Habitat modification and gene flow in *Saimiri oerstedii*: Landscape genetics, intraspecific molecular systematics, and conservation

Mary Elizabeth Blair

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ABSTRACT

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Habitat modification, when it results in population fragmentation, often results in the loss of genetic diversity due to reduced gene flow, inbreeding, and genetic drift. However, the severity of these effects depends on how diminished dispersal and gene flow become between patches of suitable habitat. An empirical understanding of how habitat change affects dispersal and gene flow within and among patches is essential to predict the effects of increased habitat modification and landscape change on population persistence and processes of divergence. Recent studies in landscape ecology suggest that our understanding of dispersal in a heterogeneous landscape will improve by explicitly considering the heterogeneity of matrix habitats, or unsuitable habitats between patches of suitable habitat. In this dissertation, I describe population genetic structure and dispersal patterns in the Central American Squirrel Monkey (*Saimiri oerstedii*, Primates: Cebidae), a New World primate threatened with extinction and living in a heterogeneous, human-modified landscape, using analyses that explicitly consider matrix heterogeneity.

I focus on the more endangered *S. o. citrinellus,* whose already restricted distribution in the Central Pacific region of Costa Rica has undergone considerable anthropogenic modification since the early 1900s. I collected non-invasive fecal samples from *S. o. citrinellus* across the Central Pacific region, obtaining full genotypes from 233 individuals. I also obtained 11 samples

from *S. o. oerstedii* in the Southern Pacific region of Costa Rica from a collaborator, as well as fine-scale landscape data for the Central Pacific.

I analyzed the data using molecular systematics, population genetics, and landscape genetic techniques. In this dissertation, first I explore whether molecular genetic support exists for the subspecies distinction between *S. o. citrinellus* and *S. o. oerstedii*. Second, I describe population genetic structure and recent migration patterns within *S. o. citrinellus* using traditional population genetic methods and Bayesian models. I also compare population genetic structure among males versus females to test for sex-biased dispersal patterns in *S. o. citrinellus*. Then, using landscape genetic approaches, I describe the relationship between landscape heterogeneity and genetic structure in *S. o. citrinellus*, and inferred which matrix habitats are costly to dispersal. Finally, I offer explicit recommendations for the conservation management of *S. oerstedii*.

My results provide genetic support for *S. o. citrinellus* and *S. o. oerstedii* as separate taxa referred to as subspecies. Also, I found evidence of population genetic structure in *S. o. citrinellus*, with two genetically distinct populations and lower genetic diversity in the western population. I did not find genetic evidence for female-biased dispersal in *S. o. citrinellus* as expected. Instead, my results suggest that both sexes disperse, with males dispersing over longer distances. The landscape genetic analysis suggests that landscape heterogeneity is important in determining local population genetic structure in *S. o. citrinellus* in the Central Pacific region of Costa Rica. Specifically, oil palm plantations are moderate barriers to gene flow between populations, but not other matrix habitats. However, these inferences are specific to the composition and configuration of the Central Pacific landscape, and should not be generalized to all *S. oerstedii* populations.

This study generated important information for conservation management. Based on my results, I recommend that conservation managers house the two *S. oerstedii* subspecies separately in captive facilities, and only transfer, reintroduce, or translocate among groups of the same subspecies. However, transfers, reintroductions, or translocations of either males or females are both likely to be successful for *S. o. citrinellus* in the Central Pacific region, pending further behavioral study. I also recommend that, in order to augment dispersal to the isolated western population of *S. o. citrinellus,* conservation efforts should focus on building biological corridors through or around adjacent oil palm plantations. Also, managers should prioritize the maintenance of existing forest connectivity in the Central Pacific region.

The results also have important implications for future studies of evolutionary and ecological processes in heterogeneous landscapes. This study contributes to a growing body of research that finds differences in dispersal patterns among local primate populations of the same taxon. My results suggest that predictive models for variation in dispersal patterns should consider both variation among the environments of local populations within a species and temporal variation in local environments (e.g. recent habitat disturbance). Finally, this dissertation also supports the idea that matrix heterogeneity should be considered explicitly in studies of dispersal and gene flow, as opposed to assuming that all non-suitable habitats have a uniform effect on these processes. In the future, agent-based simulation approaches combined with ecological niche models and data on adaptive genetic diversity could expand upon this work to inform predictive models for population divergence and speciation under different climate and landscape change scenarios.

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DEDICATION

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CHAPTER 1.

Introduction: Landscapes, habitat modification, and population genetic structure, a literature review

Abstract

Habitat modification, and in particular habitat fragmentation, causes drastic changes to the natural landscape and threatens biodiversity worldwide (Crooks, Sanjayan, 2006; Fahrig, 2003). In particular, habitat fragmentation can result in the loss of genetic diversity due to reduced gene flow, inbreeding, and genetic drift. However, the severity of these effects depends on the degree to which dispersal and gene flow are diminished between patches of suitable habitat. An empirical understanding of how habitat change affects the movement of individuals within and among patches will be essential in predicting the effects of increased habitat fragmentation on population persistence. Landscape genetics, an approach that is individually based and spatially explicit, offers more powerful tools to study the effects of habitat fragmentation on gene flow than traditional population genetics. Recent studies on matrix processes in landscape ecology reject simplistic models where the matrix between habitat patches uniformly inhibits movement between patches. Instead, the matrix is dynamic, rarely uniform, and can have both positive and negative effects on dispersal, depending on species characteristics. Landscape genetic tools can be used to infer the effects of matrix heterogeneity on population genetic structure by correlating genetic discontinuities with landscape features, including different types of matrix habitat. Least-cost distances and Bayesian clustering analyses are some of the most commonly used tools in landscape genetic studies.

Introduction

Habitat modification includes habitat loss, fragmentation, and change at the landscape-scale. One of the most well studied processes of habitat modification is habitat fragmentation, or the splitting of one large contiguous habitat into multiple, discontinuous semi-isolated patches, which in total encompass less suitable habitat area and have greater edge perimeter (Debinski, Holt, 2000; Fischer, Lindenmayer, 2007a). In the past, and to some extent currently, studies of habitat modification are hampered by inconsistent terminology (Arroyo-Rodriguez, Mandujano, 2009; Fahrig, 2003; Fischer, Lindenmayer, 2007a; Lindenmayer, Fischer, 2007; Villard, 2002). Although many researchers call all processes that fall within habitat modification "habitat fragmentation," others make a clear distinction between habitat loss and habitat fragmentation and use these terms to represent separate concepts. Fahrig (2003) shows that habitat loss and fragmentation are fundamentally different processes; while habitat loss has consistently negative effects on species persistence, habitat fragmentation may cause positive, negative, or no effects. However, because habitat loss often occurs at the same time as fragmentation, the effects of both become conflated (Fahrig, 2003).

Other researchers suggest eliminating the phrase "habitat fragmentation" altogether. Instead, they advocate for research on the effects of "habitat modification," with fragmentation as one subject of study within a sub-discipline called the "ecology of modified landscapes" (Fischer & Lindenmayer 2007a). They argue that the traditional link between the terms habitat loss and habitat fragmentation and general landscape properties (i.e. native vegetation cover) ignores recent advances in landscape ecology that emphasize species-specific definitions of habitat (Fischer, Lindenmayer, 2007a; Fischer, Lindenmayer, 2007b; Lindenmayer, Fischer, 2007).

Throughout this dissertation, I will use the term habitat modification to refer to the interacting and simultaneously occurring processes of anthropogenic habitat loss, change, and fragmentation in terrestrial landscapes, where habitat refers to species-specific habitat preferences and not necessarily a measure of native vegetation coverage.

Theoretical frameworks in studies of habitat modification

Here I summarize two theoretical frameworks that have greatly influenced the study of species' responses to habitat modification: metapopulation ecology and landscape ecology. These frameworks differ in critical ways, largely related to how they consider heterogeneous landscapes, or landscapes that consist of many patches of suitable and matrix habitat, with the latter defined as the unsuitable habitat between patches of suitable habitat.

Metapopulation ecology

Levins first coined the term "metapopulation" in the late 1960s as "a population of populations" inhabiting discrete habitat patches and linked by dispersal (Levins, 1969; Levins, 1970). Each individual population within a metapopulation is unstable, and its persistence depends on a balance of local extinction and colonization (Levins 1969, 1970; Hanski 1998). Antecedents of Levins' model include Wright's shifting balance theory and MacArthur and Wilson's island biogeography theory (Hanski, Gilpin, 1991; Hanski, Simberloff, 1997; Hastings, Harrison, 1994; MacArthur, Wilson, 1967; Wright, 1940). Metapopulation ecologists often consider their approach as intermediate between the too idealized and simplistic "theoretical ecology" and the too realistic and complicated "landscape ecology," described below, making their theoretical framework the most applicable to conservation management strategies (Hanski, 1998; Hanski, Simberloff, 1997). In particular, the metapopulation approach has been used to

identify the critical amount of habitat below which a species is expected to go extinct, called the extinction threshold (Fahrig, 2002).

In order to fit the assumptions of the metapopulation approach, a population must fulfill the following conditions: 1) patches must be connected by migration; 2) patches must show local extinctions and recolonizations over time; and 3) patch dynamics across the metapopulation must be asynchronous (Elmhagen, Angerbjorn, 2001; Hanski *et al.*, 1995). Despite the theoretical applicability of metapopulation theory to studies of habitat modification, most real populations do not fulfill these conditions (Elmhagen, Angerbjorn, 2001; Harrison, 1991). Large mammals, and primates in particular, receive a lot of attention as threatened species, and it is especially tempting to use the metapopulation approach to inform conservation management plans for these taxa without providing evidence that these populations fulfill the conditions (Anderson *et al.*, 2007a; Ferreras, 2001; Gaona *et al.*, 1998; Lawes *et al.*, 2000; Mbora, Meikle, 2004; Swart, Lawes, 1996). Many spatially structured populations are more likely to be non-equilibrium, remnant, or naturally patchy populations, not necessarily metapopulations (Baguette, 2004; Freckleton, Watkinson, 2002; Harrison, 1991; Lawton, 1993), and the application of the metapopulation approach to these populations is not valid, unless the populations meet the three conditions described above.

Even if a population does fit the assumptions of metapopulation theory, the most commonly used metapopulation models, known as "stochastic patch-occupancy models" (SPOMs; Gotelli, 2001; Ovaskainen, Hanski, 2004), are criticized for their assumption that patch area accurately represents population size, and for ignoring the importance of matrix heterogeneity and patch quality in predicting population persistence (Baum *et al.*, 2004; Bender, Fahrig, 2005; Dunford, Freemark, 2005; Fahrig, 2002; Jules, Shahani, 2003; Kupfer *et al.*, 2006;

Lindenmayer *et al.*, 1999; Pellet *et al.*, 2007; Ricketts, 2001). The simplistic nature of SPOMs should caution against their application to the study of fragmented populations in very heterogeneous landscapes.

Landscape ecology

Landscape ecology is the study of how landscape structure affects the abundance and distribution of organisms (Fahrig, 2005; Turner, 1989). One important advantage of using the landscape ecology framework to look at species' responses to habitat modification is that this framework places an increased emphasis on landscape heterogeneity, including the matrix.

Both metapopulation ecology and landscape ecology have a common root in MacArthur and Wilson's (1967) island biogeography theory (Bowers, Barrett, 1999; Forman, 1997; Forman, Godron, 1986; Naveh, Lieberman, 1984). MacArthur and Wilson's (1967) monograph includes a metaphor of terrestrial habitat patches as islands and the unsuitable habitat between them (the matrix) as the sea. In Levins' (1969) metapopulation model, he describes a binary landscape with patches of suitable habitat in a sea of unsuitable habitat. Similarly, landscape ecologists Forman and Godron (1986) developed the "patch-corridor-matrix" model which assumes that all landscapes share a common structure made of three elements: patches, corridors, and matrix (Forman, 1997; Forman, Godron, 1986). A patch in this model is a "nonlinear surface area differing in appearance from its surroundings," embedded in a matrix, or a "surrounding area that has a different species structure or composition," while corridors are narrow strips of land that differ from the matrix on either side, which may be isolated or connected to a patch (Forman, Godron, 1986).

A major difference between the two approaches, however, is that landscape ecology, unlike metapopulation ecology, has recently moved past the simplistic patch-corridor-matrix

model and begun to incorporate patch quality, matrix quality, and matrix heterogeneity into models of species abundance and distribution (Haila, 2002). This new framework takes a more realistic perspective than earlier, more simplistic models where the matrix between habitat patches uniformly inhibits movement between patches. Instead, the matrix is considered dynamic, rarely uniform, and can have both positive and negative effects on dispersal and thus the long-term persistence of a species (Baum *et al.*, 2004; Crooks, Sanjayan, 2006; Dunford, Freemark, 2005; Fischer, Lindenmayer, 2007a; Hilty *et al.*, 2006; Jules, Shahani, 2003; Kindlmann *et al.*, 2005; Kupfer *et al.*, 2006). For some species, the matrix may include suitable habitat and thus enhances connectivity between forest patches. Such species are often called matrix tolerant, and their probability of persistence in a human-modified landscape is likely to be greater as compared to species with low matrix tolerance (Hilty *et al.*, 2006).

Matrix habitats are particularly important for many mammalian taxa (Peles *et al.*, 1999). Secondary and regenerating forests help to maintain small mammal abundance and diversity in the Atlantic forest, Brazil (Pardini *et al.*, 2005). In Australia, many mammals use secondary regrowth matrix, depending on their denning requirements, diet, and degree of arboreality (Cox *et al.*, 2004; Laurance, 1990). And, Angola black-and-white colobus monkeys *(Colobus angolensis*) travel and forage daily in some matrix habitats, including live fences (Anderson *et al.*, 2007b).

It is important to note that responses to matrix quality and heterogeneity are species-specific (Beier, Noss, 1998). Gehring and Swihart (2003) found very strong interspecific differences in matrix tolerance in a study of several mammalian predators in an agricultural landscape in Indiana. Variation in matrix tolerance within this guild correlates strongly with body size, ecoregion breadth, diet breadth, and habitat breadth (Gehring, Swihart, 2003).

Because species have different responses to changing matrix quality and heterogeneity, species therefore often have different responses to habitat modification.

To complicate matters further, a species may also respond to the matrix indirectly, through the effects of the matrix on its competitors, predators, or in the case of some plants, pollinators. For example, the matrix may include a habitat required for one of a pollinator's life stages, and so management of that matrix habitat is critical to the persistence of both pollinator and plant populations. Alternatively, the matrix may harbor alternative plant populations that may "steal" pollinators from intact habitat, or the matrix can be impermeable to pollinators, causing self-fertilization by plants (Jules, Shahani, 2003). Kareiva (1987) shows that although matrix habitat has no direct effect on the persistence a prey species (aphids), prey density increases in fragmented habitats because of low matrix tolerance in the predator species (ladybird beetles; Kareiva, 1987).

The matrix may also affect organisms indirectly through “edge effects,” or the penetration of abiotic and biotic conditions from the surrounding matrix into patch interiors (Chen *et al.*, 1992). There is increasing evidence that edge effects lead to the degradation of forest fragments (Laurance *et al.*, 2002), and negatively affect several organisms, including the greater dwarf lemur *Cheirogaleus major,* which is found at lower densities at forest edges because of lower fruit and liana abundance (Lehman *et al.*, 2006). By contrast, some species are quite edge-tolerant. For example, howler monkeys (*Alouatta* spp.) are more tolerant of edge habitats and can be found in smaller forest fragments than spider monkeys (*Ateles* spp.; Arroyo-Rodriguez, Dias, 2010; Estrada, Coates-Estrada, 1996; Gilbert, 2003). Certain generalist species may even prefer edges to interior habitats because edge habitats may be more productive.

Despite growing evidence suggesting the importance of matrix quality and heterogeneity, some recent studies still fail to consider the matrix in their research design. Michalski and Peres (2007) show that large mammals are present only in large patches (>100 ha) of habitat in Amazonian forest fragments. From their data the authors conclude that large, undisturbed forest patches are needed to maximize large mammal persistence (Michalski, Peres, 2007). This conclusion is based on the assumption that large mammals are unable to move through disturbed matrix habitats to get from one large patch to another. Recent research would instead suggest that many mammals, including large ones, are able to move across at least some types of matrix habitat (Anderson *et al.*, 2007b; Cox *et al.*, 2004; Gehring, Swihart, 2003; Laurance, 1990). If Michalski and Peres (2007) had included data on matrix quality or heterogeneity in their analysis, they might have instead concluded that large mammals can persist in a landscape with large habitat patches connected by certain types of matrix habitat. Such an assessment would probably be more accurate and also more practical in the management of an already modified landscape.

Overall, the landscape ecology framework offers many advantages over metapopulation ecology in that it allows the incorporation of matrix heterogeneity when inferring responses to habitat modification.

Population fragmentation

Very generally, habitat modification causes negative effects on species persistence when it results in population fragmentation. Population fragmentation can be defined as a process whereby one previously contiguous population splits into multiple, isolated populations, typically of a lower overall population size (Frankham, 2006; Frankham *et al.*, 2002). Population

fragmentation or "structuring" may be a consequence of anthropogenic habitat modification, but may also occur due to natural habitat patchiness, large geographic barriers such as rivers, or characteristics of social and mating systems. Disturbance regimes or other environmental processes can cause natural structure in populations. Anthropogenic habitat modification, by contrast, often occurs at spatial and temporal scales much more drastic than natural fragmentation, and thus may have negative effects even on naturally structured populations.

However, anthropogenic habitat modification does not always result in population fragmentation. If a species has certain characteristics that allow it to maintain high dispersal rates despite a discontinuous habitat (e.g. terrestrial primates), habitat modification may not result in population fragmentation for that species. Whether or not habitat modification causes population fragmentation depends on species ecology and life history, the scale of fragmentation, and the nature of the matrix habitat (Debinski, Holt, 2000; Kareiva, 1987; Villard, 2002). For example, the tuatara (*Sphenodon punctatus*) is particularly susceptible to population fragmentation because it is a relatively sedentary species with low dispersal rates and as a reptile is sensitive to changes in environmental conditions (Moore *et al.*, 2008).

Some conservation biologists argue that although the process of habitat modification may have negative effects in the short term, habitat modification may produce new species in the long term from repeated founder events (Meffe *et al.*, 1997). This argument seems unfounded, however, because speciation is likely only when founder events are followed by rapid population growth due to increasing ecological opportunities, which is unlikely in a scenario of anthropogenic habitat modification (Templeton *et al.*, 2001).

The negative effects that fragmentation can have on a population depend critically on the degree of isolation between subpopulations, or in other words the level of dispersal and gene

flow that occurs among fragments. When rates of dispersal are low, the effective size of a population decreases, giving it a higher risk of extinction from demographic stochasticity and inbreeding depression (Frankham, 2006; Frankham *et al.*, 2002). I discuss this process in more detail below.

Habitat modification and population genetic structure

Two key processes of habitat modification are the reduction of total habitat area (loss) and the separation of habitat patches from one another (isolation). These processes have confounding and mutually reinforcing effects on population genetic structure, or the pattern of genetic differentiation among and within groups in a population that results from an uneven distribution of genotypes over the area where the population lives (de Jong *et al.*, 1994).

Habitat loss affects the population genetic structure of an organism by lowering its effective population size (N_e), causing a loss in heterozygosity and overall genetic diversity due to genetic drift and inbreeding. Patch isolation, by contrast, causes isolated subpopulations to differentiate from one another genetically because of reduced gene flow among patches, genetic drift, and localized selection. This process occurs in two steps: 1) initial genetic sub-division, and 2) cumulative diversification over time. Initial genetic sub-division refers to the distribution of alleles among fragments, which will be different even in equally sized fragments just by chance (e.g. founder effect). Cumulative diversification occurs over time due to allele fixation (caused by genetic drift or localized selection) and loss of heterozygosity (caused by inbreeding) in each fragment (Frankham, 2006; Frankham *et al.*, 2002).

Genetic diversity will be lost more rapidly in smaller populations, and in populations with more isolated fragments. As shown in equation 1 below, the retention of heterozygosity (H) at

time *t* relative to time *0* depends not only on the size of the population (N) but the number of isolated fragments (*f*) in the population (Frankham *et al.*, 2002).

$$\frac{H_t}{H_0} = \left[1 - \frac{1}{\left(\frac{2N}{f}\right)}\right]^t \sim e^{\frac{-tf}{2N}} \qquad (1)$$

A single population with 500 individuals in each generation, over 50 generations, loses 5% of its initial heterozygosity, while 20 populations of 25 individuals each (also totaling 500) lose 64% of their initial heterozygosity (Frankham et al. 2002; 319). Many recent studies confirm this relationship in real populations; in the golden brown mouse lemur (*Microcebus ravelobensis*), for example, mitochondrial DNA haplotype diversity is significantly less in smaller populations as compared to larger ones (Guschanski *et al.*, 2007). Simulations also confirm this relationship, showing that after a habitat fragmentation event, a large part of variance in median time to local extinction is explained by initial population size (Jaquiery *et al.*, 2009).

Alleles are also more likely to be fixed by genetic drift in smaller populations. As shown in equation 2 below, the divergence in allele frequencies (of alleles *p* and *q*) as measured by variance (σ_p^2) will increase with generations (*t)* and increase faster in smaller populations (N) (Frankham *et al.*, 2002).

$$\sigma_p^2 = p_0 q_0 \times \left[1 - \left(1 - \frac{1}{2N}\right)^t\right] \qquad (2)$$

The degree to which inbreeding, genetic drift, and population differentiation may cause the loss of genetic diversity in a fragmented population is influenced by several species- and population-specific parameters that influence the degree of isolation among fragments. These

parameters include: the spatial pattern of fragments in the landscape, migration rates, matrix type, time since modification, dispersal ability of the species, distance between fragments, number of fragments, the distribution of population sizes among fragments, underlying historical genetic structure from natural barriers (Frankham, 2006; Frankham *et al.*, 2002; Mills, Tallmon, 1999).

Measuring population genetic structure

To infer changes in population genetic structure caused by habitat modification, one must measure population genetic structure. Sewall Wright (1931, 1943) put forward what is referred to as the "classic" model of population genetic structure, which includes three hierarchical levels: a large total population (T), discrete subpopulations (S), and individuals (I) (Nei, 1973; Nei, Chesser, 1983; Nei, Tajima, 1981; Weir, Cockerham, 1984; Wright, 1931; Wright, 1943). Mating occurs within subpopulations, which are connected to each other by gene flow. Additional hierarchical levels can also be described and analyzed under this framework such as social groups or regional populations (Melnick, 1988; Melnick, Hoelzer, 1993).

Wright's F-statistics are used in traditional population genetics to characterize population genetic structure, or the partitioning of genetic variation among the different hierarchical levels described above. F_{ST}, also called an inbreeding coefficient or fixation index, is widely used in studies of population genetic structure. F_{ST} measures inbreeding due to the differentiation among sub-populations relative to the total population, specifically by measuring deviation from the expectations of Hardy-Weinberg equilibrium due to population subdivision. F_{ST} is directly proportional to the reduction in heterozygosity in the total population that occurs due to population differentiation. Originally developed for allozyme loci, there are now estimators of

F_{ST} for microsatellites (R_{ST}) and even mitochondrial markers (Φ_{ST}) (Excoffier *et al.*, 1992; Nei, 1973; Slatkin, 1985; Slatkin, 1995; Weir, Cockerham, 1984). The value of F_{ST} ranges from 0 to 1, with a higher value corresponding to greater population structure.

F-statistics are commonly used to determine the effects of habitat modification on population genetic structure. For example, a recent study compares the F_{ST} values of two arboreal gecko species (*Oedura reticulata* and *Gehyra variegata*) living in the same fragmented landscapes to show higher genetic structure for the species with lower dispersal ability, *O. reticulata* (*O. reticulata* $F_{ST} = 0.044$, *G. variegata* $F_{ST} = 0.003$, $P < 0.05$; Hoehn *et al.*, 2007).

F_{ST} can also estimate the number of migrants between subpopulations, an application particularly relevant to studies of organisms in fragmented habitats. As shown in equation 3 below, F_{ST} increases rapidly with less than one migrant per generation.

$$F_{ST} = \frac{1}{4Nm + 1} \qquad (3)$$

However, attempts to relate F_{ST} to gene flow and drift using this equation are often inappropriate because this equation assumes a drift-flow equilibrium and most real populations, especially recently fragmented ones, are not in equilibrium (Hutchison, Templeton, 1999; McCauley, 1993). Thus, F-statistics may not be particularly applicable to the measurement of gene flow in studies of the effects of habitat modification. Moreover, F-statistics are summary statistics that average across population values, and offer no spatial information. F-statistics effectively measure spatial *variance* in gene frequencies, while an approach that instead measures spatial *patterns* of gene frequencies would be more suited for studies of habitat modification (Epperson, Li, 1996).

Landscape genetics

Landscape genetic approaches are increasingly used to understand the influence of landscape patterns on dispersal patterns and population genetic structure (Balkenhol *et al.*, 2009a; Holderegger, Wagner, 2008; Segelbacher *et al.*, 2010; Sork, Waits, 2010; Storfer *et al.*, 2010). This emerging methodological approach uses spatially explicit models to examine how landscape features affect the spatial distribution of genetic variation (Holderegger, Wagner, 2006; Manel *et al.*, 2003; Storfer *et al.*, 2007). Landscape genetics differs from traditional population genetics in that it does not require an *a priori* identification of discrete populations. Instead, the individual is the operational unit of analysis within the population (Manel *et al.*, 2003; Pritchard *et al.*, 2000; Wright, 1931). Although there are analyses based on F-statistics that incorporate spatial information, such as analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992), these are not rigorous when non-Euclidean geographic distances are used and therefore have limited application in heterogeneous landscapes (see discussion of least-cost distances below).

The landscape genetic approach attempts to detect genetic discontinuities in the spatial patterning of genetic variation, i.e. population genetic structure, and then correlates those discontinuities with heterogeneous landscape features, making it particularly relevant to studies of habitat modification. In particular, landscape genetic approaches can be used to quantify the relative effects of different matrix habitat classes instead of lumping all non-suitable habitat into one category (Balkenhol *et al.*, 2009a; Cushman *et al.*, 2006; Watling *et al.*, 2011). Specific analyses used in the landscape genetic approach are described below.

Isolation-by-distance and least-cost distances

The isolation-by-distance (IBD) framework was first introduced by Wright (1943). IBD refers to the process whereby populations differentiate because of impeded gene flow due only to an increase in geographic distance (Chesser, 2003). IBD analyses examine the correlation between measures of genetic distance among populations and the geographic distance separating those populations, typically using Mantel tests for matrix correspondence (Epperson, 2003; Mantel, 1967; Smouse *et al.*, 1986). The statistical significance and tightness of the correlation can reveal the relative influences of genetic drift and gene flow in the population genetic structure of a species. Genetic distance in IBD analyses is measured using several different statistics, which can be applied to a variety of molecular data types, including microsatellite data: e.g. Nei's D_S (Nei, 1987) or Goldstein's $\delta\mu^2$ (Goldstein *et al.*, 1995). In traditional population genetics, these pairwise distances are measured among populations, but in landscape genetics these distances are measured among individuals, typically using Rousset's $\hat{a}$ (Goncalves da Silva, 2007; Rousset, 2000).

Under drift-flow equilibrium, genetic distance is expected to have a positive and monotonic relationship with geographic distance. If genetic drift has the stronger influence on population genetic structure, as it would in very isolated populations, the relationship between genetic and geographic distance will be weak, with very large residuals. If gene flow has a stronger influence, the relationship between genetic and geographic distance will also be weak, but with small residuals. A typical pattern caused by habitat modification would be a positive relationship between geographic and genetic distance that levels off past a certain distance due to genetic drift overwhelming gene flow beyond that point (Hutchison, Templeton, 1999). It is important to remember that IBD analyses only explain drift and flow at the scale over which they

are measured. If IBD analyses are performed at too small a scale, a pattern suggesting a stronger influence of gene flow may be seen, when at a larger scale genetic drift may have a stronger influence. Scale is an important issue in all studies of habitat modification, which I will discuss in more detail later in this chapter.

The IBD framework can also be used to infer the effects of different matrix habitats on gene flow. Landscape heterogeneity can be incorporated into calculations of "least-cost" dispersal routes, also called "effective" geographic distances, using prior knowledge of species dispersal ability and habitat preferences combined with detailed information of landscape features. Least-cost behavioral distances can also be calculated by taking into account social structuring and other social barriers to movement. Least-cost geographic distances are then compared to Euclidean linear geographic distances as to how well they correlate with genetic distances. If genetic distances correlate more strongly with least-cost distances than with Euclidean measures, one can infer that landscape heterogeneity has some effect on gene flow. This approach has been used in several recent studies of populations in fragmented habitats. Most find a significant effect of landscape heterogeneity on gene flow, or different patterns of gene flow for the same species in fragmented versus unfragmented landscapes (Broquet *et al.*, 2006; Coulon *et al.*, 2004; Coulon *et al.*, 2006; Keyghobadi *et al.*, 1999; Verbeylen *et al.*, 2003; Watts *et al.*, 2006).

For example, least-cost distances that incorporate a cost to high altitude and agricultural land correlate more strongly than Euclidean distances in European damselflies, showing that these types of habitat matrix are barriers to gene flow (Watts *et al.*, 2006). In the American marten, a distance incorporating the relative dispersal costs of different landscape features correlates strongly with genetic distance, while Euclidean distance does not correlate at all

(Broquet *et al.*, 2006). Least-cost distances incorporating the connectivity of woodland habitat correlate more strongly with genetic distance than Euclidean distance in the European roe deer (Coulon *et al.*, 2004). Indeed, least-cost distances have been shown to correlate more strongly with genetic distances than Euclidean distances in many recent studies of terrestrial mammals, birds and herpetofauna (Frantz *et al.*, 2010; Greenwald *et al.*, 2009; Hokit *et al.*, 2010; Lada *et al.*, 2008), including sea turtles, where least-cost distances took ocean current data into account (Blumenthal *et al.*, 2009).

Although several studies of primate molecular ecology employ Mantel tests to infer isolation-by-distance in patterns of localized gene flow (Di Fiore, Fleischer, 2005; Eriksson *et al.*, 2006; Huck *et al.*, 2007), far fewer have used least-cost distances that incorporate landscape heterogeneity to examine matrix permeability. Long, overlapping generations affect the power of these tests to detect effects of current or even historical landscape characteristics on genetic variation in primates. In addition, extensive sampling is difficult as many primates are arboreal and have low population densities. Also, as most primates reside in tropical regions, fine-scale landscape data are often very difficult to obtain (Storfer *et al.* 2010). However, some recent studies using the least-cost distance approach do show that landscape features may be driving spatial genetic structure in some primates. In *Rhinopithecus bieti,* non-Euclidean distances incorporating the presence or absence of habitat gaps explained genetic variation better than Euclidean distances, although the landscape composition of those gaps was not considered (Liu *et al.*, 2009). In sifakas (*Propithecus tattersalli*), large rivers but not roads are important barriers to gene flow (Quemere *et al.*, 2010).

Although IBD analyses make fewer assumptions than F_{ST} and other summary statistics, IBD analyses do rely on assumptions that may limit their explanatory value in some cases. For

example, IBD analyses are subject to the same assumptions as the metrics used to calculate genetic distance. Many of these metrics rely on stepwise mutation models of microsatellite evolution, which assume the number of repeats in an allele is directly related to evolutionary time. Goldstein's $\delta\mu^2$ (Goldstein *et al.*, 1995) is a measure of genetic distance that attempts to model microsatellite evolution without relying on these assumptions, but it is not widely used.

Simulations show that although the Mantel test has less power than other tests including regression and canonical analyses, Mantel tests should be used to test relationships when hypotheses are formulated in terms of genetic distances (Legendre, Fortin, 2010). However, it is important to also use partial Mantel tests in the least-cost IBD approach, because least-cost and Euclidean distances are not independent of one another (Cushman, Landguth, 2010). A particular disadvantage to both simple and partial Mantel tests, however, is the inherent difficulty in choosing among closely related models or models with only slightly different Mantel's *r*-values, which may be within a reasonable margin of error of one another (Guillot *et al.*, 2009).

It is critical to consider the assumptions, limitations, and account for uncertainties inherent in the least-cost IBD method when interpreting results. In particular, simulations show that the power and accuracy of least-cost IBD analyses depend on the spatial structure of the landscape. The greater the contrast between the permeability of different landscape elements, the greater the predictive power and accuracy of the least-cost IBD analyses (Jaquiery *et al.*, 2011; Rayfield *et al.*, 2010). Thus, least-cost distances might not predict variation in genetic distance well because of several species- or landscape-specific characteristics. Or, least-cost distances might not explain genetic variation because of problems with sample design, such as biased sampling (Segelbacher *et al.*, 2010; Storfer *et al.*, 2010) or failure to identify and analyze biologically relevant potential barriers (Cushman *et al.*, 2006).

Spatial autocorrelation

Spatial autocorrelation analyses were introduced in the 1970s (Sokal, Oden, 1978) and work within the same framework as isolation-by-distance, but use a measure of genetic similarity instead of genetic distance (Bocquet-Appel, Sokal, 1989; Epperson, Li, 1996; Smouse, Peakall, 1999). The most common measure of genetic similarity used in spatial autocorrelation analyses is Moran's *I* (Epperson, 2003; Epperson, Li, 1996; Moran, 1950). Measures of genetic similarity such as Moran's *I* make different assumptions about the data than measures of genetic distance, and Moran's *I* actually has a lower variance than Rousset's *â* (Goncalves da Silva, 2007; Hardy, Vekemans, 2006). Thus, studies of isolation-by-distance may be most convincing when measures of both distance and similarity yield consistent results (Fredsted *et al.*, 2005; Goncalves da Silva, 2007; Peakall *et al.*, 2003).

Spatial autocorrelation analyses specifically test whether pairs of observed genotypes are more similar at closer geographical distance classes. This approach is robust and quite useful as long as the sampling scale is smaller than the scale of the spatial autocorrelation (Epperson, Li, 1996; Slatkin, Arter, 1991). New approaches using Moran's *I* including spatial principal components analysis (sPCA) are also quite robust (Jombart *et al.*, 2008). Because spatial autocorrelation statistics use geographic distance classes, however, spatial autocorrelation cannot identify the specific location of a genetic discontinuity (Manel *et al.*, 2003).

Isolation-by-resistance

Recently, McRae (2006) has critiqued least-cost distances as less theoretically sound for landscape genetic analyses than "resistance distances," the products of a new theory coined "isolation-by-resistance." Isolation-by-resistance incorporates aspects of graph theory and electrical circuit theory (i.e. electronic resistance) to predict spatial genetic structure in complex

landscapes (McRae, 2006; McRae, Beier, 2007; McRae *et al.*, 2008), and is implemented in the software CIRCUITSCAPE (McRae, Shah, 2009).

Instead of looking at one least-cost distance at a time, CIRCUITSCAPE evaluates all possible inter-patch paths at once. Multiple and wider conductors (paths) connecting electrical nodes (individuals or groups) allow for greater current flow (gene flow) than would a single, narrow conductor, and may better characterize potential movements across heterogeneous landscapes. Indeed, effective resistance distances correlate more strongly with genetic distances than least-cost distances in wolverines (*Gulo gulo*) in Idaho and Manitoba and big leaf mahogany (*Swietenia macrophylla*) in Central America (McRae, Beier, 2007).

Bayesian clustering

Bayesian clustering approaches group individuals into populations of random mating individuals that minimize Hardy-Weinberg and gametic disequilibrium across the dataset (Beaumont, Rannala, 2004; Dawson, Belkhir, 2001; Manel *et al.*, 2003; Pritchard *et al.*, 2000). Bayesian clustering methods search likelihood space using Markov chain Monte Carlo (MCMC) searches, so burn-in periods and other parameters must be carefully set to ensure convergence and avoid reaching local maxima instead of the global optimum (Beaumont, Rannala, 2004; Mank, Avise, 2004). Individuals of unknown natal localities can also be assigned to their most likely population of origin, allowing the estimation of migration rates and even dispersal distances, assuming the population of origin has been sampled (Cornuet *et al.*, 1999). Bayesian analyses have several advantages over F_{ST}-based analyses in studies of population structure because they identify individuals, are geographically explicit, and can incorporate genotyping error (Corander *et al.*, 2004; Kalinowski *et al.*, 2007; Lawson Handley, Perrin, 2007; Piry *et al.*, 2004).

However, different Bayesian clustering software programs require accepting different assumptions, and the use of more than one program may increase the accuracy of this approach (Excoffier, Heckel, 2006; Faubet *et al.*, 2007; Goncalves da Silva, 2007; Latch *et al.*, 2006). For example, the program PARTITION (Dawson, Belkhir, 2001) incorrectly estimates the number of population clusters at low levels of genetic differentiation ($F_{ST} < 0.09$; Latch *et al.*, 2006). BAPS (Corander *et al.*, 2004) and STRUCTURE (Falush *et al.*, 2003; Pritchard *et al.*, 2000) do slightly better, correctly estimating the number of population clusters until $F_{ST} < 0.04$ (Goncalves da Silva, 2007; Latch *et al.*, 2006). Different assumptions also apply to programs that use Bayesian clustering methods to identify recent migrants. BAYESASS (Wilson, Rannala, 2003) assumes low migration rates between population clusters. If this assumption is met by the study population, the program will correctly estimate migration rates, but only if $F_{ST} > 0.05$. If the assumption of low migration rates is violated, BAYESASS will only correctly estimate migration rates if $F_{ST} > 0.10$ (Faubet *et al.*, 2007). Another simulation study showed that several Bayesian clustering programs perform poorly when there is strong isolation-by-distance in the dataset (Safner *et al.*, 2011).

A general drawback to Bayesian approaches is the necessity of specifying prior parameter distributions, a problem similar to that of specifying populations *a priori* in frequency-based methods (Beaumont, Rannala, 2004; Mank, Avise, 2004). Researchers attempt to bypass this issue by specifying non-informative or uniform priors, often the default for many clustering software programs, which hold little or no prior information about the parameters. However, priors should be specified when there is concrete prior knowledge about parameters, and can be particularly useful when testing whether any individuals are migrants to their supposed

populations. When using priors, researchers should systematically examine the effects of different priors on the parameters estimated by the model (Beaumont, Rannala, 2004).

If a study population fits the assumptions of Bayesian clustering methods, they can be extremely useful in studies of habitat modification and landscape genetics. For example, Bayesian clustering methods outperformed other edge detection methods to correctly estimate the boundaries of spatially structured populations in a recent simulation study (Safner *et al.*, 2011). Also, many Bayesian clustering software programs now explicitly include geographic information (including STRUCTURE, BAPS, GESTE, TESS, and GENELAND), making them even more useful for landscape genetic questions (Chen *et al.*, 2007; Falush *et al.*, 2003; Foll, Gaggiotti, 2006; Francois, Durand, 2010; Frantz *et al.*, 2009; Guillot *et al.*, 2005).

Important considerations in landscape genetic studies

Sampling: Spatial and Temporal Scales

The success of landscape genetic analyses of fragmented populations largely depends on the scale at which samples were collected from the study population. In particular, the spatial and temporal scales at which populations are sampled have important effects on the correct inference of population genetic structure.

It is extremely important to identify the appropriate spatial scale at which to sample the population of interest in studies of habitat modification (Debinski, Holt, 2000; Segelbacher *et al.*, 2010). Many studies of habitat modification examine its effects at the patch scale instead of the landscape scale, rendering it impossible to make inferences regarding the effects of modification on landscape-scale movements among habitat patches (Fahrig, 2003). A recent study of dispersal in the western lowland gorilla (*Gorilla gorilla gorilla*) revealed an extremely different dispersal

pattern than suggested previously by studies conducted on smaller scales (Bradley *et al.*, 2004; Douadi *et al.*, 2007). There is general agreement that genetic samples should be taken at a spatial scale at least as large as the dispersal distance of the study species. However, landscape geneticists should also consider extended spatial scales given that they may not fully understand the scale of gene flow in their study species (Segelbacher *et al.*, 2010).

Also, results from a particular landscape are not necessarily translatable to a different landscape. Researchers should ideally consider multiple study areas with a range of variability in landscape features before projecting results from one landscape to another. Also, if landscape features are not found to influence gene flow in one landscape, researchers should not automatically conclude that those features are unimportant in all landscapes for a given species (Short Bull *et al.*, 2011).

The temporal scale of the study, in terms of time since a habitat modification event has occurred, is also critical. Especially in long-lived species, there can be a time lag in responses to modification (Brooks *et al.*, 1999). As such, if samples are collected too soon after initial habitat modification, one may infer from a genetic study that there are no effects of modification on the study population, even if there might be an effect sometime later. In general, samples should be collected several generations after habitat modification to correctly infer its effects on a study population. Simulations suggest 1-15 generations are necessary to detect barriers to gene flow using Mantel's *r* (Landguth *et al.*, 2010). However, samples could be collected immediately after or prior to a modification event if they are viewed as baseline data for future comparison with a sample taken a number of generations later.

The potential mismatch of temporal and spatial scales of landscape and genetic data may also be an issue. Genetic markers convey a combination of historical and current data, while

landscape data can be either historical or current. Genetic markers with high rates of subsitution such as microsatellites should correspond best with current, fine-scale landscape data (Balkenhol *et al.*, 2009b).

Non-invasive sampling

Non-invasive sampling techniques have made genetic sampling of rare and endangered species possible (DeSalle, Amato, 2004); however, DNA extracted from non-invasive samples is typically of low quality and thus comes with several drawbacks. In studies using microsatellite data, allelic dropout is a particularly important issue. One study finds that only 70% of non-invasively collected samples from great apes yield extracts with enough nuclear DNA to produce an accurate genotype (Vigilant, 2002). However, results often depend on the repeat type of the microsatellite marker being amplified (Broquet *et al.*, 2007) and pre-amplification methods (Piggott *et al.*, 2004). Many studies have had considerable success genotyping from non-invasive samples (Fernando *et al.*, 2003; Di Fiore, 2009; McGrew *et al.*, 2004; Miller *et al.*, 2005; Nsubuga *et al.*, 2004; Satkoski *et al.*, 2007; Vigilant, Bradley, 2004). In any study that uses non-invasively collected samples, thorough error-checking protocols should be used to prevent allelic dropout and other potential problems, including mixed sampling error and other types of contamination (Roon *et al.*, 2005).

Neutral versus adaptive genetic diversity

It is important to note that most landscape genetic studies use measures of neutral genetic diversity, not adaptive genetic diversity. Neutral genetic markers provide information about gene flow, migration, and dispersal without the bias of selective pressure (Holderegger *et al.*, 2006). Although there are theoretical connections between reduced dispersal and gene flow caused by

habitat modification and decreased fitness through inbreeding depression, the approaches discussed here only directly measure dispersal and gene flow, not fitness.

Measures of neutral genetic variation across species or populations do not consistently correlate with measures of adaptive genetic variation and should not be used to make arguments about a population's fitness (Reed, Frankham, 2001; Reed, Frankham, 2002). However, on an individual level, there is evidence that variation in neutral genetic diversity correlates strongly with adaptive genetic diversity (also called quantitative genetic diversity or Q_{ST}; Johansson *et al.*, 2007; Reed, Frankham, 2002). A recent study compares the neutral genetic diversity of the common frog *Rana temporaria* with the quantitative genetic diversity of the same species as measured in the laboratory in response to pesticide treatment. Tadpoles collected from fragmented populations show both lower neutral genetic diversity and lower fitness compared to tadpoles collected from continuous populations (Johansson *et al.*, 2007). Such studies support the theoretical connections between reduced gene flow and susceptibility to extinction in fragmented populations. However, it is important to remember that landscape genetic analyses measuring neutral genetic diversity cannot directly support this connection (Holderegger *et al.*, 2006).

However, as genomic data become more readily available, future landscape genetic studies could and should incorporate adaptive variation. Such studies could help answer critical questions about the evolution of populations and the consequences of global change (Holderegger, Wagner, 2008; Manel *et al.*, 2010).

Implications for Conservation Management

As habitat modification continues to threaten biodiversity worldwide, the landscape genetic approach will be extremely important in informing *in situ* conservation management of organisms in modified habitats. An increased focus on landscape genetic approaches will also

enhance our general understanding of persistence, ecology, and evolutionary processes in heterogeneous and human-dominated landscapes. Landscape genetic analyses will be most useful in identifying isolated populations and specific barriers to dispersal in order to inform science-based conservation management plans that include the placement of biological corridors and management of matrix habitats (Beier *et al.*, 2008).

However, when attempting to translate the results of any landscape genetic analysis to patterns of functional connectivity, it is important to recognize that gene flow does not equate to individual movement patterns. Simulations that model the sums of individual behavioral decisions will be necessary to best inform the conservation management of taxa in heterogeneous landscapes (Bowler, Benton, 2005; Knowlton, Graham, 2010; Lowe, Allendorf, 2010; Nabe-Nielsen *et al.*, 2010; Spear *et al.*, 2010; Tracey, 2006).

Future studies in landscape genetics may shift towards simulation approaches such as the method implemented in the software CDPOP (Landguth, Cushman, 2010). Using a simulation approach such as CDPOP might allow the creation of a predictive model of genetic population structure under different climate and landscape change scenarios (Balkenhol *et al.*, 2009b).

Goals and Organization of the Dissertation

In this dissertation, I describe population genetic structure and dispersal patterns in the Central American Squirrel Monkey (*Saimiri oerstedii*, Primates: Cebidae), an endangered New World primate living in a heterogenous, human-modified landscape.

S. oerstedii is one of the most vulnerable primates in Latin America and a top priority for conservation according to the IUCN Red List of Threatened Species (IUCN, 2010). The most recent survey estimates that there were 7,000 *S. oerstedii* remaining in 1995, of which only

1,500-1,700 represent the subspecies *S. o. citrinellus* in the Central Pacific region of Costa Rica (Boinski *et al.*, 1998; Boinski, Sirot, 1997; PRMVS, 1996; Sierra *et al.*, 2003). *S. oerstedii* live in groups of 22 to 66 or more individuals and their diet includes arthropods, flowers, fruits, and small vertebrates (Wong, 1990). *S. oerstedii* can be found only in the Pacific moist forests of Costa Rica and northern Panama below ~500m asl (Arauz, 1993; Boinski, 1999; Boinski *et al.*, 1998; Boinski, Sirot, 1997). This range area is characterized by frequent landscape disturbance from high rainfall, wind, hurricanes, and rugged topography, which in combination with the other factors leads to mudslides (Boinski, 1999; Boinski *et al.*, 2005; Wallace, 1997). Despite an already restricted distribution, *S. o. citrinellus* habitat has undergone considerable anthropogenic modification since the 1930s. Manuel Antonio National Park (MANP) is the only protected forest within the distribution of *S. o. citrinellus*. Outside the park, most forests and mangroves in the Central Pacific region (approximately 80%) were replaced with rice and banana plantations in the 1930s, and around 1948 were converted into oil palm plantations (Mattey, 1992; Mattey, 1994; PRMVS, 1996).

Thus, to manage *S. o. citrinellus* populations into the future, it is essential to understand how historical habitat loss, modification, and landscape heterogeneity has affected population structure in this taxon. However, very little work has been done to determine the effects of habitat loss and modification on the behavior, demography, or genetic structure of *S. oerstedii* populations. Thus far, the only published study attempting to characterize genetic diversity in *S. o. citrinellus* included eight samples from in and around MANP (Zaldivar *et al.*, 2004). Zaldivar et al. (2004) show a relatively high level of genetic variation in *S. o. citrinellus,* but their small and spatially restricted sample offers little information regarding population structure. A larger

sample collected across a wider range is necessary to determine the effects of habitat modification on *S. o. citrinellus* dispersal patterns, gene flow, and population genetic structure.

The goals of this dissertation were to address the following questions: 1) Is there genetic support for the subspecies distinction between *S. o. citrinellus* and *S. o. oerstedii*? 2) Is there population genetic structure within *S. o. citrinellus*? 3) Do male and female patterns of dispersal and population genetic structure differ in *S. o. citrinellus*? 4) Is there a relationship between landscape heterogeneity and genetic structure in *S. o. citrinellus*? and 5) How can genetic analyses inform the conservation management of this endangered taxon?

To accomplish these goals, I collected an extensive number of non-invasive genetic samples and fine-scale landscape data, and analyzed these data using the methods of population genetics, molecular systematics, and landscape genetics. Chapter 2 explores whether there is molecular genetic support for the subspecies distinction between *S. o. citrinellus* and *S. o. oerstedii* and describes population genetic structure and recent migration patterns within *S. o. citrinellus*. Chapter 3 compares population genetic structure among males versus females to test for sex-biased dispersal patterns in *S. o. citrinellus*. Chapter 4 uses landscape genetic approaches to explore the relationship between landscape heterogeneity and genetic structure in *S. o. citrinellus* and infer which matrix habitats are costly to dispersal. Chapter 5 offers explicit management recommendations for the conservation of *S. oerstedii* based on the results from previous chapters, and Chapter 6 offers a summary of conclusions from each chapter and final thoughts about the implications of this dissertation for future studies.

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CHAPTER 2.

Population genetic structure with recent dispersal across the fragmented range of the Central American Squirrel Monkey (*Saimiri oerstedii citrinellus)*

Abstract

I estimated an intraspecific molecular phylogeny and examined population genetic structure of the endangered Central American Squirrel Monkey (*Saimiri oerstedii*) in Costa Rica. I collected fecal samples non-invasively from 244 individuals and analyzed them for 16 microsatellite markers and 880bp of the mtDNA d-loop. I found moderate levels of genetic diversity and significant population genetic structure. Within the subspecies *S. o. citrinellus*, I inferred two geographically separate genetic clusters using the Bayesian clustering programs STRUCTURE and BAPS, with evidence of recent dispersal among clusters. These clusters correspond to two mtDNA d-loop haplogroups identified in a median-joining network and phylogenetic analyses. In addition, I found statistically significant pairwise F_{ST} values among populations (0.09) and among 14 social groups within populations (mean = 0.10, range = 0.016 - 0.19), and significant variance among all hierarchical levels tested using AMOVAs. Microsatellite and mtDNA data support the two currently recognized subspecies of *S. oerstedii* as evolutionarily significant units (ESUs), but not the two disjunct populations of *S. o. citrinellus*. Conservation management should monitor and promote dispersal to and from the western population of *S. o. citrinellus*, which is more isolated and less genetically diverse than the eastern population.

Introduction

Many species exist in spatially structured populations linked by dispersal and gene flow, which can influence evolutionary, demographic, and ecological processes (Hanski, Gilpin, 1997; MacArthur, Wilson, 1967; Wright, 1951). Studies of population genetic structure are important to conduct for species living in complex, heterogeneous landscapes, which may affect that structure (Bergl, Vigilant, 2007; Goossens *et al.*, 2005; Keyghobadi *et al.*, 1999; Liu *et al.*, 2009; Manel *et al.*, 2003; Wang *et al.*, 2009).

Understanding population genetic structure is also critical to inform conservation management (Simberloff, 1988). Conservation managers need to measure the extent and distribution of genetic diversity to accurately predict population persistence, especially for small, fragmented populations (Coulon *et al.*, 2004; Frankham, 2006; Frankham *et al.*, 2002; Lacy, 1997; Lande, 1995; Lande, Barrowclough, 1987; Sherwin, Moritz, 2000). It is particularly valuable to study spatially structured populations to better understand processes of human-induced rapid evolutionary change (Smith, Bernatchez, 2008; Stockwell *et al.*, 2003). In socially diverse organisms, like primates, genetic information is especially crucial as these organisms may respond to disturbance and changes in habitat in many different ways (Cowlishaw, Dunbar, 2000).

Although many primates inhabit old-growth forests, others are found in naturally disturbed habitats with extreme topography, high rainfall, and hurricanes. Species living in these habitats may exhibit underlying historical genetic structure from natural barriers (Melnick *et al.*, 1993). Alternatively, anthropogenic habitat fragmentation may occur at spatial and temporal scales that differ from natural fragmentation, potentially causing further population fragmentation in the affected species (Frankham, 2006; Frankham *et al.*, 2002; Mills, Tallmon,

1999). Species that have historically occupied landscapes with natural barriers may also have certain characteristics that allow them to maintain high dispersal rates despite a discontinuous habitat (Debinski, Holt, 2000; Kareiva, 1987; Villard, 2002).

Primatologists have genetically studied dispersal for over 30 years to describe the complex social systems of many primates (Cheney, Seyfarth, 1983; Cheverud *et al.*, 1978; Di Fiore, Fleischer, 2005; Eriksson *et al.*, 2006; Huck *et al.*, 2007; Melnick, Hoelzer, 1992; Melnick, Kidd, 1983; Packer, 1979). However, studies of genetic patterns of dispersal and structure at the landscape level are more limited (as reviewed in Di Fiore, 2003; Di Fiore, 2009). Further, Bayesian approaches to detecting population genetic structure were uncommon in studies of nonhuman primates until quite recently (Bergl, Vigilant, 2007; Ghobrial *et al.*, 2010; Liu *et al.*, 2009; Quemere *et al.*, 2010). Individual-based Bayesian models are particularly useful for studies of potentially structured populations (Beaumont, Rannala, 2004; Manel *et al.*, 2003; Safner *et al.*, 2011) because they allow the inference of structure with greater precision and without *a priori* information about population membership. Traditional population genetics, by contrast, relies on idealized population models and summary statistics (Corander *et al.*, 2004; Kalinowski *et al.*, 2007; Lawson Handley, Perrin, 2007; Piry *et al.*, 2004).

In this study, I examine the population genetic structure of the Central American Squirrel Monkey (*Saimiri oerstedii*), with a focus on the endangered subspecies *S. o. citrinellus* in the Central Pacific region of Costa Rica (IUCN, 2010). *S. oerstedii* live in troops of 22 to 66 or more individuals and can only be found in the Pacific moist forests of Costa Rica and northern Panama below ~500m altitude (Arauz, 1993; Boinski, 1999; Boinski *et al.*, 1998; Boinski, Sirot, 1997; PRMVS, 1996; PRMVS, 2002; Wong, 1990). Frequent landscape disturbances characterize this area, including high rainfall, wind, hurricanes, and rugged topography (Boinski, 1999; Boinski *et*

al., 2005; Wallace, 1997). A study based on interviews estimated there were 200,000 *S. oerstedii* in 1983 (Vaughan, 1983), but the most recent survey estimated that there were only 7,000 *S. oerstedii* remaining in 1995, of which only 1,500-1,800 belong to the subspecies *S. o. citrinellus* (Sierra *et al.*, 2003). Their total distribution covers an area of approximately 1500km^2 (Arauz, 1993). In the 1930s, banana, oil palm, rice plantations, and cattle pasture replaced 80% of the forest in this area (Mattey, 1992; Mattey, 1994).

To date, the only study attempting to characterize genetic diversity in *S. o. citrinellus* included eight samples (Zaldivar *et al.*, 2004) from in and around Manuel Antonio National Park, the smallest national park in Costa Rica (ICT, 2005) and the only protected area in its range. Zaldivar *et al.*'s small and spatially restricted sample offers little information regarding population structure. There is also a dirth of information about the effects of a naturally heterogeneous landscape or anthropogenic habitat modification on population structure in any taxon in the Central Pacific region of Costa Rica.

The two subspecies of *S. oerstedii* have been recognized for some time (Hershkovitz, 1984; Rylands, Mittermeier, 2008) and can be distinguished by their geographic separation on either side of the large Térraba River (Figure 2.1; Arauz, 1993), and by slight differences in size and coloration (Carrillo *et al.*, 2002; Hershkovitz, 1984). There is limited support from genetic data (Boinski, Cropp, 1999; Cropp, Boinski, 2000) that the subspecies represent Evolutionarily Significant Units (ESUs), or monophyletic groups of genetically differentiated populations within a larger monophyletic species (Ryder, 1986; Vogler, Desalle, 1994). ESUs are often characterized by reciprocal monophyly at mitochondrial (mtDNA) loci and significant divergence of allele frequencies at nuclear loci (Moritz, 1994). Crandall *et al.* (2000) suggest that ESUs should be defined more broadly using the concepts of genetic and ecological

exchangeability. Exchangeability is rejected when there is evidence for ecological or genetic differentiation between populations, and those populations should be recognized as separate ESUs (Crandall *et al.*, 2000). Confirming the distinctiveness of units within a species is essential to informed conservation management (Cracraft, 1994; May, 1990). ESUs should be prioritized in conservation planning because they are historically isolated lineages that cannot be recovered if lost (Moritz, 2002).

To examine population genetic structure in *S. o. citrinellus*, I collected fecal samples non-invasively from across their distribution in Costa Rica, and analyzed them for 16 microsatellite markers using Bayesian clustering in addition to traditional population genetic methods, including a comparison of allelic diversity between populations and an analysis of molecular variance (AMOVA). I also inferred an intra-specific molecular phylogenetic tree and a median-joining haplotype network with mtDNA d-loop data. I use the results from both mtDNA and autosomal markers to infer ESUs for *S. oerstedii* following Moritz (1994) and Crandall *et al.* (2000).

Methods

Sampling and DNA extraction

Fecal samples were collected non-invasively from *S. o. citrinellus* in the Central Pacific region of Costa Rica from September 2008 – April 2009 (sampled sites were separated by 1 – 49 km; Figure 2.2). From 304 fecal samples, I successfully extracted DNA, genotyped, and verified genotypes for 233 individuals, comprised of 10 to 20 adult individuals from each of 14 groups. The average size of sampled groups was 39 individuals (range 18 – 67). Whenever possible (N=13 groups), more than 10 individuals per group were sampled to increase the precision of

genetic analyses in detecting dispersal and migration (Goudet *et al.*, 2002). Date, time, group composition, and GPS location (at an error of less than 10m) were recorded for each sample. When possible, the sex of the sampled individual was recorded at the time of sample collection, but all sex identifications were confirmed using a PCR-based sexing assay upon return to the laboratory (Di Fiore, 2005). Samples were stored in 8 ml plastic tubes with *RNAlater* buffer (Ambion) at -4^{o}C in the field and -20˚C in the laboratory. Eleven additional DNA samples from individuals of the southern subspecies *S. o. oerstedii* were contributed by G. Gutierrez (University of Costa Rica).

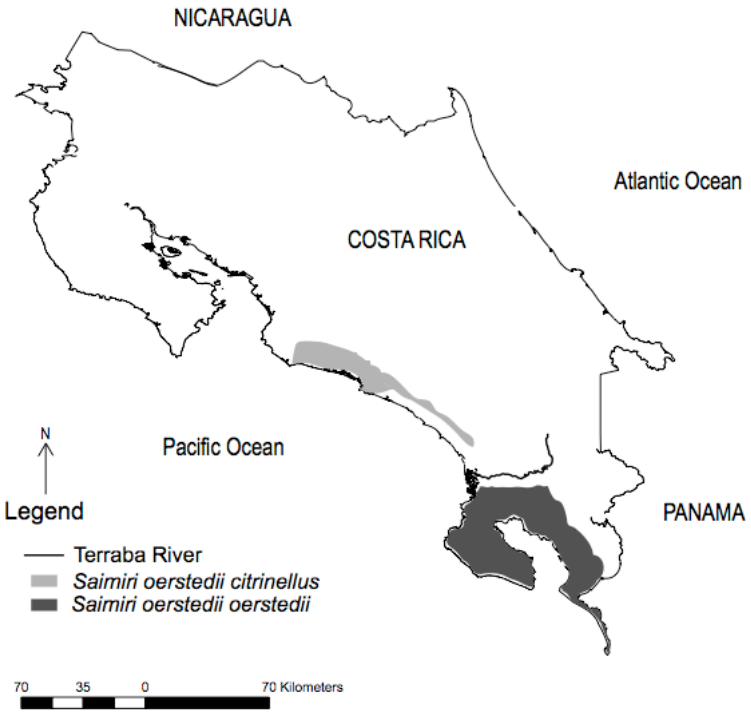

Figure 2.1. Ranges of both subspecies of *S. oerstedii* in Costa Rica, following Arauz (1993).

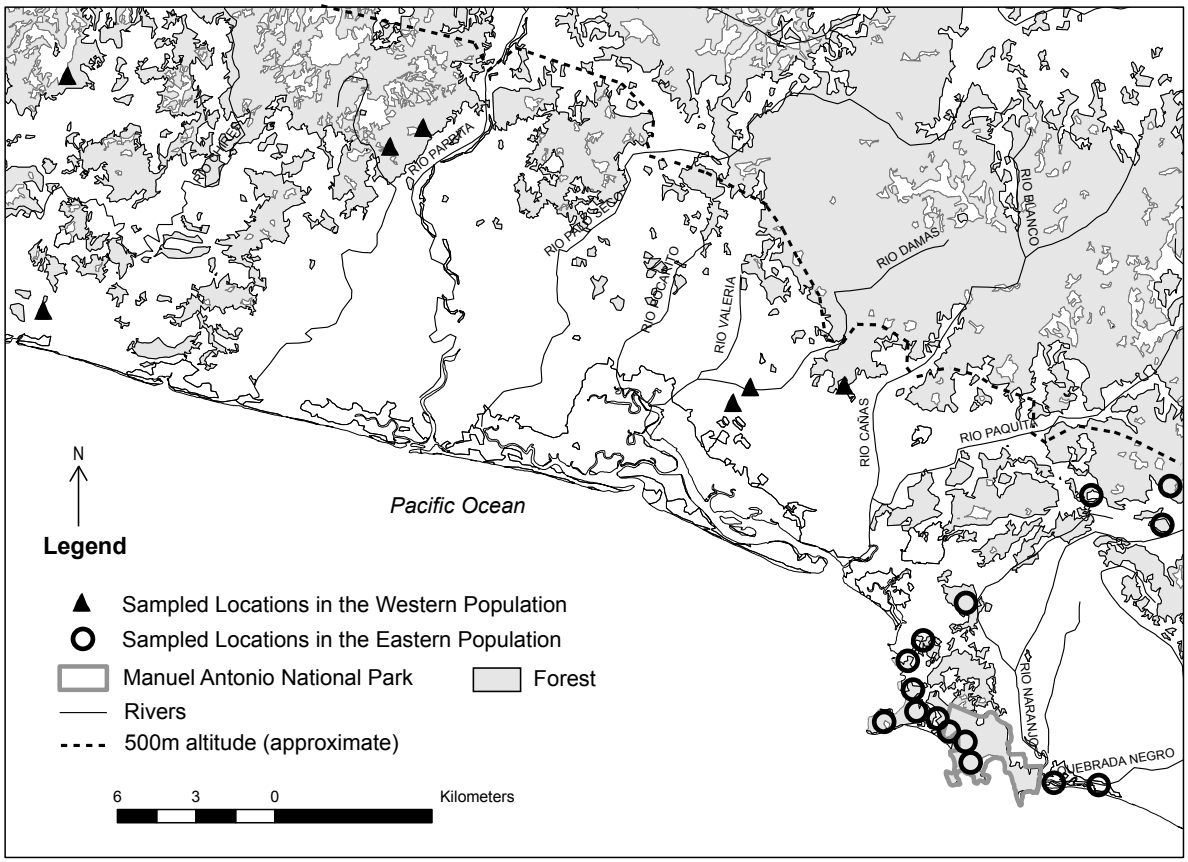

Figure 2.2. Sampled locations of *S. o. citrinellus* in the Central Pacific region of Costa Rica, showing the putative limit of 500m altitude to their distribution. Forest data are based on land cover data from the year 2000 generated by EOSL, CCT and FONAFIFO (2002) with Landsat 7 TM satellite imagery.

All labwork was carried out at the Molecular Primatology Laboratory at New York University. Total DNA was extracted using QIAamp DNA Stool Minikits (Qiagen) with minor modifications to the manufacturer's protocol "Isolation of DNA from Stool for Human DNA Analysis" (July, 2007) to increase DNA yield from nonhuman primate fecal samples (step 2: extended for 3-20 hours, step 6: extended to 6 min, step 8: increased to 35 μl proteinase K, step 11: extended to 30 min, and step 19: incubation extended to 15 min). After extraction, I used a real-time quantitative PCR method to quantify the amount of primate nuclear DNA in each sample. I used iQ SYBR Green Supermix (Bio-Rad) in 15 μl reactions using 1.5 μl template

DNA and with universal primate primers amplifying a 2-300bp length of nuclear DNA (F 5'-GCCAGAGGAGGAACGAGCT -3', R 5'- GGGCCTTTTCATTGTTTTCCA -3'; Morin *et al.*, 2001). I used the standard nuclear quantification protocol provided with the iQ5 2.0 Optical System (Bio-Rad) against two standard samples of 1 ng/μl and 10 ng/μl DNA. Samples with greater than 0.5 ng/μl DNA concentrations (averaged over two replicate runs) were used in genotyping analyses. Negative controls were used at every step.

Microsatellite genotyping and mtDNA sequencing

Seventeen autosomal microsatellite markers were PCR amplified in multiplex panels of three or four markers. These markers were isolated from several different taxa [*Callithrix*: CJ7 (Nievergelt *et al.*, 1998), *Leontopithecus*: Leon 15, Leon 21 (Perez-Sweeney *et al.*, 2005), Lr.P2BH6 (Grativol *et al.*, 2001), *Lagothrix*: LL 1-1#18, LL 1-5#7, LL 3-1#1 (Di Fiore, Fleischer, 2004), *Saguinus*: SB38 (Bohle, Zischler, 2002), human: D13s160, D17s804, D3s1210, D3s1229, D3s1766, D4s111, D5s111, D8s165, D8s260 (ResGen, Invitrogen Corp.)]. All markers are dinucleotide repeats except LL 3-1#1, which is a trinucleotide repeat, and D3s1766, which is a tetranucleotide repeat. Markers were amplified in 5 μl reactions using Multiplex PCR Kits (Qiagen) consisting of 1 μl template DNA (of greater than 0.5 ng/μl DNA), 2.5 μl 2x Multiplex PCR Master Mix (Qiagen), and a final concentration of each primer at 0.1 μ_M. Amplification conditions were: initial denaturation at 95˚C for 15 min; 37 cycles of 30 s at 94˚C, 1 min 30 s at an annealing temperature of 55˚C, 1 min at 72˚C; final extension of 30 min at 60˚C. PCR products were electrophoresed on an ABI 3730 DNA Analysis System with the size standard GENESCAN 500 ROX. Genotypes were called using GeneMapper software (ABI). Because allelic dropout is often a problem when amplifying microsatellite markers from fecal samples (Broquet *et al.*, 2007; DeSalle, Amato, 2004; Piggott *et al.*, 2004; Roon *et al.*, 2005; Vigilant,

2002), heterozygous genotypes were confirmed by scoring alleles at least four times and homozygous genotypes at least seven times. In addition, I ran MICROCHECKER (van Oosterhout *et al.*, 2004) to test for null alleles. One of the 17 markers was found to possibly contain null alleles (D13s160) and this marker was removed from the analysis, for a total of 16 markers. I tested for linkage disequilibrium and deviations from Hardy-Weinberg equilibrium (HWE) across markers in ARLEQUIN v 3.1 (Excoffier *et al.*, 2005).

I sequenced a geographically representative subset of individuals (N=43 *S. o. citrinellus* and N=7 *S. o. oerstedii*) for 880 bp of the mtDNA d-loop, a section that falls within approximately 15,500-16,400 bp in the whole mtDNA genome according to the human reference sequence. Sections of the d-loop mutate faster than any other part of the mtDNA genome, making them especially useful for studies of geographic differences among populations of the same species (Andayani *et al.*, 2001; Aquadro, Greenberg, 1983; Vigilant *et al.*, 1989). To reduce the chance of amplifying nuclear pseudogenes, I initially produced long range amplicons of 2500-7000 bp for all *S. o. citrinellus* samples included in the analysis, in addition to species-specific d-loop primers (Thalmann *et al.*, 2004). I also compared the sequences to whole mtDNA genomes generated for *S. o. oerstedii* and *S. o. citrinellus* (Chiou *et al.*, 2011), and confirmed monophyly of *S. oerstedii* sequences by comparing them to d-loop sequences from *S. o. oerstedii* and other *Saimiri* species. Two outgroup d-loop sequences from GenBank were also included for phylogenetic analyses (*Cebus albifrons* and *S. sciureus*, Table 2.1).

Long range PCRs were amplified in 25 μl reactions using the Expand Long Template PCR System (Roche) with 2.5 μl template DNA and Buffer 1 (with species specific primers developed for *S. oerstedii* from Chiou *et al.*, 2011). Amplification conditions were: initial denaturation at 93˚C for 3 min; 50 cycles of 15 s at 93˚C, 30 s at an annealing temperature of

58˚C (decreased by 0.1˚C each cycle), 4 min at 68˚C; 30 cycles of 15 s at 93˚C, 30 s at 53˚C, 3 min at 68˚C; final extension of 4 min at 68˚C. Before sequencing mtDNA, amplification products were purified of excess nucleotides, primers, enzymes and other leftover PCR reagents using the ExoSAP-IT protocol (USB Corp.). PCR products were cycle-sequenced with ABI BigDye 3.1 Terminator Ready Reactions kits using species-specific d-loop primers (Chiou *et al.*, 2011) and electrophoresed on an ABI 3730 DNA Analysis System. I carried out base calling using Sequencing Analysis v 5.2 (ABI), verified base calls by eye and assembled sequences using the software Sequencher v 4.7 (Gene Codes Corp.). Sequences were aligned with CLUSTAL W2 (Chenna *et al.*, 2003; Thompson *et al.*, 1994) and locations of incorrectly placed gaps were edited by eye in MacClade (Maddison, Maddison, 1992). Sequences included in subsequent analyses were verified with triple or quadruple coverage in both directions.

Table 2.1. Mitochondrial d-loop sequences included in the analyses and their corresponding GenBank Accession numbers. All Central American Squirrel Monkey sequences were produced in this study.

Outgroups
Cebus albifrons (pale-fronted capuchin, AJ309866)
Saimiri sciureus (common squirrel monkey, AB371091)
Central American Squirrel Monkey
Saimiri oerstedii oerstedii (HQ906794-906800)
Saimiri oerstedii citrinellus (HQ906788-906793, HQ906801-906837)

Data analyses

Population genetic structure of microsatellite data

I ran the multilocus genotypes in STRUCTURE v 2.2 (Falush *et al*., 2003) and BAPS v 2 (Corander *et al.,* 2004) to identify the number of genetic clusters among the samples using a Bayesian model. Because different Bayesian clustering software programs require accepting different assumptions, I used more than one program in order to increase the accuracy of this

approach (Excoffier, Heckel, 2006; Faubet *et al.*, 2007; Goncalves da Silva, 2007; Latch *et al.*, 2006). BAPS and STRUCTURE do slightly better, correctly estimating the number of population clusters until $F_{ST} < 0.04$ (Goncalves da Silva, 2007; Latch *et al.*, 2006).

I ran 10 independent iterations of K=1-16 in STRUCTURE for 2,000,000 Markov Chain Monte Carlo (MCMC) generations with a 200,000 burn-in period, assuming correlated allele frequencies and admixture. I identified K using the ΔK method (Evanno *et al.*, 2005), where optimum K has the highest ΔK value, or rate of change in the log probability of the data between successive K-values. I ran BAPS with the default settings (stochastic optimization) also for 10 separate iterations for a maximum K of 1-21. Both programs were run without spatial information.

I detected first generation migrants and admixed individuals using STRUCTURE and GENECLASS v 2.0 (Cornuet *et al.*, 1999; Piry *et al.*, 2004). I chose to use a combination of methods because STRUCTURE assumes all potential source populations have been sampled, which can lead to mis-assignment of migrant individuals (Bergl, Vigilant, 2007). To detect first generation migrants in STRUCTURE, I ran the program using the cluster memberships inferred as described above using the ΔK method as prior population information. I conducted several runs using a range of values for MIGRPRIOR (0.001- 0.1) following Pritchard *et al.* (2000). Because choice of MIGRPRIOR did not significantly affect program outputs, I present results from MIGRPRIOR = 0.09, the average migration rate between populations of *S. o. citrinellus* found using the software BAYESASS (Wilson, Rannala, 2003), following Liu *et al.* (2009). Burn-in and run length were the same as earlier runs of STRUCTURE without prior population information. I also performed an exclusion test and used the 'Detect first generation migrants' option in GENECLASS (Paetkau *et al.*, 2004; Piry *et al.*, 2004), using both L_h and L_h/L_{max},

which represent, respectively, the most appropriate statistic when all potential source populations have not been sampled and when they have (Paetkau *et al.*, 2004). The probability of individual genotypes coming from each population was calculated by comparing individual genotypes to 10,000 simulated individuals per population (Paetkau *et al.*, 2004).

I also examined genetic structure in the *S. o. citrinellus* microsatellite data with a locus-by-locus analysis of molecular variance (Excoffier *et al.*, 1992), implemented in ARLEQUIN v 3.1 (Excoffier *et al.*, 2005). Locus-by-locus AMOVAs are useful when sample sizes for collection sites are small (Michalakis, Excoffier, 1996). Permutation tests (of 10,000 iterations) were carried out at four hierarchical levels for the *S. o. citrinellus* data: among populations, among groups within populations, among individuals within groups, and within individuals. I performed pairwise tests for differentiation among groups and populations using F-statistics (Wright, 1978) calculated with Weir and Cockerham's (1984) estimators in FSTAT v 2.9 (Goudet, 2001; Weir, Cockerham, 1984), for 10,000 randomizations not assuming HWE. I also calculated allelic diversity at each microsatellite marker for each population of *S. o. citrinellus* and tested the differences using the likelihood G test of genotypic differentiation implemented in FSTAT, for 10,000 randomizations not assuming HWE (Goudet *et al.*, 1996). For this analysis, I defined populations for *S. o. citrinellus* using the results from the Bayesian clustering analyses.

Analyses of mtDNA data

I estimated mtDNA sequence divergence among subspecies, populations of *S. o. citrinellus*, and groups within populations of *S. o. citrinellus* by comparing the number of fixed differences and shared substitutions, and the average nucleotide substitutions and number of net substitutions per site (D_{xy} and D_a, respectively, with Jukes-Cantor correction), using DNASP v 5.1 (Librado, Rozas, 2009). A permutation test (10,000 randomizations) of genetic differentiation

was also carried out using the nearest-neighbor statistic (S_{nn}) implemented in DNASP. S_{nn} estimates how often the most similar sequences in a dataset are from the same population.

Phylogenetic relationships among mtDNA sequences were inferred using ML analyses implemented in PALM (Chen *et al.*, 2009) and Bayesian analyses implemented in MrBayes v 3.1.2 (Ronquist, Huelsenbeck, 2003). The model of sequence evolution was chosen using the Akaike Information Criterion (AIC), which compared 56 models in PALM, and 28 models for Bayesian analyses in MrModeltest v 2.3 (Nylander, 2004). Bootstrap values for ML were calculated with 1000 replicates, with a 50% consensus level, and the remaining parameters at default settings in PALM. In the Bayesian analyses, four Markov Chain Monte Carlo (MCMC) chains were run for 1,000,000 generations, sampled every 100 generations, with 25,000 samples discarded as the burn-in. I also ran an ML analysis in PAUP 4.0 and found very similar results to those from PALM (data not shown; Swofford, 2004).

I inferred a haplotype network of the mtDNA sequences using a median-joining algorithm (Bandelt *et al.*, 1999) implemented in NETWORK v 4.5 (Fluxus) with an epsilon value = 0 and all variable sites weighted equally. I defined haplogroups as groups of haplotypes with 4 or more shared substitutions.

I examined genetic structure in *S. o. citrinellus* mtDNA sequences using a standard AMOVA for haplotype data in ARLEQUIN v 3.1 (Excoffier *et al.*, 2005). Permutation tests of 10,000 iterations were carried out at three hierarchical levels: among populations of *S. o. citrinellus*, among groups within populations, and among individuals within groups (a level for within individuals is not possible in an haplotype AMOVA where only one marker is included).

Finally, I analyzed the mtDNA data using the discrete character-based methodology of Population Aggregation Analysis (Davis, Nixon, 1992). Population Aggregation Analysis groups

taxa together based on the presence of shared, fixed traits such that they are diagnosably distinct from one another (Cracraft, 1983). To evaluate the *a priori* hypothesis that *S. o. citrinellus* and *S. o. oerstedii* are distinct units, a character matrix was generated from mtDNA sequences using MacClade v 4 (Sinaur Associates Inc.). The matrix was then screened for the presence or absence of fixed and alternate character differences among putative units.

Population demographic history

I tested for genetic signatures of a recent population bottleneck in the microsatellite data of *S. o. citrinellus* using BOTTLENECK (Piry *et al.*, 1999). I tested the data under the Infinite Alleles Model (IAM), the Stepwise Mutation Model (SSM), and the Two Phase Model (TPM) with 10,000 replications using a sign test. The sign test compares observed and expected heterozygosity excess. If excess is higher than expected (based on equilibrium) for a large majority of markers in a population, the population may have recently experienced a genetic bottleneck (Cornuet, Luikart, 1996; Luikart *et al.*, 1998; Luikart, Cornuet, 1998; Piry *et al.*, 1999).

Results

I successfully extracted DNA, genotyped, and verified the genotypes of 233 individuals from 14 groups of *S. o. citrinellus* at 16 microsatellite markers. Eleven individuals from *S. o. oerstedii* were successfully genotyped for a total sample of 244 individuals (Table 2.2). I included no genotypes in the analyses with missing data, and found no evidence for linkage disequilibrium across markers. I did find violations of Hardy-Weinberg equilibrium (HWE) when samples were analyzed at the species and population levels. These results are consistent with a Wahlund effect, or disequilibrium caused by treating several separate populations as one

(Wahlund, 1928), arising from the presence of population substructure (Goossens *et al.*, 2005). At the level of the group, one marker in each of three groups (D3s1766 at Gamalotillo, Leon21 at Chirraca, and Leon15 at MANP) was significantly out of HWE. Such mild deviations from HWE are expected given the likely presence of related individuals in the sample (Bergl, Vigilant, 2007; Bourgain *et al.*, 2004; Lukas *et al.*, 2004).

Bayesian clustering

Across the 10 independent runs in STRUCTURE and BAPS, ΔK was maximized at K = 4 (Figure 2.3,2.4). K=2,3, or 4 likely represent the most probable number of clusters within the sample, but not K > 4 (Figure 2.3). For K=4, one cluster represents samples from the subspecies *S. o. oerstedii*. The other three clusters are within *S. o. citrinellus*. The first cluster within *S. o. citrinellus* includes almost all individuals from western groups. The other two clusters do not seem to be geographically separated and include mostly members from eastern groups (Figure 2.4). The two eastern clusters likely represent ancestral polymorphism that has not been sorted out in this population. For K=3, one cluster included all of the samples from the subspecies *S. o. oerstedii*, one cluster includes almost all individuals from western groups of *S. o. citrinellus*, and one cluster includes mostly individuals from eastern groups of *S. o. citrinellus*. K=2 separates the subspecies *S. o. oerstedii* and *S. o. citrinellus.* I defined a western population and an eastern population of *S. o. citrinellus* based on the strong geographic clustering inferred using STRUCTURE and BAPS.

Table 2.2. Microsatellite markers amplified in 244 *S. oerstedii* samples.

Marker	Repeat Type	No. of Alleles	Size Range	H_o	Reference
CJ7	di	11	130-150	0.668	Nievergelt *et al.*, 1998
D17s804	di	18	132-202	0.414	ResGen Human MapPair
D3s1210	di	7	117-131	0.119	ResGen Human MapPair
D3s1229	di	18	84-132	0.652	ResGen Human MapPair
D3s1766	tetra	8	187-226	0.557	ResGen Human MapPair
D4s111	di	18	130-168	0.398	ResGen Human MapPair
D5s111	di	7	155-179	0.402	ResGen Human MapPair
D8s165	di	13	137-163	0.467	ResGen Human MapPair
D8s260	di	10	219-241	0.676	ResGen Human MapPair
Leon15	di	7	262-280	0.488	Perez-Sweeney *et al.*, 2005
Leon21	di	14	326-386	0.480	Perez-Sweeney *et al.*, 2005
LL118	di	14	110-158	0.373	Di Fiore and Fleischer, 2004
LL157	di	10	207-239	0.443	Di Fiore and Fleischer, 2004
LL311	tri	31	212-317	0.730	Di Fiore and Fleischer, 2004
Locus5	di	9	102-118	0.316	Grativol *et al.*, 2001
SB38	di	6	133-145	0.475	Bohle and Zischler, 2002

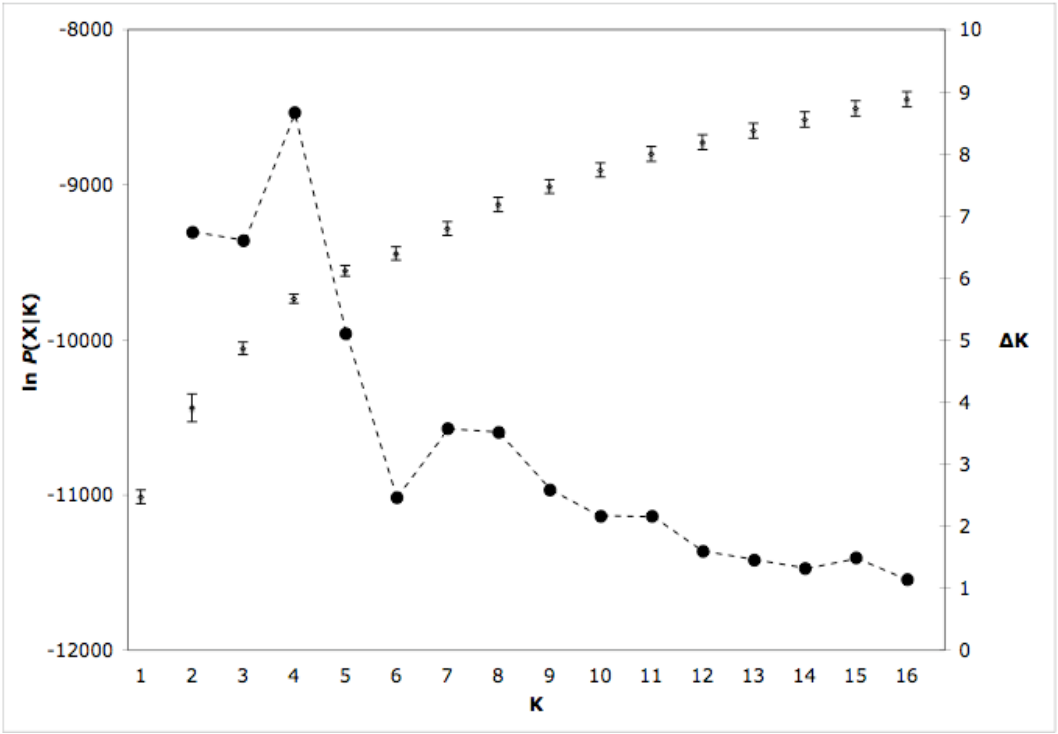

Figure 2.3. Inference of the number of genetic clusters (K) estimated using STRUCTURE. Both ln P(X | K) (the likelihood of the data given K; small diamonds) and ΔK (the standardized second order rate of change of ln P(X | K); black circles) are plotted as a function of K. Error bars of ln P(X | K) represent standard deviations.

Migration and admixture analysis

I looked for recent migrants (the product of current dispersal) and potentially admixed individuals (the product of past dispersal) between the western and eastern populations of *S. o. citrinellus*. I did not look for admixture or recent migration of individuals between the two eastern population clusters, since they are not separated geographically. Analyses using STRUCTURE and GENECLASS estimated 7 likely migrants and 10 potentially admixed individuals (Table 2.3). STRUCTURE estimated 1 potential migrant (individual G1, P=0.019), while GENECLASS estimated the same individual as a migrant in addition to 6 others (P<0.01) using both likelihood methods (L_h and L_h/L_{max}). These 7 migrants were assigned to their non-origin cluster in GENECLASS and also had lower probabilities of belonging to their origin cluster compared to other individuals in STRUCTURE, although these differences were not significant (Table 2.3).

In STRUCTURE, potentially admixed individuals do not assign with the majority of individuals from their sampled cluster and therefore have values of Q that indicate membership in more than one cluster. I used the ranking and plotting approach of Beaumont *et al.* (2001) to delineate a set of samples that did not clearly group into any one cluster (Figure 2.5). There were breaks in mean Q-values at Q = 0.2 and 0.8, similar to Bergl and Vigilant (2007) and Liu *et al.* (2009). I therefore defined individuals with mean Q-values from 0.2 to 0.8 as potentially admixed (Beaumont *et al.*, 2001; Bergl, Vigilant, 2007; Lecis *et al.*, 2006; Liu *et al.*, 2009; Vaha, Primmer, 2006). There were 20 individuals with mean Q values between 0.2 and 0.8. Ten of these individuals also had lower probabilities of the individual belonging to the origin cluster as estimated in STRUCTURE, and were assigned to >1 cluster with a high probability (>0.2) of assignment to a cluster other than the origin in GENECLASS (Table 2.3). In one case (individual

K1), an individual had low probability of being in either cluster as estimated by GENECLASS, but STRUCTURE identified it as a potential migrant (*P*=0.046). Thus, I interpreted this individual as potentially admixed, or a potential migrant from a ghost population. Of the likely migrants, 5 are male and 2 are female. Ten to 40 km separated sampled sites and inferred populations of origin.

AMOVA of microsatellite data within S. o. citrinellus

The microsatellite data yielded evidence of significant population structure (Table 2.4). Although the highest percentage of variation is within individuals, a significant percentage of genetic variation was found at all hierarchical levels, with the second largest percentage of variation among populations of *S. o. citrinellus.*

Table 2.4. Locus-by-locus AMOVA of 16 microsatellite markers for 233 *S. o. citrinellus* individuals from two populations and 14 groups, 10,000 iterations

Variance component	**Percentage of total variation**	***P***
Among populations	0.081	<0.00001
Among groups within populations	0.057	<0.002
Among individuals within groups	0.037	<0.00001
Individuals within the total sample	0.825	<0.0005

Pairwise F_{ST} of microsatellite data within S. o. citrinellus

Pairwise F_{ST} values among groups of *S. o. citrinellus* ranged from 0.016 - 0.19, with a mean of 0.103 (Table 2.5). Pairwise F_{ST} values among groups from the same population (mean = 0.06, range 0.016 - 0.11) were smaller than pairwise F_{ST} values among groups from different populations (mean = 0.14, range 0.070 - 0.19, Table 2.5). Consistent with the AMOVA results, there was significant population differentiation between populations of *S. o. citrinellus* (F_{ST} = 0.0903, P = 0.05)

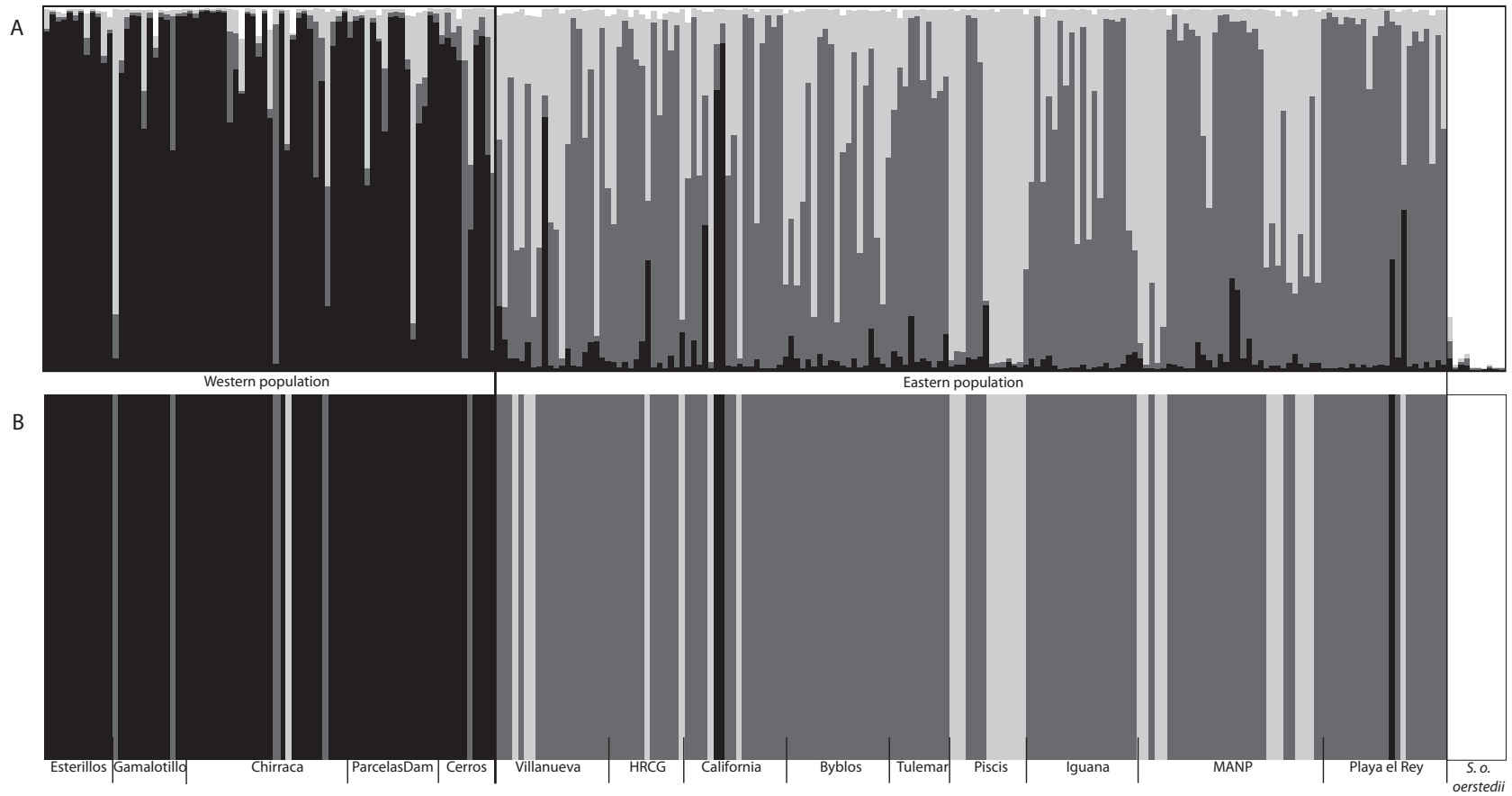

Figure 2.4. Distribution of four genetic clusters estimated in STRUCTURE (A) and BAPS (B). Vertical lines are broken into colored segments showing the proportion of each individual assigned to each K (within *S. o. citrinellus*: western cluster – black, eastern cluster 1 – light grey, eastern cluster 2 – grey; *S. o. oerstedii* – white). Sample locations are listed at the bottom of the figure, and are arrayed in west to east order (left to right).

Table 2.3. Results of migrant detection analyses.

	Geographic origin	STRUCTURE *Q* (N/S clusters)	GENECLASS cluster of highest probability assignment-exclusion test	GENECLASS highest assignment probability	GENECLASS F_0 migrant probability (L_h;L_h/L_{max} indicated with ^, **P*<0.01)	STRUCTURE probability belongs to origin cluster	Final migrant (M) / admixture (AD) classification
G1	Western	0.034/0.964	Eastern	0.0575	0.9943^*	0.019	M
O16	Western	0.033/0.963	Eastern	0.8108	0.9994^*	0.061	M
PD7	Western	0.086/0.891	Eastern	0.0491	0.9949^*	0.743	M
O6	Western	0.595/0.402	Eastern	0.7748	0.9947^*	0.828	M
O2	Western	0.388/0.608	Western/Eastern	0.1092/0.2380	0.9866	0.506	AD
K1	Western	0.018/0.979	Western/Eastern	0.0532/0.0817	NS	0.046	AD
PD8	Western	0.680/0.307	Western/Eastern	0.8376/0.7533	NS	0.877	AD
K11	Western	0.606/0.388	Western/Eastern	0.0968/0.1132	NS	0.879	-
K4	Western	0.176/0.814	Western/Eastern	0.1794/0.2430	NS	0.945	AD
K17	Western	0.531/0.467	Western/Eastern	0.0426/0.0234	NS	0.948	-
PD2	Western	0.658/0.339	Western/Eastern	0.2370/0.2449	NS	0.951	AD
D17	Western	0.683/0.313	Western	0.0812	NS	0.967	-
G14	Western	0.665/0.328	Western/Eastern	0.0410/0.0587	NS	0.977	-
PD13	Western	0.509/0.485	Western/Eastern	0.0389/0.0146	NS	0.992	-
G6	Western	0.608/0.387	Western	0.0217	NS	0.993	-
PD9	Western	0.728/0.263	Western/Eastern	0.2002/0.1078	NS	0.993	-
D8	Western	0.697/0.242	Western	0.0131	NS	0.999	-
C21	Eastern	0.903/0.094	Western	0.6139	0.9946^*	0.242	M
C19	Eastern	0.400/0.589	Western	0.2295	0.9976^*	0.508	M
L4	Eastern	0.698/0.299	Western	0.0162	0.9985^*	0.62	M
C20	Eastern	0.772/0.223	Western/Eastern	0.5680/0.6632	0.9795	0.474	AD
RB3	Eastern	0.442/0.552	Western/Eastern	0.0080/0.1104	0.9864	0.831	AD
H26	Eastern	0.305/0.643	Western/Eastern	0.0001/0.0001	0.9874	0.95	-
RB11	Eastern	0.305/0.692	Western/Eastern	0.3095/0.4535	NS	0.547	AD
MP2	Eastern	0.221/0.772	Western/Eastern	0.1232/0.3765	NS	0.766	AD
MP17	Eastern	0.253/0.743	Western/Eastern	0.2001/0.5669	NS	0.849	AD

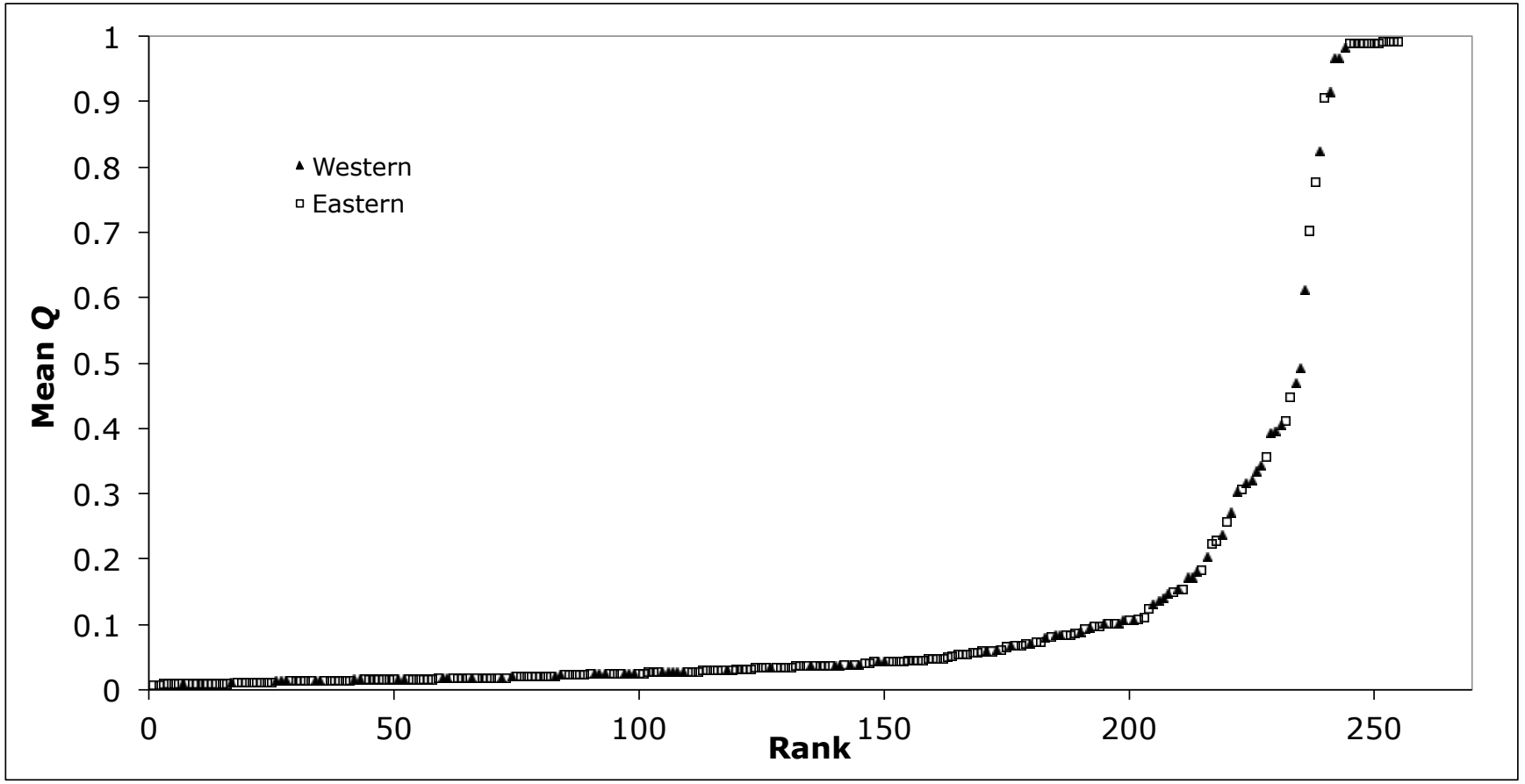

Figure 2.5. Ranked mean *Q* (proportional membership in each cluster) for each individual in each *S. o. citrinellus* cluster (Western – black triangles, or Eastern – white squares) estimated in STRUCTURE. Admixed individuals have mean *Q* values between 0.2 and 0.8.

Table 2.5. Pairwise F_{ST} values among groups of *S. o. citrinellus*. The last row shows sample sizes (*N)* for each group.

	Western Groups					Eastern Groups								
	E	G	K	O	PD	B	C	H	I	M	P	R	T	V
E	-	0.0502	0.0436	0.0783	0.0971	0.1621	0.1374	0.1375	0.145	0.1529	0.1781	0.1834	0.1679	0.1247
G	*	-	0.0656	0.0992	0.1128	0.1299	0.1375	0.1293	0.1233	0.1183	0.1569	0.1515	0.1433	0.1294
K	NS	*	-	0.0705	0.1095	0.1544	0.138	0.1513	0.1505	0.1353	0.173	0.1747	0.1595	0.1398
O	*	*	NS	-	0.0584	0.1222	0.0756	0.1152	0.1013	0.1032	0.1535	0.1436	0.1041	0.07
PD	*	*	*	*	-	0.1345	0.1207	0.1262	0.1171	0.1385	0.1919	0.1909	0.1368	0.0997
B	*	*	*	*	*	-	0.0502	0.0529	0.0438	0.0533	0.0972	0.0659	0.0453	0.0475
C	*	*	*	NS	*	*	-	0.0629	0.0351	0.0246	0.0647	0.0643	0.0405	0.0364
H	*	*	*	*	*	*	*	-	0.0193	0.061	0.1088	0.0714	0.0428	0.0312
I	*	*	*	*	*	*	*	NS	-	0.0306	0.0888	0.056	0.0163	0.0299
M	*	*	*	*	*	*	NS	*	*	-	0.0638	0.0671	0.0171	0.0517
P	*	*	*	*	*	*	*	*	*	NS	-	0.1123	0.1002	0.0911
R	*	*	*	*	*	*	*	*	*	*	*	-	0.078	0.0844
T	*	*	*	*	*	NS	*	*	NS	NS	*	*	-	0.0424
V	*	*	*	*	*	*	*	*	*	*	*	*	*	-
N	12	13	28	10	14	18	18	13	20	22	13	21	11	20

NS = non significant *P* value

* = $P < 0.05$ (under a Bonferroni corrected threshold of 0.0005)

Allele frequency comparisons

Microsatellite allelic diversity in populations of *S. o. citrinellus* ranged from 3 to 15 alleles (mean=8.1) in the western population and from 5 to 27 (mean=10.6) in the eastern population. Genotypic differentiation between the two populations was highly significant overall ($P < 0.0001$), as well as for 14 of the 16 markers individually (Table 2.6).

Table 2.6. Comparison of number of alleles between western (N=77) and eastern populations (N=156) of *S. o. citrinellus*. *P* values correspond to 10,000 randomizations of log-likelihood *G* tests of population differentiation for each marker. The test of population differentiation over all markers was highly significant ($P < 0.0001$).

	No. alleles			***G* test**
Marker	Western	Eastern	Total	*P* value
CJ7	9	10	11	<0.0001
D17s804	9	15	18	0.002
D3s1210	3	7	7	0.04
D3s1229	11	14	18	<0.0001
D3s1766	4	8	8	<0.0001
D4s111	13	17	18	0.25
D5s111	4	7	7	<0.0001
D8s165	6	9	11	<0.0001
D8s260	9	9	10	<0.0001
Leon15	5	6	7	0.006
Leon21	11	12	14	0.03
LL118	11	11	14	<0.0001
LL157	7	7	10	0.04
LL311	15	27	30	<0.0001
Locus5	8	7	8	0.08
SB38	5	5	6	<0.0001

Genetic differentiation in mtDNA sequences

In the mtDNA sequence data there were a total of 88 substitutions, 17 haplotypes, and 20 polymorphic sites. There were 5 fixed differences and two shared substitutions between subspecies, with 11 substitutions polymorphic in *S. o. oerstedii* but monomorphic in *S. o. citrinellus* and 18 substitutions polymorphic in *S. o. citrinellus* but monomorphic in *S. o.*

oerstedii. There were 5 fixed differences and one shared substitution between populations of *S. o. citrinellus*, with 14 substitutions polymorphic in the eastern population but monomorphic in the western population (and zero vice versa).

Genetic differentiation was significant between subspecies and populations of *S. o. citrinellus*, but was not consistently significant among groups within the same population (Table 2.7). In the eastern population, there were no fixed differences and 3 shared substitutions among groups. In the western population, there were no fixed differences and no shared substitutions among groups. Similarly, the AMOVA for haplotype data shows strong differentiation among populations of *S. o. citrinellus* but not among groups within populations (Table 2.8).

Phylogenetic analyses of mtDNA data

In ML analyses, a transversion (TVM) model of nucleotide evolution with a gamma (G) distribution of rates was identified as the most appropriate model. In Bayesian analyses, a Hasegawa-Kishino-Yano (HKY) model of nucleotide evolution with a gamma (G) distribution of rates was used (Hasegawa *et al.*, 1985). Bayesian and ML analyses support the monophyly of *S. oerstedii* and of *S. o. oerstedii* and *S. o. citrinellus* (Figure 2.6). Although the Bayesian posterior probability values supporting the monophyly of subspecies are high (0.83 and 0.85, respectively), they are not as high as many other studies using Bayesian inference. Reanalysis of the data with only the most conserved 200bp of the analyzed d-loop sequence did not improve support. Within *S. o. citrinellus*, Bayesian and ML analyses support the monophyly of the western population, but not the eastern population (Figure 2.6). Within *S. o. oerstedii*, there are longer branch lengths and some strongly supported intrasubspecific phylogenetic relationships.

Table 2.7. Estimates of mitochondrial sequence divergence between subspecies, populations, and groups. D_{xy} = the average number of nucleotide substitutions per site, D_a = net substitutions per site, S_{nn} = nearest-neighbor statistic, calculated with 10,000 permutations in DNASP.

Between:	D_{xy}**±SD**	D_a**±SD**	S_{nn}	***P***
Subspecies	0.015±0.003	0.0094±0.003	1.00	<0.0001
Populations of *S. o. citrinellus*	0.0071±0.002	0.0061±0.002	1.00	<0.0001
Groups in the eastern population of *S. o. citrinellus*	0.0021±0.0007	0.00088±0.0007	0.91	<0.0001
Groups in the western population of *S. o. citrinellus*	0.00013±0.0002	0.00±0.0002	0.47	1.00

Table 2.8. Standard AMOVA for haplotype data, 10,000 iterations, of mtDNA d-loop 880bp sequence (N =43).

Variance component	**Percentage of total variation**	***P***
Among populations of *S. o. citrinellus*	0.852	<0.00001
Among groups within populations of *S. o. citrinellus*	0.048	0.07406
Among individuals within groups of *S. o. citrinellus*	0.100	<0.0025

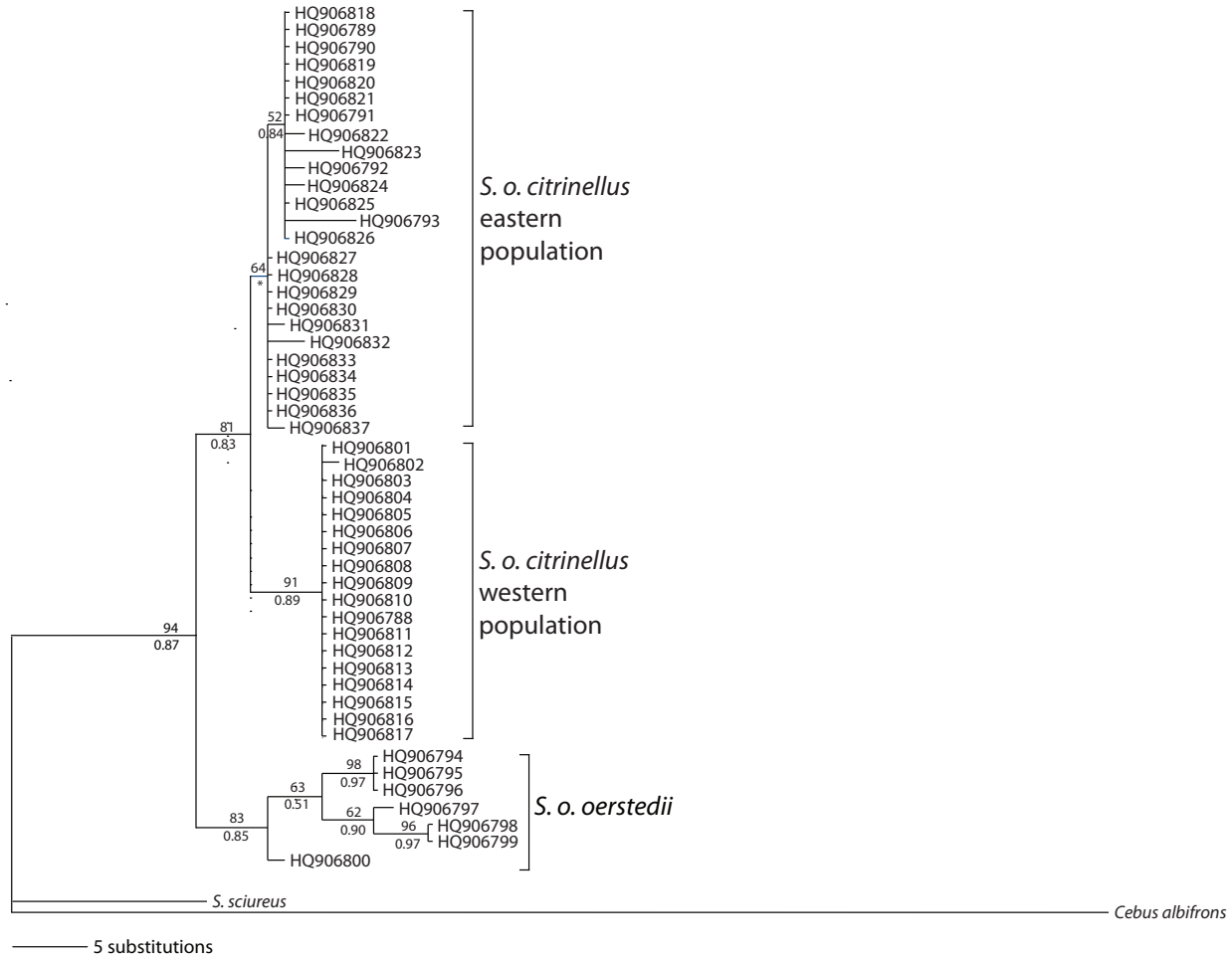

Figure 2.6. Phylogenetic tree (with ML branch lengths) inferred from mtDNA dloop sequences based upon likelihood and Bayesian analyses. Numbers above the line represent ML bootstrap values (1000 replicates); numbers below the line represent Bayesian clade credibility values (1,000,000 MCMC generations, sampled every 100 generations). * represents a Bayesian clade credibility value of less than 50.

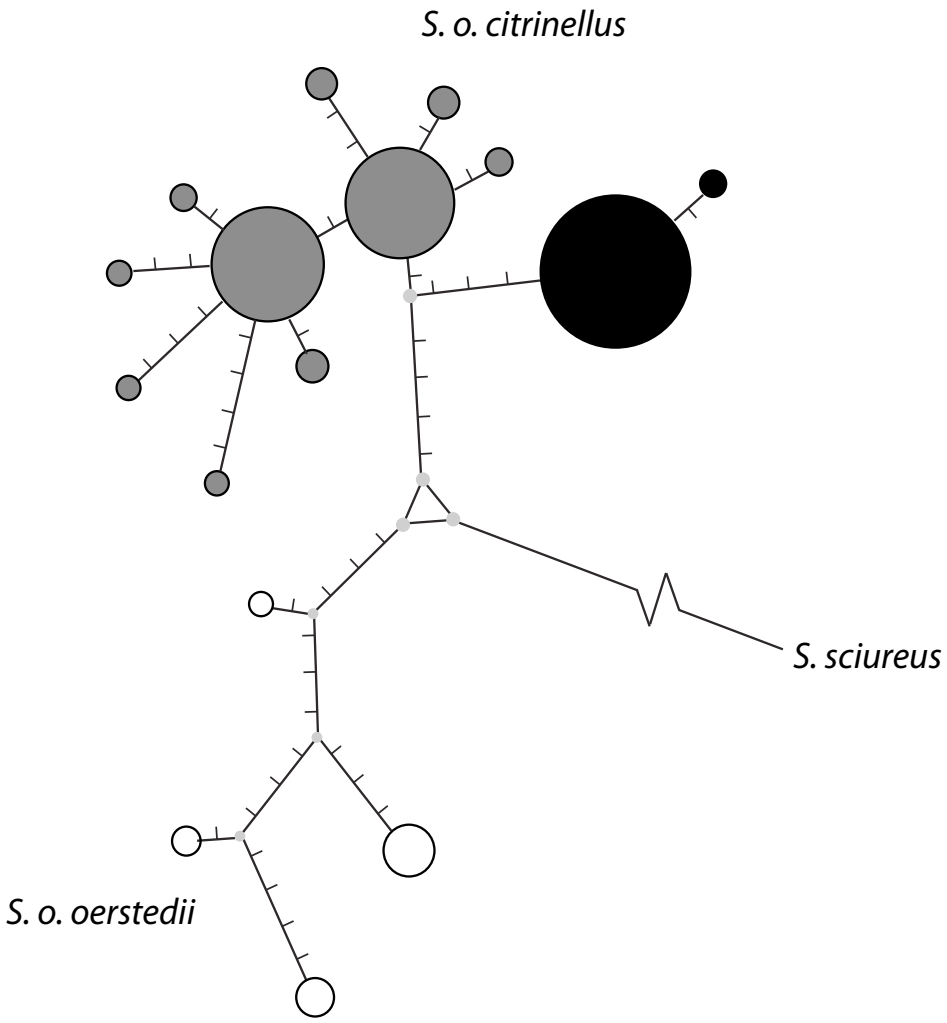

Figure 2.7. Haplotype network of *S. oerstedii* mtDNA d-loop sequences created using a median-joining algorithm (Bandelt *et al.*, 1999) implemented in NETWORK (Fluxus) with epsilon = 0 and all variable sites weighted equally. Color shading represents different sampled populations (grey = eastern population of *S. o. citrinellus*, black = western population of *S. o. citrinellus*, white = *S. o. oerstedii*). Size of the node corresponds to the frequency of that haplotype among sampled individuals. Internal nodes (light grey) represent reconstructed median haplotypes. Notches represent nucleotide differences between haplotypes. Numbers refer to individuals. Network is rooted with *S. sciureus* – nucleotide differences along this root lineage are not shown.

Haplotype network of mtDNA data

Haplotypes clearly clustered into three major haplogroups: the western population of *S. o. citrinellus*, the eastern population of *S. o. citrinellus*, and *S. o. oerstedii* (Figure 2.7). There are

greater distances overall among samples from *S. o. oerstedii* than among samples from *S. o. citrinellus*. There are also more haplotypes and greater distances among haplotypes in the eastern population of *S. o. citrinellus* as compared to the western population (Figure 2.7).

Population Aggregation Analysis

Five diagnostic characters and 14 informative traits separate the western (N=25) and eastern (N=18) populations of *S. o. citrinellus*, while there are 6 diagnostic characters and 29 informative sites between the subspecies *S. o. citrinellus* (N=43) and *S. o. oerstedii* (N=7; see Appendix, Supplementary Table S1).

Population demographic history

I found no evidence of a bottleneck from the microsatellite data. None of the sign or Wilcoxon tests across any models suggested heterozygosity excess. Similarly, all mode-shift tests showed normal L-shaped distributions.

Discussion

Support for ESUs

I found consistent results across different markers and different tests of genetic structure supporting the genetic distinctiveness of the two currently recognized subspecies of *S. oerstedii* (*S. o. citrinellus* and *S. o. oerstedii*) as ESUs. Reciprocal monophyly at mtDNA loci and significant divergence at microsatellite markers support the distinction of these subspecies as ESUs (Moritz, 1994; Moritz, 2002). Population aggregation analysis also supports the separation of *S. o. citrinellus* and *S. o. oerstedii,* although I would expect to find many more diagnostic characters with a larger sample size of *S. o. oerstedii*.

The genetic data add to other support for the subspecies distinction and suggest a lack of ecological exchangeability (sensu Crandall *et al*., 2000), including disjunct geographic distributions (Arauz 1993) and differences in pelage and morphology (Boinski, Sirot, 1997; Carrillo *et al.*, 2002). The results also confirm previous genetic studies that gave some support for reciprocal monophyly of the two subspecies at two mtDNA loci and one autosomal marker based on 4 samples from a restricted area (Boinski, Cropp, 1999; Cropp, Boinski, 2000). Previous analyses date the split of *S. o. oerstedii* and *S. o. citrinellus* at 110-160 KYA using whole mtDNA genomes (Chiou *et al.*, 2011).

Although I present support for *S. o. oerstedii* and *S. o. citrinellus* as ESUs and therefore valid subspecies, there may not be sufficient evidence to elevate these taxa to the species level. I present evidence that *S. o. oerstedii* and *S. o. citrinellus* diverge significantly across several autosomal markers and form reciprocally monophyletic units with diagnostic characters at a mtDNA marker. However, *S. o. oerstedii* and *S. o. citrinellus* are known to hybridize in captivity (Boinski *et al.*, 1998; Müller, pers comm). Thus, *S. o. oerstedii* and *S. o. citrinellus* would not represent distinct species under the biological species concept (Mayr, 1963), but would under the phylogenetic species concept (Cracraft, 1983). I suggest that *S. o. oerstedii* and *S. o. citrinellus* remain subspecies until there are additional lines of evidence that present stronger corroboration for lineage separation (de Queiroz, 2007), such as reciprocal monophyly at additional mtDNA and nuclear loci. Regardless, these subspecies represent ESUs and should be managed separately to preserve evolutionary processes and sustain genetic diversity (Moritz, 2002; DeSalle and Amato, 2004; Hendry *et al*., 2010).

S. o. citrinellus *populations*

The data do not support treating the two populations of *S. o. citrinellus* as separate ESUs. Although the western and eastern populations of *S. o. citrinellus* did form different mtDNA haplogroups and were separated by population aggregation analysis, they were not found to be reciprocally monophyletic by all analyses (Figure 2.6) and there is gene flow between these populations as evidenced by recent migrants inferred using STRUCTURE and GENECLASS (Table 2.3). Stronger structure in the mtDNA compared to the autosomal markers is likely the result of greater male dispersal between populations as compared to females (Chapter 3).

Although it is possible that human-induced habitat modification played a part in the split between western and eastern populations of *S. o. citrinellus* (the area was a settlement for the Quepo Indians who arrive around 900 A.D., a mission for Spanish colonists from 1571-1746, and a hub for British pirates in the 1700s; Melton & Myketuk, Unpublished data), the split was more likely caused by a natural barrier. There are two rivers separating the populations today, the Cañas and the Paquita (Figure 2.2), which feed into a mangrove that runs northwest from where these rivers intersect the coastline. Although they are both small rivers (the Parrita and Naranjo Rivers are much larger and do not result in genetic barriers) there is a history of natural landscape disturbance in the area (Wallace, 1997), and a series of hurricanes or excessive rainfall over several years could have made the two rivers, which are situated close together, impassable. Since then, the area between the Cañas and Paquita rivers has been modified into cattle pasture and palm plantations, which may have maintained low levels of gene flow between populations up to the present day. *Saimiri* have relatively high reproductive rates compared to other primates, and a generation time of approximately 3-6 years (Jack, 2007). As such, low levels of gene flow

between populations could be sufficient to result in the fixation of some diagnostic characters via genetic drift as found here, especially if one or both populations are small (Kimura, 1983).

Genetic diversity is lower in the western *S. o. citrinellus* population (2 mtDNA haplotypes, mean of 8 microsatellite alleles) as compared to the eastern population (10 mtDNA haplotypes, mean of 10 microsatellite alleles; Figure 2.7, Table 2.6). Depletion of genetic diversity has occurred more quickly in the mtDNA, as would be expected given the smaller effective population size of maternally-inherited mtDNA compared to autosomal, bi-parentally inherited markers such as microsatellites (Avise 1994). Although I did find evidence of recent migration between populations, I suggest that migration should be augmented and monitored between the populations to ensure that the western population is not further isolated.

Conservation Management

The levels of microsatellite genetic diversity I report are similar to other ongoing studies by Costa Rican researchers of microsatellite diversity in squirrel monkeys (G. Gutierrez, Pers. Comm.) and a recent study of isozyme diversity (Zaldivar *et al.*, 2004). Compared to the other primates in Costa Rica (*Alouatta palliata, Ateles geoffroyi,* and *Cebus capucinus*), *S. oerstedii* consistently has the highest genetic diversity, despite its endangered status (Zaldivar *et al.*, 2004; G. Gutierrez, Pers. Comm.). Such high levels of diversity may reflect certain life history characteristics of *Saimiri*. Compared to other primates, *Saimiri* have short generation times, which can result in high population growth rates and the accumulation of genetic diversity (Zaldivar *et al*., 2004).

Many genetic studies identify primate populations that should be targeted for conservation efforts citing low heterozygosity levels. However, this strategy assumes that low levels of heterozygosity are always associated with danger of extinction. Many species of

primates have naturally low levels of heterozygosity. For example, the Callitrichidae have naturally low variation at MHC and allozyme loci because of their unique reproductive physiology and social system (Cowlishaw, Dunbar, 2000; Dixson *et al.*, 1992; Meireles *et al.*, 1992; Melo *et al.*, 1992; Pope, 1996; Watkins *et al.*, 1991), although recent studies have found a range of values for variation in microsatellite markers in *Leontopithecus rosalia* (mean observed heterozygosity ranged from 0.34-0.65; Grativol *et al*., 2001) and *Saguinus mystax* (mean observed heterozgyosity = 0.75; Huck *et al.*, 2007). Similarly, many Central American howler monkeys (*Alouatta* spp.) have naturally low levels of genetic variation from repeated population bottlenecks (as reviewed in Pope, 1996). By contrast, several primate populations that are in danger of extinction have high heterozygosity. The genetic variability of muriquis (*Brachyteles* spp.) has remained quite high despite its endangered status, perhaps because the extreme fragmentation of its range has occurred relatively recently in comparison to this species' generation time (Fagundes *et al.*, 2008; Pope, 1998).

The high levels of neutral genetic diversity reported here should be confirmed with further studies of potentially adaptive genetic diversity (such as MHC loci, Watkins *et al*., 1991). High levels of genetic diversity in adaptive loci could be considered as buffers against outside extinction pressures (such as disease) to which *S. oerstedii* are vulnerable due to their dwindling habitat, small population size, and contact with humans and domestic animals. Conservation managers should work to preserve their naturally high levels of genetic diversity. In particular, managers should monitor and augment migration to the western population of *S. o. citrinellus*, which is more isolated and less genetically diverse than the eastern population.

I found significant genetic differentiation among several groups of *S. o. citrinellus* using pairwise F_{ST} comparisons. These groups are in fragments of secondary forest separated by

varying types of unsuitable habitats such as cattle pasture, commercial African palm oil plantations, and rice plantations. F-statistics effectively measure spatial *variance* in gene frequencies, while an approach that instead measures aspects of spatial *patterns* of gene frequencies would be ideal in a study of habitat fragmentation (Epperson, Li, 1996). I have conducted a complementary study that measures spatial patterns of genetic variation in *S. o. citrinellus* while taking into account landscape heterogeneity (Chapter 4) to better understand the forces behind the patterns of population genetic structure shown here.

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CHAPTER 3.

Genetic evidence for dispersal by both sexes in the Central American Squirrel Monkey, *Saimiri oerstedii citrinellus*

Abstract

Sex-biased dispersal (SBD) is common in many vertebrates, including primates. However, dispersal patterns in New World primates may vary among closely related taxa or populations in different local environments. Here I test for SBD in an endangered New World primate, the Central American Squirrel Monkey (*Saimiri oerstedii citrinellus*). Previous studies of behavioral ecology suggest predominantly female dispersal in *S. o. oerstedii* in the Southern Pacific region of Costa Rica. However, the genetic data do not support female-biased dispersal in *S. o. citrinellus* in the Central Pacific region. My tests for SBD using microsatellite data including comparisons of isolation-by-distance, AI_c, and F_{ST} values between males and females were not significant. Also, I found greater population genetic structure in mitochondrial markers than in microsatellite markers, indicative of predominantly male dispersal. However, there is evidence of recent dispersal by both males and females among populations of *S. o. citrinellus*, and therefore I conclude that both sexes disperse in *S. o. citrinellus,* but it is probable that males disperse over longer distances. I discuss how spatial and temporal variation among local populations should be taken into account when studying dispersal patterns and especially sex bias.

Introduction

Many vertebrate taxa, in particular birds and mammals, exhibit sex-biased dispersal (SBD) patterns, where males and females differ in their age at dispersal, dispersal distance, or other characteristics [for review see Lawson Handley and Perrin 2007]. Complete bias, where one sex remains philopatric almost without exception, is rare in mammals but generally more common in primates [Greenwood 1980; Johnson and Gaines 1990; Lawson Handley and Perrin 2007; Melnick and Pearl 1987; Pusey and Packer 1987]. In cercopithecine social groups with more than one female, males emigrate and females are predominantly philopatric [Melnick and Pearl 1987]. However, in many other primates, females are the predominant dispersers, or both sexes disperse but at varying distances or ages [Baker and Dietz 1996; Boinski 1986; Di Fiore 2009; Di Fiore et al. 2009; Douadi et al. 2007; Huck et al. 2007; Moore 1984; Nishimura 2003; Pope 1992; Pusey and Packer 1987; Strier 1994].

Theoretical models for the ultimate and proximate causes of SBD invoke inbreeding avoidance [Clutton-Brock 1989; Dobson 1982; Gandon 1999; Packer 1979; Pusey 1987; Shields 1982; Waser et al. 1986] or kin selection arguments including local mate competition [Dobson 1982; Hamilton 1967; Moore and Ali 1984] and local resource competition [Clarke 1978; Greenwood 1980; Greenwood 1983, Pusey and Packer 1987; Shields 1987]. Primate socioecological models have built upon these models to explain complex patterns of primate social behavior including cooperative behavior and affiliative relationships, as well as patterns of female philopatry. Generally, these models predict that female primates should be philopatric when they need to form coalitions in response to competition for food resources, i.e. when food resources are distributed in high quality patches and within-group or between-group contest competition for food resources is high. When food resources are low quality, highly scattered or

very large and scramble competition dominates, females do not need to form coalitions and there should be either facultative or obligate female dispersal [Isbell 1991; Moore 1992; Sterck et al. 1997; van Hooff and van Schaik 1992; van Hooff and van Schaik 1994; van Schaik 1989; Wrangham 1980]. These socioecological models do not explain all of the variation in nonhuman primate SBD, however [as reviewed in Isbell and Young 2002; Koenig 2002].

Indeed, there is growing evidence that dispersal patterns and other aspects of primate socioecology can be quite variable even among closely related taxa or different local populations of the same taxon, especially in New World monkeys [Boinski et al. 2005; Di Fiore 2009; Stone 2004; Zimbler-DeLorenzo and Stone 2011]. In howler monkeys, for example, both sexes typically disperse but there are interesting differences in dispersal patterns and mating systems between red (*Alouatta seniculus*) and mantled howlers (*Alouatta palliata*). In red howlers, females disperse farther than males and, because they are seldom able to enter established groups, tend to form new social groups with related females [Crocket 1984; Pope 1990, 1992, 2000]. In mantled howlers, where groups are larger and include multiple males, females are often able to join established groups and hence do not typically disperse with related females [Ellsworth 2000, Glander 1992].

Within squirrel monkeys (genus *Saimiri*), behavioral observations suggest that in *S. oerstedii* females predominantly disperse, in *S. bolivensis* males predominantly disperse, and in *S. sciureus*, both males and females disperse [Boinski 1986; Boinski 1999; Boinski et al. 2002; Mitchell et al. 1991]. Boinski [1999] and Boinski et al. [2002] explain variation in *Saimiri* dispersal patterns following van Schaik and van Hooff's [1992, 1994] and Sterck et al.'s [1997] socioecological model. In Peru, *S. boliviensis* experience strong within-group food competition, strong female social bonds, female philopatry, and female dominance over males. By contrast, in

Costa Rica, fruit is distributed in small patches, with few ripe fruits available in any patch. This pattern corresponds with low levels of contest competition within groups and weak *S. oerstedii* female social bonds, ubiquitous female dispersal, and egalitarian social structure [Boinski 1999; Boinski et al. 2002].

Central American squirrel monkeys (*S. oerstedii*) inhabit only the Pacific moist forests of Costa Rica and northern Panama below ~500m altitude [Boinski 1999; Boinski et al. 1998; Boinski and Sirot 1997]. They live in troops of 22 to 66 or more individuals and their diet includes arthropods, flowers, fruits, and small vertebrates [Arauz 1993; IUCN 2010; PRMVS 1996; PRMVS 2002; Wong 1990]. These troops have home ranges of approximately 200 ha often with extensive home range overlap between groups [Boinski 1999; Boinski et al. 2005; Mitchell et al. 1991; Wong 1990]. Frequent landscape disturbance (e.g. mudslides) from high rainfall, wind, hurricanes, and rugged topography characterize the Pacific wet lowlands of Costa Rica [Boinski 1999; Boinski et al. 2005; Wallace 1997]. The most recent survey estimated that there were only 7,000 *S. oerstedii* remaining in 1995, of which only 1,500-1,800 were *S. o. citrinellus* in the Central Pacific region of Costa Rica [IUCN 2010; Sierra et al. 2003]. The total distribution of *S. o. citrinellus* covers an area of approximately 1500km^2 [Arauz 1993]. Fruit and rice plantations and cattle pasture replaced approximately 80% of the forest in this area in the 1930s [Mattey 1992; Mattey 1994].

As noted above, behavioral studies of the southern subspecies of *S. oerstedii* (*S. o. oerstedii*) in the Osa Peninsula in the Southern Pacific region of Costa Rica suggested female-biased dispersal [Boinski 1986; Boinski 1999]. However, the use of genetic data may allow the detection of rare dispersal events by either sex not directly observed [Di Fiore 2003; Lawson Handley and Perrin 2007]. Also, habitat modification or other differences in the local ecology of

the Central Pacific may result in different dispersal patterns for *S. o. citrinellus* compared to their close relatives [Goossens et al. 2006]. To date there have been few genetic studies of dispersal in primates, and no genetic studies of dispersal in *Saimiri* [see Di Fiore 2003, 2009]. In this study, I examined whether there is molecular genetic evidence of SBD in *S. o. citrinellus*, expecting to find evidence of female-biased dispersal following behavioral studies of *S. o. oerstedii* [Boinski 1986; Boinski 1999].

SBD patterns can be inferred using a combination of genetic markers. In populations with male-biased dispersal and female philopatry, genetic structure is expected in mitochondrial (mtDNA) markers, which are matrilineally inherited, but not in autosomal markers since males disperse this material. In populations with female-biased dispersal and male philopatry, little structure would be expected in either mtDNA or autosomal markers because females disperse both, but significant structure would be expected in Y-chromosomal markers [Avise 1995; Di Fiore 2003; Di Fiore 2009; Melnick and Hoelzer 1992; Melnick and Hoelzer 1993; Morin et al. 2001]. Also, if dispersal is female-biased, females should show lower mean corrected assignment indices (mAI_c) and greater variance in those scores when compared to males, because a sample of the dispersing sex will theoretically contain a mix of immigrant and resident individuals [Favre et al. 1997; Goudet et al. 2002]. The assignment index is the probability that an individual's genotype occurred by chance in a population, and Favre et al. [1997] have applied a correction that produces mAI_c values of zero for each population. If both males and females disperse, both mtDNA and autosomal markers should show little to no structure [Avise 1995], and mAI_c values should be similarly low for both sexes. Alternatively, a pattern of high structure across all markers would suggest low levels of dispersal by both sexes, perhaps due habitat modification or other high costs of dispersal [Goossens et al. 2006]. In this study, I compare patterns of genetic

structure in mtDNA and autosomal markers, and mAI_c scores between males and females to infer patterns of SBD in *S. o. citrinellus.*

Methods

For a complete discussion of methods used for sampling and DNA extraction, microsatellite genotyping, and mtDNA sequencing, see Chapter 2.

Tests of population genetic structure

I used a locus-by-locus analysis of molecular variance and a standard AMOVA for haplotype data to compare population genetic structure in the microsatellite markers and mtDNA (for a complete discussion of methods for AMOVAs, see Chapter 2).

I inferred haplotype networks of male and female *S. o. citrinellus* mtDNA sequences using a median-joining algorithm [Bandelt et al. 1999] implemented in NETWORK v 4.5 [Fluxus] with an epsilon value = 0 and all variable sites weighted equally. In each network I included one *S. o. oerstedii* sample (a male for the male network and a female for the female network) to root the network.

Tests for sex-biased dispersal

I used the "biased dispersal" module in FSTAT [Goudet 2001] to compare mean corrected assignment indices (mAI_c) and F_{ST} values between adult males and adult females. I calculated two-tailed P values using 10,000 randomizations. Negative AI_c values characterize individuals with multilocus genotypes less likely than average to occur in a population sample, and thus lower mAI_c values for one sex implies SBD [Goudet et al. 2002; Mossman and Waser 1999; Paetkau et al. 1995]. I also compared variance in assignment indices because of the expectation that the dispersing sex should have larger variance in AI_c values [Favre et al. 1997;

Goudet et al. 2002]. F_{ST} is a statistic expressing the proportion of the total genetic variance that resides among populations [Weir and Cockerham 1984]. Allelic frequencies for individuals of the dispersing sex should be more homogeneous than those for individuals of the more philopatric sex. I therefore expected F_{ST} to be lower for the dispersing sex. FSTAT uses Weir & Cockerham's [1984] F_{ST} estimator (Φ_{ST}) because it is commonly used and unbiased [Goudet 2001]. I included 51 adult females and 136 adult males in this analysis from 11 groups. Because of the difference in sample size between males and females, I repeated these analyses three times with randomly selected samples of 51 males. The results of these permutations of the data were very similar to the reported results (see Appendix Supplementary Table S2).

To further test for sex-bias in dispersal patterns, I compared mAI_c between adult males and adult females within a few well-sampled groups with comparable sample sizes of males and females. I also compared F_{ST} values among real groups to F_{ST} values among randomized groups of females and males, respectively. I created randomizations of the genotypes into groups of the same sizes as the real groups, and compared F_{ST} values of randomized and real groups using a G-test with 10,000 permutations in FSTAT.

I also compared isolation-by-distance relationships in males and females by performing separate Mantel tests for males and females using Rousset's $\hat{a}$ [Rousset 2000], a measure of genetic distance, against Euclidean linear distance in SPAGeDi [Hardy and Vekemans 2002] with 10,000 permutations of individuals and spatial locations, and jackknifing of markers. I expected a weaker isolation-by-distance relationship, meaning smaller slope and r^2 values, for the dispersing sex.

Results

Population genetic structure of microsatellite markers and mtDNA sequences

I successfully genotyped 233 individuals from 14 groups of *S. o. citrinellus* at 16 microsatellite markers (see Appendix, Supplementary Table S3) and found significant population structure in the microsatellite data (see Chapter 2, Table 2.4). Although the highest percentage of variation is within individuals, likely due to high levels of heterozygosity, there is significant variation apportioned to all hierarchical levels in *S. o. citrinellus*.

Aligning 43 *S. o. citrinellus* mtDNA sequences (N=18 from the western population and N=25 from the eastern population), there were 17 haplotypes and 20 polymorphic sites. There were 5 fixed differences and one shared substitution among populations. In median-joining network analyses, haplotypes of both males (N=22) and females (N=21) clearly clustered into two haplogroups, which correspond to the western and eastern populations (Figure 3.1). In females, however, there are only 3 haplotypes in the eastern population (males have 8) and one haplotype in the western population (males have 2).

As discussed in Chapter 2, the AMOVA for haplotype data shows strong differentiation among populations of *S. o. citrinellus*, but not among groups within populations (Chapter 2, Table 2.8). Population differentiation is much stronger in mtDNA as compared to microsatellite markers, with variation among populations explaining 85.2% of total variation in mtDNA, while only 8.1% of total variation in the microsatellite markers is explained by variation among populations (Table 2.4,2.8).

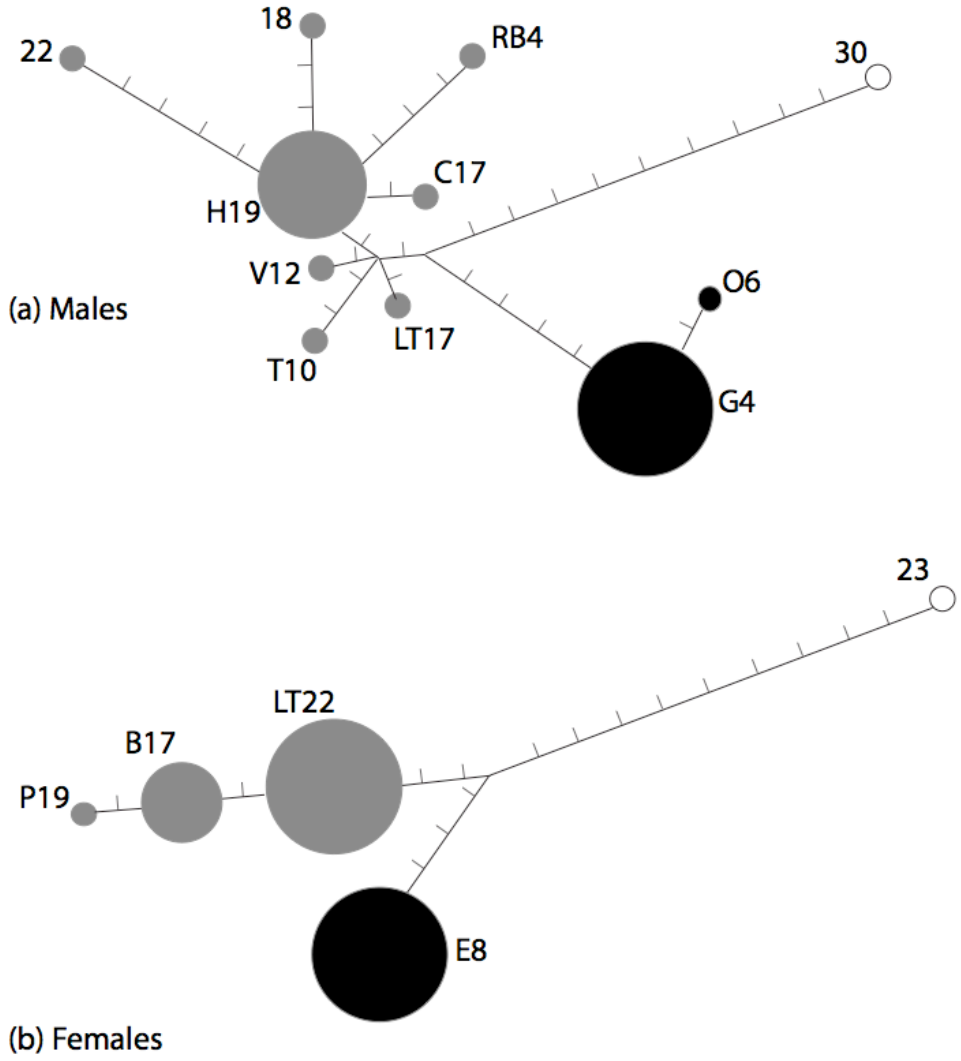

Figure 3.1. Haplotype networks of (a) male (N=22) and (b) female (N=21) *S. o. citrinellus* mtDNA d-loop sequences created using a median-joining algorithm [Bandelt et al. 1999] implemented in NETWORK [Fluxus] with epsilon = 0 and all variable sites weighted equally. Shading represents different sampled populations (grey = eastern population of *S. o. citrinellus*, black = western population of *S. o. citrinellus*). Size of the node corresponds to the frequency of that haplotype among sampled individuals. Internal nodes represent reconstructed median haplotypes. Notches represent nucleotide differences between haplotypes. Network is rooted with *S. o. oerstedii* (white).

Tests for sex-biased dispersal

Across all samples, mAI_c values calculated from the microsatellite data were not significantly different between males and females (Table 3.1). Although mean AI_c was negative for females (mean=-0.39) and positive for males (mean=0.15), variance in AI_c values was very large in both sexes (Table 3.3). Differences in F_{ST} values were also not significant (Table 3.1).

Table 3.1. Mean values and tests of sex-biased dispersal in the microsatellite data. The fourth column gives the expected value for the more dispersing sex as compared to the less dispersing sex (+ for larger value expected, - for smaller value expected).

Test	**Male**	**Female**	**Expected value of more dispersing sex**	***P***
mAIc	0.1468	-0.3915	-	0.591
variance in *AIc*	35.249	40.623	+	0.520
FST	0.1006	0.0979	-	0.903

When comparing mAI_c values between males and females in three well-sampled groups (Byblos: N_{males}=10, $N_{females}$=8; California: N_{males}=8, $N_{females}$ =10; Villanueva: N_{males}= 13, $N_{females}$=7), mean AI_c was negative for females (Byblos mAI_c=-0.448; California mAI_c=-0.196; Villanueva mAI_c=-1.766) and positive for males (Byblos mAI_c=0.359; California mAI_c=0.245; Villanueva mAI_c=0.951). However, these differences were not significant, although the comparison for the Villanueva group approached significance (Byblos: P=0.404; California: P=0.696; Villanueva: P=0.075).

F_{ST} values calculated from the microsatellite data for both males and females were not significantly different from F_{ST} values among randomized groups (males: P=0.335; females: P=0.321). In addition, there was weak but significant isolation-by-distance in both males and females in the microsatellite data, with a slightly stronger relationship in females (females: slope=0.050, r^2=0.203, P<0.0001; males: slope=0.044, r^2=0.103, P<0.0001).

Discussion

Previous studies using behavioral observations suggest that in *S. o. oerstedii*, males are philopatric and females predominantly disperse [Boinski et al. 1999, 2002; Mitchell et al. 1991]. However, the genetic results suggest dispersal by both sexes in *S. o. citrinellus*. Tests for SBD on the microsatellite data did not support a strong bias between the sexes in dispersal patterns. Although the mAI_c value comparison was in the expected direction for female-biased dispersal, the comparison was not statistically significant. Further, I found weak but significant isolation-by-distance in both males and females and F_{ST} values among groups for both males and females were not significantly different from F_{ST} values among randomized groups, also suggesting that both sexes are dispersing.

Despite the tests for SBD failing to support a strong bias in dispersal for either sex, there was stronger population genetic structure in the mtDNA sequence data as compared to the microsatellite data, and greater haplotype diversity among males as compared to females. The mtDNA AMOVA showed that 85% of the total genetic variation could be found among populations, while in the microsatellite AMOVA only 8.1% of the total variation was apportioned to among population differences (Chapter 2, Table 2.4,2.8). This pattern, disregarding the tests of SBD, would suggest predominantly male dispersal [Avise 1995; Melnick and Hoelzer 1992]. However, the significant population structure I found in mtDNA sequences was only at the population level, and is not consistently supported at the group level (see Chapter 2), suggesting that although females are moving less than males between populations, they are moving among groups.

As a part of a concurrent study, I performed a Bayesian inference of population genetic structure that identified 7 individuals as likely to be recent migrants between the western and

eastern populations of *S. o. citrinellus*. Five of these individuals were sexed as males and 2 as females (see Chapter 2). These results, similar to the tests of SBD presented here, suggest that both sexes are dispersing in *S. o. citrinellus.*

My results also suggest that females are dispersing shorter distances than males. In particular, there was significant population structure in mtDNA at the population level but not at the group level, in addition to a slightly stronger isolation-by-distance relationship among females. Also, the 5 likely migrant males identified in the Bayesian analysis traveled 10-40 km, while the 2 likely migrant females traveled only 10-12 km.

Although females are dispersing shorter distances than males, they may be dispersing more often than males. This would explain females' consistently lower mAI_c values compared to males, although differences in mAI_c values were not statistically significant. To better test this hypothesis, I would need to increase my sample size for each sampled group and repeat the Bayesian analysis to identify likely recent migrants among groups, instead of only among populations (see Chapter 2). I could then infer whether females are indeed dispersing more often than males among groups within populations, even though males are dispersing more often between populations.

The understanding of *S. o. citrinellus* dispersal patterns could be further improved by including additional genetic data from Y-chromosomal markers and a longitudinal behavioral study to confirm behavioral similarities with *S. o. oerstedii.* A behavioral study would be particularly interesting, as differences in dispersal patterns may result in different patterns of affiliative within-group social behavior between *S. o. citrinellus* and *S. o. oerstedii*. Also, recent work in black and white colobus monkeys (*Colobus guereza*) shows that in some cases neither

genetic data nor behavioral data alone can reveal true dispersal patterns, especially when complex events such as group fission have occurred [Harris et al. 2009].

This study adds to a growing body of research that finds differences in dispersal patterns and other aspects of primate socioecology among local populations within the same taxon or closely related taxa [Boinski et al. 2002; Di Fiore 2009; Stone 2004; Zimbler-DeLorenzo and Stone 2011]. Indeed, in some primate taxa where behavioral evidence suggests a strong sex bias in dispersal patterns, genetic data are revealing that the philopatric sex sometimes disperses. For example, in the Atelinae (genera *Lagothrix, Ateles,* and *Brachyteles*), female-biased dispersal seems to be the norm [Di Fiore and Campbell 2007], but genetic evidence has revealed that males disperse sometimes as well [Di Fiore 2009; Di Fiore et al. 2009].

Combining genetic and behavioral data will be necessary to build better predictive models for why dispersal patterns differ among species or populations of the same species. I hypothesized here that differences in local ecology may have resulted in the differences I found between *S. o. citrinellus* dispersal patterns and that of their close relatives. Similarly, Boinski et al. [2005] predicted that *S. oerstedii* females should have shorter dispersal distances when there are local surges in food abundance, while longer dispersal distances would be expected with declines in local food abundance.

Other studies have found that stochastic demographic variation [Pope 1998, Di Fiore et al. 2009] and recent environmental change such as anthropogenic habitat disturbance can alter dispersal patterns [Goossens et al. 2006]. These studies combined with theoretical expectations [e.g. Boinski et al. 2005] suggest that spatial and temporal variation in local environments and demography should be taken into account when studying variation in dispersal patterns, especially with respect to sampling design and scale. In *Saimiri,* it is likely that both sexes

disperse across species, with variation in the local environment and demographic factors driving spatial and temporal variation in the symmetry of dispersal patterns between the sexes.

Finally, *S. oerstedii* is threatened with extinction and a top priority for conservation in Latin America according to the IUCN Red List of Threatened Species [IUCN 2010]. Characterization of dispersal patterns, including dispersal timing, distance, and sex-bias, is important for the development of conservation management. Dispersal is the mechanism whereby genetic diversity is distributed among groups within a population, therefore studies of dispersal patterns such as this one may inform management plans that strive to maximize genetic diversity [Melnick et al. 2000; Perez-Sweeney 2002].

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CHAPTER 4.

Tracking population processes in a heterogeneous, human-dominated landscape: landscape genetics of the Central American Squirrel Monkey

Abstract

Central American Squirrel Monkeys (*Saimiri oerstedii citrinellus*) are endangered primates restricted to fragmented, human-modified habitats in the Central Pacific region of Costa Rica. Landscape genetic studies can provide a fine-scale understanding of how habitat fragmentation and heterogeneity influence gene flow and population genetic structure, allowing more detailed recommendations to conservation managers about how to maintain natural population processes in human-modified and heterogeneous landscapes. However, these studies are rare in primates because of long generation lengths and difficulties in sampling. I examined the effects of landscape heterogeneity on gene flow in *S. o. citrinellus*, analyzing 233 individuals from 14 groups for 16 microsatellite markers. I analyzed the data using both least-cost and isolation-by-resistance frameworks to characterize localized gene flow patterns for *S. o. citrinellus* while incorporating landscape heterogeneity. I found that large, commercial oil palm plantations represent moderate barriers to gene flow among populations, but cattle pastures, rivers and residential areas do not represent barriers for these secondary forest specialists in the Central Pacific region. These results stress the importance of considering the relative effects of different matrix habitat classes in landscape genetic studies instead of assuming that all "non-suitable" habitats have a uniform effect on gene flow. When landscape genetic methods are applied rigorously and at the right scale, they are sensitive enough to track population processes even in species with long, overlapping generations like primates. Thus landscape genetic approaches are extremely valuable for the conservation management of a diverse array of

endangered species in heterogeneous, human-modified habitats, including the Central American Squirrel Monkey.

Introduction

Habitat loss and modification can affect population genetic structure if these landscape changes alter dispersal patterns and fragment once contiguous populations (Frankham et al. 2002; Frankham 2006). If dispersal rates become very low, the effective size of the resulting subpopulations will decline, giving each a higher risk of extinction from demographic stochasticity and inbreeding depression (Frankham et al. 2002; Frankham 2006). Thus, the negative effects that habitat modification can have on a population depend critically on the degree of isolation between subpopulations, which reflects the decline of dispersal and gene flow among groups occupying patches of suitable habitat. The degree of isolation among fragments is influenced by several species- and landscape-specific parameters, including the spatial pattern of habitat patches in the landscape, types of unsuitable "matrix" habitats between patches of suitable habitat, time since fragmentation, dispersal ability of the species, distribution of population sizes among fragments, and underlying historical genetic structure (Mills & Tallmon 1999; Frankham et al. 2002; Frankham 2006). An empirical understanding of how habitat change affects the movement of individuals within and among patches in a given population is essential in predicting the effects of habitat modification on population persistence.

Landscape genetic approaches are increasingly used to understand the influence of landscape characteristics on population genetic structure and dispersal patterns (Balkenhol et al. 2009a; Segelbacher et al. 2010; Sork & Waits 2010; Storfer et al. 2010). This emerging approach combines population genetics, spatial statistics, and landscape ecology to measure the effects of

landscape features on gene flow (Manel et al. 2003; Holderegger & Wagner 2006; Storfer et al. 2007). Landscape genetic approaches differ from traditional population genetics because they do not require *a priori* identification of discrete populations, and the individual is the operational unit of analysis instead of the population (Wright 1931; Pritchard et al. 2000; Manel et al. 2003). Landscape genetic approaches attempt to detect genetic discontinuities among individuals and then correlate those discontinuities with landscape features (Manel et al. 2003).

Although landscape genetics is a rapidly expanding field with new statistical approaches arising quite frequently (Balkenhol et al. 2009b), many studies work within an isolation-by-distance (IBD) framework, where populations within a species diverge genetically due to geographic distance, gene flow, and genetic drift (Wright 1943; Mantel 1967; Smouse et al. 1986; Chesser 2003). In the traditional IBD framework, pairwise genetic distance is regressed against pairwise Euclidean (straight-line) geographical distance, estimated by among-population averages. In a landscape genetic IBD framework, pairwise distances are calculated among individuals (Rousset 2000) and geographic information systems (GIS) are used to calculate non-Euclidian geographic distances that incorporate aspects of landscape heterogeneity (Broquet et al. 2006). These distances are often called "least-cost" distances, where costs are assigned to different habitat classes and the least costly path between individuals is calculated. If genetic distances have a stronger correlation to least-cost distances than to Euclidean distances, one can infer some effect of landscape heterogeneity on gene flow in the study population. Such least-cost distances have shown a stronger correlation to genetic distances than to Euclidean distances in many recent studies of birds, herpetofauna, terrestrial mammals, and primates (Lada et al. 2008; Blumenthal et al. 2009; Greenwald et al. 2009; Liu et al. 2009; Frantz et al. 2010; Hokit et al. 2010; Quemere et al. 2010).

One particular challenge in landscape genetics is to quantify the relative effects of various landscape parameters on gene flow (Cushman et al. 2006; Balkenhol et al. 2009a). Recent studies in landscape ecology reject simplistic models where the matrix, or the unsuitable habitat between patches of suitable habitat for a given species, uniformly inhibits movement among patches. Instead, the matrix is dynamic, heterogeneous, and can have both positive and negative effects on dispersal and thus the long-term persistence of a species (Jules & Shahani 2003; Baum et al. 2004; Dunford & Freemark 2005; Kindlmann et al. 2005; Crooks & Sanjayan 2006; Hilty et al. 2006; Kupfer et al. 2006; Fischer & Lindenmayer 2007). Responses to matrix quality and heterogeneity are species-specific, often correlating with body size, degree of arboreality, dietary specialization, and habitat breadth (Laurance 1997; Beier & Noss 1998; Gehring & Swihart 2003; Goncalves da Silva 2007). Thus, landscape genetic studies should compare different classes of matrix habitats at multiple scales to understand their relative effects on genetic variation and better predict processes of population divergence in modified landscapes.

The endangered Central American Squirrel Monkey (*Saimiri oerstedii*, Primates: Cebidae) provides an ideal opportunity to investigate gene flow and intraspecific patterns of divergence in a human modified, heterogeneous landscape. *S. oerstedii* live in groups of 22 to 66 or more individuals, which have home ranges of approximately 200 ha. Their diet includes arthropods, flowers, fruits, and small vertebrates (Wong 1990; Boinski 1999), and they are restricted to the Pacific moist forests of Costa Rica and northern Panama below ~500 m asl (Arauz 1993; Boinski & Sirot 1997; Boinski et al. 1998; Boinski 1999). This range area is characterized by frequent landscape disturbance from high rainfall, wind, hurricanes, and rugged topography (Wallace 1997; Boinski 1999; Boinski et al. 2005). The subspecies *S. o. citrinellus*

inhabits a particularly heterogeneous landscape in the Central Pacific region of Costa Rica, where fruit and rice plantations and cattle pasture replaced approximately 80% of the natural forest in the early 1900s (Mattey 1992, 1994).

Despite this heterogeneity, little work has been done to determine the effects of such drastic natural and anthropogenic landscape change on the behavior, demography, or genetic structure of *S. o. citrinellus* populations. To date the only published study of genetic diversity in *S. o. citrinellus* was based on a small sample (N=8) from Manuel Antonio National Park (Zaldivar et al. 2004), which is the smallest national park in Costa Rica and the only protected area within the range of *S. o. citrinellus*. A much larger sample collected across a broader spatial scale and wider range of habitats is necessary to determine the effects of landscape heterogeneity on *S. o. citrinellus* dispersal patterns. I analyzed a large number of non-invasively collected molecular samples and fine-scale landscape data within a least-cost distance framework to determine whether there is a relationship between landscape heterogeneity and patterns of gene flow and which, if any, matrix habitats are barriers to gene flow in *S. o. citrinellus.*

Long, overlapping generations affect the power of landscape genetic approaches to detect the effects of current or even historical landscape patterns on genetic structure, and this issue is particularly important for primate taxa. A recent simulation study showed that it would take 1-15 generations to detect barriers to gene flow using Mantel's *r* (Landguth et al. 2010). *Saimiri* have an average generation time of 3-6 years (Jack 2007), meaning that at least 12 and up to 25 generations have likely transpired since the major transformation of the Central Pacific landscape in the early 1900s. Thus, I should be able to detect barriers to gene flow caused by landscape features in this study.

Several aspects of *S. oerstedii* behavioral ecology suggest that some types of matrix habitat will affect patterns of gene flow more than others. For example, *S. oerstedii* are known to traverse some matrix habitats such as small fruit plantations and live fences, while they likely do not traverse others such as large commercial oil palm plantations and rice plantations (Wong 1990; Boinski & Sirot 1997). If some matrix habitats represent barriers to gene flow while others do not, geographic distances that weight barrier matrix habitats with high costs should correlate more strongly with genetic distance than geographic distances that weight passable matrix habitats with high costs. By contrast, if all matrix habitats prevent gene flow, different least-cost measures of distance through matrix habitat should not differ in the strength of their associations with genetic distance.

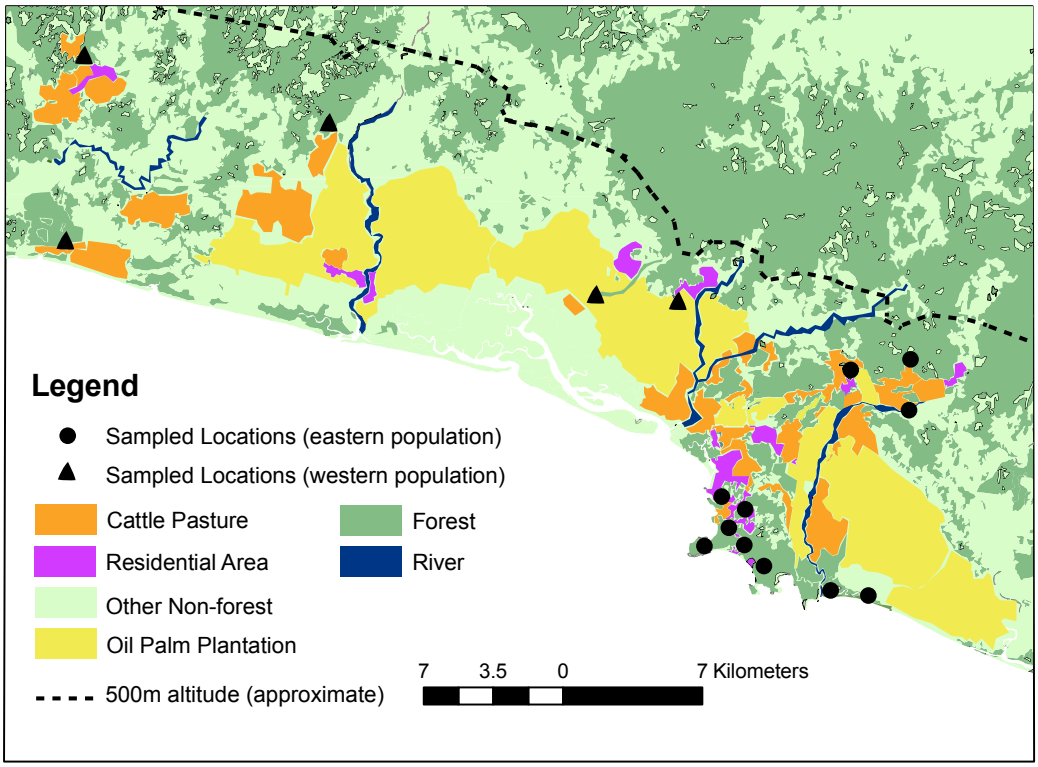

Figure 4.1. Sampled *S. o. citrinellus* groups in the Central Pacific region of Costa Rica, showing the limit of 500m asl to their distribution and different classes of matrix habitat as defined by a manual land cover classification.

Methods

For details on sample collection, DNA extraction, and microsatellite genotyping, see Chapter 2.

Land cover classification

I delineated five habitat classes (forest, cattle pasture, rivers, oil palm plantations, and residential areas) within the study region using a manual land cover classification method (Figure 4.1). I used 32 georeferenced aerial photographs taken with a DCS camera, each 10 x 10.5 km, as well as a 90 x 7 km multispectral MASTER line image, combined to cover the approximately 1,800 km^2 study area, with considerable overlap among images. Aerial photographs and the MASTER line were all taken in the year 2005 and were obtained from Misión CARTA at the Centro Nacional de Alta Technología (CENAT) in Costa Rica. I drew polygons delineating the five habitat classes manually in ArcGIS v 9.3 (ESRI) using 131 ground reference points collected during fecal sample collection, and using as a reference a forest cover dataset generated by EOSL, CCT and FONAFIFO (2002) with Landsat 7 TM satellite imagery from the year 2000. My polygons updated the forest polygons from the EOSL dataset to reflect forest regeneration and loss that took place in the study area between 2000 and 2005, and delineated four new matrix habitat classes (cattle pasture, rivers, oil palm plantations, and residential areas). Ninety-eight percent of the ground reference points, which represented all five habitat classes, were accurately reflected in the manual classification. Errors were likely due to small landscape changes that occurred since 2005. I defined residential areas as clusters of human residences less than 100m apart and consisting of a total area greater than 3 hectares. I did not delineate among forested habitats as the study area includes mostly secondary forest, with little primary forest, and *S. oerstedii* are secondary forest specialists. The classification also

included an “other non-forest” category, which included shrimp farms, rice plantations, abandoned lots, and residences that were not concentrated enough to fit my definition of a residential area.

Landscape genetic analyses

I estimated pairwise genetic relationships between individuals using Rousset’s *â* (Rousset 2000), a measure of genetic distance, and Moran’s *I* (Moran 1950; Epperson 2003), a measure of genetic similarity, calculated in SPAGeDi v 1.3 (Hardy & Vekemans 2002). I calculated both measures because they make different assumptions about the data, and Moran’s *I* has a lower variance than Rousset’s *â* (Hardy & Vekemans 2006). My confidence in the results would increase if the results were consistent with independent measures of both distance and similarity (Peakall et al. 2003; Fredsted et al. 2005; Goncalves da Silva 2007).

I measured two types of geographic distances: Euclidean distances, which are straight-line distances on a map, and least-cost geographic distances, where the costs of dispersing across different habitat classes were incorporated into the measure of geographic distance (Figure 4.2). I calculated Euclidean geographic distances among samples in ArcMap v 9.3 (ESRI) using the sampled coordinates.

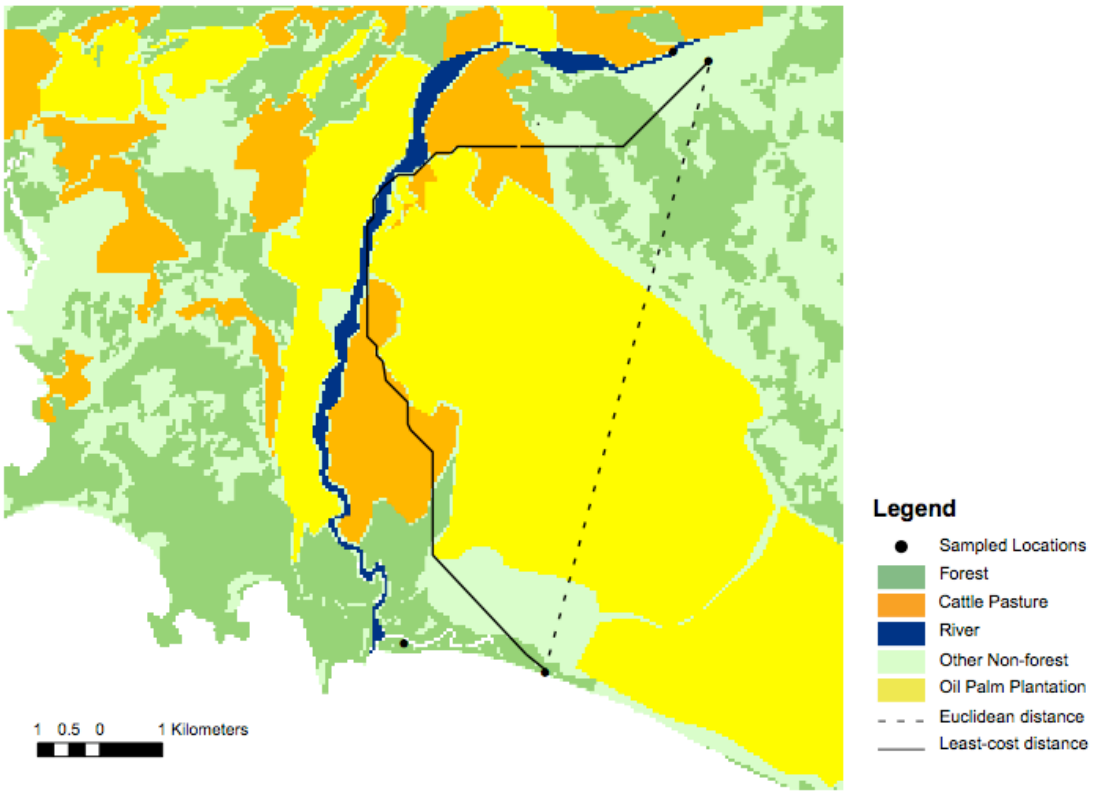

Figure 4.2. Example of the difference between the Euclidean distance (dotted line) and least-cost distance (solid line) between two sampled groups. In this example, oil palm plantations (yellow) were weighted with a cost of 1,000 in the calculation of least-cost distance.

I calculated least-cost geographic distances through the five habitat classes identified above (forests, oil palm plantations, cattle pastures, residential areas, and rivers) using the COSTDISTANCE function in ArcGIS v 9.3 (ESRI), automated with a python script. The results of this script were identical to those of a toolkit recently published for the same purpose (Etherington 2010). Although *S. oerstedii* groups are rarely seen above 500m altitude (Arauz 1993), I decided not to constrain the analyses to areas that are below 500m altitude, because there is no evidence to suggest that individuals do not traverse higher altitudes during dispersal.

The COSTDISTANCE function incorporates a cost to crossing specified habitat classes. Here, I varied the cost of one class while keeping all others at an equal, low cost (1) and then repeated this process for each habitat class. I assessed least-cost distances for a range of 6

arbitrary cost values (10, 50, 100, 1,000, 5,000, 10,000). Assessing several different potential cost patterns in this way helped to account for the sensitivity of least-cost distance analyses (Perez-Espona et al. 2008; Rayfield et al. 2010).

To examine the effect of each habitat class on squirrel monkey dispersal in the study area, I performed simple Mantel tests of matrix correspondence (Mantel 1967) between genetic distances and geographic distances in *zt mantel* (Bonnet & Van de Peer 2002). Tests were run with 10,000 permutations of pairwise genetic distances across locations in order to calculate statistical significance. If a habitat class is a barrier to gene flow, the absolute value of Mantel's *r* would be greater (more positive for Rousset's $\hat{a}$ and more negative for Moran's *I*) when that habitat class was assigned a cost. The absolute value of Mantel's *r* would peak at the least-cost distance that most closely approximates the path used on average to traverse through that habitat class. If the habitat class is not a barrier to gene flow, the absolute value of Mantel's *r* would be the same or lower than the Mantel's *r*-value that describes the relationship between genetic and Euclidean distance matrices, and would decrease with increasing cost assigned to that habitat class. This was the pattern expected for the forest habitat class. If the absolute Mantel's *r*-value did not increase or decrease with changes in the assigned cost, the habitat class either has no effect on gene flow, or its effect on gene flow could not be detected using the least-cost framework because of the configuration of that particular habitat class in the landscape.

Because least-cost distances and Euclidean distances are not independent, I also performed partial Mantel tests (Smouse et al. 1986) in *zt mantel* to test the strength of relationships between genetic and least-cost distance matrices while controlling for the effect of Euclidean distance (Broquet et al. 2006; Cushman & Landguth 2010). Partial correlations show high power and accuracy in their ability to infer the effect of landscapes on dispersal when there

is a strong contrast between the permeability of different landscape elements (Jaquiery et al. 2011). I expected significant, positive Mantel's *r*-values (for Rousset's $\hat{a}$, negative for Moran's *I*) from partial Mantel tests if least-cost distances had strong relationships with genetic distances. If partial Mantel tests were not significant or were not in the expected direction (positive for Rousset's $\hat{a}$, negative for Moran's *I*), least-cost distances are not more strongly correlated to genetic distances than are Euclidean distances. By contrast, if partial Mantel's *r*-values for a least-cost distance were both significant and greater (in absolute value) than Mantel's *r*-values for Euclidean distance, I would be confident that the least-cost distance has a stronger relationship with genetic distance than Euclidean distance, and that it differs from Euclidean distance in the information that it contributes to the analysis.

Recent studies have suggested that the spatial scale of landscape genetic analyses can have important effects on results, especially inferences about which landscape features affect gene flow (Anderson et al. 2010; Short Bull et al. 2011). To test the stability of inferences across spatial scales, I repeated the above analyses including only pairs of samples within the eastern or western population of *S. o. citrinellus* (Figure 4.1), excluding between population pairs. Populations were defined as in Chapter 2. Sampled locations across the eastern population spanned approximately 200 km^2 while sampled locations across the western population spanned approximately 600 km^2 (Figure 4.1).

I used the results of the least-cost distance analyses to generate a resistance surface characterizing the cumulative effects of landscape heterogeneity on gene flow in *S. o. citrinellus*, implemented in the software CIRCUITSCAPE 3.5 (McRae & Shah 2009). Instead of calculating a single least-cost path, CIRCUITSCAPE incorporates aspects of electronic circuit theory (i.e. electronic resistance) to visualize resistance patterns across the landscape (McRae 2006; McRae

& Beier 2007; McRae et al. 2008). I tested for relationships between pairwise resistance distances (generated using the "pairwise" mode in CIRCUITSCAPE) and genetic distances using simple and partial Mantel tests as described above. Also, I produced cumulative current flow maps in the "all to one" mode, with focal points as sampled groups, and the source current for each group scaled to group size. The habitat grid encompassed a 5-25km buffer around peripheral focal points, with forests, rivers, residential areas and cattle pastures at very low costs (<10) and oil palm plantations at a moderate cost of 20, following recommendations from the CIRCUITSCAPE manual (resistance values above 20 are considered moderate, while values above 200 are considered high). I ran the program under several other parameterizations with very similar results.

Results

Mantel tests revealed significant isolation-by-distance, meaning strong relationships between Euclidean and genetic distance matrices, both within populations and when the entire sample was considered (Tables 4.1,2,3). Across the entire sample, oil palm plantations at a cost of 10 were the only habitat class for which Mantel's *r*-values between least-cost and genetic distance matrices were consistently larger than Mantel's *r*-values between genetic and Euclidean distance matrices for both Moran's *I* and Rousset's *â* and in both simple and partial Mantel tests (Figure 4.3, Table 4.1). However, when only within-population pairs were considered, Mantel's *r*-values between least-cost and genetic distance matrices for oil palm plantations did not differ greatly from Mantel's *r*-values between genetic and Euclidean distance matrices (Figure 4.4, Table 4.2,3). For both the eastern and western within-population pairs there were large, significant partial Mantel's *r*-values for Rousset's *â* when costs of 5,000 and 10,000 were given

to oil palm plantations, but they were not greater than the Mantel's *r*-value between genetic and Euclidean distance matrices, and Moran's *I* did not show the same pattern (Figure 4.4, Table 4.2,3).

Least-cost distances for forests showed relationships that were the closest to the expected relationship for non-barrier habitat classes when all sample pairs were considered, with absolute Mantel's *r*-values almost consistently decreasing with increasing cost in both simple and partial Mantel tests (Figure 4.3, Table 4.1). This pattern was less clear when only within-population pairs were considered (Figure 4.4, Table 4.2,3).

Least-cost distances for cattle pastures and rivers did not change much in the strength of their relationship with genetic distance as cost changed, although within-population pairs from the western population had consistently high partial Mantel's *r*-values between genetic and least-cost distances for cattle pastures with a cost of 10, they were not greater than the Mantel's *r*-value between genetic and Euclidean distance matrices (Figure 4.4, Table 4.2,3).

Residential areas gave inconsistent trends when all samples were considered; least-cost distances for residential areas had weaker relationships with Rousset's $\hat{a}$ as cost increased, but showed little change in their relationship with Moran's *I* as cost increased (Figure 4.3, Table 4.1). When only within-population pairs were considered, least-cost distances had weaker relationships with genetic distances as cost increased for the eastern population, but showed little change with increasing costs in the western population (Figure 4.4, Table 4.2,3).

Pairwise resistance distances calculated in CIRCUITSCAPE showed strong relationships with genetic distance when all samples were considered (simple Mantel, $\hat{a}$: $r = 0.29$, $P < 0.0001$; *I*: $r = -0.25$, $P < 0.0001$; partial Mantel, $\hat{a}$: $r = 0.24$, $P < 0.0001$; *I*: $r = -0.21$, $P < 0.0001$). Relationships were not as strong when only within-population pairs were considered (eastern

population, simple Mantel $\hat{a}$: $r = 0.17$, $P = 0.001$; I: $r = -0.11$, $P < 0.0001$; partial Mantel $\hat{a}$: $r = 0.09$, $P > 0.05$; I: $r = -0.04$, $P = 0.042$; western population, simple Mantel $\hat{a}$: $r = 0.14$, $P = 0.02$; I: $r = -0.23$, $P < 0.0001$; partial Mantel $\hat{a}$: $r = 0.07$, $P > 0.05$; I: $r = -0.08$, $P > 0.05$).

The resistance surface output from CIRCUITSCAPE showed that even with a moderate cost, oil palm plantations cause an extensive area of low current flow in the middle of the landscape due to the cumulative costs of traversing through this expansive matrix habitat type (Figure 4.5). Although this area of low current flow is dominated by oil palm plantations, the area also encompasses other non-forest habitats, including shrimp and rice farms, some houses, and abandoned lots (Figure 4.1). The resistance surface also shows that current flow is stronger among sites in eastern population than the western population (Figure 4.5), which is consistent with there being consistently larger Mantel's *r*-values between genetic and geographic distance matrices in the western population (Figure 4.4).

Table 4.1. Results of simple and partial Mantel tests between genetic distances (Moran's *I* and Rousset's *â*) and cost distances. Partial Mantel tests controlled for the effect of Euclidean distance.

		Mantel Tests				**Partial Mantel Tests**			
		Moran's *I* Mantel's		**Rousset's *â* Mantel's**		**Moran's *I* Mantel's**		**Rousset's *â* Mantel's**	
		r	***P***	***r***	***P***	***r***	***P***	***r***	***P***
Euclidean Distance:		-0.1821	0.0001	0.2072	0.0001				
Cost Distances:									
	Cost								
Oil	10	-0.2759	0.0001	0.3074	0.0001	-0.2201	0.0001	0.2442	0.0001
Palm	50	-0.1830	0.0001	0.2125	0.0001	-0.0186	0.0024	0.0638	0.0229
Plantations	100	-0.1819	0.0001	0.2127	0.0001	-0.0102	NS	0.0578	0.0423
	1k	-0.1465	0.0001	0.2126	0.0001	0.0125	0.0397	0.0724	0.0353
	5k	-0.0963	0.0001	0.1215	0.0001	-0.0613	0.0001	0.0825	0.0005
	10k	-0.1561	0.0001	0.2169	0.0001	-0.1078	0.0001	0.1654	0.0001
Cattle	10	-0.1824	0.0001	0.2082	0.0001	-0.0207	0.0004	0.0266	0.0155
Pastures	50	-0.1809	0.0001	0.2075	0.0001	-0.0206	0.0004	0.0298	0.0081
	100	-0.1838	0.0001	0.2103	0.0001	-0.0270	0.0001	0.0367	0.0036
	1k	-0.1812	0.0001	0.2123	0.0001	-0.0043	NS	0.0546	0.0324
	5k	-0.1586	0.0001	0.2097	0.0001	0.0278	0.0001	0.0479	NS
	10k	-0.1327	0.0001	0.1970	0.0001	0.0330	0.0001	0.0677	NS
Forest	10	-0.1871	0.0001	0.2097	0.0001	-0.0670	0.0001	0.0378	0.0408
	50	-0.1755	0.0001	0.2058	0.0001	0.0111	NS	0.0161	NS
	100	-0.1651	0.0001	0.1962	0.0001	0.0138	0.0186	0.0080	NS
	1k	-0.0649	0.0001	0.1154	0.0001	-0.0358	0.0001	0.0839	0.0019
	5k	0.0262	0.0001	0.0380	0.0001	0.0405	0.0001	0.0231	NS
	10k	0.0419	0.0001	-0.0462	0.0001	0.0471	0.0001	-0.0522	0.0100
Rivers	10	-0.1844	0.0001	0.2094	0.0001	-0.0415	0.0001	0.0404	0.0138
	50	-0.1844	0.0001	0.2088	0.0001	-0.0441	0.0001	0.0322	0.0209
	100	-0.1840	0.0001	0.2087	0.0001	-0.0360	0.0001	0.0303	0.0395
	1k	-0.1852	0.0001	0.2122	0.0001	-0.0421	0.0001	0.0617	0.0115
	5k	-0.1846	0.0001	0.2198	0.0001	-0.0402	0.0001	0.0750	0.0086
	10k	-0.1702	0.0001	0.2112	0.0001	-0.0388	0.0001	0.0751	0.0085
Residential	10	-0.1747	0.0001	0.2019	0.0001	-0.0056	NS	0.0164	0.0863
Areas	50	-0.1851	0.0001	0.2052	0.0001	-0.0471	0.0001	-0.0185	NS
	100	-0.1851	0.0001	0.2044	0.0001	-0.0466	0.0001	-0.0284	NS
	1k	-0.1843	0.0001	0.1869	0.0001	-0.0295	0.0001	-0.0946	0.0058
	5k	-0.1495	0.0001	0.0903	0.0001	-0.0181	0.0022	-0.1040	0.0040
	10k	-0.0963	0.0001	0.0005	NS	-0.0144	0.0134	-0.1091	0.0024

Table 4.2. Results of simple and partial Mantel tests between genetic distances (Moran's *I* and Rousset's *â*) and cost distances, including only sample pairs within the eastern population. Partial Mantel tests controlled for the effect of Euclidean distance.

		Mantel Tests				**Partial Mantel Tests**			
		Moran's *I* Mantel's		**Rousset's *a* Mantel's**		**Moran's *I* Mantel's**		**Rousset's *a* Mantel's**	
		r	***P***	***r***	***P***	***r***	***P***	***r***	***P***
Euclidean Distance		-0.1032	0.0001	0.1420	0.0021				
Cost Distances:									
	Cost								
Oil	10	-0.1049	0.0001	0.1420	0.0018	-0.0275	0.0356	0.0052	NS
Palm	50	-0.1040	0.0001	0.1484	0.0015	-0.0128	NS	0.0643	0.0230
Plantations	100	-0.1039	0.0001	0.1523	0.0011	-0.0119	NS	0.0897	0.0251
	1k	-0.0862	0.0002	0.1808	0.0019	-0.0037	NS	0.1133	0.0438
	5k	-0.0390	NS	0.1522	0.0219	-0.0022	NS	0.1098	0.0482
	10k	-0.0275	NS	0.1399	0.0256	-0.0020	NS	0.1092	0.0543
Cattle	10	-0.1057	0.0001	0.1465	0.0012	-0.0393	0.0184	0.0687	NS
Pastures	50	-0.1006	0.0001	0.1337	0.0050	0.0283	NS	-0.0992	0.0148
	100	-0.1006	0.0001	0.1337	0.0045	0.0283	NS	-0.0992	0.0155
	1k	-0.1006	0.0001	0.1337	0.0043	0.0283	NS	-0.0992	0.0152
	5k	-0.1006	0.0001	0.1337	0.0050	0.0283	NS	-0.0992	0.0163
	10k	-0.1006	0.0001	0.1337	0.0044	0.0283	NS	-0.0992	0.0159
Forest	10	-0.1016	0.0001	0.1400	0.0023	0.0227	0.0514	-0.0279	NS
	50	-0.1183	0.0010	0.1763	0.0001	-0.0598	0.0016	0.1056	0.0164
	100	-0.1082	0.0001	0.1631	0.0001	-0.0613	0.0017	0.1021	0.0248
	1k	-0.0559	0.0061	0.0816	NS	-0.0585	0.0057	0.0856	NS
	5k	-0.0478	0.0165	0.0686	NS	-0.0578	0.0046	0.0828	NS
	10k	-0.0468	0.0159	0.0669	NS	-0.0578	0.0040	0.0825	NS
Rivers	10	-0.1048	0.0001	0.1438	0.0012	-0.0342	0.0202	0.0393	NS
	50	-0.1019	0.0001	0.1420	0.0025	0.0251	NS	0.0037	NS
	100	-0.1019	0.0001	0.1430	0.0020	0.0152	NS	0.0189	NS
	1k	-0.0988	0.0001	0.1510	0.0018	0.0035	NS	0.0538	NS
	5k	-0.0743	0.0009	0.1412	0.0128	0.0010	NS	0.0559	NS
	10k	-0.0591	0.0067	0.1274	0.0271	0.0007	NS	0.0561	NS
Residential	10	-0.1020	0.0001	0.1450	0.0031	0.0233	NS	0.0666	0.0523
Areas	50	-0.1062	0.0001	0.1371	0.0029	-0.0462	0.0044	-0.0655	NS
	100	-0.1073	0.0001	0.1343	0.0028	-0.0489	0.0080	-0.0772	NS
	1k	-0.1079	0.0001	0.0681	NS	-0.0419	0.0321	-0.0792	NS
	5k	-0.0556	0.0074	-0.0533	NS	-0.0400	0.0397	-0.0776	NS
	10k	-0.0404	0.0384	-0.0754	NS	-0.0397	0.0425	-0.0773	NS

Table 4.3. Results of simple and partial Mantel tests between genetic distances (Moran's *I* and Rousset's *â*) and cost distances, including only sample pairs within the western population. Partial Mantel tests controlled for the effect of Euclidean distance.

		Mantel Tests				**Partial Mantel Tests**			
		Moran's *I*		**Rousset's *a***		**Moran's *I***		**Rousset's *a***	
		Mantel's		**Mantel's**		**Mantel's**		**Mantel's**	
		r	***P***	***r***	***P***	***r***	***P***	***r***	***P***
Euclidean Distance		-0.3170	0.0001	0.1658	0.0001				
Cost Distances:									
	Cost								
Oil Palm	10	-0.3118	0.0001	0.1691	0.0001	0.0304	NS	0.0395	NS
Plantations	50	-0.3229	0.0001	0.1725	0.0001	-0.0652	NS	0.0507	NS
	100	-0.3213	0.0001	0.1715	0.0002	-0.0646	NS	0.0447	NS
	1k	-0.2803	0.0001	0.1895	0.0021	-0.1015	0.0331	0.1086	NS
	5k	-0.2067	0.0001	0.1729	0.0130	-0.1094	0.0256	0.1250	0.0573
	10k	-0.1908	0.0007	0.1675	0.0154	-0.1104	0.0266	0.1272	0.0574
Cattle	10	-0.3255	0.0001	0.1726	0.0001	-0.1690	0.0001	0.1259	0.0267
Pastures	50	-0.3154	0.0001	0.1640	0.0001	0.0466	0.0384	-0.0532	NS
	100	-0.3141	0.0001	0.1627	0.0001	0.0779	0.0054	-0.0826	0.0309
	1k	-0.2878	0.0001	0.1390	0.0005	0.1331	0.0005	-0.1302	0.0310
	5k	-0.1758	0.0003	0.0549	NS	0.1331	0.0008	-0.1290	0.0352
	10k	-0.1027	NS	0.0065	NS	0.1329	0.0007	-0.1287	0.0354
Forest	10	-0.3180	0.0001	0.1640	0.0001	-0.0284	NS	-0.0238	NS
	50	-0.3289	0.0001	0.1794	0.0001	-0.0927	0.0245	0.0750	NS
	100	-0.3297	0.0001	0.1816	0.0001	-0.1031	0.0144	0.0752	NS
	1k	-0.2432	0.0001	0.1371	0.0124	-0.1249	0.0054	0.0739	NS
	5k	-0.1994	0.0001	0.1132	0.0427	-0.1275	0.0055	0.0735	NS
	10k	-0.1929	0.0001	0.1096	0.0519	-0.1279	0.0060	0.0734	NS
Rivers	10	-0.3203	0.0001	0.1688	0.0001	-0.1079	0.0010	0.0918	0.0480
	50	-0.3183	0.0001	0.1661	0.0001	-0.0463	NS	0.0104	NS
	100	-0.3189	0.0001	0.1656	0.0001	-0.0617	0.0302	-0.0035	NS
	1k	-0.3190	0.0001	0.1655	0.0001	-0.0644	0.0284	-0.0045	NS
	5k	-0.3190	0.0001	0.1655	0.0001	-0.0644	0.0274	-0.0045	NS
	10k	-0.3190	0.0001	0.1655	0.0001	-0.0644	0.0267	-0.0045	NS
Residential	10	-0.3190	0.0001	0.1688	0.0001	-0.0557	0.0191	0.0715	0.0182
Areas	50	-0.3179	0.0001	0.1670	0.0001	-0.0320	NS	0.0329	NS
	100	-0.3179	0.0001	0.1670	0.0001	-0.0320	NS	0.0329	NS
	1k	-0.3179	0.0001	0.1670	0.0001	-0.0320	NS	0.0329	NS
	5k	-0.3179	0.0001	0.1670	0.0001	-0.0320	NS	0.0329	NS
	10k	-0.3179	0.0001	0.1670	0.0001	-0.0320	NS	0.0329	NS

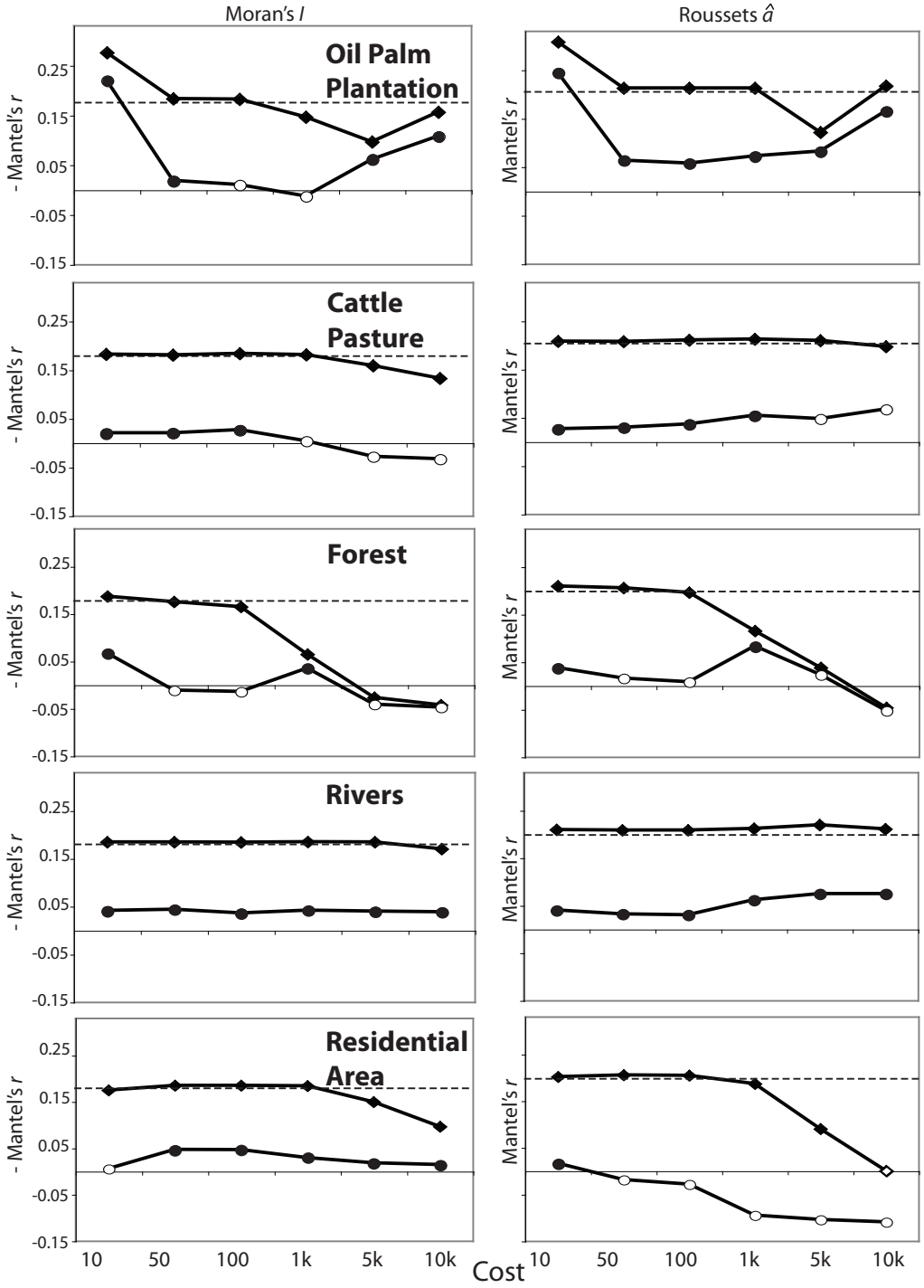

Figure 4.3. Results of Mantel tests of least-cost distances against genetic relationships. Negative Mantel's *r*-values are given (left) for Morans' *I* for easier comparison with trends in Rousset's *â* (right). Filled symbols (diamonds for simple Mantel tests and circles for partial Mantel tests) represent statistically significant Mantel's *r*-values in the expected direction (positive for *â* and negative for *I*). Dotted lines represent the Mantel's *r*-value for Euclidean distance against genetic distance (Table 4.1).

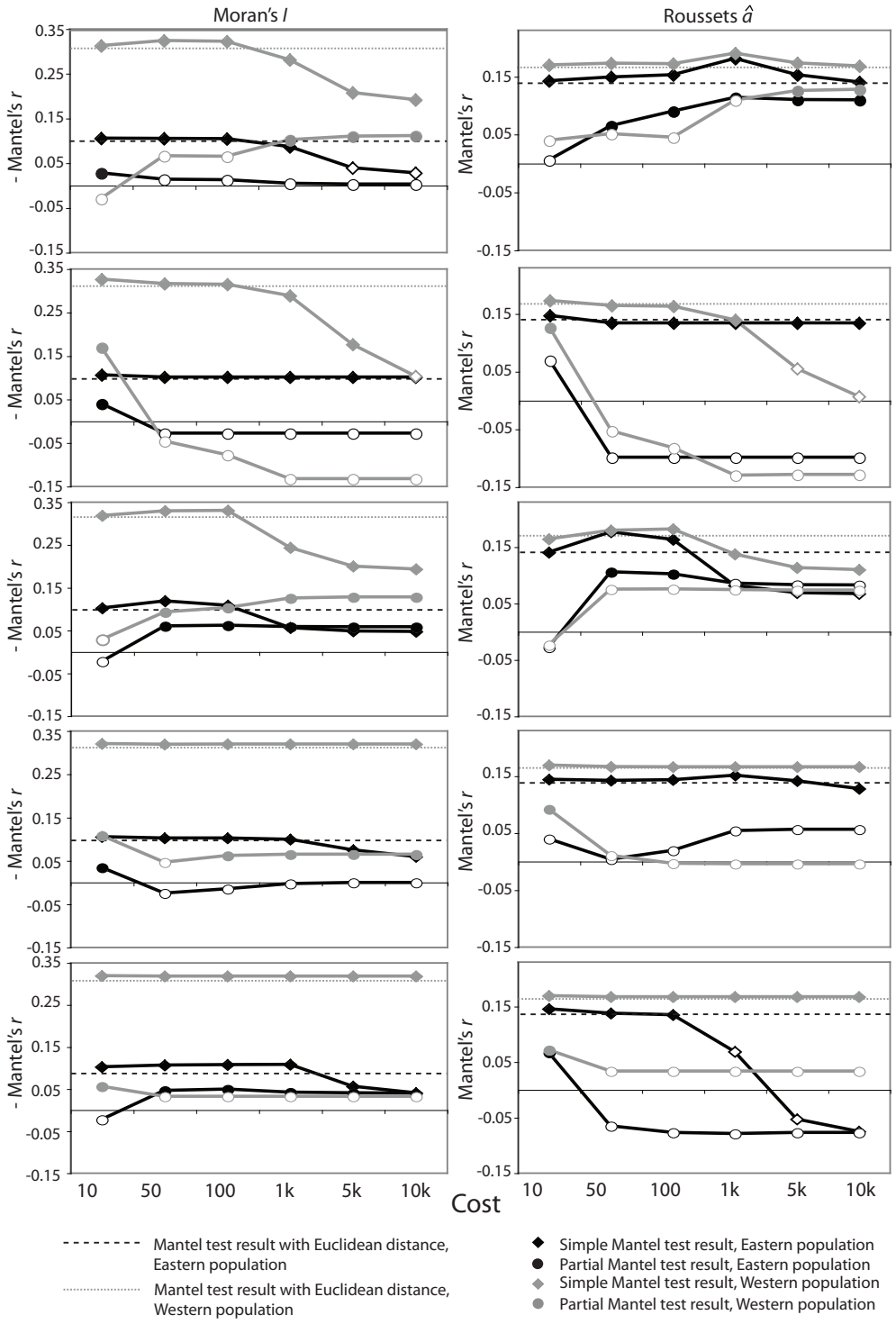

Figure 4.4. Results of Mantel tests of least-cost distances against genetic relationships, including only pairs within the eastern population (black) or western population (grey). Least-cost distances are for oil palm plantations, cattle pastures, forests, rivers, and residential areas (top to bottom). Negative Mantel's *r*-values are given (left) for Morans' *I* for easier comparison with trends in Rousset's *â* (right). Filled symbols represent statistically significant Mantel's *r*-values in the expected direction (positive for *â* and negative for *I*).

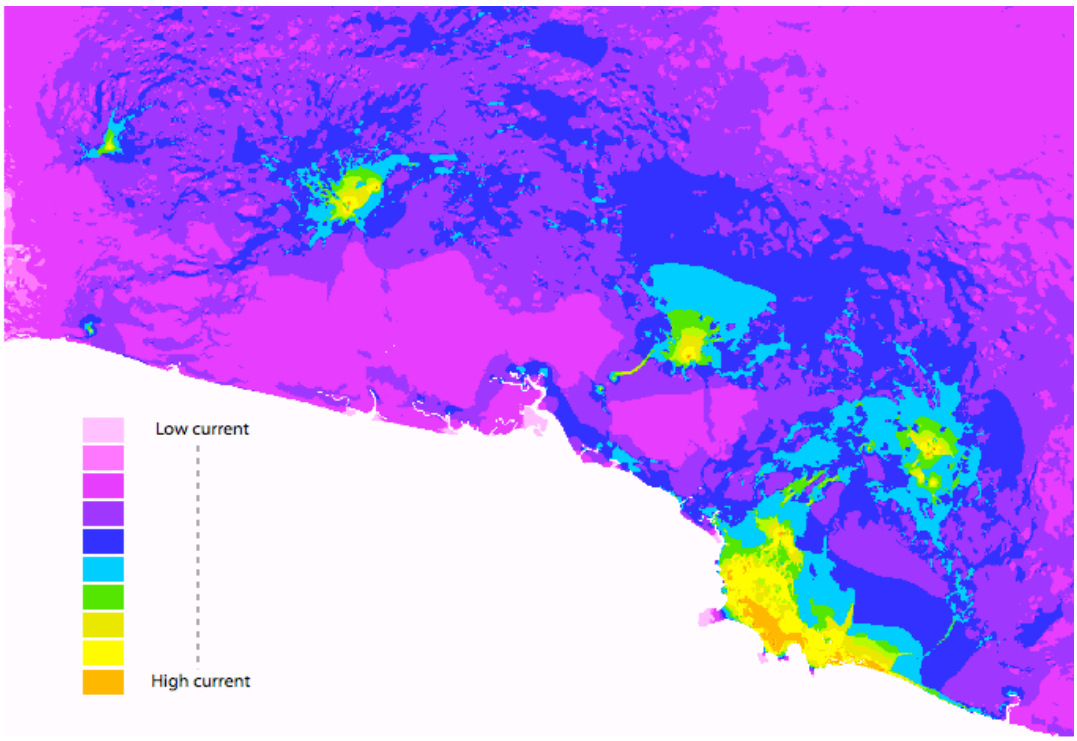

Figure 4.5. Cumulative resistance surface created in CIRCUITSCAPE. Forests, rivers, residential areas and cattle pastures were given very low resistance values (<10) and oil palm plantations were given a moderate resistance of 20.

Discussion

The results suggest that landscape heterogeneity affects genetic relationships in *S. o. citrinellus* and that different matrix habitat classes have different effects on dispersal in the studied landscape. Least-cost distances for oil palm plantations at a cost of 10 had stronger relationships with genetic distances than Euclidean distances in both simple and, more importantly, partial Mantel tests, suggesting that these least-cost distances contributed different information from Euclidean distances and that oil palm plantations represent moderate barriers to gene flow. When analyses were restricted to within population pairs, none of the least-cost distances had stronger effects than Eucidean distances, suggesting that the effect of oil palm plantations on gene flow is mainly between populations.

Similarly, the resistance surface showed how oil palm plantations, even when given only moderate resistance values, impede current flow because they dominate the landscape and the cost of crossing them accumulates over large distances. The strongest current flow in the resistance surface was in the eastern population near Manuel Antonio National Park, where there is not only the highest density of natural forest and monkey groups in the region, but also a break in the oil palm plantations due to complex topography. There was weaker isolation by distance in the eastern population as compared to the western population, also likely due to this break in the oil palm plantations.

By contrast, I found that cattle pastures, rivers, and residential areas do not differ greatly from Euclidean distance in their effects on genetic distance. Cattle pastures, rivers, and residential areas in this region are often surrounded by live fences of fruiting trees, which might explain why they did not show strong negative effects on *S. o. citrinellus* gene flow in this landscape. Alternatively, the landscape composition and configuration of these habitat types is quite different fom that of oil palm plantations, which occur as large, contiguous expanses. Cattle pastures, rivers, and residential areas, by contrast, are smaller and more isolated from one another. In this particular landscape, these features did not influence gene flow, but if multiple landscapes were tested with a range of variability in the composition and configuration of landscape features, one might find different results (Rayfield et al. 2010; Jaquiery et al. 2011; Short Bull et al. 2011).

For example, in the western population, increasing cost for residential areas did not change Mantel's *r*-values between least-cost and genetic distances, but in the eastern population, residential areas showed a non-barrier pattern, with increasing costs resulting in decreasing Mantel's *r*-values. It is likely that residential areas are non-barriers in both populations, but this analysis was not sensitive enough to pick up the signal in the western population, where

residential areas are smaller in number and more spread out in comparison to the eastern population.

A disadvantage to the Mantel test framework is that it is difficult to choose among closely related models or models with only slightly different Mantel's *r*-values (Guillot et al. 2009). Here, pairwise resistance distances created in CIRCUITSCAPE produced similar Mantel's *r*-values to least-cost distances for oil palm plantations at a cost of 10. Other studies have found that resistance distances outperform least-cost distances in how they characterize gene flow (McRae & Beier 2007; Munshi-South pers. comm.), but here I cannot say definitively which had the stronger relationship with genetic distance because the Mantel's *r*-values were within a reasonable margin of error of one another. Both assigned moderate costs to oil palm plantations, however, and had stronger relationships with genetic distances than Euclidean distance matrices when between population pairs were included in the analysis.

In addition, I acknowledge the possibility that the temporal scale of the landscape data has influenced the results (Brooks et al. 1999; Landguth et al. 2010). Genetic distance measures such as Rousset's $\hat{a}$ are based on F_{ST} and as such may reflect processes that are more likely to be apparent in historical landscape data (Balkenhol et al. 2009a). Although I have shown some effect of landscape heterogeneity on gene flow using current landscape data, historical data from the early 1900s may show a stronger relationship. Unfortunately such data are not available for the early 1900s, but aerial photographs from 1953 are available from the Instituto Geográfico Nacional in Costa Rica and I hope to incorporate these data in future analyses.

Another related issue common in many landscape genetic studies is that the different focal matrix habitats used in the analysis are likely of different ages. Rivers are older than the other four habitat classes I considered, and some residential areas are likely younger than the cattle

pastures and oil palm plantations, most of which were established in the early 1900s. Future analyses might address this issue by including different sets of models and molecular markers that pinpoint different temporal scales in order to distinguish between the effects of historical and recent landscape changes on population genetic structure (Chiucchi & Gibbs 2010).

Implications for Conservation Management

This study exemplifies how important it is to quantify the relative effects of different matrix habitat classes in landscape genetic analyses (Cushman et al. 2006; Balkenhol et al. 2009a; Watling et al. 2011), instead of assuming that all non-suitable habitats have a uniform effect on dispersal and gene flow. Because this study distinguished among matrix habitat classes, the results offer a finer understanding of what does and does not constitute a barrier to *S. o. citrinellus* gene flow in the Central Pacific Costa Rican landscape. The results also allow more detailed recommendations to conservation managers regarding the types of matrix habitat that *S. o. citrinellus* may or may not use to disperse among patches of forest in the Central Pacific. In concurrent study, I used resistance surfaces to test different biological corridor configurations for their ability to augment gene flow through oil palm plantations (see Chapter 5), which could be expanded upon further. Another option to augment gene flow through oil palm plantations might be to plant understory vegetation, which has been shown to increase bird richness in oil palm plantations in eastern Guatemala (Nájera & Simonetti 2010).

However, I must recognize that these results are specific to the Central Pacific landscape, and conservation managers should be careful not to apply these results in other landscapes or for other populations of *S. oerstedii*. For example, in a landscape where cattle pastures dominate instead of oil palm plantations, a separate landscape genetic study would be necessary to measure the relative effects of each matrix habitat to determine whether it might be more

important for conservation managers to augment gene flow through cattle pastures rather than oil palm plantations.

When attempting to translate the results of any landscape genetic analysis to patterns of functional connectivity, we must also acknowledge that measures of genetic distance do not equate to animal movement patterns. Simulations that model the sums of individual behavioral decisions are likely necessary to best inform conservation management of taxa in heterogeneous landscapes (Bowler & Benton 2005; Tracey 2006; Knowlton & Graham 2010; Lowe & Allendorf 2010; Spear et al. 2010). A possible next step would be to incorporate a recently published new form of population viability analysis that uses individual-based models (simulations) that incorporate behavioral decisions alongside models of landscape change over time (Nabe-Nielsen et al. 2010).

Conservation management of *S. o. citrinellus* and other species could be further informed by combining landscape genetics with ecological niche models to help predict the potential effects of future climate and landscape change on the evolutionary potential of populations. For example, in species like *S. o. citrinellus* where landscape genetics has shown an effect of current landscape features on gene flow, models that predict how species distributions will change in response to future climate scenarios (such as ecological niche models) could help to predict whether a species' landscape may become highly fragmented in the future, potentially causing a further loss of connectivity and gene flow (Tolley et al. 2009). In particular, novel simulation approaches such as CDPOP (Landguth & Cushman 2010) and GESTE (Foll & Gaggiotti 2006) could allow for the creation of predictive models of genetic population structure under different climate and landscape change scenarios (Balkenhol et al. 2009b).

Conclusions

Landscape genetic analyses aid in our understanding of fine-scale population and evolutionary processes, especially in heterogeneous, human-modified landscapes. These results confirm that landscape genetic approaches are highly dependent on study scale; if my study had been limited to just the eastern or western population of *S. o. citrinellus*, I would not have inferred any effect of oil palm plantations on gene flow because the effect only emerged when between population pairs were included in the analysis. This study also stresses that matrix heterogeneity should be considered explicitly in studies of dispersal and gene flow, as opposed to assuming that all non-suitable habitats have a uniform effect on these processes. Finally, this study shows that when landscape genetic methods are applied rigorously and at the right scale, they are sensitive enough to track population processes even in species with long, overlapping generations like primates. Thus, landscape genetic approaches are extremely valuable for the conservation management of a diverse array of endangered species in heterogeneous, human-modified habitats.

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CHAPTER 5.

Genetics-based conservation management recommendations for *Saimiri oerstedii*

Abstract

Incorporating genetic information into the conservation management of wild populations is extremely important yet limited by poor communication among evolutionary geneticists and conservation managers. Here I present the results of genetic research on a wild population of primates, Central American Squirrel Monkeys (*Saimiri oerstedii*), using non-technical language that will allow clear and effective communication of scientific results and related management recommendations to conservation managers and policy-makers. The most crucial recommendations include: 1) the two *S. oerstedii* subspecies should be housed separately in captive facilities and not allowed to hybridize, 2) transfers, reintroductions, or translocations should only involve groups of the same subspecies, 3) transfers, reintroductions, or translocations of either males or females are equally likely to be successful in *S. o. citrinellus*, 4) the western population of *S. o. citrinellus* is isolated and in order to augment dispersal to this population and maintain its genetic health, efforts should focus on building biological corridors through or around adjacent oil palm plantations, and 5) managers should prioritize maintaining existing forest connectivity by engaging with municipal governments to ensure that any plans for development explicitly promote connectivity of forests through forest easements.

Introduction

Genetic information has been incorporated into the design and implementation of conservation efforts since the late 1970s (Frankel & Soulé 1981; Frankham 2006; Frankham

2010). However, the practical application of this information has been largely restricted to the management of populations in captivity, not in the wild. For populations in captivity, genetic information is used, for example, to estimate relatedness among individuals to prevent inbreeding, outbreeding, or hybridization (DeSalle & Amato 2004). Genetics-based management of wild populations is less common, but not because of a lack of information; genetic studies on wild populations have increased exponentially in recent years owing to technical advances in high-throughput DNA analysis from low-quality, non-invasively collected samples including feces (DeSalle & Amato, 2004). These studies could be extremely useful to on-the-ground management efforts, as they often identify populations of concern, define biologically relevant management units, and estimate population sizes and sex ratios (Duagherty et al. 1990; Melnick et al. 2000; Andayani et al. 2001; Frankham et al. 2002; Reed et al. 2003; DeSalle & Amato 2004; Frankham 2010).

It may be that managers are not making full use of extensive genetic data available because of weak lines of communication among researchers and managers (Vernesi et al. 2008; Laikre 2010). International conservation efforts and policies at a broader level are also lacking in input from genetic findings. International efforts typically focus on habitats, landscapes, and species, but not gene level variation or its decline. Thus, important conservation genetic findings are generally not translated to concrete conservation actions as a part of international policy development. As such, genetic variation is not monitored, and there is no strategy for how genetic information can be included in international biodiversity targets (Laikre 2010).

Communication among conservation geneticists, managers, and policymakers needs to improve, as many of the most pressing issues in biodiversity conservation include those that would benefit from the input of genetic data. Chief among them is the management of

populations in fragmented and degraded habitats (Frankham 2010). Habitat fragmentation threatens species' survival by decreasing dispersal rates among populations, which can lead to drastic reductions in genetic diversity, higher susceptibility to external pressures including disease, and rapid loss of individuals and populations (Frankham et al. 2002; Frankham 2006).

Here I present a case study in how the results of highly technical genetic research on a wild population of primates can be communicated clearly and effectively to a non-technical audience of conservation managers and policy-makers. Management recommendations based on genetic research on the Central American Squirrel Monkey (*Saimiri oerstedii*) are outlined below, as they will be presented to Costa Rican non-governmental organizations, park managers, and government officials at the Ministry of the Environment and Energy. Recommendations are grouped by the type of genetic analyses undertaken: 1) Analyses of genetic variation within and among populations, 2) Analyses of dispersal by male and female monkeys, and 3) Analyses of the impact of land use and habitat type on the movement of monkeys and their genes across the complex landscape of the Central Pacific of Costa Rica. Recommendations are clear, relate to specific management actions, and are linked directly to data and results.

In my analyses, 233 individual monkeys from across the entire distribution of the subspecies *S. o. citrinellus* in the Central Pacific region of Costa Rica and 11 individuals from the subspecies *S. o. oerstedii* in the Southern Pacific region of Costa Rica were genotyped at 16 microsatellite markers in the nuclear genome and sequenced for a 880 base pairs in the control (d-loop) region of the mitochondrial genome. These markers were chosen because they evolve rapidly and neutrally, and are commonly used in studies seeking to describe patterns of individual dispersal.

National and International Conservation Importance of Saimiri oerstedii

The two subspecies of the Central American Squirrel Monkey, *S. o. citrinellus* and *S. o. oerstedii* have been listed as endangered and threatened with extinction, respectively, since 1996 on the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species, due to their restricted and fragmented ranges of occurrence, and continuing habitat loss (IUCN 2010). Because *S. oerstedii* eat primarily fruit and disperse the seeds of those fruits, they are likely to play important roles in the long-term survival of Costa Rican forests, which are some of the most diverse in the world (Hartshorn 1983; Harmon 2004). Further, *S. oerstedii* are very charismatic and considered by many as a flagship species for biodiversity conservation in the region, and are a major draw for Costa Rica's tourism industry, which represents over 15% of the Gross National Product (ICT 2005). Thus, the conservation of *S. oerstedii* is extremely important to the international conservation community, but more importantly it represents a key component of a major driver of Costa Rica's economy.

The Distribution of Genetic Variation

Analyses of the distribution of genetic variation within and among populations of an organism in the wild can be used to inform conservation management both on the ground (*in situ)* and in captivity (*ex situ*). Here, I present analyses of the distribution of genetic variation among wild populations of *S. oerstedii* in order to 1) confirm that the two recognized subspecies are truly distinct (evolutionarily significant units or ESUs), and thus should be kept separated from one another in captivity and in the wild, and 2) identify isolated populations that would benefit from receiving migrants from nearby populations through on the ground efforts.

I examined the distribution of genetic variation among populations using standard methods, with nuclear microsatellite markers that are inherited from both parents, and mitochondrial DNA, which is inherited only from mothers. A complete description of methods and results are presented in Chapter 2.

The levels of nuclear microsatellite genetic diversity I found are similar to other ongoing studies of microsatellite diversity (G. Gutierrez, Pers. comm.) and a recent study of blood protein diversity (Zaldivar et al. 2004) in *S. oerstedii*. When comparing genetic diversity across the different Costa Rican primate species, *S. oerstedii* consistently has the greatest amount of diversity despite its endangered status (Zaldivar et al. 2004). Such high levels of diversity may reflect certain life history characteristics of *Saimiri* compared to other primates. *Saimiri* have short generation times, which can result in high population growth rates and the accumulation of genetic diversity (Zaldivar et al. 2004).

Major result 1:

- The two *S. oerstedii* subspecies are well supported by genetic data as biologically distinct.

Management recommendation:

- The two *S. oerstedii* subspecies should be housed separately in captive facilities and not allowed to hybridize.
- Transfers, reintroductions, or translocations should only involve groups of the same subspecies

Analyses of both the microsatellite and mitochondrial data support strong genetic differentiation between the two *S. oerstedii* subspecies. Managers can tell the two subspecies apart by slight differences in coloration and size: *S. o. citrinellus* are slightly larger in size and males have grey pelage on the crown of their heads as opposed to black pelage in *S. o. oerstedii* (Boinski & Sirot 1997; Carrillo et al. 2002). In addition, I have identified distinct mitochondrial d-loop haplotypes for each subspecies to facilitate genetic identification of individuals of unknown geographic origin in captivity (see Appendix, Supplementary Table S1).

Major result 2:

- There are two genetically different populations, western and eastern, within *S. o. citrinellus,* and the western population is less genetically diverse than the eastern population.

Management recommendation:

- Conservation of the western population of *S. o. citrinellus* should be considered a priority and movement of individuals to this population from the eastern population should be augmented and monitored closely.

All analyses of microsatellite and mitochondrial data support there being two genetically distinct populations, western and eastern, within *S. o. citrinellus*. The western population extends from Esterillos/Gamalotillo to Cerros/Parcelas de Damas and the eastern population consists of groups in Villanueva, Londres, Naranjito, and in and around Manuel Antonio National Park including Playa el Rey (Figure 5.1). In landscapes with historical and ongoing habitat loss and modification such as *S. o. citrinellus* habitat, populations often become fragmented and certain landscape features prevent individuals in one fragment from moving to another fragment,

resulting in ever growing genetic differences among the fragmented populations. To maintain the naturally high levels of genetic diversity I found within these populations, conservation managers should monitor and augment dispersal to the western population, which is more isolated and less genetically diverse than the eastern population of *S. o. citrinellus*. The results of my landscape genetics analyses (presented below) are more specific about what landscape features are preventing dispersal, and how and where dispersal to the western population could be augmented.

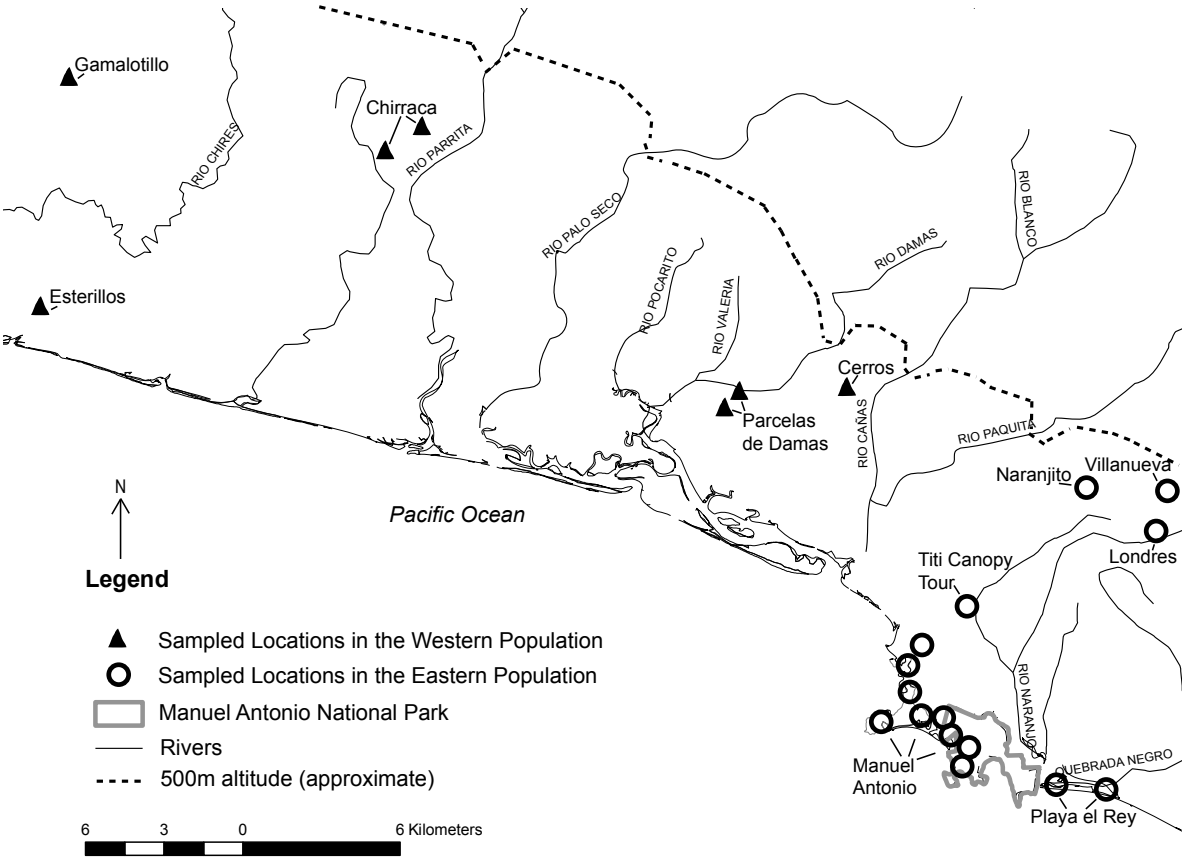

Figure 5.1. Groups of *S. o. citrinellus* sampled for the analysis of the distribution of genetic variation, showing the putative 500m altitude limit to their distribution. Groups identified as part of the western population are black triangles and groups identified as part of the eastern populations are hollow circles.

The Dispersal of Males and Females

In some primates, particularly many old-world monkeys, males leave the group into which they were born before becoming sexually mature, while females remain in their birth group throughout their lives (Melnick & Pearl 1987). However, in some species, females are more likely to disperse, or both sexes are equally likely to disperse, but do so over different distances (Moore 1984; Boinski 1986; Pusey & Packer 1987; Pope 1992; Strier 1994; Baker & Dietz 1996; Nishimura 2003; Douadi et al. 2007; Huck et al. 2007; Di Fiore 2009; Di Fiore et al. 2009). Information on whether males, females, or both disperse has important implications for conservation management activities both on the ground and in captivity. For example, in a predominantly female-dispersing species, females can be moved among captive facilities, reintroduced to wild populations, or translocated among populations, but it is unlikely that any of these activities would be successful with males.

I examined male and female dispersal patterns in *S. o. citrinellus* by separating the dataset into male and female components and comparing the association of genetic differences between populations with geographic distances between those same populations in each component. This association should be stronger among males than females if females are the principal dispersing sex. If females disperse, degrees of relatedness should also be lower and have greater variation among females as compared to males (Morin et al. 2001; Di Fiore 2003). Finally, if females disperse, the distribution of genetic variation should be similar for mitochondrial and nuclear genetic markers (Avise 1995). Because mitochondrial DNA is inherited only from mothers, patterns in the distribution of variation in mitochondrial DNA reflect the dispersal of females and the genes they pass on, while patterns in nuclear DNA reflect the dispersal of both males and females because they both pass on genes from the nuclear genome to their offspring (Melnick

1988; Melnick & Hoelzer 1993; Avise 1995; Favre et al. 1997; Di Fiore & Fleischer 2005; Eriksson et al. 2006). A complete description of methods and results are presented in Chapter 3.

Major result 3:

- There is genetic evidence that both males and females disperse in *S. o. citrinellus* in the Central Pacific landscape.

Management recommendation:

- Transfers, reintroductions, or translocations of either males or females are equally likely to be successful in this region. However, it would be safest to engage in further behavioral study to ensure successful transfers by males before broadly applying this recommendation.

Previous studies of behavioral ecology observed predominantly female dispersal in *S. o. oerstedii* in the Osa Peninsula in the Southern Pacific region of Costa Rica (Boinski & Sirot 1997; Boinski et al. 1998; Boinski 1999). However, the genetic data do not support predominantly female dispersal in *S. o. citrinellus* in the Central Pacific region. Specifically, females and males did not differ in the association of genetic difference and geographic distance, or in values of relatedness. Further, I detected recent migrants of both sexes among populations using the genetic data, and I found greater population differences in the mitochondrial DNA as compared to nuclear DNA, suggesting that although some females disperse, many do not.

It is possible that my genetic research was able to detect rare dispersal events not identifiable by direct observation (Di Fiore 2003; Lawson Handley & Perrin 2007), which may explain why *S. o. citrinellus* dispersal patterns differ from behavioral observations of *S. o.*

oerstedii. However, differences in local ecological factors in their respective habitats may also be driving differences in dispersal patterns.

My results suggest that transfers, reintroductions, and translocations of either sex are equally likely to be successful in *S. o. citrinellus*. However, I urge conservation managers to use caution; because studies of behavioral ecology only support female dispersal in *S. o. oerstedii*, managers should engage in further behavioral study to confirm successful male transfers in *S. o. citrinellus* before broadly applying this recommendation.

Landscape Genetics and Connectivity

The distribution of *S. o. citrinellus* includes only one protected area, which is also the smallest and most heavily visited protected area in the country, Manuel Antonio National Park (ICT 2005). Thus, the management of *S. o. citrinellus* outside the national park is essential to its persistence. However, outside the park the majority of forests (approximately 80%) were replaced with fruit plantations in the 1930s (Mattey 1992, 1994; PRMVS 1996). I used a "landscape genetic" approach to determine what type of habitats in between patches of natural forest are contributing the most to the isolation, identified earlier in this paper, between the western and eastern populations of *S. o. citrinellus*. I then used this information to explore whether several potential biological corridor configurations across the landscape might achieve connectivity among populations in a preliminary, qualitative analysis.

Landscape genetic analyses were used to determine which of several habitat types prevent dispersal among forest patches. In this approach, genetic differences are correlated with different measures of geographic distance. Geographic Information Systems (GIS) software then assigns costs to different habitat types and calculates the path between groups that is "least

costly", or most likely to be successful. Several least-cost paths are calculated for each habitat type with the cost of one habitat varying, and all other habitat types held at an equal, low cost. All possible cost combinations are tested against each other for the least-cost path that has the strongest association with genetic differences. Hypothetically, this path best describes how *S. o. citrinellus* moves through its landscape, and the habitat type with the highest cost in this path represents the greatest barrier to *S. o. citrinellus* dispersal. I tested five different habitat types: cattle pastures, oil palm plantations, residential areas, and rivers, with forests as a control (see Chapter 4, Figure 4.1). A complete description of methods and results are presented in Chapter 4.

Major result 4:

- Oil palm plantations represent a moderate barrier to dispersal in *S. o. citrinellus* in the Central Pacific landscape, but cattle pastures, rivers, and residential areas do not.

Management recommendation:

- To increase the movement of individuals to the isolated western population of *S. o. citrinellus*, efforts must focus on improving movement through or around oil palm plantations.

Oil palm plantations represent a moderate barrier to dispersal, but cattle pastures, rivers, and residential areas do not affect landscape-scale dispersal patterns in *S. o. citrinellus* in the Central Pacific landscape. It is important to note, however, that my results are specific to the Central Pacific landscape, and conservation managers should be careful not to apply these results in other landscapes or for other populations of *S. oerstedii* (Short Bull et al. 2011).

An additional analysis, "isolation-by-resistance," further supports that oil palm plantations have an effect on genetic relationships in the Central Pacific landscape. Isolation-by-resistance analysis generates a resistance surface characterizing the cumulative effects of different habitat types on dispersal (McRae & Shah 2009). Instead of calculating a single least-cost path, it incorporates aspects of electronic circuit theory (i.e. electronic resistance) to visualize resistance patterns across the landscape (McRae 2006; McRae & Beier 2007; McRae et al. 2008). A complete description of methods and results are presented in Chapter 4.

The resistance surface (Figure 5.2a) shows that even with a moderate cost, oil palm plantations cause a rather large area of high resistance in the middle of the landscape due to the cumulative costs of traversing through the expansive plantation. The least resistance (or strongest current flow) in the resistance surface was near Manuel Antonio National Park, where there is not only the highest density of natural forest and monkey groups in the region, but also a break in the oil palm plantations due to complex topography.

Major result 5:

- Existing connectivity is strong around Manuel Antonio National Park and up into Naranjito, Londres, and Villanueva.

Management recommendation:

- Given that development in this area is increasing rapidly, managers should prioritize maintaining existing natural forest connections by engaging with municipal governments to ensure that any plans for development explicitly address natural forest connectivity through conservation easements.

One possible solution to augmenting dispersal through or around the oil palm plantations would be the construction of a biological corridor of diverse native fruiting trees through or around the plantations. Successful implementation of such a corridor would involve negotiation with the palm oil company, Palma Tica, to develop a plan that results in minimal profit loss while still augmenting *S. o. citrinellus* dispersal. I explored how different potential corridors could augment dispersal across the landscape by generating a set of resistance surfaces with varying configurations of biological corridors (Figures 5.2b-d). Stepping stone configurations were also tested; these consisted of 5-20 patches of fruiting trees 1-5 ha in size, oriented close together but not in a continuous line. However, none of these configurations affected current flow (dispersal) among patches of habitat in my simulations.

Major result 6:

- A 100m wide corridor of diverse, native fruiting trees along the northern edge of the oil palm plantation could successfully augment dispersal with the smallest area of impact.

Management recommendation:

- Managers should engage in further scientific analysis and work with an economist to conduct a cost-benefit analysis of the suggested corridor (Corridor 3, Figure 5.2d) and present a corridor construction plan to Palma Tica.

Corridors 1 and 2 result in increased current flow at the eastern side of the corridor (Figure 5.2b,c). Corridor 3, however, results in a moderate but consistent increase in current flow all along the length of the corridor (Figure 5.2d), because it directly connects several large groups of monkeys and is close to more forest patches than the other corridors, minimizing the

inter-patch distance that dispersing animals would have to traverse along the corridor. Because Corridor 3 represents the smallest total area and facilitates the most consistent amount of current flow, it is the most likely to be successful at augmenting dispersal and also the most economically feasible option for Palma Tica. However, my analysis was qualitative and preliminary, and so managers should engage in further scientific analysis of the potential utility of this corridor, perhaps through agent-based simulation models (Balkenhol et al. 2009), and work with an economist to conduct a cost-benefit analysis of this corridor.

Although biological corridors have several potential drawbacks, including unintended travelers such as invasive species and disease (Crooks & Suarez 2006), the positive effects of corridors on increasing movement among patches and thereby increasing population size, survivorship, and growth may outweigh any potential negatives (Beier & Noss 1998; Coffman et al. 2001; Berggren et al. 2002; Tewksbury et al. 2002; Haddad et al. 2003). More studies on the effects of corridors on population viability are necessary (Haddad & Tewksbury 2006; Hilty et al. 2006), but a recent analysis of corridor effectiveness shows that corridors increase movement between patches by approximately 50% as compared to patches not connected by corridors, and are especially successful and important for maintaining connectivity in invertebrate, non-avian vertebrate, and plant communities (Gilbert-Norton et al. 2010).

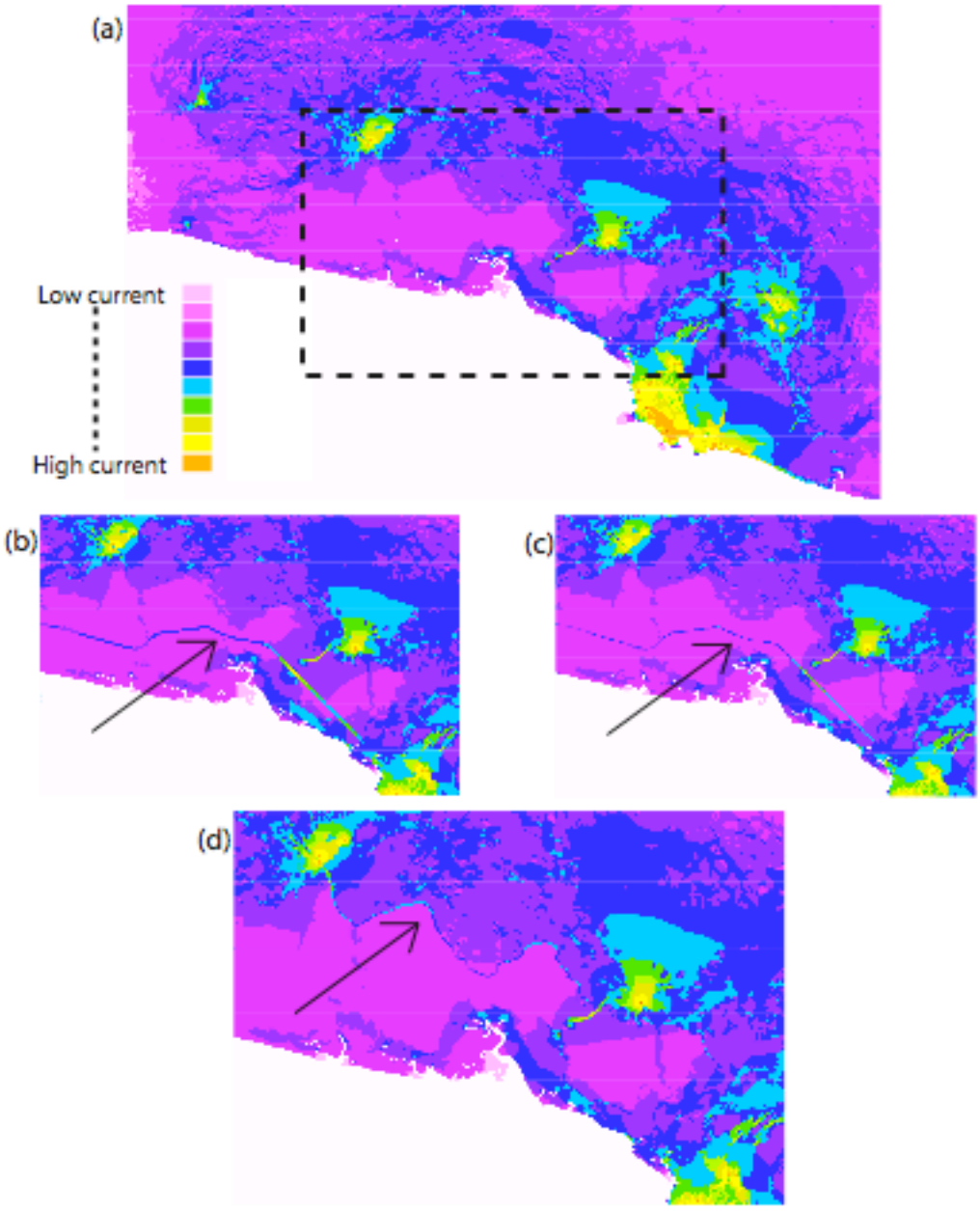

Figure 5.2. Resistance surfaces generated in CIRCUITSCAPE for *S. o. citrinellus* in the Central Pacific region of Costa Rica. Forests, rivers, residential areas and cattle pastures were given very low resistance values (<10) and oil palm plantations a moderate resistance of 20, although other parameterizations produced very similar results to those shown here. (a) Original, unchanged landscape (b) Corridor 1, a 200m wide corridor along major highway that runs through the middle of the oil palm plantation (589.3 ha total) (c) Corridor 2, a 100m wide corridor also along the highway (281.8 ha total) (d) Corridor 3, a 100m wide corridor along the northern edge of the oil palm plantation (265.3 ha total). (b-d) are zoomed in to the black box outlined in (a).

Summary of Recommendations

1. The two *S. oerstedii* subspecies, *S. o. citrinellus* and *S. o. oerstedii*, should be housed separately in captive facilities and not allowed to hybridize.
    a. Transfers, reintroductions, or translocations should only involve groups of the same subspecies.
2. Transfers, reintroductions, or translocations of either males or females are equally likely to be successful in the Central Pacific landscape. However, it would be safest to engage in further behavioral study to ensure successful transfers by males before broadly applying this recommendation.
3. Conservation of the western population of *S. o. citrinellus* should be considered a priority and movement of individuals to this population from the eastern population should be augmented and monitored closely.
    a. In order to increase the movement of individuals to the isolated western population of *S. o. citrinellus*, efforts must focus on improving movement through or around oil palm plantations.
    b. Managers should engage in further scientific analysis and work with an economist to conduct a cost-benefit analysis of the suggested corridor (Corridor 3, Figure 5.2d) and present a corridor construction plan to Palma Tica.
4. Given that development in this area is increasing rapidly, managers should prioritize maintaining the existing strong connections between Manuel Antonio National Park and Naranjito, Villanueva and Londres by engaging with municipal governments to ensure that any plans for development explicitly address natural forest connectivity through conservation easements.

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CHAPTER 6.

Summary and Conclusions

In this dissertation, I investigated dispersal patterns, population genetic structure, and intraspecific molecular systematics in an endangered New World primate, the Central American Squirrel Monkey (*Saimiri oerstedii*, Primates: Cebidae), with a special focus on the more northern subspecies, *S. o. citrinellus,* in the heterogeneous Central Pacific region of Costa Rica. The specific goals of this dissertation were to answer the following questions: 1) Is there genetic support for the subspecies distinction between *S. o. citrinellus* and *S. o. oerstedii*? 2) Is there population genetic structure within *S. o. citrinellus*? 3) Do male and female patterns of dispersal and population genetic structure differ in *S. o. citrinellus*? 4) Is there a relationship between landscape heterogeneity and genetic structure in *S. o. citrinellus*? and 5) How can genetic analyses inform the conservation management of *S. oerstedii* and in particular *S. o. citrinellus*?

To answer these questions rigorously, I collected non-invasive genetic samples and fine-scale landscape data from across the Central Pacific landscape of Costa Rica and analyzed these data using molecular systematics, population genetics, and landscape genetic methods. Chapter 2 explored whether molecular genetic support exists for the subspecies distinction between *S. o. citrinellus* and *S. o. oerstedii*. Chapter 2 also described population genetic structure and recent migration patterns within *S. o. citrinellus*. Chapter 3 compared population genetic structure among males versus among females to test for sex-biased dispersal patterns in *S. o. citrinellus*. Chapter 4 described the relationship between landscape heterogeneity and genetic structure in *S. o. citrinellus*, and inferred which matrix habitats are costly to dispersal. Chapter 5 offered

explicit management recommendations for the conservation of *S. oerstedii* based on the results from previous chapters.

The main results of the dissertation were in general agreement with my hypotheses as outlined in each chapter, except for Chapter 3 (see below). In Chapter 2, I hypothesized that I would find genetic support for *S. o. citrinellus* and *S. o. oerstedii* as separate taxa referred to as subspecies based on their disjunct geographic distributions, separated by the large Térraba River, and previously cited morphological differences (Carrillo *et al.*, 2002; Hershkovitz, 1984). Indeed, both autosomal and mitochondrial markers supported the two subspecies as genetically distinct. Also in Chapter 2, I expected to find population genetic structure within *S. o. citrinellus* due to the historically heterogeneous landscape. I did discover population genetic structure in *S. o. citrinellus*, finding two genetically distinct populations. I also found evidence of recent migration between these populations, but genetic diversity was much lower in the western population as compared to the eastern population.

In Chapter 3, I expected to find genetic evidence of female-biased dispersal in *S. o. citrinellus* based on behavioral observations of *S. o. oerstedii* in the Osa Peninsula in the Southern Pacific region of Costa Rica (Boinski, 1999). However, instead I found evidence that both sexes disperse in *S. o. citrinellus* in the Central Pacific region, and males probably disperse over longer distances. These results contribute to a growing body of evidence that primate dispersal patterns can vary among closely related taxa and even among different local populations of the same taxon (Di Fiore, 2009). However, my results would be greatly augmented by the addition of Y-chromosome genetic markers and longitudinal behavioral research on *S. o. citrinellus*.

In Chapter 4, I directly explored the effect of landscape heterogeneity on genetic relationships in *S. o. citrinellus*, expecting to find that landscape heterogeneity did have an effect on genetic structure, and that some matrix habitats would be more costly to dispersal than others. As expected, some geographic distances incorporating landscape heterogeneity correlated more strongly with genetic distances than Euclidean geographic distances, supporting landscape heterogeneity as an important factor in determining local population genetic structure in *S. o. citrinellus* in the Central Pacific region of Costa Rica. Specifically, I found that oil palm plantations are costly to dispersal, but not other matrix habitats such as cattle pastures and residential areas. However, my analysis was sensitive to the composition and configuration of the Central Pacific landscape. In another landscape where cattle pastures or other matrix habitats dominate instead of oil palm plantations, I might find that these other matrix habitats are costly to dispersal in addition to or instead of oil palm plantations (Jaquiery *et al.*, 2011; Rayfield *et al.*, 2010; Short Bull *et al.*, 2011). I can therefore only infer that oil palm plantations are costly to *S. o. citrinellus* dispersal in the Central Pacific landscape, and not necessarily across all populations of *S. oerstedii*. Also, although I was able to show some effect of landscape heterogeneity on gene flow using current landscape data, historical landscape data may better characterize these patterns of gene flow in *S. o. citrinellus* (Balkenhol *et al.*, 2009a; Brooks *et al.*, 1999; Landguth *et al.*, 2010).

In Chapter 5, I incorporated results from the previous chapters into science-based management recommendations for the conservation of *S. oerstedii,* with a special focus on *S. o. citrinellus* in the Central Pacific landscape. Based on the results of Chapter 2, I recommended that the two *S. oerstedii* subspecies should be housed separately in captive facilities and not allowed to hybridize, and that transfers, reintroductions, or translocations should only be done

among groups of the same subspecies. Based on Chapter 3, I recommended that transfers, reintroductions, or translocations of either males or females are equally likely to be successful in the Central Pacific landscape. Based on the results of Chapters 2 and 4, I suggested that in order to augment dispersal to the isolated western population of *S. o. citrinellus,* conservation efforts should focus on building biological corridors through or around adjacent oil palm plantations. Also, I recommended that managers prioritize maintaining existing forest connectivity in the Central Pacific region by engaging with municipal governments to ensure that any plans for development explicitly promote connectivity of forests through forest easements.

Implications of this work

In addition to the conservation implications of this study as discussed in Chapter 5, this dissertation has many implications for future studies of evolutionary and ecological processes in heterogeneous landscapes.

In particular, these results contribute to a growing body of research that finds differences in dispersal patterns among local primate populations of the same taxon (Di Fiore, 2009). Combining genetic and behavioral data will be necessary to build better predictive models for why dispersal patterns differ among species or populations of the same species. I hypothesized here that differences in local ecology may have resulted in the differences I found between *S. o. citrinellus* dispersal patterns and that of their close relatives, *S. o. oerstedii*. Other studies have found that recent events such as anthropogenic habitat disturbance can also alter dispersal patterns (Goossens *et al.*, 2006). Based on these results, predictive models for variation in dispersal patterns should consider both 1) variation among the environments of local populations within a species and 2) temporal variation of local environments (e.g. recent habitat disturbance).

This dissertation also adds to an accumulating list of meta-analyses and individual research studies that support the consideration of varying effects of different matrix types on patterns of gene flow and dispersal, instead of assuming uniform effects of all non-suitable habitat (Balkenhol *et al.*, 2009a; Cushman *et al.*, 2006; Watling *et al.*, 2011). The spatial and temporal scale of sampling will also influence whether or not a landscape genetic study will be able to detect differences in how different matrix habitats influence patterns of dispersal and gene flow. Short Bull *et al*. (2011) investigated multiple study areas of the same species (*Ursus americanus*), and found that different features influenced gene flow in different study areas, due to variation in landscape composition and configuration among areas. Temporal landscape variation could also be quite important, as different matrix habitats may vary in their effect on dispersal and gene flow seasonally or over longer periods of time.

This study is also one of the first to apply a landscape genetic approach in a primate population. Long, overlapping generation times in primates are expected to affect the power of landscape genetic approaches to detect the effects of current or even historical landscape heterogeneity on patterns of gene flow. In addition, the extensive amount of sampling necessary to conduct a landscape genetic study is quite difficult in certain primate taxa because of their arboreal habits and low population densities. However, this study and some others have successfully used a landscape genetic approach in primate populations (Liu *et al.*, 2009; Quemere *et al.*, 2010), owing to recent advances in technology and non-invasive genetic sampling techniques (DeSalle, Amato, 2004; Piggott *et al.*, 2004).

Because landscape change and heterogeneity are hypothesized to play central roles in primate and human evolution (e.g. Kingston, 2007), these approaches may become more common as researchers strive to understand the influence of landscape heterogeneity on

evolutionary processes in the past, and also to project the influence of these processes into the future. Examining adaptive genetic diversity across landscapes, in addition to neutral genetic diversity, will allow us to better understand the genetic basis of local adaptation and speciation processes (Holderegger *et al.*, 2006). As genomic data become more readily available, landscape genetic studies of adaptive variation may become increasingly feasible, allowing the exploration of questions about the evolution of populations and the consequences of global change (Holderegger, Wagner, 2008; Manel *et al.*, 2010). Novel agent-based simulation approaches combined with ecological niche models, paleoenvironmental reconstructions, and projected future climate scenarios could in the future inform the generation of predictive models for population divergence and speciation under different climate and landscape change scenarios (Balkenhol *et al.*, 2009b).

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APPENDIX

This research was performed in accordance with the laws of the United States and Costa Rica: research and collection permit for *S. o. citrinellus* fecal samples from the Costa Rican Ministry of Energy and the Environment No. ACOPAC-INVN-14-08, import permit from the Centers for Disease Control and Prevention No. 2008-08-151. Also, an IACUC animal care protocol was approved by Columbia University for this research project (AC-AAAA5583).

Supplementary Table S1. Diagnostic characters and informative traits for distinguishing between mitochondrial d-loop haplogroups of *S. o. citrinellus* (western and eastern populations) and between the subspecies *S. o. citrinellus* and *S. o. oerstedii*. Base pair site positions represent their location within the 880 bp fragment of the mitochondrial d-loop sequenced in this study; position 1 corresponds to approximately position 15,351 in the mitochondrial genome (human reference sequence). – represents a gap in the sequence at that site, Y = C or T, R = A or G, W = A or T, S = C or G, K = T or G, M = A or C.

	Diagnostic Sites					Informative Traits													
Population	186	224	294	315	651	251	266	320	470	515	688	719	729	784	820	845	847	854	864
West (N=25)	C	T	A	T	A	A	A	A	C	C	T	G	T	A	A	A	T	A	C
East (N=18)	T	C	G	C	G	M	M	R	Y	Y	Y	R	Y	W	W	R	K	W	M

	Diagnostic Sites					
Subspecies	77	113	132	195	663	742
citrinellus (N=43)	G	A	--	A	G	C
oerstedii (N=7)	A	G	T	G	A	T

	Informative Traits																					
Subspecies	100	186	246	251	261	266	315	318	320	323	348	351	373	382	442	470	515	651	688	719	729	743
citrinellus	G	Y	C	M	C	M	Y	A	R	C	T	T	C	A	C	Y	Y	R	Y	R	Y	T
oerstedii	R	T	Y	A	Y	A	C	R	A	Y	Y	Y	Y	R	Y	C	C	A	T	G	T	Y

	Informative Traits						
Subspecies	778	784	820	845	847	854	864
citrinellus	Y	W	W	R	K	W	M
oerstedii	T	A	A	A	T	A	C

Supplementary Table S2. Mean values and tests of sex-biased dispersal in the microsatellite data from permutations of the male samples such that N=51 for both males and females in each sample set. The fourth column gives the expected value for the more dispersing sex as compared to the less dispersing sex (+ for larger value expected, - for smaller value expected).

Test	**Male**	**Female**	**Expected value of more dispersing sex**	***P***
Random Male Sample 1				
mAIc	-0.4169	0.4169	-	0.436
variance in *AIc*	30.666	32.089	+	0.877
FST	0.0897	0.0979	-	0.772
Random Male Sample 2				
mAIc	-0.1459	0.1459	-	0.785
variance in *AIc*	31.313	35.079	+	0.720
FST	0.838	0.0979	-	0.581
Random Male Sample 3				
mAIc	0.3595	-0.3595	-	0.497
variance in *AIc*	26.240	33.883	+	0.371
FST	0.0777	0.0979	-	0.402

Supplementary Table S3. Microsatellite markers amplified in 233 *Saimiri oerstedii citrinellus* samples.

Marker	Repeat Type	No. of Alleles	Size Range	H_o	Reference
CJ7	di	11	130-150	0.682	Nievergelt et al., 1998
D17s804	di	18	132-202	0.412	ResGen Human MapPair
D3s1210	di	7	117-131	0.116	ResGen Human MapPair
D3s1229	di	18	84-132	0.670	ResGen Human MapPair
D3s1766	tetra	8	187-226	0.558	ResGen Human MapPair
D4s111	di	18	130-168	0.412	ResGen Human MapPair
D5s111	di	7	155-179	0.412	ResGen Human MapPair
D8s165	di	11	137-163	0.459	ResGen Human MapPair
D8s260	di	10	219-241	0.691	ResGen Human MapPair
Leon15	di	7	262-280	0.511	Perez-Sweeney et al., 2005
Leon21	di	14	326-386	0.498	Perez-Sweeney et al., 2005
LL118	di	14	110-158	0.365	Di Fiore and Fleischer, 2004
LL157	di	10	207-239	0.433	Di Fiore and Fleischer, 2004
LL311	tri	30	212-317	0.742	Di Fiore and Fleischer, 2004
Locus5	di	8	102-116	0.309	Grativol et al., 2001
SB38	di	6	133-145	0.481	Bohle and Zischler, 2002

Supplementary Table S4. Full genotypes for 233 *S. o. citrinellus* and 11 *S. o. oerstedii* samples at 16 microsatellite markers.

		Marker									
Sample	**Subspecies**	CJ7	CJ7	D17s804	D17s804	D3s1210	D3s1210	D3s1229	D3s1229	D3s1766	D3s1766
B10	*citrinellus*	140	150	178	178	123	123	116	116	195	203
B14	*citrinellus*	140	144	178	180	123	123	112	114	203	203
B17	*citrinellus*	140	140	178	180	123	125	112	114	195	199
B18	*citrinellus*	140	150	178	178	119	123	114	116	195	199
B19	*citrinellus*	142	142	178	180	123	123	114	118	203	203
B2	*citrinellus*	140	150	176	176	123	123	112	116	195	203
B20	*citrinellus*	142	150	178	178	123	123	114	116	203	203
B21	*citrinellus*	142	144	178	180	123	123	114	114	195	203
B22	*citrinellus*	140	150	178	178	121	123	114	116	195	199
B24	*citrinellus*	142	150	176	178	123	123	114	116	203	203
B25	*citrinellus*	130	150	178	178	123	123	112	114	195	199
B26	*citrinellus*	150	150	178	178	123	123	112	114	195	199
B3	*citrinellus*	140	150	178	178	123	123	112	114	203	203
B4	*citrinellus*	140	150	176	178	123	123	112	116	195	218
B5	*citrinellus*	136	150	178	178	123	123	112	116	195	199
B6	*citrinellus*	136	142	178	178	123	123	114	118	199	203
B8	*citrinellus*	140	150	176	178	123	123	112	116	195	203
B9	*citrinellus*	140	150	178	178	123	123	112	116	195	203
C11	*citrinellus*	144	150	178	178	123	123	110	112	195	203
C13	*citrinellus*	142	150	178	178	123	123	110	114	191	203
C17	*citrinellus*	136	144	178	178	123	123	110	112	203	203
C18	*citrinellus*	136	144	176	178	123	123	114	116	195	195
C19	*citrinellus*	130	142	178	180	123	123	110	112	199	203
C2	*citrinellus*	144	144	178	178	123	123	114	114	195	203
C20	*citrinellus*	142	142	178	180	123	123	110	112	199	203
C21	*citrinellus*	142	142	178	180	123	123	110	112	199	203
C22	*citrinellus*	136	144	176	178	123	123	114	116	195	203

		Marker									
Sample	**Subspecies**	D4s111	D4s111	D5s111	D5s111	D8s165	D8s165	D8s260	D8s260	Leon15	Leon15
B10	citrinellus	138	144	161	161	159	159	233	239	268	268
B14	citrinellus	132	144	161	161	155	159	231	233	272	272
B17	citrinellus	144	144	159	161	155	159	233	233	268	272
B18	citrinellus	144	144	161	161	141	159	233	239	268	272
B19	citrinellus	144	144	161	161	155	159	233	241	272	272
B2	citrinellus	144	144	157	161	159	159	233	239	268	272
B20	citrinellus	144	144	159	161	159	159	233	233	272	272
B21	citrinellus	144	144	161	161	159	159	231	233	272	272
B22	citrinellus	144	144	161	161	159	159	233	239	268	272
B24	citrinellus	144	144	159	161	155	159	233	239	272	272
B25	citrinellus	144	144	161	161	159	159	233	239	268	272
B26	citrinellus	144	144	161	167	159	159	233	239	268	272
B3	citrinellus	144	160	159	161	159	159	233	241	268	272
B4	citrinellus	144	144	161	161	159	159	233	239	268	272
B5	citrinellus	144	144	161	161	159	159	231	233	272	272
B6	citrinellus	144	148	159	161	157	159	231	233	268	272
B8	citrinellus	144	144	159	161	159	159	233	239	268	268
B9	citrinellus	144	160	159	161	159	159	233	239	268	268
C11	citrinellus	144	148	155	159	155	159	233	239	268	270
C13	citrinellus	144	144	159	161	137	159	233	237	268	272
C17	citrinellus	144	144	159	161	159	159	233	239	272	272
C18	citrinellus	144	144	159	161	155	159	231	239	268	272
C19	citrinellus	144	144	159	161	159	161	231	239	268	272
C2	citrinellus	144	144	161	167	157	159	231	233	266	270
C20	citrinellus	144	144	159	161	157	159	233	239	268	272
C21	citrinellus	144	144	159	161	159	159	231	239	268	272
C22	citrinellus	144	144	159	161	155	159	231	239	268	272

		Marker									
Sample	**Subspecies**	Leon21	Leon21	LL118	LL118	LL157	LL157	LL311	LL311	Locus5	Locus5
B10	*citrinellus*	364	368	148	152	231	233	221	284	114	114
B14	*citrinellus*	368	368	148	148	231	231	231	245	114	114
B17	*citrinellus*	368	372	148	148	231	231	269	284	114	114
B18	*citrinellus*	368	372	148	148	231	233	266	269	114	114
B19	*citrinellus*	368	372	148	148	231	231	245	311	112	114
B2	*citrinellus*	368	368	148	148	231	231	269	272	114	114
B20	*citrinellus*	372	372	148	148	231	233	275	290	114	114
B21	*citrinellus*	368	372	148	148	231	235	224	272	108	114
B22	*citrinellus*	368	370	148	148	231	233	224	269	114	114
B24	*citrinellus*	350	372	148	148	231	233	242	287	114	114
B25	*citrinellus*	368	374	148	148	231	233	269	269	114	114
B26	*citrinellus*	372	374	148	148	231	233	269	269	114	114
B3	*citrinellus*	370	374	148	148	231	231	269	275	114	114
B4	*citrinellus*	368	368	148	148	231	233	269	272	114	114
B5	*citrinellus*	368	374	148	148	231	233	269	290	114	114
B6	*citrinellus*	372	386	146	148	231	231	268	272	114	114
B8	*citrinellus*	368	368	132	148	231	233	224	272	110	114
B9	*citrinellus*	368	368	148	152	231	233	284	284	114	114
C11	*citrinellus*	372	374	148	148	229	229	251	269	112	114
C13	*citrinellus*	372	374	148	148	233	233	212	221	112	114
C17	*citrinellus*	372	372	148	148	231	233	245	245	114	114
C18	*citrinellus*	368	372	148	148	231	231	248	293	104	114
C19	*citrinellus*	338	372	132	148	231	231	245	275	114	114
C2	*citrinellus*	372	372	148	148	229	229	266	266	104	114
C20	*citrinellus*	372	372	148	148	229	231	245	275	110	114
C21	*citrinellus*	370	372	148	148	229	231	245	275	110	114
C22	*citrinellus*	368	372	148	148	231	233	248	272	114	114

		Marker									
Sample	**Subspecies**	CJ7	CJ7	D17s804	D17s804	D3s1210	D3s1210	D3s1229	D3s1229	D3s1766	D3s1766
C23	*citrinellus*	136	144	176	178	123	123	114	116	195	203
C3	*citrinellus*	144	150	176	178	123	125	112	112	203	203
D10	*citrinellus*	142	142	178	180	123	123	110	112	203	203
D11	*citrinellus*	142	142	178	180	123	123	112	112	203	203
D12	*citrinellus*	142	142	178	180	123	123	112	112	203	203
D13	*citrinellus*	142	142	180	180	123	123	112	112	203	203
D14	*citrinellus*	142	142	178	178	123	123	108	112	195	199
D15	*citrinellus*	142	142	178	180	123	123	112	112	203	203
D16	*citrinellus*	142	142	178	180	123	123	112	112	203	203
D17	*citrinellus*	142	142	180	180	123	123	112	114	203	203
D19	*citrinellus*	136	142	178	180	123	123	112	114	199	203
D2	*citrinellus*	140	140	178	178	125	125	112	112	199	199
D3	*citrinellus*	144	144	178	178	123	123	112	112	199	203
D5	*citrinellus*	142	144	178	180	123	123	112	112	203	203
D6	*citrinellus*	140	142	178	202	123	123	112	132	191	191
D7	*citrinellus*	144	144	178	202	123	123	112	112	199	203
D8	*citrinellus*	140	142	178	178	125	125	112	114	195	195
E11	*citrinellus*	140	142	178	178	123	123	112	116	203	203
E13	*citrinellus*	140	142	178	178	121	123	112	112	203	203
E14	*citrinellus*	142	144	178	180	123	123	112	132	199	203
E16	*citrinellus*	140	144	178	178	123	123	112	112	195	203
E18	*citrinellus*	140	142	178	178	123	123	112	112	203	203
E2	*citrinellus*	144	144	178	178	123	123	108	112	195	199
E20	*citrinellus*	140	142	178	178	123	123	112	112	203	203
E21	*citrinellus*	140	142	176	178	123	123	112	112	195	195
E6	*citrinellus*	142	144	178	180	123	123	112	112	199	203
E7	*citrinellus*	140	140	180	180	123	123	112	112	199	199

		Marker									
Sample	**Subspecies**	D4s111	D4s111	D5s111	D5s111	D8s165	D8s165	D8s260	D8s260	Leon15	Leon15
C23	*citrinellus*	144	144	159	161	155	159	231	239	268	272
C3	*citrinellus*	144	144	161	161	159	159	239	241	266	270
D10	*citrinellus*	144	144	159	159	159	159	229	231	268	272
D11	*citrinellus*	144	152	159	159	159	159	229	229	272	272
D12	*citrinellus*	144	148	159	159	159	159	229	231	268	272
D13	*citrinellus*	144	162	159	159	159	159	229	229	272	272
D14	*citrinellus*	144	166	159	159	159	159	229	231	272	272
D15	*citrinellus*	144	162	159	159	159	159	229	231	272	272
D16	*citrinellus*	144	148	157	159	159	159	229	231	272	272
D17	*citrinellus*	144	162	159	159	159	159	231	235	272	272
D19	*citrinellus*	144	158	159	159	159	159	231	233	272	272
D2	*citrinellus*	144	144	159	159	159	159	229	231	268	272
D3	*citrinellus*	144	154	159	159	159	159	229	231	272	272
D5	*citrinellus*	144	156	159	159	159	159	231	231	272	272
D6	*citrinellus*	144	148	159	159	159	159	225	231	268	272
D7	*citrinellus*	144	148	159	159	159	159	231	231	272	272
D8	*citrinellus*	144	158	159	159	157	159	231	233	272	272
E11	*citrinellus*	144	144	159	159	159	159	231	239	266	272
E13	*citrinellus*	144	166	157	159	159	159	231	239	268	272
E14	*citrinellus*	144	154	159	161	159	163	239	239	268	272
E16	*citrinellus*	144	144	159	159	159	159	231	239	268	268
E18	*citrinellus*	144	144	159	159	157	159	231	239	272	272
E2	*citrinellus*	144	144	159	159	161	163	231	239	272	272
E20	*citrinellus*	144	148	159	159	159	159	231	239	272	272
E21	*citrinellus*	144	144	159	159	159	163	233	239	268	272
E6	*citrinellus*	144	148	159	159	159	163	231	239	272	272
E7	*citrinellus*	144	144	159	159	159	159	231	231	272	272

		Marker									
Sample	**Subspecies**	Leon21	Leon21	LL118	LL118	LL157	LL157	LL311	LL311	Locus5	Locus5
C23	*citrinellus*	368	372	132	148	231	233	248	296	114	114
C3	*citrinellus*	368	372	148	148	229	231	242	245	114	114
D10	*citrinellus*	368	372	148	148	231	231	251	251	114	114
D11	*citrinellus*	372	372	146	148	229	231	245	245	112	116
D12	*citrinellus*	368	372	148	148	231	231	245	251	114	114
D13	*citrinellus*	368	368	146	148	225	231	245	251	114	114
D14	*citrinellus*	372	372	148	152	229	231	275	278	114	114
D15	*citrinellus*	368	368	148	152	231	231	245	251	114	114
D16	*citrinellus*	368	372	140	148	229	231	245	245	114	114
D17	*citrinellus*	382	372	146	148	231	231	269	269	114	114
D19	*citrinellus*	368	368	148	148	231	233	272	272	114	114
D2	*citrinellus*	326	368	148	148	231	231	242	245	114	116
D3	*citrinellus*	372	372	148	148	229	231	245	245	114	114
D5	*citrinellus*	372	372	148	148	229	231	272	272	114	114
D6	*citrinellus*	370	370	146	148	227	233	245	272	114	114
D7	*citrinellus*	372	372	146	148	231	233	251	272	114	114
D8	*citrinellus*	376	376	140	152	231	231	245	245	114	114
E11	*citrinellus*	372	372	148	150	231	231	242	251	114	114
E13	*citrinellus*	372	372	148	152	231	231	242	242	114	114
E14	*citrinellus*	368	374	148	150	231	231	224	275	114	114
E16	*citrinellus*	370	372	148	150	233	233	242	242	114	114
E18	*citrinellus*	372	372	148	150	231	231	242	251	114	114
E2	*citrinellus*	370	372	148	152	231	233	224	245	114	114
E20	*citrinellus*	372	372	148	150	231	231	242	251	102	114
E21	*citrinellus*	372	372	140	148	231	237	242	251	108	114
E6	*citrinellus*	372	372	148	158	231	233	224	242	114	114
E7	*citrinellus*	372	372	148	148	233	233	251	251	114	114

		Marker				Marker				Marker	
Sample	**Subspecies**	SB38	SB38	**Sample**	**Subspecies**	SB38	SB38	**Sample**	**Subspecies**	SB38	SB38
B10	*citrinellus*	141	143	C23	*citrinellus*	143	143	E8	*citrinellus*	139	139
B14	*citrinellus*	139	143	C3	*citrinellus*	143	143	E9	*citrinellus*	139	139
B17	*citrinellus*	143	143	D10	*citrinellus*	137	141	G1	*citrinellus*	141	143
B18	*citrinellus*	143	143	D11	*citrinellus*	137	141	G10	*citrinellus*	143	143
B19	*citrinellus*	141	143	D12	*citrinellus*	137	141	G11	*citrinellus*	141	143
B2	*citrinellus*	139	143	D13	*citrinellus*	137	141	G12	*citrinellus*	141	143
B20	*citrinellus*	137	143	D14	*citrinellus*	137	143	G13	*citrinellus*	141	141
B21	*citrinellus*	143	143	D15	*citrinellus*	137	143	G14	*citrinellus*	143	143
B22	*citrinellus*	141	143	D16	*citrinellus*	137	141	G15	*citrinellus*	141	141
B24	*citrinellus*	143	143	D17	*citrinellus*	143	143	G2	*citrinellus*	139	141
B25	*citrinellus*	143	143	D19	*citrinellus*	137	137	G3	*citrinellus*	141	141
B26	*citrinellus*	143	143	D2	*citrinellus*	141	143	G4	*citrinellus*	139	141
B3	*citrinellus*	141	143	D3	*citrinellus*	141	143	G6	*citrinellus*	137	141
B4	*citrinellus*	139	143	D5	*citrinellus*	141	143	G7	*citrinellus*	137	143
B5	*citrinellus*	139	143	D6	*citrinellus*	143	143	G9	*citrinellus*	137	143
B6	*citrinellus*	143	143	D7	*citrinellus*	137	137	H10	*citrinellus*	139	139
B8	*citrinellus*	143	143	D8	*citrinellus*	143	143	H15	*citrinellus*	141	143
B9	*citrinellus*	143	143	E11	*citrinellus*	143	143	H16	*citrinellus*	143	143
C11	*citrinellus*	143	143	E13	*citrinellus*	139	141	H19	*citrinellus*	139	143
C13	*citrinellus*	143	143	E14	*citrinellus*	137	143	H2	*citrinellus*	139	139
C17	*citrinellus*	143	143	E16	*citrinellus*	137	139	H21	*citrinellus*	139	143
C18	*citrinellus*	143	143	E18	*citrinellus*	143	143	H22	*citrinellus*	137	139
C19	*citrinellus*	143	143	E2	*citrinellus*	143	143	H26	*citrinellus*	137	137
C2	*citrinellus*	139	143	E20	*citrinellus*	137	143	H31	*citrinellus*	143	143
C20	*citrinellus*	139	143	E21	*citrinellus*	143	143	H4	*citrinellus*	141	141
C21	*citrinellus*	143	143	E6	*citrinellus*	139	143	H6	*citrinellus*	139	139
C22	*citrinellus*	133	143	E7	*citrinellus*	143	143	H8	*citrinellus*	139	143

		Marker									
Sample	**Subspecies**	CJ7	CJ7	D17s804	D17s804	D3s1210	D3s1210	D3s1229	D3s1229	D3s1766	D3s1766
E8	*citrinellus*	140	142	178	186	123	123	98	112	195	203
E9	*citrinellus*	140	144	176	178	123	123	112	112	199	203
G1	*citrinellus*	136	140	176	178	123	123	112	112	203	203
G10	*citrinellus*	142	142	178	178	121	121	110	112	199	203
G11	*citrinellus*	136	138	178	178	123	123	108	112	195	199
G12	*citrinellus*	140	142	178	178	123	123	112	112	191	199
G13	*citrinellus*	140	140	178	180	123	123	112	132	203	203
G14	*citrinellus*	136	140	178	178	123	123	112	112	199	199
G15	*citrinellus*	140	140	178	180	123	123	112	112	203	203
G2	*citrinellus*	130	136	178	180	123	125	112	112	203	203
G3	*citrinellus*	140	140	178	180	123	123	112	112	203	203
G4	*citrinellus*	136	140	178	180	123	123	112	112	203	203
G6	*citrinellus*	136	136	170	170	121	123	112	114	203	203
G7	*citrinellus*	140	142	178	180	123	123	112	112	203	203
G9	*citrinellus*	136	140	178	178	123	123	112	112	199	199
H10	*citrinellus*	140	146	178	178	123	125	110	114	195	199
H15	*citrinellus*	140	150	178	178	123	123	112	114	195	203
H16	*citrinellus*	146	150	178	178	123	123	112	114	195	203
H19	*citrinellus*	142	150	178	178	123	123	112	112	195	199
H2	*citrinellus*	146	146	176	178	123	123	110	112	203	203
H21	*citrinellus*	140	144	178	178	123	123	114	116	195	199
H22	*citrinellus*	140	140	178	178	123	123	112	116	195	203
H26	*citrinellus*	140	140	132	132	121	123	114	116	195	195
H31	*citrinellus*	146	146	178	178	123	123	110	116	195	195
H4	*citrinellus*	140	142	178	178	123	123	110	112	191	195
H6	*citrinellus*	140	146	178	178	123	123	114	114	195	203
H8	*citrinellus*	142	142	178	178	121	123	114	118	195	199

		Marker									
Sample	**Subspecies**	D4s111	D4s111	D5s111	D5s111	D8s165	D8s165	D8s260	D8s260	Leon15	Leon15
E8	*citrinellus*	140	144	159	159	159	159	231	237	268	272
E9	*citrinellus*	144	144	159	159	159	159	239	239	272	272
G1	*citrinellus*	132	144	159	161	159	159	233	241	266	272
G10	*citrinellus*	144	154	157	157	159	159	229	231	266	268
G11	*citrinellus*	142	144	159	159	159	159	231	231	268	272
G12	*citrinellus*	144	144	159	159	159	163	231	239	268	272
G13	*citrinellus*	144	144	159	161	159	163	231	239	268	272
G14	*citrinellus*	144	144	161	161	159	163	239	239	272	272
G15	*citrinellus*	144	144	159	161	159	159	231	239	268	272
G2	*citrinellus*	144	162	161	161	163	163	231	239	268	272
G3	*citrinellus*	144	144	159	161	159	163	231	239	268	272
G4	*citrinellus*	144	156	159	161	157	163	231	231	268	272
G6	*citrinellus*	144	144	159	159	159	159	229	229	272	272
G7	*citrinellus*	144	144	159	161	159	163	229	231	268	272
G9	*citrinellus*	144	144	159	159	159	159	231	239	272	272
H10	*citrinellus*	144	144	161	161	159	159	233	239	272	272
H15	*citrinellus*	144	152	159	159	155	155	233	233	268	268
H16	*citrinellus*	144	154	159	161	155	159	233	233	262	272
H19	*citrinellus*	144	144	161	161	155	159	231	233	268	272
H2	*citrinellus*	144	154	161	161	159	159	233	239	272	272
H21	*citrinellus*	144	154	159	161	159	159	231	233	268	272
H22	*citrinellus*	144	144	161	161	159	159	231	233	268	272
H26	*citrinellus*	144	144	159	161	157	159	231	231	270	280
H31	*citrinellus*	142	142	161	161	155	159	233	233	272	272
H4	*citrinellus*	144	144	161	161	155	159	231	233	268	268
H6	*citrinellus*	144	144	161	161	159	159	233	239	272	272
H8	*citrinellus*	144	144	159	161	159	159	233	239	268	272

		Marker									
Sample	**Subspecies**	Leon21	Leon21	LL118	LL118	LL157	LL157	LL311	LL311	Locus5	Locus5
E8	*citrinellus*	372	372	148	148	231	231	242	242	114	114
E9	*citrinellus*	372	374	146	148	229	231	245	275	114	114
G1	*citrinellus*	350	372	132	148	231	231	242	284	108	114
G10	*citrinellus*	372	374	148	148	231	231	251	251	114	114
G11	*citrinellus*	372	372	148	148	231	233	251	251	114	114
G12	*citrinellus*	364	368	148	148	231	231	245	278	114	114
G13	*citrinellus*	368	372	148	148	231	231	251	278	114	114
G14	*citrinellus*	368	374	152	158	231	231	245	269	112	112
G15	*citrinellus*	372	372	148	148	231	231	251	278	114	114
G2	*citrinellus*	372	368	146	148	231	231	251	251	114	114
G3	*citrinellus*	368	372	148	148	231	231	251	278	114	114
G4	*citrinellus*	368	372	148	148	231	231	251	278	114	114
G6	*citrinellus*	372	372	142	148	233	233	245	245	114	114
G7	*citrinellus*	368	372	148	148	229	231	251	278	114	114
G9	*citrinellus*	372	372	148	152	231	231	245	272	114	114
H10	*citrinellus*	372	372	148	148	231	231	242	248	112	114
H15	*citrinellus*	368	374	148	148	233	233	269	287	112	114
H16	*citrinellus*	372	372	136	142	231	233	269	269	112	114
H19	*citrinellus*	368	372	148	148	233	233	269	275	108	114
H2	*citrinellus*	328	372	142	148	231	233	317	317	112	114
H21	*citrinellus*	372	386	148	148	231	231	245	272	112	114
H22	*citrinellus*	372	372	148	148	231	233	269	290	114	114
H26	*citrinellus*	328	372	148	148	231	233	239	275	112	114
H31	*citrinellus*	328	328	148	148	231	231	275	275	106	108
H4	*citrinellus*	368	372	144	148	227	233	248	248	110	110
H6	*citrinellus*	372	372	148	148	231	231	248	254	114	114
H8	*citrinellus*	372	374	148	148	233	233	269	275	114	114

		Marker									
Sample	**Subspecies**	CJ7	CJ7	D17s804	D17s804	D3s1210	D3s1210	D3s1229	D3s1229	D3s1766	D3s1766
H9	*citrinellus*	140	146	178	178	123	123	114	114	195	203
I1	*citrinellus*	140	142	178	178	123	123	106	110	195	203
I10	*citrinellus*	140	142	144	178	123	123	112	126	195	203
I11	*citrinellus*	150	150	178	178	117	123	112	122	195	195
I13	*citrinellus*	140	142	178	178	123	125	100	104	195	203
I14	*citrinellus*	140	142	178	178	123	123	112	118	195	203
I15	*citrinellus*	142	150	172	178	123	123	114	120	195	203
I16	*citrinellus*	150	150	178	178	123	123	114	116	199	203
I17	*citrinellus*	136	140	140	178	123	123	102	116	203	203
I18	*citrinellus*	150	150	178	178	123	123	116	116	195	195
I19	*citrinellus*	136	136	178	178	123	123	102	114	207	207
I2	*citrinellus*	138	142	178	178	123	123	110	112	195	203
I20	*citrinellus*	136	138	178	178	123	123	100	114	191	195
I22	*citrinellus*	136	146	178	178	119	123	114	114	195	203
I25	*citrinellus*	136	136	178	178	123	123	112	114	195	195
I26	*citrinellus*	146	150	178	178	123	123	110	116	195	199
I3	*citrinellus*	142	146	178	178	117	123	110	116	195	199
I4	*citrinellus*	138	150	178	178	123	123	110	110	195	203
I6	*citrinellus*	142	142	178	178	123	123	114	116	195	207
I7	*citrinellus*	138	140	178	178	123	123	112	116	199	199
I9	*citrinellus*	142	150	176	178	119	123	108	112	195	203
K1	*citrinellus*	148	148	178	178	123	123	106	112	195	199
K10	*citrinellus*	142	142	178	178	123	123	112	112	199	199
K11	*citrinellus*	142	142	178	178	123	123	112	112	199	199
K13	*citrinellus*	142	142	178	178	121	123	112	114	199	199
K14	*citrinellus*	136	142	178	186	123	123	112	112	199	203
K15	*citrinellus*	142	142	178	178	123	123	112	112	203	203

		Marker									
Sample	**Subspecies**	D4s111	D4s111	D5s111	D5s111	D8s165	D8s165	D8s260	D8s260	Leon15	Leon15
H9	*citrinellus*	144	154	159	161	159	159	233	239	268	272
I1	*citrinellus*	144	144	157	161	155	155	229	237	268	268
I10	*citrinellus*	144	144	159	161	159	159	231	241	268	272
I11	*citrinellus*	144	144	161	161	155	157	233	241	272	272
I13	*citrinellus*	144	144	159	161	159	159	231	241	268	272
I14	*citrinellus*	144	152	159	161	159	159	231	241	268	272
I15	*citrinellus*	132	144	159	161	159	159	233	239	272	272
I16	*citrinellus*	144	144	161	161	155	155	239	241	268	272
I17	*citrinellus*	144	152	161	161	155	155	231	233	266	272
I18	*citrinellus*	144	144	159	161	155	157	233	241	268	272
I19	*citrinellus*	144	144	159	161	155	159	233	233	268	272
I2	*citrinellus*	144	144	159	161	155	159	233	241	268	272
I20	*citrinellus*	144	144	161	161	155	155	233	233	268	272
I22	*citrinellus*	144	144	161	161	155	159	231	233	268	272
I25	*citrinellus*	144	154	161	161	155	157	233	233	268	272
I26	*citrinellus*	144	162	159	161	155	159	233	233	272	272
I3	*citrinellus*	144	144	161	161	155	155	233	233	268	272
I4	*citrinellus*	144	144	161	161	155	159	233	239	268	272
I6	*citrinellus*	144	154	161	161	159	159	231	233	272	272
I7	*citrinellus*	144	144	161	161	159	159	233	239	266	268
I9	*citrinellus*	144	154	159	161	155	159	233	239	268	272
K1	*citrinellus*	144	144	161	161	159	159	231	233	268	272
K10	*citrinellus*	144	144	157	159	159	159	231	233	272	272
K11	*citrinellus*	144	144	159	161	159	159	239	239	270	270
K13	*citrinellus*	144	144	159	159	159	159	239	239	272	272
K14	*citrinellus*	144	164	159	159	159	159	233	239	268	272
K15	*citrinellus*	144	144	159	159	159	159	227	231	272	272

		Marker									
Sample	**Subspecies**	Leon21	Leon21	LL118	LL118	LL157	LL157	LL311	LL311	Locus5	Locus5
H9	*citrinellus*	328	328	148	148	231	231	248	248	114	114
I1	*citrinellus*	368	372	138	148	233	233	248	266	114	114
I10	*citrinellus*	372	372	132	148	231	231	248	248	112	114
I11	*citrinellus*	372	372	148	150	233	233	245	275	114	114
I13	*citrinellus*	372	372	148	148	231	231	248	269	114	114
I14	*citrinellus*	372	372	148	148	229	231	248	269	114	114
I15	*citrinellus*	372	372	148	148	231	233	248	269	114	114
I16	*citrinellus*	368	372	142	148	231	233	248	275	114	114
I17	*citrinellus*	328	372	142	148	227	233	275	275	114	114
I18	*citrinellus*	372	372	142	148	233	233	245	272	112	114
I19	*citrinellus*	368	368	140	148	223	231	266	275	112	114
I2	*citrinellus*	372	372	148	148	231	233	248	269	114	114
I20	*citrinellus*	368	368	138	148	227	229	221	275	110	110
I22	*citrinellus*	372	372	148	148	231	233	260	260	114	114
I25	*citrinellus*	368	368	148	148	231	231	245	269	114	114
I26	*citrinellus*	328	368	148	148	231	231	227	269	108	114
I3	*citrinellus*	372	386	148	148	231	231	248	278	112	114
I4	*citrinellus*	328	328	146	148	231	231	248	248	114	114
I6	*citrinellus*	372	372	148	148	231	231	269	278	114	114
I7	*citrinellus*	368	372	140	148	231	233	245	269	114	114
I9	*citrinellus*	372	372	140	148	229	231	248	269	114	114
K1	*citrinellus*	368	372	144	148	231	233	245	278	108	114
K10	*citrinellus*	372	372	146	148	231	233	245	275	114	114
K11	*citrinellus*	364	374	148	148	233	233	272	275	114	114
K13	*citrinellus*	372	372	148	154	231	233	245	275	114	114
K14	*citrinellus*	372	372	148	148	231	233	275	275	114	114
K15	*citrinellus*	364	364	148	148	233	233	245	245	114	114

		Marker									
Sample	**Subspecies**	CJ7	CJ7	D17s804	D17s804	D3s1210	D3s1210	D3s1229	D3s1229	D3s1766	D3s1766
K16	*citrinellus*	142	142	178	178	123	123	112	112	199	199
K17	*citrinellus*	134	142	178	186	123	123	112	112	195	199
K3	*citrinellus*	140	144	178	178	123	123	112	112	195	203
K4	*citrinellus*	130	140	178	182	123	123	112	112	195	195
K5	*citrinellus*	140	144	180	180	123	123	112	112	195	203
K7	*citrinellus*	142	142	178	178	123	123	112	114	203	203
K8	*citrinellus*	142	142	178	178	123	123	112	112	199	203
L1	*citrinellus*	140	144	178	180	119	123	114	116	195	203
L10	*citrinellus*	140	144	178	180	123	125	114	120	203	203
L11	*citrinellus*	140	140	178	202	123	123	112	116	195	195
L12	*citrinellus*	144	144	178	178	123	123	116	116	195	203
L14	*citrinellus*	138	144	178	178	123	131	114	116	191	195
L15	*citrinellus*	138	144	134	178	123	123	86	114	203	207
L16	*citrinellus*	136	142	178	178	123	123	114	116	195	203
L2	*citrinellus*	140	142	178	178	123	123	114	116	195	195
L3	*citrinellus*	134	138	178	178	123	125	116	120	195	199
L4	*citrinellus*	140	144	178	186	123	123	116	116	199	199
L5	*citrinellus*	140	144	178	178	123	123	114	116	195	195
L7	*citrinellus*	138	142	178	180	123	123	116	120	195	195
LT10	*citrinellus*	136	136	178	178	123	123	110	114	195	199
LT17	*citrinellus*	136	142	178	178	123	123	110	110	195	195
LT18	*citrinellus*	142	144	176	178	123	123	110	114	195	199
LT19	*citrinellus*	136	150	178	178	123	123	106	120	195	199
LT21	*citrinellus*	136	150	178	178	123	123	114	114	195	199
LT22	*citrinellus*	136	144	162	178	123	123	114	116	195	195
LT23	*citrinellus*	136	150	170	178	121	123	114	114	195	199
M1	*citrinellus*	136	150	178	180	123	123	114	116	199	203

		Marker									
Sample	**Subspecies**	D4s111	D4s111	D5s111	D5s111	D8s165	D8s165	D8s260	D8s260	Leon15	Leon15
K16	*citrinellus*	144	154	159	161	157	159	239	241	274	274
K17	*citrinellus*	144	144	159	159	155	159	241	241	272	272
K3	*citrinellus*	144	144	159	161	159	159	231	231	272	272
K4	*citrinellus*	144	144	159	161	159	159	231	231	272	272
K5	*citrinellus*	144	154	159	159	159	159	231	231	272	272
K7	*citrinellus*	138	144	159	159	159	159	231	231	272	272
K8	*citrinellus*	144	164	159	159	159	159	231	239	272	272
L1	*citrinellus*	144	144	157	161	155	159	229	233	272	272
L10	*citrinellus*	144	144	159	161	159	159	233	239	268	272
L11	*citrinellus*	144	144	161	161	155	159	231	233	268	272
L12	*citrinellus*	144	148	161	161	149	155	233	233	268	272
L14	*citrinellus*	144	164	157	159	157	159	239	239	268	272
L15	*citrinellus*	144	148	159	161	159	159	233	233	268	272
L16	*citrinellus*	144	144	159	159	137	159	233	239	268	272
L2	*citrinellus*	144	144	157	161	155	159	233	233	268	268
L3	*citrinellus*	144	144	157	161	155	159	233	233	272	272
L4	*citrinellus*	144	144	157	163	153	159	229	233	272	272
L5	*citrinellus*	144	154	159	161	159	159	231	233	272	272
L7	*citrinellus*	144	144	157	161	155	155	233	233	268	272
LT10	*citrinellus*	144	144	159	159	155	159	233	233	272	272
LT17	*citrinellus*	144	144	161	161	159	159	219	233	268	272
LT18	*citrinellus*	144	144	161	161	157	159	233	233	268	268
LT19	*citrinellus*	144	144	161	161	159	159	233	239	272	272
LT21	*citrinellus*	144	144	161	161	159	159	233	239	272	272
LT22	*citrinellus*	144	148	159	161	155	159	233	233	268	272
LT23	*citrinellus*	144	144	161	161	159	159	233	239	272	272
M1	*citrinellus*	144	154	157	161	159	159	231	233	266	270

		Marker									
Sample	**Subspecies**	Leon21	Leon21	LL118	LL118	LL157	LL157	LL311	LL311	Locus5	Locus5
K16	*citrinellus*	372	372	148	150	233	233	245	245	114	114
K17	*citrinellus*	342	372	146	148	233	233	245	278	114	114
K3	*citrinellus*	372	372	146	148	231	231	242	269	102	114
K4	*citrinellus*	374	374	132	148	231	231	245	245	114	114
K5	*citrinellus*	370	372	148	152	207	231	248	275	114	114
K7	*citrinellus*	370	372	146	148	231	231	245	245	114	114
K8	*citrinellus*	372	372	148	148	231	231	245	251	114	114
L1	*citrinellus*	338	372	148	148	231	231	275	275	114	114
L10	*citrinellus*	372	372	146	148	231	233	221	275	112	114
L11	*citrinellus*	372	372	148	152	231	233	287	287	112	114
L12	*citrinellus*	372	372	146	148	231	231	272	272	114	114
L14	*citrinellus*	372	372	132	148	233	233	272	272	112	114
L15	*citrinellus*	368	368	148	148	231	231	272	272	104	114
L16	*citrinellus*	368	372	148	148	233	233	245	248	104	114
L2	*citrinellus*	372	374	148	148	231	233	284	287	114	116
L3	*citrinellus*	368	368	148	148	231	239	269	272	114	114
L4	*citrinellus*	368	372	146	148	231	231	287	290	114	114
L5	*citrinellus*	368	372	148	148	231	233	272	272	112	114
L7	*citrinellus*	368	372	132	148	231	231	248	272	114	114
LT10	*citrinellus*	372	372	148	148	231	233	248	296	114	114
LT17	*citrinellus*	368	372	148	148	231	231	245	245	108	114
LT18	*citrinellus*	372	372	148	152	231	231	245	269	114	114
LT19	*citrinellus*	372	372	148	148	231	233	248	254	114	114
LT21	*citrinellus*	372	372	148	148	231	233	248	254	114	114
LT22	*citrinellus*	372	372	148	148	227	231	254	296	114	114
LT23	*citrinellus*	372	372	148	148	231	233	248	254	114	114
M1	*citrinellus*	372	372	148	152	229	231	272	272	112	114

		Marker				Marker				Marker	
Sample	**Subspecies**	SB38	SB38	**Sample**	**Subspecies**	SB38	SB38	**Sample**	**Subspecies**	SB38	SB38
H9	*citrinellus*	139	139	K16	*citrinellus*	137	143	M2	*citrinellus*	143	143
I1	*citrinellus*	139	143	K17	*citrinellus*	143	143	M3	*citrinellus*	143	143
I10	*citrinellus*	143	143	K3	*citrinellus*	143	143	MB	*citrinellus*	143	143
I11	*citrinellus*	143	143	K4	*citrinellus*	143	143	MC	*citrinellus*	141	143
I13	*citrinellus*	143	143	K5	*citrinellus*	141	143	ME10	*citrinellus*	143	143
I14	*citrinellus*	143	143	K7	*citrinellus*	137	137	ME11	*citrinellus*	141	143
I15	*citrinellus*	139	143	K8	*citrinellus*	143	143	ME13	*citrinellus*	143	143
I16	*citrinellus*	139	139	L1	*citrinellus*	143	143	ME14	*citrinellus*	139	143
I17	*citrinellus*	143	143	L10	*citrinellus*	141	143	ME15	*citrinellus*	139	143
I18	*citrinellus*	143	143	L11	*citrinellus*	139	143	ME16	*citrinellus*	139	143
I19	*citrinellus*	141	143	L12	*citrinellus*	143	143	ME18	*citrinellus*	143	143
I2	*citrinellus*	139	143	L14	*citrinellus*	141	143	ME3	*citrinellus*	143	143
I20	*citrinellus*	141	143	L15	*citrinellus*	139	143	ME7	*citrinellus*	143	143
I22	*citrinellus*	137	143	L16	*citrinellus*	141	143	MP1	*citrinellus*	143	143
I25	*citrinellus*	139	141	L2	*citrinellus*	143	143	MP13	*citrinellus*	143	143
I26	*citrinellus*	139	143	L3	*citrinellus*	141	143	MP17	*citrinellus*	143	143
I3	*citrinellus*	139	143	L4	*citrinellus*	143	143	MP2	*citrinellus*	143	143
I4	*citrinellus*	143	143	L5	*citrinellus*	139	143	MP3	*citrinellus*	143	143
I6	*citrinellus*	143	143	L7	*citrinellus*	143	143	MP5	*citrinellus*	137	143
I7	*citrinellus*	143	143	LT10	*citrinellus*	143	143	MP7	*citrinellus*	139	143
I9	*citrinellus*	139	139	LT17	*citrinellus*	143	143	MP8	*citrinellus*	139	143
K1	*citrinellus*	143	143	LT18	*citrinellus*	143	143	N1	*citrinellus*	143	143
K10	*citrinellus*	137	143	LT19	*citrinellus*	143	143	O10	*citrinellus*	143	143
K11	*citrinellus*	137	143	LT21	*citrinellus*	143	143	O11	*citrinellus*	141	143
K13	*citrinellus*	137	143	LT22	*citrinellus*	143	143	O13	*citrinellus*	143	143
K14	*citrinellus*	137	143	LT23	*citrinellus*	143	143	O14	*citrinellus*	141	143
K15	*citrinellus*	137	143	M1	*citrinellus*	143	143	O15	*citrinellus*	143	143

		Marker									
Sample	**Subspecies**	CJ7	CJ7	D17s804	D17s804	D3s1210	D3s1210	D3s1229	D3s1229	D3s1766	D3s1766
M2	*citrinellus*	136	150	176	178	123	123	114	116	199	195
M3	*citrinellus*	136	150	178	178	123	123	114	116	195	199
MB	*citrinellus*	136	140	176	178	123	123	114	114	195	195
MC	*citrinellus*	136	150	178	176	123	123	112	114	203	203
ME10	*citrinellus*	136	136	178	178	123	123	114	114	199	199
ME11	*citrinellus*	144	150	178	178	123	123	112	118	203	203
ME13	*citrinellus*	136	136	178	178	123	123	110	116	195	203
ME14	*citrinellus*	136	136	176	178	123	123	112	116	195	203
ME15	*citrinellus*	136	142	162	162	123	123	112	116	195	195
ME16	*citrinellus*	136	142	178	178	123	123	110	110	191	195
ME18	*citrinellus*	144	144	178	178	123	123	112	114	199	199
ME3	*citrinellus*	136	136	178	178	123	123	112	112	195	195
ME7	*citrinellus*	140	150	174	178	123	123	110	114	195	195
MP1	*citrinellus*	136	142	178	178	123	123	110	112	199	203
MP13	*citrinellus*	136	142	178	178	123	123	110	112	199	203
MP17	*citrinellus*	142	142	176	178	123	123	110	110	195	203
MP2	*citrinellus*	136	142	178	178	123	123	110	112	199	203
MP3	*citrinellus*	136	142	178	178	123	123	110	112	199	203
MP5	*citrinellus*	140	142	178	178	123	123	110	116	195	203
MP7	*citrinellus*	136	142	150	178	123	123	110	110	191	195
MP8	*citrinellus*	136	144	178	178	123	123	112	116	199	203
N1	*citrinellus*	140	140	162	178	123	123	112	112	191	195
O10	*citrinellus*	142	142	178	180	123	123	112	112	195	199
O11	*citrinellus*	142	142	178	180	123	123	112	112	199	199
O13	*citrinellus*	142	144	178	180	123	123	112	112	195	195
O14	*citrinellus*	142	144	178	180	121	123	112	116	195	195
O15	*citrinellus*	142	144	178	180	123	123	112	116	195	195

		Marker									
Sample	**Subspecies**	D4s111	D4s111	D5s111	D5s111	D8s165	D8s165	D8s260	D8s260	Leon15	Leon15
M2	*citrinellus*	144	144	155	161	159	159	233	233	266	270
M3	*citrinellus*	132	144	161	161	159	159	233	233	262	266
MB	*citrinellus*	142	144	161	161	155	159	239	241	266	270
MC	*citrinellus*	144	154	161	161	155	159	231	233	266	270
ME10	*citrinellus*	144	148	161	161	155	159	231	231	272	272
ME11	*citrinellus*	144	144	161	161	151	155	233	233	272	272
ME13	*citrinellus*	144	144	161	161	159	159	231	233	272	272
ME14	*citrinellus*	144	148	161	161	155	159	231	241	272	272
ME15	*citrinellus*	144	144	161	161	159	159	231	241	272	272
ME16	*citrinellus*	148	148	161	179	159	159	239	239	272	272
ME18	*citrinellus*	144	148	161	161	155	159	231	231	268	268
ME3	*citrinellus*	146	146	159	161	157	157	231	231	272	272
ME7	*citrinellus*	144	144	159	161	157	159	231	233	268	272
MP1	*citrinellus*	144	148	161	161	151	159	231	233	272	272
MP13	*citrinellus*	144	154	161	161	151	159	231	233	272	272
MP17	*citrinellus*	144	158	159	161	151	159	231	231	272	272
MP2	*citrinellus*	144	156	161	161	151	159	231	233	272	272
MP3	*citrinellus*	144	168	161	161	151	159	231	233	272	272
MP5	*citrinellus*	144	144	159	161	159	159	233	233	272	272
MP7	*citrinellus*	144	148	161	161	155	157	239	241	268	272
MP8	*citrinellus*	144	144	161	161	155	159	233	239	272	272
N1	*citrinellus*	144	144	155	161	155	157	231	233	266	270
O10	*citrinellus*	144	156	159	161	157	159	229	239	268	268
O11	*citrinellus*	144	148	159	161	159	159	233	239	268	272
O13	*citrinellus*	144	148	159	159	157	159	239	239	272	272
O14	*citrinellus*	144	154	159	159	157	159	239	239	272	272
O15	*citrinellus*	144	144	159	159	157	159	231	239	272	272

		Marker									
Sample	**Subspecies**	Leon21	Leon21	LL118	LL118	LL157	LL157	LL311	LL311	Locus5	Locus5
M2	*citrinellus*	368	372	148	152	229	231	266	272	114	114
M3	*citrinellus*	368	372	148	152	231	233	269	275	114	114
MB	*citrinellus*	368	374	148	148	229	229	272	275	114	114
MC	*citrinellus*	372	372	132	148	231	229	272	311	112	114
ME10	*citrinellus*	374	374	148	148	231	233	224	278	114	114
ME11	*citrinellus*	372	372	148	148	231	231	245	296	114	114
ME13	*citrinellus*	368	372	148	148	231	233	272	275	114	114
ME14	*citrinellus*	368	372	148	148	233	233	269	317	114	114
ME15	*citrinellus*	372	372	148	148	233	233	269	269	112	114
ME16	*citrinellus*	372	372	148	148	231	231	245	272	114	114
ME18	*citrinellus*	372	372	148	148	231	231	272	272	114	114
ME3	*citrinellus*	374	374	148	148	231	231	269	269	110	114
ME7	*citrinellus*	372	372	148	148	231	231	254	269	108	114
MP1	*citrinellus*	368	372	148	148	231	233	245	245	114	114
MP13	*citrinellus*	368	372	148	148	231	233	245	248	114	114
MP17	*citrinellus*	372	372	148	148	231	231	245	272	114	114
MP2	*citrinellus*	368	372	148	152	231	233	245	248	110	114
MP3	*citrinellus*	368	372	110	148	231	233	245	245	114	114
MP5	*citrinellus*	372	372	148	148	231	233	269	275	114	114
MP7	*citrinellus*	370	372	148	148	231	233	245	269	114	114
MP8	*citrinellus*	368	372	148	148	231	233	257	275	110	114
N1	*citrinellus*	372	374	148	148	229	229	287	287	114	114
O10	*citrinellus*	326	372	148	148	231	231	245	245	114	114
O11	*citrinellus*	368	372	148	148	231	231	245	287	114	114
O13	*citrinellus*	372	372	148	148	231	233	245	257	114	114
O14	*citrinellus*	366	368	148	148	231	233	245	245	114	114
O15	*citrinellus*	370	372	126	148	231	233	236	245	106	114

		Marker									
Sample	**Subspecies**	CJ7	CJ7	D17s804	D17s804	D3s1210	D3s1210	D3s1229	D3s1229	D3s1766	D3s1766
O16	*citrinellus*	136	144	178	178	123	123	112	114	195	195
O2	*citrinellus*	140	142	156	176	123	123	112	112	195	199
O3	*citrinellus*	140	142	178	178	123	123	112	116	195	199
O4	*citrinellus*	140	142	178	178	123	123	112	116	199	199
O6	*citrinellus*	142	144	178	180	123	123	112	116	199	199
P10	*citrinellus*	144	150	176	178	123	123	114	116	187	203
P11	*citrinellus*	142	144	178	178	123	123	114	114	199	203
P12	*citrinellus*	142	142	174	178	123	123	110	112	195	203
P14	*citrinellus*	142	148	178	180	123	123	112	114	195	195
P15	*citrinellus*	142	150	180	180	123	123	112	114	195	203
P19	*citrinellus*	136	142	178	178	123	123	112	114	195	203
P2	*citrinellus*	136	142	176	178	123	123	112	116	199	203
P3	*citrinellus*	144	150	176	178	123	123	112	114	195	203
P4	*citrinellus*	142	144	176	178	123	123	114	114	195	203
P5	*citrinellus*	142	150	176	178	123	123	106	114	195	203
P7	*citrinellus*	140	150	178	178	123	123	110	112	195	199
P8	*citrinellus*	144	150	160	178	123	123	114	114	199	203
P9	*citrinellus*	144	150	176	178	123	123	112	120	195	203
PD1	*citrinellus*	140	142	178	178	123	123	84	112	199	199
PD10	*citrinellus*	134	144	176	178	121	123	112	112	199	203
PD12	*citrinellus*	142	144	178	202	123	123	112	112	199	203
PD13	*citrinellus*	142	144	156	178	123	123	112	124	199	203
PD14	*citrinellus*	142	142	178	178	123	123	116	116	199	199
PD15	*citrinellus*	132	140	178	178	123	125	108	112	195	199
PD2	*citrinellus*	138	142	178	178	123	123	86	112	199	203
PD3	*citrinellus*	142	144	178	178	123	123	112	112	199	203
PD4	*citrinellus*	142	144	178	178	123	123	112	112	199	203

		Marker									
Sample	**Subspecies**	D4s111	D4s111	D5s111	D5s111	D8s165	D8s165	D8s260	D8s260	Leon15	Leon15
O16	*citrinellus*	144	148	161	161	159	159	233	239	268	268
O2	*citrinellus*	144	144	159	161	155	159	239	241	268	268
O3	*citrinellus*	144	144	159	159	157	163	233	239	272	272
O4	*citrinellus*	144	144	159	159	157	163	233	239	272	272
O6	*citrinellus*	144	144	157	159	159	159	231	233	268	272
P10	*citrinellus*	144	144	161	161	155	159	229	231	266	266
P11	*citrinellus*	144	144	161	161	159	159	231	233	270	270
P12	*citrinellus*	144	144	161	161	155	157	231	241	266	270
P14	*citrinellus*	144	148	161	161	159	159	231	231	268	272
P15	*citrinellus*	144	144	161	161	159	159	233	233	268	272
P19	*citrinellus*	144	144	161	161	159	159	231	231	272	272
P2	*citrinellus*	140	144	159	161	157	159	231	239	266	270
P3	*citrinellus*	144	144	161	161	159	159	233	239	270	270
P4	*citrinellus*	144	150	161	161	159	161	231	231	270	270
P5	*citrinellus*	144	164	155	161	159	159	229	233	266	270
P7	*citrinellus*	144	144	159	161	157	159	231	239	266	270
P8	*citrinellus*	144	144	161	161	157	159	229	233	270	270
P9	*citrinellus*	144	144	161	161	159	159	229	231	266	270
PD1	*citrinellus*	144	156	159	159	155	159	233	239	268	272
PD10	*citrinellus*	144	144	159	159	157	157	231	233	268	272
PD12	*citrinellus*	144	144	157	159	157	163	233	233	268	272
PD13	*citrinellus*	132	144	159	159	139	157	233	233	268	272
PD14	*citrinellus*	142	144	159	163	157	159	239	239	268	272
PD15	*citrinellus*	142	144	157	159	157	159	233	239	268	268
PD2	*citrinellus*	142	144	159	159	155	159	231	233	268	272
PD3	*citrinellus*	144	148	159	159	157	157	233	233	268	272
PD4	*citrinellus*	144	158	159	159	157	157	233	233	268	272

		Marker									
Sample	**Subspecies**	Leon21	Leon21	LL118	LL118	LL157	LL157	LL311	LL311	Locus5	Locus5
O16	*citrinellus*	326	372	148	148	231	231	245	272	114	114
O2	*citrinellus*	370	372	148	148	229	231	245	245	108	114
O3	*citrinellus*	368	372	148	148	231	231	242	245	104	114
O4	*citrinellus*	372	372	148	148	231	231	242	245	108	110
O6	*citrinellus*	372	372	148	148	231	233	248	248	114	114
P10	*citrinellus*	368	372	148	148	229	231	245	275	114	114
P11	*citrinellus*	368	372	148	148	231	231	242	248	114	114
P12	*citrinellus*	368	372	148	148	229	231	242	242	114	114
P14	*citrinellus*	372	372	148	148	231	231	245	275	114	114
P15	*citrinellus*	372	372	148	148	231	231	245	275	114	114
P19	*citrinellus*	368	372	148	148	231	231	245	269	114	114
P2	*citrinellus*	372	374	148	148	229	231	245	287	114	114
P3	*citrinellus*	372	372	148	148	229	229	242	311	114	114
P4	*citrinellus*	368	372	148	148	229	231	242	266	114	114
P5	*citrinellus*	372	372	148	148	229	229	242	269	114	114
P7	*citrinellus*	368	372	148	148	229	229	242	272	114	114
P8	*citrinellus*	366	368	148	148	229	229	242	272	114	114
P9	*citrinellus*	372	372	148	148	229	229	242	269	114	114
PD1	*citrinellus*	372	372	148	148	231	231	245	245	112	114
PD10	*citrinellus*	372	372	148	150	231	231	218	245	114	114
PD12	*citrinellus*	366	372	148	150	231	231	245	251	114	116
PD13	*citrinellus*	368	374	132	148	231	231	245	251	114	114
PD14	*citrinellus*	368	368	150	152	231	233	245	251	114	114
PD15	*citrinellus*	368	372	152	152	231	231	245	245	114	114
PD2	*citrinellus*	372	372	148	148	231	231	224	245	112	114
PD3	*citrinellus*	368	372	148	150	231	231	245	251	114	114
PD4	*citrinellus*	368	372	148	150	231	231	245	251	114	114

		Marker									
Sample	**Subspecies**	CJ7	CJ7	D17s804	D17s804	D3s1210	D3s1210	D3s1229	D3s1229	D3s1766	D3s1766
PD5	*citrinellus*	136	142	178	202	123	123	112	112	199	203
PD6	*citrinellus*	136	140	176	178	123	123	108	116	195	199
PD7	*citrinellus*	136	142	178	178	123	125	116	116	199	199
PD8	*citrinellus*	140	142	178	178	121	123	112	114	195	203
PD9	*citrinellus*	136	140	178	196	123	123	86	116	195	199
RA10	*citrinellus*	150	150	178	180	123	123	110	114	195	203
RA11	*citrinellus*	150	150	178	180	123	123	110	114	195	203
RA12	*citrinellus*	150	150	178	180	123	123	110	114	195	203
RA13	*citrinellus*	150	150	178	180	123	123	114	116	195	203
RA14	*citrinellus*	144	150	178	178	123	123	110	116	195	195
RA15	*citrinellus*	144	150	178	178	123	123	112	114	203	203
RA6	*citrinellus*	150	150	178	178	123	123	112	114	203	203
RA7	*citrinellus*	150	150	178	178	123	123	112	114	203	203
RA8	*citrinellus*	130	150	178	180	123	123	110	114	195	203
RA9	*citrinellus*	150	150	178	180	123	123	110	114	203	203
RB1	*citrinellus*	144	150	178	180	123	123	110	112	195	203
RB10	*citrinellus*	142	150	180	180	123	123	110	112	203	203
RB11	*citrinellus*	142	142	178	178	123	123	112	116	195	203
RB12	*citrinellus*	150	150	180	180	123	123	110	110	203	203
RB3	*citrinellus*	142	142	132	176	123	123	110	112	226	226
RB4	*citrinellus*	150	150	178	178	123	123	112	116	195	203
RB5	*citrinellus*	142	142	178	178	123	123	114	116	199	203
RB6	*citrinellus*	136	150	178	178	123	123	112	114	195	195
RB7	*citrinellus*	142	150	178	178	123	123	112	116	203	203
RB8	*citrinellus*	130	150	178	180	123	123	114	116	203	203
RB9	*citrinellus*	150	150	176	178	123	123	112	114	203	203
T1	*citrinellus*	140	140	176	178	123	123	114	114	195	195

		Marker									
Sample	**Subspecies**	D4s111	D4s111	D5s111	D5s111	D8s165	D8s165	D8s260	D8s260	Leon15	Leon15
PD5	*citrinellus*	144	144	159	159	157	157	233	233	268	272
PD6	*citrinellus*	144	144	157	163	157	159	239	239	268	268
PD7	*citrinellus*	144	144	157	161	157	159	239	239	268	272
PD8	*citrinellus*	144	144	159	161	159	159	233	239	272	272
PD9	*citrinellus*	144	144	159	159	159	159	231	239	272	272
RA10	*citrinellus*	144	144	161	161	159	159	239	231	272	272
RA11	*citrinellus*	144	144	161	161	157	159	233	241	272	272
RA12	*citrinellus*	144	144	159	161	159	159	233	233	272	272
RA13	*citrinellus*	144	144	161	161	159	159	241	241	268	268
RA14	*citrinellus*	144	144	161	161	159	159	241	241	268	272
RA15	*citrinellus*	144	148	161	161	157	159	231	241	268	272
RA6	*citrinellus*	144	144	161	161	155	159	233	241	268	272
RA7	*citrinellus*	144	144	161	161	155	159	233	241	268	272
RA8	*citrinellus*	142	144	157	161	151	159	233	241	272	272
RA9	*citrinellus*	144	144	155	161	159	159	233	241	272	272
RB1	*citrinellus*	144	144	161	161	159	159	233	241	272	272
RB10	*citrinellus*	144	152	161	161	151	151	233	241	272	272
RB11	*citrinellus*	144	156	159	161	159	159	235	241	268	272
RB12	*citrinellus*	144	144	161	161	155	159	225	231	272	272
RB3	*citrinellus*	144	148	159	161	159	159	235	239	268	268
RB4	*citrinellus*	144	144	161	161	157	157	241	241	268	272
RB5	*citrinellus*	144	144	159	161	155	159	233	233	268	272
RB6	*citrinellus*	144	160	161	161	159	159	241	241	268	272
RB7	*citrinellus*	144	144	159	161	155	159	233	233	268	272
RB8	*citrinellus*	132	144	161	161	159	159	233	233	268	272
RB9	*citrinellus*	144	144	161	161	159	159	233	239	268	272
T1	*citrinellus*	130	144	161	161	155	159	231	233	268	272

		Marker									
Sample	**Subspecies**	Leon21	Leon21	LL118	LL118	LL157	LL157	LL311	LL311	Locus5	Locus5
PD5	*citrinellus*	368	372	148	150	231	231	245	251	114	114
PD6	*citrinellus*	370	370	148	152	231	233	245	251	112	114
PD7	*citrinellus*	368	368	148	152	229	231	272	272	110	114
PD8	*citrinellus*	368	372	148	152	231	231	242	245	114	114
PD9	*citrinellus*	368	372	148	152	231	231	215	245	114	114
RA10	*citrinellus*	328	372	148	148	231	231	266	272	108	108
RA11	*citrinellus*	372	372	148	148	231	233	269	272	108	114
RA12	*citrinellus*	372	372	148	148	231	233	266	269	108	114
RA13	*citrinellus*	338	372	146	148	231	233	248	269	108	112
RA14	*citrinellus*	372	374	142	148	231	233	245	248	114	114
RA15	*citrinellus*	372	372	148	148	231	231	224	248	108	114
RA6	*citrinellus*	372	372	148	148	231	231	245	269	108	114
RA7	*citrinellus*	372	372	148	148	231	231	245	269	108	114
RA8	*citrinellus*	342	372	132	148	231	233	269	272	108	114
RA9	*citrinellus*	364	372	148	148	231	233	257	272	108	114
RB1	*citrinellus*	372	372	148	148	231	231	221	245	114	114
RB10	*citrinellus*	370	372	148	148	231	233	269	272	108	114
RB11	*citrinellus*	372	372	148	148	231	233	245	248	108	114
RB12	*citrinellus*	372	372	148	148	231	233	272	281	114	114
RB3	*citrinellus*	372	372	148	148	231	233	242	242	108	114
RB4	*citrinellus*	372	372	148	148	231	231	245	248	114	114
RB5	*citrinellus*	372	372	148	148	233	233	248	269	108	114
RB6	*citrinellus*	372	374	148	148	231	231	248	269	114	114
RB7	*citrinellus*	326	372	148	148	233	233	269	272	108	114
RB8	*citrinellus*	374	374	132	148	233	233	224	248	114	114
RB9	*citrinellus*	372	372	148	148	231	233	245	278	106	114
T1	*citrinellus*	372	372	148	148	231	231	269	290	114	114

		Marker				Marker				Marker	
Sample	**Subspecies**	SB38	SB38	**Sample**	**Subspecies**	SB38	SB38	**Sample**	**Subspecies**	SB38	SB38
O16	*citrinellus*	143	143	PD5	*citrinellus*	139	143	T10	*citrinellus*	139	143
O2	*citrinellus*	143	143	PD6	*citrinellus*	139	143	T11	*citrinellus*	139	143
O3	*citrinellus*	145	145	PD7	*citrinellus*	139	139	T12	*citrinellus*	139	143
O4	*citrinellus*	143	143	PD8	*citrinellus*	139	143	T13	*citrinellus*	143	143
O6	*citrinellus*	143	143	PD9	*citrinellus*	139	139	T15	*citrinellus*	139	143
P10	*citrinellus*	143	143	RA10	*citrinellus*	141	143	T3	*citrinellus*	143	143
P11	*citrinellus*	139	143	RA11	*citrinellus*	141	143	T4	*citrinellus*	139	143
P12	*citrinellus*	143	143	RA12	*citrinellus*	141	143	T7	*citrinellus*	139	143
P14	*citrinellus*	139	143	RA13	*citrinellus*	139	141	T8	*citrinellus*	143	143
P15	*citrinellus*	139	143	RA14	*citrinellus*	139	143	T9	*citrinellus*	137	143
P19	*citrinellus*	139	143	RA15	*citrinellus*	141	143	V10	*citrinellus*	139	143
P2	*citrinellus*	141	143	RA6	*citrinellus*	143	143	V11	*citrinellus*	143	143
P3	*citrinellus*	139	143	RA7	*citrinellus*	143	143	V12	*citrinellus*	143	143
P4	*citrinellus*	139	143	RA8	*citrinellus*	141	143	V15	*citrinellus*	139	143
P5	*citrinellus*	143	143	RA9	*citrinellus*	141	141	V16	*citrinellus*	139	143
P7	*citrinellus*	139	143	RB1	*citrinellus*	143	143	V6	*citrinellus*	139	143
P8	*citrinellus*	143	143	RB10	*citrinellus*	139	141	V9	*citrinellus*	143	143
P9	*citrinellus*	143	143	RB11	*citrinellus*	143	143	SO23	*oerstedii*	139	139
PD1	*citrinellus*	143	143	RB12	*citrinellus*	137	141	SO25	*oerstedii*	139	141
PD10	*citrinellus*	143	143	RB3	*citrinellus*	143	143	SO27	*oerstedii*	139	141
PD12	*citrinellus*	139	143	RB4	*citrinellus*	143	143	SO30	*oerstedii*	139	139
PD13	*citrinellus*	139	143	RB5	*citrinellus*	139	143	SO31	*oerstedii*	139	143
PD14	*citrinellus*	139	143	RB6	*citrinellus*	141	141	SO33	*oerstedii*	139	139
PD15	*citrinellus*	143	143	RB7	*citrinellus*	139	143	SO34	*oerstedii*	139	139
PD2	*citrinellus*	143	143	RB8	*citrinellus*	141	143	SO35	*oerstedii*	139	139
PD3	*citrinellus*	139	143	RB9	*citrinellus*	143	143	SO36	*oerstedii*	139	139
PD4	*citrinellus*	139	143	T1	*citrinellus*	143	143	SO38	*oerstedii*	139	139
								SO39	*oerstedii*	139	141

		Marker									
Sample	**Subspecies**	CJ7	CJ7	D17s804	D17s804	D3s1210	D3s1210	D3s1229	D3s1229	D3s1766	D3s1766
T10	*citrinellus*	142	142	178	178	123	123	116	116	195	195
T11	*citrinellus*	142	150	178	178	123	123	114	116	195	195
T12	*citrinellus*	136	142	178	178	123	123	114	116	203	203
T13	*citrinellus*	136	136	180	180	123	123	110	114	199	199
T15	*citrinellus*	142	150	178	178	123	123	116	116	195	199
T3	*citrinellus*	140	142	178	178	123	123	116	116	195	203
T4	*citrinellus*	142	150	178	178	123	123	102	116	195	199
T7	*citrinellus*	136	136	178	178	123	123	116	118	203	203
T8	*citrinellus*	136	140	178	178	123	123	112	114	195	195
T9	*citrinellus*	136	140	178	178	123	123	112	114	195	195
V10	*citrinellus*	140	144	174	178	123	129	112	114	195	195
V11	*citrinellus*	144	150	178	178	123	123	114	116	195	195
V12	*citrinellus*	144	150	178	178	123	123	114	116	195	195
V15	*citrinellus*	140	144	176	176	123	123	112	114	195	195
V16	*citrinellus*	140	144	178	186	123	123	112	114	195	203
V6	*citrinellus*	142	150	178	178	123	123	108	114	199	203
V9	*citrinellus*	144	150	178	178	123	123	114	116	195	195
SO23	*oerstedii*	146	146	176	178	125	125	116	118	195	203
SO25	*oerstedii*	140	140	176	178	123	125	114	114	191	195
SO27	*oerstedii*	140	140	178	178	123	125	114	116	191	195
SO30	*oerstedii*	142	146	176	178	125	125	116	116	199	203
SO31	*oerstedii*	136	140	176	178	123	123	110	110	195	203
SO33	*oerstedii*	140	140	176	176	125	125	114	114	203	203
SO34	*oerstedii*	146	146	176	178	125	125	114	114	203	203
SO35	*oerstedii*	146	146	178	178	125	125	114	114	203	203
SO36	*oerstedii*	140	146	178	178	125	125	114	114	191	203
SO38	*oerstedii*	140	146	178	178	125	125	108	114	203	203
SO39	*oerstedii*	146	146	178	178	125	125	114	114	203	203

		Marker									
Sample	**Subspecies**	D4s111	D4s111	D5s111	D5s111	D8s165	D8s165	D8s260	D8s260	Leon15	Leon15
T10	*citrinellus*	140	144	159	161	155	159	231	233	272	272
T11	*citrinellus*	144	144	161	163	155	159	231	233	268	272
T12	*citrinellus*	144	144	161	161	155	159	233	239	268	272
T13	*citrinellus*	144	160	161	161	151	159	231	233	272	272
T15	*citrinellus*	144	152	159	161	159	159	233	233	272	272
T3	*citrinellus*	144	144	159	161	155	155	231	233	272	272
T4	*citrinellus*	144	144	159	161	159	159	233	233	272	272
T7	*citrinellus*	144	144	161	161	155	157	231	239	268	272
T8	*citrinellus*	144	144	161	161	159	159	231	239	268	272
T9	*citrinellus*	144	144	161	161	155	159	231	239	268	272
V10	*citrinellus*	144	164	159	161	157	159	233	239	268	272
V11	*citrinellus*	144	148	159	161	159	159	231	233	272	272
V12	*citrinellus*	144	144	159	161	159	159	231	233	268	272
V15	*citrinellus*	144	148	157	161	157	159	233	239	268	272
V16	*citrinellus*	144	148	161	161	157	159	231	233	268	272
V6	*citrinellus*	144	150	159	159	159	159	233	239	270	270
V9	*citrinellus*	144	144	159	161	159	159	231	231	272	272
SO23	*oerstedii*	144	144	161	161	143	143	229	229	268	268
SO25	*oerstedii*	144	144	161	161	143	149	231	231	268	268
SO27	*oerstedii*	144	144	159	161	143	149	231	231	268	268
SO30	*oerstedii*	144	144	161	161	137	137	225	231	268	268
SO31	*oerstedii*	144	144	159	161	159	161	233	233	272	272
SO33	*oerstedii*	144	144	161	161	145	145	231	231	268	268
SO34	*oerstedii*	144	144	161	161	143	143	225	231	268	268
SO35	*oerstedii*	144	144	161	161	143	145	231	231	268	268
SO36	*oerstedii*	144	148	161	161	143	145	225	231	268	268
SO38	*oerstedii*	144	144	161	161	143	145	231	231	268	268
SO39	*oerstedii*	144	144	161	161	143	145	225	231	268	268

		Marker									
Sample	**Subspecies**	Leon21	Leon21	LL118	LL118	LL157	LL157	LL311	LL311	Locus5	Locus5
T10	*citrinellus*	368	372	148	148	231	233	245	269	104	114
T11	*citrinellus*	372	372	148	148	231	231	245	269	114	116
T12	*citrinellus*	368	372	148	148	231	231	245	269	114	114
T13	*citrinellus*	372	374	148	148	231	233	245	251	114	114
T15	*citrinellus*	372	372	148	150	233	233	245	269	114	114
T3	*citrinellus*	368	372	148	148	231	231	224	269	114	114
T4	*citrinellus*	372	372	148	150	233	233	245	269	114	114
T7	*citrinellus*	368	372	148	148	231	231	269	269	114	114
T8	*citrinellus*	372	372	148	148	231	231	245	269	110	114
T9	*citrinellus*	364	372	148	148	231	231	245	269	114	114
V10	*citrinellus*	372	372	148	148	231	231	248	296	114	114
V11	*citrinellus*	370	372	148	148	231	231	248	296	114	114
V12	*citrinellus*	372	372	148	148	231	231	248	296	114	114
V15	*citrinellus*	370	372	148	148	231	231	248	248	108	114
V16	*citrinellus*	326	372	148	148	231	231	248	296	104	114
V6	*citrinellus*	368	372	148	148	229	229	242	245	114	114
V9	*citrinellus*	372	372	148	150	231	231	248	272	114	114
SO23	*oerstedii*	372	374	154	154	231	233	236	275	110	118
SO25	*oerstedii*	376	376	150	154	233	239	266	266	114	116
SO27	*oerstedii*	376	376	154	154	233	233	266	266	114	114
SO30	*oerstedii*	376	376	152	154	233	237	239	254	114	116
SO31	*oerstedii*	372	372	148	148	231	233	248	311	114	114
SO33	*oerstedii*	374	374	152	154	231	233	263	263	114	116
SO34	*oerstedii*	374	374	152	154	231	233	263	263	114	114
SO35	*oerstedii*	374	374	154	154	233	233	263	263	114	114
SO36	*oerstedii*	374	374	154	154	231	233	236	263	114	114
SO38	*oerstedii*	374	374	150	152	233	233	263	263	114	116
SO39	*oerstedii*	374	374	150	152	233	233	236	263	114	114