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# Conservation genetics of two gecko species in a fragmented landscape

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# Conservation genetics of two gecko species in a fragmented landscape

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# Summary

Rising empirical evidence suggests that some species are at greater risk of extinction in fragmented landscapes than others. Identification of these species and the characteristics that make them sensitive to habitat fragmentation would have important implications for the management of these species. Specialization is one ecological trait that is linked to extinction due to its impact on variables such as population abundance, population and genetic variability, and dispersal ability. Due to their more specific habitat preferences, specialists may have a low tolerance for the modified habitat matrix in fragmented landscapes, which may result in a limited dispersal ability .

Here, we present a comparative study of the population genetic structure of two gecko species that differ in their degree of habitat specialization. *Oedura reticulata* (reticulated velvet gecko), a habitat specialist and *Gehyra variegata* (tree dtella), a habitat generalist, occur sympatrically in the Western Australian wheatbelt, where they have been subjected to severe habitat fragmentation. We used microsatellite DNA markers and long-term demographic data to address questions relating to the role of dispersal and specialization in the two species.

In this fine-scale genetic study of the two gecko species, which compared fragmented habitat with continuous forest sites, we detected a reduction of genetic diversity and an increase in genetic differentiation among subpopulations in the fragmented landscape. The reduction in genetic variation and the increase in genetic differentiation was higher for the habitat specialist species *O. reticulata*. In addition, the habitat generalist species *G. variegata* seems to have higher rates of dispersal, and it managed to migrate over larger distances of matrix in the fragmented landscape than the habitat specialist *O. reticulata*. Despite the fact that the census population sizes stayed stable over a ten year period in the specialist *O. reticulata*, genetic diversity was lost and genetic differentiation among populations increased. Our results suggest that the specialist *O. reticulata* is less able to move through the modified landscape than the generalist *G. variegata*. We discuss how life history characteristics, and particularly specialization, may have a great influence on the dispersal ability of a species.

Life history traits may also have an impact on the effective population size of species. In this study we comprehensively compared demographic and genetic estimates of the effective population size, $N_e$. We identified the most important demographic variables contributing to reduction of $N_e/N$ ratios. The $N_e/N$ ratio was lower in *O. reticulata* than in *G. variegata*, presumably because the age structure, sex ratio and fluctuating population sizes reduced the effective population size to a higher extent in the specialist. We discuss how differences in the demographic parameters might be related to the difference in dispersal ability of the two species.

In conclusion, both species of geckos were affected by landscape fragmentation, but the habitat specialist species *O. reticulata* appears more prone to extinction than the habitat generalist *G. variegata* due to smaller effective population sizes, higher genetic erosion and lower dispersal ability. From a conservation perspective, this study indicates that the specialist species *O. reticulata* is likely to be a good genetic indicator species for monitoring the impact of anthropogenic perturbations in Western Australian woodlands.

# Introduction

Habitat loss and fragmentation threaten species throughout the world and are a major threat to biodiversity (Groombridge 1992, WCMC 1992, Henle 2004a). As a result of habitat fragmentation, many species are limited to habitat remnants, which are reduced in size and isolated by a matrix of less suitable habitat. Species with limited dispersal capabilities are particularly vulnerable to the negative impact habitat fragmentation has on genetic and demographic factors (Henle et al. 1996, Davies et al. 2000, Henle et al. 2004a). Habitat fragmentation can affect genetic structure through its effects on gene flow by restricting dispersal and by reducing effective population size and increasing the effects of genetic drift in small habitat patches (Young & Clarke 2000, Frankham et al. 2002). This can lead to genetic isolation, reduced genetic diversity, and increased inbreeding. Ultimately these genetic changes may result in a decline in fitness and extinction, because of the accumulation of mildly deleterious alleles (Frankham 1995a, Lande 1995, Saccheri et al. 1998, Frankham et al. 2002).

Empirical genetic and demographic studies have shown that habitat fragmentation has negative impact on the persistence of reptiles (Sarre et al. 1995, Boudjemadi et al. 1999, Stow et al. 2001, 2004a, b, Templeton et al. 2001, Driscoll 2004, Lecomte et al. 2004). Many reptile species have a low absolute mobility, which increases their vulnerability to local extinction to a greater extent than for birds, mammals, and some insects (Henle & Streit 1990, Webb & Shine 1997; Coddington & Cree 1998; Hokit et al. 1999, Mac Nally & Brown 2001).

In this study we investigated the effects of habitat fragmentation on the genetic structure and dispersal rates of two species of gecko, *Oedura reticulata* (reticulated velvet gecko) and *Gehyra variegata* (tree dtella). Neither species is currently threatened with extinction. *O. reticulata* is also common throughout the Goldfield area, where its habitat is more intact than in the study area, the Western Australian wheatbelt. *G. variegata* is widespread throughout the southern half of Australia. Since these species are not close to extinction, they are useful models for analyzing the effects of habitat fragmentation on genetic structure, gene flow, migration, survival, and extinction, and may give us an insight into the influence of habitat specialization on the persistence of species in fragmented landscapes.

It is often difficult to evaluate broader generalities from single-species studies. There have been very few population genetic studies (Brouat et al. 2003, 2004) that compare two or more sympatric species, which contrast in their ecology, life-history traits, and rates of dispersal. Yet multi-species studies can represent independent data points and provide important information. More relevant hypotheses can be tested, which provide links between questions in ecology and the evolution of species (Bohonak 1999). In our study, we

hypothesized that the habitat specialist would be more affected by habitat fragmentation because of its inability to move through the modified landscape.

In conservation biology there has often been a lack of integration between the traditionally rival fields of ecology and genetics. Ecologist have investigated the roles of demographic processes such as changes in habitat quality and quantity, population growth rates, breeding structure and migration on species extinction, whereas population geneticists have examined loss of genetic diversity, inbreeding and changes in fitness, with little interaction between the two fields (Young & Clarke 2000). The lack of integration between these two fundamentally related areas is surprising, because only by the interaction of these two fields can we hope to achieve effective conservation management and long-term population and species survival (Nunney & Campbell 1993, Soulé & Mills 1998, Young & Clarke 2000). Therefore, one of our major goals was to examine the genetic and demographic consequences of habitat fragmentation simultaneously. We integrated and combined demographic and genetic data to gain additional knowledge of the extinction processes of the two gecko species.

The goal of the first chapter was to develop microsatellite DNA marker. Their high information content makes them the preferred genetic marker to conduct a fine-scale genetic study. In the second chapter we used the developed microsatellite DNA markers to answer questions relating to the roles of dispersal and specialization. We expected that a higher level of specialization leads to a lower level of dispersal. Thus, we expected higher levels of genetic differentiation and lower rates of dispersal and gene flow in the specialist *O. reticulata* than in the generalist *G. variegata*. To understand the genetic structure of both species, we initially used several genetic methods to determine the degrees of genetic diversity, genetic differentiation, and isolation-by-distance. Then, we more comprehensively investigated comparative rates of dispersal and gene flow. We expected a higher level of male dispersal in both species since both are territorial, and also because more male than female `floaters´ (dispersers) were found within a population of *G. variegata* (Gruber & Henle subm.).

The goal of the third chapter was to compare the fine-scale genetic structure of fragmented versus continuous populations of the two gecko species. Previous empirical and modelling approaches (Sarre et al. 1995, Hoehn unpublished data) demonstrated a marked reduction in the persistence of the specialist species in fragmented landscapes. It is unclear whether the fact that the species is under-represented in the fragments has any impact on the genetic diversity and structure of the populations. We expected that fragmentation is responsible for reduced genetic diversity in both gecko species in remnant populations, in comparison with continuous (unfragmented) populations. Theoretically, population substructure should be higher among fragments than among continuous forest sites. These changes following habitat fragmentation might be related to the difference in the level of specialization and dispersal ability; i.e. the specialized species is presumably more strongly affected.

In fragmented landscapes small population size can lead to loss of neutral genetic variation, fixation of mildly deleterious alleles, and thereby reduce the population fitness (Frankham 1995a, Frankham et al. 2002). The rate of this process depends on the effective size of a population ($N_e$), rather than the actual number of living individuals, N (Wright 1938, Lande & Barrowclough 1987, Waples 1989, 2002, Nunney 1993, Kalinowski & Waples 2002). The effective population size is an important parameter in evolutionary and conservation biology as it is an indicator of long-term risk of extinction from genetic factors. In chapter 4 we have used long-term extensive demographic information and microsatellite DNA markers to directly and indirectly estimate the effective population size $N_e$ and the effective number of breeders $N_b$ for both gecko species over a period of ten years. In addition, we calculated the ratios $N_e/N$ and $N_b/N$ and tried to identify the most important variables contributing to the reduction of the ratios estimated. Finally, we comprehensively compared the demographic and genetic estimates of $N_e$.

In chapter 5 we describe an interactive genetic and demographic study that presents a temporal analysis of microsatellite allele frequencies over a period of ten years (1990-2000), and also a mark-recapture study which estimates the census and effective population sizes over the same time. We expected that the allelic richness and genetic diversity would have declined, while the genetic differentiation among the isolated populations would have increased. Over the same time census and effective population sizes probably declined. Again, these changes might be related to the difference in the level of specialization and dispersal ability; i.e. the specialized species is more strongly affected.

In the discussion chapter we present an overview of the questions that are relevant in conservation genetics, especially at the population level in fragmented landscapes. Furthermore, we discuss how innovative molecular genetic techniques and analytical tools may be used to address conservation related questions and provide examples of the use of molecular techniques in conservation. We do not aim to present an exhaustive review but rather to summarize our own research. Our primary goals are the management of biodiversity and the conservation genetic diversity in isolated populations. Accordingly, in each of these chapters genetic and demographic information is used to suggest guidelines for the management of fragmented populations and specialized species.

*Oedura reticulata*, habitat specialist

*Gehyra variegata*, habitat generalist

# Chapter I

## Microsatellite DNA markers for Australian geckos

Microsatellites consist of tandemly repeated units of short sequence motifs (no more than six bases long) (Goldstein & Schlötterer 1999). They are highly polymorphic due to variation in the number of repeat units, especially if long and uninterrupted. Therefore they are useful hypervariable, codominant, nuclear markers (Amos et al. 1993, Bruford et al. 1996). The length variation among alleles can be revealed via polymerase chain reaction (PCR). Genetic and other studies suggest that slipped-strand mispairing during replication is the predominant mechanism of mutation of microsatellites, but recombination is also discussed as a potential mechanism (Goldstein & Schlötterer 1999). Microsatellites appear to be more or less uniformly distributed across eukaryotic genomes, but are under-represented in coding regions, and possibly also telomeres. So far, they have been found in every organism investigated. Repeats of poly(A)/poly(T) are the most common microsatellites in all genomes, but beyond that different genomes show subtly different frequency distributions. (Goldstein & Schlötterer 1999). Their high information content makes them the preferred genetic marker in many studies. Microsatellites provide data for individual identification, which is important for studies in human and animal forensics, parentage, relatedness, population genetics, dispersal, migration and effective population size. Despite the fact that the development of the loci is laborious, they are the basis of modern population genetics (Luikart & England 1999, Sunnucks 2000).

Geckos are a major part of the Australian fauna. They comprise over 60 species in 17 genera and, are distributed throughout the mainland with the exception of the very south of Victoria (Glasby et al. 1993, Cogger 2000). Despite the important ecological role of geckos, microsatellite markers for conservation genetic studies have been developed for only two gecko species worldwide, the parthenogenetic species *Lepidodactylus lugubris* (mourning gecko) (Wilmhoff et al. 2003) and *Heteronotia binoei* (Bynoe`s gecko), which is endemic to Australia (Straßburg 2004).

The goal of the present study was to develop microsatellite loci for a conservation genetic study (chapters 2 to 5) on two sympatric gecko species known to differ in their dispersal ability and specialization. *O. reticulata* (reticulated velvet gecko), a habitat specialist, is endemic to the southwest of Western Australia. *G. variegata* (tree dtella) is a habitat generalist and widespread throughout the southern half of Australia. Restriction Fragment Length Polymorphism analysis of mtDNA has been used to study the history of *O. reticulata* (Sarre 1995b), but the resolution of this approach is insufficient at an individual and small geographical scale. In contrast, the high polymorphic loci typical of microsatellite DNA should enable parentage analysis and detailed estimation of dispersal.

A microsatellite-enriched genomic library was developed for both species separately following the method of Armour et al. (1994). We isolated tetranucleotide motifs due to their abundance in lizards and because they are less prone to PCR stutter and easier to genotype (Berry et al. 2003). Total DNA was extracted from tail tissue that had been taken from field specimens (ten individuals of each species) and stored in liquid nitrogen. DNA was digested with *Sau3A*, size selected (300-800bp), and gel purified (Biolab Kit). The fragments were ligated to SAUL linkers, which provided primer (SAULA) binding sites for pre-enrichment PCR amplification (95°C, 5min; 30 x (56°C 20s, 72°C 30s, 95°C 30s); 72°C 5min).

Nylon membranes (5mm$^2$) saturated with GATA/CTAT and AAAG/TTTC target repeats were hybridized to the DNA at 42°C in DIG Easy Hyb solution (Roche). The membranes were washed to remove unbound DNA. The enriched DNA was recovered with 100 µl 50mM KOH/0.01% SDS, followed by 100 µl 50mM TrisHCl pH 7.5/0.01%, and then PCR amplified again using SAULA as the primer (95°C, 5min; 35 x (67°C 30s, 72°C 30s, 95°C 30s); 72°C 4min). Finally, the linkers were removed by digestion with *Sau3A* and PEG-purification. The enriched, purified DNA was then ligated into pUC18 cut with *Bam HI* (GibcoBRL), and the plasmid transformed into XLI Blue Competent cells (Stratagene). Recombinant clones were identified by blue-white selection, cultured, transferred onto nylon filters and then probed by hybridisation to DIG-labeled $(GATA)_n$ and $(AAAG)_n$ repeat sequences (Roche). Of the cultured clones, about 15 % (for each species) successfully hybridized to the probe, and were sequenced using Beckman Coulter Quick Sequencing Kit on a Beckman Coulter CEQ-8000 sequencer. Primers for PCR were designed using the program OLIGO version 4.0.

PCR conditions were optimized for each primer pair with the intention that 2-3 primer pairs can be multiplexed in one reaction. The reaction mix (10 µl) consisted of 1 x PCR buffer, 2.5 mM $MgCl_2$, 0.8 mM dNTPs, 0.4 µg/µl bovine serum albumin, 1M Betaine, 0.5 U *Taq polymerase* (Roche), 20-100ng gecko genomic DNA, and primers. Annealing temperature and primer concentrations that gave the most even multiplex amplifications are shown in Table 1. PCR thermocycling was performed in an Eppendorf Mastercycler. Cycling conditions were initiated with one cycle of 95°C for 5 minutes, followed by 30 cycles of (95°C 30s, $T_{annealing}$ 20s, 72°C 30s) and a final extension at 72°C for 5 minutes.

We genotyped individuals using DNA extracted from toe clips or tail tips by a Chelex extraction procedure (Sigma). Ten loci for *O. reticulata* and nine loci for *G. variegata* yielded repeatable and scorable results, their characteristics and primer sequences are shown in Table 1. Individuals were genotyped on a Beckman Coulter CEQ-8000 using Fragment Analysis software version 5.0 (Beckman Coulter). All but one loci were highly polymorphic. Locus OR11B6 was almost monomorphic, with only two alleles, and was therefore excluded from further analysis. Cross-species amplification with primers developed on each species did not give reliable results for either of the two species (no bands or no polymorphism). In summary, nine polymorphic loci for *O. reticulata* and nine polymorphic loci for *G. variegata* are available for population genetic studies in the Western Australian wheatbelt.

*Table 1 Characteristics of primer pairs that amplify microsatellites from* O. reticulata *(OR) and* G. variegata *(GV). $T_A$ is annealing temperature*

| Locus | Primer sequence (5´-3´) | Multiple | Primer conc (mM) | $T_A$ (°C) | Size (bp) |
|---|---|---|---|---|---|
| OR205 | F GTTTTATTCCTGCCTGTATG<br>R GAAACAAATACCACATTGATG | 1 | 0.4 | 48 | 299-339 |
| OR220 | F TTTCACACAGCAGCCAGTCAG<br>R GATGCGTGTTATGTGGTGTTG | 2 | 0.8 | 55 | 281-317 |
| OR266 | F CCACATGGCACGGCAGGGG<br>R AACTCCTCCGCAGCCCAGATG | 3 | 0.8 | 62 | 335-418 |
| OR6F4 | F GTGGTATTTGGACCCTGCTTC<br>R CAATGAACCTGGGAGCAGCC | 3 | 0.3 | 62 | 273-321 |
| OR10H7 | F TGGGGTTGGATACAAAAGCG<br>R GGAAAATGGTTGTGAAGGACAG | 2 | 0.4 | 55 | 290-357 |
| OR11B6 | F GGAAGGACTCTCAGGTTTAGC<br>R AGTGGTATGGTCCCTGTGAC | 1 | 0.4 | 48 | 224-264 |
| OR11G3 | F GGAGTTCCCAGATGAGAGTC<br>R CCACTAAGTCGTACCTAAGAC | 3 | 0.6 | 62 | 462-522 |
| OR12D7 | F TATGTTGTTGAGCACTATATG<br>R AGGATTAGAACTGGAAGAGAG | 1 | 0.8 | 48 | 202-494 |
| OR12D9 | F CGTATCTCAAATGGAAAGTGC<br>R GGACTGGTGTTGGTCAAATG | 4 | 0.3 | 49 | 183-330 |
| OR14A7 | F TGTGTGCCATTTTGACTGTAG<br>R GATGTTTCACACTATTGATTG | 4 | 1.0 | 49 | 211-251 |
| GV1C5 | F CTCATTCTCAGGATTTTTGC<br>R TACTGTGGGTTGGATTTGTG | 6 | 0.6 | 55 | 132-259 |
| GV1C10 | F ACTTTTCTTATTTATGCTGAC<br>R ATCACAGTTCCATTAGATGC | 7 | 0.4 | 55 | 292-423 |
| GV1F1 | F CATTCCAGTTTTTGATGTTTC<br>R TTGGCAGAAGAACACTATTTA | 5 | 0.4 | 56 | 392-460 |
| GV3B5 | F TTTAAATGCAATTGACATATG<br>R GTGGCTAATGCTTGTAAAAC | 7 | 0.2 | 55 | 135-233 |
| GV3C6 | F GCTTTCCTCTTGTTTTGATTA<br>R GCTCACTTCCAATTCTGTGC | 5 | 0.2 | 56 | 189-273 |
| GV3E10 | F TTATCAAATGTCAAGCAGGC<br>R GCAATGAAAATCTGAAAGCAA | 7 | 0.4 | 55 | 219-354 |
| GV4B6 | F TCCAAAGATGAGTGTGAAAGC<br>R AGAAAGAAAGGGGGGCATTC | 7 | 0.8 | 55 | 316-467 |
| GV4C9 | F GTCTCTGTTCTCAGCCTCAG<br>R CTCAAGGAATGACTGGACAC | 6 | 0.8 | 55 | 230-321 |
| GV4G6 | F TTTACACTATGTGAACAGCC<br>R AAAATCCATAAAAGTCCTCAT | 5 | 0.4 | 56 | 281-345 |

# Chapter II

## Dispersal tendencies of two gecko species with contrasting levels of habitat specialization

### *Introduction*

Rising empirical evidence suggests that some species are at greater risk of extinction in fragmented landscapes than others (Margules et al. 1994, Sarre et al. 1995, Davies et al. 2000, 2001, 2004, Mac Nally et al. 2000, Henle et al. 2004a,b). Identification of these species and the characteristics that make them sensitive to habitat fragmentation would have important implications for the management of species as well as for our understanding of ecological and evolutionary theory (Davies et al. 2000, 2001, 2004, Henle et al. 2004a).

It has long been known that life-history traits are related to the dynamics of populations, including colonization ability and extinction risk, which means that traits may act as indicators for extinction risk when we lack better data (MacArthur & Wilson 1967, Fagan et al. 2001, Davies et al. 2004). Specialization, for example, is one ecological trait that is linked to extinction due to its impact on variables such as population abundance, population and genetic variability, and dispersal ability. The resources used by specialist species are likely to be more patchy than those used by generalists leading to a more discontinuous distribution for the specialist species. Following habitat fragmentation the essential resources required by specialists are therefore less likely to be represented in the remaining fragments than the essential resources required by generalists. (Terborgh & Winter 1980, Patterson 1987). In addition, habitat fragmentation leads to biotic and abiotic changes (Saunders et al. 1991), and since specialist species are less likely to maintain stable populations under environmentally fluctuating conditions, they are therefore more prone to extinction (Leigh 1981, Lande & Orzack 1988, Lande 1993, Wissel et al. 1994, Henle 2004a). Finally, due to their more specific habitat preferences specialists may have a low tolerance for the modified habitat matrix, which results in limited dispersal abilities (Sarre et al. 1995). Alternatively, species depending on naturally patchily distributed resources could also have evolved strong dispersal power (Henle et al. 2004a). Theory predicts that such species should have lower extinction risks than species with limited dispersal power (Henle et al. 2004a).

Dispersal, or the amount of dispersion between an individual´s birthplace and that of its offspring (Koenig et al. 1996), is one of the most important life history traits in species persistence (Clobert et al. 2001). Metapopulation theory predicts that subpopulations living in isolated patches periodically go extinct and are recolonized by individuals migrating from other patches (Levins 1970, Hanski 1998). Species with sufficiently high dispersal power to rescue and recolonize distant patches usually have lower extinction risk in fragmented habitats (Frank et al. 1994, Hanski 1998).

Obtaining direct estimates of dispersal from field data has long been plagued by biases (Sarre 1995a, Rousset 2001). Consequently, genetic markers are a powerful tool for obtaining indirect genetic estimates of dispersal and gene flow in natural populations. Recent advances in the analysis of microsatellite data promise to simplify studies of dispersal and colonization. Tests have been developed that use the high information content of hypervariable markers to assign an individual to the population where its genotype has the greatest probability of occurring (Paetkau et al. 1995, Rannala & Mountain 1997, Cornuet et al. 1999, Pritchard et al. 2000).

Here, we present a comparative study of the genetic structure of two gecko species that occur sympatrically, but differ in their degree of habitat specialization. *G. variegata* (tree dtella) is abundant and widespread throughout the southern half of Australia. It is a habitat generalist, and can be found on trees, logs, fallen timber, shrubs, rocks and in highly disturbed habitat (How & Kitchener 1983, Kitchener et al. 1988). *O. reticulata* (reticulated velvet gecko) is endemic to the southwest of Western Australia and is a habitat specialist. It is limited in its range of habitat, being exclusively arboreal and restricted to smooth-barked *Eucalyptus* woodlands (How & Kitchener 1983, Kitchener et al. 1988, Sarre et al. 1995). Both species have been subjected to severe population fragmentation in the Western Australian wheatbelt (How & Kitchener 1983, Kitchener et al. 1988, Sarre 1995 a, b, 1998, Sarre et al. 1995).

Previous empirical and modelling approaches (Sarre et al. 1995, Wiegand et al. 2001, 2002, Hoehn unpublished data) demonstrated that the habitat generalist *G. variegata* showed a markedly higher persistence than the habitat specialist *O. reticulata* (97% remnant occupancy vs 72%) in habitat remnants (Sarre et al. 1995). In addition, over the last decade we found a yearly extinction rate of about 1% for the specialist, whereas the generalist persisted in the remnants (Hoehn unpublished data). Finally, the spatial structure of the populations considerably influenced the extinction risk (Wiegand et al. 2001, 2002). However, it is unclear whether the specialist species is under-represented in the remaining habitat fragments because of the discontinuous distribution of its habitat prior to habitat fragmentation, or because of an inability to disperse and recolonize remnants.

Although the dispersal capability of these geckos have been studied (Sarre 1995 a, b), the demographic data on precise dispersal rates is unsatisfying, as both species are difficult to track with conventional direct methods. A pitfall trap survey in our study area demonstrated that even at a time of peak activity in summer only a single individual of the species *G. variegata* could be caught beyond the boundaries of the remnants (Sarre 1995b). In addition, in a mark-recapture study (chapter 4) none of the individuals were caught in a neighbouring patch. Consequently, we assumed that the dispersal rates are unlikely to be high. Using indirect genetic markers and recently developed innovative analytical and statistical tools to study dispersal, we hoped to shed light on the role of dispersal and specialization in the two gecko species.

We used microsatellite DNA markers to answer questions relating to the roles of dispersal and specialization in the two gecko species. We proposed that a higher level of specialization leads to a lower level of dispersal. Thus, we expected to find higher levels of genetic differentiation and lower rates of dispersal and gene flow in the specialist *O. reticulata* than in the generalist *G. variegata*. To understand the genetic structure of both species we initially used several genetic methods to determine the levels of genetic diversity, genetic differentiation, and isolation-by-distance. Then, using the assignment test, we more comprehensively investigated comparative rates of dispersal and gene flow. We expected a higher level of male dispersal in both species since both are territorial, and also because more male than female 'floaters' (dispersers) were found within a population of *G. variegata* (Gruber & Henle subm.). From this, we draw conclusions about the mating system in these species.

## *Material and methods*

### *Study area and sampling*

The study area was located between Kellerberrin and Trayning in the Western Australian wheatbelt. Large areas of native vegetation have been removed from the region, and replaced by agricultural crops, pastures, and livestock. Since 1900, approximately 93% of the original vegetation has been cleared, and the remnant vegetation is distributed over thousands of patches of varying size (Saunders & Hobbs 1991, Hobbs 1993, Hobbs & Saunders 1993, Saunders et al. 1993). Tissue samples were collected from the two gecko species during the summer months, from November 2000 until March 2001 and in December 2003. Lizards were spotted at night using head-torches and were captured by hand. A total of 174 *O. reticulata* individuals from six habitat fragments and a total of 184 *G. variegata* individuals from seven habitat fragments were captured for genetic analysis. The tip of the tail of each individual was removed and stored in liquid nitrogen. In all fragments 25-30 samples were collected with the exception of fragment population 7 (N=13) for *G. variegata*, where no further individuals could be located. The sample populations were labelled independently for each species, with one habitat fragment (or 'patch' or 'remnant') equivalent to one sample population. The *O. reticulata* populations were labelled F1-6, and the *G. variegata* populations F1-7. In two cases a habitat remnant was used as a sample population for both species, i.e. *G. variegata* population F3 inhabited the same fragment as *O. reticulata* population F5, and similarly, *G. variegata* population F6 occupied the same habitat patch as *O. reticulata* population F4. All other sample populations were from separate fragments, with no overlapping between the two species. Locations of the study areas are displayed in Figure 1 and listed in Table 1. We employed the following sampling strategies (semi-experimental approach) to study assignment and dispersal between pairs of habitat patches on a fine geographic scale. Three pairs of populations separated by distances of 150m, 550m, and 580m for *O. reticulata* and four pairs separated by distances of 150m, 300m, 300m, and 1000m for *G. variegata* were selected. Two neighbouring *O. reticulata*

populations were close to roadside vegetation (150m and 230m distant). Two neighbouring *G. variegata* populations were also close to roadside vegetation (0m and 350m distant), but in this case the roadside vegetation did not create a connection between the neighbouring habitat fragments. All other sample populations, for both species, were separated from roadside vegetation by distances exceeding 400m (Table 1).

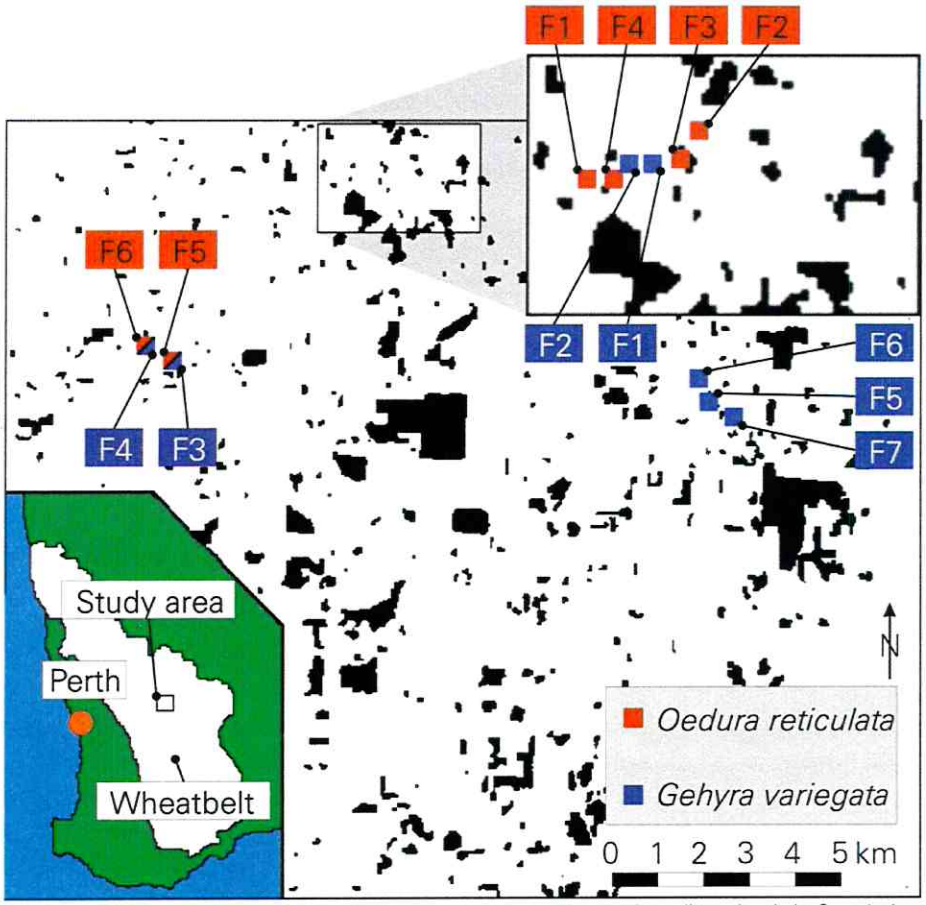



*Figure 1 Map of the study area and location of sites in the Western Australian wheatbelt.* O. reticulata *populations inhabit fragments labelled in red F1-6,* G. variegata *fragments are labelled in blue F1-7*

*Table 1 Habitat fragments sampled and the number of individuals genotyped (samples), fragment size (in ha), distances to neighbouring fragments (distance NF) and the road side vegetation (distance RSV) and, approximate time since isolation from Sarre (1995a)*

| Fragment | Species | Samples | Size | Distance NF | Distance RSV | Isolation |
|---|---|---|---|---|---|---|
| F1 | *Oedura* | 30 | 0.5 | 580m | 230m | 1912 |
| F4 | *Oedura* | 27 | 0.4 | 580m | 150m | 1912 |
| F2 | *Oedura* | 30 | 0.8 | 550m | 1000m | 1936 |
| F3 | *Oedura* | 27 | 0.4 | 550m | 500m | 1936 |
| F5 | *Oedura* | 30 | 5.4 | 150m | 450m | 1910-20 |
| F6 | *Oedura* | 30 | 2 | 150m | 450m | 1910-20 |
| F1 | *Gehyra* | 30 | 0.3 | 300m | 350m | 1912 |
| F2 | *Gehyra* | 30 | 1.4 | 300m | 0m | 1912 |
| F3 | *Gehyra* | 30 | 5.4 | 150m | 450m | 1910-20 |
| F4 | *Gehyra* | 30 | 2 | 150m | 450m | 1910-20 |
| F5 | *Gehyra* | 30 | 0.3 | 1000m, 300m | 400m | 1920 |
| F6 | *Gehyra* | 25 | 0.5 | 1000m | 1000m | 1920 |
| F7 | *Gehyra* | 13 | 0.6 | 300m | 250m | 1920 |

## *Laboratory methods*

DNA was extracted from the tip of the tail of each individual using the Chelex extraction method. We genotyped individuals of *O. reticulata* using nine tetranucleotide microsatellite loci developed from an enriched library for this species (OR205, OR220, OR266, OR6F4, OR10H7, OR11G3, OR12D7, OR12D9, OR14A7) (Hoehn & Sarre subm.). For *G. variegata*, we genotyped individuals using nine tetranucleotide microsatellite markers cloned from an enriched library for this species (GV1C5, GV1C10, GV1F1, GV3B5, GV3C6, GV3E10, GV4B6, GV4G6, GV4C9) (Hoehn & Sarre in prep.). Polymerase chain reaction (PCR) amplification and genotyping on the Beckman Coulter Sequencer CEQ-8000 were performed according to conditions described in Hoehn & Sarre (subm.) and Hoehn & Sarre (in prep.).

*Statistical analysis*

Descriptive statistics – the allelic richness ($A_n$) (number of alleles corrected by sample size) and the observed ($H_o$) and expected ($H_e$) heterozygosity per locus – were calculated using *FSTAT 2.9.3*. (Goudet 1995, 2001). Global tests for deviation from Hardy-Weinberg were performed. In addition, we tested heterozygote deficits per locus and habitat fragment employing a sequential Bonferroni correction (*FSTAT 2.9.3*.). No significant deviations from linkage equilibrium between loci and habitat fragments were detected.

The population genetic structure was investigated by estimating $F_{ST}$ using the method of Weir & Cockerham (1984) in *FSTAT 2.9.3*. The significance of mean F-statistics was assessed by constructing the 95% confidence intervals (CI) by jackknifing across loci (Weir 1990) using *GENETIX 4.01* (Belkhir et al. 2001). In comparing between species, values with non-overlapping 95% CIs were considered to be significantly different. In addition, isolation by distance was tested through linear regression of $F_{ST}/(1- F_{ST})$ against the geographical distance between pairs of habitat fragments (Rousset 1997). The Mantel test option in *FSTAT 2.9.3*. was used to assess the significance of correlation between matrices of genetic differentiation and geographical distance between sampled populations. Geographical distance between fragments within the study area was determined using the map generated by M. G. Brooker, CSIRO, Division of Wildlife and Ecology, Perth and Department of Agriculture, Western Australia.

As a measure of disperal rates (distinctiveness) between populations, assignments were conducted using the programs *STRUCTURE* (Pritchard et al. 2000) and *GENECLASS* (Cornuet et al. 1999). Using the Bayesian clustering method implemented in *STRUCTURE* (Pritchard et al. 2000), we assumed that our sample consisted of six populations (three population pairs) for *O. reticulata* and seven populations (four population pairs) for *G. variegata,* and that prior migration between populations was rare. The length of the initial burn-in period was set at 10,000 iterations and subsequently data was collected for 100,000 Markov chain Monte Carlo repetitions. No prior population information about the potential source populations was incorporated into the analysis, while individuals were assigned to the population in which their posterior probability was highest.

Using *GENECLASS* (Cornuet et al. 1999), two different types of likelihood based assignment tests were used; the frequency method (Paetkau et al. 1995) and a Bayesian method (Rannala & Mountain 1997, Cornuet et al. 1999). For each individual a probability of belonging to its source population was calculated to assess the accuracy with which individuals could be classified to their known sample population. The frequency method simply assigns individuals to the population in which their probability of belonging is highest. Using the Bayesian method, individuals were assigned to the population in which their probability of belonging was highest and in which it exceeded the arbitrary threshold values $p \geq 0.05$. Two different simulations were performed, because different levels of stringency are required to answer different questions (Eldridge et al. 2001, Berry et al. 2004). To estimate an overall

dispersal probability it is more important to assign a high number of individuals with a lower accuracy to their most likely population of origin. In contrast, to identify an individual as a disperser a higher accuracy is required. Unfortunately, only a relatively low number of individuals can be assigned due to their undefined origin.

To test for sex-biased dispersal, *FSTAT 2.9.3.* (Goudet 1995, 2001, Goudet et al. 2002) was used to calculate five different tests: $F_{IS}$, $F_{ST}$, relatedness, mean Assignment Index (AIc) and variance of Assignment Indices (vAIc). Positive $F_{IS}$ values were predicted for the dispersing sex because $F_{IS}$ is a measure of how well the genotype frequencies within the population match Hardy-Weinberg expectations (Hartl & Clark 1997). The dispersing sex at one site will be a mixture of immigrants and residents and thus consist of two separate populations at any one site. This hidden subdivision will lead to a heterozygote deficit and a positive $F_{IS}$ (Wahlund effect). A lower relatedness is also predicted for the dispersing sex (Knight et al. 1999, Surridge et al. 1999, Lampert et al. 2003). Lower $F_{ST}$ values for the dispersing sex follow because $F_{ST}$ expresses the proportion of the total genetic variance attributable to among-population differentiation (Hartl & Clark 1997). The dispersing sex should be less differentiated in its allelic frequencies. Finally, we chose Assignment Indices to test for differences in the mean values and in variance of Assignment Indices between the sexes. The Assignment Index statistic calculates the probability for each genotype to be represented in the sampled population (Paetkau et al. 1995; Favre et al. 1997; Goudet et al. 2002). AIc values are centred around zero. Resident individuals probably have genotypes that are more likely than average to occur in the sample and should therefore have positive AIc values, while immigrant genotypes are less likely to occur in the sample and therefore have negative AIc values (Goudet et al. 2002). The mean Assignment Index should be higher in the philopatric sex, while the variance of AIc (vAIc) should be higher in the dispersing sex, because sampled members of this sex should include dispersed and resident genotypes with positive and negative vAIc values. A randomisation process in *FSTAT 2.9.3.* (Goudet et al. 1995, 2001) with 1000 randomisations was used to test for significant differences in vAIc between the sexes.

## *Results*

### *Genetic diversity*

Table 2a, b show the summary statistics for genetic diversity observed in each remnant for the two gecko species. All microsatellite loci were highly polymorphic and the genetic variability did not differ considerably among loci. Allelic richness ($A_n$) per site ranged from 4.00 to 22.71 for *O. reticulata* and from 4.74 to 16.00 for *G. variegata*. The expected heterozygosity ($H_e$) per locus across all sites ranged from 0.71 to 0.87 for *O. reticulata* (mean $H_e$: 0.79) and from 0.80 to 0.92 for *G. variegata* (mean $H_e$: 0.88). Allelic richness and gene diversity were higher for the habitat generalist *G. variegata* than for the specialist *O. reticulata* (Mann-Whitney U-tests, $p < 0.01$).

*Table 2a Allelic richness ($A_n$), mean observed ($H_o$) and expected ($H_e$) heterozygosity for each locus at each site for the species* O. reticulata. **Deviation from Hardy-Weinberg expectations ($p < 0.05$). Site codes follow Tab. 1*

| Locus | Site | F1 | F2 | F3 | F4 | F5 | F6 | Mean |
|---|---|---|---|---|---|---|---|---|
| OR205 | $A_n$ | 8.25 | 4.95 | 4.00 | 7.81 | 8.71 | 7.47 | 6.87 |
| | $H_e$ | 0.77 | 0.59 | 0.76 | 0.83 | 0.83 | 0.77 | 0.76 |
| | $H_o$ | 0.80 | 0.63 | 0.67 | 0.85 | 0.63 | 0.70 | 0.71 |
| OR220 | $A_n$ | 4.77 | 5.72 | 4.85 | 5.85 | 8.93 | 6.88 | 6.17 |
| | $H_e$ | 0.74 | 0.69 | 0.60 | 0.59 | 0.87 | 0.76 | 0.71 |
| | $H_o$ | 0.70 | 0.63 | 0.63 | 0.56 | 0.63* | 0.63 | 0.63 |
| OR266 | $A_n$ | 7.48 | 5.66 | 4.96 | 6.85 | 8.47 | 9.70 | 7.19 |
| | $H_e$ | 0.82 | 0.70 | 0.70 | 0.82 | 0.81 | 0.88 | 0.79 |
| | $H_o$ | 0.93 | 0.70 | 0.67 | 0.74 | 0.53* | 0.80 | 0.73 |
| OR6F4 | $A_n$ | 5.71 | 5.95 | 5.00 | 6.96 | 9.65 | 7.75 | 6.84 |
| | $H_e$ | 0.58 | 0.77 | 0.56 | 0.73 | 0.84 | 0.82 | 0.72 |
| | $H_o$ | 0.41 | 0.57 | 0.57 | 0.41* | 0.90 | 0.87 | 0.62 |
| OR10H7 | $A_n$ | 8.42 | 10.43 | 7.00 | 11.37 | 13.89 | 11.24 | 10.39 |
| | $H_e$ | 0.81 | 0.86 | 0.83 | 0.83 | 0.87 | 0.89 | 0.85 |
| | $H_o$ | 0.83 | 0.83 | 0.89 | 0.70 | 0.93 | 0.90 | 0.85 |
| OR11G3 | $A_n$ | 5.99 | 13.41 | 6.70 | 6.83 | 10.23 | 10.43 | 8.93 |
| | $H_e$ | 0.80 | 0.92 | 0.72 | 0.72 | 0.85 | 0.88 | 0.82 |
| | $H_o$ | 0.70 | 0.93 | 0.85 | 0.63 | 0.77 | 0.73 | 0.77 |
| OR12D7 | $A_n$ | 8.47 | 9.41 | 5.54 | 9.81 | 22.71 | 12.17 | 11.35 |
| | $H_e$ | 0.83 | 0.79 | 0.60 | 0.86 | 0.96 | 0.86 | 0.82 |
| | $H_o$ | 0.90 | 0.83 | 0.67 | 0.89 | 0.97 | 0.87 | 0.85 |
| OR12D9 | $A_n$ | 11.24 | 11.35 | 7.94 | 10.63 | 21.50 | 13.49 | 12.69 |
| | $H_e$ | 0.89 | 0.87 | 0.77 | 0.82 | 0.96 | 0.90 | 0.87 |
| | $H_o$ | 0.90 | 0.83 | 0.85 | 0.81 | 0.93 | 0.83 | 0.86 |
| OR14A7 | $A_n$ | 5.76 | 6.00 | 5.96 | 5.85 | 8.76 | 8.85 | 6.86 |
| | $H_e$ | 0.69 | 0.75 | 0.71 | 0.76 | 0.87 | 0.86 | 0.77 |
| | $H_o$ | 0.63 | 0.70 | 0.81 | 0.85 | 0.93 | 0.93 | 0.81 |
| Mean | $A_n$ | 7.34 | 8.10 | 5.77 | 7.99 | 12.54 | 9.78 | 8.59 |
| | $H_e$ | 0.77 | 0.77 | 0.69 | 0.77 | 0.87 | 0.85 | 0.79 |
| | $H_o$ | 0.76 | 0.74 | 0.73 | 0.72 | 0.80 | 0.81 | 0.76 |

*Table 2b Allelic richness ($A_n$), mean observed ($H_o$) and expected ($H_e$) heterozygosity for each locus at each site for the species* G. variegata. **Deviation from Hardy-Weinberg expectations ($p < 0.05$). Site codes follow Tab. 1*

| Locus | Site | F1 | F2 | F3 | F4 | F5 | F6 | F7 | Mean |
|---|---|---|---|---|---|---|---|---|---|
| GV1C10 | $A_n$ | 11.31 | 8.61 | 8.81 | 10.82 | 9.24 | 7.55 | 11.00 | 9.62 |
| | $H_e$ | 0.92 | 0.81 | 0.88 | 0.89 | 0.85 | 0.87 | 0.92 | 0.88 |
| | $H_o$ | 0.80 | 0.57 | 0.87 | 0.80 | 0.73 | 0.81 | 0.77 | 0.76 |
| GV1C5 | $A_n$ | 11.82 | 8.50 | 14.21 | 13.71 | 10.64 | 12.95 | 16.00 | 12.55 |
| | $H_e$ | 0.88 | 0.86 | 0.94 | 0.93 | 0.91 | 0.92 | 0.96 | 0.92 |
| | $H_o$ | 0.93 | 0.57* | 0.87 | 0.83 | 0.90 | 0.95 | 0.77 | 0.83 |
| GV1F1 | $A_n$ | 9.54 | 9.92 | 9.29 | 9.33 | 10.08 | 9.94 | 8.00 | 9.44 |
| | $H_e$ | 0.86 | 0.88 | 0.88 | 0.87 | 0.85 | 0.90 | 0.80 | 0.86 |
| | $H_o$ | 0.80 | 0.90 | 0.90 | 0.83 | 0.87 | 1.00 | 0.85 | 0.88 |
| GV3B5 | $A_n$ | 9.87 | 6.82 | 8.76 | 9.08 | 9.78 | 9.45 | 11.00 | 9.25 |
| | $H_e$ | 0.87 | 0.83 | 0.87 | 0.85 | 0.90 | 0.89 | 0.90 | 0.87 |
| | $H_o$ | 0.67 | 0.77 | 0.93 | 0.87 | 0.93 | 0.81 | 0.92 | 0.84 |
| GV3C6 | $A_n$ | 8.10 | 8.68 | 10.29 | 9.84 | 7.61 | 8.40 | 10.00 | 8.99 |
| | $H_e$ | 0.87 | 0.86 | 0.91 | 0.89 | 0.85 | 0.83 | 0.88 | 0.87 |
| | $H_o$ | 0.93 | 0.93 | 0.87 | 0.93 | 0.90 | 0.81 | 0.92 | 0.90 |
| GV3D10 | $A_n$ | 12.47 | 8.97 | 13.72 | 12.10 | 9.82 | 7.44 | 10.00 | 10.65 |
| | $H_e$ | 0.92 | 0.87 | 0.94 | 0.92 | 0.90 | 0.85 | 0.93 | 0.90 |
| | $H_o$ | 0.93 | 0.83 | 0.97 | 0.93 | 0.90 | 0.95 | 1.00 | 0.93 |
| GV4B6 | $A_n$ | 12.67 | 9.60 | 15.15 | 15.38 | 11.38 | 11.83 | 11.00 | 12.43 |
| | $H_e$ | 0.92 | 0.86 | 0.95 | 0.94 | 0.90 | 0.88 | 0.91 | 0.91 |
| | $H_o$ | 0.97 | 0.90 | 0.97 | 0.93 | 0.83 | 0.81 | 1.00 | 0.91 |
| GV4C9 | $A_n$ | 8.45 | 6.35 | 4.74 | 7.28 | 6.36 | 7.12 | 6.00 | 6.61 |
| | $H_e$ | 0.83 | 0.78 | 0.76 | 0.86 | 0.77 | 0.79 | 0.79 | 0.80 |
| | $H_o$ | 0.89 | 0.78 | 0.50 | 0.52* | 0.70 | 0.81 | 0.62 | 0.69 |
| GV4G6 | $A_n$ | 10.12 | 8.39 | 10.07 | 12.01 | 9.39 | 10.05 | 10.00 | 10.00 |
| | $H_e$ | 0.87 | 0.88 | 0.89 | 0.92 | 0.87 | 0.87 | 0.89 | 0.88 |
| | $H_o$ | 0.83 | 0.90 | 0.83 | 0.93 | 0.93 | 0.81 | 0.92 | 0.88 |
| Mean | $A_n$ | 10.48 | 8.43 | 10.56 | 11.06 | 9.37 | 9.41 | 10.33 | 9.95 |
| | $H_e$ | 0.88 | 0.85 | 0.89 | 0.90 | 0.87 | 0.87 | 0.89 | 0.88 |
| | $H_o$ | 0.86 | 0.79 | 0.86 | 0.84 | 0.86 | 0.86 | 0.86 | 0.85 |

Three of the 54 tests for Hardy-Weinberg equilibrium in *O. reticulata* and two of the 63 tests in *G. variegata* showed significant deviation from expected allele frequencies after Bonferroni correction; all were due to a deficiency of heterozygotes. The heterozygote deficiency may be due to null alleles; although there does not appear to be any consistent pattern of Hardy-Weinberg deviation among loci, or populations (Table 2a, b). No linkage disequilibrium between loci occurred at any locality and therefore independence among loci was assumed in the subsequent analysis.

### *Population differentiation*

Microsatellites revealed significant genotypic differentiation (Table 3) among populations both in *G. variegata* ($p < 0.01$) and in *O. reticulata* ($p < 0.01$). The level of differentiation was significantly higher in *O. reticulata* ($F_{ST} = 0.101$; 95% CI 0.089-0.112) than in *G. variegata* ($F_{ST} = 0.045$; 95% CI 0.035-0.055), the 95% confidence intervals of the $F_{ST}$ estimates being mutually exclusive. Tests for divergence were significant for all pairs of populations with pairwise $F_{ST}$ estimates ranging from 0.041 to 0.163 for *O. reticulata* and from 0.007 to 0.081 for *G. variegata*.

### *Isolation-by-distance*

Figure 2 shows the regression of $F_{ST}/(1-F_{ST})$ against the ln of the geographical distance. A Mantel test showed that the positive association between the genetic differentiation and geographical distance separating samples was significant ($p < 0.01$) for the generalist *G. variegata*. No pattern of isolation by distance was apparent for *O. reticulata* when total geographical distance was taken into account, suggesting that isolation had no influence on the genetic structure in that species.

### *Assignment*

Both the Bayesian clustering method (Pritchard et al. 2000) and the likelihood based frequency method (Paetkau et al. 1995) performed well, meaning that individuals were assigned either to the one or the neighbouring population due their undefined origin. In the absence of a significant threshold, individuals were simply assigned to the population for which their probability of belonging was highest (Table 4). Both methods produced consistent results that were used to estimate dispersal probabilities. The performance of the Bayesian method (Rannala & Mountain 1997, Cornuet et al. 1999) at a $p \leq 0.05$ significance threshold was weak. Despite the higher accuracy fewer individuals (*O. reticulata* $N = 8$; *G. variegata* $N = 15$) could be assigned due to their undefined origins.

*Table 3a Pairwise $F_{ST}$ estimates for* O. reticulata *for all loci on the lower matrix. The upper matrix indicates p-values for pairwise comparisons: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Site codes follow Table 1*

| Site | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| F1 | ------- | ** | ** | ** | ** | ** |
| F2 | 0.089 | ------- | ** | ** | ** | ** |
| F3 | 0.163 | 0.144 | ------- | ** | ** | ** |
| F4 | 0.089 | 0.091 | 0.138 | ------- | ** | ** |
| F5 | 0.084 | 0.068 | 0.114 | 0.074 | ------- | ** |
| F6 | 0.109 | 0.098 | 0.135 | 0.088 | 0.041 | ------- |

*Table 3b Pairwise $F_{ST}$ estimates for* G. variegata *for all loci on the lower matrix. The upper matrix indicates p-values for pairwise comparisons: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Site codes follow Table 1*

| Site | F1 | F2 | F3 | F4 | F5 | F6 | F7 |
|---|---|---|---|---|---|---|---|
| F1 | -------- | ** | ** | ** | ** | ** | ** |
| F2 | 0.036 | -------- | ** | ** | ** | ** | ** |
| F3 | 0.034 | 0.045 | -------- | ** | ** | ** | ** |
| F4 | 0.042 | 0.055 | 0.011 | -------- | ** | ** | ** |
| F5 | 0.057 | 0.081 | 0.055 | 0.045 | -------- | ** | * |
| F6 | 0.036 | 0.058 | 0.030 | 0.044 | 0.049 | -------- | ** |
| F7 | 0.045 | 0.063 | 0.040 | 0.044 | 0.007 | 0.048 | -------- |

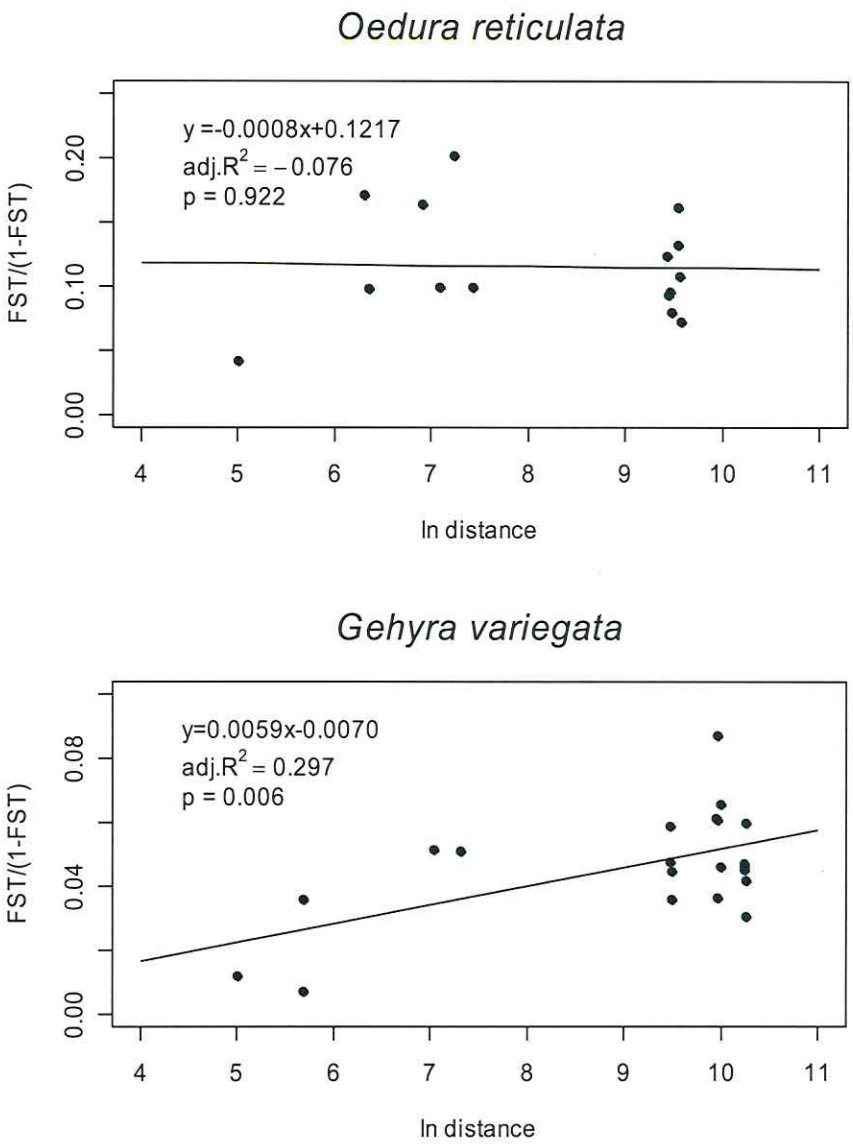



*Figure 2 Relationship between the logarithms of geographical distances and genetic differentiation estimated as* $F_{ST}/(1-F_{ST})$ *for* O. reticulata *(above) and* G. variegata *(below)*

In *O. reticulata*, the Bayesian clustering method (Pritchard et al. 2000) and the likelihood based frequency method (Paetkau et al. 1995) demonstrated that the two populations, which were in closest proximity (F5 and F6: 150m) revealed the highest percentage of misassigned individuals (27-50 % depending on the method). Population F1 and F4 and population F2 and F3 are isolated by 580 and 550m respectively, and the assignment tests revealed that there were dispersal probabilities of 6-19% (depending on the method) between the first population pair and of 1-4 % (depending on the method) between the second pair. The slightly higher proportion of dispersers in the second pair might have been due to the close proximity (150m) to the roadside vegetation (Figure 3a and Table 4a).

In *G. variegata*, both assignment methods demonstrated that the two populations F3 and F4, which were in closest distance (150m) has a high percentage of misassigned individuals (40-50 % depending on the method). Populations F1 and F2 are isolated by 300m and had a dispersal probability of 17-27 %. Finally, population F5 and F6 were 1000m apart from each other and still showed relatively high misassignment rates (8-40%). Populations F5 and F7, which are isolated by 300m, had astonishingly high dispersal probabilities (27-83%). The results are illustrated in more detail in Figure 3b and Table 4b. In summary, we found a higher percentage of misassignments in *G. variegata* (mean of 35-38%, depending on the method) than in *O. reticulata* (mean of 20-29%, depending on the method), despite the fact that the populations of *O. reticulata* were in slightly closer proximity to each other.

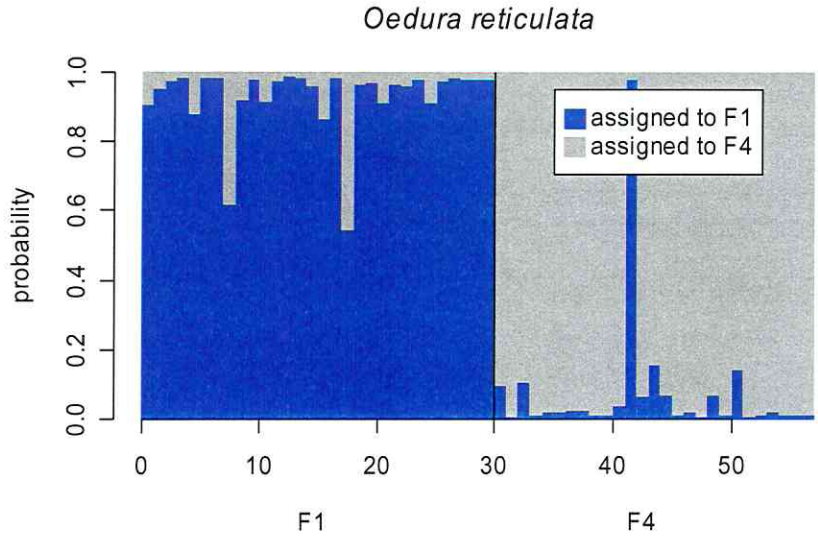
In *GENECLASS* a significance threshold ($p \leq 0.05$) is implemented within the Bayesian method to identify an individual as a disperser at a higher accuracy. Using this method for *O. reticulata*, we detected three disperser in population pair 1/4, one in population pair F2/F3, and five in population pair F5/F6 (at a 95% level of confidence). In *G. variegata* there were two dispersers in population pair F1/F2, three in population pair F3/F4, one in population pair F5/F6 and two in population pair F5/F7 (at a 95% level of confidence). On average we identified 3.0 dispersers per population pair in *O. reticulata* and 2.0 dispersers per population pair in *G. variegata* (Table 4a, b).

*Table 4a Average probability for the O. reticulata individuals of a population having originated in the source (assigned) population or a neighbouring population (misassigned) and the number of identified dispersers (#) between the source and the neighbouring population. STRUCTURE refers to the Bayesian clustering method (Pritchard et al. 2000) and GENECLASS to the likelihood based frequency method (Paetkau et al. 1995). Average probabilities are derived without a threshold level, the number of identified dispersers (#) is derived at a threshold criteria of $p \leq 0.05$. Neighbouring population pairs are F1/F4; F2/F3; F5/F6; Dist. is the distance between neighbouring populations in (m). Site codes followTabel 1*

| **Assigned** | | | | **#** | **Dist** |
|---|---|---|---|---|---|
| | | F1 | F4 | | |
| Structure | F1 | 0.94 | 0.06 | | |
| | F4 | 0.07 | 0.93 | | |
| Geneclass | F1 | 0.87 | 0.13 | | |
| | F4 | 0.19 | 0.81 | 3 | 580 |
| | | F2 | F3 | | |
| Structure | F2 | 0.99 | 0.01 | | |
| | F3 | 0.04 | 0.96 | | |
| Geneclass | F2 | 0.97 | 0.03 | | |
| | F3 | 0.00 | 1.00 | 1 | 550 |
| | | F5 | F6 | | |
| Structure | F5 | 0.50 | 0.50 | | |
| | F6 | 0.50 | 0.50 | | |
| Geneclass | F5 | 0.73 | 0.27 | | |
| | F6 | 0.27 | 0.73 | 5 | 150 |
| Misassigned (mean) | | | | | |
| Geneclass | | | 0.20 | 3 | |
| Structure | | | 0.15 | | |

*Table 4b Average probability for the* G. variegata *individuals of a population having originated in the source (assigned) population or a neighbouring population (misassigned) and the number of identified dispersers (#) between the source and the neighbouring population.* STRUCTURE *refers to the Bayesian clustering method (Pritchard et al. 2000) and* GENECLASS *to the likelihood based frequency method (Paetkau et al. 1995). Average probabilities are derived without a threshold level, the number of identified dispersers (#) is derived at a threshold criteria of $p \leq 0.05$. Neighbouring population pairs are F1/F2; F3/F4; F5/F6; F5/F7, Dist. is the distance between neighbouring populations in (m). Site codes follow Table 1*

| **Assigned** | | | | **#** | **Dist** |
|---|---|---|---|---|---|
| | | F1 | F2 | | |
| Structure | F1 | 0.75 | 0.25 | | |
| | F2 | 0.27 | 0.73 | | |
| Geneclass | F1 | 0.80 | 0.20 | | |
| | F2 | 0.17 | 0.83 | 2 | 300 |
| | | F3 | F4 | | |
| Structure | F3 | 0.50 | 0.50 | | |
| | F4 | 0.50 | 0.50 | | |
| Geneclass | F3 | 0.6 | 0.4 | | |
| | F4 | 0.4 | 0.6 | 3 | 150 |
| | | F5 | F6 | | |
| Structure | F5 | 0.82 | 0.18 | | |
| | F6 | 0.08 | 0.92 | | |
| Geneclass | F5 | 0.60 | 0.40 | | |
| | F6 | 0.24 | 0.76 | 1 | 1000 |
| | | F5 | F7 | | |
| Structure | F5 | 0.56 | 0.44 | | |
| | F7 | 0.83 | 0.17 | | |
| Geneclass | F5 | 0.73 | 0.27 | | |
| | F7 | 0.69 | 0.31 | 2 | 300 |
| Misassigned (mean) | | | | | |
| Geneclass | | | 0.38 | 2 | |
| Structure | | | 0.35 | | |


Oedura reticulata
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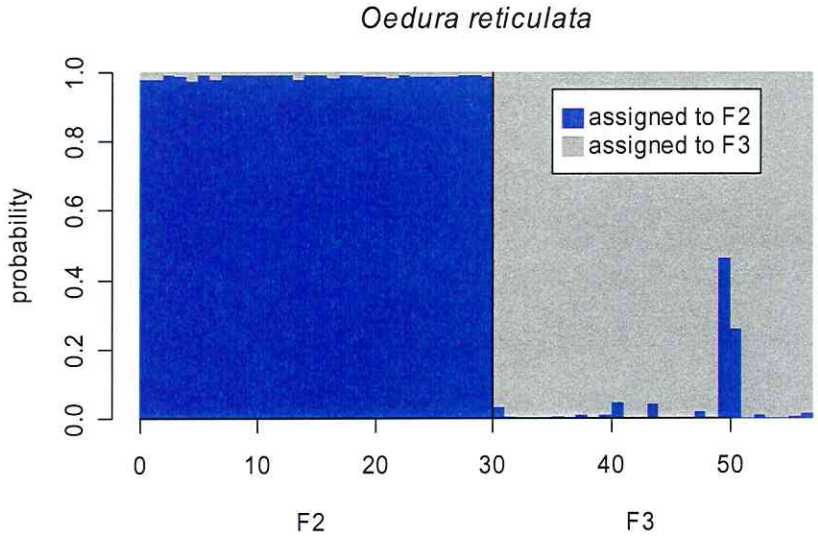
0.2
0.4
0.6
0.8
1.0
assigned to F1
assigned to F4
0
10
20
30
40
50
F1
F4


Oedura reticulata
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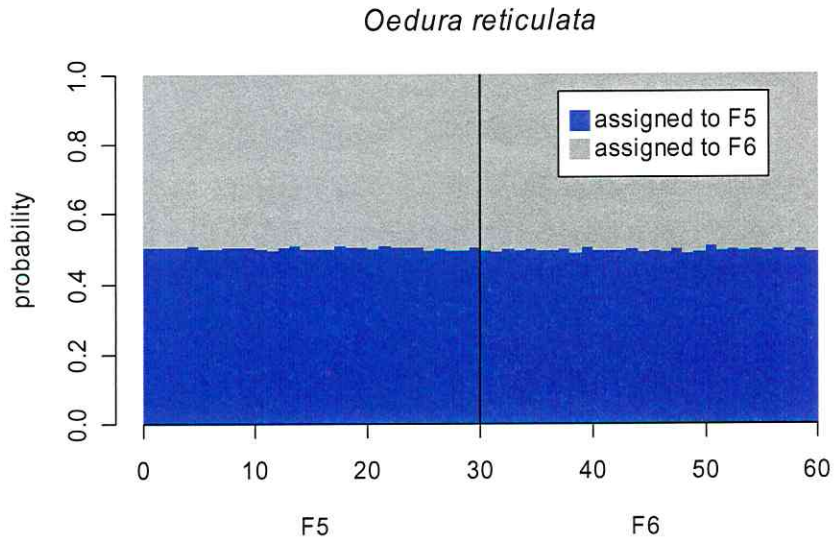
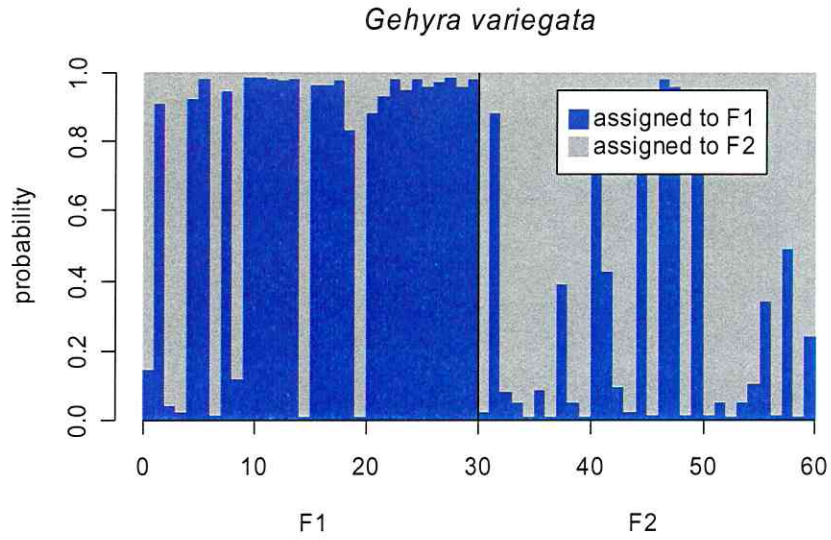
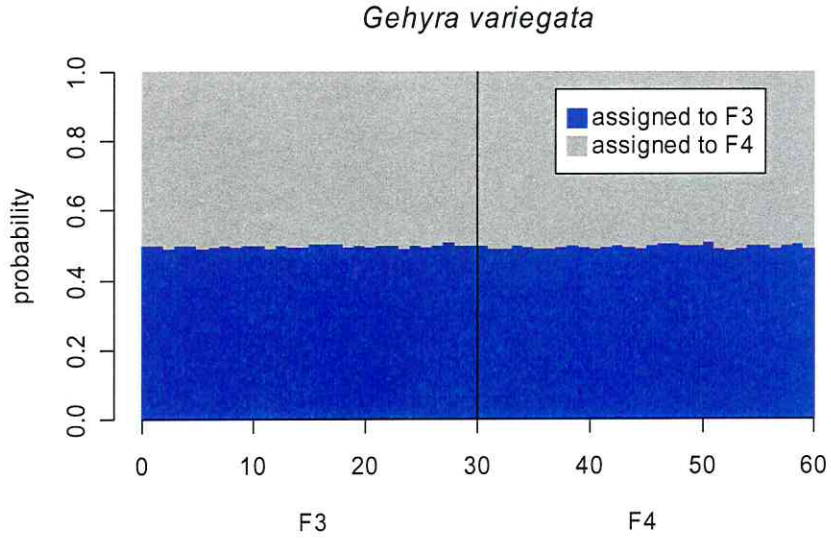



*Figure 3a Average probability for the individuals of a* O. reticulata *population having originated in the source population or a neighbouring population derived from data of the Bayesian clustering method (Pritchard et al. 2000). Neighbouring population pairs are F1/F4; F2/F3; F5/F6*


Gehyra variegata
probability
0.0
0.2
0.4
0.6
0.8
1.0
assigned to F1
assigned to F2
0
10
20
30
40
50
60
F1
F2


Gehyra variegata
probability
0.0
0.2
0.4
0.6
0.8
1.0
assigned to F3
assigned to F4
0
10
20
30
40
50
60
F3
F4

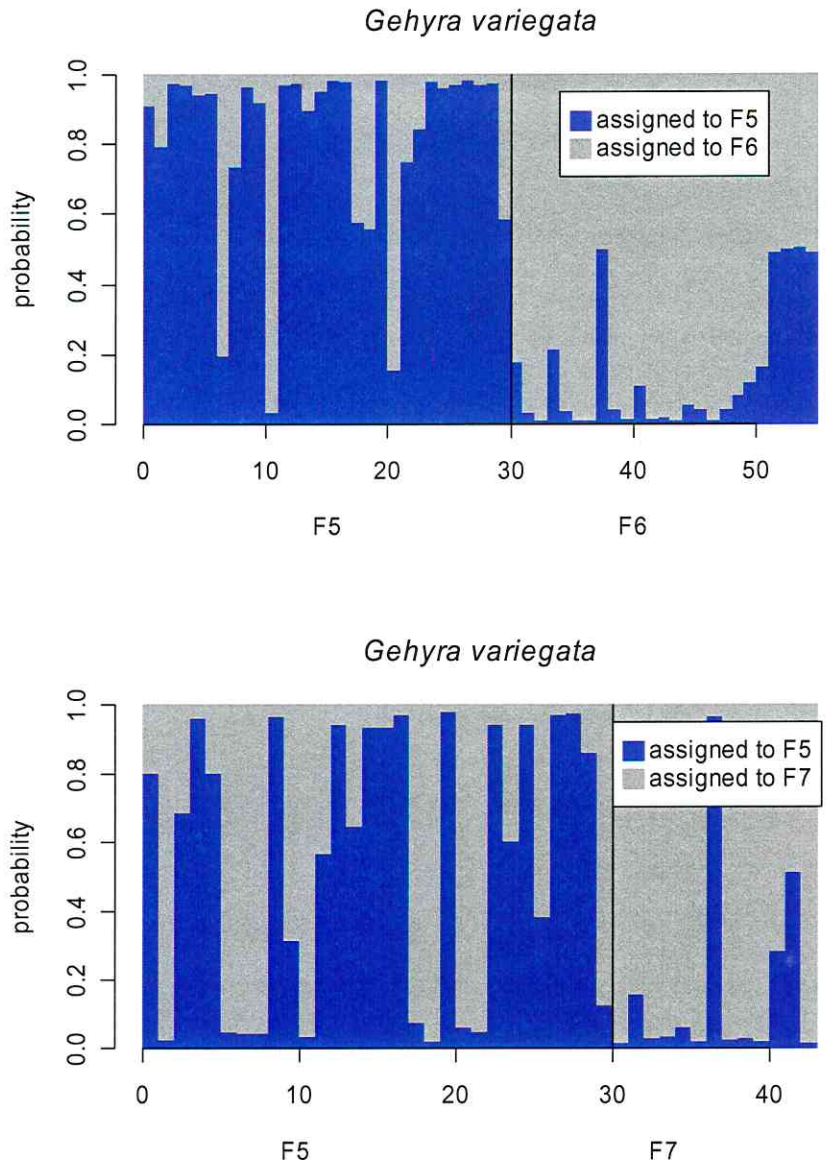



*Figure 3b Average probability for the individuals of a* G. variegata *population having originated in the source population or a neighbouring population derived from data of the Bayesian clustering method (Pritchard et al. 2000). Neighbouring population pairs are F1/F2; F3/F4; F5/F6; F5/F7*

## *Sex-biased dispersal*

In *G. variegata*, all calculations with the exception of $F_{IS}$ indicated a male biased dispersal. These include higher $F_{ST}$ in females, higher relatedness values among females, lower mean assignment in males and higher variance of assignment indices in males. However, only two of these tests were significant: the mean AIc ($p < 0.05$) and the vAIc ($p < 0.05$) revealed a significant difference between male and female geckos, identifying males as the predominantly dispersing sex in *G. variegata* (Table 5 and Figure 4).

In the case of *O. reticulata*, once again all calculations besides AIc (higher $F_{IS}$ in males, higher $F_{ST}$ in females, higher relatedness values among females and higher variance of assignment indices in males) suggested a male biased dispersal. Only one test turned out to be significant: the variance of Assignment Index test revealed a significant difference between male and female geckos ($p < 0.05$), identifying males as the predominantly dispersing sex in *O. reticulata* (Table 5 and Figure 4).

*Table 5 Test results for sex-biased dispersal in* O. reticulata *and* G. variegata. *N is the number of individuals, Rel. the relatedness, AIc the mean assignment index, vAIc the variance of the assignment index*

| ***Gehyra*** | **n** | **$F_{IS}$** | **$F_{ST}$** | **Rel** | **AIc** | **vAIc** |
|---|---|---|---|---|---|---|
| Females | 77 | 0.060 | 0.047 | 0.085 | 0.414 | 9.275 |
| Males | 67 | 0.056 | 0.043 | 0.078 | -0.476 | 16.176 |
| p-value | | 0.629 | 0.354 | 0.375 | 0.05 | 0.026 |
| ***Oedura*** | **n** | **$F_{IS}$** | **$F_{ST}$** | **Rel** | **AIc** | **vAIc** |
| Females | 70 | 0.040 | 0.097 | 0.172 | -0.082 | 9.601 |
| Males | 41 | 0.068 | 0.093 | 0.161 | 0.140 | 15.819 |
| p-value | | 0.299 | 0.618 | 0.579 | 0.615 | 0.021 |

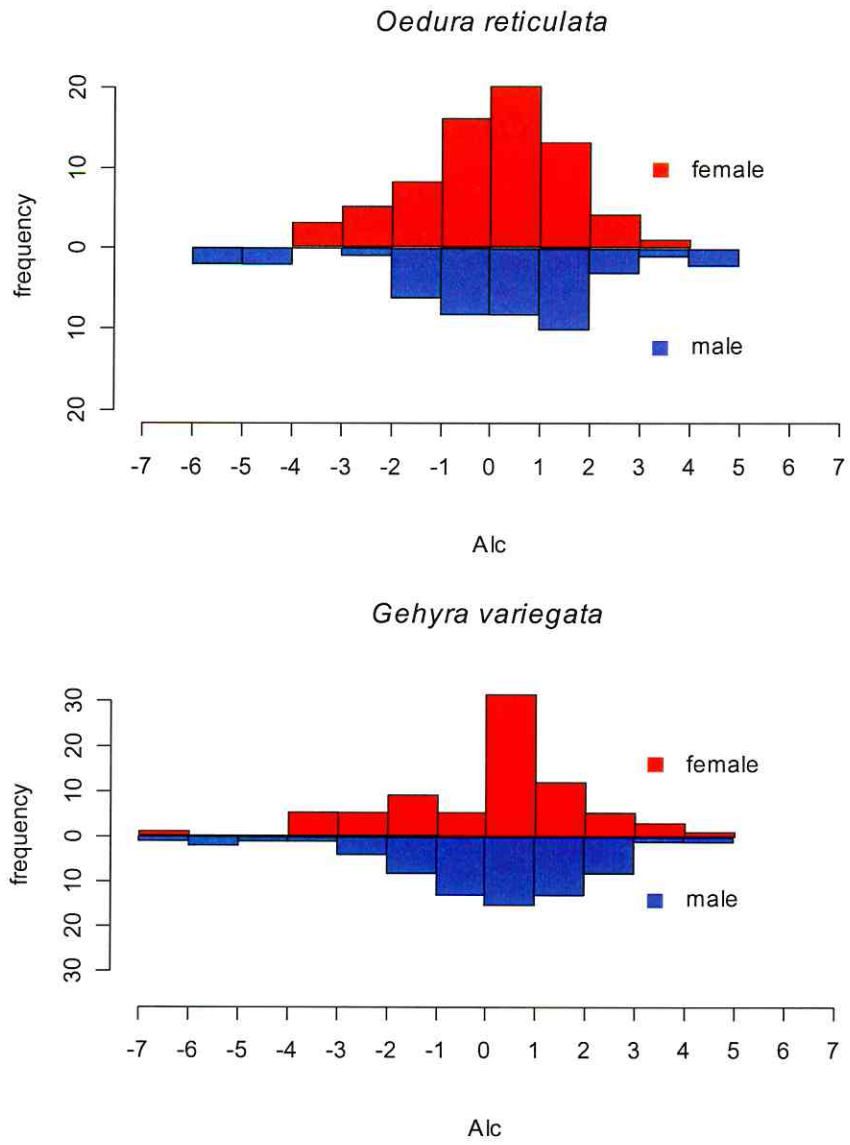



*Figure 4 Frequency distribution of the assignment index (Alc) for males and females in* O. reticulata *(above) and for males and females in* G. variegata *(below)*

## Discussion

The two species of gecko, *G. variegata* and *O. reticulata*, vary in their levels of specialization. *G. variegata* has been described as a habitat generalist, whereas *O. reticulata* is limited in its range of habitat, being exclusively arboreal and restricted to smooth-barked *Eucalyptus* woodlands (How & Kitchener 1983, Kitchener et al. 1988, Sarre 1995 a, b, 1998, Sarre et al. 1995). These species should differ in their dispersal capability in fragmented habitats. Our fine-scale genetic study supports this expectation. 1. Allelic richness and heterozygosity were higher for the generalist *G. variegata* than for the specialist *O. reticulata*, suggesting a higher genetic diversity for the former species. However, the fact that two different sets of microsatellite loci were used for each of the species renders the comparison of polymorphism levels difficult. 2. The genetic differentiation among populations of the specialist species was two times higher than for the generalist, suggesting that the gene flow between populations of *G. variegata* is higher. 3. In addition, we observed an isolation-by-distance effect in *G. variegata*. The levels of differentiation significantly increased with increasing geographical distance. In contrast, geographical distance explained almost none of the variation of the genetic differentiation among populations in *O. reticulata*. Thus, no isolation-by-distance effect was discovered in this species. 4. The assignment test revealed that *G. variegata* has higher rates of dispersal compared to *O. reticulata* in fragmented landscapes. For both species, more individuals dispersed between fragments separated by only small distances (150m), than between fragments separated by larger distances. We discovered that *G. variegata* is able to disperse over distances of 1000m, whereas in *O. reticulata* there was hardly any dispersal between fragments separated by 500m. 5. Finally, the dispersal in both gecko species is slightly biased towards male-dispersal.

### *Isolation-by-distance*

Understanding how genetic differentiation between populations varies with geographical distance can help to determine whether genetic differentiation is primarily due to limited dispersal or more complex demographic processes (Leblois et al. 2000). At mutation-migration-drift equilibrium, and for species with limited dispersal in space (such as those studied here), genetic differentiation is expected to increase with geographical distance (Slatkin 1993, Rousset 1997). However, only one of the two species that we studied clearly conformed to these theoretical expectations. For the habitat generalist *G. variegata*, genetic differentiation is positively correlated with total geographic distance. In contrast, for the specialist *O. reticulata*, no relationship was found between genetic differentiation and total geographical distance throughout the fragmented landscape. The absence of an observable pattern of isolation-by-distance may suggest that populations of *O. reticulata* are not in an equilibrium between genetic drift and migration. Dispersal of *O. reticulata* between the fragments is limited by the lack of shelter and suitable microhabitat. The gene pools of the isolated populations therefore randomly lose or gain alleles through genetic drift and mutation, without any contribution of alleles from immigrants from other populations.

*Assignment methods*

Results from assignment methods need to be interpreted with care. Both assignment methods provide a level of confidence in the assignment of an individual. The level of confidence or stringency required to accept an individual as a disperser or stayer is thus able to be varied. The level of stringency selected is dependent on the purpose of the analysis. A very high level of confidence may be required if the information is to be used to forensically determine the origin of a smuggled animal or trophy. A lower level of confidence may be sufficient in studies of wildlife dispersal (Pritchard et al. 2000, Eldridge et al. 2001, Berry et al. 2004). For the estimation of dispersal probabilities every single individual does not necessarily need to be assigned with a high accuracy. Instead the benefit is an overall estimate of the dispersal probability for the species. We initially calculated assignment at a threshold of $p \leq 0.05$. Despite the higher accuracy only a very low number of individuals could be assigned. Thus, we chose the most likely option, which assigns the individual to the population, where its genotype is most likely to occur, and calculated a probability of belonging from the results. Other empirical studies provided evidence that this is an appropriate approach (Eldridge et al. 2001, Berry et al. 2004). As a second approach for identifying an exact number of dispersers, the assignment test was performed at a threshold of $p \leq 0.05$.

In spite of the potential methodological limitations, we were able to estimate the percentage of misassignments, which was on average lower for *O. reticulata* (15-20%, depending on the method) than for *G. variegata* (35-38%, depending on the method), suggesting that the number of dispersers in *G. variegata* is nearly twice the number in *O. reticulata*. With the semi-experimental approach, we were also able to determine a rough distance of separation. For *O. reticulata* a distance of 150m through cleared habitat did not appear to be a barrier, whereas it appeared that movement over a distance of 550m occurs only very rarely (with only a single migrant found for populations this distant). In the one case where two neighbouring populations of *O. reticulata* were relatively close to roadside vegetation (distances of 150m and 230m respectively), three immigrants were detected. This would involve dispersal over a distance of 580m of matrix or instead 150m of matrix, 580m of roadside vegetation and 230m of matrix between the two habitat fragments. Given that migration of *O. reticulata* individuals through a matrix distance of 550m appeared rare, as described above, it would thus appear that dispersing individuals are able to use roadside vegetation as 'stepping stones' for migration to other habitat fragments. This finding raises hopes for the restoration of 'stepping stones' or habitat corridors. However, the debate about their effectiveness and their possible negative aspects continues (Hobbs 1992, Simberloff et al. 1992, Orrock et al. 2003, Driscoll 2004, Hoyle & Gilbert 2004).

For *G. variegata,* the isolation of remnants by 150 to 300m of cleared matrix did not stop migrants from moving through the modified landscape. Some individuals of the generalist species even covered distances of 1000m between habitat patches. This is remarkable for a

animal measuring less than 6cm in snout-vent-length. Dispersal requires individuals to pass through rural landscapes in which the native vegetation has been removed and replaced by crop or stubble after harvest. The environment contains a high density of introduced predators, and suitable hiding places (even rocks and fences) for the geckos are scarce. Hence, successful dispersal under these circumstances should be a rare event.

It was not surprising to find that the number of identified dispersers by assignment test at a threshold ($p \leq 0.05$) was similar for the two gecko species or even slightly higher in the specialist species. Theoretical and empirical research implies that there is a positive relationship between the level of differentiation ($F_{ST}$) and the ability to correctly assign individuals to their natal population (Hansen et al. 2001, Berry et al. 2004). For moderate levels of differentiation (FST ~ 0.07), the accuracy of assigning individuals correctly to their natal population is higher (100% accuracy) than for low levels of genetic subdivision (FST ~ 0.04; 78% accuracy) (Berry et al. 2004). We suggest that the assignment test was more likely to correctly identify the dispersers for *O. reticulata*, which had an $F_{ST}$ of 0.10, than for *G. variegata* with an $F_{ST}$ of 0.04. Therefore, we assume that the actual number of disperers would be higher than estimated in the species *G. variegata*. The same is true for populations of both species in remnants, which are in close proximity. The differentiation is lower between those populations and consequently the number of dispersers might be underestimated.

The direction of gene flow between habitat fragment pairs was mostly unbiased in both species. The exceptions were population F5 and F6 as well as F5 and F7 in *G. variegata* and population F1 and F4 in *O. reticulata*. We found a higher percentage of gene flow (19% vs 13%) from population F1 to F4 (*O. reticulata*) as well as a higher percentage (69% vs 31%) from population F5 to F7 and from F6 to F5 (*G. variegata*)(40% vs 24%). There are several possible explanations for these findings. First, in two cases the immigrants came from a population (F1 in *O. reticulata* and F5 in *G. variegata*) of high density. Consequently the asymmetry might be the result of density-dependent induced migration (see Stenseth et al. 1998, Gundersen et al. 2001, Nathan 2001, Wiegand et al. 2002, Henle et al. 2004a). Second, environmental changes can lead to directional dispersal events. A catastrophic event, more specifically a fire occurred recently in fragment F7, and exterminated part of population F7. As a response, the dispersal rate might have increased and individuals from population F5 (300m distance) might have recolonized fragment F7. Third, the low number of sampled individuals in population F7 might have biased the result. None of the three explanations sufficiently accounts for the pattern of directional dispersal that has been observed in several fragments. Thus, more research for example on the role of density-dependent dispersal in the two gecko species is required.

## *Sex-biased dispersal*

Most of the tests for sex-biased dispersal failed to detect significant differences between females and males in their dispersal range. However, the Alc and/or vAlc suggested a significant higher dispersal rate for males. On this basis, we consider males to be the dispersing sex in *O. reticulata* and *G. variegata*. What does the disparity in dispersal between sexes mean in terms of mating systems and ecological traits in the two gecko species? Dispersal presents both costs and benefits to an individual, and therefore should be optimized by natural selection. That the optimum differs among sexes suggests asymmetries in costs/benefits. Three main hypotheses have been proposed to account for this bias and all three attribute a key role to mating systems (summarized in Favre et al. 1997). 1. The resource-competition hypothesis (Greenwood 1980, 1983) builds on the benefits brought by philopatry or familiarity with the natal area. This hypothesis predicts male-biased dispersal in polygynous species, and female-biased dispersal in monogamous species. 2. The local mate competition hypothesis (Dobson 1982) builds on the observation that philopatry induces competition for mates among kin (Hamilton 1972). This hypothesis therefore predicts that in polygynous species, males should be the dispersing sex, while no difference in dispersal among sexes is expected in monogamous species. 3. The inbreeding hypothesis (Wolff 1993, 1994) holds that philopatry puts an animal at the risk of inbreeding with close kin. In polygynous species only females live in close contact with their progeny, so that females may safely remain philopatric, while males should emigrate. By contrast, this asymmetry does not exist in monogamous species, so that no dispersal bias is expected. One obvious problem with these models lies in the convergence of their predictions. However, all three models predict a male-biased dispersion merely for polygynous species. For the two gecko species, the result of the assignment indices implied male-biased sex-dispersal. Under these circumstances the prediction of all three models suggest a polygynous mating system.

There are other studies that discovered male-biased dispersal in reptiles (Clobert et al. 1994, lizard; Gardner et al. 2001, lizard; Stow et al. 2001, lizard; Casale et al. 2002, turtle) and other taxa (Knight et al. 1999, cichlids; Dallimer et al. 2002, quelea; Hutchings & Gerber 2002, trout; Lampert et al. 2003, frog; Bekkevold et al. 2004, trout). Female-biased dispersal has mainly been found in monogamous birds and mammals (Favre et al. 1997, Yáber & Rabenold 2002) and also in amphibians (Austin et al. 2003, Palo et al. 2004), but to the best of our knowledge has never been discovered in lizards.

Gruber & Henle (2004, subm.) observed a population of *G. variegata* in Kinchega National Park, in eastern inland Australia. Females and males both appeared to be territorial, but both sexes consisted of so-called 'floaters' - individuals that move between territories. The majority of 'floaters' are male, which could lead to male-biased dispersal in the species. This result is in agreement with our conclusions. The difference between male and female dispersal indicated by our genetic data is not large. The dispersal of just a few more floating males could explain this situation. It should be noted that the study of Gruber & Henle (2004,

subm.) dealt with movement over small distances (between trees) and was conducted in undisturbed, riverine woodlands, whereas our study examines long-distance dispersal between habitat fragments of a highly fragmented landscape. Deforestation may inhibit female dispersal to a greater extent than it does for males (as described for skinks in Stow et al. 2001). Thus, more research on sex-biased dispersal is required in continuous forest sites, which is one of our future intentions.

## *Conclusions*

Comparative genetic studies of two or more species living in the same area with contrasting life history traits are rare and have been restricted to insects (Brouat et al. 2003), mammals (Matocq et al. 2000, Ehrich et al. 2001a, b), and species living in aquatic systems (Monaghan et al. 2002). Brouat et al. (2003) studied two carabid species on a fine genetic scale. In agreement with our study, it was found that the forest specialist appeared more spatially structured than the generalist. Ehrich et al. (2001a) found a difference in the genetic structure of collared and brown lemming populations according to their dispersal abilities. Collared lemmings disperse over larger distances, which resulted in a lower local genetic differentiation. Their findings are in agreement with our results. The habitat generalist species *G. variegata* seems to have lower levels of differentiation, higher rates of dispersal, and manages to migrate over larger distances of matrix in the fragmented landscape than the habitat specialist *O. reticulata*. Both species obviously contrast in their levels of specialization, but are similar in other life history traits. Our data suggests that dispersal in both species is slightly male-biased and it is likely that both species have the same mating system. Both species live to an approximately equivalent age, have two offspring per year (*O. reticulata*: 1 clutch of 2 eggs; *G. variegata*: 2 cluches of 1 egg), and live in the same area (characteristics summarized in Sarre et al. 1995; How & Kitchener 1983, Kitchener et al. 1988). *O. reticulata* is larger than *G. variegata* and reaches maturity later (4.8 years vs. 2.8), which leads to approximately 19 generation since isolation for the specialist vs. 32 generations for the generalist. One could argue that the specialist species did not have enough time since isolation to reach an equilibrium between mutation and migration. On the other hand, it is rather likely that a long generation time leads to the fact that the erosion of genetic variability might not be detected and the missing differentiation might be misjudged as gene flow (Srikwan & Woodruff 2000). We can not exclude the possibility that other life history characteristics may influence the dispersal of these two gecko species, but given the number of intensive studies conducted on these species and their failure to identify alternatives (Sarre et al. 1995; How & Kitchener 1983, Kitchener et al. 1988), we suggest that the most likely reason for their variation in dispersal capability is their different level of habitat specialization. In the future, we intend to test this finding in other taxa, and search for general rules that assist prediction of vulnerability of species to habitat fragmentation based on their ecological attributes (see Davies et al. 2000, 2004, Mac Nally et al. 2000, Henle et al. 2004a,b). From a conservation perspective, this study indicates that the specialist species

*O. reticulata* is likely to be a good genetic indicator species for monitoring the impact of anthropogenic perturbations in Western Australian *Eucalyptus* woodlands. Usually conservation managers have to deal with a minimum of management costs and a minimum time available, but genetic monitoring is still quite expensive and time consuming. As a result it does not seem to be practicable to genetically observe every species that might be vulnerable to extinction. Instead, monitoring indicator species like *O. reticulata*, which are especially sensitive to habitat fragmentation could be a feasible approach to reduce costs and maximize the information content of the system.

# Chapter III

## Population genetic structure of two gecko species in fragmented versus continuous landscapes

### *Introduction*

Habitat loss and fragmentation threaten species throughout the world and are a major threat to biodiversity (Groombridge 1992, WCMC 1992, Henle 2004a). As a result of habitat fragmentation, many species are limited to habitat remnants, which are reduced in size and isolated by a matrix of less suitable habitat. Species with a limited dispersal capability are particularly vulnerable to the negative impact habitat fragmentation has on genetic and demographic factors (Henle et al. 1996, Davies et al. 2000, Henle et al. 2004a).

Conservation genetic studies have typically inferred the effects of habitat fragmentation by documenting patterns of genetic differentiation and levels of genetic diversity among potentially isolated patches (Young et al. 1996, Young & Clarke 2000). Ideally, such studies should take additional factors into account. First, levels of differentiation and genetic diversity among fragmented populations should be compared to undisturbed populations. Surveys of genetic diversity in fragmented versus continuous populations play an important role in the understanding of the effect of habitat fragmentation (Van Dongen et al. 1998, Sumner et al. 2004). Two complementary strategies have been used to examine the genetic effects of fragmentation; comparison of a particular set of populations through time under more or less fragmented states (Srikwan & Woodruff 2000), or comparison at a single point in time of different sets of populations inhabiting more or less fragmented landscapes (Paetkau et al. 1998, Stow et al. 2001, Caizergues et al. 2003, Sumner et al. 2004). The former is typically constrained by sampling and the latter sometimes by confounding effects such as habitat differences or spatially varying population histories (Cunningham & Moritz 1998). In both cases, investigators face the general problem of distinguishing between historical connectivity and current migration as a cause of observed genetic similarity among populations, which is especially acute when using a small number of genetic loci and traditional approaches (Slatkin 1987, Nielsen & Slatkin 2000). In addition, finding both fragmented and non-fragmented populations can be difficult among naturally occurring populations, often because species are not of conservation concern until only a few, isolated populations remain.

Second, historical levels of isolation and differentiation among populations should be determined prior to investigation (Bermingham & Avise 1986, Cunningham & Moritz 1998). Historical population structure can have a profound influence on the distribution of genetic variation among contemporary populations such that any differentiation observed may be the result of long-term isolation, rather than recent, anthropogenic fragmentation (Cunningham & Moritz 1998). Alternatively, a lack of differentiation among populations could be the result of

shared ancestry among populations, rather than ongoing gene flow among them (Avise et al. 1987, Avise 1994). A solution to those problems might be the use of microsatellite DNA markers. Their high mutation rate makes them less prone to confounding effects of population history, and also a high number of alleles makes them more sensitive for fine-scale analysis of populations and for studies on a fine spatial scale (Estoup et al. 1998, Broaut et al. 2003).

Third, population genetic processes are complex and difficult to generalize from single-species studies. These problems can be overcome through multispecies studies, in which comparative hypotheses are tested and ways to evaluate broader generalities are provided. Closely related, ecologically comparable species with dispersal differences will function as independent data points (Bohonak 1999). Species with different life-history traits commonly possess associated differences in dispersal capability (Davies et al. 2000, Henle et al. 2004b), which in turn produces differences in genetic exchange and variation. Although comparisons of these types of species have been conducted for some taxa (Waples 1987; fish, Hellberg 1996; coral), only a limited number of studies have used microsatellite markers (Ehrich et al. 2001a; lemmings, Brouat et al. 2003; carabids), or have used this approach in human fragmented landscapes (Brouat et al. 2003; carabids).

The goal of the present study was to compare the fine-scale genetic structure of fragmented versus continuous populations of two sympatrical gecko species known to differ in their dispersal ability and specialization. Previous empirical and modelling approaches (Sarre et al. 1995, Hoehn unpublished data) demonstrated that the habitat generalist in comparison to the specialist showed a marked higher persistence in habitat remnants after habitat fragmentation. It is unclear whether the fact that the specialist species is under-represented in the fragments has any impact on the genetic diversity and genetic structure of the species. Habitat fragmentation can affect genetic structure through its effects on gene flow by restricting dispersal and by reducing effective population size and increasing the effects of genetic drift in small habitat patches (Young & Clarke 2000, Frankham et al. 2002). This can lead to genetic isolation, reduced genetic diversity, and increased inbreeding. Ultimately these genetic changes may result in a decline in fitness and extinction, because of the accumulation of mildly deleterious alleles (Frankham 1995a, Lande 1995, Saccheri et al. 1998, Frankham et al. 2002).

We used microsatellite DNA markers to evaluate and compare the genetic structure of gecko populations in habitat fragments with that of populations in continuous forest sites. We tested the hypothesis that fragmentation is responsible for reduced genetic diversity in both gecko species in habitat fragment populations in comparison with continuous (unfragmented) populations. We also expected that fragmentation is responsible for an increase in the population substructure among fragments compared with continuous populations. These changes following habitat fragmentation may well be related to the difference in the level of specialization and dispersal ability of these two species; i.e. the

specialized species is presumably more strongly affected. We incorporated a variety of different measures to determine if the observed pattern of genetic differentiation was consistent across methodologies

### *Model species and model system*

Neither *O. reticulata* nor *G. variegata* are currently threatened with extinction. *O. reticulata* is also common throughout the Goldfield area, where its habitat is much more intact than in the Western Australian wheatbelt. *G. variegata* is widespread throughout the southern half of Australia. Since both species are not threatened with extinction, they are useful models for analyzing the effects of habitat fragmentation on genetic structure, gene flow, migration, survival, and extinction and give us an insight into the influence of habitat specialization on the persistence of species in fragmented landscapes. Many reptile species have much lower absolute mobility, which increases their vulnerability to local extinction to a greater extent than for birds, mammals, and some insects (Henle & Streit 1990, Webb & Shine 1997, Coddington & Cree 1998, Hokit et al. 1999, Mac Nally & Brown 2001).

Both gecko species occur sympatrically in the study area, the Western Australian wheatbelt, but contrast in their degree of habitat specialization and dispersal capability (Sarre et al. 1995, chapter 2). *G. variegata* (tree dtella) is a habitat generalist, whereas *O. reticulata* (reticulated velvet gecko) is a habitat specialist and is limited in its range of habitat. The specialist is exclusively arboreal and restricted to smooth-barked *Eucalyptus* woodlands (How & Kitchener 1983, Kitchener et al. 1988, Sarre 1995a, 1998, Sarre et al. 1995). The two species have been subjected to severe population fragmentation in the Western Australian wheatbelt, a region that has undergone rapid development for agriculture. Large areas of native vegetation have been removed and replaced with agricultural crops, pastures, and livestock. Since 1900 approximately 93% of the original vegetation has been cleared, and the remnant vegetation is distributed as thousands of patches of varying size. These changes have resulted in alterations to ecosystem processes and biotic impoverishment. Clearing of vegetation has changed the hydrologic balance of the wheatbelt, the climate, and the native biota (Saunders & Hobbs 1991, Hobbs 1993, Hobbs & Saunders 1993, Saunders et al. 1993).

In a previous study, phylogenetic analysis of mitochondrial DNA (mtDNA) variation among populations of the species *O. reticulata* demonstrated little regional structure (Sarre 1995b). Several haplotypes were present in three different regions and no population or group of populations could be considered to be a separate phylogenetic unit. This implied that there had been no major historical barrier to gene flow in the study area. In contrast to the variation at a regional scale, a high degree of independence of remnant populations and low diversity within the remnants indicated that there should be modest gene flow among remnant populations (Sarre 1995b). Our research extends this work by using hypervariable microsatellite DNA markers to compare the levels of genetic diversity and the genetic differentiation among habitat fragment populations to the genetic structure of populations in

a Nature Reserve. In addition, we compared the results from *O. reticulata* to those from the species *G. variegata*, which has a lower level of specialization and higher dispersal rates. Comparison of the results will improve the understanding of complex population genetic dynamics.

## *Method*

### *Sample collection*

The study area was located between Kellerberrin and Trayning in the Western Australian wheatbelt. Few non-fragmented populations remained in that area, but a set of three sites per species were located within a range of one kilometer inside the continuous Northbandee Nature reserve, which encompasses 50 ha of gimlet woodlands. The second set of six to seven populations per species were located in habitat that has been highly fragmented since potentially as long ago as 1900. Each fragment is about 0.25-5.5 ha in size. This setup provided the opportunity to compare levels of genetic differentiation and diversity between fragmented and continuous woodland sites. The sample populations were labelled independently for each species, with one habitat fragment (or 'patch' or 'remnant') equivalent to one sample population. The *O. reticulata* populations were labelled F1-6 and C1-3, and the *G. variegata* populations F1-7 and C1-3. In two cases a habitat remnant was used as a sample population for both species, i.e. *G. variegata* population F3 inhabited the same fragment as *O. reticulata* population F5, and similarly, *G. variegata* population F6 occupied the same habitat patch as *O. reticulata* population F4. All other sample populations were from separate fragments, with no overlapping between the two species. Continuous forest sites (C1-3) were used as sample populations for both species. Locations are listed in Table 1 and displayed in Figure 1.

Tissue samples were collected during the summer months, from November 2000 to March 2001 and also in December 2003. Lizards were spotted at night using head-torches and were captured by hand. A total of 251 *O. reticulata* and 269 *G. variegata* were collected from the fragments and the continuous forest sites. The tip of the tail of each individual was removed and stored in liquid nitrogen. In all fragments or forest sites 25-30 samples were collected with the exception of fragment population F7 (N=13) for *G. variegata* and forest population C3 (N=17) for *O. reticulata*. At these sites no more individuals could be located despite concerted attempts.

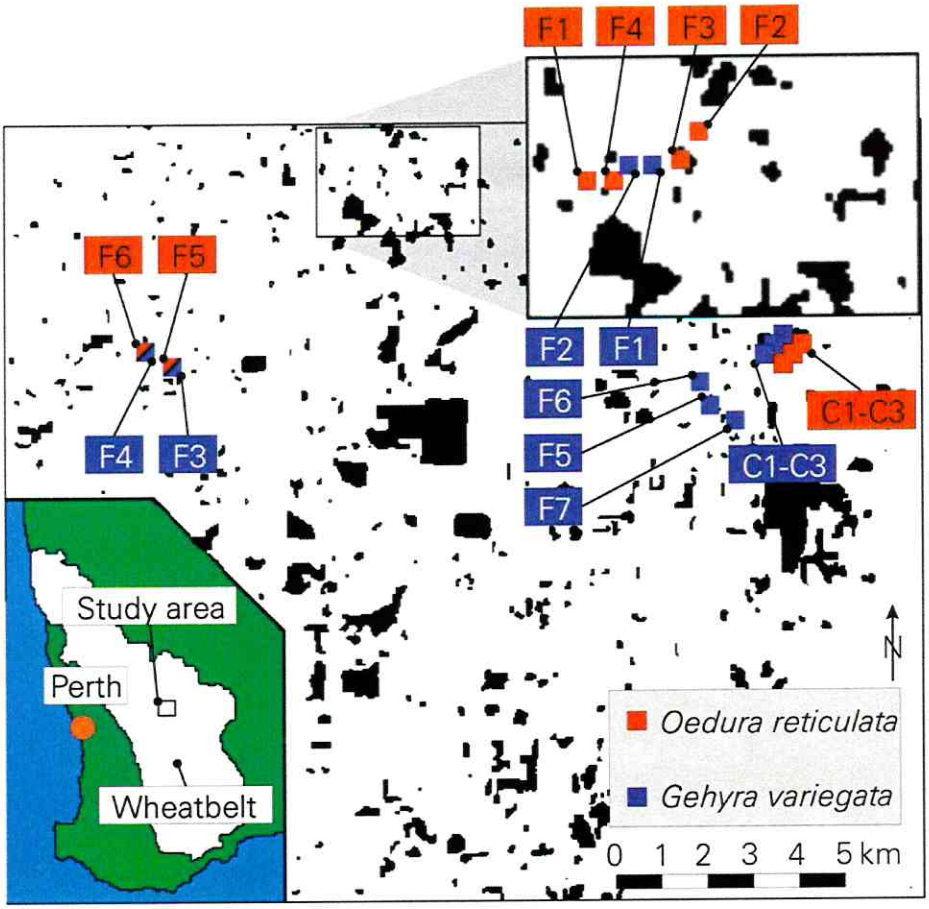



*Figure 1 Map of the study area and location of sites in the Western Australian wheatbelt.* O. reticulata *populations inhabit fragments labelled in red F1-6 and continuous sites labelled C1-3,* G. variegata *fragments are labelled in blue F1-7 and continuous sites C1-3*

*Table 1 Fragments sampled and the number of individuals genotyped (samples), the site type, fragment size (in ha), the number of* Eucalyptus salubris *(preferred habitat of* O. reticulata*), approximate time since isolation from Sarre (1995a)*

| Fragment | Species | Samples | Site type | Size | *E. salubris* | Isolation |
|---|---|---|---|---|---|---|
| F1 | *Oedura* | 30 | Fragment | 0.5 | 89 | 1912 |
| F2 | *Oedura* | 30 | Fragment | 0.8 | 60 | 1936 |
| F3 | *Oedura* | 27 | Fragment | 0.4 | 6 | 1936 |
| F4 | *Oedura* | 27 | Fragment | 0.4 | 0 | 1912 |
| F5 | *Oedura* | 30 | Fragment | 5.4 | 350 | 1910-20 |
| F6 | *Oedura* | 30 | Fragment | 2.0 | 159 | 1910-20 |
| C1 | *Oedura* | 30 | Continuous | | ------- | ------- |
| C2 | *Oedura* | 17 | Continuous | | ------- | ------- |
| C3 | *Oedura* | 30 | Continuous | | ------- | ------- |
| F1 | *Gehyra* | 30 | Fragment | 0.3 | ------- | 1912 |
| F2 | *Gehyra* | 30 | Fragment | 1.4 | ------- | 1912 |
| F3 | *Gehyra* | 30 | Fragment | 5.4 | ------- | 1910-20 |
| F4 | *Gehyra* | 30 | Fragment | 2.0 | ------- | 1910-20 |
| F5 | *Gehyra* | 30 | Fragment | 0.3 | ------- | 1920 |
| F6 | *Gehyra* | 25 | Fragment | 0.5 | ------- | 1920 |
| F7 | *Gehyra* | 13 | Fragment | 0.6 | ------- | 1920 |
| C1 | *Gehyra* | 30 | Continuous | | ------- | ------- |
| C2 | *Gehyra* | 25 | Continuous | | ------- | ------- |
| C3 | *Gehyra* | 30 | Continuous | | ------- | ------- |

### *Laboratory methods*

DNA was extracted from the tip of the tail of each individual using the Chelex extraction technique. We genotyped individuals of *O. reticulata* using nine tetranucleotide microsatellite loci developed from an enriched library for this species (OR205, OR220, OR266, OR6F4, OR10H7, OR11G3, OR12D7, OR12D9, OR14A7) (Hoehn & Sarre subm.). For *G. variegata*, we genotyped individuals using nine tetranucleotide microsatellite markers cloned from an enriched library for this species (GV1C5, GV1C10, GV1F1, GV3B5, GV3C6, GV3E10, GV4B6, GV4G6, GV4C9) (Hoehn & Sarre in prep.). Polymerase chain reaction (PCR) amplification and genotyping on the Beckman Coulter Sequencer CEQ-8000 were performed according to conditions described in Hoehn & Sarre (subm.) and Hoehn & Sarre (in prep.).

### *Statistical analysis*

Tests for deviation from Hardy-Weinberg equilibrium (HWE) were performed for each locus-population combination using an exact test. P-values were estimated using a Markov chain method implemented in *GENEPOP 3.2a* (Raymond & Rousset 1995). Tests for genotypic linkage disequilibrium for all combinations of locus pairs within populations were also performed using Markov chain methods. Sequential Bonferroni multiple test adjustment were made for significance tests (Rice 1989).

*FSTAT 2.9.3* (Goudet 1995, 2001) was used to calculate descriptive statistics for populations including the mean allelic richness ($A_n$) (number of alleles corrected for sample size), observed ($H_o$) and expected ($H_e$) heterozygosity. Of these, allelic diversity is expected to be most sensitive to recent reduction in population size (Nei et al. 1975, Garza & Williamson 2001). Significance of differences in allelic richness, observed ($H_o$) and expected ($H_e$) heterozygosity between fragments and continuous forest sites was tested using the program *FSTAT 2.9.3*. To assess significance a permutation scheme of 15,000 permutations was used.

The ratio of the total number of alleles to the overall range in allele size (defined as sequence or repeat length) can be used to detect a population bottleneck (Garza & Williamson 2001). The average M ratio ($M = k/r$) was calculated for each site, where k is the number of alleles and r is the range in allele size $+1$ at each locus (Garza & Williamson 2001). Rather than testing for significant deviation from mutation-drift equilibrium, which requires an a priori estimate of $4N_e\mu$, we tested for greater deviation (lower M) in fragments versus continuous forest, assuming that equilibrium $4N_e\mu$ was the same across the sampled sites prior to habitat fragmentation (Sumner et al. 2004). T-tests (*STATISTICA 4.1*) were used to test for differences in M ratios between fragments and continuous forests.

We have incorporated a variety of different measures to determine if the observed pattern of genetic differentiation are consistent across methodologies. Wright's F-statistics (1951),

calculated following Weir & Cockerham (1984), were used to measure population structure between sites using *FSTAT 2.9.3* (Goudet 1995, 2001) ($\theta$ was calculated, hereafter referred to as $F_{ST}$) and *GENEPOP 3.2a* (Raymond & Rousset 1995). Population differentiation was further determined using the $R_{ST}$ values (Slatkin 1995) estimated with *GenAlEx V5* (Smouse & Peakall 1999, Peakall & Smouse 2001). Whereas $\theta_{ST}$ is consistent with an infinite allele model (IAM), $R_{ST}$ is consistent with a step-wise mutation model (SMM). Assumptions concerning either the IAM or the SMM can influence the estimates of genetic variation and, which model is appropriate for a given level of inquiry has been a topic of much debate and largely unresolved (Goldstein et al. 1995).

The continuous sites were located within a range of one kilometer inside the Northbandee Nature Reserve. The geographical distance among populations was on average smaller for the populations in the Nature Reserve than for the populations in the fragmented landscape. To take this into consideration a second calculation of genetic differentiation among fragment populations was performed. In this calculation only populations of fragments were included, which were within a range of one kilometer of each other.

## *Results*

A total of 251 *O. reticulata* from nine sites (six fragments and three continuous forest sites) and 269 *G. variegata* from ten sites (seven fragments and three continuous forest sites) were genotyped at nine microsatellite loci. Three of the 81 tests for Hardy-Weinberg equilibrium in *O. reticulata* and four of the 90 tests in *G. variegata* showed significant deviation from expected allele frequencies after Bonferroni correction; all were due to a deficiency of heterozygotes. The heterozygote deficiency may be due to null alleles; although there does not appear to be any consistent pattern of Hardy-Weinberg deviation among loci, or populations (Table 2a, b).

### *Comparisons of within-site diversity*

Allelic richness ($A_n$) per site ranged from 4.00 to 19.26 for *O. reticulata* and from 4.74 to 16.42 for *G. variegata* (Table 2). We found a difference in the mean allelic richness between fragmented and continuous forest sites for *O. reticulata*, which approached significance (mean 7.97 and 9.81 respectively, $p = 0.09$). For *G. variegata* the difference in the allelic richness between fragments and continuous forest sites was significant (mean 9.95 and 11.15 respectively, $p < 0.05$) (Figure 2).

*Table 2a The allelic richness ($A_n$), the and observed ($H_o$) and expected ($H_e$) heterozygosity for each locus at each site in the species* O. reticulata. **Deviation from Hardy-Weinberg expectations ($p < 0.05$). Site codes follow Table 1*

| Locus | Site | F1 | F2 | F3 | F4 | F5 | F6 | C1 | C2 | C3 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| OR205 | $A_n$ | 7.48 | 4.80 | 4.00 | 7.36 | 8.33 | 6.86 | 6.31 | 9.00 | 7.12 | 6.81 |
| | $H_e$ | 0.77 | 0.59 | 0.76 | 0.83 | 0.83 | 0.77 | 0.79 | 0.87 | 0.81 | 0.78 |
| | $H_o$ | 0.80 | 0.63 | 0.67 | 0.85 | 0.63 | 0.70 | 0.70 | 0.94 | 0.73 | 0.74 |
| OR220 | $A_n$ | 4.57 | 5.37 | 4.57 | 5.52 | 8.67 | 6.53 | 8.19 | 8.00 | 6.62 | 6.45 |
| | $H_e$ | 0.74 | 0.69 | 0.60 | 0.59 | 0.87 | 0.76 | 0.86 | 0.84 | 0.82 | 0.75 |
| | $H_o$ | 0.70 | 0.63 | 0.63 | 0.56 | 0.63* | 0.63 | 0.83 | 0.65 | 0.77 | 0.67 |
| OR266 | $A_n$ | 6.94 | 5.20 | 4.73 | 6.60 | 7.83 | 9.26 | 9.53 | 10.00 | 10.22 | 7.81 |
| | $H_e$ | 0.82 | 0.70 | 0.70 | 0.82 | 0.81 | 0.88 | 0.85 | 0.89 | 0.88 | 0.82 |
| | $H_o$ | 0.93 | 0.70 | 0.67 | 0.74 | 0.53* | 0.80 | 0.87 | 0.82 | 0.83 | 0.77 |
| OR6F4 | $A_n$ | 5.25 | 5.81 | 4.61 | 6.67 | 9.03 | 7.41 | 8.34 | 8.00 | 8.70 | 7.09 |
| | $H_e$ | 0.58 | 0.77 | 0.56 | 0.73 | 0.84 | 0.82 | 0.83 | 0.82 | 0.83 | 0.75 |
| | $H_o$ | 0.41 | 0.57 | 0.57 | 0.41* | 0.90 | 0.87 | 0.72 | 0.71 | 0.62 | 0.64 |
| OR10H7 | $A_n$ | 7.69 | 9.69 | 6.97 | 10.19 | 12.46 | 10.43 | 13.70 | 15.00 | 11.69 | 10.87 |
| | $H_e$ | 0.81 | 0.86 | 0.83 | 0.83 | 0.87 | 0.89 | 0.88 | 0.90 | 0.89 | 0.86 |
| | $H_o$ | 0.83 | 0.83 | 0.89 | 0.70 | 0.93 | 0.90 | 0.90 | 0.94 | 0.87 | 0.87 |
| OR11G3 | $A_n$ | 5.92 | 12.55 | 6.19 | 6.47 | 9.38 | 9.72 | 7.90 | 9.00 | 8.06 | 8.35 |
| | $H_e$ | 0.80 | 0.92 | 0.72 | 0.72 | 0.85 | 0.88 | 0.80 | 0.87 | 0.83 | 0.82 |
| | $H_o$ | 0.70 | 0.93 | 0.85 | 0.63 | 0.77 | 0.73 | 0.70 | 0.76 | 0.70 | 0.75 |
| OR12D7 | $A_n$ | 7.84 | 8.58 | 4.76 | 9.29 | 19.26 | 11.11 | 13.84 | 15.00 | 13.24 | 11.43 |
| | $H_e$ | 0.83 | 0.79 | 0.60 | 0.86 | 0.96 | 0.86 | 0.93 | 0.94 | 0.93 | 0.86 |
| | $H_o$ | 0.90 | 0.83 | 0.67 | 0.89 | 0.97 | 0.87 | 1.00 | 0.88 | 0.97 | 0.89 |
| OR12D9 | $A_n$ | 10.38 | 10.35 | 7.55 | 9.71 | 18.99 | 11.93 | 10.26 | 12.00 | 11.36 | 11.39 |
| | $H_e$ | 0.89 | 0.87 | 0.77 | 0.82 | 0.96 | 0.90 | 0.88 | 0.88 | 0.90 | 0.87 |
| | $H_o$ | 0.90 | 0.83 | 0.85 | 0.81 | 0.93 | 0.83 | 0.87 | 0.94 | 0.90 | 0.87 |
| OR14A7 | $A_n$ | 5.48 | 5.95 | 5.69 | 5.62 | 8.46 | 8.43 | 9.33 | 6.00 | 8.44 | 7.05 |
| | $H_e$ | 0.69 | 0.75 | 0.71 | 0.76 | 0.87 | 0.86 | 0.85 | 0.81 | 0.85 | 0.79 |
| | $H_o$ | 0.63 | 0.70 | 0.81 | 0.85 | 0.93 | 0.93 | 0.80 | 0.82 | 0.67 | 0.80 |
| Mean | $A_n$ | 6.84 | 7.59 | 5.45 | 7.49 | 11.38 | 9.07 | 9.71 | 10.22 | 9.49 | |
| | $H_e$ | 0.77 | 0.77 | 0.69 | 0.77 | 0.87 | 0.85 | 0.85 | 0.87 | 0.86 | |
| | $H_o$ | 0.76 | 0.74 | 0.73 | 0.72 | 0.80 | 0.81 | 0.82 | 0.83 | 0.78 | |

*Table 2b The allelic richness ($A_n$), the and observed ($H_o$) and expected ($H_e$) heterozygosity for each locus at each site in the species G.variegata. *Deviation from Hardy-Weinberg expectations ($p < 0.05$). Site codes follow Table 1*

| Locus | Site | F1 | F2 | F3 | F4 | F5 | F6 | F7 | C1 | C2 | C3 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GV1C10 | $A_n$ | 11.31 | 8.61 | 8.81 | 10.82 | 9.24 | 7.55 | 11.00 | 12.23 | 11.57 | 12.12 | 10.33 |
| | $H_e$ | 0.92 | 0.81 | 0.88 | 0.89 | 0.85 | 0.87 | 0.92 | 0.92 | 0.92 | 0.92 | 0.89 |
| | $H_o$ | 0.80 | 0.57 | 0.87 | 0.80 | 0.73 | 0.81 | 0.77 | 0.73 | 0.71 | 0.59* | 0.74 |
| GV1C5 | $A_n$ | 11.82 | 8.50 | 14.21 | 13.71 | 10.64 | 12.95 | 16.00 | 16.42 | 14.77 | 14.34 | 13.34 |
| | $H_e$ | 0.88 | 0.86 | 0.94 | 0.93 | 0.91 | 0.92 | 0.96 | 0.96 | 0.93 | 0.94 | 0.92 |
| | $H_o$ | 0.93 | 0.57* | 0.87 | 0.83 | 0.90 | 0.95 | 0.77 | 0.83 | 0.88 | 0.73* | 0.83 |
| GV1F1 | $A_n$ | 9.54 | 9.92 | 9.29 | 9.33 | 10.08 | 9.94 | 8.00 | 8.51 | 11.14 | 8.92 | 9.47 |
| | $H_e$ | 0.86 | 0.88 | 0.88 | 0.87 | 0.85 | 0.90 | 0.80 | 0.85 | 0.91 | 0.88 | 0.87 |
| | $H_o$ | 0.80 | 0.90 | 0.90 | 0.83 | 0.87 | 1.00 | 0.85 | 0.97 | 1.00 | 0.83 | 0.89 |
| GV3B5 | $A_n$ | 9.87 | 6.82 | 8.76 | 9.08 | 9.78 | 9.45 | 11.00 | 12.10 | 10.15 | 12.52 | 9.95 |
| | $H_e$ | 0.87 | 0.83 | 0.87 | 0.85 | 0.90 | 0.89 | 0.90 | 0.92 | 0.88 | 0.92 | 0.88 |
| | $H_o$ | 0.67 | 0.77 | 0.93 | 0.87 | 0.93 | 0.81 | 0.92 | 0.87 | 0.96 | 0.93 | 0.87 |
| GV3C6 | $A_n$ | 8.10 | 8.68 | 10.29 | 9.84 | 7.61 | 8.40 | 10.00 | 10.66 | 11.24 | 10.35 | 9.51 |
| | $H_e$ | 0.87 | 0.86 | 0.91 | 0.89 | 0.85 | 0.83 | 0.88 | 0.91 | 0.92 | 0.90 | 0.88 |
| | $H_o$ | 0.93 | 0.93 | 0.87 | 0.93 | 0.90 | 0.81 | 0.92 | 0.97 | 0.96 | 0.87 | 0.91 |
| GV3D10 | $A_n$ | 12.47 | 8.97 | 13.72 | 12.10 | 9.82 | 7.44 | 10.00 | 8.45 | 9.26 | 10.15 | 10.24 |
| | $H_e$ | 0.92 | 0.87 | 0.94 | 0.92 | 0.90 | 0.85 | 0.93 | 0.88 | 0.87 | 0.89 | 0.90 |
| | $H_o$ | 0.93 | 0.83 | 0.97 | 0.93 | 0.90 | 0.95 | 1.00 | 0.87 | 0.96 | 0.93 | 0.93 |
| GV4B6 | $A_n$ | 12.67 | 9.60 | 15.15 | 15.38 | 11.38 | 11.83 | 11.00 | 14.37 | 12.96 | 12.64 | 12.70 |
| | $H_e$ | 0.92 | 0.86 | 0.95 | 0.94 | 0.90 | 0.88 | 0.91 | 0.94 | 0.93 | 0.93 | 0.91 |
| | $H_o$ | 0.97 | 0.90 | 0.97 | 0.93 | 0.83 | 0.81 | 1.00 | 0.90 | 0.92 | 0.87 | 0.91 |
| GV4C9 | $A_n$ | 8.45 | 6.35 | 4.74 | 7.28 | 6.36 | 7.12 | 6.00 | 7.44 | 9.27 | 8.74 | 7.17 |
| | $H_e$ | 0.83 | 0.78 | 0.76 | 0.86 | 0.77 | 0.79 | 0.79 | 0.85 | 0.88 | 0.88 | 0.82 |
| | $H_o$ | 0.89 | 0.78 | 0.50 | 0.52* | 0.70 | 0.81 | 0.62 | 0.87 | 0.67 | 0.87 | 0.72 |
| GV4G6 | $A_n$ | 10.12 | 8.39 | 10.07 | 12.01 | 9.39 | 10.05 | 10.00 | 10.90 | 11.06 | 8.65 | 10.06 |
| | $H_e$ | 0.87 | 0.88 | 0.89 | 0.92 | 0.87 | 0.87 | 0.89 | 0.91 | 0.92 | 0.87 | 0.89 |
| | $H_o$ | 0.83 | 0.90 | 0.83 | 0.93 | 0.93 | 0.81 | 0.92 | 0.87 | 0.92 | 0.90 | 0.89 |
| Average | $A_n$ | 10.48 | 8.43 | 10.56 | 11.06 | 9.37 | 9.41 | 10.33 | 11.23 | 11.27 | 10.93 | |
| | $H_e$ | 0.88 | 0.85 | 0.89 | 0.90 | 0.87 | 0.87 | 0.89 | 0.90 | 0.91 | 0.90 | |
| | $H_o$ | 0.86 | 0.79 | 0.86 | 0.84 | 0.86 | 0.86 | 0.86 | 0.87 | 0.89 | 0.84 | |

The expected heterozygosity ($H_e$) per locus across all sites (Table 2a, b) ranged from 0.75 to 0.87 for *O. reticulata* (mean $H_e$: 0.81) and from 0.82 to 0.92 for *G. variegata* (mean $H_e$: 0.89). For *O. reticulata* we found a difference in mean heterozygosity between fragments (mean $H_e$: 0.79; range: 0.69-0.87 per site) and continuous forest sites (mean $H_e$: 0.86; range: 0.85-0.87 per site; $p = 0.09$), which approached significance. This trend was stronger in *G. variegata*, where we found a significant difference between fragments (mean $H_e$: 0.88; range: 0.85-0.90 per population) and continuous forest site (mean $H_e$: 0.91; range: 0.90-0.91; $p < 0.01$) (Figure 2).

M ratios (Table 3) averaged across loci ranged from 0.191 to 0.225 for *O. reticulata* and from 0.156 to 0.217 for *G. variegata*. For *O. reticulata* there was no significant difference in the M ratio between fragments (mean M: 0.206) and continuous forest sites (mean M: 0.211, $p = 0.77$). In contrast, populations of *G. variegata* had lower M ratios on average in the fragments (mean M: 0.184) than in the continuous forest sites (mean M: 0.210, $p = 0.09$), but the difference only approached significance.

*Table 3 M ratios for* O. reticulata *and* G. variegata *for each site. Site codes follow Table 1*

| | **M ratio** | |
|---|---|---|
| **Site** | ***Oedura*** | ***Gehyra*** |
| F1 | 0.185 | 0.176 |
| F2 | 0.191 | 0.156 |
| F3 | 0.214 | 0.211 |
| F4 | 0.213 | 0.201 |
| F5 | 0.211 | 0.203 |
| F6 | 0.222 | 0.165 |
| F7 | -------- | 0.177 |
| C1 | 0.205 | 0.206 |
| C2 | 0.225 | 0.217 |
| C3 | 0.204 | 0.207 |

### *Comparisons of population structure*

Values of pairwise $F_{ST}$ calculated between sites ranged from 0.003 to 0.163 for *O. reticulata* and from 0.000 to 0.081 for *G. variegata* (Table 4a, b). Most pairs of sites exhibited a fairly similar range of values, although for *O. reticulata* population F3 had larger values on average than the other sites. $F_{IS}$ scores calculated separately for fragments and continuous forest sites provided no indication of a significant difference in nonrandom mating within habitat fragments (*O. reticulata*: $F_{IS}$: 0.052; *G. variegata*: $F_{IS}$: 0.054) and continuous forest sites (*O. reticulata*: $F_{IS}$: 0.060, $p = 0.33$; *G. variegata*: $F_{IS}$: 0.046, $p = 0.56$).

For *O. reticulata* there was a significant difference (Table 4a) in overall population subdivision among fragments compared to continuous forest sites: fragments had $F_{ST} = 0.101$; 95%-CI: 0.089-0.112; continuous forest sites had $F_{ST} = 0.005$; 95%-CI: -0.001-0.011, with the 95% confidence intervals of the $F_{ST}$ estimates being mutually exclusive. The same trend was apparent for *G. variegata* (Table 4b): fragments had $F_{ST} = 0.045$; 95%-CI: 0.035-0.055; continuous forest sites had $F_{ST} = 0.001$; 95%-CI: -0.002-0.004. In addition, we performed another calculation of the genetic differentiation including only the populations of habitat fragments, which were in close proximity to each other. The population subdivision obtained from that restricted analysis was similar to our overall result above: $F_{ST} = 0.101$ for *O. reticulata* and $F_{ST} = 0.030$ for *G. variegata*. The mean geographical distance between these fragments was 696m for *O. reticulata* and 680m for *G. variegata*, in comparison to a mean geographical distance of 666m among the continuous forest sites for both species. In general, the $F_{ST}$ values were nearly three times higher for *O. reticulata* than for *G. variegata* in fragmented landscapes. In both species pairwise $F_{ST}$ values were not significantly different from zero in the continuous forest sites.

The $R_{ST}$ values (Table 4b) for *G. variegata* did not vary considerably from the $F_{ST}$ values, and there was a significant difference in overall $R_{ST}$ population subdivision among fragments compared to continuous forest sites: fragments had $R_{ST} = 0.049$ and continuous forest sites had $R_{ST} = 0.011$. In contrast, there was a noticeable difference between the $F_{ST}$ values and the $R_{ST}$ values in *O. reticulata* (Table 4a), again with a significant difference in overall $R_{ST}$ subdivision: fragments had $R_{ST} = 0.177$ and continuous forest sites had $R_{ST} = 0.002$. Again we performed a second calculation including only populations of habitat fragments, which were in close proximity to each other (≤ 1000m), and once more the population subdivision was similar to the overall result above: $R_{ST} = 0.127$ for *O. reticulata* and $R_{ST} = 0.031$ for *G. variegata*. In both species pairwise $R_{ST}$ values were not significantly different from zero in the continuous forest sites.

*Table 4a Pairwise $F_{ST}$ estimates (above) and $R_{ST}$ estimates (below) for* O. reticulata *for all loci on the lower matrix. The upper matrix indicates p-values for pairwise comparisons: NS indicates no significant differences, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Site codes follow Table 1*

| Site | F1 | F2 | F3 | F4 | F5 | F6 | C1 | C2 | C3 |
|---|---|---|---|---|---|---|---|---|---|
| F1 | ------- | ** | ** | ** | ** | ** | ** | ** | ** |
| F2 | 0.089 | ------- | ** | ** | ** | ** | ** | ** | ** |
| F3 | 0.163 | 0.144 | ------- | ** | ** | ** | ** | ** | ** |
| F4 | 0.089 | 0.091 | 0.138 | ------- | ** | ** | ** | ** | ** |
| F5 | 0.084 | 0.068 | 0.114 | 0.074 | ------- | ** | ** | ** | ** |
| F6 | 0.109 | 0.098 | 0.135 | 0.088 | 0.041 | ------- | ** | ** | ** |
| C1 | 0.089 | 0.075 | 0.125 | 0.066 | 0.047 | 0.055 | ------- | NS | NS |
| C2 | 0.073 | 0.062 | 0.114 | 0.045 | 0.027 | 0.040 | 0.006 | ------- | NS |
| C3 | 0.027 | 0.054 | 0.105 | 0.051 | 0.036 | 0.053 | 0.007 | 0.003 | ------- |

| Site | F1 | F2 | F3 | F4 | F5 | F6 | C1 | C2 | C3 |
|---|---|---|---|---|---|---|---|---|---|
| F1 | ------- | ** | ** | * | ** | ** | NS | NS | NS |
| F2 | 0.083 | ------- | ** | NS | ** | ** | ** | ** | ** |
| F3 | 0.106 | 0.194 | ------- | ** | ** | ** | ** | ** | ** |
| F4 | 0.031 | 0.021 | 0.171 | ------- | ** | ** | * | * | * |
| F5 | 0.318 | 0.303 | 0.405 | 0.319 | ------- | ** | ** | ** | ** |
| F6 | 0.114 | 0.102 | 0.230 | 0.080 | 0.218 | ------- | ** | ** | ** |
| C1 | 0.000 | 0.072 | 0.139 | 0.022 | 0.281 | 0.097 | ------- | NS | NS |
| C2 | 0.013 | 0.112 | 0.167 | 0.038 | 0.279 | 0.105 | 0.000 | ------- | NS |
| C3 | 0.000 | 0.079 | 0.122 | 0.028 | 0.303 | 0.113 | 0.000 | 0.002 | ------- |

*Table 4b Pairwise $F_{ST}$ estimates (above) and $R_{ST}$ estimates (below) for* G. variegata *for all loci on the lower matrix. The upper matrix indicates p-values for pairwise comparisons: NS indicates no significant differences, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Site codes follow Table 1*

| Site | F1 | F2 | F3 | F4 | F5 | F6 | F7 | C1 | C2 | C3 |
|---|---|---|---|---|---|---|---|---|---|---|
| F1 | -------- | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| F2 | 0.036 | -------- | ** | ** | ** | ** | ** | ** | ** | ** |
| F3 | 0.034 | 0.045 | -------- | ** | ** | ** | ** | ** | ** | ** |
| F4 | 0.042 | 0.055 | 0.011 | -------- | ** | ** | ** | ** | ** | ** |
| F5 | 0.057 | 0.081 | 0.055 | 0.045 | -------- | ** | * | ** | ** | ** |
| F6 | 0.036 | 0.058 | 0.030 | 0.044 | 0.049 | -------- | ** | ** | ** | ** |
| F7 | 0.045 | 0.063 | 0.040 | 0.044 | 0.007 | 0.048 | -------- | ** | ** | * |
| C1 | 0.024 | 0.043 | 0.015 | 0.021 | 0.030 | 0.028 | 0.017 | -------- | NS | NS |
| C2 | 0.021 | 0.037 | 0.013 | 0.015 | 0.035 | 0.025 | 0.027 | 0.004 | -------- | NS |
| C3 | 0.023 | 0.044 | 0.017 | 0.024 | 0.025 | 0.026 | 0.011 | 0.000 | 0.002 | -------- |

| Site | F1 | F2 | F3 | F4 | F5 | F6 | F7 | C1 | C2 | C3 |
|---|---|---|---|---|---|---|---|---|---|---|
| F1 | ------- | NS | * | NS | ** | * | NS | NS | NS | ** |
| F2 | 0.009 | ------- | NS | NS | ** | NS | NS | ** | NS | ** |
| F3 | 0.035 | 0.026 | ------- | NS | ** | * | * | ** | ** | ** |
| F4 | 0.005 | 0.000 | 0.004 | ------- | * | NS | NS | * | NS | NS |
| F5 | 0.064 | 0.058 | 0.131 | 0.054 | ------- | ** | NS | * | * | NS |
| F6 | 0.031 | 0.022 | 0.094 | 0.010 | 0.055 | ------- | ** | NS | NS | NS |
| F7 | 0.030 | 0.015 | 0.065 | 0.015 | 0.020 | 0.068 | ------- | * | * | NS |
| C1 | 0.028 | 0.036 | 0.118 | 0.027 | 0.029 | 0.003 | 0.044 | ------- | NS | NS |
| C2 | 0.019 | 0.006 | 0.045 | 0.004 | 0.022 | 0.003 | 0.025 | 0.013 | ------- | NS |
| C3 | 0.043 | 0.026 | 0.097 | 0.027 | 0.003 | 0.000 | 0.022 | 0.000 | 0.000 | ------- |

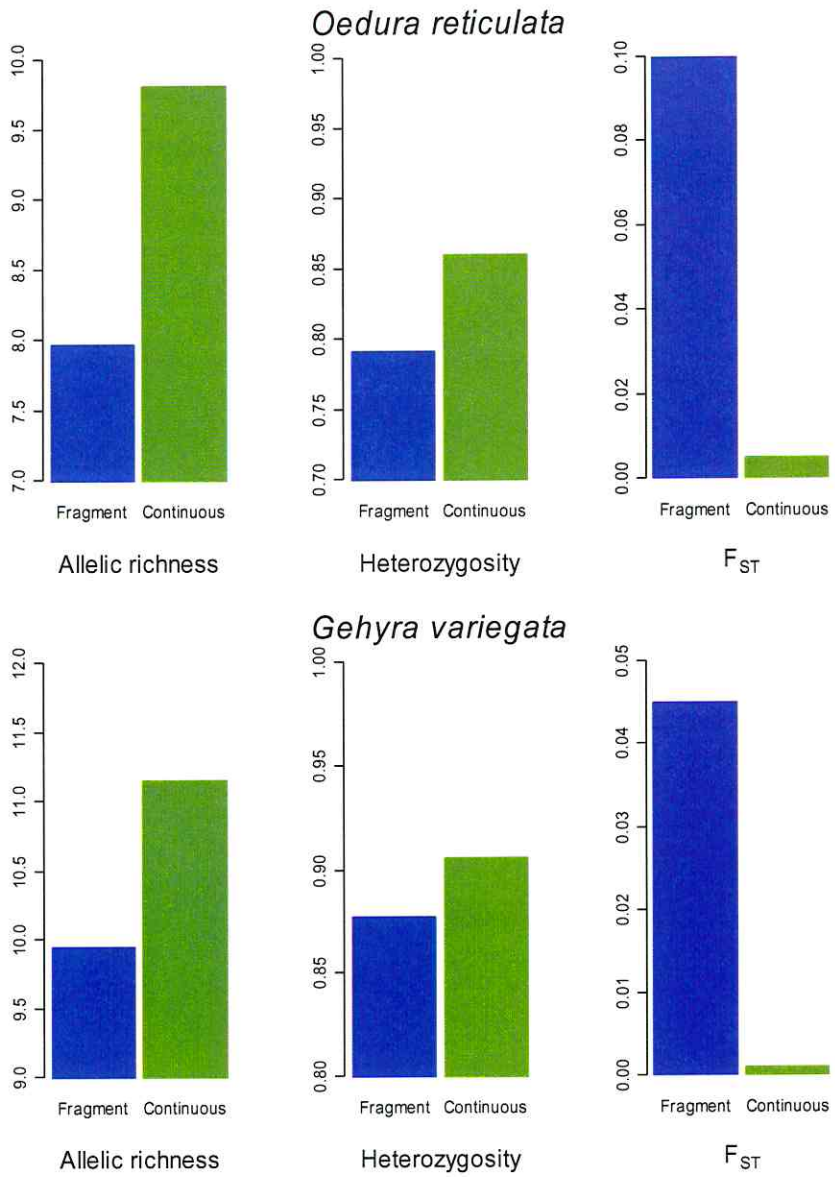



*Figure 2 Comparison of allelic richness ($A_n$), expected ($H_e$) heterozygosity and $F_{ST}$ estimates between fragments and continuous forest site for* O. reticulata *(above) and for* G. variegata *(below)*

## *Discussion*

The general patterns observed at all nine microsatellite loci in both species are consistent with the predicted genetic effects of recent habitat fragmentation. Both theoretical and experimental studies have outlined patterns of genetic differentiation expected from habitat fragmentation (Lande & Barrowclough 1987, Harrison & Hastings 1996, Templeton 1998, Spencer et al. 2000, Frankham et al. 2002). First, populations in fragmented habitats may experience restricted gene flow among populations, resulting in higher levels of genetic differentiation among populations (Harrison & Hastings 1996, Hutchison & Templeton 1999). Second, small, isolated populations may be more likely to suffer from genetic drift and inbreeding, which in turn reduces genetic diversity (Frankham 1995a, Westemeier et al. 1998, Frankham et al. 2002). Given that microsatellites exhibit several alleles per locus, a reduction in genetic variability is likely to be manifested as a reduction in allelic diversity (Spencer et al. 2000). Also, the expected levels of heterozygosity should be lower in small populations and heterozygosity levels will vary depending on the severity and length of the genetic bottleneck experienced by a population, as well as the mating system and the life history characteristics (Charlesworth & Charlesworth 1987, Spencer et al. 2000, Frankham et al. 2002).

Our research indicates that the populations of the two gecko species in the Western Australian wheatbelt have undergone severe changes in the genetic structure as a result of habitat fragmentation. Each of the three changes detected is in the direction predicted by theory. First, in both species we observed a decrease in the allelic richness within the fragmented habitats (significant for *G. variegata* and approaching significance for *O. reticulata*). Second, in both species the genetic diversity (He) was significantly lower in habitat fragments than in continuous forest sites. Both the reduction in allelic richness and genetic diversity is presumably due to increased genetic drift. Third, in both species the genetic differentiation ($F_{ST}$ and $R_{ST}$) among populations in the fragments was ten to nearly 100 times higher than between populations in the continuous forest. The latter observation can be interpreted as a recent reduction of gene flow among fragment populations with reference to the absence of historical difference in the genetic structure among regions (Sarre 1995b).

### *Population differentiation*

In accordance with our expectations the reduction of gene flow has been higher for the specialist species, which is incapable of using the matrix extensively (Sarre 1995b, chapter 2). The population genetic differentiation estimated using Slatkin`s $R_{ST}$ (Slatkin 1995) was slightly higher than $F_{ST}$ in *G. variegata* and considerably higher *in O. reticulata*. Since microsatellites are highly variable and subject to high mutation rates, they usually display high levels of within-population heterozygosity (Hedrick 1999). Therefore, statistics based on an infinite allele model that consider alleles to be identical by descent, such as $\theta$ and $F_{ST}$,

tend to underestimate population differentiation (Slatkin 1995), so that higher values of $R_{ST}$ compared to $\theta$ can be expected (Collevatti et al. 2001). The assumption of identity by decent in $\theta$ imply that differences at the locus are due to migration. In contrast, under the assumption of identity by state ($R_{ST}$), differences are due to mutations. Gaggiotti et al. (1999) performed simulations which indicated that $R_{ST}$ based estimates for Nm are more precise than $\theta$ estimates at low migration rates, but are less reliable at moderate to high migration rates. Here, we assume the specialist species to have a much lower dispersal ability than the generalist species, and as a result an $R_{ST}$ based estimation of population differentiation should be more accurate for the former, but similar for the latter as was the case in our study. However, the simulations of Gaggiotti et al. (1999) further demonstrated that $F_{ST}$ based estimates performed better than $R_{ST}$, when sample size were moderate or small ($n < 10$), and the number of loci scored was low ($l < 20$). These are the conditions under which many real investigations are carried out. In our special case, we used a considered number of samples ($n \sim 30$), but only nine loci have been scored. Therefore, the most conservative approach would be to use estimates derived from $F_{ST}$.

### *M-ratio and bottleneck*

Greater reduction in the number of alleles relative to allele size range was not detected in habitat fragments in either of the species. We predicted lower values of M-ratio for the habitat fragments compared with continuous habitats and lower values for the habitat specialist compared with the generalist species. However, our results indicate that there are no differences among habitat types and/or species. The values of M retrieved in our study are much lower than those identified across a range of taxa by Garza & Williamson (2001), but they are similar to those found for skinks by Sumner et al. (2004). This result might reflect a strong bottleneck and a reduction in population size in the fragments as well as in the Nature Reserve in both gecko species. Alternatively, the results of both studies might represent a general exception for lizards. However, it is likely that some of the assumptions of the model were violated in our analysis. For example: First, some of our loci did not fit the step-wise mutation model; they were either odd-sized and had large gaps between alleles resulting in a bimodal distributions or they were multiples of the repeat units and had ideal distribution. Second, the number of individuals was not always twice the number of alleles at the most variable locus. Third, population subdivision might have artificially reduced the value of M. The first violation would lead to an increase in r (the range between the largest and smallest allele), the second to a reduction in k (the number of alleles) and as a result to a reduction in M. To remove potential sources of violations of model assumptions, we omitted the odd-sized loci and took the data from only one population to fit the model assumptions, but we were not able to achieve any change in the result. In summary, we suggest that the violation of the model assumption might have biased our results towards a low M ratio.

## *Fragmented and continuous habitats*

Despite the concern over the impact of habitat fragmentation on biodiversity and the rapidly growing literature in this field, only a small proportion of studies include reptiles and amphibians (Mac Nally & Brown 2001). There is a great need to increase knowledge of the effects of habitat fragmentation on taxa other than birds, mammals, and insects. Many reptile species have much lower absolute mobility, which increases their vulnerability to local extinction to a greater extent than for birds, mammals, and insects (Henle & Streit 1990, Webb & Shine 1997, Coddington & Cree 1998, Hokit et al. 1999, Mac Nally & Brown 2001).

Microsatellite studies on the genetic structure of reptile populations after habitat fragmentation are few and controversial. Stow et al. (2001) found evidence for lowered dispersal between groups of Cunningham's skinks (*Egernia cunninghami*) in cleared sites. In contrast, Sumner et al. (2004) discovered that the populations of prickly forest skinks (*Gnypetoscincus queenslandia*) in rain forest remnants have only undergone small genetic changes as a result of habitat fragmentation. The impact of habitat fragmentation on the genetic structure of mammal, bird, and insect populations has received more attention than the effect on reptiles. For those taxa, higher genetic structure in fragmented compared to continuous forest populations has been reported for red-backed vole (*Clethrionomys californicus*), black grouse (*Tetrao tetrix*), capercaillie (*T. urogallus*), butterflies (*Speyeria idalia*), and beetles (*Carabus punctatoauratus; C. nemoralis*) (Tallmon et al. 2002, Brouat et al. 2003, Caizergues et al. 2003, Segelbacher et al. 2003, Williams et al. 2003). In accordance with our findings, the genetic differentiation of these species in habitat fragments was higher and/or the genetic diversity was lower than in continuous forest sites. A decrease in $H_e$ has also been documented in a large number of cases of long-isolated islands versus continental populations (Frankham et al. 2002). In a few special cases habitat fragmentation can lead to an increase in gene flow among fragmented populations of plant species, contrary to the expected pattern, because gene flow among fragmented populations separated by an open matrix may be enhanced in species that exhibit wind pollination (Young et al. 1993).

Significant levels of population differentiation and low levels of genetic variation are commonly reported in other genetic studies, but the inclusion of control populations is still more an exception than the rule. However, studies without controls cannot adequately determine whether the observed genetic patterns are the result of recent habitat fragmentation, population history, or are indicative of natural levels. Fortunately, the number of studies which include control populations and/or which determine the level of historic population structure is growing (Knutsen et al. 2000, Mossman & Waser 2001, Tallmon et al. 2002, Brouat et al. 2003, Caizergues et al. 2003, Segelbacher et al. 2003, Williams et al. 2003).

### *Multi-species studies*

There have been very few population genetic studies that include the comparison of two or more species, which are sympatrical, but contrast in their ecology, life-history traits, and rates of dispersal (Brouat et al. 2003, 2004). Such species can represent independent data points and provide important information that is difficult to gain through single-species studies. More relevant hypotheses can be tested, which provide links between questions in ecology and the evolution of species (Bohonak 1999). In our study, we predicted that a habitat specialist would be more affected by habitat fragmentation than a generalist because of its inability to move through the modified landscape. Whereas the $F_{ST}$ and $R_{ST}$ values in the forest sites were not significantly different from zero for both species, the level of genetic structure in the fragments was two times higher in the specialist than in the generalist, which indicates lower gene flow and lower levels of dispersal in the specialist (chapter 2). Nevertheless, both species showed a decrease in the number of alleles and the level of heterozygosity. Alone with a multi-species approach it was possible to draw those conclusions. Otherwise we merely would have found a significant or a non-significant decline in genetic diversity for any one species.

### *Historical levels of differentiation*

According to Sarre (1995b) the absence of clear historical isolation among populations of *O. reticulata* shows that none of the populations were phylogenetically distinct. Consequently the change in the genetic structure is due to recent, anthropogenic alterations in the habitat and not due to historical barriers (Cunningham & Moritz 1998).

### *Conservation implications*

Over the last decade human-induced fragmentation has been increasingly perceived as one of the major factors influencing the persistence of species across the planet. With increased research efforts our understanding of the processes and their impact on biota has developed considerably. Despite these advances, experimental approaches have not yet provided clear insights into the ecological mechanisms and effects of fragmentation. Fragmentation has different effects in different places, making it difficult to make generalized predictions (Norton et al. 1995, Hobbs & Yates 2003, Henle et al. 2004b).

Nevertheless, the effect of fragmentation on the two gecko species has management implications. Both species exhibited a significant decrease in allelic richness and genetic diversity and a significant increase in genetic differentiation in habitat remnants when compared with continuous forest sites in the Western Australian wheatbelt. The change in genetic differentiation was even stronger in the habitat specialist, which may indicate that specialist species are more affected due to their inability to utilize the matrix between fragments. This is also in accordance with a lower incidence of the specialist in fragments

compared to the generalist (Sarre et al. 1995) and with predictions from population viability models (Wiegand et al. 2001, 2002).

Habitat linkage and corridors appear to be the most obvious management implication in our case study. Given that they are expensive to manage and to maintain, the debate about their effectiveness and the possible negative aspects still continues (Hobbs 1992, Simberloff et al. 1992, Orrock et al. 2003, Driscoll 2004, Hoyle & Gilbert 2004). Comprehensive restoration programs and guidelines (Yates & Hobbs 1997) have been developed for the Western Australian wheatbelt, and some farmers are planting up to 100,000 trees per year (MacFarlane, pers. communications). Yet barely one percent of the land has been restored to date (pers. observation). Moreover, plants used for revegetation programs often are fast growing *Eucalyptus* species instead of native species. Improvement in this respect is required, since the restoration of original *Eucalyptus* woodland species is essential for the conservation of endemic specialist fauna species. There is a clear need to develop a general understanding of the processes involved in habitat fragmentation, with the aim of increasing our ability to answer specific management questions about particular species. This study demonstrated that the use of microsatellite markers to examine fine-scale genetic structure of fragmented populations can provide qualitative information relevant to conservation management issues. Coupled with demographic studies, this approach should enhance our understanding of complex population dynamic processes.

# Chapter IV

## Demographic and genetic estimates of effective population size ($N_e$) in two gecko species

### *Introduction*

In fragmented landscapes small population size can lead to reduced population fitness, due to loss of neutral genetic variation and fixation of mildly deleterious alleles. (Frankham 1995a, Frankham et al. 2002). The rate of these processes depends on the effective size of a population ($N_e$), rather than the actual number of living individuals, N (Wright 1938, Lande & Barrowclough 1987, Waples 1989, 2002, Nunney 1993, Kalinowski & Waples 2002). Effective population size is therefore an indicator of long-term risk of extinction from genetic factors, and thus an important parameter in evolutionary and conservation biology. $N_e$ is inversely related to the rate of loss of genetic diversity, the rate of inbreeding and the rate of extinction (Lande & Barrowclough 1987, Frankham 1995b, Saccheri et al. 1998, Luikart & Cornuet 1999). Despite the importance of $N_e$ for understanding evolutionary processes, it is notoriously difficult to estimate in natural populations (Waples 1989, Frankham 1995b, Berthier et al. 2002).

Wright (1931, 1938) originally described the concept of genetic effective size under idealized conditions: These include random mating, an even sex ratio, non-overlapping generations, variance in reproductive success, and temporally stable population size. Subsequent development of the theory for estimating $N_e$ in real populations has emerged from evaluating effects of violations of idealized assumptions (Caballero 1994, Whitlock & Barton 1997, Nunney 1999). Three variables are responsible for reducing $N_e$ below the number of sexually mature adults in a population (N): fluctuations in population size, variance in individual reproductive success above binomial expectations, and an unequal sex ratio. Frankham (1995b) suggested that fluctuation in population size was the largest variable reducing $N_e/N$ in natural populations. Others have argued that variance in reproductive success caused by high fecundity or polygamy is the major contributing factor to reducing $N_e/N$ (Nunney 1999, Storz et al. 2001).

Nunney (1993) examined the theoretical basis of the $N_e/N$ ratio and suggested it should be approximately 0.5 under most natural conditions. In a review of studies that estimated $N_e$, Frankham (1995b) found the average $N_e$ to N ratio across 102 species was 0.11, and concluded that wildlife populations generally have smaller effective population sizes than predicted by theory. Vucetich et al. (1997) resolved this apparent discrepancy by pointing out that the theory of Nunney (1991, 1993, 1996) assumes constant population size. When Vucetich et al. (1997) accounted for population fluctuation, empirical estimates were in rough agreement with theoretical expectations. To the best of our knowledge there have been few published studies that estimate the $N_e/N$ ratio in lizards (Hranitz 2000: $N_e/N$ ratio of

0.42). Among vertebrates, fish species have often been used to study $N_e/N$ ratios, due to their economic value, their high incidence of overexploitation, and because of the relatively easy access to archived scales. In fish population ratios between 0.04-0.29 have been found for Pacific salmon (*Oncorhynchus spec.*), chinook salmon (*Oncorhynchus tschawytascha)* and steelhead trout (*Oncorhynchus mykiss*) (Bartley et al. 1992, Allendorf et al. 1997, Heath et al. 2002, Ardren & Kapuscinski 2003).

Many methods for estimation of $N_e$ exist, and these fall into two categories: direct estimates of the effective size of populations requiring demographic data (reviewed by Caballero 1994) and indirect estimates requiring genetic data (reviewed by Neigel 1996). Demographic estimates of $N_e$ utilize the concepts and equations introduced by Wright (1931, 1938) and later modified by others (reviewed by Caballero 1994). These equations use extensive demographic data to account for the three effects, 1. fluctuations in population size, 2. variance in individual reproductive success above bionomial expectations, and 3. an unequal sex ratio.

Numerous procedures for estimating $N_e$ from genetic data have been developed (Hill 1981, Campton 1987, Waples 1989, 1990 a, b, 2002, Bartley et al. 1992, Pudovkin et al. 1996, Luikart & Cornuet 1999, Williamson & Slatkin 1999, Anderson et al. 2000, Wang 2001, Berthier et al. 2002, Wang & Whitlock 2003). Two commonly used indirect genetic estimators are the linkage disequilibrium and heterozygote excess methods. These methods give point estimates and have one advantage over temporal methods in that only one sample is required, provided that the sample is truly representative of the population. Effective size of a population can be estimated as soon as representative genotype data are available, which might be more time effective than mark-recapture studies. The linkage disequilibrium method of estimating $N_e$, developed by Hill (1981) and modified by Waples (1991), is based on the principle that in closed finite populations, associations between alleles at different neutral loci are a function of the population´s $N_e$. Therefore, measuring these associations between alleles should allow estimation of $N_e$. The heterozygote excess method (Pudovkin et al. 1996, Luikart & Cornuet 1999) is based on the principle that the allele frequencies will by chance be different in males and females, when the effective number of breeders in a population is small.

The temporal methods are based on the logic that if $N_e$ is the only parameter needed to determine rates of change in genetic variation at neutral loci, then a measure of genetic change over time should allow the estimation of $N_e$ (Waples 1989). In addition to the moment based temporal method (Waples 1989), recently Bayesian approaches (Berthier et al. 2002) and likelihood-based methods (Williamson & Slatkin 1999, Anderson et al. 2000, Wang 2001, Wang & Whitlock 2003) have been developed. These also require temporally spaced samples, but are more precise, using more information of the data (Edwards 1972, Berthier et al. 2002).

This chapter presents a comparative study of historical population dynamics and associated genetic changes in two populations of the gecko species *O. reticulata* (reticulated velvet gecko) and in two populations of the gecko species *G. variegata* (tree dtella). Both of these species have been subjected to severe population fragmentation in the Western Australian wheatbelt and knowledge of their effective population sizes would be extremely useful for developing management strategies. We have used long-term extensive demographic information and developed microsatellite DNA markers to directly and indirectly estimate the effective population size $N_e$ and the effective number of breeders $N_b$ for both gecko species over a period of ten years. Demographic long-term information was gained from a previous study (Sarre 1995 a, b, 1998, Sarre et al. 1995) and genetic information was assessed at two points in time by analyzing DNA from tissue samples of geckos that were collected ten years ago.

The goals of this study were to estimate $N_e$ and $N_b$ for the two populations of both gecko species, and to calculate the $N_e/N$ and $N_b/N$ ratios. We identified the most important variables contributing to reduction of the ratios derived from demographic data. In addition, we comprehensively compared the demographic and genetic estimates of $N_e$. We incorporated a variety of different measures to determine if the genetic estimates were consistent across methodologies. We expected to find that the Bayesian and the likelihood-based methods are more precise.

## *Methods*

### *Study species*

*Gehyra variegata* (tree dtella) is abundant and widespread over the southern half of Australia. It is a habitat generalist, and can be found on trees, logs, fallen timber, shrubs, rocks and in highly disturbed habitat (How & Kitchener 1983, Kitchener et al. 1988). *Oedura reticulata* (reticulated velvet gecko) is endemic to the southwest of Western Australia and is a habitat specialist. It is limited in its range of habitat, being exclusively arboreal and restricted to smooth-barked *Eucalyptus* woodlands (How & Kitchener 1983, Kitchener et al. 1988, Sarre et al. 1995). Both species have been subjected to severe population fragmentation in the Western Australian wheatbelt (How & Kitchener 1983, Kitchener et al. 1988, Sarre 1995 a, b, 1998, Sarre et al. 1995).

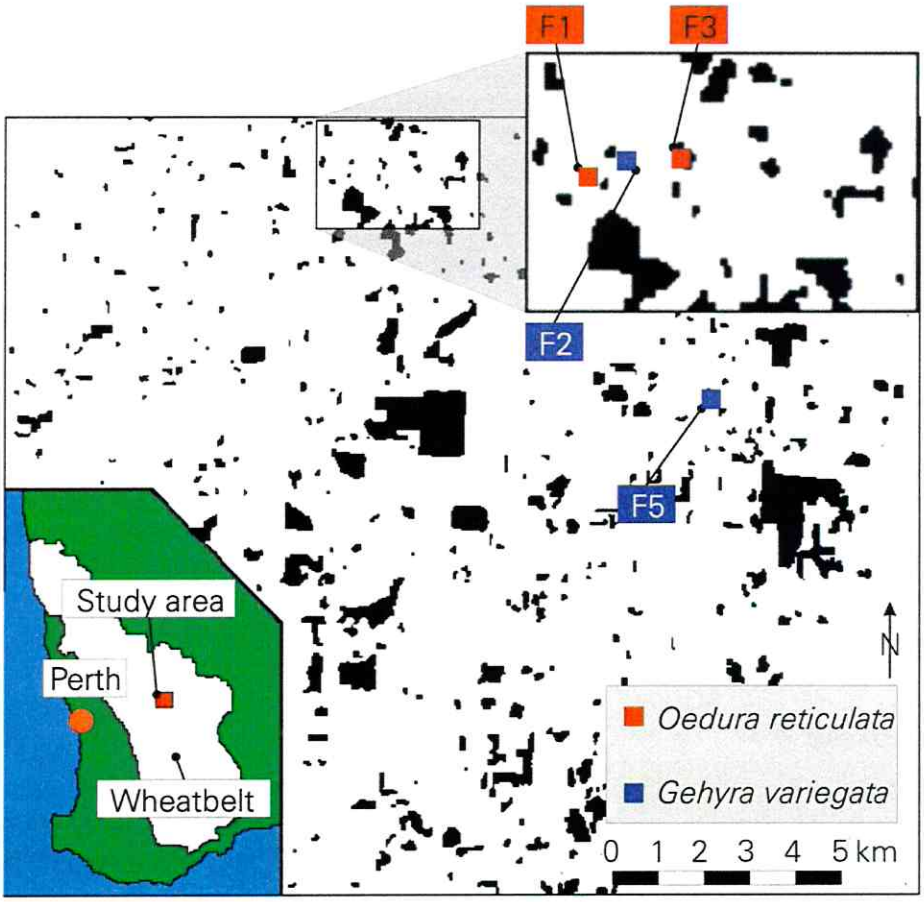



*Figure 1 Map of the study area and location of sites in the Western Australian wheatbelt.* O. reticulata *populations inhabit fragments labelled in red F1 and F3,* G. variegata *fragments are labelled in blue F2 and F5*

### *Study area*

The study area is located between Kellerberrin and Trayning in the Western Australian wheatbelt. Large areas of native vegetation have been removed from this region, and replaced by agricultural crops, pastures, and livestock. Since 1900, approximately 93% of the original vegetation has been cleared, and the remnant vegetation is distributed over thousands of patches of varying size (Saunders & Hobbs 1991, Hobbs 1993, Hobbs & Saunders 1993, Saunders et al. 1993). The gecko populations are located in these small woodland remnants that are separated from other woodland by land cleared for agriculture (details in chapter 2 and 3).

Two populations (F1 and F3) of the species *O. reticulata* and two populations (F2 and F5) of the species *G. variegata* were chosen for inclusion in the present study based on the fact that tissue samples and demographic data were collected from these populations in 1989-1991 (Sarre 1995a, b, 1998, Sarre et al. 1995). Each of these populations was re-sampled in 2000. Locations of the study habitat fragments are displayed in Figure 1.

Tissue samples were collected during the summer months, from November 2000 to March 2001. Lizards were spotted at night using head-torches and were captured by hand. The tip of the tail of each individual sampled was removed and stored in liquid nitrogen, and the snout-vent length, body mass, sex and age of each individual sampled were recorded. For each population, the sex ratio and adult: subadult ratio were calculated from the percentage of adult females and subadults plus juveniles sampled respectively.

### *Census population size*

A capture-mark-recapture study was conducted to estimate the census populations N of the two gecko species. The individuals were captured, marked by toe-clipping and released. Individuals were then recaptured during four to eight subsequent sessions (the number depending on the population size). Population size was estimated using the program *CAPTURE* (Otis et al. 1978). *CAPTURE* allows moderation of the often unrealistic assumption of equal capture probabilities over the period of the study (Model $M_0$). Otis et al. (1978) distinguished between three different sources of variability in capture probabilities: individual heterogeneity (model $M_h$), behavioural response to capture (model $M_b$), and temporal variation (model $M_t$). In *CAPTURE*, all possible combinations of these models (except $M_{thb}$) provide estimates for the numbers of individuals in a population.

### *Demographic estimates of $N_b$ and $N_e$*

We used the method of Lande & Barrowclough (1987) for combining $N_e$ estimates gained from each demographic variable (sex ratio, variance in reproductive success, fluctuating population size) into a single comprehensive $N_e$ estimate. The Lande and Barrowclough

(1987) estimator assumes discrete generations and was therefore modified in the method of Waples (2002), which accounts for overlapping generations (Ardren & Kapuscinski 2003). Our data on the variance in the reproductive success of male and female geckos is limited. Thus, in the present study only the unequal sex ratio and the fluctuation in population size were considered.

The reduction in $N_b$ caused by an **unequal sex ratio** is defined as :

$$N_{b(demo)} = \frac{4(N_f \cdot N_m)}{N_f + N_m}, \qquad (1)$$

where $N_f$ represents the number of sexually mature females, and $N_m$ represents the number of sexually mature males in any given year (Waples 2002).

The method of Waples (2002) provides a comprehensive way to account for the influence of **fluctuating population size** on $N_e$. This method incorporates the information from $N_b$ estimates from equation 1 into a single-generation $N_e$ estimate for populations that fluctuate in size. The following equation provides an estimate of effective population size over one full generation:

$$N_e = \frac{1}{\sum(X_i^2 / N_{b(demo)i})}, \qquad (2)$$

where $N_b$ is the effective population size of all breeding animals in brood year $i$ and $X_i$ is the proportional contribution of individuals in year $i$ to the next generation (Waples 2002, Ardren & Kapuscinski 2003).

In the present study we considered the estimation of the number of effective breeders in 1990 and 2000 as an approximation for the single generation effective size. Due to limitations in the data the proportional contribution $X_i$ and the brood year was considered to equal 1. Thus, the effective number of breeders equals the effective populations size of a single generation.

Effective population size can be defined for different time frames, for a single generation and, for several generations (Kalinowski & Waples 2002). Whereas equation 2 calculates the effective population size of a single generation, the effective size over $k$ generations, called the multigeneration effective size, is the harmonic mean of the single generation effective size (Wright 1938, Kalinowski & Waples 2002). In our study the harmonic mean was used to calculate the multigeneration effective population size over two generations.

$$N_e = \frac{1}{\left(\frac{1}{N_{b1990}} + \frac{1}{N_{b2000}}\right)/2} \quad (3)$$

where $N_b$ is the effective number of breeders in 1990 and 2000.

### *Genetic analysis*

DNA was extracted from the tip of the tail of each individual using the Chelex extraction method. We genotyped individuals of *O. reticulata* using nine tetranucleotide microsatellite loci developed from an enriched library for this species (OR205, OR220, OR266, OR6F4, OR10H7, OR11G3, OR12D7, OR12D9, OR14A7) (Hoehn & Sarre subm.). For *G. variegata*, we genotyped individuals using nine tetranucleotide microsatellite markers cloned from an enriched library for this species (GV1C5, GV1C10, GV1F1, GV3B5, GV3C6, GV3E10, GV4B6, GV4G6, GV4C9) (Hoehn & Sarre in prep.). Polymerase chain reaction (PCR) amplification and genotyping on the Beckman Coulter Sequencer CEQ-8000 were performed according to conditions described in Hoehn & Sarre (subm.) and Hoehn & Sarre (in prep.).

### *Genetic estimates of the effective population size*

We used the program *NeEstimator* (Peel et al. 2004) to obtain a genetic estimate of $N_e$ using five different methods: linkage disequilibrium method, heterozygote excess method, moment-based method, Bayesian method and likelihood-based method. The first two methods are point estimates and have one advantage over the temporal methods (method 3 to 5) in that only one sample is required, provided that the sample is truly representative of the population. Effective size of a population can be estimated as soon as representative genotype data are available.

1. The linkage disequilibrium method (Hill 1981, Campton 1987, Bartley et al. 1992) implies that the linkage disequilibrium and the correlation among alleles at different loci are related to the effective size of a population. In an ideal, infinite, randomly-mating population, both linkage disequilibrium and the correlation among alleles at different loci will be zero. However, in a real, finite population, both values depart from zero due to genetic drift, migration, selection and physical linkage.

2. The heterozygote excess method implemented in *NeEstimator* (Peel et al. 2004) examines the excess of heterozygotes in a sample compared to the proportion predicted under Hardy-Weinberg equilibrium. It is based on the principle that the allele frequencies will by chance be different in males and females, when the effective number of breeders in a population is small (Pudovkin et al. 1996, Luikart & Cornuet 1999).

3. The temporal moment-based method (Waples 1989) implemented in *NeEstimator* is an F statistic estimator of the variance effective size (Berthier et al. 2002). In general, the temporal method estimates the effective population size from examining allele frequency variation across generations. When the number of generations between the samples is larger than one, the result is the harmonic mean of each generation's effective population size. Allele frequencies can be calculated for all observed alleles for each sampling year within a population. Standardized allele variance $F_c$, can be estimated for each locus following Nei & Tajima (1981) (summarized in Arden & Kapuscinski 2003):

$$\hat{F}_c = \frac{1}{L}\sum_{i=1}^{L}\frac{(X_{oi} - X_{ti})^2}{(X_{oi} + X_{ti})/2 - X_{oi} + X_{ti}} \qquad (4)$$

where $L$ is the number of alleles and the values of $X_{0i}$ and $X_{ti}$ are the frequencies of allele $i$ ($i = 1,2,...L$) at sampling time $0$ and $t$ respectively. For multiple loci, the weighted mean $F_c$ is calculated as:

$$\hat{F}_c = \frac{\sum(L_j F_{cj})}{\sum L_j} \qquad (5)$$

where the value $j$ is the index of the different loci (Nei & Tajima 1981). The 95% confidence limits can be determined according to the method of Waples (1989).

4. In addition to the moment based method, *NeEstimator* has been designed to interact with a third party method called TM3 (Berthier et al. 2002). This is a Bayesian approach based on coalescence and also requires two temporally spaced samples (i.e. a difference of one or more generations between samples).

5. Finally, recently published likelihood-based methods are also incorporated into *NeEstimator* (Williamson & Slatkin 1999, Anderson et al. 2000, Wang 2001, Wang & Whitlock 2003). Likelihood-based estimators provide higher precision compared with moment-based estimators, because they use more of the information of the data (Edwards 1972, Berthier et al. 2002).

## Results

### Demographic estimates

**Census population size**: Estimates of N are mark-recapture estimates of the entire population (including juveniles and subadults). The number of individuals in the species *O. reticulata* stayed stable in both populations: estimates for fragment F1 were 194 individuals in 1990 and 197 in 2000, and for fragment F3, 22 individuals in 1990 and 33 in 2000. In *G. variegata*, the population size N increased in both populations over the same time period, from 36 to 76 in fragment F2 and from 32 to 50 in fragment F5 (Table 1).

**Influence of age structure**: In *O. reticulata* the mean number of adults $N_a$ (harmonic mean over the ten year period) was 106 in fragment F1 and 13 in fragment F3. The age structure of the population had a large influence on the $N_a/N$ ratio, which was on average (harmonic mean) 0.55 in F1, 0.50 in F3, and 0.53 over both populations. This indicates that nearly half of the population was subadult or juvenile individuals, which do not contribute to the effective number of breeding individuals. In *G. variegata*, the mean number of adults was 40 in F2 and 29 in F5. Lower proportions of subadults and juveniles accounted for a $N_a/N$ ratio of 0.77 in F2, 0.74 in F5, and 0.76 over both populations. Consequently, a higher percentage of individuals contributed to the effective number of breeders in *G. variegata* (Table1).

**Influence of sex ratio**: In *O. reticulata* the mean number of females $N_f$ (harmonic mean over the ten year period) is 65 in F1 and ten in F3. The mean number of breeding individuals $N_b$ (harmonic mean over the ten year period) was 97 in F1 and nine in F3. The biased sex ratio resulted in some reduction in the number of effective breeders in *O. reticulata*. The resulting $N_b/N_a$ ratio was 0.93 in F1, 0.72 in F3, and 0.83 over both populations. The reduction was higher in fragment F3 with a very small census population size and a biased sex ratio in both years (particularly in 1990). In *G. variegata* the mean number of females was 18 in F2 and 14 in F5, whereas the mean number of breeding individuals was 39 and 28, respectively. The $N_b/N_a$ ratio was 0.98 in F2, 0.97 in F5, and 0.98 over both populations. This resulted from a sex ratio that was nearly equal (40-56% females) in both fragments in both sampling years, and which had little effect on the effective number of breeders in *G. variegata* (Table 1).

**Influence of fluctuating population size**: To isolate the impact of the fluctuating population size on the effective population size (Table 2), we calculated the harmonic mean of the effective number of breeders over the entire time period ($N_e$) and compared it to the arithmetic mean ($N_{b\ arith}$). For *O. reticulata* the harmonic mean of the effective number of breeders ($N_e$) was 97 individuals in fragment F1 and nine individuals in fragment F3. The influence of the fluctuation in population size contributed to a $N_e/N_{b\ arith}$ ratio of 0.92 in F1 and 0.82 in F3, with a mean of 0.87. For *G. variegata* the effective number of breeders (harmonic mean) was 39 individuals in F2 and 28 individuals in F5. The influence of the fluctuation in

population size over the time period was lower in *G. variegata* than in *O. reticulata* with a overall $N_e/N_{b\ arith}$ ratio of 0.94 (0.95 in F2 and 0.93 in F5).

*Table 1 Estimates of $N_a$ and $N_b$ for two population of* O. reticulata *and* G. variegata *over a period of ten years. N is the population size of the entire population with lower and upper 95% confidence interval (LCI and UCI), $N_a$ is the number of the adults, $N_f$ is the number of females, % F is the percentage of females in the population and $N_b$ the effective number of breeders based on bias in sex ratio*

| **Species** | **Fragment** | **Year** | **N** | **LCI** | **UCI** | **$N_a$** | **$N_a/N$** | **% F** | **$N_f$** | **$N_b$** | **$N_b/N_a$** | **$N_b/N$** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *O. reticulata* | F1 | 1990 | 194 | 152 | 235 | 89 | 0.46 | 69 | 61 | 77 | 0.87 | 0.40 |
| *O. reticulata* | F1 | 2000 | 197 | 162 | 261 | 132 | 0.67 | 53 | 70 | 132 | 1.00 | 0.67 |
| Harmonic Mean | | | 195 | | | 106 | 0.55 | | 65 | 97 | 0.93 | 0.50 |
| *O. reticulata* | F3 | 1990 | 22 | 12 | 32 | 10 | 0.45 | 78 | 8 | 6 | 0.60 | 0.27 |
| *O. reticulata* | F3 | 2000 | 33 | 30 | 42 | 18 | 0.55 | 67 | 12 | 16 | 0.89 | 0.48 |
| Harmonic Mean | | | 26 | | | 13 | 0.50 | | 10 | 9 | 0.72 | 0.35 |
| Mean | | | | | | | 0.53 | | | | 0.83 | |
| *G. variegata* | F2 | 1990 | 36 | 33 | 43 | 33 | 0.92 | 53 | 17 | 33 | 1.00 | 0.92 |
| *G. variegata* | F2 | 2000 | 76 | 65 | 101 | 50 | 0.66 | 40 | 20 | 48 | 0.96 | 0.63 |
| Harmonic Mean | | | 49 | | | 40 | 0.77 | | 18 | 39 | 0.98 | 0.75 |
| *G. variegata* | F5 | 1990 | 32 | 26 | 54 | 24 | 0.75 | 40 | 10 | 23 | 0.96 | 0.72 |
| *G. variegata* | F5 | 2000 | 50 | 41 | 82 | 37 | 0.74 | 56 | 21 | 36 | 0.97 | 0.72 |
| Harmonic Mean | | | 39 | | | 29 | 0.74 | | 14 | 28 | 0.97 | 0.72 |
| Mean | | | | | | | 0.76 | | | | 0.98 | |

**Comprehensive $N_e$ estimates**: The comprehensive $N_e/N_a$ estimate over the entire time and averaged for both populations in *O. reticulata* was 0.76. This was a result of the cumulative effect of the sex ratio and the fluctuating population size. If we include the effect of age structure the $N_e/N$ estimate over the entire time and averaged for both populations was 0.40. For *G. variegata* the influence of the sex ratio and fluctuating population size was lower with a comprehensive $N_e/N_a$ estimate of 0.92 over the entire time and averaged for both populations. Likewise, the age structure had less influence than in the other species with an $N_e/N$ estimate of 0.69 over the entire time and averaged for both populations (Table 2).

*Table 2 Estimates of the demographic $N_e$ for two population of* O. reticulata *and* G. variegata *as a harmonic mean over a period of ten years. $N_{b\ (arith)}$ is the arithmetric mean of the effective number of breeders and $N_a$ is the arithmetric mean of the adults*

| Species | Fragment | Mean N | Mean $N_a$ | $N_{b\ arith}$ | $N_e$ | $N_e/N_{b\ arith}$ | $N_e/N_a$ | $N_e/N$ |
|---|---|---|---|---|---|---|---|---|
| *O. reticulata* | F1 | 196 | 111 | 105 | 97 | 0.92 | 0.87 | 0.49 |
| *O. reticulata* | F3 | 28 | 14 | 11 | 9 | 0.82 | 0.64 | 0.32 |
| Mean | | | | | | 0.87 | 0.76 | 0.40 |
| *G. variegata* | F2 | 56 | 42 | 41 | 39 | 0.95 | 0.93 | 0.70 |
| *G. variegata* | F5 | 41 | 31 | 30 | 28 | 0.93 | 0.90 | 0.68 |
| Mean | | | | | | 0.94 | 0.92 | 0.69 |

## *Genetic estimates*

Estimated effective population size was compared for five different methods (Table 3). In general, the estimates differ greatly between methods. In *O. reticulata* the estimates of the temporal methods (moment based, Bayesian, and pseudo-likelihood) delivered relatively consistent results (86.9-264.3 in F1 and 79.7-186.8 in F3). The estimates derived from the linkage disequilibrium were totally different from the other estimates, but very similar to the demographic estimates. (38.5 and 14.8, respectively). The estimates derived from this method were much lower for the populations of both species than the estimates from the temporal method. The heterozygote excess method provided imprecise, infinite estimates in all populations. This method usually requires relatively high numbers of samples to produce high quality estimates. For example, in Bartley et al. (1992) the minimum average sample size was 92.3 individuals.

In *G. variegata* the Bayesian and likelihood-based approaches provided unusually high $N_e$ estimates (7145.4-9953.4 in F2 and 2334.4 to 6117.1 in F5). In comparison, the results from the linkage disequilibrium method were much lower (29.0 and 25.7, respectively) and the results from the heterozygote excess method infinite.

*Table 3 Estimates of the genetic $N_e$ for two population of* O. reticulata *and* G. variegata *based on several methods: linkage disequilibrium, heterozygote excess, moment based temporal, Bayesian method TM3 and pseudo-likelihood MLNE*

| | ***O. reticulata*** | | ***G. variegata*** | |
|---|---|---|---|---|
| | F1 | F3 | F2 | F5 |
| Linkage disequilibrium | 38.5 | 14.8 | 29.0 | 25.7 |
| Heterozygote excess | ∞ | ∞ | ∞ | ∞ |
| Moment based temporal | 243.3 | 79.7 | ∞ | 256.5 |
| Bayesian method TM3 | 264.3 | 186.8 | 7145.4 | 6117.1 |
| Pseudolikelihood MLNE | 86.9 | 88.6 | 9953.4 | 2334.4 |

## *Comparison of genetic and demographic based estimates of $N_e$*

Differences in the ratios of effective population size derived from genetic and demographic methods are shown in Table 4. There was no general relationship between $N_{e(gen)}$ and $N_{e(demo)}$ or $N_a$ or N over all methods. The genetic method that best matched the estimates derived from the demographic data was the linkage disequilibrium method. The $N_{e(gen)}/N_a$ ratios (0.3-1.1) were in agreement with the ratios predicted from theory and other empirical studies. The result is surprising due to the fact that the linkage disequilibrium method relies on one set of samples in time and is predicted to be less precise than the temporal methods. In *O. reticulata* the genetic based estimates (derived from all methods) are of about the same order of magnitude as the demographic results in fragment F1 ($N_{e(gen)}/N_a$ ratios 0.3-2.4), but vary to a higher extent in fragment F3 ($N_{e(gen)}/N_a$ ratios 1.1-13.3). In *G. variegata* genetic and demographic estimates diverge considerably ($N_{e(gen)}/ N_a$ ratios 0.7-237.0).

*Table 4 Comparison of the genetic-based estimate of $N_{egen}$ with the number of adults (derived from demographic data) and the demographic estimates of $N_{ademo}$ for two population of* O. reticulata *and* G. variegata

| | ***O. reticulata*** | | ***G. variegata*** | |
|---|---|---|---|---|
| | F1 | F3 | F2 | F5 |
| $N_{egen}/N_a$ | | | | |
| Linkage disequilibrium | 0.3 | 1.1 | 0.7 | 0.8 |
| Heterozygote excess | ----- | ----- | ----- | ----- |
| Moment based temporal | 2.2 | 5.7 | ----- | 8.27 |
| Bayesian method TM3 | 2.4 | 13.3 | 170.1 | 197.3 |
| Pseudolikelihood MLNE | 0.8 | 6.3 | 237.0 | 75.3 |
| $N_{egen}/N_{ademo}$ | | | | |
| Linkage disequilibrium | 0.4 | 1.6 | 0.7 | 0.9 |
| Heterozygote excess | ----- | ----- | ----- | ----- |
| Moment based temporal | 2.5 | 8.9 | ----- | 9.16 |
| Bayesian method TM3 | 2.7 | 20.8 | 183.2 | 218.5 |
| Pseudolikelihood MLNE | 0.9 | 9.8 | 255.2 | 83.4 |

## Discussion

### Demographic-based estimates

The connection between $N_e$ and life history, behavioural ecology, and demography is becoming better understood (Wang & Cabellero 1999, Turner et al. 2002). $N_e$ is usually lower than N due to a variety of factors, including fluctuating population size, small initial population, unequal sex ratio and variance in family size. When each demographic variable was examined independently in the two populations of *O. reticulata*, the age structure resulted in the largest reduction of $N_e/N$, followed by the bias in the sex ratio, and then the fluctuation in population size between 1990 and 2000. The impact of fluctuating populations and sex ratio did not differ to a great extent. Both populations consisted of an unusually high number of subadults and juveniles and showed an odd sex ratio. Sarre (1995a) suggested that the inability of natal dispersal to other remnants might have caused that bias. This lack of dispersal ability is due to the fact that most of the vegetation surrounding the habitat remnants has been cleared, and the habitat specialist species has only a limited ability to utilize the remaining matrix for dispersal.

The influences of demographic parameters on the reduction of $N_e/N$ was comparable in *G. variegata*. The age structure had the highest impact, followed by the fluctuation in population sizes between 1990 and 2000, whereas the sex ratio did not have much impact. The reduction of $N_e/N$ due to the age structure was much lower in the generalist species than in the specialist species, suggesting that different life-history characteristics and external factors might lead to differences in the reduction of the census population size. Jehle et al. (2001) reported that newts differ to toads in having an even sex ratio, because female limit their risk of reproductive failure by distributing eggs over pond areas. These characteristics probably account for $N_b/N$ values that were 20 times higher for newts than for toads (Jehle et al. 2001). Our results may reflect the fact that the two gecko species contrast in their level of specialization and also that the study was carried out in fragmented landscapes. Fragmentation limits dispersal of the specialist species to a greater extent than it does in the generalist species (chapter 2). *G. variegata* populations may be able to form a metapopulation that allows offspring to disperse and immigrants to contribute to equal sex ratios. The opposite is probably true for the specialist species *O. reticulata,* which is reflected in a high proportion of juveniles in the population and an unequal sex ratio. Another explanation for the high rates of juveniles and subadults could be that in *O. reticulata* adults reach maturity later (4.8 years) than in *G. variegata* (2.8 years). Consequently, more immature individuals accumulate in the populations contributing to a biased juvenile/subadult: adult ratio.

Ardren & Kapuscinski (2003) found that variation in reproductive success had the most substantial impact on the reduction of demographic $N_e/N$ in a population of steelhead trout. Our data is limited in terms of estimating the variation in reproductive success. However, in

comparison to steelhead trout, gecko females produce a smaller number of offspring (two eggs per year). This would suggest there is low variability in the reproductive success of females. Nevertheless, some variation exists due to differences in the territoriality and longevity of females and mortality rates of the offspring (Henle 1990). In gecko males a higher number of 'floaters' that lack a territory might result in a higher variation in the reproductive success. Future research should improve our understanding of the variation in the reproductive success of these species.

Small initial population size, or population bottlenecks, can also lead to reduced $N_e$ (Franklin 1980, Garza & Williamson 2001, Shrimpton & Heath 2003). In addition, several authors state that the mating system has a high impact on the reduction in $N_e/N$ (Hilborn & Walters 1992, Nunney 1993, Nunney 1996). In chapter 2 we hypothesized that both species show similarities in their mating system. Gruber & Henle (subm.) primarily described territoriality for males and females in the species *G. variegata* in a study in the Kinchega NP, Australia.

### *Genetic-based estimates*

The genetic-based estimates of $N_e/N$ were quite different from the demographical estimates. The genetic and demographic estimates are in general agreement in only one population of *O. reticulata*. In the other population of this species the genetic estimate is much higher than the actual census population size. In *G. variegata* genetic and demographic estimates diverge considerably. This raises the question as to whether the genetic based methods are intrinsically biased. In contrast to our results, studies that have compared genetic and demographic estimates of the effective population size have found that genetic-based estimates are often substantially smaller than ecological estimates (Husband & Barrett 1992, Shrimpton & Heath 2003). We also found the different genetic estimation methods produced rather inconsistent results. The discrepancy may be due to differences in the mutation model used in different methods. In addition, the assumption of discrete generations and constant population size may have been violated. Surprisingly, the linkage disequilibrium method best matched the estimates derived from the demographic data (Hill 1981, Waples 1991). The linkage disequilibrium method is based on the principle that in closed finite populations, associations between alleles at different neutral loci are a function of the population´s $N_e$. Therefore, measuring these associations between alleles should allow estimation of $N_e$ (Hill 1981, Waples 1991). In contrast to the temporal methods, the linkage disequilibrium method uses less data from only one genetic sample in time and is predicted to be less precise. Estimating $N_e$ by likelihood and Bayesian methods should have advantages over estimating $N_e$ using F-statistics. Until recently it has been impractical to compute the likelihood using all the data when loci with more than two alleles were available. This approach discards some important information, whereas the likelihood methods allow full use of the data and are a preferable option for analyzing temporal data (Williamson & Slatkin 1999, Anderson et al. 2000, Wang 2001, Wang & Whitlock 2003).

While it may be that the genetic methods were biased, there could be an alternative explanation for the high estimates of the *G. variegata* populations. Consideration of the assumptions underlying the temporal methods (no mutation, neutral alleles, no migration, random mating and random sampling) suggests that the assumption of no migration was probably violated. In chapter 2 we found that populations of *G. variegata* are less differentiated, disperse larger distances and have higher dispersal rates than *O. reticulata*. While the remnants had very defined edges and the effective population size derived from the demographic data had a defined size, the genetic neighbourhood size of these populations could have been much larger. Such a difference could have created smaller demographic estimates relative to genetic-based estimates of $N_e$. Another factor that could lead to low census estimates but high genetic estimates, is if current population size is small relative to the size in historic populations (Gerber & Templeton 1996, Matocq 2004). Since we found an increase in both *G. variegata* populations over the entire time period this explanation seems rather unlikely. Finally, just as differentiation among subpopulations can contribute to the maintenance of genetic variation in the total population, differentiation among breeding groups can influence maintenance of variation within a subpopulation. This could result in higher levels of genetic diversity than would be expected based on the assumption of random mating in the subpopulation (Matocq 2004). The existence of breeding groups would be an important consideration in estimates of effective size, but we have limited data on the existence of such groups.

### *$N_e$/N ratios in other taxa*

Because census sizes are the only demographic data available for most populations, the ratio $N_e$/N or $N_b$/N is a critical parameter for monitoring changes in genetic diversity within populations. Kalinowski & Waples (2002) and Ardren & Kapuscinski (2003) suggested that taxon-specific $N_e$/N or $N_b$/N ratios would be useful because it would be possible to predict the rate of genetic loss in populations by simply estimating N. However, their studies demonstrated that a constant ratio over time cannot be assumed in wild populations. Additionally, our study showed that the ratio is not constant across populations. Still, the ratio of $N_e$/N is an important parameter to understand genetic drift in natural populations. Nunney (1993) demonstrated theoretically that the ratio of $N_e$/N should be close to 0.5 in an ideal population, and that it is unusual for $N_e$/N to be outside the range of 0.25-1.0. The ratios calculated from the demographic data in our study are within that range, whereas the genetic-based estimates are outside the range, which is probably due to violations of assumptions. Frankham (1995b) calculated $N_e$/N-values for 102 species from published sources of demographic and genetic data, and found an average value of 0.11. In this analysis, the main factors responsible for low $N_e$/N estimates were fluctuation in population size, variance in family size, and to a lesser extent unequal sex-ratio. Vucetich et al. (1997) theoretically derived $N_e$/N for 44 populations to obtain an average of 0.21. There have been extremely few studies on the effective population size in lizard species. Hranitz (2000) found

a $N_e/N$ ratio of 0.42 and an effective population size of 46 in the collared lizard. Estimates of the $N_e/N$ ratio in amphibian populations are in a similar range or below: 0.09-0.16 in the newts *Triturus cristatus* and *T. marmoratus* (Jehle et al. 2001), 0.44 in *Rana sylvatica* (Berven & Grudzien) and 0.005-0.012 in *Bufo bufo* (Scribner et al. 1997).

### *Conservation implications*

All finite populations lose genetic diversity and increase their rate of inbreeding at the rate of ½ $N_e$ per generation. In our study the populations had an demographically estimated $N_e$ of 9 and 97 in *O. reticulata* and 39 and 28 in *G. variegata*. This indicates that most of the populations had an effective size below 50. At these levels, the populations are theoretically losing genetic diversity at a rate fast enough to permit immediate concerns about inbreeding or loss of heterozygosity. The consequences of such low $N_e$ may be profound, as inbreeding may lead to reduced fecundity, juvenile survival and lifespan (Frankham 1995b). Waples (1990a, b) showed that low-frequency alleles are subject to rapid extinction in Pacific salmon where $N_e$ is less than 500. Theory suggests low $N_e$ leads to inbreeding depression and a lack of response to stochastic events. Thus, populations with small $N_e$ are at a much greater risk of extinction, but also may give little warning of impending extinction (Frankham 1995b).

# Chapter V

## Temporal changes in demographic and genetic structure of gecko populations

### *Introduction*

Genetic information provided by microsatellite data and innovations in analytical and statistical tools that deal with large quantities of genetic information have improved our understanding of genetic diversity, genetic differentiation, gene flow, dispersal and migration related issues, relatedness and parentage (Luikart & England 1999, Hansen et al. 2002). However, single genetic surveys of a population or series of populations are limited in their explanatory power because of the number of factors that may influence the way in which the genetic variation is distributed. A deeper understanding of the biological significance of the genetic structure of populations, beyond the mere demonstration of genetic differentiation, requires supplementary information. Temporal surveys involving separate surveys of the same populations can provide such additional insights into the dynamics of interconnected populations beyond those offered by spatial studies from a single snapshot in time (Heath et al. 2002, Guinand et al. 2003, Arnaud et al. 2004).

First, historical samples can serve as a valuable source of reference with which to interpret contemporary levels of genetic diversity (Arnaud et al. 2004, Guinand et al. 2003). For example, knowledge of historical patterns of genetic diversity facilitates assessment of the loss of genetic diversity. Second, many natural populations are unstable over time, and may consist of local populations subjected to more or less frequent extinction and recolonization events (Frank et al. 1994, Hanski 1999, Ives & Whitlock 2002). At the same time, studies involving both genetic structure and genetic diversity rely on point samples, thus they implicitly assume that genetic structure or diversity are relatively stable over time (Leberg 1992, Waples 1998, Garant et al. 2000, Heath et al. 2001, chapter 3). Theoretically, this can not be accurate due to genetic drift in all but infinite populations. Such studies interpret outliers in the genetic diversity as evidence for genetic bottlenecks, and assume that observed differences are due to relatively recent changes in genetic diversity as a result of human mediated environmental processes. These assumptions are unlikely to be true in all cases and require the interpretation of genetic variation in terms of contemporary and historical processes, which are often difficult to tease apart. Ideally, researchers should assess historic samples to test assumptions concerning the history of genetic changes (Nielsen et al. 1997, 1999a, b, Queney et al. 2000, Heath et al. 2002). Accordingly, population-level studies using DNA from historical samples have become increasingly common (Hansen et al. 2002, Heath et al. 2002, Ardren & Kapuscinski 2003, Guinand et al. 2003, Shrimpton & Heath 2003). Among vertebrates, fish species have often been used to study historical patterns because of their economic value, their high incidence of

overexploitation, and because of the relatively easy access to archived scales. In contrast, few studies have included amphibians or reptiles (Jehle et al. 2001).

The interpretation of genetic surveys of natural populations can also be improved through combination with demographic analysis (Clarke & Young 2000). The integration of genetics and demography and their interaction is particularly pertinent to understanding extinction processes and achieving effective conservation management and long-term population and species survival by producing more realistic population viability analysis (PVA) (Menges 2000). Although the demographic (Saunders et al. 1991, Hobbs 1993) and genetic consequences (Young et al. 1996, Frankham et al. 2002) of habitat fragmentation have been documented, very few attempts have been made to examine these simultaneously (Nunney & Campell 1993, Lindenmayer & Peakall 2000, Young & Clarke 2000). The effects of habitat fragmentation are complicated because many impacting factors do not act in isolation, but are in fact interactive or cumulative in their influence on the dynamics of populations (Gilpin & Soulé 1986, Lindenmayer & Peakall 2000, Young & Clarke 2000). Habitat loss and fragmentation reduce population size and change the spatial distribution of remaining subpopulations by confining them to remnant patches. Reduced population size and the isolation of subpopulations, in return, result in increased genetic drift and inbreeding, leading to loss of heterozygosity and genetic variation, thereby increasing genetic differentiation among populations. Under increased inbreeding the interplay between inbreeding depression and demography (e.g. juvenile fitness and mortality rates among offspring) (Lacy 1993, Lacy & Lindenmayer 1995, Lindenmayer & Peakall 2000) may reduce population growth rates and overall population size, problems further exposing populations to increased inbreeding and genetic drift (Gilpin & Soulé 1986). In addition, there may have been pre-fragmentation isolation effects, which distort the interpretation of the distribution of genetic variation. These complicated effects of habitat fragmentation can only be disentangled by an integration of demographic and genetic surveys (Lindenmayer & Peakall 2000).

We have conducted a long-term, interdisciplinary study that combines population demography and population genetics to examine the response of two gecko species to habitat fragmentation in the Western Australian wheatbelt. Large areas of native vegetation have been removed from this region, and replaced by agricultural crops, pastures, and livestock. Since 1900 approximately 93% of the original vegetation has been cleared, and the remnant vegetation is distributed over thousands of patches of varying size (Saunders & Hobbs 1991, Hobbs 1993, Hobbs & Saunders 1993, Saunders et al. 1993). Here, we describe an interactive genetic and demographic study that presents a temporal analysis of microsatellite allele frequencies at nine highly polymorphic loci in two populations of the specialist gecko species *O. reticulata* (reticulated velvet gecko) and two populations of the generalist species *G. variegata* (tree dtella) over a period of ten years (1990-2000). We also present a temporal mark-recapture study, which estimates the census population sizes in five populations of each species over time. With supplementary information about sex ratio

and fluctuations in population size, the effective population size has been calculated and compared to the census population size.

We used the historic genetic and demographic data to test the hypothesis that genetic and demographic erosion has occurred in isolated gecko populations over a period of ten years. We expected to find a decline in allelic richness and genetic diversity and an increase in genetic differentiation among fragmented populations. In addition, we considered it likely that there would have been a decline in the census and effective population sizes over the same time period. These declines were related to the difference in the level of specialization and dispersal ability between these two species (see chapter 2 and 3 for detailed information). For example, we suggested that genetic diversity would decline more strongly in the specialist species.

## *Method*

### *Study area and sampling*

The study area was located between Kellerberrin and Trayning in the Western Australian wheatbelt. The field study was carried out during the summer months, from 1989 until 1991, and from November 2000 until March 2001. Lizards were spotted at night using head-torches and were captured by hand. Locations of the study remnants are provided in Table 1 and displayed in Figure 1. Two populations (F1 and F3) of the species *O. reticulata* and two populations (F2 and F5) of the species *G. variegata* were chosen for inclusion in this study based on the fact that tissue samples and demographic data were collected from these populations in 1989-1991 (Sarre 1995a, b, 1998, Sarre et al. 1995). Three additional populations for each species with data on demographics from 1989-1991 were available and were also incorporated in the current study (F5, F7 and F8 in *O. reticulata* and F3, F7 and F8 in *G. variegata*). Each of these populations was resampled. The tip of the tail of each individual was removed and stored in liquid nitrogen. Snout-vent length, body mass, sex, and age were recorded for all individuals. The percentage of mature females and the percentage of subadults and juveniles were used to calculate the sex ratio and adult: subadult ratio, respectively. The effective number of breeding adults and the effective population sizes were calculated with the equations in chapter 4 (Lande & Barrowclough 1987, Waples 1989, 2002, Caballero 1994) using the information collected in 1990 and in 2000 on the census population size (see below), the sex ratio and the adult: subadult ratio.

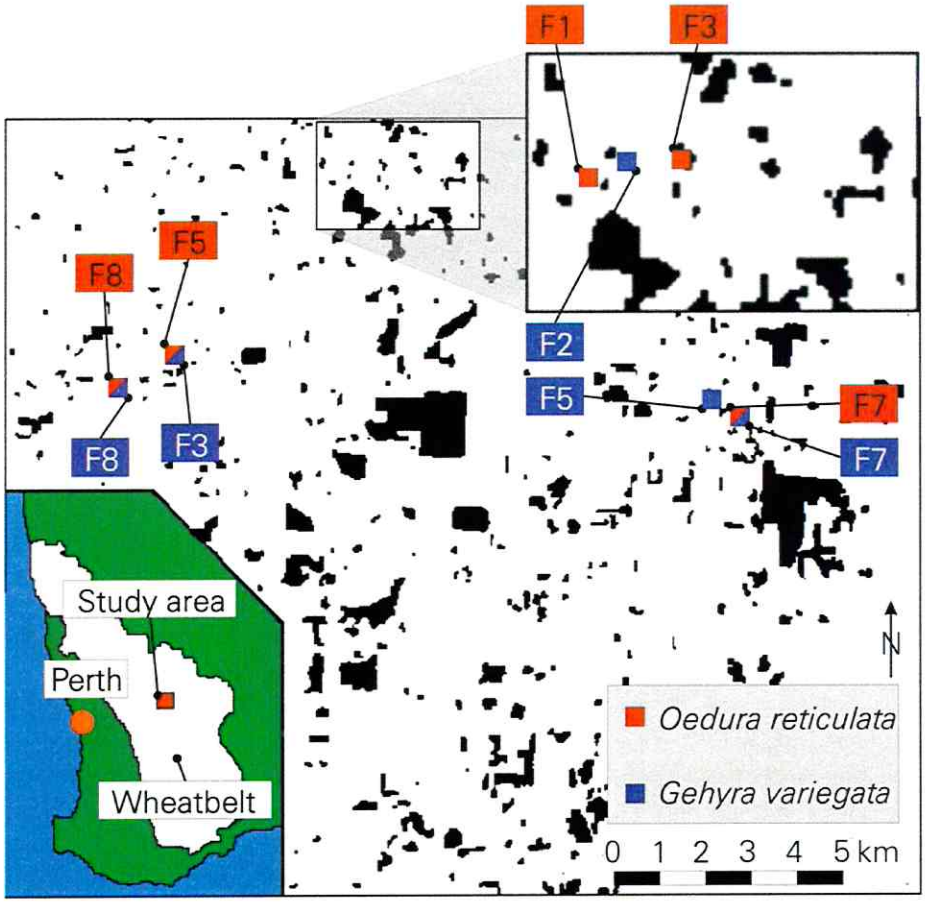



*Figure 1 Map of the study area and location of sites in the Western Australian wheatbelt.* O. reticulata *populations inhabit fragments labelled in red F1, F3, F5, F7 and F8.* G. variegata *fragments are labelled in blue F2, F3, F5, F7, F8*

*Table 1 Sites sampled, mark-recapture model used for size estimation (model), census population size N, effective number of breeders $N_b$ and the effective population size $N_e$ all with lower and upper 95% confidence interval (LCI and UCI) in the species* O. reticulata *and* G. variegata.

| Species | Patch | N 1990 | LCI | UCI | $N_b$ 1990 | LCI | UCI | Model | N 2000 | LCI | UCI | $N_b$ 2000 | LCI | UCI | $N_e$ | LCI | UCI | $N_e/N$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Gehyra* | F2 | 36 | 33 | 43 | 33 | 30 | 40 | M(o) | 76 | 65 | 101 | 48 | 41 | 64 | 39 | 35 | 49 | 0.70 |
| *Gehyra* | F5 | 32 | 26 | 54 | 23 | 19 | 39 | M(th) | 50 | 41 | 82 | 36 | 30 | 59 | 28 | 23 | 47 | 0.68 |
| *Gehyra* | F7 | 26 | 21 | 41 | 23 | 18 | 36 | M(th) | 18 | 15 | 34 | 10 | 8 | 19 | 14 | 11 | 25 | 0.64 |
| *Gehyra* | F8 | 55 | 51 | 65 | 42 | 39 | 49 | M(th) | 22 | 19 | 40 | 16 | 14 | 29 | 23 | 21 | 36 | 0.59 |
| *Gehyra* | F3 | 545 | 397 | 694 | 478 | 349 | 611 | M(h) | 325 | 264 | 411 | 254 | 206 | 321 | 332 | 259 | 421 | 0.76 |
| Mean | | 139 | | | 120 | | | | 98 | | | 73 | | | 87 | | | 0.67 |
| *Oedura* | F1 | 194 | 152 | 235 | 77 | 61 | 94 | M(h) | 197 | 162 | 261 | 132 | 109 | 175 | 97 | 78 | 122 | 0.49 |
| *Oedura* | F3 | 22 | 12 | 32 | 6 | 3 | 9 | M(t) | 33 | 30 | 42 | 16 | 14 | 20 | 9 | 5 | 12 | 0.32 |
| *Oedura* | F7 | 55 | 41 | 69 | 21 | 16 | 26 | M(th) | 104 | 85 | 148 | 66 | 54 | 93 | 32 | 25 | 41 | 0.40 |
| *Oedura* | F8 | 50 | 37 | 64 | 34 | 25 | 44 | M(th) | 211 | 169 | 289 | 127 | 101 | 173 | 54 | 40 | 70 | 0.41 |
| *Oedura* | F5 | 430 | 248 | 578 | 309 | 179 | 416 | M(th) | 231 | 174 | 340 | 182 | 137 | 269 | 229 | 155 | 327 | 0.69 |
| Mean | | 150 | | | 89 | | | | 155 | | | 105 | | | 84 | | | 0.46 |

### *Demography and population size estimates*

To estimate the census populations in the two gecko species a capture-mark-recapture study was conducted. Individuals were captured, marked by toe-clipping and released. Individuals were then recaptured in 4-8 following sessions (depending on the population size). Population size was estimated using the program *CAPTURE* (Otis et al. 1978). *CAPTURE* allows moderation of the often unrealistic assumption of equal capture probabilities over the period of the study (Model $M_0$). Otis et al. (1978) distinguished between three different sources of variability in capture probabilities: individual heterogeneity (model $M_h$), behavioural response to capture (model $M_b$), and temporal variation (model $M_t$). *CAPTURE* provides estimates for the numbers of individuals in a population for all possible combinations of these models (except $M_{thb}$). For each estimates the most appropriate model was chosen and is listed in Table 1. In the rare cases where the most appropriate model appeared unrealistic from a biological point of view, the model $M_{th}$ was chosen. Individual heterogeneity and temporal variation are the most likely factors affecting the capture probability for geckos (B. Gruber pers. communication)

### *Microsatellites*

DNA was extracted from the tip of the tail of each individual using the Chelex extraction method. We genotyped individuals of *O. reticulata* using nine tetranucleotide microsatellite loci developed from an enriched library for this species (OR205, OR220, OR266, OR6F4, OR10H7, OR11G3, OR12D7, OR12D9, OR14A7) (Hoehn & Sarre subm.). For *G. variegata*, we genotyped individuals using nine tetranucleotide microsatellite markers cloned from an enriched library for this species (GV1C5, GV1C10, GV1F1, GV3B5, GV3C6, GV3E10, GV4B6, GV4G6, GV4C9) (Hoehn & Sarre in prep.). Polymerase chain reaction (PCR) amplification and genotyping on the Beckman Coulter Sequencer 8000 were performed according to conditions described in Hoehn & Sarre (subm.) and Hoehn & Sarre (in prep.).

### *Genetic analysis*

*FSTAT 2.9.3* (Goudet 1995, 2001) and *GENEPOP 3.2a* (Raymond & Rousset 1995) were used to calculate descriptive statistics for the populations including the mean number of alleles (A), and observed ($H_o$) and expected ($H_e$) heterozygosity. Of these, allelic diversity is expected to be the most sensitive to recent reduction in population size (Nei et al. 1975, Garza & Williamson 2001). Differences in the number of alleles (A), and observed ($H_o$) and expected ($H_e$) heterozygosity between populations in 1990 and 2000 were tested for using the Wilcoxon test. Wright's F-statistics (1951), calculated following Weir & Cockerham (1984), implemented in *FSTAT 2.9.3* (Goudet 1995, 2001) were used to measure population structure between sites ($\theta$ was calculated, hereafter it is referred to as $F_{ST}$).

## Results

### Genetic analysis

The nine microsatellite loci for each species showed high levels of variation in the populations of *O. reticulata* (8-16 alleles) and *G. variegata* (9-27 alleles). The DNA amplification had a high success rate, even for the tail samples taken in 1990. We used 24 and 30 samples for the two population of *O. reticulata* and 28 samples for each *G. variegata* population. All samples successfully amplified at all loci. One of the 36 tests for Hardy-Weinberg equilibrium in *O. reticulata* and two of the 36 tests in *G. variegata* showed significant deviation from expected allele frequencies after Bonferroni correction; all were due to a deficiency of heterozygotes. The heterozygote deficiency may have been due to null alleles, although there did not appear to be any consistent pattern of Hardy-Weinberg deviation among loci, years, or populations (Table 2a, b). Note that the number of alleles and expected and observed heterozygosity differs slightly from the calculations in chapter 2 and 3. The inconsistency is due to the fact that different set of samples (randomly chosen) have been used to equal the number of samples collected in the earlier study.

Figure 2 presents the allele frequency distribution of the nine loci for *O. reticulata* and *G. variegata* during the two separate sampling periods (1990 and 2000). In both species there were more alleles in the low-frequency class than in the intermediate-frequency classes, indicating that these populations have not recently undergone a severe genetic bottleneck.

*Table 2a The number of alleles (A), and the observed ($H_o$) and expected ($H_e$) heterozygosity for each locus at each site and each year in two populations of the species* O. reticulata. **Deviation from Hardy-Weinberg expectations ($p < 0.05$). Site codes follow Table 1*

| **Locus** | **F1** | | | **F3** | | |
|---|---|---|---|---|---|---|
| | Year | 1990 | 2000 | Year | 1990 | 2000 |
| | N | 30 | 30 | N | 24 | 24 |
| OR205 | A | 10 | 9 | | 4 | 4 |
| | $H_e$ | 0.80 | 0.77 | | 0.65 | 0.76 |
| | $H_o$ | 0.70 | 0.80 | | 0.63 | 0.71 |
| OR220 | A | 7 | 5 | | 6 | 4 |
| | $H_e$ | 0.77 | 0.74 | | 0.72 | 0.54 |
| | $H_o$ | 0.80 | 0.70 | | 0.50 | 0.33 |
| OR266 | A | 10 | 8 | | 5 | 5 |
| | $H_e$ | 0.83 | 0.82 | | 0.66 | 0.71 |
| | $H_o$ | 0.80 | 0.93 | | 0.58 | 0.67 |
| OR6F4 | A | 9 | 6 | | 5 | 4 |
| | $H_e$ | 0.83 | 0.58 | | 0.69 | 0.51 |
| | $H_o$ | 0.57* | 0.41 | | 0.68 | 0.55 |
| OR10H7 | A | 10 | 9 | | 9 | 7 |
| | $H_e$ | 0.84 | 0.81 | | 0.83 | 0.83 |
| | $H_o$ | 0.83 | 0.83 | | 0.88 | 0.92 |
| OR11G3 | A | 7 | 6 | | 7 | 5 |
| | $H_e$ | 0.83 | 0.80 | | 0.75 | 0.71 |
| | $H_o$ | 0.90 | 0.70 | | 0.83 | 0.83 |
| OR12D7 | A | 10 | 9 | | 6 | 4 |
| | $H_e$ | 0.83 | 0.83 | | 0.70 | 0.56 |
| | $H_o$ | 0.93 | 0.90 | | 0.71 | 0.67 |
| OR12D9 | A | 14 | 12 | | 7 | 7 |
| | $H_e$ | 0.91 | 0.89 | | 0.77 | 0.76 |
| | $H_o$ | 0.93 | 0.90 | | 0.75 | 0.83 |
| OR14A7 | A | 7 | 6 | | 6 | 5 |
| | $H_e$ | 0.73 | 0.69 | | 0.74 | 0.70 |
| | $H_o$ | 0.70 | 0.63 | | 0.79 | 0.79 |
| Mean | A | 9.33 | 7.78 | | 6.11 | 5.00 |
| | $H_e$ | 0.82 | 0.77 | | 0.72 | 0.68 |
| | $H_o$ | 0.80 | 0.76 | | 0.71 | 0.70 |

*Table 2b The number of alleles (A), and the observed ($H_o$) and expected ($H_e$) heterozygosity for each locus at each site and each year in two populations of the species* G. variegata. **Deviation from Hardy-Weinberg expectations ($p < 0.05$). Site codes follow Table 1*

| **Locus** | **F2** | | | **F5** | | |
|---|---|---|---|---|---|---|
| | Year | 1990 | 2000 | Year | 1990 | 2000 |
| | N | 28 | 28 | N | 28 | 28 |
| GV1C10 | A | 9 | 10 | | 10 | 13 |
| | $H_e$ | 0.79 | 0.83 | | 0.87 | 0.85 |
| | $H_o$ | 0.63 | 0.57* | | 0.86 | 0.71 |
| GV1C5 | A | 10 | 10 | | 15 | 14 |
| | $H_e$ | 0.84 | 0.85 | | 0.90 | 0.90 |
| | $H_o$ | 0.82 | 0.61* | | 0.86 | 0.89 |
| GV1F1 | A | 10 | 12 | | 9 | 14 |
| | $H_e$ | 0.85 | 0.88 | | 0.84 | 0.85 |
| | $H_o$ | 0.82 | 0.89 | | 0.89 | 0.86 |
| GV3B5 | A | 8 | 7 | | 11 | 12 |
| | $H_e$ | 0.84 | 0.83 | | 0.89 | 0.90 |
| | $H_o$ | 0.93 | 0.75 | | 0.89 | 0.93 |
| GV3C6 | A | 11 | 10 | | 8 | 10 |
| | $H_e$ | 0.89 | 0.87 | | 0.84 | 0.85 |
| | $H_o$ | 0.86 | 0.93 | | 0.96 | 0.89 |
| GV3D10 | A | 12 | 11 | | 11 | 11 |
| | $H_e$ | 0.87 | 0.87 | | 0.89 | 0.90 |
| | $H_o$ | 0.89 | 0.86 | | 0.86 | 0.89 |
| GV4B6 | A | 11 | 13 | | 14 | 15 |
| | $H_e$ | 0.86 | 0.86 | | 0.92 | 0.91 |
| | $H_o$ | 0.86 | 0.89 | | 0.96 | 0.86 |
| GV4C9 | A | 6 | 7 | | 6 | 7 |
| | $H_e$ | 0.75 | 0.78 | | 0.73 | 0.78 |
| | $H_o$ | 0.69 | 0.78 | | 0.64 | 0.68 |
| GV4G6 | A | 8 | 9 | | 10 | 11 |
| | $H_e$ | 0.83 | 0.88 | | 0.89 | 0.87 |
| | $H_o$ | 0.89 | 0.93 | | 0.93 | 0.93 |
| Mean | A | 9.44 | 9.89 | | 10.44 | 11.89 |
| | $H_e$ | 0.84 | 0.85 | | 0.86 | 0.86 |
| | $H_o$ | 0.82 | 0.80 | | 0.87 | 0.85 |

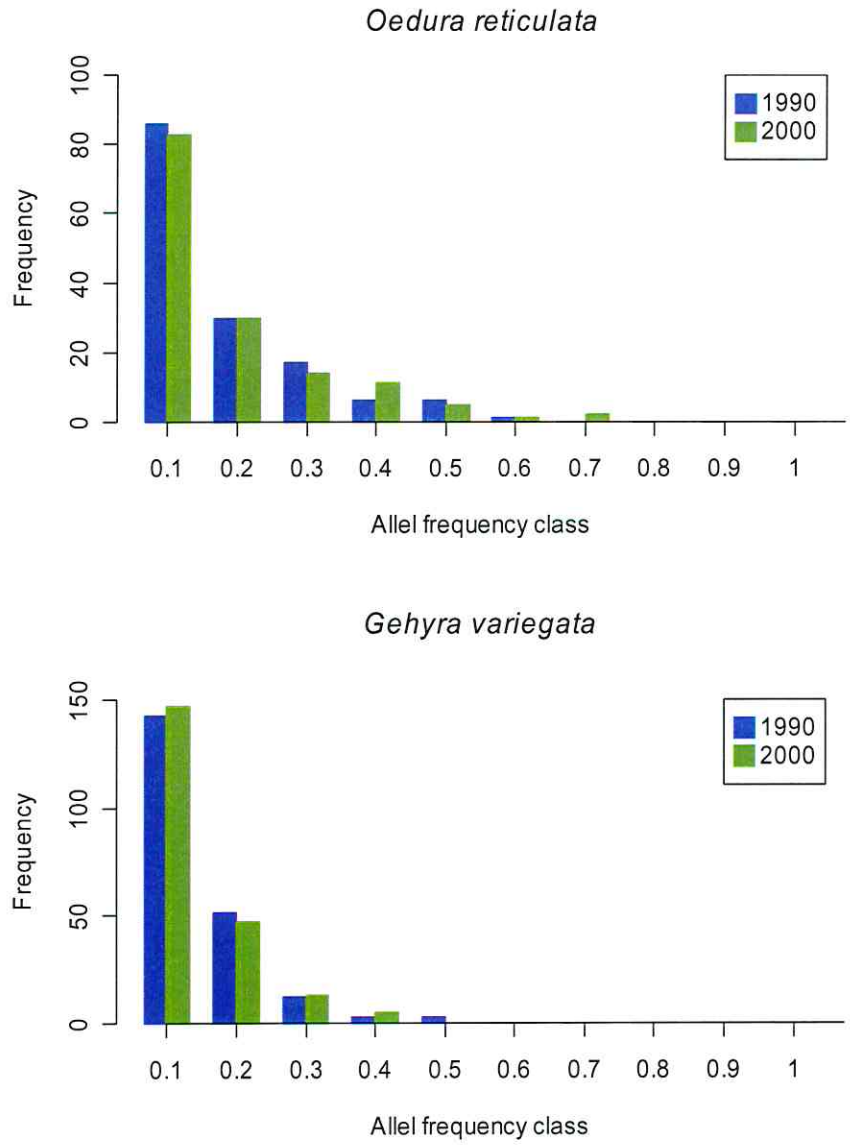



*Figure 2 Histogram of the historical comparison (1990 and 2000) of the allele frequency distribution at nine microsatellite loci in two populations of the species* O. reticulata *(above) and two populations of the species* G. variegata *(below)*

The number of alleles per locus across all samples and years ranged from four to 14 for *O. reticulata* and from six to 15 for *G. variegata*. The frequency of the most common allele at a locus ranged from 0.20 to 0.65 for *O. reticulata* with a mean frequency of 0.38. In *G. variegata* the frequency ranged from 0.16 to 0.41 with a mean frequency of 0.26. For some loci, the most common allele changed over the ten year period (Figure 3).

There was a significant reduction in the total numbers of alleles observed over time in both populations of *O. reticulata*. In the historic sample there were 84 alleles detected in population F1 and 55 alleles in population F3, compared with 70 (F1) and 45 alleles (F3) detected in the recent sample. The mean number of alleles declined significantly from 9.33 to 7.78 in population F1 (Wilcoxon: $p < 0.01$) and from 6.11 to 5.00 in population F3 (Wilcoxon: $p < 0.05$). An opposite trend was discovered in the two populations of *G. variegata*. In 1990 we detected 85 alleles in population F2 and 94 alleles in population F5 whereas there were 89 and 107 alleles found in 2000, respectively. The mean number of alleles increased from 9.44 to 9.89 in population F2 (not significant) and from 10.44 to 11.89 in population F5 (significant: Wilcoxon: $p < 0.05$) (Figure 4).

The expected heterozygosity (Figure 4) ranged from 0.51 to 0.91 in *O. reticulata* and from 0.73 to 0.92 in *G. variegata* among loci, populations, and years. Genetic diversity showed a similar pattern of change over time compared with the allelic diversity. *O. reticulata* was characterized by a reduction in mean gene diversity over time in both populations (from 0.82 to 0.77 in F1 and from 0.72 to 0.68 in F3), which was significant in F1 (Wilcoxon: $p < 0.01$), but only indicating a trend in F3 (Wilcoxon: $p = 0.14$). *G. variegata* showed a slight increase in mean genetic diversity in F2 (from 0.84 to 0.85), which was approaching significance (Wilcoxon: $p = 0.07$), but showed no increase in F5 (from 0.86 to 0.86).

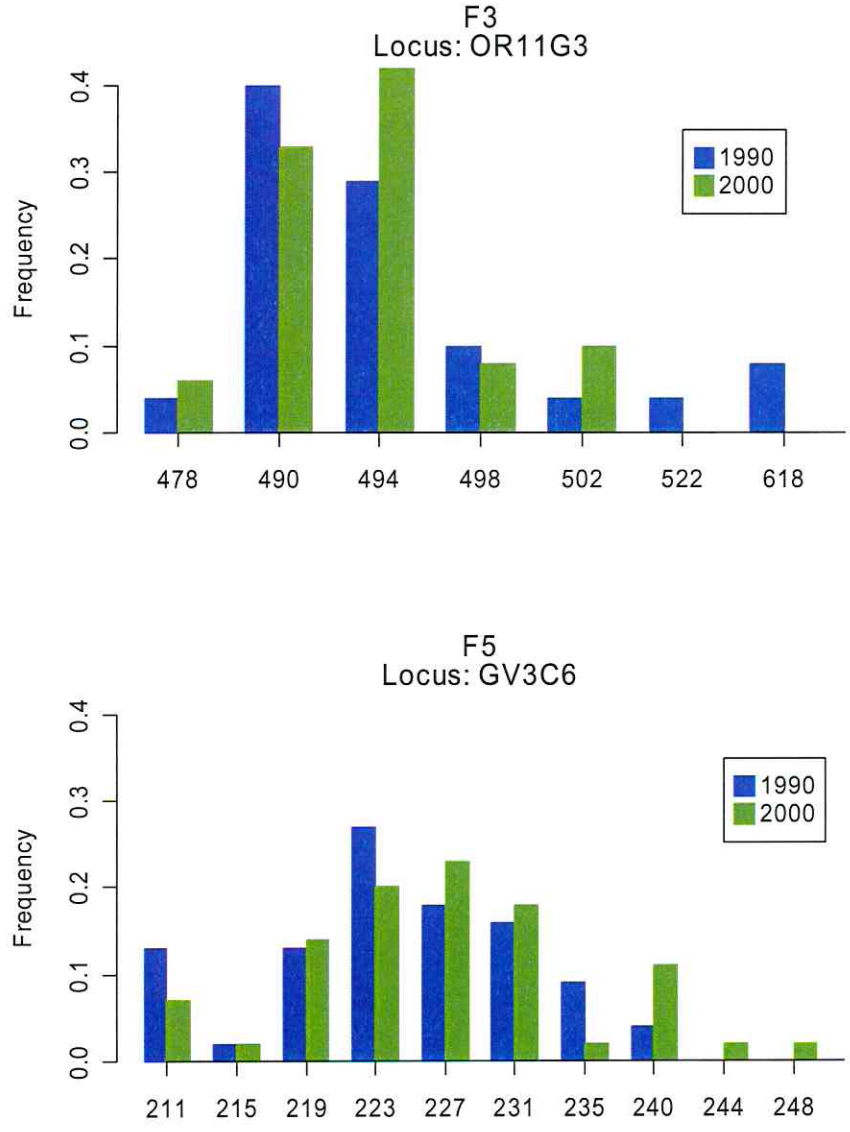



*Figure 3 Allele frequency distribution at one microsatellite locus (OR11G3) for two time points (1990 and 2000) in one population of the species* O. reticulata *(above) and another microsatellite locus (GV3C6) in one population of the species* G. variegata. *Site codes follow Table 1*

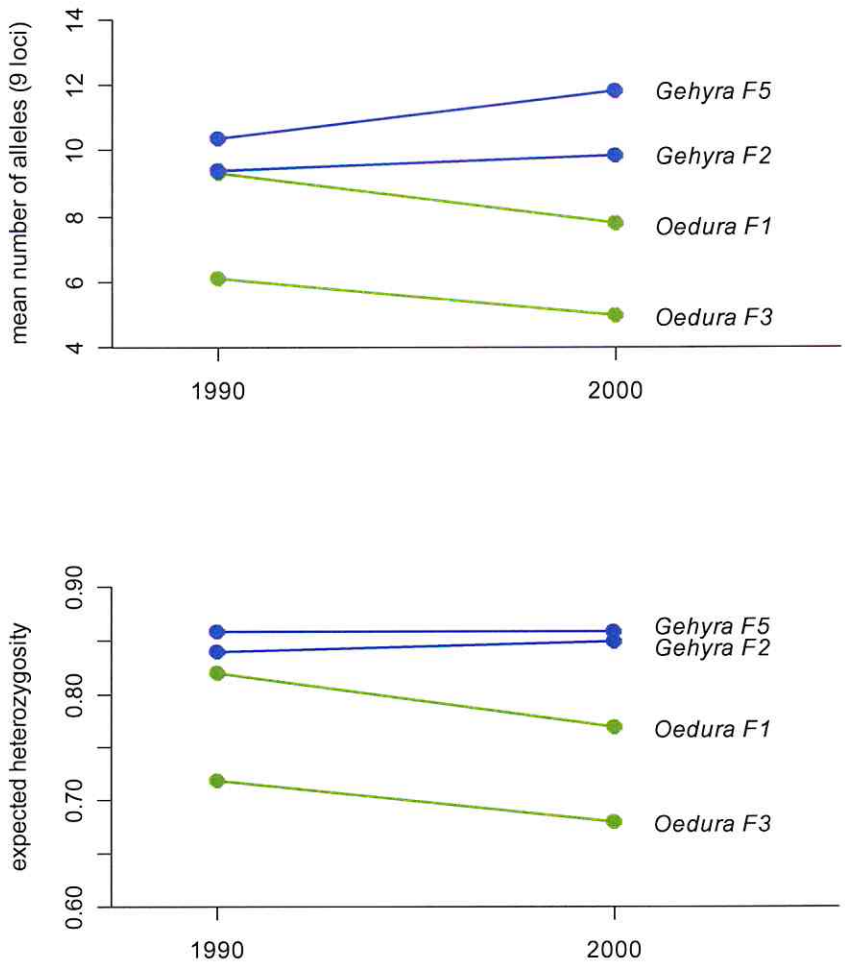



*Figure 4 Comparison of the number of alleles (A) (above) and expected ($H_e$) heterozygosity (below) between years for two populations of* O. reticulata *and two populations of* G. variegata*. Site codes follow Table 1*

## Temporal changes

We found no significant difference for all pairwise comparisons of allele frequency distributions among years in either species. Pairwise $F_{ST}$ estimates, between temporally comparable samples, were not significant after Bonferroni correction (Table 3). In contrast, the differentiation between the two spatially separated *O. reticulata* populations was substantial and increased over time (0.123 in 1990 vs 0.179 in 2000). The opposite was true for *G. variegata*. The two spatially separated populations were not particularly differentiated and $F_{ST}$ values decreased slightly over time (0.083 in 1990 vs. 0.079 in 2000).

*Table 3 Pairwise $F_{ST}$ estimates among temporal and spatial populations of the species* O. reticulata *(above) and* G. variegata *(below) for all loci on the upper matrix. The lower matrix indicates p-values for pairwise comparisons: NS indicates no significant differences, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Site codes follow Table 1*

| **Population** | **Sampling year** | **F1** | | **F3** | |
|---|---|---|---|---|---|
| | | 1990 | 2000 | 1990 | 2000 |
| F1 | 1990 | ------- | 0.009 | 0.123 | 0.152 |
| | 2000 | NS | ------- | 0.152 | 0.179 |
| F3 | 1990 | ** | ** | ------- | 0.009 |
| | 2000 | ** | ** | NS | ------- |

| **Population** | **Sampling year** | **F2** | | **F5** | |
|---|---|---|---|---|---|
| | | 1990 | 2000 | 1990 | 2000 |
| F2 | 1990 | ------- | 0.006 | 0.083 | 0.087 |
| | 2000 | NS | ------- | 0.073 | 0.079 |
| F5 | 1990 | ** | ** | ------- | 0.000 |
| | 2000 | ** | ** | NS | ------- |

### *Demographic analysis*

There was no general trend for the census population size of either species over the ten year period. In *O. reticulata* two populations increased, one declined, and two stayed stable over time. In *G. variegata* two populations increased, two declined, and one stayed stable over time (Figure 5). The effective number of breeders ($N_b$) and the effective population sizes ($N_e$) were calculated from demographic data (chapter 5). Demographic estimates of $N_b$ and $N_e$ use the concepts and equations introduced by Wright (1931, 1938) and later modified by others (reviewed by Caballero 1994). These equations use extensive demographic data to account for the effects of: 1. fluctuations in population size, 2. variance in individual reproductive success above binomial expectations, and 3. an unequal sex ratio on the variance in allele frequency change and inbreeding in the population under consideration. Although we lack data on the individual reproductive success, we accounted for the other two effects and also took account of the age structure. The changes in the effective number of breeders over time showed the same pattern as for the census population sizes with the exception of the increase in population F1 in *O. reticulata* (Figure 6). The effective population sizes in *O. reticulata* and *G. variegata* were generally low in comparison with the census population sizes. Even the *O. reticulata* populations that showed an increase in census size tended to have a small effective size (Figure 5).

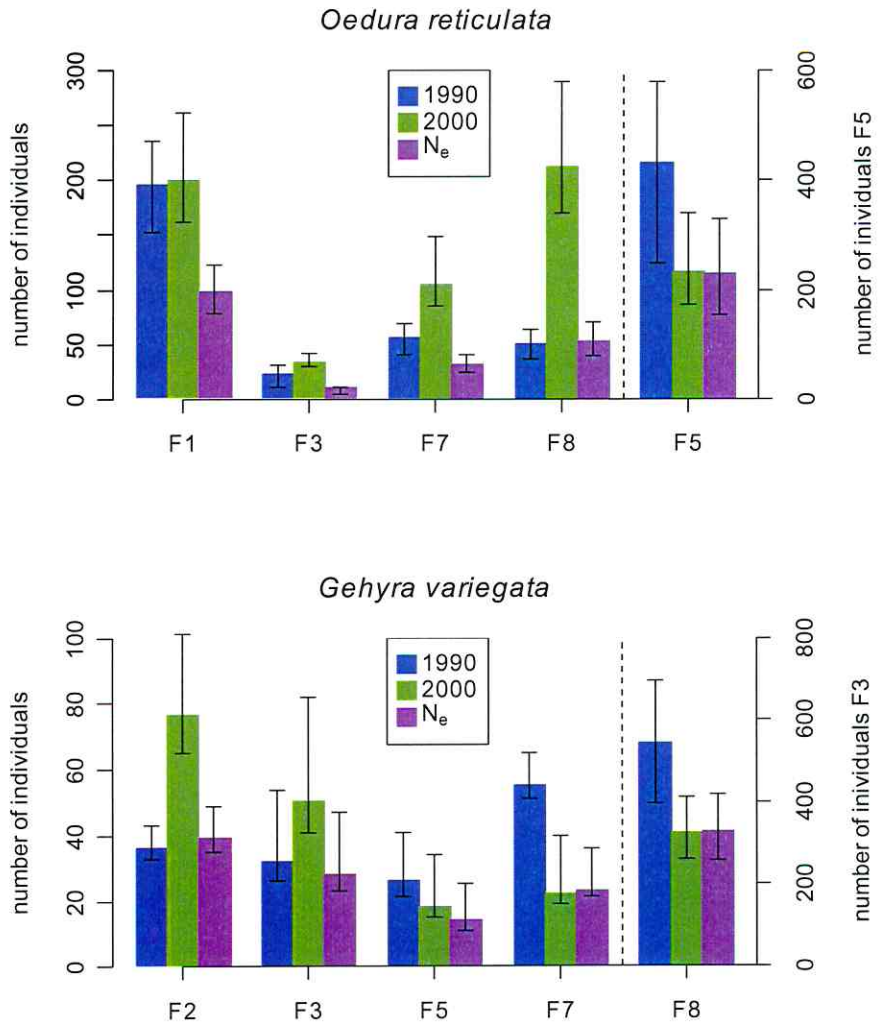



*Figure 5 Comparison of the census population size N in 1990 and 2000 and the effective population size $N_e$ of five populations of the species* O. reticulata *(above) and five populations of the species* G. variegata *(below). Site codes follow Table 1. Note: the values of fragment F5 in* O. reticulata *and F3 in* G. variegata *are shown at the right y-axis*

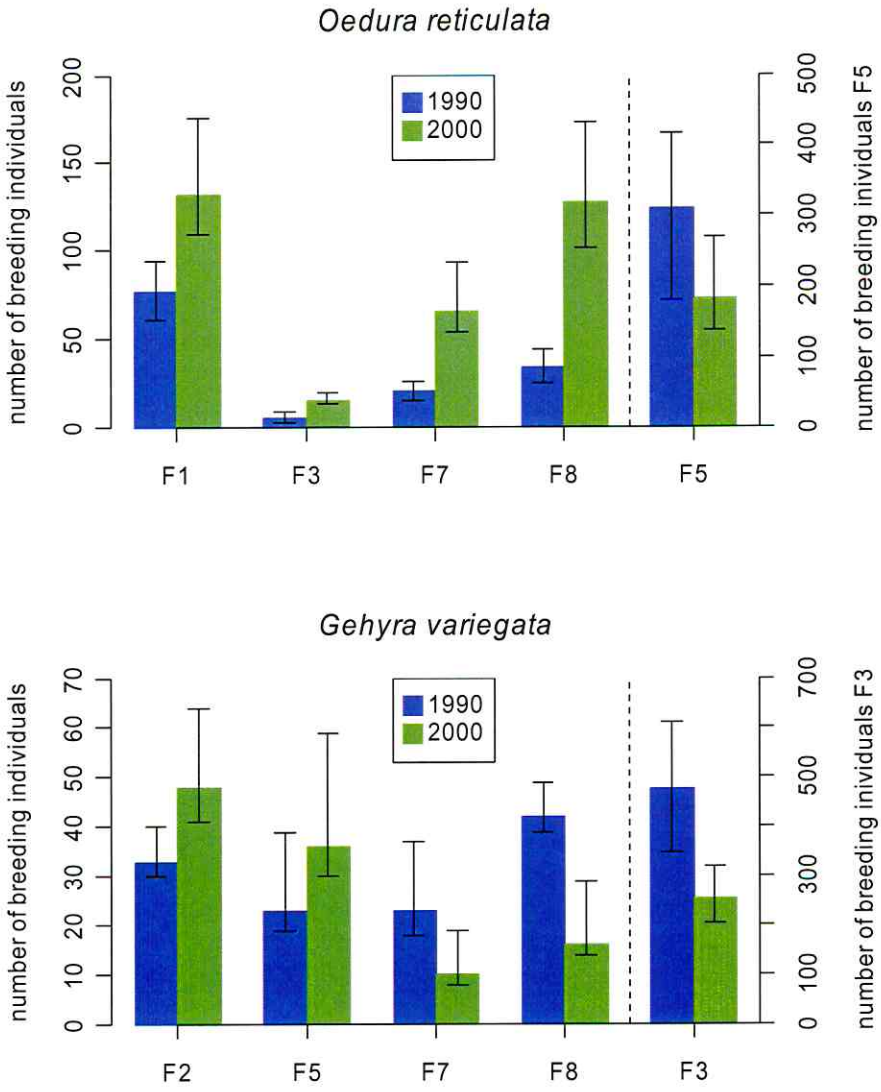



*Figure 6 Comparison of the effective number of breeders in 1990 and 2000 of five populations of the species* O. reticulata (above) *and five populations of the species* G. variegata (below). *Site codes follow Table 1. Note: the values of fragment F5 in* O. reticulata *and F3 in* G. variegata *are shown at the right y-axis*

## *Discussion*

Temporal population genetic studies are scarce compared with studies on spatial population structure. Yet our attempts to integrate the results of demographic and genetic studies have identified some potentially important outcomes and a more complete picture than would have been possible from an investigation confined to any single approach. Our findings also highlight the complexity of modified landscape systems, and the difficulty of disentangling the consequences of habitat fragmentation.

### *Temporal genetic results*

This study showed that genetic erosion occurred in the species *O. reticulata* in a fragmented landscape. This could have remained undetected had we relied solely on the traditionally used measure of overall population heterozygosity. For example, the smallest population (F3), with an effective population size of ten, showed only a slight trend towards loss of expected heterozygosity ($p = 0.14$) whereas the larger population F1 ($N_e = 97$) significantly lost genetic diversity ($p < 0.01$). Although the declines in heterozygosity were not always significant we found that alleles were significantly lost from F1 and F3 over the ten year period. The loss of rare alleles might have led to the maintenance of high-frequency alleles in the small population. That in turn might have resulted in a slower decline in heterozygosity than in allele number (Srikwan & Woodruff 2000). Accordingly, allelic diversity appears to be a more sensitive way of monitoring the loss of genetic variation (Nei et al. 1975, Garza & Williamson 2001). The rate of loss of heterozygosity occurs at a constant rate of $1/(2\ N_e)$ per generation, which should lead to a higher loss of heterozygosity in small populations. We predicted a heterozygosity loss of 11% for the small *O. reticulata* population F3 ($N_e = 9$), assuming only two generations. A loss of 1% was predicted for the large population F1 ($N_e = 97$). Instead, we discovered a real loss of 5.5% in F3 and 6% respectively in F1. With our data set it is only possible to make approximate estimates of the effective population size and the generation time. Thus, 11% compared with 5.5% appears a fairly good approximation. On the other hand, there has been a dramatic loss of heterozygosity in the larger population over a short period of time. Future research will indicate if the result can be confirmed in additional populations.

In contrast to Jehle et al. (2001) and Heath et al. (2002), we were not able to find high $F_{ST}$ values between temporal samples in either of the two species. In the species *O. reticulata* however, the genetic differentiation among remnants (F1 and F3) increased over time. This indicates that there was limited gene flow in the species, but the time and the number of generations for genetic drift to occur might have been limited. Several studies have reported remarkable temporal stability in genetic structure over time (Taylor et al. 1994, Miller & Kapuscinski 1997, Nielsen et al. 1999a, Tessier & Bernatchez 1999). Among the few studies that have found changes, Jorde & Ryman (1996) and Heath et al. (2002) concluded that the large genetic variation among temporal samples was due mostly to genetic drift. Therefore,

future studies of population genetic structure should include a temporal component, whenever possible, particularly if the results are to be used to evaluate conservation management.

### *Temporal demographic results*

Our demographic results show that there was no consistent decline or increase in the population size of *O. reticulata* and *G. variegata* over the entire time period. The effective population size was smaller than the census population size as we expected. In chapter 4 we concluded that the reduction in $N_e/N$ in two populations of the generalist species was much lower than in the specialist species. In this chapter, we extended our study to five populations and arrived at the same conclusion. In chapter 4 we suggested that different life-history characteristics and external factors might lead to differences in the reduction of the census population size. Our results may reflect the fact that the two gecko species contrast in their level of specialization and also that the study was carried out in fragmented landscapes. Fragmentation limits dispersal of the specialist species to a greater extent than it does in the generalist species (chapter 2). *G. variegata* populations may be able to form a metapopulation that allows offspring to disperse and immigrants to contribute to equal sex ratios. The opposite is probably true for the specialist species *O. reticulata,* which is reflected in a high proportion of juveniles in the population and an unequal sex ratio. Another explanation for the high rates of juveniles and subadults could be that in *O. reticulata* adults reach maturity later (4.8 years) than in *G. variegata* (2.8 years). Consequently, more immature individuals accumulate in the populations contributing to a biased juvenile and subadult: adult ratio.

In terms of maintaining genetic variation, *G. variegata* has appeared to cope better with the changes of habitat fragmentation than *O. reticulata*. The *G. variegata* populations increased and the level of genetic variation was stable or increased over the time period. The persistence of high genetic variation is probably due to high immigration rates and the ability of the species to form a metapopulation. Srikwan & Woodruff (2000) argued that a long generation time and high tolerance of habitat alteration may cause the erosion of genetic variability to go undetected, and furthermore, that the undetected differentiation might be misjudged as gene flow. However, in our case *G. variegata* has a shorter generation time than *O. reticulata,* and since the latter species clearly exhibited genetic erosion, it seems unlikely that genetic erosion would escape detection in the shorter-lived species.

Few other studies have investigated demography and genetic patterns of species in fragmented landscapes on a local scale (Lindenmayer & Peakall 2000, Sumner et al. 2001, Brouat et al. 2003, 2004). Many genetic studies of species subjected to habitat fragmentation tend to focus on larger geographic areas by comparing island-like fragments with continuous mainland populations (Gaines et al. 1997, Cunningham & Moritz 1998). In addition to their larger geographic scale, many of these studies have been predominantly

concerned with assessing the relationship between population size and genetic diversity and with considering the genetic differentiation at a single point in time. While the loss of genetic diversity associated with reductions in population size and the formation of genetic differentiation associated with reductions in gene flow are important and serious long-term problems of habitat fragmentation (Lindenmayer & Peakall 2000), knowledge of historical patterns of the genetic structure might facilitate the assessment of the loss of genetic diversity. Historical samples are a valuable source of reference with which to interpret contemporary levels of genetic diversity and ideally should be part of future research in the field of habitat fragmentation.

### *Conservation implications*

The results of our integrated historic, demographic and genetic study of the two species of geckos in the Western Australian wheatbelt have some potential implications for nature conservation. Habitat fragmentation and the disturbance of species can lead to genetic changes in their populations. Since genetic variation can affect fitness and adaptive potential, one important goal in precautionary conservation is to prevent erosion of genetic variation within species. Since adaptive genetic variation is difficult to analyze directly, management and monitoring strategies usually involve indirect methods. These indirect approaches include for example minimizing the reduction of effective population size and increasing the level of gene flow (Sherwin & Moritz 2000). Translated into ecological terms this means increasing the size of habitat and linking isolated remnants by woodland corridors, a strategy generally suggested to mitigate the effects of habitat fragmentation (Hobbs 1992, Hobbs & Yates 2003).

Demographic effects of habitat fragmentation may be observed before the effects of genetic erosion, but the reverse may also be true (Srikwan & Woodruff 2000). In our study, habitat fragmentation resulted in the loss of alleles and gene diversity (genetic erosion) in the species *O. reticulata* even though a decline in the census or effective population sizes was not yet apparent. This is a important finding, given that genetic studies are often performed after demographic studies have indicated that there is a problem. This asynchrony between demographic collapse and genetic erosion has also been found by others (Srikwan & Woodruff 2000, Shrimpton & Heath 2003). Clearly, managers must monitor both demography and genetics, and their interaction, if they are to assess accurately the viability of a fragmented population.

# Discussion

## Conservation genetics in fragmented landscapes

### *Conservation genetics*

Conservation genetics operates at many levels, from taxa and species down to individual organisms. At each of these levels, molecular genetic techniques provide appropriate tools to evaluate processes and to develop management strategies (Smith & Wayne 1996). Here, we present an overview of the questions that are relevant at the population level in fragmented landscapes, discuss how innovative molecular genetic techniques and analytical tools may be used to address these questions and provide examples of the use of molecular techniques in conservation. We do not aim to present an exhaustive review but rather to summarize our own research. Our primary goals are the management of biodiversity and the conservation of genetic diversity in isolated populations. These may be achieved through the maintenance of genetic diversity within subpopulations and the reduction in genetic differentiation among subpopulations. The maintenance of genetic diversity will allow populations to adapt to novel environmental conditions and avoid the potential negative effects of inbreeding. Specifically, we describe genetic variability, genetic erosion, effective population size, genetic differentiation, dispersal and gene flow of two gecko species in a fragmented landscape. In addition, genetic information will be used to make recommendations for these fragmented populations.

### *Habitat fragmentation*

Habitat fragmentation and habitat loss induced by humans through land clearing, forestry and damming of rivers, are recognized as the primary causes of the loss of biodiversity (Groombridge 1992, WCMC 1992). Habitat fragmentation includes two processes, a reduction in total habitat area and the creation of separate isolated habitat patches from a larger continuous distribution. These changes lead to a reduction in the population size of species and to reduction in dispersal (gene flow) among patches (Sih et al. 2000, Frankham 2002). As a result, many species are limited to habitat remnants, which are reduced in size and isolated by a matrix of less suitable habitat. Both effects (reduction in population size and reduction in dispersal) and their genetic consequences are discussed in detail below.

### *Reduction in population size*

Small and declining populations of threatened and endangered species are more prone to extinction than large stable populations. Population size is the most influential of the criteria for listing species as endangered. Species whose adult population size is less than 50, 250 or 1000 are critically endangered, endangered or vulnerable, respectively (Frankham et al.

2002, Keller & Waller 2002). Small population size is a pervasive concern in conservation genetics. Such populations usually suffer from a reduced number of alleles, reduced heterozygosity or reduced genetic diversity and thus often have an increased probability of extinction (Frankham et al. 2002, Keller & Waller 2002). In addition, the evolutionary process in small populations is fundamentally different from that in large populations. The role of genetic drift predominates and the effect of selection is typically reduced or even eliminated. Small populations also become inbred at a faster rate than do large populations, as inbreeding is unavoidable. Inbreeding increases the probability that an individual is homozygous at a locus. Since naturally outbreeding populations contain deleterious alleles (mostly partially recessive) at low frequencies in mutation-selection balance, inbreeding increases the risk of exposing them as homozygotes. In addition, inbreeding also slowly increases homozygosity and therefore reduces genetic diversity in a population. As a consequence, inbreeding reduces reproductive fitness (inbreeding depression) as observed in well-studied populations of outbreeding animal and plant species, and increases the risk of extinction (Thornhill 1993, Frankham 2002, Keller & Waller 2002).

Our research indicates that the populations of the two gecko species, *O. reticulata* (reticulated velvet gecko) and *G. variegata* (tree dtella) have undergone severe changes in their genetic structure as a result of habitat fragmentation in the Western Australian wheatbelt. In the present study we compared fragmented to continuous populations and showed that fragmentation caused a reduction in the genetic diversity of populations. In both species we observed a decrease in the allelic richness within the fragmented habitats. The expected heterozygosity was significantly lower in habitat fragments than in continuous forest sites. Both the reduction in allelic richness and genetic diversity is presumably due to increased genetic drift.

In addition, we compared the present results to historical data from ten years ago and demonstrated that genetic erosion occurred in the species *O. reticulata* in a fragmented landscape. The smaller population (F3) with an effective population size of nine showed a loss of expected heterozygosity and the larger population (F1: $N_e = 97$) significantly lost genetic diversity. Although declines in the heterozygosity were not significant in every remnant we found that alleles were significantly lost from fragmented populations over the ten year period. The loss of rare alleles might have led to the maintenance of high-frequency alleles in the small population. That in turn might have resulted in the slower decline in heterozygosity than in the number of alleles.

To the best of our knowledge, the changes in the genetic structure of these populations are due to recent, anthropogenic alterations in the habitat and not to historical barriers (Cunningham & Moritz 1998). According to Sarre (1995b), the absence of clear historical isolation among populations of *O. reticulata* suggests that none of the populations are phylogenetically distinct. Moreover, we analyzed the genetic structure of these populations using hypervariable microsatellite DNA marker, which are less prone to the confounding

effects of population history, because of their high mutation rate. Finally, to minimize any effects of differing habitat types or landscape history, our study was carried out on a fine spatial scale with some fragments isolated by only a few hundred meters.

### *Reduction in dispersal*

Dispersal – the movement of organisms away from their parent source – is a fundamental biological process that operates at multiple temporal and spatial scales. The process therefore has overwhelmingly important implications at multiple scales of organization: for the survival, growth and reproduction of individuals, for the composition, structure and dynamics of populations and communities, and for the persistence, evolution and geographical distribution of species. Consequently, the study of dispersal has evolved to become a major theme in biology, unifying and incorporating fields of research as diverse as ecology, evolutionary biology, molecular biology, mathematics, physics, modelling, engineering, epidemiology, agricultural science, and geography. The most fundamental task in studying the process of dispersal is describing the patterns that it generates. There is an immense difficulty in measuring dispersal, especially long-distance dispersal (Koenig et al. 1996, Dieckmann et al. 1999, Ferriere et al. 2000, Clobert et al. 2001, Nathan 2001). Despite the importance of dispersal, obtaining direct estimates of dispersal from field data on natural populations has remained challenging. Often dispersal rates are low and species cryptic, so the probability of observing an immigration event is low. Moreover, even if all migration was observable, the migration rate might be over-estimated if immigrants fail to contribute to reproductive output (Sarre 1995). In addition, if the scale over which dispersal is measured is smaller than the scale over which individuals actually move, average dispersal can be underestimated (Koenig et al. 1996, Dieckmann et al. 1999). Given these constraints, recent innovations in the analytical and statistical analysis of conservation genetic methods provide a high potential for indirect estimates of dispersal in terms of gene flow. Most of these developments are summarized below. Parallel to the field of genetics, technology is also advancing rapidly in the field of demography. For example, an automatic activity monitoring system was developed to study the activity of geckos (*G. variegata*). This system recorded activity using passive integrated transponder (PIT) technology. The task of the system was to record movement of individual geckos when they left or returned to their tree (Gruber 2004). In another example, bumblebees (*Bombus terrestris*) were tagged with diode transponders of just a few milligrams that could be detected hundreds of meters away by harmonic radar (Osborne summarized in Nathan 2001).

The two gecko species occur sympatrically in the Western Australian, but differ in their degree of habitat specialization. *G. variegata* is a habitat generalist, whereas *O. reticulata* is a habitat specialist. In the present study we hypothesized that the species should differ in their dispersal capability in fragmented habitats. Our fine-scale genetic study supported this expectation. The genetic differentiation among populations of the specialist species was two times higher than for the generalist, suggesting that the gene flow between populations of

the generalist *G. variegata* is higher. In addition, in both species the genetic differentiation among populations in the fragments was ten to nearly 100 times higher than between populations in the continuous forest. That reveals that deforestation of natural habitat caused a barrier to the gene flow of both species. We also found that for the specialist *O. reticulata*, genetic differentiation among remnants increased over time. Again this is an indication of limited gene flow in this species. The assignment test revealed that *G. variegata* has higher rates of dispersal compared to *O. reticulata* in fragmented landscapes. For both species, more individuals dispersed between fragments separated by only small distances (150m), than between fragments separated by larger distances. We discovered that *G. variegata* is able to disperse over distances of 1000m, whereas in *O. reticulata* there was hardly any dispersal between fragments separated by 500m. It would thus appear that life history characteristics have an important influence on the dispersal ability of gecko species (chapter 2).

### *Landscape genetics*

Recent improvements in molecular genetic tools, combined with existing or new statistical tools (e.g. geostatistics, maximum likelihood, Bayesian approaches) and powerful computers have led to the combination of molecular population genetics and landscape ecology. The discipline of landscape genetics aims to provide information about the interaction between landscape features and microevolutionary processes, such as gene flow, genetic drift and selection. It will facilitate our understanding of how geographical and environmental characteristics structure genetic variation at both the population and individual levels, and has implications for ecology, evolution and conservation biology. It will also aid in identifying boundaries, which are either breaks in the gene flow across populations with no obvious reason, or secondary contact among previously isolated populations. Landscape genetics can resolve population substructure across different geographical scales at fine taxonomic levels. It differs from other genetic approaches, such as phylogeography, in that it tends to focus on finer spatial and temporal scales (Manel et al. 2003).

Our research integrated molecular population genetic data and landscape features by testing isolation by distance through linear regression of genetic distance against the geographical distance between pairs of habitat fragments. The genetic differentiation between fragments is expected to increase with geographical distance (Slatkin 1993, Rousset 1997). The habitat generalist *G. variegata* followed these theoretical expectations and genetic differentiation was positively correlated with total geographic distance. In contrast, no relationship between genetic and geographical distance was apparent for the specialist *O. reticulata*. We suggest that populations of *O. reticulata* are not at an equilibrium between genetic drift and migration due to their limited dispersal ability. That is, the gene pools of the isolated populations of *O. reticulata* are randomly losing or gaining alleles through genetic drift and mutation without any contribution of alleles through immigrants from other populations.

### *Genetic methods*

As it is not yet feasible or practical to measure the entire genome of an individual, a given set of genetic markers is used in conservation genetics to measure the genetic variation within individuals of a population. Different regions of the genome have different characteristics and the data derived from them may have quite different applications. The sequence of some DNA regions evolves at a rate that is sufficiently high to identify individuals and trace genealogies. Other DNA regions are conserved and resolve genetic distances at taxonomic levels only (Burgman & Lindenmayer 1998).

In the past decade, several key advances in molecular genetics have greatly increased the impact of population genetics on biology: Most important have been: 1) the development of the polymerase chain reaction (PCR), which amplifies specified stretches of DNA to useable concentrations; 2) the application of evolutionarily conserved sets of PCR primers; 3) the discovery of hypervariable microsatellite loci; and 4) improvements in routine DNA sequencing (Sunnucks 2000).

Microsatellites have become the preferred marker in many population genetic studies because of their high level of variability, ease and reliability of scoring, codominant inheritance and short lengths, making them useful for studies of DNA from animal or plant tissues but also from fossils, hair, or faeces collected in the field (Luikart & England 1999). In parallel with the exceptional quantity of data produced by microsatellite marker considerable innovations in the analytical and statistical methods have been developed. Among the most promising analytical methods for retrieving information from microsatellite data are the coalescent, maximum likelihood and Bayesian statistical approaches. These methods have been used for some time for phylogeny reconstruction and analyzing DNA sequence data, but only in recent years have they been applied to markers with high allelic diversity, such as microsatellites. These methods allow more detailed inference about both evolutionary parameters and historical events. They also generally provide more precise and accurate estimates of population parameters such as migration rates, effective population size and intra- and interlocus disequilibrium (Ayres & Balding 1998, Beerli & Felsenstein 1999, 2001, Luikart & England 1999, Kuhner et al. 2000).

In addition to estimating the current and long-term historical $N_e$, several statistical tests and computer programs are now available for detecting changes in $N_e$. It is important to detect changes in recent $N_e$ because such changes can severely bias estimates of phylogenies, divergence times and migration rates. Detecting drastic reductions in $N_e$ is critical in conservation biology where population declines can increase the risk of extinction. Genetic tests are now available for identifying a recently bottlenecked population when no information exists on the current or historical population size (Luikart et al. 1998, Luikart & England 1999, Waples 2002).

Our research demonstrated that estimates of the effective population size $N_e$ from demographic and genetic data diverge significantly. In only one *O. reticulata* population were the genetic and demographic estimates in general agreement. In the other population the genetic estimate was much higher than the actual census population size. In *G. variegata* the genetic and demographic estimates diverged considerably. This raises the question as to whether the genetic based methods are intrinsically biased. In contrast to our results, studies that have compared genetic and demographic estimates of the effective population size have found that genetic-based estimates are often substantially smaller than ecological estimates (Husband & Barrett 1992, Shrimpton & Heath 2003). We also found the different genetic estimation methods produced rather inconsistent results. The discrepancy may be due to differences in the mutation model used in different methods. In addition, the assumption of discrete generations and constant population size may have been violated. Surprisingly, the linkage disequilibrium method best matched the estimates derived from the demographic data (Hill 1981, Waples 1991).

Statistical methods that estimate dispersal were summarized in Rousset (2001). These methods are based on well-defined theoretical and statistical frameworks. The most traditionally used methods is the island model, in which $F_{ST}$ (Wright 1951) is defined as the standardized variance in the frequency of an allele within and among populations. It is possible to derive an estimate of demographic parameter values, such as dispersal, from an estimate of $F_{ST}$. The usual example is the infinite island model with subpopulations of $N$ adults and dispersal rate $m$, for which $F_{ST} \approx 1/(1+4Nm)$, so that the estimate of $Nm$ may be derived from the estimate of $F_{ST}$. In addition to $F_{ST}$, analogues of $F_{ST}$ (e.g. $R_{ST}$ sensu Slatkin 1995) have also been defined taking into account microsatellite allele size. Approaches such as this, may have poor statistical properties in comparison with more conventional F statistics, when the assumptions in making use of the additional information are not exactly valid (Rousset 2001).

There is increasingly interest in likelihood methods for the estimation of demographic parameters from genetic data. Such attempts do have theoretical and practical problems including the difficulty of computing the likelihood of a sample under theoretical models and the difficulty of finding the maximum likelihood estimate. Two slightly different algorithms have been proposed: one has been described by Bahlo & Griffiths (2000) and is incorporated in the GENETREE software, the other is described by Beerli & Felsenstein (1999) and is incorporated in the LAMARC software.

A specific class of alternatives to the island model is the isolation by distance model. These models embody the hypothesis that dispersal occurs preferentially between nearby subpopulations. Such models were first discussed by Wright (1943, 1946), but Slatkin (1991, 1993) developed a more coherent interpretation of the model and studied approximation of $F_{ST}$ as a function of geographical distance. In addition, it is possible to estimate the neighbourhood size $D\sigma^2$ from the increase of pairwise $F_{ST}$ values with distance,

where D is the population density and $\sigma^2$ is the mean axial square of parent-offspring dispersal rate. Neighbourhood size estimates allow the evaluation of either dispersal if density is known, or alternatively of density if dispersal is known (Rousset 1997, 2001).

The potential aim of the assignment method is to find the origin of an immigrant (Paetkau et al. 1995, Rannala & Mountain 1997, Pritchard et al. 2000). It is then possible to estimate dispersal rates between all pairs of subpopulations. The performance of such an approach has been investigated (Eldridge et al. 2001, Berry et al. 2004) and seems to be more efficient than other methods and allows estimation of parameters not estimable by other methods, particularly in cases where dispersal has been rare or nonexistent up to the last few generations (Rousset 2001).

In the present study both the Bayesian clustering method (Pritchard et al. 2000) and the likelihood based frequency method (Paetkau et al. 1995) performed well, meaning that individuals were assigned either to the one or the neighbouring population. In the absence of a significant threshold, individuals were simply assigned to the population in which their probability of belonging was highest. Both methods produced consistent results that were used to estimate dispersal probabilities. The performance of the Bayesian method (Rannala & Mountain 1997, Cornuet et al. 1999) at a $p \leq 0.05$ significance threshold was weak. Despite the higher accuracy fewer individuals could be assigned due to their undefined origins. In order to increase the number of individuals assigned to a population, we suggest varying the level of confidence or stringency required to accept an individual as a disperser or stayer. The chosen level of stringency depends on the purpose of the analysis. A very high level of confidence may be required if the information has to be used in forensics to find the origin of a smuggled animal or trophy. In contrast, a lower level of confidence may be sufficient in studies of wildlife dispersal (Pritchard et al. 2000, Eldridge et al. 2001, Berry et al. 2004).

For any of the above methods, assumptions are made relative to the temporal stability or the specific demographic background involving fluctuations in population size (Rousset 2001). Estimators are only robust when such assumptions are valid. In other cases some important aspect might be missed. Thus, it is important to test the methods against independent estimates of dispersal parameters obtained by demographic methods such as mark-recapture. Several surveys have concluded the existence of some rough agreement, but many have indicated inconsistency in specific cases (e.g. see Hastings & Harrison 1994, Slatkin 1994, Koenig et al. 1996 for reviews). While many studies have compared their estimates of genetic differentiation to a priori expectations based on various arguments, few have compared them to independent estimates obtained by statistical analysis of demographic observations from the same study (Rousset 2001).

Discrepancies between genetic and demographic estimates have been attributed to several different causes. Rousset (2001) argues that the dispersal parameters in the genetic models are backward dispersal rates (defined by considering where the parent of an adult was),

while demographic studies may estimate rates with different interpretation, such as forward dispersal rate (which considers where the juveniles go). The genetic models have some other deficiencies. The neutrality of genetic markers is often assumed, but for microsatellites neutrality has not yet been proven. A second assumption is demographic stability. The impact of demographic fluctuations is complex and at present extensive research goes into the impact of changes in effective population size $N_e$ (Waples 1989, 1990a, b, 2002, Luikart & Cornuet 1999). A third category of assumptions relates to mutation rates and mutational processes. The effect of mutational processes on the value of $F_{ST}$ and other measures has been discussed and attempts have been made to construct statistics allowing for supposed mutational processes at microsatellite loci (Slatkin 1995). For F statistics high mutation rate is a more important feature than the mutational process, because the quantitative impact of a $10^3$ variation in mutation rate is larger than the variation resulting from different mutational processes. What may have been most critical to many inferred incongruities between direct and indirect estimates is an inadequate use of the models. The genetic estimates are derived under the assumption of low dispersal rates between well-defined subpopulations and may be of dubious value for many studies (Rousset 2001).

# Summary

To summarize, in the present study of two gecko species we detected a reduction of genetic diversity and an increase in genetic differentiation among subpopulations in fragmented landscapes compared with continuous forest sites. The reduction in genetic variation and the increase in genetic differentiation was higher for the habitat specialist species *O. reticulata*. Despite the fact that the census population sizes stayed stable over a ten year period, genetic diversity was lost and genetic differentiation among populations increased. In addition, individuals of this species showed lower dispersal rates. Our results suggest that the specialist *O. reticulata* is less able to move through the modified landscape than the generalist *G. variegata*. Obviously, life history characteristics have great influence on the dispersal ability of species. Life history traits also have an impact on the effective population size of species. The $N_e/N$ ratio was lower in *O. reticulata* than in *G. variegata*, presumably because the age structure, sex ratio and fluctuating population sizes reduced the effective population size to a higher extent in the specialist. In conclusion, both species of geckos were affected by landscape fragmentation, but the habitat specialist species *O. reticulata* appears more prone to extinction than the habitat generalist *G. variegata* due to smaller effective population sizes, higher genetic erosion and higher reduction in dispersal ability.

# Conclusion

Modern molecular genetics provides such large quantities of precise data that there is a tendency to overlook its limitations. Certainly genetic studies can provide much evidence that is relevant to conservation biology, including indications of past fluctuations in population size, the extent of gene flow between populations, and relatedness within populations. Nevertheless, this genetic evidence can only be interpreted in conjunction with demographic data and knowledge of the biology of the species concerned. Conservation geneticists need to be encouraged to make full use of their knowledge rather than to rely on the genetic information alone (Nichols 1996). Recent combined applications of genetic markers, demographic monitoring and computer simulation modeling has provided some significant insights into the structure of fragmented populations (Young & Clarke 2000).

On the other hand, genetics will probably play an even more important role in conservation in the future than it does now. The utilization of information from the human and other genome projects will provide substantially more background understanding and appropriate use of these data, should be of great benefit to conservation. In particular, techniques to screen and analyze large amounts of data, will be available to provide an even deeper understanding of how individuals interact on a population level. However, to complement the high statistical power from these data, an evolutionary perspective is required to evaluate their biological importance. In addition, these data will allow an understanding of past evolutionary events, such as bottleneck or gene flow in endangered species (Hedrick 2001).

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