FINE-SCALE POPULATION STRUCTURE OF MASKED SHREWS (*Sorex cinereus)* IN AN EXPERIMENTALLY FRAGMENTED FOREST

by

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Abstract

Habitat fragmentation is perhaps the most important issue facing conservation efforts today. Forests in BC are becoming increasingly fragmented and knowledge about how species respond to different types of forest fragmentation is important for effective management of biological resources. This thesis attempts to use genetic techniques to address this issue. The population genetic structure of masked shrews, *Sorex cinereus*, was measured for a population in an experimentally fragmented forest near Sicamous Creek, BC. Two highly polymorphic microsatellite markers revealed a significant deficit of heterozygotes in the population (F_{IS}=0.31), which has not previously been reported for shrew populations but may be partly due to the presence of null alleles at these loci. Estimates of pairwise relatedness reveal family structure in the population, wherein related individuals tend to be sampled near one another. This may explain the significant difference that is observed in allelic and genotypic distributions between forest treatment blocks, despite the low F_{ST} over the study site (0.006). No significant difference in population structure between types of harvest treatments and the uncut controls was observed. Since only 3 generations of shrews have passed since logging of the study site, these results describe the short-term impact of forest harvest on the population structure of masked shrews and should be supplemented with future studies in order to gauge the long-term effects of habitat fragmentation on the population.

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1.1 Habitat Fragmentation

Habitat loss and fragmentation are considered "perhaps the single most significant challenge to development of models applicable to wildlife management, if not ultimately to the survival of wildlife altogether" (Temple and Wilcox 1986). Habitat fragmentation occurs primarily as a result of human development, agriculture and forestry practices. The two most direct effects of habitat loss are a decrease in the area of suitable habitat available to organisms, and increasing isolation of suitable habitat fragments resulting in decreased connectivity, accompanied by an increasing amount of edge habitat (Bowers *et al.* 1996). Edge is the boundary of two distinct habitat types (Sekgororoane and Dilworth 1995).

Possible detrimental effects of habitat fragmentation include reducing the available amount of habitat below a critical amount required to support a species, which is especially important for species with large home ranges such as many large mammals. Increasing isolation of habitat patches may also limit migration between patches, which would threaten migratory species, as well as species which may exist as part of a metapopulation, where the viability of the metapopulation can depend on the rates of migration between subpopulations (Hanski 1997). Different population sizes may be supported by continuous and fragmented habitats, even when the total area is the same, particularly for species with large home ranges (Bowers *et al.* 1996).

Although there is no disagreement that habitat loss is detrimental to wildlife, there is not a unanimous consensus on the impact of habitat fragmentation, which

can be both detrimental and beneficial. Possible benefits of fragmentation include a decreased susceptibility of the spread of contagions, and assistance in the evolution of locally adapted genotypes. Often as long as some migration between habitat patches is still present, the separation of populations can be beneficial (Simberloff 1988).

Components of ecosystems respond differently to habitat fragmentation (Robinson *et al.* 1992). Increasing amounts of edge often results in increased vegetation complexity and cover for some species, and an increase in the number of habitat types available (Sekgororoane and Dilworth 1995). This can be accompanied by increased species diversity, but also changes the composition of species, where some species may suffer in edge habitats (Harris 1988; Robinson *et al.* 1992). Still, some authors consider edge habitats to be generally beneficial for wildlife (Kremsater and Bunnell 1992). It may also be important to distinguish between hard edge, which is the boundary between suitable and unsuitable habitats, and soft edge, which is the boundary between two suitable but distinctly different types of habitat. Data on soft edge effects are relatively sparse (Nupp and Swihart 1996).

Conflicting views on the effects of habitat fragmentation are at the heart of the still debated SLOSS (single large or several small) issue, regarding the size and distribution of habitat reserves (Simberloff 1988) and the related issue of the benefits and hazards associated with the use of corridors between habitat patches (Downes *et al.* 1997).

Habitat fragmentation can affect a wide range of population parameters including size (and effective size) (Bjornstad *et al.* 1998), social structure and home range sizes (Bowers *et al.* 1996; Collins and Barrett 1997), movement and dispersal (Stamps *et al.* 1987), age structure and sex ratios (Nupp and Swihart 1996), genetic structuring (Bjornstad *et al.* 1998), and ultimately extinction rates (Gilpin and Hanski 1991; Bjornstad *et al.* 1998). It is difficult to generalize the predicted effects since they are often species-specific (Opdam 1991; Mills 1995). Even for a single species there can be considerable contradiction between the proposed effects of habitat fragmentation. For instance, some researchers believe large patches should support higher densities of small mammals because they have greater habitat diversity (Foster and Gaines 1991), while others suggest smaller patches should support higher densities (Nupp and Swihart 1996). The effects on the population dynamics are still undetermined for most species (Peacock and Smith 1997).

A large portion of the literature regarding the negative effects of habitat fragmentation and edge effects documents the impact on avian species. Many bird species are negatively affected by habitat fragmentation. In smaller habitat patches and near edges, many species suffer increased predation and parasitism (Yahner 1988; Paton 1994). Many nesting songbirds decrease in abundance as the ratio of edge to interior habitat increases (Temple and Wilcox 1986), and for several species nest success is positively correlated with habitat patch area. Paton reviewed the literature on edge effects on avians and concluded that the effects of edge habitat extend at least 50 m into the interior of habitat patches (Paton 1994).

For small mammals however the effects of habitat fragmentation are more equivocal. It is difficult to generalize the effects of habitat fragmentation on mammals, since species are affected in different ways. Some small mammal species actually increase in population density as patch size decreases (Adler and Levins 1994), such as the white-footed mouse, *Peromyscus leucopus* (Nupp and Swihart 1996). Ascertaining the effect of fragmentation on the abundance of small mammal populations is difficult, however, since population sizes are naturally highly variable over both space and time (Klenner 1997; Bayne and Hobson 1998). In fact, the abundance of most species doesn't seem to be affected by attributes of edge or patch size in forests fragmented with clear cuts (Heske 1995; Nupp and Swihart 1996). To further complicate matters, the effects of edge may vary over time as cleared habitats go through succession, and may not be visible until 6-10 years after forest harvest in some cases (Heske 1995; Nupp and Swihart 1996).

Some species show clear habitat preferences, such that forest patches are almost entirely isolated from one another, with little or no migration between habitat patches across logged areas. This is so for red-backed voles (*Clethrionomys californicus*), which avoid clear cuts in favour of forest patches (Mills 1995). White-footed mice also show a preference for forest patches over clear cuts, evidenced by the fact that residents of clear cut areas were far more likely to colonize vacated forest habitats than residents of forest patches were to move into vacated clear cut areas (Linzey 1989). Other mammals show no clear preference for forest or clear cut patches. The masked shrew, *Sorex cinereus,* shows no clear attraction or avoidance of edge habitats (Sekgororoane and Dilworth 1995), but has been shown

to decrease in abundance in clear cut areas as well as in areas where only a fraction of the trees are removed (Huggard and Klenner 1997).

The social structure in many species can be affected by habitat fragmentation, especially in species that exhibit habitat selection. Juvenile and sub-adult individuals may be excluded from optimal habitat patches, as documented for the meadow vole, *Microtus pennsylvanicus*, in which the size of home ranges was also affected by the type of habitat occupied (Collins and Barrett 1997). Home range effects have been reported for a number of small mammal species (Ims *et al.* 1993), which may lead to genetic structuring within the population due to non-random mating. This may be particularly relevant in this study, where the scale of habitat fragmentation corresponds approximately to the home range size of *S. cinereus*.

Another population dynamic that may be affected by habitat fragmentation, which could lead to genetic structuring, is the extent of movement and the frequency and magnitude of dispersal. Both movement and dispersal are likely to decrease with an increasing amount of hard edge (Stamps *et al.* 1987), but soft edge effects are less certain. Surprisingly there are few studies documenting within-species variation in dispersal due to habitat spatial structure (Peacock and Smith 1997). Gray-tailed voles (*Microtus canicaudus*) move less often among small habitat patches than among larger ones (Wolff *et al.* 1997). One study concluded that species of rats, mice and voles tended to move into large habitat patches more often than crossing into small habitat patches, and that as the degree of fragmentation increased, the proportion of individuals moving between patches decreased,

although individuals that did move between patches tended to move greater distances (Diffendorfer *et al.* 1995). Some species move frequently between clear cut and forest interior habitat patches, while others exist more as discrete populations within a single habitat patch (Kirkland Jr. *et al.* 1985).

A review of the literature suggests that there are few consistent effects of habitat fragmentation on the population dynamics of small mammals, but instead these effects tend to be species-specific. The effects of habitat fragmentation on one species can occasionally be used to infer likely effects on other species (Ims *et al.* 1993), when care is taken to consider particulars of species ecology, the types of habitats involved, the scale of habitat fragmentation and the spatial arrangement of habitat patches.

1.2 Conservation Genetics

Genetic information is increasingly used in conservation efforts to assist in making management decisions. Generally the goal of conservation efforts has been to preserve biological diversity. More recently the focus of efforts has been extended to include the preservation of genetic diversity and the ongoing process of evolution.

Genetic diversity itself may be important in the survival of populations and species, either in a short term where viability is potentially jeopardized by a lack of variation due to inbreeding depression, as some suggest may be the case for cheetah populations (O'Brien *et al.* 1985), or over a much longer term, where a lack of diversity may limit or constrain evolutionary opportunity. In this light, maintaining

a maximum amount of heterozygosity is generally considered desirable, though several authors point out that many successful species have low heterozygosity (Laikre and Ryman 1991). An important caveat in studies suggesting direct associations between heterozygosity and fitness is that much of the genetic variation that is commonly scored today, using markers such as microsatellites or RAPDs, is not itself adaptive.

In the short term the survival of populations and species is much more likely to depend on environmental and demographic factors than on a lack of genetic variability. Genetic information can, however, be a powerful tool in revealing demographic processes, in particular elements of population structure and migration, where they might otherwise be difficult or impossible to detect. Historic evolutionary processes may also leave a signature detectable in the assay of current levels of genetic diversity, as in the case of population bottlenecks (O'Brien *et al.* 1987; Menotti-Raymond and O'Brien 1993; Hedrick 1996).

Conservation efforts at a range of scales can benefit from genetic information (Mace *et al.* 1996). At an individual level, genetics can refine breeding programs by identifying the relatedness between individuals, which is often beneficial when it is desirable to have matings between individuals of low relatedness. Genetics also allows the identification of quantitative trait loci, which can greatly benefit breeding, programs and stock management for species that are harvested and important economically.

Within a species, genetics can identify the relationship between populations, detecting migration and gene flow between populations (Avise 1994) and defining

allopatric populations that may be of special conservation interest. This is done largely by identifying fixed allele differences, but also by detecting significantly different allele frequency distributions. For dangerously small populations of very rare species, genetic relatedness can suggest which (if any) candidate populations are most appropriate for use in breeding programs to restore impoverished genetic variability (Roelke *et al.* 1993). Likewise it is possible to screen individuals to distinguish between 'pure' individuals and those whose lineages involve hybridization and introgression, to better manage breeding programs (Rieseberg *et al.* 1989).

The pattern and magnitude of dispersal and gene flow can be estimated using genetic markers as an alternative to mark-recapture techniques or direct observation. Rare or unique alleles found primarily in one locale that are then detected in other populations can reveal otherwise inconspicuous gene flow. This can be problematic if the allele frequencies in the recipient population are not well known, or if markers such as microsatellites are used where a high mutation rate might lead to the generation of novel alleles in a population. In these situations maximum likelihood estimates can assist in the estimation of the probability of rare alleles arriving in a population due to gene flow (Beerli and Felsenstein 1999).

Genetic information can distinguish species from one another in cases where morphological differences are inconspicuous or lacking, and can also detect evidence for hybridization which can be crucial in the enforcement of endangered species policies. For example, genetic analysis has revealed introgression of coyote DNA into gray wolf populations in Ontario (Lehman *et al.* 1991).

Beyond the level of species, phylogeny reconstruction can be used to identify genetically distinct lineages which might deserve special attention in conservation efforts. This is relevant when the goal is to preserve maximum diversity of evolutionarily lineages (Faith 1994), which may be the case when decisions need to be made about how to best distribute resources for conservation endeavours. This may direct efforts in different areas than if the aim is simply the preservation of the maximum number of species, with no consideration given to the relationships between those species.

An active field of conservation genetics, due in part to increasing amounts of habitat fragmentation, is concerned with metapopulation dynamics (Levins 1969; McCullough 1996). Generally metapopulations are a group of separate populations connected to some degree by dispersal between populations, and subject to local extinctions and recolonizations from other populations. This definition is extended by some to include any populations that have spatial structure, but generally groups of populations or subpopulations that are not substantially isolated are considered to be continuous populations (McCullough 1996). One important result of population subdivision is that, in general, the effective size of the population is less than that of a single panmictic population (Maruyama and Kimura 1980; Gilpin 1991; Harrison and Hastings 1996; Whitlock and Barton 1997). Populations with smaller effective sizes are subject to stronger genetic drift and are more likely to lose advantageous alleles and have deleterious alleles rise to fixation (Lynch *et al.* 1995).

At a finer spatial scale, genetic information can discern patterns of relatedness between individuals separated by different geographic distances, which

can reveal neighbourhoods in which families of individuals are clustered together, or elucidate patterns of dispersal and gene flow (McFadden and Aydin 1996). For example, fine-scale clustering of related genotypes has been observed in house mice (*Mus musculus*), where family structure existed between different barns on a farm (Selander 1970). Vagile organisms tend to have less structured populations (Avise 1994), but patchy environments or barriers to dispersal can lead to significant structuring in these species, even on small spatial scales.

Certainly, effective conservation efforts require more than just genetic information. Morphological data may be capable of differentiating between populations without the need of genetic information, and may be correlated with adaptive differences. Demographic and ecological factors probably have a greater impact on the survival of most populations than their level of genetic variability. Particularly, small populations are greatly impacted by demographic factors, which may threaten survival in a much more immediate manner than a decrease in genetic variation. Nonetheless, genetic information can illuminate underlying demographic processes or events that are otherwise difficult or impossible to detect.

1.3 Shrews

Shrews comprise the family Soricidae, in which there are two subfamilies, Soricinae and Crocidurinae. Some authors recognize a third subfamily, Scutisoricinae, as being distinct from the Crocidurinae (Ohdachi *et al.* 1997). Within the family Soricidae there are 20 genera totaling approximately 266 species (Corbet

and Hill 1980; Churchfield 1990a; Dannelid 1991). This discussion describes shrews in the Soricinae subfamily.

The Soricinae are comprised of approximately 110 species, about 60 of which are in the genus *Sorex* (Dannelid 1991; Ohdachi *et al.* 1997). Most of the roughly 35 *Sorex* species in North America, including *Sorex cinereus*, are in the subgenus *Otisorex*, while most species in Eurasia, including the common shrew, *S. araneus*, are in the subgenus *Sorex*. Only *S.cinereus* and *S.tundrensis* are found in both North America and Eurasia (Hoffmann 1971). Ancestors of *Sorex* and *Otisorex* were probably separated after the flooding of the Bering Land Bridge either in the late Miocene (Diersing 1980) or perhaps more recently in the Pliocene (Findley 1955; George 1988).

Shrews are short-legged mouse-like mammals with long, pointed snouts. Their fur is short and dense, and their tails, approximately half the length of their bodies, are covered in short bristles. Their eyes are very small, and are often nearly hidden amongst the fur. They are among the smallest mammals in the world, and have extremely high metabolisms (Churchfield 1990a).

Shrews are among the most ancient mammals and retain several primitive characteristics including a plantigrade locomotion, a very small cerebral hemisphere and undescended testes. Shrews have large incisors and unlike rodents their teeth do not grow as they wear away. Instead, the Soricinae have characteristic red caps on their teeth, which are iron deposits that probably reduce tooth wear (Dotsch and Koenigswald 1978; Churchfield 1990a).

Shrews generally have poor vision, but highly developed senses of hearing

and smell. Most shrews will however respond quickly with flight to a shadow moving over them. Vision may also be important in encounters between individuals, where distinctive postures are important in communication (Churchfield 1990a; Churchfield 1990b).

Scent is extremely important to shrews and they have large nasal passages and well-developed olfactory lobes in the brain. Scent is used both in foraging and in communication. Scent glands produce highly odiferous secretions that are used to mark territories and probably aid in mate location, particularly the glands on the flanks of males which are only active in the breeding season (Churchfield 1990a). In addition, these secretions make shrews largely unpalatable to most mammalian predators, though they are still prey to weasels, martens, owls and raptors (Erlinge 1981; Churchfield 1990f).

Acoustic and tactile information are both important to shrews in prey location. Some shrews, notably *S. cinereus*, show evidence of echolocation like that found in bats and in many marine mammals, though apparently not as highly sophisticated (Saarikko 1989; Churchfield 1990b). Tactile sensations are also important in navigation, which is aided by the long vibrissae on the snout which relay information as the shrew weaves its way through the often cluttered vegetation without the benefit of acute vision.

Shrews have extremely high metabolic rates, particularly the species that live in northern climates (Hanski 1984), consuming several times more oxygen per gram body weight than even small rodents such as mice (Churchfield 1990d). To meet their energy demands they must feed every 2-3 hours to prevent starvation. This

requires that they be active night and day. During the course of a 24-hour period, shrews can consume 1-2 times their own weight (Churchfield 1982; Hanski 1984; Churchfield 1990e). They are opportunistic insectivores, feeding on a wide variety of ants, beetles, spiders, earthworms and various insect larvae. In one study, 42 different taxa were identified in their diet (French 1984). Prey are not captured entirely indiscriminately, however, and shrews exhibit preferences for certain prey regardless of their abundance (Churchfield 1990e).

Shrews play an important role in many ecosystems, and may in fact function as keystone predators in some terrestrial ecosystems due to their voracious appetites and preference for carnivorous invertebrates. Increased predation by *S. cinereus* has been shown to increase invertebrate species diversity, since masked shrews tend to reduce the numbers of the more dominant species (Platt and Blakley 1973). Masked shrews increase in numbers as prey availability increases, and have been used as biological control agents in efforts to reduce larch sawfly populations in Newfoundland and New York State. In areas of high shrew density, larch sawfly populations were reduced by as much as 50% (Churchfield 1990g).

1.3.1 Shrew Life Cycle

Shrews tend to be annual species in northern temperate regions with a life span around 12 or 13 months, and show a seasonal cycle in population size. The gestation period for *S. araneus* is 24-25 days, and varies little among other species. Litters of 5-7 young are born in the summer, 3-4 of which typically survive to weaning. The young develop rapidly and are fully-grown in 18 days, at which time

they begin to make occasional ventures outside of the nest. The average mass of *S. cinereus* at adulthood is 3.6 g. Young are fully weaned after 22-25 days, when they begin to disperse and occupy their own nest sites (Churchfield 1990f).

Shrews remain sexually immature throughout the winter. Winter survivorship is facilitated by weight loss, during which even internal organs and skeletal features shrink in size. Although a higher surface area to volume ratio usually leads to greater heat loss, this weight loss probably decreases food requirements, meaning less activity and time spent foraging and perhaps actually reduces heat loss. This is particularly important because shrews cannot hibernate (Merritt 1995). Interestingly, some of the smallest species have ranges that extend the furthest north, contradictory to Bergmann's Rule (Churchfield 1990f).

Rapid sexual maturation proceeds in the spring, when shrews become more active on the ground surface. Breeding begins in May in most northern regions and continues through as late as September, during which time males wander more widely in search of mates. In northern habitats such as those in Canada, females usually have only one litter per season (Chuchfield 1990f; D. Huggard, personal communication, Sept. 1997). Breeding is highly efficient, and more than 90% of females may become pregnant. Breeding success is facilitated by a synchrony of estrus in *S. araneus* (Stockley 1996).

Mortality is high in the first month or so of life, due to competition for resources with littermates, competition for nest sites after dispersal from the nest, and predation. Mortality is also high during the breeding season, due to competition and increased activity leading to greater predation. The most important predators of

shrews are owls, raptors, weasels, martens, foxes and snakes. Finally, mortality is also significant as shrews near the end of their one year life spans and succumb to competition with juveniles for access to resources, injuries, predation, or to the elements as the winter approaches (Churchfield 1990f).

1.3.2 Habitat Preferences, Home Ranges and Dispersal

Soricinae inhabit mainly cool, moist environments with abundant vegetation cover and invertebrate prey. They thrive in a variety of habitats from scrublands and grasslands to woodlands and forests. Most species live primarily on the ground surface, though some spend time in the underground burrows made by other small mammals. As generalists and with their opportunistic feeding habits, shrews are highly adaptive and generally do well as long as there is ample vegetation (Churchfield 1990a).

Shrews are highly territorial and live solitary lives. After weaning, juveniles leave the nest and disperse to establish their own home ranges. This dispersal is encouraged by hostility from the mother and fellow siblings. Home ranges are fiercely defended and there is little or no overlap of home ranges through the autumn and the winter (Michielsen 1966; Churchfield 1990h). Juveniles generally occupy the nearest vacant territory they encounter. At this time adults may be displaced from their territories. Territoriality partially breaks down during the breeding season as mature males expand their territories and wander in search of mates. Breeding females maintain a strong site-attachment, and their home ranges change little in size (Churchfield 1990h).

S. cinereus is particularly intolerant of infringements on home ranges, regardless of the population density. Though other species become more accepting of home range overlap at high population densities, masked shrews instead contract their home ranges and continue to exclude competitors (Buckner 1966; Churchfield 1990h). Home range size estimates for masked shrews range from 0.01 ha (Klenner and Huggard 1997) up to 0.5 ha (Buckner 1966), but are usually considered to be about 0.1 ha in size (Klenner and Huggard 1997). It has been suggested that masked shrews have territories larger than many other shrew species because they forage almost exclusively on the ground surface, while common shrews also forage underground and thus make use of resources in three dimensions. In addition, North American species tend to have larger territories than Eurasian species, perhaps due to living in comparatively resource-poor habitats (Churchfield 1990h).

Home range size varies depending on the type of habitat and the availability of prey. Smaller territories have been found in partly wooded areas than in grassy areas. Of great importance in this study, home ranges are often found to coincide with vegetational and topographical features (Churchfield 1990h). Since territories are actively defended, vegetational boundaries may represent a sort of barrier to shrew movement and dispersal. This might result in population genetic structure, where related individuals congregate in distinct habitat patches.

Smaller juveniles are forced to disperse more widely as larger individuals secure territories closer to their natal site, perhaps only a few metres away. The greatest distance reportedly traveled by a masked shrew in a natural population is

800 m, traversed by a male from juvenile to adulthood (Churchfield 1990h). Travel across greater distances is possible in the absence of competition however, and a population of masked shrews that was introduced in Newfoundland to combat the larch sawfly population managed to expand 11-19 km per year (Churchfield 1990g).

1.3.3 Shrews in the Study of Habitat Fragmentation

Shrews are a useful and convenient organism for the purpose of studying the effects of habitat fragmentation on the genetic structure of populations. They are abundant in the forests of British Columbia and are also very important in the energy dynamics and integrity of ecosystems due to their role as the predators of small invertebrates, and due to their voracious appetites and large numbers (Churchfield 1990e). In northern latitudes shrews comprise a large percentage of the species of small mammals (Churchfield 1990c). Their importance in the functioning of ecosystems is evidenced by their use as biological control agents in the past (Churchfield 1990g).

There are also a number of indications that shrews exhibit habitat selection. Studies have shown that shrews prefer damp, moist habitats over drier areas (Spencer and Pettus 1966), and that food availability was the primary factor influencing the distribution of shrews, which is a secondary effect of both moisture and vegetation cover (Getz 1961). Another study showed that shrews preferred undisturbed grassland to grazed or periodically burned areas (Delany 1964). In addition to habitat preferences the extent of activity and movement of shrews is also

dependent on vegetation cover and moisture levels in natural habitats (Spencer and Pettus 1966) as well as in experimental forest preparations (McCay 1996).

The masked shrew, *Sorex cinereus*, is the most common shrew species in North America, as well as at the Sicamous Creek study site near Kamloops, BC (Virtanen 1989; Hanski 1990; Dannelid 1991).

1.3.4 Effect of Forest Harvest on Population Size

Estimating the impact of different forestry practices on the abundance of shrews is difficult because shrew populations show large annual fluctuations in size. The abundance of shrews, as estimated by the number of shrews captured in pitfall traps before and after tree harvest, decreased by about 40% in clear cut patches (regardless of their size) at the Sicamous Creek study site (D. Huggard, personal communication, Aug. 6, 1999). No difference in abundance was observed in uncut leave strips or controls, and a slight increase in shrew abundance was observed in single-tree selection treatments. Abundance increased in treatments with 0.1 ha cut patches by approximately 30%, and decreased by about 20% in 1 ha and 10 ha patch cut treatments (D. Huggard, personal communication, Aug. 6, 1999).

Population density may affect the distance individuals disperse before establishing home ranges, which would have an impact on fine scale population structure. How dispersal is affected by population density is unclear, however. Increased population density could decrease the distance traveled by dispersing shrews if they encounter more opposition from shrews defending their territories.

Alternatively, high population density could force shrews to disperse greater distances in search of suitable unoccupied habitat.

1.3.5 Population Genetic Effects of Forest Fragmentation

The effects of forest fragmentation on the population structure of shrew species are unknown. The territoriality of masked shrews persists (and intensifies) at high population densities (Churchfield 1990c), but whether this leads to increased or decreased dispersal distances is unclear. Apart from its effect on population density, habitat fragmentation could potentially decrease the likelihood and magnitude of dispersal if shrews avoid habitat edges, but this is apparently not the case (Sekgororoane and Dilworth 1995), despite the fact that shrew home ranges tend to conform to vegetational boundaries (Churchfield 1990c).

Decreased dispersal generally leads to an increase in genetic structure in a population. If forest fragmentation has lead to decreased dispersal of shrews at Sicamous Creek, this may be evidenced by a departure from Hardy-Weinburg Equilibrium genotypic frequencies due to non-random mating of individuals. Family structure in the population might result if related juveniles (siblings, half-siblings, etc.) tend to establish home ranges near to one another, and if these individuals are then more likely to interbreed the result will be decreased heterozygosity in the population. A departure from HWE can occur quickly, after just one generation of non-random mating. Conversely, populations differentiate from one another through genetic drift only after many generations of isolation. The rate at which populations differentiate through drift is dependent on their size and the rate of migration

between populations (Whitlock and McCauley 1999). This suggests that any effects of population subdivision by forest fragmentation will first be evident in departures from HWE in the population, and only later will subpopulation differentiation occur.

1.4 Genetic Markers

Previous studies of shrew population structure using allozyme markers have detected very low levels of heterozygosity (Heikkila 1989), and 26 allozyme loci studied in *Sorex cinereus* showed an average heterozygosity of just 0.04 (George 1988). Even with a large number of loci this level of variation would make the estimation of the relatedness of individuals very difficult, and little genetic variation would be expected within the small spatial scale of this study.

Mitochondrial markers are an alternative and primers exist that amplify the variable D-loop in *S. cinereus* (Stewart and Baker 1994). These markers have relatively high mutation rates but are maternally inherited (in most species and all known mammals) and segregate as a single non-recombining locus. Here the estimation of relatedness is difficult not because of the number of unique alleles, since the number of different sequence haplotypes can be large (Stewart and Baker 1997), but rather by the limited inference possible from a single, uniparentally inherited locus.

Randomly amplified polymorphic DNA (RAPD) markers offer a potentially large number of loci without prior sequence knowledge in the species of interest since non-specific primers are used to amplify PCR products. Drawbacks of RAPD markers include the time and labour required in screening sets of primers, optimizing

primer conditions and verifying that amplification results are reproducible, as well as the fact that RAPDs are dominantly inherited and can be scored only by their presence or absence. One cannot distinguish between homozygotes and heterozygotes at a locus, so these loci carry much less information than codominant loci in studies of relatedness or kinship.

Microsatellite markers are advantageous for studies of population structure for a number of reasons. Perhaps most importantly they can be highly polymorphic, which lends higher statistical power to any attempt to detect population structuring or isolation by distance, which is important on a spatial scale as small as the Sicamous Creek study site. Unlike mtDNA markers or RAPDs, they are codominantly inherited, and PCR primers designed to amplify microsatellite loci in the genus *Sorex* are available. Initial results suggested that cross-species amplification was possible for most species in the genus (F. Balloux, personal communication, Jan.20, 1997).

1.5 Microsatellites

Microsatellites, also referred to as short tandem repeats (STRs), simple sequence repeats (SSRs) and variable number tandem repeats (VNTRs), are DNA sequences containing motifs of 1-6 base pairs, repeated up to 60 times or more in some cases (Ashley and Warren 1995; Goldstein and Pollock 1997), usually with a repeat region of length less than 200 base pairs (Lehmann *et al.* 1996). They are found in all eukaryotic genomes studied to date and are densely distributed in mammalian genomes, which contain hundreds of thousands of them (Weber and

Wong 1993). The most common repeat motifs are $[GT]_n$, $[GA]_n$ and poly-A sequences (Tautz *et al.* 1986; Henderson and Petes 1992).

Microsatellites are often highly polymorphic, with alleles distinguished by a variable number of repeat units. Commonly 10 or more alleles are present at a single locus, even with relatively small samples sizes, and loci frequently have heterozygosities in excess of 0.60 (Bowcock *et al.* 1994; Deka *et al.* 1995). They are quickly becoming the genetic marker of choice due to their high variability, codominant inheritance, abundance in eukaryotic genomes and the ease with which they are scored on electrophoretic gels (Lehmann *et al.* 1996; Goldstein and Pollock 1997). Furthermore, loci are amplified by PCR so only minute amounts of template DNA are required, permitting non-invasive studies sometimes impossible with other markers such as allozymes. Microsatellites are now widely used in studies of population genetic structure (Bruford and Wayne 1993; Bowcock *et al.* 1994; Slatkin 1995), conservation genetics (Gotelli *et al.* 1994), kinship (Morin *et al.* 1994), and forensics (Hagelberg *et al.* 1991). They are less commonly used in phylogenetic reconstruction, however, owing to constraints on their evolution and the peculiarity of their mutation process (Bowcock 1994; Goldstein 1997).

Microsatellites are generally regarded as selectively neutral (or nearly neutral) genetic markers. Most occur in non-coding regions of the genome (Coote and Bruford 1996). Notable exceptions exist in the case of a number of human diseases associated with trinucleotide repeat expansion, including myotonic dystrophy, fragile-X disease and Huntington's disease (Weber and Wong 1993), and possibly in cases where the mutation rate of microsatellites increases in some human cancers

(Wooster *et al.* 1994). They are also present in the SRY gene, which is sex-determining, and in the period locus in *Drosophila*, which is involved in the establishment of circadian rhythms. This coupled with the fact that poly-[GT] sequences are non-randomly distributed in *Drosophila* (being associated with transcriptional activity), and form non-standard helical structures (z-DNA) *in vitro* which alter gene expression, suggests that at least some microsatellites are not neutral markers (Karl and Streelman 1998). In cases where selection is operating, the integrity of genetic analyses assuming neutrality would thus be jeopardized.

1.5.1 Microsatellite Evolution

The abundance of genetic variation at microsatellite loci is a product of their high mutation rates. Mutations within the region of repeats occur with frequencies measured between 10^{-2} and 10^{-5} per locus per gamete per generation (Henderson and Petes 1992; Weber and Wong 1993; Goldstein and Pollock 1997). Trinucleotide and tetranucleotide repeat loci tend to have higher mutation rates than dinucleotide repeat loci. Point mutations in the flanking regions of non-repetitive DNA surrounding the region of repeats occur with a frequency of about 10^{-9}, which is typical for most non-coding regions in the genome (Goldstein and Pollock 1997).

The reason for this high rate of mutation is most commonly thought to be slippage of DNA polymerase during replication (Levinson and Gutman 1987). Experimental evidence suggests this slippage does occur *in vitro* (Schlotterer and Tautz 1992). The majority of these mutations result in either the addition or deletion of one repeat unit (referred to as a single-step mutation), but multi-step mutations

also occur (Henderson and Petes 1992; Weber and Wong 1993; Di Rienzo *et al.* 1994).

Several researchers have found evidence of directionality in the mutation process of microsatellites, which tends to generate alleles of increasing size (Weber and Wong 1993; Ashley and Warren 1995; Rubinsztein *et al.* 1995). A tendency towards deletions has been observed in microsatellite sequences inserted into bacteriophage M13 in *Escherichia coli*, where constraints on genome size may have been the cause of selection (Levinson and Gutman 1987). Ascertainment bias is likely the cause of apparent directionality in the mutation process in some cases, since cloning procedures tend to select for large stretches of repeats. Reciprocal comparisons between species show that microsatellites tend to be larger in the focal species in which they were originally identified (Ellegren *et al.* 1997). Similarly, some authors contend that homologous microsatellite loci evolve at significantly different rates in different species (Rubinsztein *et al.* 1995), while others feel interspecific length differences are also the result of ascertainment bias (Ellegren *et al.* 1997).

If directionality in the mutation process does exist, it makes the apparent constraint on microsatellite lengths even more curious. With the exception of trinucleotide expansion loci, microsatellites with more than 60 repeats are rarely observed (Goldstein and Pollock 1997). Given such high rates of mutation one would expect to commonly find very large alleles for some loci, but this is not the case. It is possible that large alleles are unstable and frequently undergo deletions (Goldstein and Pollock 1997), or that there is selection against large alleles (Epplen

et al. 1993). Regardless of the cause, constraints on the length of alleles limit the utility of microsatellites for phylogenetic inferences (Bowcock *et al.* 1994; Lehmann *et al.* 1996).

Another feature of microsatellite evolution is that mutations are more likely to occur in heterozygotes, particularly those in which there is a large difference in length between the two alleles (Amos *et al.* 1996). It has been suggested that this is due to events of unequal exchange during recombination (Goldstein and Pollock 1997), which becomes more likely due to the repetitiveness in the repeat regions (Henderson and Petes 1992). The combined frequency of recombination events between the repeat regions of two alleles, and of point mutations in the flanking region bounding the repeat domain, has been estimated at approximately 10^{-6} (Lehmann *et al.* 1996). This makes such events much more common than ordinary point mutations, but less common than slippage events within the repeat region.

Since the mutation process of microsatellites usually changes the size of alleles by one or two repeat units, and since there seems to be a constraint on the length of microsatellite alleles, several assumptions of the Infinite Alleles Model (IAM) of evolution (Kimura and Crow 1964) used for other genetic markers are violated. The IAM assumes that mutations generate only novel alleles, and that alleles arise with equal probability, independent of the frequencies of existing alleles. Both of these assumptions are clearly incompatible with microsatellite evolution. As an alternative to the IAM, microsatellite evolution is often modeled after the Stepwise Mutation Model (SMM) of Ohta and Kimura (Ohta and Kimura 1973). This model accounts for the aforementioned peculiarities of microsatellite evolution, assuming

that all mutations result in either the addition or deletion of a single repeat unit. More detailed models allowing for multi-step mutations are also used (Goldstein *et al.* 1995). The SMM is consistent with the observed allele distributions at microsatellite loci (Shriver *et al.* 1993; Valdes *et al.* 1993), which can be unimodal, bimodal, multi-modal or complex (Edwards *et al.* 1992). Distance measures designed to increase linearly in time under the IAM such as Nei's distance (Nei 1972) are non-linear for microsatellites (Goldstein *et al.* 1995), and distance measures assuming the SMM have now been developed (Slatkin 1995; Goldstein 1995). Likewise, measures of population subdivision based specifically on microsatellite allele frequencies have been developed (Slatkin 1995; Michalakis and Excoffier 1996; Rousset 1996).

1.5.2 Cross-Species Amplification and Null Alleles

Since microsatellites are amplified by PCR, locus-specific primers are required in order to utilize them as genetic markers. Primers have been developed for a vast array of species, but fortunately it is not always necessary to use primers specifically developed for the species of interest. Owing to the relatively low rate of mutations in the flanking regions of microsatellites, primers developed for one species will sometimes also amplify microsatellite loci in other closely related species, in which the flanking regions have undergone sufficiently little change such that primers may anneal to template DNA under PCR conditions. Often, however, microsatellites evolve too quickly even in the flanking regions to be of use in species other than the focal species (Coote and Bruford 1996).

Human microsatellites have been found to amplify homologous loci in apes

and Old World monkeys, which share a most recent common ancestor some 30 million years ago (Coote and Bruford 1996). Whales exhibit perhaps the highest conservation in microsatellite flanking regions, averaging just 3.2% divergence over 35-40 million years (Schlotterer *et al.* 1991). In other species results can be less encouraging. For example, in a study attempting cross-species amplification in mice (*Mus musculus musculus*) and rats (*Rattus norvegicus*) just 23 of 153 rat primers amplified loci in mice, while only 20 of 166 mouse primers gave amplification products in rats (Kondo *et al.* 1993). Primers developed for *S. araneus* are variably successful in amplifying products in other *Sorex* species, and entirely unsuccessful in amplifying products in shrews in different genera (Wyttenbach *et al.* 1997).

1.5.3 Null Alleles

A problem with some microsatellites which is particularly relevant to cross-species amplification is the presence of null alleles at some loci. Null alleles are alleles that cannot be detected on a gel because of insufficient (or lacking) PCR product due to mutations in the flanking sequence complementary to the primers being used (Chakraborty *et al.* 1992; Lehmann *et al.* 1996). They are common even in the species for which the primers were originally designed. One study demonstrated that 30% of human microsatellite loci (7 of 23) had null alleles (Callen *et al.* 1993). Nulls can be present at high frequency for any given locus (Scribner *et al.* 1996), and are expected more often when primers are used in non-focal species, since flanking sequences will have had time to diverge since the existence of the most recent common ancestor between the species. Since the mutation rate in the

repeat domain remains high after mutations in the flanking regions have prohibited PCR amplification, there is in fact a distribution of alleles associated with each unique set of flanking regions that may bound a microsatellite locus. These alleles are indistinguishable unless primers complementary to the flanking regions of the series of null alleles are made, which allow them to be resolved on gels.

Null alleles can be a major factor in the depression of observed heterozygosity in populations, compared with expectations under Hardy-Weinberg equilibrium, since individuals carrying a null allele are mistakenly scored as homozygotes for their other allele (Callen *et al.* 1993). This causes inaccuracy in the estimation of population structure, relatedness or any other estimate making use of genotypic information.

1.6 Objectives

The objectives of this thesis are to:

1) Measure the level of population structure at a small spatial scale for a population of masked shrews (*Sorex cinereus*).
2) Compare the population structure within different forest harvest treatments to determine whether the type of tree harvest has an effect on local population structure.

It is unlikely that a large amount of population differentiation will be apparent between harvest treatments due to the small spatial scale of this study, the potential for frequent gene flow between treatments, and the short amount of time that has

passed since forest harvest. Closely related individuals are expected to be trapped proximate to one another. If forest fragmentation affects shrew dispersal, levels of inbreeding among sampled individuals might be expected to vary between treatments. The effects of the different harvest treatments on local population structure are difficult to predict, however. If forest fragmentation and soft edge effects result in decreased dispersal, then the most fragmented treatments might be expected to show higher levels of homozygosity. Fragmentation could conceivably increase dispersal, however, if shrews disperse greater distances in search of the highest quality habitats for use as their home range. Dispersal may also be partially dependent on the population density, but again, whether dispersal will increase with population density as shrews range widely in search of unoccupied territories, or decrease as shrews encounter greater opposition crossing defended territories, is uncertain. Small mammals show species-specific responses to habitat fragmentation, and the effects forest harvest will have on masked shrew populations are by no means clear.

Section 2 - Materials and Laboratory Methods

2.1 Study Site Description

The Sicamous Creek study site is an experimental high-elevation forest located near Kamloops, B.C., in the Salmon Arm Forest District. It is in the Engelmann Spruce – Subalpine Fir wet cold subzone, as described by Lloyd *et al.* (1990). Studying the effects of forest fragmentation in this type of old growth forest is of particular interest, as it is the target of current logging operations (Vyse 1997). The site is roughly rectangular in shape, approximately 3.5 km in length and 1.5 km in width. Logging of the area was done as part of a project to study the response of high elevation forest ecosystems of southern Interior British Columbia to various harvesting techniques (Vyse 1997). The project is supported by the Forest Renewal BC research program and involves a large number of researchers studying elements of forest ecology (Vyse 1997).

Pitfall traps were used to capture shrews at the study site. These traps are plastic cups (500 ml) set in the ground with their tops flush with the ground surface, shielded from rain and debris by cover boards that are supported by three stakes. Each cup contains 100 ml of the preservative, proplyene glycol. Figure 1 is a diagram of the study site and Figure 2 shows a rough map of the area, with pitfall trap locations drawn as either small black circles or squares. These symbols represent a circle of 5 pitfall traps, and there are a total of 855 pitfall traps in the study area. The precise locations of the traps relative to one another are shown in Figure 3, where they have been plotted according to their Universal Transverse Mercator (UTM) coordinates, which are accurate to within approximately 15 m.

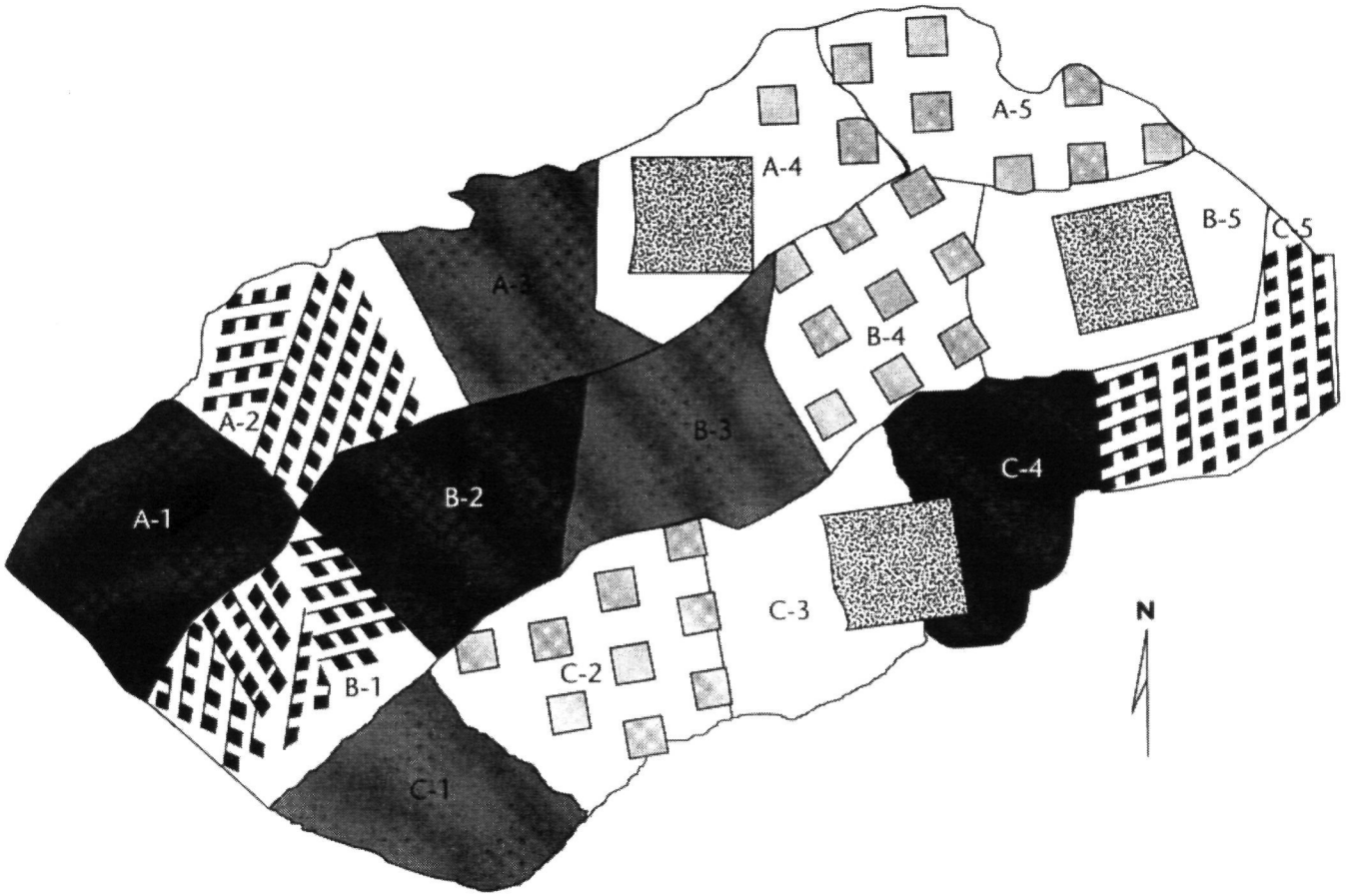

Figure 1. The Sicamous Creek study site, showing the uncut control (A-1, B-2, C-4), 0.1-ha patch cuts (A-2, B-1, C-5), 1-ha patch cuts (A-5, B-4, C-2), single-tree selection (partial cut) (A-3, B-3, C-1) and 10-ha clearcut (A-4, B-5, C-3) treamtents, from Vyse (1997).

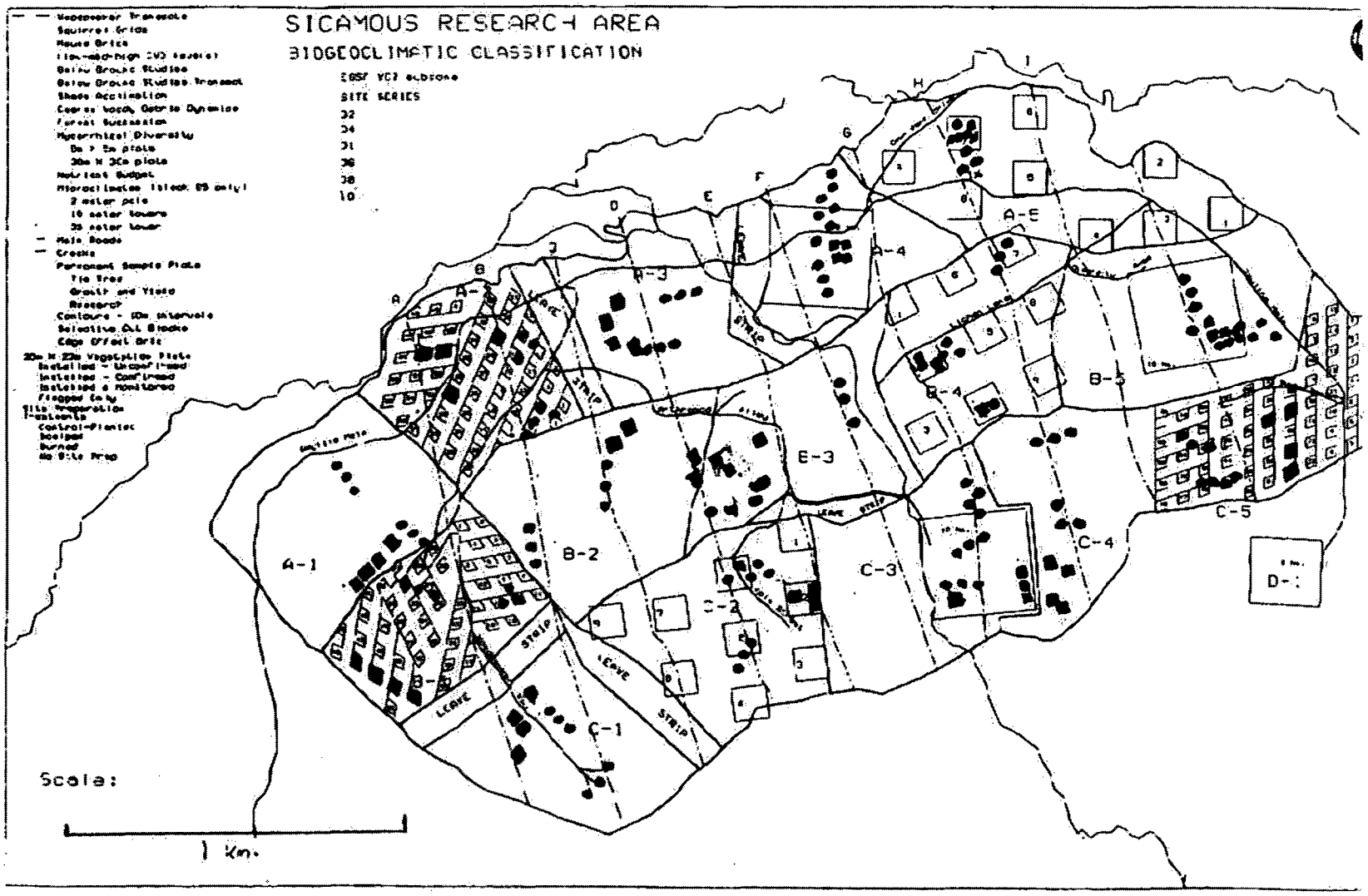

Figure 2. A rough map of the Sicamous Creek study site showing the approximate location of trap circles.

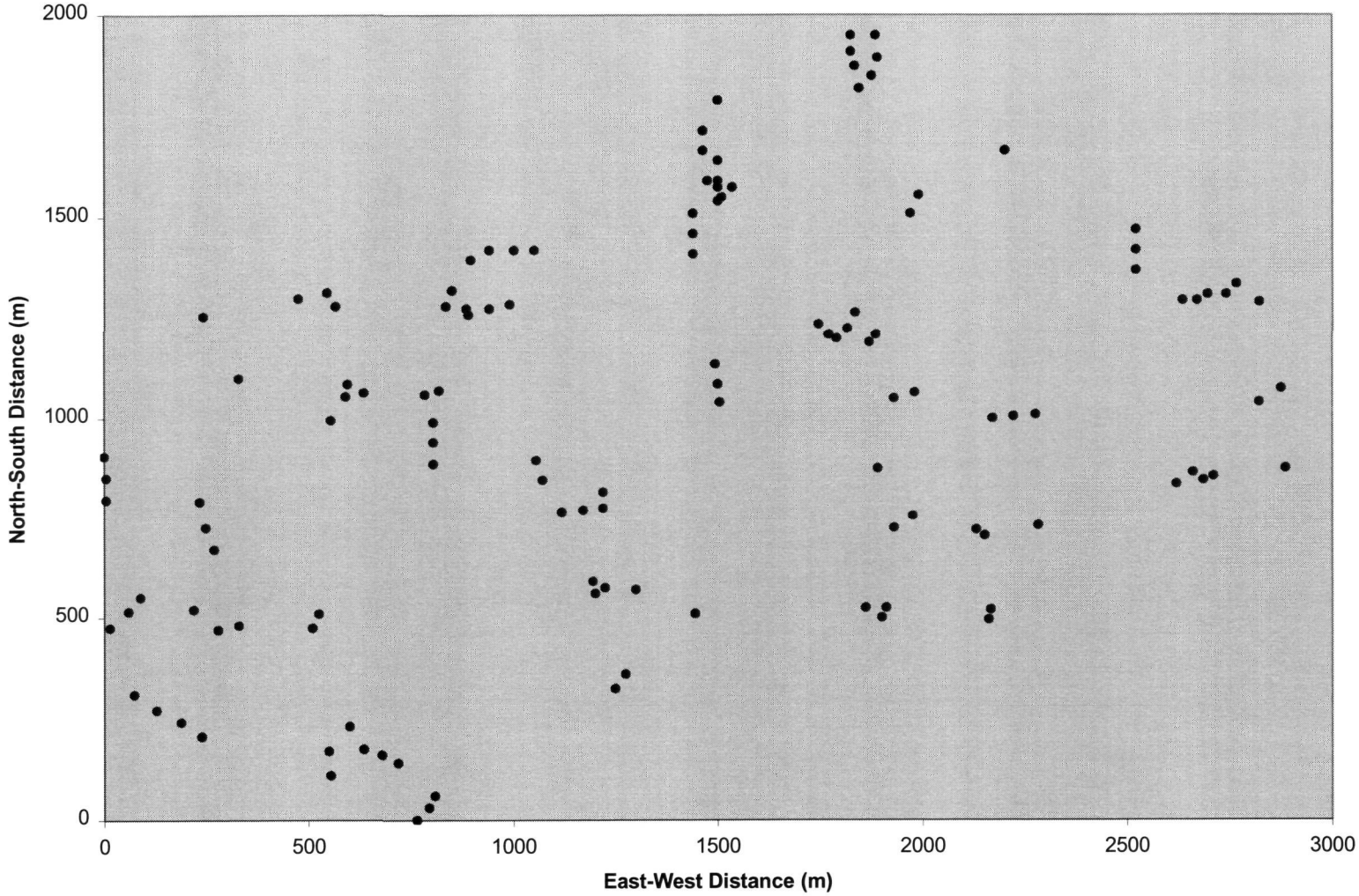

Figure 3. A plot of the location of all trap circles at the Sicamous Creek study site. Distances are relative to the most easterly and southerly trap circles.

Adjacent trap circles are typically separated by 50-75 m, and each treatment contains at least 10 trap circles.

The site is divided in a randomized block design, with three replicates of 5 treatment blocks, labeled A1-A5, B1-B5, and C1-C5, as shown in Figure 1. Each block is approximately 30 ha in area, and with the exception of the uncut controls, approximately 33% of the trees were cleared in each treatment. Leave strips of standing trees 100 m wide were left around each block so that they might be considered independent to one another. Tree harvest was done in the winter of 1994-1995.

2.2 Tissue Collection

Shrews were collected from pitfall traps at the Sicamous Creek experimental forest by the Ministry of Forestry, under the direction of Dave Huggard. Trapping was done in 4 sessions, 2 weeks at a time. Collections took place on July 14-15, July 28-29, August 25-26 and September 8-9 of 1997. Pitfall traps contained a small amount of glycol so that bug collections taken from the same traps could be preserved. Most of the samples analyzed in this study were collected on the July 14-15 and August 25-26 collections, although samples from all collection dates are represented.

After shrews were retrieved from the pitfall traps, they were stored in freezers at approximately -15°C until their tails were clipped and stored in cryovials in a liquid nitrogen cooled storage tank, up until either DNA extraction, or transfer to a -80°C freezer preceding DNA extraction.

2.3 DNA Isolation

Different types of tissue were evaluated as sources of DNA from the captured shrews, including heart, liver, kidney, muscle and tail tissue. Tail tissue consistently yielded the highest DNA concentrations and the largest DNA fragments, perhaps due in part to DNA degradation in other tissues with greater enzyme activity.

DNA was extracted from tail clips approximately 5-8 mm long. Tail clippings were incubated at 55°C overnight in a solution containing STE buffer (0.1 M NaCl, 20 mM Tris-HCL pH 8.0, 25 mM EDTA pH 8.0) and 1% SDS, as well as approximately 0.2 mg/μl of proteinase K to digest proteins.

DNA extraction proceeded by adding 500 μl of phenol, mixing with a rotator for 5 minutes, spinning in a microcentrifuge at 12,000 RPM for at least 5 minutes, and then transferring the aqueous layer to a fresh tube. This phenol step was then repeated, and followed by the addition of 500 μl of chloroform : isoamyl alcohol (24:1), mixing for 5-10 minutes with a rotator, then spinning for 10 minutes in a microcentrifuge at 12,000 RPM. The aqueous layer was then transferred to a fresh tube and DNA was precipitated by adding 1/25 volume of 5 M NaCl and 2 volumes of 100% ethanol chilled to –20°C, followed by gentle mixing for approximately 5 minutes and finally by spinning in the microcentrifuge at 12,000 RPM for 15 minutes. This yielded a pellet of DNA which was then washed with 1 ml of 70% ethanol during a final spin in a microcentrifuge at 12,000 RPM for 10 minutes. Ethanol was then poured off and the pellet was allowed to air dry, before DNA was resuspended in 100-200 μl of distilled water.

The resulting DNA concentration was determined for each sample by

measuring the absorbance of light by diluted DNA samples relative to the absorbance of distilled water, using a spectrophotometer (Pharmacia Biotech Ultrospec 3000, Cambridge, England). The molecular weight of DNA extracted was assayed for approximately 15% of all DNA samples by running DNA on 2% agarose gels with a TBE (Tris, Boric acid, EDTA) running buffer, along with a DNA ladder consisting of lambda phage DNA digested with HindIII restriction enzyme (Gibco BRL). The DNA purity was also assayed for several samples using a spectrophotometer, to verify that protein contamination was minimal after the extraction procedure.

2.4 Primer Screening

Primers developed for 17 microsatellite loci in *S. araneus* were screened for their ability to amplify polymorphic products in the *S. cinereus*. Of these, 12 were dinucleotide $(AC)_n$ repeats, 3 were trinucleotide repeats and the remaining 2 were tetranucleotide repeats. Previous studies had determined that only 5 of these loci gave amplification products in *S. cinereus*, and only 3 were expected to give polymorphic products (Wyttenbach *et al.* 1997). Two loci that were initially considered to be polymorphic in *S. cinereus* were later rejected as useful markers since they could not be reliably amplified (F. Balloux, personal communication, Jan.20, 1997).

PCR amplification was tested over a wide range of temperature and reaction conditions in an attempt to optimize conditions for each locus individually, as summarized in Table 1. Initially thermal cycling was done in a Perkin Elmer cycler

Table 1. Results of primer screening in *S. cinereus* (+/- indicates inconsistent amplification success).

Locus	Core Sequence	Amplification	Polymorphic	Product Size	Temperature
L2	(GGA)12	-	-	-	45-55
L9	(AC)29	-	-	-	50-55
L16	(AC)16	-	-	-	45-55
L45	(AC)10	+	-	98	45-55
L57	(AC)10	-	-	-	50-55
L62	(AC)16	+	+	216	55
L67	(AC)17	+	-	108	50-55
L69	(AC)17	-	-	-	50-55
L92	(AC)7	+	-	122	50-55
L16b	(GGAA)22	-	-	-	45-55
L68	(AC)14	-	-	-	50-55
L13	(AATT)6	-	-	-	50-55
L33	(AC)19	-	-	-	50-55
L97	(AC)56	+	+	263	55
L8	(GAA)20	-	-	-	50-55
L14	(AC)14	+/-	+	210	45-55
L39	(GGA)?	-	-	-	50-55

(Perkin Elmer DNA Thermal Cycler Model #480, Norwalk CT. USA), which required a drop of mineral oil in each reaction tube, and typically reaction volumes of 20 μl. Later an MJ Research PTC-100 Thermal Controller (MJ Research, Inc) was used, with 32 cycles of 45 seconds at 94°C, 45 seconds at 45-55°C, and 45 seconds at 72°C, preceded by a 3 minute denaturation step at 94 °C, and followed by a 10 minute elongation step at 72°C. Reactions were typically 10 μl in volume, containing 50-100 ng of DNA template, 0.5 μM each of forward and reverse primers (1 μM total), 1x Reaction Buffer (Gibco BRL),1-3 mM $MgCl_2$, 80 μM dNTPs (dATP, dCTP, dGTP, dTTP), and 0.5 units of *Taq* polymerase (Gibco BRL). Initially amplification products were run on 2% agarose gels with 1X TBE buffer, along with a 100 bp DNA ladder as a molecular weight standard (Gibco BRL). Products were visualized by staining the gels with ethidium bromide and illumination with UV light. Finer resolution of product sizes was performed on polyacrylamide gels to screen for polymorphic loci, which were stained and illuminated as with the agarose gels. Finally, four loci (L14, L62, L67, L97) were amplified with IR-labeled primers and run on 6-7% polyacrylamide gels on a Licor DNA sequencer (Licor Model 4200-S2 DNA Sequencer, Licor Inc., Lincoln, NE, USA), which uses a laser to cause the IR-labels to fluoresce and then generates a digital image of the gel.

Loci behaved as previously reported in terms of the presence or absence of amplification products, as well as in terms of being either monomorphic or polymorphic. One further locus (locus L14) that had previously shown low levels of polymorphism in *S. cinereus* was rejected as a useful marker since it would not

amplify reliably. Variation in product size for this locus is apparent on polyacrylamide gels, but no more than about 20% of samples analyzed for screening purposes yielded amplification products so this locus was not used as a marker for the remaining samples in the study. This result may be due to primer site mutations having occurred since *S. cinereus* and *S. araneus* shared a common ancestor, and suggests that polymorphism exists at the primer site within *S. cinereus*, i.e. the presence of null alleles. Locus L45 appeared to yield a monomorphic 98 bp product, apparently one repeat unit smaller than the most common product size in *S. araneus*. Locus L45 was originally considered polymorphic in *S. cinereus* (with 2 different alleles scored) but was later rejected as a useful marker due to inconsistent amplification success (F. Balloux, personal communication, Jan.20, 1997). The amplification products for both loci L62 and L97 were larger than reported in *S. araneus*, where the average product size was 181 bases for locus L62 and 235 bases for locus L97.

2.5 Genotyping

Genotypes at each of loci L62 and L97 were determined by multiplex amplification of PCR products which were then resolved on a Licor DNA sequencer. Table 2 lists the primer sequences for both loci. One of the primers for each locus carried a tail M13 sequence containing IR-labeled nucleotides, which allows visualization of products on the sequencer. Fluorescent intensity depends on the amount of product present and not on the products molecular weight or sequence (which would be the case if IR-labeled nucleotides were incorporated into the

Table 2. Primer sequences for the loci L62 and L97. Accession codes can be used to retrieve sequences from Genbank. The first primer listed for each locus is the forward primer, followed by the reverse primer.

Locus	Accession	Primer Sequence (5' - 3', forward, reverse)
L62	U82715	CACGACGTTGTAAAACGACCAGTCTCTCACTGTGGCACTATG
		GTCATTCTGGATAAGAACCATATGC
L97	AF032915	ATTCTCGTGGGTAGACCGTG
		GGATAACAATTTCACACAGGATAAATGTGGGAAATGGACAGG

amplification products by *Taq* polymerase). The forward primer for locus L62 carried an M13F sequence and fluoresced under illumination with an 800 nm laser, while the reverse primer for locus L97 carried an M13R sequence and fluoresced under illumination by a 700 nm laser. This allows the sequencer to generate a separate gel image for the products of each locus, avoiding ambiguity in determining the identity of fragments of similar molecular weights. This was necessary since the distribution of allele sizes at the two loci partially overlap.

PCR was conducted with an MJ Research PTC-100 Thermal Controller as described above, except with the addition of 5 μg of BSA in each 10 μl reaction, which was found to improve amplification results. All products were wrapped and stored in foil, since the fluorescent labels are light-sensitive. Separation of products was performed on vertical, denaturing 6-7% polyacrylamide gels (30 ml volume containing 12.6 g urea, 5.25 ml 40% polyacrylamide, 7.2 ml 5X TBE buffer, dH_2O, 200 μl ammonium persulfate, and 15 μl TEMED), with a 1X TBE running buffer. Prior to loading, 2 μl of stop dye was added to all PCR products, which were then denatured at 90°C for 3 minutes and immediately put on ice to prevent re-annealing. Gels were run at a constant temperature of 50°C, at 1500 V and 25 W.

Each gel was loaded with a molecular weight ladder (Licor, Inc.), and where possible 1-3 positive control samples of known genotypes were included to allow the allele sizes to be determined accurately and to verify the reproducibility of previous results. The resolution of these gel images allows the detection of 1 bp differences between fragments. Both microsatellite loci in this study contain dinucleotide repeats, so alleles differing by only one repeat were discernable from each other.

DNA fragment pattern analysis software (RFLPscan, Scanalytics, CSPI. Billeria, MA. USA) was used to assist in gauging band intensity. This software plots a line graph of the intensity of the IR signal alongside the gel image, and the two bands of highest intensity were considered to be the true allele sizes.

One microsatellite allele can result in multiple amplified fragments of differing lengths, as a result of slippage of *Taq* polymerase during DNA synthesis. These spurious bands are often referred to as shadow bands or stutter steps, and were usually shorter in length, presumably because the polymerase tended to skip ahead on the template by one or more repeat units. Consequently, many bands may appear for a given individual at a single diploid locus. Often these bands form 2 distinct groups of bands on the gel, where one band in each group represents an actual allele, while the remaining bands are shadow bands.

In cases where an individual is heterozygous at a locus for alleles of similar size, the bands may form one group on the gel instead of two. It is important to be able to distinguish between such heterozygous individuals and homozygotes, which also give only one group of bands. Most often the polymerase accurately copies the template, so the fragment corresponding to the true allele size will register the strongest IR signal. Individuals with one group of bands in which the most intense signal was not the largest sized product were often scored as heterozygotes. The higher intensity of these smaller bands is presumably due to contributions of both alleles to products of the same size, due to polymerase slippage.

Occasionally a faint shadow band was detected that was one repeat larger in size than a very intense band. This was assumed to be the result of polymerase

slippage backwards on the template, resulting in the insertion of an additional repeat during amplification.

The precise molecular weight of each band was determined using the known molecular weights of both the ladder and the positive control samples, with the assistance of the RFLPscan software. Non-amplifying samples were used in one or more further amplification reactions, in an attempt to generate amplification products. Figure 4 is a sample gel image showing products at locus L97, and Figure 5 is an image of the products scored at locus L62.

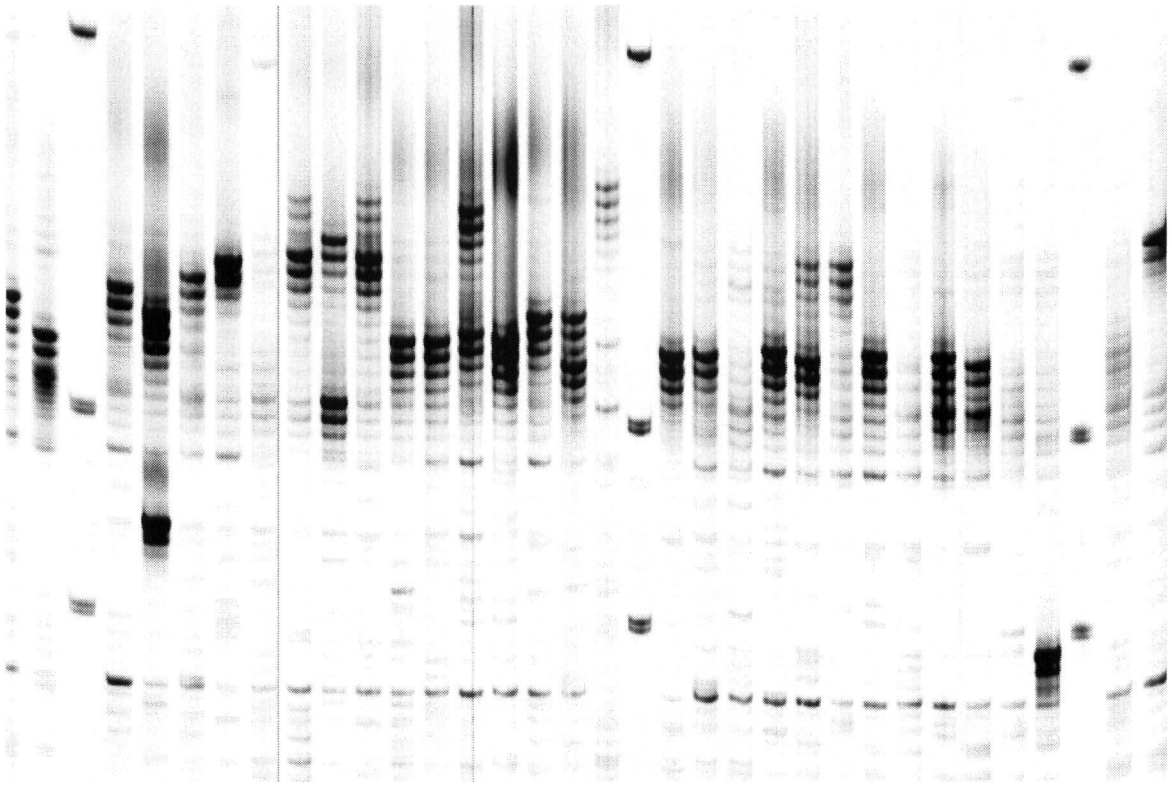

Figure 4. Sample digital gel image of products at locus L97. The three lanes with only 3 bands contain molecular weight standards.

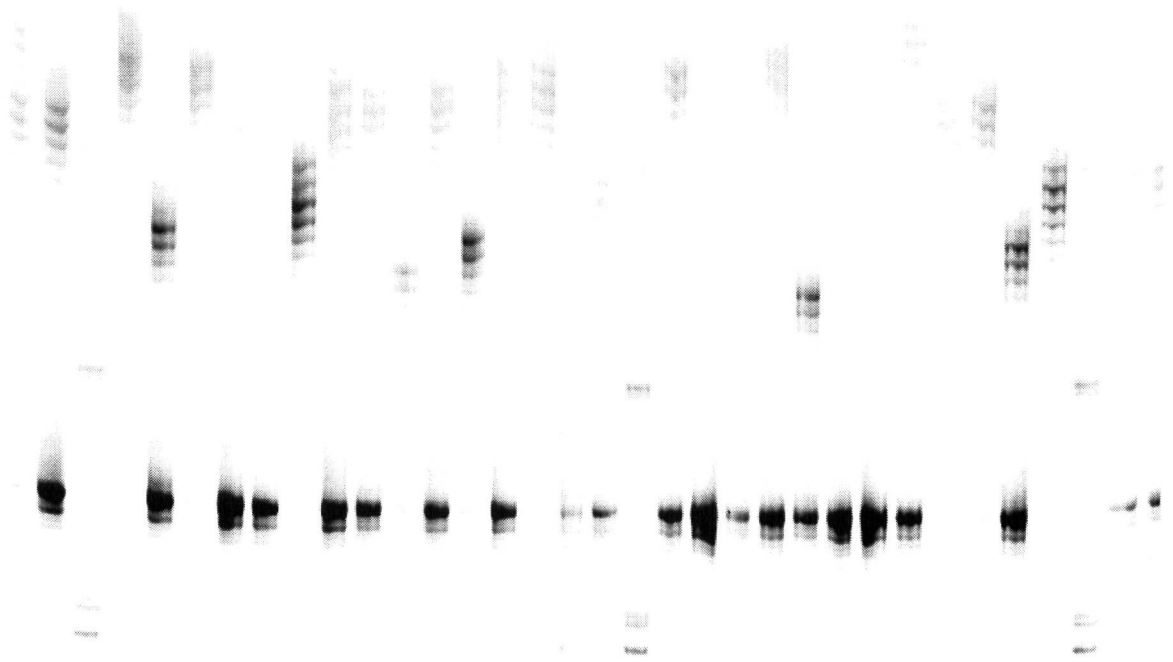

Figure 5. Sample digital gel image of products at locus L62. Three lanes contain molecular weight standards.

Section 3 - Genetic Analyses and Results

3.1 PCR Amplification Results

Of 379 individual shrew DNA samples, 340 (90%) gave amplification products at locus L97, and only 188 individuals (50%) amplified for locus L62. The number of samples amplifying within each treatment block is shown in Table 3. To ascertain whether DNA quality was a cause of the dropouts at each locus, the ratio of dropouts among all samples at loci L62 and L97 were compared. Among all 379 samples, 191 dropouts at locus L62 were observed (frequency = 0.504). Among the 39 samples that failed to amplify at locus L97, 22 individuals also failed to amplify at locus L62 (frequency = 0.560). A χ^2 test failed to reject the null hypothesis that failing to amplify at locus L62 was independent of amplification success at locus L97 (χ^2=0.5911, P>0.44). That amplification success at one locus is independent of success at the other locus argues that the quality of DNA is not a major factor in preventing successful amplification.

Among the 379 samples, 218 were identified as males, 139 were identified as females and 22 individuals were of unknown sex. A χ^2 test identifies this as a significant departure from a 50/50 ratio (χ^2=17.485, df=1, P=0.00003).

3.2 Test for Genotypic Disequilibrium

Prior to proceeding with the analysis of genotypes at the two loci, a test to verify that the loci are in genotypic equilibrium was performed using the program

Table 3. Number of samples giving PCR amplification products for loci L97 and L62.

Treatment	Replicate	L97	L62
Uncut	A	21	13
Uncut	B	18	5
Uncut	C	29	10
0.1 ha patches	A	34	23
0.1 ha patches	B	18	5
0.1 ha patches	C	18	9
1 ha patches	A	24	14
1 ha patches	B	27	20
1 ha patches	C	13	8
Partial Cut	A	37	20
Partial Cut	B	19	9
Partial Cut	C	23	12
10 ha clearcut	A	23	14
10 ha clearcut	B	25	19
10 ha clearcut	C	11	7
Total Population	**-**	**340**	**188**

GENEPOP 3.1a (Raymond and Rousset 1995). The program constructs a contingency table with all of the observed genotype combinations between the two loci, and then performs a probability test using a Markov chain algorithm (Guo and Thompson 1992), testing the null hypothesis H_0: "Genotypes at locus L97 are independent from the genotypes at locus L62". The Markov chain algorithm is an adaptation of the Metropolis algorithm (Metropolis *et al.* 1953) and is useful in the analysis of sparse contingency tables, which often result where the number of alleles is large and several observed genotype frequencies are zero. The algorithm is normally used to estimate the probability of genotypic distributions under the null hypothesis of Hardy-Weinburg Equilibrium (HWE) genotypic frequencies, by constructing a chain with an equilibrium distribution matching that expected under HWE and with the same allelic counts as observed in the data (Guo and Thompson 1992). The algorithm returns estimates the P-value of the probability test under the null hypothesis, which represents the probability of a genotype distribution departing from HWE expectation at least as much as the observed data does. Across all populations the test returned a χ^2 value of 15.773 (df=26), which gives a P-value of 0.942. The test also gives high P-values suggesting genotypic equilibrium for each treatment, with 3 exceptions. In two treatments, the 0.1 ha patch cut treatment and the uncut treatment in replicate B, no multilocus genotypes were scored and so a test for genotypic equilibrium could not be performed. Each of these treatments had only 5 individuals that amplified for locus L62, and none of them successfully amplified for locus L97. A third treatment, the 10 ha clear cut treatment in replicate B, returned a P-value of 0.011. This could very well be a Type I error, and in fact is

not quite a significant result after a Bonferroni correction to the required α-value, which would then require a P-value no more than 0.0038 (α=0.05/13 treatments). This test confirms that genotypes at each locus are independent of genotypes at the other locus, thus locus L97 and locus L62 are subsequently treated as two independently-segregating loci.

3.3 Allele Frequency Distributions

3.3.1 Locus L97

The distribution of allele sizes for all alleles scored at locus L97 is shown in Figure 6. The distribution is roughly normal and bell-shaped, centered on the most common allele, which is 263 nucleotides in length and occurs at a frequency in the total population of 0.178. The distribution of alleles is continuous apart from a single rare allele at each end of the distribution (with lengths 227 and 297 nucleotides), which were scored just once each. For the rest of the range of allele sizes between 239 and 289 bases in length, alleles were scored for each possible product size, in increments of one dinucleotide repeat, yielding a total of 28 different alleles.

The allele frequencies scored in each treatment block are shown in Table 4. A plot of these frequencies is shown in Figure 7. It is evident from both the table and the plot that marked differences exist between treatment blocks in their allele frequency distributions. All treatment blocks show a unimodal distribution of alleles but the allele frequencies vary greatly between blocks. In several treatment blocks the median of the distribution is an allele other than allele 263, and treatment blocks vary in the frequency of rare alleles (such as alleles 241, 277, and 279), which were

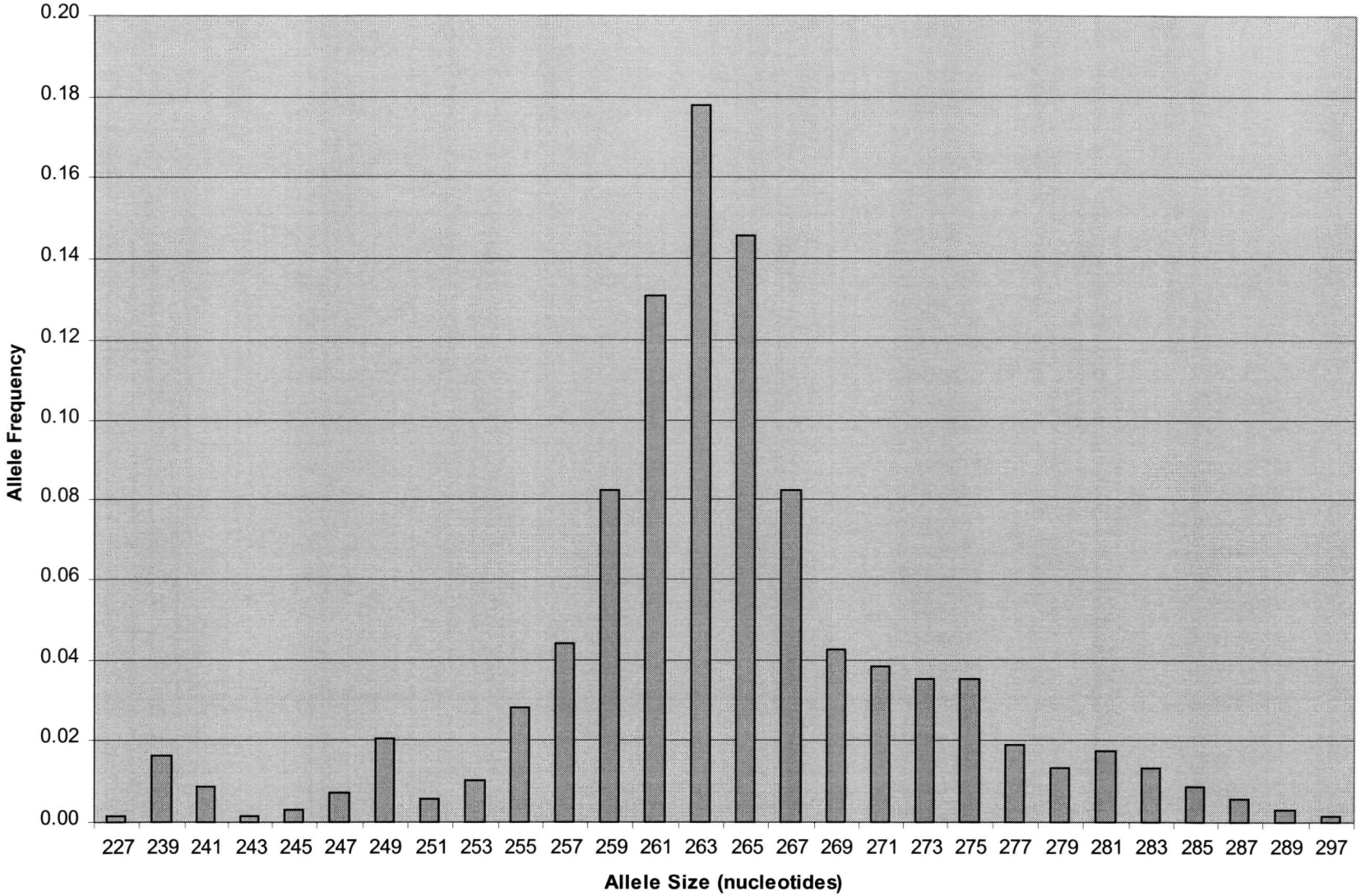

Figure 6. Locus L97 Allele Frequency Distribution (Total Population)

Table 4. Locus L97 allele frequencies in each treatment block. Listed values represent the percentage of alleles scored in that treatment that were of the specified size. Some rows do not total 100 percent because values are rounded off to the nearest 1 percent. (Allele Numbers are equal to actual PCR product size in nucleotides minus 200 bp. Treatments labeled as "Uncut" = Uncut control, "0.1 ha" = 0.1 ha patch cuts, "1 ha" = 1 ha patch cuts, "Partial Cut" = Single-Tree Selection, "10 ha" = 10 ha clear cut)

	Allele (#bp - 200)																											
	27	39	41	43	45	47	49	51	53	55	57	59	61	63	65	67	69	71	73	75	77	79	81	83	85	87	89	97
Treatment Block																												
Uncut A	0	0	0	0	0	5	5	2	0	2	7	7	5	19	14	12	5	0	0	7	7	0	0	0	0	0	0	2
Uncut B	0	0	0	0	0	0	0	0	3	11	0	6	11	14	17	17	0	0	11	3	0	3	3	3	0	0	0	0
Uncut C	0	0	0	0	0	2	0	0	0	2	2	5	9	26	17	12	7	5	7	2	0	0	0	3	0	2	0	0
0.1 ha A	0	0	6	0	0	0	4	0	0	0	6	6	21	16	13	7	4	6	4	4	1	0	0	0	0	0	0	0
0.1 ha B	0	0	0	0	0	0	6	0	0	6	0	3	11	22	3	0	8	8	3	3	6	6	3	3	3	6	3	0
0.1 ha C	0	0	0	0	0	0	0	0	3	0	11	25	19	8	22	0	3	6	0	0	3	0	0	0	0	0	0	0
1 ha A	0	4	2	0	0	0	0	0	4	0	4	4	13	6	13	19	10	0	2	8	0	2	6	2	0	0	0	0
1 ha B	0	0	0	0	0	0	0	0	0	4	11	28	11	17	7	2	2	2	6	4	7	0	0	0	0	0	0	0
1 ha C	0	8	0	0	0	8	0	0	0	0	4	8	31	19	8	4	4	4	0	0	0	0	0	4	0	0	0	0
Partial Cut A	0	1	0	0	0	0	3	0	0	3	1	9	5	23	18	8	7	3	3	4	3	5	4	0	0	0	0	0
Partial Cut B	0	5	0	3	0	0	3	0	8	11	0	0	13	24	18	0	0	0	5	3	0	0	0	0	3	3	3	0
Partial Cut C	0	4	2	0	0	0	2	0	0	0	13	2	13	13	17	9	0	15	2	0	0	0	0	2	4	0	0	0
10 ha A	2	0	0	0	0	0	2	0	0	4	2	13	22	11	11	15	0	4	2	9	0	0	2	0	0	0	0	0
10 ha B	0	4	0	0	4	0	4	6	0	2	2	2	8	18	18	4	8	2	4	2	0	2	4	4	2	0	0	0
10 ha C	0	0	0	0	0	0	0	0	0	0	0	0	18	36	23	14	0	0	0	0	0	0	5	0	5	0	0	0

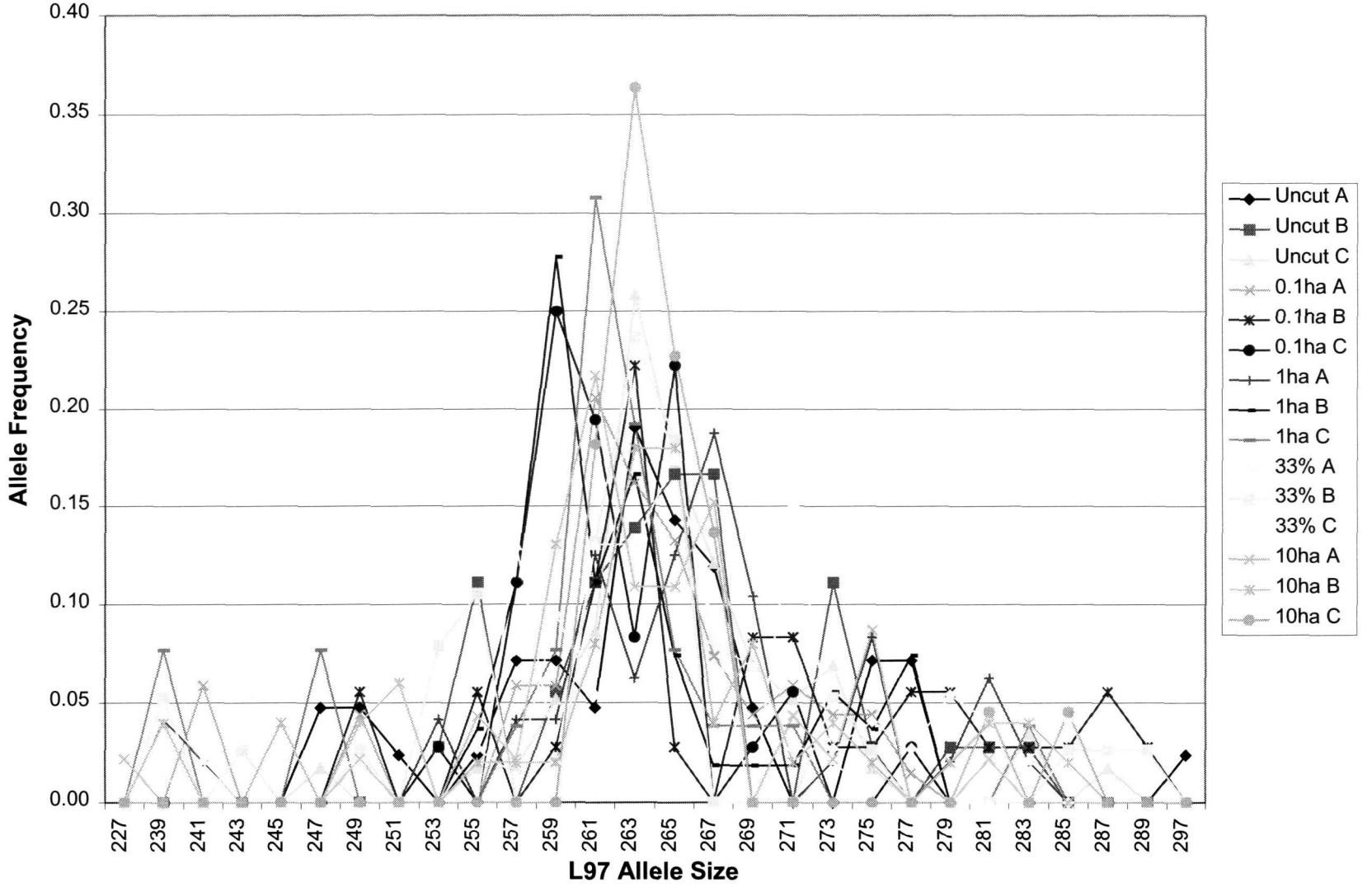

Figure 7. Locus L97 allele frequency distribution for all subpopulations.

often not found in many of the treatment blocks despite being present at appreciable frequencies in others.

In order to test whether the allele distributions in the treatment blocks were significantly different from one another, an exact test using a modified Markov chain was used to give an unbiased estimate of the probability of the observed allelic distributions under the null hypothesis H_0: "the allele distribution is identical among treatment blocks". Here, the program GENEPOP 3.1a was used in the analysis of the allele frequency data, using Markov chain parameters of 1000 dememorization steps and 1000 batches, each consisting of 1000 iterations. The null hypothesis was strongly rejected ($P<0.00001$, S.E.=0.00000), showing that the allelic distributions in the treatment blocks are significantly different from each other. Thus, the treatment blocks are hereafter referred to as subpopulations.

3.3.2 Locus L62

The distribution of alleles scored at locus L62 is shown in Figure 8. The smallest allele scored was by far the most common, with a size of 216 nucleotides and occurring at a frequency of 0.566. Other than this common allele, the rest of the allele distribution is approximately normal, centered around an allele 256 nucleotides in length. To increase the resolution of this figure, the scale on the Y-axis only extends to a frequency of 0.15. In all, 22 different alleles were scored at locus L62.

The high frequency of the 216 allele is curious, especially since it is much smaller in size than the rest of the alleles scored at this locus. This product does appear to segregate as an allele at locus L62, however. No individuals carrying two

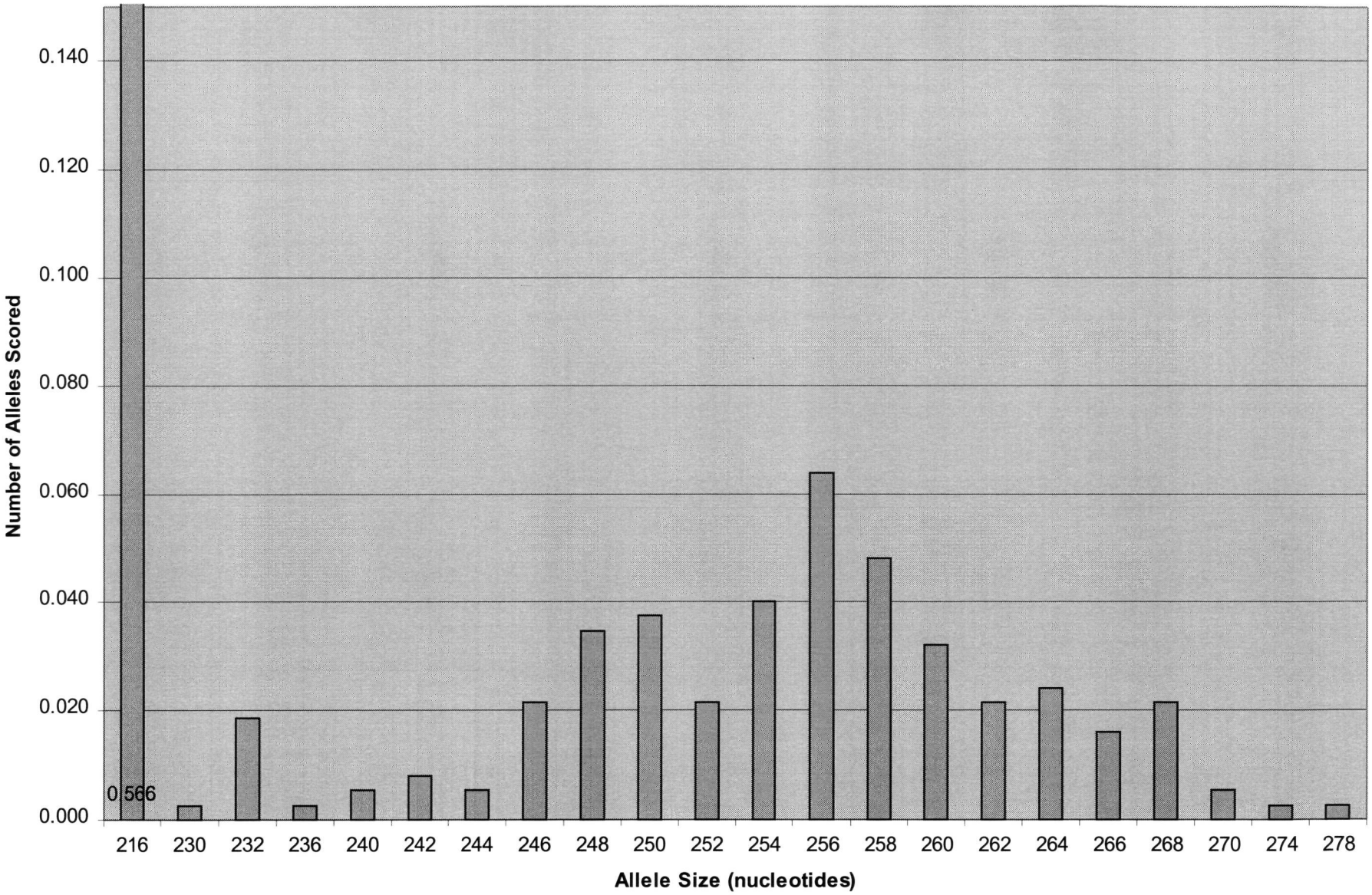

Figure 8. Locus L62 allele frequency distribution (Total population).

alleles from the rest of the allele distribution ever amplified the 216-nucleotide product, but many heterozygotes were scored that carried just one allele other than the 216 allele. When the 216 allele was scored it was almost always the most intense signal in the lane, but reducing the intensity of the digital image allowed the resolution of an apparent shadow band associated with this product. The pattern of bands associated with the product was unique, however, in that fewer shadow bands were detected than for the other alleles. It is possible that this product represents an allele which has all but lost the microsatellite repeat region and has risen to high frequency in the population. The intensity of the product may be a result of primer competition, which tends to favour smaller-sized products.

Another possible explanation for this common product is that there has been an insertion or duplication of the reverse primer-binding site, 3' of the SSR region. This would explain the lack of size variation about products 216 bp in length, since the variable repeat region would lie 5' to the amplified region. If this is the case, individuals which carry different alleles (numbers of repeats) would be indistinguishable from one another if they carried the duplicated primer binding site, and this product would therefore represent a number of different alleles. This scenario seems unlikely, since the insertion of the second primer binding site would have to have occurred in a narrow region of the genome. Only 21 bp separates the forward primer binding site from the beginning of the repeat region in *Sorex araneus*.

As in the analysis of subpopulation allele frequencies for locus L97, GENEPOP was used to test the null hypothesis H_0: "the L62 allele distribution is identical among subpopulations". Here the data do not reject the null hypothesis,

and thus do not suggest that there is a significant difference in the allele distributions at locus L62 among subpopulations (P=0.57572, S.E.=0.01156).

3.3.3 Combined Results of Allele Frequency Distribution Comparisons

Combining the results of the tests of the differentiation in allele frequency distributions using Fisher's method (Raymond and Rousset 1995), a χ^2 test suggests that there is strong evidence that the subpopulations differ in their allelic distributions ($\chi^2=\infty$, df=4). This is an important finding, as evidence of population structuring over such a small spatial scale has not been previously reported for *S. cinereus*.

3.4 Tests for Hardy-Weinburg Equilibrium

Table 5 and Table 6 list the observed and expected heterozygosity levels (H_o and H_e) for all subpopulations and for the total population, as well as the number of individuals for which products were scored and the number of different alleles scored at loci L97 and L62 respectively. For locus L97, all subpopulations show lower observed levels of heterozygosity than expected under Hardy-Weinburg Equilibrium. At locus L62 most subpopulations have $H_o<H_e$, with the exception of the Uncut controls in replicates A and C.

A χ^2 test shows that the sex ratio among individuals which are homozygous (or non-amplifying) for either locus is not significantly different from the sex ratio among the entire sample of shrews (for locus L97, P=0.367; for locus L62, P=0.192).

Table 5. Observed heterozygosity (H_o) and expected heterozygosity (H_e) for locus L97, listing the number of individuals giving amplification products (N) and the number of distinct allele sizes scored (A).

Treatment	Replicate	H_o	H_e	L97 N	L97 A
Uncut Control	A	0.381	0.898	21	14
Uncut Control	B	0.500	0.881	18	12
Uncut Control	C	0.517	0.864	29	14
0.1 ha patches	A	0.618	0.887	34	13
0.1 ha patches	B	0.500	0.903	18	17
0.1 ha patches	C	0.722	0.826	18	9
1 ha patches	A	0.708	0.899	24	15
1 ha patches	B	0.481	0.853	27	12
1 ha patches	C	0.308	0.837	13	11
Partial Cut	A	0.703	0.883	37	16
Partial Cut	B	0.474	0.866	19	13
Partial Cut	C	0.609	0.882	23	13
10 ha Clearcut	A	0.565	0.875	23	13
10 ha Clearcut	B	0.440	0.905	25	19
10 ha Clearcut	C	0.455	0.760	11	6
Total Population	**-**	**0.550**	**0.906**	**340**	**28**

Table 6. Observed heterozygosity (H_o) and expected heterozygosity (H_e) for locus L62, listing the number of individuals giving amplification products (N) and the number of distinct allele sizes scored (A).

Treatment	Replicate	H_o	H_e	L62 N	L62 A
Uncut Control	A	0.846	0.825	13	11
Uncut Control	B	0.400	0.480	5	4
Uncut Control	C	0.500	0.485	10	5
0.1 ha patches	A	0.609	0.743	23	13
0.1 ha patches	B	0.200	0.340	5	3
0.1 ha patches	C	0.444	0.463	9	6
1 ha patches	A	0.357	0.429	14	8
1 ha patches	B	0.600	0.648	20	11
1 ha patches	C	0.375	0.578	8	6
Partial Cut	A	0.500	0.694	20	11
Partial Cut	B	0.111	0.377	9	4
Partial Cut	C	0.667	0.823	12	13
10 ha Clearcut	A	0.643	0.747	14	10
10 ha Clearcut	B	0.579	0.663	19	9
10 ha Clearcut	C	0.429	0.612	7	5
Total Population	**-**	**0.527**	**0.664**	**188**	**22**

A test of HWE was performed for each locus in each subpopulation using the program GENEPOP. GENEPOP performed this test using the alternative hypothesis H_1: "There is a deficit of heterozygotes", which if true would indicate an inbreeding-like effect of population structuring. The program performs a U test, again using a Markov chain to estimate the P-value of the null hypothesis. In the uncut, 0.1 ha patch cut and partial cut (single-tree selection) treatments in replicate B, less than 5 distinct alleles were scored at locus L62. Here GENEPOP performs a complete enumeration to generate an exact P-value (Louis and Dempster 1987). The results of these tests are shown in Table 7. Both loci showed a significant deficit of heterozygotes over the entire study site ($P<0.0001$). For locus L97 the null hypothesis was rejected for all 15 subpopulations ($\alpha=0.05$) after a sequential Bonferroni correction (Rice 1989) was used to adjust the critical P-values due to the multiple tests performed. For locus L62 only 4 replicates (all of the single-tree selection treatments, and the 0.1 ha patch cut treatment in replicate A) had a P-value under the null hypothesis of less than 0.05, and only the partial cut treatment in replicate A had a P-value less than the critical P-value after a sequential Bonferroni correction of the critical P-values. The power of this test on locus L62 is somewhat reduced because several subpopulations had only a few individuals which gave amplification products for this locus.

Table 7 also displays the results of a multilocus test of HWE for each subpopulation, performed as described above for the single locus tests. Here all subpopulations showed evidence of a significant deficit of heterozygotes ($\alpha=0.05$), after a sequential Bonferroni adjustment to the critical P-values for multiple tests. A

Table 7. P-values and standard errors associated with a test of H_0:"The genotype frequencies are at HWE expectations" for all loci. An asterisk indicates a complete enumeration of (n) contingency tables.

Treatment	Replicate	Locus L97 P-value	S.E.	Locus L62 P-value	S.E.	Multi-locus P-value	S.E.
Uncut Control	A	0.0000	0.0000	0.6299	0.0100	0.0000	0.0000
Uncut Control	B	0.0000	0.0000	0.3333	0* (4)	0.0000	0.0000
Uncut Control	C	0.0000	0.0000	0.6574	0.0046	0.0000	0.0000
0.1 ha patches	A	0.0000	0.0000	0.0145	0.0020	0.0000	0.0000
0.1 ha patches	B	0.0000	0.0000	0.1111	0* (2)	0.0000	0.0000
0.1 ha patches	C	0.0182	0.0016	0.5344	0.0073	0.0239	0.0064
1 ha patches	A	0.0000	0.0000	0.1501	0.0081	0.0028	0.0028
1 ha patches	B	0.0000	0.0000	0.1496	0.0072	0.0000	0.0000
1 ha patches	C	0.0000	0.0000	0.0691	0.0026	0.0000	0.0000
Partial Cut	A	0.0000	0.0000	0.0254	0.0028	0.0000	0.0000
Partial Cut	B	0.0000	0.0000	0.0039	0* (7)	0.0000	0.0000
Partial Cut	C	0.0000	0.0000	0.0227	0.0035	0.0000	0.0000
10 ha Clearcut	A	0.0000	0.0000	0.2507	0.0083	0.0000	0.0000
10 ha Clearcut	B	0.0000	0.0000	0.0556	0.0032	0.0025	0.0025
10 ha Clearcut	C	0.0027	0.0003	0.2341	0.0032	0.0009	0.0005
Total Population	-	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**

multilocus test of HWE, treating the entire study site as a single population, strongly rejects the null hypothesis (where H_1:"there is a deficit of heterozygotes"; P<0.0001, S.E.=0.0000).

3.5 Genotypic Differentiation between Subpopulations

A test for population differentiation in the form of differing genotypic distributions among subpopulations was performed using GENEPOP, again using a Markov chain (2000 batches, 1000 iterations per batch) as described earlier in the test for genotypic disequilibrium. This test gives an unbiased estimate of the P-value of a log-likelihood (G) based exact test (Raymond and Rousset 1995; Goudet *et al.* 1996). The program calculates G for genotype tables with the same marginal values as observed in the actual data, and returns the probability of observing genotype counts equal or lesser in likelihood than those observed, when the allele frequencies are held constant. Here, the null hypothesis H_0: "the genotypic distribution is identical across subpopulations" was strongly rejected for locus L97 (P=0.0001, S.E.=0.0001), but was not rejected for locus L62 (P=0.6577, S.E.=0.0086). Again, the power of the test for locus L62 is reduced due to the small sample sizes, though this is not necessarily the cause of this discrepancy. Combining the results from both loci using Fisher's method (Raymond and Rousset 1995), the null hypothesis is rejected (χ^2=18.688, df=4, P=0.0009), suggesting that there is differentiation among the subpopulations in their genotypic distributions.

3.6 Population Differentiation: F-statistics

As a measure of population structure, estimates of Wright's F-statistics (Wright 1951) were calculated using GENEPOP, following the method of Weir and Cockerham (Weir and Cockerham 1984). F_{ST} was estimated using a weighted analysis of variance, and multilocus estimates of F-statistics were made by weighting the loci, giving more weight to locus L97 because of its higher expected heterozygosity.

Wright's F-statistics (F_{IS}, F_{IT} and F_{ST}) measure the heterozygosity observed at different scales of population subdivision relative to the expected heterozygosities under HWE. Positive values result when the observed heterozygosity is lower than expected under HWE. The relationship between these statistics is shown in the formula:

$$(1-F_{IT}) = (1-F_{IS})\,(1-F_{ST})$$

F_{IS} is a measure comparing the observed heterozygosity within a subpopulation to the expected heterozygosity given the allele frequencies within the subpopulation, and is can be estimated by the equation:

$$F_{IS} = (H_S-H_I)/H_S$$

Here, H_S is the expected heterozygosity under HWE, and H_I is the observed heterozygosity in the subpopulation. F_{IT} is a measure comparing the observed

heterozygosity in a population to the heterozygosity expected under HWE where there is no differentiation between subpopulations, as shown in the formula:

$$F_{IT} = (H_T - H_I)/H_T$$

In this case, H_T represents the expected heterozygosity under HWE given the observed allele frequencies in the total population. F_{ST} is commonly used to measure population subdivision, and relates the difference between observed and expected heterozygosity levels owing to non-random mating between subpopulations. Using the terms defined above, F_{ST} is given by the formula:

$$F_{ST} = (H_T - H_S)/H_T$$

Table 8 shows F-statistic estimates for each locus as well as a multilocus estimate. The standard errors surrounding these estimates were calculated using the program FSTAT (Goudet 1999), which jackknifes over subpopulations for each locus, omitting successive samples and recalculating the resulting F-statistics in order to calculate the variance surrounding the estimation of each statistic. The multilocus F_{ST} of 0.0061 is not significantly different from zero, suggesting there is little differentiation between the treatment blocks. This results in estimates of F_{IS} and F_{IT} that are nearly identical.

Estimates of F_{IS} for each subpopulation are shown in Table 9 and multilocus estimates are plotted in Figure 9. The error bars in Figure 9 represent an

Table 8. F-statistic estimates for the total population of shrews at Sicamous Creek. Individual loci are weighted according to their expected heterozygosity to derive the multilocus estimate. Standard errors are in parentheses.

Locus	F_{IS}	F_{IT}	F_{ST}
L97	0.3897 (S.E.=0.036)	0.3944 (S.E.=0.035)	0.0076 (S.E.=0.005)
L62	0.2082 (S.E.=0.034)	0.2101 (S.E.=0.032)	0.0024 (S.E.=0.009)
Multilocus	0.3127	0.3169	0.0061

Table 9. F_{IS} estimates for all treatment blocks.

Treatment	Replicate	L97	L62	Multilocus
Uncut Control	A	0.5918	0.0149	0.3155
Uncut Control	B	0.4555	0.2727	0.3910
Uncut Control	C	0.4159	0.0217	0.2742
0.1 ha patches	A	0.3169	0.2031	0.2650
0.1 ha patches	B	0.4687	0.5000	0.4773
0.1 ha patches	C	0.1533	0.0986	0.1336
1 ha patches	A	0.2326	0.2025	0.2229
1 ha patches	B	0.4504	0.0988	0.2986
1 ha patches	C	0.6559	0.4085	0.5548
Partial Cut	A	0.2174	0.3028	0.2550
Partial Cut	B	0.474	0.7333	0.5526
Partial Cut	C	0.3297	0.2314	0.2822
10 ha Clearcut	A	0.3735	0.1761	0.2826
10 ha Clearcut	B	0.5286	0.1538	0.3700
10 ha Clearcut	C	0.4413	0.3684	0.4088
Total Population	-	0.3897	0.2082	0.3127

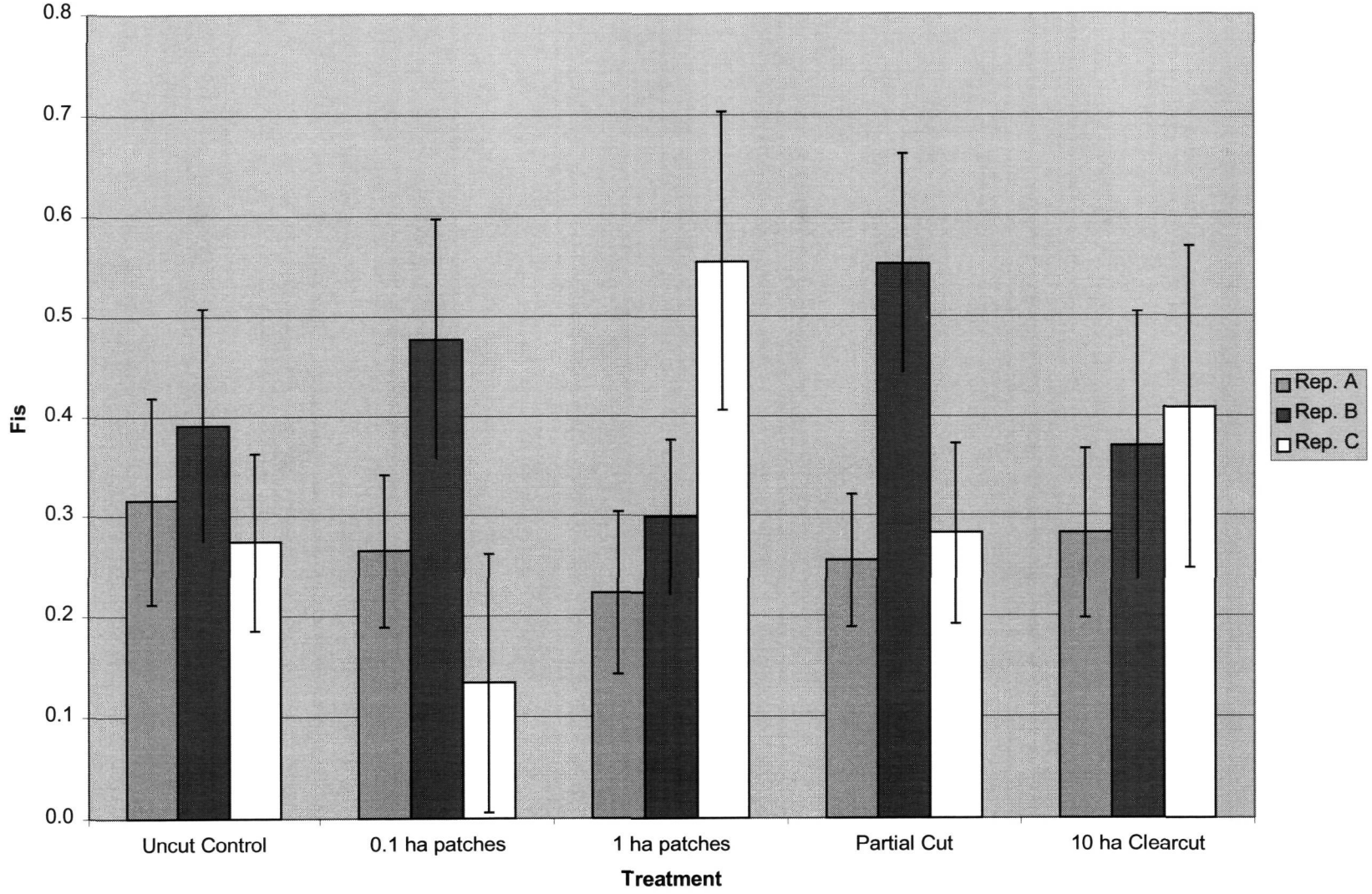

Figure 9. Multilocus F_{IS} estimates for all treatment blocks. Error bars represent the standard error of each estimate.

approximation of the standard error surrounding these F_{IS} estimates. This value was calculated by measuring the variance surrounding the F_{IS} estimates among 100 bootstrap samples for each subpopulation using a program written in FORTRAN by Kermit Ritland, which generated the same estimates of F_{IS} as did both GENEPOP and FSTAT.

Each treatment was replicated 3 times, giving 3 independent estimates of F_{IS} for each treatment. An analysis of variance (ANOVA) was performed testing the null hypothesis H_0: "The mean F_{IS} values for each treatment are equal", testing the F_{IS} estimates for loci L97 and L62 as well as the multilocus estimate. The results of these tests are shown in Table 10, Table 11 and Table 12. In each case the majority of variation in F_{IS} values is present within treatments as opposed to between treatments, and the null hypothesis was not rejected.

3.7 Relationship Between Geographic Distance and Relatedness

A method-of-moments estimator of relatedness presented by Ritland (Ritland 1999) was used to estimate pairwise relatedness between individuals. This estimator has the advantage of reduced bias for pairwise individual estimates where there is an inherently small sample size (Ritland 1996), as compared to alternative maximum likelihood estimators (Currie-Cohen 1981), and is also superior for use with highly polymorphic markers such as microsatellites (Ritland 1996). The estimator defines relationship as the probabilities of identity by descent of alleles (Jacquard 1974), assuming that estimated allele frequencies from Section 3.3 are the true allele frequencies, which is a valid assumption since these estimates are

Table 10. Analysis of variance results comparing testing H_0:"The mean F_{IS} is the same among different treatments", using F_{IS} estimates from locus L97.

Source of Variation	SS	df	MS	F	P-value	F critical
Between Treatments	0.069027793	4	0.017257	0.856037	0.521937	3.47805
Within Treatments	0.201591160	10	0.020159			
Total	0.270618953	14				

Table 11. Analysis of variance results comparing testing H_0:"The mean F_{IS} is the same among different treatments", using F_{IS} estimates from locus L62.

Source of Variation	SS	df	MS	F	P-value	F critical
Between Treatments	0.156238883	4	0.039060	1.10062	0.407995	3.47805
Within Treatments	0.354888413	10	0.035489			
Total	0.511127296	14				

Table 12. Analysis of variance results comparing testing H_0:"The mean F_{IS} is the same among different treatments", using multilocus F_{IS} estimates.

Source of Variation	SS	df	MS	F	P-value	F critical
Between Treatments	0.010672545	4	0.002668	0.140296	0.963300	3.47805
Within Treatments	0.190179111	10	0.019018			
Total	0.200851656	14				

made from a fairly large sample population. The relatedness (r) is then the probability that two alleles, one randomly sampled from each individual, are identical by descent. In an outbred population r=1/4 for parent-offspring and full-sibling pairs, r=1/8 for half-siblings, etc.

Here the relatedness between two individuals is estimated independently for all possible alleles at each locus. The single-locus estimate of r is given by

$$r = ((\delta_{ik}+\delta_{il})/P_i + (\delta_{jk}+\delta_{jl})/P_j - 1) / 4(n-1)$$

where individual #1 has alleles i and j, individual #2 has alleles k and l, P_i and P_j are frequencies of alleles i and j respectively, δ is 1 if alleles are identical and 0 if alleles are different, and n is the number of different alleles at that locus (Ritland 1999). Multilocus estimates of r are simply the average of the single locus estimates, weighted by the number of alleles at each locus. A property of this estimate of relatedness is that it can give estimates less than 0, or greater than 1 in cases where individuals share rare alleles.

Pairwise comparisons were analyzed consisting of pairs of individuals that were trapped in the same treatment block. This limits the analysis to comparisons between individuals separated by no more than 529 metres. The distance between the centers of trap circles from which each individual was collected was used as an estimate of the distance separating the individuals. Individuals trapped in the same trap circle were considered to have been trapped in the same location even in cases where they were collected from separate pitfall traps.

Figure 10 plots 8878 pairwise comparisons of individuals within subpopulations. Figure 11 displays the same data but only up to a maximum estimated relatedness of 4.0, simply in order to allow better resolution of the points.

A linear regression of relatedness on the logarithm of distance was performed to approximate the relationship between the two variables. Figure 12 shows the results of this regression, showing that the relationship between the two variables is weak (r^2=0.0361). Confidence intervals were determined by bootstrapping individuals to form replicate data sets. (Identical comparisons, in which the same individual was compared to itself, were omitted from analysis). The 95% confidence interval of the slope of the regression line is –0.1034 +/- 0.0111, and the Y-intercept is 0.2354 +/- 0.0231.

In fact, this relationship is not truly linear, as suggested by the low value of r^2. The mean estimated relatedness between pairs sampled in different trap circles is approximately zero regardless of the distance between the traps. This is true even for pairs of individuals separated by as little as 14-50 m. In fact, if pairs of individuals sampled in the same trap circle are excluded from the analysis, a linear regression suggests that the slope of the regression line and its Y-intercept are not significantly different from zero (P=0.595 and P=0.645, respectively).

A linear regression of relatedness on the logarithm of distance for each individual subpopulation was also done, to ascertain whether a difference in the relationship between these two measurements existed depending on the type of forest harvest. Such a difference might be expected if the type of forest harvest affects the level of dispersal. A reduction in the degree of dispersal in a treatment

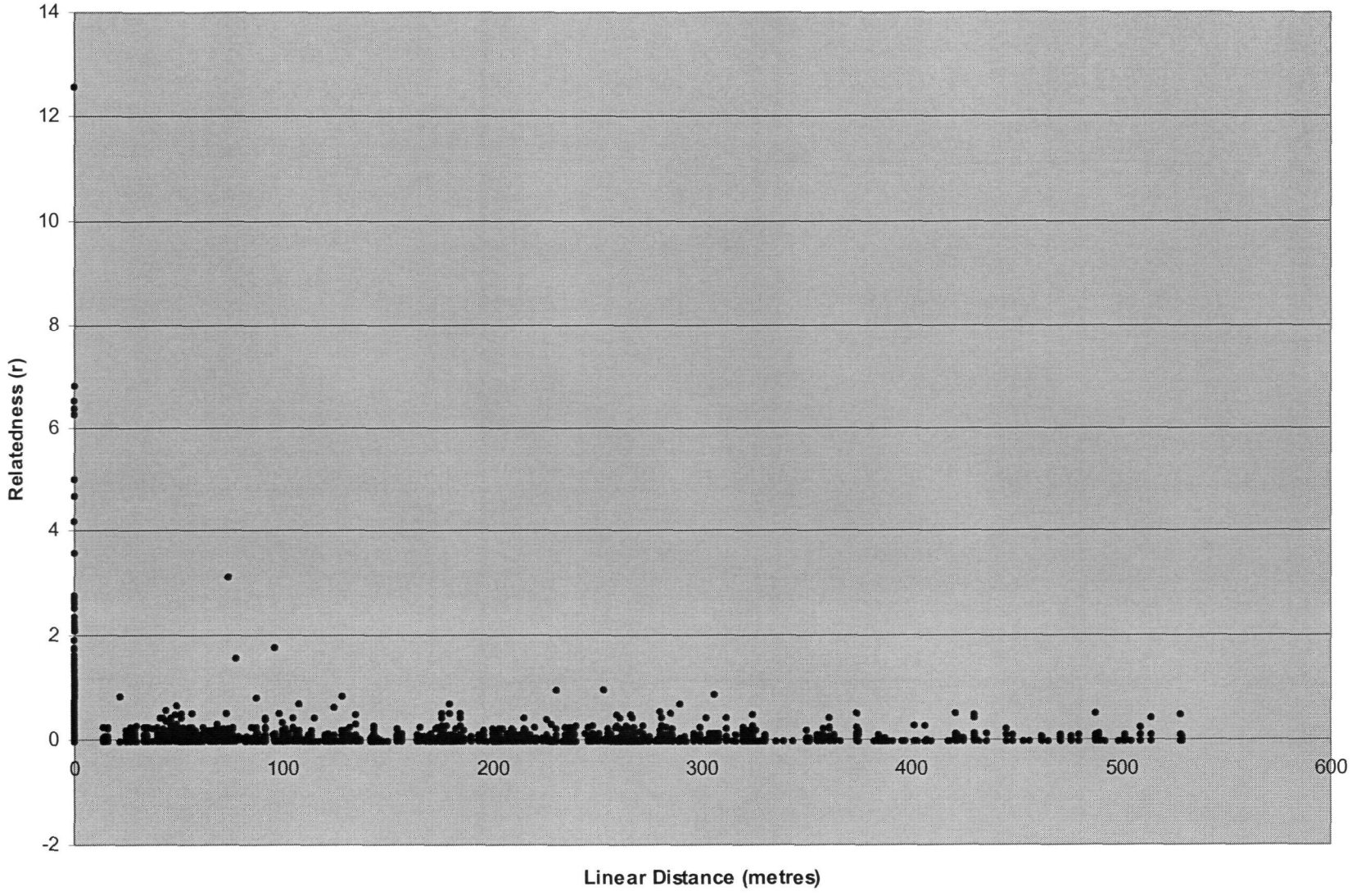

Figure 10. Pairwise relatedness vs. linear distance for all pairs of individuals within the same subpopulation.

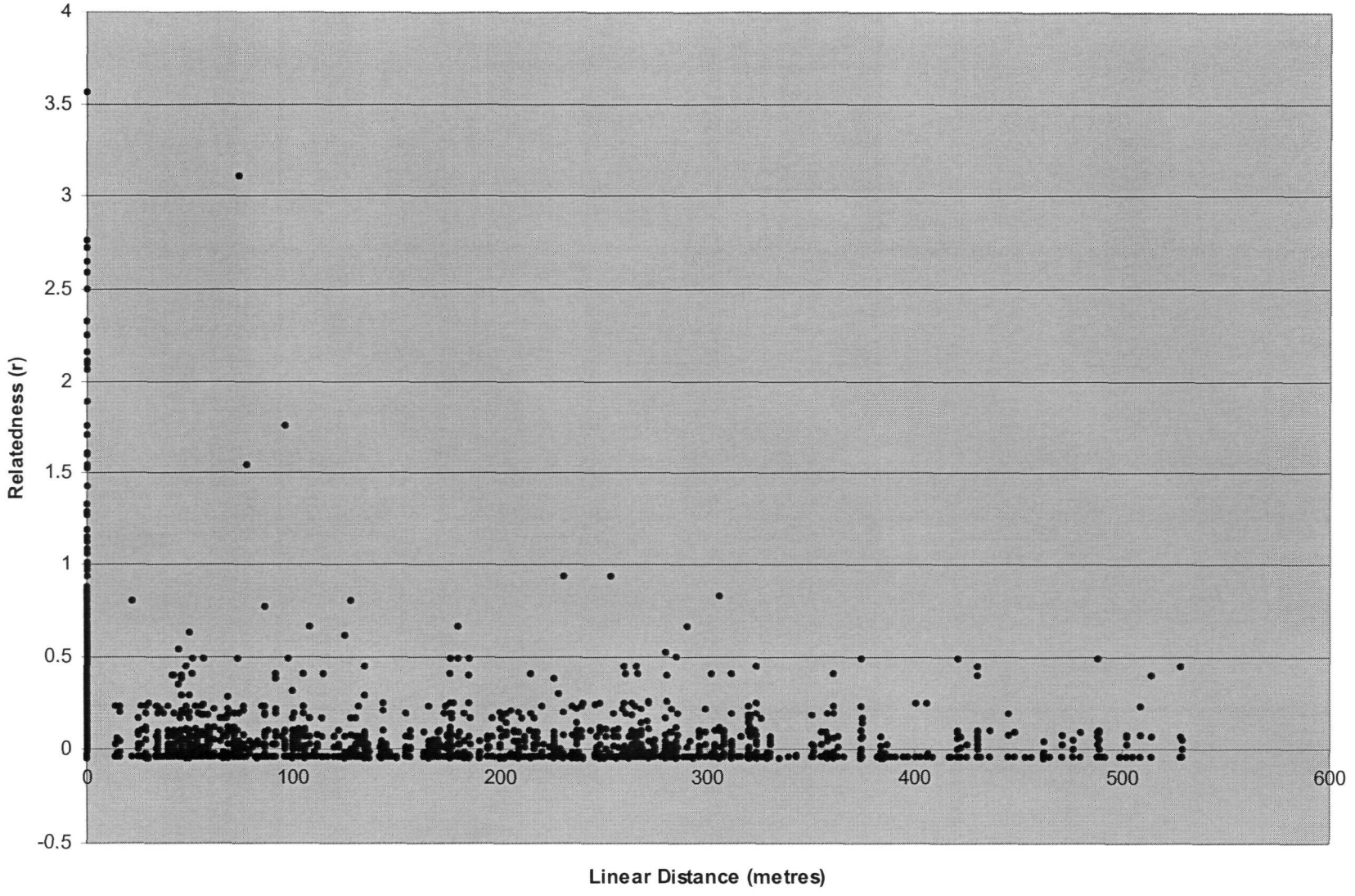

Figure 11. Pairwise relatedness vs. linear distance for all possible pairs of individuals within the same subpopulation. Pairs with an estimated relatedness greater than 4.0 are not shown.

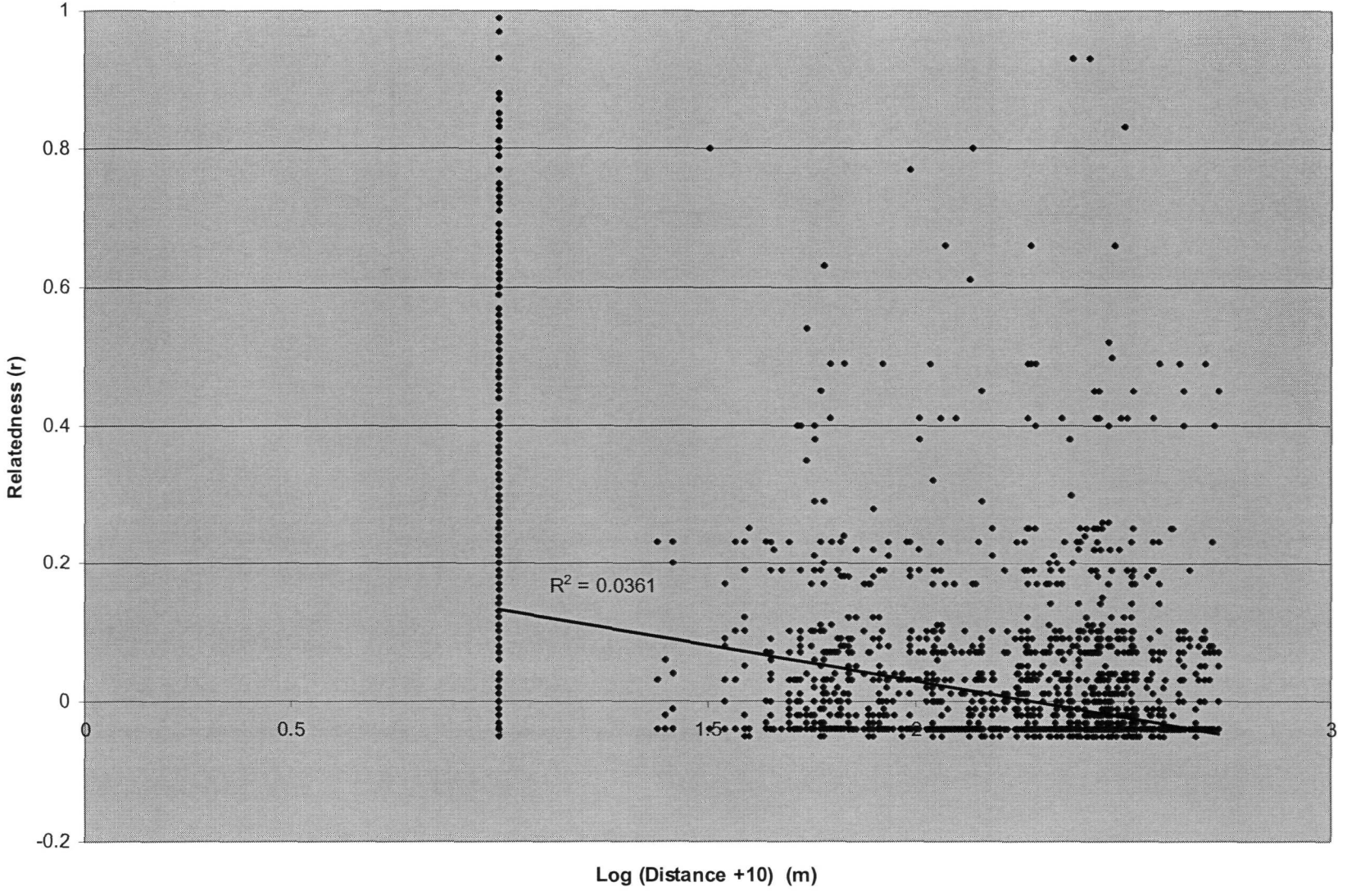

Figure 12. Linear regression of relatedness (r) on the logarithm of linear distance (+10 m) separating all pairs of individuals in the same subpopulation. Only pairs with a relatedness of 1.0 or less are shown.

could lead to a stronger coefficient of relationship between pairwise distance and relatedness. A difference in the slope of the regression lines for two treatments might result if the degree of dispersal varies between treatments, with treatments in which dispersal occurs over greater distances yielding regression lines with a more gradual slope. Likewise the Y-intercepts may vary between treatments if marked differences exist in family structure. ANOVA analyses on the coefficients of relationship, estimated slopes and Y-intercepts for the regression lines for each subpopulation suggest that there is no significant difference (α=0.05) between the different types of treatment, as shown in Tables 13-15.

The mean estimated relatedness of all pairs of individuals trapped in the same trap circle is 0.177, which is comparable to the expected relatedness between a pair of half-siblings, which have a relatedness of 0.125, or to the average relatedness of a group comprised of two mutually unrelated full-sibling families. The mean pairwise relatedness within each subpopulation of pairs trapped within the same trap circle is shown in Figure 13. An ANOVA failed to reject the null hypothesis H_0:"The mean pairwise relatedness within treatments are equal for all types of treatments" (P=0.997). The mean relatedness of all 8878 pairs of individuals was 0.027 (roughly the expected relatedness between 2nd cousins), while the average among pairs of individuals not sampled in the same trap circle is -.001, or roughly zero.

Table 13. Analysis of variance testing H_0:"The mean of the coefficients of determination (r^2) for the linear regressions of pairwise relatedness on log(distance + 10 m) in each treatment are equal", showing no significant difference among treatments.

Source of Variation	SS	df	MS	F	P-value	F critical
Between Treatments	0.000068	4	0.000017	1.414365	0.298249	3.47805
Within Treatments	0.000121	10	0.000012			
Total	0.000189	14				

Table 14. Analysis of variance testing H_0:"The mean of the slopes of the linear regression lines from the regression of pairwise relatedness on log(distance+10m) in each treatment are equal", showing no significant difference among treatments.

Source of Variation	SS	df	MS	F	P-value	F critical
Between Treatments	0.000197	4	0.000049	0.235651	0.911898	3.47805
Within Treatments	0.002091	10	0.000209			
Total	0.002288	14				

Table 15. Analysis of variance testing H_0:"The mean of the Y-intercepts from the regressions of pairwise relatedness on log(distance+10m) in each treatment are equal", showing no significant difference among treatments.

Source of Variation	SS	df	MS	F	P-value	F critical
Between Treatments	0.003188	4	0.000797	0.121509	0.971555	3.47805
Within Treatments	0.065600	10	0.006560			
Total	0.068788	14				

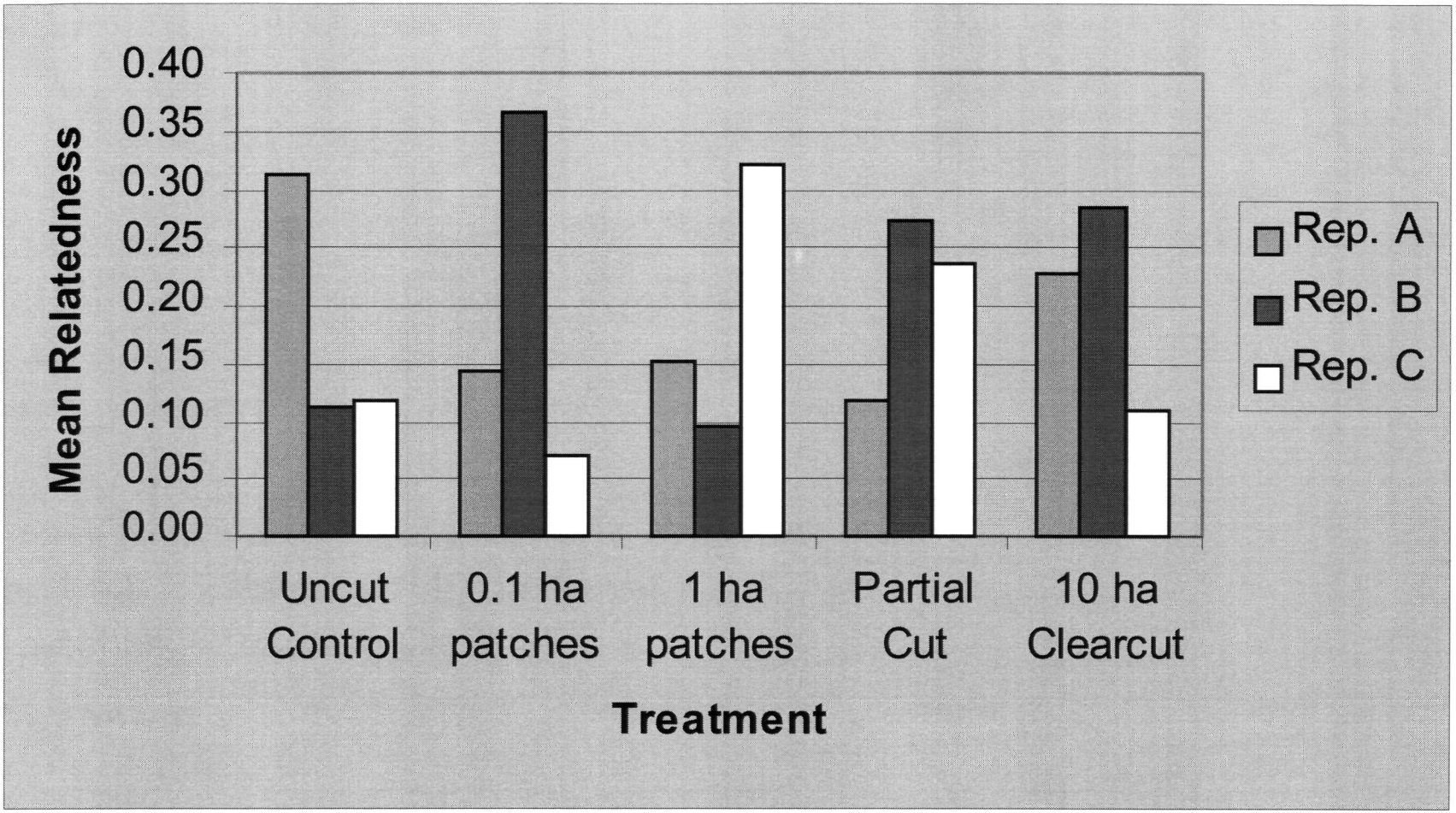

Figure 13. Mean pairwise relatedness of individuals trapped in the same trap circle, for each treatment block.

3.8 Estimation of Null Allele Frequency

Locus L97 shows a very large deficit of heterozygotes relative to the number expected of a population at HWE. The presence of null alleles at this locus may partly explain this discrepancy. Locus L62 also shows a heterozygote deficit in some treatment blocks but it is much less pronounced.

Since individuals that are homozygotes for a certain allele are indistinguishable from individuals heterozygous for the allele and a null allele, estimation of the frequency of null alleles in a sample requires first an approximation of the true inbreeding coefficient in the population. Typically methods to estimate the frequency of null alleles operate under the assumption that the population is in HWE (where F_{IS}=0), although it is also possible to jointly estimate the null allele frequency and F_{IS} where the expected value of F_{IS} is non-zero (K. Ritland, personal communication, Feb. 1999). Since other studies of population genetic structure in shrews have generally reported F_{IS} values near zero (Stewart and Baker 1992; Lugon-Moulin *et al.* 1999), for the purpose of estimating the frequency of null alleles at locus L97 the population was assumed to be at HWE.

Two alternative methods of estimating the null allele frequency were used, both of which gave very similar results. First, the program GENEPOP was used to estimate null allele frequency, assuming the population was at HWE. Here the program generates a maximum likelihood estimate using the EM algorithm (Dempster *et al.* 1977). This method considers the proportion of non-amplifying samples in estimating the frequency of null alleles. A second method which does not consider the number of non-amplifying samples is incorporated in the program

NULLTEST (Amos 1996), which simply estimates the number of individuals that were scored as homozygotes for each allele which are in fact heterozygotes for that allele and a null allele assuming HWE. This program then estimates the number of samples that would have dropped out because they carried two null alleles, given its final estimate of the frequency of null alleles. Here the program slightly underestimated the number of dropouts that were actually observed for locus L97, which may mean the program has underestimated the frequency of null alleles or simply that some samples failed to amplify for another reason.

Figure 14 shows the estimated effect the presence of null alleles had on the estimates of allele frequency at locus L97 according to the results of the analysis done by NULLTEST, which estimated the frequency of null alleles to be 0.246. If null alleles are present at this frequency in the data set, then the expected apparent heterozygosity at locus L97 under Hardy-Weinburg Equilibrium would be 0.577, which is close to the actual observed heterozygosity of 0.550.

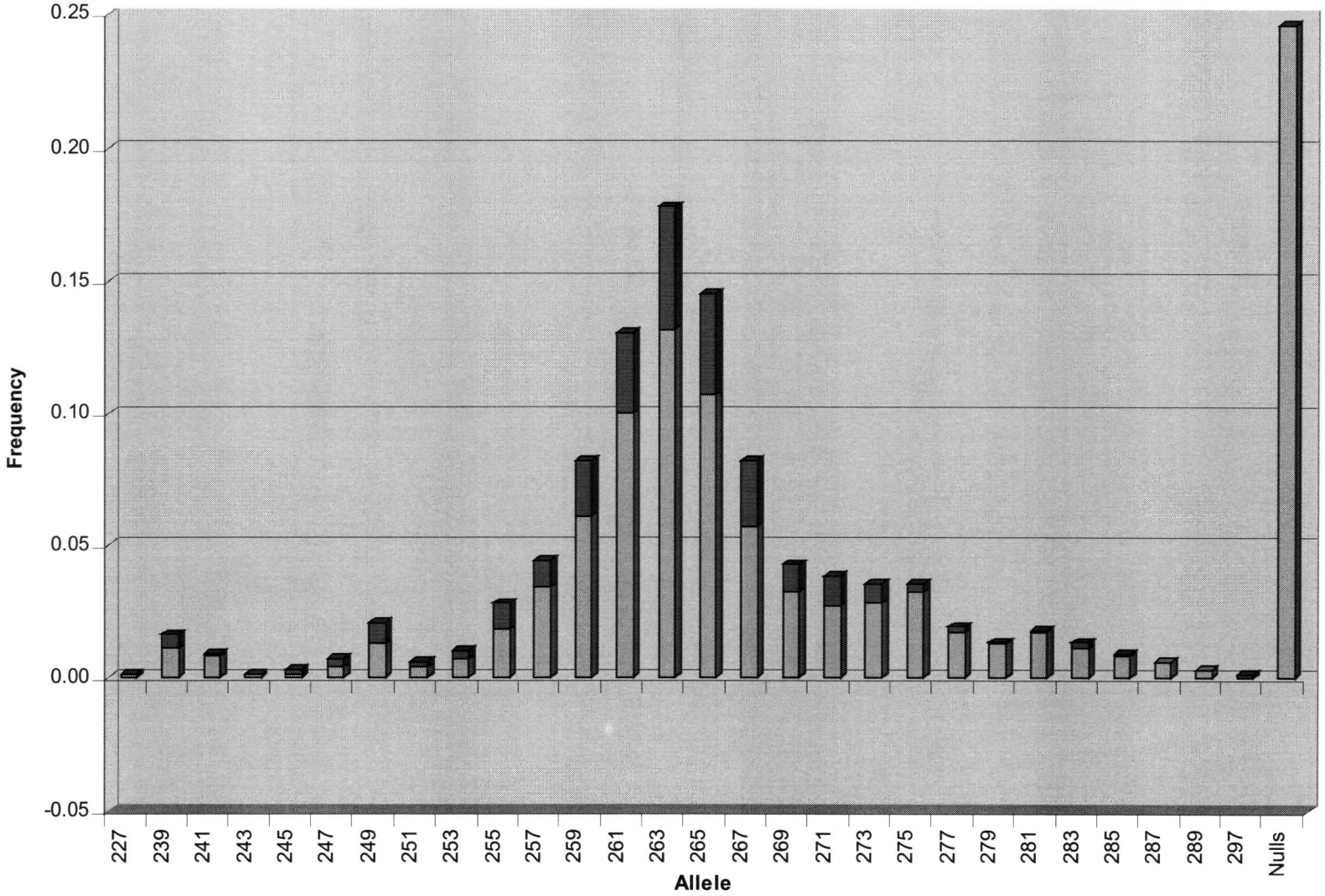

Figure 14. Estimated allele frequencies at locus L97 with (light) and without (dark) the presence of null alleles.

Section 4 - Discussion

4.1 Amplification Results

There are a number of explanations for dropouts during DNA amplification. Some DNA may have been of poor quality due to degradation in the field prior to collection, since up to 2 weeks would have passed between the time of mortality and collection for some samples. Errors during DNA isolation may lead to a low yield of DNA, or contamination with proteins or other cellular products that may inhibit PCR or degrade DNA. Since proteinase K was used during DNA extraction, failure to fully clean up the DNA preparations with the phenol washes could result in leftover proteinase K digesting *Taq* polymerase and inhibiting amplification. However, since amplification success at one locus is independent of amplification success at the other locus, these problems are not likely to be a major cause of non-amplifying samples.

Another possible cause of dropouts is simply the use of suboptimal PCR conditions, despite efforts to optimize them. As previously mentioned, poor results in cross-species amplification in *S. cinereus* using primers designed to amplify loci in *S. araneus* have been previously observed (Wyttenbach *et al.* 1997; Balloux *et al.* 1998). It is also possible that part of the reason for individuals failing to amplify at these loci is that they carry null alleles, with primer site mutations that prevent proper annealing of the PCR primers during the thermal cycling. It has been suggested that primer site mutations in mammals with short generation times like those of shrews have a higher transmission probability in comparison to species with longer generation times such as bovines, primates or cetaceans (Wyttenbach *et al.* 1997).

Of the two loci, null alleles seem more likely present for locus L97, where the difference between observed and expected heterozygosity is most pronounced.

4.2 Allele Frequency Distributions

Significant variation between the allele frequency distributions in treatment blocks was found for locus L97 but not for locus L62. If genetic drift is the cause of the difference between allele frequency distributions, loci are expected show different patterns since drift affects allele frequencies at random. It is also possible that a Type II error was made in the test for locus L62, particularly since the power of this test is reduced due to the small number of alleles scored in several of the subpopulations for that locus. The expected heterozygosity is lower for locus L62 than locus L97 because it has less allele diversity, with fewer alleles as well as one allele that is very common. Alternatively (or in addition), the discrepancy between the results for the two loci could be the result of null alleles at locus L97, which would introduce error in the estimates of the true allele frequencies in each subpopulation (see Section 3.8) and potentially exaggerate differences among estimates of allele frequencies.

The allele frequency distributions for locus L97 vary among treatment blocks for both common and rare classes of alleles, but all of the distributions are roughly normal and bell-shaped (see Figure 7). The significance of the difference in the allele frequency distributions for locus L97 rests on the assumption that alleles were sampled at random and independent of on another in each of the treatments. This is not strictly true, since we have observed that related individuals tend to be clustered

to some extent in the trap circles. This means that once we have sampled a given allele in a trap circle, the chance of sampling that same allele again in the same trap circle is somewhat greater than expected by chance and the alleles frequency in the total population. This is unavoidable due to the sampling design and the use of pitfall traps, and may explain why the allele frequency distributions for locus L97 vary significantly among treatments, despite that fact that the overall F_{ST} at the site is low.

4.3 Tests of Hardy-Weinburg Equilibrium

Hardy-Weinburg Equilibrium describes the genotypic frequencies expected in an ideal population of infinite size (not subject to genetic drift) in which there is random mating (random union of gametes), no selection, no migration, no mutation, an equal sex ratio, and non-overlapping discrete generations. Clearly not all of these criteria are met in this study. Although finite, the shrew population is certainly very large and is probably not greatly affected by genetic drift unless discrete patches of individuals exist with little migration connecting them to other areas of the site, which seems unlikely. Microsatellite loci have high mutation rates, but this does not pose a problem in the interpretation of population subdivision unless the mutation rate is large relative to the migration rates between populations (Whitlock and McCauley 1999). This is unlikely given the area of the study site and the range of shrew movement and dispersal, particularly during the breeding season.

The shrew population at Sicamous Creek may nearly meet the HWE criterion of non-overlapping discrete generations, although there are reports of shrews in other areas breeding in the first calendar year of their life (Teferi *et al.* 1992).

As discussed earlier, microsatellite loci are generally assumed to be selectively neutral. Tests of HWE genotypic frequencies are fairly robust to minor violations of the assumptions of lack of selection, equal sex ratio and discrete generations. Departures from HWE genotypic frequencies in this study are likely due primarily to the presence of null alleles, which are suggested by both the large heterozygote deficiency and the presence of non-amplifying individuals in the data set (see Section 4.6). Other violations of HWE assumptions, namely those requiring no migration and random mating, are also potentially partly responsible for deviations from HWE. Migration between treatments as well as from without the study is probably quite common, but migration is expected to decrease levels of homozygosity and here we observe greater than expected homozygosity. Non-random mating between relatives increases homozygosity, since related individuals often carry alleles that are identical by descent, which are then passed on to their offspring.

Figure 10 suggests that related individuals are non-randomly distributed throughout the study site, but since most of the individuals that are trapped are likely to be dispersing individuals, it is not clear whether sexually mature relatives that have completed dispersal also tend to be found near one another. Individuals in this study were trapped during the summer, towards the end of the breeding season, and many samples were probably caught towards the end of the season in which dispersal is occurring. There is very little overlap of home ranges over autumn and winter (Michielsen 1966; Churchfield 1990h), so many of the shrews in this study may have been trapped towards the end of their opportunity for dispersal. If this is

the case, then it may in part account for the reduced heterozygosity since related individuals may well establish home ranges and territories quite close to one another and come to breed in the spring.

Another possible explanation for observing heterozygosity much lower than expected might be that the locus in question is on one of the sex chromosomes. Although neither locus has been found to segregate as if it were on a sex chromosome in any other shrew species, it is conceivable that a translocation could have transferred a locus onto a sex chromosome in *S. cinereus*. If a locus were on the X chromosome then all males should be scored as homozygotes, and if on a Y chromosome then females should fail to amplify products at that locus. This is not the case, and in fact there is no significant difference between heterozygosity or amplification success between the two sexes, so this possibility can be rejected.

It is interesting that 3 of the 4 subpopulations that show significant heterozygote deficits for locus L62 are from the partial cut treatments. The probability of selecting 4 of the 15 subpopulations at random and happening to select 3 from any one type of treatment is 0.044. In addition, these 4 subpopulations are among the 2 types of treatments which represent perhaps the greatest degree of disturbance due to logging. Although all of the treatments (other than the uncut controls) have roughly the same percentage of trees removed, these 2 types of treatments have had trees removed over a greater percentage of the total area of the treatment block, leaving less undisturbed habitat. Logging was not found to significantly change the volume, diameter or profile class composition of coarse woody debris in the treatments (Huggard and Klenner 1997), but the results of this

test at least suggest that types of logging activity may have variable effects on the population structure of masked shrews. Curiously, the partial cut treatments and the 0.1 ha patch cut treatments saw a slight increase in the abundance of *S. cinereus*, while abundance in treatments with larger patch sizes decreased slightly. This is consistent with the hypothesis that at higher population densities, shrew home ranges become smaller and shrew dispersal becomes more difficult, since a dispersing individual must cross an increasing number of defended territories. If this serves to reduce the distance traveled by dispersing individuals it could increase the likelihood of relatives breeding with one another, and in part account for the increased homozygosity in these treatments. Likewise, 3 of the 4 subpopulations with the smallest deviations from HWE at locus L62 are the 3 uncut control treatments.

If forest harvest temporarily isolated groups of individuals from one another by reducing the level of dispersal within or between subpopulations, non-random mating would lead to decreased heterozygosity in the next generation. One generation of random mating is sufficient to restore HWE genotype frequencies, however, if the rest of the assumptions underlying the model hold true. Here the results suggest the possibility of non-random mating during the previous seasons breeding period. It is important to note, however, that this is the first test of HWE for the shrew population at Sicamous Creek. Any departure from HWE expectations cannot simply be attributed to logging, of course, since we do not know whether the population was at HWE prior to logging.

4.4 F-statistic Estimation

The F_{IS} values reported here are quite high, reflecting the observed heterozygote deficit (see Table 5 and Table 6). A previous study of genetic variation in *S. cinereus* populations using allozyme markers reported F_{IS} values near zero (0.004) (Stewart and Baker 1992). Although this value is near zero, allozymes are often not polymorphic enough to detect genetic variation at small spatial scales. Although no studies of *S. cinereus* population structure using highly polymorphic markers have been conducted, results from a study of *S. araneus* also suggest F_{IS} values near zero (0.012) (Lugon-Moulin *et al.* 1999). The results in this study are likely largely due to the presence of null alleles at these loci, in particular at locus L97, which has larger F_{IS} values and a more significant deficit of heterozygotes. However, if juveniles disperse a minimum distance from the natal nest to occupy individual territories some level of inbreeding might be expected during the breeding season. Estimates of pairwise relatedness show that related individuals (likely siblings) are often trapped in the same location.

Despite the large estimate of F_{IS} the estimated F_{ST} is near zero. An inflated estimate of F_{IS} due to null alleles is one explanation for this observation. It is also possible that despite the propensity for inbreeding, migration between subpopulations maintains a low F_{ST}. The well-known one migrant per generation rule of thumb of conservation genetics (Spieth 1974; Mills and Allendorf 1996) suggests that even low levels of migration between populations can prevent genetic drift from differentiating populations, and here we can certainly expect appreciable numbers of migrants between subpopulations. Thus some level of inbreeding could

occur within a treatment over many generations without differentiation among treatments arising due to genetic drift, due to both the large population size of shrews and continued migration between treatments.

An interesting alternative explanation is that the population has not yet reached an equilibrium value of F_{ST}, since inbreeding has only occurred recently. F_{IS} can reach a new equilibrium after just one generation but it takes time for population structure to accumulate due to genetic drift between inbreeding patches of individuals. For instance, Whitlock has shown that the time (in generations) it takes a metapopulation to reach a half-way point between an old F_{ST} and a new equilibrium is $\ln(1/2)/\ln[(1-m)^2(1-1/2N)]$ (Whitlock 1992). This time increases as the rate of migration (m) decreases, but even with relatively high migration rates between subpopulations it will take a number of generations to reach the new equilibrium value of F_{ST}. For instance, if we arbitrarily assume the migration rate (m) to be 0.1, and assume that the population size is large, we arrive at a time of roughly 3 generations to reach an F_{ST} halfway to the new equilibrium. Since only 3 generations of breeding occurred between forest harvest and sample collection, it is possible that with the large population size of shrews, we may not expect the population to have reached a new equilibrium F_{ST} since the harvesting.

Possible inflations in the estimates of F_{IS} due to the presence of null alleles do not preclude the comparison of F_{IS} values between treatments, with the goal of discerning whether the type of forest harvest has an effect on the level of inbreeding within a subpopulation. Here we are more interested in the estimates relative to one another than we are in the magnitude of the F_{IS} estimates.

Due to the high level of variance in estimates of F_{IS} for any one treatment and the limited number of replicates, the ANOVA tests have low power and may not reject the null hypothesis even if there is a real difference in F_{IS} values between treatments. Of the three ANOVA tests the one performed on F_{IS} values estimated from locus L97 has the highest power, which is only about 0.136. These power estimates violate the assumption that the sample sizes used to generate the F_{IS} values for each subpopulation are the same, but nonetheless suggest that ANOVA analyses are unlikely to detect a real difference in inbreeding between the treatments if one does exist.

4.5 Relationship between Geographic Distance and Relatedness

Although little or no population subdivision is evident over the entire study site (F_{ST}=0.006), shrew life history suggests that the pitfall traps used for sampling might capture related individuals near to one another, since many individuals that are trapped are juveniles dispersing from the natal nest. Individuals trapped a great distance apart are less likely to be close relatives, due to spatial structure in the population leading to a sort of isolation by distance (Epperson 1995a; Epperson 1995b). If such a relationship between relatedness and the distance separating individuals exists it should be detectable given the high polymorphism of the two loci used in this study. Since most of the shrews trapped in this study were juveniles, it is uncertain whether the observed pattern of relatedness would hold strictly for sexually mature adults, where related individuals might be sampled greater distances from one another, having completed dispersal and established their home ranges.

An important consideration in this analysis is that the points plotted on Figures 10-12 are not independent from one another, since each individual has its distance and relatedness calculated for comparisons with all other individuals trapped in the same subpopulation. A clear trend is that large estimates of relatedness (r>1) are made only for individuals sampled near to one another, most often from traps in the same trap circle. In fact, no estimates of r>1 were made for individuals trapped at least 100 m apart. This pattern is not simply a result of a greater number of comparisons being made between individuals trapped in the same trap circle, since only 1402/8878 pairs of individuals within the same treatment were trapped in the same trap circle. As previously mentioned, these large values of r result from comparisons of individuals that share rare alleles. The obvious explanation of these results is that sibling pairs or parent-offspring pairs sharing rare alleles by descent were often trapped within the same trap circle. This family structure probably explains the significant difference between observed subpopulation allelic distributions, despite the low overall F_{ST}.

As for estimating F-statistics, estimation of relatedness would also be affected by the presence of null alleles. If a pair of individuals shares one allele at a locus and each individual carries a null allele their relatedness may be overestimated, whereas pairs of individuals which each carry the same null allele but different scored alleles will have their relatedness underestimated. The overall bias may be negligible, but this has not been documented in the literature. If sampling is done at random, the effect of null alleles on estimates of relatedness should not vary between treatments, nor between pairs of individuals separated by different

distances. That is, if two individuals share a single rare allele at a locus and each carries a null allele, their relatedness will be overestimated and be largely positive regardless of the distance between the individuals. Figure 10 shows that large estimates of *r* were only made for pairs of individuals separated by short distances. Even if null alleles were responsible for some of the large estimates of *r*, say where $r>1$, these overestimates only occur for nearby pairs of individuals. This further emphasizes that alleles are not distributed randomly throughout the population, likely due to the clustering of related individuals (parents, offspring and siblings).

Presumably siblings can establish home ranges separated by large distances. In fact, many pairs of individuals scored values of $r>0.25$ (the expected relatedness between full-siblings), and two such pairs when separated by distances greater than 500 m. Estimates of $r>1$ occur only when individuals share rare alleles. This illustrates the power of highly polymorphic markers such as microsatellites in revealing even very fine-scale population structure, here family structure between related siblings. Rare alleles, which were sampled only a few times in the entire sample of shrews, were only found in individuals that were trapped near one another. This level of resolution would not be possible with less polymorphic markers or with a smaller sample size, which would reduce the likelihood of sampling very rare alleles, unless perhaps sampling were confined to an even finer spatial scale.

4.6 Null Alleles

The large deficit of heterozygotes at locus L97 suggests the presence of null

alleles in some individuals. The difference between the observed allele frequency and the estimated allele frequency in the presence of null alleles is largest for alleles which were often scored in homozygotes. Figure 14 shows that this difference is greatest for the more common alleles, but is also significant for a number of the rarer alleles for which homozygotes were scored. Homozygotes for rare alleles are very unlikely to occur by chance in a randomly-mating population, but cannot simply be dismissed outright as heterozygotes carrying a null allele, particularly due to the observed degree of relatedness between individuals sampled within the same trap circle. This suggests the possibility of non-random mating. Inbreeding of relatives could also result in individuals homozygous for rare alleles, and this may occur if siblings come to establish home ranges near to one another after limited dispersal from the natal nest.

Although null alleles add a source of error to the estimation of many statistics, they do not preclude comparisons of estimates among treatments. Future efforts to measure population structure in *S. cinereus* populations would benefit from the use of PCR primers designed specifically for the flanking regions of the microsatellite loci in the *S. cinereus* genome, but even this does not rule out the presence of null alleles, since a significant portion of microsatellite loci carry polymorphisms in their flanking regions even in the species in which they are first characterized (Callen *et al.* 1993). Only sequencing each individual can definitively verify or rule out the presence of null alleles at a locus. As in this study, it may be best to compare the results of HWE tests of independent loci to detect differences which may be attributable to null alleles at a locus.

4.7 Limitations of Experimental Design

A number of limitations were imposed on this study by the experimental design. The small sample sizes within several treatment blocks, resulting from the number of shrews trapped and the failure of some samples to give PCR amplification products, introduce high levels of variance surrounding the estimated F-statistics. This variance, coupled with the limited number of treatment replications, reduces the power of tests to discern differences among the harvest treatments.

Increasing the number of marker loci used in the study would have helped to decrease the standard errors surrounding the estimated F-statistics. It would also be helpful to use markers characterized for *S. cinereus*, to minimize the impact of null alleles on these analyses and to increase the number of amplifying individuals, thus increasing the sample size.

Even with just two loci, the estimation of relatedness can be quite powerful if the loci are highly polymorphic, as in this study, since the variance surrounding this estimation varies inversely with the number of alleles at a locus (Ritland 1996). This makes just one highly polymorphic microsatellite locus more informative in the estimation of relatedness than several diallelic loci. Thus, apart from the error introduced by the presence of null alleles, the loci used in this study allow the resolution of fine-scale structure in the population.

This study would have benefited from an increased degree of replication of the harvest treatments and increased sample sizes within the treatments, both of which are practically difficult or infeasible. Increasing the number of marker loci would

reduce the need for more intense sampling of individuals within the treatment blocks, and improve the power to discern differences between treatment types by reducing the variance surrounding the estimation of F-statistics.

Section 5 - Conclusion

A deficit of heterozygotes at two highly polymorphic microsatellite loci exists for the *S. cinereus* population at Sicamous Creek. The multilocus estimate of F_{IS} (0.31) departs from values of nearly zero measured in populations of other shrew species. This deficit may in part be due to the presence of null alleles for at least one of these loci, but may also be due in part to non-random mating, which is suggested by the observation that related individuals tend to be trapped near to one another.

Allelic and genotypic distributions show significant variation between the treatment blocks, but the multilocus estimate of F_{ST} over the entire study site is just 0.006. The differentiation between treatment blocks in allelic and genotypic distributions may be partly due to the increased likelihood of trapping related individuals in the same trap circle. This results in a non-random sample of individuals from each treatment block, which may inflate the estimated frequency of certain alleles or genotypes in one treatment while underestimating their frequency in another.

Even with just 2 microsatellite loci we have the resolution to detect spatial structure based on the proximity of sampled rare alleles. Individuals sharing rare alleles were sampled only from the same trap circle, or from trap circles separated by short distances. These individuals are likely siblings that share rare alleles by common descent. Since most of the trapped shrews are juveniles, it is uncertain whether this spatial association persists after dispersal and the establishment of home ranges. Sampling during the winter or early spring might help to clarify this

point. If shrews do not disperse great distances then inbreeding between related individuals may account for the depressed level of heterozygosity.

There is no obvious difference between the types of forest harvest in their impact on local shrew population structure. Logging was done in the winter to reduce the disturbance of coarse woody debris on the forest floor, which is probably an important habitat feature for shrews, so it is possible that logging has had little effect on the population structure of masked shrews. There is some suggestion that increased population density, which occurs in the partial cut (individual-tree selection) treatments and in the 0.1 ha patch cut treatments, may lead to greater likelihood of inbreeding but this will have to be confirmed in future studies.

Since forest harvest occurred only 2.5 years prior to sampling it is unlikely that F_{ST} has reached a new equilibrium, therefore it is possible that the shrew population has become more structured due to fragmentation and that F_{ST} will increase over future generations. The results of this study describe the short term effects of logging on the population of *S. cinereus* at Sicamous Creek, and temporal studies tracking F_{ST} estimates over a number of years will be required to more accurately measure the impact of logging on the population structure of masked shrews.

Section 6 – References

Adler, G. H., and R. Levins, 1994 The island syndrome in rodent populations. Quarterly Review of Biology **69:** 473-490.

Amos, W., 1996 NULLTEST - a program for estimating the frequency of null alleles. Department of Genetics, Downing St., Cambridge, UK .

Amos, W., S. J. Sawcer, R. W. Feakes and D. C. Rubinsztein, 1996 Microsatellites show mutational bias and heterozygote instability. Nature Genetics **13:** 391-392.

Ashley, C. T., and S. T. Warren, 1995 Trinucleotide repeat expansion and human disease. Annual Review of Genetics **29:** 703-728.

Avise, J. C., 1994 *Molecular markers, natural history and evolution.* Chapman and Hall, New York, NY.

Balloux, F., E. Ecoffey, L. Fumagalli, J. Goudet, A. Wyttenbach *et al.*, 1998 Microsatellite conservation, polymorphism, and GC content in shrews of the genus Sorex. Molecular Biology and Evolution **15:** 473-475.

Bayne, E. M., and K. A. Hobson, 1998 The effects of habitat fragmentation by forestry and agriculture on the abundance of small mammals in the southern boreal mixedwood forest. Canadian Journal of Zoology **76:** 62-69.

Beerli, P., and J. Felsenstein, 1999 Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics **152**: 763-773.

Bjornstad, O. N., H. P. Andreassen and R. A. Ims, 1998 Effects of habitat patchiness and connectivity on the spatial ecology of the root vole *Microtus oeconomus*. Journal of Animal Ecology **67:** 127-140.

Bowcock, A. M., A. Ruiz-Linares, J. Tomfohrde, E. Minch, J. R. Kidd *et al.*, 1994 High resolution of human evolutionary trees with polymorphic microsatellites. Nature **368:** 455-457.

Bowers, M. A., S. F. Matter, J. L. Dooley Jr., J. L. Dauten and J. A. Simkins, 1996 Controlled experiments of habitat fragmentation: a simple computer simulation and a test using small mammals. Oecologia **108:** 182-191.

Bruford, M. W., and R. K. Wayne, 1993 Microsatellites and their application to population genetic studies. Current Opinion in Genetics and Development **3:** 939-943.

Buckner, C. H., 1966 Populations and ecological relationships of shrews in tamarack bogs of southwestern Manitoba. Journal of Mammalogy **47:** 181-194.

Callen, D. F., A. D. Thompson, Y. Shen, H. A. Phillips, R. I. Richards *et al.*, 1993 Incidence and origin of "null" alleles in the $(AC)_n$ microsatellite markers. American Journal of Human Genetics **52:** 922-927.

Chakraborty, R., M. de Andrade, S. P. Daiger and B. Budowle, 1992 Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. American Journal of Human Genetics **56:** 34-57.

Churchfield, S., 1982 Food availability and the diet of the common shrew, *Sorex araneus*, in Britain. Journal of Animal Ecology **51:** 15-28.

Churchfield, S., 1990a Characteristics of shrews, pp. 1-23 in *The Natural History of Shrews*, edited by E. Neal. Cornell University Press, Ithaca, NY.

Churchfield, S., 1990b Communication and orientation, pp. 72-88 in *The Natural History of Shrews*, edited by E. Neal. Cornell University Press, Ithaca, NY.

Churchfield, S., 1990c Community structure and habitat preferences, pp. 131-147 in *The Natural History of Shrews*, edited by E. Neal. Cornell University Press, Ithaca, NY.

Churchfield, S., 1990d Energetics of shrews, pp. 111-130 in *The Natural History of Shrews*, edited by E. Neal. Cornell University Press, Ithaca, NY.

Churchfield, S., 1990e Food and foraging behaviour, pp. 89-110 in *The Natural History of Shrews*, edited by E. Neal. Cornell Universtiy Press, Ithaca, NY.

Churchfield, S., 1990f The life history of shrews, pp. 24-54 in *The Natural History of Shrews*, edited by E. Neal. Cornell University Press, Ithaca, NY.

Churchfield, S., 1990g Shrews and man, pp. 148-166 in *The Natural History of Shrews*, edited by E. Neal. Cornell University Press, Ithaca, NY.

Churchfield, S., 1990h Social organisation, pp. 55-71 in *The Natural History of Shrews*, edited by E. Neal. Cornell University Press, Ithaca, NY.

Collins, R. J., and G. W. Barrett, 1997 Effects of habitat fragmentation on meadow vole (*Microtus pennsylvanicus*) population dymanics in experiment landscape patches. Landscape Ecology **12:** 63-76.

Coote, T., and M. W. Bruford, 1996 Human microsatellites applicable for analysis of genetic variation in apes and Old World monkeys. Journal of Heredity **87:** 406-410.

Corbet, G. B., and J. E. Hill, 1980 A world list of mammalian species. British Museum (Natural History), London.

Currie-Cohen, M., 1981 Estimates of inbreeding in a natural population: a comparison of sampling properties. Genetics **100:** 339-358.

Dannelid, E., 1991 The genus *Sorex* (Mammalia, Soricidae) - distribution and evolutionary aspects of Eurasian species. Mammalian Review **21:** 1-20.

Deka, R., L. Jin, M. D. Shriver, L. M. Yu, S. Decroo *et al.*, 1995 Population genetics of dinucleotide $(dC\text{-}dA)_n(dG\text{-}dT)_n$ polymorphisms in world populations. American Journal of Human Genetics **56:** 461-474.

Delany, M. J., 1964 An ecological study of the small mammals in the Queen Elizabeth Park, Uganda. Rev. Zool. Bot. Afr. **70:** 129-149.

Dempster, A. P., N. M. Laird and D. B. Rubin, 1977 Maximum likelihood from incomplete data via the EM algorithm. Journal of the Royal Statistical Society B **39:** 1-38.

Di Rienzo, A., A. C. Peterson, J. C. Garza, A. M. Valdes, M. Slatkin *et al.*, 1994 Mutational processes of simple sequence repeat loci in human populations. Proceedings of the National Academy of Sciences **91:** 3166-3170.

Diersing, V. E., 1980 Systematics and evolution of the pygmy shrews (subgenus *Microsorex*) of North America. Journal of Mammalogy **61:** 76-101.

Diffendorfer, J. E., M. S. Gaines and R. D. Holt, 1995 Habitat fragmentation and movements of three small mammals (*Sigmodon*, *Microtus*, and *Peromyscus*). Ecology **76:** 827-839.

Dotsch, C., and W. V. Koenigswald, 1978 Zur Rotfarbung von Soricidenzahnen. Z. Saugetierk **43:** 65-70.

Downes, S. J., K. A. Handasyde and M. A. Elgar, 1997 Variation in the use of corridors by introduced and native rodents in south-eastern Australia. Biological Conservation **82:** 379-383.

Edwards, A., H. A. Hammond, L. Jin, C. T. Caskey and R. Chakraborty, 1992 Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics **12:** 241-253.

Ellegren, H., S. Moore, N. Robinson, K. Byrne, W. Ward *et al.*, 1997 Microsatellite evolution - A reciprocal study of repeat lengths at homologous loci in cattle and sheep. Molecular Biology and Evolution **14:** 854-860.

Epperson, B. K., 1995a Fine-scale spatial structure: Correlations for individual genotypes differ from those for local gene frequencies. Evolution **49:** 1022-1026.

Epperson, B. K., 1995b Spatial structure of two-locus genotypes under isolation by distance. Genetics **140:** 365-375.

Epplen, C., G. Melmer, I. Siedlaczck, F. W. Schwaiger, W. Maueler *et al.*, 1993 On the essence of "meaningless" simple repetitive DNA in eukaryote genomes., pp. 29-46 in *DNA Fingerprinting: State of the Science*, edited by S. D. J. Pena, Chakraborty, R., Epplen, J.T., and Jeffreys, A.J. Birkhauser Verlag, Basel.

Erlinge, S., 1981 Food preference, optimal diet and reproductive output in stoats *Mustela erminea* in Sweden. Oikos **36:** 303-315.

Faith, D. P., 1994 Genetic diversity and taxonomic priorities for conservation. Biological Conservation **68:** 69-74.

Findley, J. S., 1955 Speciation of the wandering shrew. University of Kansas Museum of Natural History, Kansas.

Foster, J., and M. S. Gaines, 1991 The effects of a successional habitat mosaic on a small mammal community. Ecology **72:** 1358-1373.

French, T. W., 1984 Dietary overlap of *Sorex longirostris* and *S. cinereus* in hardwood floodplain habitats in Vigo County, Indiana. American Midland Naturalist **111:** 41-46.

George, S. B., 1988 Systematics, historical biogeography and evolution of the genus *Sorex*. Journal of Mammalogy **69:** 443-461.

Getz, L. L., 1961 Factors influencing the local distribution of shrews. American Midland Naturalist **65:** 67-88.

Gilpin, M., 1991 The genetic effective size of a metapopulation. Biological Journal of the Linnean Society **42:** 165-175.

Gilpin, M. E., and I. Hanski, 1991 Metapopulations dynamics: empirical and theoretical investigations. Biological Journal of the Linnean Society **42:** 1-323.

Goldstein, D. B., A. R. Linares, L. L. Cavalli-Sforza and M. W. Feldman, 1995 An evaluation of genetic distances for use with microsatellite loci. Genetics **139:** 463-471.

Goldstein, D. B., and D. D. Pollock, 1997 Launching microsatellites: A review of mutation processes and methods of phylogenetic inference. Journal of Heredity **88:** 335-342.

Gotelli, D., C. Sillero-Zubiri, G. D. Applebaum, M. S. Roy, D. J. Girman *et al.*, 1994 Molecular genetics of the most endangered canid: the Ethiopian wolf *Canis simensis*. Molecular Ecology **3:** 301-312.

Goudet, J., 1999 FSTAT vers. 2.8, updated from Goudet (1995). Fstat: a computer program to calculate F-statistics. Journal of Heredity **86:** 485-486.

Goudet, J., M. Raymond, T. De Meeüs and F. Rousset, 1996 Testing differentiation in diploid populations. Genetics **144:** 1933-1940.

Guo, S. W., and E. A. Thompson, 1992 Performing the Exact Test of Hardy-Weinburg Proportion for Multiple Alleles. Biometrics **48:** 361-372.

Hagelberg, E., I. C. Gray and A. J. Jefferys, 1991 Identification of the skeletal remains of a murder victim by DNA analysis. Nature **352:** 427-429.

Hanski, I., 1984 Food consumption, assimilations and metabolic rate in six species of shrew (*Sorex* and *Neomys*). Annales Zoologici Fennici **21:** 157-165.

Hanski, I., 1990 *Insectivorous mammals*. Oxford University Press, Oxford.

Hanski, I., 1997 Metapopulation Dynamics, pp. 69-91 in *Metapopulation Biology: ecology, genetics and evolution*, edited by I. A. a. G. Hanski, M.E. Academic Press, San Diego, California.

Harris, L. D., 1988 Edge effects and conservation of biotic diversity. Conservation Biology **2:** 330-332.

Harrison, S., and A. Hastings, 1996 Genetic and evolutionary consequences of metapopulation structure. Trends in Ecology and Evolution **11:** 180-183.

Hedrick, P. W., 1996 Bottleneck(s) or metapopulation in cheetahs. Conservation Biology **10:** 897-899.

Heikkila, J., 1989 Population genetics of north temperate shrews (Soricidae). A review. Annales Zoologici Fennici **26:** 459-467.

Henderson, S. T., and T. D. Petes, 1992 Instability of simple sequence DNA in *Saccharomyces cerevisiae.* Molecular and Cellular Biology **12:** 2749-2757.

Heske, E. J., 1995 Mammalian abundances on forest-farm edges versus forest interiors in southern Illinois: Is there an edge effect? Jounal of Mammology **76:** 562-568.

Hoffmann, R. S., 1971 Relationships of certain holarctic shrews, genus *Sorex.* Zeitschrift fur Saugetierkunde **36:** 193-200.

Huggard, D., and W. Klenner, 1997 Initial Effects of Harvesting on Shrews, Immature Rodents, and Forest Floor Habitat at Sicamous Creek, pp. 200-208 in *Sicamous Creek Silvicultural Systems Project: Workshop Proceedings.*, edited by C. Hollstedt, and Vyse, A. Research Branch, B.C. Ministry of Forestry, Kamloops, British Columbia, Canada.

Ims, R. A., J. Rolstad and P. Wegge, 1993 Predicting space use responses to habitat fragmentation: Can voles *Microtus oeconomus* server as an experimental model system (EMS) for capercaillie grouse *Tetao urogallus* in boreal forest? Biological Conservation **63:** 261-268.

Jacquard, A., 1974 *The Genetic Structure of Populations.* Springer, Berlin.

Karl, S. A., and T. Streelman, 1998 Are microsatellite loci really neutral genetic markers?, in *Annual Meeting of the Society for the Study of Evolution, the American Society of Naturalists, and the Society of Systematic Biologists*, The University of British Columbia, Vancouver, BC, Canada.

Kimura, M., and J. F. Crow, 1964 The number of alleles that can be maintained in a finite population. Genetics **49:** 725-738.

Kirkland Jr., G. L., T. R. Johnston Jr. and P. F. Steblein, 1985 Small mammal exploitation of a forest-clearcut interface. Acta Theriologica **30:** 211-218.

Klenner, W., 1997 Effects of harvest treatments on the abundance of small mammals at Sicamous Creek., in *Sicamous Creek Silvicultural Systems Project: Workshop Proceedings.*, edited by C. Hollstedt, and Vyse, A. Research Branch, B.C. Ministry of Forestry, Kamloops, British Columbia, Canada.

Klenner, W., and D. Huggard, 1997 Faunal biodiversity studies at Sicamous Creek: Background and rationale for the choice of species indicator groups., in *Sicamous Creek Silvicultural Systems Project: Workshop Proceedings.*, edited by C. Hollstedt, and Vyse, A. Research Branch, B.C. Ministry of Forestry, Kamloops, British Columbia, Canada.

Kondo, Y., M. Mori, T. Kuramoto, J. Yamada, J. S. Beckman *et al.*, 1993 DNA segments mapped by reciprocal use of microsatellite primers between mouse and rat. Mammalian Genome **4:** 571-576.

Kremsater, L. L., and F. L. Bunnell, 1992 Testing responses to forest edges: The example of the black-tailed deer. Canadian Journal of Zoology **70:** 2426-2435.

Laikre, L., and N. Ryman, 1991 Inbreeding depression in a captive wolf (*Canis lupus*) population. Conservation Biology **5:** 33-40.

Lehman, N., A. Eisenhawer, K. Hansen, D. L. Mech, R. O. Peterson *et al.*, 1991 Introgression of coyote mitochondrial DNA into sympatric North American gray wolf populations. Evolution **45:** 104-119.

Lehmann, T., W. A. Hawley and F. H. Collins, 1996 An evaluation of evolutionary constraints on microsatellite loci using null alleles. Genetics **144:** 1155-1163.

Levins, R., 1969 Some demographic and genetic consequences of environmental heterogeneity for biological control. Bulletin of the Entomological Society of America **15:** 237-240.

Levinson, G., and G. A. Gutman, 1987 High frequencies of short frameshifts in poly-CA/TG tandem repeats borne by bacteriophage M13 in *Escherichia coli* K-12. Nucleic Acids Research **15:** 5323-5338.

Linzey, A. V., 1989 Response of the white-footed mouse (*Peromyscus leucopus*) to the transition between disturbed and undisturbed habitats. Canadian Journal of Zoology **67:** 505-512.

Lloyd, D. A., K. Angove, G. Hope and C. Thompson, 1990 A guide to site identification and interpretation for the Kamloops Forest Region., in *Land Management Handbook No. 23.* B.C. Ministry of Forests, Victoria, B.C.

Louis, E. J., and E. R. Dempster, 1987 An exact test for Hardy-Weinberg and multiple alleles. Biometrics **43:** 805-811.

Lugon-Moulin, N., H. Brunner, A. Wyttenbach, J. Hausser and J. Goudet, 1999 Hierarchical analyses of genetic differentiation in a hybrid zone of *Sorex araneus* (Insectivora: Soricidae). Molecular Ecology **8:** 419-431.

Lynch, M., J. Conery and R. Burger, 1995 Mutation accumulation and the extinction of small populations. American Naturalist **146:** 489-518.

Mace, G. M., T. B. Smith, M. W. Bruford and R. K. Wayne, 1996 An overview of the issues., in *Molecular genetic approaches in conservation.*, edited by T. B. Smith, and Wayne, R.K. Oxford University Press, New York, NY.

Maruyama, T., and M. Kimura, 1980 Genetic variability and effective population size when local extinction and recolonization of subpopulations are frequent. Proceedings of the National Academy of Sciences **77:** 6710-6714.

McCay, T., 1996 Response of masked shrews (*Sorex cinereus*) to precipitation in irrigated and nonirrigated forests. American Midland Naturalist **135:** 178-180.

McCullough, D. R., 1996 *Metapopulations and Wildlife Conservation*. Island Press, Washington, DC.

McFadden, C. S., and K. Y. Aydin, 1996 Spatial autocorrelation analysis of small-scale genetic structure in a clonal soft coral with limited larval dispersal. Marine Biology **126:** 215-224.

Menotti-Raymond, M., and S. J. O'Brien, 1993 Dating the genetic bottleneck of the African cheetah. Proceedings of the National Academy of Sciences **90:** 3172-3176.

Merritt, J. F., 1995 Seasonal thermogenesis and changes in body mass of masked shrews, *Sorex cinereus*. Journal of Mammalogy **76:** 1020-1035.

Metropolis, N., A. W. Rosenbluth, M. N. Rosenbluth, A. H. Teller and E. Teller, 1953 Equations of state calculations by fast computing machines. Journal of Chemical Physics **21:** 1087-1092.

Michalakis, Y., and L. Excoffier, 1996 A generic estimation of population subdivision using distances between alleles with special reference to microsatellite loci. Genetics **142:** 1061-1064.

Michielsen, N. C., 1966 Intraspecific and interspecific competition in the shrews *Sorex araneus* L. and *Sorex minutus* L. Archives Neerlandaises de Zoologie **17:** 73-174.

Mills, L. S., 1995 Edge effects and isolation: Red-backed voles on forest remnants. Conservation Biology **9:** 395-403.

Mills, L. S., and F. W. Allendorf, 1996 The One-Migrant-per-Generation Rule in conservation and management. Conservation Biology **10:** 1509-1518.

Morin, P. A., J. Wallis, J. J. Moore and D. S. Woodruff, 1994 Paternity exclusion in a community of wild chimpanzees using hypervariable simple sequence repeats. Molecular Ecology **3:** 469-478.

Nei, M., 1972 Genetic distance between populations. American Naturalist **106:** 283-292.

Nupp, T. E., and R. K. Swihart, 1996 Effect of forest patch area on population attributes of white-footed mice (*Peromyscus leucopus*) in fragmented landscapes. Canadian Journal of Zoology **74:** 467-472.

O'Brien, S. J., M. E. Roelke, L. Marker, A. Newman, C. A. Winkler *et al.*, 1985 Genetic basis for species vulnerability in the cheetah. Science **227:** 1428-1434.

O'Brien, S. J., D. E. Wildt, M. Bush, T. M. Caro, C. FitzGibbon *et al.*, 1987 East African cheetahs: Evidence for two population bottlenecks? Proceedings of the National Academy of Sciences **84:** 508-511.

Ohdachi, S., R. Masuda, H. Abe, J. Adachi, N. E. Dokuchaev *et al.*, 1997 Phylogeny of Eurasian soricine shrews (Insectivora, Mammalia) inferred from the mitochondrial cytochrome *b* gene sequences. Zoological Science **14:** 527-532.

Ohta, T., and M. Kimura, 1973 The model of mutation appropriate to esitmate the number of electrophoretically detectable alleles in a genetic population. Genetical Research **22:** 201-204.

Opdam, P., 1991 Metapopulation theory and habitat fragmentation: A review of holarctic bird studies. Landscape Ecology **5:** 93-106.

Paton, P. W. C., 1994 The effect of edge on avian nest success: How strong is the evidence? Conservation Biology **8:** 17-26.

Peacock, M. M., and A. T. Smith, 1997 The effect of habitat fragmentation on dispersal patterns, mating behavior, and genetic variation in a pika (*Ochotona princeps*) metapopulation. Oecologia **112:** 524-533.

Platt, W. J., and N. R. Blakley, 1973 Short-term effects of shrew predation upon invertebrate prey sets in prairie ecosystems. Proceedings of the Iowa Academy of Science **80:** 60-66.

Raymond, M., and F. Rousset, 1995 GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity **86:** 248-249.

Rice, W. R., 1989 Analyzing Tables of Statistical Tests. Evolution **43:** 223-225.

Rieseberg, L. H., S. Zona, L. Aberbom and T. D. Martin, 1989 Hybridization in the island endemic, Catalina mahogany. Conservation Biology **3:** 52-58.

Ritland, K., 1996 Estimators for pairwise relatedness and individual inbreeding coefficients. Genetical Research **67:** 175-185.

Ritland, K., 1999 Detecting inheritance with inferred relatedness in nature., in *Adaptive genetic variation in the wild*, edited by T. Mousseau. Oxford University Press, Oxford.

Robinson, G. R., R. D. Holt, M. S. Gaines, S. P. Hamburg, M. L. Johson *et al.*, 1992 Diverse and contrasting effects of habitat fragmentation. Science **257:** 524-526.

Roelke, M. E., J. S. Martenson and S. J. O'Brien, 1993 The consequences of demographic reduction and genetic depletion in the endangered Florida panther. Current Biology **3:** 340-350.

Rousset, F., 1996 Equilibrium values of measures of populations subdivision for stepwise mutation processes. Genetics **142:** 1357-1362.

Rubinsztein, D. C., W. Amos, J. Leggo, S. Goodburn, S. Jain, Li S. *et al.*, 1995 Microsatellite evolution - evidence for directionality and variation in rate between species. Nature Genetics **10:** 337-343.

Saarikko, J., 1989 Foraging behaviour of shrews. Annales Zoologici Fennici **26:** 411-423.

Schlotterer, C., B. Amos and D. Tautz, 1991 Conservation of polymorphic simple sequence loci in cetacean species. Nature **354:** 63-65.

Schlotterer, C., and D. Tautz, 1992 Slippage synthesis of simple sequence DNA. Nucleic Acids Research **20:** 211-215.

Scribner, K. T., J. R. Gust and R. L. Fields, 1996 Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. Canadian Journal of Fisheries and Aquatic Sciences **53:** 833-841.

Sekgororoane, G. B., and T. G. Dilworth, 1995 Relative abundance, richness, and diversity of small mammals at induced forest edges. Canadian Journal of Zoology **73:** 1432-1437.

Selander, R. K., 1970 Behavior and genetic variation in natural populations. American Zoologist **10:** 53-66.

Shriver, M. D., L. Jin, R. Chakraborty and E. Boerwinkle, 1993 VNTR allele frequency distributions under the stepwise mutation model: a computer simulation approach. Genetics **134:** 983-993.

Simberloff, D., 1988 The contribution of population and community biology to conservation science. Annual Review of Ecological Systematics **19:** 473-511.

Slatkin, M., 1995 A measure of population subdivision based on microsatellite allele frequencies. Genetics **139:** 457-462.

Spencer, A. W., and D. Pettus, 1966 Habitat preferences in five sympatric species of long-tailed shrews. Ecology **47:** 677-683.

Spieth, P. T., 1974 Gene flow and genetic differentiation. Genetics **78:** 961-965.

Stamps, J. A., M. Buechner and K. V.V., 1987 The effects of edge geometry on territorial defense costs: intruder pressure in bounded habitats. American Zoologist **27:** 307-325.

Stewart, D. T., and A. J. Baker, 1992 Genetic differentiation and biogeography of the masked shrew in Atlantic Canada. Canadian Journal of Zoology **70:** 106-114.

Stewart, D. T., and A. J. Baker, 1994 Patterns of sequence variation in the mitochondrial D-loop region of shrews. Molecular Biology and Evolution **11:** 9-21.

Stewart, D. T., and A. J. Baker, 1997 A phylogeny of some taxa of masked shrews (*Sorex cinereus*) based on mitochondrial-DNA, D-loop sequences. Journal of Mammalogy **78:** 361-376.

Stockley, P., 1996 Synchrony of estrus in common shrews. Journal of Mammalogy **77:** 383-387.

Tautz, D., M. Trick and G. Dover, 1986 Cryptic simplicity in DNA is a major source of genetic variation. Nature **322:** 652-666.

Teferi, T., T. B. Herman and D. T. Stewart, 1992 Breeding biology of an insular population of the masked shrew (*Sorex cinereus* Kerr) in Nova Scotia. Canadian Journal of Zoology **70:** 62-66.

Temple, S. A., and B. A. Wilcox, 1986 Effects of forest fragmentation on vertebrates in douglas-fir forests., pp. 261-262 in *Modeling habitat relationships of terrestrial vertebrates.*, edited by J. Verner, Morrison, M.L., and Ralph, C.J. University of Wisconsin Press, Madison, Wisconsin.

Valdes, A. M., M. Slatkin and N. B. Freimer, 1993 Allele frequencies at microsatellite loci: the stepwise mutation model revisited. Genetics **133:** 737-749.

Virtanen, H., 1989 Morphological variation in Eurasian and North American shrews, in *Zoology*. Universtiy of Helsinki, Helsinki, Finland.

Vyse, A., 1997 The Sicamous Creek Silvicultural Systems Project: How the Project Came To Be and What It Aims to Accomplish., pp. 4-14 in *Sicamous Creek Silvicultural Systems Project: Workshop Proceedings.*, edited by C. Hollstedt, and Vyse, A. Research Branch, B.C. Ministry of Forestry, Kamloops, British Columbia, Canada.

Weber, J. L., and C. Wong, 1993 Mutation of human short tandem repeats. Human Molecular Genetics **2:** 1123-1128.

Weir, B. S., and C. C. Cockerham, 1984 Estimating F-statistics for the analysis of population structure. Evolution **38:** 1358-1370.

Whitlock, M. C., 1992 Temporal fluctuations in demographic parameters and the genetic variance among populations. Evolution **46:** 608-615.

Whitlock, M. C., and N. H. Barton, 1997 The effective size of a subdivided population. Genetics **146:** 427-441.

Whitlock, M. C., and D. E. McCauley, 1999 Indirect measures of gene flow and migration: Fst≠1/(4Nm+1). Heredity **82:** 117-125.

Wolff, J. O., E. M. Schauber and W. D. Edge, 1997 Effects of habitat loss and fragmentation on the behaviour and demography of gray-tailed voles. Conservation Biology **11:** 945-956.

Wooster, R., A. M. Cleton-Jansen, N. Collins, J. Mangion, R. S. Cornelis *et al.*, 1994 Instability of short tandem repeats (microsatellites) in human cancers. Nature Genetics **6:** 152-156.

Wright, S., 1951 The genetical structure of populations. Ann. Eugen. **15:** 323-354.

Wyttenbach, A., L. Favre and J. Hausser, 1997 Isolation and characterization of simple sequence repeats in the genome of the common shrew. Molecular Ecology **6:** 797-800.

Yahner, R. H., 1988 Changes in wildlife communities near edges. Conservation Biology **2:** 333-339.