



'Acacia cyclops' (Rooikrans) tree. 'Acacia paradoxa' (Kangaroo wattle) in Table Mountain National Park.



This weed is currently only found in one moderately invasive population on the slopes of Table Mountain and may represent an incipient weed that is emerging from a lag phase.



'Acacia saligna' (Port Jackson) tree. Photographs: John Wilson

What can genes tell us about **INVASIVE SPECIES?**

Identifying alien plant species accurately is crucial to their management in the South African environment. *Jaco le Roux* from the Centre for Invasion Biology at the University of Stellenbosch tells how molecular genetics can be used.

Invasive species in South Africa

If he was still around, Charles Darwin may not have been surprised by the extent and impact of alien invasive species around the globe today, as he noted in his 1859 publication *The Origin of Species*, the ease by which non-indigenous species naturalize in their new non-native environments. Invasive species are those species that occur, as a result of unnatural dispersal and movement, beyond their accepted and historical normal distribution and which threaten valued environmental, agricultural or other social resources. South Africa is home to an estimated 9000 alien plant species, some of which contribute to R30 billion in annual losses in the agricultural industry. It is difficult to obtain similar estimates that accurately reflect damages and losses of "natural" capital. For example, what is the economic value attached to the reduction of species biodiversity or the alteration of ecosystem functions? A single ecosystem can supply numerous services. For example, a wetland can offer fishing, act as a bio-filtration system for human-produced wastes, be home to diverse groups of species or protect surrounding areas from flooding.

It was nevertheless estimated in 2001 that South Africa loses up to R21 billion annually as a result of losses of ecosystem services and environmental impacts caused by invasive species. Given the difficulty of quantifying such monetary values and the extensive number of invasive species in South Africa, this is likely to be a very conservative figure. The South African government realizes the urgency of these "natural" capital losses and has established programmes specifically aimed at reducing and countering the impacts of weeds and pests.

While WfW has an outstanding track record, the current and projected extent of invasive species and their impacts in South Africa call for integrating all possible management approaches. Maintaining appropriate levels of invasive species management and protecting South Africa's natural resources over the long term will require increased resources. More importantly, management programmes will have to be innovative and will have to come up with novel approaches to keep pace with the anticipated increases in the numbers of introduced species in the future. >>



Students and a professor (*John Hoffmann*) discussing biological control of *Acacia longifolia*. A gall-forming wasp, intentionally introduced from Australia, has greatly reduced seed production of this invasive tree, thus reducing its invasive capacity. Photograph: Dave Richardson.

A water management programme

As an example, the Working for Water (WfW) programme was started in 1995 to conduct and coordinate alien plant management throughout South Africa. WfW is a multidisciplinary initiative with a primary focus on social upliftment and job creation throughout the country. Hailed both locally and internationally for its socio-economic achievements, the programme has also proved highly successful at managing certain invasive species that are threatening South Africa's natural resources, particularly fresh water. WfW is currently one of the single largest conservation efforts in the world and since its inception has cleared more than one million hectares of invasive alien plants, providing jobs and training more than 30 000 people annually.

Invasive species management – a genetic approach

A novel approach to enhance the efficiency by which invasive species is managed involves the use of molecular genetics and DNA technology.

Charles Darwin would have been, no doubt impressed and maybe even surprised by the scientific progress that stands firmly on the foundations laid by his 1859 publication. A mere century later James Watson and Francis Crick would published the double helix structure of deoxyribonucleic acid (DNA), an achievement that gave a name and description to the variation that was invisible to Darwin and won them the Nobel prize. This variation is what underlies every single biological feature that we know. Today, little over half a century after the momentous discovery of DNA, research and technology have reached horizons stretching far beyond the imagination of any of these pioneering naturalists and scholars at the time of their discoveries. Biotechnologists now rearrange many biological features of different species in a copy and paste fashion of DNA. Genes for freeze tolerance copied from the deep sea flounder have been pasted into strawberries, creating frost resistant varieties. Genes sensitive to low levels of nitrogen dioxide have been inserted into plants, creating transgenic plant species that are capable of “sniffing” out unexploded landmines!

The double helix structure of DNA



An agreement between WfW and Stellenbosch University's DST-NRF Centre of Excellence for Invasion Biology (C-I-B) has set the platform for a comprehensive research programme in South Africa to investigate the molecular ecology of plant invasions.

Taxonomy and the native origins of weeds

Many organisms, in particular plants, create unique taxonomic obstacles that make fast and reliable species identification difficult. Many plant species show great morphological variability within and among species, implying that it is not always easy and sometimes actually impossible to distinguish between closely related species on the basis of morphology alone. This becomes particularly problematic when we know that there are variants that occur below the species level, such as subspecies. For example, Port Jackson (*Acacia saligna*), a widespread noxious weed in South Africa, has at least four known subspecies in its native Australia. These subspecies are not easily distinguished in the field and it is currently not known how many of these are present and invasive in South Africa.

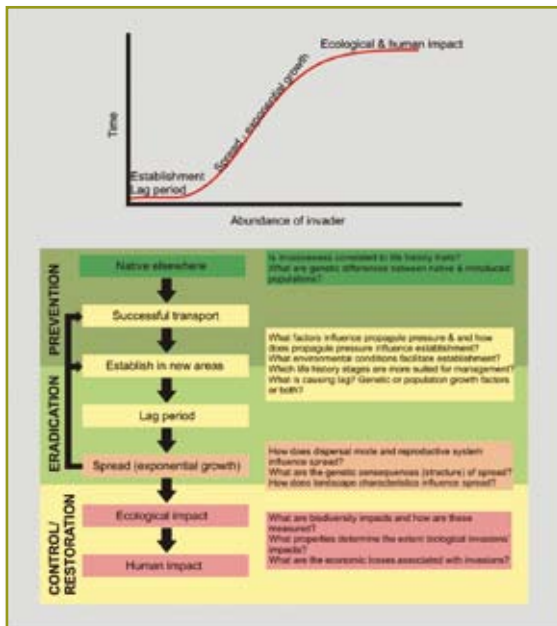
This genetic distinctness shown by subspecies will lead to distinctness for various adaptations such as habitat preference, physiological requirements and interactions and associations with other organisms. Species that

▷ To date, most molecular genetics research, especially biotechnology, has been aimed at improving food supply (agriculture) and human health (e.g. pharmaceuticals and cancer research) with comparably fewer efforts focused on the wellbeing of natural ecosystems and resources. But this is slowly changing as research programmes around the world are now also applying advances in

molecular genetics in a way that allows us to better understand biodiversity, global climate change, impacts of invasive species and the conservation of rare and endangered species.

Effective management of invasive plant species requires knowledge of basic biological processes that were, prior to the recent advances in molecular genetics, hard to clarify or quantify under field conditions. For example: How far do seeds disperse and how do populations move and disperse into particular environments? How long does seed survive in seed banks and what are the sizes of seed banks? What landscape and environmental characteristics make invasions easier? A better understanding of these processes will not only help in identifying the best integrated approaches to combat invasive species but will also assist in strategic and policy making decisions for programmes such as WfW.

The basic steps that occur during biological invasions



This figure shows the basic steps during biological invasions. Species are transported deliberately or accidentally into their new environment. Species then need to establish or form self sustained populations. Following establishment many species often remain in low numbers without spread into their new environment. This may reflect genetic constraints on local adaptation or the consequences of absolute population growth rates. This lag phase is normally followed by exponential growth phase; rapid dispersal and spread into favourable habitat. Growth reaches a saturation point (asymptote) that represents the point when most available and suitable habitat has been occupied. A series of questions related to management at these steps where molecular genetics may aid is shown. This figure has been modified from Sakai et al. 2001.

Part of a long-term collaborative research agreement that was signed between WfW and the DST-NRF C-I-B aims at improving the effectiveness of the alien plant management using genetic approaches. In terms of the agreement DST-NRF C-I-B will establish a research group to investigate the molecular ecology of various Australian *Acacia* species such as Rooikrans (*A. cyclops*), Black Wattle (*A. mearnsii*) and Port Jackson (*A. saligna*) that are invasive in South Africa. More specifically, it was agreed that the DST-NRF C-I-B will:

1. Develop capacity in South Africa to undertake co-ordinated work on the genetics of invasive plants.
2. Improve the success rate of biological control programmes by identifying the source populations of invasive alien plant species in their native range.
3. Improve strategic weed management decisions by understanding regional variations in weed populations in South Africa, in particular by identifying hybrids and new mutations.
4. Quantify the invasion risk posed by introducing new genotypes of non-native species by exploring the role of genetics in the lag phase of invasions and the link to climate.
5. Establish a plant material library for use in future research.
6. Quantify the risks of local invasion pathways by examining regional patterns of spread.
7. Identify possible mechanisms and sources of the spread of invasive plants at a local scale by determining the provenance of invasive populations.
8. Analyse the genetic consequences of biological control programmes on the agents released and compare the source of the agents released with the source of the plants.

For further details go to: <http://www.sun.ac.za/cib>

From test tubes to invasive species management

Taxonomy is the science of classifying organisms, i.e. naming living things. Biologists use the Linnaean taxonomic system, named after Swedish biologist Carolus Linnaeus (1707–1778), that breaks down organisms into seven major divisions, called taxa. The divisions are: Kingdom, Phylum, Class, Order, Family, Genus and Species, becoming more specific as you progress from kingdom to species so that many organisms belong to the same kingdom, fewer belong to the same phylum, and so on, with species being the most specific classification. These classifications also tell us something about the degree of relationship between different organisms. For example, two animals that belong to the same family and genus are more closely related than two animals that simply belong to the same family.

The most widely accepted definition for a species was proposed by the biologist Ernst Mayr, which defines a species as a group of genetically distinct, interbreeding organisms. However, this definition would not include organisms that reproduce asexually such as most fungi, some plants, some insects and so on.

In addition, morphology has been one of the most important taxonomic tools for centuries. However, biologists often do not know whether two morphologically similar groups of organisms can “potentially” interbreed and thus whether they represent a single or multiple species. Recent genetic data reveal that genetically distinct populations may appear very similar morphologically while large morphological divergences may be found within the same population of a single species. Furthermore, there is considerable variation in the degree to which different species can outcross, especially plants. Taxonomy is thus complicated by numerous biological processes and not always straightforward.



A forest of invasive trees, with ‘Acacia saligna’ (Port Jackson willow) in the foreground, and Eucalyptus species in the background. Dense stands like this totally transform natural fynbos communities by radically changing nutrient cycling regimes and changing other processes. Photograph: Dave Richardson.



A lone shrub of ‘Hakea sericea’ in the fynbos in the Slanghoek Valley in the Western Cape. This species often forms extremely dense stands that suppress the natural fynbos. Photograph: Dave Richardson.



A flowering branch of *Acacia podalyriifolia* (Queensland Silver Wattle). Queensland Silver Wattle is among the most widely cultivated wattle species due to its attractive flowers and fast growth rate.

Photograph: Dave Richardson.

How do subspecies arise?

Subspecies normally result from geographic isolation over historical time, whereby populations of the same species become isolated so that in the absence of interbreeding, they become genetically distinct.

are genetically quite distinct but which cannot be distinguished on morphology alone are also known as *cryptic species*. On the flipside of the coin, genetic distinctness in *non-cryptic species* is normally shown by the fact that these species do look different, thus making reliable identification on morphology alone straightforward.

Some plant species also frequently interbreed with closely related species or subspecies, a process known as *hybridization*. The occurrence of hybridization can, in some instances, readily be seen when offspring are morphologically “intermediate” between their parental species or lineages. However, in many cases hybrids remain undetected because they look like their parent species.

Taxonomy and the management of invasive species

So how can all of these taxonomic obscurities be linked to or contribute to the management of invasive species in general? The short answer is that sound taxonomy is the very first step towards making appropriate management decisions that may drastically influence the outcome of particular management strategies. For example, biological control is currently the most efficient and effective way to control weeds that are widespread in natural ecosystems. >>

Biological control

Biological control makes use of natural enemies (control agents) such as fungi or herbivorous insects that parasitize or feed on the target species (hosts). Control agents are selected and collected from *native* range areas of the host plant. For example, exploration for control agents against invasive acacias in South Africa would thus be done in Australia. This is because the associations and interactions between host plants and natural enemies evolved over historical time.

▷ The evolution of tightly knit interactions such as *mutualisms*, antagonisms or commensalisms is known as co-evolution, and has important implications for the success of biological control programmes.

In native ranges, geographic and reproductive isolation will, in a way similar to the way that subspecies arise (discussed earlier), influence the underlying genetic signature of co-evolutionary processes so that genetic signatures for co-evolved organisms become distinct. In other words, an insect species that is specialized to exist on only certain subspecies of a particular *Acacia* species would show genetic distinctness that mirrors that of their host subspecies. Historic geographical isolation that led to subspeciation in the host plant would have led to reproductive isolation among the insect species specialized for each subspecies. This would imply that host-enemy relationships will become genetically distinct with the potential of being cryptic and thus left unnoticed in the field.

This clearly illustrates the need for sound taxonomy when dealing with biological control as a management approach. For example, a particular insect variant may have evolved to recognize one particular Port Jackson subspecies that it is associated with more easily than to recognise other subspecies. Such preferential associations between control agents and host species is known as *host-specificity*. Host-specificity is not only an important factor that will influence the effectiveness of potential control. It will also influence the likelihood that control agents will have harmful and damaging effects on beneficial plants, a phenomenon known as non-target impacts. For example, South Africa is home to numerous indigenous *Acacia* species, which means that to find the most host-specific control

Mutualism – a symbiotic relationship in which both species benefit from the association.

Antagonism – a symbiotic relationship where one species benefits and while the other is harmed.

Commensalism – a symbiotic relationship in which one species benefits from a relationship, while the other species neither benefits nor is harmed.



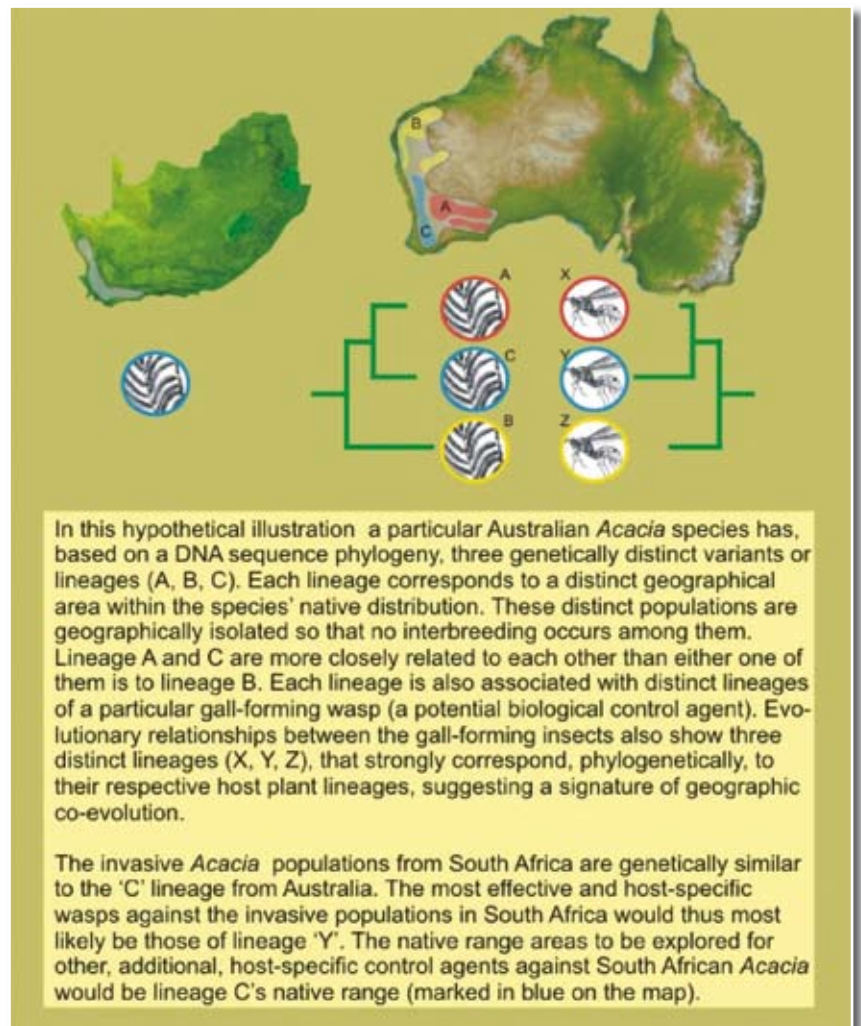
agents for Australian acacias we need to eliminate potential non-target impacts on these indigenous species.

We can do this by looking for co-evolutionary relationships using a phylogenetic comparison of DNA sequence data for both the host plant and the natural enemy of interest in their native ranges and matching that to genetic information (DNA) of

invasive populations.

Phylogenetics is the study of evolutionary relationships between organisms based on genealogy, that is, their shared characteristics due to common ancestry.

The rust fungus *Uromycladium tepperianum* was deliberately introduced to South Africa from Australia as a biological control agent



In this hypothetical illustration a particular Australian *Acacia* species has, based on a DNA sequence phylogeny, three genetically distinct variants or lineages (A, B, C). Each lineage corresponds to a distinct geographical area within the species' native distribution. These distinct populations are geographically isolated so that no interbreeding occurs among them. Lineage A and C are more closely related to each other than either one of them is to lineage B. Each lineage is also associated with distinct lineages of a particular gall-forming wasp (a potential biological control agent). Evolutionary relationships between the gall-forming insects also show three distinct lineages (X, Y, Z), that strongly correspond, phylogenetically, to their respective host plant lineages, suggesting a signature of geographic co-evolution.

The invasive *Acacia* populations from South Africa are genetically similar to the 'C' lineage from Australia. The most effective and host-specific wasps against the invasive populations in South Africa would thus most likely be those of lineage 'Y'. The native range areas to be explored for other, additional, host-specific control agents against South African *Acacia* would be lineage C's native range (marked in blue on the map).

against Port Jackson. This fungus is known to have different genetic variants. But because at the time of introduction, we lacked current molecular technologies, we don't know whether the best suited variant against Port Jackson was released in South Africa. Extensive host-specificity trials did show that the released strain was specific to *Acacia saligna* only, with no non-target effects. But, we still don't know how many different strains of Port Jackson exist in South Africa.

However, now that we can reconstruct phylogenetic relationships between *A. saligna* variants from Australia and those found in South Africa we can find out the number of subspecies present in South Africa. These data will also show us the geographical origins of South African variant(s) within Australia. It is these Australian regions that would be most likely to provide us with host-specific (co-evolved) and more damaging natural enemies of *A. saligna*. Phylogenetic analysis for all *Uromycladium tepperianum* variants associated with native Port Jackson variants will also confirm whether intraspecific variants are found in association only with certain Port Jackson subspecies. Research to show these relationships is currently underway at the DST-NRF C-I-B and will contribute to untangling co-evolutionary relationships and improve biological control programmes against Port Jackson in South Africa.

Hybridization and managing invasive aliens

Hybridization also has important consequences for the management of invasive species. Making crosses between closely related species and invasives (outcrossing) can lead to offspring that have characteristics of both parents that can potentially lead to higher invasiveness, a process that leads to *hybrid vigour*.

Hybridization normally also inflates genetic variation in hybrids, enhancing their ability to adapt to new environments. Genetic approaches are the fastest and most reliable way to accurately identify such events, especially when offspring show

Hybrid vigour is the superiority of a hybrid that is produced by crossing two different types of parent species that have one or more inferior characteristics compared to their offspring.



Introgressive hybridisation is the spread of genes from one species into the germ plasma of another species by hybridisation.

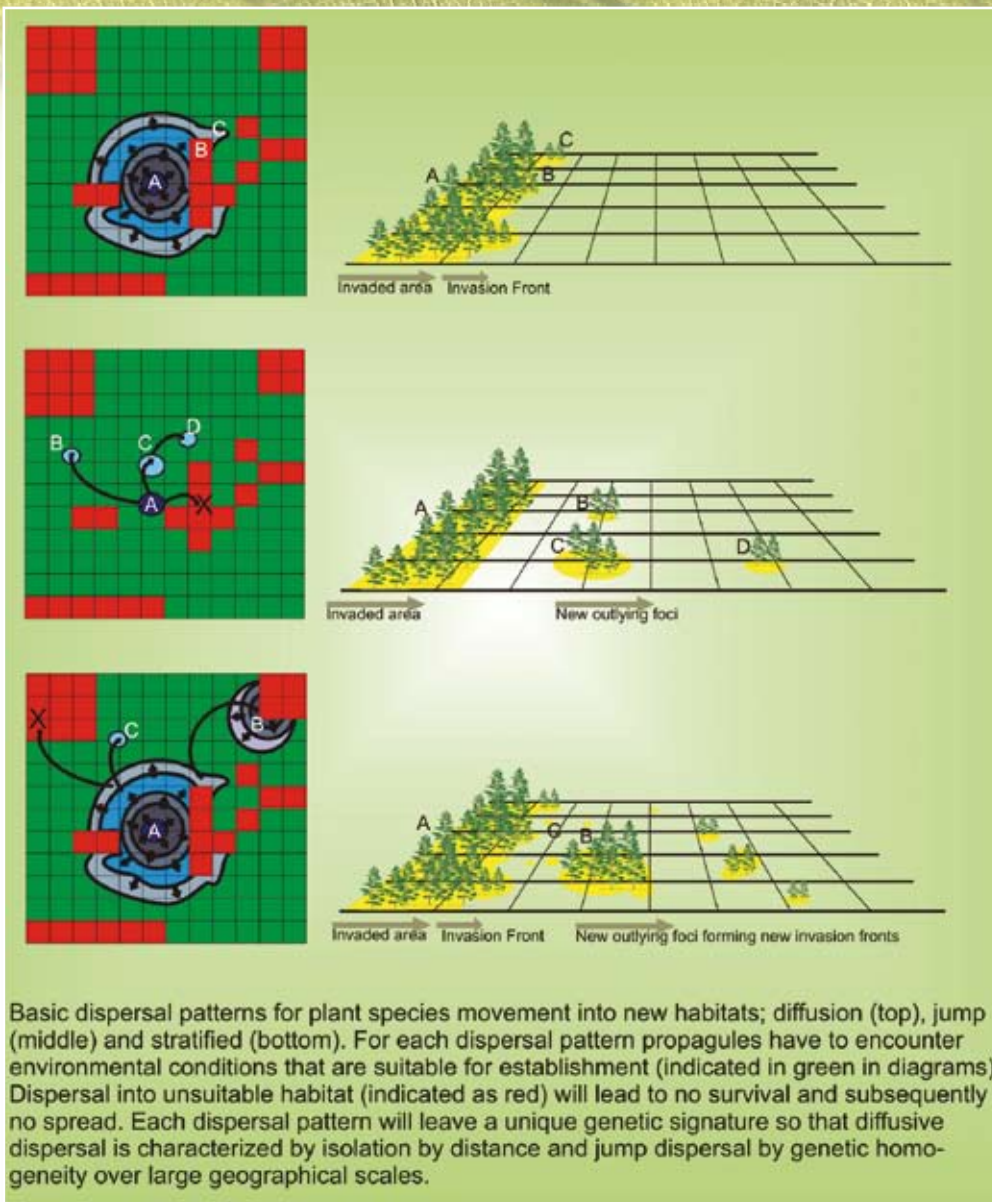
morphological characteristics that show that they come from one line of parents.

In most plant species particular genetic regions are only inherited from one of the parents. Chloroplast DNA (found in the cell's cytoplasm) is usually inherited from the mother as it is absent in pollen, while nuclear DNA (found in the cell's nucleus) is inherited from both parents. DNA sequencing from nuclear and cytoplasmic regions will, in hybrids, lead to dissimilar evolutionary relationships. Chloroplast and nuclear gene regions in *non*-hybridizing species leave the same genetic signature so that, when individually analysed, they show the same relationships within and between different species. Placing hybrid species in an evolutionary line using chloroplast genes will show relationships similar to the maternal parent's relationships to other species. On the other hand, evolutionary relationships based on nuclear genetic information will be derived from both parents as a result of *introgression*.

The genetic signatures of differently inherited genes, when considered

separately, will thus be different; showing that hybrids came from different genetic lines (species). For invasive species management such data can be valuable because it shows us the areas that need to be eradicated first because hybridization has already occurred or where different parental species are likely to outcross. This needs a sound understanding of what pollinators are visiting plants and how far pollen is likely to move and how far and by what mechanism(s) do seeds disperse.

Some Australian acacias that are invasive in South Africa such as the Black Wattle (*A. mearnsii*), Cootamundra Wattle (*A. baileyana*) and Silver Wattle (*A. dealbata*) are known to hybridize under plantation conditions, and that this may potentially also occur under field conditions. Novel morphological variants of Black Wattle have been observed in wild invasive populations in South Africa, raising the question of whether these are the result of hybridization or whether they are new genetic variants as a result of mutation. >>



▷ Spread and dispersal of weeds

To better understand biological invasions and how to manage them, we need to understand landscape processes and the way in which they shape weed dispersal. For animals the direction, frequency and distance of dispersal are relatively easy to measure by mark-and-recapture studies that are carried out over long periods of time. For sessile terrestrial plants the situation is more complicated because movement is through the dispersal of seeds or vegetative plant parts. It is hard to quantify seed dispersal, and so the frequency, direction and distance of spread, in the field. There are three major types of seed dispersal: diffusive, long-distance and stratified. During diffusive dispersal, populations expand in a concentric fashion over short distances with movement into suitable habitats next to the currently occupied range, in the same way that an army advances across a front. Jump dispersal, as the name indicates, involves occasional long jumps from source

populations into the new environment, with new populations being established whenever suitable habitats are encountered. Stratified dispersal is a combination of diffusive and jump dispersal so that the initial (source) and distant (outlier) populations that result from long-distance jumps expand in a concentric fashion.

Each of these patterns has implications for invasive species management. Each dispersal pattern also has characteristic genetic consequences that can easily be differentiated from one another using molecular genetic approaches. Dispersed seeds take all the genetic information derived from their source population along with them. The spatial and temporal distribution of numerous unlinked genes, also known as genetic structure, should thus give a good substitute for seed dispersal. A diffusive dispersal pattern would result in genetic structure that shows a gradient in genetic similarity when measured from the core of the source population to its border. Such a genetic pattern, also known as

isolation by distance, would show a strong correlation between geographic distance and genetic similarity, so that neighbouring populations are more genetically similar than geographically distant populations. Long-distance dispersal, on the other hand, would be characterized by a genetic signature indicating high similarities over much larger landscape areas (between source and outlier populations for instance). Seed movement and thus gene flow over such large temporal scales stop the formation of a strong population genetic structure. Spatial distribution of genetic diversity (the genetic structure) can be related to various environmental conditions such as rainfall, temperature, altitude, and vegetation to identify those habitats suitable for establishment and at highest risk of being invaded based on dispersal.

Knowledge of dispersal would help conservationists in identifying areas for management prioritization, defining the appropriate size of areas to be surveyed for the presence of outlying foci, and in determining the risk of re-colonization from neighbouring populations following removal of given stands. Looking at dispersal distances would also identify how connected different populations are and so would help to identify “units” where simultaneous removal efforts would yield the best results. This would minimize re-colonization risks. Dispersal distances are also interesting from a biological control point of view. Those invasive species that disperse over long distances would need a control agent capable of dispersing over equally long distances in order to keep up with its target host.

Genetic diversity

Evolutionary theory predicts that populations with high levels of genetic diversity and correspondingly high levels of phenotypic variation are better equipped for adaptation to new environments. This is because higher levels of diversity will, by chance alone, increase the likelihood of harbouring individuals with traits that are better suited for survival in any particular environment. Indeed, various weedy species show high genetic diversities that have contributed to their success in their new environments.

Molecular genetics can now be used to assess the amount and distribution of genetic diversity within and among invasive populations. Characterization of gene diversity will allow the

identification of populations with high genetic diversity that should be the initial focus of eradication programmes. This will lead to a decline of gene diversity over time and higher levels of inbreeding that may hamper the species' ability to spread and adapt. If genetic changes, and thus evolution, are common during biological invasions then it is important to understand the role of genetic diversity, and evolutionary analyses may need to be a major focus of work on invasive species biology. For example, studies of genetic variation may help to predict the potential for populations of invasive species to evolve in response to management practices such as resistance to herbicides or biological control agents. Genetically poor populations of invaders would be easier to manage since their chances of evolving resistance against management practices are lower.

The importance of genetic diversity also cautions against multiple introductions and subsequent genetic augmentation of exotic species. Many species that are not currently invasive such as escaped ornamentals may be considered "safe". Invasions can generally be depicted by a logistic growth curve so that, initially, species go through a lag phase followed by exponential growth. Lag phases may reflect the consequences of genetic constraints such as inbreeding, low genetic diversity and adaptive ability. Many species have only become serious weeds after a long lag phase and after genetic augmentation via separate, multiple introductions, which increase their genetic diversity, and thus their ability to adapt to their new environment.

On the other hand the consequences of genetic diversity can guide biological control programmes to evaluate the genetic diversity present in the control agents' native populations. Such estimates should give insight into the minimum number of individuals (known as the *effective population size*) that need to be introduced to sustain sufficient levels of genetic diversity in order to avoid inbreeding. This will insure maximum adaptive potential for control agents in their new environment and thus effectiveness against their target hosts.

What does the future hold

The age of functional genomics, the study of gene expression throughout whole genomes, is a new and rapidly

expanding field of enquiry in molecular genetics research. Whole genome sequences are becoming available for well-studied model organisms. Soon, major invasive weeds may be added to the list of species for which full-genome sequences are available; this will give researchers insight into the sub cellular processes associated with biological invasions. This would allow for gene expression levels to be estimated under certain environmental conditions so that candidate genes for "invasiveness" can be identified. For example comparing genome-wide gene expression in invasive and native populations of weeds may lead to the identification of genes whose expression is associated with invasiveness. In the near future such genes could potentially be used as "invasiveness barcodes" to screen closely related species for their potential to become invasive.

Similarly, comparing different levels of gene expression between "more" host-specific and "less" host-specific variants of potential biological control agents may lead to the identification of important functional genes. Functional genes that directly influence host-specificity, host recognition, confer virulence or pathogenicity, for example, can be valuable tools in novel and future approaches to controlling invasive species. Just as biotechnologists rearrange traits by copying and pasting their underlying genetic information between different organisms, next generation biological control agents may include transgenic pathogens or arthropods with enhanced virulence and host-specificity genes cloned into them. Such transgenic biological control agents may emerge soon as research using genome-wide gene expression to identifying virulence and pathogenicity associated genes is currently being done in laboratories around the world.

Genetically inferior populations of plants, i.e. those harbouring traits that are detrimental or that significantly lower population fitness, can be created through classic artificial selection experiments and inbreeding. These populations can then be used to "swamp" gene pools of invasive populations allowing introgression of inferior traits to the detriment of invasive populations. Traits such as terminator seeds (seed incapable of germinating) have been introduced into agricultural transgenic varieties and may also prove useful against plant invaders; transgenic plants fertilize invasive plants to create



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Above: PIC 6: P1010049.jpg

terminator seeds, lowering propagule pressure and invasive ability. Analogous approaches have been used to combat some invasive insects whereby millions of sterile males are released into invasive populations to allow competition with wild males to fertilize females. This approach has been successfully applied to eradicate highly invasive Mediterranean fruit flies (*Ceratitidis capitata*) in California in the USA.

As global trade and travel increase every year, so does the number of potentially invasive species hitching a free ride. Genetic approaches will play an increasingly important role in solving problems associated with invasions and in finding improved and novel ways to deal with them. □

Further reading

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