What genes can tell us about ecology and evolution.

Professorial Lecture

by

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17 July 2003

Introduction

Ecology is the study of the relationships of individual organisms to one another and to their physical environment and genes play a role in these relationships. I am basically an ecologist and my interests lie in answering ecological questions. Patterns of genetic variation reflect many ecological processes and I have become fascinated with the power of genetic techniques to address questions about ecology. In the past, many ecological questions have been addressed using conventional ecological techniques, but when genetic techniques have been applied to the same questions, the answers have often been extremely surprising. Tonight I am going to talk about how genes, or genetic markers, can be useful to ecologists. I am going to address four broad areas in which genetic markers are used in understanding questions about ecology and evolution and where I believe the use of genetic techniques has dramatically changed our understanding of processes. These are: mating systems, life-histories and dispersal patterns, microevolution and species and speciation. I will use examples from my own work and that of some of my students.

Genes in individuals

In 1866, Gregor Mendel, a Franciscan monk, discovered how heredity worked. He tended the garden in the monastery and noticed that certain traits in offspring were inherited, though not necessarily blended between those of the mother and father. He worked with peas and snapdragons to discover the laws that we know as Mendelian Genetics. Basically, this means that for each gene (or gene locus) an individual possesses two copies, one of which is inherited from the mother and one from the father. Although Mendelian Genetics has been understood for nearly 100 years, it was not until recent developments in molecular biology that we have been able to use these laws generally in ecological studies.

Molecular techniques provide a method for identifying differences in DNA composition between individuals. We can determine whether two individuals differ from each other at particular points on their chromosomes (although we do not necessarily know what parts we are looking at). In the same way as for the morphological traits that Mendel worked with, individuals possess two copies of each gene (or fragment of DNA). These can be the same (homozygous) or different (heterozygous). On an electrophoretic gel, the different bands denote differences in DNA composition, such that each individual has two different alleles at a locus (two bands) or two copies of the same allele (one band) (Figure 1).

By looking at potential mothers and fathers for each offspring, we can determine which is the mother and which is the father.

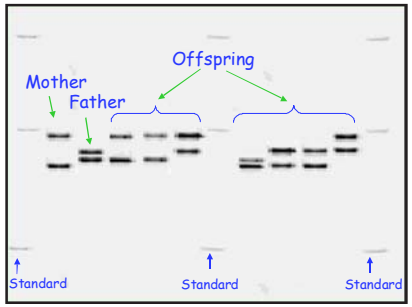

Figure 1. Microsatellite gel, showing banding patterns of mother, father and resulting offspring. Note: all individuals are heterozygous (two-banded).

Mating systems in birds

These techniques have been used to study mating systems in a number of animal and plant species. Until recently, birds were thought to be the best example of species that had a truly monogamous mating system. Many species of birds develop long-term pair-bonds, which may last for life. However new techniques of DNA fingerprinting and microsatellite analysis have shown that, while most species appear to be socially monogamous, females may mate with males outside the pair bond, called 'extra-pair copulations' or EPC's. A recent review of published studies reported that 86% of the 200 or more bird species examined so far exhibit at least some EPC's within their populations (Griffith *et al.*, 2002).

The Australian Magpie is a highly territorial species whose social behaviour appears to vary among geographic regions. In south east Queensland, magpies tend to live in pairs, with adult birds ousting young from the previous year before breeding begins again the following season (Hughes *et al.,* 1996). In south-eastern Australia, magpies live in larger groups. Female juveniles may remain and breed in the territory in which they are born, but males tend to disperse away from their territory and join non-territorial flocks (Veltman and Carrick 1990). In south western Australia, magpies live in very large territorial groups, up to 15 birds in a territory. Both male and female birds may remain in their natal territory and there are no territorial flocks (Hughes *et al*., in review). In all regions, magpies in groups are very aggressive towards any other magpies

that enter their territory, and groups tend to remain stable over a number of years. In a population in Victoria that we have been examining since 1992, some of the same dominant males and females remain even 11 years later. Given their highly territorial nature, we expected that magpies would show limited evidence of extra-group copulations.

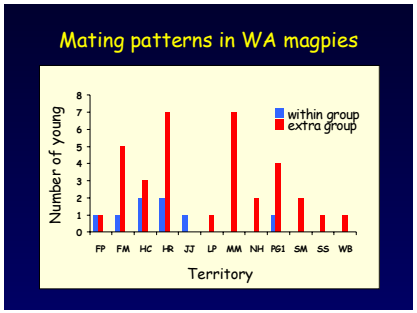

Figure 2. Numbers of juvenile magpies fathered by males from outside their territorial group in a population of the Australian magpie in Perth, WA.

We have used microsatellite analysis to examine the levels of EPC's (or extra-group young) in a population at Guilford, just out of Perth. Most of the groups consisted of multiple males and females. The largest had five adult males and five adult females, all of which had nests in all sampling years. We used 8 independent microsatellite loci to determine the parents of 43 fledglings born over a three-year period. All magpies in 12 territories were caught, individually colour-banded and their blood sampled. The results were really striking. Of the twelve territories examined, all but one showed evidence of extra-group young (Figure 2). In most territories, there were more extra-group young than within-group young. Overall, 88% of the fledglings had fathers that came from outside their own territory (Hughes *et al.*, in press).

This is the highest level of extra-group young reported for any bird species. The only other similar result is for Andrew Cockburn's fairy wrens in Canberra, which showed 76% extra-group young (Double and Cockburn 2000). The result is especially surprising given the highly territorial nature of the species. From years of watching banded magpies, we have seldom seen a banded magpie away from its home territory, except during border disputes, which usually only occur at the boundary of the territory.

Given the particular behaviour of south-western magpies, it is clear that this high level of outside-group mating will reduce the potential for inbreeding. For example, females that mate with males from within their

own territory are likely to be with their fathers or their brothers. Our result implies that, contrary to conventional views as to the notion of territory, in these magpies, the territories function for feeding and for rearing young, but not for breeding. We are currently examining three eastern Australian magpie populations to determine whether similar patterns occur there. Given the different dispersal patterns, we predict that the levels of extra-group paternity in these populations will be lower in south eastern populations and lowest in Queensland populations.

Genes in populations

Population genetics is a much more recent discipline than Mendelian genetics, especially its application to ecology. Although Sewell-Wright developed the theory of population genetics in the 1920's, it was not until the advent of allozyme electrophoresis in 1956, that geneticists were able to apply the theory to real populations. Basically, population genetics involves the study of the proportion of different genes or alleles at a single gene locus. For any locus, an individual possesses two alleles (these may or may not be the same). In a population, the relative proportion of each allele is determined by working out the genotype of each individual and then calculating frequencies across the whole population. Gene frequencies are the relative frequencies of each of a number of possible genes or alleles at a single locus.

Dispersal and life-histories

Population genetic techniques can be used to infer the mechanisms and patterns of dispersal of animal and plant species. To do this, we rely on two very simple principles. First, if populations are isolated from one another, over time they will tend to diverge and become genetically differentiated. This will occur, even if the populations are not affected by different selection pressures, i.e. just as a result of chance. This process is called genetic drift. If dispersal occurs between populations, this will tend to homogenise gene frequencies and make them more similar genetically. This process is called gene flow.
By using genetic markers, it is possible to calculate gene frequencies at a number of gene loci and thus to infer the levels of dispersal and evaluate potential mechanisms. For example, in our early work in the Conondale Range, we used allozymes to assess the mechanisms of dispersal in a

number of aquatic species. Our sampling design included streams on either side of the Conondale Range, flowing into the Brisbane River on one side of the range and into the Mary River on the other side of the range. We were interested in determining, not only the levels of dispersal among streams, but also the major mechanisms of dispersal for each species. For example, the shrimp *Paratya australiensis* lives in the stream channel, but it was thought that it could possibly disperse across land during particularly damp conditions. Using seven different gene loci, we were able to demonstrate quite clearly that terrestrial dispersal across catchment boundaries did not occur (Hughes *et al.*, 1995). Populations on either side of the range were highly differentiated from one another (Figure 3a). We also examined a number of aquatic insect species. They spend most of their life-cycle in the stream as larvae, but each has a short adult winged stage. However, in many cases the adults appear to be very poor flyers and also mark-recapture studies on many stream insects have suggested that individuals tend to remain near to the stream, possibly heading up-stream (Muller, 1982). Our data however, were able to show that clearly flight is an important mechanism for dispersal in these species (Figure 3b, 3c). Allele frequencies were homogeneous across the study area, suggesting substantial mixing each generation (Schmidt *et al*., 1995, Hughes *et al.*, 1998, 2000). This result also suggests that if populations of these insect species undergo local extinctions due to either natural or human disturbance, that recolonization is very likely. The same could not be said for the shrimp.

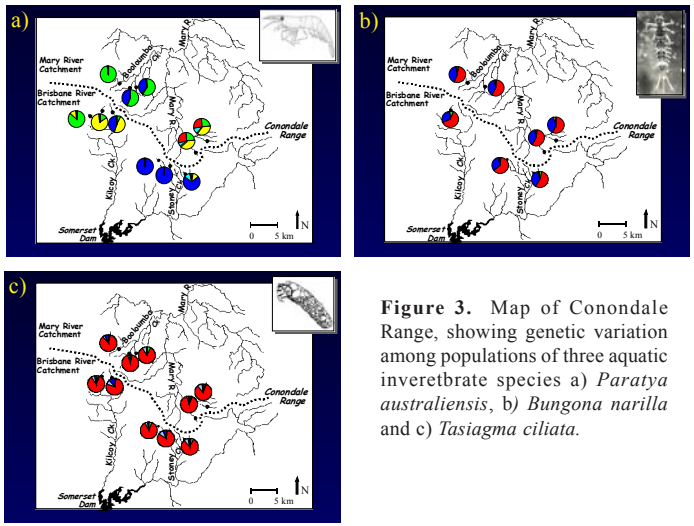

Figure 3. Map of Conondale Range, showing genetic variation among populations of three aquatic inveretbrate species a) *Paratya australiensis*, b) *Bungona narilla* and c) *Tasiagma ciliata.*

Non-Mendelian inheritance

While Mendelian genetics describes the patterns of inheritance of nuclear genes, we now know that there are some genes that are not inherited in this manner. Mitochondria are organelles within the cytoplasm of animal cells. They are responsible for cellular respiration and each mitochondrion has its own circular strand of DNA, which contains about 27 genes. The really interesting aspect of mtDNA is its mode of inheritance. Whereas an individual receives nuclear DNA from both parents, mitochondrial DNA is only passed on by the mother. From the point of view of the population geneticist, this is extremely useful, because mtDNA is passed directly from mother to daughter and so on, and mitochondrial genes are passed through generations, effectively with no mixing. This makes it relatively easy to trace evolutionary history of populations, because the only process producing variation is mutation.

By sequencing fragments of mtDNA from a number of individuals, it is possible to assess the evolutionary relationships among the sequences. The direct sequence data can be used to infer the evolutionary process. Haplotype networks show how many mutations have occurred between each pair of sequences (Figure 4). Each different sequence is referred to as a haplotype. From the network we can infer that haplotypes 1, 3 and 4 each arose from haplotype 2 by a single mutation. Haplotype 5 arose from haplotype 4. Thus we can infer the 'ancestral haplotypes', 2 and 4, and the recent or 'derived' haplotypes (1,3 and 5). The same haplotypes can be shown as a phylogenetic tree. The tree also shows relationships among haplotypes but trees are more useful when examining samples for evolutionary processes that have occurred in the past. In addition to being able to determine relationships among sequences, phylogenetic trees can be used to examine relationships among populations and species, where different populations possess different DNA sequences. Moreover, mitochondrial DNA has been shown to evolve in a clock-like fashion (Page and Holmes, 1998). A particular mitochondrial gene will evolve at a roughly constant rate. This observation is particularly useful because it allows us to estimate the timing of divergence between populations and species. This has been calibrated for various taxonomic groups from known timing of geological events, such as the separation of populations on either side of the Isthmus of Panama (Bermingham *et al*. 1997).

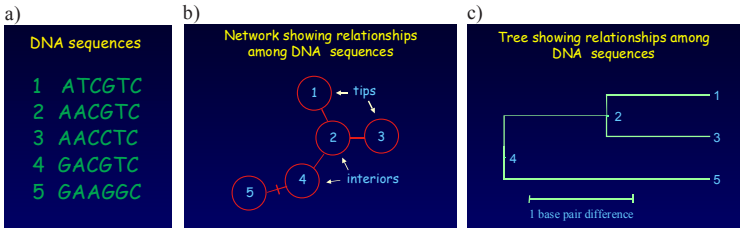

Figure 4. Methods to show relationships among DNA sequences, a) five different sequences, b) haplotype network , and c) neighbour joining tree

Separating historical from contemporary processes

MtDNA is particularly useful to population geneticists because it can be used in the same way as nuclear markers, to look at contemporary patterns of connectivity among populations, by just assessing haplotype frequency differences among populations, but also it can be used to infer historical patterns of gene flow. I want to show two examples of the usefulness of mtDNA sequence data. The first is with magpies, the second with a freshwater crayfish species.

Plumage variation in the Australian magpie

The Australian Magpie shows substantial plumage variation across its natural range. A recent publication suggests that there are eight different plumage forms described as subspecies, although there are really only three major forms (Schodde and Mason, 1999). The black-backed forms occur across northern Australia. In the south-east and Tasmania, magpies are white-backed, and in the south-west, males are white-backed and females are black-backed (Figure 5). The widely accepted view, which is derived largely from work on North American bird species, is that geographical variation in plumage patterns commonly results from past periods of separation/isolation, when forms diverged from one another, followed by subsequent range expansions. In other words, the current distribution patterns are thought to reflect historical isolation, rather than current differences in selection pressures across the species range. In the case of magpies, the conventional view would be that the three back colour

forms were isolated from one another in refugia in more arid times during the Pleistocene. While isolated, the three forms diverged from one another, possibly just by chance, i.e. by genetic drift. Subsequently, when times became wetter and populations expanded out of the refugia, the ranges of the different forms may have recontacted, as we see today. An alternative hypothesis would be that the distributions we see today reflect differences in current selection pressures in different parts of the range. That is, in northern Australia, black-backed magpies have a higher fitness than white-backed birds, while in the south, white-backed birds have the highest fitness.

Figure 5. Map showing distribution of major plumage forms of the Australian Magpie. Magpies are very rare in central Australia and plumage forms are virtually unknown.

We can use mtDNA variation to distinguish between these two hypotheses. If the present-day distribution is merely the result of historical isolation, then we should expect the mtDNA variation to reflect the same history as the plumage patterns. Alternatively, if dispersal is currently occurring between different parts of the distribution while the distribution of plumage patterns is being maintained by natural selection, then the mtDNA would be expected to show quite different patterns of variation from the plumage distribution.

When we look at frequencies of mtDNA haplotypes across Australia, we see that there is a break between eastern and western populations. The network is divided into two parts, with all western forms in one part of the network and all eastern forms in the other part (Figure 6). These two groups, or clades, are separated by 4 base pairs (Hughes *et al*., 2001). This suggests

that eastern and western forms evolved in isolation as a result of formation of the arid Nullarbor Plain over the last 60,000-100,000 years. However, what we are really interested in is looking at the patterns from north to south, where we go from all black-backed males to all white-backed males. In both eastern and western Australia, there are no clear genetic differences between north and south and common haplotypes are shared between white-backed and black-backed parts of the distribution. Statistical analysis confirms that there is more genetic variation among populations of each back colour than there is between back colours (Hughes *et al.,* 2001, Toon *et al.,* in review). This result strongly supports the idea that the distribution of black-backed and white-backed males are maintained by some form of current natural selection and are not merely the result of history.

a) b)

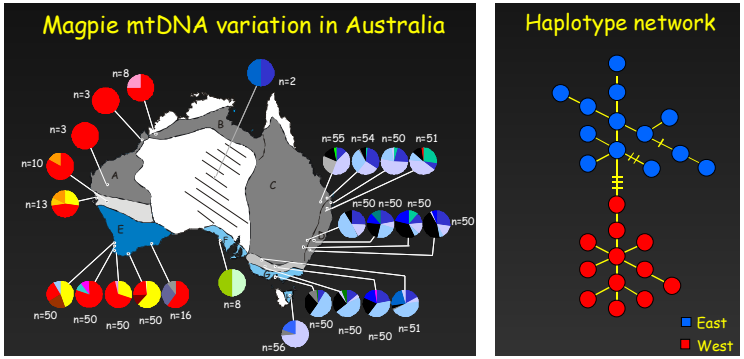

Figure 6. a) Haplotype frequencies and b) haplotype network, showing relationships among haplotypes for the Australian Magpie.

Many other Australian bird species show substantial geographic variation in plumage patterns (Schodde and Mason, 1999). We expect that many will show genetic patterns very similar to the magpie, suggesting differential selection pressures across their ranges. Determining the actual processes involved is a much more difficult task. We currently have a long-term project on a magpie population in the hybrid zone between white-backed and black-backed magpies in south-eastern Australia, in which we are looking at the fitness of different plumage forms and hybrids in different habitats (Hughes *et al.*, 2002).

Past and present patterns of connectivity in a freshwater crayfish

As part of a broad study of levels of connectivity among populations of freshwater species in central Australia and the role that waterholes play as refugia during times of drought, we have been examining patterns of mtDNA variation in the freshwater crayfish *Cherax destructor.*
C. destructor , or the freshwater yabby, is common throughout the system. As far as we know it spends its life within the waterhole, and is only able to move between waterholes at times of flood. Differences in elevation are so low in these areas that during extreme floods, it is likely that waterholes from different sub-catchments may be connected both longitudinally and laterally. Some other species in the genus *Cherax* are known to disperse across land occasionally, thus we were interested in trying to determine the extent to which this may happen in central Queensland populations and whether or not the isolated waterholes had genetically differentiated populations of the crayfish. We were also interested in the historical dispersal of the species.
By looking at frequencies of mtDNA haplotypes, it is clear that within catchments, there is a lot of sharing of haplotypes, particularly in the Cooper (Figure 7). However, there is almost no sharing of haplotypes across major catchment boundaries, indicating that currently movement is restricted to within the stream channels. The interesting result comes from viewing the network showing how the haplotypes are related to one another. Haplotypes can be grouped into 'clades' of closely related haplotypes. If dispersal across drainage boundaries has been restricted historically as well as at the present time, then recently derived haplotypes and clades should be restricted to particular drainages while ancestral (internal) haplotypes and clades will tend to be shared across drainages. Certainly, tip haplotypes, or recently derived haplotypes, should be restricted to the catchment in which they evolved. This is clearly not the case here. The network suggests that Murray-Darling and Cooper populations have been isolated from one another, allowing divergence, with new haplotypes probably having arisen during separation. But also, we see evidence that, subsequently there has been movement across drainage boundaries, in an east-west direction, from the Murray Darling to the Bulloo and from the Bulloo to the Cooper, so that in the Cooper we now see haplotypes that are part of the resident clade, but also some very recently derived haplotypes which have evolved from the major Murray-Darling clade. This example clearly shows the power of mtDNA in separating contemporary from historical patterns of dispersal and gene flow.

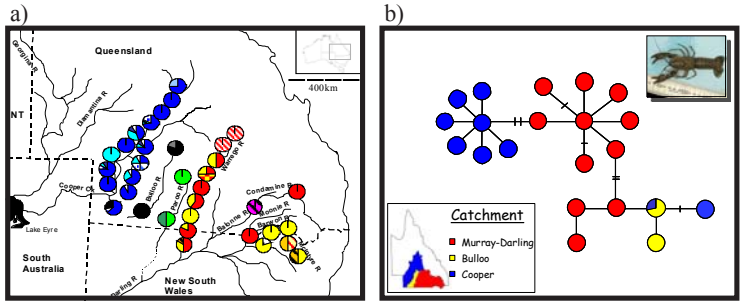

Figure 7. a) Haplotype frequencies, and b) haplotype network for the crayfish *Cherax destructor* in central Queensland.

Species and Cryptic biodiversity

Finally, I want to show how genetic markers can be used to address questions about species. There are many different concepts of species, but I am going to restrict my discussion to 'biological species', i.e. groups of populations that share a common fertilization system, as per Hugh Paterson (Paterson, 1993). Members of the same species mate with one another, exchange genes and therefore share many morphological and behavioural characteristics in common. Individuals belonging to different species do not interbreed, do not exchange genes and as a consequence are likely to have diverged in morphological and behavioural characteristics. Most taxonomists have assumed that morphology is sufficient to differentiate among species. However, this is not always the case, because the characteristics that individuals of a species use to recognise potential mates differ vastly among broad taxonomic groups. For example, birds commonly use plumage pattern and song to recognise conspecifics, whereas insects may use behavioural displays and chemical signals (pheromones). Clearly the former will be more obvious to taxonomists than the latter. Although some would argue that recognition of morphological species is sufficient to address most ecological questions, I would argue strongly against this view. Even though two different species may differ little in morphology, they may differ in very important ecological characteristics. Classical examples are the 'cryptic' species in the *Anopheles aegypti* (Paterson, 1993) species complex, of which only some species are vectors for the malaria parasite. Another example comes from Dick Drew's work involving species of tephritid fruitflies in the *Bactrocera musae* complex. *B. musae* is a serious pest of commercial

bananas while all the other cryptic species feed on native host-plants and do not infest commercial crops. Clearly, it is critical to be able to identify these different species unambiguously.

How can genes help us to answer questions about species?

We can use principles of Mendelian genetics to conclude that interbreeding is not occurring between two co-occurring species. Similarly, information from mtDNA can indicate evidence of 'deep' lineages in a phylogenetic tree, which may also correspond to 'good' species.

As part of the refugium project, we wanted to examine patterns of connectivity among freshwater mussel populations in central Queensland drainages. We sampled mussels from waterholes in the same way as we did for the crayfish. However, when we examined allozyme (similar to microsatellites) and mtDNA variation, we found a very different pattern (Baker *et al.*, in press). We also measured morphological characteristics of the shells. From the allozymes, we were able to distinguish four discrete species. Each species had unique alleles and, even when two species occurred in the same waterhole, no hybrids were detected on the gels. This is clear evidence of lack of interbreeding. The mtDNA tree also

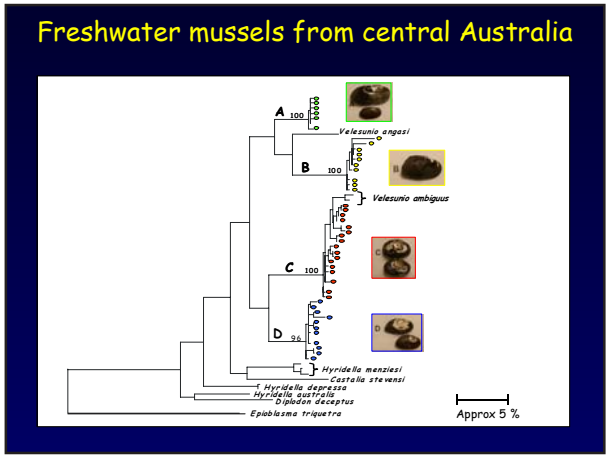

Figure 8. Phylogenetic tree showing relationships among haplotypes for the freshwater mussel.

shows deep lineages, which correspond exactly to the groups of genotypes identified from the allozymes (Figure 8). Even after we knew of the presence of the four species from the genetic work, using morphology, we could only distinguish Species C from the other three species using morpohological criteria. Species A, B and D could not be separated, even using multivariate morphometrics.

Indeed as we have collected samples for dispersal and life-history work over the last few years, we have discovered complexes of cryptic species in a wide range of aquatic invertebrate species, even those for which detailed taxonomic and ecological work has been completed. These have included mayflies from the Rocky Mountains, which had been studied ecologically for many years, caddisflies in Victoria, freshwater shrimps and freshwater beetles (Figure 9). The prevalence of cryptic species in these groups may be explained by the fact that taxonomists mostly rely on morphological differences to recognise species, but the individuals of these species may use chemical and behavioural cues to recognise potential mates.

Is all this cryptic biodiversity important?

I have already alluded to the fact that individuals belonging to different species are likely to show different behaviour, pest status, disease resistance etc. Thus, if an aim of conservation is to conserve biodiversity then this cryptic diversity is likely to be at least as important as the diversity we can see. Confusion of species can lead to quite erroneous conclusions about potential management of species and populations. For example, in the case of the mayflies in the Rocky Mountains, extensive ecological work by Bobbi Peckarsky and her colleagues had concluded that *Baetis bicaudatus* had two generations a year, a summer generation and a winter generation, which had different behaviour patterns (Peckarsky *et al*. in press) When we examined samples of *B. bicaudatus*, we discovered that, not only were the summer and winter cohorts completely different species (14% divergence in the mtDNA, suggestive of being separated for more than 5 millions years), but there were at least five species within the currently recognised species. With this additional knowledge, Bobbi and her co-workers have been able to show that each of the species has a slightly different life-history and occupies slightly different micro-habitats. For example, one of the species only occupies streams flowing out of beaver dams!

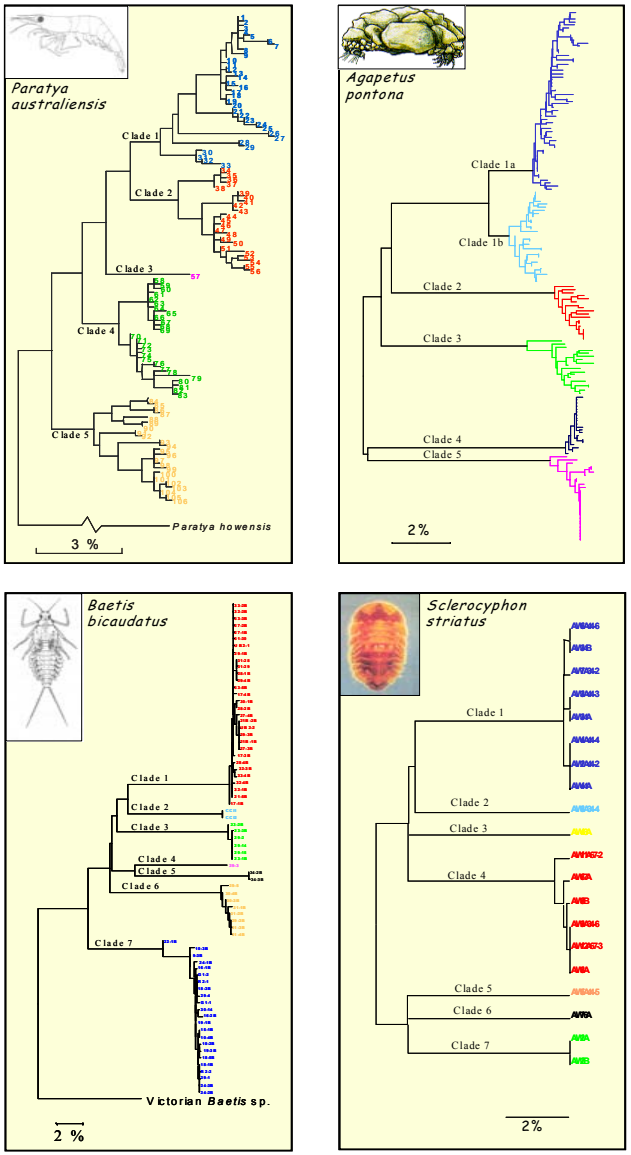

Figure 9. Phylogenetic trees based on mtDNA for four 'species' analysed in Molecular Ecology Laboratory in 2003

It is very likely that many of the invertebrate species recognised on morphological grounds actually consist of a complex of cryptic species, each with a different distribution and each with different behaviour, life-history and conservation significance. Without an understanding of the scale of this diversity it will be impossible to conserve and manage it.

An example of the need to understand and recognise cryptic species is indicated in the results of a recent experiment performed on two populations of freshwater shrimps in the *Paratya australiensis* complex. In 1993, Marty Hancock, translocated a sample of shrimp between two pools in the same drainage system in southeast Queensland (Hancock and Hughes, 1999). The initial purpose of the experiment was to use fixed allelic differences between the two populations to monitor small-scale dispersal patterns within the stream. At the time, the fact that the two populations were very divergent from one another (6% at mtDNA, Hurwood *et al.*, 2003) was not known.

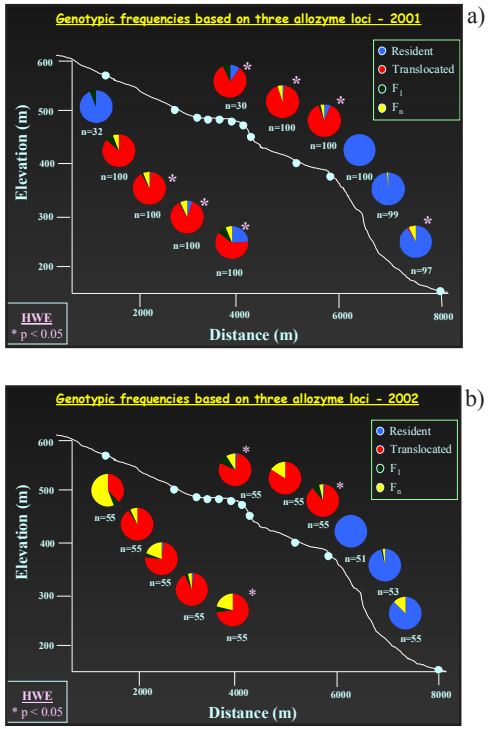

Figure 10. Profile of Branch Creek showing proportions of resident, translocated and hybrid individuals in Branch Creek, a) 7 years and b) 8 years after a translocation.

Subsequent sampling seven years after the event showed that the translocated genotype had almost sent the resident genotype extinct (Figure 10a). We suggested that this was because resident females recognised the introduced males as potential mates, but their hybrid offspring did not survive. We were able to determine this by

examining variation at three nuclear gene loci and mtDNA. After only one more year, the translocated genotypes had reached the top of the creek and had taken over all pools above the original one (Fawcett 2002). Interestingly, this did not happen to such an extent down-stream of the translocation site (Figure 10b). We suggest that translocated genotypes, which come from a higher elevation, may not be as tolerant of high temperatures. During early summer, before there has been much rain and therefore before pools in the stream are connected by flowing water, temperatures can be quite high. Preliminary laboratory experiments also indicate that translocated genotypes are less tolerant of high temperatures.

The implications of these results are obvious. Fisheries biologists worldwide are constantly being asked about translocations and re-stocking of depleted populations. Also water managers regularly transfer water between major drainages for irrigation and other purposes. Without knowledge of the amount of diversity present in natural populations, it is not possible even to predict the effects of these management options.

The results of this study also have implications for our understanding of the process of speciation. Many authors propose that if hybrids between two genetic forms are inviable or less viable than the parental types, natural selection will act to reduce the probability of mating between the forms (e.g. Dobzhansky 1970). The alternative view put forward by Paterson is that one form is likely to go extinct in the area of overlap (Paterson, 1993). Our result certainly supports the latter view. Future monitoring of the areas of co-occurrence will provide a very neat field test of the two hypotheses in a natural situation.

Conclusion

Tonight I have illustrated some of the areas in which the use of genetic techniques has enhanced or changed our understanding of ecological and evolutionary processes. Molecular markers have contributed significantly to our understanding of mating systems in animals and plants, patterns of dispersal and connectivity among populations, the importance of microevolutionary processes such as natural selection and genetic drift, the role of historical processes in shaping species distributions and the recognition of cryptic species.

I hope I have convinced you that genetics has an important role to play in developing our understanding of ecology.

Acknowledgements

I would like to acknowledge all the people in the Molecular Ecology Lab over the last 10 years. All have, in various ways, influenced my thinking and approach. Ann Robinson, Steve Chenoweth, Dave Hurwood, Dugald McGlashan, Mark Ponniah and David Gopurenko were instrumental in our move into the brave new world of molecular genetics.

I would particularly like to acknowledge the following people who collected some of the data presented tonight:
For magpies: Andrew Baker and Alicia Toon.
For freshwater invertebrates: Andrew Baker, Mia Hillyer, Dave Hurwood, Cath Cleary, Sonja Parsonage, James Fawcett, Katrina Goudkamp, Chris Bartlett, Arlene Wheatley, Ben Cook, Jemma Somerville.

I would also like to thank the many collaborators, without whom this work would not have been possible.
For magpies: Peter Mather, Corinna Lange, Ann Robinson, Ian Rowley, Eleanor Russell.
For freshwater invertebrates: Stuart Bunn, Peter Mather, Bobbi Peckarsky (Cornell University), Richard Marchant (National Museum of Victoria).
Particular thanks to Jing Ma, who runs the Molecular Ecology Lab, and is our real molecular biologist!
I would also like to thank all members of 'Geek Group' for the fabulous discussions and arguments about various ideas, techniques and concepts in genetics and ecology.

Our projects have been funded by the Australian Research Council, Australian Geographic, Griffith University Grants Schemes, the Cooperative Research Centre for Freshwater Ecology, the Cooperative Research Centre for Rainforest Ecology and the Cooperative Research Centre for Sustainable Plantation Forestry.

A long-standing collaboration with Pacific Seeds has supported many student projects over the last 10 years.

This presentation would not have been possible without the dedicated assistance of Mia Hillyer. Thanks Mia !
Thank-you also to Petney Dickson who took over organisation at the last minute and worried about the important things while I worried about what I was going to say.

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