The Role of Genetics in Understanding Forest Fragmentation

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Recent advances in genetic analysis have demonstrated clearly that population fragmentation can lead to a loss of genetic variation. Whether such loss will occur on any particular forest fragment is less certain, and will depend in part on whether the population on the fragment is completely isolated, the size of the fragment, the time since the isolation occurred, and the underlying historical genetic structure that exists due to natural barriers to movement. A loss in genetic variation can cause inbreeding depression and decreased ability to adapt to long-term changes. Inbreeding depression, a decrease in birth and/or survival rates due to a decrease in heterozygosity, has been found in a wide variety of wild plant and animal populations, and has been shown to decrease population growth rate and population persistence. Genetic tools can assay the extent to which fragmentation has occurred in a landscape by facilitating comparison between past and present levels of connectivity and population sizes. Genetic aspects of forest fragmentation cannot be ignored, because the population consequences are real. However, genetic analysis of fragmented populations is of limited utility unless placed in an ecological context based on field research.

The definition of fragmentation proposed for this conference – the process of reducing the size and connectivity of stands that compose a forest – begins to make the concept of fragmentation operational, and objectively defined. But two of the most important terms in this definition – size and connectivity -- are vague. The critical question is, how much of a reduction in the size and connectivity of wildlife populations can occur before a forest fragmentation "alarm bell" should go off? Of course, the answer will depend on the species, the place, and a host of other factors, but remarkable technological and analytical advances of the last decade have revolutionized the role that genetics can play in considering this crucial question. We will consider how changes in forest fragment size and connectivity can alter genetic structure of wildlife populations, and how those changes can alter population dynamics. We also explore how genetic tools can help us to decide how small is too small for a forest fragment, or how unconnected is too unconnected. These issues are not strictly genetic issues. A theme we hope to weave throughout is that genetic aspects are an important consideration when evaluating the consequences of forest harvest, both because genetic changes can negatively impact wildlife populations, and because they can tell us something about when a forest fragment has become small and isolated. We first define some terms that are relevant to evaluating genetic changes following fragmentation, then describe some known consequences of forest fragmentation. Finally, we discuss genetic tools that can help evaluate changes in popu-

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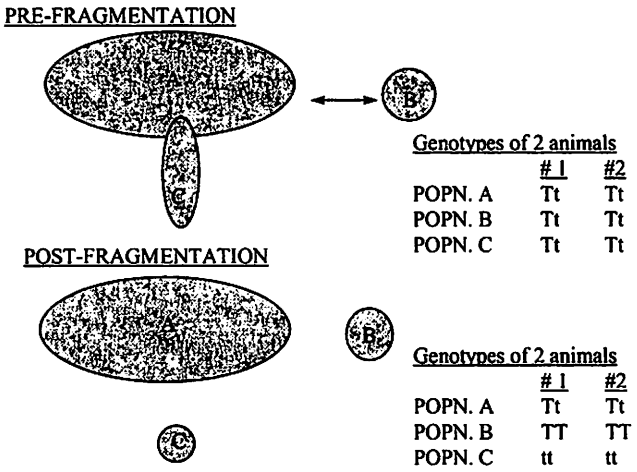

Figure 1. Cartoon demonstration of potential genetic consequences following forest fragmentation events that decrease population size and connectivity among populations. Encircled areas represent suitable habitat and contain wildlife populations. Arrows represent gene flow across a semi-hospitable habitat. The genotypes of only two individuals and one gene are shown per population, although in real studies many genes and many individuals would be sampled.

lation size and connectivity, thereby making "fragmentation" a more operational term.

1. RELATIONSHIP BETWEEN GENETIC VARIATION, POPULATION SIZE, AND CONNECTIVITY

1.1 Background

At the outset, it is important to emphasize that although we cannot see genes (or the DNA building blocks that comprise genes) with the human eye, their dynamics and impacts on populations are not merely theoretical. Both morphological characteristics and behavioral traits have some genetic component, making genetic composition as real and important to organisms as the air they breathe or the food they eat.

Genetic variation in a wildlife population can be described by its heterozygosity and allelic diversity (see Chambers, 1983 for concise description; Hartl and Clark, 1997 for very readable details). Heterozygosity is the proportion of genotypes in the population that have different alleles, or forms of a gene (e.g. genotype Tt vs. TT or tt). Allelic diversity can be described by the number of different alleles in a population.

Fragmentation can affect genetic variation in a variety of ways. We will consider two cases of special relevance to forest fragmentation, realizing that these changes would be complicated by the strength and mode of natural selection, as well as factors such as mutation rate and interactions among genes. The first case is for single populations that go from being large to being small, as might be expected from habitat loss via forest fragmentation. In a large population, the frequency of different alleles, and therefore heterozygosity and allelic diversity, will change slowly in response to natural selection under local conditions. In contrast, in a small isolated population, natural selection becomes overwhelmed by sampling error. That is, because only a small number of individuals reproduce and they produce only a limited number of offspring, only certain subsets of alleles are

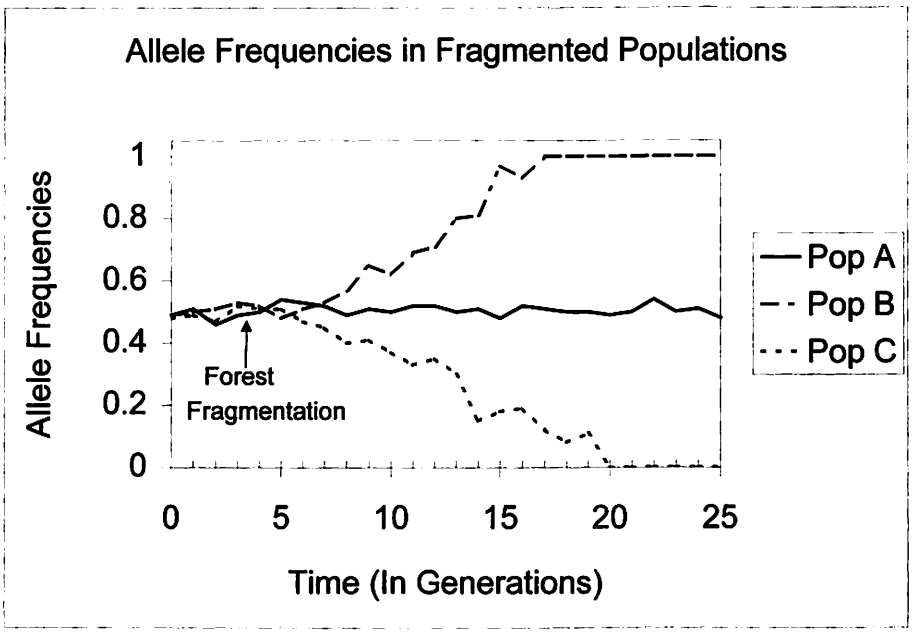

Figure 2. Changes in the frequencies of two alleles (T and t) of a single gene found in three populations following a hypothetical forest fragmentation event, such as that in Fig. 1. Here, the fragmentation event occurs in the third generation. Thereafter, allele frequencies in the reduced size (Population C) and reduced connectivity (Population B) populations diverge from each other and from population A; the time for divergence could be hundreds of generations (see text). Allele frequencies in population A remain relatively stable due to its large size.

passed on to the offspring of the next generation. This process of genetic drift, or sampling error, can lead to rapid and random changes in allele frequencies over a few generations. Consequently, heterozygosity and allelic diversity are lost, as some alleles reach a frequency of one while others vanish (Allendorf, 1983).

For example, consider a simple hypothetical situation of a fragmented landscape (Fig. 1). Fragmentation has caused population C to become small after being severed from the large population A, with changes in gene frequencies and loss of heterozygosity and allelic diversity over subsequent generations (Fig. 2). In contrast, there is little genetic drift (sampling error) each generation in population A because a relatively much larger number of offspring is created each generation from the pool of parents (Fig. 1, Fig. 2). Therefore, we see genetic drift playing a large role in eliminating genetic variation in the small population compared to the large population.

The second, related way fragmentation affects genetic variation can be seen by looking across populations transformed from being connected to isolated. Although population B in Fig. 1 is small, it is connected by movement of animals between B and A prior to fragmentation. This frequent movement of individuals between the two populations causes them to have similar allele frequencies and levels of genetic variation, because gene flow from population A counteracts genetic drift in population B. After fragmentation, population B has become isolated, and as genetic drift takes effect on these now independent populations, allele frequencies in population B will diverge over time from those of population A (Fig. 2). In addition, within the isolated populations, heterozygosity and allelic diversity decrease (Fig. 1, Fig. 2).

With these cartoon characterizations, a couple of important points emerge. First, allelic diversity can be high across suites of isolated populations, as different alleles are lost within different populations. Secondly, even though allelic diversity can be retained in sets of small, isolated populations, heterozygosity and allelic diversity within any one population will be lost eventually due to genetic drift, even if there are fairly strong selective forces acting on the population. As will be discussed shortly, genetic changes are important because they can lead to inbreeding depression in the short-term, and decreased long-term ability of a population to respond to selective pressures (Briscoe et al., 1992).

1.2 Results From Field Studies

Genetic changes in populations are real and measurable, and not merely theoretical. At the extreme of isolation, we know that genetic variation tends to be lower in populations on islands than for those on mainlands (Frankham, 1997). As just a few of many examples, genetic variation is lower on islands than the mainland for wolves (Wayne et al., 1991), Channel island foxes (Gilbert et al., 1990), and silvereye birds (Degnan, 1993). Genetic variation also tends to decrease in general as population size decreases for a wide range of wildlife species (see Frankham, 1996).

Thus, there is no doubt that genetic variation can be lost when isolation is complete and/or population sizes are small. But do these expected genetic changes occur with habitat fragmentation in general, and forest fragmentation in particular? As expected from the previous discussion, genetic consequences of habitat fragmentation will vary widely, depending on the size of the population, how much populations have become truly isolated, how long ago the isolation occurred, and other factors. But reductions in genetic variation have certainly been documented. For example, heterozygosity and allelic diversity declined in greater prairie chickens (Bouzat et al., 1998a, Bouzat et al. 1998b), hairy-nosed wombats (Taylor et al., 1994), and koalas (Houlden et al., 1996), all of whose ranges and population sizes have been reduced by habitat fragmentation, disease, and/or overharvest over the last century. Similarly, population differentiation or loss of genetic variation has accompanied habitat fragmentation and harvesting of wild turkeys (Leberg, 1991) and brown bears (Paetkau et al., 1998), as well as frogs whose dispersal has become limited by urban sprawl (Hitchings and Beebee, 1997).

Forest fragmentation plays a role in many of these instances, but studies focusing on forest fragmentation per se are limited. One example is that of Sarre (1995) on geckos in forest remnants in Australia. By examining genetic variation in nine remnant populations and three large nearby nature reserves across a landscape, Sarre (1995) found lower variation in remnant than in nature reserve populations and attributed the changes to recent forest fragmentation. Similarly, Wauters et al. (1994) found that populations of the Eurasian red squirrel on five woodland fragments had lower genetic variation than did two large control populations. On the other hand, Leung et al. (1993) found that although genetic variation in an Australian rodent was lower on a true island than in a control, there was no loss of variation for 3 forest fragments created about 60-70 years ago. Similarly, in our own work we have found strong ecological effects of forest fragmentation on California red-backed voles in Oregon, resulting in both negative edge effects and apparent isolation (Mills, 1995; Mills 1996); however, our preliminary genetic analyses of highly sensitive microsatellite DNA markers show no differences in levels of genetic variation between 5 large control and 13 small remnant populations (Tallmon et al., In Prep).

The studies that demonstrated a reduction in genetic diversity on remnants support our expectations about the effects of fragmentation. But the studies that fail to demonstrate a reduction in genetic diversity do not necessarily reject the possibility that fragmentation affects genetic variation. There are at least three reasons, probably acting together, that explain why a loss of genetic variation may not be detected in many studies of forest fragmentation. The first explanation is that isolation may not exist, despite fragmentation. Even a small amount of connectivity can minimize the loss of genetic variation. One to ten migrating individuals per generation are

sufficient to minimize the loss of genetic variation in any one population (Spielman and Frankham, 1992; Mills and Allendorf, 1996; Newman and Tallmon, In Prep), although more may be necessary if populations fluctuate greatly over time (Vucetich and Waite, In Review).

A second reason why decreased genetic variation may not be detected in a study of an apparently isolated species is that not enough time has passed to initiate a change in genetic composition. After a drastic change in population size or connectivity (say due to forest fragmentation), anywhere from several to hundreds of generations may pass before measures of variation reflect those changes (Steinberg and Jordan, 1997; Lindenmayer and Lacy, 1995; Slatkin, 1994). This "time to equilibrium" will be faster for truly isolated populations if they are relatively small (Allendorf and Phelps, 1981; Varvio et al., 1986). It will also depend on the genetic tool used to assess genetic variation; protein electrophoresis is the least likely to reveal recent changes since proteins have low levels of variation (see Leung et al., 1993), whereas DNA markers are more sensitive (Avise, 1994). The time to equilibrium explains why islands that have been isolated for hundreds or thousands of years so consistently show low levels of variation compared to relatively recently-formed habitat remnants.

The third major reason why forest fragmentation may not lead to detectable changes in genetic variation is that population differentiation can occur "naturally" across a landscape. That is, historical barriers to dispersal such as rivers and mountain ranges can contribute statistical noise that can obscure the detection of subtle, on-going genetic changes due to recent fragmentation (Avise, 1994). For example, Cunningham and Moritz (1998) found that the effects of recent clearing had less effect on genetic diversity in a rainforest lizard than long-term climatic and geological processes.

It is worth noting that the last two of these factors – time lags and statistical noise – will similarly affect ecological studies of edge effects and forest fragmentation described elsewhere in this book. Before concluding that fragmentation has "no effects," one should consider whether the time scale of the study is appropriate, and whether the study has sufficient statistical power to detect effects that do in fact exist in a "noisy" landscape.

2. POPULATION-LEVEL CONSEQUENCES OF CHANGES IN GENETIC VARIATION

2.1 Background

Although the evidence is incontrovertible that fragmentation can have consequences on genetic variation at varying time scales, genetic concerns only become relevant if they compromise population persistence. Genetic changes due to forest fragmentation can have such effects, both in the short term, primarily through inbreeding depression, and in the long term through loss of adaptive genetic variation and through the accumulation of mutations that decrease adaptive potential. Inbreeding depression is the reduction in fitness of individuals produced from mating among relatives, which becomes unavoidable in a small population. Adaptive potential is the ability to evolve to future conditions, which is constrained by underlying levels of genetic variation in a population. These genetic consequences of fragmentation can contribute significantly to population decline.

2.2 Short-Term Effects: Inbreeding Depression

Like the loss of variation in small, isolated populations, the phenomenon of inbreeding depression is real and has been observed. Awareness of the demographic consequences of inbreeding depression probably traces back to the dawn of domestication (Wright, 1977). More recently, the cost of inbreeding has been quantified in an array of wild mammal species in captivity, with varying levels of reduced juvenile survival (Laikre and Ryman, 1991; Ralls et al., 1988; Lacy, 1993), reduced reproduction (Brewer et al., 1990; Ballou, 1997; Lacy and Horner, 1997; Lacy and Ballou, 1998; Margulis, 1998), and reduced adult survival (Jiménez et al., 1994).

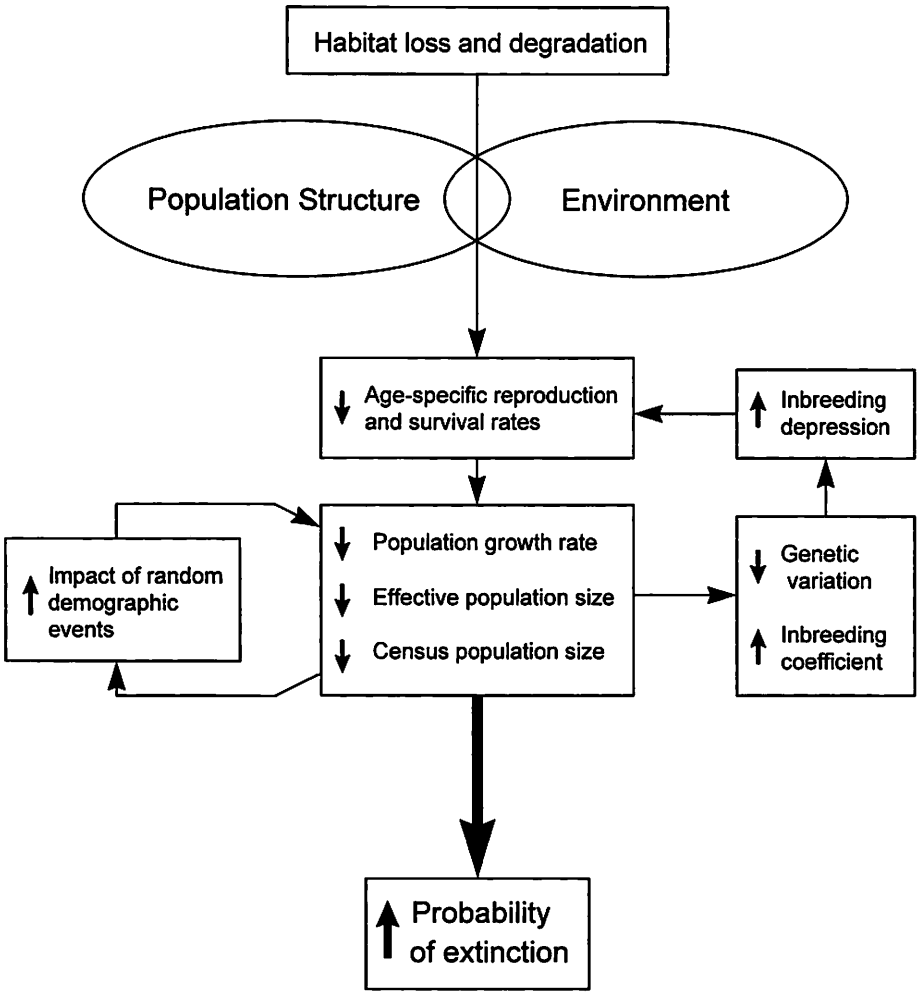

Figure 3. Simplified representation of a positive feedback extinction vortex. "Habitat loss and degradation" may include invasion of exotics, overharvest, etc. "Population structure" includes the age-structure, sex ratio, behavioral interactions, distribution, physiological status, and intrinsic birth and death rates. "Environment" includes habitat as well as extrinsic factors that vary, such as weather, competition, predators, and food abundance. Each turn of the feedback cycle increases extinction probability. The extinction vortex model predicts that some small populations are more likely to become smaller, and go extinct, each passing generation from the interaction of genetic and non-genetic factors. Adapted from Soulé and Mills (1998).

Importantly, inbreeding depression arising from small population size has also been demonstrated in free-living populations of plants, snails, fish, frogs, birds, and mammals (for reviews see Frankham, 1995; Lacy, 1997). Inbreeding depression is probably underestimated in captivity because negative fitness effects might not show up in the relatively tranquil captive environment. Field studies also tend to underestimate overall inbreeding depression because any one study typically measures only one or a few of the many aspects of fitness likely to be simultaneously affected by inbreeding depression (Keller et

al., 1994; Jiménez et al., 1994; Lacy, 1997). In two well-studied species where several measures of fitness have been measured, African cheetahs and Florida panthers, inbreeding depression affects physiological traits ranging from sperm production to immune response to heart function (O'Brien, 1994; Roelke et al., 1993). These effects translate into potential impacts on a wide range of life history characteristics. Ultimately, inbreeding depression arising from reduced genetic variation can decrease not only individual birth and death rates, but also population growth rate (Leberg, 1990) and probability of extinction (Newman and Pilson, 1997; Saccheri et al., 1998). This means inbreeding depression can have important consequences for population persistence.

Fortunately, the discussion of the consequences of inbreeding depression on the persistence of fragmented populations has matured beyond a point where genetic issues are considered apart from other factors. Following a decade where genetic factors were considered preeminent, by the late 1980's the pendulum had swung to the opposite extreme, with a prevailing view that populations would go extinct due to other factors before inbreeding translated into a substantial loss in fitness (for review see Mills and Smouse, 1994; Hedrick et al., 1996). Subsequently, a number of studies have shown that such a radical dichotomy – splitting apart "genetic" vs. "non-genetic" factors in demographic analyses – was false (Mills and Smouse, 1994; Lynch et al., 1995; Newman and Pilson, 1997; Saccheri et al. 1998). It is now widely accepted that inbreeding depression is just one of several demographic impacts that interact to increase extinction probability via an "extinction vortex" (Gilpin and Soulé, 1986) in a fragmented population (Figure 3; Soulé and Mills, 1998).

Unfortunately, not many studies are comprehensive enough to demonstrate how genetic and non-genetic factors interact to lead to population decline or to an extinction vortex following fragmentation (Figure 3). However, in a recent example, Westemeier et al. (1998) tracked demographic and genetic changes in greater prairie chickens in Illinois for 35 years. They noted a steep population decline due to habitat loss as the population became isolated and declined to a low of <50 birds. During the same time period, populations in neighboring states remained large and widespread. The Illinois population declined despite intense and somewhat successful efforts to control predators and increase the quality and quantity of habitat. The conclusion that genetics played a role in the continuing downward spiral of this population came from two observations: first, the decline in prairie chicken numbers was accompanied by a decrease in genetic variation, both for the Illinois birds compared to the larger, nearby populations (Bouzat et al., 1998b), and for the present population in Illinois compared to historical samples collected before the demographic contraction (Bouzat et al., 1998a). Second, translocations of prairie chickens from one of the neighboring states to Illinois increased egg fertility and hatching success (Westemeier et al., 1998), the life stage that surpasses all others in its importance to population growth rate in greater prairie chickens (Wisdom and Mills, 1997). This increase in egg hatching success, without any obvious changes in environmental conditions, implies that inbreeding depression on hatch rate was "broken" by the new breeders. A similar story of inbreeding depression causing subtle changes in demographic rates and exacerbating population decline has been documented for adders isolated by agricultural expansion in Sweden (Madsen et al., 1996).

2.3 Long-Term Effects

Although short-term effects of inbreeding generally receive the most attention, it is important to remember that conservation efforts and goals must be placed in an evolutionary time scale (Meffe, 1996). Long-term adaptation to climate, disease, or other changes will ultimately be limited by available genetic variation (Soulé, 1980). This is especially true if movement of individuals to track optimal habitat conditions is limited by habitat fragmentation (Lande, 1996). Populations that have been reduced in size will have decreased genetic variation as demonstrated above, and so will have decreased ability to adapt to changing conditions.

In addition to a loss of adaptive variation, isolated and small populations are threatened with a "mutational meltdown" (Lande, 1995; Lynch et al., 1995). Although mutations are the ultimate source of the genetic variation that allows populations to evolve and to adapt to changing conditions, most mutations are deleterious. As stated earlier, large populations can respond to natural selection, allowing many deleterious mutations to be removed effectively by natural selection. In contrast, small populations are dominated by random genetic drift and are therefore less able to shed deleterious mutations. As a result, over many generations small populations become less able to persist as mutations that reduce fitness become fixed by random chance (Lynch et al., 1995). This will further decrease population size, leading to increased accumulation of deleterious mutations, a decrease in fitness, again and again – a "mutational meltdown". Conversely, because large populations can respond to natural selection, those few mutations that are beneficial are more likely to be maintained in these populations than in small ones.

3. INSIGHTS INTO DEGREE OF FRAGMENTATION USING GENETIC TECHNIQUES

3.1 Background

If reductions in population size and connectivity, the twin components of forest fragmentation, can lead to genetic changes that negatively impact wildlife populations, how might genetic tools help us make operational the concepts of "fragmentation"? One approach would be to compare genetic variation in a population to a "standard" value: if the target population has lower genetic variation than the standard, it is too small; if the population has genetic structure indicating lower connectivity than the standard, it is too isolated. Unfortunately, no such standard exists for an "appropriate" level of genetic variation; in fact, as a general rule over the short term the absolute amount of heterozygosity is less important than the rate of loss of heterozygosity (Frankel and Soulé, 1981; Hedrick and Miller, 1992). As for connectivity, there is a standard, or rule of thumb, mentioned earlier: that one [to ten] breeding immigrants per generation (generation time will be species specific) will minimize the loss of heterozygosity in a population while allowing for local adaptation (Mills and Allendorf, 1996). Although this rule may be a useful starting point in the absence of other information, it is not entirely satisfactory because it is based purely on genetic considerations, and ignores many nuances of behavior and population and genetic structure.

Instead of searching for a universal rule, perhaps genetic tools can help us derive from the animals themselves, at a particular place and particular time, some operational definitions of when fragmentation has occurred. For example, just as we might evaluate population density or rates of movement using radiotelemetry or capture-recapture studies in an unfragmented landscape, and use those as goals for desired population density or rate of connectivity in a fragmented area, we may be able to do something similar using genetic techniques. A problem with this approach is that large, unfragmented "control" areas do not exist for many species and many areas; also, distinct processes may be at work in different locations (Steinberg and Jordan, 1997).

3.2 Historical Levels of Connectivity and Population Size

Fortunately, genetic techniques can provide a window into the past that other field techniques cannot. Samples collected prior to fragmentation can be compared to those collected in the same location after fragmentation to evaluate whether genetic variation has been lost. Thanks to recent advances in analysis of DNA, the potential exists to analyze very old samples from wall mounts or museum specimens (Ellegren, 1991; Mundy et al., 1997), as well as contemporary samples collected noninvasively (without having to capture the animal) from hair, feces, urine, and so on (Morin and Woodruff, 1996; Kohn and Wayne, 1997; Schwartz et al., 1998). If variation is lower in contemporary samples, increased isolation of existing populations is implied

(Bouzat et al., 1998a; Bouzat et al., 1998b; Taylor et al., 1994; Houlden et al., 1996).

It is even possible to learn about past levels of connectivity by looking at current genetic population structure across a fragmented landscape. Because isolated populations evolve independently (Fig. 1, Fig. 2), more isolated populations become more genetically differentiated compared to populations with high connectivity. This level of differentiation can be quantified with genetic analyses, and the average level of movement leading to that differentiation can be estimated (Avise, 1994; Slatkin, 1994; Slatkin, 1995; Goudet, 1995; Templeton and Georgiadis, 1995, Paetkau et al., 1998).

The most commonly used way to quantify connectivity indirectly via population differentiation is to use Wright's Fst statistic (but see Slatkin, 1985; Slatkin and Maddison, 1990; and Neigel, 1996 for other approaches). Fst is a measure of population subdivision calculated from allele frequencies in samples collected from populations across a landscape. It is based upon the distribution of genetic variation within versus between populations. If variation is distributed between populations (see Figure 1 Post-Fragmentation) then Fst is large. Conversely, if most genetic variation is found within populations (Fig. 1 Pre-Fragmentation) then Fst is small. The Fst derived from genetic samples can be used to calculate Nm, the number of individuals exchanged between populations per generation (Slatkin and Barton, 1989):

$Nm \approx (1/4)\,(1/Fst - 1)$.

A low Nm value, or low connectivity, would be indicated by a large Fst value, whereas a high Nm would be implied by a small Fst value. A Nm of one would correspond to one immigrant per generation. Estimates of population subdivision (Fst) or connectivity (Nm) should always be accompanied by estimates of error, which can be quite large (Steinberg and Jordan, 1997).

There are many analogs to Fst that can be used to estimate migration depending upon the type of genetic marker used (Slatkin, 1995; Rannala and Hartigan, 1996; Weir and Cockerham, 1984). However, all Fst and analogous measures are based upon the same general principles and are subject to the same basic assumptions. For example, these methods assume an infinite number of islands all equally accessible to each other. Although this will obviously be violated in all real-world cases, the model is relatively robust to these violations (Mills and Allendorf, 1996). More importantly, estimates of migration based on population subdivision assume equilibrium levels of divergence among populations. This is a very important assumption, because it can take hundreds of generations for equilbrium levels of divergence to be reached (Steinberg and Jordan, 1997). Therefore, levels of divergence measured for recently fragmented populations will reflect past population structure and not recent changes, unless population sizes are extremely small.

Consequently, because patterns seen in genetic markers reflect previous equilibrium levels of connectivity, genetic markers have the potential to tell us about historic movement rates. As mentioned earlier, the number of generations required to reach equilibrium will vary widely according to mutation rates and selection pressures, among other factors, but will often be on the order of 100 generations (Crow and Aoki, 1984; Slatkin, 1994; Slatkin and Barton, 1989). This is widely viewed as a criticism of these techniques for estimating current levels of gene flow (Neigel, 1996; Ims and Yoccoz, 1997; Bossart and Prowell, 1998).

However, long times to equilibrium may actually be a benefit of these approaches for operationalizing connectivity because human-caused fragmentation is typically on the order of just a few generations for most vertebrate species. Thus, historic levels of connectivity can help direct current management to maintain long term processes by facilitating comparison against current gene flow determined from direct ecological approaches or from molecular tools that reach equilibrium Nm more quickly (e.g. Avise, 1994; Slatkin, 1994; Slatkin, 1995). For example, Forbes and Boyd (1996) relate historical to current rates of gene flow for recolonizing wolves in Montana.

3.3 Current Levels of Connectivity and Population Size

The use of genetic tools is not limited to insights into past levels of fragmentation. Genetic tools can also be used to estimate important demographic components such as current connectivity among fragmented populations, recent population bottlenecks, and current population sizes. Waser and Strobeck (1998) outline an "assignment test" that can assign individuals in a population to the population from which they originated. In short, a disperser or its offspring will look genetically distinct relative to a background of individuals with very different genetic make-ups (consider how a Post-Fragmentation disperser from population B to C in Figs. 1 or 2 would "stand-out" if its genes were examined in a sample of individuals from C). The assignment test takes advantage of the genetic differences among semi-isolated populations and provides a method to detect putative dispersal events without detailed monitoring via telemetry or mark-recapture (Davies et al., 1999). For example, using this technique Favre et al. (1997) inferred sex-biased dispersal of shrews, Paekau et al. (1995) described immigration in polar bears, and Haig et al. (1997) identified the breeding population of origin for shorebirds on fall migration. A limitation of this technique is that it can only distinguish dispersers in cases where the genetic profiles of populations are differentiated enough that individuals originating from a different population "stand out" genetically or many genetic markers are used (Rannala and Mountain, 1997). Importantly, statistical power to detect immigrants can be assessed for any data set using the approach outlined by Rannala and Mountain (1997).

DNA markers can also be used to investigate whether a population has passed through a small-population bottleneck and is therefore susceptible to inbreeding depression. When a population passes through a bottleneck, allelic diversity is reduced faster than heterozygosity, because rare alleles are lost quickly and these alleles have little effect on heterozygosity (Nei et al., 1975; Allendorf, 1986; Leberg, 1992; Hartl and Pucek, 1994). Building on this, Luikart and Cornuet (1998) developed a test for detecting bottlenecks by tracking allele changes. With analysis of at least 5-10 highly polymorphic microsatellite markers in 20-30 individuals, the test successfully detected bottlenecks that occurred in the last several to tens of generations in a variety of animals (Luikart and Cornuet, 1998).

A final way genetic techniques can be used to evaluate fragmentation is in the direct estimation of current population size. On the heels of molecular tools that allow individual identification of animals using creatively-collected bits of tissue (Haig, 1998; Schwartz et al., 1998), there is now the potential to use unobtrusive or non-invasive microsatellite techniques to "mark" and then "recapture" animals. These data can be used in traditional mark-recapture estimators of population size (e.g. Woods et al., 1996; Palsboll et al., 1997). These non-invasive approaches offer advantages over conventional mark-recapture approaches, including the potential to minimize or even eliminate some of the problems that plague demographic estimates, such as tag loss, the need to handle animals, or even trap response or individual heterogeneity in capture probability (Foran et al., 1998). The technique can be limited by underlying low levels of genetic variation, however. We have evaluated the use of non-invasive DNA sampling to estimate population size for rare carnivores (Mills et al., In Press), and found that this technique will lead to a negative bias in population size estimates unless greater than about five to seven microsatellite markers are analyzed. Thus, DNA sampling holds great promise for estimating current population size of hard-to-sample forest animals, but it is not a silver bullet.

3.4 Genetic Markers

Since the development of protein markers in the late 1960's, an array of genetic markers have become available for evaluating the aspects of forest fragmentation that we have discussed (Avise, 1994; Hillis et al., 1996). The workhorse of traditional population genetics, protein electrophoresis, is relatively insensitive to fragmentation effects because the genetic markers are usually not highly variable, although the approach is relatively inexpen-

sive and can be used effectively to understand long-term evolutionary history. Unfortunately protein electophoresis often requires sacrificing individuals for genetic study, because tissues such as muscle, heart, liver, or brain are usually required.

In the past decade, the widespread adoption of PCR based techniques -- a method of making many copies of target DNA from small amounts of DNA sampled in the field -- has increased the availability of genetic approaches to wildlife studies and the number of questions that can be addressed (Parker et al., 1998). Only small amounts of DNA, as might be obtained from a few hair follicles, are sufficient for many genetic analyses. Several textbooks address the application of genetic markers to conservation issues in detail (Loeschcke et al., 1994; Avise and Hamrick, 1996; Smith and Wayne, 1996). For example, microsatellite DNA markers typically show extremely high allelic diversity, and so are very sensitive to fragmentation events that might otherwise go undetected with other markers (Davies et al., 1999). In addition, sex-biased dispersal can be identified by comparing patterns in uniparentally inherited markers, such as mitochondrial DNA or sex chromosome markers, to patterns from protein and nuclear DNA that are passed to offspring from both parents (e.g. Bowen et al., 1992). Overall, the genetic tools to address most of the questions surrounding forest fragmentation are available, but the most important step is to choose the correct tool to best answer a question (Milligan et al., 1994). Often, interpretation will be most robust when different types of molecular markers are used simultaneously, because each marker has strengths and weaknesses (Haig, 1998).

4. GENETIC ISSUES MEET OTHER FACTORS

By the time genetic changes are detected in forest fragments, populations may already be in trouble from ecological and/or demographic impacts. This does not mean that genetic factors can be dismissed, because there are plenty of instances demonstrating genetic changes over the short time scale of human-induced forest fragmentation, and those changes can further affect population persistence. Gaines et al. (1997) review several studies of the effects of habitat fragmentation on small mammals at different scales and note that extreme fragmentation can lead to loss of genetic variation and increased genetic differentiation. However, they also note that levels of fragmentation that can be demographically important do not necessarily result in genetic changes. In other words, detectable genetic changes imply severe isolation and/or population size reduction has occurred, and the genetic changes will interact with other factors to affect population dynamics and persistence (Soulé and Mills, 1998).

There is now no doubt that movement between populations that have been isolated by human activities can increase population persistence for both genetic and non-genetic reasons. In experiments, movement between populations has been shown to reduce probability of extinction due to demographic and environmental variation, and increase probability of recolonization (e.g. Forney and Gilpin, 1989; Fahrig and Merriam, 1994; Sjogren Gulve, 1994). Similarly, experiments have shown that movement between inbred small populations has decreased the chance of extinction due to inbreeding depression (Spielman and Frankham, 1992).

In sum, fragmentation becomes a threat to populations from a genetic perspective when populations become small and isolated, causing them to become inbred and to lose adequate levels of genetic variation for future adaptation to an ever-changing environment. These threats are real, well-documented, and not mere theoretical conjecture. However, genetic factors cannot be addressed adequately in the absence of knowledge of demographic and environmental changes. Genetic techniques are powerful supplements to ecological approaches to monitoring populations and can be a powerful and effective tool to direct management activities across fragmented landscapes.

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