
Effect of Vegetation Matrix on Animal Dispersal: Genetic Evidence from a Study of Endangered Skinks

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Abstract: *Maintaining connectivity in fragmented landscapes is a key principle of biological conservation. Although corridors are a widely accepted approach to connecting populations, their merits are still debated, and they may be impractical in many situations. A focus on management of the vegetation matrix between populations has been advocated as an alternative way to deal with habitat fragmentation and has theoretical support. We combined microsatellite DNA and demographic data to provide an empirical account of how two forms of agricultural land use affect the connectivity of insular populations of an endangered skink in southern New Zealand. The grand skink (*Oligosoma grande*) lives in small populations (approximately 20 individuals) on rock outcrops separated from one another by 50–150 m of inhospitable matrix vegetation (either native tussock grassland or exotic pasture). Skinks typically dispersed short distances, and the nature of the matrix both quantitatively and qualitatively affected dispersal dynamics. Skink populations in pasture were significantly more genetically structured and had less genetic variation than similar populations in tussock, implying less dispersal between populations in pasture than tussock. Furthermore, although female-biased dispersal was a feature of populations in tussock, no sex bias was evident in pasture. In addition, Bayesian individual-based genetic assignment tests that incorporated prior mark-recapture information revealed that some populations produced many emigrants but received few immigrants, whereas other populations were relatively insular. Patterns of dispersal and response to matrix vegetation were complex, and the causes of these patterns deserve attention in future studies of habitat fragmentation. Managing the vegetation matrix may be a practical way to connect animal populations in some situations.*

Key Words: dispersal, genetic assignment tests, genetic structure, habitat fragmentation, matrix, *Oligosoma grande*, skink

Efecto de la Matriz de Vegetación Sobre la Dispersión Animal: Evidencia Genética de un Estudio de Esquincos en Peligro

Resumen: *El mantenimiento de la conectividad en paisajes fragmentados es un principio clave de la biología de la conservación. Aunque los corredores son un enfoque ampliamente aceptado para conectar poblaciones, sus méritos aun son debatidos, y pueden ser imprácticos en muchas situaciones. Se ha considerado al enfoque en el manejo de la matriz de vegetación entre poblaciones como una forma alternativa de ocuparse de la fragmentación de hábitat y que tiene sustento teórico. Combinamos datos de ADN microsatélite y demográficos para proporcionar una explicación empírica del efecto de dos formas de uso de suelo agrícola sobre la conectividad de poblaciones insulares de un esquinco en peligro en Nueva Zelanda. El esquinco (*Oligosoma grande*) vive en poblaciones pequeñas (aproximadamente 20 individuos) en afloramientos rocosos separados entre sí por 50-150 m de matriz de vegetación inhóspita (ya sea pastizal nativo o exótico). Los esquincos*

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típicamente se dispersan por distancias cortas, y la naturaleza de la matriz afectó a la dinámica de dispersión tanto cualitativa como cuantitativamente. Las poblaciones de esquincos en los pastizales exóticos estuvieron genéticamente mejor estructuradas significativamente y tuvieron menos variación genética que poblaciones similares en pastizales nativos, lo que implica menos dispersión entre poblaciones en pastizales exóticos que en nativos. Más aun, aunque la dispersión sesgada hacia hembras fue una característica de las poblaciones en pastizales nativos, no fue evidente en pastizales exóticos. Adicionalmente, las pruebas Bayesianas de asignación genética basadas en individuos que incorporaron información previa de captura-recaptura revelaron que algunas poblaciones produjeron muchos emigrantes pero recibieron pocos inmigrantes, mientras que otras fueron relativamente insulares. Los patrones de dispersión y de respuesta a la matriz de vegetación fueron complejos, y las causas de estos patrones merecen ser atendidas en estudios de fragmentación de hábitat futuros. El manejo de la matriz de vegetación puede ser una manera práctica de conectar poblaciones animales en algunas situaciones.

Palabras Clave: dispersión, esquinco, estructura genética, fragmentación de hábitat, matriz, *Oligosoma grande*, pruebas de asignación genética

Introduction

The maintenance of connectivity in fragmented landscapes is a long-standing principle of conservation biology and stems from the prediction that small, isolated populations risk stochastic extinction (Lande 1988). Because many landscapes are now fragmented, determining how connectivity can best be promoted in these environments is the subject of concerted research (Beier & Noss 1998; Tewksbury et al. 2002). Much attention has been directed toward corridors, which are strips of high-quality habitat that permit animal dispersal between otherwise disconnected patches, but ecologists are increasingly focusing on the nature of the matrix outside habitat patches or corridors and investigating how the matrix influences connectivity in fragmented landscapes (Ricketts 2001; Perfecto & Vandermeer 2002).

Recent computer models that incorporate different types of matrix show that the nature of the matrix can profoundly affect the dynamics of fragmented populations. For example, Fahrig (2001) showed that increasing the quality of the matrix reduces the amount of patch habitat required for a species to persist. Other models demonstrate that by decreasing the resistance of the matrix and thus increasing interpatch dispersal, the overall likelihood of persistence of a metapopulation increases (Vandermeer & Carvajal 2001). These models indicate that managing the matrix is theoretically a useful way to maintain connectivity in a fragmented landscape but require empirical support.

In practice, the influence of the matrix on animal dispersal is likely to be species specific and depend in part on the dispersal ability of the target species (Ricketts 2001). A key to understanding how a target species responds to the matrix is to measure dispersal in different landscapes. For most organisms, however, long-term patterns of dispersal are difficult to measure by conventional means such as mark and recapture; consequently,

most studies compare only short-term patterns of dispersal (e.g., Roland et al. 2000; Jonsen et al. 2001; Ricketts 2001). Yet short-term studies may not detect subtle differences in dispersal rates and may miss important dispersal events if the species are cryptic or if the events are rare (Koenig et al. 1996).

Genetic studies based on patterns of genetic subdivision among populations are sometimes viewed as a rapid way to measure dispersal that can provide a greater temporal perspective on patterns of connectivity between populations (Steinberg & Jordan 1998). Typically, investigators compare estimators of genetic structure, such as F_{ST} , θ , or R_{ST} , and estimators of genetic diversity, such as heterozygosity between populations in different landscapes, with the expectation that the more fragmented landscape should be more genetically structured and less genetically variable (e.g., Knutsen et al. 2000; Mech & Hallett 2001). Although this approach can show evidence of qualitative differences in dispersal between fragmented and unfragmented landscapes, it has at least three limitations. First, all the estimators can be slow to respond to changes in dispersal rates (Steinberg & Jordan 1998; Whitlock & McCauley 1999), making them least useful when perturbations to the landscape are recent (e.g., land clearing). These properties and their relevance to studies of landscape connectivity have been discussed at length in the literature (Sarre 1995; Steinberg & Jordan 1998; Whitlock & McCauley 1999; Pannell & Charlesworth 2000). Second, in providing a measure of dispersal that is applied to all individuals under study, these estimators may not recover the maximum information about dispersal by individuals that is available from highly variable multilocus genotypes (e.g., Paetkau et al. 1995; Stow et al. 2001). Finally, these approaches can generally provide only a qualitative estimate of dispersal because the assumptions required to interpret them quantitatively rarely hold (Whitlock & McCauley 1999). This makes it difficult to integrate the data with the quantitative and instantaneous

demographic data such as birth and death rates that are usually obtained in ecological studies so they can be included in models of population dynamics.

Recent analytical developments in population genetics, in particular genetic assignment tests, can provide improved resolution of patterns of dispersal because they focus on the individual rather than the population (Waser & Strobeck 1998). Applications include testing for sex-biased dispersal, identifying hybrid individuals, and identifying dispersing individuals (e.g., Favre et al. 1997; Galbusera et al. 2000; Beaumont et al. 2001). A further advantage of the assignment approach is the ability to make use of existing demographic data to inform the analysis of genetic data (Pritchard et al. 2000; Gaggiotti et al. 2002), which together can provide highly accurate quantitative dispersal information (Berry et al. 2004).

We investigated how two structurally different types of vegetation matrix influenced connectivity between populations of the endangered skink *Oligosoma grande* (the grand skink) in southern New Zealand. This large lizard is well suited to study of the effects of matrix on dispersal because it lives in small easily censused groups (approximately 20 individuals) on discrete, regularly spaced rock outcrops that are separated from other groups by 50–150 m of vegetation. Two types of vegetation separate rock outcrops: native tussock grassland (*Chionochloa* spp.), which dominated southern New Zealand following Polynesian settlement but is now greatly reduced in extent (McGlone et al. 1995), and exotic pasture. Livestock graze both tussock grassland and pasture, but tussock grassland is denser and structurally more complex than pasture. Results of a previous study suggest that skink populations in pasture are less abundant, patchier, and more extinction prone than populations in tussock, possibly because pasture inhibits dispersal and recolonization of rock outcrops (Whitaker 1996). But this remains untested because long-term patterns of dispersal have not been studied in *O. grande*.

We combined demographic data and data from microsatellite DNA markers to investigate connectivity of grand skink populations in different matrix vegetation. First, we characterized levels of genetic subdivision and genetic diversity in replicate tussock and pasture landscapes to qualitatively examine patterns of connectivity. Then we combined individual-based assignment analyses and mark-recapture data to reveal that patterns of connectivity may be more complex than is obvious from the qualitative genetic analysis alone.

Methods

Field Methods

Our study area was located at Redbank, near Macraes Flat, southern New Zealand (45°25'S, 170°24'E). Part of

this area is designated as a reserve for lizard conservation and consists of a mosaic of tussock grassland and agricultural pasture regularly punctuated by house-sized schist rock outcrops. A map of the study area is in Berry et al. (2004). We used nooses of nylon fishing line to capture skinks from two pasture-dominated sites (P1 and P2) and two tussock-dominated sites (T1 and T2). Each site consisted of between two and four rock outcrops inhabited by skinks (e.g., P1.1, P1.2...T1.1, T1.2) and was chosen because it had large skink populations and the group of rocks was relatively isolated from neighboring rocks. Sites were separated by distances >800 m, which is larger than the maximum dispersal distance recorded for grand skinks (678 m; Houghton 2000), so we considered the sites independent of one another. In 1982 the entire study area was covered by tussock grassland; however, sites P1 and P2 were converted to pasture in 1983 and 1988, respectively. Skinks were captured between November 2000 and April 2001 and were marked with a unique and permanent toe code and released. We captured nearly all resident skinks on each outcrop by repeatedly visiting rock outcrops over the skink's entire active period (November–April). This was verified by estimation of the population sizes by mark-recapture analyses.

Estimation of Population Sizes

We used a mark and resight method to estimate the number of skinks resident on five rock outcrops. Each rock was visited three to six times over a period of 7 to 15 days in April (autumn). Skinks were captured on each visit and marked with a highly visible and unique number with a nontoxic marker. We also counted numbered and unnumbered skinks, noted their size class and mapped their location. Newly marked individuals were released after the completion of each survey. The combination of unique numbers, four easily distinguished size classes, fidelity to crevice retreats, and small population sizes minimized the chances of double counting individuals. Furthermore, because the survey period was brief and births are uncommon at this time, dispersal, deaths, and recruitment were highly unlikely. We analyzed the data as a closed system by the Schumacher-Eschmeyer modification of the Petersen method (Krebs 1999).

DNA and Data Analysis

Tissue samples from all captured skinks were collected for genetic analysis. Samples consisted of toe clips or tail tips (<2 mg tissue) and were placed immediately into liquid nitrogen. The DNA was extracted by a salting-out procedure (Sunnucks & Hales 1996). We genotyped 261 skinks for 15 microsatellite DNA loci following the methods described in Berry et al. (2003).

We tested the data for departures from Hardy-Weinberg and linkage equilibrium with the randomization approaches implemented in FSTAT 2.9.3 (Goudet 1995),

and calculated observed heterozygosity and gene diversity on each rock outcrop according to Nei (1987). As a measure of genetic subdivision, we used Weir and Cockerham's (1984) method to calculate the correlation of gene frequencies (F_{ST}) among all rock outcrops and between all pairs of rock outcrops in FSTAT 2.9.3 (Goudet 1995). When p was <0.05 , significance levels were adjusted with the sequential Bonferroni correction for multiple tests.

Effect of Matrix on Genetic Subdivision and Genetic Diversity

We pooled pairwise F_{ST} estimates from within the two tussock and two pasture sites and used a two-sample randomization test (resampled without replacement, 9999 iterations) implemented in Poptools 2.5.3 (Hood 2002) to test whether the mean pairwise F_{ST} was lower between rocks in pasture than tussock. We also tested whether populations in pasture matrix had lower genetic diversity than those in tussock based on two measures of genetic diversity: (1) allelic richness, a measure of the number of alleles in a population corrected for sample size (Petit et al. 1998) and (2) gene diversity (Nei 1987). These measures were pooled from the tussock and pasture sites, and one-sided tests were performed using the permutation procedures in FSTAT with 1000 permutations.

Isolation by Distance

We used a test for isolation by distance to determine the spatial scale at which skink dispersal was restricted. We used a Mantel test (Mantel 1967) implemented in Poptools to identify significant correlations between genetic differentiation between pairs of rock outcrops (F_{ST}) and distance. We applied the test to data transformed according to Rousset (1997), who showed that in a two-dimensional landscape a linear relationship is expected between $F_{ST}/(1-F_{ST})$ and \ln distance. We used Pearson's correlation coefficient, r , as the test statistic and 9999 randomizations of the distance matrices. We performed this test for all pairwise comparisons and for pairs of populations within sites only. Minimum distances between rocks were estimated using a Garmin GPS (± 5 -m accuracy) and 50-m measuring tapes. Tests for isolation by distance were not conducted within each of the matrix types because of small sample sizes.

Sex-Biased Dispersal

Grand skinks have four easily distinguished age classes (≤ 1 , ≤ 2 , ≤ 3 years, adult; Whitaker 1996), and adults can be sexed by the obvious presence of subcutaneous hemipenes in males. We tested for sex-biased dispersal by comparing the mean corrected assignment index of adult males and females (mAlc; Favre et al. 1997) and F_{ST} between adult males and females. The tests were implemented in FSTAT 2.9.3 and tested for significance with a permutation procedure in which the test statistic (t ;

Goudet et al. 2002) was compared with a null distribution of the statistic generated by randomizing gender with respect to genotype 10,000 times and recalculating the statistic. We carried out separate tests for populations in tussock (six rock outcrops) and pasture (six rock outcrops). Two-sided tests were used because there was no a priori expectation for one sex to disperse more than the other.

Analyses of Individual Multilocus Genotypes

We estimated the probability that individual skinks were born on each of the candidate rocks at each site with a Bayesian-model-based clustering approach implemented with the program Structure 2.0 (Pritchard et al. 2000). Structure allows prior information to be incorporated into the model, and to aid clustering we used mark-recapture records of the natal rocks of a subset of the skinks under study (131 of 261 individuals). These individuals had been studied as part of a long-term mark-recapture investigation (M.D.T., unpublished data). In the model, we specified that we had high confidence in the natal rock outcrop of these skinks but allowed a small probability ($v = 0.03$) that these classifications were wrong (0.03 is the empirically determined error rate expected from our definition of natal rock; see Berry et al. [2004] for details). We used this prior information to foster clustering of the remaining individuals whose natal rocks were unknown. The Structure program estimates the posterior probability (Q) that each skink was born on each of K candidate rock outcrops at each site. Because our mark-recapture data showed that few individuals (3 of 131) originated from outside the study sites (M.D.T., unpublished data), we specified K to equal the number of rocks at the site. We also specified that allele frequencies on different candidate rock outcrops were correlated (FREQSCORR = 1) and left the remainder of the input parameters at default values. We ran the clustering for a burn-in of 50,000 iterations followed by a run for 1×10^5 iterations of the Markov chain. We ran 10 replicates for each site to check for multimodality in the clustering process.

Results

Sample and Population Sizes

A total of 261 skinks were genotyped from the study rocks. The number of skinks genotyped was very close to the estimated population size on the five rock outcrops for which population estimates were made (Table 1). Because capture effort was more than twice as high at rocks where population estimates were not made, it is likely that we genotyped nearly all the skinks inhabiting each rock outcrop.

Table 1. Summary statistics for 12 grand skink populations genotyped for 13 microsatellite loci.

Site	Rock	Distance to nearest rock (m)	Population estimate ^a (95% CI)	Minimum population index ^b	No. genotyped ^c (\pm SE)	A ^d (\pm SE)	H _{exp} ^e (\pm SE)	H _{obs} ^f (\pm SE)
P1	1	71	13.09 (8.85–25.13)	12	12.0 \pm 0.0	6.5 \pm 0.4	0.787 \pm 0.004	0.769 \pm 0.123
	2	71	—	14	12.8 \pm 0.2	5.8 \pm 0.7	0.744 \pm 0.007	0.669 \pm 0.236
	3	164	—	38	37.4 \pm 0.3	9.0 \pm 0.8	0.776 \pm 0.020	0.803 \pm 0.108
T1	1	61	—	32	22.7 \pm 0.4	8.2 \pm 0.7	0.793 \pm 0.045	0.800 \pm 0.180
	2	34	—	22	21.1 \pm 0.3	8.1 \pm 0.8	0.794 \pm 0.022	0.816 \pm 0.082
	3	89	—	25	22.0 \pm 0.0	8.4 \pm 0.6	0.800 \pm 0.040	0.860 \pm 0.178
	4	34	—	16	15.9 \pm 0.1	8.2 \pm 0.7	0.800 \pm 0.042	0.820 \pm 0.193
P2	1	223	28.24 (23.17–36.15)	29	28.6 \pm 0.2	7.9 \pm 0.5	0.780 \pm 0.026	0.834 \pm 0.122
	2	184	24.24 (19.45–32.17)	27	26.4 \pm 0.1	6.8 \pm 0.4	0.728 \pm 0.051	0.766 \pm 0.214
	3	184	17.57 (13.36–25.65)	19	18.3 \pm 0.2	6.2 \pm 0.0	0.741 \pm 0.045	0.791 \pm 0.188
T2	1	135	22.88 (20.74–25.51)	24	22.0 \pm 0.7	7.5 \pm 0.5	0.768 \pm 0.045	0.811 \pm 0.176
	2	135	—	15	15.0 \pm 0.0	6.8 \pm 0.4	0.760 \pm 0.050	0.744 \pm 0.207

^aMark-recapture estimate of population size (— indicates a mark-recapture estimate was not made).

^bTotal number of skinks captured on a rock outcrop.

^cAverage number of skinks genotyped at each locus.

^dAverage number of alleles at each locus.

^eAverage gene diversity at each locus (Nei 1987).

^fAverage observed heterozygosity at each locus.

Characteristics of Microsatellite Markers

The 15 microsatellite loci typed were highly variable. The average number of alleles per locus was 15.73 (\pm 1.16 SE), and the average observed heterozygosity was 0.76 (\pm 0.03 SE). Populations on each rock outcrop also exhibited high genetic diversity (Table 1). Based on a global test, there was no significant overall departure from Hardy-Weinberg equilibrium ($p > 0.05$). But tests per locus and per rock outcrop showed that two loci had significant deficits of heterozygotes, indicating the existence of null alleles on some rock outcrops. We excluded these loci from the remaining analyses. Loci were not significantly in linkage disequilibrium.

Genetic Subdivision

Skink populations were significantly genetically structured. The overall level of genetic subdivision (F_{ST}) among all 12 rock outcrops sampled was 0.097 ± 0.006 SE, which was significantly different from zero ($p < 0.0002$). Furthermore, all pairwise tests for differentiation between rock outcrops were significant except between rocks P1.1 and P1.2.

Effect of Matrix Type on Genetic Subdivision and Genetic Diversity

The F_{ST} was lower at the two-tussock dominated sites than at the two pasture-dominated sites (Fig. 1). In addition, the mean pairwise F_{ST} was significantly lower at tussock-dominated sites than at pasture-dominated sites ($p = 0.03$), and both allelic richness and gene diversity were significantly lower on rocks in pasture than in tussock ($p < 0.05$; Fig. 2).

Isolation by Distance and Sex-Biased Dispersal

There was a positive and significant association between genetic differentiation ($F_{ST}/1-F_{ST}$) and \ln distance between rock outcrops both overall and within sites (overall $r = 0.669$, $p < 0.001$; within sites $r = 0.502$, $p = 0.041$).

In tussock-dominated sites, the test based on mean corrected assignment index detected significant female-biased dispersal (male mAIC = 1.52, $n = 15$, female mAIC = -1.14 , $n = 20$, $p = 0.05$), but no evidence for sex-biased dispersal was detected among the populations in pasture (male mAIC = -0.638 , $n = 21$, female mAIC = 0.216, $n = 26$, $p = 0.46$). Similarly, tests based on F_{ST} detected a significant female-biased dispersal in tussock (male $F_{ST} = 0.089$, female $F_{ST} = 0.006$, $p = 0.02$) but not in pasture (male $F_{ST} = 0.069$, female $F_{ST} = 0.113$, $p = 0.23$).

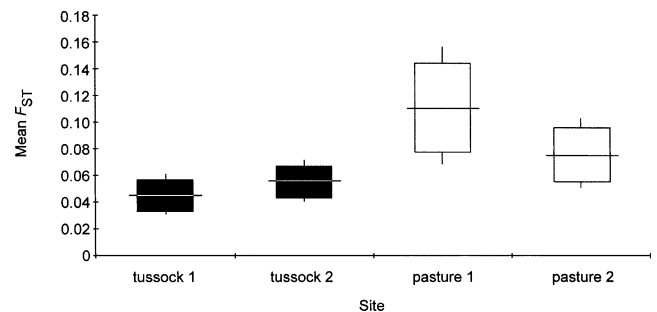


Figure 1. Comparison of genetic differentiation (mean F_{ST}) of grand skinks between rock outcrops at each study site (horizontal line), and showing 95% and 99% CI derived by bootstrapping over loci (box and whiskers, respectively).

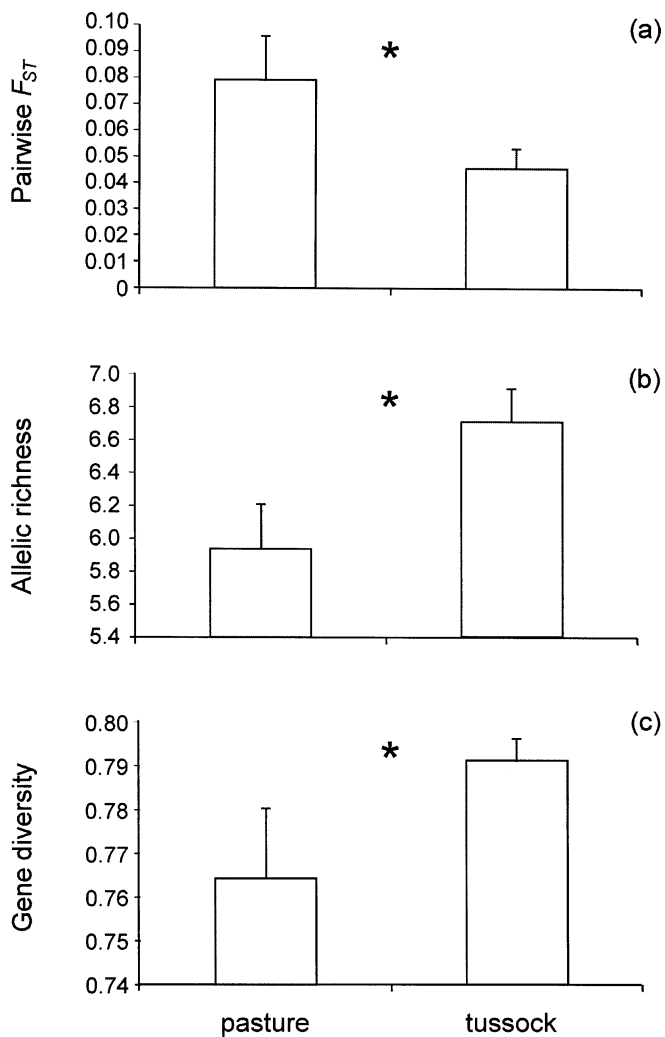


Figure 2. Comparison of (a) mean pairwise F_{ST} , (b) allelic richness, and (c) gene diversity of grand skinks in pasture and tussock-dominated sites (with SE). An asterisk (*) indicates a significant difference ($p < 0.05$).

Analyses of Individual Multilocus Genotypes

Examination of the range of values of Q for individuals in tussock and pasture revealed patterns of dispersal that were sometimes not obvious from population-level analyses illustrated in Figs. 1 and 2 (Fig. 3). At pasture site P2 seven skinks had higher probabilities of belonging to rocks other than their capture rocks, marking these individuals as possible dispersers. These assignments are given strong support because Berry et al. (2004) showed that assignments of skinks at this site were 100% correct relative to known mark-recapture data.

Clustering of the data for pasture site P1 converged at two different modes in replicate runs, indicating that the loci did not have sufficient power to consistently re-

veal population structure. This multimodality occurred because of the failure to resolve genetic structure between rocks P1.1 and P1.2, which lacked significant genetic structure and were effectively a single population. Combining these populations and rerunning the clustering consistently resulted in a single mode. In this case only five individuals had probabilities of originating on rock outcrops different from their capture rock (Fig. 3). These individuals are likely to be dispersers because Berry et al. (2004) showed that assignments of skinks at this site relative to mark-recapture data were accurate in 95% of cases.

More complex patterns were evident for the two tussock sites, with some rock outcrops appearing to be highly admixed and others showing little admixture (Fig. 3). At tussock site T2, 10 skinks from rock T2.1 had high probabilities of belonging to rock T2.2, but no skinks from rock T2.2 had high probabilities of belonging to rock T2.1. Similarly, at tussock site T1, few skinks from either rocks T1.1 or T1.3 had high probabilities of belonging to another rock, but many skinks from rocks T1.2 and T1.3 had high probabilities of belonging to other rock outcrops. In the case of rock T1.2, most of these individuals were assigned to rock T1.3, and in the case of rock T1.3, most individuals were assigned to rock T1.1. Most of these skinks are likely to be dispersers because Berry et al. (2004) showed that between 79% (site T1) and 65% (site T2) of skinks were assigned correctly relative to mark-recapture data at these sites.

Discussion

The global spread of intensive agriculture means that many species now occur as small populations embedded within highly modified landscapes. Although corridors are a widely accepted approach to connecting populations (Rosenberg et al. 1997), their merits are still debated (Beier & Noss 1998) and they are likely to be impractical in many situations. A focus on managing the matrix has been advocated as an alternative way to deal with habitat fragmentation (Perfecto & Vandermeer 2002) and has theoretical support. We sought to provide an empirical account of how two forms of agricultural land use affect the connectivity of populations of endangered skinks.

Study Design and Interpretation

Making inferences about landscape connectivity from genetic data poses several difficulties that must be addressed by appropriate experimental design (Steinberg & Jordan 1998). First, one must assume that the underlying patterns of genetic variation before land-use change or fragmentation were homogenous across the study area and are not the result of historical effects such as range changes (cf. Cunningham & Moritz 1998). In our case, because of the

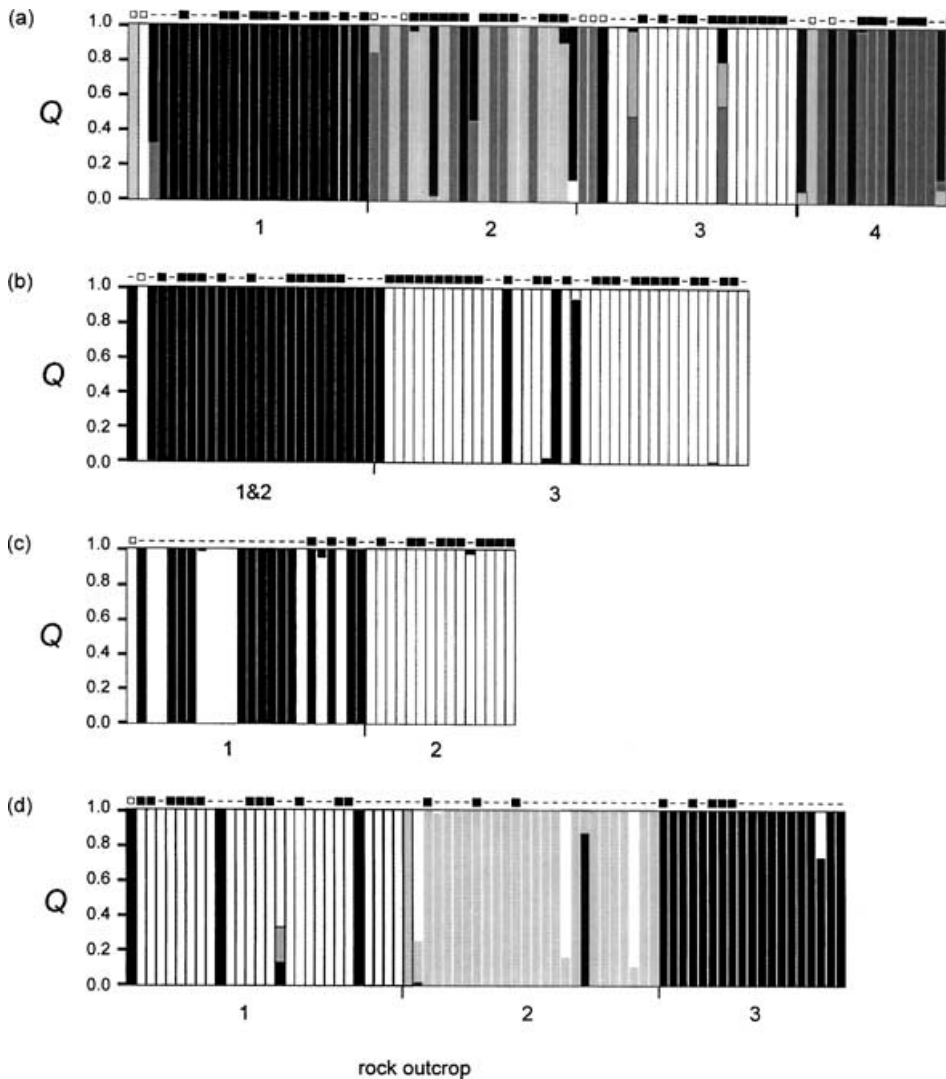


Figure 3. Results of Bayesian clustering of individual skink genotypes with Structure 2.0. Each site is shown separately: (a) site T1, (b) site P1, (c) site T2, (d) site P2. Individuals are represented across the x-axis by a vertical bar that may be divided into shaded segments that represent that individual's probability of originating (Q) from each of the rocks at a study site. Skinks are also grouped across the x-axis according to the rock they were captured on. Filled squares above an individual indicate that the natal rock was known and the individual did not disperse, open squares indicate that the individual was a known disperser, and dashes indicate that the natal rock was not known for that individual.

small spatial scale of the study and interspersed replicate sites, the observed differences are unlikely to be the result of such effects.

Second, estimators of genetic structure and genetic variation can be biased by small sample size or nonrandom sampling (Hansen et al. 1997; Mossman & Waser 2001; Tallmon et al. 2002), meaning that samples do not adequately represent the genetic variation present in populations. We used mark-recapture population estimates to show that genetic sampling of the populations was nearly complete, thus allowing us to examine the effects of the matrix with little sampling error.

Third, it is important to match the characteristics of populations in the two types of landscapes because the rate of loss of genetic variation within populations and increase in genetic divergence between populations may result from three factors: differences in the size of populations, the rate of migration, and the rate of mutation (Wright 1969). Because only rate of migration is of interest, the other two characters must be matched. A central

feature of our design was that populations in pasture and tussock were replicated and about the same size (based on minimum population size, t test, $p > 0.05$), making any observed treatment effects the result of dispersal rate (we also assumed that mutation rate is equal in pasture and tussock populations). Although we used replicate sites in an attempt to account for inherent differences that could confound our results, populations in pasture were on average farther apart than those in tussock ($205 \text{ m} \pm 38.3 \text{ SE}$ vs. $92 \text{ m} \pm 20.7 \text{ SE}$). This should increase genetic subdivision in pasture relative to tussock, and without additional study sites we could not determine the extent to which this contributes to our results. This difference, however, was largely the result of widely separated populations at pasture site P2 because site P1, which had been pasture for the longest period (over 18 years), had the most genetically subdivided populations, but its populations were not separated by distances significantly different from those in tussock (t test, $p > 0.05$). This suggests that the matrix-limited dispersal is a more critical factor.

Environmental and Genetic Patchiness

Grand skinks live in a naturally patchy environment, and this patchiness is reflected in the fine-scaled genetic structure detected. Several lines of evidence indicate that dispersal is very localized in this species. First, a pattern of isolation by distance existed at both small (<350 m) and moderate (<2.5 km) spatial scales, indicating that skinks disperse more often between nearby rock outcrops than more distant rock outcrops (even at scales ≤ 350 m). Second, neighboring populations were genetically distinct in nearly all cases, even though some were only 35 m apart (e.g., between rocks T1.2 and T1.3). These observations are supported by mark-recapture data showing some skinks to have remained on a single rock for over 12 years (M.D.T. unpublished data.).

Although the fine scale of genetic structuring indicated that dispersal was restricted between rock outcrops, it is unlikely that the high levels of genetic diversity recorded on each rock outcrop could be sustained in such small populations without recurrent dispersal (cf Tallmon et al. 2002). Completely isolated populations with population sizes similar to those recorded here ($n \sim 25$) would probably have effective population sizes (N_e) 10–50% of that size (Frankham 1995) and would lose neutral genetic variation rapidly. For example, without immigration, they would on average lose 50% of their heterozygosity within $1.39 \times N_e$ generations, which is between 3.5 and 17.4 generations (Hartl & Clark 1997). The high levels of genetic variation observed indicate that despite the patchiness of the rocky habitat, regular short-distance dispersal must also be a characteristic of this species. This provides independent evidence that grand skinks persist in this patchy environment through the formation of a metapopulation (Whitaker 1996). Further, it makes focus on dispersal particularly relevant in this species because species with limited dispersal abilities may be more responsive to the nature of the matrix than more vagile species (Ricketts 2001).

Importance of Matrix

Theory predicts that small isolated populations are more subject to genetic drift than populations experiencing dispersal and gene flow, should feature less genetic variation, and should be more genetically structured (Wright 1969). Our results are consistent with these expectations—populations in pasture were predicted to be more insular than those in tussock (Whitaker 1996)—and we showed that they had less genetic variation and were more genetically structured than similar populations in tussock. This result is notable because results from short-term (≤ 2 years) studies of dispersal based on mark-recapture data have not shown any significant effect of the matrix on rates of dispersal (Whitaker 1996; Houghton 2000). Importantly, reduced dispersal in pasture-dominated land-

scapes means that populations should be more subject to stochastic processes (Hanski 1998) and explains why populations are patchier and more extinction prone (Whitaker 1996).

Skink Sources and Sinks?—Individual-Based Analysis

Individual-based genetic analyses revealed that patterns of dispersal relative to the vegetation matrix were more complex than indicated by the standard qualitative genetics analyses. Based on the qualitative genetic results from tussock study sites, we expected more skinks to be identified as likely dispersers than in the pasture sites. Yet, we observed that some rock outcrops were highly admixed as expected, whereas others were relatively insular. For example, rocks T1.1 and T2.2 both received a few or no immigrants but contributed many to neighboring rock outcrops. It is unclear whether this pattern is indicative of source-sink dynamics (Harrison 1991) or what features of these rock outcrops promote greater emigration than immigration, but density-dependent emigration resulting from resource limitation merits investigation (e.g., Gaggiotti et al. 2002).

The assignment results from the pasture sites were more in line with the expectations from the qualitative genetic analysis, with few skinks from any rocks identified as likely dispersers. The one exception was between two rocks that were highly admixed at site P1. These populations were separated by 71 m, but two small rock outcrops inhabited by a handful of skinks (≤ 3) were situated in the intervening space and may have facilitated the high level of dispersal by acting as stepping stones.

Sex-Biased Dispersal and the Matrix

Sex-biased dispersal has been identified in a handful of lizard species and in most cases has been male biased (e.g., Doughty et al. 1994; Gardner et al. 2001). Our results show that (1) neither sex is strictly philopatric because both male and female individuals had low mAlc scores (data not shown), (2) female-biased dispersal was a feature of populations in tussock, and (3) sex bias was absent in pasture. The first observation is supported by data from a mark-recapture study that detected both male and female dispersers between rock outcrops (M.D.T., unpublished data; Houghton 2000). The effect of the matrix on sex-biased dispersal (observations 2 and 3) is important because it indicates that not only did the pasture matrix reduce levels of dispersal, but it also may have qualitatively affected the individuals that disperse. This effect of habitat fragmentation is seldom studied (see Stow et al. 2001; Stow & Sunnucks 2004), and it is unclear whether it might detrimentally alter the demography or reproductive behavior of isolated populations. The detection of a sex bias in dispersal is also notable because no consistent sex bias in interpopulation dispersal was detected in

earlier mark-recapture studies of this species (Whitaker 1996; Houghton 2000; Marshall 2000).

Implications for Skink and Tussock Grassland Conservation

Most studies of habitat fragmentation focus on forest-dwelling mammals or birds and rarely on reptiles or grasslands (McGarigal & Cushman 2002). Yet in some cases these groups represent major components of regional biodiversity. In New Zealand, where native terrestrial mammals are absent, reptiles form a large proportion of vertebrate biodiversity, but most species have suffered major range declines or extinctions since human settlement (Towns et al. 2001). Similarly, montane tussock grasslands were historically a prominent vegetation assemblage in southern New Zealand but have been greatly reduced in area by agricultural development (McGlone et al. 1995; McGlone 2001). Our results have relevance to the conservation of both the grand skink and native tussock grasslands. The grand skink has suffered a massive range collapse (Whitaker & Loh 1995). Because this coincided with the widespread loss of native vegetation it has been speculated that the processes are related (Whitaker & Loh 1995; Whitaker 1996). We have demonstrated an effect of matrix vegetation that has the potential to alter landscape connectivity for grand skink populations and possibly even alter social dynamics. This is cause for concern because it is well established that small populations such as those studied here are vulnerable to stochastic extinctions resulting from both demographic and genetic causes should they become isolated (Lande 1988). Linking landscape change to the decline of this species, however, is complicated by the simultaneous pressures of multiple introduced mammalian predators (Towns et al. 2001), which may act in combination with landscape change to endanger skink populations. The effects of introduced predators on skink populations are currently under study (M.D.T., unpublished data).

Summary

We aimed to provide an empirical example of how different agricultural land uses affect connectivity of animal populations. Our combined genetic and demographic data enabled us to make four main observations about the significance of vegetation matrix for skink dispersal: (1) grand skinks live in small groups on rock outcrops and usually disperse short distances; (2) on average, dispersal was less frequent between populations in pasture than between populations in tussock; (3) female skinks were more likely than males to disperse between rock outcrops in tussock but not in pasture; and (4) despite observation 2, not all rocks in tussock were characterized by high emigration and immigration—some appeared to function as sources of immigrants to neighboring populations and received relatively few immigrants themselves. Together, these results suggest that the nature of the matrix can both

quantitatively and qualitatively affect dispersal, but characteristics of individual populations may also play a role in determining dispersal dynamics and warrant further attention in studies of habitat fragmentation. Managing the vegetation matrix may be a practical way to connect fragmented populations in some situations.

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Literature Cited

- Beaumont, M., E. Barratt, D. Gottelli, A. C. Kitchener, M. J. Daniels, J. K. Pritchard, and M. W. Bruford. 2001. Genetic diversity and introgression in the Scottish wildcat. *Molecular Ecology* **10**:319–336.
- Beier, P., and R. F. Noss. 1998. Do habitat corridors provide connectivity? *Conservation Biology* **12**:1241–1252.
- Berry, O., D. M. Gleeson, and S. D. Sarre. 2003. Microsatellite DNA markers for New Zealand skinks. *Conservation Genetics* **4**:411–414.
- Berry, O., M. D. Tocher, and S. D. Sarre. 2004. Can assignment tests measure dispersal? *Molecular Ecology* **13**:551–561.
- Cunningham, M., and C. Moritz. 1998. Genetic effects of forest fragmentation on a rainforest restricted lizard (Scincidae: *Gnypetoscincus queenslandiae*). *Biological Conservation* **83**:19–30.
- Doughty, P., B. Sinervo, and G. M. Burghardt. 1994. Sex-biased dispersal in a polygynous lizard, *Uta stansburiana*. *Animal Behaviour* **47**:227–229.
- Fahrig, L. 2001. How much habitat is enough? *Biological Conservation* **100**:65–74.
- Favre, L., F. Balloux, J. Goudet, and N. Perrin. 1997. Female biased dispersal in the monogamous mammal *Crocidura russula*: evidence from field data and microsatellite patterns. *Proceedings of the Royal Society of London B* **264**:127–132.
- Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: a review. *Genetical Research* **66**:95–107.
- Gaggiotti, O. E., F. Jones, W. M. Lee, W. Amos, J. Harwood, and R. A. Nichols. 2002. Patterns of colonisation in a metapopulation of grey seals. *Nature* **416**:424–427.
- Galbusera, P., L. Lens, T. Schenck, W. Waiyaki, and E. Matthysen. 2000. Genetic variability and gene flow in the globally, critically-endangered Taita thrush. *Conservation Genetics* **1**:45–55.
- Gardner, M. G., C. M. Bull, S. J. B. Cooper, and G. Duffield. 2001. Genetic evidence for a family structure in stable social aggregations of the Australian lizard *Egernia stokesii*. *Molecular Ecology* **10**:175–183.
- Goudet, J. 1995. FSTAT (vers. 1.2): a computer program to calculate F-statistics. *Journal of Heredity* **86**:485–486.

- Goudet, J., N. Perrin, and P. Waser. 2002. Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Molecular Ecology* **11**:1103–1114.
- Hansen, M. M., E. E. Nielsen, and K.-L. D. Mensburg. 1997. The problem of sampling families rather than populations: relatedness among individuals in samples of juvenile brown trout *Salmo trutta* L. *Molecular Ecology* **6**:469–474.
- Hanski, I. 1998. Metapopulation dynamics. *Nature* **396**:41–49.
- Harrison, S. 1991. Local extinction in a metapopulation context: an empirical evaluation. *Biological Journal of the Linnean Society* **42**:73–88.
- Hartl, D. L., and G. C. Clark. 1997. Principles of population genetics. 3rd edition. Sinauer Associates, Sunderland, Massachusetts.
- Hood, G. 2002. Poptools 2.5.3. CSIRO, Clayton, Victoria, Australia. Available from <http://www.cse.csiro.au/poptools/index.htm> (accessed March 2003).
- Houghton, C. 2000. The dispersal and metapopulation dynamics of two skink species, *Oligosoma grande* and *O. ottagense* at Macraes Flat, Otago. M.S. thesis. Department of Zoology, University of Otago, Otago, New Zealand.
- Jonsen, I. D., R. S. Bouchier, and J. Roland. 2001. The influence of matrix habitat on *Aphthona* flea beetle immigration to leafy spurge patches. *Oecologia* **127**:287–294.
- Knutsen, H., B. Arne, P. Jorde, and R. Ims. 2000. Genetic differentiation among populations of the beetle *Bolitophagus reticulatus* (Coleoptera: Tenebrionidae) in a fragmented and a continuous landscape. *Heredity* **84**:667–676.
- Koenig, W. D., D. V. Vuren, and P. N. Hooge. 1996. Detectability, philopatry, and the distribution of dispersal distances in vertebrates. *Trends in Ecology & Evolution* **11**:514–517.
- Krebs, C. J. 1999. Ecological methodology. 2nd edition. Benjamin/Cummings, Menlo Park, California.
- Lande, R. 1988. Genetics and demography in biological conservation. *Science* **241**:1455–1460.
- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**:209–220.
- Marshall, L. J. 2000. Home ranges and activity patterns of sympatric grand and Otago skinks. M.S. thesis. Department of Zoology, University of Otago, Otago, New Zealand.
- McGarigal, K., and S. A. Cushman. 2002. Comparative evaluation of experimental approaches to the study of habitat fragmentation effects. *Ecological Applications* **12**:335–345.
- McGlone, M. S. 2001. The origin of the indigenous grasslands of southeastern South Island in relation to pre-human woody ecosystems. *New Zealand Journal of Ecology* **25**:1–15.
- McGlone, M. S., A. F. Mark, and D. Bell. 1995. Late Pleistocene and Holocene vegetation history, central Otago, South Island, New Zealand. *Journal of the Royal Society of New Zealand* **25**:1–22.
- Mech, S. G., and J. G. Hallett. 2001. Evaluating the effectiveness of corridors: a genetic approach. *Conservation Biology* **15**:467–474.
- Mossman, C. A., and P. M. Waser. 2001. Effects of habitat fragmentation on population genetic structure in the white-footed mouse (*Peromyscus leucopus*). *Canadian Journal of Zoology* **79**:285–295.
- Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.
- Paetkau, D., W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite analysis of population structure of Canadian polar bears. *Molecular Ecology* **4**:347–354.
- Pannell, J. R., and B. Charlesworth. 2000. Effects of metapopulation processes on measures of genetic diversity. *Philosophical Transactions of the Royal Society of London B* **355**:1851–1864.
- Perfecto, I., and J. Vandermeer. 2002. Quality of agroecological matrix in a tropical montane landscape: ants in coffee plantations in southern Mexico. *Conservation Biology* **16**:174–182.
- Petit, R. J., A. E. Mousadik, and O. Pons. 1998. Identifying populations for conservation on the basis of genetic markers. *Conservation Biology* **12**:844–855.
- Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**:945–959.
- Ricketts, T. H. 2001. The matrix matters: effective isolation in fragmented landscapes. *The American Naturalist* **158**:87–99.
- Roland, J., N. Keyghobadi, and S. Fownes. 2000. Alpine *Parnassius* butterfly dispersal: effects of landscape and population size. *Ecology* **81**:1642–1653.
- Rosenberg, D. K., B. R. Noon, and E. C. Meslow. 1997. Biological corridors: form, function, and efficacy. *BioScience* **47**:677–687.
- Rousset, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* **145**:1219–1228.
- Sarre, S. 1995. Mitochondrial DNA variation among populations of *Oedura reticulata* (Gekkonidae) in remnant vegetation: implications for metapopulation structure and population decline. *Molecular Ecology* **4**:395–405.
- Steinberg, E. K., and C. E. Jordan. 1998. Using molecular genetics to learn about the ecology of threatened species: the allure and the illusion of measuring genetic structure in natural populations. Pages 440–460 in P. L. Fiedler and P. M. Kareiva, editors. *Conservation biology: for the coming decade*. 2nd edition. Chapman & Hall, New York.
- Stow, A. J., P. Sunnucks, D. A. Briscoe, and M. G. Gardner. 2001. The impact of habitat fragmentation on dispersal of Cunningham's skink (*Egernia cunninghami*): evidence from allelic and genotypic analyses of microsatellites. *Molecular Ecology* **10**:867–878.
- Stow, A. J., and P. Sunnucks. 2004. High mate and site fidelity in Cunningham's skinks (*Egernia cunninghami*) in natural and fragmented habitat. *Molecular Ecology* **13**:419–430.
- Sunnucks, P., and D. F. Hales. 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution* **13**:510–524.
- Tallmon, D. A., H. M. Draheim, L. S. Mills, and F. W. Allendorf. 2002. Insights into recently fragmented vole populations from combined genetic and demographic data. *Molecular Ecology* **11**:699–709.
- Tewksbury, J. J., D. J. Levey, N. M. Haddad, S. Sargent, J. L. Orrock, A. Welton, B. J. Danielson, J. Brinkerhoff, E. I. Damschen, and P. Townsend. 2002. Corridors affect plants, animals, and their interactions in fragmented landscapes. *Proceedings of the National Academy of Sciences of the United States of America* **99**:12923–12926.
- Towns, D. R., C. H. Daugherty, and A. Cree. 2001. Raising the prospects for a forgotten fauna: a review of 10 years of conservation effort for New Zealand reptiles. *Biological Conservation* **99**:3–16.
- Vandermeer, J., and R. Carvajal. 2001. Metapopulation dynamics and the quality of the matrix. *The American Naturalist* **158**:211–220.
- Waser, P. M., and C. Strobeck. 1998. Genetic signatures of interpopulation dispersal. *Trends in Ecology & Evolution* **13**:43–44.
- Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38**:1358–1370.
- Whitaker, A. H. 1996. Impact of agricultural development on grand skink (*Oligosoma grande*) (Reptilia: Scincidae) populations at Macraes Flat, Otago, New Zealand. Science for conservation report 33. New Zealand Department of Conservation, Wellington.
- Whitaker, A. H., and G. Loh. 1995. Otago skink and grand skink recovery plan (*Leiopisma ottagense* and *L. grande*). Threatened species recovery plan 14. New Zealand Department of Conservation, Wellington.
- Whitlock, M. C., and D. E. McCauley. 1999. Indirect estimates of gene flow and migration: $F_{st} \neq 1/(4Nm + 1)$. *Heredity* **82**:117–125.
- Wright, S. 1969. The theory of gene frequencies. University of Chicago Press, Chicago.