle latitudinal regions where the refugial zones meet (Petit *et al.* 2003).

Whilst most studies of historical colonization have used organelle genomic regions (including cpSSR variation), some studies have successfully applied nuclear microsatellite (and in some cases allozyme) variation to reconstruct post-glacial colonization pathways and dynamics; including beech (Comps *et al.* 2001), oak (Muir *et al.* 2001), ash (Heuertz *et al.* 2004), red squirrel (Hale *et al.* 2001), silver fir (Liepelt *et al.* 2002), butterflies (*Erebia medusa* and *Polyommatus coridon*; Schmitt and Seitz 2001a & b). Most of these studies have corroborated organelle findings but added the extra dimension of male or pollen mediated gene flow.

Interestingly almost none of the above-described studies indicate that refugial populations ever existed within the British Isles and it appears that almost all of the contemporary flora and fauna of the British Isles has established from a central or southern European source. One possible exception is Scots pine, where the distribution of variation for mitochondrial DNA could be consistent with the existence of a refugium off the west coast of Scotland (Ennos *et al.* 1997; Sinclair *et al.* 1999). Interestingly the pine beauty moth, whose native host is Scots pine, also exhibits mitochondrial variation consistent with a western British Isles refugium (Lowe *et al.*, In Press). This area is one of the 'cryptic refugia' postulated by Stewart and Lister (2001) to exist within the British Isles (including one in SW Ireland and another at Kent's Cavern in Cornwall). The verification of these regions as important refugial locations for at least some biota will require further fossil and phylogeographic work. Another recent development that may shed light on the historical colonization of species is the possibility of extracting DNA from ancient macrofossils (Dumolin-Lapègue *et al.* 1999), which provides exciting avenues of research for the future.

### ***Gene dynamics within and between contemporary woodland systems***

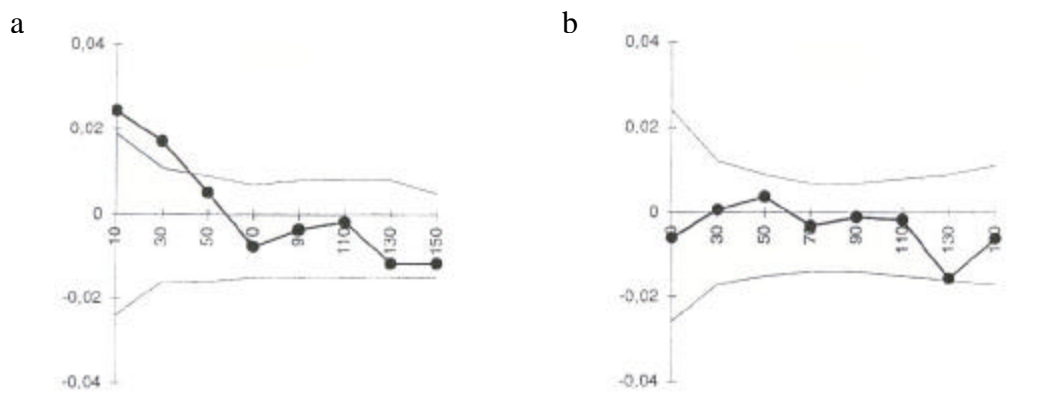
Gene dispersal dynamics have a profound impact on the magnitude of contemporary gene flow and the structuring of genetic diversity within and between populations. For species with low vagility, the effect of restricted dispersal will produce genetic structuring within populations. Advances in the analysis of fine-scale genetic structure have resulted from the availability of highly polymorphic markers (e.g. microsatellites) and concurrent development of statistical analyses for dealing with specific requirements and

assumptions of the data and have helped to infer contemporary gene dispersal dynamics within native forest communities (Sork *et al.* 1999).

### Case study of oak population genetics - a well studied forest community

At the scale of a single population, local gene flow dynamics (seed and pollen dispersal mechanisms), population density (Gram and Sork 1999), regeneration ecology and selection, all influence fine-scale genetic structure. Whilst several studies have documented the level of diversity within and between tree populations, only relatively few have examined fine-scale genetic structure. Part of the reason for the lack of such studies is that sufficiently polymorphic markers and easily applicable statistical methods of analysis have only recently been available (e.g. Epperson and Allard 1989; Perry and Knowles 1991; Berg and Hamrick 1995; Degen 2000).

For oak species more than 50 polymorphic microsatellite loci have now been isolated (see Section 2). In a study of an intensively sampled plot in France (Petite Charnie), 355 mature *Q. robur* and *Q. petraea* trees were screened for 6 microsatellite and 4 allozyme loci (Bacilieri, Labbé and Kremer 1994; Streiff *et al.* 1998). For both species, microsatellites highlighted stronger spatial structure than allozymes. The discrepancy in results between the two marker sets are believed to be mainly due to allelic richness in the two marker types, as higher numbers of rare alleles (typical for SSR data sets) increase differentiation estimates. Comparison of fine-scale genetic structure within the *Q. robur* and *Q. petraea* components of the wood hint at interesting biological differences between the two species. For both marker sets, the *Q. petraea* component of the wood exhibited significantly higher spatial structure than the *Q. robur* component. Streiff *et al.* (1998) suggest that differences in seed dispersal ability between the two species are mainly responsible for the observed differences in genetic structure. The acorns of *Q. robur* occur on long peduncles which project them clear of leaf whorls making them more obvious to birds such as jays (Bossemma, 1979) and thus more likely to be dispersed. However, the acorns of *Q. petraea* are nestled within the leaf whorls on short peduncles, and are thus less conspicuous.

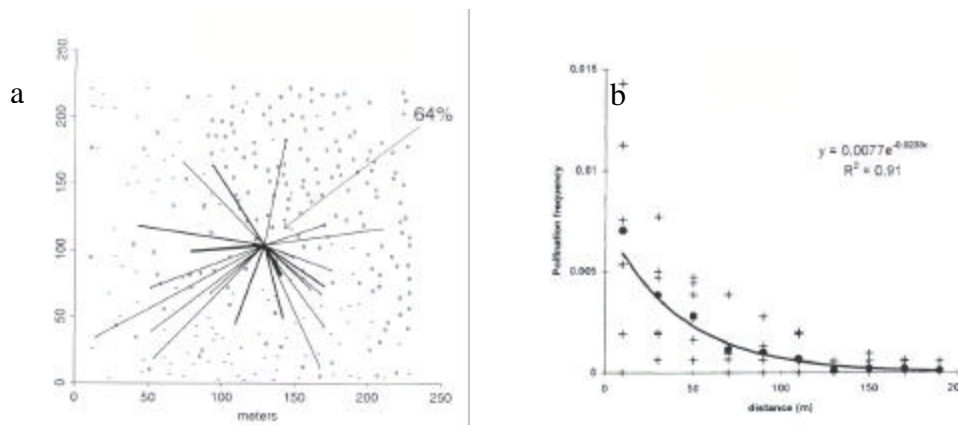


**Figure 12.** Fine-scale spatial genetic structure and pollen-mediated gene flow within the French forest stand, Petite Charnie of 166 *Q. petraea* trees, 183 *Q. robur* trees and 6 intermediate mapped individuals. a

and b) Spatial autocorrelation analysis within the wood, where black circles indicate observed values for ordered allele analysis and thin lines are 95% confidence limits, for *Q. petraea* and *Q. robur* respectively (from Streiff *et al.* 1998).

Another microsatellite study of fine scale genetic structure, this time in a British oak wood (Cottrell *et al.* 2003), also found significantly stronger spatial structuring in the *Q. petraea* component compared to the *Q. robur* component of the wood. The authors of this study speculate that in addition to seed dispersal differences, variation in the regeneration ecology of the two species has a significant influence on genetic structure. At Roudsea, *Q. robur* tends to grow and regenerate at low density in open woodland surrounded by many other herb and tree species, and where non-local colonizers have a good chance of establishing and disrupting local genetic structure. However, *Q. petraea* tends to occur as a mono-dominant stand where local, heavy seed rain would be likely to swamp out non-local colonizers, and thus maintain strong fine-scale spatial structure. A third study of a *Q. petraea* woodland in Denmark (Jensen *et al.* 2003) found that spatial genetic structure was stronger in juvenile compared to ancient trees and this is thought to reflect selective forces acting across the life time of individuals. Habitat type and forest structure have also been found to have a significant influence on the spatial genetic structure in other oak species (e.g. *Q. alba*, Gram and Sork 2001).

For oaks, direct estimation of gene flow is possible due to the availability of highly polymorphic marker sets (e.g. microsatellites). Exclusion and maximum likelihood statistics can be applied to identify seed contaminants, infer seed parents and calculate the number of pollen donors (Lexer *et al.* 2000; Smouse *et al.* 2001). One of the most biologically informative estimates of direct gene flow has been made at the Petite Charnie stand in France using microsatellites (Streiff *et al.* 1999). In this study, 984 offspring were collected from 13 mother trees within a natural stand of 296 previously genotyped mature trees (Streiff *et al.* 1998). In all families, a high percentage of offspring (65% for *Q. robur* and 69% for *Q. petraea*) were pollinated by fathers from outside the study area (5.76 ha; Streiff *et al.* 1999). By pooling data from all progeny arrays, pollen dispersal curves within the stand were inferred. Whilst proximate trees are the most likely to act as pollen donors, the proportion of long-distance pollination events is very high, and the pollen dispersal curves suggest that pollination events could be over several km (Fig. 13; Streiff *et al.*, 1999). Overall direct estimates of pollen-mediated gene flow for oaks correlate well with the indirect estimates made from population genetic structure (Zanetto and Kremer 1995). Both types of study indicate a substantial level of pollination involving very long distance transfer (km not m), which is probably the main mechanism maintaining connectivity between even widely separated populations.



**Figure 13.** a) Direction, distance and frequency (indicated by thickness of line) of inferred pollinations for a *Q. petraea* mother tree. The proportion of incoming pollen (64%) is indicated by the arrow. b) Distribution of mating frequencies as a function of the distance between parental trees. Mating frequencies, i.e. number of matings observed over the total number of matings analyzed is plotted as a function of the distance between the maternal and paternal parents. Black circles represent average values of mating frequencies for each maternal tree (crosses) from Streiff *et al.* (1999).

Microsatellite analysis has also been used to examine parentage of seedlings within populations of *Q. macrocarpa* in the USA (Dow and Ashley 1996). Of the 100 saplings analysed, 94 matched at least one of 62 adult bur oaks within the sample stand. In addition, matched saplings tended to grow in dense half-sib clusters around the presumed maternal parent, although some long distance seed dispersal events were recorded. The mast-seeding (Sork 1993; Sork and Bramble 1993) and secondary transfer of acorns by birds (Bossema, 1979) and small mammals (Sork, Stacey and Averett 1983; Sork 1984), all have a role in producing this effective reproductive and dispersal strategy for oaks. The correspondence between seed dispersal estimates, inferred from genetic structure of maternally inherited markers (cpDNA), and actual measures of seed-mediated gene flow in natural populations, appear to be mostly concordant for oaks. Such empirical validation provides further support for results of simulation models on the influence of dispersal on genetic structure (e.g. Davies *et al.* 2004). There does appear to be a consistent bias between these estimates though and direct seed flow estimates are usually shorter than those based on indirect (genetic structure) calculations. One explanation for this observation is that seed flow estimates based on cpDNA genetic structure represent seed dispersal in an open landscape whereas direct estimates of seed flow are conducted in mature wooded landscapes, where colonization is more limited.

### **Other studies of gene structure and dynamics within forest dwelling species**

Several studies of population genetic structure, gene flow and fine scale genetic structure demonstrate further ecological behaviours for other tree, animal and fungal species inhabiting forest ecosystems. The specialized nature of the studies does not allow wider generalizations of the biology of the organisms within a forest ecosystem context. However, they remain interesting case studies and as further studies are compiled underlying patterns of spatial genetic structure and gene flow may become apparent for a range European forest species.

Natural populations of common ash *Fraxinus excelsior* (although outside the UK) have been subject to population genetic studies using microsatellites. In common with other temperate forest trees, populations of ash in France and Bulgaria support very high levels of genetic diversity and have low differentiation between populations (Morand *et al.* 2002; Heuertz *et al.* 2001). European populations also exhibited significant levels of inbreeding and may be connected with the complex mating system (where individuals can be male, female or both) and fine scale genetic structure. Analysis of spatial autocorrelation was also performed on a Romanian population and whilst significant spatial structuring was evident, restricted seed dispersal (< 14 m calculated from spatial genetic structure analysis) rather than the more moderate pollen dispersal was the probable cause (between 70 and 140 m according to spatial genetic structure analysis; Heuertz *et al.* 2003).

A survey of microsatellite variation of trees and seedlings in 22 riparian forest fragments of black poplar, *Populus nigra*, along the Drome River in southern France revealed a strong isolation by distance effect (Imbert and Lefevre 2003). Gene flow parameters were high throughout the river system and there was no accumulation of diversity in the lower portions of the river indicating symmetrical gene flow both up and down stream, which may be differentially mediated by seed and pollen.

Genetic structure of a population of the ectomycorrhizal basidiomycete *Laccaria amethystine* was assessed using a modified microsatellite probing technique in a 150-year-old beech forest in northeastern France (Gherbi *et al.* 2003). 388 different genotypes were resolved amongst 634 sporophores analyzed and genet density was as high as 130 per 100 m. The largest clone covered >1 m, but most genets covered a few cm and produced only one to three sporophores. Only eight genotypes identified in 1994 were found in 1997. Although *L. amethystine* has the capacity for vegetative persistence, the study indicated that sexual reproduction occurs at a high frequency and that beech trees can be recolonized by new genotypes each year (Gherbi *et al.* 2003).

The genetic structure of populations of two carabid species of ground beetle, one described as a generalist and the other known as a forest specialist, have been described using microsatellite analysis in southern France (Brouat *et al.* 2003). Genetic differentiation was substantial for both species studied, but populations of the forest specialist, *Carabus punctatoauratus*, was more spatially structured than those of *C. nemoralis*. Isolation by distance analyses showed that nonforested areas are partial barriers to gene flow for both species studied, although more clearly for the forest specialist. In addition, the between and within forests dispersal capacity of the generalist *C. nemoralis* was higher than that of the specialist *C. punctatoauratus* (Brouat *et al.* 2004).

### ***Impacts of woodland clearance and potential for restoration***

#### **Clearance, fragmentation and habitat disturbance**

Habitat disturbance and fragmentation occurs in natural ecosystems, notably through fire, wind and flooding (Andr en 1994). However, human activities, such as deforestation for commercial wood products and land-use changes for agriculture, are the most important,

large-scale causes of fragmentation and loss of natural habitat. For the last 20 years, a great deal of attention has been given to the sudden deforestation of the tropics which is occurring at an alarming rate of 100 000 to 200 000 km<sup>2</sup> per year (Katzman and Cale 1990; White *et al.* 1999), threatening the existence of the world's reservoir of biodiversity (Sih *et al.* 2000). Less commonly considered is the more ancient and continuing deforestation of the temperate zone (Wilcove *et al.* 1986). For example, in Great Britain, forest fragmentation began over 5000 years ago with permanent clearances by Neolithic farmers and was well advanced by the 11<sup>th</sup> century (Wilcove *et al.* 1986), and in Ireland less than 0.2% of the land area of the great Atlantic woodland remains today (Muir *et al.* 2004).

### **Theoretical consequences of fragmentation**

Habitat fragmentation reduces the size and increases the spatial isolation of suitable sites for a species (Wilcox and Murphy 1985). Three major component processes can be distinguished (Andren 1994): loss of original habitat (pure habitat loss), reduction in habitat patch size and increased isolation of habitat patches. Such 'insularization' (Wilcove *et al.* 1986) of habitats produces a patchwork-like landscape, in a mosaic, where small patches of similar habitats are isolated from one another by a matrix of different habitats. Theoretical predictions are that habitat fragmentation leads to species extinctions due to reduced patch size and reduced species colonization with increasing distance between patches (MacArthur and Wilson 2001). Similar predictions are made for genetic diversity and gene flow but fragmentation is also predicted to be accompanied by dramatic changes in population genetics, affecting population and species viability in the short and long term (Young *et al.* 1996).

Habitat fragmentation is predicted to reduce genetic diversity within population and increase interpopulation genetic differentiation (Young *et al.* 1996). It is thought that in the long term, loss of genetic variation will lead to a reduction in the ability of populations to respond to changing selection pressures (Young *et al.* 1996). In the short term, it is expected to result in increased susceptibility to pest and diseases (Barrett and Kohn 1991) and to a reduction in population viability associated with the fixation of deleterious alleles (Huenneke 1991). For plant populations, these changes are likely to be complicated by their sessile habit (Loveless and Hamrick 1984), and therefore, their prefragmentation abundance. Species population remnants are expected to experience genetic effects, associated with reduced population size, as follows:

#### *Loss of genetic variation*

As a consequence of habitat fragmentation, only a sample of the individuals and alleles within the original population will remain in the remnants. The loss of genetic variation associated with a sudden reduction in population size is referred to as a genetic bottleneck (Barrett and Kohn 1991), and its intensity depends on the severity and duration of habitat fragmentation. Only a sample of the original individuals and their alleles will survive. The severity of genetic loss associated with a sudden population size reduction, a genetic bottleneck (Barrett and Kohn 1991), will depend on the severity of habitat fragmentation or disturbance, that is to say, the severity of reduction in population size. Whilst the first alleles to be lost following a genetic bottleneck will be rare ones, extended periods of

drift, acting over many generations, are expected to result in additional loss of more common alleles, and result in severe depletion of genetic diversity (Lande 1988).

Populations that remain small for many generations undergo increased random genetic drift and loss of allelic variation (Ellstrand and Elam 1993; Barrett and Kohn 1991). Population viability may be affected within a relatively short period, since random genetic drift acts equally on neutral and selected genes and deleterious alleles may become fixed by chance (Huenneke 1991).

#### *Increased inbreeding and inbreeding depression*

Increased inbreeding is one expected outcome of fragmentation and is thought to arise following changes in mating patterns within populations. In hermaphrodite species, increased inbreeding may arise if the proportion of selfing is increased. In normally dioecious or outcrossing species, inbreeding can increase due to increased mating between close relatives (biparental inbreeding). As a result of increased isolation between populations (and between individuals within populations), gamete exchange between more distant individuals or populations is reduced and mating between spatially proximate individuals becomes more likely. As proximate individuals in most species are often more likely to be relatives, such mating patterns can result in biparental inbreeding (Barrett and Kohn 1991).

Increased inbreeding translates into a reduction of individual heterozygosity, which can lower individual fitness, a phenomenon referred to as inbreeding depression (Charlesworth and Charlesworth 1987). Inbreeding depression will affect individual fecundity, establishment and survival and ultimately population viability (Hedrick 1994; Keller and Waller 2002). These effects are likely to be greater for plant than animal species (Barrett and Kohn 1991). However, how population viability is affected depends on the composition of the genetic load (i.e. the population component of inbreeding depression). Genetic load is in turn influenced by population size and history (Keller and Waller 2002) and the nature of mutations causing inbreeding depression (Bataillon and Kirkpatrick 2000).

Historically, populations of dioecious or outcrossing species are expected to harbour a high genetic load. A shift to mating patterns, which increase inbreeding after fragmentation, is therefore expected to have the most severe fitness effects on these species (Reinhartz and Les 1994). In contrast, chronically small populations are likely to exhibit lower levels of inbreeding depression as deleterious mutations are purged by selection, although this depends on the genetic basis of inbreeding depression (Crnokrak and Barrett 2003). Successive generations of inbreeding will purge lethal alleles while the genetic load resulting from mildly deleterious alleles will persist (Hedrick 1994; Bataillon and Kirkpatrick 2000).

#### *Reduced migration*

By increasing isolation between patches, initial predictions are that habitat fragmentation reduces gene flow among populations. A reduction in the proportion of immigrant genes combined with increased genetic drift and inbreeding, increases genetic differentiation

among fragmented remnants (Young *et al.* 1996). Gene flow is best known as a force homogenising the distribution of genetic variation among populations. However, gene flow in the field is often idiosyncratic, varying greatly between species, populations and seasons (Young *et al.* 1996). The ultimate effect of gene flow on local population diversity will depend on the nature of genetic variation in source relative to sink populations (Ellstrand and Elam 1993) and population genetic connectivity (Couvet 2002).

The actual relationship between inbreeding depression and gene flow has been addressed theoretically only very recently (Couvet 2002). It appears that the viability of individuals is reduced in populations of small size when gene flow is limited. Levels of gene flow greater than one migrant per generation (sufficient to balance the effect of genetic drift) may be required to prevent the accumulation of deleterious mutations in small populations (Couvet 2002).

In species with historically high levels of gene flow, habitat fragmentation will most likely provoke a drop in the amount of gene flow (by increasing the distance between populations and by subsequently disturbing pollinator and seed disperser guilds). This enhances the detrimental effects associated with genetic drift and inbreeding associated with small population size. However, if the disturbance created by fragmentation reduces the size of a population so that the fraction of seed fertilized by immigrant pollen or the fraction of immigrant seed increases, gene flow is enhanced even if the absolute number of migrants may be reduced (Ellstrand and Elam 1993).

When a species' demography is characterized by frequent extinction of local populations and colonization of new sites (i.e. a metapopulation), it is under threat of local extinction, if colonization cannot balance extinction in a fragmented habitat (Lande 1988). Nevertheless, colonization of new sites represents founder events, which have similar population genetic consequences to bottlenecks (Barrett and Kohn 1991). Founder effects may be particularly strong in a fragmented habitat because a source population may itself be suffering the detrimental effects of increased drift and inbreeding.

### **Empirical studies documenting genetic impacts of fragmentation**

A large number of studies have examined the genetic impact of habitat fragmentation on a range of woodland and forest dwelling species. Many of these studies have been conducted in European and British systems, but interesting results also come from studies conducted in North America and the neotropics. In most cases there is a genetic consequence to species inhabiting fragmented compared to non-fragmented landscapes, which is manifest as one or a combination of; loss of genetic diversity, increased inbreeding and/or reduced migration.

#### *Decreased genetic diversity*

Most empirical data confirm that the amount of genetic variation maintained within a fragment is usually related to population size. For instance, Prober and Brown (1994) found a significant positive relationship between population size and both the percentage

of polymorphic loci and allelic richness measured at isozyme loci within fragments of *Eucalyptus albens*.

Similar dynamics of lowered genetic diversity fragmented compared to continuous habitat populations have been observed for a range of animal, bird, insect and herb species using a range of marker systems. One of these studies include: analysis of Belgian populations of the winter moth, *Operophtera brumata* (Vandongen *et al.* 1994); mitochondrial allelic diversity of forest dwelling California red-backed vole, *Clethrionomys californicus* (Tallmon *et al.* 2002); isozyme analysis of the common understory herb, *Viola pubescens* (Violaceae), in Ohio, USA (Culley and Grubb 2003); mitochondrial DNA variation in the highly endangered Spanish imperial eagle, *Aquila adalberti*, compared to the closely related eastern imperial eagle, *Aquila heliaca* (Martinez-Cruz *et al.* 2004). For the rare Mediterranean orchid, *Anacamptis palustris*, chloroplast microsatellites not only detected a reduction of genetic diversity in fragmented compared to continuous populations, but signature colonization bottlenecks were also observed in the smallest populations (Cossolino *et al.* 2003);

Habitat degradation activities, e.g. logging, can also reduce the genetic diversity of an impacted habitats biota. For example lower genetic diversity, measured using microsatellites and AFLPs, was found for British Columbian populations of the salamander, *Dicamptodon tenebrosus*, inhabiting recently clearcut sites compared to old growth forest (Curtis and Taylor 2004).

The level of genetic variation maintained at microsatellite markers in adult and juvenile cohorts of continuous and fragmented stands have been compared for several neotropical tree species. In these recently fragmented forests, the level of genetic variation sampled in the adult cohort represents a prefragmentation abundance while sapling and seedling cohorts should reflect the genetic effects of habitat fragmentation. Dayanandan *et al.* (1999) found that allelic richness was lower in saplings of isolated fragments of *Carapa guianensis*. For *Swietenia humilis*, White *et al.* (1999) also found that rare alleles present in the continuous stand were lost in fragments and that such allelic loss increased as fragment size decreases. However, Aldrich *et al.* (1998) found a similar number of alleles in adult and sapling cohorts of both continuous and fragmented stands of *Symphonia globulifera* and increased genetic diversity was observed in fragments of *Acer saccharum* compared to continuous stands (Young *et al.* 1993). These studies are informative to European and British taxa as the spatial scale of fragmentation is similar. However, one possible explanation for the discrepancy between the abovementioned studies is that the populations of interest may have been fragmented for only a few generations, which means that genetic drift is unlikely to have had sufficient time to cause additional diversity loss (i.e. heterozygosity, Petit *et al.* 1998). It is likely that native British forest/woodland species will have been fragmented for much longer periods of time.

A study by Prober and Brown (1994) found that the most isolated remnants of *E. albens* were also the genetically most depauperate, suggesting a possible fragmentation threshold (Young *et al.* 1996). A threshold of fragmentation was also suggested by the study of Cruzan (2001) on the endemic North American herbaceous perennial,

*Scutellaria montana* (Lamiaceae). Cruzan found that genetic diversity decreased significantly in fragmented populations with a census of less than 100 individuals and isolated by more than 8km, compared to larger more proximate sites (Andren 1994).

#### *Increased inbreeding*

In contrast to a reduction in genetic variation, which will affect population viability over the longer term, changes in mating parameters respond more rapidly to habitat fragmentation.

Increased inbreeding (presumably due to selfing and/or biparental inbreeding) following fragmentation has been reported for several species. In the daisy, *Rutidosia leptorrhynchoides*, Young and Brown (1999) observed a paternal bottleneck (i.e. greater divergence between the allele frequencies in the populations than those in the pollen pool estimated from progeny arrays) and higher correlation of outcrossed paternity (i.e. production of more full-sibs) in small isolated populations. Similarly, Fuchs *et al.* (2003) found that the progeny of *Pachira quinata* trees from continuous stands, experienced lower levels of relatedness as a result of higher outcrossing rate or higher number of sires than the progeny of isolated trees. Aldrich *et al.* (1998) compared genetic structure at microsatellite loci of adults, saplings and seedlings cohorts of continuous and fragmented stands of *Symphonia globulifera*. A significant inbreeding coefficient was associated with the fragmented stands and seedlings. The critically endangered Taita thrush was found to exhibit significant inbreeding and development instability (measured by fluctuating asymmetry) in its current distribution in highly fragmented habitat (Lens *et al.* 2000). Central European populations of roe deer, *Capreolus capreolus*, surveyed with microsatellites and isozymes exhibited a high significant correlation between inbreeding coefficient and the density of human settlement (Wang and Schreiber 2001).

For plant species, the effects on mating parameters may be increased by association with pollinators and seed dispersers. Franceschinelli and Bawa (2000) found that outcrossing rate in populations of *Helicteres brevispera* was directly correlated with plant density, which is low in forest areas and high in the savannah. Increased selfing rate in areas of low density was explained by pollinators tending to visit several flowers per plant (Franceschinelli and Bawa 2000). However, the response of pollinators may be very different if the variation in plant density results from a disturbance of habitat.

#### *Decreased gene flow and genetic connectivity*

An impressive body of literature exists that describes the genetic structure (from which gene flow and genetic connectivity estimates can be made) at neutral marker loci within and among fragmented and non fragmented populations. Studies on a range of animals, birds and insects indicate that fragmentation may more profoundly impact on dispersal patterns than on reproductive output and survival.

Example studies include direct observations of dispersal and genetic connectivity for squirrels, small passerines and butterflies which were higher within continuous compared to fragmented forest population in Belgian (Matthysen *et al.* 1995). Genetic differentiation, as measured by isozymes, of the beetle *Bolitophagus reticulatus*

(Coleoptera: Tenebrionidae), was 4 times higher for fragmented compared to continuous forest populations (Knutsen *et al.* 2000). Differentiation, as measured by isozymes, for two weakly flying geometric moths, *Epirrita* species, was higher within fragmented than continuous woodland populations in southern England (Wynne *et al.* 2003). Isozyme differentiation over the same spatial scale was higher for two Australian lizard species within fragmented compared to non-fragmented woodland systems (Driscoll 2004). Fragmented populations of *Speyeria idalia* (Nymphalidae) had lower diversity and higher differentiation, as measured by microsatellites, compared to populations in a continuous habitat, despite this being a species with high effective gene flow (Williams *et al.* 2003). Populations of black grouse, *Tetrao tetrix*, sampled in Finland, France, Switzerland and Italy and surveyed with microsatellites were found to be significantly more differentiated in fragmented compared to continuous habitat (Caizergues *et al.* 2003). The European tree frog, *Hyla arborea*, was screened for variation at 12 microsatellite loci, which revealed lower genetic diversity, higher inbreeding and higher differentiation within the most fragmented of comparative populations sampled in Denmark (Andersen *et al.* 2004). Hall *et al.* (1996) compared genetic structure at isozyme loci of populations from a large reserve and several fragmented stands for the neotropical tree, *Pithecellobium elegans*. They found a positive relationship between level of differentiation and the distance of the fragment from the reserve, thus suggesting that gene flow decreases as fragments become increasingly isolated. Dayanandan *et al.* (1999) also found greater genetic differentiation among sapling cohorts than adult cohorts in fragments of *C. guianensis* and concluded that this was a result of restricted gene flow as a consequence of fragmentation.

Studies that have attempted to measure contemporary gene flow directly are now also increasing in number due to the recent availability of highly polymorphic markers and new statistical methods which have rendered the task more achievable (Sork *et al.* 1999). Sork *et al.* (2002) found that for the North American wind pollinated oak, *Quercus lobata*, very few trees contribute to the pollen pool of individuals in a severely fragmented habitat. This is likely to be a similar situation of fragmentation and response for British woodland trees. Koenig and Ashley (2003) observe that whilst pollen from wind-pollinated trees has traditionally been assumed to be abundant and to travel long distances, resulting in extensive gene flow, a review of recent empirical work indicates that short-distance dispersal of limited pollen might be common and play an important role in causing the highly variable seed production (e.g. masting). They also speculate that pollen movement might be sufficiently restricted that increased fragmentation could ultimately bring about reproductive failure in some species.

#### *Consequences of human infrastructure*

Several studies have highlighted the potential impact of linear infrastructure (e.g. roads, and railways) and their role in subdividing and fragmenting the habitat. It appears that species inhabiting systems subdivided by roads can suffer quite severe genetic effects associated with fragmentation effects.

Example studies that found a genetic impact due to linear infrastructures include, isozyme and RAPD analysis of Belgian populations of *Coelotes terrestris* (Araneae,

Agelenidae; Gurdebeke *et al.* 2000); direct dispersal and migratory observation of eight species of amphibians along road sides and in continuous forest of Central Maine, USA (De Maynadier and Hunter 2000); microsatellite analysis of the flightless ground beetles *Carabus violaceus* L. and *Abax parallelepipedus* in Swiss forests fragmented into different compartment sizes by roads (Keller and Lurgiader 2003; Keller *et al.* 2004).

A study of the bank vole, *Clethrionomys glareolus*, from Germany and Switzerland found that populations separated by a major highway or rail line were significantly more differentiated compared to those occurring across similar spatial dimensions of continuous habitat or with an intervening country road (Gerlach and Musolf 2000). Significant genetic differentiation has even been observed for populations of six plant species separated by the Juyong-guan Great Wall (Su *et al.* 2003). *Ulmus pumila*, a wind-pollinated woody species, showed less genetic differentiation than four insect-pollinated species: *Prunus armeniaca*, *Ziziphus jujuba*, *Vitex negundo*, and *Heteropappus hispidus*; and *Cleistogenes caespitosa*, a wind-pollinated perennial herb, displayed the highest level of genetic differentiation between subpopulations separated by the Great Wall of China. In general it appears that the smaller and less mobile the species, the greater the impact of habitat fragmentation due to linear infrastructure.

#### **Mitigating against fragmentation; gene flow, connectivity and corridors**

An increasing number of studies that have aimed to document genetic diversity, inbreeding or connectivity consequences of fragmentation have found that some species are resilient to the impacts of fragmentation. Most studies indicate that particular life history traits (usually propagule dispersal or migration abilities) or that certain landscape features (e.g. corridors and stepping stones) can dramatically reduce the impact of fragmentation by maintaining population connectivity, and thereby reducing inbreeding and genetic diversity loss. Synthesis of results from such studies is invaluable for conservation efforts. Such information will help identify which species groups (and life history characteristics) are most prone to suffer genetic effects of fragmentation, and what types of landscape should be preserved or regenerated for maximum biodiversity conservation.

For plants, an increasing number of studies suggest that, at least for some species, gene flow mediated by pollen and/or seed may actually increase following fragmentation. For *Acer saccharum*, Fore *et al.* (1992) found that habitat fragmentation led to decreased genetic differentiation and increased gene flow in juvenile cohorts compared to the adult cohort. These results are in agreement with another study of *A. saccharum* (Young *et al.* 1993), suggesting that gene flow increased following logging as the disruption of the canopy allowed seed dispersal by wind to occur across greater distances (Fore *et al.* 1992; Young *et al.* 1993). Estimates from recently fragmented stands of several neotropical tree species suggest similar results and that, contrary to theoretical expectations, isolated trees and fragments are part of a large reproductive network. Gene flow by pollen has been documented to occur over very great distance in *Pithecellobium elegans* (Chase *et al.* 1996), in *Spondias monbini*, and in *Ficus* spp. (Nason and Hamrick 1997). Isolated pasture trees of *Swietenia humilis* (White *et al.* 2002) and *Dinizia excelsa* (Dick 2001; Dick *et al.* 2003) receive pollen gene flow from distant sources and are

reproductively active. In *D. excelsa*, such long distance pollen-mediated gene flow is possible because an alien pollinator, African honeybees, visit isolated trees in pastures although native pollinators do not (Dick 2001). Murren (2003) found that genetic diversity was high and differentiation low across fragmented and continuous populations of the epiphytic orchid, *Catasetum viridiflavum*, measured at 17 isozyme loci. The small breeding population, high frequency of mating among relatives and high rates of seed dispersal, which is normally found for this species help, maintain a large genetic population across disparate sites, making the species resilient to the genetic impacts of fragmentation.

Increased gene flow in fragmented systems may not be all good news though. For example, parentage analysis of seedlings established in fragmented stands of *Symphonia globulifera*, demonstrated that the increased gene flow came from just a few source trees in neighbouring pasture (Aldrich and Hamrick 1998). Furthermore, significant inbreeding and genetic differentiation was found in seedling cohorts from fragmented stands and the reduction in effective breeding population size was consistent with the effects of a genetic bottleneck (Aldrich *et al.* 1998).

For animals habitat specificity and the spatial arrangement of the landscape appear to be key features determining their response to fragmentation. Within Washington, USA, a microsatellite study of the red-backed vole, *Clethrionomys gapperi*, a closed canopy specialist, found that woodland fragments connected by corridors exhibited lower genetic differentiation than those in unconnected fragments, but that diversity was still lower than that in continuous populations (Mech and Hallett 2000). Within the same landscape, microsatellite analysis of the deer mouse, *Peromyscus maniculatus*, a habitat generalist, found no difference in genetic diversity between continuous, connected or isolated populations, and was presumed to be due to the ease with which these animals moved across both closed and open habitats. Habitat corridors that linked forest patches fragmented by agriculture and motorway networks in Austria were found to significantly improve landscape permeability of red deer, moose and large carnivores as evaluated by remote tracking and genetic mixing (Woess *et al.* 2002). A study of the woodland dependent European roe deer, found that genetic differentiation between isolated patches was best correlated with a geographic distance that considered habitat corridors rather than a Euclidean metric, deducing that corridors influence landscape connectivity, particularly for females (Coulon *et al.* 2004). Populations of the capercaillie from Central Europe, Alps and Pyrenees, which have experienced different patterns of recent and ancient fragmentation and connectivity, were assessed using microsatellites (Segelbacher *et al.* 2003). Results agreed with the concept of a gradual increase in genetic differentiation from connectivity to isolation and from recent to historical isolation.

Some evidence indicates that gender-specific dispersal may differentially affect nuclear and organelle genomes for animals inhabiting fragmented landscapes. The presence of corridors linking otherwise isolated habitat fragments were found to significantly increase interbreeding, as measured by allelic heterozygosity, compared to isolated treatments for populations of the root vole (Aars and Ims 1999; Ims and Andreassen 1999). Direct observations confirmed that it was the increased movement of females through corridor-

linked habitats that was mainly responsible for increased interbreeding, where males roamed widely across all landscapes. A similar gender bias was observed by Tallmon *et al.* (2002), who had previously found that mitochondrial DNA variation, which is maternally inherited in the forest dwelling California red-backed vole, *Clethrionomys californicus*, suggested a loss of genetic diversity due to fragmentation. However, microsatellite allelic variation (which is biparentally inherited), indicated no consequence of genetic diversity due to fragmentation, and very high levels of gene flow occurred between fragmented populations, and is presumably mediated by males (Tallmon *et al.* 2002).

In addition to corridors, habitat stepping stone patches can also significantly contribute to genetic connectivity and species permeability within a fragmented landscape. The red squirrel in Britain has experienced a significant ‘defragmentation’ of its landscape due to the planting of large conifer forests which have served to provide stepping stones (the animals can move some distance in open habitat) between large remnant forest blocks in northern England and southern Scotland (Hale *et al.* 2001). A survey of microsatellite variation sampling contemporary populations and individuals from museum collections identified that mixing of Cumbrian and Scottish genetic types, which had previously been differentiated due to ancient habitat isolation, had occurred only within the last couple of decades.

### **Genetic restoration in a degraded landscape**

The development of species or habitat recovery plans, whether they utilize methods to increase the connectivity of remnant patches or promote new areas for rehabilitation, must of course operate within a social and economic system that supports such actions. This review does not tackle those issues and solely concentrates on reviewing the biodiversity evidence.

A number of key areas of critical importance to restoration biology that can use research methodologies have been highlighted by Montalvo *et al.* (1997). These are:

- The influence of numbers of individuals and genetic variation in the initial population on population colonization, establishment, growth, and evolutionary potential
- The role of local adaptation and life history traits in the success of restored populations;
- The influence of the spatial arrangement of landscape elements on metapopulation dynamics and population processes such as migration
- The effects of genetic drift, gene flow, and selection on population persistence within an often accelerated, successional time frame
- The influence of interspecific interactions on population dynamics and community development.

Most of these topics are also highlighted as necessary for informing forest genetic resource planning (Rajora and Mosseler 2001), and whilst they have been addressed by some of the case studies described above, they should remain the principle focus of work that aims to understand the successes of previous restoration and rehabilitation programs and those that aim to plan future efforts. In particular large scale experiments that

examine the response of a range of species (animal, bird, insect and plant), using comparable genetic markers, within the same landscape that offers a combination of undisturbed and fragmented habitats which differ in size, spatial isolation, duration of fragmentation, degree of habitat degradation and recovery methods to facilitate secondary contact (e.g. corridors and stepping stones) need to be undertaken.

#### *Optimising landscapes to maximise genetic diversity*

Based on their results, the authors of several of the above case studies, but particularly Segelbacher *et al.* (2003), note that securing connectivity between spatially distinct populations should be the main objective of conservation programs that seek to maintain high levels of genetic variation for species inhabiting fragmented habitats. They argue that it is this action that can best alleviate the most significant genetic and evolutionary consequences of fragmentation within surviving populations. However it is difficult to derive best practice strategies from the results of single case studies that use different methodologies, spatial scales and landscape histories. A recent study by Tewsbury *et al.* (2002) has tried to redress this balance by undertaking a large-scale experiment targeting key plant and animal groups in the same landscape. In eight large-scale experimental landscapes that control for patch area and corridor function, Tewsbury *et al.* (2002) found that corridors not only increase the exchange of animals between patches, but also facilitate two key plant-animal interactions: pollination and seed dispersal. Thus the beneficial effects of corridors extend beyond the area they add, and suggest that increased plant and animal movement through corridors will have positive impacts on animal and plant populations and community interactions in fragmented landscapes.

Using a simulation approach Gibbs (2001) examined how primary demographic and habitat factors affect secondary population processes and thereby influence population genetic structure. The model revealed that both genetic diversity and divergence were most affected by the proportion of patches in a landscape that remain occupied, which in turn was affected primarily by patch disturbance frequency. Habitat availability influenced dispersal success and thereby secondarily influenced genetic divergence among populations. Based on these results, Gibbs (2001) recommends that conservation of genetic diversity in wild populations should be based on both habitat and population management and can best be achieved by maintaining healthy sized, local populations well-distributed among a network of infrequently disturbed habitats

#### *Sourcing material for habitat rehabilitation and reintroduction*

In conjunction with the preservation and improved connectivity of remnant habitat fragments, there are issues about the sourcing of species and populations for such activities.

Where sufficient genetic diversity exists within species inhabiting degraded remnant patches, the rehabilitation of species and habitats is often left to natural reproductive processes of dispersal/migration and recruitment/rearing. One study that examined the genetic diversity of stands of lodgepole pine, *Pinus contorta*, reforested by various natural and plantation means in the USA found no significant difference between methods for diversity and heterozygosity measures of RAPD and microsatellite markers

(Thomas *et al.* 1999), and thus appeared to indicate that diversity was fairly robust to stand regeneration type.

Remnant stands have also been highlighted as potential sources for repopulating a fragmented landscape once landuse pressures that drive habitat clearance subside. In Europe the possibility that large areas of farmland will be abandoned due to global shifts in primary production and disease incidence, may offer tremendous potential to reassess land usage and may drive reclamation of native habitats to satisfy recreational, biodiversity and global carbon budget drivers. Several projects are underway to assess the potential of native species that are remnant within agricultural landscape to colonize into abandoned areas (e.g. EU funded OAKFLOW project to examine oak recolonization of abandoned farm land). These projects aim to assess both the potential speed and diversity impacts of natural vegetation regeneration and animal colonization processes, but few results have so far been published. A study by Jacquemyn *et al.* (2003) who examined part of this issue, recommend that new areas intended for restoration or regeneration should be classified according to their distance from remnant and potential source populations. Those new stands which are located less than 100m distance from remnant patches are likely to experience substantial restocking due to seed dispersal and animal migration compared to those located at a greater distance.

In many situations, where genetic diversity is highly degraded or species have gone locally extinct, reintroduction and restocking programmes need to be considered. For such plans the sourcing of material for these activities has been the subject of intense research.

For animals, many flagship reintroduction programmes have been successful and appear to be a useful strategy where no other options exist. One example is the white tailed deer, *Odocoileus virginianus*, which was the subject of a successful conservation and reintroduction program in the southeastern USA that started in the early 1900s. Microsatellite analysis indicated that whilst 9 of the 19 contemporary populations surveyed, exhibited evidence of a significant population bottleneck, all populations maintained high diversity and heterozygosity, and exceeded diversity reports for cervid species experiencing similar population declines (DeYoung *et al.* 2003). The authors speculate that their results confirm the importance of rapid population expansion and habitat continuity in retaining genetic variation in restored populations. However, the use of diverse transplant stocks and the varied demographic histories of populations appear to have resulted in fine-scale genetic structuring, although this is not necessarily a bad thing for the long term viability of the species.

For plants, issues of local provenance and the source/context of plants from which to collect reproductive material have been tested. For Scottish plant species, Ennos *et al.* (1998) make a number of recommendations, which are probably more generally applicable to European and global systems, including:

- Existing populations probably still retain significant elements of adaptive variation; though this will vary according to species, with some showing close correlation

- between patterns of adaptive variation and environmental factors and others being more plastic.
- Indigenous gene pools have been modified by fragmentation, changes in the forest environment, and introduction of foreign material, much of which is maladapted to upland sites.
  - With some exceptions, the levels of genetic variation in existing indigenous populations comprise an adequate basis for restoring and expanding native woodland.
  - The current indiscriminate use of continental provenances needs to be strongly discouraged; but equally restricting choice of seed sources to the most local provenances can also be inappropriate.
  - Use of Scottish material from the same region and similar site type as the planting site needs to be encouraged.
  - Current genetic management measures are rudimentary and fail to address adequately key issues of provenance choice, genetic conservation, seed supply or provision of selected or improved material, especially in broadleaves.
  - Changes in legislation, regulation, seed supply, advice and research are proposed to address these shortcomings.
  - The need for more provenance research is highlighted.

It is also clear now from recent research that the number and context of seed sourcing plants is important for the diversity and fitness of stock to use for rehabilitation and reintroduction. These issues also apply to animals but not so much work has been undertaken in this area. The genetic diversity of half sib families collected from two large remnant trees of European oak, *Quercus robur*, in the Netherlands was found to be low compared to ancient remnant forest patches (Bakker *et al.* 2003). In order that seed for native woodland reforestation be as diverse as possible, the authors recommended collecting reproductive material from as many trees as possible spread over a local geographic range.

Much work on these issues has been conducted in the neotropics and is informative to European practice. Isolated trees of Spanish Cedar, *Cedrela odorata*, showed a full complement of seed set (James *et al.* 1988), but in a common garden experiment using material sourced from across Central America, progeny grown from seed collected from such trees showed reduced vigour compared to that of progeny from trees in primary or secondary forest (Navarro 2002). A similar situation was also found for the progeny of mahogany trees, *Swietenia macrophylla*, under similarly isolation conditions (Navarro 2002). For both situations, reduced diversity of pollen reaching isolated trees is probably the cause, rather than increased inbreeding. Other studies suggest that reductions in the vigour of progeny may be due to more complex factors than simply an increase in inbreeding. In *Enterolobium cyclocarpum* trees from continuous forest were almost six times more likely to set fruits and produce more seeds per fruit than trees in pastures (Rocha and Aguilar, 2001). Moreover, progeny from trees in continuous forests were more vigorous than the progeny from trees in pastures. Rocha and Aguilar (2001) suggest that as no evidence of inbreeding was found, the mechanisms that regulate progeny

vigour are probably being disrupted in trees from pastures, such that less fit seed are allowed to mature.

Population bottlenecks, through reproductive dominance, were also seen in *Symphonia globulifera*, despite abundant and superficially healthy regeneration (Aldrich and Hamrick 1998). A study of Brazil nut showed that populations subject to persistent levels of harvest lack sufficient juvenile recruitment and may succumb to a recruitment bottleneck (which would also be genetic), failing to maintain populations over the long term (Perez et al 2003). In dioecious species in which the fruit is valued, any tendency to favour retention of female trees may similarly lead to a reduced effective population size. The context of individuals from which reproductive material is sourced may thus be an important and under evaluated factor for reintroduction and rehabilitation.

Jelinski (1997), in reviewing the literature on plant genetic diversity, recommends that a more comprehensive assessment of genetic variation in natural plant populations at various hierarchical levels is essential in view of concerns for loss of species owing to habitat fragmentation and global change. Indeed with climate change scenarios varying dramatically there is however some uncertainty with what scenarios to plan for. With this in mind Ledig and Kitzmiller (1992) make several suggests:

- Reforestation strategies should emphasize conservation, diversification, and broader deployment of species, sources, and families
- Reintroduction/replanting programs may have to deploy non-local sources, imported from further south or from lower elevations, which necessitates a system for conserving native gene pools
- Introducing/planting a diverse array of species or sources is a hedge against the uncertainty inherent in current projections of warming
- Many tree improvement programs already stress genetic diversity and deployment of multi-progeny mixes, but may better prepare for climate change by testing selections in an even wider set of environments than is now the case.

### **Case study – genetic survival and recovery of ash in a chronically fragmented landscape**

The Southern Uplands of Scotland is one of the regions of Britain most lacking in natural woodland cover and although areas have been planted with non-indigenous conifer species, it remains highly deforested. For instance, in Peeblesshire (one region in this area), only 0.1% of the land-area supports long-established natural woodlands (Newton and Ashmole 1998). Major human impact in this region has been estimated to have begun as early as 6000 years ago (Trust 2000). Ancient land use, for pasture and much more recent implementation of conifer plantations, has greatly fragmented the landscape. Many native tree species are confined to steep, narrow riversides, which are inaccessible to grazing herds. Remnants are very small, comprising ten to 30 mature individuals with no natural regeneration in grazed areas. Typically, forest patches are separated by hundreds of meters although some can be isolated from others by more than 1 km. As a result of continuous exposure to wind in a dramatically deforested open landscape, trees are undersized and display a convoluted shape (Bacles *et al.* 2004).

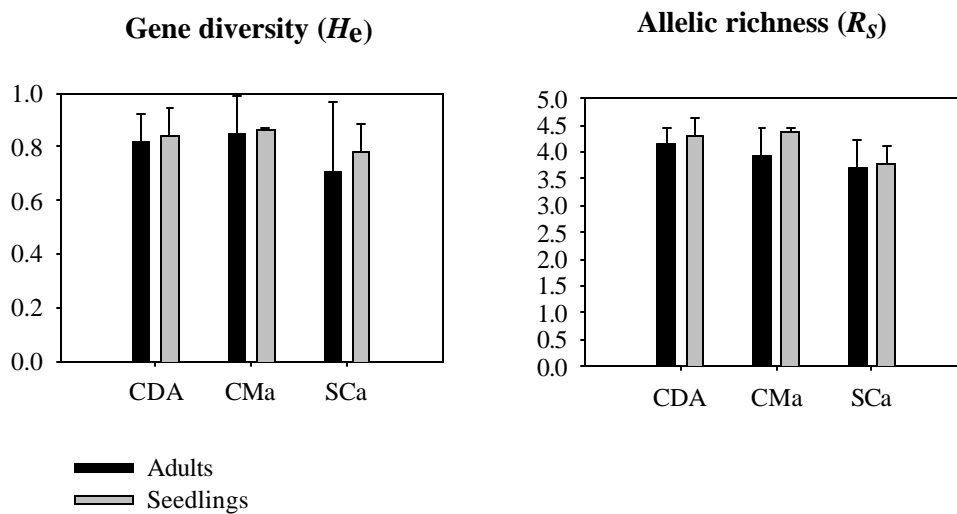
Ash is a post-pioneer tree species widespread in temperate Europe and native throughout the British Isles. In the highly fragmented landscape of southern Scotland, individuals of the species grow commonly along riverbanks where they rarely exceed 8-10 m in height. The species' polygamous sexual system is complex and poorly known (Wallander 2001). Sex expression varies at the floral, inflorescence and tree level, as well as between years, and individuals may be classified phenotypically in a continuum from purely male to purely female with a range of hermaphroditic intermediates (Wallander 2001). Fruits are wind dispersed and regular fruit bearing begins around 20 years of age. The species displays masting behaviour with irregular fruiting between years.

The complex mating system of ash and the long-term fragmentation and size reduction of Scottish populations suggests that detrimental genetic effects should be observed. Furthermore, as forest remnants of the Southern Uplands of Scotland are being considered for native woodland restoration in the region, there is an immediate need to assess their population genetics. Extensive planting, using seed collected from such stands, is planned by the Carrifran Wildwood Trust (Trust, 2000), a conservation project aiming to recreate native forest ecosystem within one of the Southern Scottish catchments, Carrifran. Thus there is also a requirement to understand the mating patterns of trees within and between forest patches to determine whether the genetic quality of such planting stock would be suitable for sustainable forest restoration.

The genetic diversity of five ash remnant populations (a total of only 88 mature trees) in the severely deforested catchment of Carrifran (in the Scottish southern Uplands) was surveyed at eight microsatellite loci (Bacles *et al.* In Press). Diversity was found to be very high, with between six and 40 alleles sampled at each marker locus, and gene diversity ( $H_e$ ) ranging from 0.649 to 0.940. These figures are remarkably similar to values reported for five of the same microsatellite loci by Heuertz *et al.* (2003) for a Romanian population. The latter was a large population (152 individuals), composed of continuous mixed deciduous forest, with between 10 and 42 alleles sampled and gene diversity values of between 0.663 and 0.918 (Heuertz *et al.* 2003). Furthermore, for continuous stands sampled across Bulgaria, Heuertz *et al.* (2001) observed between 10 and 59 alleles at five microsatellite loci for a much larger sample ( $N=322$ ) and overall gene diversity ( $H_e=0.793$ ) was in the range of those estimated in the Moffat Dale. The comparison with such findings shows that populations of *F. excelsior* in the Southern Uplands of Scotland harbour exceptionally high genetic diversity, which is comparable with levels from continuous populations in southeastern Europe. It is also important to note that this area of the Balkans has been identified as a hotspot of genetic diversity and the refugial colonization source of Scottish ash material (Petit *et al.* 2003; Heuertz *et al.* 2004). It seems that despite small remnant size and severe isolation, chronic habitat fragmentation for over 6000 years genetic variation has not decreased within populations of this species.

Analysis of contemporary pollen immigration into Moffat Dale found that gene exchange between remnants has not been limited. In fact contemporary pollen-mediated gene flow into the catchment appears to be extensive, with 57% of pollinations involving trees from outside the sample area (Bacles *et al.* In Press). Gene exchange between remnants thus

appears to occur at a very large spatial scale, much larger than the sampled area (>2900 ha), and is maintaining genetic diversity in newly produced individuals across remnants. On average, within the Moffat Dale less than a quarter of the sampled trees contribute to the pollen pool of mother trees, and effective pollen dispersal is limited, on average, to 328 m. Interestingly, this estimated pollen dispersal distance is at least 2.5 times greater than the distance inferred from the spatial genetic structure of *F. excelsior* trees in a continuous forest population in Romania (<140 m; Heuertz *et al.* 2003). Such an increase in pollen dispersal distance in a deforested catchment may be the result of greater dispersal of airborne pollen in an open landscape as has been suggested for fragmented populations of the wind pollinated tree *Acer saccharum*, (Foré *et al.* 1992; Young *et al.* 1993).



**Figure 14.** Comparison of gene diversity ( $H_e$ , left) and allelic richness ( $R_s$ , right) between adult and seedling cohorts averaged for three *F. excelsior* remnants (CDA, CMa and SCa) in the Moffat Dale.

A similarly high level of seed dispersal was noted from parentage analysis of a sample of seedlings growing within the fragmented catchment. For 63% of seedlings no parent could be identified within the catchment (distance > 3 km) and for 35% of seedlings only a single adult could be identified (Bacles 2004). Therefore 98% of the seedlings within the catchment had been derived by some component of extra-catchment gene flow (either by pollen or seed). The extent of both pollen and seed mediated gene flow for ash remnants inhabiting this chronically fragmented landscape highlights the responsive nature of some species under extreme habitat pressures. Indeed, a survey of genetic variation within trees and seedlings sampled from valley remnants (Fig. 14) found similar levels of genetic diversity across life stage. It would thus be appropriate to restock the valley with material sourced locally from the site, although Bacles (2004) recommends that several different trees from different remnants are sampled to capture different components of incoming pollen diversity. This strategy is now the sampling and regeneration aim of the Carrifran Wildwood Trust.

## Conclusions

Microsatellites are a very powerful molecular marker tool. They can and have been used extensively to understand the contemporary population dynamics and connectivity of individuals within different landscape settings. Indeed microsatellites are arguably the best molecular marker currently available for such purposes. The literature on conservation genetics has ballooned since the introduction of these markers. Numerous studies now exist examining the dynamics of genetic diversity within species across changing landscapes, including those subject to fragmentation. Using specialized statistical methods and surveying genetic variation within nuclear and organelle genomes, microsatellites also offer insights into historical colonization processes and population evolutionary history. One of the additional strengths of this marker type is that it can be used with very small quantities of living and/or dead tissue of the study organism. Thus it is possible to use historically collected material (e.g. from herbaria or museums), and this capability has added a temporal context to many studies.

Whilst not a universal marker (one which can be applied to any organism), microsatellites can be developed for almost any species with even modest research budgets. Microsatellites have been isolated from a range of organisms, including, plants, mammals, birds, insects and fungi. Microsatellites do not give the researcher direct access to community or habitat assessments of species richness. Rather by applying a range of recently developed statistical methods, they inform on the processes of propagule, individual and population movement and dynamics for specific species.

Microsatellites are being applied to an increasing number of studies of European flora and fauna, and this review has sought to document a representative sample of this research to examine; historical colonization, contemporary gene dynamics and the impacts and restoration of fragmented landscapes.

### *Historical colonization*

Sequence data from organelle genomes is the best type of variation to reconstruct past colonization dynamics due to the power of phylogenetic interpretations to indicate historical connectivity. However, microsatellite variation within organelle (e.g. cpSSRs for plants) and nuclear genomes have highlighted useful polymorphism for studies examining historical colonization and are often used in concert with sequence data. Using such variation from contemporary populations, it has been possible to determine the direction and pattern of postglacial colonization (since the last glacial maximum, approximately 20,000 years ago) for a range of plants and animals. Fossil records indicate that many plants and animals survived the last ice in refugial populations in southern Europe. These putative refugial locations are also the location of high allelic diversity within contemporary populations, and identify them as the genetic sources for material that colonized central and northern Europe (including the British Isles) following climate warming. British biota are derived from a range of refugial sources (Iberian, Italian and Balkan), and the relative contribution of each or a combination of sources appears to be related to the migration ability, successional cohort and ecological tolerance of individual species. Such studies can also be used to infer the relative rate of migration and dynamism of species under future global climate change scenarios.

### *Contemporary gene dispersal dynamics*

Microsatellites have been used most extensively to document propagule, individual and population dynamics. Such inferences are usually set with a framework of population genetic theory. For example, using the spatial distribution of genetic variation it is possible to estimate migration/dispersal rates between populations of a species. At the individual level it is also possible to use exclusion analyses to work out familial relationships, and identify parent-offspring, full sib and half sib relations. Such information is valuable for documenting the migratory ability of progeny compared to adults and is used to make direct estimations of gene dispersal by propagules and individuals. A range of typical studies has been reviewed here, including herb, animal and fungal systems. A detailed case study is also reviewed that describes the gene dynamics inferred from spatial genetic structure and direct dispersal estimation for European oak species.

Empirical studies indicate that the level and distribution of genetic diversity is strongly linked to the life history traits of individual species. In general species that have outcrossing mating systems, have highly vagile propagules and/or individuals and have high population density support high levels of genetic diversity due to a higher effective reproductive population size. In addition, this diversity is more evenly spread amongst populations than species that have more limited dispersal systems, mating systems that promote inbreeding and/or occur at low population densities. In addition, environmental selection, habitat type and structure were found to exert a significant effect on the spatial genetic structure of forest dwelling species.

### *Genetic effects of habitat fragmentation*

An impressive body of literature exists describing genetic structure (from which gene flow and genetic connectivity estimates can be made) at neutral marker loci from fragmented and non fragmented populations. A review of this literature is made and individual results are presented for a range of species within European habitats. In addition an integrated case study of ash growing within a chronically fragmented landscape of the southern Scottish uplands is described.

Overall habitat fragmentation serves to disrupt the normal processes of population connectivity and reduces effective population size of individual species inhabiting degraded landscapes. For most species, fragmented remnants manifest lower diversity, higher population inbreeding, higher population differentiation and lower interpopulation gene flow, than continuous populations. The consequences of fragmentation have been documented for a whole range of plant and animal species. Indeed such responses ought to be considered the default assumption for species inhabiting such degraded systems. Evidence suggests that threshold levels may exist and that the diversity of impact is most extreme for very small very isolated fragments.

Despite these general conclusions, an increasing number of studies that have aimed to document genetic diversity, inbreeding or connectivity consequences of fragmentation have found that some species are resilient to the impacts of fragmentation. Most studies

indicate that particular life history traits (usually propagule dispersal or migration abilities) or that certain landscape features (e.g. corridors and stepping stones) can dramatically reduce the impact of fragmentation by maintaining population connectivity, and thereby reducing inbreeding and genetic diversity loss. In addition, for some plant species, gene flow mediated by pollen and/or seed may actually increase following fragmentation. Increased gene flow in fragmented systems may not be all good news though as relatively few trees act as pollen sources and genetic bottlenecks can result. Synthesis of results from such studies is invaluable for conservation efforts. Such information will help identify which species groups (and life history characteristics) are most prone to suffer genetic effects of fragmentation, and what types of landscape should be preserved or regenerated for maximum biodiversity conservation.

#### *Improving landscape connectivity*

Increasing connectivity between isolated remnant patches can ameliorate the impacts of fragmentation. Microsatellite studies documenting gene flow have identified corridor and stepping stone habitat linkages as good methods for increasing reproductive connectivity between patches and increasing genetic diversity. However, such connected remnant networks are still not as effective at maintaining diversity within species as large continuous habitat blocks.

#### *Mode and material for reintroduction and rehabilitation*

Microsatellites have been used to examine the genetic outcomes of, and to advise best strategies for, reintroduction and rehabilitation programmes. Some reintroduction programmes have been very successful and managed to restore high levels of genetic diversity within previously denuded areas. However, gene flow assessment indicates that the source of material for reintroduction may be an important issue that has been under evaluated. For example many plant species demonstrate high gene flow even amongst isolated trees. However the diversity of trees contributing to these progeny may be low compared to trees within continuous forest, and thus genetic diversity within the source will also be low. For species where the fitness of isolated tree progeny has been compared to that source from continuous forest trees the former is often lower.

Another rehabilitation and restocking debate is how 'local' should material for reintroduction be. Gene flow estimates can be made using microsatellites to understand how genetically connected populations are and to inform decisions concerning the distance over which material should be transferred. Ultimately the relative survival, growth and performance of introduced compared to local material needs to be assessed using quantitative methods. Some general guidelines are made. The current indiscriminant use of continental provenances should be strongly discouraged. Equally, restricting choice of seed sources to the most local provenances can also be inappropriate. The use of material from the same region and similar site type as the rehabilitation site should be encouraged.

### **Suggested future research priorities**

A number of key areas of critical importance to restoration biology have been highlighted previously by Montalvo *et al.* (1997). These are:

- The influence of numbers of individuals and genetic variation in the initial population on population colonization, establishment, growth, and evolutionary potential
- The role of local adaptation and life history traits in the success of restored populations;
- The influence of the spatial arrangement of landscape elements on metapopulation dynamics and population processes such as migration
- The effects of genetic drift, gene flow, and selection on population persistence within an often accelerated, successional time frame
- The influence of interspecific interactions on population dynamics and community development.

These priorities are still valid and yet to be fully addressed. They should therefore remain the principle focus of work that aims to understand the successes of restoration and rehabilitation programs and those that aim to plan future efforts.

Some further priorities can also be emphasized.

1. At present results for a range of studies are difficult to combine due to differences in scale, history and context of landscapes and analyses undertaken. It is thus very difficult to fully appreciate which species are likely to be most sensitive and resilient to fragmentation. There is a need to conduct large-scale experiments that examine the genetic diversity response of a representative portion of forest dwelling species (animal, bird, insect and plant), using comparable genetic markers marker, within the same landscape that offers a combination of undisturbed and fragmented habitats which differ in size, spatial isolation, duration of fragmentation, degree of habitat degradation and recovery methods to facilitate secondary contact (e.g. corridors and stepping stones). If no single landscape is available then a series of studies that controls for scale and history of habitat degradation could be used. Microsatellites are best marker to undertake such comparative assessments.
2. The genetic quality of remnants and individuals as sources of reproductive material for reintroduction and habitat rehabilitation needs to be considered in more detail. In particular the relative diversity of progeny from individuals in different landscape contexts (isolated vs. continuous habitat), and the number of individuals to sample reproductive material from needs more consideration. Such studies need to be undertaken in conjunction with assessments of fitness and performance of progeny.
3. The potential to harness natural processes of dispersal for recolonization and rehabilitation outcomes needs more examination. Microsatellites should be used to survey the diversity of regeneration resulting from different methods (natural and assisted) across a range of species and landscapes.

4. Finally where sourcing options are available for reintroduction and rehabilitation, the use of environmental matching methods and natural genetic connectivity neighbourhoods needs more consideration. The importance of maintaining and using regionally adapted stock should be tested using fitness experiments to examine the relative performance of local vs. imported material. However the relative gene mobility across the range of a species needs more consideration, and can be estimated using microsatellites. If species have high natural dispersal or motility capabilities, it makes little sense to restrict the importation of rehabilitation stock according to distance when such areas may be the source of naturally occurring contemporary gene flow.

## Glossary

<b>AFLP:</b>	See Amplified Fragment Length Polymorphism.
<b>Allele:</b>	One of two or more alternative forms of a gene, locus or DNA sequence.
<b>Allele frequency:</b>	The abundance of a particular allele in a population or species (expressed as a proportion of 1).
<b>Allogamy:</b>	Cross fertilization
<b>Allopatry:</b>	Geographically separate populations or taxa.
<b>Allozyme:</b>	The alternative forms of a particular protein visualized on a gel as bands of different mobility. Each separate allozyme is coded for by a different allele of the same gene.
<b>Amplified Fragment Length Polymorphism (AFLP):</b>	The selective amplification, using PCR, of a set of DNA fragments produced by a frequently cutting (usually 4 bp recognition site) and rarely cutting (usually 6 bp recognition site) restriction enzyme.
<b>Apomictic:</b>	Clonal reproduction, producing exact genetic copy of maternal parent.
<b>Assortative mating:</b>	Deviation from mating patterns expected by random assortment of genes due to increased incidence of particular phenotype to mate with another individual of the same genotype (positive assortative mating), or another individual of a different phenotype/genotype (negative assortative mating).
<b>Autogamy:</b>	Self fertilization.
<b>Backcrossing:</b>	Crossing between interspecific hybrid and one or both parental taxa (see also hybrid zone and introgression).
<b>Biparental Inheritance:</b>	Characteristics that are controlled by a nuclear gene (or genes) which have two copies, one on each homologous chromosome, each parent contributing one set of chromosomes (see also maternal and paternal inheritance).
<b>Bottlenecks:</b>	Sudden reduction in population size (see also genetic bottleneck).
<b>Cluster:</b>	A group of genetically similar samples positioned together in a pictorial representation such as a dendrogram.
<b>Coalescence:</b>	The principle of using ordered data to trace allele changes back through time.
<b>Chloroplast DNA:</b>	Double stranded, haploid circular DNA molecule found in the chloroplast organelle of plants. Usually maternally inherited in Angiosperms and paternally inherited in Gymnosperms.
<b>Codominant marker:</b>	A genetic marker in which both alleles are expressed, thus heterozygous individuals can be distinguished from either homozygous state.
<b>CpDNA:</b>	See Chloroplast DNA.
<b>CpSSRs:</b>	See Microsatellite.

<b>Dendrogram:</b>	A method of representing genetic similarities by a branching tree. The more closely related individuals are, the shorter the branch lengths separating them.
<b>Dioecy:</b>	Breeding system in which male and female gametes are borne in separate individuals (dioecous).
<b>Diploid:</b>	A nucleus or individual having two copies of each chromosome.
<b>DNA:</b>	Deoxyribo Nucleic Acid, molecular which encodes genetic information of cell/organism.
<b>Dominant marker:</b>	A marker that shows dominant inheritance with homozygous dominant individuals indistinguishable from those which are heterozygous possessing a recessive allele.
<b>Drift-Migration Equilibrium:</b>	The maintenance of genetic diversity in a population whereby the loss of alleles by genetic drift is offset by the gain of alleles into the population by migration.
<b>Effective Population Size:</b>	The number of individuals in a population reproducing and contributing to the alleles present in the next generation.
<b>Enrichment (SSR library):</b>	Laboratory method which is used to increase the proportion of clones within a library which contain microsatellite repeat units.
<b>EST:</b>	See Expressed Sequence Tag
<b>Expressed Sequence Tag (EST)</b>	Genetic marker developed from expressed portion of genome (usually nuclear) using specialist molecular techniques.
<b>Gamete:</b>	A typically haploid cell produced by meiosis which may unite with another such cell to form a zygote.
<b>Gene diversity:</b>	A measure of the genetic variation found in a population or species based on the mean expected heterozygosity.
<b>Gene flow:</b>	The proportion of newly immigrant genes moving into a given population, implies successful dispersal of genes. Can be estimated using a variety of parameters but using genetic differentiation measures, $Nm$ , can be derived, the number of migrants per generation.
<b>Genetic bottleneck:</b>	Loss of genetic variation in a population due to a fall in population numbers. While population size may recover relatively quickly, genetic variation remains low until restored by mutation and/or gene flow.
<b>Genetic differentiation:</b>	A measure of the allocation of genetic variation within a species and among populations. Species with a high level of genetic differentiation show high variation between populations. A number of genetic differentiation parameters can be estimated, including; $F_{ST}$ , $G_{ST}$ and $Q$ .

<b>Genetic distance:</b>	The degree of genetic similarity between a pair of individuals, population or species. Values typically vary between 0 (identical) and 1 (completely different).
<b>Genetic drift:</b>	Change in allele frequencies within a population over time due to the sampling effects of small population size.
<b>Genetic marker:</b>	A sequence of DNA or protein that can be screened to reveal key attributes of its state or composition and thus used to reveal genetic variation.
<b>Genome:</b>	The full complement of genes present in a haploid set of chromosomes in an organism.
<b>Genomics:</b>	The process of accessing gene function or relative gene position or interactions.
<b>Genotype:</b>	State (e.g. allelic composition) for a particular genetic locus of an organism.
<b>Haploid:</b>	One copy of each chromosome, typically found in a gamete.
<b>Heterozygote:</b>	An individual with two different alleles at a locus. Heterozygous is the state of a locus/individual with 2 or more alleles.
<b>Homologous:</b>	Identical characters that share a common evolutionary origin (identical by descent), and can be in different taxa.
<b>Homoplasy:</b>	Identical characters that have evolved separately in independent evolutionary lineages (i.e. not by descent).
<b>Homozygote:</b>	An individual with two copies of the same allele at a locus. Homozygous is the state of a locus/individual with 1 allele.
<b>Hybrid zone:</b>	Area where two closely related species are in reproductive contact producing a range of hybrid and backcrossed entities.
<b>Inbreeding:</b>	Reproduction between closely related individuals, includes self-fertilization. Population level inbreeding can be calculated using Wright's F statistic, $F_{IS}$ .
<b>Inbreeding depression:</b>	Reduction of fitness due to expression of deleterious alleles in homozygous state following breeding with close relatives.
<b>Indel:</b>	A form of mutation where a sequence of DNA is inserted and/or deleted.
<b>Introgression:</b>	The transfer of genes or alleles from one taxa to another due to hybridization between the taxa followed by repeated backcrossing of hybrids with one of the parents.
<b>Isolation by distance:</b>	Reduction of gene flow in species with low dispersal powers due to spatial separation of one population from another.
<b>Isozyme:</b>	A form of an enzyme separated through a gel matrix by an electronic current then visualized with an appropriate stain (see also allozyme).
<b>ISSR:</b>	See Microsatellite.

<b>Lineage:</b>	A particular monophyletic group of genotypes that are evolutionarily more closely related than other such groups (i.e. related by descent). Can be in a taxon, population or individual.
<b>Linkage:</b>	Where two loci have a lower rate of recombination between them than the infinite expectation between two unlinked loci. Such loci are found on the same chromosome and tightly linked loci are closely proximate.
<b>Linkage disequilibrium:</b>	The situation where two alleles from different loci co-occur at a significantly greater frequency than that expected by random association due to linkage between the loci.
<b>Locus (<i>pl</i>, Loci):</b>	A specific region or position on the genome or chromosome, sometimes corresponding to a coding gene.
<b>Maternal inheritance:</b>	Inheritance of a trait or genome solely through the female gamete, often of organelle origin.
<b>Metapopulation:</b>	A population consisting of a network of partially isolated smaller populations.
<b>Microsatellite:</b>	Short tandem repeats of a short sequence of (typically 2 – 4) nucleotides randomly distributed throughout the genome. Markers are also often referred to as Simple Sequence Repeats (SSRs). Sometimes markers are highlighted between simple sequence repeat (SSR) loci, these are known as inter-SSRs or ISSRs. Microsatellite loci can also be found in the chloroplast genome (cpSSRs), where they are normally mononucleotide repeats.
<b>Minimum Viable Population:</b>	The minimum effective population required to persist despite genetic drift, demographic and environmental stochasticity.
<b>Minisatellites:</b>	Highly repetitive sequences, typically occurring near telomeres, consisting of nucleotides 10 – 100 bp long repeated in tandem arrays.
<b>Mitochondrial DNA:</b>	Haploid, double stranded circular DNA molecule located in the mitochondria, usually maternally inherited in both animals and plants.
<b>Monoecy:</b>	Breeding system in which male and female gamete producing structures are in different locations on the same organism.
<b>Monomorphic:</b>	Absence of more than one allele at a particular locus or gene, leading to a uniform phenotype in the population.
<b>MtDNA:</b>	See Mitochondrial DNA.
<b>Multiallelic locus:</b>	Presence of two or more alleles at a particular locus.
<b>Multilocus:</b>	Multiple genomic regions (see locus).
<b>Mutation:</b>	Change in nucleotide sequence of an organism.
<b>Mutation rate:</b>	The frequency at which a particular mutation occurs in a genome.
<b>nDNA:</b>	See Nuclear DNA.

<b>Nuclear DNA:</b>	Sequence of DNA nucleotides located in the nucleus.
<b>Null allele:</b>	An allele that is not expressed and therefore does not produce a band on a gel.
<b>Ordered data:</b>	Data in which the evolutionary direction can be identified, with original characters (termed primitive or ancestral) mutating into novel characters (termed derived). See also Unordered data.
<b>Organelle:</b>	Membrane bound structure within a eukaryotic cell responsible for a particular function or functions.
<b>Orthology:</b>	Divergence between two homologous characters following speciation.
<b>Outbreeding depression:</b>	Reduction in fitness due to break up of adaptive gene complexes following reproduction between widely disparate individuals.
<b>Outcrossing:</b>	Breeding between two individuals that are not closely related genetically.
<b>Panmixia:</b>	Mating between individuals in a population that is random with respect to phenotype/genotype. (see also Assortative Mating).
<b>Parapatric:</b>	Occupying adjacent contiguous areas.
<b>Parology:</b>	Divergence between two homologous characters or loci following genome or locus duplication.
<b>Paternal inheritance:</b>	Transfer of an inherited characteristic only through the male line/gamete.
<b>PCR:</b>	See Polymerase Chain Reaction.
<b>Pelagic:</b>	Free floating larvae usually dispersed in oceanic currents (e.g. corals).
<b>Phenology:</b>	Time to maturity or to flowering
<b>Phenotype:</b>	The observable characteristics of an organism, often an interactions between genotype and environment.
<b>Phylogeny:</b>	A hypothesized evolutionary relationships of descent and ancestry between genotypes, individuals or species, often presented as a dichotomous tree.
<b>Phylogeography:</b>	The study of the geographical distribution or pattern of evolutionary lineages.
<b>Pollen Core Analysis:</b>	Stratigraphic sampling and identification of pollen to reveal ecological information, typically climate.
<b>Polymerase Chain Reaction (PCR):</b>	A technique for increasing the number of target DNA sequences by several orders of magnitude by repeated cycles of denaturation, primer annealing and fragment extension using natural properties of a thermostable DNA polymerase.
<b>Polymorphic:</b>	loci with more than one allele leading to different phenotypes in the population (see also monomorphic).

<b>Polyploid:</b>	Cell or individual containing more than two copies of the diploid genome complement (see Allopolyploidy and Autopolyploidy).
<b>Population:</b>	<i>Ecological Population</i> - Group of individuals of the same species within the same habitat at the same time. <i>Statistical Population</i> – All the items under study. <i>Genetical Population</i> - All the individuals connected by gene flow, i.e. the gene pool.
<b>Private alleles:</b>	The possession of unique alleles within a sampling unit (e.g., population).
<b>Progeny Array:</b>	Group of progeny derived from a single maternal parent, often used to infer outcrossing or inbreeding rates in plant populations.
<b>Provenance:</b>	The origin of a sample individual.
<b>Qualitative variation:</b>	Discrete variation with phenotypes being classifiable into clear distinctions without intermediates.
<b>Quantitative variation:</b>	Continuous variation with phenotypes falling at a point on a continuum.
<b>RAPD:</b>	See Random Amplified Polymorphic DNA.
<b>Random amplified polymorphic DNA (RAPD):</b>	The selective amplification, using PCR, of a set of DNA fragments produced by amplification of genomic regions delineated by primers of random sequence (usually decamers).
<b>Rarification:</b>	Method of addressing unequal sample sizes (usually in measures of allelic richness) by recording the frequency of alleles in a large population and estimating the number of each allele that would occur at these frequencies in smaller sample sizes.
<b>Replication:</b>	The copying of both strands within a DNA molecule to produce two identical pairs of DNA sequence.
<b>Recombination:</b>	Exchange of genetic material between homologous chromosomes to break up linkage groups and yield allelic combinations not recorded in parental generations.
<b>Repetitive DNA:</b>	Repeated nucleotide sequences found throughout the genome. Distinguished into two types corresponding to the frequency with which the sequences appear in the genome; moderately repetitive DNA (present in a few to about $10^5$ copies in the genome), and highly repetitive DNA (present in about $10^5$ to $10^7$ copies in the genome).
<b>Reproductive isolating Mechanism:</b>	A barrier to reproduction between groups of organisms that prevents gene flow between them. These may be pre-zygotic (operating before fertilization can occur) or post-zygotic (operating after fertilization, e.g. infertile offspring).
<b>Restriction Fragment Length Polymorphism</b>	Fragments of DNA of varying size produced by cutting sample DNA with restriction enzymes.

<b>(RFLP): Restriction Site Variation:</b>	The basis of the generation of polymorphisms revealed by restrictive enzymes. Such enzymes cleave DNA where specific DNA sequences are recognized. Thus mutations within a previously recognized and cleaved sequence will cause the enzyme to leave the section of the genome uncut.
<b>RFLP: Selection:</b>	See Restriction Fragment Length Polymorphism. The influence of the environment (in its broadest sense) in determining which individuals will breed and pass their genes on to the next generation and those who will not breed.
<b>Self-incompatibility:</b>	The inability of a hermaphrodite or monoecious individual to fertilize itself and produce viable offspring.
<b>Selfing:</b>	Successful fusion of male and female gametes produced by the same individual.
<b>Sequence analysis:</b>	Determination of the sequence of DNA bases in a specific region.
<b>Simple Sequence Repeat</b>	See Microsatellite.
<b>Single Nucleotide Polymorphism: (SNP)</b>	Genetic marker based on surveying single base variation within sequences of (usually) expressed genes.
<b>SNP:</b>	See Single Nucleotide Polymorphism.
<b>SSR:</b>	See Microsatellite.
<b>Sympatry:</b>	Occupying the same geographic area.
<b>Tandem repeats:</b>	A form of repetitive DNA with units of usually short sequences repeated hundreds of thousands of times (see also Interspersed repeats).
<b>Taxon (<i>pl</i> Taxa):</b>	The organisms comprising a particular taxonomic entity (e.g., species or sub-species).
<b>Unordered data:</b>	Data in which the evolutionary direction of genetic change cannot be identified (see also Ordered Data).
<b>Uniparental inheritance:</b>	A genetic character that is passed on solely along the male or female lines as often found for organellar genomes (see also biparental inheritance).
<b>Vagile</b>	Mobile
<b>Wahlund effect:</b>	The sampling of a single population that is actually two or more genetic populations with limited gene flow between them. Such sampling leads to a deficit of heterozygotes for alleles that differentiate the two genetic populations.

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## Appendix

Listing of some of the SSR loci available for British woodland species  
(N.B. List not exhaustive, and citations are not repeated in reference list)

Common name (alphabetical)	Scientific name (higher order classification)	Geographic range and habitat	Number of polymorphic loci (range, mean or max. no. number of alleles per locus)	Heterozygosity (H) or inbreeding (f)	Full reference
Trees					
Ash	<i>Fraxinus excelsior</i>	Europe; forest/woodland	-	-	Vendramin GG, Frascaria-Lacoste N, Vekemans X (2004) A new set of mono- and dinucleotide chloroplast microsatellites in Fagaceae. <i>Molecular Ecology Notes</i> <b>4</b> :259. Heuertz M, Hausman J-F, Hardy OJ (2004) Nuclear microsatellites reveal contrasting patterns of genetic structure between western and southeastern european populaiotns of the common ash ( <i>Fraxinus excelsior</i> L.). <i>Evolution</i> <b>58</b> :976-988.
Birch	<i>Betula pendula</i> , transferred to related <i>Betula</i> and <i>Alnus</i> spp. (Betulaceae)	Eurasia; forest	23 (2-20 alleles)	H = 0.17-0.92	Kulju KKM, Pekkinen M, Varvio S (2004) Twenty-three microsatellite primer pairs for <i>Betula pendula</i> (Betulaceae). <i>Molecular Ecology Notes</i> , In Press.
Black poplar and other poplar species	<i>Populus nigra</i> L. ( <i>P. deltoides</i> , <i>P. tricarpa</i> , <i>P. tremula</i> , <i>P. tremuloides</i> , <i>P. candicans</i> , <i>P. lasiocarpa</i> )	Europe; pioneer tree species of riparian ecosystems	6 (up to 12 alleles)	H = 0.32 - 0.91	Van der Schoot J, Pospiskovam M, Vosman B, Smulders MGM (2000) Development and characterization of microsatellitemarkers in black poplar ( <i>Populus nigra</i> L.) <i>Theoretical and Applied Genetics</i> <b>101</b> :317-322 Smulders MJM, Van Der Schoot J, Arens P, Vosman B (2001) Trinucleotide repeat microsatellite markers for black poplar ( <i>Populus nigra</i> L.) <i>Molecular Ecology Notes</i> <b>1</b> .

					Also see: Dayanandan S, Rajora OP, Bawa KS (1998) Isolation and characterization of microsatellites in trembling aspen ( <i>Populus tremuloides</i> ). <i>Theoretical and Applied Genetics</i> <b>96</b> :950-956. <a href="http://poplar2.cfr.washington.edu/pmgc">http://poplar2.cfr.washington.edu/pmgc</a>
European beech and other Fagaceae	<i>Fagus sylvatica</i> L. and transferred to <i>Fagus orientalis</i> Lipsky, <i>Castanea sativa</i> , and <i>Quercus petraea</i> (Fagaceae)	Eurasia; forest	20 (3-21 alleles)	H = 0.58-0.85	Pastorelli R, Smulders MJM, Van'T Westende WPC, Vosman B, Giannini R, Vettori C, Vendramin GG (2003) Characterization of microsatellite markers in <i>Fagus sylvatica</i> L. and <i>Fagus orientalis</i> Lipsky. <i>Molecular Ecology Notes</i> <b>3</b> :76. Sebastiani F, Carnevale S, Vendramin GG (2004) A new set of mono- and dinucleotide chloroplast microsatellites in Fagaceae. <i>Molecular Ecology Notes</i> <b>4</b> :259.
European chestnut	<i>Castanea sativa</i> Mill.	Europe; woodland	13 (2-14 alleles, mean = 5.15)	-	Buck EJ, Hadonou M, James CJ, Blakesley D, Russell K (2003) Isolation and characterization of polymorphic microsatellites in European chestnut ( <i>Castanea sativa</i> Mill.). <i>Molecular Ecology Notes</i> <b>3</b> :239.
European oak	<i>Quercus robur</i> , <i>Q. petraea</i> (some transferable from <i>Q. macrocarpa</i> )	Europe; forest/woodland	More than 50 (some more than 50 alleles)	-	Dow BD, Ashley MV, Howe HF (1995) Characterization of highly variable (GA/CT) <sub>n</sub> microsatellites in the bur oak, <i>Quercus macrocarpa</i> . <i>Theoretical and Applied Genetics</i> <b>91</b> :137-141. Kampfer S, Lexer C, Glössl J, Steinkeelner H (1998) Characterization of (GA) <sub>n</sub> microsatellite loci from <i>Quercus robur</i> . <i>Hereditas</i> <b>129</b> :183-186. Steinkellner H, Fluch S, Turetschek E, Lexer C, Streiff R, Kremer A, Burg K, Glössl J (1997) Identification and characterization of (GA/CT) <sub>n</sub> microsatellite loci from <i>Quercus petraea</i> . <i>Plant Molecular Biology</i> <b>33</b> :1093-1096.

European white elm	<i>Ulmus laevis</i> Pall., also transferred to <i>U. americana</i> , <i>U. glabra</i> , <i>U. minor</i> , and <i>U. pumila</i>	Eurasia; riparian forest	6 (2-9 alleles)	-	Whiteley RE, Black-Samuelsson S, Clapham D (2003) Development of microsatellite markers for the European white elm ( <i>Ulmus laevis</i> Pall.) and cross-species amplification within the genus <i>Ulmus</i> . <i>Molecular Ecology Notes</i> <b>3</b> :598.
Rowan/ mountain ash and related species	<i>Sorbus torminalis</i> (Rosaceae), and 36 other species in the Maloideae	Eurasia; woodland	9 (mean 10.7 alleles)	Exclusion probability for paternity > 0.993	Oddou-Muratorio S, Aligon C, Decroocq S, Plomion C, Lamant T, Mush-Demesure B (2001) Microsatellite primers for <i>Sorbus torminalis</i> and related species. <i>Molecular Ecology Notes</i> <b>1</b> :297.
Scots pine	<i>Pinus sylvestris</i>	Europe; forest/woodland	17 cpSSRs	-	Provan J, Soranzo N, Wilson NJ, McNicol JW, Forrest GI, Cottrell J, Powell W (1998) Gene-pool variation in Caledonian and European Scots pine ( <i>Pinus sylvestris</i> L.) revealed by chloroplast simple -sequence repeats. <i>Proceedings of the Royal Society, London B Series</i> <b>265</b> :1697-1705.
Subarctic willow	<i>Salix lanata</i> , <i>S. lapponum</i> and <i>S. herbacea</i> (Salicaceae)	Subarctic Europe; open woodland	7	-	Stamati K, Blackie S, Brown JWS, Russell J (2003) A set of polymorphic SSR loci for subarctic willow ( <i>Salix lanata</i> , <i>S. lapponum</i> and <i>S. herbacea</i> ). <i>Molecular Ecology Notes</i> <b>3</b> :280.
Sweet cherry and related species	<i>Prunus avium</i> , transferred to black cherry ( <i>P. serotina</i> ), sour cherry ( <i>P. cerasus</i> ), almond ( <i>P. dulcis</i> ), peach ( <i>P. persica</i> ), <i>Prunus</i> hybrids and apricot ( <i>P. armeniaca</i> ) (Rosaceae)	Eurasia; woodland	23 (2-7 alleles)	-	Clarke JB, Tobutt KR (2003) Development and characterization of polymorphic microsatellites from <i>Prunus avium</i> 'Napoleon'. <i>Molecular Ecology Notes</i> <b>3</b> :578. Dirlwanger E, Cosson P, Tavaud M <i>et al.</i> (2002) Development of microsatellite markers in peach ( <i>Prunus persica</i> (L.) Batsch) and their use in genetic diversity analysis in peach and sweet cherry ( <i>Prunus avium</i> L.). <i>Theoretical and Applied Genetics</i> , <b>105</b> , 127-138. Vaughan SP, Russell K (2004) Characterization of novel microsatellites and development of multiplex PCR for large-

					scale population studies in wild cherry, <i>Prunus avium</i> . <i>Molecular Ecology Notes</i> <b>4</b> .
Willows	<i>Salix burjatica</i> and 31 other <i>Salix</i> species	Eurasian; woodland	17 (2-22 alleles)	-	Barker JHA, Pahlich A, Trybush S, Edwards KJ, Karp A (2003) Microsatellite markers for diverse <i>Salix</i> species. <i>Molecular Ecology Notes</i> <b>3:4</b> .
Spruce (Norway and Sitka)	<i>Picea abies</i> , also transferred to <i>P. glauca</i> , <i>P. engelmannii</i> and <i>P. omorika</i> (Pinaceae)	Europe; forest	Many (large numbers of alleles)	-	Besnard G, Acheré V, Faivre Rampant P, Favre JM, Jeandroz S (2003) A set of cross-species amplifying microsatellite markers developed from DNA sequence databanks in <i>Picea</i> (Pinaceae). <i>Molecular Ecology Notes</i> <b>3:380</b> . Pfeiffer A, Olivieri AM, Morgante M (1997) Identification and characterization of microsatellites in norway spruce ( <i>Picea abies</i> K.). <i>Genome</i> <b>40:411-419</b> . A'Hara S Cottrell JE (in press) A set of microsatellite markers for use in Sitka spruce ( <i>Picea sitchensis</i> ) developed from <i>Picea glauca</i> EST sequences. <i>Molecular Ecology Notes</i> . van de Ven WTG, McNicol RJ (1996) Microsatellites as DNA markers in sitka spruce. <i>Theoretical and Applied Genetics</i> <b>93:613-617</b> .
Sycamore	<i>Acer pseudoplatanus</i> L., transferred to 21 species of the genus <i>Acer</i>	Central Europe; mixed montane forest	8	-	Pandey M, Gailing O, Fischer D, Hattemer, HH, Finkeldey R (2004) Characterization of microsatellite markers in sycamore ( <i>Acer pseudoplatanus</i> L.). <i>Molecular Ecology Notes</i> <b>4:253</b> .
Shrubs and herbs					
Angiosperms	Dicotyledonous plants	-	cpSSRs	-	Weising K, Gardner RC (1999) A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in the chloroplast genomes of dicotyledonous plants. <i>Genome</i> , <b>42</b> , 9-19.
Blackcurrant	<i>Ribes nigrum</i> L., and	Europe; open	11 (2-18 alleles)	H = 0.18-0.91	Brennan R, Jorgensen L, Woodhead M,

	related species	woodland			Russell J (2002) Development and characterization of SSR markers in <i>Ribes</i> species. <i>Molecular Ecology Notes</i> 2:327.
Bladder campion	<i>Silene vulgaris</i> (Caryophyllaceae)	Eurasia and North America; open woodland and edges	7 (9- 40 alleles, mean = 22.1)	H = 16.2-77.4	Juillet N, Freymond H, Degen L, Goudet J (2003) Isolation and characterization of highly polymorphic microsatellite loci in the bladder campion, <i>Silene vulgaris</i> (Caryophyllaceae). <i>Molecular Ecology Notes</i> 3:358.
Grass	Poaceae	Global; open woodland	cpSSRs - 5	Polymorphism in <i>Anthoxanthum odoratum</i> at two loci with haplotype diversity of 0.495	Provan J, Biss PM, McMeel D Mathews S (2004) Universal primers for the amplification of chloroplast microsatellites in grasses (Poaceae). <i>Molecular Ecology Notes</i> 4:262.
Hound's-tongue	<i>Cynoglossum officinale</i> (Boraginaceae)	Europe; open woodland	9 (2-4 alleles)	-	Korbecka G, Wolff K (2004) Characterization of nine microsatellite loci in <i>Cynoglossum officinale</i> (Boraginaceae). <i>Molecular Ecology Notes</i> 4:229.
Raspberries	<i>Rubus idaeus</i> and other <i>Rubus</i> species	Eurasia; woodland	10	-	Graham J, Smith K, Woodhead M, Russell J (2002) Development and use of simple sequence repeat SSR markers in <i>Rubus</i> species. <i>Molecular Ecology Notes</i> 2:250.
Western and mountain hemlock	<i>Tsuga heterophylla</i> (Raf.) Sarg, and transferred to mountain hemlock ( <i>Tsuga mertensiana</i> [Bong] Carr.)	Eurasia; forest	15 (5-30 alleles)	Mean H = 0.88	Wellman H, Pritchard E, Benowicz A, Ally D, Ritland C (2003) Microsatellite markers in western hemlock ( <i>Tsuga heterophylla</i> [Raf.] Sarg). <i>Molecular Ecology Notes</i> 3:592.
Wild strawberry	<i>Fragaria viridis</i> , also transferred to <i>F. vesca</i> (mapping population)	Eurasia; open woodland	22 (mean = 4.95 alleles)	Mean H = 0.68	Sargent DJ, Hadonou AM, Simpson DW (2003) Development and characterization of polymorphic microsatellite markers from <i>Fragaria viridis</i> , a wild diploid strawberry. <i>Molecular Ecology Notes</i> 3:550.
Wood avens	<i>Geum urbanum</i> , and 19 other <i>Geum</i> species and two <i>Waldsteinia</i> species	Eurasia; woodland/ forest	13	-	Arens P, Durka W, Wernke-Lenting JH, Smulders MJM (2004) Isolation and characterization of microsatellite loci in

	(Rosaceae)				<i>Geum urbanum</i> (Rosaceae) and their transferability within the genus <i>Geum</i> . <i>Molecular Ecology Notes</i> 4:209.
Birds and mammals					
Boreal owls	<i>Aegolius funereus</i> , transferred to 7 other Strigidae species.	Holarctic boreal and subalpine forest	22 (1-11 alleles)	H = 0.00 to 0.81	Koopman ME, Schable NA Glenn TC (2004) Development and optimization of microsatellite DNA primers for boreal owls ( <i>Aegolius funereus</i> ). <i>Molecular Ecology Notes</i> 4.
Brown bear	<i>Ursus arctos</i>	Europe (extinct in Britain); woodland/ forest	-	-	Bellemain E, Taberlet P (2004) Improved noninvasive genotyping method: application to brown bear ( <i>Ursus arctos</i> ) faeces. <i>Molecular Ecology Notes</i> 4.
Capercaillie	<i>Tetrao urogallus</i>		-	-	Segelbacher G, Paxton R, Steinbruck G, Trontelj P, Storch I (2000) Characterization of microsatellites in capercaillie <i>Tetrao urogallus</i> (AVES). <i>Molecular Ecology Notes</i> 9:1934-1935. Piertney SB, Höglund J (2001) Polymorphic microsatellite DNA markers in black grouse ( <i>Tetrao tetris</i> ). <i>Molecular Ecology Notes</i> 1:303-304.
Eurasian badger	<i>Meles meles</i> (Carnivora: Mustelidae)	Eurasia; woodland and forest	39	-	Carpenter PJ, Dawson DA, Greig C, Parham A, Cheeseman CL, Burke T (2003) Isolation of 39 polymorphic microsatellite loci and the development of a fluorescently labelled marker set for the Eurasian badger ( <i>Meles meles</i> ) (Carnivora: Mustelidae). <i>Molecular Ecology Notes</i> 3:610.
European roe deer	<i>Capreolus capreolus</i>	Europe; open woodland	34	-	Vial L, Maudet C, Luikart G (2003) Thirty-four polymorphic microsatellites for European roe deer. <i>Molecular Ecology Notes</i> 3:523.
Great tit	<i>Parus major</i> (Avies)	Europe; woodland	22 (3-19 alleles)	H = 0.45-0.77	Kawano KM (2003) Isolation of polymorphic microsatellite markers in the great tit ( <i>Parus major minor</i> ). <i>Molecular Ecology Notes</i> 3:314.

					Saladin V, Bonfils D, Binz T, Richner H (2003) Isolation and characterization of 16 microsatellite loci in the Great Tit <i>Parus major</i> . <i>Molecular Ecology Notes</i> 3:520.
Greater horseshoe bat	<i>Rhinolophus ferrumequinum</i> and 8 other <i>Rhinolophus</i> species (Rhinolophidae, Chiroptera)	Europe; tree roosts	14 (2-12 alleles)	-	Dawson DA, Rossiter SJ, Jones G, Faulkes CG (2004) Microsatellite loci for the greater horseshoe bat, <i>Rhinolophus ferrumequinum</i> (Rhinolophidae, Chiroptera) and their cross-utility in 17 other bat species. <i>Molecular Ecology Notes</i> 4:96.
House wren	<i>Troglodytes aedon</i>	Europe; woodlands	5	-	Cabe PR, Marshall KE (2001) Microsatellite loci from the House Wren ( <i>Troglodytes aedon</i> ) <i>Molecular Ecology Notes</i> 1:8.
Red squirrel and American grey squirrel	<i>Sciurus vulgaris</i> and <i>S. carolinensis</i>	Europe; woodland	21	13 loci had significant f values	Hale ML, Bevan R, Wolff K (2001) New polymorphic microsatellite markers for the red squirrel ( <i>Sciurus vulgaris</i> ) and their applicability to the grey squirrel ( <i>S. carolinensis</i> ). <i>Molecular Ecology Notes</i> 1:47. Todd R (2000) Microsatellite loci in the Eurasian red squirrel, <i>Sciurus vulgaris</i> L. <i>Molecular Ecology</i> 9:2165-2166.
Schreibers' long-fingered bat	<i>Miniopterus schreibersii</i> (Chiroptera: Vespertilionidae)	Europe, Africa, Madagascar, Australia and Asia; tree roosts	5 (17-20 alleles)	-	Miller-Butterworth CM, Jacobs DS, Harley EH (2002) Isolation and characterization of highly polymorphic microsatellite loci in Schreibers' long-fingered bat, <i>Miniopterus schreibersii</i> (Chiroptera: Vespertilionidae). <i>Molecular Ecology Notes</i> 2:139.
Song wren	<i>Cyphorhinus phaeocephalus</i>	Europe; woodland	6 (4-12 alleles)	H = 0.21-0.90	Hughes CR, Robinson TR (2001) Characterization of microsatellite loci developed for song wrens <i>Cyphorhinus phaeocephalus</i> . <i>Molecular Ecology Notes</i> 1:4.
Song sparrow and related species	<i>Melospiza melodia</i> (Aves), and other Emberizidae	Europe; woodlands	6	H > 0.7	Jeffery KJ, Keller LF, Arcese P, Bruford MW (2001) The development of microsatellite loci in the song sparrow, <i>Melospiza melodia</i> (Aves) and genotyping errors associated with good quality DNA.

					<i>Molecular Ecology Notes</i> 1:11.
Reptiles and amphibians					
Common frog	<i>Rana temporaria</i> (Amphibia, Ranidae)	Europe; woodland ponds	18 (2-27 alleles)	H = 0.28 - 0.96	Rowe G, Beebee TJC (2001) Polymerase chain reaction primers for microsatellite loci in the common frog <i>Rana temporaria</i> . <i>Molecular Ecology Notes</i> 1:6. Pidancier N, Gauthier P, Miquel C, Pompanon F (2002) Polymorphic microsatellite DNA loci identified in the common frog ( <i>Rana temporaria</i> , Amphibia, Ranidae). <i>Molecular Ecology Notes</i> 2:304.
Common toad	<i>Bufo bufo</i>	Eurasia; woodland ponds	15	-	Brede EG, Rowe G, Trojanowski J, Beebee TJC (2001) Polymerase chain reaction primers for microsatellite loci in the Common Toad <i>Bufo bufo</i> . <i>Molecular Ecology Notes</i> 1:308.
Adder	<i>Vipera berus</i> and other species in the Viperidae	Eurasia; open woodland	6 (2-38 alleles)	-	Carlsson M, Isaksson M, Höggren M, Tegelström H (2003) Characterization of polymorphic microsatellite markers in the adder, <i>Vipera berus</i> . <i>Molecular Ecology Notes</i> 3:73.
Insects					
Aphid	<i>Rhopalosiphum padi</i>	Europe; on plants	8	-	Simon J-C, Leterme N, Delmotte F, Martin O, Estoup A (2001) Isolation and characterization of microsatellite loci in the aphid species, <i>Rhopalosiphum padi</i> . <i>Molecular Ecology Notes</i> 1:4.
Ant	<i>Formica exsecta</i> (Hymenoptera: Formicidae)	Europe; woodland edges	14 (3-18 alleles)	-	Gyllenstrand N, Gertsch PJ, Pamilo P (2002) Polymorphic microsatellite DNA markers in the ant <i>Formica exsecta</i> . <i>Molecular Ecology Notes</i> 2:67.
Ant	<i>Myrmica scabrinodis</i> Nylander 1846 (Myrmicinae)	Transpalearctic; woodland edges	5	-	Henrich K-O, Sander A-C, Wolters V, Dauber J (2003) Isolation and characterization of microsatellite loci in the ant <i>Myrmica scabrinodis</i> . <i>Molecular Ecology Notes</i> 3:304.
Ant - fungus	<i>Cyphomyrmex longiscapus</i>	Europe; open	5	-	Villesen P, Gertsch PJ, Boomsma JJ (2002)

growing	and <i>Trachymyrmex</i> cf. <i>zeteki</i> , and five species of higher attine ants	woodland			Microsatellite primers for fungus-growing ants. <i>Molecular Ecology Notes</i> 2:320.
Beetle, ground	<i>Carabus nemoralis</i> (Coleoptera, Carabidae)	Europe; open woodland	10 (3-12 alleles)	-	Brouat C, Mondor-Genson G, Audiot P, Sennedot F, Lesobre L, Rasplus J-Y (2002) Isolation and characterization of microsatellite loci in the ground beetle <i>Carabus nemoralis</i> (Coleoptera, Carabidae). <i>Molecular Ecology Notes</i> 2:119.
Beetle, ground	<i>Abax parallelepipedus</i> (Coleoptera, Carabidae)	Eurasian; forests and hedges	5 (2-19 alleles)	H = 0.10-0.97	Keller I, Largiadèr CR (2003) Five microsatellite DNA markers for the ground beetle <i>Abax parallelepipedus</i> (Coleoptera, Carabidae). <i>Molecular Ecology Notes</i> 3:113.
Beetle, ground	<i>Carabus problematicus</i> (Coleoptera, Carabidae)	Europe; old forests to heather upland	6 (2-17 alleles)	H = 0.14-0.71	Gaublomme E, Dhuyvetter H, Verdyck P, Mondor-Genson G, Rasplus J-Y, Desender K (2003) Isolation and characterization of microsatellite loci in the ground beetle <i>Carabus problematicus</i> (Coleoptera, Carabidae). <i>Molecular Ecology Notes</i> 3:341.
Beetle, ground	<i>Pterostichus oblongopunctatus</i> F.	Palaearctic region; forest floor	6 (3-6 alleles)	H = 0.20-0.67	Lagisz M, Wolff K (2004) Microsatellite DNA markers for the ground beetle <i>Pterostichus oblongopunctatus</i> F. <i>Molecular Ecology Notes</i> 4:113.
Beetle, pine shoot	<i>Tomicus piniperda</i> (Coleoptera: Scolytidae)	Eurasian; pest of pine forest	5	-	Kerdelhué C, Mondor-Genson G, Rasplus J-Y, Robert A, Lieutier F (2003) Characterization of five microsatellite loci in the pine shoot beetle <i>Tomicus piniperda</i> (Coleoptera: Scolytidae). <i>Molecular Ecology Notes</i> 3:100.
Beetle, spruce bark	<i>Ips typographus</i> (Coleoptera: Scolytinae)	Europe; pest of spruce forest, particularly Norway spruce ( <i>Picea abies</i> )	5 (3-11 alleles)	H = 0.3-0.97	Sallé A, Kerdelhué C, Breton M, Lieutier F (2003) Characterization of microsatellite loci in the spruce bark beetle <i>Ips typographus</i> (Coleoptera: Scolytinae). <i>Molecular Ecology Notes</i> 3:336.
Honeybee	<i>Apis mellifera</i> L., some transferred to <i>A. cerana</i> (58%), <i>A. dorsata</i> (59%)	Europe; open woodland	552	Loci genomically mapped	Solignac M, Vautrin D, Loiseau A, Mougél F, Baudry E, Estoup A, Garnery L, Haberl M, Cornuet J-M (2003) Five hundred and fifty

	and <i>A. florum</i> (38%).				microsatellite markers for the study of the honeybee ( <i>Apis mellifera</i> L.) genome. <i>Molecular Ecology Notes</i> 3:307.
Ladybird, 2-Spot	<i>Adalia bipunctata</i>	Europe; open woodland	10 (high polymorphism)	-	Hadrill PR, Majerus MEN, Mayes S (2002) Isolation and characterization of highly polymorphic microsatellite loci in the 2-Spot Ladybird, <i>Adalia bipunctata</i> . <i>Molecular Ecology Notes</i> 2:316.
Moth, peppered	<i>Biston betularia</i>	Europe; woodland	14 (3-22 alleles)	-	Daly D, Waltham K, Mulley J, Watts PC, Rosin A, Kemp SJ, Saccheri IJ (2004) Trinucleotide microsatellite loci for the peppered moth ( <i>Biston betularia</i> ). <i>Molecular Ecology Notes</i> 4:179.
Springtail, soil-dwelling	<i>Orchesella cincta</i> (Insecta; Collembola)	Holarctic; soil dwelling	5 (2-5 alleles)	H = 0.1 - 0.9	Van Der Wurff AWG, Gols R, Ernsting G, Van Straalen NM (2001) Microsatellite loci in the soil-dwelling collembolan, <i>Orchesella cincta</i> . <i>Molecular Ecology Notes</i> 1.
Spider	<i>Pholcus phalangioides</i>	Cosmopolitan	14 (3-11 alleles)	-	Rütten KB, Schulz I, Olek K, Uhl G (2001) Polymorphic microsatellite markers in the spider <i>Pholcus phalangioides</i> isolated from a library enriched for CA repeats. <i>Molecular Ecology Notes</i> 1:255.
Spider, fen raft	<i>Dolomedes plantarius</i>	Britain; open woodland	10 (4-15 alleles)	H = 0.62-0.9	Ji Y-J, Smith H, Zhang D-X, Hewitt GM (2004) Ten polymorphic microsatellite DNA loci for paternity and population genetics analysis in the fen raft spider ( <i>Dolomedes plantarius</i> ). <i>Molecular Ecology Notes</i> 4:274.
Termite, European subterranean	<i>Reticulitermes santonensis</i> Feytaud	Europe; pest of forest trees	11 (2-8 alleles)	H = 0.10-0.48	Dronnet S, Bagnères A-G, Juba TR, Vargo EL (2004) Polymorphic microsatellite loci in the European subterranean termite, <i>Reticulitermes santonensis</i> Feytaud. <i>Molecular Ecology Notes</i> 4: 127.
Wasps	<i>Vespula vulgaris</i> and other species within the Vespidae, including <i>V.</i>	Cosmopolitan	19 (2-14 alleles)	H = 0.04-0.93	Daly D, Archer ME, Watts PC, Speed MP, Hughes MR, Barker FS, Jones J, Odgaard K, Kemp SJ (2002) Polymorphic microsatellite

	<i>austriaca</i> , <i>Dolichovespula sylvestris</i> , <i>V. germanica</i> , <i>Vespa crabro</i> , <i>V. rufa</i> , <i>D. media</i> and <i>D. norwegica</i> (Hymenoptera: Vespidae)				loci for eusocial wasps (Hymenoptera: Vespidae). <i>Molecular Ecology Notes</i> 2:273.
Wasp, gall	<i>Eurytoma brunniventris</i> (Eurytomidae)	Eurasia; parasitoid of a wide range of insect hosts within galls induced by oak gallwasps (Hymenoptera, Cynipidae and Cynipini)	9	-	Hale ML, Acs Z, Stone GN (2004) Polymorphic microsatellite loci in <i>Eurytoma brunniventris</i> , a generalist parasitoid in oak cynipid galls. <i>Molecular Ecology Notes</i> 4:197.
Wasp - Jewel/ Nasonia	<i>Nasonia vitripennis</i> , and cross-amplified in <i>N. longicornis</i> and <i>N. giraulti</i>	Cosmopolitan and nearctic; parasitoids of fly pupae in bird nests	11 (2-7 alleles)	H = 0.07-0.47	Pietsch C, Rütten K, Gadau J (2004) Eleven microsatellite markers in <i>Nasonia</i> , Ashmead 1904 (Hymenoptera; Pteromalidae). <i>Molecular Ecology Notes</i> 4:43.
Wasp, parasitic	<i>Cotesia melitaearum</i> (Wilkinson) (Braconidae: Microgastrinae) and <i>Hyposoter horticola</i> (Gravenhost) (Ichneumonidae: Campopleginae), and transferred to <i>C. acuminata</i> (Reinhard), <i>C. bignellii</i> (Marshall), <i>C. congregata</i> (Say), <i>C. cynthiae</i> (Nixon) and <i>C. koebelei</i> (Riley)	Eurasia; parasites of <i>Melitaea cinxia</i> (L.)	8	-	Kankare M, Jensen MK, Kester KM, Saccheri IJ (2004) Characterization of microsatellite loci in two primary parasitoids of the butterfly <i>Melitaea cinxia</i> , <i>Cotesia melitaearum</i> and <i>Hyposoter horticola</i> (Hymenoptera). <i>Molecular Ecology Notes</i> 4:231.
Wasp, parasitic	<i>Aphidius ervi</i> (Hymenoptera: Braconidae)	Europe; parasite of aphids	6 (2-37 alleles)	-	Hufbauer RA, Bogdanowicz SM, Perez L, Harrison RG (2001) Isolation and characterization of microsatellites in <i>Aphidius ervi</i> (Hymenoptera: Braconidae) and their applicability to related species. <i>Molecular Ecology Notes</i> 1.
Fungi, microbes and parasites					
Apple scab, a	<i>Venturia inaequalis</i> ,	Cosmopolitan;	21 (2-24 alleles,	-	Guérin F, Franck P, Loiseau A, Devaux M,

phytopathogenic fungus	transferable to mainly <i>Spilocaea pyracanthae</i> and <i>S. eriobotryae</i>	causal pathogen of apple scab, open woodland	mean = 9.1)		Le Cam B (2004) Isolation of 21 new polymorphic microsatellite loci in the phytopathogenic fungus <i>Venturia inaequalis</i> . <i>Molecular Ecology Notes</i> 4:268.
<i>Armillaria</i> fungus and relatives	<i>Armillaria ostoyae</i> and related species	Root pathogen of conifers, particularly maritime pine ( <i>Pinus pinaster</i> Ait.)	12	-	Langrell SRH, Lung-Escarmant B, Decroocq S (2001) Isolation and characterization of polymorphic simple sequence repeat loci in <i>Armillaria ostoyae</i> . <i>Molecular Ecology Notes</i> 1:305.
Sapstain fungus	<i>Ophiostoma ips</i>	Cosmopolitan; carried by the bark beetle, <i>Orthotomicus erosus</i> , and infesting <i>Pinus</i> spp.	12	-	Zhou XD, Burgess T, de Beer ZW, Wingfield BD, Wingfield MJ (2002) Development of polymorphic microsatellite markers for the tree pathogen and sapstain agent, <i>Ophiostoma ips</i> . <i>Molecular Ecology Notes</i> 2:309.
White truffle	<i>Tuber magnatum</i>	Europe; forest	8 (2-18 alleles)	-	Rubini A, Topini F, Riccioni C, Paolocci F, Arcioni S (2004) Isolation and characterization of polymorphic microsatellite loci in white truffle ( <i>Tuber magnatum</i> ). <i>Molecular Ecology Notes</i> 4:116.