Genetic diversity and the dynamics of metapopulations

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Summary

Habitat fragmentation has important consequences for the genetics and dynamics of populations. As such, ideas concerning the effects of fragmentation have become an important part of conservation biology, often under the name of metapopulation biology. A metapopulation is a group of subpopulations that live in discrete habitat patches, but may be connected by dispersal. While much work on metapopulations has looked at genetics and dynamics separately, there is a growing body of literature examining the relationship between these two aspects of metapopulation biology. In this study I examined how patterns of genetic diversity can be used to gain insights into various aspects of metapopulation dynamics.

The thesis can essentially be divided into two sections. In the first section I considered the relationship between the rate at which individuals enter a population, and their impact in terms of gene flow. In particular, I addressed one of the assumptions involved when using genetic diversity data to infer immigration rates between habitat patches: that immigrants have the same fitness as population residents. For this I performed a laboratory experiment, using *Drosophila melanogaster,* to measure the genetic contribution of single male immigrants to small, inbred populations. Genetic contribution was assessed by measuring the relative frequency of immigrant marker alleles. When immigrants were outbred, the mean frequency of the immigrant allele was significantly higher than its initial frequency, as early as one generation after immigration. There was no significant change in allele frequency for populations receiving inbred immigrants. The increase in allele frequency for outbred immigrants was attributed to an initial outbred vigour fitness advantage of immigrant males over resident males experiencing inbreeding depression. Furthermore, hybrid vigour of immigrant progeny and the rare male effect did not have a statistically significant role in the fitness advantage of the immigrant allele. These results, based initially on parametric analyses, were also supported by randomisation tests and bootstrap analyses.

The results of this experiment add to our understanding of the complex relationship between the rate at which immigrants arrive into a population and their impact in terms of gene flow. This work also has implications for understanding the rescue effect, whereby immigrants may

rescue extant populations from extinction. In particular, this study suggests that large, outbred populations may be valuable for their contribution to the genetic diversity of small, inbred populations.

In the second section of the thesis I explored the value of using genetic diversity data to make qualitative "rules of thumb" decisions when managing the dynamics of metapopulations. In particular, I examined whether ranking patches based on genetic diversity provides a good estimate of the relative value of those patches in terms of their contribution to metapopulation persistence. The logic behind this approach is that the same features that make a patch valuable for metapopulation persistence also tend to increase the genetic diversity of the subpopulation occupying that patch. Thus, a large, centrally located patch is expected to (1) be valuable for maintaining metapopulation persistence, and (2) support a genetically diverse subpopulation. I explored this potential link between genetic diversity and relative patch value using an individual-based computer simulation model for two taxa with very different life history properties: owls and rodents. The model was run over a number of scenarios: three-patch and eight-patch metapopulations, with and without catastrophes, with sex-biased and unbiased dispersal, and over a range of dispersal rates. For each scenario, the relative value of two patches to the metapopulation was assessed by measuring the effect of patch removal on the metapopulation's 100-year extinction probability, as determined by simulation. The question was whether a measure of the relative genetic diversity for the two patches could reliably identify which of the two was most valuable for metapopulation persistence. The probability of correctly ranking patches was then estimated by simulation, based on a number of measures of nuclear and mitochondrial DNA diversity.

In some scenarios, genetic diversity provided very good predictions of relative patch value, with a greater than 90% chance of correctly ranking the two patches in question. However, in other scenarios this predictive accuracy was as low as (or sometimes lower than) the null hypothesis of 50% (equivalent to randomly assigning relative patch value). Importantly, this variation in predictive accuracy appears to be related to how different two patches are in value; the greater the difference in value between two patches, the higher the probability that we will rank them correctly using genetic diversity. This pattern suggests that biologists could make statements about the probability of correctly ranking patches as a function of the difference in patch value.

A relatively tight, positive relationship between relative patch value and the predictive accuracy of genetic rankings was found for owl metapopulations whose genetic diversity (mean number of alleles per locus) was sampled 40 years after the system was fragmented. It appears that with earlier samples there was too little time for the subpopulations to diverge in genetic diversity, while with later samples there was too great a chance that one or more of the patches would be unoccupied. Unfortunately it was not possible to explore the link between relative patch value and predictive accuracy of genetic rankings for rodent metapopulations beyond 10 years after fragmentation, as too many metapopulations contained unoccupied habitat patches. This limitation is attributable to the higher extinction probabilities of rodent metapopulations compared to owl metapopulations, which, in turn, is a reflection of the highly stochastic nature of rodent population dynamics. As such, one avenue for further study would be to model rodent metapopulations with larger, more extinction-resistant subpopulations. In summary, this second section of the thesis suggests that genetic diversity may, in some circumstances, provide a useful way of assessing the relative value of the various patches in metapopulations.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Stephen J. Ball
June 2001

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Publications

Part of the work described in this thesis has been published:

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CHAPTER 1

General introduction

Fragmented populations abound in nature, over a broad range of spatial scales and wide variety of organisms. Examples of naturally fragmented populations include birds on oceanic islands (Reed *et al.*, 1998), plants on rocky outcrops (Holderegger and Schneller, 1994), and parasites living in their host "islands" (Ferrara and Cook, 1998). Extensive fragmentation has also occurred as a result of human activity; one widespread example is the fragmentation of previously continuous forest habitat into patches of forest surrounded by farmland (Wilcove *et al.*, 1986; Skole and Tucker, 1993; Quammen, 1996).

Biologists have known for many years that subdivision has important implications for the genetics and dynamics of populations (Wright, 1931; Andrewartha and Birch, 1954; Levins, 1970), and over time, ideas concerning the effects of fragmentation have become an integral part of conservation biology (Hanski and Gilpin, 1991). A key element in managing fragmented populations lies in understanding the amount of dispersal that occurs between habitat fragments. Dispersal has been described as the "glue" that binds fragmented populations together (Hansson, 1991), and those systems loosely connected by such dispersal glue are commonly referred to as metapopulations (Hanski, 1991).

While much work on metapopulations has dealt with genetics and dynamics separately, there is a growing body of literature that examines the relationship between these two aspects of metapopulation biology (Hastings and Harrison, 1994; Lacy and Lindenmayer, 1995; Saccheri *et al.*, 1998). My thesis also considers this relationship. In particular, I am interested in how patterns of genetic diversity can be used to understand aspects of metapopulation dynamics. Following the definitions of Moritz (1994), the emphasis of this study is on "molecular ecology" (the use of genetic variation to guide and assist demographic studies), rather than "gene conservation" (the identification and management of genetic diversity *per se*).

The thesis can essentially be divided into two distinct sections. In the first section I examine the reliability of using genetic diversity data to estimate dispersal rates in fragmented populations. Using a laboratory system, I address one of the assumptions involved in using genetic diversity data to infer immigration rates between patches: namely that immigrants have the same fitness as population residents.

In the second section of the thesis I explore the value of using genetic diversity data to make qualitative "rules of thumb" decisions when managing the dynamics of metapopulations. In particular I consider a question that might be asked by the managers of specific fragmented populations: What is the relative importance of the different patches in a system, in terms of their contribution to long term metapopulation persistence? I examine whether ranking patches based on genetic diversity provides a good estimate of the relative value of those patches.

With the focus of this thesis on metapopulations, it is important to recognise that the term metapopulation has come to mean many things to many people. Therefore, before further discussion I will define its use in this thesis. I then consider the role of metapopulation theory in conservation management, and discuss how genetic diversity data can potentially be used to help make specific management decisions about metapopulations. Finally I outline the structure of this thesis, presenting the questions that will be addressed.

1.1 The many meanings of "metapopulation"

The metapopulation concept can be traced back to Andrewartha and Birch (1954), who argued that local extinction and recolonisation were important aspects of many natural populations. Later, Den Boer (1968) developed the concept of the "spreading of risk", identifying dispersal as a key process stabilising the dynamics of patchy populations. However it wasn't until Levins (1970) that the term "metapopulation" first entered the literature. He defined a metapopulation as a fragmented population that could persist indefinitely in a balance between local extinction and colonisation of subpopulations, assuming that:

- All occupied patches contribute equally to a common pool of colonists and are equally likely to go extinct in a given time step.

- All unoccupied patches are equally likely to be colonised in a given time step.
- There are so many patches in the system that the dynamics of patch occupancy can be described deterministically.

The metapopulation concept gained widespread popularity after its introduction by Levins (1970), despite a time lag of 20 or so years (Hanksi and Simberloff, 1997). However, this popularity has been associated with much distortion of the original definition. While it is unrealistic to expect any natural system to fit Levins' assumptions exactly, many uses of the term metapopulation do not even vaguely resemble the sort of system Levins was describing. In its broadest sense, the term "metapopulation" has come to refer to any population that can conceptually be broken down into spatially smaller units, with or without any reference to local extinction or colonisation events.

Harrison (1991) presented a well-needed review of the semantics of metapopulations, and identified four types of fragmented populations to which the term metapopulation has been applied in the literature. These are:

- *Patchy populations*, which have high enough dispersal between patches that any extinction events are rendered trivial, in the sense that empty habitat patches are colonised extremely quickly.
- *Non-equilibrium metapopulations*, which are fragmented populations in which local extinction occurs, and in which so little dispersal occurs that empty patches remain empty. These systems are non-equilibrium in the sense that they are in transition towards global extinction.
- *Classical metapopulations*, which fit Levins' (1970) description (as described earlier).
- *Mainland-island metapopulations* (and *source-sink metapopulations*) in which one or a few extinction-resistant populations are largely responsible for the long-term persistence of the system, and provide most of the colonists for recolonisation of the smaller (or sink) habitat patches.

While Harrison's (1991) definitions provide a clear basis for naming the different types of fragmented populations, they place the onus on biologists knowing what name to apply to the particular system being studied. The problem is that in many cases, without adequate time to observe dynamics, it isn't clear whether a system is a patchy population, classical metapopulation, mainland-island metapopulation or non-equilibrium metapopulation.

Accordingly, in this thesis I use the terms *metapopulation* and *fragmented population* to refer collectively to all of Harrison's categories (including patchy populations which she identifies as not being metapopulations). This is similar to Spellerberg and Sawyer's (1999) use of the term metapopulation to refer to "a group of populations that are possibly but not necessarily interconnected." However, while using such a general definition, it is important to recognise that I am focussing on systems for which subpopulation turnover (extinction and recolonisation) is at least suspected. Thus, I am placing subpopulation turnover as a major management concern, even if it never actually occurs. This contrasts with the more extinction-resistant fragmented systems for which the overriding concern would be one of gene conservation rather than demographic persistence, such as those systems for which biologists define evolutionary significant units (see Moritz, 1994).

1.2 The utility of metapopulation theory

Many systems of management concern are described as metapopulations in the literature. Examples include work on marsupial gliders (Possingham *et al.*, 1994), spotted owls (Lahaye *et al.*, 1994), butterflies (Hanksi and Thomas, 1994) and plants (Ouborg, 1993). Although metapopulation theory is often seen as a valuable framework for managing such populations (Simberloff, 1988; Hanski and Gilpin, 1991; Caughley, 1994), it is important to recognise that metapopulation theory offers management support at two very different levels - the general and the specific.

At a general level, metapopulation theory has been quite valuable, providing many useful principles and guidelines. For example:

- The Levins (1970) model provided a basis for (1) placing value on empty habitat patches, and (2) placing value on dispersal between patches.
- Day and Possingham (1995) showed that the most important patches in a system (in terms of their contribution to metapopulation persistence) are those patches that are most frequently occupied.
- Hanski's core-satellite hypothesis (Hanski, 1982) stated that metapopulations are unlikely to exist in a state of intermediate patch occupancy in nature.
- Gilpin (1991) showed that metapopulation dynamics can drastically reduce the effective genetic size of a fragmented population.

- Saccheri *et al.* (1998) reported that inbreeding depression can influence metapopulation dynamics.
- Lande (1987) found that metapopulation persistence requires a threshold, minimum amount of suitable habitat in a region.

While these insights have certainly helped change the way biologists think about fragmented populations, they nonetheless fail to address some of the specific questions that managers of specific metapopulations may ask. For example, a manager may ask:

- Which patches are the most important for the long-term persistence of a particular metapopulation?
- How quickly will a particular metapopulation lose genetic diversity?
- How much effect does inbreeding depression have on the persistence of a particular metapopulation?
- Which set of patches should be connected by habitat corridors in order to maximise a particular metapopulation's persistence?

The aim of this thesis is to explore how genetic diversity data can help to answer specific questions such as these. In particular, I consider how genetic diversity data can be used to gain the sort of specific insights that help make management decisions that concern the dynamics of metapopulations.

Unfortunately many of the biological processes that influence metapopulation dynamics are inherently difficult to observe, and not surprisingly biologists have tried to make the most out of the limited data available. In the absence of good demographic data, patterns of genetic diversity potentially offer a valuable indirect insight into many of the biological processes underlying metapopulation dynamics. Importantly, genetic diversity is influenced by local population dynamics, and immigration - two key processes in the dynamics of metapopulations. For instance, population bottlenecks cause reductions in genetic diversity (Nei *et al.*, 1975; Packer *et al.*, 1991; Glenn *et al.*, 1999), while immigration increases genetic diversity (Hartl and Clarke, 1989; Hedrick, 1995). Given that genetic diversity is influenced by the processes underlying metapopulation dynamics, it is tempting to think that genetic diversity data will in turn reflect those processes in some resolvable way (Milligan *et al.*, 1994). The aim of this thesis is to consider the utility of genetic diversity data in this regard.

1.3 Thesis outline

1.3.1 First section

The first section of this thesis considers how genetic diversity data can be used to estimate the amount of dispersal occurring among the subpopulations of a metapopulation. A popular approach in this regard is to estimate Wright's (1931) *Nm*, the number of migrants exchanged between subpopulations per generation (*e.g.*, Driscoll *et al.* (1995); Seppa and Laurila, (1999)). In this section of the thesis, I explored one of the key assumptions involved when using genetic diversity data to infer immigration between patches: namely that immigrants have the same fitness as population residents. The focus of this section is a laboratory experiment where I used *Drosophila melanogaster* to measure the genetic contribution of single male immigrants (either inbred or outbred) to small, inbred populations. The aim of this experiment was to examine whether inbreeding can influence the impact that an immigrant has on the genetic diversity of a population. In so doing, this experiment adds another element to our understanding of the relationship between the rate at which individuals immigrate into populations, and their impact in terms of gene flow. The experiment was designed in such a way that it was possible to explore the relative importance of the different components of immigrant fitness including (i) the rare-male effect, (ii) initial outbred vigour of immigrants and (iii) hybrid vigour of immigrant progeny. The experiment also has implications for understanding the "rescue effect" (Brown and Kodric-Brown, 1977) whereby the arrival of immigrants may rescue populations from extinction.

The first section of the thesis is structured as follows. Chapter 2 is an experiment-based chapter where I examine the genetic contribution of immigrants arriving into inbred populations of *Drosophila melanogaster*. Non-normality of the data in Chapter 2 throw concerns over the robustness of analyses; therefore in Chapter 3, I explore alternate analyses using bootstrapping and randomisation tests. The primary issue in Chapter 3 is whether the parametric analyses in Chapter 2 (based on the normal distribution) concur with their non-parametric analogues. I also assess the validity of the parametric analyses of Chapter 2 through considerations based on the central limit theorem.

1.3.2 Link between the first and second sections

Chapter 4 is a general discussion of the limitations of using genetics to make insights into dispersal rates and colonisation probabilities - two processes underlying metapopulation dynamics. This is largely a review chapter, and forms something of a link between the first and second sections of the thesis.

1.3.3 Second section

The aim of the second section of the thesis is to examine the utility of using genetic diversity data to estimate the relative value of patches in metapopulations, where the value of a patch is defined as its contribution to metapopulation persistence.

At present, patch value tends to be estimated through the use of population viability analysis, or PVA (Hanski, 1994b; Day and Possingham, 1995; Lindenmayer and Possingham, 1996). Among metapopulation PVAs, biological processes are modelled in very different ways according to the size of the system in question. In large metapopulations (containing more than 30 patches), a statistical procedure based on patch occupancy data can be used to parameterise "incidence functions", which describe subpopulation extinction and colonisation probabilities as functions of patch area and isolation (Hanski, 1994a; Hanski, 1994b). These can then be used to run numerical iterations of metapopulation dynamics, and thereby explore relative patch value (Hanski, 1994a; Hanski, 1994b).

In contrast, small metapopulations (containing relatively few patches) are better suited to a style of simulation that incorporates more biological detail than is used in the incidence function approach (Beissinger and Westphal, 1998). There are two reasons for this. Firstly, the presence of fewer patches offers too little patch occupancy data to use the incidence function approach with confidence (Hanski, 1994a). Secondly, with fewer patches (and fewer individuals) computer models can deal with greater complexity, and thereby follow the fate of individuals in each population within reasonable run times. Accordingly, PVAs for small metapopulations often include descriptions of many biological processes, including reproduction, mortality, dispersal, and disturbance events. Thus, PVAs for small metapopulations attempt a bottom-up approach, describing the mechanisms that underlie metapopulation dynamics (and sometimes genetics). PVA packages well suited to modelling

small metapopulations include VORTEX, ALEX and RAMAS/space (Lindenmayer *et al.*, 1995).

The focus in the second section of the thesis is on small metapopulations, where PVA predictions depend on good descriptions of many aspects of an organism's biology. There are certainly many small metapopulations of management concern. Examples include the Mauritius Fody (a bird) with five subpopulations (Safford, 1997), Shenandoah salamanders with six subpopulations (Griffis and Jaeger, 1998), the fern *Asplenium septentrionale* with three subpopulations (Holderegger and Schneller, 1994), and Nepalese tigers with four subpopulations (Smith *et al.*, 1998). While biologists may hope to obtain good descriptions of some of the processes incorporated into PVAs for small metapopulations, many processes such as dispersal and mortality are difficult to measure (Beissinger and Westphal, 1998).

In light of these difficulties for estimating relative patch value for small metapopulations, I have explored a new approach that simply involves ranking patches according to the genetic diversity of the subpopulations they contain. The logic behind this approach is that the same features that make a patch valuable for metapopulation persistence also tend to increase the genetic diversity of the subpopulation occupying that patch. Thus, large, centrally located patches are expected to (1) be valuable for maintaining the long-term persistence of a metapopulation, and (2) support genetically diverse subpopulations. I explore this potential link between genetic diversity and relative patch value using an individual-based computer simulation model. This model is based on two taxa with very different life history properties: owls and rodents.

Chapter 5 provides an introduction and detailed description of the model I use to explore the link between genetic diversity and the relative value of patches. This can essentially be considered a methodological chapter. In Chapter 6, I present and analyse the results of the metapopulation model, while Chapter 7 is a general discussion of the model results, with some final comments for the thesis.

CHAPTER 2

The genetic contribution of single male immigrants to small, inbred populations: a laboratory study using *Drosophila melanogaster*

2.1 Introduction

One of the major challenges in metapopulation biology lies in the integration of two very different forms of information: genetic and demographic (Hastings and Harrison, 1994). An important part of this challenge involves using patterns of genetic diversity to try to estimate the rate at which immigrants move among subpopulations. A popular model in this regard is Wright's infinite-island model (1931) which states that the number of immigrants, *Nm*, entering each subpopulation per generation can be predicted from the degree of genetic subdivision among subpopulations, $F_{ST,}$ using the equation $F_{ST} = 1/(4Nm + 1)$.

The use of Wright's model recently received criticism from Whitlock and McCauley (1999) on the grounds that many real systems do not meet its assumptions. They argued that Wright's (1931) model is unlikely to produce reliable estimates of the true rate at which immigrant individuals arrive into populations, as compared to the effective genetic rate of immigration. Furthermore, Whitlock and McCauley (1999) point out that although alternative models exist for estimating *Nm* (using different measures of genetic differentiation), those models make the same fundamental assumptions as Wright's (1931), and are therefore open to the same criticisms. Essentially this means that biologists may have been putting false hopes in using Wright's model (and others like it) to link the genetic and ecological impacts of immigration.

Importantly, Wright's model assumes that immigrants have the same fitness as population residents. For real systems however, there are several reasons why this assumption may be

violated. For example immigrants may differ from population residents in both age and social status (Gaines and McClenaghan, 1980). Genetic factors may also be important, and in this chapter I describe an experiment where I examined the effect of inbreeding on immigrant fitness. In particular, I examined the genetic contribution of single male immigrants (both inbred and outbred) arriving into inbred populations. Essentially this study addressed two assumptions implicit in Wright's model: 1) that immigrants have the same fitness as residents of the populations they arrive into, and 2) that an immigrant's fitness is independent of whether it originated from a large outbred source population or a small inbred source population. As such, the purpose of this chapter is to contribute to the questioning of the way in which biologists use measures of genetic subdivision to estimate the rate at which individuals immigrate into subpopulations.

I initially identified three reasons why immigrants arriving into inbred populations might have higher fitness than residents. These were:

(1) Higher fitness over resident males due to a rare male effect.

(2) Higher fitness over resident males experiencing inbreeding depression. I refer to this as "initial outbred vigour".

(3) Higher fitness due to hybrid vigour of immigrant progeny over inbred competitors.

The rare male hypothesis states that genetically rare males have greater mating success over their non-rare competitors due to rareness *per se*. While the underlying mechanisms are poorly understood, it has been suggested for *Drosophila* that females assess the rarity of the different males in a population using a range of cues, and subsequently choose rare males to mate with them (Petit and Ehrman, 1969). This rare male effect has been reported in a number of *Drosophila* species (Petit and Ehrman, 1969), as well as a number of other insect species (Sinnock, 1970; Grant *et al.*, 1974). Rareness was expected to arise in the present study due to the random drift of allele frequencies whenever populations were inbred.

Inbreeding depression is the decrease in fitness due to the mating of closely related individuals. The prevailing belief is that this drop in fitness is caused by increased homozygosity associated with inbreeding, which in turn causes more frequent expression of rare recessive (or partially recessive) deleterious alleles (Charlesworth and Charlesworth, 1987; Lande, 1988). In this study inbreeding had the potential to favour the immigrant genome at two points in time. Firstly it could give outbred immigrants an initial outbred

vigour advantage over inbred residents. Secondly, it could give immigrant progeny a hybrid vigour advantage over inbred competitors. Hybrid vigour arises when the crossing of two inbred lines (or an outbred line with an inbred line) produces individuals that are heterozygous at loci that were previously homozygous for deleterious alleles (Falconer, 1981).

I studied the impact of these mechanisms by measuring the genetic contribution of single male immigrants arriving into inbred populations of *Drosophila melanogaster* in the laboratory. This species was chosen because of its fast generation time, the ease with which sexes can be distinguished, evidence that the species is highly polymorphic at allozyme loci (Singh and Rhomberg, 1987), and ease of rearing. Furthermore, indirect evidence suggested that single *D. melanogaster* immigrants are capable of making a substantial genetic contribution to small, inbred populations. Spielman and Frankham (1992) found that single *D. melanogaster* immigrants caused a marked decrease in the inbreeding depression of inbred laboratory populations. My experiment differs from that of Spielman and Frankham (1992) by directly considering the implications of immigration on allele frequencies *per se*, rather than any effects on inbreeding depression.

The experimental design involved two treatments: one where the immigrant was inbred and one where the immigrant was outbred. Inbred immigrants received the same inbreeding regime as the populations they arrived into. Such immigrants may experience a fitness advantage over population residents because (a) they are genetically rare to their recipient population, and (b) their progeny experience a hybrid vigour advantage over the progeny of residents. Importantly however, there can be no initial outbred vigour in this case, as the immigrant males would, on average, have the same level of inbreeding depression as the resident males.

Outbred immigrants were much less inbred than their recipient populations. As with inbred immigrants, outbred immigrants may also be genetically rare to their recipient population, and their offspring may also experience hybrid vigour. However in addition, outbred immigrants may themselves experience an initial outbred vigour advantage as a result of inbreeding depression amongst their recipient population. By comparing the two treatments I was therefore able to assess the importance of any initial outbred vigour.

Importantly, both a rare male effect and hybrid vigour potentially give the immigrant genome a fitness advantage in the generations following immigration (compared with initial outbred vigour, which is restricted to the generation in which the immigrant arrives). Given this potential, I decided to measure the fate of the immigrant allele in both the first and the second generations after immigration.

The genetic contribution of single immigrants into populations has received some attention in the past. Kaufmann and Wool (1992) used flour beetles to measure the frequency of a phenotypically-dominant marker in the first generation after immigration. While immigrant beetles had significantly higher fitness than residents, it is possible that this immigrant advantage was due entirely to selection associated with the marker used. The use of a dominant marker restricts such an experiment to being performed in one direction, with homozygous dominant immigrants arriving into homozygous recessive populations. Importantly, I controlled for any effect of selection at the marker locus. By using a co-dominant allozyme polymorphism (*Adh* with two alleles *F* and *S*) I was able to perform the experiment reciprocally, producing one set of results with an *FF* immigrant arriving into an *SS* population, and another set *vice versa*. This reciprocal design is particularly important given that there is evidence of selection at the *Adh* locus in *D. melanogaster* (Oakshott, 1979; Gilbert and Richmond, 1982). Secondly I controlled for the experience of immigrants by rearing all experimental individuals (immigrant and resident) under equivalent conditions. The structure of this study can be summarised as follows:

(1) The fate of an immigrant allele was measured in the two generations following immigration.
(2) There were two main treatments: inbred immigrant and outbred immigrant.
(3) The entire experiment was performed reciprocally to account for any selection associated with the allelic marker used.

2.2 Methods

This study involved collecting a wild-type population from the field, developing two marked base populations (each fixed for a different allele at the same locus), and then using these marked populations as the source of individuals for the experiment. Figure 2.1 provides an overview of the methods that are detailed below.

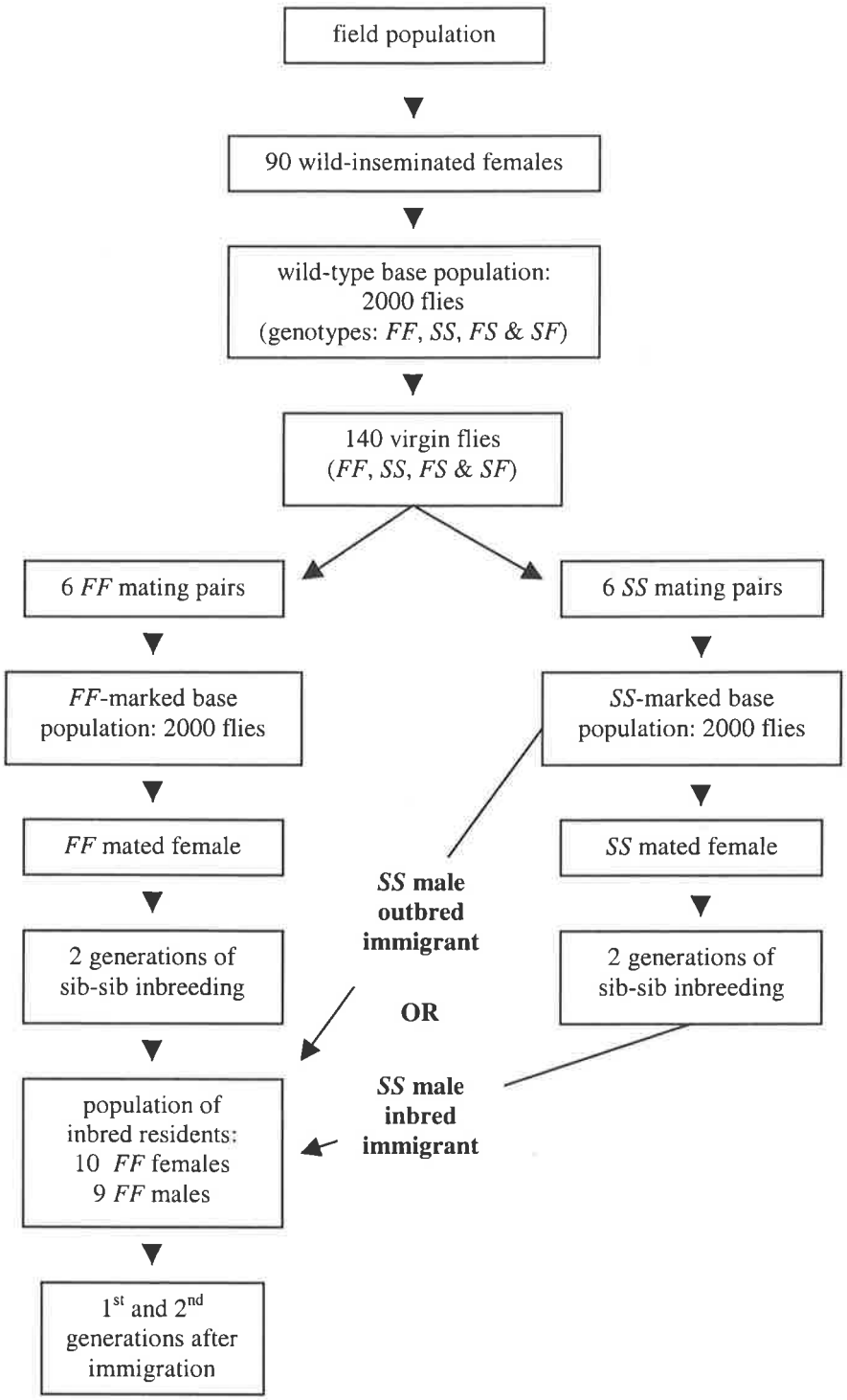

Figure 2.1 Experimental design. This shows the processes leading from the initial collection of wild-type flies to the introduction of either an outbred *SS* or inbred *SS* immigrant into an inbred *FF* population.

2.2.1 Establishment and maintenance of wild-type laboratory population

A prerequisite for this experiment was to have a population which, when inbred, would have the potential to experience both a rare male effect and inbreeding depression. This meant obtaining a base population that contained as much genetic diversity as possible. I therefore made a field collection of *D. melanogaster* and maintained as large a population as was practical. The population was collected in September 1996 from the Penfolds winery in Nuriootpa (approximately 60 km northeast of Adelaide, South Australia). Ninety wild-inseminated females were placed in separate vials, and each female was confirmed as being *D. melanogaster* by examining the genetalia of her male progeny (using the description in McNamee and Dytham (1993)). Twenty progeny from each of 90 wild-inseminated females were combined to create a total population of 1800 individuals. This was maintained at approximately 2000 individuals in subsequent generations.

Efforts were made to limit the rate at which genetic diversity was lost in the laboratory population. Briscoe *et al.* (1992) demonstrated that even relatively large populations of *D. melanogaster* can rapidly lose genetic diversity in the laboratory. While Briscoe *et al.* (1992) were unable to identify the mechanism responsible for this effect, it is clear from theoretical considerations (Falconer, 1981) that variation in fitness between individuals can greatly increase the loss of genetic diversity in populations. Given the polygamous mating structure of *D. melanogaster* (Gromko *et al.*, 1984), the potential exists for large variation in mating success. Ideally I would have ensured that every individual had the same number of offspring, however this was clearly not practical in a large population. Therefore an approach of population subdivision was used (based on pers. comm., R. Frankham). Every generation the population was subdivided into 32 jars (64 flies per jar) and allowed to oviposit for three days on fresh agar. The adults were removed, and two weeks later the progeny were moved onto fresh agar (to avoid another generation of flies emerging in the same jars). One to two weeks later, 64 flies were removed from each jar, and all 2048 flies were combined in a single jar, where they were allowed to mix (for no more than one hour). The population was once again subdivided into 32 jars of 64 flies, thereby completing a full generation of subdivision, panmixis and subdivision. While this is far from ensuring equal fitness of individuals, it does mean that at an individual's fitness is restricted to 1/32th (3%) of the total population in any given generation.

The culture was maintained at 25°C as discrete generations with approximately 30 days per generation. The flies were reared on a boiled mixture of water (910 ml), cornmeal (130 g), agar (12 g), treacle (150 ml) and fresh baker's yeast (240 g), with approximately 30 mls of Tegosept mould inhibitor per litre of medium (Tegosept is a 10% w/w solution of methylparaben in 95% ethanol). The flies were handled under anesthesis using CO_2 and moved using the flared tip of a small paint brush.

2.2.2 Development of marked outbred base populations

Approximately 16 generations after the field collection, the wild-type laboratory population was used to set up two marked base populations – each one fixed for a different *Adh* allele (*F* and *S*). These base populations then became the source of all individuals (both inbred and outbred) in the experiments. Electrophoresis was carried out on cellulose acetate gels using methods described by Richardson *et al.* (1986). While I found a number of polymorphic loci in the wild-type population, *Adh* was chosen because it had two common alleles and was the least expensive stain for large-scale screening. Although there is evidence of selection at this locus (Oakeshott, 1979; Gilbert and Richmond, 1982), I considered it better to use *Adh* than a poorly studied locus - in this way I would at least have some insights into any marker effect.

I founded the two marked base populations with as many individuals as possible in order to maximise their genetic diversity, and therefore maximise their potential to produce outbred and inbred individuals that would differ in fitness. The important issue here is that any outbred experimental fly could only be as outbred as the base population from which it was drawn. Therefore, a total of 140 wild-type virgin flies were non-destructively genotyped by removing a middle leg (using jeweler's forceps) from individuals immobilised in a cold room (10°C), and squashing the leg directly onto the gel loading position. Individuals homozygous for the same allele were then arranged into mating pairs, which were placed in separate vials and allowed to reproduce. This provided a total of six *SS* pairs and six *FF* pairs. Each marked population was increased to 180 individuals in the following generation (30 individuals from each of the founding pairs), and maintained at approximately 2000 individuals in subsequent generations using the same method of subdivision-panmixis-subdivision described above for the wild-type population. The inbreeding imposed by this founding bottleneck is considered negligible relative to the two generations of full sib-sib mating imposed during experiments. If we set the inbreeding coefficient, F, to zero for the

wild-type base population, then F = 0.04 for outbred immigrants, and F = 0.46 for both inbred residents and immigrants (based on Falconer (1981)). The purity of the two marked base populations was tested and confirmed both before and after individuals were removed for the experiments by sampling 20 individuals from each population.

2.2.3 The experiment

The units of replication in this study were single populations. Each replicate involved creating an inbred population founded by a mated female from one of the marked base populations. This is similar to founding each population with a mating pair of virgins given that *D. melanogaster* has a strong last male advantage, with 85% of a female's progeny being sired by her last mate (Gromko *et al.,* 1984). Each population was then inbred for two generations by removing a single female every generation and placing her in a vial of fresh medium. Given the last male advantage this approximates full sib-sib mating in each generation.

In the third generation (20 days after oviposition from the previous generation), 10 females and nine males were removed from the population, and placed on fresh medium together with a single male immigrant that was marked with the alternate allele to the resident flies. Importantly, immigrants were given similar experience to resident flies, whereby each immigrant was drawn from a vial in which a mated female had been allowed to oviposit 20 days beforehand. The mother of each outbred immigrant was drawn directly from the appropriate marked base population. In contrast, inbred immigrants had experienced the same inbreeding regime as the populations they arrived into. After the immigrant was introduced, each population was kept on fresh medium for 10 days to allow remating, and then placed in a jar with fresh medium for three days to allow females to oviposit.

When the immigrant arrived, the immigrant marker allele made up 1 in 20 (i.e. 0.05) of the total alleles in each replicate population. The null hypothesis was that the mean allele frequency would not change from this initial frequency over time. Allele frequency was measured by sampling up to 20 individuals in the two generations following immigration. Each generation was discrete, and was separated by 30 to 33 days. On day 20 of the first generation, 10 males and 10 females were removed from each population and placed on fresh medium (the remaining flies were discarded). Ten days later these 20 flies were placed on

fresh medium and allowed to oviposit for three days. These same flies were then frozen and genotyped. This meant that the flies that parented the second generation were the same individuals genotyped in the first generation. Although less than 20 individuals were available for some replicates, very few (only 2.5% of all replicates) provided fewer than 15 individuals.

A total of 40 replicate populations were established with *FF*-marked immigrants arriving into *SS* populations. In 20 of these the immigrant was outbred and in the other 20 the immigrant was inbred. Because allele frequency was measured over two generations, this experimental design provided four data sets. While these will be referred to as four separate "treatments", the data sets from the two generations are essentially repeated measures rather than separate experimental treatments. A reciprocal experiment (with equal replication) was performed in which *SS* immigrants were introduced into *FF* populations. The entire experiment of eight treatments was carried out simultaneously. All replicates were independent in the sense that each replicate was derived from a separate female drawn from one of the marked base populations. The populations of all treatments were thoroughly shuffled to minimise bias due to microclimatic variation in laboratory conditions.

Two sets of questions were addressed in the data analyses:

(1) Was there a change in the mean allele frequency from the null level of 0.05 (one immigrant fly in a population of 20) for each of the treatments?

(2) Were there differences in the mean allele frequencies between certain pairs of treatments (see results for details)?

All data were analyzed using $\alpha = 0.05$ as the significance level, and all analyses were performed as two-tailed tests to include the possibility that immigrants could somehow be at a selective disadvantage in inbred populations. Although a number of tests were performed, no adjustment was made of the test-wise α value in order to maintain the experiment-wise α value of 0.05, since each test was planned *a priori* as a separate hypothesis, each with a different biological meaning.

Importantly, the data were not pooled over markers. The concern was that in the presence of a strong marker effect, pooling would produce a mean immigrant allele frequency greater

than the null frequency of 0.05, and thereby give a false impression of an immigrant fitness advantage. To illustrate the basis for this concern, consider a hypothetical extreme marker effect where SS-marked males have complete mating dominance over FF males (i.e. when the two strains are together, only SS males sire the next generation's progeny). Assume here that there are no treatment effects. In this case, all first generation progeny from the SS immigrant replicates would have FS genotypes (the FF resident females mate only with the SS immigrant male), giving an immigrant allele frequency of 0.50. In contrast, all progeny from the FF immigrant replicates will be SS (the resident SS females mate only with the SS resident males), giving an immigrant allele frequency of 0.00. If we pooled these data we would obtain the misleading result that the immigrant allele frequency was 0.25, and incorrectly infer that this was greater than the null allele frequency of 0.05 due to an immigrant fitness advantage. In contrast, by not pooling the data, we are able to detect that the changes in allele frequency were due (at least in part) to a marker effect.

2.3 Results

2.3.1 Statistical tests

A total of 3066 flies were genotyped in the eight treatments (for raw data see Table 2.1). The frequency distributions of the immigrant allele frequency in the first and second generations after immigration were not normally distributed (Figure 2.2), a feature confirmed using the Shapiro and Wilk (1965) test. This test showed that the data in six of the eight treatments diverged significantly from normality (Table 2.2). The use of several data transformations was unsuccessful in reducing this non-normality; not surprising given the large numbers of zeros in each distribution (Figure 2.2). This threw initial concerns on using parametric analyses that assume data are normally distributed.

2.3.2 The binomial test as an alternative to parametric analyses

As an alternative to parametric analyses, I initially considered performing a set of binomial tests and binomial-based confidence intervals. To appreciate the logic behind this approach, consider the case of testing whether observed allele frequencies differ significantly from the null allele frequency of 0.05. Two forms of binomial tests could be used to address this issue.

One approach is to pool results across all individuals and all replicates in each treatment. Thus, for the first generation progeny of FF-marked inbred immigrants (the first column of data in Table 2.1), there were 46 individuals from a total of 360 that carried the immigrant allele. The expectation according to the null hypothesis of no change in allele frequency is that 18 individuals (i.e. 0.05 x 360) should carry the immigrant allele. It is then simply a matter of using the binomial distribution to determine the probability that a result as extreme or more extreme than the observed 46 individuals could have occurred by chance. The other approach is to perform a binomial test on each replicate population, giving 20 *P*-values for each treatment. A combined probability test would then have to be performed across replicates to give an overall *P*-value for each treatment (Sokal and Rohlf, 1981).

Table 2.1 Raw data showing the number of immigrant alleles per replicate population (in bold), together with the number of individuals sampled (not in bold). The frequency of the immigrant allele is calculated as the number of immigrant alleles / (2 x the number of individuals). The first and second generation results are paired for each replicate. In first generation replicates, individuals possessing the immigrant allele must be heterozygous, while second generation results include immigrant-heterozygous and immigrant-homozygous individuals (data not shown).

	FF Inbred		**FF Outbred**		**SS Inbred**		**SS Outbred**	
	1st	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**
replicate								
1	**0** 20	**0** 20	**0** 20	**0** 20	**0** 19	**0** 20	**0** 20	**0** 20
2	**0** 20	**0** 20	**0** 20	**0** 20	**0** 18	**0** 20	**0** 20	**0** 20
3	**0** 17	**0** 20	**0** 20	**0** 20	**0** 19	**0** 20	**0** 19	**0** 20
4	**0** 20	**0** 20	**0** 15	**0** 20	**0** 19	**0** 20	**0** 19	**0** 20
5	**0** 16	**0** 20	**0** 4	**0** 20	**0** 18	**0** 20	**3** 20	**7** 20
6	**0** 18	**0** 20	**0** 20	**0** 20	**0** 20	**0** 20	**5** 20	**12** 20
7	**0** 19	**0** 20	**3** 20	**5** 20	**0** 18	**0** 20	**5** 20	**11** 20
8	**0** 17	**0** 20	**3** 19	**2** 20	**0** 18	**0** 20	**5** 20	**8** 20
9	**0** 19	**0** 20	**3** 19	**6** 20	**0** 19	**0** 20	**5** 19	**7** 20
10	**0** 15	**0** 20	**3** 17	**6** 20	**0** 20	**0** 20	**7** 20	**12** 20
11	**0** 18	**0** 20	**6** 20	**5** 20	**2** 20	**2** 20	**9** 20	**13** 20
12	**0** 8	**0** 5	**9** 19	**8** 20	**2** 19	**2** 20	**9** 19	**14** 20
13	**1** 20	**0** 11	**10** 20	**18** 20	**4** 19	**8** 20	**9** 17	**10** 20
14	**1** 18	**4** 20	**10** 19	**10** 20	**4** 19	**3** 20	**11** 19	**21** 20
15	**2** 18	**15** 20	**11** 20	**21** 20	**4** 18	**6** 20	**12** 19	**17** 19
16	**4** 18	**4** 19	**11** 20	**11** 20	**7** 20	**9** 20	**13** 19	**19** 20
17	**5** 19	**10** 20	**12** 20	**15** 20	**8** 20	**11** 17	**13** 19	**22** 20
18	**6** 20	**13** 20	**11** 18	**20** 20	**11** 19	**18** 20	**14** 20	**18** 20
19	**13** 20	**14** 20	**14** 19	**19** 18	**12** 20	**14** 20	**14** 19	**20** 20
20	**14** 20	**18** 20	**15** 19	**15** 20	**19** 20	**16** 20	**15** 19	**17** 20

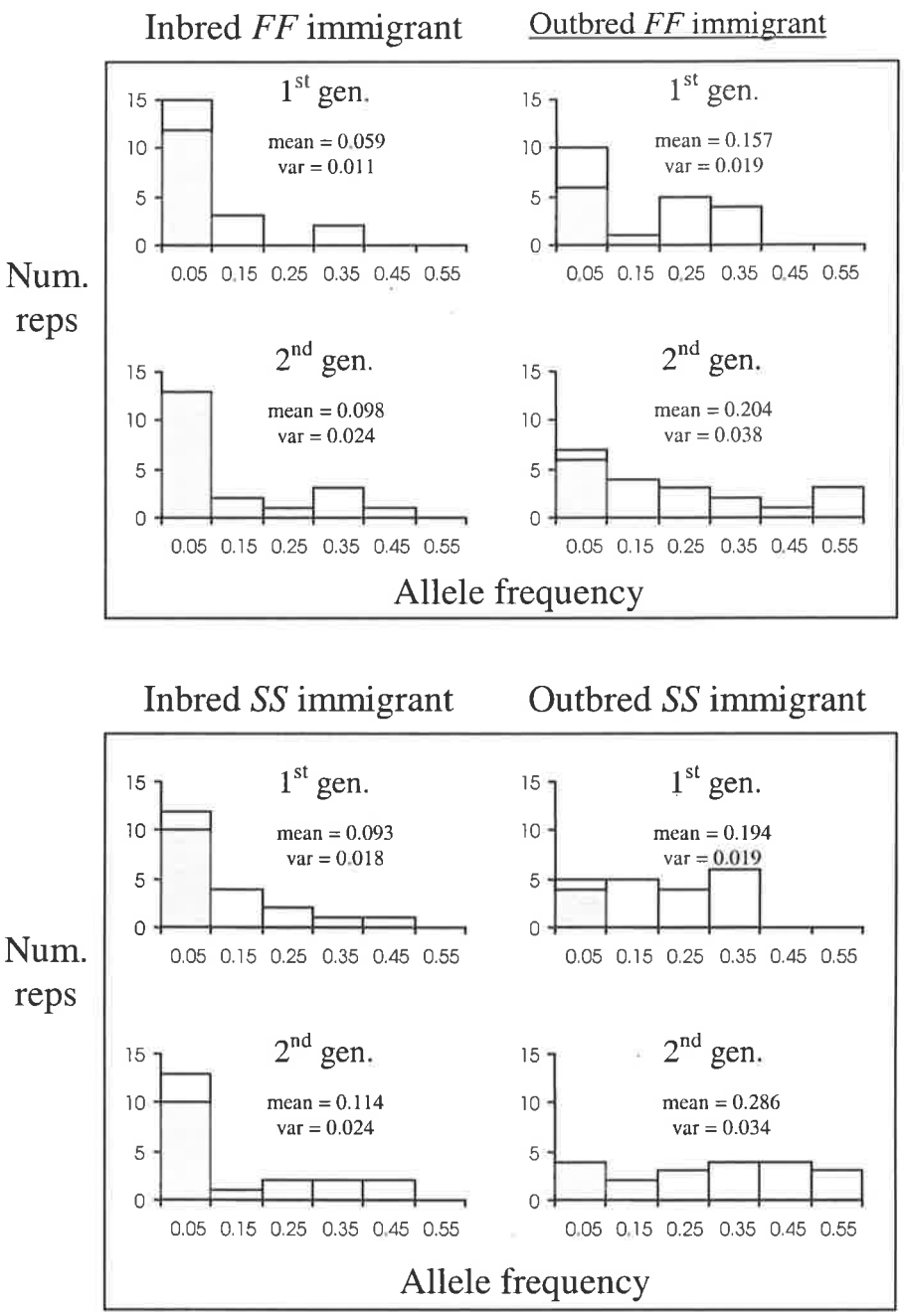

Figure 2.2 Distributions of immigrant allele frequency. Shown for each treatment are the numbers of replicates (from n = 20) belonging to different allele frequency categories. Each category has a width of 0.10, and each label on the x axis refers to the midpoint of a category. The grey portion of the 0.05 category is the number of values that were zeros. The sample mean and sample variance are given for each treatment.

Table 2.2 Results of the Shapiro and Wilk (1965) test for normality. Smaller values of the test statistic W represent greater deviations from normality. $W_{critical(0.05)} = 0.905$, and significance ($\alpha = 0.05$) is indicated by an asterisk.

Marker	Treatment	W	*P*-value
FF	1st gen. inbred	0.624	< 0.01*
FF	1st gen. outbred	0.873	< 0.02*
FF	2nd gen. inbred	0.670	< 0.01*
FF	2nd gen. outbred	0.872	< 0.02*
SS	1st gen. inbred	0.751	< 0.01*
SS	1st gen. outbred	0.913	< 0.10
SS	2nd gen. inbred	0.756	< 0.01*
SS	2nd gen. outbred	0.918	< 0.10

The problem with both these approaches is that they assume that each individual represents an independent event with a 0.05 chance of carrying the immigrant allele. I believe this assumption is incorrect. Importantly, the design of the experiment would have encouraged non-independence among the progeny of each replicate population. Because there are only 20 flies per generation (ten males and ten females), there is likely to be some degree of clumping in the fate of the immigrant genome. *D. melanogaster* females remate approximately every two to five days on average (Fukui and Gromko, 1989; Pitnick, 1991), and as stated earlier, most of a female's progeny are sired by her most recent mate (Gromko *et al.*, 1984). Given that the females in this experiment were only given three days in which to oviposit, this means that there may have been somewhere close to ten independent opportunities (i.e. ten females) for the immigrant male to sire offspring. With variation in female fitness there would be even fewer opportunities. For example if for some reason only five females dominated a population's reproduction, there would essentially be only five independent opportunities for the immigrant male to pass on his genes. Similarly, variation in male fitness (other than that due to treatment or marker effects, or chance alone) may also have contributed to non-independence among progeny genotypes.

The number of independent events involved in a binomial test can have a large effect on whether or not we reject the null hypothesis. Consider a replicate population where four of the 20 first generation progeny carry the immigrant allele. If we assume these progeny originated from 20 independent events each with a 0.05 probability of resulting in an immigrant individual, a binomial test would tell us there was a 0.016 probability of observing a result as extreme or more extreme than four immigrant progeny. If the data actually came from only ten independent events (*e.g.,* ten females each producing two offspring from a given mating event), the probability of obtaining a result as extreme or more extreme than the observed result is somewhat higher, at 0.086. With only five independent events the probability is 0.226. Therefore, by assuming that all individuals originate from independent events we are likely to underestimate the likelihood of extreme events according to the null model.

Given that there is reason to believe the progeny in this experiment did not arise from independent events, and that it is not clear how many independent events were actually involved, I believe it is inappropriate to use binomial tests for the data in this chapter.

2.3.3 Justification for using parametric analyses

Despite the non-normality of the raw data, I nonetheless decided to use parametric analyses. The saving grace of parametric analysis when data are not normally distributed is the central limit theorem, which states that even if a distribution is highly skewed, the probability distribution of sample means will approximate a normal distribution as the number of replicates in the sample increases (Hays, 1988). The validity of relying on the central limit theorem for the analyses in the present chapter is assessed in detail in Chapter 3.

2.3.4 Comparison of treatment means to the null allele frequency

The mean allele frequency was compared to the null allele frequency of 0.05 for each of the eight treatments. The test in each case was whether a 95% confidence interval around the sample mean would include the null allele frequency of 0.05. While the sample mean was greater than the null allele frequency for all treatments, it was significantly greater only when the immigrant was outbred (Figure 2.3). This was true for both the first and second generations after immigration, and for both marker combinations.

2.3.5 Comparison of allele frequency between treatment means

Treatment means were compared in a pair-wise manner using *t*-tests. The four types of comparisons were:

(1) first generation (outbred vs. inbred immigrant)

(2) second generation (outbred vs. inbred immigrant)

(3) inbred immigrant (second generation vs. first generation)

(4) outbred immigrant (second generation vs. first generation)

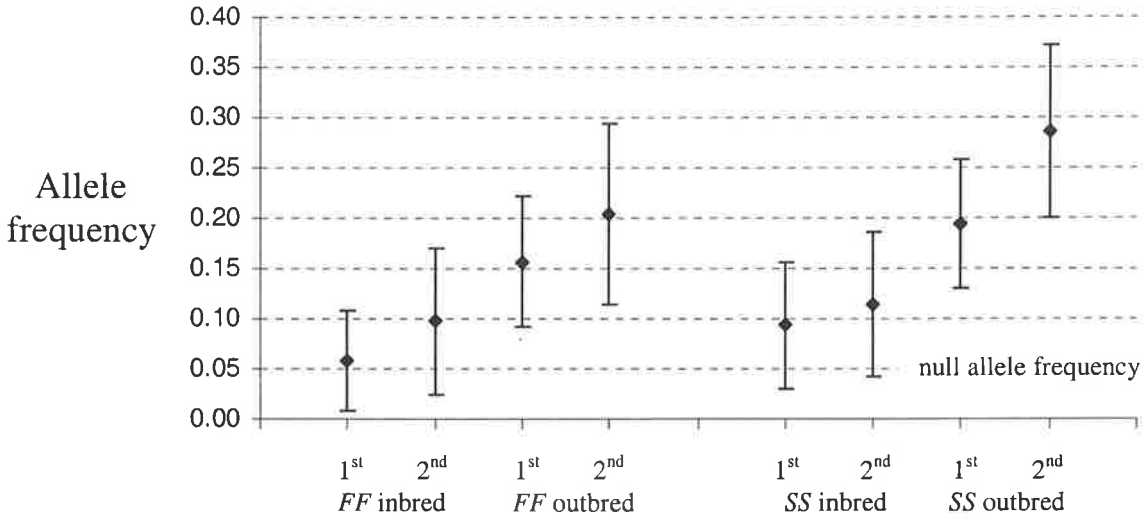

Figure 2.3 Sample mean ± 95% confidence interval for all treatments. 1st and 2nd refer to generations after immigration. Note the position of confidence intervals relative to the null allele frequency.

Non-normality and heterogeneity of variances (Figure 2.2) initially threw concerns on using *t*-tests (with data transformation doing little to reduce the heterogeneity of variances). Nonetheless, I considered *t*-tests to be a robust approach for the data at hand. In regards to normality the central limit theorem once again applies (Sokal and Rohlf, 1981 - pg. 414), while the heterogeneity of variances is compensated by the equality of sample sizes – an important component of *t*-test robustness (Zar, 1984 - pg. 130).

Of the four *t*-tests performed on *FF* immigrant data, only first generation offspring (outbred vs. inbred immigrant) showed a significant difference between treatment means (Table 2.3). Of the four tests applied to the *SS* immigrant data, two showed a significant difference between treatment means (Table 2.3). These were first generation offspring (outbred vs. inbred immigrant), and second generation offspring (outbred vs inbred immigrant). Thus, unlike the *SS* immigrant data, the *FF* immigrant data did not show a significant difference for second generation offspring (outbred vs. inbred immigrant). Notably however, this comparison was only marginally non-significant for *FF* immigrants, with $P = 0.064$ (Table 2.3).

Table 2.3 Results of *t*-tests. "Marker" refers to the immigrant genotype. For each comparison d.f. = 38. Significance ($\alpha = 0.05$) is indicated by an asterisk.

Marker	Comparison	*t*-stat.	*P*-value
FF	1st gen. (outbred vs. inbred)	2.502	0.017*
FF	2nd gen. (outbred vs. inbred)	1.907	0.064
FF	inbred (2nd vs. 1st gen.)	0.922	0.363
FF	outbred (2nd vs. 1st gen.)	0.879	0.385
SS	1st gen. (outbred vs. inbred)	2.372	0.023*
SS	2nd gen. (outbred vs. inbred)	3.202	0.003*
SS	inbred (2nd vs. 1st gen.)	0.499	0.656
SS	outbred (2nd vs. 1st gen.)	1.789	0.082

2.3.6 Results summary

The reciprocal marker experiments produced very similar results (Figure 2.3). In summary:

(1) When the immigrant was outbred, the mean frequency of the immigrant allele was significantly higher than the null allele frequency of 0.05 in the first and second generations after immigration.

(2) When the immigrant was inbred, the mean frequency of the immigrant allele was not significantly different from the null allele frequency in either the first or second generations after immigration.

(3) The mean allele frequency of both inbred and outbred immigrants did not change significantly from the first generation to the second generation, for both markers.

(4) In the first generation after immigration, the mean allele frequency of outbred immigrants was significantly higher than that of inbred immigrants.

(5) In the second generation after immigration, the mean allele frequency of outbred immigrants was still significantly higher than that of inbred immigrants when the immigrant carried the *SS* marker. This difference was marginally non-significant for *FF* immigrants.

2.4 Discussion

This experiment showed that inbreeding can have a considerable impact on the contribution of single male immigrants to the genetic diversity of inbred populations.

At the same time, the rare male effect and hybrid vigour of immigrant progeny seemed to have had little impact on immigrant fitness in this system. This is evident in the observation that the genetic contribution of inbred immigrants did not differ significantly from the initial null allele frequency of 0.05. However, although non-significant, the fact that the sample means for this treatment were above the null frequency for both markers suggests a trend worthy of further study.

The apparent lack of a rare male effect in this study lends support to suggestions that this effect either (a) does not exist or (b) is not of universal importance. In terms of the former, a number of authors have argued that the rare male effect may be an artifact of experimental design (Bryant *et al.*, 1980; Knoppien, 1987). In relation to the latter, it has been argued that the rare male effect is not as strong for *D. melanogaster* as for another well studied species in this regard - *Drosophila pseudoobscura* (Markow *et al.*, 1980). Alternately, it could be true that a weak rare male effect was operating in this study, but could not be detected due to inadequate statistical power.

The observation that inbred immigrants had no significant fitness advantage suggests the rare male effect and hybrid vigour also made an insignificant contribution to the fitness of outbred immigrants. This inference relies on the assumption that both the rare male effect and hybrid vigour operate with equal strength irrespective of whether the immigrant is inbred or outbred. If this assumption is valid, the results imply that the fitness advantage of outbred immigrants over residents is attributable to initial outbred vigour, as this was the only mechanism identified as giving outbred immigrants an advantage over inbred immigrants. There are several ways in which this initial outbred vigour could occur. Outbred immigrants may have been (a) more active in courting mates, (b) more attractive to females by appearing fitter than resident males (*e.g.*, by being larger or more active), or (c) more successful in fertilising females (*e.g.*, by producing semen that is more competitive than that of resident males). Exploring these three mechanisms warrants further study.

This experiment did not detect a significant change in the frequency of the immigrant allele from the first generation to the second generation after immigration. This was true for both markers and both inbred and outbred immigrants. This result is somewhat surprising given that first generation populations should include male immigrant progeny that, due to hybrid vigour, have the same advantage over their inbred competitors that gave the original male immigrants their initial outbred vigour advantage. One potential explanation is that the difference in fitness between immigrant progeny and inbred individuals was not as strong for females as it was for males. This would clearly reduce the average impact of hybrid vigour. Recombination of genes may also have reduced the impact of hybrid vigour, a mechanism that potentially applies to both sexes. The effect of recombination is that when immigrant progeny reproduced, the marker allele would have become disassociated from part of the immigrant genome that previously gave it a selective advantage.

Another issue to consider is that an increase in allele frequency between the first and second generations may have been obscured by increased variance. Indeed, in every situation (inbred and outbred immigrants, and both marker situations) the sample variance increased from the first to the second generation (Figure 2.2). This is understandable when we consider the design of this experiment - from all treatments initially having the same immigrant allele frequency of 0.05, the variance in allele frequencies would tend to accumulate over the generations as population allele frequencies diverge. Although non-significant, there were consistent increases in the sample means from the first to second generations for all treatment combinations it is perhaps unwise to say that any increase can be ruled out. Therefore I would recommend further testing of the ability of immigrant genes to increase in frequency over the generations following immigration.

Although this study controlled for both 1) a marker effect, and 2) the experience of male immigrants relative to population residents, there remains the potential for inherited environmental effects to have influenced the results. Inherited environmental effects are those components of an individual's phenotype that are derived from either parent, apart from nuclear genes (Rossiter, 1996). In this regard it is important to acknowledge that the parents of outbred immigrants were reared in jars containing many hundreds of individuals, whereas the parents of all inbred immigrants were reared in small vials containing approximately 20 to 40 individuals. While I believe the density of flies was comparable in each case, I cannot

dismiss the possibility that inherited environmental effects influenced the results to some degree.

This experiment has demonstrated a situation where Wright's (1931) assumptions of 1) immigrants having the same fitness as residents, and 2) immigrants from different source populations having the same mean fitness as each other, are not met. Essentially this adds another element to our understanding of the complex relationship between the rate at which individuals immigrate into populations, and their impact in terms of gene flow. As such, this experiment lends support to notion that biologists should be careful when using measures of population subdivision such as F_{ST} to infer Nm – the number of migrants entering each population per generation (Whitlock and McCauley, 1999).

This experiment also has implications for understanding the "rescue effect". It is thought that the occasional arrival of immigrants may rescue populations from extinction (Brown and Kodric-Brown, 1977), and that this may play a crucial role in the dynamics of metapopulations (Hanski and Gyllenberg, 1993). If the loss of genetic diversity increases the probability that populations become extinct (Lande and Barrowclough, 1987; Caughley, 1994; Frankham, 1995a; Saccheri *et al.*, 1998), the rescue effect may have an important genetic component (as compared to a purely demographic component). Given the potential for such genetic rescue, this study suggests that for conservation purposes, greater value should be placed on large mainland populations that act as a source of outbred immigrants to small, inbred populations.

Finally, in this chapter I have raised concerns about the adequacy of the statistical tests performed. These tests were based on the assumption of normality, but used data that were not normally distributed. As such, there is reason to doubt some of the conclusions made. At the same time, the central limit theorem provides some indication that the effect of such departures from normality may be trivial. Therefore, in the next chapter I assess the robustness of the analyses presented here in Chapter 2.

CHAPTER 3

Additional analyses of immigrant fitness experiment data

3.1 Introduction

The purpose of this chapter is to assess the robustness of the analyses performed in Chapter 2. Those analyses, based on an assumption of normality, involved data that were clearly not normally distributed. As such, the inferences in that chapter depend on the central limit theorem, which states that although a distribution (with mean μ and variance σ^2) may be highly skewed, the probability distribution of sample means will approximate a normal distribution (with mean μ and variance σ^2/N) as the sample size (N) increases (Hays, 1988).

The key question here is: were the sample sizes in Chapter 2 large enough to achieve sample mean distributions that were acceptably close approximations to normality? Unfortunately there is no golden rule for deciding what sample size is adequate, with the degree of approximation to normality depending on the shape of the underlying probability distribution of the data (Pagano, 1994, p. 286). Nonetheless, it is suggested in the literature that a sample size of 30 is large enough in most cases to effectively normalise a distribution of sample means (Madsen and Moeschberger, 1980; Hays, 1988; Pagano, 1994). Given that the samples in Chapter 2 each comprised of 20 replicates, this throws some doubt on whether the conclusions in that chapter depend critically on the shape of the underlying distributions.

This chapter has two components. In the first component I use bootstrap analysis to assess the degree to which the sample means of Chapter 2 are normally distributed. Secondly, I use data-driven techniques (bootstrap analysis and randomisation tests) as alternatives to the parametric analyses in Chapter 2. While these represent very different ways to analysing data, this chapter demonstrates that the two approaches may in fact compliment each other, thereby providing a valuable cross-check of the robustness of analyses such as those performed in Chapter 2. Throughout this chapter I will use the term "parametric" to refer to

analyses that rest on specific assumptions about the form of the population distribution - in this case the normal distribution (Gibbons, 1976).

3.2 How close are the sample means of Chapter 2 to being normally distributed?

While the central limit theorem tells us that the distribution of sample means approximates normality if our sample size is large enough, the data in Chapter 2 provide only one sample mean per treatment, and thus no direct insight into how sample means are distributed. A highly impractical way to gain this insight would be to perform hundreds of replicate experiments, obtain hundreds of sample means, and use these to construct sample mean distributions. Bootstrap analysis provides a powerful alternative in this regard by enabling us to ask: How would sample means be distributed if drawn from a population of data points having a probability distribution similar in shape to the frequency distribution of the observed data set?

The philosophy behind bootstrap analysis is to treat a sample frequency distribution as the best estimate (in the absence of other information) of the underlying probability distribution of some population of data points (Manly, 1997). Accordingly, the observed frequency distribution is treated as an infinite pool of data points from which a large number of replicate bootstrap samples are drawn. Each bootstrap sample is drawn by randomly sampling the observed data set with replacement a certain number of times (generally the number of data points in the original sample) to obtain a value for some parameter of interest such as the sample mean. Thousands of replicate bootstrap samples are drawn to create a frequency distribution of that parameter (a relatively straightforward task given modern computing power) and this frequency distribution is then used either to test hypotheses or create confidence intervals. In this way, we can mimic the act of performing replicate experiments. Appropriately, the term "bootstrap" is said to represent someone pulling themselves out of the [statistical] mud by their bootstraps (Manly, 1997).

By creating an artificial infinite population of data points, the bootstrap approach offers biologists the versatility of being able explore the properties of a wide range of parameters and data structures (Young, 1994a). Essentially the approach allows us to "play" with a data set as if we had the true probability distribution (bearing in mind that we don't, and probably

never will, have access to the true probability distribution). One application of bootstrapping is as an alternative to traditional parametric analyses, such as the calculation of confidence intervals (undertaken in a later section of this chapter). While there is generally no need to perform bootstrapping when the assumptions of parametric analogues are valid (Efron and Tibshirani, 1993, p. 171), it can provide a useful alternative in situations when those assumptions are broken. Bootstrap analysis is also valuable in situations where parametric analogues do not exist, as is the case for example, when trying to analyse phylogenetic relationships (Hillis and Bull, 1993). Manly (1997) provides a summary of some of the diverse ways in which bootstrapping has been used. For a thorough description of the theory of bootstrap analysis see Efron and Tibshirani (1993), Young (1994a) and Manly (1997).

While bootstrapping is generally used to generate confidence intervals and test hypotheses (Manly, 1997), I believe the approach is also well suited for assessing how well the central limit theorem applies to samples whose normality is in question. In this context, bootstrapping may represent a useful support, rather than an alternative, to parametric analyses. A clear precedent for this approach is provided by Pitt and Kreutzweiser (1998) who used a frequency distribution of 5000 bootstrap sample means to illustrate the central limit theorem for a sample of 30 data points drawn from an exponential distribution. They demonstrated the approximate normality of this bootstrap distribution by comparing the mean, variance and proportion of data points within one standard deviation of the mean, to those values expected under the assumption of normality. This connection between the central limit theorem and bootstrapping is also made by Efron and Tibshirani (1993, p. 171). I have essentially adopted the same approach as Pitt and Kreutzweiser (1998), but used 10,000 bootstrap samples instead of their 5000, and have taken a more direct approach to measuring the normality of bootstrap distributions.

Using a program written in Turbo Pascal 7.0 (see Appendix 2 - A:\APP2\CHAPTER3.PAS on the accompanying disk) I calculated 10,000 bootstrap sample means for each of the eight treatments in Chapter 2. This and other programs written for this thesis used the predefined pseudo-random number generator available in Turbo Pascal 7.0 (see Appendix 1 for confirmation that this produces an effectively random sequence of numbers uniformly distributed between 0 and 1). The frequency distributions of bootstrap sample means closely approximate normality in each case (Figure 3.1). To measure how close the approximation was, I calculated the maximum absolute difference between the bootstrap cumulative

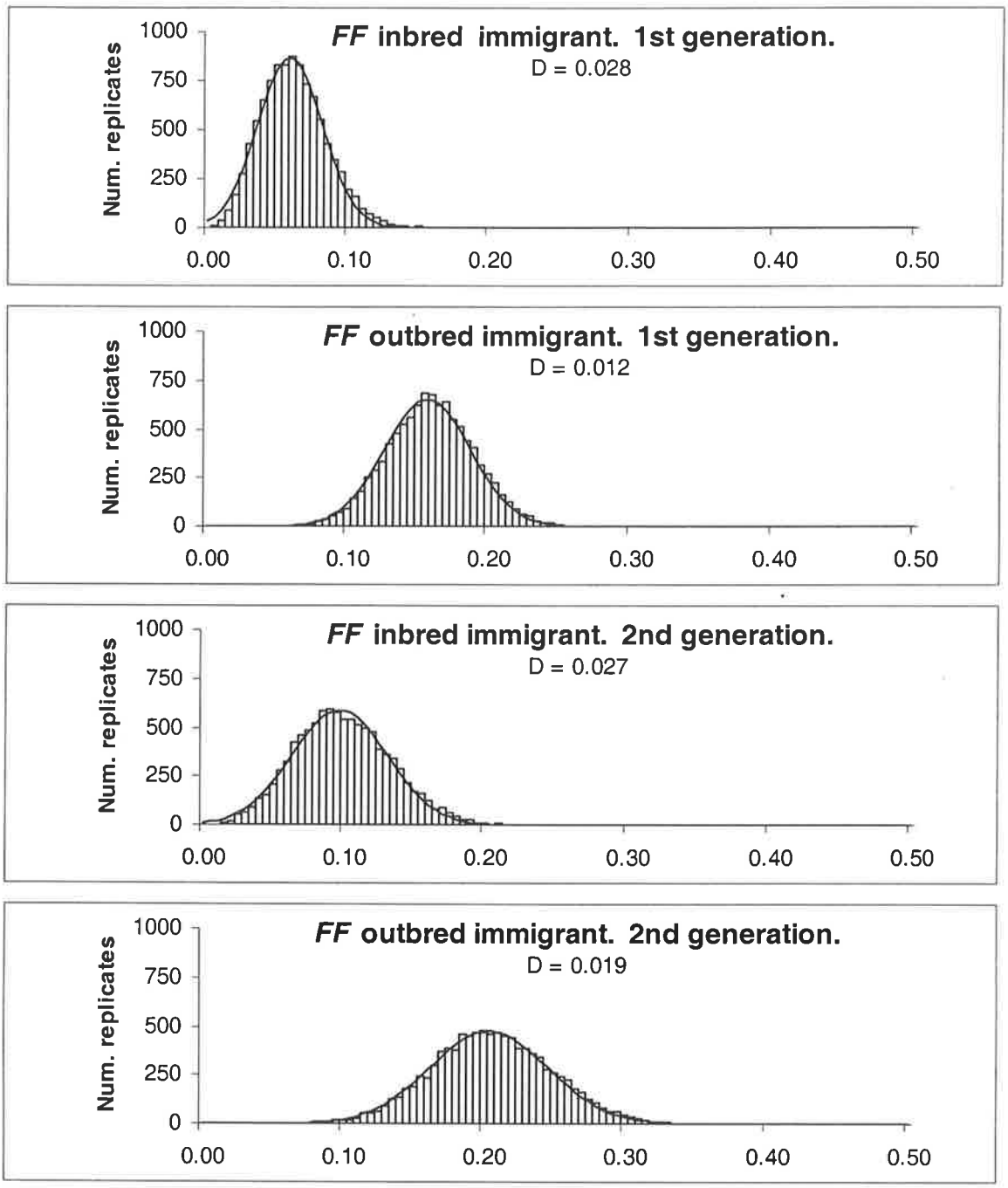

Figure 3.1a Distribution of bootstrapped sample means for each of the four treatments where the immigrant was ***FF***-marked. Each distribution is from 10,000 bootstrap samples. Also shown is the normal curve having the same mean and variance as that calculated among the 10,000 bootstrap samples. D is the maximum absolute difference between the cumulative frequency distribution of bootstrap samples and the cumulative probability distribution for the associated normal curve.

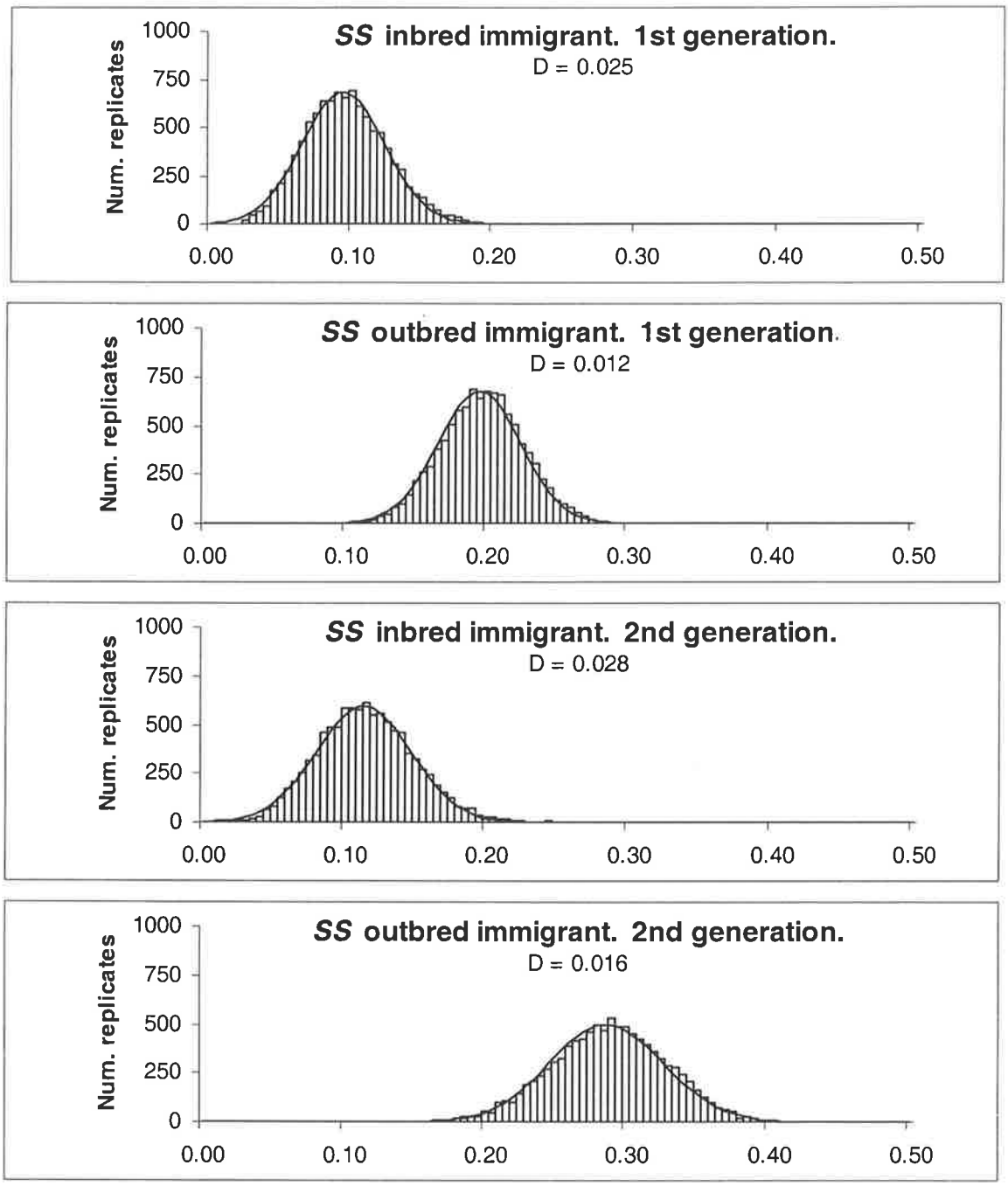

Figure 3.1b Distribution of bootstrapped sample means for each of the four treatments where the immigrant was *SS*-marked. Each distribution is from 10,000 bootstrap samples. Also shown is the normal curve having the same mean and variance as that calculated among the 10,000 bootstrap samples. D is the maximum absolute difference between the cumulative frequency distribution of bootstrap samples and the cumulative probability distribution for the associated normal curve.

frequency distribution and the associated normal cumulative frequency distribution. In all cases there was little difference between the two distributions (see "D" values in Figure 3.1) – the greatest absolute difference being 2.8 %.

In one sense, using bootstrapping to explore the distribution of sample means has simply led to another question – how close an approximation to normality is tolerable? Clearly the value of this approach lies in providing an explicit, quantitative measure of how closely a distribution of sample means approximates normality. The decision then lies with the reader as to how close an approximation is acceptable. While the literature offers no guidelines, I would suggest that the differences observed here are negligible.

3.3 Non-parametric alternatives to the analysis of immigrant experiment data

Another way to assess the robustness of the analyses in Chapter 2 is to perform alternate, yet analogous tests. In the remainder of this chapter I explore such alternatives. As with Chapter 2, two sets of analyses were carried out: (1) estimation of 95% confidence intervals around the mean allele frequency for each treatment to test for a significant difference from the null allele frequency of 0.05, and (2) pairwise comparisons between treatment means.

I estimated confidence intervals using bootstrap analysis, and compared means using randomisation tests. Both these methods use some form of resampling of the observed data as the basis for drawing inferences (Manly, 1997). Importantly they are non-parametric in the sense that they do not assume the data come from a particular probability distribution, and for this reason they are particularly useful when we have reason to doubt the assumptions of parametric analyses (Potvin and Roff, 1993).

3.3.1 Calculation of confidence intervals using bootstrap analysis

Bootstrap confidence intervals were estimated using a modification of Efron's (1979) percentile method. This method involves simply delineating 95% confidence limits as the 2.5 and 97.5 percentiles of a frequency distribution of bootstrapped values. This is shown in Figure 3.2 for a set of 10,000 bootstrap sample means calculated from the data of *FF* inbred immigrants, first generation offspring (using the same set of values presented in the topmost graph in Figure 3.1a).

Importantly though, Efron's (1979) approach does not take into account the bias introduced by bootstrapping. Bias occurs when an estimator is, on average, higher than or lower than the true value of that parameter. This is the reason why statisticians use "n - 1" instead of "n" as the denominator when estimating sample variance (Zar, 1984). Bias may arise in bootstrapping when a parameter value (as estimated from the original sample) is not the median of a distribution of bootstrap estimates of that parameter (Manly, 1997). To illustrate the importance of this discrepancy, consider the example of trying to estimate the mean. The starting point here is to state that the mean from our original sample is our best estimate of the true population mean. Accordingly, with traditional parametric statistics, the sample mean is automatically included in any confidence interval we make. This is because the symmetry of the normal distribution dictates that the mean and median of a parameter coincide. Thus, when we calculate 95% confidence limits for a normal distribution we include the 47.5 percentiles lying either side of the mean. The problem with Efron's (1979) percentile method is that when bias exists, the mean of the original sample is displaced from the centre of the percentile confidence interval. This means that instead of a 95% confidence interval including the 47.5 percentiles lying symmetrically either side of the original sample mean, we may unwittingly state the confidence interval as including the 42 percentile below and the 53 percentile above the sample mean. In this way, using Efron's (1979) original percentile method may bias our estimate of the confidence limits towards one particular side of our best estimate of the true population mean. Accordingly, Efron (1981) introduced a method for calculating bias-corrected percentile confidence limits, and this is the approach I have used.

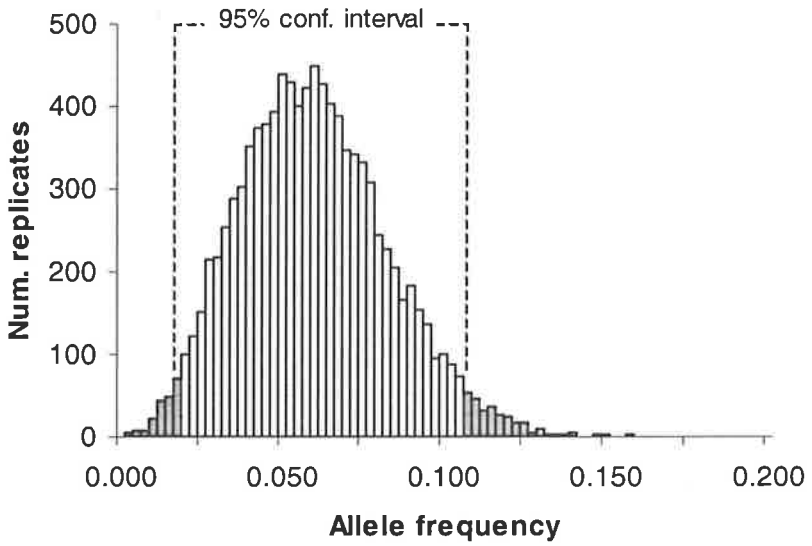

Figure 3.2 A demonstration of Efron's (1979) percentile confidence limits as applied to the data of *FF* inbred immigrant, first generation offspring. In this case a sample of 10,000 bootstrap sample means were drawn, and the confidence interval delineated by the values that exclude the 250 largest and 250 smallest sample means (shaded). Because the histogram is composed of discrete categories, there are slightly fewer than 250 values included in the shaded portion of each tail of the histogram.

To perform this analysis I used the same sets of 10,000 bootstrap samples calculated earlier (Figure 3.1). This level of replication is likely to be more than adequate, given Manly's (1997) recommendations of having a minimum of 1000 bootstrap samples to resolve significance at the 5% level.

Bootstrap analysis produced strikingly similar 95% confidence intervals to the parametric analyses (Table 3.1 and Figure 3.3). Importantly, the bootstrap confidence intervals lead to the same inferences that were drawn in Chapter 2.

Table 3.1 95% confidence limits of immigrant allele frequencies calculated using Efron's (1981) bias-corrected percentile method ("bootstrap" in the table headings), and traditional parametric analysis. The greatest absolute difference between the two approaches was 0.010.

Marker	Immigrant	Generation	Limit	Bootstrap	Parametric	Difference
Fast	inbred	1	upper	0.103	0.109	-0.006
			lower	0.016	0.009	+0.007
Fast	inbred	2	upper	0.163	0.171	-0.008
			lower	0.033	0.025	+0.008
Fast	outbred	1	upper	0.217	0.222	-0.005
			lower	0.097	0.092	+0.005
Fast	outbred	2	upper	0.287	0.295	-0.008
			lower	0.123	0.113	+0.010
Slow	inbred	1	upper	0.153	0.155	-0.002
			lower	0.040	0.031	+0.009
Slow	inbred	2	upper	0.179	0.186	-0.007
			lower	0.049	0.041	+0.008
Slow	outbred	1	upper	0.254	0.258	-0.004
			lower	0.138	0.130	+0.008
Slow	outbred	2	upper	0.364	0.372	-0.008
			lower	0.208	0.200	+0.008

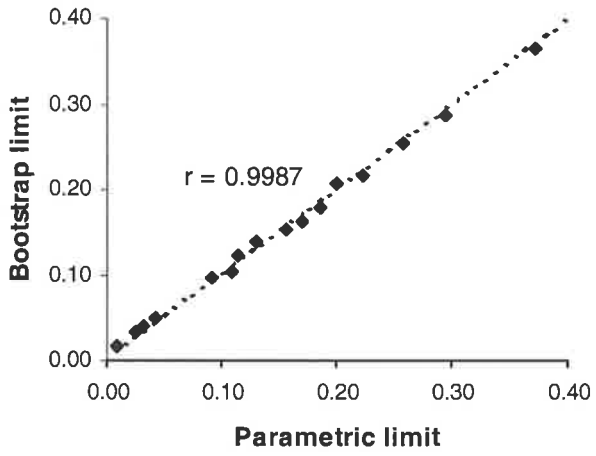

Figure 3.3 The relationship between bootstrap and parametric estimates of 95% confidence limits. These data are the upper and lower limits presented in Table 3.1. The hatched line is NOT a line of best fit, but the line along which bootstrap and parametric estimates would be equal (y-intercept = 0, slope = 1). The value r is the correlation coefficient.

3.3.2 Comparison of treatment means using randomisation tests

Treatment means were compared using randomisation tests, an approach pioneered by Fisher (1935). As well as facilitating the comparison of means, randomisation tests are useful for addressing a wide range of statistical problems (Potvin and Roff, 1993; Manly, 1997). As with parametric tests, the general question behind randomisation tests is: "Can an observed pattern be explained by chance?" To compare means between two samples, we start with the null hypothesis that the two samples were drawn randomly from a single population - the same assertion we would make for the null hypothesis of a *t*-test. However, where parametric analyses rely on the concept of theoretical populations of data points, randomisation tests treat the observed data as if it were the entire population of data points.

The starting point of a randomisation test is to combine the data from two treatments into a common pool. The question then is – if the data from the two treatments came from a common pool (i.e. a single population of data), how likely is it that we would have obtained the observed difference in treatment means, or a more extreme difference? If the probability of obtaining the observed difference by chance is too low (less than 0.05) then we would reject the null hypothesis that the data were randomly drawn from a single population, and state that there is a significant difference between means. This probability is estimated by obtaining a set of randomised differences between means. Each randomised difference is calculated by allocating the data points without replacement from the combined data pool into two "treatments", each having the same sample size as the observed samples. After obtaining a number of randomised differences, the test is then simply whether the observed difference lies in the 5% most extreme of these values. For two-tailed tests, we are interested in whether the observed difference lies among the 2.5% largest or 2.5% smallest randomised differences. Alternately we can present the *P*-value for such a test as the proportion of randomised difference between means that are as extreme as, or more extreme than the observed difference between means (Figure 3.4).

Two approaches to randomisation can be used – complete enumeration and random sampling (Manly, 1997). With complete enumeration, the difference between means is calculated for every possible permutation of the combined data pool. While this is well suited to analysing the data from small sample sizes, complete enumeration becomes cumbersome for larger data sets - for example comparing two treatments using the data in Chapter 2 would involve

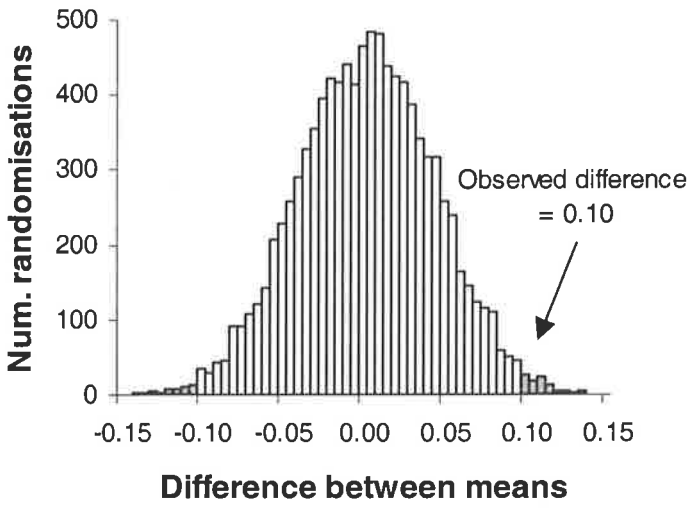

Figure 3.4 A randomisation test - the method used to generate *P*-values to compare means. 10,000 randomised differences between means were calculated and sorted. The *P*-value is the proportion of randomised differences that are as extreme as or more extreme than the observed difference (shaded regions).

complete enumeration of more than 137 billion permutations! Fortunately random sampling, when performed with adequate replication, provides a good approximation to complete enumeration (Manly, 1997), and for this reason it was used for the data at hand. A small point worth noting is that the observed difference between means was included as one of the randomised values. This technicality is simply in keeping with the null hypothesis that the observed difference is one of a random sample of differences between means (Manly, 1997).

One of the major attractions of randomisation tests over their parametric analogues is that they do not assume the data fall into any particular theoretical distribution (Pitt and Kreutzweiser, 1998). Accordingly, such tests are frequently referred to as "distribution-free", a term that is appropriate if we simply test for the equality of distributions - that is, if we simultaneously test for differences in skewness, variance, and the location of the mean. Importantly though, not all randomisation tests are entirely free of assumptions. This is certainly the case if we test the equality of single "location parameters" such as the mean. Indeed, using a randomisation test to compare means is only appropriate when variances are equal among treatments (Boik, 1987). Thus, the randomisation tests employed in this section have the same assumption of homogeneity of variances that is important in *t*-tests and ANOVAs. Although a bootstrapping alternative exists for comparing sample means when variances differ (Efron and Tibshirani, 1993), I have chosen to use randomisation tests here. While the comparisons performed in this chapter involve samples whose variances do indeed differ (see Figure 2.2 - previous chapter), the effect of such heteroscedasticity (variance of variances) is likely to be trivial. Fortunately several features of the data are favourable in this regard (see Boik, 1987): (i) the heteroscedasticity is moderate; (ii) the sample sizes are equal; and (iii) the sample sizes (n = 20) are moderately high.

For each of the eight pairwise comparisons of Chapter 2, random sampling was performed using a program written in Turbo Pascal 7.0 (see Appendix 2 - A:\APP2\CHAPTER3.PAS on the accompanying disk) to obtain 10,000 values of the test statistic - the difference between means. As with bootstrapping, this level of replication exceeds Manly's (1997) recommendations of a minimum of 1000 randomisations to resolve significance at the 5% level (and 5000 at the 1% level). The results from randomisation tests and the previously-employed *t*-tests were strikingly similar, and the same inferences would be drawn using α = 0.05 (Table 3.2 and Figure 3.5).

Table 3.2 *P*-values from comparisons of treatment means using randomisation tests and parametric analysis (*t*-tests). The greatest absolute difference in *P*-values between the two approaches is 0.07. In every comparison the two approaches lead to the same inference when using $\alpha = 0.05$.

Marker	Comparison	Randomisation	Parametric
Fast	Outbred 1st gen. - Inbred 1st gen.	0.017	0.017
Fast	Outbred 2nd gen. - Inbred 2nd gen.	0.063	0.064
Fast	Inbred 2nd gen. - Inbred 1st gen.	0.370	0.363
Fast	Outbred 2nd gen. - Outbred 1st gen.	0.383	0.385
Slow	Outbred 1st gen. - Inbred 1st gen.	0.023	0.023
Slow	Outbred 2nd gen. - Inbred 2nd gen.	0.002	0.003
Slow	Inbred 2nd gen. - Inbred 1st gen.	0.658	0.656
Slow	Outbred 2nd gen. - Outbred 1st gen.	0.085	0.082

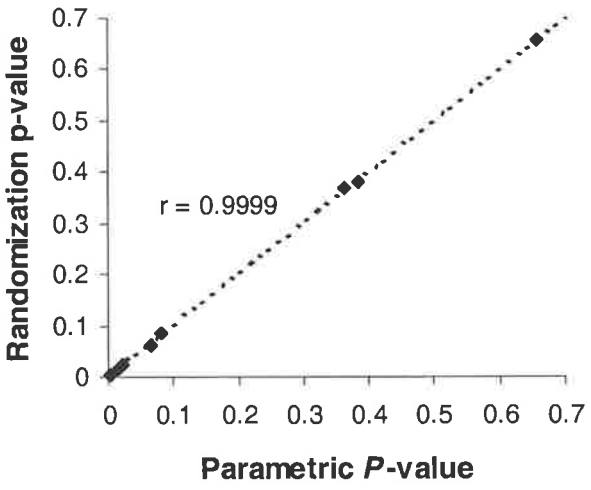

Figure 3.5 Relationship between *P*-values calculated using randomisation tests and parametric *t*-tests. The hatched line is NOT a line of best fit, but the line along which randomisation and parametric *P*-values would be equal (y-intercept = 0, slope = 1). The value r is the correlation coefficient.

3.4 Conclusions

In summary:

- Although the frequency distributions of the data in Chapter 2 were highly skewed, bootstrap sampling suggests the distributions of sample means associated with these data closely approximate normality. As such, the parametric analyses of Chapter 2 are likely to be robust to the non-normality of the raw data.
- Bootstrap confidence intervals and randomisation tests produced very similar *P*-values to their parametric analogues.

To some extent the two points above are related. It turns out that both bootstrap confidence intervals and randomisation tests produce very similar results to their parametric analogues when the assumptions of parametric analysis are met (Efron and Tibshirani, 1993, p. 171; Manly, 1997). Thus, there is some degree of overkill by (1) showing that parametric analyses are likely to be valid by virtue of the central limit theorem, and (2) providing alternatives to those parametric analyses. It is therefore worth considering which of these approaches should be employed whenever there are concerns about data not meeting the assumptions of normality for parametric tests.

The value of using bootstrap sampling to initially explore the normality of sample means clearly lies in being able to support the use of parametric analysis. The potential advantage of this is that parametric analyses are widely accepted and understood. The disadvantage however, is that it is not clear how closely a distribution of sample means must approximate normality in order for inferences to be robust. Until guidelines exist to help make this judgement, using bootstrap analysis to demonstrate the central limit theorem is limited to guesses about how close an approximation to normality is acceptable.

In order to remain strictly quantitative, any serious doubts about data not meeting the assumptions of normality should lead to non-parametric analogues, such as the bootstrapping and randomisation techniques employed in this chapter. It is important to point out however, that the data-driven analyses used in this chapter are not the only alternatives to traditional parametric analysis. For example, the Mann-Whitney test provides a non-parametric alternative to the *t*-test (Potvin and Roff, 1993). However this and many other forms of non-parametric analyses are based on ranks rather than actual data (Potvin and Roff, 1993).

Another form of non-parametric analysis is the sign test (Zar, 1984), which requires that the difference between two, paired data points is recorded as +, 0, or -. While ranks-based and sign-based methods are simple to perform and offer a useful alternative for hypothesis testing, they do have the disadvantage of "destroying" data by converting numbers to ranks or signs. This reduces the amount of information in the data, and leads to less powerful tests. Because of the way they simplify data, many non-parametric tests have less statistical power than their bootstrapping and randomisation analogues (Pitt and Kreutzweiser, 1998), and are unable to facilitate parameter estimation.

At the same time, it is important to recognise that bootstrapping and randomisation techniques are not without their drawbacks. As discussed earlier, the bootstrap method does have the complication of bias (Efron, 1981), and some randomisation tests must satisfy the assumption that variances are equal (Boik, 1987). Clearly each form of analysis has its own advantages and disadvantages, and deciding which approach to use requires carefully weighing the advantages and disadvantages in relation to the questions being asked, the computer tools available and the features of the data being analysed.

While this chapter has provided a useful opportunity to explore a number of issues concerning the analysis of non-normally distributed data, the most important point here is that all conclusions made in Chapter 2 still hold. That is, the mean allele frequencies of outbred immigrant males (and not inbred immigrant males) were found to increase significantly within the first generation upon arriving into inbred populations. This, in turn, reaffirms the more general statement that inbreeding can effect the contribution made by immigrants to the genetic diversity of populations.

This concludes the experiment-based section of my thesis. So far I have considered the effects of immigration on population genetics, and have thereby focussed on one of many aspects of metapopulation biology. At this point the direction of my thesis changes considerably, with the emphasis shifting towards entire metapopulations, taking into account a large number of biological processes. It is important to appreciate that there is no direct connection between these two halves of the thesis - that is, I do not include the results of the earlier chapters into the later, modelling chapters. Nonetheless the next chapter does provide something of a link between the two sections of the thesis, in the sense that I discuss the

relationship between different metapopulation processes. In particular, I consider the limitations of using gene flow data to estimate colonisation probabilities.

CHAPTER 4

The limitations of using genetics to make insights into colonisation probabilities in metapopulations: a discussion

4.1 Introduction

Just as it is useful to study population dynamics in terms of birth and death rates, it is also useful to study metapopulation dynamics in terms of colonisation and extinction probabilities. By knowing the colonisation probabilities of the empty habitat patches in a metapopulation, we can potentially make valuable insights into how well the system will recover from local extinctions. Unfortunately colonisation probabilities are not easy to measure. In most systems of management concern there is little time available to wait and observe colonisation directly. Even if we are lucky enough to observe one or more colonisations, it is unlikely that a handful of stochastic events will allow us to make reliable estimates of colonisation probabilities, let alone predict how those probabilities vary as patch occupancy in the metapopulation changes. One option is to measure the process that drives colonisation - dispersal. However, detecting dispersal is itself quite a challenge, often requiring considerable effort in radio-tracking or mark-release-recapture techniques to try to detect what may be very rare events. The amount of time and money required to make reliable estimates of dispersal rates from such data is probably prohibitive in most cases. An indirect alternative to estimating dispersal in metapopulations is to use genetic diversity data.

A number of studies have used insights into gene flow to make statements concerning the likelihood of colonisation within metapopulations. For example, in a study of the frog *Geocrinia alba*, Driscoll *et al.* (1995) used Wright's *Nm* (1931) to state that "with less than one individual per generation ... recolonization of vacant habitat patches is unlikely to occur". Other studies that made similar statements include Sarre (1995), Hitchings and Beebee (1997) and Hoole *et al.* (1999). Despite such assessments, no one to my knowledge has ever tried to use genetic diversity data to make quantitative estimates of colonisation

probabilities. However, such a quantitative approach is necessary if statements about colonisation are to make a valuable contribution to understanding the dynamics of particular metapopulations.

In this chapter I explore the reliability of using genetic diversity data to estimate both dispersal rates and colonisation probabilities. I first define gene flow, dispersal and colonisation, and describe the relationship between these three processes. I then consider the limitations of using genetic diversity data to estimate dispersal rate, focussing firstly on Wright's (1931) infinite island model, and then on the relatively new "assignment method". I then discuss the limitations of using estimates of dispersal rate to predict colonisation probabilities, and argue that ultimately there are unavoidable limitations in using genetic diversity data to predict colonisation probabilities. Nonetheless, it is important to recognise that genetic diversity data can provide valuable insights into the dynamics of metapopulations, and I describe some examples of this. Finally, as a prelude to Chapter 5, I suggest a qualitative "rule of thumb" approach for using genetics to make management decisions about the dynamics of metapopulations.

4.2 Gene flow, dispersal and colonisation

Initially it is important to distinguish between gene flow as the movement of genes, dispersal as the movement of individuals between habitat patches, and colonisation as the initiation of a new population in a previously empty habitat patch. In many organisms (*e.g.* vertebrates), gene flow can only occur when individuals disperse. In other organisms however, gene flow may occur through the movement of gametes. For example, much gene flow in plant populations involves pollen transfer (Chase *et al.*, 1996; Nason *et al.*, 1998) while gene flow in many marine organisms may occur via the movement of gametes through the water column (Yund, 1995). Hence, in some organisms, evidence of gene flow does not necessarily indicate that individuals have dispersed.

Here it is important to define what is meant by the term "individual". Clearly this is open to interpretation, since there is essentially no reason why a pollen grain should be considered any less of an individual than a mature tree. Both are essential parts of a life cycle. In this thesis however, I am defining an individual as any part of an organism's life cycle that can

directly give rise to a life history stage that could be sampled by a metapopulation biologist. Accordingly, I would consider a plant seed be an individual, as it can directly give rise to a seedling or mature plant, either of which could be sampled by a metapopulation biologist. In contrast a pollen grain is not considered here to be an individual, since it cannot give rise to a metapopulation sampling unit (seedling or mature plant) without first fertilising an ovule. Furthermore, a pollen grain is unlikely to ever be a metapopulation sampling unit itself, since biologists tend not to describe the metapopulation dynamics of pollen! Following this definition, I use the term "dispersal" to refer exclusively to the movement of individuals between habitat patches.

While gene flow can occur without the dispersal of individuals, the reverse is also true. That is, individuals can disperse without having an impact on gene flow, simply because they fail to reproduce into their new patch.

Dispersal into empty habitat patches can, but doesn't necessarily result in colonisation. For example, a female brown bear may disperse into an unoccupied habitat patch but fail to reproduce, through lack of males. Here it is important to define the term "colonisation". So far I have defined colonisation as the initiation of a new population in a previously empty habitat patch, but it is important to recognise that the term "initiation" is itself open to interpretation. Clearly it would be inappropriate to say that a population has been initiated if a patch contains only a single male. Therefore, I am defining a patch to be colonised only if it contains a potentially reproducing set of individuals, a definition that clearly depends on the breeding system of the organism in question. For selfing plants and parthenogenic animals, this would simply require the presence of a single individual, while for other plants and animals colonisation would require the presence of at least one individual of each strain or sex, or a mated female. Admittedly other definitions of population initiation are possible. For example, we could say that a population has only been initiated when at least one full life cycle has been completed in the patch, or when the population has passed through its initially high extinction probability (Ebenhard, 1991).

To summarise the relationship between these different processes, we can say that gene flow can occur without individuals dispersing (in some organisms), individuals can disperse without having an impact on gene flow, and individuals can disperse into empty habitat patches without necessarily resulting in colonisation (Figure 4.1).

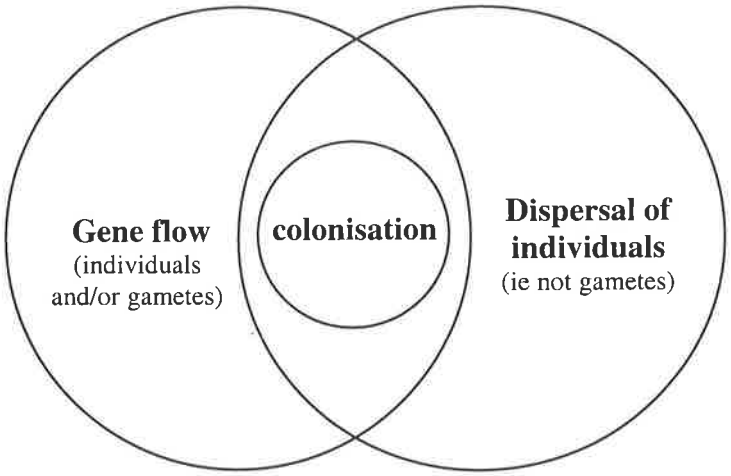

Figure 4.1 Venn diagram representing the relationships between gene flow, dispersal and colonisation. Not all gene flow is due to the dispersal of individuals, not all dispersal events result in gene flow, and not all dispersal results in the colonisation of previously empty habitat patches. For some organisms (*e.g.* vertebrates), gene flow only occurs when individuals disperse. Therefore, the left-hand side of this figure (gene flow without dispersal) does not apply to all organisms.

4.3 Using genetic diversity data to estimate dispersal rates

4.3.1 Wright's *Nm*

A number of studies have used estimates of gene flow to infer the rate at which individuals disperse between the patches in metapopulations (Driscoll *et al.*, 1995; Hitchings and Beebee, 1997; Vrijenhoek, 1997; Seppa and Laurila, 1999). The motivation for using this approach may be partly related to the popularity of Wright's "*Nm*" (1931), which provides a convenient way of conceptualising gene flow as equivalent to the number of individuals moving from one subpopulation to another per generation. Wright's model states that *Nm* can be estimated from the degree of genetic subdivision, F_{ST}, using the equation $Nm = (F_{ST}^{-1} - 1) / 4$. Importantly though, a particular value of *Nm* does not necessarily mean that individuals are actually immigrating at that same rate.

The distinction between gene flow and dispersal underlies two very different ways in which the results of Wright's model can be interpreted. One major use of Wright's model is for gene conservation - the study and conservation of genetic diversity for its own sake (following the definition used by Moritz (1994)). Among the many examples of such studies are Van Dongen *et al.* (1998), Broadhurst *et al.* (1999) and Durka (1999). In this context the process of interest is gene flow, and the dispersal rate responsible for a particular level of gene flow is essentially irrelevant. The other potential use of Wright's model is for molecular ecology - to provide insights into the demographics of populations (following Moritz (1994)). I make this distinction so it is clear that the criticisms I have are not of using genetic diversity data to describe gene flow and genetic structure *per se*, but of using genetic diversity data to estimate dispersal rates and the likelihood of colonisation.

The results in Chapter 2 (as supported by Chapter 3) represent one reason why it is potentially misleading to use measures of genetic subdivision to estimate dispersal rates in metapopulations. Those chapters demonstrated that inbreeding can influence the impact that an immigrant has on population genetic diversity. This means that if a metapopulation contains inbred subpopulations, Wright's island model (1931) may lead to poor estimates of the true rate at which immigrants arrive into those subpopulations (in this case leading to an overestimate of dispersal rate).

The effect of inbreeding is only one of a number of potential problems of using Wright's (1931) model to estimate dispersal. Other problems with the model's assumptions are discussed in detail by Mills and Allendorf (1996), and by Whitlock & McCauley (1999), who state that their criticisms apply not only to Wright's model but also to similar models which use measures of genetic subdivision to estimate dispersal rate. Below is a summary of the frequently violated assumptions involved in using Wright's model to relate genetic subdivision to dispersal rate (taken from Mills and Allendorf (1996)):

(1) *Island model of migration*: There is no spatial pattern to migration - a migrant is equally likely to have originated from any subpopulation. This implies that the system is made up of a large number of similar-sized subpopulations.

(2) *Selective neutrality and no mutation*: There are no selective differences among genotypes, and no new mutations entering the population. That is, gene-frequency dynamics are determined entirely by the interaction between genetic drift and gene flow.

(3) *Ideal populations*: The subpopulations have the characteristics of an ideal genetic population, so that the census number of individuals equals the effective population size. For general purposes, the ideal population consists of a constant number of N diploid individuals (N/2 females and N/2 males) in which all parents have an equal probability of contributing offspring to the next generation.

(4) *Demographic equality*: Immigrants have the same demographic attributes (in terms of survival and reproduction probabilities) as resident individuals.

(5) *Equilibrium*: Subpopulations persist long enough to reach steady-state or equilibrium gene frequencies.

In the "real world" metapopulations that biologists try to understand and manage, many of these assumption are likely to be violated. Perhaps the only assumption we can easily accept is that of *selective neutrality and no mutation.* Neutrality is not a major issue given the availability of selectively neutral markers such as microsatellites (Queller *et al.*, 1993). And although mutation occurs, in many cases it is probably negligible relative to the effects of gene flow and drift (Hartl and Clarke, 1989).

In contrast, the other assumptions of Wright's (1931) model should be of considerable concern when applied to real metapopulations. The assumption of an *island model of migration* is clearly incompatible with the present views of metapopulations. Although the early work on metapopulations described them as large collections of similar-sized habitat patches (Levins, 1970), it is now believed that such systems are quite rare in nature (Harrison, 1991). Instead, there is now a greater appreciation that the subpopulations of many systems vary considerably in their relative importance as sources of immigrants to other patches (Harrison, 1991).

Cases where the assumption of *ideal populations* is upheld are probably best thought of as the exceptions rather than the rule, with most natural populations showing a large discrepancy between the effective size of a population (N_e) and its census size (N_c) (Frankham, 1995b). Furthermore, the ratio of N_e to N_c varies considerably among species (Frankham, 1995b).

There are several reasons why the assumption of *demographic equality* of immigrants and residents may be violated in real systems. As described earlier in relation to Chapters 2 and 3, immigrants may differ from residents in fitness due to inbreeding depression. Non-genetic

effects may also be important. For example, immigrants may have reduced fitness if the act of dispersal is itself costly. Importantly, these processes operate in different directions, the former increasing the genetic contribution of immigrants over population residents, and the latter decreasing their impact. As such, it may be unclear whether a particular value of *Nm* is an overestimate or underestimate of the true rate at which immigrants arrive into a population.

Finally, the assumption of *equilibrium* requires that the system in question has reached a stable state with respect to gene frequencies. While this may apply to naturally occurring metapopulations that have been existence for a long time, this may be an invalid assumption for the many metapopulations that have arisen in recent history due to human-induced habitat fragmentation.

In summary, many aspects of the biology of metapopulations throw doubt on the assumptions of Wright's (1931) model. Following Whitlock and McCauley (1999), I have focussed on Wright's model because of its popularity. Nonetheless, it is important to recognise that many of the criticisms above also apply to other methods that, like Wright's model, also estimate gene flow from measures of genetic differentiation (Whitlock and McCauley, 1999).

4.3.2 The assignment method

An alternative to using gene flow to estimate dispersal involves the relatively new "assignment method", which uses genetic diversity data (typically microsatellites) to determine which subpopulation is the most likely birthplace of the different individuals in a metapopulation (Waser and Strobeck, 1998). Essentially this is the same type of analysis that a forensic scientist would use to estimate the probability that a particular animal trophy originated from a protected population rather than a non-protected population (Waser and Strobeck, 1998). This method allows biologists to estimate both the number and sex of immigrants entering each subpopulation, as well as identifying the source subpopulation of each immigrant. In this way, the assignment method may provide useful insights into the biology and spatial patterns of dispersal within metapopulations. Although studies based on ecological methods such as radio-tracking and mark-recapture may attempt to collect similar data, those methods may influence the dispersal behaviour of their subjects. In contrast

genetics-based approaches such as the assignment test have the advantage of being potentially non-invasive, by using samples of hair, feather or faeces (Tablerlet *et al.*, 1999).

While the level of detail provided by the assignment method is on the one hand quite appealing, such detail is also a challenge to interpret. One problem is that there may be high levels of error involved when using individual records of dispersal to estimate dispersal rates - the same problem that would be encountered with dispersal data from ecological studies. For instance: How would we interpret evidence of only one individual moving between two subpopulations? While estimation error could be reduced through repeated measures, this may be impractical for the many metapopulations where management decisions are required over a short time frame.

4.4 The relationship between dispersal rate and colonisation probability

While the reliability of using genetic diversity data to estimate dispersal rates is clearly one issue of concern, it is also important to question how useful it is to know dispersal rate for the purposes of estimating colonisation probability. To address this issue, it is useful to consider a hypothetical metapopulation where our estimates of dispersal rate are entirely reliable.

Given that dispersal is the process driving colonisation, it follows that there should be a positive relationship between dispersal rate and colonisation probability. This does not however, mean that if we know the dispersal rate into a habitat patch that we can make a reliable estimate of its colonisation probability if it were empty. To do so would obviously require that we have a good understanding of the relationship between these processes. Factors likely to affect this relationship are:

- *The demographic composition of dispersers*: What proportion of dispersers are males, unmated females and mated females? If dispersal into a patch is strongly male-biased, we would expect colonisation probability to be relatively low compared to a situation with the same overall dispersal rate where the majority of dispersers are mated females.
- *The temporal distribution of dispersal events*: How is the arrival of individual dispersers distributed over time? For example, if birds disperse between habitat islands in flocks (Diamond, 1975), this may have a large effect on colonisation probabilities. A patch receiving a single group of five individuals once in fifty years may have a higher

colonisation probability than a patch receiving the same overall rate of dispersers (five in fifty years), but spread out as one individual every ten years.

It is easy to imagine that the above factors vary considerably among species. Accordingly, if estimates of dispersal rates are to be used to estimate colonisation probabilities, the challenge then lies with biologists in being able to describe both the demographic composition of dispersers and temporal distribution of dispersal events for each system being studied. Estimates of gene flow can, to some extent, be used to describe the demographic composition of dispersers. Because organellar DNA is maternally inherited, and nuclear DNA is biparentally inherited (Birky *et al.*, 1989), a comparison of organellar and nuclear gene flow can be used to estimate the sex-ratio of gene flow (Birky *et al.*, 1989; FitzsSimmons *et al.*, 1997). However, this approach only tells us about the sex-ratio of gene flow, and not the sex-ratio of dispersal; these may differ if male and female immigrants vary in their demographic relationship to population residents, as in point (4) of section 4.3.1. Estimates of gene flow do not tell us the proportion of dispersing females that are mated before they arrive at a patch; nor do they provide insights into the temporal distribution of dispersal events, since they only describe an average rate of gene flow over time (Whitlock and McCauley, 1999). In summary, gene flow data is of limited value for describing the link between dispersal rates and colonisation probabilities.

In contrast, the assignment method does potentially provide the basis for a comprehensive description of the processes linking dispersal rates to colonisation probabilities. By allowing biologists to identify particular individuals as dispersers, the assignment method provides the opportunity to determine the relative proportion of dispersers that are males, unmated females and mated females. For example, Mossman and Waser (1999) used the assignment method to detect sex-biased dispersal in the white-footed mouse. Importantly though, the accuracy of this approach will be limited to systems with relatively high rates of dispersal. By facilitating a real-time description of dispersal, the assignment method could also be used to estimate the temporal distribution of dispersal events. Once again however, the same feature that makes the assignment method appealing (i.e. its detailed output data) also presents a difficulty in terms of estimation error. Although this could be overcome through replication over time, the amount of time and money required may be prohibitive in many cases.

4.5 Conclusion

The relationships between genetic diversity, dispersal rate and colonisation probability are influenced by many biological processes, the nature of which may vary considerably among species. Unless biologists are able to describe those processes accurately for each system in question, genetic diversity data is unlikely to provide a reliable basis for estimating colonisation probabilities.

Nonetheless, the analysis of genetic diversity data does have an important role to play in metapopulation biology. For biologists interested in preserving genetic diversity *per se*, describing genetic diversity patterns is essential, and analyses such as those based on Wright's island model provide a valuable way of summarising genetic structure.

There are also ways in which genetic diversity data can assist biologists studying metapopulation dynamics, albeit not for making statements about colonisation probabilities. Firstly, genetic diversity can be used to help identify the spatial scale at which populations interact. Isolation by distance models allow biologists to describe how genetic "distance" varies as a function of geographic distance between subpopulations (Slatkin, 1993; Allegrucci *et al.*, 1997; Becher and Griffiths, 1998), thereby essentially providing an insight into the spatial scale at which gene flow occurs. This, in turn, may provide a good approximation of the spatial scale at which dispersal occurs. Although gene flow may be an unreliable indication of dispersal rate, it may provide valuable insights into the spatial scale of dispersal. Genetic diversity patterns may also be useful in indicating directionality of gene flow, and hence dispersal (Gornall *et al.*, 1998); thereby giving biologists valuable insights into the potential for source-sink metapopulation structure (Pulliam, 1988; Harrison, 1991).

In this chapter I have focussed on the utility of genetics for making the *quantitative* insight of trying to estimate colonisation probabilities. An alternate approach is to use genetics to make *qualitative* insights into metapopulation dynamics. For example, the assignment method can tell us if there is *any* evidence of dispersal, and hence any potential for colonisation. Similarly, for organisms where gene flow only occurs through the dispersal of individuals (*e.g.* vertebrates), estimates of gene flow may be taken as evidence that at least some dispersal has occurred, even if the rate of dispersal is open to debate. In this regard, perhaps

we should treat evidence of high rates of gene flow as strong evidence that dispersal has occurred, rather than as an indication of a high dispersal rate.

Genetic diversity data may also be valuable in providing evidence of population turnover (extinction and colonisation). Importantly, population turnover is known to influence genetic structure (Wade and McCauley, 1988; Whitlock and McCauley, 1990; Milligan *et al.*, 1994), and it has been suggested that patterns of genetic diversity may therefore reflect population turnover in some resolvable way (Milligan *et al.*, 1994). Unfortunately it is difficult to disentangle the confounding effects of migration and population turnover, and as such, methods for detecting turnover from genetic diversity data are currently not available (Milligan *et al.*, 1994).

Finally, genetic diversity data may be used to make qualitative "rules of thumb" insights into the dynamics of metapopulations. In the next two chapters I explore the utility of such an approach. In particular I examine whether the genetic diversity of each subpopulation can provide a good indication of the relative value of habitat patches to metapopulation persistence.

CHAPTER 5

Using genetics to rank the value of patches: introduction and model description

5.1 Introduction

Metapopulations were initially described as collections of equivalent habitat patches contributing equally to overall metapopulation persistence (Levins, 1970). Over recent years however, there has been a growing appreciation that many metapopulations possess large variation in the relative value of individual patches (Gilpin, 1987; Hanski, 1994a; Day and Possingham, 1995). An extreme form of this variation occurs in mainland-island metapopulations where persistence may depend almost entirely on the presence of one large patch (Harrison, 1991). Appreciating that variation in patch value exists is one thing; actually describing that variation in a way that can be used to manage metapopulations is another challenge altogether. In this chapter I introduce a method for estimating the relative importance of the different patches in a metapopulation using genetic diversity information.

Initially it is important to define patch value. I am considering patch value based purely on demographic concerns (i.e. maintaining metapopulation persistence), rather than genetic concerns (i.e. preserving the genetic diversity of a metapopulation). The definition I use is that the demographic value of a patch is a measure of its contribution to a metapopulation's likelihood of persistence (Hanksi, 1994a; Hanski, 1994b; Day and Possingham, 1995; Lindenmayer and Possingham, 1996). Thus, the patch whose removal would cause the greatest increase in metapopulation extinction probability is ranked as the most important patch.

There are several reasons why understanding variation in patch value is potentially useful for managers of metapopulations. In metapopulations where the impact of threatening processes can be reduced by human intervention, a ranking of patches could help managers focus their

limited resources on the patches of greatest value. This might involve reducing the impacts of predation, weeds, pathogens, or disturbances such as fire. In the extreme case of patches being destroyed (*e.g.*, due to vegetation clearance), it would clearly be important to avoid losing one of the more valuable patches.

The value of a patch to metapopulation persistence is likely to depend on a combination of four patch attributes - the tendency to (1) remain occupied, (2) become recolonised in the event of extinction, (3) help keep other extant subpopulations occupied (by facilitating a "rescue effect"), and (4) contribute to the recolonisation of other habitat patches if they become unoccupied. In turn, these attributes are likely to be influenced by patch size and location (i.e. size and location are the ultimate causes of patch value, while the four attributes are proximate causes). A large patch should have a relatively high chance of remaining occupied (attribute 1) by supporting a large subpopulation that is resistant to the extinction risks imposed by demographic and environmental stochasticity. A large patch may be more likely to intercept dispersing individuals by virtue of its longer perimeter (thereby imparting attributes 1 and 2), and a large patch is also likely to act as a large source of immigrants capable of facilitating any rescue effect and recolonisation in other patches (attributes 3 and 4). In terms of location, a centrally located patch is likely to be relatively well connected to other patches by dispersal, thereby facilitating any rescue effect and recolonisation of both the patch in question and the surrounding patches (attributes 1, 2, 3, and 4).

Admittedly the patch area/location framework for understanding metapopulations makes a number of simplifying assumptions. Using area to predict the tendency of a subpopulation to resist extinction and act a source of dispersers assumes that all patches have the same quality per unit area, and experience the same disturbance regimes. Using location to represent connectivity assumes that landscape features such as dispersal corridors and dispersal barriers are of little importance to the interaction between patches. Despite these assumptions, the patch area/location approach serves as a useful framework for visualising the processes responsible for variation in patch value, and has been widely used as a best-guess approach for modelling metapopulations (Hanksi and Thomas, 1994; Hanski, 1994a; Day and Possingham, 1995; Possingham and Davies, 1995). I use the patch area/location paradigm throughout this thesis, for both its simplicity and appeal as a visual aid in understanding variation in patch value.

Ranking patches according to their value to metapopulation persistence may initially seem a trivial exercise. That is, we expect large patches to be more valuable than small ones, and centrally located patches to be more valuable than isolated ones. Combining these two criteria, we would clearly expect large, centrally located patches to be more valuable than small, isolated ones. A challenge arises however, when we want to compare a small, centrally located patch with a large, isolated one. In this case relative patch value is not immediately obvious, as it depends on a tradeoff between patch size and location. Thus, a key point to draw from the patch area/location approach is that connectivity can compensate for patch size. Although a patch may be small, if it is close enough to another patch it may nonetheless be valuable to the metapopulation due to its tendency to interact with that neighbouring patch (Figure 5.1).

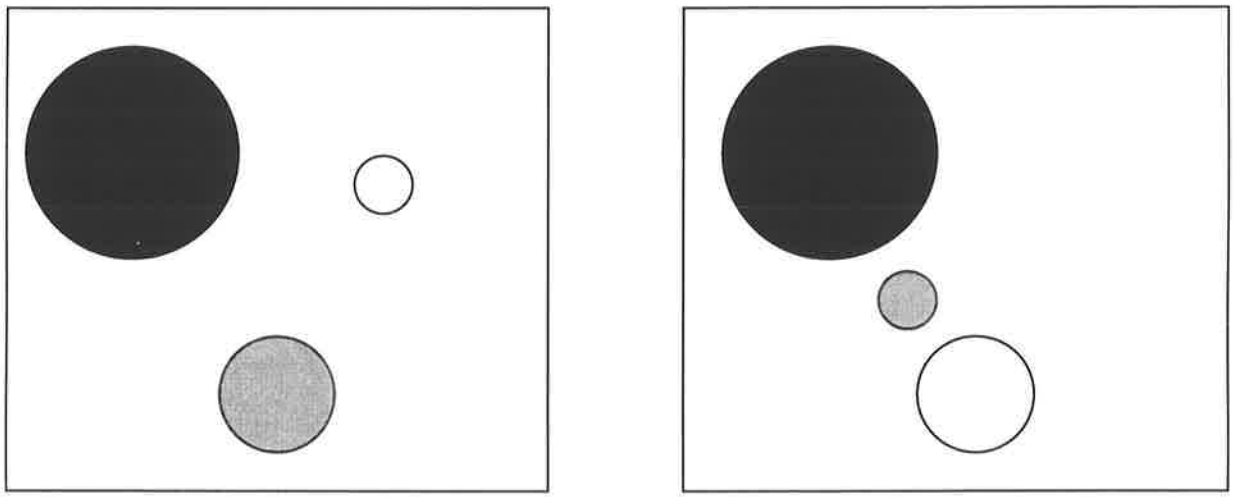

Figure 5.1 An example of how connectivity may cause changes in patch value. In these two metapopulations, relative patch value is represented by different shades: black = most valuable patch; grey = patch of intermediate value; white = least valuable patch. This figure suggests how even a small patch may be valuable if it is well placed in relation to other patches (as in the metapopulation on the right).

Ecologists have already developed a number of tools for assessing the value of patches in metapopulations. The "incidence function" approach (Hanksi, 1994a; Hanski, 1994b) uses a snapshot of a metapopulation's spatial pattern of patch occupancy to assign parameter values

to functions that describe patch extinction and colonisation probabilities on the basis of patch area and isolation. Once these functions have been parameterised, it is then possible to use numerical iterations of metapopulation dynamics to determine the relative value of the different patches in a system (Hanski, 1994a; Hanski, 1994b). Importantly however, the incidence function approach requires that a metapopulation contains a relatively large number of patches, with Hanski (1994a) suggesting that 30-50 patches are sufficient. This raises the question: How are we to determine the relative value of patches in metapopulations that contain relatively few patches? There are certainly many examples of metapopulations of conservation concern that contain too few subpopulations to use the incidence function approach. Examples include the fern *Asplenium septentrionale* with three subpopulations (Holderegger and Schneller, 1994); the Mauritius fody (a bird) with five subpopulations (Safford, 1997); Shenandoah salamanders with six subpopulations (Griffis and Jaeger, 1998), and Nepalese tigers with four subpopulations (Smith *et al.*, 1998).

While small metapopulations are not suitable for the use of incidence functions, they are amenable, by virtue of their size, to computer simulation. Modern computing power has facilitated the widespread use of stochastic models of population viability analysis (PVA) for studying the dynamics of small metapopulations, with programs such as ALEX, VORTEX and RAMAS/space (Lindenmayer *et al.*, 1995). Another potential approach is to use deterministic numerical modelling (Day and Possingham, 1995).

Although PVA allows biologists to ask a range of valuable questions, it is unlikely to provide reliable estimates of the relative value of the patches in a system. Ultimately the predictive accuracy of PVA packages is limited by the ability of ecologists to either directly estimate colonisation and extinction probabilities, or estimate the biological processes that determine those probabilities. It is difficult to imagine many small metapopulations (i.e those containing few patches) where ecologists will ever be able to make good direct estimates of extinction and colonisation probabilities. Unless there happens to be enough long term data on population turnover events (without the system going extinct in the meantime) any attempt to directly estimate extinction and colonisation probabilities can at best be an educated guess. Perhaps for this reason, many PVAs (including the widely-used VORTEX and ALEX packages (Lindenmayer *et al.*, 1995)) attempt a bottom-up approach to predicting metapopulation dynamics, whereby the emphasis is not on estimating extinction and colonisation probabilities *per se*, but on parameterising the many biological processes that

determine those probabilities. Accordingly, the reliability of many PVAs rests on how well ecologists can estimate the processes underlying metapopulation dynamics. This includes trying to estimate birth rates and death rates, spatial and temporal variability in those rates, the impact of genetic diversity on demographic processes, and the rates and spatial patterns of dispersal between patches. While data is often readily available for some of these parameters, many parameters are extremely difficult to measure accurately. Descriptions of inter-patch dispersal are particularly difficult in this regard, requiring large amounts of time and money to try to detect what in many cases may be very rare events. Even if a researcher is lucky enough to record a handful of stochastic dispersal events, it is another challenge to be able to use that data for making reliable estimates of the rates and patterns of inter-patch dispersal.

Given the challenges that ecologists face when trying to assess the relative value of patches in small metapopulations, I decided to explore a new approach that would avoid the theoretical and empirical problems of trying to directly estimate extinction and colonisation probabilities, or the processes that underlie those probabilities. In particular, I examined whether genetic diversity information could provide a reliable basis for ranking the patches in a metapopulation. The logic behind this approach is that the same features that make a patch valuable for metapopulation persistence also tend to increase the genetic diversity of the subpopulation occupying that patch. Thus, where a large patch is valuable due to its resistance to extinction and its role as a source of immigrants to other patches, it will also have higher levels of genetic diversity than a small population, by having lower levels of drift. Patch location and connectivity are also important. Where a well-connected patch is valuable for its ability to receive and send immigrants, it should also have higher genetic diversity due to the influx of genes from the surrounding subpopulations. Therefore, both genetic diversity and patch value are expected to vary in similar ways as functions of both patch area and connectivity (Figure 5.2). Accordingly we can imagine that Figure 5.1 applies to genetic diversity in the same way that it applies to patch value. It is this logic that lead me to the idea that ranking the patches in a metapopulation according to their genetic diversity may provide a good estimate of their relative contribution to metapopulation persistence. Dunham *et al.* (1999) presented a similar argument to this, suggesting that genetic diversity may reflect the relative extinction risk of the different subpopulations in a metapopulation. My approach differs from that of Dunham *et al.* (1999) by considering how genetic diversity reflects relative patch value, rather than the relative risk of subpopulation extinction.

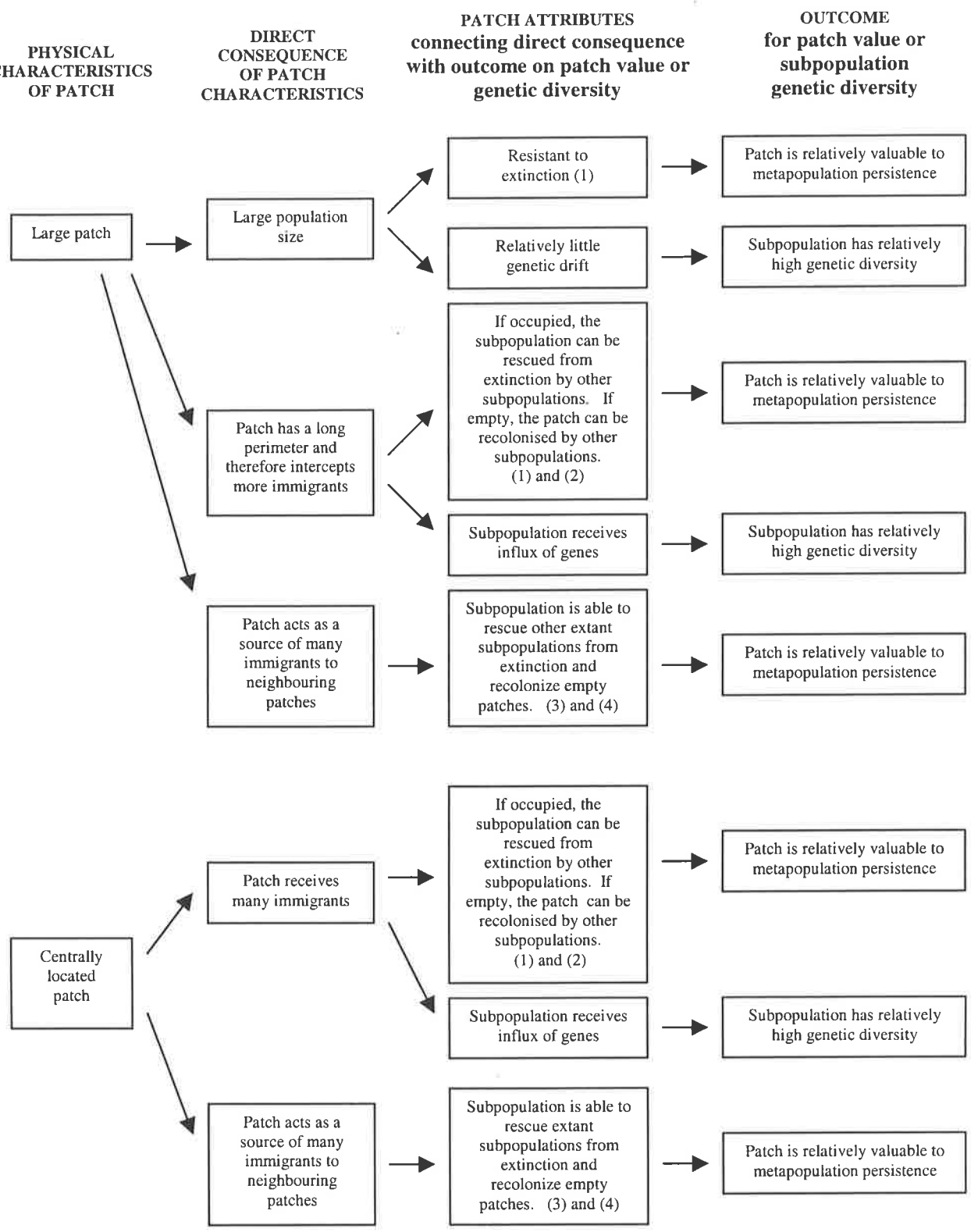

Figure 5.2 Illustration of how genetic diversity and patch value are influenced in similar ways by patch area and isolation. Note that population size, patch perimeter and the reception of immigrants influence both genetic diversity and patch value. In contrast, emigration out of a patch affects only its value. The numbers in brackets are the four patch attributes described earlier in this chapter (see second page).

While there is good reason to believe that genetic diversity data provide a basis for estimating the relative values of patches in a metapopulation, there are also reasons why the relationship between genetic diversity and patch value may be unreliable.

1) The processes driving both genetic diversity (drift, mutation and immigration) and colonisation and extinction are inherently stochastic. Therefore any relationship between patch value and genetic diversity is subject to a certain amount of noise.
2) Patch size and isolation will affect patch value and genetic diversity in different ways (Figure 5.2). Although these relationships may have the same general trends, subtle differences between them might mean that the combined effect of patch size and isolation are quite different for ranking patch value than for ranking genetic diversity. In particular, note that while the value of a patch may be increased by its tendency to act as a source of immigrants to other patches (Figure 5.2), this will not increase the genetic diversity of the patch.

The aim of the present study was to explore the utility of using genetic diversity data to estimate the relative value of the patches in metapopulations, and to explore the conditions under which the approach is most reliable. I addressed these aims using a Monte Carlo computer simulation model. This was necessary because of the highly stochastic nature of metapopulations, and the subsequent need to run thousands of replicate metapopulations to obtain accurate parameter estimates for the questions being asked.

5.2 Model description

In this section I describe the computer simulation model used to examine the utility of using genetic diversity to estimate the demographic value of the different patches in metapopulations. Essentially the model (referred to as "MultiPop") is an individual-based stochastic simulation model of metapopulation dynamics and genetics. It is individual-based in the sense that it follows the fate of individual organisms – each with an age, a sex and a genome. It is stochastic in the sense that all demographic processes (reproduction, dispersal and mortality) are modelled stochastically using by a pseudo-random number generator. The model also allows environmental stochasticity to be included, whereby subpopulations may independently experience random years of unusually high mortality. Both forms of stochasticity are capable of causing subpopulation extinctions. It is a metapopulation model

since it involves modelling a set of subpopulations, with dispersal between habitat patches. Dispersing individuals are capable of recolonising empty habitat patches and rescuing extant subpopulations from extinction. And finally, the model can be used to examine both population dynamics (in terms of the time to metapopulation extinction), and genetics (using several measures of subpopulation genetic diversity).

The structure of MultiPop is not unlike that of VORTEX - a model widely used for population viability analysis (Lacy, 1993; Lindenmayer *et al.*, 1995). The motivation for writing MultiPop rather than using an existing model such as VORTEX was that I could have full control over the various scenarios that were run, and the type of output produced. Writing MultiPop also meant that I was able to avoid using computer run time to model processes that I was not interested in modelling. MultiPop was written in Turbo Pascal 7.0, and the code is presented as a Pascal file on the enclosed floppy disc (A:\APP3\MULTIPOP.PAS). All stochastic processes modelled in MultiPop use the Turbo Pascal 7.0 built-in pseudo-random number generator (see Appendix 1 for an examination of the quality of this number generator).

The initial aim of using this model was to create a system where the ranking of patches (based on their demographic value to a metapopulation) could be manipulated by changing the connectivity between patches. With low connectivity, the value of patches in a metapopulation should be closely associated with patch size, while with increased connectivity, isolation becomes an important component of patch value. I then aimed to test the utility of using genetic diversity data to predict patch value over a wide range of connectivity scenarios, in order to assess how robust the approach might be.

MultiPop was used to model two types of metapopulations: three-patch systems and eight-patch systems. Carrying capacities are assigned to the different patches in a system, and individuals only reproduce within the habitat patches. Nonetheless, juveniles are able to leave patches and disperse to other patches, and this dispersal can lead to the recolonisation of empty habitat patches, and can facilitate the rescue of extant subpopulations from extinction.

Each individual in the model has the following attributes:

- **Habitat patch**: A single integer value that represents the habitat patch in which the individual lives.
- **Sex**: Male or female.
- **Age**: This is an integer value representing the individual's age in years from 0 up to a set maximum age.
- **Genotype**: There are both nuclear and mitochondrial components to each individual's genome. The nuclear component is comprised of 10 loci, each with 10 possible alleles. This number of loci and number of alleles per locus were chosen to represent the amount of variation commonly found using microsatellites (Burland *et al.*, 1998; Dallas and Piertney, 1998; Hughes *et al.*, 1998; Kumari and Kemp, 1998; Piertney *et al.*, 1998). Each individual in the model carries two copies (effectively homologous chromosomes) for each locus. All loci assort with complete independence as if they occur on separate chromosomes. The mitochondrial genome is comprised of a single integer value representing one of 100 possible haplotypes. An individual's haplotype is essentially its mitochondrial DNA fingerprint. Because mitochondrial DNA is maternally inherited without recombination, the only way an individual's haplotype can differ from its mother's haplotype is by mutation. I chose to initialise the model with 100 possible haplotypes - a conservatively high representation of the number of haplotypes found in natural populations (Edwards, 1993; Sarre, 1995; Moritz *et al.*, 1997; Lovejoy and De Araujo, 2000; Trewick, 2000). For both the nuclear and mitochondrial genotypes I assumed that there is no mutation during the running of the model.
- **Mating partner**: For monogamous organisms, each individual is described as either paired or single, and any paired individual carries the identity of its mating partner.

The amount of computer memory required for each individual imposed considerable limitations on the maximum number of individuals allowable within the model. For a system with three habitat patches, the maximum allowable subpopulation size was 735, while for an eight-patch system the maximum was 273 individuals. To remain safely under these population ceilings, it was necessary to restrict use of the model to organisms with relatively low fecundity, such as mammals and birds, rather than more highly fecund organisms such as plants and insects.

Within the constraints of low population ceilings and low fecundity, I decided to model two organisms with very different life history properties, as a form of sensitivity analysis at the species level. Using this approach, a large difference in the output of the two organisms would be interpreted as the model's sensitivity to life history properties.

The two organisms I modelled were based on the life history properties of owls (to represent a slow breeding monogamous organism) and rodents (to represent a fast breeding polygamous organism), (Table 5.1). Importantly these model organisms are not intended to represent particular species, and should be thought of as a generic owl and a generic rodent.

Table 5.1 Summary of the life history properties of owls and rodents as modelled in this study.

Life history property	Owl	Rodent
Maximum life span	20 years	2 years
First year survivorship	50%	50%
Yearly survivorship (after first year)	95%	50%
Age at sexual maturity	3 years	1 year
Mating system	Monogamous	Polygamous
Offspring/female/year: mean (max)	0.6 (4)	6 (8)
Sex ratio at birth	0.5	0.5

These properties were based loosely on examples in the literature. For owls, see Lundberg and Westman (1984), Wilson *et al.* (1986), Lande (1988), Bull *et al.* (1989), Pientiainen (1989) and Gerhardt *et al.* (1994); and for rodents see French *et al.* (1967), Watts and Aslin (1981), Millar and Zammato (1983), Kenagy and Bartholomew (1985) and Strahan (1998). While the values used for some of these parameters are clearly open to debate, the importance lies not in the values themselves, but in how much they vary between the owl and rodent.

5.2.1 The model structure (flow of demographic processes)

The model starts by filling the metapopulation with individuals. The metapopulation then enters a yearly cycle of non-overlapping demographic events: aging, pairing, breeding, dispersal and mortality (Figure 5.3). The model continues through this cycle until either (1) the entire metapopulation becomes extinct, or (2) the metapopulation has been in existence for as many years as the user has specified. In the sections below I describe each of the model processes in the annual cycle.

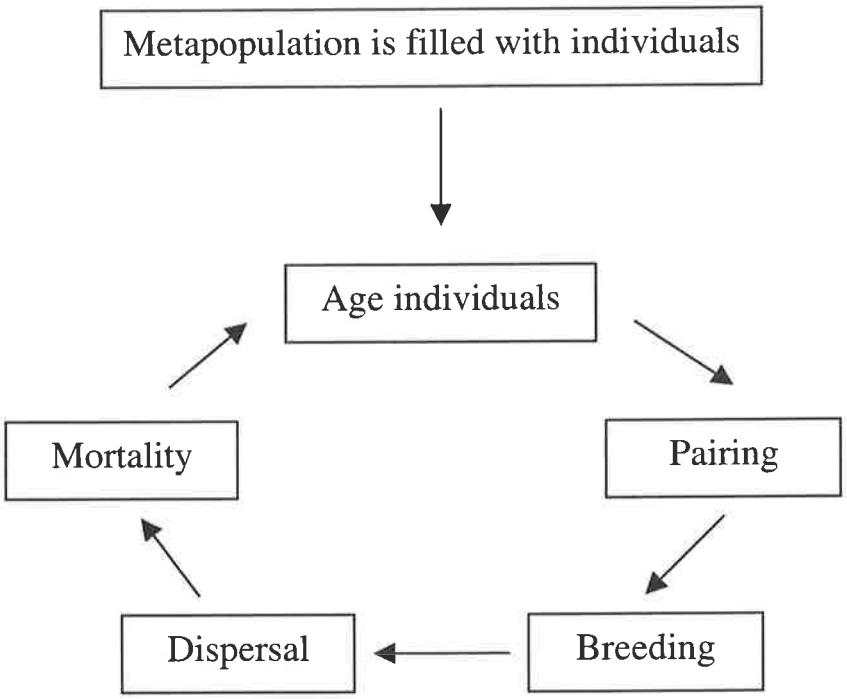

Figure 5.3 The basic model structure. Each replicate metapopulation starts by filling the system with individuals. The metapopulation then enters an annual cycle of demographic events.

5.2.2 Metapopulation is filled with individuals

Summary: The patches are filled to carrying capacity with individuals drawn randomly from what was previously a large continuous panmictic population.

- Each replicate metapopulation is initiated as if a previously panmictic population living in continuous habitat has suddenly been subdivided into a set of subpopulations restricted to habitat islands.

- All patches are filled to a predetermined carrying capacity. The assumption here is that in the previously continuous landscape any local variation in densities due to demographic stochasticity was reduced by dispersal - giving a uniform density of individuals over space and time.
- The genome of each individual is drawn randomly as if from a genetically panmictic population containing maximum levels of heterozygosity. Thus for the nuclear genome it is assumed that the 10 alleles at each of the 10 loci occurred in equal proportions in the previously continuous population, and that the 100 mitochondrial haplotypes also occurred in equal proportions. Individuals are simply allocated genomes by drawing randomly from this gene pool.
- Individuals are randomly allocated a sex, assuming a 1:1 sex ratio.
- All individuals are initially unpaired (to be assigned mates in the pairing procedure).
- The age of each individual is assigned by drawing randomly from a stable age distribution. The use of stable age distributions is based on the condition set earlier that densities in the previously panmictic population were constant over space and time. These stable age distributions (Figure 5.4) were determined on the basis of mortality rates that will be described in the section 5.2.7.

5.2.3 Age individuals

Summary: One year is added to the age of each individual every year.

- Each individual's age is stored as an integer value.
- This procedure simply adds one year to the age of each individual.
- Notice that aging is performed immediately prior to the courting and breeding procedures. Thus a newborn individual turns one-year-old just before the following year's pairing/breeding season.

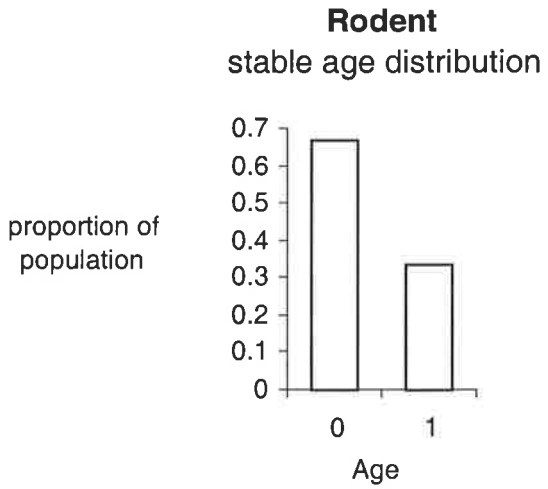

Figure 5.4 Stable age distributions used to initialise the age of individuals at the start of each metapopulation. These distributions are each based on a hypothetical large, continuous population at equilibrium, with no net immigration or emigration. The owl age distribution assumes 95% survival each year and a maximum age of 20 years; the rodent age distribution assumes 50% survival each year and a maximum age of two years (these conditions are described in the section on mortality). Note that these distributions describe the ages of individuals immediately prior to one year being added (this is why there are no 20-year-old owls, or two-year-old rodents).

5.2.4 Pairing (applies only to owls)

Summary: All sexually mature individuals are randomly allocated mates until the supply of potential mates is exhausted for one sex. This procedure applies only to owls (the rodents are polygamous and do not form lasting pair bonds).

- Only sexually mature individuals can form mating pairs (the age of maturity is set at three years for owls).
- Individuals are arranged into pairs until the supply of unpaired sexually mature individuals is exhausted for at least one sex. Any remaining, unpaired individuals simply remain in the patch.
- Pair bonds last for life.
- Widowed individuals may form new pairs.
- Pair allocation is performed using a pseudo-random number generator, whereby any unpaired sexually mature male has an equal probability of pairing with any of the unpaired sexually mature females. Thus, pair allocation is independent of age (as long as individuals are sexually mature), and very old individuals may pair with newly matured individuals.

5.2.5 Breeding

Summary: If subpopulation size is less than or equal to carrying capacity, every female produces a set number of zygotes – each of which has the same probability of developing into an independent juvenile. If subpopulation size is larger than carrying capacity, no individuals reproduce.

- Only sexually mature individuals reproduce, and for owls, only paired individuals reproduce.
- If subpopulation size is smaller than or equal to carrying capacity then reproduction can occur. Each female's expected fecundity is independent of population size (as long as the population size is smaller than or equal to carrying capacity). If subpopulation size is greater than carrying capacity, no individuals reproduce. Thus, fecundity is a step function of population size (Figure5.5).

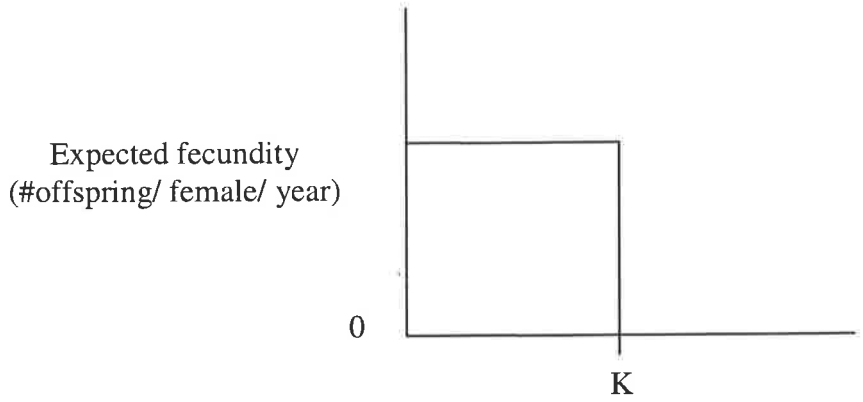

Figure 5.5 The fecundity of females as a function of population size.

- Whether or not each individual reproduces is fixed at the start of the breeding season. That is, although breeding may cause the size of a subpopulation to exceed carrying capacity part of the way through the model dealing with a list of breeding females, this does not change the fecundity of the remaining females in that list.
- In a given breeding season, all breeding females produce the same number of zygotes and all zygotes have the same survivorship probability. The model specifies that a breeding owl produces 4 zygotes, each with a 0.15 probability of surviving to birth, while a breeding rodent produces 8 zygotes, each with a 0.75 probability of surviving to birth. Table 5.2 shows the probability distribution of different clutch/litter sizes for each reproducing female owl and rodent per year.

Table 5.2 Probabilities of different clutch/litter sizes for each reproducing female owl and rodent per year.

Clutch/litter size	owl	rodent
0	0.5220	0.0000
1	0.3685	0.0004
2	0.0975	0.0038
3	0.0115	0.0231
4	0.0005	0.0865
5		0.2076
6		0.3115
7		0.2670
8		0.1001
total	1.0000	1.0000

- Once an individual is old enough to reproduce, its yearly fecundity is independent of age.
- For owls, there are no extra-pair matings.
- For rodents, each zygote has an equal probability of being sired by any of the breeding males in the population. Age does not influence the fitness of males. Although unlikely, one male alone could sire all the offspring in an entire subpopulation in a given generation (the probability of a given male, from a population of m breeding males, siring all n possible offspring in a given generation equals m^{-n}).
- New offspring are born with an age of zero, and their sex is determined randomly, assuming a 1:1 sex ratio.
- Each offspring's mitochondrial genome is maternally inherited. This is simply a matter of giving each offspring the same haplotype as its mother.
- Each offspring's nuclear genome is assigned according to the laws of Mendelian inheritance. Thus, an individual inherits two copies of each gene - one from its mother and one from its father. Importantly, the mother herself has two copies of each gene. A pseudo-random number generator is used to determine which of her copies is passed on - each having an equal probability of being inherited. The same applies to the father's copies.

5.2.6 Dispersal

Summary: Only juveniles disperse. If subpopulation size is less than or equal to carrying capacity there is a 5% chance that a given individual will disperse. If subpopulation size is greater than carrying capacity there is a 30% chance of an individual dispersing.

- Only juveniles (i.e. individuals who have not yet reached the age of sexual maturity) can disperse. One important implication of this is that only unmated individuals disperse.
- If the subpopulation size is smaller than or equal to the patch's carrying capacity, there is a 5% chance of each juvenile leaving the patch in that year. If the subpopulation size is greater than the patch's carrying capacity there is a 30% chance of each juvenile leaving the patch in that year. Thus, dispersal probability is a step function of population size (Figure 5.6).
- All the juveniles in a subpopulation have the same probability of leaving their patch in a given year.
- Whether or not a particular juvenile leaves a patch in a given year is determined by a pseudo-random number generator.

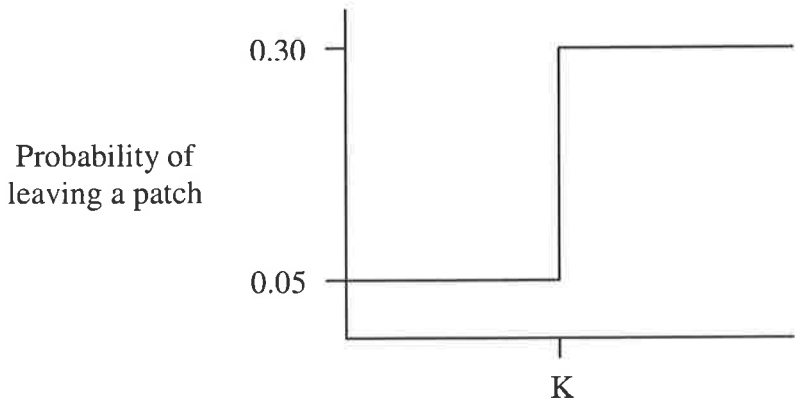

Figure 5.6 Dispersal probability of juveniles as a function of population size.

- The probability of each individual leaving its patch is set at the start of the dispersal season. Thus, even if emigration causes a subpopulation to fall below carrying capacity, or if immigration causes a subpopulation to rise above carrying capacity part of the way through the model dealing with a list of juveniles, this does not change the dispersal probabilities of the remaining juveniles in the list. This is equivalent to having all dispersal occur simultaneously.
- Not all dispersing individuals arrive at other patches - some effectively die in transit. This "dispersal mortality" occurs in addition to the normal mortality experienced within each patch (described below in section 5.2.7). Mortality during dispersal has been reported in many empirical studies (Bowman and Robel, 1977; Gaines and McClenaghan, 1980; Woollard and Harris, 1990; Matthysen, 1999) and has been included in a number of metapopulation models (Adler and Nuernberger, 1994; Possingham and Davies, 1995; Ruxton *et al.*, 1997).
- Dispersal does not impose any lasting (post-disperal) effects on an individual's fitness. That is, an immigrant has the same potential fitness as a resident individual of the same age and sex.
- The probability that a dispersing individual immigrates into a particular patch is independent of the size of the subpopulation in the recipient patch.
- In the baseline case males and females have the same probability of dispersing. Later I present the special case of sex-biased dispersal.
- If an individual leaves a certain patch, its destination is determined using a pseudo-random number generator and referring to a matrix that describes the pairwise dispersal probabilities between patches. Each value in such a matrix is the probability that an individual emigrating from one patch will arrive in a particular other patch. These probabilities are fixed for the duration of each replicate metapopulation.
- Dispersal is reciprocal between pairs of patches, in the sense that an individual leaving patch x has the same probability of arriving at patch y as an individual leaving patch y has of arriving at patch x. This of course means that the dispersal *rate* is not necessarily the same in both directions. For example if patch x has a larger carrying capacity than patch y, then on average more individuals are expected to move from patch x to patch y than *vice versa*.
- Each individual can disperse only once in a given year. Thus, an individual cannot disperse from one patch to another and then disperse again in the same year.

- Owls (which experience three dispersal seasons before they mature), may disperse more than once over a number of years, potentially back to a patch they were previously in.

5.2.7 Mortality

Summary: Owls and rodents have a 50% chance of dying in their first year of life; thereafter, owls have a 5% chance of dying each year and rodents a 50% chance; no owls live beyond 20 years of age, and no rodents beyond two years.

- The chance of dying is independent of population size.
- First year mortality probability is set at 0.50 for both owls and rodents.
- The yearly mortality probability for owls older than one year of age is set at 0.05. For rodents the yearly mortality probability it is set at 0.50.
- Whether or not an individual dies is determined by drawing a pseudo-random number.
- There is a maximum life span (20 years for owls, two years for rodents). Any individual that reaches that age has a mortality probability of 1.00.
- Mortality probability is independent of whether or not an individual is in a mating pair, and whether or not the individual has produced offspring.
- *A note on the MultiPop code*: For newborn individuals, the model inflicts first year mortality in the same procedure that allows breeding. This avoids having to create complete records for individuals that are likely to die soon anyway. This approach has two advantages. Firstly it helps to reduce the model's running time (by requiring fewer calculations and less storage of unnecessary information). Secondly it reduces the risk that when a subpopulation breeds, the addition of new individuals will overflow the amount of memory allowed by the program. This essentially means that I was able to model (1) patches with larger carrying capacities and/or (2) a greater number of patches without crashing the program. This preemptive application of first year mortality is only possible because first year mortality probability is fixed across all patches, irrespective of local population dynamics. Thus, the chance of each newborn individual dying is 0.5, irrespective of whether it disperses to another patch or stays in the patch it was born in.

5.2.8 Optional feature 1: catastrophic disturbance events

Summary: Catastrophic disturbance events represent an optional feature that was used in some scenarios. When this disturbance feature is switched on, each subpopulation has a 5% chance per year of experiencing a disturbance event. A disturbance event applies a strong bout of mortality in addition to the normal yearly mortality. For owls, a disturbance event gives every individual in the subpopulation a 50% chance of dying, while for rodents there is a 97.5% chance of dying.

- Catastrophes represent one of the "treatments" I apply using the model.
- When a disturbance regime is applied to a metapopulation, each subpopulation has a 5% chance per year of experiencing a disturbance event. Disturbance events occur independently among subpopulations.
- Each catastrophe lasts for just one year, and disturbance events occur independently among years and patches.
- A catastrophe applies a strong bout of mortality in addition to normal yearly mortality. For owls, a disturbance event means that any individuals surviving normal yearly mortality have a 50% chance of dying due to the disturbance, while for rodents there is a 97.5% chance of dying due to disturbance.
- It is an important feature that the catastrophes are not modelled as causing 100% mortality. Firstly, this rarely occurs in nature: usually some individuals survive catastrophes (Patterson, 1984; Stiles, 1992; Wauer and Wunderle, 1992; Young, 1994b; Hailey, 2000). Secondly, if disturbance caused 100% mortality, all patches would end up having similar extinction probabilities, particularly in systems with carrying capacities high enough that demographic stochasticity is a negligible cause of subpopulation extinctions. In contrast, with disturbance mortality less than 100%, subpopulations with larger carrying capacities are more likely to survive disturbance events. This in turn means that the patches in the model are more likely to differ in their demographic value to their metapopulation.

5.2.9 Optional feature 2: sex-biased dispersal

Summary: Sex-biased dispersal is an optional feature that was used in some scenarios. When sex-biased dispersal is applied, a female owl is four times more likely to disperse than a male owl, while a male rodent is four times more likely to disperse than a female rodent.

- Sex-biased dispersal represents one of the "treatments" I apply using the model. The owls are modelled as having female-biased dispersal, and the rodents as having male-biased dispersal. This follows the patterns reported in the literature for female-biased dispersal among birds (Greenwood, 1980; Clarke *et al.*, 1997) and male-biased dispersal among mammals (Greenwood, 1980).
- For owls, a juvenile female has four times the odds of dispersing than a juvenile male. The dispersal tendency of females is set to the same as that for individuals in populations where there is no sex-bias in dispersal. Thus, the probability that a juvenile female will disperse is 0.05 when N is less than or equal to K, and 0.30 when N is greater than K. This means that the probability of a juvenile male dispersing is 0.013 when N is less than or equal to K, and 0.097 when N is greater than K. These values were calculated by rearranging the equation:

$$\text{Odds ratio} = \frac{P_{FEMALE} / (1 - P_{FEMALE})}{P_{MALE} / (1 - P_{MALE})} = 4 \qquad \text{(Eqn 5.1)}$$

  where the odds ratio describes how many times more likely an individual of one sex is to disperse compared to an individual of the other sex. In this equation, P_{FEMALE} and P_{MALE} are the probabilities that a juvenile female and juvenile male will disperse.
- For rodents, juvenile males are four times more likely to disperse than juvenile females, the opposite to owls. This means that the probability of a juvenile male dispersing is 0.05 when N is less than or equal to K, and 0.30 when N is greater than K, and that the probability of a juvenile female dispersing is 0.013 when N is less than or equal to K, and 0.097 when N is greater than K.

5.3 The net effect of breeding, dispersal and mortality

The demographic properties of owls and rodents as specified in this model clearly differ. Owls produce fewer offspring per year, have female-biased instead of male-biased dispersal (when sex biased dispersal is specified), experience much lower mortality and have a longer potential lifespan. A useful basis for understanding the net effect of these differences between owls and rodents is to consider R, the fundamental net per capita rate of increase ($N_{(t+1)} / N_{(t)}$). There are several important points to note when using R. Firstly, we need to define the time step which separates $N_{(t+1)}$ from $N_{(t)}$. For the MultiPop model the obvious choice is one year. Secondly, R depends on the distribution of age classes in a population. Changes in population size influence the recruitment of new individuals, and this causes changes in the abundance of different age classes over time. Because the different age classes in MultiPop vary in their demographic profiles (reproduction, dispersal and to a limited extent mortality), R is sensitive to changes in the shape of the age class distribution.

For this reason it is perhaps most meaningful to report the equilibrium R - when the frequency distribution of age classes has effectively stabilised. In reality this equilibrium will never be reached, since recruitment, mortality and dispersal will vary over time as a population bounces above and below carrying capacity. Nonetheless we can determine the equilibrium R value that would be approached if demographic properties were held constant for long enough. Accordingly, I estimated R for both owls and rodents by iterating their respective life-history tables (using a Microsoft Excel spreadsheet) until population growth rate had stabilised to the third decimal point. This approach involved setting an arbitrary initial frequency distribution of age classes (I gave all age classes the same initial abundance), and allowing population dynamics to unfold deterministically as if the population was so large that demographic stochasticity was zero. In every case R was calculated by considering females as the limiting sex.

The equilibrium R value for owls in this model is 1.053, while that for rodents is 1.896. After including the effect of catastrophes (by averaging their impact over years), R becomes 1.030 and 1.880 respectively. These values assume that immigration and emigration are equal. With only emigration (no immigration) and sex-biased dispersal, R = 1.035 for owls and R = 1.875 for rodents, while including catastrophes changes these to 1.012 and 1.859. With only

emigration (no immigration) and unbiased dispersal, R = 1.035 for owls and R = 1.817 for rodents. With catastrophes included these become 1.012 and 1.801.

5.4 The three-patch metapopulation

The model was first applied to a hypothetical three-patch metapopulation. This is the smallest system in which it is possible to manipulate the demographic value of patches by altering connectivity. The baseline case does not include catastrophes or sex-bias in dispersal.

The three patches were given carrying capacities of 20, 15 and 10 individuals, and the patches will be referred to as $Patch_{20}$, $Patch_{15}$ and $Patch_{10}$ (or as P_{20}, P_{15} and P_{10}). Admittedly these carrying capacities are quite low, and for any real system we would hardly think of this as the basis for a long-lived metapopulation. Nonetheless, it is important to remember that in this situation the only cause of extinction being modelled is demographic stochasticity. Therefore the subpopulations are in fact quite extinction-resistant. If we were to increase their carrying capacities by much, there would be so little turnover of patch occupancy that it would be quite inappropriate to be concerned about metapopulation extinction.

While it is difficult to imagine any natural system free of environmental stochasticity, its exclusion from the three-patch metapopulation provides the basis for some potentially useful insights. Importantly, environmental stochasticity reduces the link between a patch's genetic diversity and its value to a metapopulation. Remember that one component of the value of a patch is its tendency to contain an extinction-resistant subpopulation. Ideally we would like the genetic diversity of a subpopulation to reflect its resistance to extinction. If we consider a scenario of extreme environmental stochasticity where each disturbance event causes 100% mortality, extinction essentially strikes without warning, and there is no opportunity for genetic diversity to reflect the extinction-resistance of each patch. In contrast, when the only cause of extinction is demographic stochasticity, each subpopulation may have a number of bottleneck "warnings" before actually going extinct, and these may be reflected by the genetic diversity of that subpopulation. Therefore, we might expect the genetic diversity of a system free of environmental stochasticity to provide relatively good insights into the

demographic value of patches. Although the environmental stochasticity used in this thesis is catastrophic mortality that is less than 100% (50% for owls and 97.5% for rodents), this stochasticity may nonetheless cause considerable decoupling of genetic diversity data from patch value. For this reason I have included the special case of the three-patch system without environmental stochasticity.

In the three-patch metapopulation, I create what is effectively a dispersal corridor between $Patch_{20}$ and $Patch_{10}$, while keeping $Patch_{15}$ isolated using dispersal barriers (Figure 5.7). I then vary the amount of connectivity between $Patch_{20}$ and $Patch_{10}$, and run replicate metapopulations. I use six different connectivity levels: 0%, 20%, 40%, 60%, 80% and 100%. A connectivity of 40% means that an individual leaving $Patch_{20}$ has a 40% chance of arriving at $Patch_{10}$, and *vice versa* (the remaining 60% of individuals effectively die en route). The different connectivity levels can be thought of as different risks of predation or starvation while travelling through the dispersal corridor. This in turn could be visualised as changes in the quality of the corridor environment, or by having different distances between $Patch_{20}$ and $Patch_{10}$. When the connectivity is 0%, all three patches are isolated and the relative value of each patch is determined purely by its resistance to extinction, and hence carrying capacity. In this no-dispersal case $Patch_{10}$ is of the least demographic value to the metapopulation. With 100% connectivity however, individuals dispersing from $Patch_{20}$ and $Patch_{10}$ are able to rescue their neighbouring subpopulation from extinction, and recolonise their neighbouring patch in the event of extinction. While $Patch_{20}$ should not change from being the most important patch, we might expect $Patch_{10}$ to become more valuable than $Patch_{15}$, due to its ability to interact with $Patch_{20}$. This approach - changing the level of connectivity to change patch ranking - is the basis for much of the analyses I perform using the MultiPop model.

One "treatment" applied to the initially simple three-patch metapopulation was the inclusion of environmental stochasticity in the form of catastrophic disturbance events. This has two implications. Firstly, as stated earlier, environmental stochasticity may reduce the link between a patch's genetic diversity and its demographic value. Secondly, because disturbance increases metapopulation extinction probabilities, it becomes feasible (in terms of the program's run time) to model patches with much larger carrying capacities than what is practical for a system without environmental stochasticity. Thus, patch carrying capacities that were previously set at 20, 15 and 10, are increased to 80, 60 and 40, and the three patches are referred to as $Patch_{80}$, $Patch_{60}$ and $Patch_{40}$. In fact if the carrying capacities were not

increased, the metapopulation would have such a high extinction probability that it would be quite meaningless to try to conserve such a system. An important consequence of increasing patch carrying capacities is that subpopulation genetic diversity is likely to decay at a slower rate and with less stochasticity (until a disturbance event strikes).

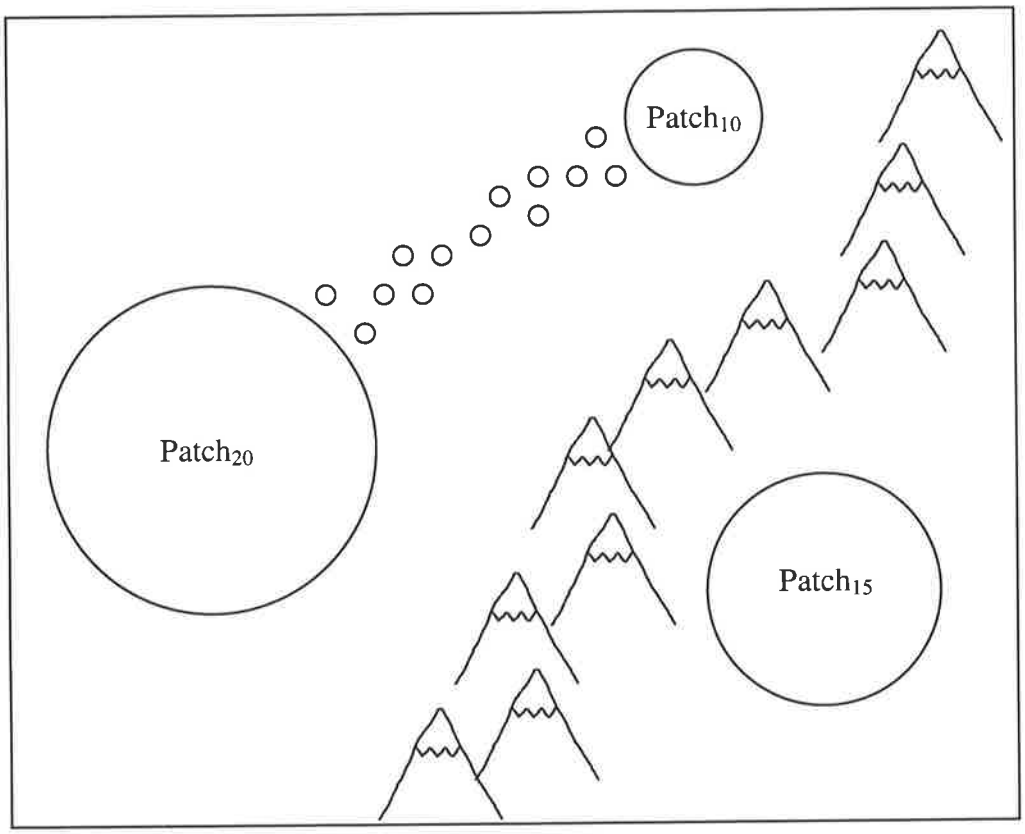

Figure 5.7 Hypothetical three-patch metapopulation. $Patch_{20}$ and $Patch_{10}$ are linked by a dispersal corridor, represented here by small circles (as if they were tiny fragments of habitat). $Patch_{15}$ is completely isolated from the other patches by a dispersal barrier, here represented by a mountain range.

Another treatment applied to the three-patch metapopulation was to have sex-biased dispersal. This was applied to metapopulations with and without environmental stochasticity. The three-patch metapopulation was therefore analysed separately under a range of different scenarios:

- ORGANISM (owl and rodent)
- ENVIRONMENTAL STOCHASTICITY (excluded and included)
- SEX-BIASED DISPERSAL (yes and no)
- CONNECTIVITY (0%, 20%, 40%, 60%, 80%, 100%)

5.5 The eight-patch metapopulation

The model was also applied to an eight-patch system. This increase in complexity was undertaken to address concerns that the results from a three-patch system may be too simplistic to generalise to the larger systems that commonly occur in nature. This particular number of patches was chosen as a compromise between trying to increase complexity while working within the constraints of how much computer memory was available. Importantly, there are many examples of metapopulations of management concern with similar numbers of patches (*e.g.,* Gottelli *et al.*, 1994; Gaona *et al.*, 1998; Griffis and Jaeger, 1998; Reed *et al.*, 1998; Baguette *et al.*, 2000).

The eight patches were given different carrying capacities: 15, 20, 25, 30, 35, 40, 45 and 50. Unlike the three-patch system where only two patches were connected by dispersal, the eight-patch system involves a "web" of connectivity, with all patches being connected to all seven other patches to varying degrees. The connectivity in the eight-patch system is based on an isolation-by-distance model of connectivity. Initially I placed the eight patches on a two dimensional landscape (Figure 5.8). Having created a number of random computer-generated patch arrangements, the arrangement shown in Figure 5.8 was selected on the basis of having a relatively wide range of inter-patch distances (and hence connectivities).

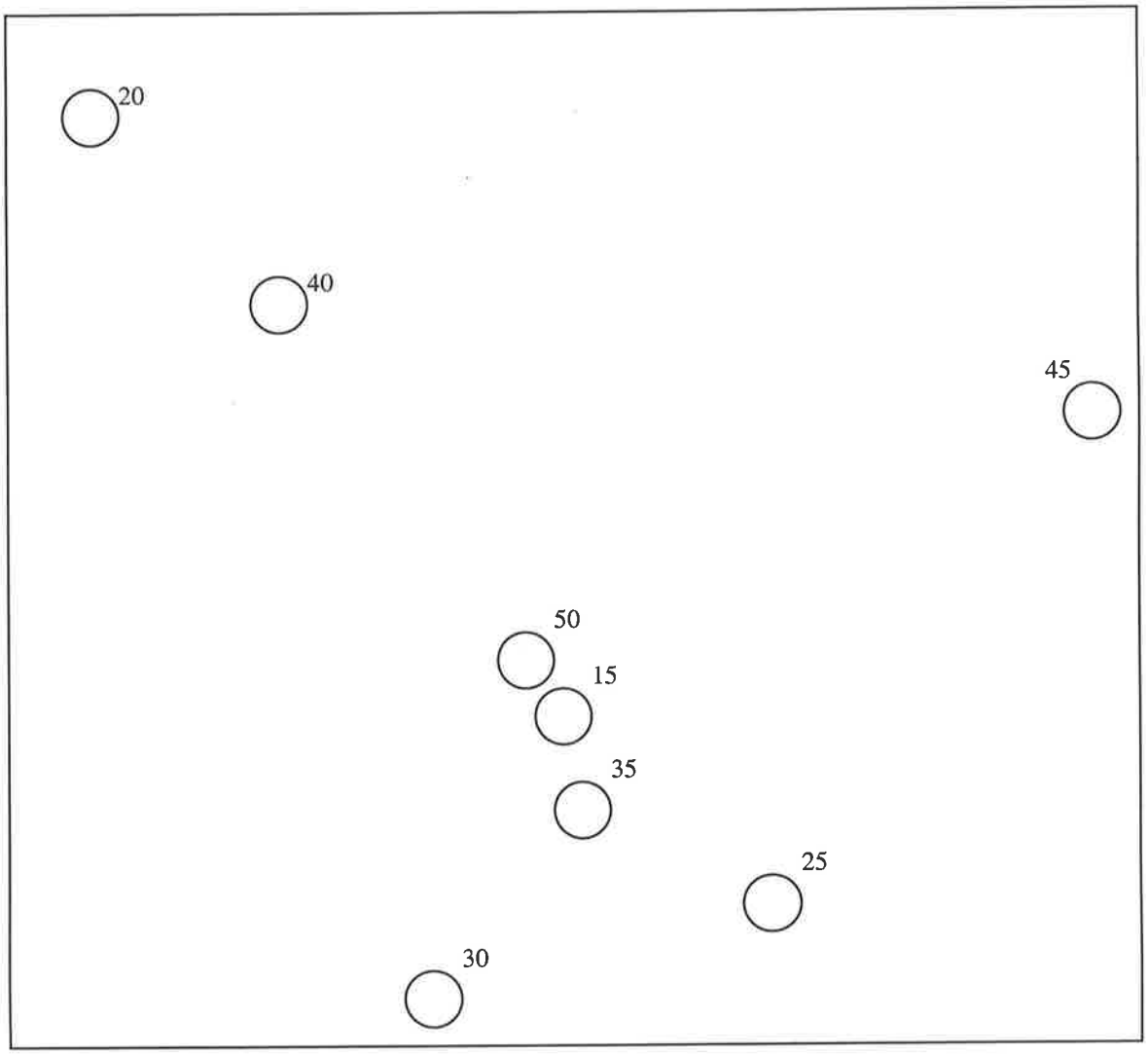

Figure 5.8 Arrangement of patches used for the eight-patch metapopulations. Each number represents patch carrying capacity.

I used this patch arrangement for all analyses of the eight-patch metapopulation. I modelled the pairwise interaction between patches by assuming that the probability that an immigrant arrives at patch A, given that it has left patch B, decreases as an exponential function of the distance between the two patches. For this I used the equation:

$$\Pr(ArriveAtPatchA \mid LeavePatchB) = \frac{\exp(-DispersalMortality \times DISTANCE_{AB})}{NumberOfPatches - 1} \quad \text{(Eqn 5.2)}$$

based loosely on Hanski (1994a). The denominator ((NumberOfPatches-1) = 7) was included to maintain the condition that the probability of the emigrants of a particular patch arriving at any of the seven other patches is less than or equal to 1. Note that when dispersal mortality is zero, there is an equal 14% chance that an emigrant will arrive at one of the seven other patches. As dispersal mortality increases, the connectivity between patches becomes more strongly influenced by inter-patch distances.

The biological meaning of Equation 5.2 can be considered as follows. Imagine that the large square in Figure 5.8 represents an oceanic island. On that island, there are eight habitat patches in a matrix of inhospitable habitat. Individuals can disperse (but not breed) between the eight patches, but no individuals leave the island. As such, the boundaries of the square are reflective so that an individual reaching the water's edge returns inland. Patches arranged in a line do not "shadow" each by intercepting potential dispersers. This is essentially as if the patches were so small that their alignment does not come into play, and individuals disperse by random walk - thereby allowing them to move around patches that would otherwise intercept straight-line dispersers. Note that equation 5.2 does not give larger patches a higher tendency to intercept dispersers. Accordingly, the patches in Figure 5.8 are represented with equal size, and in this system it is best to imagine that higher carrying capacities are caused by differences in patch quality which allow more individuals per area (without any effects on the demographic properties of individuals).

Six different levels of dispersal mortality were used for this eight-patch system, and these are analogous to the different connectivity levels used in the three-patch system. These are a measure of the probability that an individual dies per unit distance travelled between two patches. Clearly the lower the dispersal mortality the greater connectivity between patches. Preliminary runs of the model showed that the persistence of rodent metapopulations was

more sensitive to low levels of dispersal than was the persistence of owl metapopulations. Therefore different ranges of dispersal mortality were chosen for the two organisms: for owls they were 0.08, 0.04, 0.02, 0.01, 0.005, and 0.0025, and for rodents they were 0.12, 0.10, 0.08, 0.06, 0.04, and 0.02

For each connectivity level, a matrix of pairwise dispersal probabilities between patches was calculated using equation (5.2). Below is the exact patch arrangement used (Table 5.3), the matrix of pairwise distances between patches (Table 5.4), and example matrices of the pairwise dispersal probabilities between patches (Tables 5.5 and 5.6).

Table 5.3 Exact patch arrangement in the eight-patch metapopulation. The x and y coordinates are integer values in a 100 by 100 grid, and these axes correspond to the horizontal and vertical dimensions of Figure 5.8.

	Patch identity (in terms of carrying capacity)							
	15	**20**	**25**	**30**	**35**	**40**	**45**	**50**
x-coordinate	51	6	66	39	52	24	97	48
y-coordinate	30	91	16	3	24	70	63	35

Table 5.4 Matrix of pairwise straight-line distances between patches. Patch identities are represented by their carrying capacities (bold). "Departure" and "destination" refer which patch a dispersing individual is leaving and which patch it is arriving into. The distances are taken from the centre of the departure patch to the centre of the destination patch (i.e. treating patches as points on the landscape).

		DESTINATION PATCH							
		15	**20**	**25**	**30**	**35**	**40**	**45**	**50**
DEPARTURE PATCH	**15**	0.00	75.80	20.52	29.55	6.08	48.26	56.61	5.83
	20	75.80	0.00	96.05	93.98	81.27	27.66	95.21	70.00
	25	20.52	96.05	0.00	29.97	16.12	68.41	56.30	26.17
	30	29.55	93.98	29.97	0.00	24.70	68.66	83.45	33.24
	35	6.08	81.27	16.12	24.70	0.00	53.85	59.55	11.70
	40	48.26	27.66	68.41	68.66	53.85	0.00	73.33	42.44
	45	56.61	95.21	56.30	83.45	59.55	73.33	0.00	56.44
	50	5.83	70.00	26.17	33.24	11.70	42.44	56.44	0.00

Table 5.5 High mortality during dispersal. This is a matrix of pairwise immigration probabilities between patches when dispersal mortality is high (0.12). This is the highest dispersal mortality used for rodents. Patch identities are represented by their carrying capacities (bold). Each value is the probability that an individual that has left a particular departure patch arrives at a particular destination patch. "Total" refers to the probability that an individual leaving a particular departure patch arrives at any of the other patches.

		DESTINATION PATCH								
		15	**20**	**25**	**30**	**35**	**40**	**45**	**50**	**total**
DEPARTURE PATCH	**15**	0.000	0.000	0.012	0.004	0.069	0.000	0.000	0.071	0.157
	20	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.005
	25	0.012	0.000	0.000	0.004	0.021	0.000	0.000	0.006	0.043
	30	0.004	0.000	0.004	0.000	0.007	0.000	0.000	0.003	0.018
	35	0.069	0.000	0.021	0.007	0.000	0.000	0.000	0.035	0.132
	40	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.001	0.007
	45	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
	50	0.071	0.000	0.006	0.003	0.035	0.001	0.000	0.000	0.116

Table 5.6 Low mortality during dispersal. This is a matrix of pairwise immigration probabilities between patches when dispersal mortality is low (0.0025). This is the lowest dispersal mortality used for owls. Patch identities are represented by their carrying capacities (bold). Each value is the probability that an individual that has left a particular departure patch arrives at a particular destination patch. "Total" refers to the probability that an individual leaving a particular departure patch arrives at any of the other patches.

		DESTINATION PATCH								
		15	**20**	**25**	**30**	**35**	**40**	**45**	**50**	**total**
DEPARTURE PATCH	**15**	0.000	0.118	0.136	0.133	0.141	0.127	0.124	0.141	0.919
	20	0.118	0.000	0.112	0.113	0.117	0.133	0.113	0.120	0.826
	25	0.136	0.112	0.000	0.133	0.137	0.120	0.124	0.134	0.896
	30	0.133	0.113	0.133	0.000	0.134	0.120	0.116	0.131	0.880
	35	0.141	0.117	0.137	0.134	0.000	0.125	0.123	0.139	0.916
	40	0.127	0.133	0.120	0.120	0.125	0.000	0.119	0.128	0.873
	45	0.124	0.113	0.124	0.116	0.123	0.119	0.000	0.124	0.843
	50	0.141	0.120	0.134	0.131	0.139	0.128	0.124	0.000	0.917

The run times required for the eight-patch system were extremely long under some scenarios. Therefore eight-patch metapopulations were analysed over a much smaller range of scenarios than was used for the three-patch metapopulations. The scenarios used were:

- ORGANISM (owl or rodent)
- ENVIRONMENTAL STOCHASTICITY (included)
- SEX-BIASED DISPERSAL (yes)
- CONNECTIVITY (Owl dispersal mortality: 0.08, 0.04, 0.02, 0.01. 0.005, 0.0025.
  Rodent dispersal mortality: 0.12, 0.10, 0.08, 0.06. 0.04, 0.02)

The different scenarios analysed for the three-patch and eight-patch systems are summarised in Figure 5.9.

5.6 Using the model to determine the demographic value of patches

The relative value of the different patches to each metapopulation was determined using a method of single patch removal (Day and Possingham, 1995; Lindenmayer and Possingham, 1996) - first running the metapopulation with all patches included, and then running it with each of the patches removed. Each patch was removed by setting its carrying capacity to zero and preventing it from receiving any immigrants (in the model the removal of a patch does not have any effect on the dispersal probabilities between the other patches remaining in the system). A total of 10,000 replicate metapopulations were run in each patch removal scenario. This level of replication offered an acceptable compromise between (1) obtaining reliable parameter estimates and (2) the model having reasonable run times. With each replicate metapopulation, the patches were initially filled to carrying capacity, and the time (in years), was set to zero. Each metapopulation was then allowed to pass through yearly cycles of demographic events until no more individuals remained in the system. The output variable for each replicate was the number of years taken to reach metapopulation extinction. The 10,000 extinction times for each patch removal scenario were then sorted, and the probability of extinction by 100 years was calculated as the proportion of replicates whose extinction time was less than or equal to 100 years (for code see A:\APP3\SORTER.PAS on

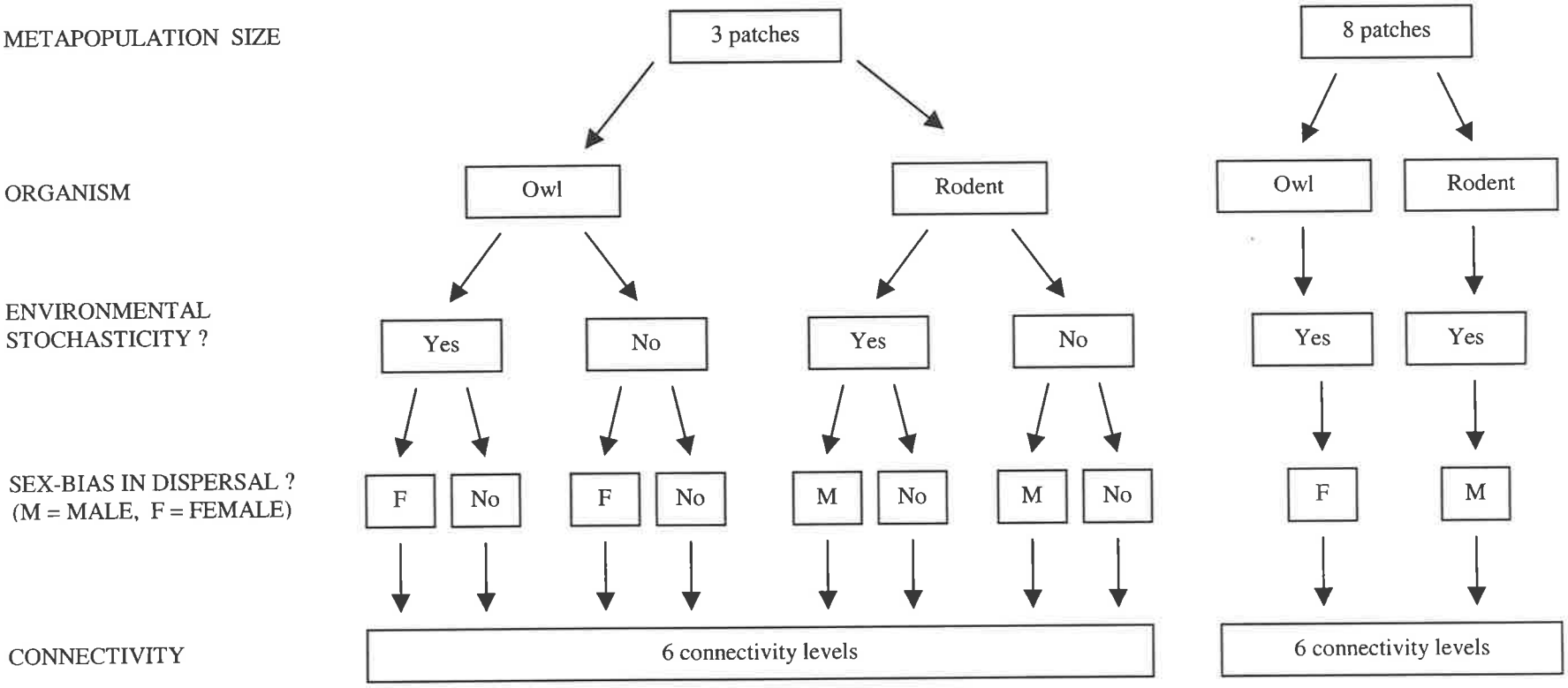

Figure 5.9. Summary of the different scenarios examined using the MultiPop model.

the accompanying disc). Patches were ranked using probabilities of extinction within 100 years, $P[E]_{100}$, given that this is commonly used as a measure of population viability by conservation biologists (Mace and Lande, 1991; Day and Possingham, 1995; Maguire *et al.*, 1995; Green *et al.*, 1996; Hiraldo *et al.*, 1996).

The data from Multipop were analysed using a two-patch approach. Thus, I tested whether ranking two patches on the basis of their genetic diversity provides a good estimate of their ranking in terms of demographic value to the metapopulation. The measure in this case is simply the probability of correctly ranking the two patches, estimated from the proportion of simulations when genetic diversity correctly ranked the two patches according to their demographic value. This is then compared to the null hypothesis that our estimate of patch ranking was randomly assigned (i.e. probability of correct ranking = 0.5). The logical alternative to this approach would involve measuring the association between the two complete lists of ranks (in this case genetic versus demographic patch rankings), using a measure such as Kendall's coefficient of rank correlation, τ (Sokal and Rohlf, 1981). While a multi-patch approach allows the use of a convenient summary statistic for an entire metapopulation, the interpretation of such a statistic for metapopulation management is not immediately clear. In contrast, a two-patch comparison allows us to express the predictive accuracy of ranking patches as a probability, which has a clear and direct meaning for metapopulation management.

The two-patch comparison is also valuable in allowing us to measure the magnitude of differences in patch value. For this I have chosen to measure the value of each patch as the percent decline in metapopulation viability caused by the removal of that patch, as used by Lindenmayer and Possingham (1996). The viability of a metapopulation is simply the probability that the metapopulation will be extant by some chosen time (*e.g.*, 100 years). Thus if $P[E]_{100[COMPLETE]}$ is the probability that the complete metapopulation (no patch removals) goes extinct within 100 years, then

$$\text{Viability} = 1 - P[E]_{100[COMPLETE]} \qquad \text{(Eqn 5.3)}$$

Upon removing $Patch_A$ we have $P[E]_{100[WITHOUT\ A]}$, the probability that the metapopulation, now missing $Patch_A$, goes extinct within 100 years. This gives a new viability of $1-P[E]_{100[WITHOUT\_A]}$, giving us

$$\text{decline in viability} = (1- P[E]_{100[COMPLETE]}) - (1- P[E]_{100[WITHOUT\ A]}) \quad \text{(Eqn 5.4a)}$$

$$= P[E]_{100[WITHOUT\ A]} - P[E]_{100[COMPLETE]}. \quad \text{(Eqn 5.4b)}$$

And from this,

$$\text{\% decline in viability (value of a patch)} = \frac{100 \times (P[E]_{100[WITHOUT\ A]} - P[E]_{100[COMPLETE]})}{(1- P[E]_{100[COMPLETE]})} \quad \text{(Eqn 5.5)}$$

Thus a patch of no value (i.e. whose removal caused no change in the metapopulation's extinction probability) is given a patch value of zero, while a patch of infinite value (whose removal caused the metapopulation's extinction probability to drop to zero) is given a patch value of 100. Using this measure, the relative value of two patches A and B can be expressed as

$$RV_{AB} = \frac{\text{\% decline in viability caused by removing } Patch_A}{\text{\% decline in viability caused by removing } Patch_B} \quad \text{(Eqn 5.6a)}$$

which reduces to

$$RV_{AB} = \frac{(P[E]_{100[WITHOUT\ A]} - P[E]_{100[COMPLETE]})}{(P[E]_{100[WITHOUT\ B]} - P[E]_{100[COMPLETE]})} \quad \text{(Eqn 5.6b)}$$

To assess the ability of RV_{AB} to provide a consistently meaningful measure of the relative value of two patches, I identified a number of different "test" scenarios (Table 5.7). Each scenario is represented by a set of three extinction probabilities - one for a complete

metapopulation, one with Patch A removed and one with Patch B removed. In each case there are logical arguments for expecting RV_{AB} to have a certain property. In one case RV_{AB} is predicted to equal exactly 1; in one case it should be infinite; and in another case the value should be meaningless (0 divided by 0). There are also two sets of paired scenarios where logic tells us which scenario should have the highest RV_{AB} value. If the calculated values were to go against any of these predictions, we would clearly have reason to doubt RV_{AB} as a meaningful measure of relative patch value. The measure was able to meet the prediction in each case (Table 5.7).

Table 5.7 Test scenarios used to assess the robustness of using RV_{AB} to measure the relative value of patch A to patch B. In scenario (1), the relative value of the patches should equal one because the removal of both patches gave the same metapopulation extinction probability. In scenario (2) patch B is of no value at all, so patch A should be infinitely more valuable. The only difference between scenarios (3a) and (3b) is that patch A has greater value in scenario (3a) - and should therefore have higher relative value over B in (3a) than in (3b). The only difference between scenarios (4a) and (4b) is that the extinction probability of the complete metapopulation is higher in (4a). The closer this baseline probability gets to $P[E]_{100[WITHOUT\ B]}$, the higher the relative value of Patch A (if we increased the baseline probability even further, we would approach the situation we see in scenario (2) where the relative value of Patch A $= \infty$). In scenario (5) both patches have no value to the metapopulation, and reporting a relative value is meaningless. The calculation in this case is $RV_{AB} = 0/0$, which is also meaningless.

Scenario	$P[E]_{100[COMP.]}$	$P[E]_{100[W/OUT\_A]}$	$P[E]_{100[W/OUT\_B]}$	Prediction	RV_{AB}
(1)	0.4	0.8	0.8	RV(1) = 1	1
(2)	0.4	0.8	0.4	RV(2) = ∞	∞
(3a)	0.4	0.9	0.5	RV(3a) > RV(3b)	5
(3b)	0.4	0.6	0.5		2
(4a)	0.4	0.6	0.5	RV(4a) > RV(4b)	2
(4b)	0.3	0.6	0.5		1.5
(5)	0.4	0.4	0.4	meaningless	0/0

By allowing us to measure the relative value of patches, the two-patch approach opens up potentially useful opportunities in the analysis of MultiPop's output. In particular, we can examine whether the probability of correctly ranking patches increases as a function of the difference in value between patches. The same issue could not be easily addressed with a multi-patch approach to patch ranking. While it may be possible to measure the differences among a number of patches using a measure analogous to variance, such a summary statistic would clearly ignore valuable information.

5.7 Using the model to measure genetic diversity in patches

Subpopulation genetic diversity was measured at 5, 10, 20, 40 and 80 years after the model was initialised. A total of 1000 replicate metapopulations were run independently for each of these sampling times, and these were independent of the runs used to determine the demographic value of patches. There are two reasons why I chose to use less replication for the genetic diversity data than was used to determine the demographic value of patches (i.e 1000 compared to 10,000). Firstly, the genetics replicates had much greater run times than the dynamics replicates. The reason for this was that the genetics replicates required the inclusion of the full genome (ten nuclear loci with ten alleles and 100 mitochondrial haplotypes), whereas this information was unnecessary and therefore omitted when running the dynamics replicates. Secondly, the genetic replicates required less replication simply because they were being used for a different purpose to the dynamics replicates. The purpose of the dynamics replicates was one of parameter estimation - to identify the demographic value of the patches as accurately as possible. In contrast, the purpose of the genetics replicates was one of hypothesis testing - to test whether using genetics to rank patches is better than making a random guess of the demographic ranking of patch values.

Genetic diversity was measured in six different ways, to account for the possibility that some measures may provide more information than others. A separate set of 1000 replicates was run for each of these six measurements of genetic diversity (i.e. they were independently sampled). Genetic diversity was measured across all individuals in each subpopulation.
For the nuclear genome I measured:

(1) The mean number of alleles per locus.

(2) The number of polymorphic loci in the subpopulation.

(3) Mean observed heterozygosity across all 10 loci and all individuals in a subpopulation.

(4) Mean expected heterozygosity across all 10 loci. This is the heterozygosity we would expect to observe if the population was very large and at Hardy-Weinberg equilibrium, and is calculated as

$$Expected\_Heterozygosity = \frac{\sum^{l}\left(1-\sum^{a} p_i^2\right)}{l} \qquad \text{(Eqn 5.7)}$$

where l = the number of loci, a = the number of alleles per locus, and p_i = the frequency of allele i in the population (Crow, 1986).

The difference between the observed and expected heterozygosity can be appreciated by considering a population with only two individuals that are homozygous for different alleles (A and a) at the same locus. While we would record the observed heterozygosity as zero for this locus, it is intuitive that a population of two individuals (AA and aa) is genetically more diverse than a population entirely fixed for one allele (*e.g.*, both individuals are AA and AA). Expected heterozygosity takes this into account, and a population of two individuals AA and aa would give an expected heterozygosity of 0.5.

For the mitochondrial genome I measured:

(1) The number of haplotypes in the subpopulation.

(2) Haplotype diversity. This is a measure of both (1) how many different haplotypes are in a population and (2) how even the haplotype frequencies are. It is calculated as

$$Haplotype\_Diversity = 1-\sum^{h} p_i^2 \qquad \text{(Eqn 5.8)}$$

where h = the number of haplotypes in the population, and p_i = the frequency of haplotype i in the population (see Crow (1986)).

To understand this measure it is useful to consider some examples. Firstly, if a population contains only one haplotype, the haplotype diversity equals zero, irrespective of how many individuals are in the population. In a population with two haplotypes in equal abundance, the haplotype diversity = 0.5, while with three

haplotypes in equal abundance the diversity = 0.67. If we double population size, but still have only three haplotypes in equal proportion, the haplotype diversity remains at 0.67. In a population where there are three haplotypes in unequal abundance, the diversity is less than 0.67.

5.8 Testing the success of using genetics to rank patches

The two patches compared within the three-patch metapopulation were $Patch_{15}$ and $Patch_{10}$, based on the expectation that the ranking of these patches would change as a function of connectivity between $Patch_{10}$ and $Patch_{20}$. With the eight-patch system, four pairs of patches were chosen for comparison:

Pair 1 = $Patch_{50}$ and $Patch_{20}$;
Pair 2 = $Patch_{45}$ and $Patch_{15}$;
Pair 3 = $Patch_{30}$ and $Patch_{25}$;
Pair 4 = $Patch_{40}$ and $Patch_{35}$;

Genetic diversity was used to rank the two patches in each pair, and the patch with the highest level of genetic diversity was given the highest ranking of the two patches. This genetic rank was then compared to the ranking of the patches according to their demographic value to the metapopulation, as calculated using the dynamics data. Each genetic rank was labeled as correct or incorrect, and this was repeated 1000 times to give the proportion of ranks based on genetic diversity data that were correct (for code see A:\APP3\ANALYST.PAS on the accompanying disc). This analysis was performed separately for each of the six measures of genetic diversity.

In some cases the two patches in question will have equal genetic diversity - particularly with integer measures such as the number of polymorphic loci and the number of mitochondrial haplotypes. In these cases the data point was excluded from the analysis. Importantly, this doesn't represent an incorrect guess of patch ranking, but simply non-informative data. This is because the question being asked here is not "Do the two patches differ in value?", but "Which of the two patches is most valuable?". An ecologist trying answer the latter would be unable to use data where the two genetic diversity values are equal. Accordingly, I am not

interested in knowing the proportion of all occasions where genetics correctly ranks two patches, but the proportion *attempted* patch rankings that are correct.

5.9 Comments on the simplifications in MultiPop

MultiPop clearly simplifies a number of biological processes, and as such, would probably provide an inappropriate description of the dynamics or genetics of any real species. At the same time, care was taken to include the general features associated with the real populations. Importantly the model includes stochasticity in a range of demographic processes, as well as environmental disturbance events. Although fecundity and dispersal are described in the model as very simple functions of population size (and mortality described as being independent of population size), this set of demographic properties nonetheless creates density dependence, with population growth varying either side of carrying capacity.

Some aspects of MultiPop's simplicity actually contribute to its value as a tool for asking questions about metapopulations. Many parameters such as carrying capacities and mortality probabilities were fixed for each scenario, and the metapopulation started from the hypothetical situation of a previously large panmictic population being fragmented essentially instantly. These simplifications are likely to have eliminated much of the random variation that may otherwise have scrambled the link between the value of a patch and its genetic diversity. Accordingly, if we do not find a good link between patch value and genetic diversity using the model, it would seem unlikely that such a link would be found in real systems.

Finally, a key reason for maintaining the model's simplicity was to enhance its transparency.

5.10 A key assumption: genetics does not influence demographics

One assumption of MultiPop is that genetic diversity and inbreeding does not influence demographic processes. Clearly if such effects were included, this would strengthen the link between genetic diversity and the demographic value of a patch, for the simple reason that patches with higher genetic diversity (and therefore less inbreeding) would be more resistant

to extinction than patches with low genetic diversity. The main reason for excluding such effects from the model is that the relative importance of genetics to metapopulation dynamics is still hotly debated in the literature (Lande, 1988; Caro and Laurenson, 1994; Caughley, 1994; Mills and Smouse, 1994; Frankham, 1995a). Even if biologists can demonstrate that genetic processes are important in the dynamics of some metapopulations (*e.g.,* Saccheri *et al.*, 1998), the magnitude of such effects may vary considerably between systems (Ralls *et al.*, 1988; Simberloff, 1988). Therefore I took the stance that if genetic diversity data is to provide a robust approach to assessing the demographic value of patches, it must be successful in the baseline scenario of genetics having no impact on demographic processes.

5.11 The following chapter

In the next chapter I present the results of the MultiPop model for the scenarios outlined in Figure 5.9. After initially presenting the results for dynamics and genetics separately, I then link these two aspects of metapopulation biology with an analysis of how well genetics-based ranks estimate the relative demographic value of patches in owl and rodent metapopulations.

CHAPTER 6

Using genetics to rank patches: model results and summary

6.1 Introduction

The model of metapopulation genetics and dynamics used in this thesis (MultiPop) was developed as a simulation model because of the need to include a complex combination of stochastic genetic and demographic processes. Unlike many analytic models whose logic and behaviour is often readily accessible as a set of equations, simulation models may fall into the trap of becoming something of a black box - accepting one data set as input and delivering another set as output. To understand how such a simulation model works, it can be valuable to examine the patterns produced by a number of its component processes. Accordingly, this chapter is structured so that before presenting results of the MultiPop model relevant to the central questions of the thesis, I offer a range of insights into the way the model works, firstly in terms of population dynamics, and then in terms of population genetics. Although the final results are described for a wide range of scenarios (Figure 5.9), many of the initial results used to demonstrate the model's behaviour are restricted to a limited range of all possible scenarios. In some instances I have chosen to only focus on the simple case of a three-patch owl metapopulation where there are no catastrophes and no sex-bias in dispersal. While some of these results can be interpreted quantitatively, some are intended purely to provide a general impression of how the model works. Finally the main results are presented and I focus on the central question of whether genetic information is likely to be useful for determining the relative value of patches in metapopulations.

6.2 Population Dynamics

6.2.1 The population dynamics of individual replicate metapopulations

Starting at one of the model's most basic levels we can look at the dynamics of individual replicate metapopulations (Figures 6.1 to 6.4). Figure 6.1 presents six replicate metapopulations for the scenario of a three-patch owl metapopulation with no catastrophes and no dispersal between patches. Note that the population size trajectories are quite erratic (in this case purely as a result of demographic stochasticity), and that the population with the largest carrying capacity (thickest line) is often, but not always, the last to go extinct. In some instances the metapopulation is still extant 100 years after the model is initiated. Figure 6.2 presents six replicates for the same scenario, except that now there is 100% dispersal (with no sex-bias) between the large and small patches (the very thick and very thin lines respectively). Although it is difficult to appreciate how increased dispersal benefits the large patch, it is clear to see that the small patch is more likely to be occupied by virtue of recolonisation - a feature evident in Figures 6.2.c. and 6.2.e. Also of importance is the existence of a rescue effect, evident in Figure 6.2.d., where the subpopulation in the smallest patch dropped to a single individual before being rescued from extinction by individuals dispersing from the large patch.

The dynamics of rodent populations is quite different to that of the owls. This is evident in Figure 6.3, which shows six replicates for a three-patch rodent metapopulation with no catastrophes and no dispersal between patches (i.e. the equivalent scenario to that for owls in Figure 6.1). The overwhelming feature of the rodent population dynamics is the strong, rapid cycling (every 3 or 4 years) as population size bounces over and under carrying capacity. Given the structure of the model (Chapter 5), a plausible explanation is that each cycle involves the following phases:

(1) One or two years of high fecundity and low dispersal (5%), when the population is below carrying capacity but increasing in size.

(2) A year of high fecundity, followed by high dispersal (30%) as the population exceeds carrying capacity.

(3) Possibly (if the subpopulation is still above carrying capacity) a year of zero fecundity and high dispersal (30%).

(4) A return to phase (1), after phases (2) and (3) have decreased the population below carrying capacity.

As such, the strong cycling in rodent population dynamics is to some extent a function of the model's simplicity, with both fecundity and dispersal being step functions of population size (Figures 5.5 and 5.6). Nonetheless, 4-5 year cycling is known to be a feature of some rodent populations in nature (Finerty, 1980; Heikkila *et al.*, 1994), and is therefore not an entirely unrealistic feature for the model to produce.

Figure 6.4 demonstrates the effect of catastrophes and increased carrying capacities on owl population dynamics. These replicates are for a three-patch owl metapopulation with catastrophes and no dispersal between patches. Thus, the only differences between this and the scenario in Figure 6.1 are (1) the presence of catastrophes (occasional years of 50% mortality on top of normal yearly mortality), and (2) the fact that the carrying capacities (and hence the scale of the y-axis) have been increased four-fold. The effect of catastrophes is clearly evident in the sporadic crashes in population size - as in, for example, the large patch of Figure 6.4.a. at the 15-year mark.

6.2.2 Frequency distributions of the time to global extinction

Moving a level up from the detail of population dynamics, each replicate metapopulation provides us with a time to global extinction (i.e. extinction of all subpopulations). Thus for the replicate in Figure 6.1.c., the time to global extinction is 81 years. From 10,000 replicates, we can obtain a frequency distribution of extinction times. Figure 6.5 shows a set of frequency distributions for the extinction times of a three-patch owl metapopulation with no catastrophes, over varying levels of connectivity. There are two key features to note here:

(1) The time to metapopulation extinction increases as a function of connectivity. Thus, the frequency distributions of extinction times shift to the right hand side as connectivity increases.

(2) The per year metapopulation extinction probabilities are initially low - a feature which is attributable to the model's starting condition of having all patches fully occupied (and therefore less likely to go extinct) at year zero.

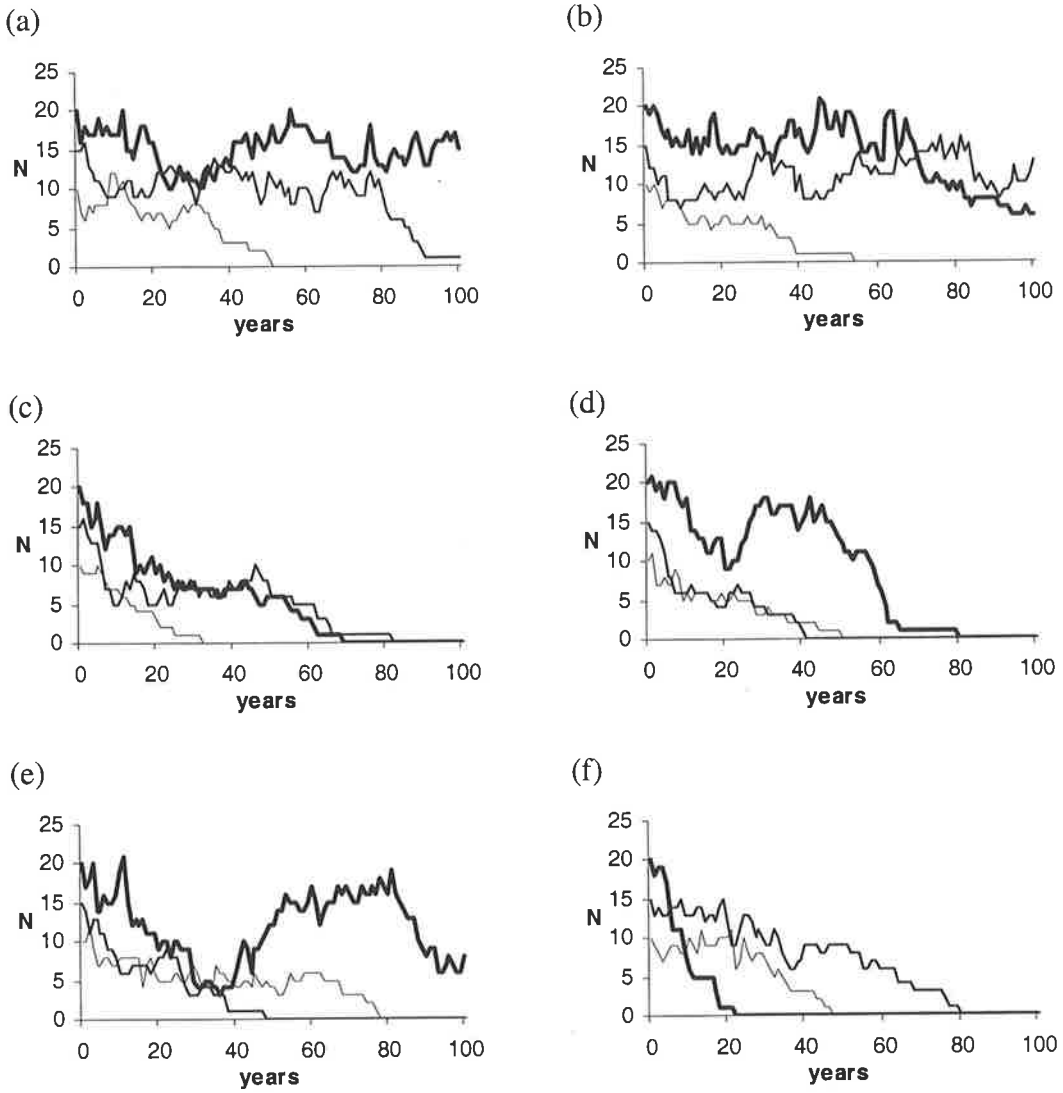

Figure 6.1 Population dynamics of six replicate **three-patch owl metapopulations with no catastrophes and no dispersal**. Each graph shows how population size (N) changes over the first 100 years for each of the three subpopulations. The thickness of each line reflects the carrying capacity of the associated habitat patch: thick (K=20); medium (K=15); thin (K=10).

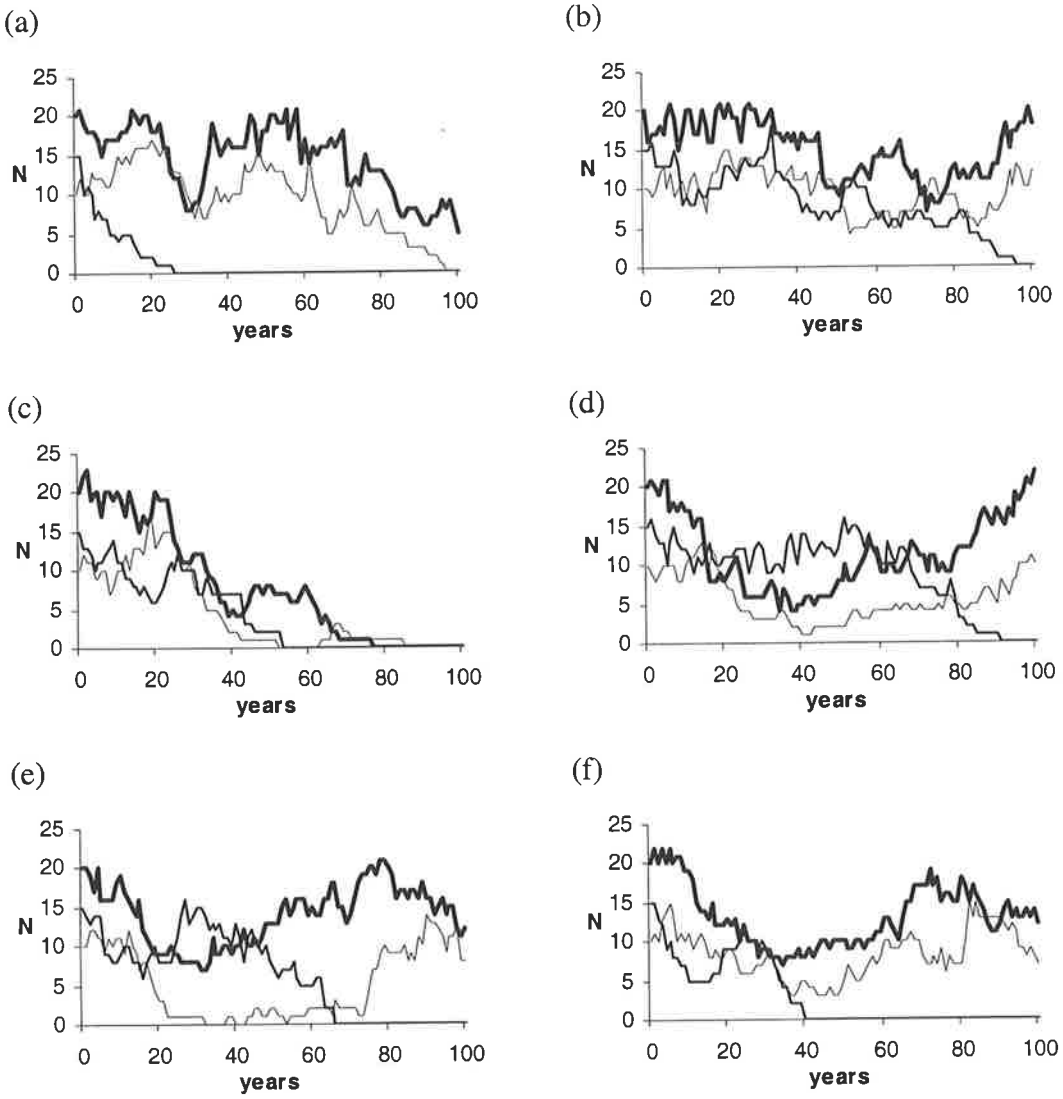

Figure 6.2 Population dynamics of six replicate **three-patch owl metapopulations with no catastrophes, and 100% dispersal between the large and small habitat patches**. Each graph shows how population size (N) changes over the first 100 years for each of the three subpopulations. The thickness of each line reflects the carrying capacity of the associated habitat patch: thick (K=20); medium (K=15); thin (K=10). Note the recolonisation events in (c) and (e), and the rescue from extinction in (d).

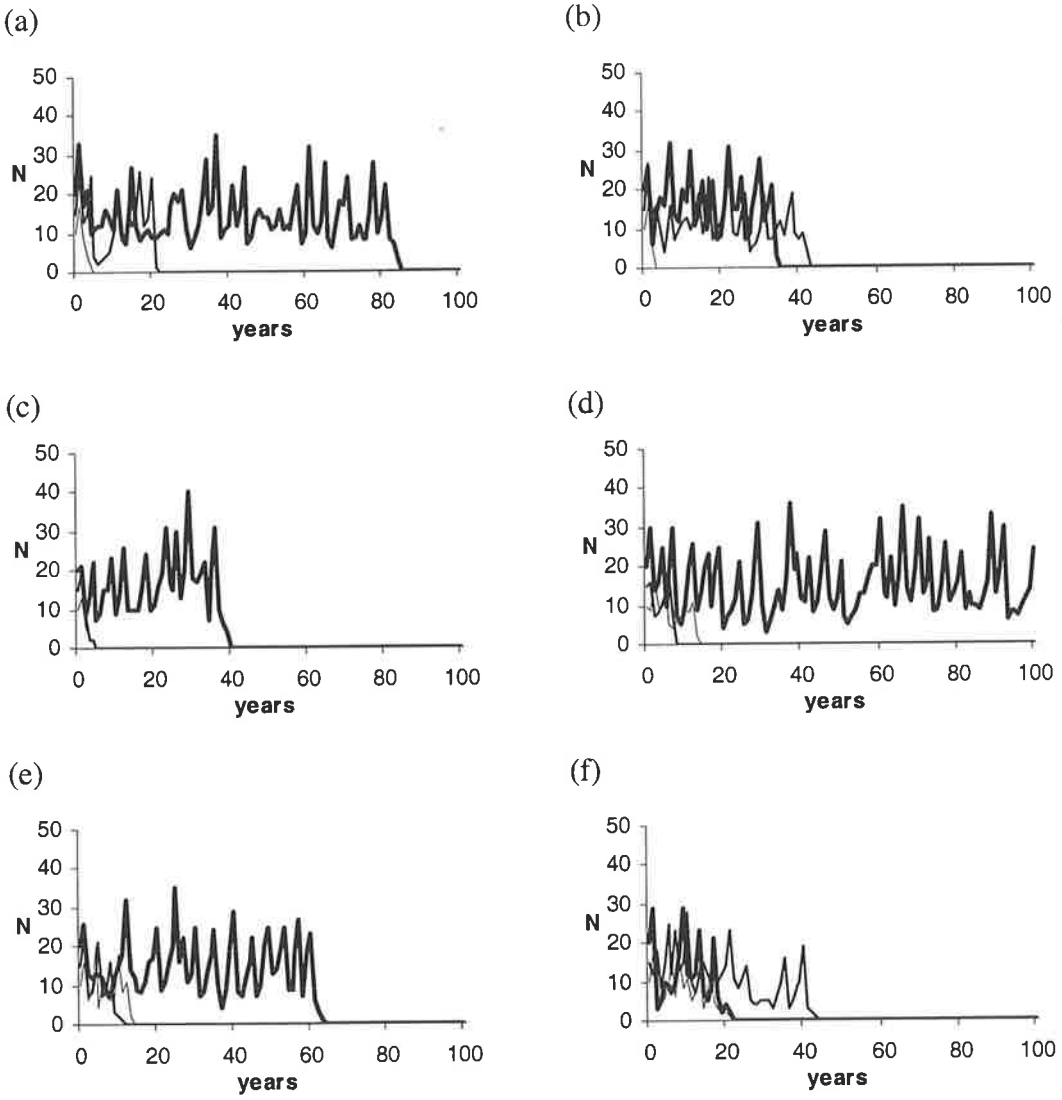

Figure 6.3 Population dynamics of six replicate **three-patch rodent metapopulations with no catastrophes and no dispersal**. Each graph shows how population size (N) changes over the first 100 years for each of the three subpopulations. The thickness of each line reflects the carrying capacity of the associated habitat patch: thick (K=20); medium (K=15); thin (K=10).

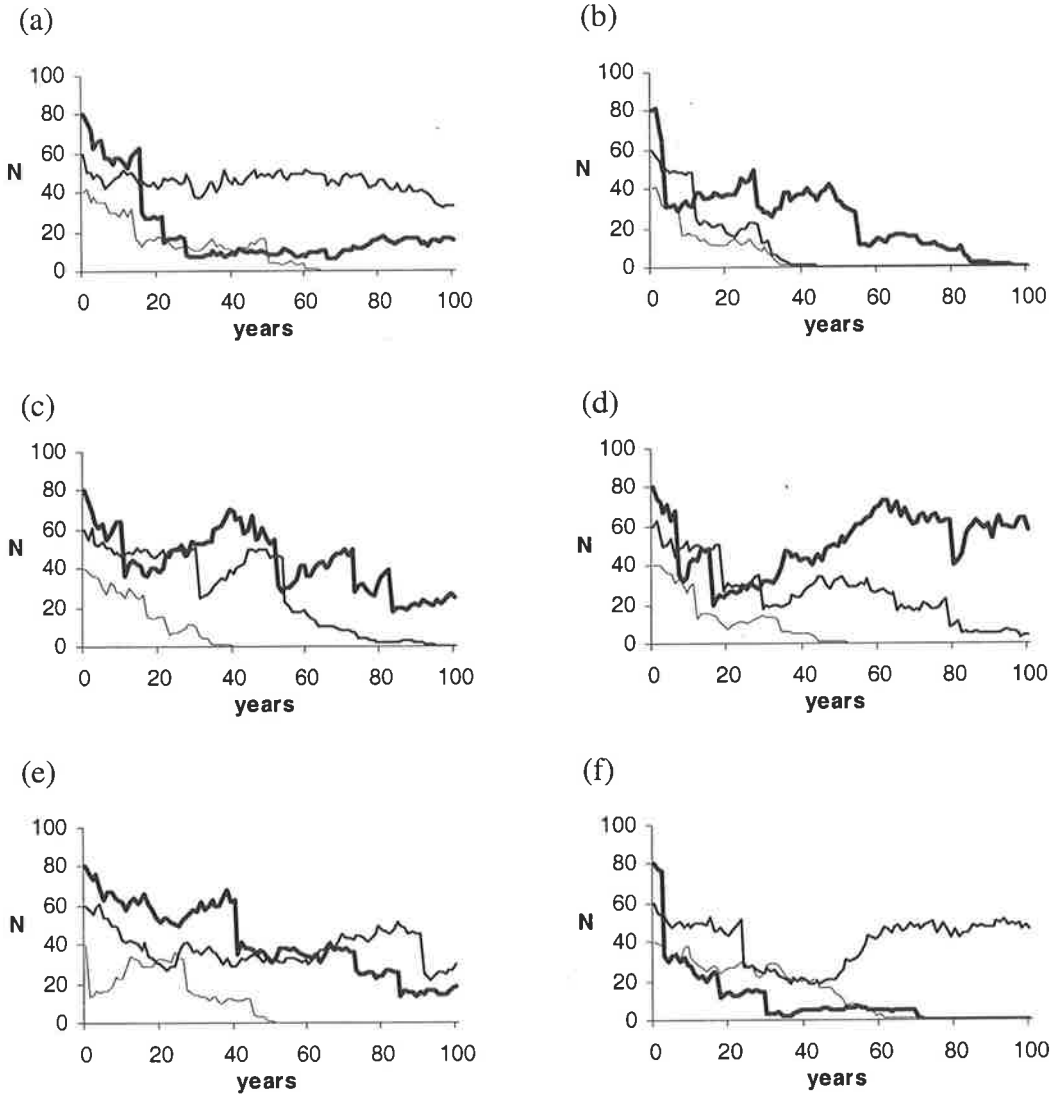

Figure 6.4 Population dynamics of six replicate **three-patch owl metapopulations with catastrophes and no dispersal**. Each graph shows how population size (N) changes over the first 100 years for each of the three subpopulations. The thickness of each line reflects the carrying capacity of the associated habitat patch: thick (K=80); medium (K=60); thin (K=40). Note the large, sudden drops in population size (i.e. catastrophes), as has occurred at the 15-year mark in the large habitat patch in (a).

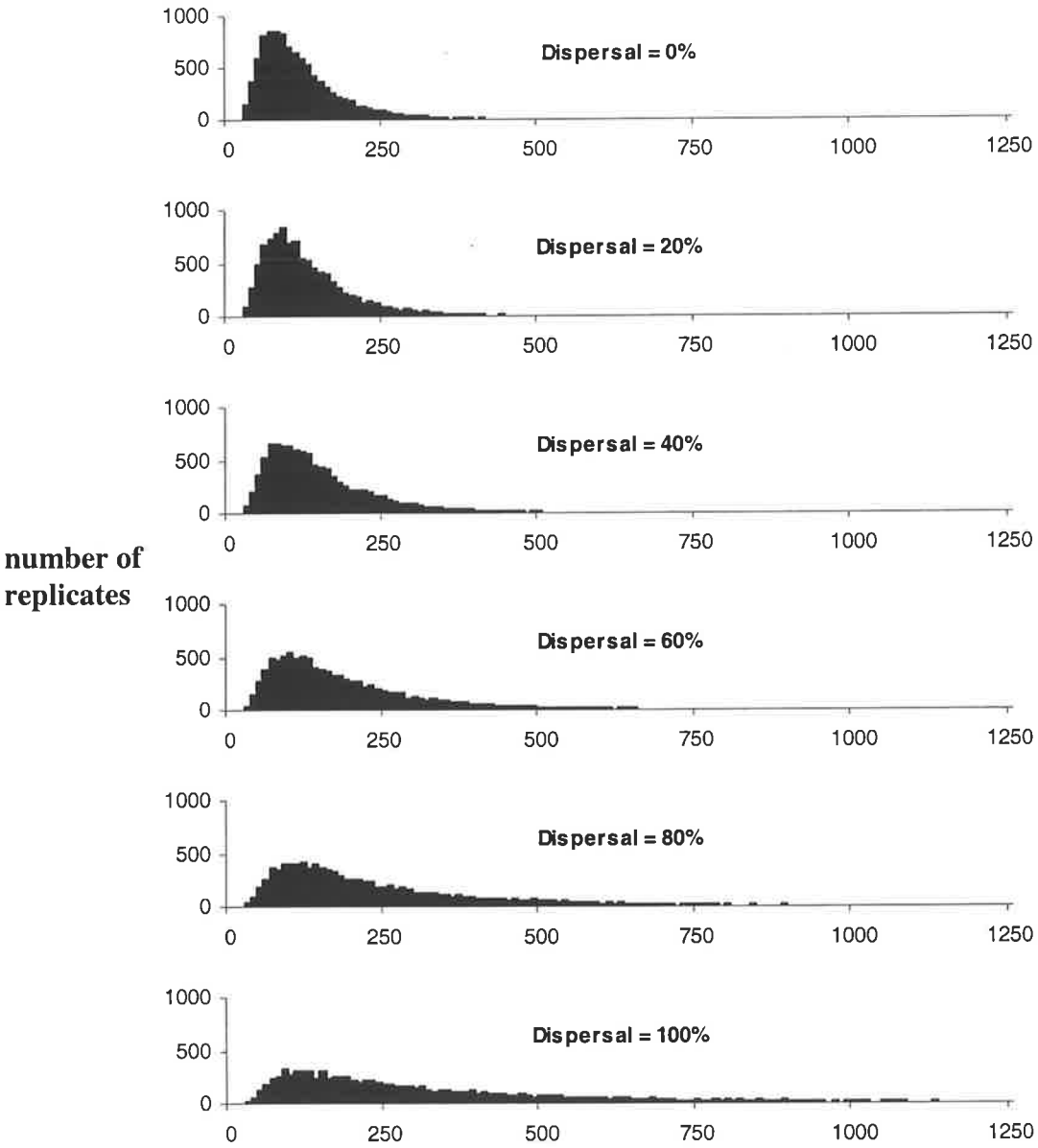

Figure 6.5 Frequency distributions of extinction times for three-patch owl metapopulations with no catastrophes over different connectivity levels. Each distribution is taken from 10,000 replicate metapopulations.

6.2.3 Estimating the probability of metapopulation extinction

Each frequency distribution of extinction times provides the basis for estimating the probability of extinction within some time frame. This is simply calculated as the proportion of values lying to the left of some chosen time, such as 100 years. Thus, in Figure 6.6.a., where a total of 44.6% of all extinction times (shaded values) were less than or equal to 100 years, the estimate of $P[E]_{100}$ is 0.446.

6.2.4 The effect of patch removal

Figure 6.6 demonstrates the effect of patch removal on metapopulation viability for a three-patch owl metapopulation with no catastrophes and no dispersal between patches. The baseline case against which to measure patch removal is the complete three-patch system (Figure 6.6.a.), with $P[E]_{100} = 0.446$. On removing the smallest patch and performing 10,000 simulations, the probability of metapopulation extinction increases very slightly to 0.455 (Figure 6.6.b.). Replacing the small patch and removing the medium patch gives an even higher extinction probability of 0.551 (Figure 6.6.c.), while the large patch is clearly the most important, with its removal causing the extinction probability to increase to 0.761 (Figure 6.6.d.). Using equation 5.5, these extinction probabilities can then be converted into patch value measures. Accordingly, the value of the small, medium and large patches are 0.016, 0.190, and 0.569 respectively. Finally, this can be used to estimate the value of the medium patch relative to the small patch, as 0.190 / 0.016 = 11.9.

Table 6.1 and Figure 6.7 present summary results for the three-patch owl metapopulation with no catastrophes and no dispersal. This includes the results for all six connectivity levels. The very first line of Table 6.1 (connectivity = 0%) is the same data as that in the paragraph above (with slightly different values because significant figures were preserved for the calculations in the table). Note that there are two versions of relative patch value. The version on the left hand side of the relative value measures of Table 6.1 are the same as that described above - calculated as the relative value of the largest patch in a pair over the smallest patch in the pair. In contrast, the version on the right hand side is calculated as the relative value of the most important patch in a pair over the least important patch in the pair. In future these will be referred to as RV1 and RV2 respectively. Thus, if RV1 is less than

one, RV2 equals the inverse of RV1, while if RV1 is greater than or equal to one, RV1 equals RV2.

Tables 6.2 to 6.10 present the equivalent results to Table 6.1, for all other scenarios examined using the model. In the eight-patch metapopulations (Tables 6.9 and 6.10), the connectivity values represent different levels of mortality during dispersal.

6.2.5 Some relative value measures are unreliable

It is important to note that some of the relative value measurements are negative (Tables 6.2, 6.8 and 6.10). Although it is possible to conceive of patches with negative values in nature (*e.g.*, a poor quality sink patch which attracts immigrants but sends out few emigrants), no such mechanisms were included in this model. Therefore these results would seem to be an artifact of sampling error, and indeed there is some indication that this is the case. The problem here is not in our confidence in each $P[E]_{100}$ value *per se*. For example, with 10,000 simulations the 95% confidence interval around an estimated $P[E]_{100}$ of 0.5000 is 0.4902 to 0.5098 (based on Zar (1984)). Instead, the real problem is that relative patch value is calculated as a ratio of the difference between $P[E]_{100}$ values:

$$RV_{AB} = \frac{P[E]_{100[\text{WITHOUT A}]} - P[E]_{100[\text{COMPLETE}]})}{(P[E]_{100[\text{WITHOUT B}]} - P[E]_{100[\text{COMPLETE}]})}$$

This means that the closer two $P[E]_{100}$ values are to each other, the greater the impact of sampling error. To illustrate this point, consider the data in the second row of Table 6.2 (a three-patch rodent metapopulation, with 20% dispersal), where the relative value of the medium patch over the small patch is reported as -0.01. This in turn is attributable to the very small yet negative value of the medium patch of -0.0035. The problem here is that the value of this patch was calculated as:

$$\text{Value of medium patch} = \frac{(P[E]_{100[\text{WITHOUT P15}]} - P[E]_{100[\text{COMPLETE}]})}{(1 - P[E]_{100[\text{COMPLETE}]})}$$

$$= \frac{(0.6519 - 0.6531)}{(1 - 0.6531)}$$

Thus, the difference between the two proportions in the numerator (0.0012) involved only 12 replicate metapopulations out of 10,000. Clearly the potential effects of sampling error in this case gives considerable cause for concern. For this reason I have chosen to exclude from future analyses all cases where the relative value measure involved a difference of less than 0.01 (i.e. 100 replicate metapopulations out of 10,000). These are printed in bold in Tables 6.1 to 6.10. Although this is a rather crude approach for which there may be more elegant alternatives, this criterion was successful in excluding all negative patch value measures, as well as many suspiciously large positive values where sampling error may have been equally misleading (*e.g.*, Tables 6.2, 6.6 and 6.8). Unfortunately the effect of this exclusion was that some scenarios are poorly represented, particularly among rodent metapopulations (Tables 6.2, 6.8 and 6.10).

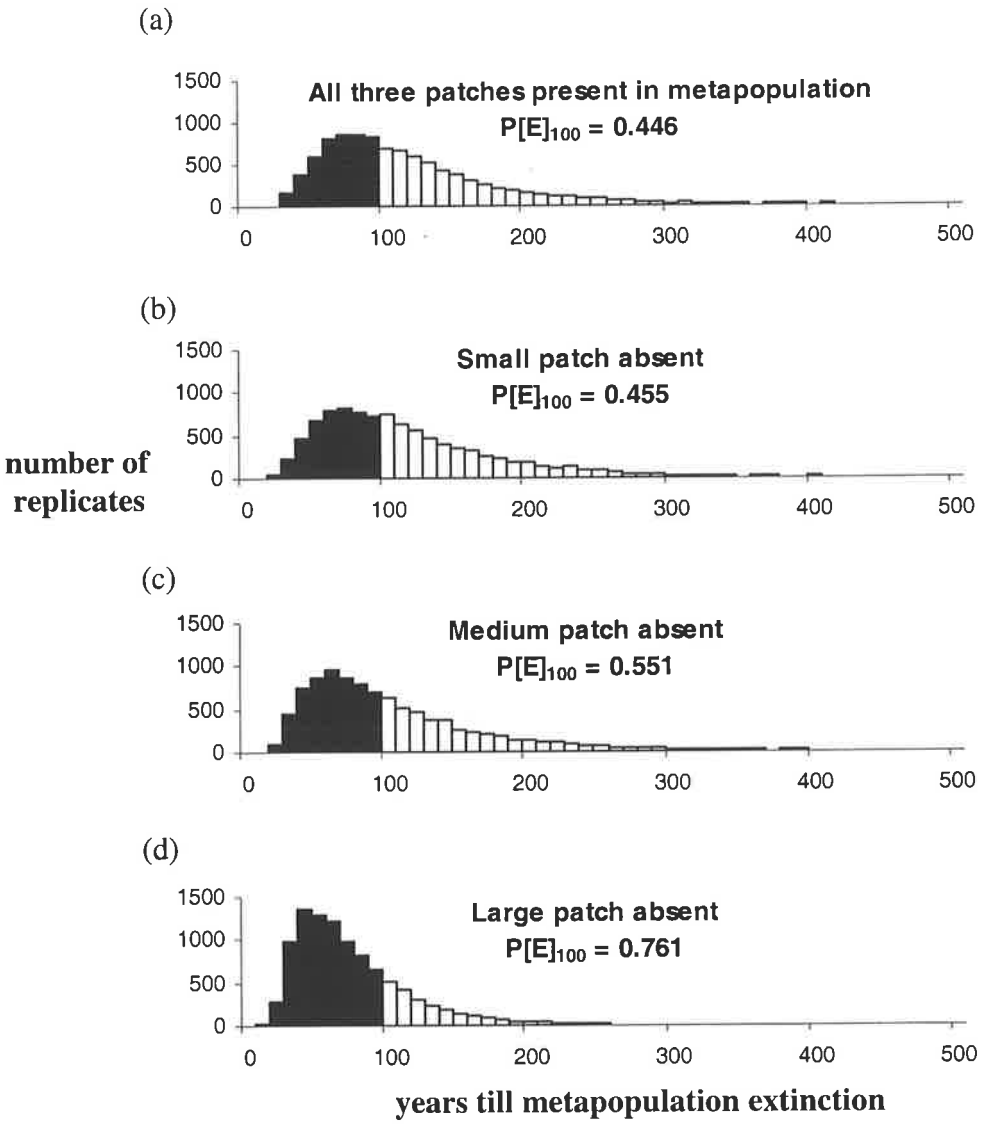

Figure 6.6 The effect of patch removal on the 100-year extinction probability of a three-patch owl metapopulation with no catastrophes and no dispersal. The 100-year extinction probability ($P[E]_{100}$) is calculated as the proportion of each frequency distribution to the left of the 100-year mark (shaded region). Note how patch removal increases $P[E]_{100}$.

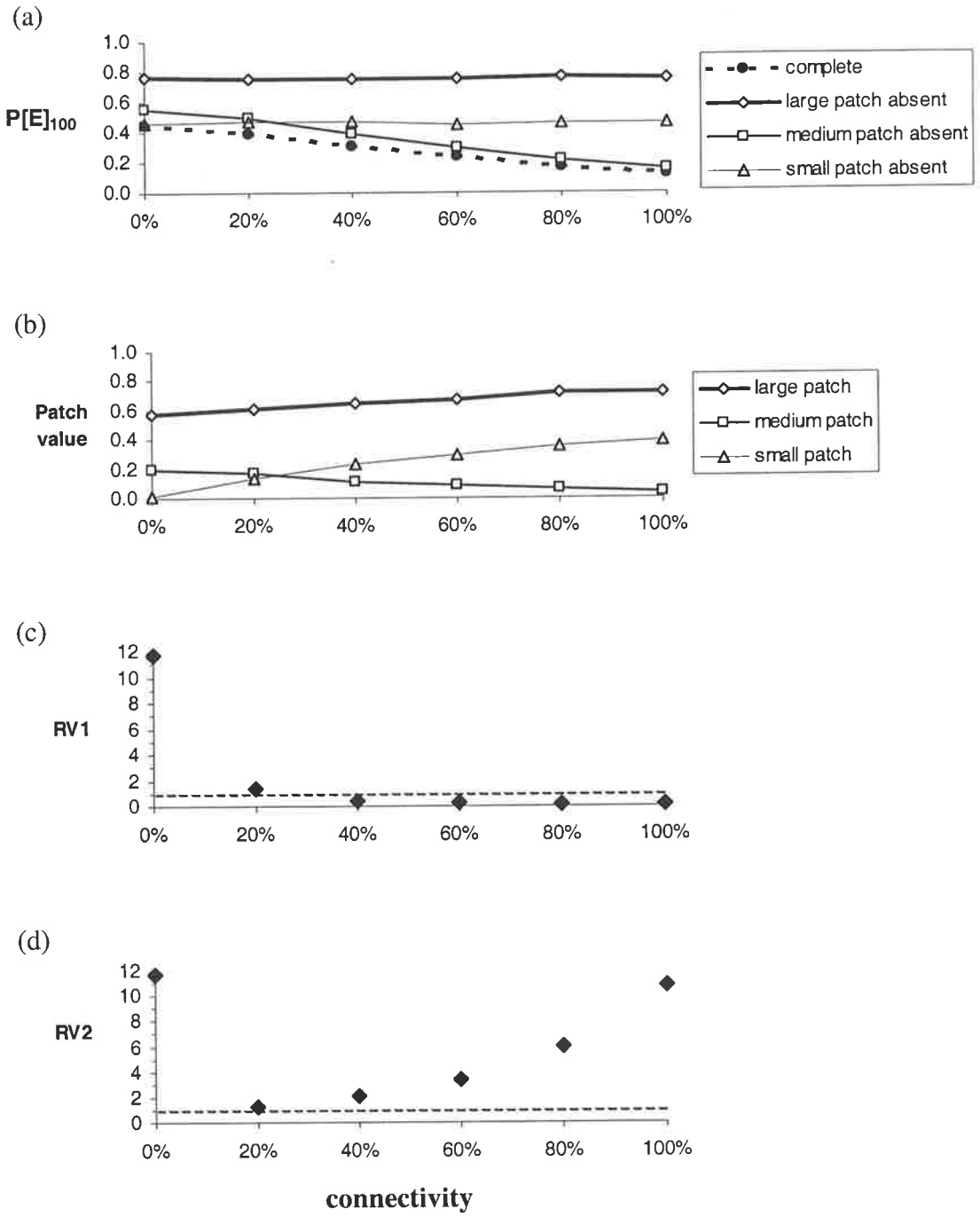

Figure 6.7 Summary results for three-patch owl metapopulations with no catastrophes, over various levels of unbiased dispersal. (a) Probability of extinction within 100 years under different scenarios of patch removal. (b) The value of each of the three patches (calculated using the data in (a)). (c) RV1 - the relative value of the medium patch to the small patch. (d) RV2 - same as RV1 but expressed as the most valuable patch relative to the least valuable patch in the pair. The hashed line in (c) and (d) shows where RV equals one.

Table 6.1 Summary data for 3-patch owl metapopulation ; no catastrophes ; unbiased dispersal.

	$P[E]_{100}$				Patch value			Relative value (P_{15} : P_{10})	
Conn.	Complete	P_{20}	P_{15}	P_{10}	P_{20}	P_{15}	P_{10}		
0%	0.4458	0.7611	0.5513	0.4548	0.5689	0.1904	0.0162	**11.72**	**11.72**
20%	0.3857	0.7576	0.4898	0.4650	0.6054	0.1695	0.1291	1.31	1.31
40%	0.3117	0.7565	0.3873	0.4684	0.6462	0.1098	0.2277	0.48	2.07
60%	0.2315	0.7493	0.2954	0.4519	0.6738	0.0831	0.2868	0.29	3.45
80%	0.1657	0.7618	0.2145	0.4602	0.7145	0.0585	0.3530	0.17	6.03
100%	0.1209	0.7560	0.1521	0.4598	0.7224	0.0355	0.3855	0.09	10.86

Table 6.2 Summary data for 3-patch rodent metapopulation ; no catastrophes ; unbiased dispersal.

	$P[E]_{100}$				Patch value			Relative value (P_{15} : P_{10})	
Conn.	Complete	P_{20}	P_{15}	P_{10}	P_{20}	P_{15}	P_{10}		
0%	0.8076	0.9846	0.8266	0.8101	0.9200	0.0988	0.0130	**7.60**	**7.60**
20%	0.6531	0.9845	0.6519	0.8147	0.9553	-0.0035	0.4658	**-0.01**	**-134.67**
40%	0.4781	0.9850	0.4726	0.8209	0.9713	-0.0105	0.6568	**-0.02**	**-62.33**
60%	0.4054	0.9843	0.4087	0.8135	0.9736	0.0055	0.6863	**0.01**	**123.67**
80%	0.3675	0.9855	0.3709	0.8139	0.9771	0.0054	0.7058	**0.01**	**131.29**
100%	0.3486	0.9851	0.3531	0.8121	0.9771	0.0069	0.7115	**0.01**	**103.00**

- Conn. = connectivity level.
- **$P[E]_{100}$** = probability of metapopulation extinction within 100 years.
- "Complete" means the metapopulation contains all patches.
- "P_{20}" under the **$P[E]_{100}$** heading means the metapopulation is missing the patch which has a carrying capacity of 20 individuals.
- "P_{20}" under the **Patch value** heading refers to the value of that patch.
- "(P_{15} : P_{10})" refers to the relative value of these two patches - the left hand column is RV1 (the value of the large patch relative to the small patch), while the right hand column is RV2 (the value of the most important patch relative to the least important in the pair of patches).

Table 6.3 Summary data for 3-patch owl metapopulation ; no catastrophes ; female-biased dispersal

	$P[E]_{100}$				Patch value			Relative value	
Conn.	Complete	P_{20}	P_{15}	P_{10}	P_{20}	P_{15}	P_{10}	(P_{15} : P_{10})	
0%	0.3277	0.6634	0.4641	0.3625	0.4993	0.2029	0.0518	3.92	3.92
20%	0.2952	0.6619	0.4059	0.3579	0.5203	0.1571	0.0890	1.77	1.77
40%	0.2393	0.6770	0.3298	0.3622	0.5754	0.1190	0.1616	0.74	1.36
60%	0.1950	0.6707	0.2703	0.3618	0.5909	0.0935	0.2072	0.45	2.22
80%	0.1532	0.6647	0.2061	0.3634	0.6040	0.0625	0.2482	0.25	3.97
100%	0.1174	0.6700	0.1635	0.3668	0.6261	0.0522	0.2826	0.18	5.41

Table 6.4 Summary data for 3-patch rodent metapopulation ; no catastrophes ; male-biased dispersal.

	$P[E]_{100}$				Patch value			Relative value	
Conn.	Complete	P_{20}	P_{15}	P_{10}	P_{20}	P_{15}	P_{10}	(P_{15} : P_{10})	
0%	0.7230	0.9692	0.7530	0.7317	0.8888	0.1083	0.0314	**3.45**	**3.45**
20%	0.6731	0.9655	0.6942	0.7268	0.8945	0.0645	0.1643	0.39	2.55
40%	0.5825	0.9611	0.6015	0.7286	0.9068	0.0455	0.3499	0.13	7.69
60%	0.5261	0.9646	0.5462	0.7289	0.9253	0.0424	0.4279	0.10	10.09
80%	0.4898	0.9655	0.5111	0.7296	0.9324	0.0417	0.4700	0.09	11.26
100%	0.4739	0.9642	0.4964	0.7280	0.9320	0.0428	0.4830	0.09	11.29

Table 6.5 Summary data for 3-patch owl metapopulation ; catastrophes ; unbiased dispersal.

	$P[E]_{100}$				Patch value			Relative value ($P_{15} : P_{10}$)	
Conn.	Complete	P_{20}	P_{15}	P_{10}	P_{20}	P_{15}	P_{10}		
0%	0.2953	0.5501	0.4480	0.3735	0.3616	0.2167	0.1110	1.95	1.95
20%	0.2471	0.5439	0.3715	0.3793	0.3942	0.1652	0.1756	0.94	1.06
40%	0.1884	0.5541	0.2801	0.3701	0.4506	0.1130	0.2239	0.50	1.98
60%	0.1382	0.5384	0.2110	0.3768	0.4644	0.0845	0.2769	0.31	3.28
80%	0.0969	0.5492	0.1455	0.3707	0.5008	0.0538	0.3032	0.18	5.63
100%	0.0702	0.5452	0.1052	0.3703	0.5109	0.0376	0.3228	0.12	8.57

Table 6.6 Summary data for 3-patch rodent metapopulation ; catastrophes ; unbiased dispersal.

	$P[E]_{100}$				Patch value			Relative value ($P_{15} : P_{10}$)	
Conn.	Complete	P_{20}	P_{15}	P_{10}	P_{20}	P_{15}	P_{10}		
0%	0.9588	0.9792	0.9756	0.9691	0.4951	0.4078	0.2500	1.63	1.63
20%	0.4847	0.9819	0.4911	0.9726	0.9649	0.0124	0.9468	**0.01**	**76.23**
40%	0.3507	0.9799	0.3557	0.9709	0.9690	0.0077	0.9552	**0.01**	**124.04**
60%	0.3199	0.9817	0.3312	0.9727	0.9731	0.0166	0.9599	0.02	57.77
80%	0.3303	0.9810	0.3431	0.9699	0.9716	0.0191	0.9551	0.02	49.97
100%	0.3440	0.9787	0.3479	0.9725	0.9675	0.0059	0.9581	**0.01**	**161.15**

Table 6.7 Summary data for 3-patch owl metapopulation ; catastrophes ; female-biased dispersal.

	$P[E]_{100}$				Patch value			Relative value	
Conn.	Complete	P_{20}	P_{15}	P_{10}	P_{20}	P_{15}	P_{10}	(P_{15} : P_{10})	
0%	0.2245	0.4620	0.3719	0.2947	0.3063	0.1901	0.0905	2.10	2.10
20%	0.1846	0.4587	0.3125	0.3056	0.3362	0.1569	0.1484	1.06	1.06
40%	0.1420	0.4536	0.2438	0.3000	0.3632	0.1186	0.1841	0.64	1.55
60%	0.1093	0.4635	0.1830	0.2952	0.3977	0.0827	0.2087	0.40	2.52
80%	0.0888	0.4541	0.1506	0.2901	0.4009	0.0678	0.2209	0.31	3.26
100%	0.0758	0.4570	0.1176	0.2907	0.4125	0.0452	0.2325	0.19	5.14

Table 6.8 Summary data for 3-patch rodent metapopulation ; catastrophes ; male-biased dispersal.

	$P[E]_{100}$				Patch value			Relative value	
Conn.	Complete	P_{20}	P_{15}	P_{10}	P_{20}	P_{15}	P_{10}	(P_{15} : P_{10})	
0%	0.9543	0.9756	0.9701	0.9620	0.4661	0.3457	0.1685	**2.05**	**2.05**
20%	0.6483	0.9755	0.6542	0.9617	0.9303	0.0168	0.8911	**0.02**	**53.12**
40%	0.4887	0.9752	0.4931	0.9616	0.9515	0.0086	0.9249	**0.01**	**107.48**
60%	0.4348	0.9758	0.4354	0.9631	0.9572	0.0011	0.9347	**0.00**	**880.50**
80%	0.4218	0.9752	0.4213	0.9588	0.9571	-0.0009	0.9287	**0.00**	**-1074.00**
100%	0.4121	0.9752	0.4248	0.9671	0.9578	0.0216	0.9440	0.02	43.70

Table 6.9 Summary data for 8-patch owl metapopulation ; catastrophes ; female-biased dispersal. The connectivity levels are expressed in terms of dispersal mortality.

					$P[E]_{100}$				
Conn.	Complete	P_{15}	P_{20}	P_{25}	P_{30}	P_{35}	P_{40}	P_{45}	P_{50}
0.0800	0.1770	0.1843	0.1899	0.2040	0.2146	0.2433	0.2300	0.2420	0.2776
0.0400	0.1376	0.1550	0.1629	0.1731	0.1868	0.2042	0.1985	0.2029	0.2523
0.0200	0.0889	0.1097	0.1003	0.1254	0.1294	0.1555	0.1473	0.1560	0.1965
0.0100	0.0457	0.0671	0.0655	0.0767	0.0852	0.1086	0.1035	0.1089	0.1450
0.0050	0.0319	0.0463	0.0463	0.0552	0.0628	0.0709	0.0778	0.0800	0.0983
0.0025	0.0212	0.0327	0.0375	0.0409	0.0513	0.0528	0.0636	0.0636	0.0760

				Patch value				
Conn.	P_{15}	P_{20}	P_{25}	P_{30}	P_{35}	P_{40}	P_{45}	P_{50}
0.0800	0.0089	0.0157	0.0328	0.0457	0.0806	0.0644	0.0790	0.1222
0.0400	0.0202	0.0293	0.0412	0.0571	0.0772	0.0706	0.0757	0.1330
0.0200	0.0228	0.0125	0.0401	0.0445	0.0731	0.0641	0.0736	0.1181
0.0100	0.0224	0.0207	0.0325	0.0414	0.0659	0.0606	0.0662	0.1041
0.0050	0.0149	0.0149	0.0241	0.0319	0.0403	0.0474	0.0497	0.0686
0.0025	0.0117	0.0167	0.0201	0.0308	0.0323	0.0433	0.0433	0.0560

	Relative value			
Conn.	$(P_{50} : P_{20})$	$(P_{45} : P_{15})$	$(P_{30} : P_{25})$	$(P_{40} : P_{35})$
0.0800	7.80 7.80	**8.90 8.90**	1.39 1.39	0.80 1.25
0.0400	4.53 4.53	3.75 3.75	1.39 1.39	0.91 1.09
0.0200	9.44 9.44	3.23 3.23	1.11 1.11	0.88 1.14
0.0100	5.02 5.02	2.95 2.95	1.27 1.27	0.92 1.09
0.0050	4.61 4.61	3.34 3.34	1.33 1.33	1.18 1.18
0.0025	3.36 3.36	3.69 3.69	1.53 1.53	1.34 1.34

Table 6.10 Summary data for 8-patch rodent metapopulation ; catastrophes ; male-biased dispersal. The connectivity levels are expressed in terms of dispersal mortality.

					$P[E]_{100}$				
Conn.	Complete	P_{15}	P_{20}	P_{25}	P_{30}	P_{35}	P_{40}	P_{45}	P_{50}
0.12	0.9083	0.9313	0.9099	0.9199	0.9146	0.9491	0.9095	0.9125	0.9545
0.10	0.8854	0.9223	0.8807	0.8936	0.8944	0.9430	0.8975	0.8881	0.9486
0.08	0.8336	0.8905	0.8364	0.8662	0.8492	0.9358	0.8455	0.8406	0.9347
0.06	0.7297	0.8218	0.7271	0.7976	0.7723	0.9029	0.7438	0.7456	0.9100
0.04	0.4541	0.6195	0.4810	0.6246	0.5920	0.7854	0.5293	0.5065	0.8163
0.02	0.0669	0.1317	0.0835	0.1747	0.1578	0.2766	0.1616	0.1330	0.3394

Patch value

Conn.	P_{15}	P_{20}	P_{25}	P_{30}	P_{35}	P_{40}	P_{45}	P_{50}
0.12	0.2508	0.0174	0.1265	0.0687	0.4449	0.0131	0.0458	0.5038
0.10	0.3220	-0.0410	0.0716	0.0785	0.5026	0.1056	0.0236	0.5515
0.08	0.3419	0.0168	0.1959	0.0937	0.6142	0.0715	0.0421	0.6076
0.06	0.3407	-0.0096	0.2512	0.1576	0.6408	0.0522	0.0588	0.6670
0.04	0.3030	0.0493	0.3123	0.2526	0.6069	0.1378	0.0960	0.6635
0.02	0.0694	0.0178	0.1155	0.0974	0.2247	0.1015	0.0708	0.2920

Relative value

Conn.	$(P_{50} : P_{20})$		$(P_{45} : P_{15})$		$(P_{30} : P_{25})$		$(P_{40} : P_{35})$	
0.12	**28.87**	**28.87**	**0.18**	**5.48**	**0.54**	**1.84**	**0.03**	**34.00**
0.10	**-13.45**	**-13.45**	**0.07**	**13.67**	**1.10**	**1.10**	0.21	4.76
0.08	**36.11**	**36.11**	**0.12**	**8.13**	0.48	2.09	0.12	8.59
0.06	**-69.35**	**-69.35**	0.17	5.79	0.63	1.59	0.08	12.28
0.04	13.46	13.46	0.32	3.16	0.81	1.24	0.23	4.41
0.02	16.42	16.42	1.02	1.02	0.84	1.19	0.45	2.21

6.2.6 Comparison of extinction probabilites among all metapopulations

The degree of connectivity between subpopulations had a large effect on the viability of both the owl and rodent metapopulations. This is evident in Figure 6.8, which is a summary of all $P[E]_{100[COMPLETE]}$ values in Tables 6.1 to 6.10 (i.e. the left-most column in each table). One interesting feature of this figure is that rodent metapopulations experienced an extinction threshold effect, whereby extinction probability suddenly increased below critical levels of connectivity. If connectivity decreases as a function of inter-patch distance in real systems, this suggests that a similar extinction threshold might occur as a function of patch density. In other words rodent metapopulations may be sensitive to critical levels of patch density.

A similar extinction threshold effect was reported by Lande (1988), with a model that suggested minimum threshold levels of suitable habitat are required for the persistence of spotted owl metapopulations. It is interesting that a similar effect was not found for the owl metapopulations modelled in this thesis (Figure 6.8). While the basis for this discrepancy is unclear, the scale of fragmentation may somehow be important for achieving a threshold effect in owls, given that Lande (1988) modelled habitat patches at the scale of individual territories. The occurrence of extinction threshold effects is certainly worth further study, and may shed light on the apparent bimodal distribution of patch occupancy reported in many natural metapopulations (Hanski, 1982). In this regard it would be valuable to know if organisms with particular life histories are more susceptible to critical levels of fragmentation than other organisms.

The metapopulations modelled here have relatively high 100-year extinction probabilities by conservation standards (Figure 6.8). This is particularly true for the rodent metapopulations, many of which have 100-year extinction probabilities greater than 0.3, and some greater than 0.8. It is also important to recognise however, that these extinction probabilities are not dissimilar to the expectations for many of the world's endangered populations and species (*e.g.,* Maguire *et al.*, 1995; Green *et al.*, 1996; Hiraldo *et al.*, 1996; Gaona *et al.*, 1998). Although it would have been valuable to examine metapopulations with lower extinction probabilities, this would have required larger carrying capacities, which in turn would have required computing speed and memory beyond that which was available for this thesis.

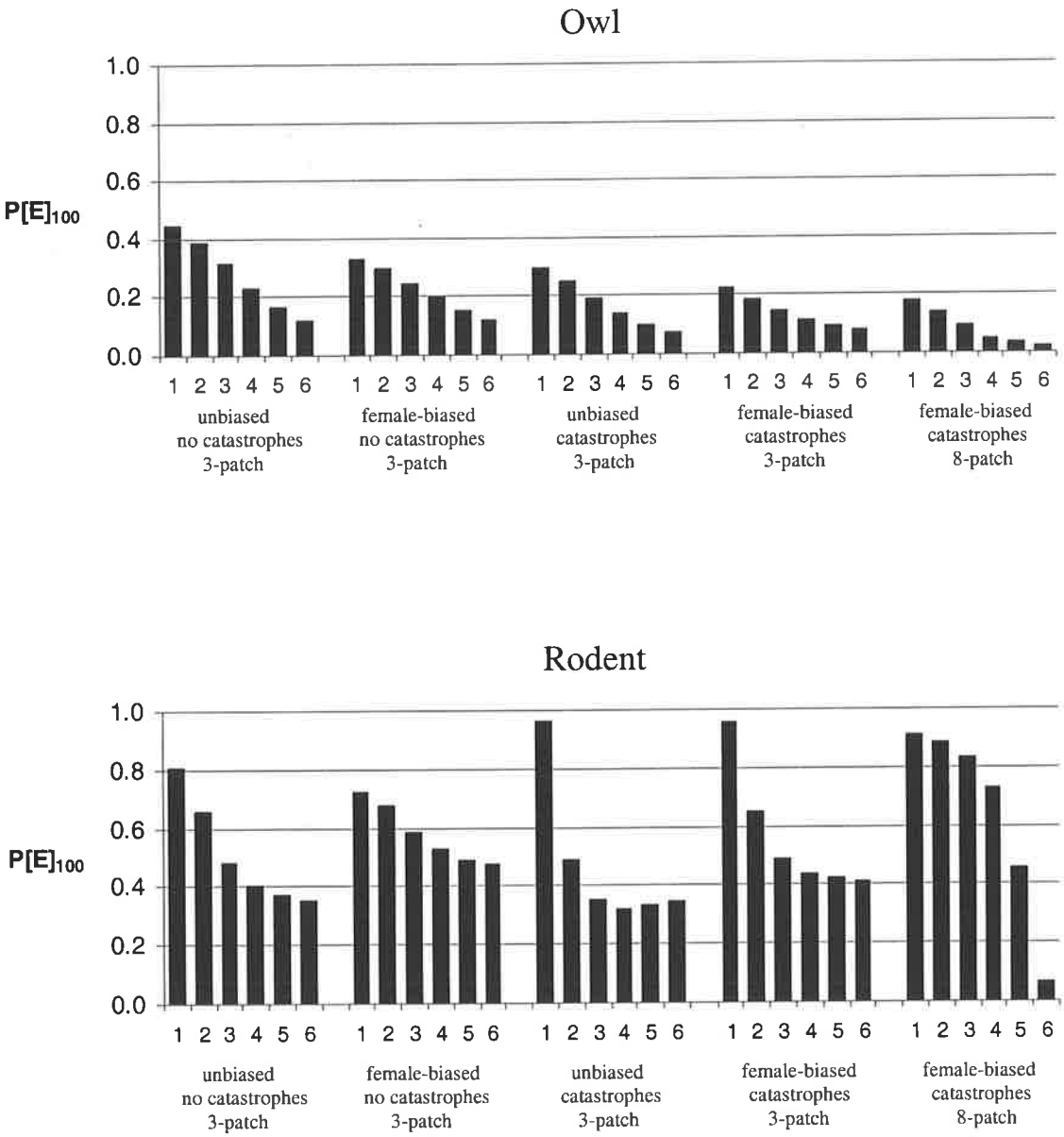

Figure 6.8 Comparison of 100-year extinction probabilities for all complete metapopulations (i.e. not missing any patches). Values 1 to 6 on the x-axis correspond to increasing levels of metapopulation connectivity (refer to Tables 6.1 to 6.10 for the details of these connectivity levels).

6.2.7 The impact of initial conditions on patch value estimates

The main purpose of using the MultiPop model in this thesis was to assess whether genetic diversity data, as measured at different times after a system's fragmentation, provides a good estimate of the relative value of different habitat patches. Later I make this assessment for genetics-based rankings of patch value that are made 5, 10, 20, 40 and 80 years after fragmentation. If we define viability over a 100-year time frame, a biologist measuring the genetic diversity of a metapopulation 40 years after fragmention would like to know the value of patches over the *next* 100 years. Accordingly, the patch value estimates for the 40-year sampling time should ideally be based on extinction probabilities measured from 40 to 140 years after fragmentation.

In contrast, all patch value estimates used in this thesis are based on extinction probabilities measured from 0 to 100 years after fragmentation (Tables 6.1 to 6.10). If patch extinction probabilities are constant over time, this discrepancy will have no effect; however, as shown earlier (Figure 6.5), the metapopulations modelled by MultiPop do have an initially low extinction probability due to the starting condition of all patches being fully occupied to carrying capacity. To some extent this may bias the estimates of relative patch value.

The ideal approach would be to determine separate patch value estimates for each genetic sampling time (5, 10, 20, 40 and 80 years), using each of these as the starting point from which the time to extinction is measured. However this was not possible because of the extremely long computer run times required. For example consider an eight patch metapopulation where we want to measure the 100-year extinction probability starting from 80 years after fragmentation. In this case the chance of obtaining metapopulations with all patches occupied at the 80-year mark is so low that the vast majority of the program's run time is wasted on replicates which end up being discarded after 80 years. Instead, I have chosen to use the patch value estimates above (Tables 6.1 to 6.10) as the best estimates of patch value available. The assumption being made here is that these measurements provide a reasonable estimate of the value of a patch in maintaining metapopulation persistence over the 100 years following the genetic sample. Nonetheless it is important to keep in mind that these estimates may include some bias due to the potential effect of initial conditions.

6.2.8 The time frame for extinction probabilities: 100 years

Although patch values were calculated using 100-year extinction probabilities, any time frame could have been used. The 100-year criterion was used here mainly to be in keeping with the conservation literature (Mace and Lande, 1991; Day and Possingham, 1995; Maguire *et al.*, 1995; Green *et al.*, 1996; Hiraldo *et al.*, 1996). While a longer time frame will clearly increase extinction probabilities, it may also have some impact on patch value estimates. This is evident in Table 6.11 where values based on both 100 and 200-year extinction probabilities are given for a three-patch owl metapopultion with no catastrophes and 20% dispersal.

Table 6.11 A demonstration of how the time frame used to measure extinction probabilities affects patch value estimates for a 3-patch owl metapopulation with no catastrophes and 20% dispersal between patches. Here, extinction probabilities (and hence patch value estimates) are based on two different time frames (100 and 200 years after the model is initiated). See the box under Table 6.2 for further explanation of column headings.

OWL : 3-PATCH : UNBIASED DISPERSAL : NO CATASTROPHES: CONN. = 20%

	P[E]				Patch value			Relative value	
Time	Complete	P_{20}	P_{15}	P_{10}	P_{20}	P_{15}	P_{10}	(P_{15} : P_{10})	
100 yr	0.3857	0.7576	0.4898	0.4650	0.6054	0.1695	0.1291	1.31	1.31
200 yr	0.8429	0.9776	0.8648	0.8696	0.8574	0.1394	0.1700	0.82	1.22

6.2.9 Summary of relative patch value estimates

Figure 6.9 shows, for owls and rodents, how RV1 (the relative value of the large patch over the small patch in a patch pair) changes with connectivity for each of the five scenarios modelled. Any value above 1 indicates that the largest patch was the most valuable in the pair of patches. Remember that in the three-patch scenario, the comparison being made is between an isolated medium-sized patch and a small patch connected by dispersal to a large patch (section 5.4 and Fig. 5.7). Notice that for all three-patch metapopulations, the relative value of the medium patch over the small patch decreases with connectivity. This is entirely

in keeping with the prediction that increased connectivity between the small and large patches would increase the value of the small patch over the isolated medium patch.

The changes in RV1 are more complex for the eight-patch metapopulations (bottom scenarios), with patch value changing erratically as a function of connectivity. Although these changes in patch value may in part be due to sampling error, it could also be the case that they are somehow attributable to subtle changes in the web of interaction involved in these eight-patch metapopulations. This interesting feature of metapopulation dynamics is potentially worth further study.

Note: some scenarios are poorly represented because of missing data (bold values in Tables 6.1 to 6.10), with no data at all for the top-most rodent scenario.

Figure 6.10 shows how RV2 values change with connectivity. Remember that this is the relative value of the most valuable patch over the least valuable patch in a pair of patches. As with RV1, any value close to 1 means that two patches in a pair were very similar in value to the metapopulation. However, large RV2 values may be due not only to differences in patch size (as for RV1) but also due to differences in connectivity. Accordingly the high RV2 values on the left hand side of these graphs (i.e. low or zero connectivity) represent differences in patch value due to patch size, while high RV2 values on the right hand side (i.e. high connectivity) represent differences due to one of the patches being well connected to another patch (or set of patches).

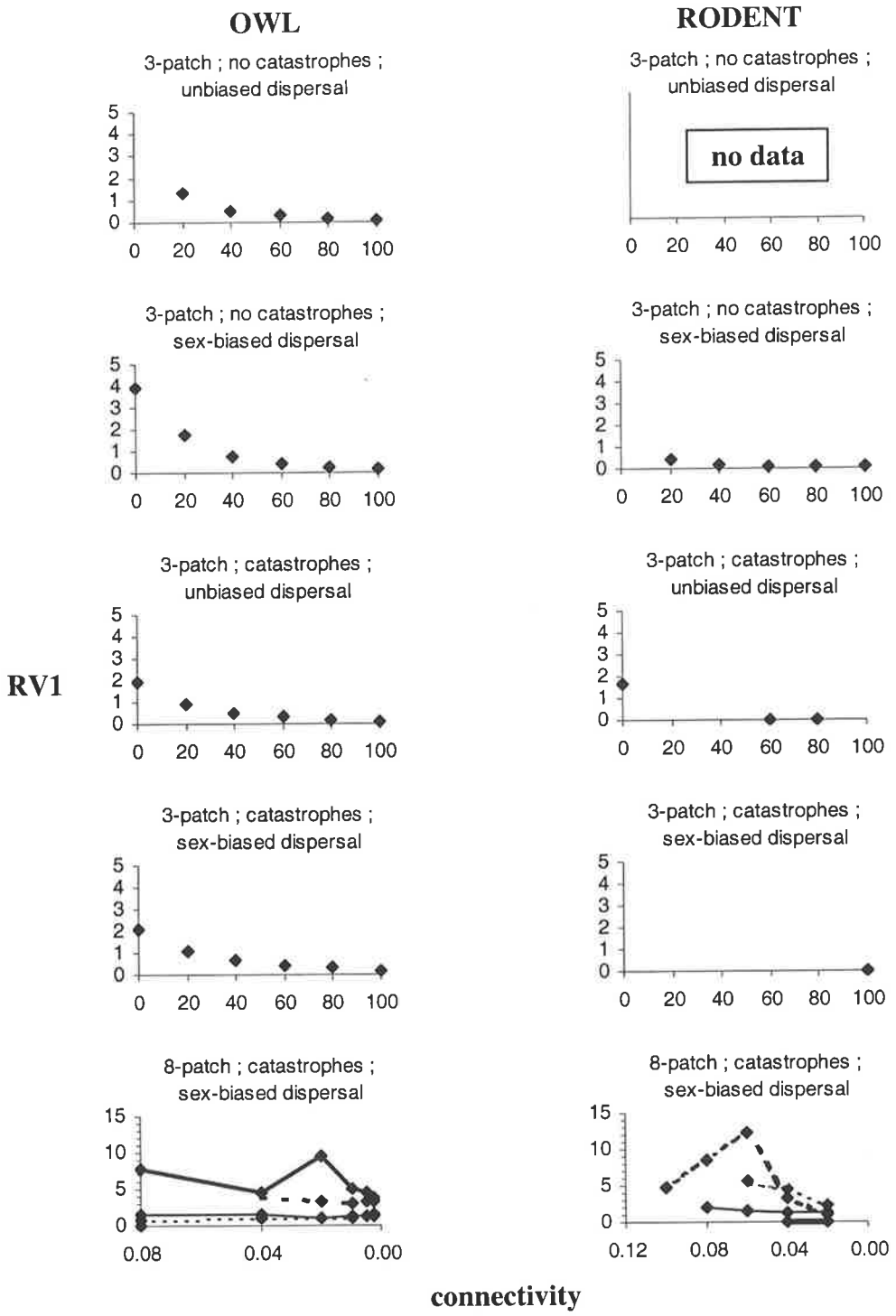

Figure 6.9 **RV1** versus connectivity for all scenarios (owl on left, rodent on right). RV1 is the value of the largest patch relative to value of the smallest patch in a pair. For the eight-patch systems the patch pairs are P_{50} & P_{20} (———), P_{45} & P_{15} (- - - - -), P_{30} & P_{25} (——), and P_{40} & P_{35} (--------). Connectivity for three-patch metapopulations is the % of dispersers successfully moving between the large and small patches; connectivity for eight-patch metapopulations is dispersal mortality per unit distance (note: the x-axis for eight-patch systems is reversed so that connectivity increases from left to right)

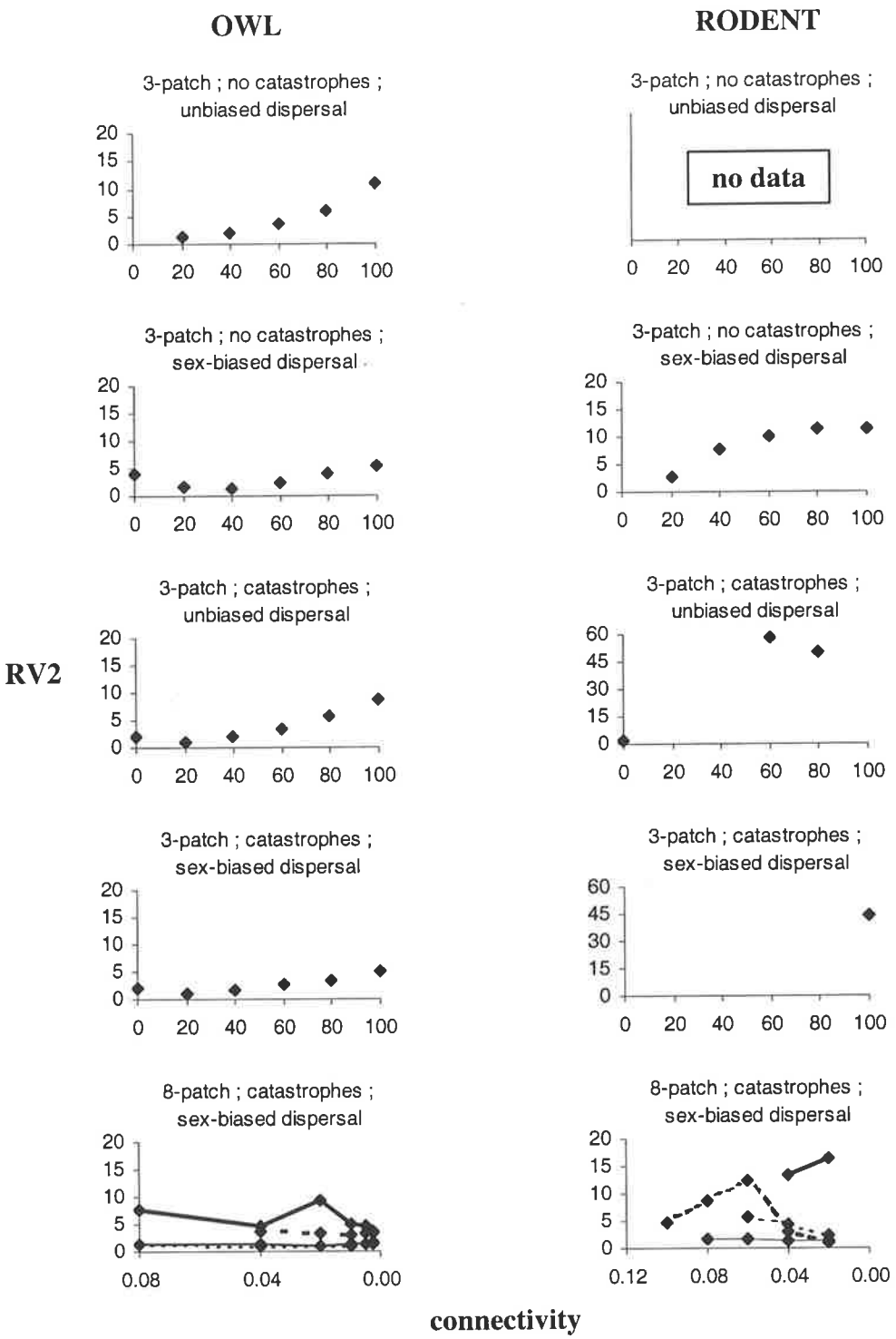

Figure 6.10 **RV2** versus connectivity for all scenarios (owl on left, rodent on right). RV2 is the relative value of the most important patch to the least important patch in the pair of patches. For the eight-patch systems the patch pairs are P_{50} & P_{20} (———), P_{45} & P_{15} (- - - - -), P_{30} & P_{25} (———), and P_{40} & P_{35} (- - - - - - - -). Connectivity for three-patch metapopulations is the % of dispersers successfully moving between the large and small patches; connectivity for eight-patch metapopulations is dispersal mortality per unit distance (note: the x-axis for eight-patch systems is reversed so that connectivity increases from left to right).

6.3 Population Genetics

6.3.1 The population genetics of individual replicate metapopulations

Just as it is useful to introduce metapopulation dynamics in terms of individual replicate metapopulations, it is equally useful to introduce genetics at the same level. Figure 6.11 shows the changes in all six measures of genetic diversity (and population size) over the first 100 years after fragmentation for a large patch (carrying capacity = 20) in a three-patch owl metapopulation with no catastrophes and no dispersal. These data were taken irrespective of whether the other patches in the system were occupied. This differs from the later treatment of genetic diversity data, where values are only used for ranking patches if all patches are occupied. Note how the different measures of genetic diversity decay at different rates. Observed heterozygosity and expected heterozygosity (i.e. expected under Hardy-Weinberg equilibrium) decay slowly, the mean number of alleles per locus has a moderate rate of decay, the number of polymorphic loci does not change at all, while both measures of mitochondrial diversity decay very quickly.

As a comparison, Figure 6.12 shows the same set of graphs but for a small patch (carrying capacity = 10) in a three-patch owl metapopulation with no catastrophes and 100% dispersal between the large and small patches. This subpopulation goes extinct soon after 20 years, and receives immigrants several times before becoming fully established again at approximately the 75-year mark. Where the patch is empty, no genetic diversity values are given. Thus, I am considering the genetic diversity of a non-existent population to be a meaningless concept. Note the effect on genetic diversity of immigrants arriving into the patch after extinction occurred. Some measures such as observed and expected heterozygosity, and the number of polymorphic loci are extremely sensitive to immigration, with the arrival of only one or two immigrants from the large patch being enough to elevate genetic diversity to almost pre-extinction levels. This sensitivity makes sense given that a single immigrant individual that is heterozygous for every locus will result in observed and expected heterozygosties of 1.0, and 10 polymorphic loci. In contrast the mean number of alleles per locus is only moderately affected by immigration, being only partially restored to its pre-extinction level of diversity. This also makes sense given that the mean number of alleles per locus depends not just on the genetic diversity of individuals, but also the number

of individuals with different alleles. Meanwhile both measures of mitochondrial diversity are almost unaffected by immigration, presumably because the rest of the metapopulation has already lost much of its mitochondrial diversity (as in Figure 6.11).

Figure 6.13 shows the changes in population size and genetic diversity for a large patch (carrying capacity = 20) in a three-patch rodent metapopulation with no catastrophes and no dispersal. Note the relatively high rates of genetic diversity decay compared to the equivalent owl population (Figure 6.11). While the number of polymorphic loci did not change for owls, it drops to zero here for rodents. The measures of mitochondrial diversity decay particularly quickly - down to their minima within 10 years. Such rapid rates of decay for the rodent population are attributable to both its fast generation time and frequent bottlenecking in numbers. Interestingly, although the changes in rodent population size are quite erratic, the decay in genetic diversity is quite smooth.

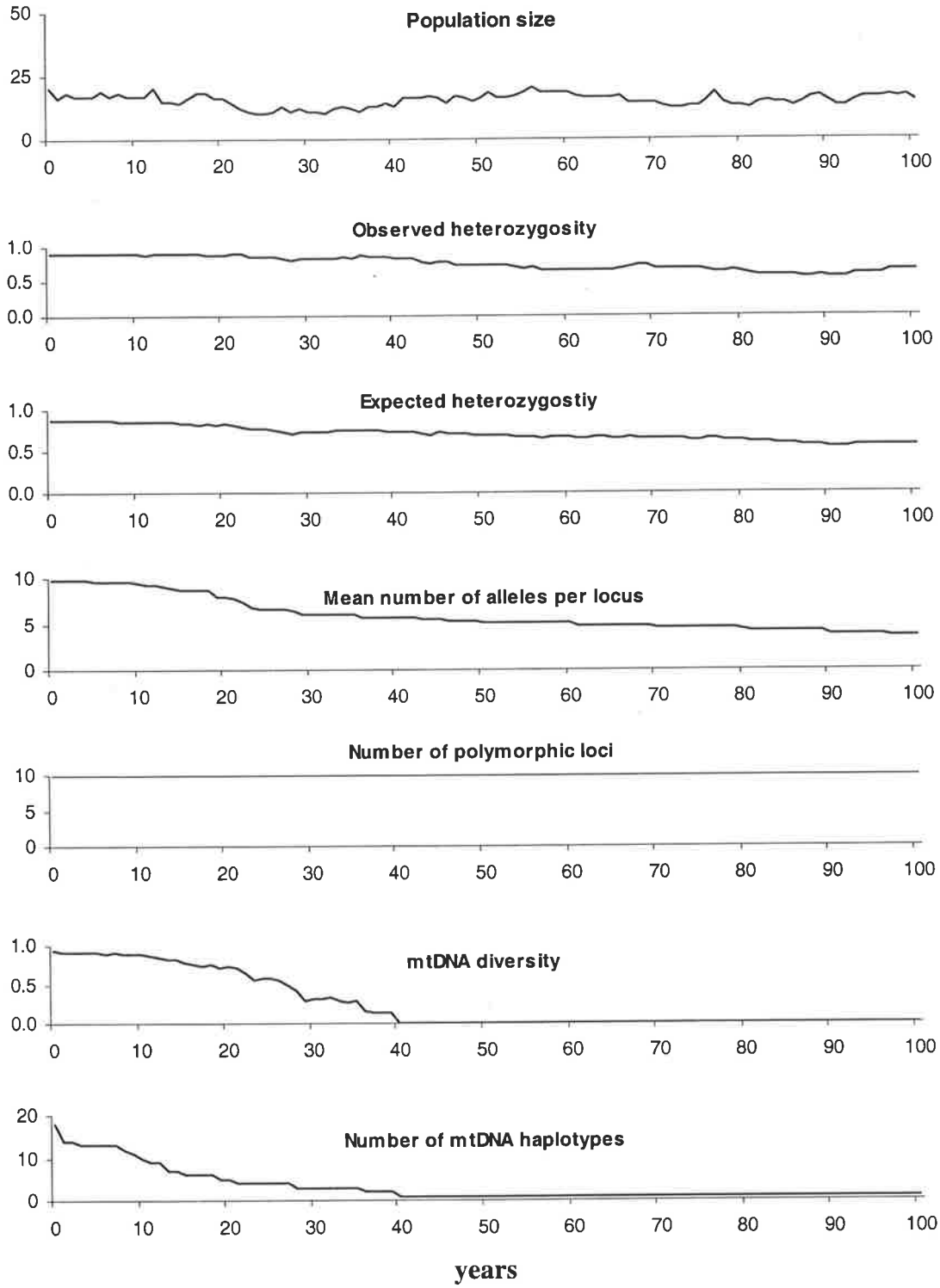

Figure 6.11 Population genetics (and dynamics) for an isolated owl subpopulation with a carrying capacity of 20 in a three-patch metapopulation with no catastrophes. Expected heterozygosity refers to the expectation at Hardy-Weinberg equilibrium.

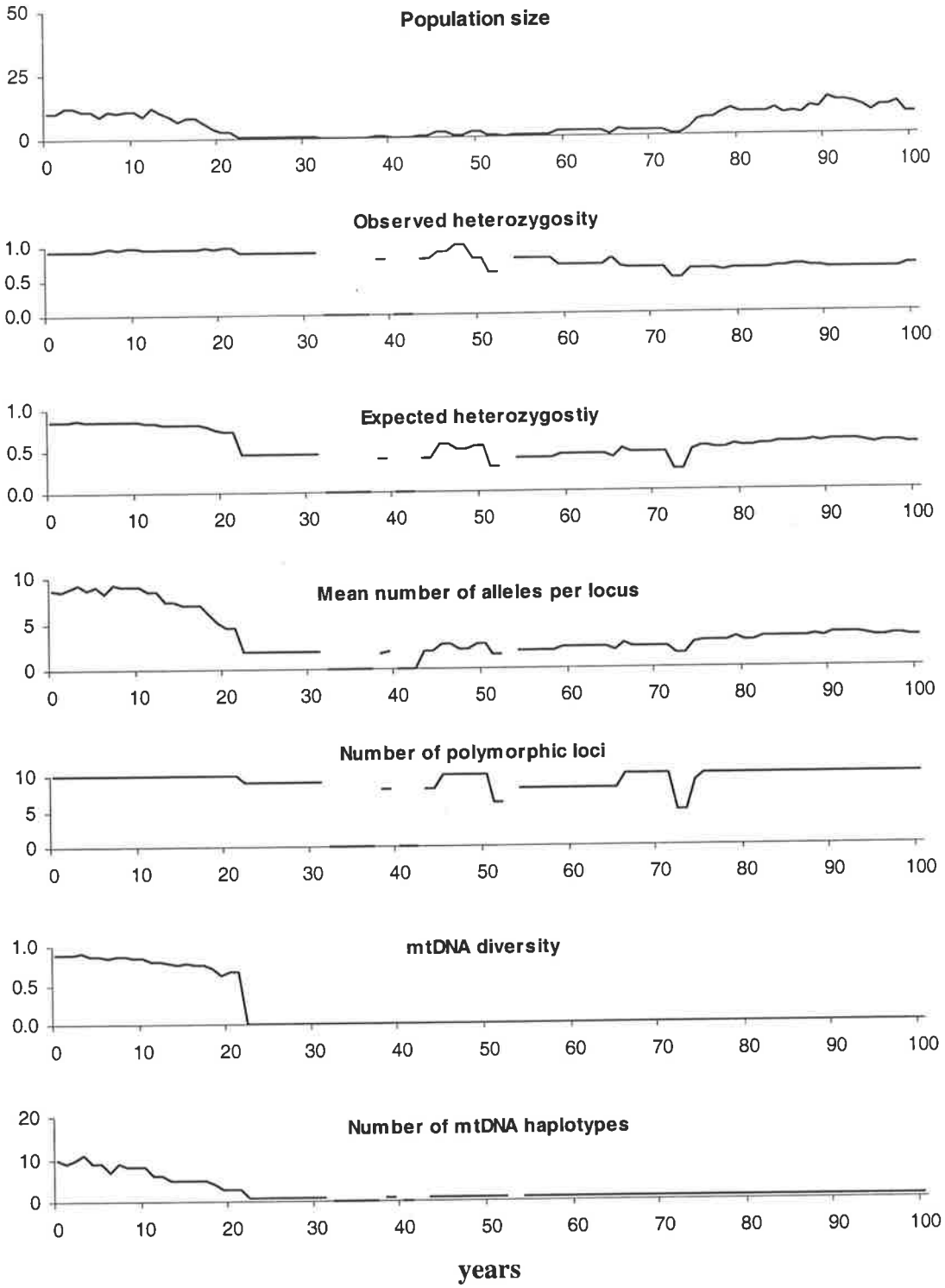

Figure 6.12 Population genetics (and dynamics) for an owl subpopulation with a carrying capacity of 10 in a three-patch metapopulation with no catastrophes and 100% dispersal. Broken sections of the genetic diversity graphs occur where population size is zero (in which case a measure of genetic diversity is meaningless). Expected heterozygosity refers to the expectation at Hardy-Weinberg equilibrium.

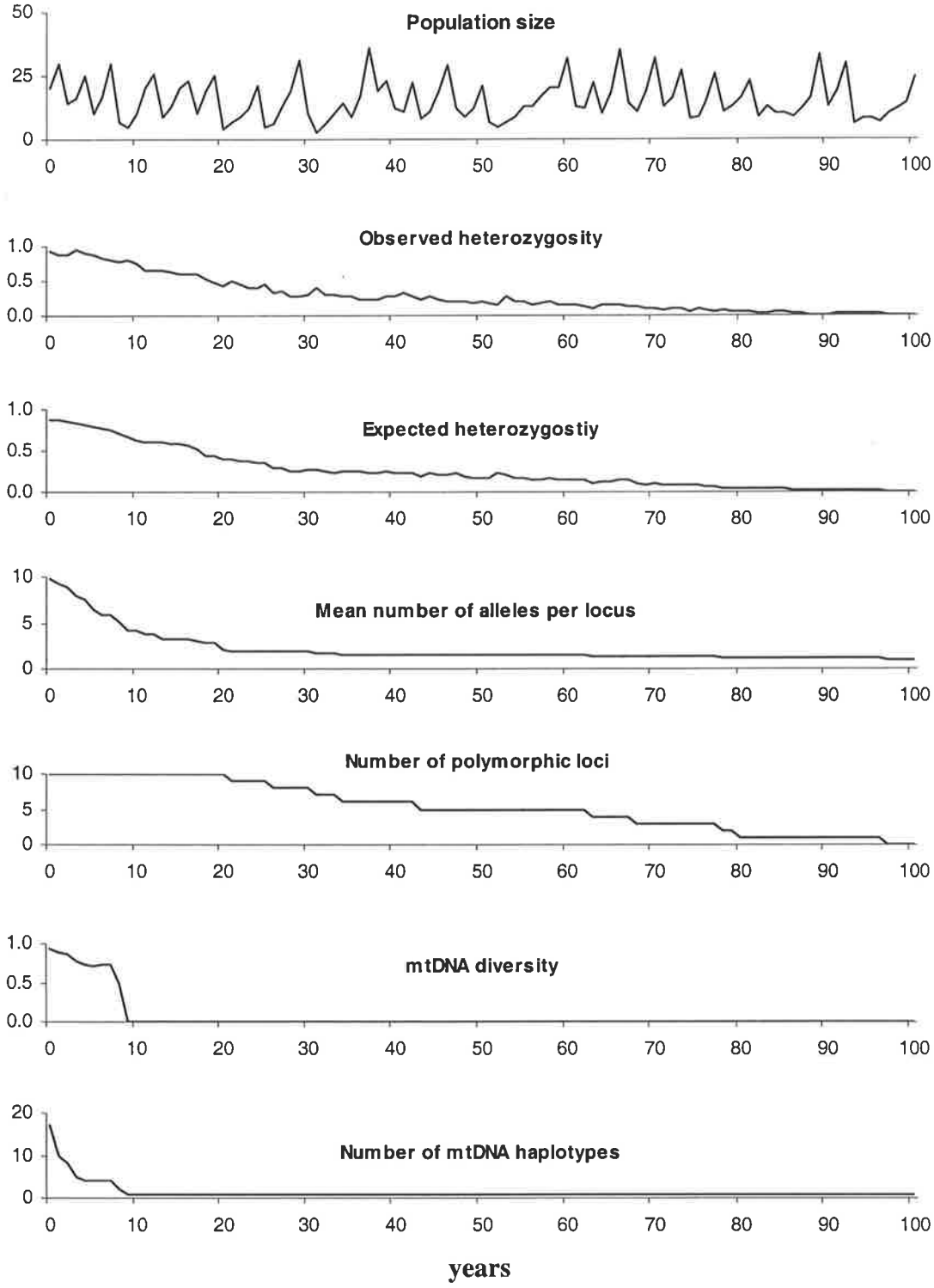

Figure 6.13 Population genetics (and dynamics) for an isolated rodent subpopulation with a carrying capacity of 20 in a three-patch metapopulation with no catastrophes. Expected heterozygosity refers to the expectation at Hardy-Weinberg equilibrium.

6.3.2 Mean changes in genetic diversity over time

Because the changes in genetic diversity of individual populations are stochastic, it is useful to examine the summary results from a large number of replicates. Figure 6.14 shows the changes in mean observed heterozygosity, expected heterozygosity (i.e. assuming Hardy-Weinberg equilibrium) and mean number of alleles per locus for owls and rodents in three-patch metapopulations with no dispersal. Each graph shows the conditional mean genetic diversity from 100 replicates for six different populations, calculated in each of the first 80 years after fragmentation. These means were conditional in the sense that replicates were only used when the population in question was extant. Therefore many more than 100 simulations were required to obtain the 100 suitable replicates. Due to long computer run times, it was not possible to perform independent replicates for each of the different measures of genetic diversity (i.e. the same 100 replicates were used for all measures). The top three lines (from top to bottom) are the large (K = 80), medium (K = 60) and small (K = 40) patches in a system with catastrophes, while the bottom three lines (from top to bottom) are the large (K = 20), medium (K = 15) and small (K = 10) patches in a three-patch system with no catastrophes. Figure 6.15 is from the same metapopulations, but describes changes in the mean number of polymorphic loci, mitochondrial diverisity, and number of mitochondrial haplotypes.

Important features to note are that:

- The rate of decline in mean genetic diversity is much higher for rodents than owls.
- The shape of genetic diversity curves varies considerably between the different measures. In particular, at different times, some measures of genetic diversity achieve greater separation among patches than other measures.

The rate of decline in genetic diversity of these populations is particularly high. Clearly these populations would be somewhere toward the hopeless end of any conservation genetics spectrum. It is important to remember however, that the purpose of this model is not explore issues of conservation genetics *per se*, but to understand how genetic diversity can help gain insights into metapopulation demographics. The high extinction probabilities and high rates of decay of genetic diversity produced by this model are an unfortunate limitation required to keep computer run times reasonably low.

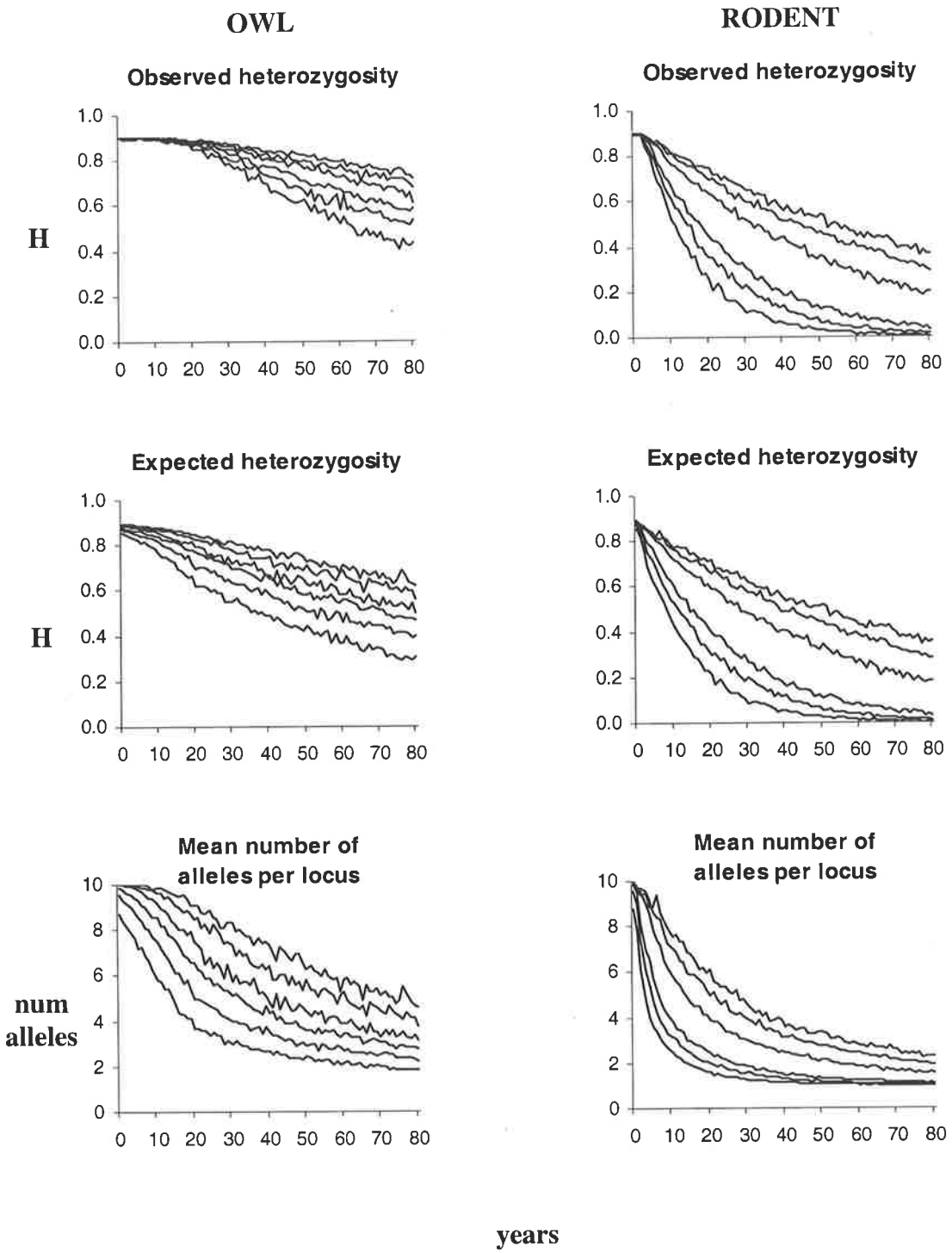

Figure 6.14 Changes in conditional mean genetic diversity over the first 80 years following fragmentation (i.e. only using data from extant populations). Shown here for owls (left) and rodents (right) are data for **observed heterozygosity, expected heterozygosity and the mean number of alleles per locus**. Expected heterozygosity refers to the expectation at Hardy-Weinberg equilibrium. All data are for subpopulations with no dispersal. From top to bottom for each graph are patches with carrying capacities of 80, 60 and 40 (with catastrophes), and carrying capacities of 20, 15 and 10 (without catastrophes).

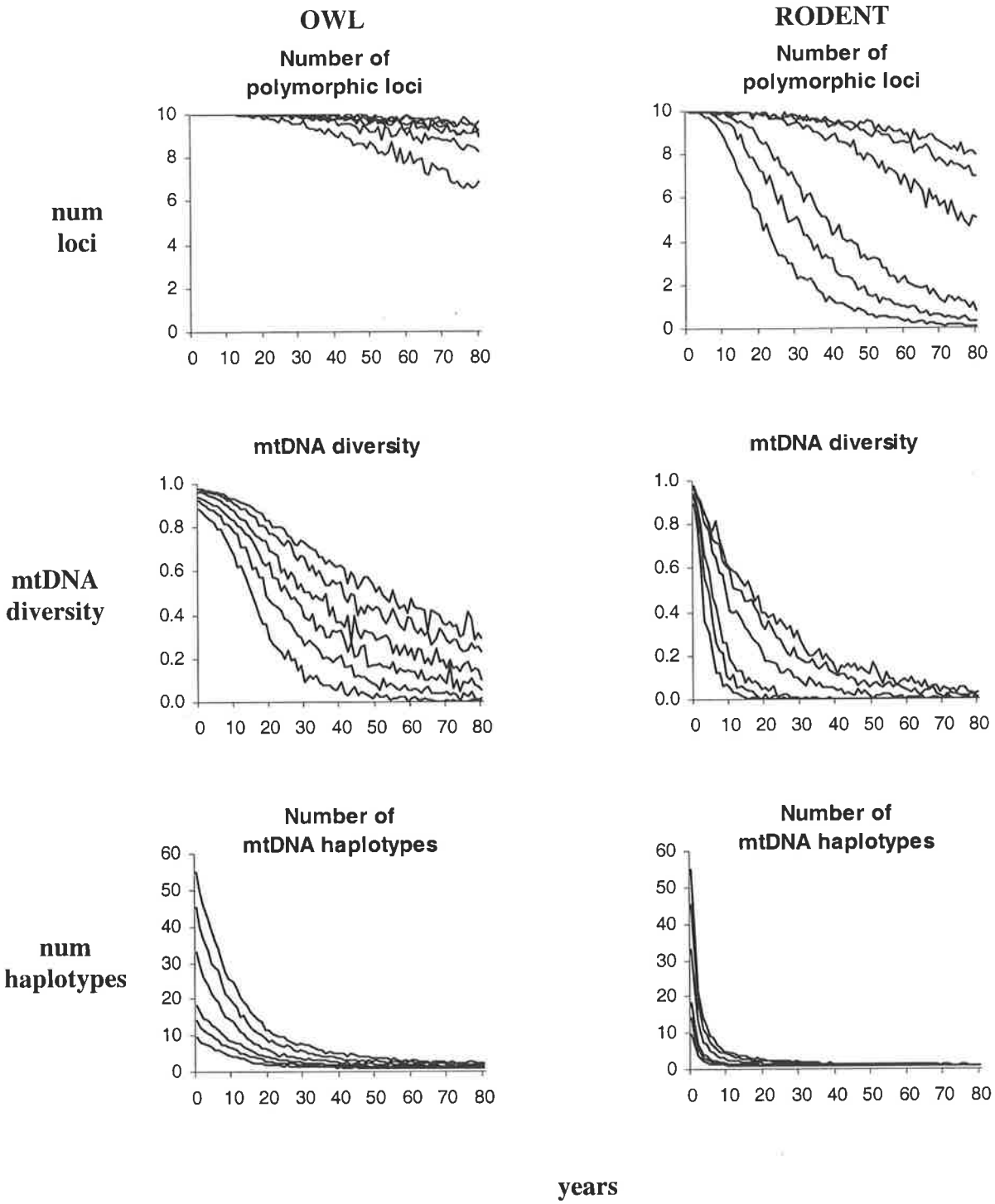

Figure 6.15 Changes in conditional mean genetic diversity over the first 80 years following fragmentation (i.e only using data from extant populations). Shown here for owls (left) and rodents (right) are data for the **number of polymporphic loci, mitochondrial diversity and number of mitochondrial haplotypes**. All data are for subpopulations with no dispersal. From top to bottom for each graph are patches with carrying capacities of 80, 60 and 40 (with catastrophes), and carrying capacities of 20, 15 and 10 (without catastrophes).

6.3.3 Changes in the variance of genetic diversity over time

As well as looking at changes in mean genetic diversity, it is also important to appreciate changes in variance over time. The higher the variance, the greater the chance that genetic diversity will provide a poor basis for estimating the relative value of patches. Figures 6.16 and 6.17 show (for both owls and rodents) how the spread in genetic diversity values changes over time for an isolated patch with a carrying capacity of 20 and no catastrophes. The line on each graph is the conditional mean from 100 data points (i.e. the same as the K = 20 line in Figures 6.14 and 6.15). Also shown are 80 scatterplot values - one for each of the 80 years after fragmentation. Each of these values was calculated from a single replicate population, and as with the means, these values were only taken conditional on the population in question being extant. Note how the patterns of variation vary among the six measures of genetic diversity.

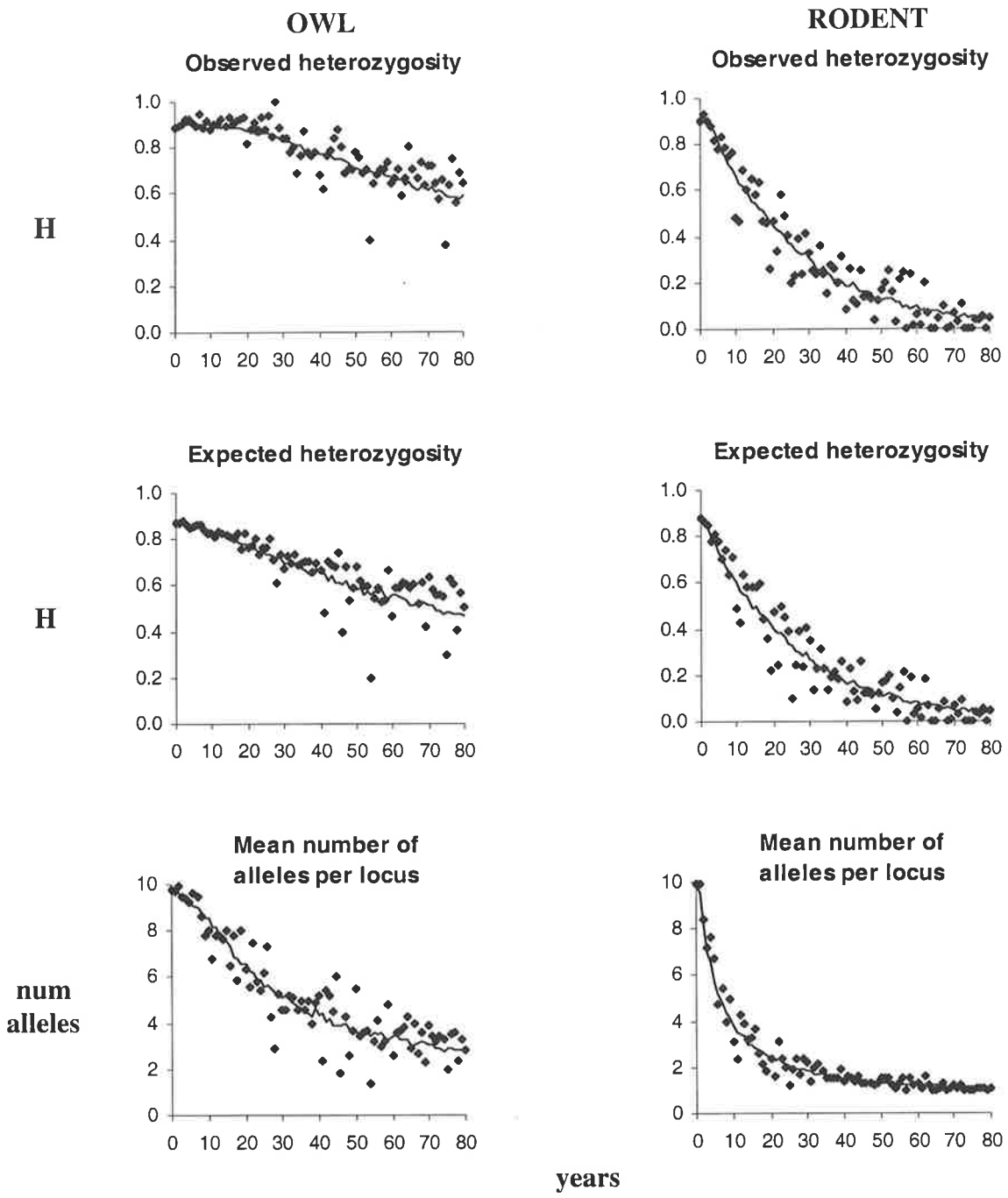

Figure 6.16 Changes in the spread of genetic diversity values over the first 80 years following fragmentation. Shown here for owls (left) and rodents (right) are data for **observed heterozygosity, expected heterozygosity and the mean number of alleles per locus**. Expected heterozygosity refers to the expectation at Hardy-Weinberg equilibrium. These data are for an isolated patch with a carrying capacity of 20 and no catastrophes. The markers correspond to individual replicates, and each line corresponds to the mean of 100 replicates. These data are conditional in the sense that they are based only on extant populations.

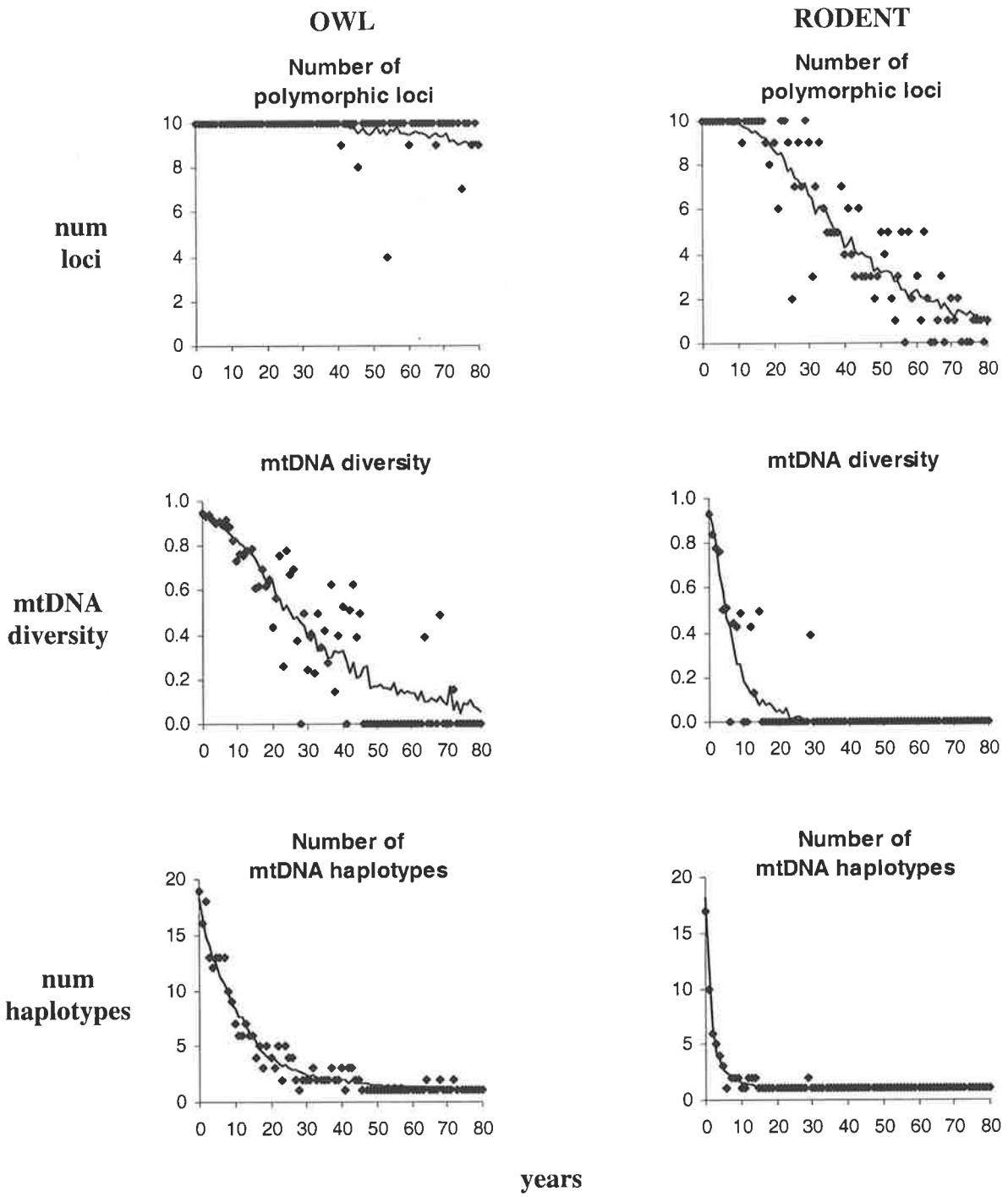

Figure 6.17 Changes in the spread of genetic diversity values over the first 80 years following fragmentation. Shown here for owls (left) and rodents (right) are data for the **number of polymorphic loci, mitochondrial diversity and the number of mitochondrial haplotypes**. These data are for an isolated patch with a carrying capacity of 20 and no catastrophes. The markers correspond to individual replicates, and each line corresponds to the mean of 100 replicates. These data are conditional in the sense that they are based only on extant populations.

6.4 Integrating population dynamics and genetics

6.4.1 Reducing the analysis to a manageable level

One of the major difficulties of trying to draw general conclusions from a simulation model lies in determining an appropriate set of scenarios over which to run the model. One extreme is to explore so few scenarios that the generality of any conclusions may come into question, while the reverse extreme is to explore so many scenarios that any synthesis is obscured by the sheer volume of results. Clearly it is important to achieve a compromise between these two extremes. In trying to assess the utility of genetic diversity data for ranking patches, I chose the set of scenarios described in Figure 5.9. The number of genetic diversity ranks produced with this design became immense following the inclusion of five sampling times and six measures of genetic diversity. Therefore, in order to restrict the analyses to a manageable level, I decided to reduce the results to what appeared to be the best sampling time, and the most informative measure of genetic diversity.

6.4.1.1. The choice of sampling time

Figures 6.18 to 6.22 show for owls and rodents the proportion of metapopulations still extant, and the proportion of metapopulations with all patches occupied, for all scenarios examined using the model. Each page represents a different sampling time (5, 10, 20, 40 and 80 years), while the ten graphs on each page correspond to the ten different scenarios (containing data for all six connectivity levels). Note that for any given scenario the proportion of metapopulations still extant decreases with time, as does the proportion of metapopulations with all patches occupied. The most important of these two variables is the proportion of metapopulations with all patches occupied, as only fully occupied metapopulations were used as replicates for ranking the genetic diversity of patches. This level of conditionality contrasts with the genetic examples above, where conditionality extended only to the subpopulation in question (i.e. not to all subpopulations in the metapopulation) (Figures 6.14 to 6.17), or where data were used without any conditions on patch occupancy (not even for the patch in question) (Figures 6.11 to 6.13).

The approach taken here was to choose a sampling time that would be long enough to have allowed the genetic diversity of subpopulations to diverge, but not be so long that (1) genetic

diversity had reached its minimum, or (2) that there were too few scenarios with high levels of patch occupancy. No single sampling time is optimal for all scenarios, with rodent metapopulations appearing to be much more sensitive to an increase in sampling time than owl metapopulations. Fully intact rodent metapopulations were very rare at the longer sampling times of 20, 40 and 80 years (Figures 6.20, 6.21 and 6.22). Not only does this reduce the number of fully intact replicates available for data analysis at these times, but also presents a situation of very little management importance. That is, if the chance of finding a fully intact rodent metapopulation at 20 years is extremely low, we would expect to only very rarely find all subpopulations extant in such a metapopulation in nature. In that case we would hardly ever expect to apply any rule that links genetic diversity to patch value. For these reasons I chose to use the sampling time of 10 years after fragmentation (see Figure 6.19) to accommodate all scenarios. Although some scenarios are still poorly represented (*e.g.,* the eight-patch rodent metapopulation), in most scenarios the proportion of metapopulations fully intact is quite high. Furthermore, Figures 6.14 and 6.15 suggest that at 10 years after fragmentation there is generally some differentiation between patches in mean genetic diversity.

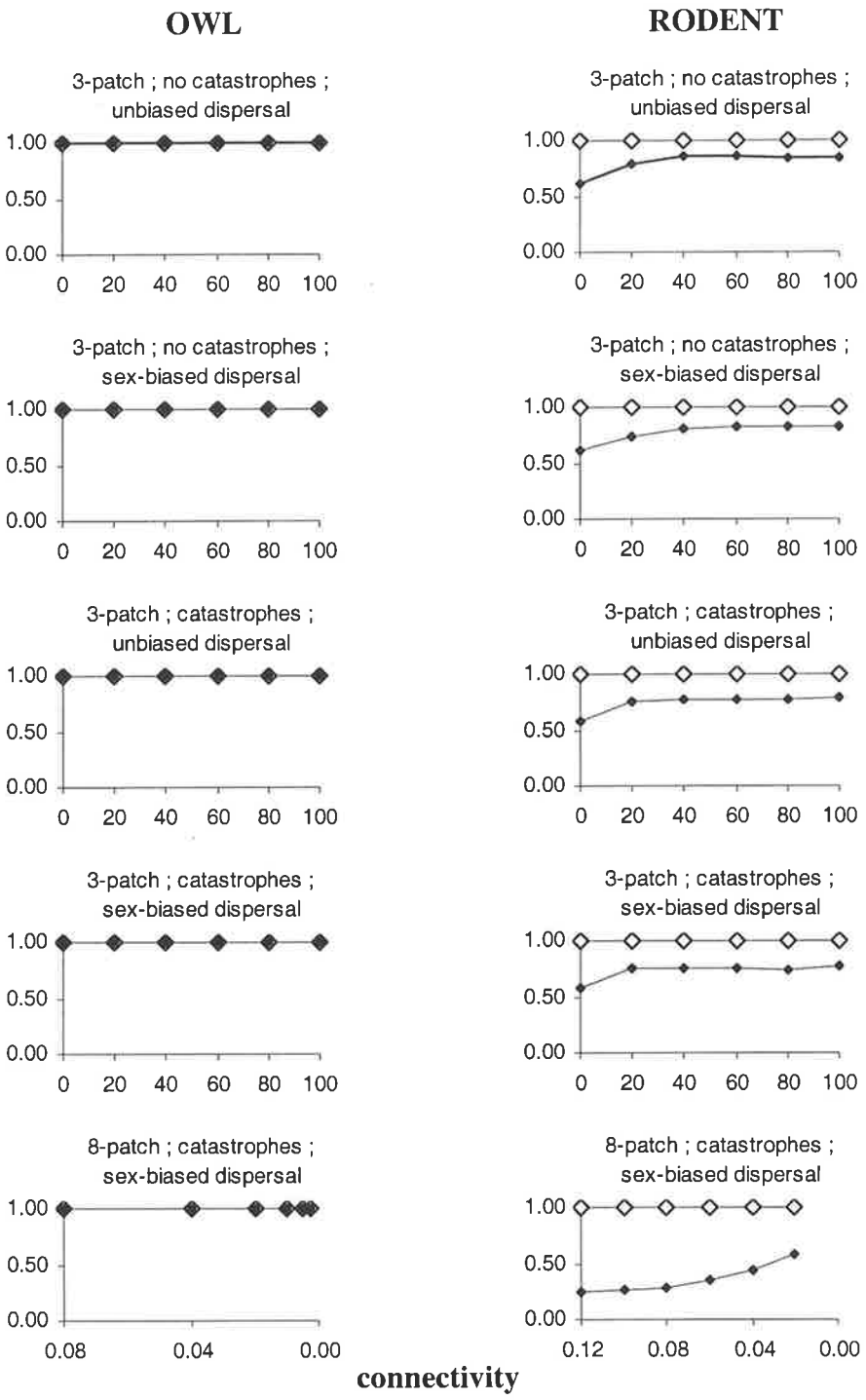

Figure 6.18 Proportion of metapopulations extant (large white markers) and proportion of metapopulations with all subpopulations extant (small black markers) **5 years after fragmentation**, for owls (left) and rodents (right). 95% confidence intervals were included but do not extend beyond the markers. Connectivity for three-patch metapopulations is the % of dispersers successfully moving between the large and small patches; connectivity for eight-patch metapopulations is dispersal mortality per unit distance (note: the x-axis for eight-patch systems is reversed so that connectivity increases from left to right).

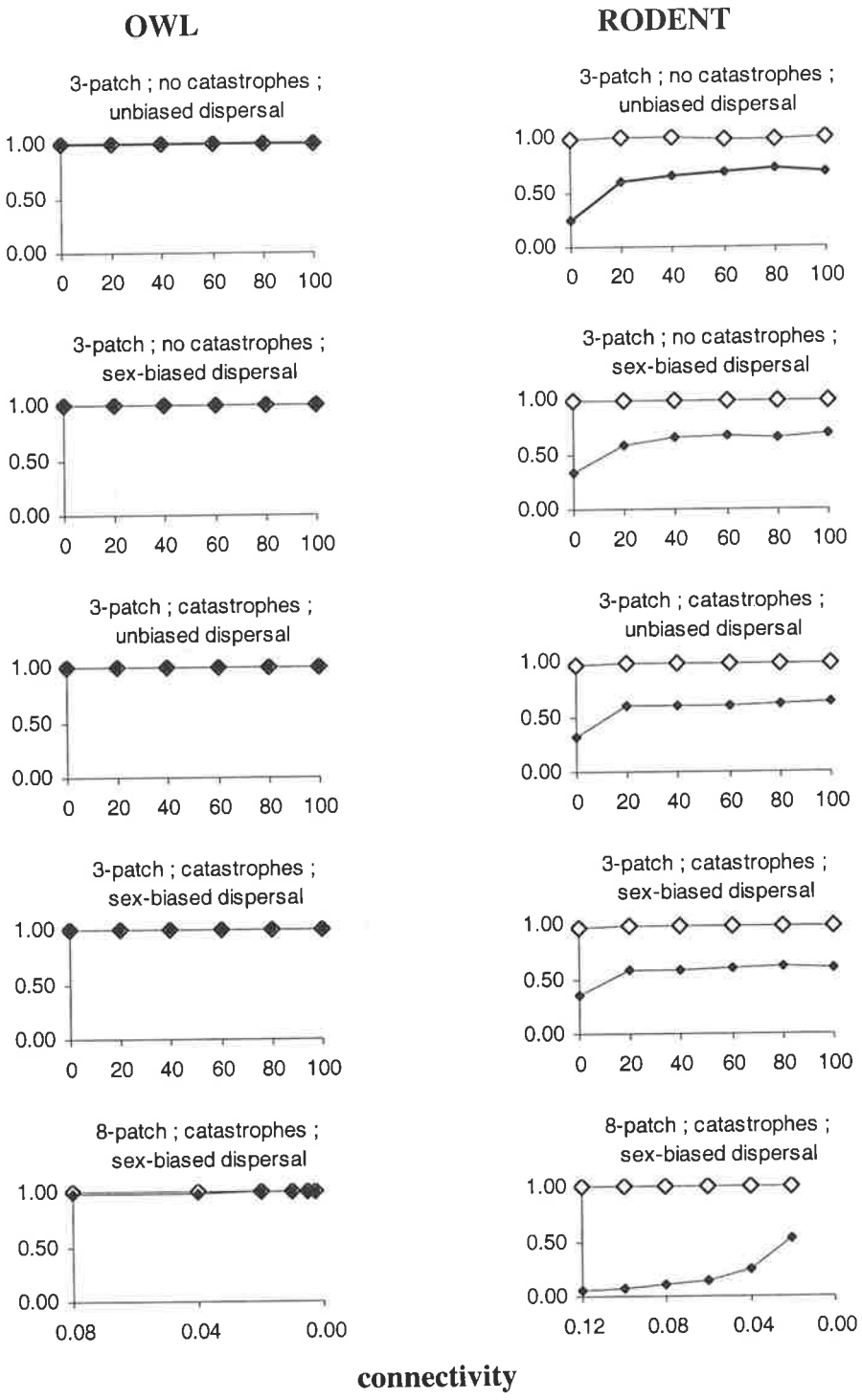

Figure 6.19 Proportion of metapopulations extant (large white markers) and proportion of metapopulations with all subpopulations extant (small black markers) **10 years after fragmentation**, for owls (left) and rodents (right). 95% confidence intervals were included but do not extend beyond the markers. Connectivity for three-patch metapopulations is the % of dispersers successfully moving between the large and small patches; connectivity for eight-patch metapopulations is dispersal mortality per unit distance (note: the x-axis for eight-patch systems is reversed so that connectivity increases from left to right).

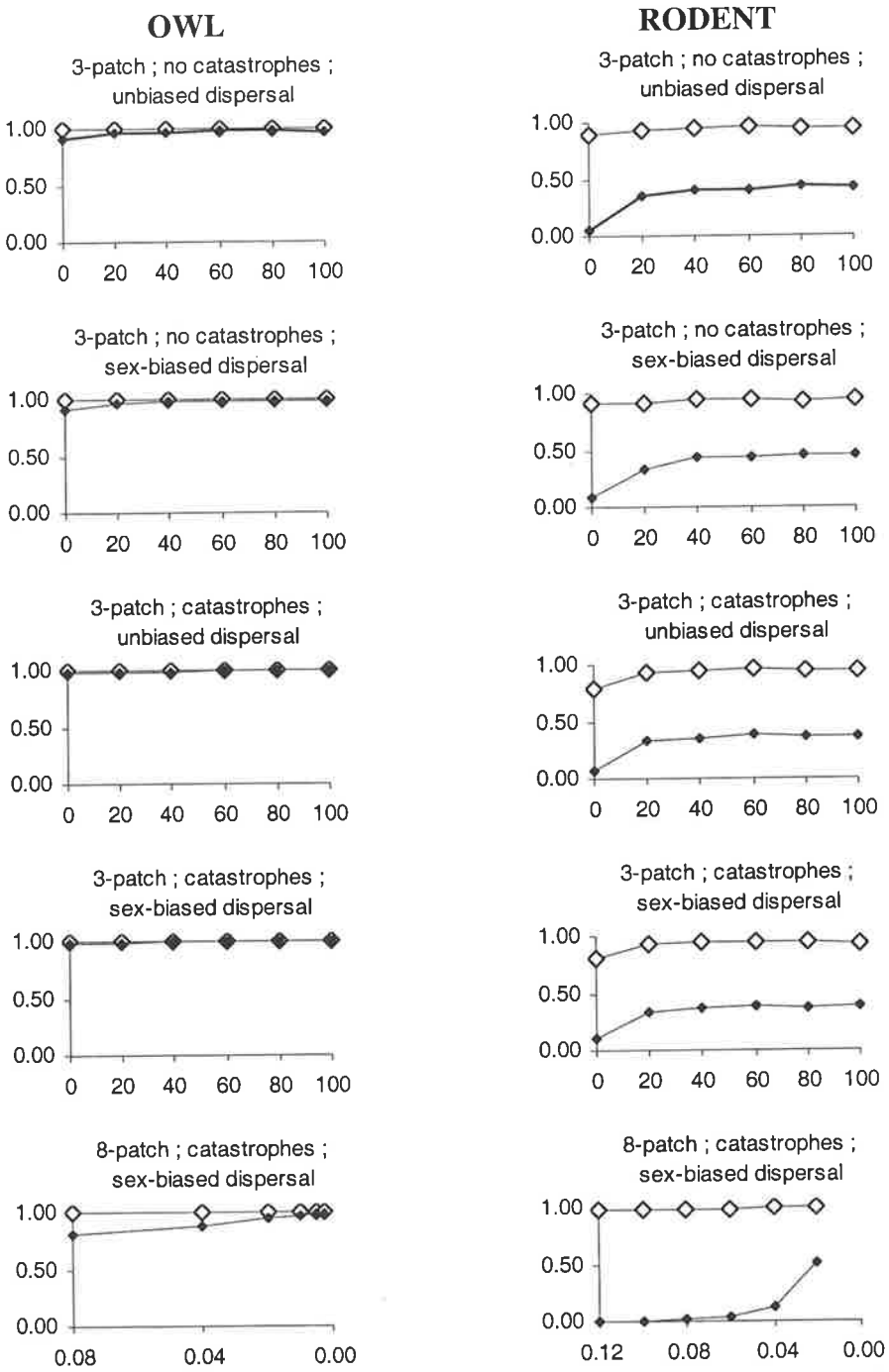

Figure 6.20 Proportion of metapopulations extant (large white markers) and proportion of metapopulations with all subpopulations extant (small black markers) **20 years after fragmentation**, for owls (left) and rodents (right). 95% confidence intervals were included but do not extend beyond the markers. Connectivity for three-patch metapopulations is the % of dispersers successfully moving between the large and small patches; connectivity for eight-patch metapopulations is dispersal mortality per unit distance (note: the x-axis for eight-patch systems is reversed so that connectivity increases from left to right).

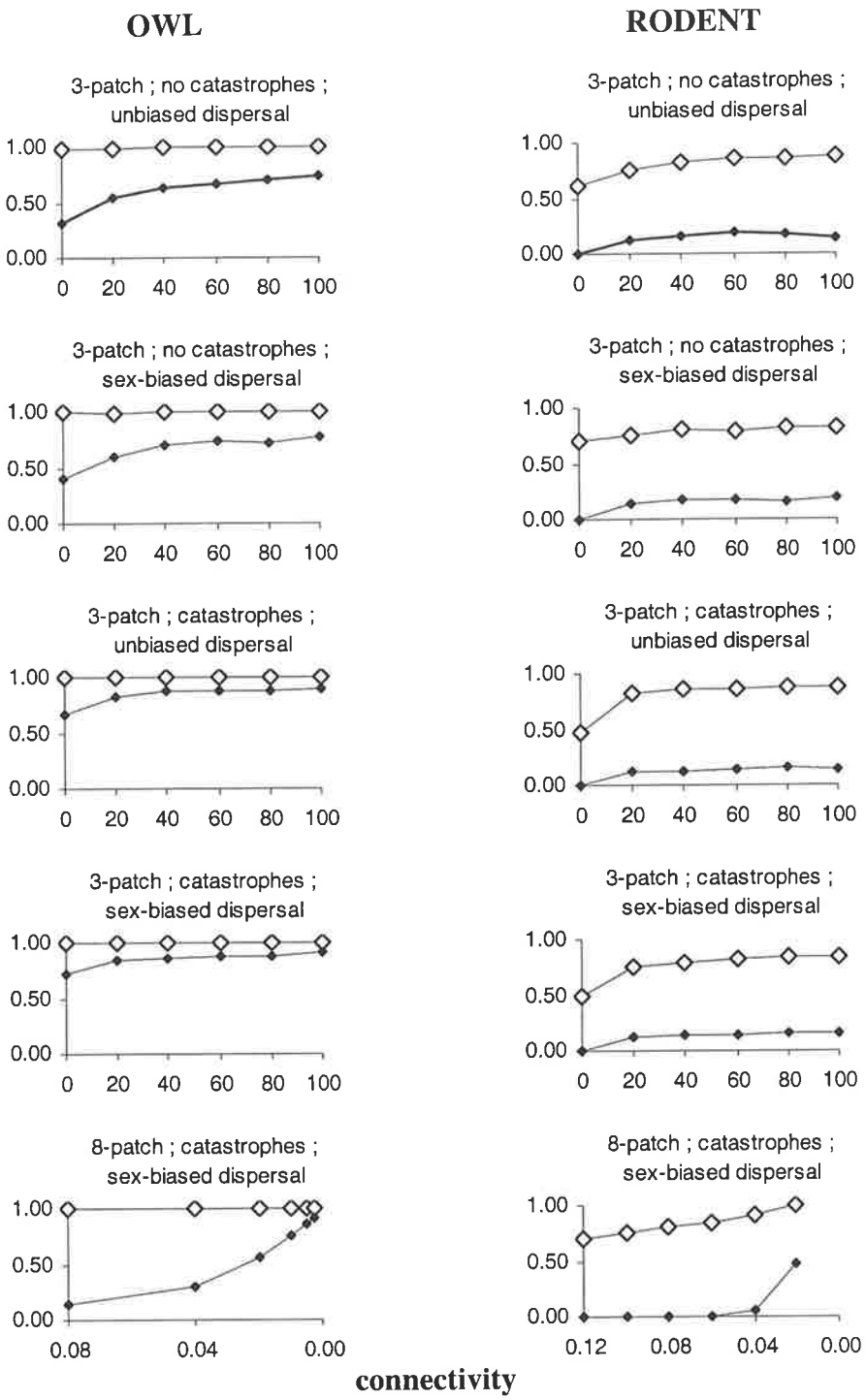

Figure 6.21 Proportion of metapopulations extant (large white markers) and proportion of metapopulations with all subpopulations extant (small black markers) **40 years after fragmentation**, for owls (left) and rodents (right). 95% confidence intervals were included but do not extend beyond the markers. Connectivity for three-patch metapopulations is the % of dispersers successfully moving between the large and small patches; connectivity for eight-patch metapopulations is dispersal mortality per unit distance (note: the x-axis for eight-patch systems is reversed so that connectivity increases from left to right).

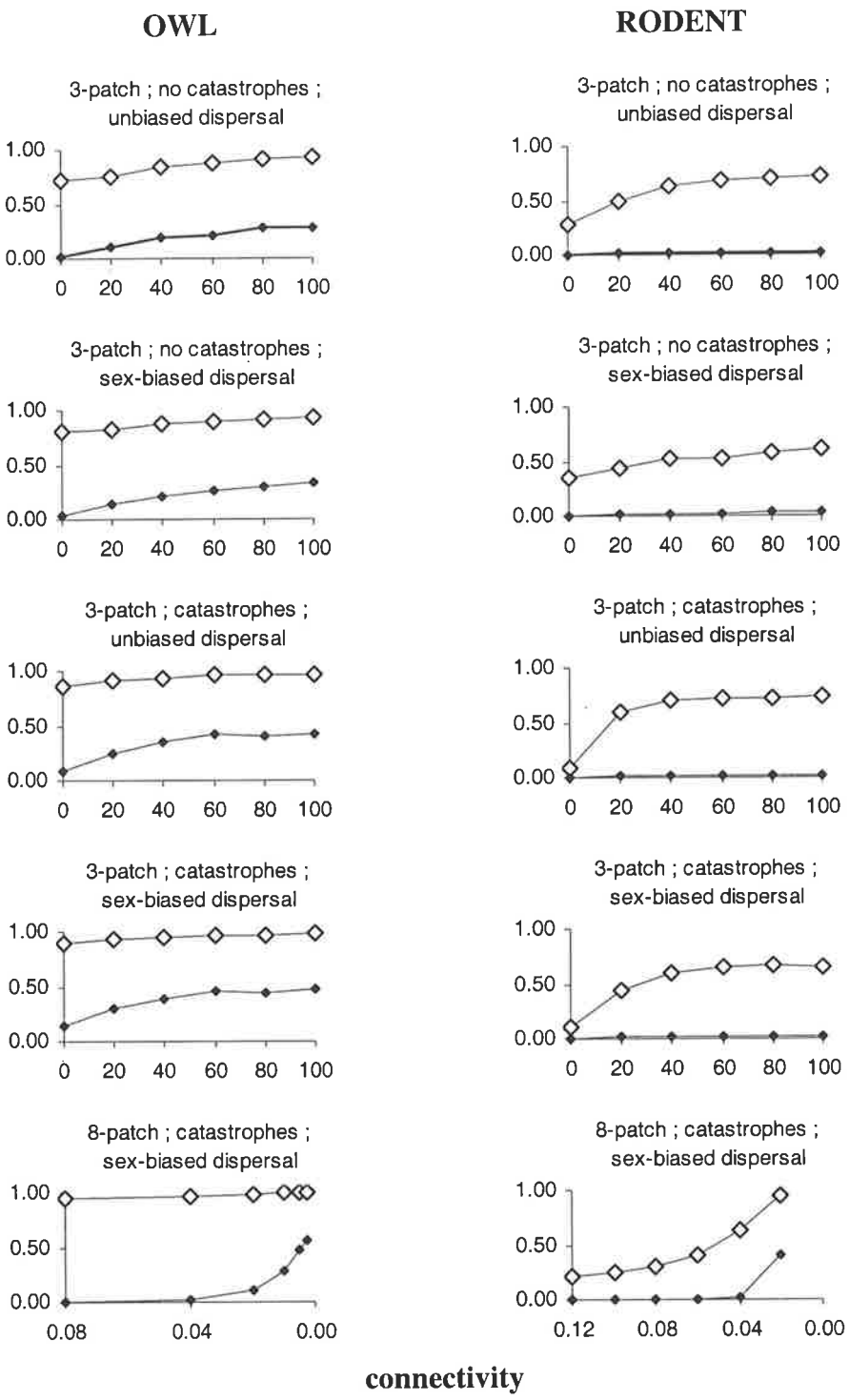

Figure 6.22 Proportion of metapopulations extant (large white markers) and proportion of metapopulations with all subpopulations extant (small black markers) **80 years after fragmentation**, for owls (left) and rodents (right). 95% confidence intervals were included but do not extend beyond the markers. Connectivity for three-patch metapopulations is the % of dispersers successfully moving between the large and small patches; connectivity for eight-patch metapopulations is dispersal mortality per unit distance (note: the x-axis for eight-patch systems is reversed so that connectivity increases from left to right).

6.4.1.2 The proportion of correct genetic rankings as a function of connectivity

Having decided upon a sampling time of 10 years after fragmentation, we can now look at how well the different measures of genetic diversity perform in determining the relative value of patches. Figures 6.23 to 6.28 show the ability of each of the six different measures of genetic diversity to detect which patch in a pair is the most valuable to a metapopulation. This is measured as the proportion of genetics rankings that correspond to the ranking of the patches based on their demographic value (as in Tables 6.1 to 6.10). So, for example, Table 6.1 tells us that when there is 100% dispersal in the 3-patch owl metapopulation with no catastrophes and no sex-bias in dispersal, the relative value of the medium patch to the small patch (RV1) is 0.09. Because this is less than the critical value of 1, we identify the small patch as the most valuable of the two patches. Next, with the data from 1000 replicate metapopulations where genetic diversity was recorded we can rank the same patches according to their genetic diversity, making the assertion that the most valuable patch will have the highest genetic diversity. For the same scenario described above, it turns out that using observed heterozygosity performs quite poorly in this regard - correctly identifying the small patch as the most important of the two patches on only slightly more than 50% of occasions (the right-most data point in Figure 6.23a). In other words this is similar to the null hypothesis of randomly choosing the most valuable patch. In contrast, expected heterozygosity provides a much better basis for predicting relative patch value for this example (the right-most data point in Figure 6.24a). In this case we correctly identified the most important patch on approximately 75% of all occasions (i.e. genetic diversity was higher in the small patch than in the medium patch for 75% of the replicates).

Although 1000 metapopulations were replicated for each proportion, not all of these were useable in the analysis. There were several reasons for this. Firstly, metapopulations were excluded if some of their patches were unoccupied at the time of sampling. This was particularly common with the rodent metapopulations (see Figure 6.19). These replicates were excluded so that the same starting condition used to estimate relative patch value by simulation (i.e. all patches being initially occupied) would correspond to the patch occupancy at the time of genetic sampling. Also, in many cases, one or more of the patches being ranked was unoccupied, in which case it was not possible to measure genetic diversity. Even when all patches were occupied, replicate metapopulations were excluded when there was no difference between the genetic diversity of the two patches in question. As stated in section

5.8, these excluded data points do not represent incorrect estimates of patch ranking, but simply non-informative instances when a conservation biologist would not attempt to rank patches.

Because many of the proportions in Figures 6.23 to 6.28 were based on less than 1000 data points, these estimates have varying levels of sampling error. Accordingly the size of the 95% confidence intervals in these figures varies somewhat among scenarios and different measures of genetic diversity. In many cases no confidence interval can be seen - this is simply because the confidence interval was too small to extend beyond the marker. Confidence limits based on less than or equal to 50 data points were calculated using binomial probabilities, while a normal approximation was used for proportions based on more than 50 data points (Steel and Torrie, 1960).

There are several important features to note from Figures 6.23 to 6.28:

- Some measures of genetic diversity are better predictors of relative patch value than others. Observed heterozygosity (Figure 6.23) seems a weak predictor of relative patch value, particularly for the owl metapopulations, for which its predictive accuracy was very close to the null hypothesis of 0.50. The number of polymorphic loci is also a poor predictor of relative patch value, for rodents as well as owls. In this case (Figure 6.26) note the large confidence intervals which are due to fact that so few of the 1000 genetics replicates were useable. This was not due to the lack of fully occupied metapopulations (Figure 6.19) but to the high frequency of metapopulations having two patches with the same number of polymorphic loci. This in turn can be attributed to this measure of genetic diversity being (1) based on a small range of integer values, and (2) having a slow initial rate of decay from the starting condition of all subpopulations having 10 polymorphic loci (Figure 6.15).
- Even the better predictors of relative patch value (expected heterozygosity, mean number of alleles per locus, mitochondrial diversity, and number of mitochondrial haplotypes) were quite unreliable. None of these measures allow us to make a general statement such as "a guess of relative patch ranking based on this measure of genetic diversity will be correct 80% of the time". While in some instances these measures of genetic diversity provide very good predictions of relative patch value (i.e. more than 90% correct), they also provide poor predictions (50% correct or lower). Although this is frustrating, it is not at all surprising, since we can expect that the ability to resolve a difference in patch

value will be low when two patches have a similar value, and higher when patches are more different in value. Indeed there is some indication that this is the case, with the proportion of genetics-based guesses that are correct being greater at the two extremes of connectivity (Figures 6.24, 6.25, 6.27, and 6.28) - a somewhat similar pattern to the way that relative patch value (RV2) varies with connectivity (Figure 6.10).

- Some predictions of patch value were misleading, whereby less than 50% of the genetics-based guesses were correct (*e.g.*, Figures 6.25a, c, e, g, and j). In such cases, using genetics to estimate relative patch value is worse than randomly assigning relative patch values. Interestingly, these misleading scenarios occurred when connectivity made the smaller patch in a pair slightly more valuable than the larger patch (see Tables 6.1, 6.3, 6.5, 6.7). This pattern provides some insight into an issue highlighted in Chapter 5 (in relation to Figure 5.2), that the link between genetic diversity and relative patch value may in part be obscured if these variables follow different functions of patch isolation and area. The data here suggest that as we increase connectivity, the switch in relative relative patch value occurs just before the switch in relative genetic diversity.

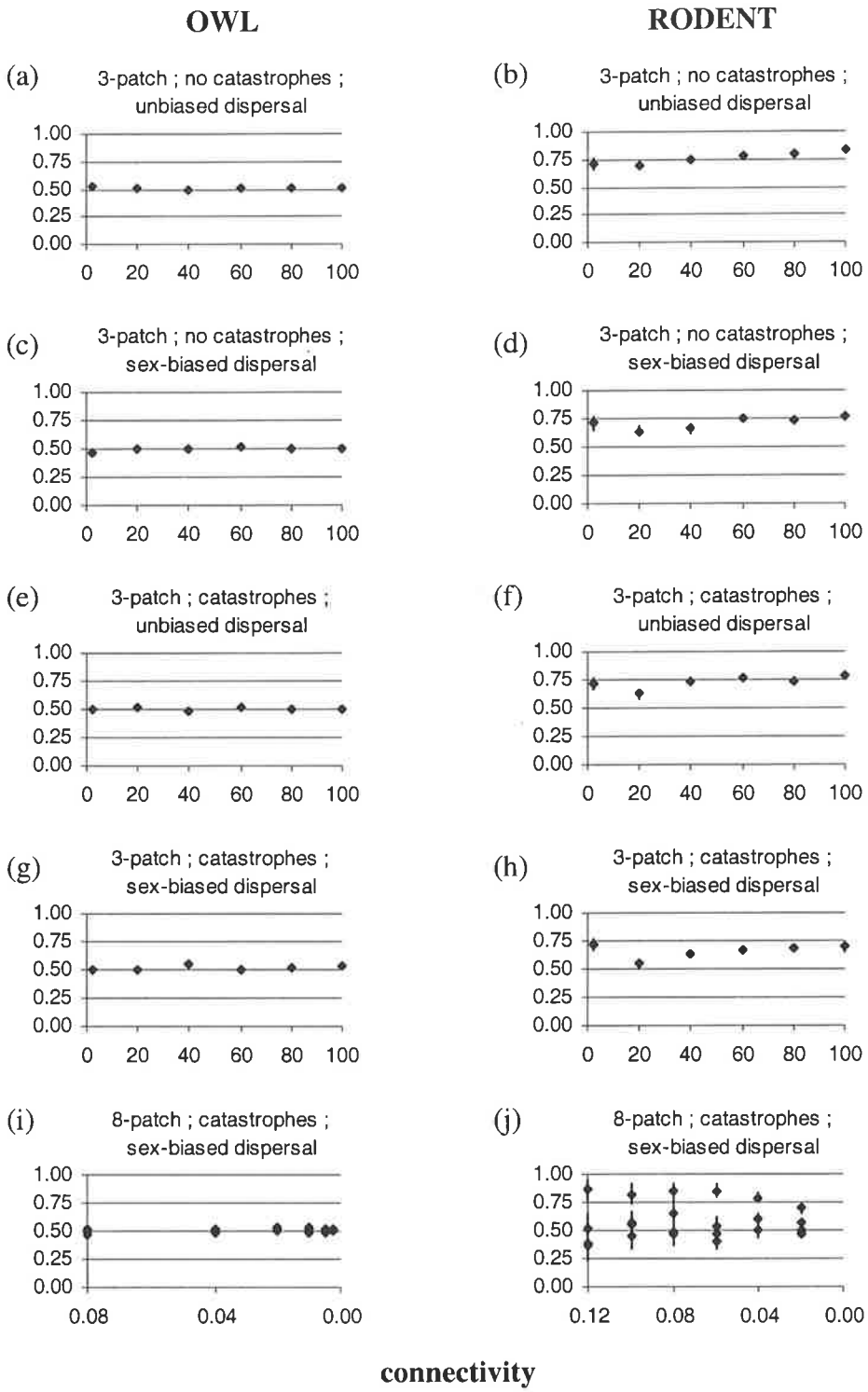

Figure 6.23 Proportion of correct genetic rankings for owls (left) and rodents (right) when using a 10-year sample of **observed heterozygosity**. Where large enough, 95% confidence intervals extend beyond markers. For the eight-patch systems the four markers at each connectivity level correspond to the following patch pairs: P_{50} & P_{20}, P_{45} & P_{15}, P_{30} & P_{25}, and P_{40} & P_{35}. Connectivity for three-patch metapopulations is the % of dispersers successfully moving between the large and small patches; connectivity for eight-patch metapopulations is dispersal mortality per unit distance (note: the x-axis for eight-patch systems is reversed so that connectivity increases from left to right).

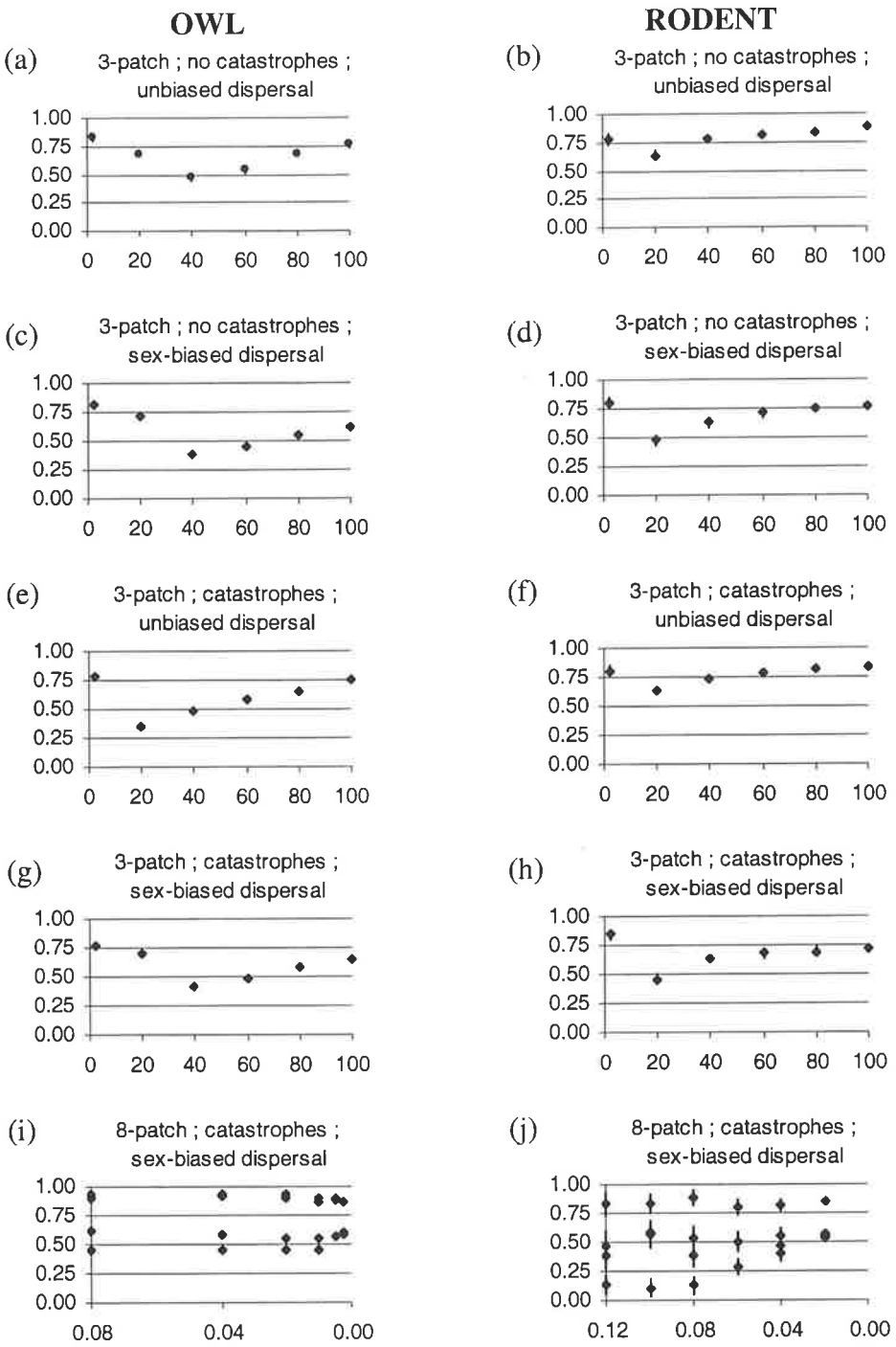

Figure 6.24 Proportion of correct genetic rankings for owls (left) and rodents (right) when using a 10-year sample of **expected heterozygosity**. Where large enough, 95% confidence intervals extend beyond markers. For the eight-patch systems the four markers at each connectivity level correspond to the following patch pairs: P_{50} & P_{20}, P_{45} & P_{15}, P_{30} & P_{25}, and P_{40} & P_{35}. Connectivity for three-patch metapopulations is the % of dispersers successfully moving between the large and small patches; connectivity for eight-patch metapopulations is dispersal mortality per unit distance (note: the x-axis for eight-patch systems is reversed so that connectivity increases from left to right).

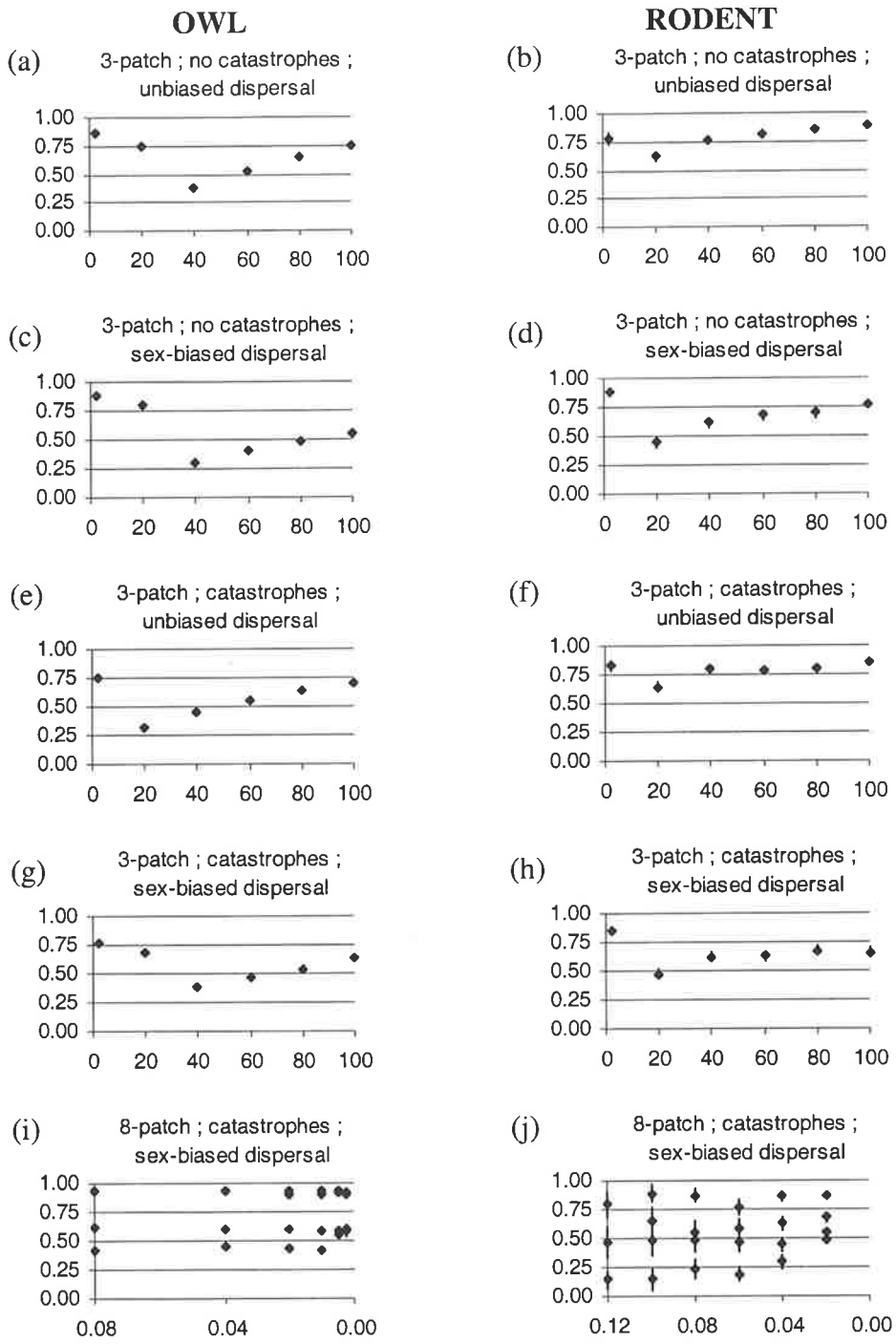

Figure 6.25 Proportion of correct genetic rankings for owls (left) and rodents (right) when using a 10-year sample of the **mean number of alleles per locus**. Where large enough, 95% confidence intervals extend beyond markers. For the eight-patch systems the four markers at each connectivity level correspond to the following patch pairs: P_{50} & P_{20}, P_{45} & P_{15}, P_{30} & P_{25}, and P_{40} & P_{35}. Connectivity for three-patch metapopulations is the % of dispersers successfully moving between the large and small patches; connectivity for eight-patch metapopulations is dispersal mortality per unit distance (note: the x-axis for eight-patch systems is reversed so that connectivity increases from left to right).

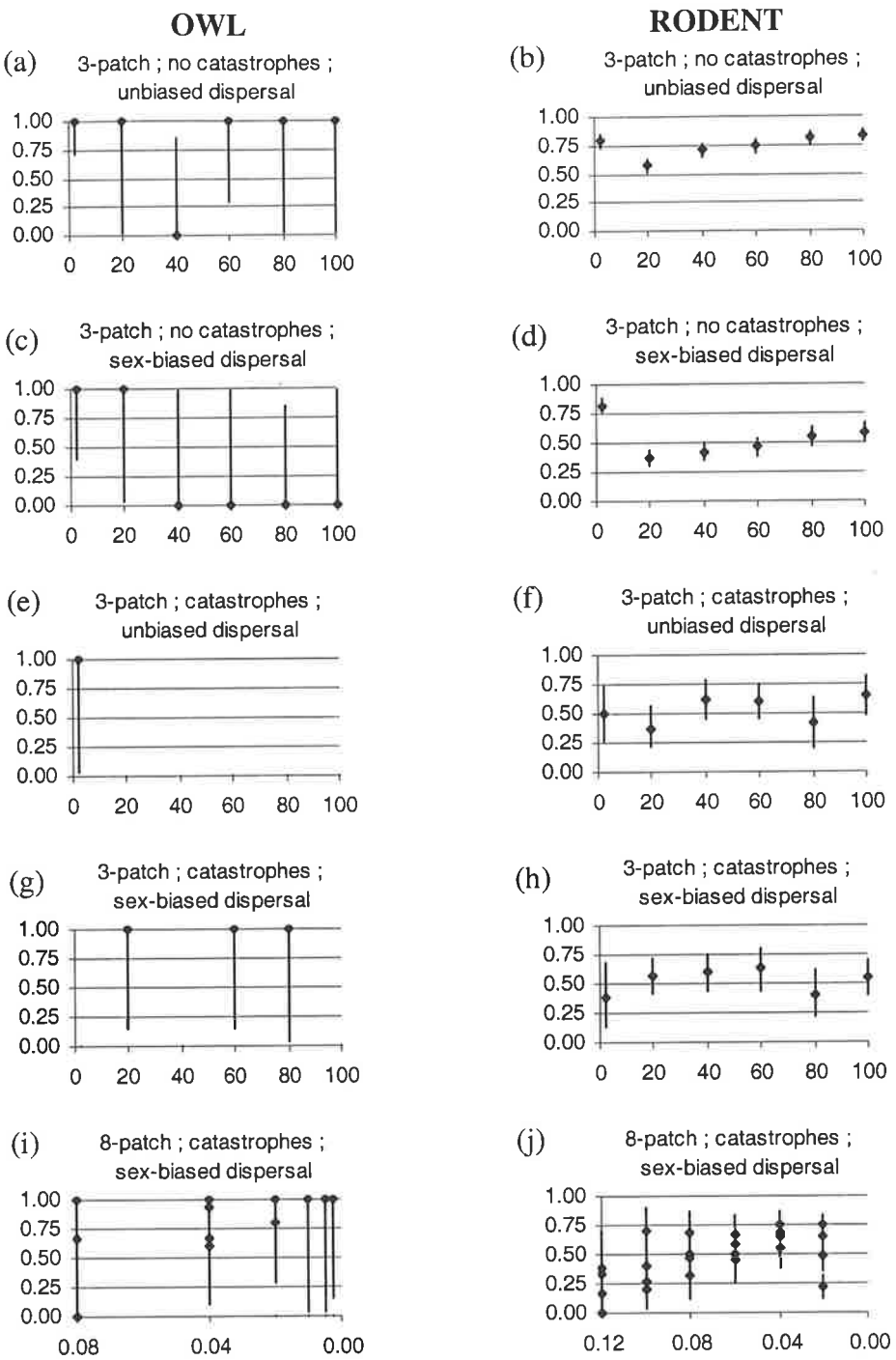

Figure 6.26 Proportion of correct genetic rankings for owls (left) and rodents (right) when using a 10-year sample of the **number of polymorphic loci**. Where large enough, 95% confidence intervals extend beyond markers. For the eight-patch systems the four markers at each connectivity level correspond to the following patch pairs: P_{50} & P_{20}, P_{45} & P_{15}, P_{30} & P_{25}, and P_{40} & P_{35}. Connectivity for three-patch metapopulations is the % of dispersers successfully moving between the large and small patches; connectivity for eight-patch metapopulations is dispersal mortality per unit distance (note: the x-axis for eight-patch systems is reversed so that connectivity increases from left to right).

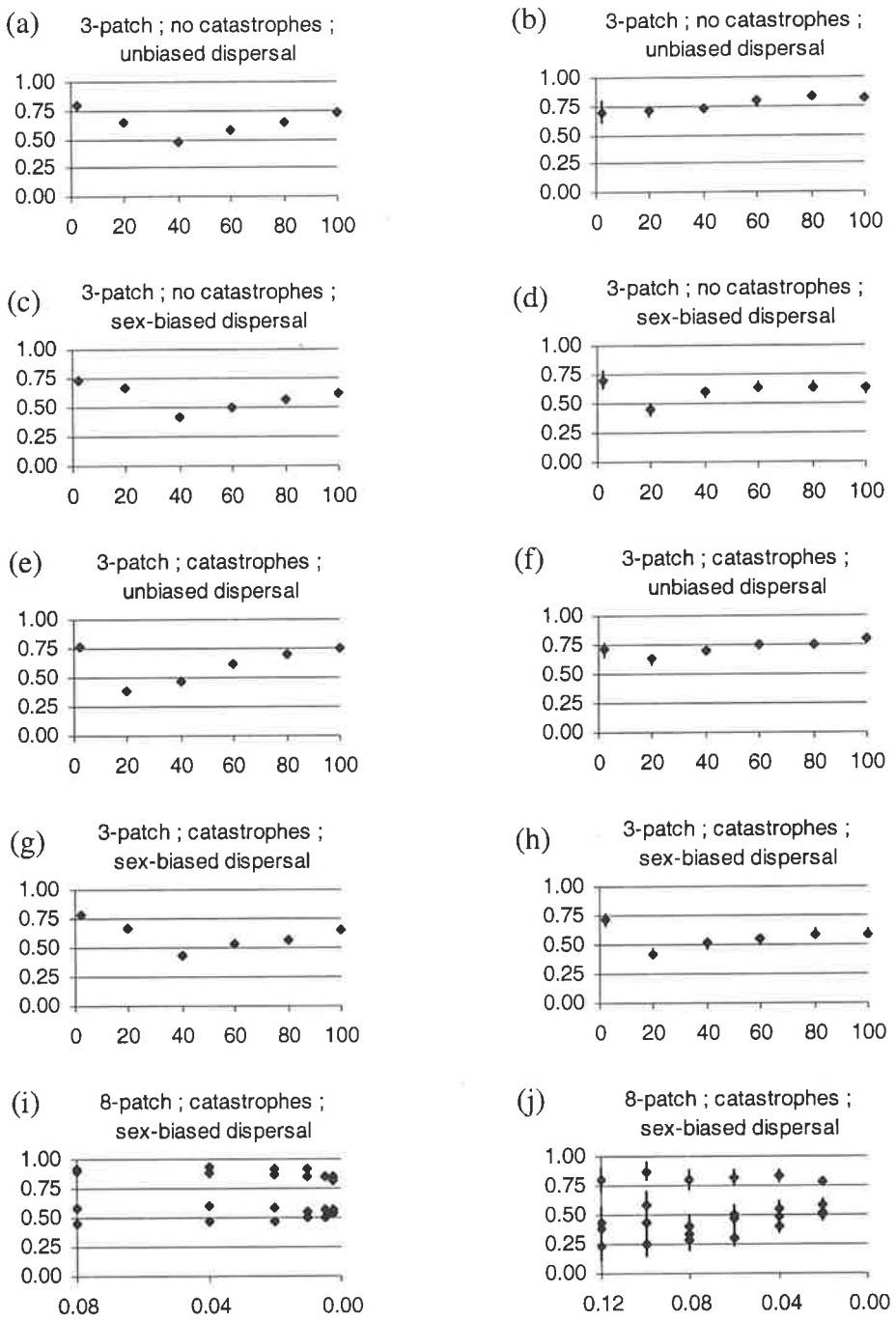

Figure 6.27 Proportion of correct genetic rankings for owls (left) and rodents (right) when using a 10-year sample of **mitochondrial diversity**. Where large enough, 95% confidence intervals extend beyond markers. For the eight-patch systems the four markers at each connectivity level correspond to the following patch pairs: P_{50} & P_{20}, P_{45} & P_{15}, P_{30} & P_{25}, and P_{40} & P_{35}. Connectivity for three-patch metapopulations is the % of dispersers successfully moving between the large and small patches; connectivity for eight-patch metapopulations is dispersal mortality per unit distance (note: the x-axis for eight-patch systems is reversed so that connectivity increases from left to right).

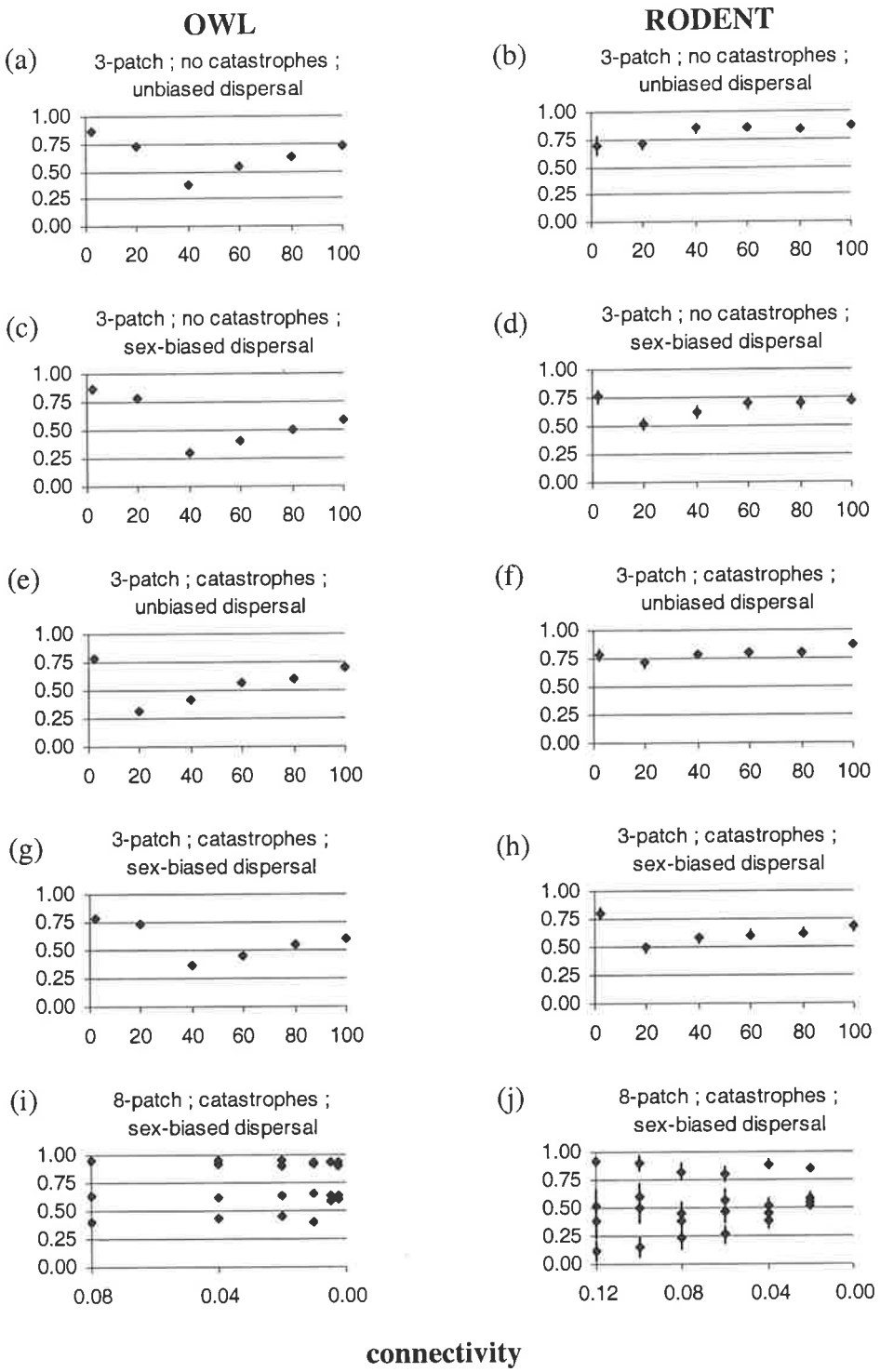

Figure 6.28 Proportion correct genetic rankings for owls (left) and rodents (right) when using a 10-year sample of the **number of mitochondrial haplotypes**. Where large enough, 95% confidence intervals extend beyond markers. For the eight-patch systems the four markers at each connectivity level correspond to the following patch pairs: P_{50} & P_{20}, P_{45} & P_{15}, P_{30} & P_{25}, and P_{40} & P_{35}. Connectivity for three-patch metapopulations is the % of dispersers successfully moving between the large and small patches; connectivity for eight-patch metapopulations is dispersal mortality per unit distance (note: the x-axis for eight-patch systems is reversed so that connectivity increases from left to right).

6.4.1.3 Choosing an appropriate measure of genetic diversity

The purpose of this section is to choose a particular measure of genetic diversity with which to base further analyses. Of the six measures, two (observed heterozygosity and the number of polymorphic loci) have already been identified as poor candidates for predicting relative patch value. When choosing among the remaining four measures of genetic diversity, the first point to note is that the proportion of guesses correct is highly correlated among the four measures. While this can be seen by comparing the shapes of Figures 6.24, 6.25, 6.27 and 6.28, Figure 6.29 summarises these data as a set of pairwise correlations, with the correlation coefficient, r, ranging from 0.95 to 0.98. Based on how tight these correlations are (and the fact that the data fall approximately around a 1:1 line), it would seem that none of the genetic measures stands out as being better than the others. Given this freedom of choice I decided to use the mean number of alleles per locus. As will become clear later, this measure is particularly useful in providing a quantitative measure of relative genetic diversity of two patches. Because the mean number of alleles per locus is greater than or equal to 1, we can always obtain a ratio of genetic diversity between two patches, and because it is a real number, the ratio can potentially take on a large number of values. In contrast, both expected heterozygosity and mitochondrial diversity can drop to zero, thereby preventing us from calculating a ratio. The number of mitochondrial haplotypes, while always greater than or equal to 1, has the disadvantage of being an integer. Accordingly any ratio of two patches will be limited to a smaller number of possible values than if we used the mean number of alleles per locus.

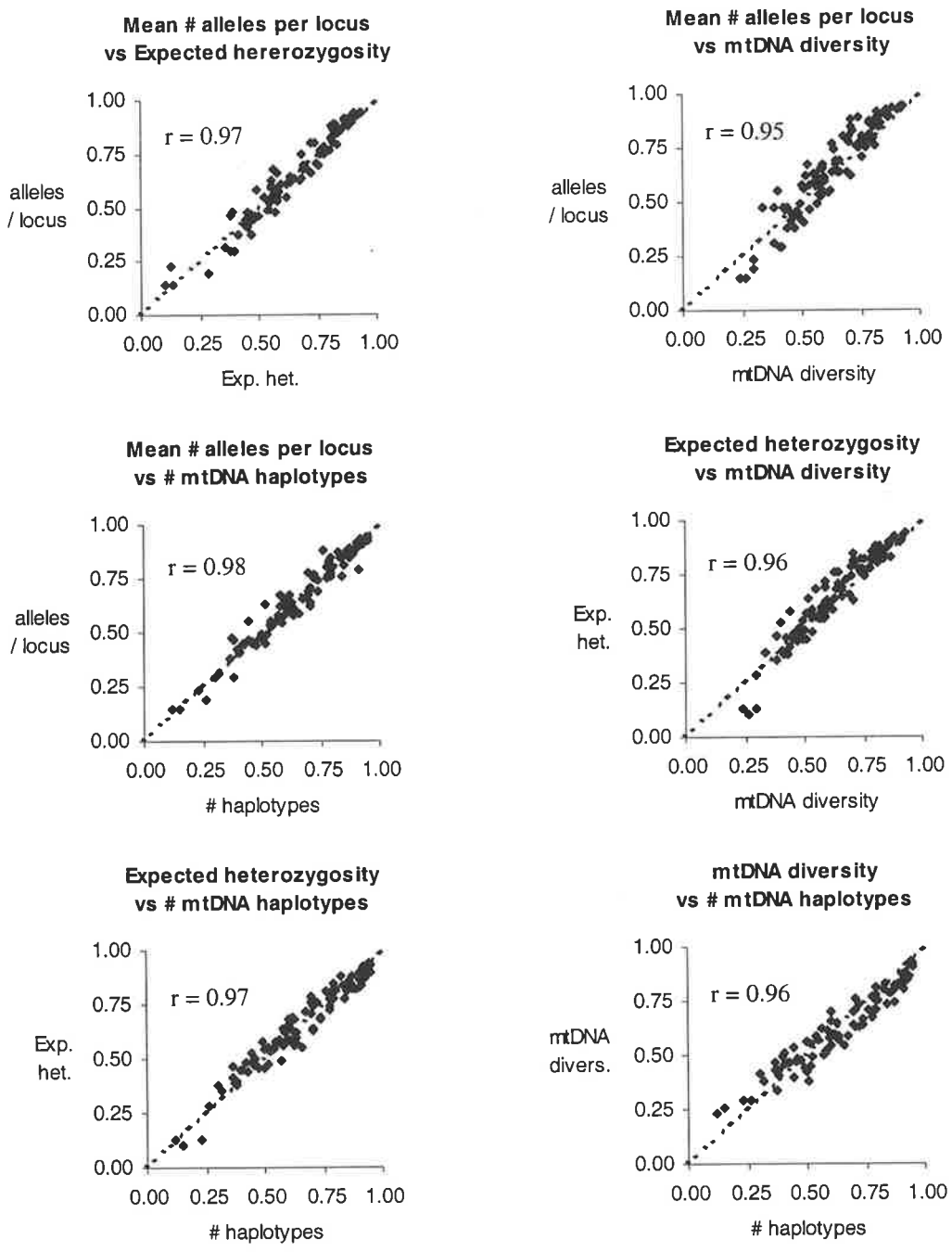

Figure 6.29 Correlations of the proportion of correct patch rankings between different measures of genetic diversity. The four measures compared here are expected heterozygosity, the mean number of alleles per locus, mitochondrial diversity and the number of mitochondrial haplotypes. Expected heterozygosity refers to the expectation under Hardy-Weinberg equilibrium.

6.5 Understanding how the proportion of correct genetic rankings varies as a function of relative patch value

6.5.1 Using 10-year samples of the mean number of alleles per locus for owls and rodents

Based on the results so far, we are limited to stating that the predictive accuracy of using genetics to guess relative patch value varies among scenarios from less than 50% (i.e. worse than a random choice) to more than 90% (see Figure 6.25 for the mean number of alleles per locus). In itself this would provide very little guidance for the managers of metapopulations, other to say that genetic ranking of patches *can* be accurate in some circumstances. Importantly however, this variation in predictive accuracy appears to be related to how different two patches are in value (i.e. compare Figures 6.25 and 6.10). For the purposes of managing metapopulations, it would certainly be useful to know if genetic rankings are more likely to be correct when the real difference in patch value is greater. This would potentially provide a useful way for dealing with variation in the proportion of genetic rankings that are correct. So although we can't make a general statement such as:

"there is an 80% chance of correctly ranking two patches"

we might be able to make the statement that:

"there is an 80% chance of correctly ranking two patches whose relative value differs by a factor of 2 or more."

In other words, we might be able to make statements about the probability of making mistakes of varying magnitude. The important point here is that the more different two patches are in value, the greater our need (from a management perspective) to be correct in ranking them. For example we don't want to give advice to clear a patch of native vegetation if there is a much less valuable patch to sacrifice in its place. In contrast, if two patches have very similar value (i.e. RV2 = 1), we should not really care if we rank them incorrectly. Even the misleading scenarios (i.e. when less than 50% of genetic rankings are correct) are not of any great concern if they occur only with pairs of patches that have similar value.

Figure 6.30 shows how the proportion of correct genetic rankings varies as a function of relative patch value (RV2). Ideally we would like the relationship between the proportion of correct genetic rankings and relative patch value to be positive, tight, steep, and reaching a high asymptote. Unfortunately this is not the case for most of the data in Figure 6.30, with the exception of the scenarios in Figures 6.30d and 6.30i. Instead, the high level of noise apparent in most of Figure 6.30 suggests that we are unable to use genetic rankings to make meaningful statements about relative patch value, at least in the set of scenarios examined here.

6.5.2 Using 40-year samples of the mean number of alleles per locus for owls: an encouraging insight

Given the lack of success in using 10-year genetic samples to predict relative patch value, I decided to perform the same set of analysis using 40-year samples, thereby giving more time for the genetic diversity of subpopulations to have diverged. Importantly however, these analyses could only be performed for the owl metapopulations, since very few rodent metapopulations were fully occupied 40 years after fragmentation (Figure 6.21). As with the 10-year samples, the mean number of alleles per locus was chosen as the measure of genetic diversity.

Figure 6.31 shows how the proportion of correct genetic rankings varies as a function of relative patch value (RV2). This is the 40-year equivalent of the left-hand column of 6.30. Importantly, note how for each scenario these relationships are now positive, tight, steep, and reach a high asymptote - thereby satisfying all the criteria we would expect of a meaningful predictor of relative patch value. Pooling the data from all owl scenarios examined in this thesis, Figure 6.32a demonstrates how a single tight relationship between the proportion of correct genetic rankings and relative patch value accommodates the different situations. Included here are three-patch metapopulations (with and without catastrophes, with and without sex-biased dispersal), eight-patch metapopulations (with catastrophes and sex-biased dispersal), and a range of patch pairs - some where relative patch value is determined by size, and some by connectivity. From this relationship we could claim that there is at least a 75% chance that a genetics-based guess will correctly rank two patches whose relative value differs by a factor of 4 or more. Note that in these 40-year data, there are none of the misleading (less than 50% correct) scenarios that occurred with the 10-year samples. This

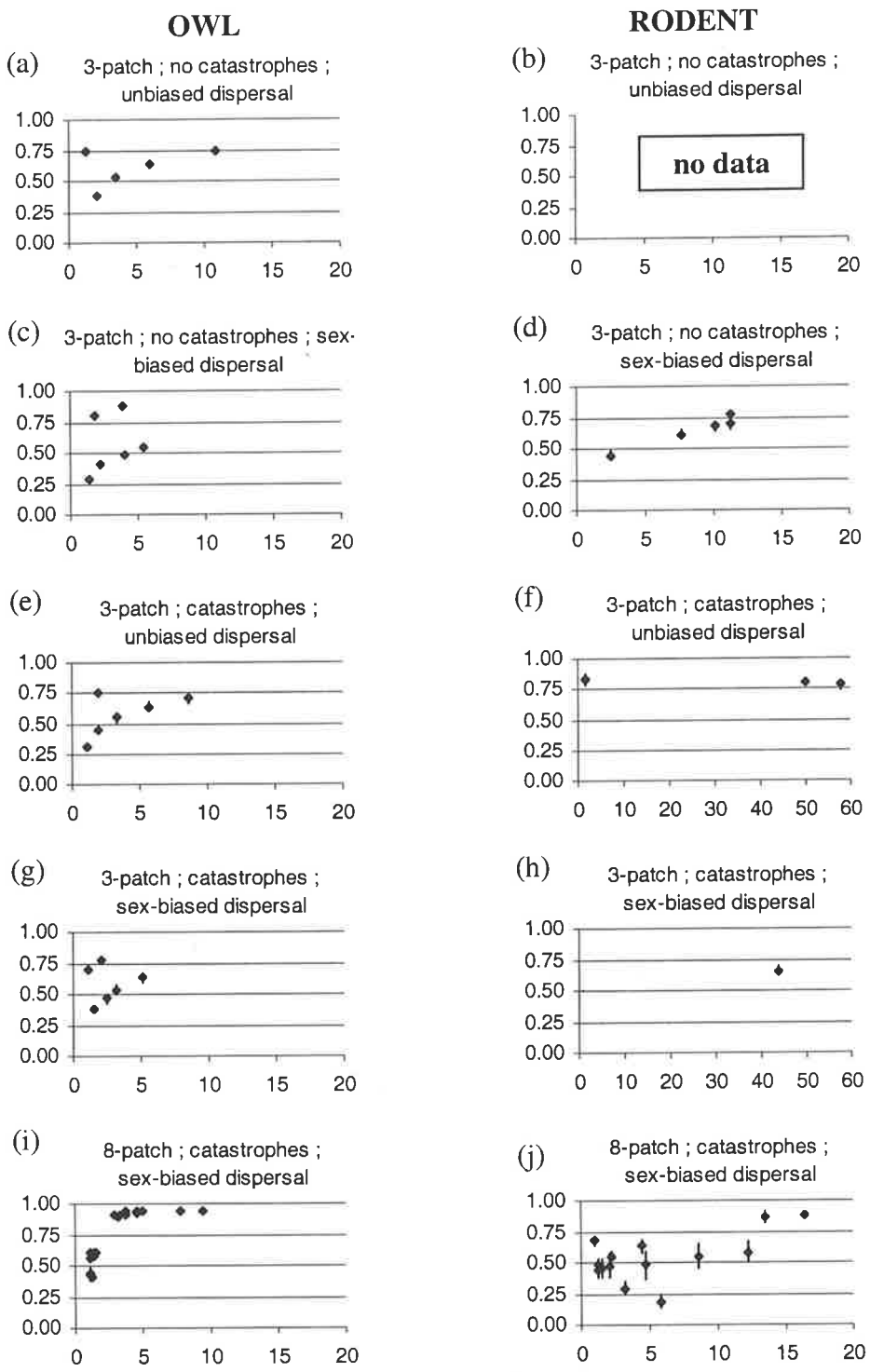

Figure 6.30 The proportion of correct genetic rankings versus relative patch value (RV2) using a **10-year sample** of the mean number of alleles per locus, for owls (left) and rodents (right). Where large enough, 95% confidence intervals extend beyond markers.

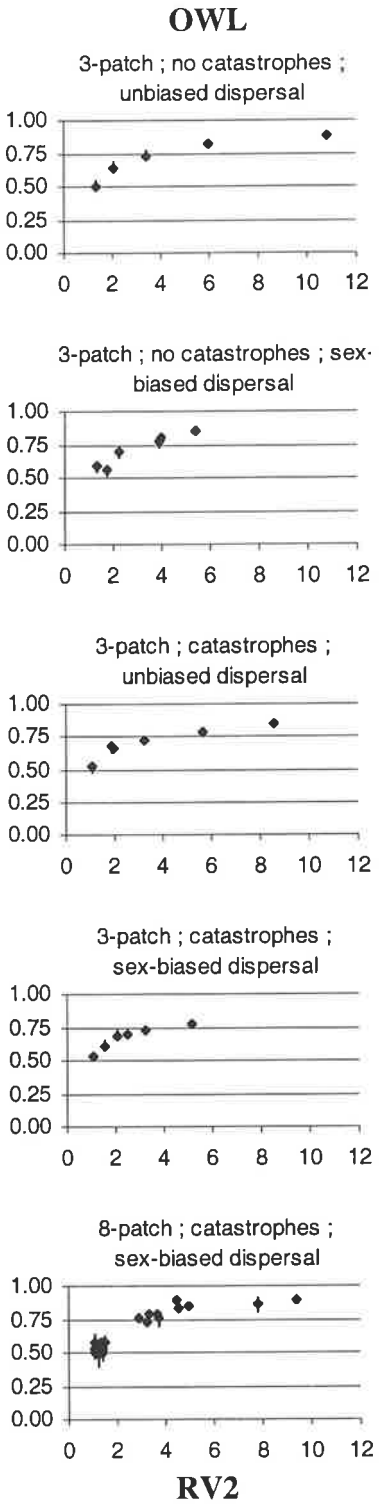

Figure 6.31 The proportion of correct genetic rankings versus relative patch value (RV2) using a **40-year sample** of the mean number of alleles per locus, for owls only. Where large enough, 95% confidence intervals extend beyond markers.

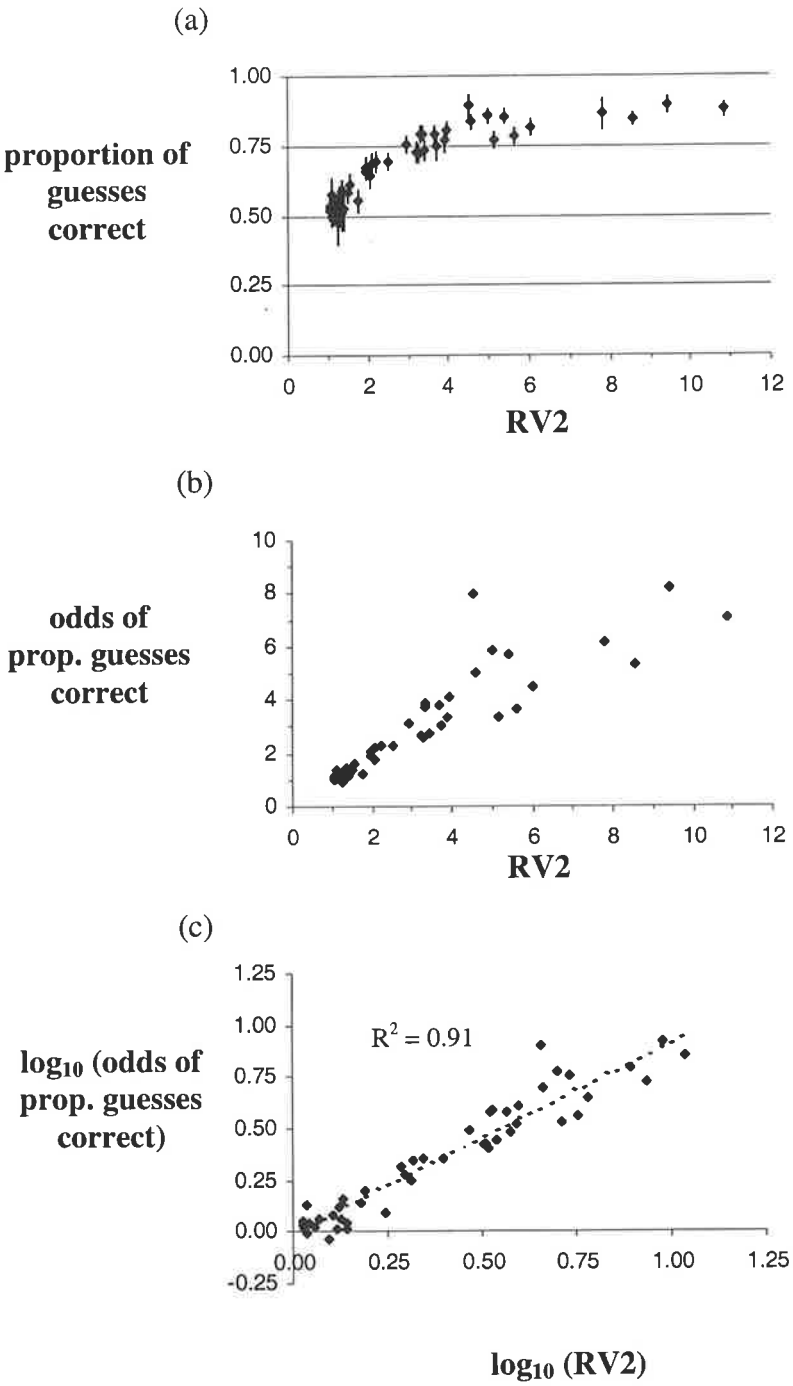

Figure 6.32 Pooled data over all owl scenarios for the proportion of correct genetic rankings vs relative patch value (RV2) using a 40-year sample of the mean number of alleles per locus. (a) untransformed data. (b) y-axis has been transformed to odds. (c) both y and x axes have been $\log_{10}$ transformed. Where large enough, 95% confidence intervals extend beyond markers.

suggests that the misleading results for 10-year samples are partly because the system has not yet approached quasi-equilibrium (i.e. equilibrium conditional on subpopulations still being extant). Remember that these misleading results occurred when small patches were not properly identified as the most important patch in a pair. Accordingly it would seem that if a system is sampled too soon after fragmentation, there has been insufficient time for dispersal to have made the genetic diversity of a small patch greater than that of a larger but less important patch to which it is being compared.

To further describe the relationship in Figure 6.32a, it is convenient to initially transform the y axis from a proportion, p, to its odds, p/(1-p). This has the desirable effect of making the relationship between y and x approximately linear (Figure 6.32b). To perform linear regression however, it is also necessary that the data are homoscedastic (equal variance around y for different values of x). This was achieved with these data by log-transforming both the x and y values, giving Figure 6.32c. The regression equation for this relationship is $y = -0.0133 + 0.93x$ (R^2=0.91, d.f. = 45, $P < 0.001$).

Perhaps most importantly, the results from this regression show that relative patch value explains 91% of the variation in the proportion of correct genetic rankings. Keeping in mind that some of the remaining 9% will be partly due to sampling error (see error bars in Figure 6.32a), this result tells us a number of things. Firstly it suggests that within a set of circumstances (*e.g.*, 40-year samples the number of alleles per locus in owl metapopulations), we may expect a high level of robustness to this method of ranking patches. That is, even including variables such as environmental catastrophes, sex-biased dispersal and the number of patches, there is a tight relationship between the proportion of correct rankings and relative patch value. To the extent that these scenarios differ, these results indicate that the approach used here has passed a form of sensitivity analysis. This in turn suggests that if we have a system with unknown parameter values, we can include a range of different, plausible scenarios and hope that that the same relationship between the proportion of correct rankings and relative patch value holds across all scenarios.

Secondly, the tightness of the regression supports the use of the measure of relative patch value used in this thesis. The fact that a pattern of genetic diversity can be so closely related to a set of metapopulation extinction probabilities suggests that the relative patch value measure used here is indeed meaningful. If, in contrast, it was a meaningless measure of relative patch value we could not expect the tight relationship in Figure 6.32. This confirms not only Lindenmayer and Possingham's (1996) equation for the value of a single patch (Eqn 5.5), but also its adaptation here into the relative value of two patches (Eqn 5.6).

6.6 Making the most of genetic diversity data

So far the data have only been analyzed in terms of which of two patches has the highest genetic diversity. This ignores some potentially useful data concerning the magnitude of the ratio of genetic diversity between two patches. The important issue here is that a large ratio of genetic diversity may be a good indication that there is a large difference in the value of the two patches. To understand how this affects our estimate of making a correct guess of patch ranking, consider the following. Figure 6.33 shows a frequency distribution of 1000 genetic ratios for the scenario of a three-patch owl metapopulation with no catastrophes, and unbiased 100% dispersal. These data are based on 40-year samples of the mean number of alleles per locus. In this scenario the medium patch is less valuable than the small patch because of the small patch's connectivity to the large patch. This is reflected in the relative patch value (RV1) of the medium patch to the small patch, of 0.09. Accordingly, most of the genetic ratio values (medium/small) are less than 1 (Figure 6.33b), the proportion being 0.87 (i.e. 874 of the 1000 replicates). Therefore, if we simply guess that the patch with the highest genetic diversity is the most important patch then there is an 87% chance of being correct.

The accuracy of this guess improves however, if we take into account the magnitude of the genetic diversity ratio. For example, we can ask what the probability of being correct is when the observed ratio of genetic diversity is greater than or equal to 2.0 (or less than or equal to its reciprocal, 0.5). In this case we exclude all genetic ratios between 0.5 and 2.0 (Figure 6.33c) and recalculate the probability of being correct as 0.94 (276/294). Thereby the confidence of making a correct guess has increased from 0.87 to 0.94.

Figure 6.34 shows how taking into account the size of genetic diversity ratios increases our confidence in correctly guessing patch value across the full set of owl metapopulation scenarios examined in this thesis. As before, this is using 40-year samples of the mean number of alleles per locus. Figure 6.34a is virtually the same as 6.32a, the only difference being the level of replication. In the case of 6.32a, proportions were based on anything up to 1000 replicates, depending on the number of metapopulations fully-occupied, and the number of replicates whose genetic diversity ratio was not exactly 1. In contrast, each of the proportions in Figures 6.34a and 6.34c was based on 100 values, so as to standardise the level or replication for 6.34a and 6.34c.

Note the higher proportion of correct rankings when patches are ranked only if the ratio of genetic diversity is greater than or equal to 2.0 (or less than or equal to 0.5). Not only does the curve in the selective case (6.35c) rise faster than the all-inclusive case (6.35a), but it also reaches a higher asymptote. The difference between the two curves can be seen clearly by comparing the regression lines in Figures 6.34b and 6.34d. These two data sets are superimposed in Figure 6.34e, with the slopes of the two regression lines being significantly different ($F = 6.89$, d.f. = 88, $P = 0.01$, using Sokal and Rohlf (1981, p. 499)).

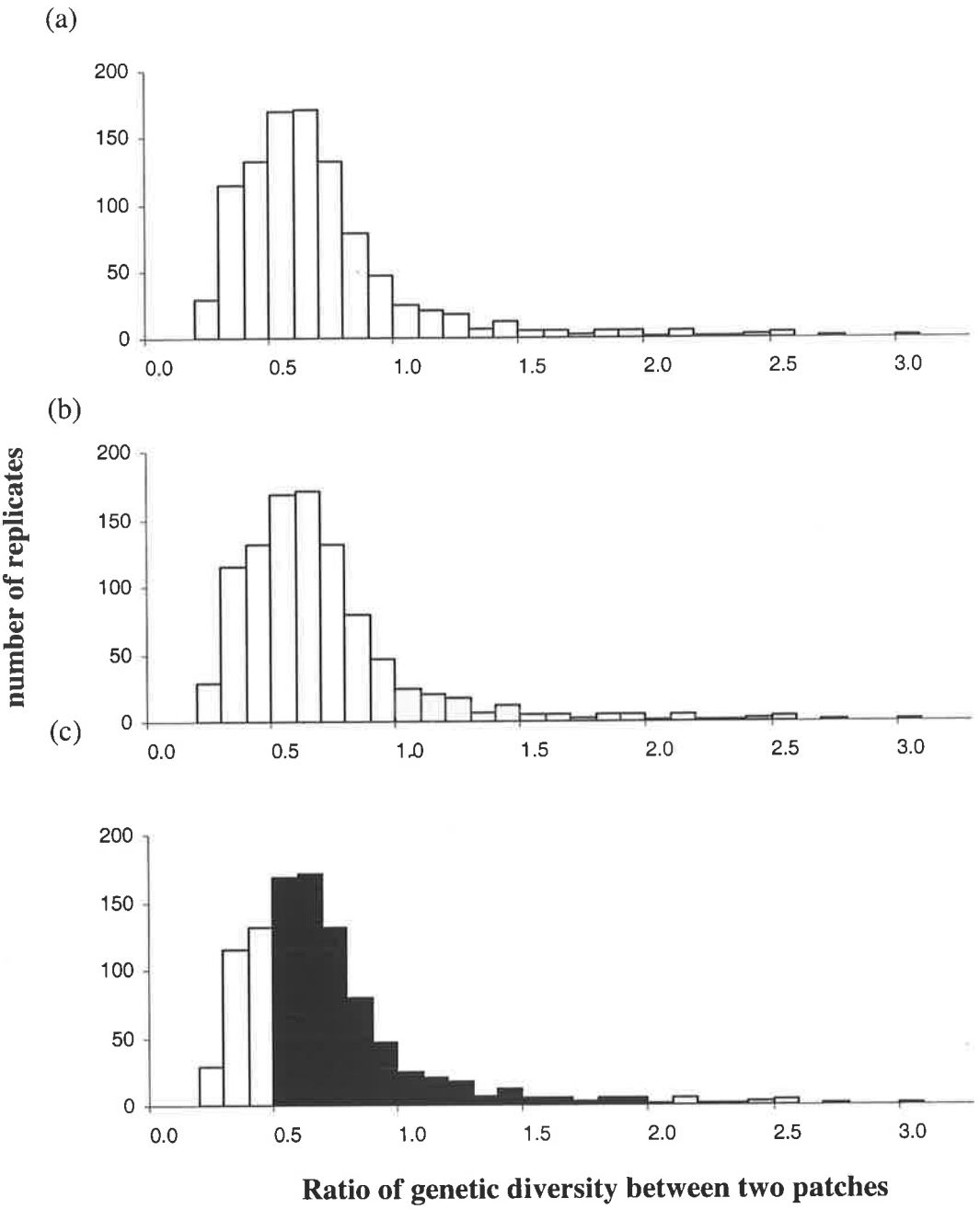

Figure 6.33 Taking into account the magnitude of the ratio of genetic diversity between two patches. These data are from 1000 replicate 40-year samples, comparing the mean number of alleles per locus (medium patch / small patch) in a three-patch owl metapopulation with no catastrophes and unbiased 100% dispersal. (a) raw data. (b) shows the proportion of correct genetic rankings (unshaded region as a proportion of total data set). (c) removal of those data not exceeding some threshold ratio (in this case data between 0.5 and 2.0).

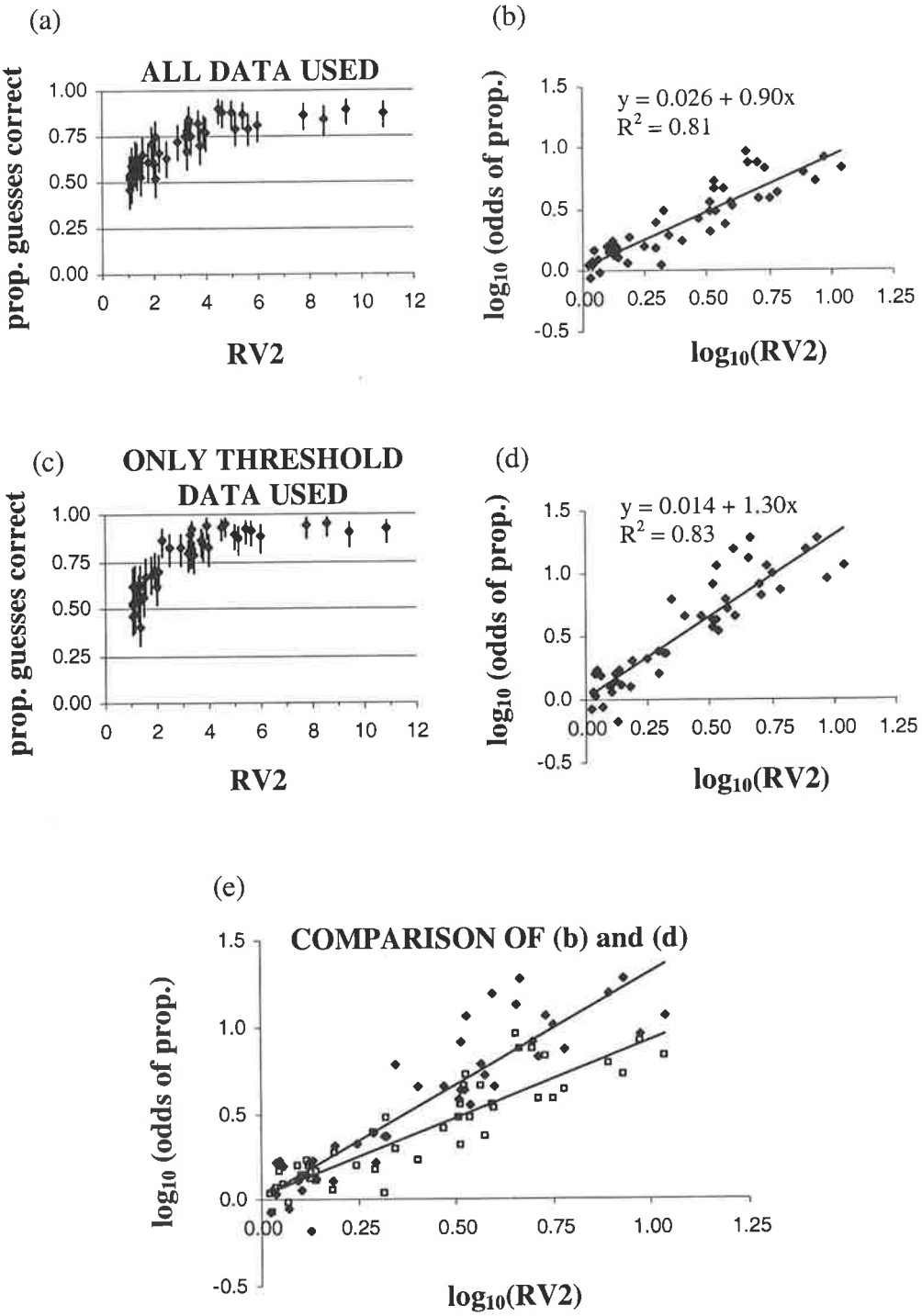

Figure 6.34 The importance of using genetic diversity ratios that lie beyond some threshold level. These data are pooled from all five owl metapopulation scenarios using 40-year samples of the mean number of alleles per locus. (a) and (b) give the proportion of correct genetic rankings when all data are used, while (c) and (d) are based only on replicates where the genetic ratio was larger than 2.0 or less than 0.5. (e) compares the two data sets, representing the values from (b) as white squares and those from (d) as black diamonds. Each value is based on 100 replicate metapopulations and 95% confidence intervals are given for (a) and (c).

6.7 Summary of model results

The MultiPop model has shown that genetic diversity data can provide a useful way for estimating the relative value of the patches in a metapopulation. Among the situations examined, a strong link between genetic diversity and patch value was found using the mean number of alleles per locus, sampled 40 years after fragmentation in owl metapopulations. In contrast, measuring genetic diversity in owl metapopulations only 10 years after fragmentation provided a very poor basis for estimating patch value. This clearly demonstrates that metapopulations created by the fragmentation of a previously panmictic population should not be sampled too soon after fragmentation or the genetic diversity of their subpopulations will not have diverged enough to distinguish patches of different value.

Unfortunately rodent metapopulations could only be studied at sampling times close to fragmentation, where there was no clear pattern between genetic diversity and relative patch value. Because of their highly stochastic dynamics (due to high fecundity and high mortality), the rodent metapopulations were so extinction-prone that there was a scarcity of fully occupied metapopulations beyond the 10-year sampling time.

The data produced by MultiPop suggest that there are no golden rules in relating genetic diversity to relative patch value; in other words, there is no single probability of correctly ranking patches. Although under some circumstances the probability of successfully ranking patches can be quite high (greater than 90%), this probability varies according to how different two patches are in value. As such, we can really only hope to talk about "accuracy versus relative patch value curves", the quality of which is determined by a combination of the tightness and steepness of the relationship, and height of its asymptote. Essentially these curves allow us to describe the probability of making mistakes of varying magnitude, where ideally we want a low probability of making a big mistake when estimating relative patch value.

Some metapopulations may lend themselves very well to this framework of analysis. This is evident in the analysis performed on 40-year samples of owl genetic diversity in this thesis. In this case the accuracy versus relative patch value curve was indeed a tight, steep relationship with a high asymptote. Importantly, the shape of this relationship seemed insensitive to details of the metapopulation's structure - disturbance regime (none or some),

dispersal (amount and whether or not it was sex-biased), and the size of the metapopulation (three versus eight patches). Even more encouraging was that both the steepness of the relationship and height of the asymptote were improved by excluding data in which the ratio in genetic diversity between the two patches was less than a nominated threshold.

If measures of genetic diversity are to be used to estimate relative patch value, it is important to identify the conditions under which the approach works. In the following chapter I suggest how the managers of specific metapopulations might incorporate a genetic-based approach to ranking the value of the different patches for a particular species. Nonetheless it is important to consider the need for general research in developing an understanding of the link between genetic diversity and relative patch value. One of the major challenges in this regard would be to try to gain an indication of what combinations of organism life histories, patch carrying capacities and sampling times provide the basis for reliable genetics-based ranking of patches. While rodent metapopulations were examined in this study, their carrying capacities were clearly too low (and hence extinction probabilities too high) to fully explore the potential link between genetic diversity and relative patch value. Increasing the carrying capacities of rodent metapopulations should result in higher levels of patch occupancy, slower and more deterministic declines in genetic diversity over time, and thereby possibly provide the basis for more successful ranking of patches at sampling times greater than the 10 years after fragmentation examined here. This however, would require making significant changes to the MultiPop model, possibly in a different language to Turbo 7.0 Pascal, which (a) accommodates more individuals, and (b) allows faster population dynamics. If possible, it would also be valuable to include organisms with very different life histories to owl and rodents, such as plants and invertebrates.

CHAPTER 7

Discussion and conclusions

The purpose of this chapter is to discuss how the findings from the model in Chapters 5 and 6 may be applied in managing real metapopulations, and how the approach presented in those chapters relates to existing methods used in metapopulation management. I then make some final comments to summarise the ideas in this thesis.

7.1 How can metapopulation managers use genetic diversity to rank patches?

Chapter 6 showed that genetic diversity data can, under some circumstances, provide a useful way for estimating the relative value of patches in a metapopulation. For the managers of any real metapopulation, the challenge lies in knowing how well to trust this potential link between genetic diversity and relative patch value. In particular, this requires system-specific knowledge of the shape of the "accuracy versus relative patch value" curve that describes how the estimated proportion of correct genetics-based patch rankings varies as a function of the relative value of the two patches being ranked.

Importantly, the owl and rodent metapopulations presented in this thesis should only be seen as examples of how to perform similar analyses, and as such, the results here are not directly applicable to any real scenario. Thus, although a strong relationship was found to link the mean number of alleles per locus to relative patch value for owls sampled 40 years after habitat fragmentation, this does not mean that this finding applies directly to any real owl metapopulation. There are simply too many parameters and assumptions specific to these simulations to have confidence they will match up closely with any particular real system. Furthermore, many of the assumptions used in this thesis are optimistic. For example the subpopulations were initialised with very high levels of genetic diversity, and all genetic diversity measurements were based on a complete census of every individual in each subpopulation being measured.

Accordingly, it is important that the link between genetic diversity and relative patch value is thoroughly explored for any new system to which it is applied. This would involve first creating a simulation model that best captures the dynamics and genetics of the system at hand, and then performing sensitivity analysis for those parameters that are poorly understood. Because population simulation models often contain a large number of processes, the most practical approach is to simply fix well-known parameters while varying the poorly known ones. In the MultiPop model presented in Chapters 5 and 6, disturbance regime and dispersal biology (with or without sex-bias) were varied as though they were unknowns, while many other components of population biology such as fecundity and mortality were treated as if they were known. In contrast, the manager of a real system may have a good description of disturbance and dispersal biology, and decide to keep those descriptions fixed while performing sensitivity analysis on poorly known aspects of the organism's reproductive biology. Biologists may also want to relax some of the many assumptions made in this thesis. This might involve:

(1) Allowing genetic diversity to influence demographic processes, whereby loss of genetic variation and inbreeding depression increase population extinction probabilities (Caughley, 1994; Frankham, 1995a; Sanjayan *et al.*, 1996; Saccheri *et al.*, 1998). This is predicted to strengthen the link between genetic diversity and patch value, given that patches with higher genetic diversity (and therefore less inbreeding depression) would be more resistant to extinction than patches with low genetic diversity.

(2) Allowing inbreeding depression to change the impact that immigrants have on genetic diversity (Ball *et al.*, 2000; Chapter 2 of this thesis).

(3) Providing a more realistic description of density dependence than the simple step functions used for the owls and rodents in Chapters 5 and 6.

(4) Including source/sink dynamics whereby habitat quality varies between patches (Pulliam, 1988; Harrison, 1991; Gaona *et al.*, 1998). It may be that centrally located sink populations have high genetic diversity due to the influx of genes, but are of very little value for the persistence of the metapopulation. In this way, source/sink dynamics may weaken the link between genetic diversity and patch value.

(5) Sampling only a proportion of the subpopulations - a situation likely to apply to cryptic organisms. This would increase the error of genetic diversity measurements, and therefore weaken the link between genetic diversity and patch value. It is also important to consider how the various genetic diversity measures are biased by sample size. In particular, the number of haplotypes, number of polymorphic loci and the mean number

of alleles per locus are all expected to increase with sample size. For example, if we only sample five individuals from a population, then we are limited to recording a maximum of five haplotypes. By using complete population censuses (as in this thesis), higher values for these variables are partly a reflection of the fact that some patches contain more individuals. This sampling effect increases the apparent genetic diversity of patches with higher carrying capacities, and would therefore strengthen the link between genetic diversity and patch value. In reality, biologists may invest similar sampling effort into all patches, and in such cases it would be appropriate to sample the same number of individuals from each patch when running the model. Note that this sampling effect does not apply to observed or expected heterozygosity, or mitochondrial diversity, which are only expected to decrease in variance as a function of sample size.

It is important that any model is set up to incorporate the history and limitations of the real system being studied. For example, if the real system in question was fragmented gradually over a period of 20 years then ideally the same patch-by-patch history of fragmentation should be incorporated into the simulation. And if genetic diversity data exist for only six loci, then this should also be included as a constraint. In many situations, the real situation may dictate the timing of genetic sampling in the model. For instance we may have a metapopulation that was fragmented 54 years ago but for which we urgently need to assess relative patch value. In that case we would need to specify that the model takes a genetic sample at the 54-year mark. Alternately, we may be trying to manage a recently fragmented population, in which case we may have the opportunity to choose which set of future sampling times will provide the best indication of relative patch values.

Any new study aiming to establish a link between genetic diversity and relative patch value should explore the utility of the different measures of genetic diversity. The mean number of alleles per locus was chosen for the organisms in this thesis because, compared to the three other measures which provided similar predictive capabilities, this measure had the added advantage of allowing calculation of the magnitude of the ratio of the genetic diversity in two patches. This in turn provided the basis for more accurate genetics-based estimates of relative patch value, whereby patches were only ranked when the ratio of the genetic diversity of their subpopulations was above some critical level. Although this same advantage may apply in other situations, some of the other measures of genetic diversity may be of greater utility under different circumstances. For example, the slow decay in the

number of polymorphic loci may mean that in some situations this measure provides more information than the other, more rapidly decaying measures of genetic diversity.

Real metapopulations are likely to have properties that were not introduced in this thesis. For instance some patches might be unoccupied. This complication was avoided in the owl and rodent metapopulations in Chapters 5 and 6 by only analyzing data from fully occupied metapopulations. Partially unoccupied metapopulations present a challenge for a number of reasons. Firstly, because unoccupied patches contain no genetic diversity they cannot be ranked using a genetics-based method. This clearly ignores one of the major lessons to be learnt from metapopulation biology - that unoccupied habitat patches can be valuable. Nonetheless it may be possible to rank occupied patches relative to each other, and where possible, assign values to empty patches of similar size and position to some of the ranked patches. Alternately, it may be appropriate to simply give empty patches a default minimum value of zero or one, depending on the measure of genetic diversity used to rank the occupied patches. Thus, the minimum for observed and expected heterozygosity, the number of polymorphic loci and mitochondrial diversity would be zero, while the minimum for the number of haplotypes and the mean number of alleles per locus would be one. This approach would effectively treat all empty patches as the least valuable patches in a metapopulation. Although this would lead to some mistakes (i.e. when highly valuable patches are unoccupied), the chance of making such mistakes may be quite low given that the more valuable a patch is to metapopulation persistence, the less likely it is to be unoccupied (Day and Possingham, 1995). Exploring different ways of ranking empty patches would certainly be an important step in being able to apply the insights of Chapters 5 and 6 to other systems.

An important simplification made in this thesis was that patch value was defined on the basis of removing a single patch at a time. Importantly, some management scenarios may require that we consider the simultaneous removal of more than one patch (Lindenmayer and Possingham, 1996). For instance the manager of a metapopulation may need to know whether it is more important to preserve one large patch than a group of six smaller patches. The potential complication here is that while each of the small patches may individually be of little value, they may collectively be of greater value than the single large patch, particularly if they are tightly linked by dispersal. Determining the value of groups of patches presents something of a challenge. Simply determining the average genetic diversity will only give us the average value of each patch, and will not represent their collective value. An alternate

approach would be to treat each group of patches as a single entity, and calculate genetic diversity across all individuals in the group of patches. However this may lead to erroneous results in many situations. For example, consider two small patches of low value at either end of a metapopulation. Although each population may have become fixed at all the loci being measured, when analysed as a pair (by pooling data) the two patches may appear to have high genetic diversity, and therefore lead to an overestimate of their value as a pair. In summary it is not clear whether there is any promising way of using genetics to estimate the value of groups of patches, and as such, this may represent one of the limitations of genetics-based ranking of patches.

7.2 The role of genetics-based patch ranking in the context of other approaches

While this thesis suggests there is a potential role for using genetic diversity data to estimate relative patch value, it is important to recognise its place as one of a number of approaches to determining the relative value of patches in metapopulations. For systems with many patches, Hanksi's incidence function provides a well established statistical basis for gaining insights into metapopulation dynamics and hence patch ranking (Hanski, 1994a; Hanski, 1994b; Nieminen, 1996; Lindenmayer *et al.*, 1999; Moilanen, 1999). In contrast, for systems with few patches, population viability analyses (PVAs) that incorporate basic details of a population's biology form a key approach to making management decisions (Beissinger and Westphal, 1998). As described earlier, the link between genetic diversity and relative patch value should be established separately for each system using what is essentially a PVA. In this way, genetics-based estimation of relative patch value should be thought of as one component to potentially emerge from of a more comprehensive PVA approach applicable to small metapopulations. With this being the case, there are two possible ways of ranking the patches in small metapopulations: (a) PVA can be used to directly calculate patch values based on the patch-removal method (Day and Possingham, 1995; Lindenmayer and Possingham, 1996), and (b) genetic diversity data can be used to rank patches if the PVA has shown a strong link between genetic diversity and relative patch value. While the first approach may suffice when patch value predictions are robust to unknown variables in the model's structure, in situations when this is not the case it may be useful to also consider estimates based on genetic diversity data (if these are robust to sensitivity analysis).

7.3 Final comments for the thesis

While metapopulation genetics and demographics are becoming more integrated disciplines with time, there remains much opportunity to develop our understanding of the link between these two aspects of metapopulation biology. Developments in molecular biology (Avise, 1994; Jarne and Lagoda, 1996) have meant that biologists can often collect large amounts of data on the genetic diversity of fragmented populations. The challenge in many cases lies in analyzing those data in ways that give meaningful insights into the dynamics of metapopulations. In this thesis I explored some aspects of how patterns of genetic diversity can be used to understand metapopulation dynamics.

I have considered how genetic diversity data can be used at different levels. The first level represents something of a bottom-up approach, by trying to understand the link between genetic diversity and one of the key processes underlying metapopulation dynamics - dispersal. For many years biologists have used patterns of genetic diversity to infer movement rates in fragmented populations (Wright, 1931; Slatkin, 1985; Neigel, 1997; Waser and Strobeck, 1998), with a popular approach involving the calculation of Wright's *Nm* (1931). Recently however, this approach has attracted the criticism that it represents an unreliable basis for estimating the true rate at which individuals move about fragmented populations (Whitlock and McCauley, 1999). In Chapters 2 and 3, I demonstrated the existence of a process lending support to this criticism. In particular, I found that the genetic contribution of an immigrant can be influenced by inbreeding, thereby adding another element to our understanding of the complex (and therefore difficult to predict) relationship between the rate at which immigrants arrive into a population and their impact in terms of gene flow.

Chapters 2 and 3 also have implications for understanding the rescue effect, whereby immigrants rescue extant populations from extinction (Brown and Kodric-Brown, 1977). If the results of those chapters apply to real metapopulations, this study suggests that large outbred populations may make a disproportionately large contribution to the genetic diversity of small, inbred populations. If populations with low genetic diversity have higher extinction probabilities through lack of adaptive potential (Frankham, 1995a; Sanjayan *et al.*, 1996), the influx of outbred immigrants may be an important component to the rescue of small inbred populations from extinction. Such a genetic rescue may thereby increase the patch

occupancy of small patches in metapopulations, and thereby increase metapopulation persistence times.

While modelling studies have shown that the rescue effect can potentially have a large effect on the dynamics of metapopulations (Hanski, 1982; Gotelli, 1991; Hanski and Gyllenberg, 1993), relatively little is known about the importance of this effect in real systems. Although a number of empirical studies have reported the presence of genetic and/or demographic rescue (Sjogren, 1991; Sinsch, 1992; Spielman and Frankham, 1992; Hanski *et al.*, 1994; Matthysen, 1999), much remains to be learned about the relative importance of these two processes. The results in Chapters 2 and 3 add further support for the existence of a genetic rescue effect; in this case due to the disproportionately large contribution of male immigrants to the population genetic diversity of inbred populations. Furthermore, I was able to demonstrate that the basis for this immigrant fitness advantage was outbred vigour of immigrant males, rather than a rare male effect or hybrid vigour of immigrant progeny. This in turn suggests that outbred source populations would provide the basis for a stronger genetic rescue effect than inbred source populations.

In Chapter 4, I considered the reliability of using genetic diversity to estimate both dispersal rates and colonisation probabilities in metapopulations. Importantly, the relationships between genetic diversity, dispersal rate and colonisation probability are influenced by many biological processes, the nature of which is likely to vary considerably among species. Although different types of analysis are available for estimating dispersal rate from genetic diversity data (*e.g.,* Wright's *Nm* and the assignment method), such methods are either burdened by questionable assumptions, or require so much data that they are of limited use for estimating dispersal rates and colonisation probabilities.

I also explored a way of using genetic diversity data that is probably best considered a top-down approach, since it treats genetic diversity as a single composite measure of the many biological processes involved in metapopulation dynamics. In this way, I believe there is benefit in not pulling this composite apart, but instead using it directly as an informative measure in its own right.

In this regard, I believe one of the key issues in managing metapopulations lies in deciding the relative value of the different patches in a system, where the value of a patch is a measure

of its contribution to metapopulation persistence. In the absence of good demographic data to parameterise population viability analyses, genetic diversity data is potentially very useful in reflecting many of the processes that determine relative patch value.

By creating a simulation model of metapopulation dynamics and genetics I was able to demonstrate in Chapters 5 and 6 that ranking pairs of patches based on the genetic diversity of their subpopulations can, under certain circumstances provide good estimates of relative patch value. Although the applicability of this approach may vary considerably between systems, this can and should be assessed for each system, and could potentially provide a useful component of population viability analyses.

Finally, if genetic diversity does concur well with relative patch value, this may represent common ground on which both metapopulation demographers and geneticists can stand. For a number of years there has been an active debate in the literature as to whether conservation biologists should be more concerned with population demographics or genetics (Lande, 1988; Caro and Laurenson, 1994; Caughley, 1994; Mills and Smouse, 1994; Frankham, 1995a). Accordingly, the patches in a metapopulation can be ranked on the basis of their ability to increase metapopulation persistence or preserve genetic diversity. Importantly, the results in Chapter 6 suggests that these two criteria may in some circumstances rank patches in a very similar way, and this may prove to be one situation where biologists studying metapopulation dynamics and genetics can agree on the same course of action.

APPENDIX 1

Assessment of Turbo Pascal's pseudo-random number generator

A1.1 Introduction

Programs written for this thesis used the built-in pseudo-random number generator available in Turbo Pascal 7.0 whenever it was necessary to produce real numbers uniformly distributed between 0 and 1. The term "pseudo-random" refers to the fact that the numbers are not generated by a truly random process, but by a deterministic algorithm (Savitch, 1993). Thus, given the same initial "seed" value, such a number generator produces the same sequence every time. For the programs in this thesis the seed value was taken from digits on the computer's clock, so that the seed values and therefore the pseudo-random sequences were likely to differ every time a program was run.

A "good" pseudo-random number generator should produce a sequence of numbers that has the same relevant statistical properties as a sequence of truly random numbers (Ripley, 1987). The purpose of this appendix is to provide some insight into whether the Turbo Pascal 7.0 pseudo-random number generator produces data with the same properties we would expect to find in a truly random sequence of real values uniformly distributed between 0 and 1. A number of descriptive statistics and tests are useful in this regard, and these are described below, with each being applied to a separate sequence of 10,000 pseudo-random values.

If the reader prefers to skip the details of these tests, the synopsis is that the Turbo Pascal 7.0 pseudo-random number generator passed every test applied in this appendix. These were the frequency (equidistribution) test, the test for correlation between sequential pairs, the serial, coupon collector's, run, gap and poker tests.

A1.2 Tests of pseudo-random number generator properties

A1.2.1 Descriptive statistics

The mean of 10,000 values was 0.4996. Importantly, 0.5000 (the mean of a uniform distribution between 0 and 1) was included in the 95% confidence interval ($0.4939 < \mu < 0.5052$). The sample variance of 0.0825 was close to the expected value of 0.0833 - the variance of a uniform distribution between 0 and 1 (Madsen and Moeschberger, 1980).

A1.2.2 Frequency test (equidistribution test)

The frequency test examines whether some sections of the range 0 to 1 are over or under-represented in terms of the number of values produced. To perform this analysis, the range 0 to 1 was divided evenly into 100 intervals. The null hypothesis (for randomly generated values uniformly distributed between 0 and 1) is that there is an equal probability (0.01 in this case) of each value falling into each of the 100 intervals. Therefore there is an expected frequency of 100 values occurring in each of the 100 size categories (from the sequence of 10,000 values). This set of expectations can be compared to the observed frequencies using a chi-square test. Importantly however, this should be performed as a two-tailed test, as there are two ways in which the pattern can diverge from randomness (Morgan, 1984). Firstly, we should be suspicious if the observed frequencies are too divergent from the expected frequencies. This is the sort of chi-square analysis generally employed for a goodness of fit test. However because we are testing randomness *per se*, we should also be suspicious if our observed frequencies are too close to the expected frequencies, as this would suggest that there is some pattern to the way that values are produced. To illustrate this concern, consider a number generator that, in a sample of 100 numbers, produces a value in each of the 100 evenly spaced categories between 0 and 1. While such a pattern would exactly match the expected frequencies, we would clearly be suspicious that the numbers are too evenly distributed. Accordingly, we should also be concerned if the observed chi-square statistic is too *small*. Using this two-tailed approach (with $\alpha = 0.05$), we should therefore reject the null hypothesis if $\chi^2_{0.975} > \chi^2_{observed} > \chi^2_{0.025}$. The logic behind this two-tailed testing applies to all of the other chi-square tests used in this appendix.

The results of the frequency test showed that the observed frequencies (Figure A1.1) were neither too close to, nor too divergent from the expected frequencies ($\chi^2_{observed}$ = 115.84, $\chi^2_{0.975}$ = 73.36, $\chi^2_{0.025}$ = 128.42, d.f. = 99).

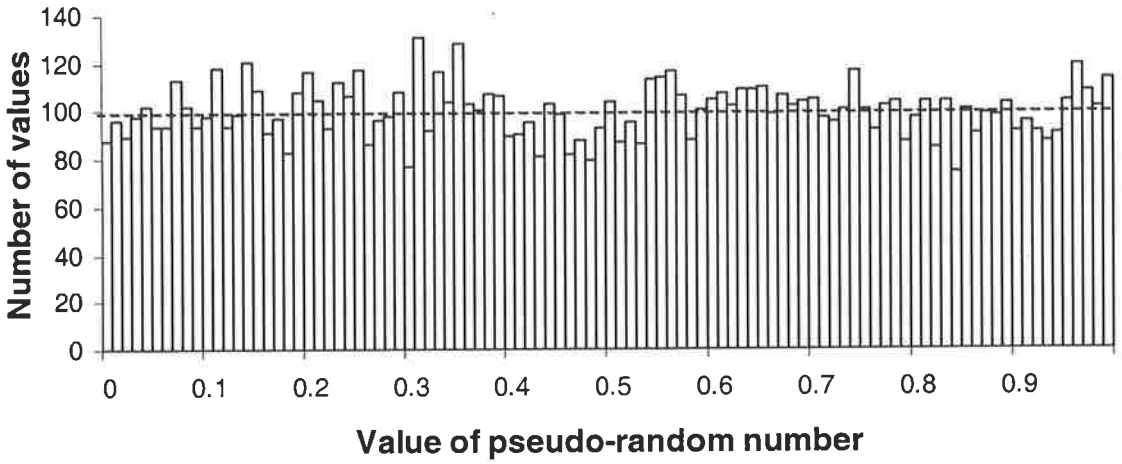

Figure A1.1 Frequency distribution of 10,000 sequential pseudo-random numbers produced by Turbo Pascal 7.0. The dashed line represents the expected frequency.

A1.2.3 Test for correlation between sequential pairs

The correlation test examines whether there is a correlation between one value and the next in a sequence of values. The null hypothesis (based on truly random sampling from a uniform distribution) is that there is no correlation between one value and the next. The 10,000 pseudo-randomly generated values were used to provide a total of 5000 non-overlapping pairs of sequential values. Figure A1.2 shows a scatterplot of 500 of these pairs. A two-tailed test (allowing for the possibility of a positive or negative correlation) showed no significant correlation between the first and second values in sequential pairs (r = -0.01124, p = 0.427, d.f. = 4998).

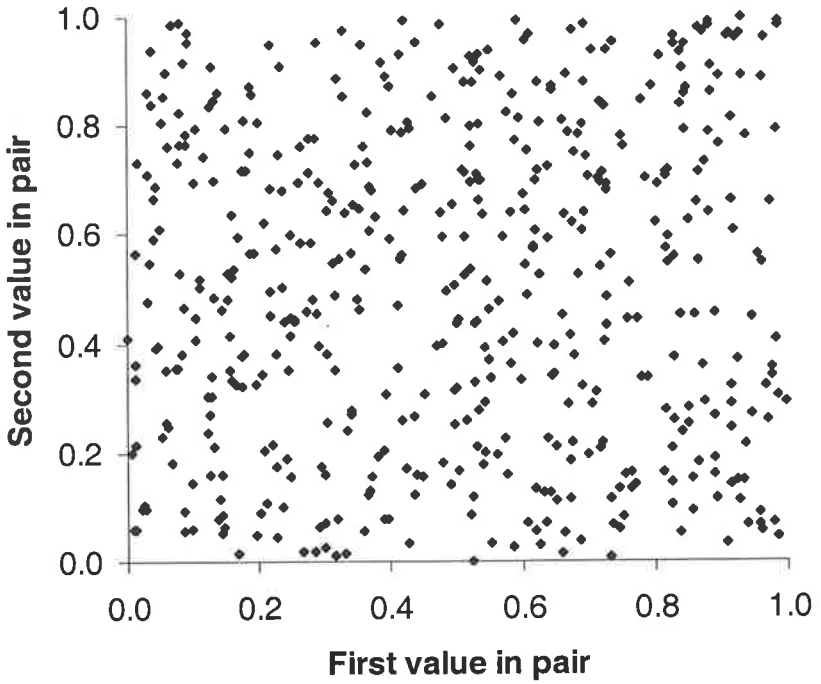

Figure A1.2 Scatterplot of the first vs second values in sequential pairs of pseudo-random values produced by Turbo Pascal 7.0 (r = -0.01124, p = 0.427, *d.f.*= 4998). To maintain visual clarity this plot shows only the first 500 of the total 5000 pairs used to calculate the correlation.

A1.2.4 Serial Test

The serial test allows us to examine whether there is non-randomness in the transition between pairs of sequential numbers. Therefore this test bares some resemblance to the correlation test. The starting point for the serial test is to convert a sequence of real numbers into integers. I have chosen to use the range 0 to 9 inclusive. This is achieved by multiplying each value by 10 and truncating the result, so that for example 0.373 becomes 3, and 0.801 becomes 8. Then, by working through the list of 10,000 pseudo-random integers, we have 5000 non-overlapping pairs for which we can record a "first number → second number" transition: *e.g.*, first number in pair = 3, second number in pair = 8. We would, for example, be suspicious of pseudo-random number generator in which every occurrence of a 3 was followed by an 8.

A 10 by 10 matrix is created to record the frequency of the 100 different possible transitions (i.e. 10 possible first numbers and 10 possible second numbers). The null hypothesis (based on a randomly generated sequence of uniformly distributed values) is that there is an equal probability for each of the 100 different transitions in the matrix. Therefore from a total of 5000 replicate transitions we have the null expectation that each transition receives 50 replicates. (Note that this null hypothesis includes the assumption that there is an equal probability of each of the 10 possible first numbers occurring – an assumption we can clearly be confident of given the results of the frequency test.)

The observed values were compared to the expected values using a two-tailed chi-square analysis. The results of the serial test (Table A1.1) showed that the observed frequencies were neither too close to, nor too divergent from the expected frequencies ($\chi^2_{observed} = 101.52$, $\chi^2_{0.975} = 73.36$, $\chi^2_{0.025} = 128.42$, d.f. = 99).

Table A1.1 Results of the serial test. 10,000 sequential values produced by the Turbo Pascal 7.0 pseudo-random number generator were converted into integers 0 to 9, giving 5000 sequential integer pairs. Shown are the numbers of pairs falling into different sequence categories according to what the first and second values in each pair were. The null hypothesis for each category is an expected frequency of 50 values.

		first value in sequential pair									
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
second value in sequential pair	**0**	39	40	56	51	43	39	46	50	48	52
	1	63	54	37	48	59	46	55	48	54	45
	2	46	51	44	52	51	50	43	49	60	42
	3	43	45	51	35	61	45	60	47	39	43
	4	65	51	41	51	45	55	39	37	55	44
	5	63	53	38	50	57	58	38	60	45	49
	6	54	51	52	59	44	52	52	48	59	56
	7	48	57	58	56	48	47	54	46	55	46
	8	41	59	56	52	43	59	65	49	59	53
	9	54	45	48	57	38	47	59	48	41	61

A1.2.5 Coupon collector's test

As its name suggests, the coupon collector's test is based around the idea of collecting a set of coupons. Knuth (1969) describes this using the analogy of a child trying to collect a set of coupons which are randomly distributed among breakfast cereal boxes - the child must keep eating cereal until he/she has collected one coupon of each type. We can perform a similar exercise with a sequence of numbers, where we are interested in how many sequential pseudo-randomly generated values we must work through before we obtain a full collection of some pre-defined set of integers.

The first step with the coupon collector's test is to define the set of numbers that are to be collected. Here I have chosen to simply convert a list of 10,000 pseudo-random real numbers into the integers 0 to 9 inclusive (as was done for the serial test). These 10 integers form the full set of coupons. The next step is to work sequentially through the list of 10,000 values and record the length of consecutive coupon collector segments (a segment is the set of sequential values required to collect a full set of "coupons"). Clearly the segments will vary in length, and we can calculate the probability distribution of segment lengths expected of a truly random number generator (Knuth, 1969). Accordingly, we can assess the apparent randomness of our pseudo-random number generator by comparing the observed and expected frequency distributions of segment lengths using a two-tailed chi-square test.

From the list of 10,000 values, there were 328 consecutive segments. The frequency distribution of the different segment lengths is shown in Figure A1.3. The probability of obtaining each segment length was calculated according to Knuth (1969, pg 58) and multiplied by 328 to give the expected frequencies of the different segment lengths. Because the calculation of these probabilities required Stirling numbers of the second kind, and these numbers were unavailable for segments greater than 25 values in length (Goldberg *et al.*, 1964), observed and expected frequencies were pooled for segment lengths $\geq$ 26. Frequencies were also pooled for segment lengths 10 to 13 inclusive so that none of the expected frequencies would be less than 1.0 and no more than 20% of the expected frequencies would be less than 5.0 - both of which are important conditions for performing a chi-square analysis (Zar, 1984, pg 50).

The results of the coupon collector's test showed that the observed frequencies of segment lengths were neither too close to, nor too divergent from the expected frequencies ($\chi^2_{observed}$ = 8.554, $\chi^2_{0.975}$ = 5.009, $\chi^2_{0.025}$ = 24.736, d.f. = 13).

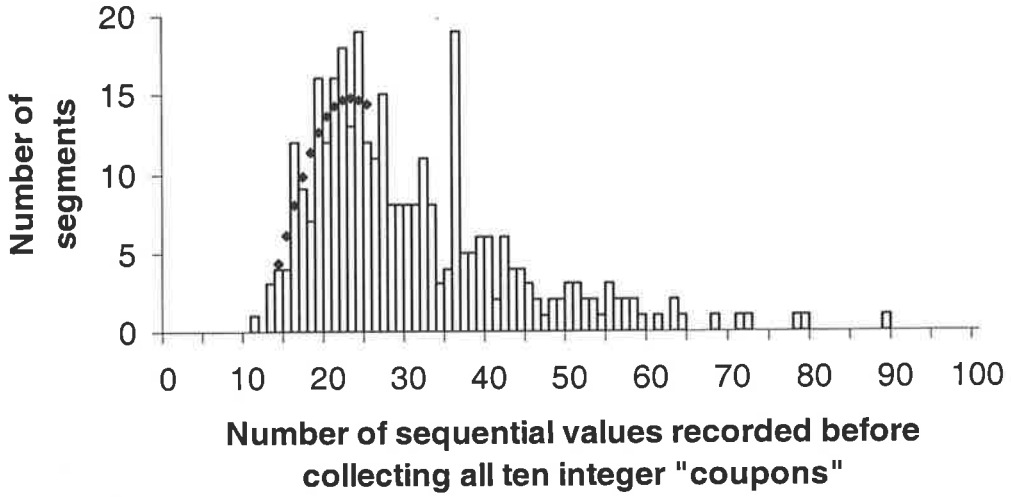

Figure A1.3 Results of the coupon collector's test: the frequency distribution of segment lengths. 10,000 sequential values produced by the Turbo Pascal 7.0 pseudo-random number generator were converted into integers 0 to 9. A segment is the collection of sequential values required to collect a full set of the integers 0 to 9. Expected frequencies are shown for segment lengths 14 to 25 inclusive (diamond markers).

A1.2.6 Run Test

A run is a sequence of values that either consistently increases (a "run up"), or consistently decreases (a "run down"). Thus, in the following sequence of 10 values, runs up are shown in brackets: (0.023, 0.302, 0.780) (0.456) (0.232, 0.985) (0.891) (0.321, 0.543, 0.634). Probability theory can be used to calculate the frequency distribution of the different run lengths expected under the null hypothesis of true randomness (Morgan, 1984), and this can be compared to an observed frequency distribution of run lengths to test the apparent randomness of our number generator. Importantly though, because runs are contiguous (as in the sequence above), they are not independent. Thus a long run will tend to be followed by a

short run, and vice versa (Knuth, 1969). This means that the usual chi-square approach is inappropriate for comparing observed and expected frequencies, and an alternate statistic, V, which takes into account the covariance of run lengths should be employed (Knuth, 1969). Using this statistic, I analysed the frequency distribution of runs up from a sequence of 10,000 pseudo-random numbers, and then the frequency distribution of runs down from a separate sequence of 10,000 numbers. The results of runs that were six or more values in length were pooled to comply with the approach used by Knuth (1969). Table A1.2 shows the observed frequency distributions of runs up and runs down, together with the frequency distribution expected under the assumption of randomness. With six data categories, the V statistic has a chi-square distribution with six degrees of freedom (not the five (k-1) degrees of freedom we would use in a standard goodness of fit test). The data were analysed as a two-tailed test (Morgan, 1984).

The run test showed that the observed frequencies of run lengths were neither too close to, nor too divergent from the expected frequencies. This was true for both runs up and runs down ($V_{up} = 12.645$, $V_{down} = 4.402$, $\chi^2_{0.975} = 14.449$, $\chi^2_{0.025} = 1.237$, d.f. = 6).

Table A1.2 Results of the run test. Shown are the frequencies of different run lengths for runs up (consecutively increasing values) taken from a sample of 10,000 pseudo-randomly generated values. Also shown are the frequencies for runs down (consecutively decreasing values) from a separate sample of 10,000 values, as well as the frequencies expected under the assumption of randomness.

Run length	Runs up frequency	Runs down frequency	Expected frequency
1	1645	1648	1666.3
2	2091	2059	2082.7
3	911	960	916.3
4	272	259	263.8
5	62	52	57.5
≥6	7	9	11.9

A1.2.7 Gap Test

A gap is a sequence of numbers in which a pre-defined numerical "event" has not occurred. For example, in a sequence of integers we could define the event as the occurrence of the number four. By working through a sequence in order, we then simply record gap lengths as the number of values before each next occurrence of a four. Thus, in the following sequence:

5 **4** 3 2 5 6 8 **4** **4** 6 7 8 **4** 6 3 0 6 2 9 8 7 **4**

we would say that the gap lengths were 1, 5, 0, 3 and 8. In a truly random sample, gap lengths are geometrically distributed (Morgan, 1984), and this forms the basis for calculating our expected frequencies. To perform the gap test I converted all pseudo-randomly generated values into the integers 0 to 9, and then carried out 10 gap tests - using a different integer 0 to 9 as the "event" in each test. A separate sequence of 10,000 pseudo-random values was generated for each of the 10 tests. Gaps greater than 10 values in length were pooled, giving a total of 11 gap length categories (0, 1, ..., 8, 9, ≥10). The observed gap length frequencies were compared to the expected frequencies using two-tailed chi-square tests. Importantly, because there were essentially 10 "replicate" gap tests, the test-wise α was adjusted from 0.05 to 0.0051 to maintain the experiment-wise α at 0.05 (Jones, 1984). Table A1.3 shows the observed frequencies and chi-square values for each of the 10 gap tests. Expected frequencies can be calculated by referring to Morgan (1984). In all 10 tests the observed frequencies were neither too close to, nor too divergent from the expected frequencies (critical chi-square values when using $\alpha = 0.0051$ are: $\chi^2_{0.99745} \doteq 1.837$, $\chi^2_{0.00255} = 27.049$, d.f. = 10).

Table A1.3 Results of the 10 gap tests. Each test used a different integer as the event marking the end of gaps. Shown for each test are the observed frequencies of different gap lengths recorded from 10,000 pseudo-randomly generated values. The expected frequencies for chi-square values were based on the null hypothesis of gap lengths being geometrically distributed. The critical chi-square values are: $\chi^2_{0.99745} = 1.837$, $\chi^2_{0.00255} = 27.049$, d.f. = 10.

	Integer used as the "event" marking the end of gaps										
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Expected**
0	119	92	104	96	101	94	83	90	103	82	100.0
1	93	101	103	121	85	99	111	94	80	82	90.0
2	81	99	88	77	85	82	82	77	81	79	81.0
3	74	69	77	83	61	73	61	78	64	83	72.9
4	75	60	70	63	62	51	57	75	64	78	65.6
5	55	47	48	58	69	64	65	59	57	60	59.0
6	49	52	43	43	47	51	60	51	61	58	53.1
7	63	60	50	41	35	43	42	54	38	52	47.8
8	44	43	48	48	42	35	30	53	48	35	43.0
9	36	43	39	39	36	41	45	35	37	32	38.7
≥10	355	348	353	366	364	350	341	330	349	349	348.7
χ^2	8.503	12.353	7.200	15.146	9.300	6.986	18.091	7.637	6.013	11.252	

A1.2.8 Poker Test

The poker test is modelled around the idea of drawing a hand of five cards from an infinite deck and recording if there are (i) no cards of the same rank, (ii) one pair, (iii) two pairs, (iv) three of a kind, (v) a full house, (vi) four of a kind, or (vii) five of a kind. Instead of using card ranks (2 to 10, jack, queen, king and ace) the poker test simply uses a set of integers, and once again I have chosen to use the range of integers 0 to 9 inclusive. Furthermore I have chosen to use the approach suggested by Knuth (1969) of simply recording the number of distinct values among each hand of five "cards". Thus there are five possible outcomes:

5 different values = all different;

4 different values = one pair;

3 different values = two pairs, or three of a kind;

2 different values = full house, or four of a kind;

1 different value = five of a kind.

Each hand is drawn as five consecutive values from a sequence. Thus, a total of 2000 hands were drawn from the sequence of 10,000 pseudo-randomly generated integers. Assuming that we have a truly random number generator producing uniformly distributed values, the probability of obtaining a hand with a particular number of unique values can be calculated based on Knuth (1969). Multiplying these probabilities by 2000 gives the expected number of hands falling into each of the five categories, and this forms the null hypothesis against which we can compare the observed frequencies (using a two-tailed chi-square test). Because the expected number of hands containing only one unique value was only 0.2, this category was pooled with the "two unique values" category to maintain the condition that all expected frequencies were greater than 1.0. The results of the poker test show that observed frequencies of the different types of "poker hands" were neither too close to, nor too divergent from the expected frequencies ($\chi^2_{observed} = 5.491$, $\chi^2_{0.975} = 0.216$, $\chi^2_{0.025} = 9.348$, d.f. = 3) (Table A1.4).

Table A1.4 Results of the poker test. 10,000 sequential values produced by the Turbo Pascal 7.0 pseudo-random number generator were converted into integers 0 to 9. A total of 2000 "hands" of cards were drawn in sequence, with five integer "cards" per hand. These hands were placed into one of four different categories depending on the number of unique integers in each hand (the results for hands with only one or two unique integers were pooled). Shown here are the observed and expected numbers of hands in different categories.

number of unique values in "hand"	observed	expected
1 or 2	22	27.2
3	393	360
4	1010	1008
5	575	604.8

A1.3 Summary

The Turbo Pascal 7.0 pseudo-random number generator passed all the empirical tests of randomness employed in this appendix. In other words, there was no significant difference (using $\alpha = 0.05$) between the statistical properties of the data produced by this number generator and the properties of data produced by a truly random number generator producing values uniformly distributed between 0 and 1. On this basis it is assumed that any artifact patterns produced by the Turbo Pascal 7.0 pseudo-number generator had a negligible impact on the results of the programs used in this thesis.

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