Genetic consequences of population subdivision: the marsupial *Micoureus paraguayanus* (Mammalia: Didelphimorphia) as a case study

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ABSTRACT. Habitat fragmentation may cause population subdivision, affecting genetic variation, leading to heterozygosity loss and increased inbreeding, and contributing to population extinction. However, some genetic models have shown that under some conditions, population subdivision can favor heterozygosity and allelic diversity, and small populations may adapt to inbreeding. Here I investigate the relationship between population subdivision and genetic diversity for the marsupial *Micoureus paraguayanus* (Tate, 1931) using the program Vortex. Hypothetical populations of 100 and 2000 individuals were partitioned into 1, 2, 5 or 10 populations that were linked by varying rates of dispersal and also by sex-biased dispersal. Results suggested that heterozygosity and allelic diversity declined rapidly when a population was subdivided. Genetic and demographic stochasticity diminished the effectiveness of selection against recessive lethal alleles. Dispersal partly reversed the impacts of population subdivision. However, even high rates of dispersal did not eliminate demographic fluctuations or prevent extinction. Although gene flow largely prevented genetic divergence between populations, dispersal did not prevent heterozygosity from being lost more rapidly in subdivided populations than in single populations of equivalent total size. The dynamics of small, fragmented populations were critically dependent on interactions between demographic and genetic processes. Populations of *M. paraguayanus* may have to be relatively large and continuous to avoid significant losses of genetic diversity.

KEYWORDS. Genetic drift; genetic load; habitat fragmentation; inbreeding; metapopulation; population viability analysis.
than by natural selection (Lacy 1987). This results in lower heterozygosity and rapid loss of alleles (Lacy 1987, 1997, Hedrick 2001, Keller & Waller 2002). Reduced heterozygosity can impact population demography and viability by diminishing other aspects of fitness, such as fecundity, mating success, disease resistance and competitive ability (Allen dore & Leary 1986, Ralls et al. 1988, Lacy 1993a, Miller & Hedrick 1993). Allele loss may reduce flexibility for future adaptive evolution (Van Valen 1973, Franklin 1980, Brito & Fernandez 2000a). Although population subdivision results in faster genetic drift within habitat patches, some of the genetic diversity that is lost from populations within patches is converted into between-patch diversity (Lacy 1987). As a result, genetic diversity within a metapopulation may be greater than that retained within a single population of comparable total size (Boecklen 1986, Lacy 1987).

Intentional, managed fragmentation of continuous populations, with low dispersal among populations has been recommended as a strategy to preserve genetic variation (Boecklen 1986, Lacy 1987). However, genetic decay, manifest in both inbreeding depression and loss of evolutionary flexibility, may cause the demise of small populations (Gilpin & Soule 1986, Lacy 1993a, Brito & Fernandez 2000a). Therefore, it is important to understand under what conditions fragmentation causes deleterious genetic consequences, and under what conditions such effects can be beneficial.

This study uses population viability analysis (PVA) as a tool to model genetic aspects in response to population subdivision for Micoureus paraguayanus (Tate, 1931), Didelphidae. Here I assess the impacts of fragmentation on genetic changes within populations of M. paraguayanus that were divided into metapopulations of varying size and dispersal levels.

**MATERIAL AND METHODS**

*Micoureus paraguayanus* is an endemic marsupial of the Atlantic Forest (Gardner 2005). This small (~130g), nocturnal, largely arboreal, solitary and polygynous didelphid is found in mature and secondary forest (Passamani 1995, 2000, Grelle 2003). The species favors dense viny vegetation with many palm trees (Moraes & Chiarello 2005b). *M. paraguayanus* feeds on arthropods, fruits and nectar (Leite et al. 1994, Carvalho et al. 1999, Caceres et al. 2002, Pinheiro et al. 2002, Fernandez et al. 2006). Demographic data of *M. paraguayanus* that were input to Vortex (Tab. I) are based on a wide range of field studies (Pires & Fernandez 1999, Pires et al. 1999, 2002, Brito & Fernandez 2000b, 2002, Quintal et al. 2001, Fernandez et al. 2003, 2006, Brito & Grelle 2004, Moraes & Chiarello 2005a, b, Brito & da Fonseca 2006, 2007, Goulart et al. 2006). The average size of its home range is estimated at 0.65 ha, home ranges of males were overlapping with those of females (Pires & Fernandez 1999, Pires et al. 1999). Adult females and males first breed at 6 months of age with a maximum of 11 young per litter. The longest lifespan recorded for the species in the wild is about 24 months. Pires et al. (2002) estimated a dispersal rate of 1.2% between Atlantic Forest fragments in southeastern Brazil for *M. paraguayanus*, crossing up to 800 m of matrix habitat. Pires & Fernandez (1999) studied a metapopulation of *M. paraguayanus* in Atlantic Forest remnants and observed that movements among forest patches were made by adult males, during the reproductive season, and that the vast majority of individuals (males and females) were restricted to a single fragment during their whole life. There is evidence for male-only dispersal in *M. paraguayanus* (Pires & Fernandez 1999, Pires et al. 2002), and in this case, local extinctions cannot be naturally replaced by recolonization. A study on the frequency of movements of small mammals among Atlantic Forest fragments in southeastern Brazil estimated an inter-fragment movement rate of 1.2% for *M. paraguayanus* (Pires et al. 2002), a value quite similar and within the range of the scenarios modeled in the present analysis. However, there is evidence that females are also capable of dispersing through grassland matrix, at least to some extent (Moraes & Chiarello 2005a).

The computer simulation package Vortex version 9.57 was used. Vortex is a Monte Carlo simulation of the effects of deterministic forces as well as demographic, environmental and genetic consequences of population subdivision:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Breeding system</td>
<td>Polygynous</td>
</tr>
<tr>
<td>Maximum age</td>
<td>12</td>
</tr>
<tr>
<td>Minimum female breeding age</td>
<td>3</td>
</tr>
<tr>
<td>Minimum male breeding age</td>
<td>3</td>
</tr>
<tr>
<td>Sex ratio (%males)</td>
<td>50%</td>
</tr>
<tr>
<td>% females producing litters</td>
<td>16.67%</td>
</tr>
<tr>
<td>% litter size 1</td>
<td>0.10%</td>
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<tr>
<td>% litter size 2</td>
<td>1.40%</td>
</tr>
<tr>
<td>% litter size 3</td>
<td>15.50%</td>
</tr>
<tr>
<td>% litter size 4</td>
<td>83%</td>
</tr>
<tr>
<td>% males in breeding pool</td>
<td>100%</td>
</tr>
<tr>
<td>Female mortality</td>
<td>8.72%</td>
</tr>
<tr>
<td>Male mortality</td>
<td>8.72%</td>
</tr>
<tr>
<td>Density dependence</td>
<td>nil</td>
</tr>
<tr>
<td>Deterministic population growth (lambda)</td>
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</tr>
<tr>
<td>Generation time (years)</td>
<td>1.08</td>
</tr>
<tr>
<td>Environmental variation as SD (fluctuation across years)</td>
<td></td>
</tr>
<tr>
<td>In fecundity</td>
<td>10% of the mean</td>
</tr>
<tr>
<td>In mortality</td>
<td>10% of the mean</td>
</tr>
<tr>
<td>Environmental variation, survival and reproduction</td>
<td>Concordant</td>
</tr>
</tbody>
</table>
Impacts of population subdivision

Increasing population subdivision led to a more rapid decline in gene diversity, particularly for the metapopulation size of 100 individuals (Figs 1 and 2). This was expected given the small population sizes in each habitat fragment. Moreover, small isolated populations undergo greater demographic fluctuations (Brito & Fonseca 2007), which would contribute to further decreases in effective population size and more rapid genetic drift (Lande & Barrowclough 1987). Gene diversity declined more rapidly in metapopulations of 100 individuals than in undivided populations of equivalent size (Fig. 1). For the larger population size (2000 individuals), gene diversity declined at the same rate in metapopulations as in the non-fragmented populations (Fig. 2). Genetic drift caused by increased demographic fluctuations in the small populations offsets the potential for population subdivision to preserve gene diversity. In addition, gene diversity declined rapidly in scenarios with 100 animals after the populations began suffering extinctions. The results suggest a subdivision threshold size, below which the effects of demographic stochasticity and extinction surpass any benefits gained from inter-population gene diversity maintained by genetic drift. For populations with 100 individuals, the number of alleles also declined more rapidly in scenarios with metapopulations than in scenarios with undivided populations (Fig. 3). A decline in allelic diversity in metapopulations was detected even before extinctions occurred. The loss of alleles resulting from the demographic instability of small populations occurred earlier than the decline in gene diversity. For the population size of 2000, the rate of decline was similar in metapopulations and undivided populations (Fig. 4). Population subdivision led to a reduction of the proportion of alleles that were recessive lethals (Figs 5 and 6). Both viable and lethal alleles were lost rapidly from the populations. However, selection against homozygotes for the recessive lethal alleles may have caused more rapid losses of lethal alleles.

Impacts of dispersal

Migration among populations slowed the negative effects of population subdivision (Figs 1 and 2). Gene diversity declined more slowly in metapopulations that were connected by dispersal than in totally isolated metapopulations (Figs 1 and 2). In metapopulations of 2000 individuals, a dispersal rate of 1% effectively prevented populations from losing gene diversity more rapidly than an undivided population (Fig. 2). This dispersal rate also limited the extent of genetic diversity among populations (Fig. 2). Conversely, in metapopulations of 100 animals, even 5% dispersal rate did not fully reverse the effects of subdivision (Fig. 1). Losses of gene diversity in highly subdivided populations (100 individuals subdivided into 2, 5 or 10 populations) that were connected by high rates of dispersal exceeded the losses from undivided populations. Many populations suffered extinction, and genetic losses resulted from a failure of dispersal to demographically stabilize the popula-
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No migration

1% migration

5% migration

Figures 1-2. Loss of gene diversity over 100 years from metapopulations of *M. paraguayanus* with carrying capacity of 100 (1) and 2000 (2), divided into 1 (○), 2 (□), 5 (△) or 10 (◇) subpopulations connected by different migration rates (0%, 1% or 5%) and by equal dispersal by both sexes (white) and male-biased dispersal (black).

Increased dispersal led to more alleles within populations (Figs 3 and 4), because immigrants periodically introduced new alleles into the populations. In the early years of the simulations, dispersal resulted in decreases in the number of alleles preserved within the metapopulation, because it reduced the ability of population subdivision to ensure the protection of different alleles in each population. However, in the later years of the simulations, dispersal resulted in more alleles being maintained in the metapopulation, presumably because the populations suffered fewer extinctions and the metapopulation remained larger than in scenarios with no dispersal. Dispersal reduced the effectiveness of removal of recessive lethal alleles from metapopulations by selection (Figs 5 and 6). Animals were less likely to mate with relatives in scenarios with high rates of dispersal among populations. This lower inbreeding helped to preserve gene diversity (Figs 1 and 2), but reduced the frequency of selection against recessive lethal alleles. The higher the dispersal rate, the lower the reduction in the recessive lethal alleles (Figs 5 and 6). In scenarios with small metapopulations (100 individuals) and high rates of dispersal, the few extant metapopulations comprised only one or two populations, which were highly inbred and were likely on the verge of extinction.

**Impacts of sex-biased dispersal**

In small metapopulations, scenarios simulating dispersal by both sexes resulted in greater preservation of gene diversity than scenarios where only males were capable of dispersal among populations (Fig. 1). However, in scenarios with only males dispersing, populations are more isolated, enhancing inbreeding. As a result, recessive lethal alleles are more rapidly eliminated from the population when compared to scenarios where both sexes are capable of dispersing among populations (Fig. 5).
DISCUSSION

The effects of population size, population subdivision, and dispersal on genetic diversity can be strongly influenced by interactions between demographic and genetic processes. In theory, population subdivision can lead to a better retention of gene diversity than in a panmitic population (Boecklen 1986, Lacy 1987). This is because some of the gene diversity that is lost from within populations is protected in the form of between population gene diversity (Lacy & Lindenmayer 1995). However, such a mechanism depends critically on the demographic stability of populations. The smaller populations that result from the process of subdivision are subjected to greater demographic stochasticity, which causes greater fluctuations in population size (Lacy & Lindenmayer 1995, Brito & da Fonseca 2007). Subdivided populations would be expected to lose genetic variation due to random drift and gene diversity rarely matched the levels in panmitic populations.

Metapopulation structure may make it difficult for natural selection to purge deleterious alleles that cause inbreeding depression (Ebert et al. 2002, Ives & Whitlock 2002). If dispersal is low, populations remain genetically distinct and weakly selected-against deleterious alleles can reach high frequencies in local populations, setting stage for inbreeding depression. The semi-isolation of populations means that they are likely to differ with respect to the deleterious alleles they harbour. Therefore, benefits accrue among the hybrid offspring of residents and immigrants, because the bad effects of any recessive alleles they receive from one parent are likely to be masked by the alleles from the other parent (Ives & Whitlock 2002). Metapopulation structure, habitat loss and fragmentation, and environmental stochasticity can be expected to greatly accelerate the accumulation of mildly deleterious mutations (Lande 1995, Higgins &
LYNCH 2001), lowering the genetic effective size to such a degree that even large metapopulations may be at risk of extinction (LANDE 1995, HIGGINS & LYNCH 2001). Because of mutation accumulation, viable metapopulations may need to be far larger and better connected than would be required under just demographic stochasticity (HIGGINS & LYNCH 2001). From a genetic perspective, a metapopulation may be much more vulnerable to extinction than a population of the same overall number of individuals (HIGGINS & LYNCH 2001). BRITO & FERNANDEZ (2000b) estimated that genetic stochasticity and inbreeding depression might be one of the most serious threats to the long-term persistence of a M. paraguayanus metapopulation composed of very small populations in southeastern Brazil. In that case, heterozygosity was proposed as one management variable to be used to measure the relative contribution of each population to the viability of the metapopulation (BRITO & FERNANDEZ 2002).

Fluctuations in population size due to demographic stochasticity may negate the presumed benefits of subdivision in preserving gene diversity. Population extinctions eliminate the inter-population component of gene diversity, and metapopulation gene diversity may approach that of the highly inbred populations (LACY & LINDENMAYER 1995). Bottlenecked populations are expected to rapidly lose rare alleles (MARUYAMA & FUERST 1985, ALLENDORF 1986, FUERST & MARUYAMA 1986). Because
different alleles can be protected in the various populations (Boecklen 1986, Lacy 1987), population subdivision may help to preserve metapopulation gene diversity. However, the results indicated that alleles were lost as fast, or faster, from subdivided populations than from single populations of the same initial total size. Even when there were no extinctions, alleles were lost more rapidly in subdivided populations. This probably occurred as a result of the smaller total population size maintained in the fragmented and greater demographic stochasticity. Eventually, a group of isolated populations would be expected to retain more alleles and gene diversity than would a panmictic population, as different alleles would become fixed in the populations while a single allele would remain in a panmictic population, in the absence of mutation. However, the approach to such an equilibrium is so slow (Boecklen 1986, Vario et al. 1986) that the equilibrium result is almost irrelevant to conservation actions dealing with the present crisis of biodiversity decline. As the case for gene diversity, the hypothetical advantage of subdivision in protecting allelic diversity is reversed if populations go extinct.

Low rates of dispersal, of approximately one individual per generation, are commonly considered to be effective for preventing deleterious losses of genetic diversity in subdivided populations (Lacy 1987). However, several authors suggest that more than one migrant per generation may be necessary to achieve genetic viability (Mills & Allendorf 1996, Vucetich & Waite 2000). The one migrant per generation rule only applies when effective population size equals actual population size (Vucetich & Waite 2000). As effective population size is usually less than actual population size (Frankham 1995), the number of migrants required for genetic viability should exceed one. Mills & Allendorf (1996) suggested that a minimum of one and a maximum of 10 migrants per generation would be an appropriate general estimate for genetic purposes. Vucetich & Waite (2000) demonstrated that even more than 20 migrants per generation may be needed to maintain genetic diversity. Dispersal in the scenarios of the present study was an order of magnitude more than one animal per generation. However, our results showed that such migration was relatively ineffective. The large demographic fluctuations that occur in highly subdivided populations can significantly depress effective population size (Maruyama & Kimura 1980). Increased dispersal in our models reduced the rate of gene and allelic diversity loss. However, even at the highest rates of dispersal, gene and allelic diversity were always lost as fast, or faster, from subdivided populations than from panmictic populations of the same initial total size. In our scenarios, one migrant per generation is not enough to rescue populations from genetic erosion.

It has been suggested that a careful balance between fragmentation and dispersal can achieve simultaneously the benefits of both population isolation and genetic mixing. However, the results presented here suggest that within a highly fragmented population linked by dispersal, heterozygosity may be lost rapidly due to the small size and instability of populations. The small populations might not be sufficiently robust to survive the intense selection needed to remove the genetic load, and alleles could be lost due to the genetic homogenization imparted by dispersing individuals. For many populations in the Atlantic Forest, including those of M. paraguayanus, the most effective conservation solution to fragmentation might be to restore large areas of habitat capable of supporting populations that are graphically and genetically stable. Until recently, there were no genetic studies for M. paraguayanus, only modelling approaches (e.g. Brito & Fernandez 2000b, 2002). However, Rodrigues et al. (2006) have recently isolated and characterized five microsatellite loci for M. paraguayanus. This would provide a useful tool for studying genetic diversity and the consequences of habitat fragmentation on population genetic structure for this species.

ACKNOWLEDGMENTS

I would like to thank Gustavo A.B. da Fonseca, Carlos E.V. Grellle, José E.C. Figueira, Cláudia M. Jacobi, Flávio H.G. Rodrigues and Adriano G. Chiarello for critical comments and suggestions on the manuscript.

LITERATURE CITED


MARUYAMA, T. & P.A. FUERST. 1985. Population bottlenecks and non-equilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. Genetics 111: 675-689.

MARUYAMA, T. & M. KIMURA. 1980. Genetic variability and effective population size when local extinction and re-colonization of sub-populations are frequent. Proceedings of the National Academy of Science USA 77: 6710-6714.


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