

## **Problems Associated With The Introduction of Non-Native Genotypes On NRS Reserves**

Committee to evaluate introduction of exotic genotypes into UC Reserves John Endler, Chair Susan Mazer, Mike Williams, Cristina Sandoval, Wayne Ferren

### **A) What is the problem with non-native genotypes?**

The reason these guidelines have to be made is the widespread practice of assuming that all individuals in a class are identical. This is entirely inappropriate in biology because a fundamental property of populations of plants, animals, and microorganisms is extensive variation among individuals. This variation is found at all biological scales from DNA sequences up to physiological, morphological, behavioral and other traits. Virtually all of this variation has a genetic component, so we will refer to this variation as genetic variation, and the variants as genotypes. Extensive documentation of genetic variation in natural and artificial populations can be found in textbooks of population genetics, quantitative genetics, conservation genetics and conservation biology. The burgeoning field of individual-based models and approaches in Ecology is another indication of the widespread acceptance of the importance of variation.

In spite of the well-known widespread genetic variation in animals, plants, and microorganisms there is a widespread implicit assumption that, if one wants to use a species in a particular study or experiment, or for habitat restoration, that it does not matter from where the individuals in that experiment originate, it doesn't matter where they are released, and it doesn't matter what happens to them after the experiment has finished, so long as the experiment takes place within the original natural range of that species. This assumption is not only wrong (because of widespread geographical genetic variation), it can be a highly dangerous assumption. It is dangerous because introductions of non-native genotypes can seriously endanger both the native populations of the same species and the entire community in which the species lives.

There are at least six documented effects of introductions of non-native genotypes:

- 1) Disruption of natural patterns of geographic variation in genotype frequencies. The measurement and study of genetic differentiation among populations within a species' geographic range has been a fruitful approach to the detection of several evolutionary processes, including natural selection, gene flow, and genetic drift. When distinct populations or subpopulations are close to evolutionary equilibrium, differences in their genetic structure (i.e., the frequencies of different alleles and genotypes) reflect the potential role of natural selection in molding phenotypic and genetic variation. Such differences among populations have been detected at all geographic scales, from meters to kilometers to regions. For examples and reviews see J. A. Endler (1977) *Geographic Variation, Speciation and Clines*, Princeton University Press, and Y. B. Linhart & M. C. Grant (1996), *Evolutionary significance of local genetic differentiation in plants*, *Annual Review of Ecology and Systematics* 27:237:277.

UC reserves provide the opportunity to compare populations within and among them to detect local adaptation in response to variation in abiotic factors (soil type, slope, aspect, temperature) as well as to variation in biotic factors (interspecific competitors, pollinators, herbivores, and predators). The reliability of such studies, however, depends on the assumption that populations (and the genotypes that comprise them) have been evolving in situ for many generations. A significant influx of alleles from exotic populations introduced to a UC reserve is that, given sufficient gene flow between introduced and resident populations, the population genetic structure of resident populations may change rapidly, no longer reflecting a stable outcome of natural selection. Populations that have been subject to gene flow from other populations in this way cannot be used in studies aiming to detect clear evidence for local adaptation.

If introductions were not documented then totally false conclusions could be made from subsequent studies assuming natural distributions. This will make it difficult for any research in ecology, ecological genetics, evolution, behavioral ecology, which is attempting to understand what happens in natural systems.

- 2) Introduction of genes which are poorly adapted to local conditions. Geographic variation in gene and genotype frequencies is caused by geographical variation in various environmental conditions (another aspect of typological thinking is to assume that the environment is the same everywhere within a species range). Geographical variation in environmental conditions causes geographical variation in natural selection and the result is that different genes and gene combinations increase in some places and decrease in others. Some new mutations will be advantageous in some places and not others. Some “bad” mutations will disappear more slowly in some places and faster in others; even the generation of variation is affected by geographically varying natural selection. Generally, the common genotypes in a natural population are those with highest fitness in that environment, but these same genotypes usually have much lower fitness in other environments. Introducing genotypes from other locations almost always introduces genotypes which are poorly adapted to local conditions. In some species the pattern is stronger the greater the distance from the source (alien) to the host (native) population and in other cases the effects are stronger the more disparate the habitats.

Note that keeping populations under domestication or semi-domestication (cages, enclosures, greenhouses, nurseries) before using them in experiments or restoration also alters gene frequencies because domestic conditions will not be identical to the original locations, nor will they be identical to where these populations are introduced. In addition, domestication often results in a large drop in the effective population size (the effective number of breeding individuals, assuming equal fertility, fecundity and mating success of breeding individuals) causing large random changes in gene frequencies in addition to any caused by differences between the source, domestic, and introduced environments.

Natural populations normally contain variants with various degrees of adaptedness, and we only call the extremes maladapted. For brevity here we will call the others semi-adapted. Introduction of individuals from other populations may change the frequencies of these semi-adapted genes and genotypes even if such introductions do not increase the frequency of maladapted genotypes. This can have damaging effects on the local population, even if less intense than effects of maladapted genotypes. Such effects can also add together over many genes and give rise to unexpected maladaptive gene combinations.

Shifting of gene frequencies can arise even if the source and host populations are identical because the number of individuals introduced is often small enough for them to be, by chance, a nonrepresentative sample of the source population's genotype frequency distribution. This is well known in population genetics where it is called the “founder principle”, or “genetic bottlenecks”, and is part of a larger phenomenon known as genetic drift. This is a particular problem in domestication, where genetic drift will proceed much faster rate because the captive populations have a much smaller effective population size than native populations.

It is sometimes assumed by people who are vaguely familiar with population genetics and evolution that natural selection will remove any maladapted genotypes caused by introductions. While this may be true in many cases the real problem is what happens to the population during the process. While natural selection is most intense, removing the maladapted genotypes, the mortality is very high, the fecundity and fertility low, and the mating success is very low. The resultant drop in effective population size can result in significant genetic drift, possibly fixing maladapted genotypes (reaching 100% instead of

being removed by natural selection). For example, if the frequency of a maladapted gene is 50% (which would happen initially if the introduced genotypes make up 50% of the local population, and would happen after hybridization with the locals with even a smaller initial proportion, through recombination), then the probability of fixing it is 0.5 (50%). Even in the absence of genetic drift, purely ecological factors increase the probability of extirpation while the population size is low. There are additional problems for animals with mating systems involving social facilitation or high local densities-- these species may fall below the minimum local density necessary for mating. The same may be true for insect-pollinated plants where pollinators may switch away from the rare species, leaving them with no pollinators, or reduced pollinators. The latter would decrease the effective population even further, causing more genetic drift. An additional risk of reduced population size is inbreeding depression.

- 3) Disruption of local patterns of gene interaction. This is also known as “coadaptation” in the older literature. Some combinations of alleles at some gene loci work well with certain combinations of alleles at other loci but very poorly with different combinations at those same loci. In natural populations the maladaptive gene combinations have been removed by natural selection. When non-native genotypes are introduced and these individuals breed with native genotypes, then recombination results in a sudden increase in the maladaptive gene combinations. This can cause extinction of the local population if the frequency of maladapted gene combinations results in high enough mortality and/or low enough fertility, fecundity and mating ability. Even if these effects are less than catastrophic the reduced fertility, fecundity, mating ability and/or increased mortality can increase the probability of extinction or extirpation by purely ecological or anthropogenic causes.
- 4) Potential to affect the population's future ability to respond to environmental change. Whenever the environment changes, either through climatic or anthropogenic change, natural selection changes in direction and intensity. The rate of response to natural selection is directly proportional to the genetic variance (this is known as Fisher's fundamental theorem). If genetic variance is lost, or is low relative to the strength of natural selection, then the species may not be able to evolve rapidly enough to adapt to the new conditions, and go extinct. Introductions always affect the genetic variation. If introductions involve large numbers of genetically similar organisms and the ratio of introduced to native individual numbers is large, then genetic variation will be reduced, endangering the population's ability to respond genetically to environmental change. Even if introductions are a small fraction of the native population and are genetically variable themselves, the genetic disruptions discussed in items 2-3 above can result in mortality and/or differential reproduction which results in only a small fraction of the original genetic variation surviving to the next generation. Either way genetic variation is lost, endangering responses to future environmental change, or even ongoing environmental change that the native population was able to respond to successfully.

It has been argued by some restoration biologists that the more genotypes the better, because this increases the genetic variation, so mixing genotypes from different populations is good. This misses all of the points made earlier (items 1-3). Increased variation is not sufficient, it must be compatible genes which are introduced. In addition, locally adapted populations may have little genetic variation and yet still have sufficient to have survived all of the previous glacial and interglacial climatic changes. They have appropriate genetic variation, and it may be maintained by stabilizing selection. Genetic variation of those species and populations could still be reduced by inappropriate introductions. Variation could also be increased, leading to genetic incompatibilities and then extinction. This is a quality versus quantity problem; more is not necessarily better.

- 5) Inadvertent introduction of a new species into the community. “Sibling species” are well known in almost all taxonomic groups. These are sets of species which are

morphologically and behaviorally identical, or differ by only small amounts in traits which humans use regularly to identify species. They are particularly common in insects, amphibians, lizards, rodents and many plant groups. Although they superficially look alike they may have very different ecological or other properties and their effects may be no different and no less dangerous than introducing any new species to a community. This is a particularly insidious danger because one may not know at the time of introduction whether or not what is being introduced is in fact the right species, or even if it consists of more than one species. This can only be checked by genetic methods.

A second source of introductions could be called “hitch-hikers”. Even if the appropriate genotypes are introduced at the appropriate frequencies, other species may come in with them. For example in an otherwise perfect experiment with introduced plant genotypes, the plants themselves may contain pathogens, miners and other herbivores, other arthropods, earthworms and microorganisms which are not native to the host locality. Even if the introduced hitch-hikers are of the appropriate species, they may still contain the inappropriate genotypes. For example, a parasitoid wasp found in eggs hidden in the introduced plants may contain genes maladapted to the local population, causing a crash in the parasitoid wasp population, causing a boom of the host caterpillars, causing defoliation if not extinction of the native (and non-native) plants on which the caterpillars feed. A similar argument could be made for pathogens which control herbivores or the other insects, or the plants themselves.

- 6) Cascading effects through the community. Any change in one species in a community will cause changes in all species with which it interacts, and this may cascade throughout the community. The ecological and conservation literature is full of examples and theory, although most of this literature concerns the effects of presence or absence of certain species, especially keystone species (species whose changes have massive effects on the community). However, any genetic changes which alter a given species' ecological properties are likely to be felt in the community, in some cases as much as if the species were removed, or a new species were added. This needs more investigation but is an obvious conclusion from ecological theory. This problem blends into the more familiar problem of introductions of non-native species, but is more insidious since it is not so obvious that a perturbation has occurred.

All of these affects also apply to well established non-native species (ferals). In some cases it may be desired to preserve pastoral habitats such as pastures for historical/archaeological or other reasons, even though they contain few or no native species. Note that even non-native species will have, after 300+ years of ranching, evolved locally-adapted genotypes, so introduction of non-local genotypes to feral populations may have all of the effects found in native species. Furthermore, these effects might shift the balance between ferals and natives. If all natives have been extirpated from an old pasture and it is desired to preserve the pasture, introductions of non-local genotypes of the pasture organisms can still disrupt local adaptation and community structure. This would be particularly unfortunate if the pasture contained, for example, native animals which depend on the feral plants and soil structure; cascading effects might cause extirpation of the remaining natives. The other side of the coin is that introductions of non-local feral genotypes might be a way of shifting the balance between ferals and natives towards the natives, as in some forms of biological control of insects.

## **B. What are the causes and levels of the problems at NRS reserves?**

Non-native genotypes have been and are being introduced for a variety of reasons, many of them laudable, but not all of them well thought out. There are at least six classes of introductions:

- 1) Mitigation by a company that uses or has used the reserve (e.g. McLaughlin), erosion control, cleanup of contaminated soil or other soil remediation, and roadside

“landscaping”. The company contracts a landscaper or other contractor with no real biological knowledge. Many acres may be planted with entirely inappropriate plants (species and genotypes). Similar events have occurred with animals.

- 2) Restoration to improve the Reserve's ecological state; removal of exotics and reintroduction of native species, or reintroduction of extirpated genes or genotypes. The scale may be sufficiently large that commercial sources are used and the companies usually do not reveal where the stock was originally collected, and/or how many generations, the stocks were kept since original collection, or what population size the stocks have been kept under. Even if the source population were local, keeping them for many generations in domestication causes large changes in genotype frequencies, means and genotypic diversity. (By domestication we mean any breeding or cultivation of animals and plants under wholly or partially controlled conditions, in locations which are not native to the stocks). Problems may occur even in small-scale restoration projects, especially those involving local community-based projects.
- 3) Use of the reserve to breed organisms for another restoration project elsewhere. Assuming the source is correct, the breeding conditions (local reserve conditions) are most likely to be different from the source and future host, causing selection for new gene frequencies and gene combinations which may be inappropriate there.
- 4) Large-scale research. If the scale of research is large enough stocks may come from commercial sources. Again, commercial sources are poor about providing information about where the stocks came from, or how long their stocks were in cultivation, and what the effective population size is during domestication. A common problem is that seed companies use the location of growing as the “source” stated to the buyer, rather than the original collection site. So even if the seeds are grown locally they could easily have genotypes from hundreds of miles away. Such change of habitats can result in rapid selection in the new growing site, making these plants different from their original source as well as different from the local populations. Example, S&S seeds, *Bromus carinatus* seed lot S7113, collected a long time ago in northern California (possibly Fresno), but grown in Oregon for many years, then used in southern California. Similar problems arise from animal breeders (particularly insects such as butterflies).
- 5) Small-scale research. Reciprocal transplants are a common and powerful technique to disentangle genetic and environmental effects, and this is well established technique for plants, invertebrates and vertebrates. However, if the introduced individuals are allowed to interbreed with the natives any or all of the problems listed in the previous section can occur. If transplants are not removed after experiments are over, there may be interbreeding or even displacement by non-native genotypes after the experiment, even if the experiment seemed harmless while it was being carried out.
- 6) Agricultural introductions. Some reserves have long histories of ranching and other agriculture. An example would be the grafting of the English Walnut (*Juglans regia*) on to trunks of the native California Walnut (*J. californica*). The walnut groves often were planted adjacent to streams along which occurred the native populations of the California Walnut. Hybrid walnuts now occur occasionally in regional riparian zones as a result of two potential sources, crossing between grafted trees and naturally occurring walnuts in the riparian zones, and crossing between the grafted trees and shoots of *J. californica* that emerge from the bases of the grafts once groves are abandoned and no longer pruned. Similar problems may occur if restoration experiments with native trees involve grafting. In addition, cows and other farm animals may introduce alien genotypes from adjacent properties. Some reserves may be used for experiments in improving pastures, etc., which can also result in the introduction of non-native genotypes. The same applies to experiments involving interactions between native and non-native pasture components.

- 7) Use of commercial sources of stock. In the American Standards for Nursery Stock (1996, American Association of Nurserymen, Washington, D.C.) there are no standards at all for the naming of plant stocks, and radically different genotypes are often found under the same stock names. Even if the stock names happen to be correct the names are vaguely defined and there are no standards for maintaining them, or of recording the original collection sites. The same is true for breeders of insects and vertebrates. Even when asked, seedsmen are not always forthcoming about the sources of their seeds, and even if they wanted to be, there may be no records.
- 8) Introgression from projects or organisms from outside the reserve. Gene flow from animals, plants or microorganisms outside the reserve may affect the populations inside the reserve. Large-scale projects within a couple of gene flow distances from the reserve will be just as damaging as if they were on the reserve. This is a particular problem with smaller reserves, where community (and adjacent landowner) awareness of the problem needs to be heightened.

### **C. Gene flow distance and population differentiation.**

Populations differentiate genetically as a result of genetic drift and natural selection, and this is counteracted by gene flow, which tends to make populations more similar. The balance between these three factors results in every species having a patchwork of populations differing in gene frequencies over all scales of geographic distance.

For those who are interested, here is a short summary of the meaning of the gene flow distance and how it relates to population differentiation. For more details see J. A. Endler (1977), *Geographic Variation, Speciation and Clines*, Princeton University Press, and M. Slatkin (1994), *Gene flow and population structure*, Pp. 3-17 in Real, L. A., (Ed.) *Ecological Genetics*, Princeton University Press. An interesting idea of the effects of gene flow on populations with respect to many genes is found in M. Slatkin (1995). *Epistatic selection opposed by immigration in multiple locus genetic systems*, *Journal of Evolutionary Biology* 8: 623-633. A more general introduction is found in the textbook by P. W. Hedrick (2000) *Genetics of Populations*, Jones & Bartlett.

Gene flow is the movement of genes between a source and a host population. For a large number of animals and plants most gene flow is short and the probability of a gene (allele) travelling from one place to another declines with distance. In other words most individuals breed with close rather than distant individuals. If we were to plot the distribution of distances travelled by individuals (animals or spores, released gametes, pollen or seed) from birth to reproduction the distribution has a peak in the middle (birthplace) and declines in all directions from the birthplace. For many organisms this distribution is similar to the bivariate normal distribution (a 2-dimensional version of the normal distribution), where the mean is at the birthplace and the standard deviation describes the average distance moved from birth to reproduction. This standard deviation is the gene flow distance, and is measured in m or km. (Some species are leptokurtic relative to the normal distribution--this means that more individuals move very short or very long distances compared to the bivariate normal distribution--however the empirically determined standard deviation is still a measure of the average distance moved). The standard deviation of distances travelled from birth to reproduction, or gene flow distance, is called  $l$ , and determines the scale of geographical variation among populations, as well as the scale of genetic incompatibility when individuals are moved between populations.

If genes are nearly or actually neutral (weak or no natural selection affects them), then genetic drift is the primary determinant of gene frequencies. In this case the important spatial unit is the neighborhood, which is defined as  $4l^2$ , the circle enclosed by a radius of  $2l$ . If gene flow is close to bivariate normal this area includes 95% of the movements. The neighborhood area and the population size within that area together determine how much differentiation can occur, and this gives rise to differentiation of groups of populations in patches of around 10-15 neighborhoods, or in patches around 3-4 neighborhoods in diameter. Taking the differentiated area width as around 4 neighborhoods each with diameter  $4l$ , this means

that, on average, populations around  $16l$  units apart will be different with respect to weakly or unselected loci. Example 1:  $l = 60\text{m}$  (Valley Oak), neighborhood diameter is  $240\text{m}$  and differentiation should occur over about  $960\text{m}$ . Example 2:  $l = 1\text{km}$  (Passerine birds), neighborhood diameter is  $4\text{km}$ , differentiation over  $16\text{km}$ . Note that, although these loci are nearly or actually neutral, local combinations of alleles at different loci may become very different among populations by chance, and may cause fitness problems when crossed. The problem is more serious when selection varies with distance.

Natural populations are subject to a variety of gradients in physical and biological factors, and all can induce natural selection on a variety of traits. In the absence of gene flow, this selection would yield a patchwork of genetically very different populations over the landscape; wherever the direction of natural selection changes direction a genetic discontinuity would evolve. This is true for both weak and strong selection. Considering only part of the landscape involving a gradient between only two different microenvironments, we can measure the strength of the selection gradient (change of selection with distance) by the slope  $b$  of the regression of selection intensity ( $s$ ) on distance. In the absence of gene flow, gene frequencies would change suddenly where the line goes through zero (selection changes direction). When there is gene flow, this counteracts the strongly differentiating effects of selection, and the result is a cline--a geographic change in gene frequency with distance. How steep the cline is (how strongly the populations differ along the cline) can be measured either by the maximum slope (steepest part) of the cline, or the distance between gene frequencies of, for example,  $0.2$  and  $0.8$ . The latter is called the cline width  $w$ ;  $w$  is larger for less population differentiation. The cline width is determined by the balance between gene flow and selection and  $w = k(12/b)^{1/3}$ , where  $k$  depends upon how  $w$  is measured ( $k = 1.66$  using the distance between  $0.2$  and  $0.8$ ). The more gene flow relative to selection the greater the  $w$  and the less the differentiation, and the more selection relative to gene flow the smaller the  $w$  and the more differentiation. If selection changes very rapidly with distance we have an ecotone and the relationship becomes even simpler,  $w = k l / s$  ( $k = 2.08$  using  $0.2 - 0.8$ ,  $s$  is the selective difference between both sides of the ecotone), but basically the same conclusion: the amount of differentiation depends upon the ratio of selection to gene flow. To compare different species with different  $l$  we can divide both equations for  $w$  by  $l$  to get  $w/l = k(1/b)^{1/3}$  for a gradient and  $w/l = k/s$  for an ecotone. The basic conclusion is that the relative cline width  $w/l$  can be small (strong population differentiation over short distances), even if there is no ecotone. Values in natural clines vary from  $1.2$  to  $1000$  with an average around  $50$ , and interestingly, smaller animals within a larger taxonomic group tend to have larger  $w/l$ . In any case, genetic incompatibility is likely when crossing individuals more than  $w/l$  apart. Examples of  $w/l$  and  $w$  (in parentheses): *Thomomys*  $1.2$  ( $630\text{m}$ ); *Melospiza*  $3.2$  ( $1\text{km}$ ); *Eucalyptus*  $5.0$  ( $100\text{m}$ ); *Linanthus*  $11.0$  ( $190\text{m}$ ); *Pinus*  $71$  ( $500\text{m}$ ); *Peromyscus*  $90.0$  ( $24\text{km}$ ); *Myrmeleotettix*  $130.0$  ( $200\text{m}$ ); *Sceloporus*  $320$  ( $57\text{m}$ ); *Drosophila*  $940$  ( $170\text{km}$ ). Note the lack of relationship between  $w/l$  and  $w$ , arising because of great variation in  $l$ . It is clear that each species has its own scale of population differentiation, and that extra care should be taken with introductions from further away than the  $w/l$  for that species. If this is not known, then  $10l$  should be used as a rule-of-thumb; if  $l$  isn't known, then  $l$  for the closest relative should be used with caution.