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Genetic and Demographic Consequences of Human-Driven Landscape Changes on Bird Populations: the Case of *Aphrastura spinicauda* (Furnariidae) in the Temperate Rainforest of South America

By

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General Abstract

Human activities, such as expansion of agricultural land and forest exploitation, have modified landscapes worldwide. Despite a large accumulation of empirical and theoretical knowledge on habitat loss and fragmentation, some aspects remain poorly understood, especially those related to the interaction between different forms of habitat degradation. The overall goal of this research was to study the effects of habitat fragmentation and changes in forest structure on bird populations in a human-modified landscape in the temperate rainforest of South America using *Aphrastura spinicauda* (Furnariidae) as a model species. Specifically, I evaluated i) if replacement of forest by open habitat leads to reduced dispersal influencing the genetic structure of populations; ii) if density and reproductive success vary across a fragmented landscape and if populations are limited because of nest-site availability in forests that differed in structure (modified by selective logging) and degree of isolation; and iii) if nest-site selection patterns and associated consequences on fitness are spatially variable in response to changes in forest structure and connectivity. First I show that forest replacement by open habitat reduced landscape connectivity and influenced the genetic structure of populations even within the time-space scale of habitat fragmentation caused by human activities. Secondly, I show that bird density varied across the fragmented landscape while nesting success remained constant. *Aphrastura spinicauda* is a non-excavator cavity-nester and thus relies on old trees or snags where most cavities form. A nest-site supplementation experiment revealed that nest-site limitation is the primary cause of density reduction in selectively-logged forests, but that other processes directly related to forest fragmentation (e.g. loss of connectivity) also influence population responses to habitat changes. Finally, I showed evidence for adaptive nest-site preferences, but also that nest-site choice is spatially variable in response to ecological gradients produced by human activities. By combining genetic and demographic responses of populations I provided evidence for unforeseen and potentially synergistic interactions among different forms of habitat degradation. These results emphasize the need of explicit and independent consideration of habitat fragmentation and other forms of habitat degradation, such as selective logging, when studying populations in human-modified landscapes. In that way, we can better understand and predict population persistence and their adaptive responses in these landscapes.

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CHAPTER I

Genetic structure of forest bird populations in naturally and human fragmented forests: a contrast between two time-space scales

Abstract. - Habitat fragmentation can disrupt dispersal of organisms and therefore it can also influence genetic processes at the population level. Time since isolation, however, can be a limiting factor to detect the full extent of genetic consequences on populations, especially when fragmentation occurs at small spatiotemporal scales as in many human-modified landscapes. Naturally fragmented systems that have been isolated for long periods of time (i.e. thousands of years), such as relict cloud-forests in north-central Chile provide a good opportunity to study large-scale and long-term effects of forest fragmentation. The goals of this study were (1) to contrast the genetic structure of *Aphrastura spinicauda* (Furnariidae) populations inhabiting naturally fragmented relict-forests with those found in human-caused fragments and (2) to determine if forest replacement by open habitat reduces landscape connectivity and therefore influences the genetic structure of populations by comparing populations in fragmented and continuous forests. Six populations were sampled at a regional scale (distance range: 100-1000 km) with two of those (one continuous forest and one anthropogenic fragmented landscape) further divided into several subpopulations to investigate genetic spatial patterns at a short and small time-space scale (distance range: 2-30 km). Individuals were genotyped at five microsatellite loci. Genetic consequences of isolation were observed at both small and large time-space scales although patterns in human-caused fragments were less pronounced. Relict forests were inhabited by two genetically distinct populations, but two continuous-forest populations separated by the same distance were not differentiated, indicating that large distances are a barrier for *A. spinicauda* dispersal only if habitat separating populations is not forest. A similar pattern was observed at the small scale. The comparison of genetic differentiation among four sites in the continuous forest revealed that these subpopulations were not genetically differentiated. In contrast, subpopulations in forest fragments that were similarly distributed across space (but not connected by forest) were significantly differentiated suggesting reduced dispersal among fragments. There was a significant isolation by distance pattern at the large spatial scale. At the small scale, however, it was significant only after controlling for the landscape context of individual fragments (i.e. number and size of fragments within the neighborhood of a focal patch). In this study, I showed that time-space scales are important in revealing the genetic consequences of habitat fragmentation and that forest replacement by open habitat, geographic distance among populations, and size of forest fragments are important in determining genetic differentiation of populations.

Key words: *Aphrastura spinicauda,* Chile, Furnariidae, habitat fragmentation, microsatellite, population genetic structure, south-temperate rainforest.

Introduction

An abrupt change in landscape connectivity, such as that resulting from forest fragmentation, may interfere with dispersal success of organisms. Reduction in size and increased isolation of habitat fragments are important causes of population decline (Davies et al. 2001) because dispersal restriction and population size reduction increase the probability of local extinction (Bender et al. 1998; Andren 1994; Brown & Kodrik-Brown 1977), disrupt important ecological processes (Kareiva & Wennergren 1995), and lead to the genetic deterioration of populations

(Avise & Hamrick 1996). Previous studies have shown that in the long term (i.e., tens of generations), habitat fragmentation can affect genetic population structure even in birds, a group considered to have high dispersal capability (Bates 2002). Furthermore, these effects may even be observed within the time frame of human-caused fragmentation in both, small mammal (Gerlach & Musolf 2000) and bird populations (Galbusera et al. 2000). However, time-since-isolation can limit expression of the full extent of genetic consequences of human-caused fragmentation because observable effects on populations usually lag behind the disturbances that caused them (Tilman et al. 1994). Moreover, these habitat changes are usually an ongoing process and in many cases equilibrium situations may not have been attained (Hutchison & Temple 1999). Therefore, systems that have been naturally fragmented and are isolated in the long-term and at large spatial scales may provide a good contrast to more recent and human-caused fragmentation.

Relict cloud-forests in north-central Chile provide good comparative sites for detecting large-scale and long-term effects of fragmentation on populations (Cornelius et al. 2000). These forests occur in patches along the coastal mountain range where fog-induced microclimatic conditions allow the forest to exist in a semiarid region (Del Val et al. 2006). Bird (Cornelius et al. 2000; Ried et al. 2002) and plant (Perez & Villagran 1985) composition of relict forests closely resemble that of Valdivian rainforests, located more than 1000 km to the south. These relict forests are remnants of an ancient forest community that became fragmented and isolated during the Pliocene and Pleistocene periods (Villagran et al. 2004). During periods in the Quaternary, these forests expanded their ranges because of wetter conditions caused by glaciation events which resulted in periods of higher connectivity among these relict forests and the southern Valdivian forests (Villagran et al. 2004; Troncoso et al. 1980).

Aphrastura spinicauda (Furnariidae), an endemic bird from the South American temperate rainforest, is a good model species to investigate population genetic consequences of habitat fragmentation because it is restricted to forest habitats and does not occur in any type of open habitat that typically surrounds forests within the study area (Cornelius et al. 2000; Diaz et al. 2005). It is an insectivorous, year-round resident bird that nests in tree cavities typically found at mid- and high-canopy levels in the forest (Diaz et al. 2005; Estades & Temple 1999; Chapter 2). To date, no studies have investigated the population genetic structure of any bird species in the south-temperate forest, and only a few studies have investigated the genetic effects of human-driven forest fragmentation on bird populations in other systems (Ellegren et al. 1999; Galbusera et al. 2000; Galbusera et al. 2004; Brown et al. 2004). In the south-temperate rainforest, however, evidence from bird movements (Sieving et al. 1996; Castellón & Sieving 2006a) and mating success (Willson 2004; Diaz et al. 2006a) have revealed dispersal restriction across open habitats for most understory bird species. Dispersal capability of *A. spinicauda* across open habitats has not been evaluated directly; however, it is likely to be higher than most understory bird species because of its non-terrestrial habits (Castellón & Sieving 2006b). *Aphrastura spinicauda* is among the few forest bird species that have been able to colonize and persist in relict-forest systems located outside the temperate forest region (Cornelius et al. 2000; Ried et al. 2002).

The goal of this study is to determine if loss of connectivity, through the replacement of forest habitat by open habitat, results in changes to the genetic structure of populations, using *Aphrastura spinicauda* as a model species. To address this question, I studied the genetic structure of populations at two different time-space scales: (1) long-term and large-scale isolation by comparing populations in two relict forests with that of populations sampled in a continuous forest, and (2) short-term and small-scale isolation driven by human-caused forest fragmentation by comparing populations among fragments within an agricultural landscape with populations within a continuous forest.

Methods

Large-scale sampling

Populations of *A. spinicauda* were sampled within two main regions across its distributional range in coastal central-southern Chile: the northern semiarid region (30° S to 32° S) and the southern region (40° S to 43° S) where the broad-leaved and evergreen Valdivian rainforest type is dominant. Within these regions, populations were sampled in six sites (Fig. 1a). Two sites were in relict forests located along a north-south gradient of aridity; the northernmost forest, Fray Jorge, is separated by 165 km of semiarid scrub from the next relict forest to the south, Santa Inés. Both relict forests are small (< 300 ha) forest remnants that are surrounded by semiarid scrub habitat. Towards the south, aridity decreases and dense scrub and sclerophyllous forests become abundant. Thus, from Santa Inés to the south, forests are immersed in a region with increasing landscape connectivity. To control for isolation by distance, in contrast to isolation caused by the inability of *A. spinicauda* to cross large expanses of non-forested habitat, two sampling sites (Chaihuín and Parga) were established in the southern Valdivian region, that were separated by a similar distance (165 km) as the two relict forests. These two sites were located within the last continuous-forest remnant (439 000 ha) of the coastal range of the Valdivian region (40°S – 43°S; Smith-Ramirez 2004) and where the larger overall forest cover results in increased landscape connectivity (Castellón & Sieving 2006b). The final two sites were established on Chiloé, a large land-bridge island separated from the mainland by a narrow 2 to 5-km wide marine channel. One site was established in Chiloé National Park (Chiloé-NP) in a large continuous-forest remnant (35,200 ha) along the western coastal range. The second site was located 80 km from the National Park in an area where the landscape is composed of several forest fragments distributed across an agricultural landscape (Chiloé-AL) in north-east Chiloé (Fig. 1a).

Small-scale sampling

To study the structure of *A. spinicauda* populations at a finer scale, the two sampling sites in Chiloé were further subdivided into several subpopulations (Fig. 1b). In the agricultural landscape (Chiloé- AL), birds were sampled in nine anthropogenic forest fragments within an area of 550 km^2; pairwise distances among fragments ranged from 1 to 22.8 km. Forest fragments in this area were surrounded by a matrix of agricultural fields, pastures and abandoned fields, habitat types not used by *A. spinicauda*. To control for isolation by distance in contrast to loss of connectivity caused by replacement of forest by agricultural land, four sampling sites were established in the continuous forest in Parque Nacional Chiloé (Chiloé-NP); pairwise distances among these sites ranged from 1.9 km to 14 km. Neither site (Chiloé-NP and Chiloé-AL) contained major barriers such as mountains or large rivers. Landscape metrics were calculated from a 2001 Landsat Thematic Mapper image with a pixel resolution of 30 m; habitat was categorized as forest or non-forest using ArcGIS 9.1. Individual fragments were identified using program FRAGSTAT (McGarigal & Marks 1995). In the agricultural landscape, birds were sampled in three large (> 1,000 ha), three medium (100 – 400 ha) and three small fragments (10 - 15 ha). To quantify the isolation of forest fragments, I used an index of habitat patch proximity (PX) calculated with FRAGSTAT. This index is a measure of patch isolation that quantifies the spatial context of a focal patch in relation to other patches within a specified buffer distance (Gustafson & Parker 1992). This index distinguishes isolated patches (i.e. small PX values) from those that are part of a cluster of patches (i.e. large PX values) by considering size and distance among patches, which are both important variables in determining the genetic structure of populations. Ideally, the buffer distance to evaluate PX should reflect the scale of movement of the organism investigated. Because no *a priori* information exists about the dispersal capability of *A. spinicauda*, I used an arbitrary buffer distance of 1 km. Proximity index values, however, remained quantitatively and qualitatively similar with buffer distances of 0.5 km to 5 km. This small variation in PX with buffer distance is expected when overall forest cover exceeds 40% (Gustafson & Parker 1994).

Sample collecting procedure

Blood samples were collected from 235 individuals in 17 sites (including relict forests, sites in continuous forests and forest fragments, as described above), during August through October of 2003, 2004 and 2005. Samples were collected before or during the early stages of the breeding season so that only adult breeding birds were included and so that, most probably, dispersal of birds hatched in the previous year already had occurred. Intensive mist-netting with playbacks was used to capture at least 10 individuals in each site (mean: 13.8; range: 10 – 18). At the fine-sampling scale (i.e. in Chiloé-AL and Chiloé-NP), the size of the collection area within the continuous-forest sites and within large fragments was equivalent (10 – 15 ha) to the collection area within smaller fragments, to compare the existing diversity of large and small forests within equivalent sampling areas. Relict forests were sampled over their entire range and sites in the Valdivian region were sampled over a comparable geographical span. Each bird captured was uniquely marked with numbered aluminum bands and released in the same capture area. A small (0.05 µl) blood sample was taken using microcapillary tubes following a small puncture of the brachial vein (McGuill & Rowan 1989); blood was stored in lysis buffer (1% SDS, 100mM Tris, 10mM EDTA pH 8.0) for later analysis at University of Missouri St. Louis.

Microsatellite analysis

DNA was extracted with a standard phenol-chloroform protocol after incubation for 12 hours at 65° C in the presence of 30 µg of proteinase K. Genotyping of individuals was based on five microsatellite loci previously developed for other bird species. Loci Man1, Man3, and Man13 were developed for *Manacus manacus*, family Pipridae (Piertney et al. 2002) and loci wbwc-28 and wbwc-58 for *Glyphorynchus spirurus*, family Dendrocolaptidae (Milá & Bardeleben 2005). Starting from the original PCR conditions, different concentrations of $MgCl_2$, DNA template, and annealing temperatures were tested using normal thermal cyclers. Optimal reaction conditions for each locus for *A. spinicauda* are described in Table 1. Fluorescently labeled forward primers were used in PCR reactions and products were analyzed on an ABI 3100 sequencer (Applied Biosystems). Fragment length was determined by comparison to an internal size standard to determine genotypes using GeneMapper 3.0 software (ABI Prism, Applied Biosystems). Genotyping accuracy was examined by visually checking each genotype; for each locus an average of 80% (range: 65% - 95%) of samples were amplified multiple times to minimize genotyping error. Samples with ambiguous or unique genotypes of low quality were reamplified until genotype could be verified. Samples that were amplified repeatedly with conflicting results were not assigned a genotype (< 1 % of samples at any locus).

Data analyses

Indices of genetic diversity and statistics such as number of alleles per locus (A_N), allelic richness per locus (A_R), a measure of allele number independent of sample size (El Mousadik & Petit 1996), Nei's gene diversity (H_S) averaged over loci (Nei 1987), observed heterozygosity (H_O), and the index F_{IS}, were estimated using program FSTAT version 2.9.3.2 (Goudet 2000). Hardy-Weinberg (HW) equilibrium departures over all loci within each population were determined by examining deficiency of heterozygotes (F_{IS}) and generating *P*-values with 600 permutations. Allelic richness and gene diversity in the northern and southern regions were compared with two sided *t*-tests with *P*-values based on 15,000 permutations using FSTAT. Observed (H_O) and expected (H_E) heterozygosities and departures from HW for each locus per population were estimated in ARLEQUIN version 3.0 (Excoffier et al. 2005). All pairs of loci were tested for linkage disequilibrium within each population using a log-likelihood ratio test with *P*-values based on 1,200 permutations in FSTAT.

Population differentiation was first examined at three different spatial scales with Weir & Cockerham (1984) measure of global population differentiation F_{ST} (Φ); 95% confidence intervals were obtained by bootstrapping and significance was evaluated based on randomizing genotypes among populations 1,000 times using a log-likelihood test (Goudet et al. 1996) implemented in FSTAT. The statistic R_{ST} that assumes a stepwise mutation model (Slatkin 1995), was also estimated for the global population differentiation analyses. Global F_{ST} (Φ) was compared at large, meso, and small spatial scales. The large spatial scale included all six populations (see Fig.1); the meso scale was a subset of all populations and consisted of the three populations from the southern region within continuous forests (Chaihuín, Parga and Chiloé-NP); and the small spatial scale included populations sampled in fragments within the agricultural landscape (Chiloé-AL). Population differentiation was also evaluated by calculating population pairwise measures of F_{ST} (Wright 1968) among populations at the large spatial scale (i.e. among the six populations) and at the small spatial scale (i.e. among the nine forest fragments and among the four sites within the continuous forest in Chiloé) with significance for pairwise values of F_{ST} determined by 120-720 permutations using FSTAT. Mantel tests (Mantel 1967) with 10,000 permutations were used to determine the relationship between pairwise geographic distance ($\log_{10}$ transformed) and genetic distances with F_{ST} / (1- F_{ST}) as a measure of genetic distance (Rousset 1997). For all tests involving multiple comparisons, a Bonferroni correction was used.

Dispersal patterns among populations at the large spatial scale were investigated with assignment tests (Waser & Strobeck 1998), implemented in ARLEQUIN 3.0, to determine the log-likelihood that each individual multi-locus genotype belongs to the population in which it was sampled. This test allows identification of individuals sampled in one population that appear genetically closer to a different population (i.e. potential migrants). Levels of mis-assignment were determined between the two relict populations (Fray Jorge and Santa Inés) in the northern region, and then contrasted to the levels of mis-assignment between the two populations in the continuous forest located in the southern region (Chaihuín and Parga) that are separated by the same distance as the two relict forests.

To determine whether there was significant population differentiation, and to estimate the distribution of genetic variation within versus among populations, analyses of molecular variance (AMOVA; Excoffier et al. 1992) were conducted, with Weir and Cockerham's (1984) F_{ST} as the measure of genetic distance, using ARLEQUIN 3.0. Different models were compared to determine whether differences between populations are better explained by geographic distance or by differences in landscape connectivity (i.e. whether habitat between populations is forest or non-forest). Significance was obtained after 10,000 permutations to determine the probability of a random F_{ST} value being greater than or equal to the observed value (Excoffier et al. 2005).

At the small scale (i.e. among fragments in the agricultural landscape), the relationship between geographic distance and genetic distance was investigated in two ways. First, the spatial genetic autocorrelation of individuals was investigated by calculating a correlogram, using Moran's index (I_q) as a measure of pairwise distance, at 2-km interval classes using Spatial Genetic Software (SGS) version 1.0c (Degen & Kremer 2001). By examination of the correlogram, it is possible to determine the spatial extent at which individuals are more or less related than expected by chance. Second, the relationship between geographic distance and genetic distance was investigated by incorporating isolation of patches as a predictor variable for genetic distance among forest fragments. To obtain a pairwise value of isolation among fragments, I used the smaller value of the proximity index (PX) between two fragments being compared. The smaller value was chosen because pairwise genetic differentiation (i.e. a high F_{ST} value between two fragments) is not necessarily a result of the isolation of both patches; it can also result from the isolation of only one of the two patches being compared when controlling for the distance separating them. To compare two variables while controlling for a third variable, partial-Mantel tests (Smouse et al. 1985) with 10,000 permutations were conducted in FSTAT.

Results

Overall genetic diversity patterns

Number of alleles ranged from 6 to 21 at the five microsatellite loci screened for *A. spinicauda* across south-central Chile (Table 2). Hardy-Weinberg (HW) equilibrium tests conducted for each locus in each population revealed a significant deviation from HW in only five cases (Appendix A). However, no locus or population had significant HW departure more than once, except for the locus wbwc-28 that had a significant departure from HW in two populations. Over all loci, there was no departure from HW equilibrium within populations except for the fragmented population in the southern region (Chiloé-AL) where a significant deficiency in heterozygotes was detected (Table 2). Tests for genotypic linkage disequilibrium revealed no significant associations between pairs of loci in any population or over all populations (Appendix B).

Observed heterozygosity (H_O), averaged over all loci, ranged from 0.7 to 0.8 among the six populations. Both relict-forest populations had the lowest values of allelic richness (A_R) and gene diversity (H_S), with Fray Jorge having the lowest level of gene diversity of all populations sampled (Table 2). Allelic richness and gene diversity, however, did not differ significantly between the relict populations in the northern region and the populations in the southern temperate-forest region ($A_R = 6.58$ and $A_R = 8.13$, $P = 0.060$, respectively; $H_S = 0.787$ and $H_S = 0.828$, $P = 0.064$, respectively).

Long-term and large-scale population structure

The regional comparison among all six populations revealed a global estimate of F_{ST} (Φ) that was low but significantly different from zero ($\Phi = 0.028$, 95% CI 0.016 – 0.041, $P < 0.001$). The meso-scale comparison of the three populations found in continuous forests within the south temperate-forest region (Chaihuín, Parga and Chiloé-NP) revealed a low Φ estimate that was not significantly different from zero ($\Phi = 0.009$, 95% CI -0.002 – 0.019, $P > 0.05$). However, at the small scale among-fragments within the agricultural landscape (Chiloé- AL), Φ was significantly different from zero ($\Phi = 0.02$, 95% CI 0.01 – 0.033, $P < 0.001$). The relationship between global F_{ST} (Φ) estimates across the three spatial scales is shown in Fig. 2. Estimates of population differentiation based on a stepwise mutation model showed similar patterns with lowest values observed at the meso scale ($R_{ST} = 0.0038$) and highest values at the large ($R_{ST} = 0.1159$) and small scales ($R_{ST} = 0.1163$). A hierarchical AMOVA at the large spatial scale, in which populations were assigned to three groups according to their time-space isolation history (G1:

Fray Jorge and Santa Inés, G2: Chaihuín, Parga, Chiloé-NP, G3: Chiloé-AL), revealed a low but significant differentiation among the three groups, with most of the variation being explained at the lowest hierarchical level (Table 3, model a).

Pairwise comparisons of genetic distance (F_{ST}) among the six populations of *A. spinicauda* (Table 4) showed that Fray Jorge, the most-northern relict forest, had a significant reduction in heterozygosity relative to all other populations (F_{ST} ranged from 0.111 to 0.073, $P < 0.003$). The population in the relict forest Santa Inés, also had significant F_{ST} values with all southern populations (F_{ST} ranged from 0.068 to 0.025, $P < 0.003$). F_{ST} between the two continuous forest populations in the Valdivian region (Chaihuín and Parga), separated by the same distance as the two relict forests, was not significant (Table 4), suggesting that dispersal of *A. spinicauda* is restricted across large distances only if the habitat separating populations is non-forested (i.e. semi-arid scrub). The correlation between all pairwise genetic and geographic distances among the six populations indicated isolation by distance at the regional scale (Mantel test $R^2 = 0.34$, $P = 0.023$). The genetic distance between the two relict forests, however, was a marked outlier in this correlation given that populations separated at equivalent distances in the southern region were much less differentiated (Fig. 3). Results from the assignment tests further confirmed that the relict populations are genetically distinct with no individuals mis-assigned between them (Fig. 4a). This is in contrast to the two southern populations in the Valdivian region where some overlap of the two likelihood distributions suggests a higher connectivity (Fig. 4b).

That populations in the southern region were not differentiated (i.e. had no significant pairwise F_{ST} values, Table 4) was supported by two hierarchical AMOVA models conducted within the southern region (Table 3 models b and c). The first model conducted between two groups of continuous forest populations (G1: Chaihuín and Parga, G2: four sites in Chiloé-NP), revealed no genetic differentiation between mainland populations (G1) and Chiloé island populations (G2), with no differentiation among populations within groups (Table 3 model b). The second model, in which the same mainland continuous forest populations (G1: Chaihuín and Parga) were compared with the fragmented forest populations in the agricultural landscape in Chiloé (G2: nine forest fragments in Chiloé-AL), again showed that there was no significant differentiation between the mainland and island populations; however contrary to the previous model, there was a small but significant differentiation of populations within groups, with 2.13 % of the total variation explained by differences among subpopulations in the anthropogenic forest fragments (Table 3 model c).

Short-term and small-scale population structure

The local comparison of genetic differentiation among the four sites in the continuous forest (Chiloé-NP) revealed that subpopulations in the continuous forest were not genetically differentiated (AMOVA, $F_{ST} = 0.011$, P = 0.178), with no significant pairwise F_{ST} values among sites (Table 5). In contrast, subpopulations in forest fragments that were similarly distributed across space (but not connected by forest) were significantly differentiated (AMOVA, $F_{ST} = 0.022$, P < 0.001). Only 2.18% of the total variation was explained, however, by differences among forest fragments; 97.82% of the variation was explained at the individual level within populations. The nine fragments sampled and their spatial relationships across the agricultural landscape in Chiloé are shown in Figure 5, in which isolation of fragments is classified according to the habitat patch proximity index (PX). Patch area was significantly related to PX value (Pearson r = 0.940, $P < 0.001$); so, small patches were in a more isolated landscape context than larger patches (Fig. 5). The analysis of pairwise genetic distances among fragments revealed that several subpopulations in fragments were differentiated in relation to other fragments in the landscape. Significant pairwise F_{ST} values (after correction for multiple comparisons) ranged from 0.045 to 0.080 ($P < 0.0014$); marginally significant values of F_{ST} ranged from 0.028 to 0.050 ($P < 0.05$). Results suggest that gene flow is restricted to a certain degree in this fragmented landscape (Table 6).

Examination of Moran's correlogram of spatial genetic autocorrelation of individuals across the fragmented landscape indicated that individuals are more related than expected by chance, at 2 km or less. At greater distances, individual relatedness is mostly within random expectations with only a few distance classes in which individuals were less or more related than expected by chance; there was no clear pattern between distance and relatedness (Fig. 6). The relationship between geographic distance and genetic distance among forest fragments showed a similar pattern. Geographic distance did not predict the genetic distance among fragments (Mantel $R^2 = 0.044$, $P = 0.222$). Although low pairwise genetic distances were observed at short geographic distances, there was high variation in genetic distances at greater geographic distances (Fig. 7a), suggesting that other landscape characteristics are probably also important in determining genetic differentiation. When the effect of geographic distance on genetic distance was examined after controlling for the effect of isolation of forest fragments (i.e. using the pairwise patch proximity index) there was a significant correlation between geographic and genetic distance, with 11.4% (partial Mantel test $P < 0.042$) of the variance explained by geographic distance alone (Fig. 7b). On the other hand, patch proximity was significantly related to genetic distance among forest fragments (Mantel $R^2 = 0.198$, $P = 0.0052$, Fig. 7c) but, after controlling for the effect of geographic distance among forest fragments, a higher percentage of the variation (25.7%, Mantel test $P < 0.0018$) was explained (Fig 7d). Therefore, patch isolation or characteristics of the immediate neighborhood of forest fragments, given by the distance and size of neighbor fragments, is more important in determining genetic differentiation among forest fragments than geographic distance alone.

Discussion

Ecological consequences of human-driven habitat fragmentation have been widely demonstrated (Saunders et al. 1991; Harrison & Bruna 1999, Davies et al. 2001), whereas the extent to which genetic diversity and structure of populations may be modified is less understood (Galbusera et al. 2000; Gerlach & Musolf 2000; Garner et al. 2005). A thorough understanding of genetic consequences of habitat fragmentation requires the study of systems whose fragmentation history is sufficiently long to allow such consequences to have become detectable. Most habitat fragmentation caused by humans is too recent and, in most cases, occurs over too limited spatial scale to reveal the full extent of genetic consequences on populations. Here, I used natural forest fragments isolated for thousands of years, and more recent human-caused fragments to contrast and demonstrate the influence of temporal and spatial scales on genetic differentiation of populations of a forest bird. Genetic consequences of habitat fragmentation were observed in both systems although patterns were less pronounced in human-caused fragments. Three main processes were associated with genetic differentiation among populations in fragmented landscapes: forest replacement by open habitat (i.e isolation), fragment size, and distance among populations.

Long-term and large-scale isolation

Populations of *A. spinicauda* in the relict forests Fray Jorge and Santa Inés were genetically distinct, with a higher pairwise F_{ST} value (0.111) than between any other pair of populations compared in this study. Lack of mis-assigned individuals in either population also suggests reduced migration between these forests (Waser & Strobeck 1998). F_{ST} values in the range of 0.05 to 0.15, however, are considered to indicate moderate divergence (Hartl & Clark 1997), suggesting that although genetic differentiation has occurred, some birds may still disperse between these forests. Based on F_{ST} statistics, the number of migrants per generation (*Nm*) between these isolated forests is two birds, which is low but exceeding the threshold considered sufficient to counteract the effects of genetic drift (Slatkin 1987). This estimate needs to be taken with caution, however, because the number of migrants estimated indirectly from F_{ST} assumes that equilibrium conditions have been attained, in addition to other assumptions that are rarely met in natural populations (Whitlock & McCauley 1999). For example, if equilibrium has not been reached, dispersal rate estimates may reflect previous conditions and not current levels of dispersal.

The two relict-forest populations were more differentiated from each other than either was from populations located more than 1,000 km to the south despite the fact that the distance between the two relict forests was much smaller (165 km). Fray Jorge and Santa Inés populations are confined to small forest remnants of less than 300 ha each, and as a consequence, fewer in number than the southern populations found in much larger forests. Therefore, the high differentiation between the two relict forests is likely because both are small populations and thus subject to genetic drift. This is a process that becomes especially important in small populations (Hartl & Clark 1997) because the relative strength of gene flow (and mutations) decreases in relation to genetic drift (Wright 1968). Furthermore, there was a significant correlation between genetic and geographic distance among the six populations studied, but it was entirely driven by genetic distances among the relict forests and the southern populations. The isolation-by-distance model considers distance as the only factor determining genetic differentiation and does not account for the presence of barriers to dispersal. For example, the large genetic distance between the two relict forests indicates the presence of a strong barrier to dispersal (i.e. semiarid habitat) because populations separated by similar distances in the southern-forest region were not genetically differentiated. Both relict forests also had lower values of allelic richness and gene diversity than did southern populations located within the temperate forest region, although these differences were only marginally significant. This suggests that, in the long term, habitat fragmentation can result in reduced genetic diversity even in a bird with moderate dispersal capability.

The two populations in the continuous forest of the Valdivian coastal region were separated by the same distance as the two relict forests. Thus, they provided a good control for the effect of isolation by distance in contrast to dispersal limitation because of the absence of forest habitat. Most parts of this coastal range remained free from glacial influence at the time glaciers reached their maximum extension in the southern hemisphere [20,000 year BP, Villagran (1990)]. Therefore, populations of *A. spinicauda* in the Valdivian region likely have been present over the same temporal scale as have relict forest populations in the northern region. The two populations in the Valdivian region were not genetically differentiated despite having been separated by the same distance as the two relict forests in the semiarid region. Lack of differentiation may indicate that gene flow can be substantial even over large distances in continuous forests and that dispersal of *A. spinicauda* is only restricted at this spatial scale if habitat separating populations is not forested.

The cross-scale comparison of global estimates of F_{ST} (Φ) showed that, as expected, the highest level of differentiation occurred at the large spatial scale (1,000 – 1,400 km). In contrast to expectations, however, the lowest level of differentiation did not occur at the smallest spatial scale (1 -30 km) that had intermediate but significant levels of differentiation, but at the meso scale (100-300 km). Populations at the meso scale were part of large and continuous forest populations, whereas populations compared at the small scale were a set of anthropogenic fragments in an agricultural landscape. Therefore, these Φ values can be re-interpreted as levels of differentiation across a temporal scale of time since isolation (Fig. 8), in which populations in continuous forests and relict forests represent two extremes of a time-since-isolation scale with human-caused fragments at an intermediate position.

Sufficient time and an expanse of hostile habitat, such as that observed between the two relict forests, are enough to produce significant genetic differentiation among populations. The role of small-scale barriers, however, is not that clear. Populations in the Valdivian region were not differentiated from populations in Chiloé Island, despite separation by a narrow marine channel. Therefore, small distances (2-5 km) of non-forest habitat are not enough to genetically differentiate very large populations, as revealed with this set of microsatellite loci. This result, however, needs to be interpreted with caution, because *A. spinicauda* from Chiloé is recognized as a subspecies based on slight differences in plumage coloration relative to the mainland populations. The historical relationships among *A. spinicauda* populations are beyond the scope of this study, but further investigations using appropriate markers are warranted. Inferences based on microsatellite markers involving very large populations need to be taken with caution because high mutation rates can result in homogenization of populations, mimicking recurrent gene flow (Nauta & Weissing 1996).

Short-term and small-scale isolation

There was an overall genetic differentiation of subpopulations of *A. spinicauda* among forest fragments in the agricultural landscape in Chiloé. At a comparable spatial scale, however, subpopulations from the continuous forest in Chiloé National Park were not genetically differentiated. This result strongly suggests that the replacement of forest habitat by agricultural land is having an effect on the genetic structure of populations in fragments, likely due to reduced dispersal across open habitats. It is important to remember that patterns of allele distribution across the landscape do not directly reveal how much gene flow is occurring, because such patterns are a result of a combination of processes, besides dispersal (Slatkin 1987). For example, gene flow does not only depend on movement of birds among fragments but also on successful breeding.

Genetic differentiation among forest fragments in the agricultural landscape is similar to the pattern obtained at the large time-space scale. Pairwise F_{ST} estimates among fragments were lower than those observed between the two relict forests and the southern populations but were, nevertheless, significantly different from zero (i.e. 0.08 to 0.027). F_{ST} values within this range are generally associated with moderate or little genetic differentiation (Hartl & Clark 1997). F_{ST} statistics, however, assume equilibrium conditions, which might not have been achieved in these fragmented forests that have been isolated for no more than 50 to 80 years. Therefore, it is possible that differentiation may increase in the long term, as currently observed among relict forests. Fragments did not show a decrease in allelic richness or gene diversity, relative to populations in continuous forests. As a whole, the population in the fragmented landscape did exhibit an overall reduction of heterozygosity, probably because this population is composed of several subpopulations and, thus, the excess of homozygotes is a consequence of pooling individuals across these subpopulations (i.e. Wahlund effect).

Only a few studies have reported genetic consequences of fragmentation of bird populations in anthropogenic fragments. For instance, Galbusera and collaborators (2000) found high levels of differentiation among populations of an endangered bird in forest fragments in Africa. Pairwise F_{ST} values among these subpopulations ranged between 0.103 and 0.238, considerably higher than those found for *A. spinicauda* in the fragmented landscape in Chiloé. High levels of differentiation in the African study were attributed to isolation of small fragments (2 – 40 ha) produced by substantial habitat loss since the 1960s and small size of remaining populations. Another study with populations of understory birds in Costa Rica also showed very high pairwise population differentiation with F_{ST} values as high as 0.256 (Brown et al. 2004). These extreme and relatively recent fragmentation situations are producing patterns similar to those observed among relict forests in this study and suggest that high levels of genetic differentiation can be observed even within the time scale of human-driven habitat fragmentation, especially after extreme levels of habitat loss.

The degree of genetic differentiation of populations across fragmented landscapes depends on the dispersal capability of individuals and on how animals perceive the landscape. Studies on non-volant small mammals that inhabit anthropogenic fragmented landscapes have demonstrated high levels of differentiation, with pairwise F_{ST} values ranging from 0.018 to 0.46 (Garner et al. 2005; Gerlach & Musolf 2000; Trizio et al. 2005). This is not surprising, given that small non-volant mammals have a lower dispersal capability than most bird species. The striking result of this study on *A. spinicauda* and that of other studies that have examined the genetic structure of bird populations in anthropogenic fragments (Galbusera et al. 2000, 2004; Brown et al. 2004; Arguedas & Parker 2000) is that even with higher dispersal capabilities, bird populations still exhibit genetic consequences of fragmentation even at the temporal and spatial scale of human-caused habitat fragmentation. This is likely a consequence of the fact that dispersal of individuals and, hence, gene flow usually occurs over much shorter distances than individuals are actually capable of moving (Ehrlich & Raven 1969; Slatkin 1987).

The role of geographic distance in reducing gene flow among populations is largely dependent on the scale of analysis (Rousset 1997) and on the landscape characteristics that promote or restrict movement of organisms (i.e. barriers, hostile habitats). In the agricultural landscape in Chiloé, relatedness of individuals did not show a clear pattern with distance. Moreover, distance among fragments was not a good predictor of genetic differentiation, indicating that other landscape features need to be accounted for to fully explain patterns of genetic differentiation. In fact, the patch proximity index PX - a measure of isolation defined by the neighborhood of each forest fragment - explained more of the variation in genetic differences among fragments than distance among them. Isolation by distance was also observed but only after controlling for the landscape context of forest fragments (i.e. patch proximity). Consequently, both the index PX and distance between fragments are important in shaping the population structure of *A. spinicauda* in the fragmented agricultural landscape.

The proximity index used (modified from Gustafson & Parker 1992) was highly correlated with size of forest fragments in this study system and, thus, subpopulations in small fragments were also highly isolated (i.e. surrounded by few and small forest fragments). Highest F_{ST} values were observed among small fragments and among small fragments and other fragments in the landscape, with the highest ($F_{ST} = 0.08$) obtained between the two smallest and isolated fragments. This result also resembles that obtained at the large-scale between relict forests, reinforcing the role of small population size and isolation in determining genetic differentiation among populations. Therefore, populations in small fragments are those contributing most to the overall genetic differentiation among fragments, which is also the case in other fragmented landscapes (Galbusera et al. 2000; Brown et al. 2004; McDonald et al. 1999). This is likely the result of the higher relative effect of gene drift over other differentiating processes that occur in small and isolated habitat fragments.

Concluding remarks

As more habitat is lost and degraded, and as more of it becomes fragmented, populations will either adapt to the novel conditions imposed by human-driven modifications or they will progressively decline until extinction. Yet, before extinction or adaptation occur, processes associated with habitat loss can have detrimental consequences on populations (Davies et al. 2001). Consequences of reduced landscape connectivity are usually difficult to measure. The use of appropriate genetic markers, however, can provide useful information by indirectly measuring movement patterns of organisms (Parker et al. 1998). In this study, I showed that populations of a forest bird in continuous forests showed high levels of dispersal even across large distances (> 200 km) but that the replacement of forest by open habitat (e.g., agricultural land and pastures) can result in moderate levels of genetic differentiation even at short temporal and small spatial scales.

Results from this study are rather conservative for three reasons. First, levels of forest fragmentation in the agricultural landscape in north-eastern Chiloé are not as extreme as in other fragmented landscapes. Current forest cover in the area ranges between 40 – 50 %, which is above the fragmentation threshold of 30 % below which detrimental consequences on populations are highest (Andren 1994). Second, *A. spinicauda* has moderate dispersal capabilities compared to most understory bird species in the south temperate forest for which several lines of evidence suggest reduced dispersal across open habitats (Sieving et al. 1996; Willson 2004; Diaz et al. 2006; Castellón & Sieving 2006a, 2006b). Therefore, based on the results of this study, it is very likely that most understory bird species in this landscape will show equal or stronger genetic effects due to habitat fragmentation. Finally, indirect estimates of gene flow based on allelic frequency data are always higher than direct estimates of real dispersal patterns of organisms (Slatkin 1987).

The temperate rainforest of southern South America has been recognized as an endangered ecosystem with high conservation priority (Dinerstein 1995); it is one of the 25 global hotspots of biological diversity (Myers et al. 2000). Human activities have intensively modified the landscape in this region, resulting in high rates of forest fragmentation characterized by an increase in the number of small and isolated fragments (Echeverria et al. 2006). Habitat loss and fragmentation has been especially high in areas north of Chiloé where only one large remnant remains on the coastal range and few small fragments remain in the central valley (Smith-Ramirez 2004). Therefore, at current rates of deforestation and forest habitat replacement, strong ecological and genetic consequences on plant and animal populations are foreseen in this region.

Literature Cited

Andren, H. (1994) Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. *Oikos* 71: 355-366.

Arguedas, N. & Parker, G. P. (2000) Seasonal migration and genetic population structure in house wrens. *The Condor* 102: 517-528.

Avise, J. & Hamrick, J. (1996) Conservation Genetics: case histories from nature. Chapman & Hall, New York.

Bates, J. M. (2002) The genetic effects of forest fragmentation on five species of Amazonian birds. *Journal of Avian Biology* 33: 276-294.

Bender, D. J., Contreras, T. A. & Fahrig, L. (1998) Habitat loss and population decline: a meta-analysis of patch size effect. *Ecology* 79: 517-533.

Brown, J. H. & Kodrik-Brown, A. (1977) Turnover rates in insular biogeography: effect of immigration on extinction. *Ecology* 58: 445-449.

Brown, L., Ramey, R. R., Tamburini, B. & Gavin, T. (2004) Population structure and mitochondrial DNA variation in sedentary Neotropical birds isolated by forest fragmentation. *Conservation Genetics* 5: 743-757.

Castellón, T. D. & Sieving, K. E. (2006a) An experimental test of matrix permeability and corridor use by an endemic understory bird. *Conservation Biology* 20: 135-145.

Castellón, T. D. & Sieving, K. E. (2006b) Landscape history, fragmentation, and patch occupancy: models for a forest bird with limited dispersal. *Ecological Applications* 16: 2233-2234.

Cornelius, C., Ried, S. & Marquet, P. A. (2003) Conservación de las comunidades de aves en los bosques relictos en Chile central, pp. 139-142. In: *Conservación y biodiversidad de los bosques de la cordillera de la costa en Chile* (eds C. Smith-Ramirez, C. Valdovinos & J. J. Armesto). Editorial Universitaria, Santiago, Chile.

Cornelius, C., Cofre, H. & Marquet, P. A. (2000) Effects of habitat fragmentation on bird species in a relict temperate forest in semiarid Chile. *Conservation Biology* 14: 534-543.

Davies, K. F., Gascon, C. & Margules, C. R. (2001) Habitat fragmentation, pp. 81-98. In: *Conservation Biology: research priorities for the next decade* (M. E. Soule & G. H. Orians, eds.). Island Press, Washington DC.

Degen, B. & Kremer, A. (2001) SGS - Spatial genetic software: A computer program for analysis of spatial genetic and phenotypic structures of individuals and populations. *Journal of Heredity* 92: 447-448.

Del Va, E., Armesto, J. J., Barbosa, O., Christie, D. A., Gutierrez, A. G., Jones, C., Marquet, P. A. & Weathers, K. C. (2006) Rain forest islands in the Chilean semiarid region: Fog-dependency, ecosystem persistence and tree regeneration. *Ecosystems* 9: 598-608.

Diaz, I. A., Armesto, J. J., Reid, S., Sieving, K. E. & Willson, M. F. (2005) Linking forest structure and composition: avian diversity in successional forests of Chiloé Island, Chile. *Biological Conservation* 123: 91-101.

Diaz, I. A., Armesto, J. J. & Willson, M. F. (2006a) Mating success of the endemic Des Murs' Wiretail (*Sylviorthorhynchus desmursii*, Furnariidae) in fragmented Chilean rainforests. *Austral Ecology* 31: 13-21.

Dinerstein, E. (1995) A conservation assessment of the terrestrial ecoregions of Latin America and the Caribbean. WWF Fund and The World Bank, Washington, DC.

Echeverria, C., Coomes, D., Salas, J., Rey-Benayas, J. M., Lara, A. & Newton, A. (2006) Rapid deforestation and fragmentation of Chilean Temperate Forests. *Biological Conservation* 130: 481-494.

Ehrlich, P. & Raven, P. (1969) Differentiation of populations. *Science* 165: 1228-1232.

El Mousadik, A. & Petit, R. J. (1996) High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. 92:832-839. *Theoretical Applied Genetics* 92: 832-839.

Ellegren, H., Carlson, A. & Stenberg, I. (1999) Genetic structure and variability of white-backed woodpecker (*Dendrocopos leucotos*) populations in northern Europe. *Hereditas* 130: 291-299.

Estades, C. F. & Temple, S. (1999) Deciduous-forest bird communities in a fragmented landscape dominated by exotic pine plantations. *Ecological Applications* 9: 573-585.

Excoffier, L., Laval, L. G. & Schneider S. (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analyses. *Evolutionary Bioinfromatics*: 47-50.

Excoffier, L., Smouse, P. E. & Quattro, J. M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial restriction data. *Genetics* 131: 479-491.

Galbusera, P., Githiru, M., Lens, L. & Matthysen, E. (2004) Genetic equilibrium despite habitat fragmentation in an Afrotropical bird. *Molecular Ecology* 13: 1409-1421.

Galbusera, P., Lens, L., Waiyaki, E., Schenck, T. & Matthysen, E. (2000) Genetic variability and gene flow in the globally, critically-endangered Taita thrush. *Conservation Genetics* 1: 45-55.

Garner, A., Rachlow, J. L. & Waits, L. P. (2005) Genetic diversity and population divergence in fragmented habitats: conservation of Idaho ground squirrels. *Conservation Genetics* 6: 759-774.

Gerlach, G. & Musolf, K. (2000) Fragmentation of landscape as a cause for genetic subdivision in bank voles. *Conservation Biology* 14: 1066-1074.

Goudet, J. FSTAT: a program to estimate and test gene diversities and fixation indices. [2.9.1]. 2000. Institute of Ecology, University of Lausanne, Switzerland.

Goudet, J., Raymond, M., Demeeus, T. & Rousset, F. (1996) Testing differentiation in diploid populations. *Genetics* 144: 1933-1940.

Gustafson, E. J. & Parker, G. R. (1992) Relationships between landcover proportion and indices of landscape spatial pattern. *Landscape Ecology* 7: 101-110.

Gustafson, E. J. & Parker, G. R. (1994) Using an index of habitat patch proximity for landscape design. *Landscape and Urban Planning* 29: 117-130.

Harrison, S. & Bruna, E. (1999) Habitat fragmentation and large-scale conservation: what do we know for sure? *Ecography* 22: 225-232.

Hartl, D. L. & Clark, A. G. (1997) Principles of population genetics. Sinauer Associates, Inc., Sunderland, MA.

Kareiva, P. & Wennergren, U. (1995) Connecting landscape patterns to ecosystem and population processes. *Nature* 373: 299-302.

Mantel, N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research* 27: 209-220.

McDonald, D. B., Potts, W. K., Fitzpatrick, J. W. & Woolfenden, G. E. (1999) Contrasting genetic structure in sister species of North American scrub-jays. *Proceedings of the Royal Society B-Biological Sciences* 266: 1117-1125.

McGarigal, K. & Marks, B. J. (1995) Fragstats: spatial pattern analysis program for quantifying landscape structure. U.S. Forest Service General Technical Report PNW 351.

McGuill, M. & Rowan, A. (1989) Biological effects of blood loss: implications for sampling volumes and techniques. *ILAR News* 31: 5-18.

Milá, B. & Bardeleben, C. (2005) Isolation of polymorphic tetranucleotide microsatellite markers for the wedged-billed woodcreeper *Glyphorynchus spirurus*. *Molecular Ecology Notes* 5: 844-845.

Myers, N., Mittermeier, R., Mittemeier, C., Fonseca, G. & Kent, J. (2000) Biodiversity hotspot for conservation priorities. *Nature* 403: 853-858.

Nauta, M. J. & Weissing, F. J. (1996) Constraints on allele size at microsatellite loci: implications for genetic differentiation. *Genetics* 143: 1021-1032.

Nei M. (1987) Molecular Evolutionary Genetics. Columbia University Press, New York.

Parker, P.G., Snow, A., Schug, M., Booton, G. & Fuerst, P. (1998) What molecules can tell us about populations: choosing and using a molecular marker. *Ecology* 79: 361-382.

Perez, C. & Villagrán, C. (1985) Distribución de la abundancia de especies en bosques relictos de la zona mediterránea de Chile. *Revista Chilena de Historia Natural* 58: 157-170.

Piertney, S. B., Shorey, L. & Höglund, J. (2002) Characterization of microsatellite DNA markers in the white-bearded manakin (*Manakus manacus*). *Molecular Ecology Notes* 2: 504-505.

Ried, S., Cornelius, C., Barbosa, O., Meynard, C., Silva, C. & Marquet, P. A. (2002) Conservation of temperate forest birds in Chile: implications from the study of an isolated forest relict. *Biodiversity and Conservation* 11: 1975-1990.

Rousset, F. (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* 145: 1219-1228.

Saunders, D. A., Hobbs, R. J. & Margules, C. R. (1991) Biological consequences of ecosystem fragmentation: a review. *Conservation Biology* 5: 18-32.

Sieving, K. E., Willson, M. F. & De Santo, T. L. (1996) Habitat barriers to movement of understory birds in fragmented south-temperate rainforest. *Auk* 113: 944-949.

Slatkin, M. (1987) Gene flow and the geographic structure of natural populations. *Science* 236: 787-792.

Slatkin, M. (1995) A measure of population subdivision based on microsatellite allele frequency. *Genetics* 139: 457-162.

Smith-Ramirez, C. (2004) The Chilean coastal range: a vanishing center of biodiversity and endemism in South American temperate rainforests. *Biodiversity and Conservation* 13: 373-393.

Smouse, P. E., Long, J. C. & Sokal, R. R. (1985) Multiple regression and correlation extensions of the mantel test of matrix correspondence. *Systematic Zoology*: 627-632.

Tilman, D., May, R. M., Lehman, C. L. & Nowak, M. A. (1994) Habitat destruction and the extinction debt. *Nature* 371: 65-66.

Trizio, I., Crestanello, B., Galbusera, P., Wauters, L. A., Tosi, G., Matthysen, E. & Hauffe, H. C. (2005) Geographical distance and physical barriers shape the genetic structure of Eurasian red squirrels (*Sciurus vulgaris*) in the Italian Alps. *Molecular Ecology* 14: 469-481.

Troncoso, A. C., Villagrán, C. & Muñoz, M. (1980) Una nueva hipótesis acerca del origen y edad del bosque de Fray Jorge (Coquimbo, Chile). *Boletin del Museo Nacional de Historia Natural (Chile)* 37: 117-152.

Villagrán, C. (1990) Glacial climates and their effect on the history of vegetation of Chile: A synthesis based on palynological evidence from Isla de Chiloé. *Review of Paleobotany and Palynology* 65: 17-24.

Villagrán, C., Armesto, J. J., Hinojosa, F. C., Cuevertino, J., Perez, C. & Medina, C. (2004) El enigmático origen del bosque relicto de Fray Jorge, pp. 3-43. In: *Historia natural del parque* *nacional bosque Fray Jorge* (eds F. Squeo, J. R. Gutierrez & I. R. Hernández). Universidad de la Serena, La Serena.

Waser, P. M. & Strobeck, C. (1998) Genetic signatures of interpopulation dispersal. *Trends in Ecology & Evolution* 13: 43-44.

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

Whitlock, M. C. & McCauley, D. E. (1999) Indirect measures of gene flow and migration: F_{st} not equal to 1 / (4Nm +1). *Heredity* 82: 117-125.

Willson, M. F. (2004) Loss of habitat connectivity hinders pair formation and juvenile dispersal of Chucao Tapaculos in Chilean rainforest. *Condor* 106: 166-171.

Wright, S. (1968) Evolution and genetics of populations Vol. 1 Genetic and biometric foundations. University of Chicago Press, Chicago, IL.

Table 1. Product size range, number of alleles and optimal reaction conditions per microsatellite primer set for *A. spinicauda.*

Locus	PCR product size (bp)	Number of alleles	Annealing temp. (C°)	$MgCl_2$ (mM)
Man 1	143 – 165	6	54	2.5
Man 3	195 – 280	15	52	2
Man 13	123 – 151	14	52	2
WbWc 28	236 – 293	21	53	1
WbWc 58	236 - 271	13	51	0.75

Table 2. Measures of genetic diversity and population statistics for *Aphrastura spinicauda* screened at five microsatellite loci in six study sites in north-central Chile.

Population	**Region**	**Forest**	N_{SP}	**N**	A_N	A_R	H_S	H_O	F_{IS}	**HW (*P*)**
Fray Jorge	North	Relict	1	17	6.8 (1.4)	6.6 (1.4)	0.776	0.776	-0.001	0.547
Santa Inés	North	Relict	1	14	6.6 (1.3)	6.6 (1.3)	0.801	0.700	0.126	0.032
Chaihuín	South	Continuous	1	15	8.4 (1.6)	8.2 (1.6)	0.807	0.773	0.042	0.278
Parga	South	Continuous	1	16	8.2 (1.4)	7.8 (1.4)	0.819	0.800	0.023	0.375
Chiloé- NP	South	Continuous	4	42	10.4 (1.9)	8.2 (1.5)	0.816	0.780	0.084	0.005
Chiloé- AL	South	Fragments	9	131	12.8 (2.2)	8.3 (1.4)	0.834	0.778	0.068	**< 0.002**

The number of subpopulations sampled (N_{SP}) and individuals genotyped for all five microsatellite loci are indicated (N). Mean with SE in brackets is indicated for number of alleles (A_N) and allelic richness (A_R). Gene diversity (H_S) and values for observed heterozygosity (H_O) are reported as mean values over all loci. Multilocus estimates of F_{IS} are reported with corresponding Hardy-Weinberg equilibrium *P* values for deficiency of heterozygotes (bold denotes significance after corrections for multiple comparisons based on 600 randomizations).

Table 3. Analysis of molecular variance (AMOVA) results to compare the genetic variation in microsatellite data from *A. spinicauda* using three models (a,b, and c). Populations were assigned to different groups according to their fragmentation status at two different spatial scales.

Structure **Source of variation**	**d.f.**	**% of total variation**	**Fixation indices**	***P* value**
Large scale				
a) Relicts - Continuous – Fragments				
Among regions	2	1.10	0.011 (F_{CT})	< 0.02
Among populations within regions	14	2.67	0.027 (F_{SC})	< 0.001
Within populations	453	96.23	0.038 (F_{ST})	< 0.001
Total	469			
Meso scale				
b) Continuous – Continuous				
Among regions	1	0.00	0.000 (F_{CT})	NS
Among populations within regions	4	1.32	0.013 (F_{SC})	NS
Within population	140	98.68	0.013 (F_{ST})	< 0.03
Total	145			
c) Continuous – Fragments				
Among regions	1	-0.35	-0.003 (F_{CT})	NS
Among populations within regions	9	2.13	0.021 (F_{SC})	< 0.001
Within populations	313	98.22	0.018 (F_{ST})	< 0.001
Total	323			

Table 4. Pairwise F_{ST} (lower diagonal) and pairwise geographic distance in km (upper diagonal) among the six sample sites (R= relict, C = continuous, FR = fragments).

Population		R1	R2	C1	C2	C3	FR
Fray Jorge	(R1)		167.5	1049.0	1212.9	1332.3	1257.4
Santa Inés	(R2)	0.111*		888.3	1051.9	1171.2	1095.2
Chaihuín	(C1)	0.077*	0.068*		164.0	283.3	209.8
Parga	(C2)	0.080*	0.025*	0.016		119.8	49.1
Chiloé-NP	(C3)	0.090*	0.056*	0.003	0.012		81.1
Chiloé-AL	(FR)	0.073*	0.044*	0.008	0.004	0.005	

Asterisks indicate significant pairwise F_{ST} with P values obtained after 300 permutations ($P <$ 0.003 adjusted nominal level for multiple comparisons). Grey shading indicates comparison between the two relict forests and the two sites in continuous forest.

Table 5. Pairwise F_{ST} (lower diagonal) and pairwise geographic distance in km (upper diagonal) between the four sites in the continuous forest in Chiloé National Park.

	NP-1	**NP-2**	**NP-3**	**NP-4**
NP-1		3.1	13.0	14.3
NP-2	0.0055		11.2	12.3
NP-3	0.0341	-0.0002		1.9
NP-4	0.0007	0.0064	-0.0041	

All F_{ST} values were not significant ($P > 0.05$ after 120 permutations).

Table 6. Pairwise F_{ST} (lower diagonal) and pairwise geographic distance in km (upper diagonal) between the nine forest fragments in an agricultural landscape in Chiloé (L = large, M = medium, S = small).

Fragment	L1	L2	L3	M1	M2	M3	S1	S2	S3
L1		12.1	22.8	21.1	24.7	18.5	18.9	21.9	13.6
L2	0.037**		10.7	10.4	13.2	8.2	8.0	10.1	7.0
L3	0.006	0.007		7.4	3.9	7.8	6.8	4.2	12.6
M1	0.006	0.027*	0.006		11.3	2.5	2.0	10.9	8.0
M2	0.034*	0.012	0.004	0.028**		11.7	10.7	3.4	16.4
M3	0.018	0.018	0.000	-0.007	0.000		1.1	10.4	5.7
S1	0.036**	0.050**	0.025	0.016	0.030**	-0.006		9.6	6.5
S2	0.004	0.026	-0.003	0.004	0.017	0.000	0.017		14.3
S3	0.060***	0.041*	0.031**	0.059***	0.045***	0.050**	0.080***	0.042**	

Asterisks indicate significant pairwise F_{ST} estimates with P values obtained after 720 permutations (*** $P < 0.003$ adjusted nominal level for multiple comparisons, ** $P < 0.01$, * $P < 0.05$).

APPENDIX A

Observed (H_{obs}) and expected (H_{exp}) heterozygosity per locus within each population and Hardy-Weinberg (H-W) equilibrium departure *P* value (exact test using a Markov chain), bold values indicate significant departure from H-W equilibrium. N = number of individuals genotyped, A_N = number of alleles.

Population	Locus	N	A_N	H_{obs}	H_{exp}	H-W *P* value
Fray Jorge						
	Man 1	17	6	0.824	0.761	0.966
	Man 3	17	10	0.765	0.923	0.090
	Man 13	17	5	0.647	0.613	1.000
	wbwc-28	17	7	0.882	0.811	0.839
	wbwc-58	17	6	0.765	0.815	0.949
Santa Inés						
	Man 1	14	4	0.500	0.772	0.354
	Man 3	14	8	0.929	0.873	0.696
	Man 13	14	6	0.786	0.720	0.753
	wbwc-28	14	7	0.714	0.839	0.136
	wbwc-58	14	8	0.571	0.841	**0.013**
Chaihuín						
	Man 1	15	5	0.667	0.763	0.487
	Man 3	15	7	0.600	0.828	**0.000**
	Man 13	15	8	0.800	0.699	0.800
	wbwc-28	15	10	0.867	0.883	0.875
	wbwc-58	15	12	0.933	0.871	0.902
Parga						
	Man 1	16	5	0.625	0.843	**0.031**
	Man 3	16	10	0.813	0.865	0.064
	Man 13	16	8	0.688	0.770	0.665
	wbwc-28	16	8	0.875	0.788	0.848
	wbwc-58	16	10	1.000	0.855	0.662
Chiloé-NP						
	Man 1	42	5	0.619	0.801	0.141
	Man 3	42	9	0.857	0.835	0.707
	Man 13	42	11	0.690	0.791	0.057
	wbwc-28	42	15	0.810	0.885	**0.009**
	wbwc-58	42	12	0.762	0.773	0.314
Chiloé-AL						
	Man 1	131	6	0.695	0.794	0.218
	Man 3	131	12	0.763	0.855	0.148
	Man 13	131	14	0.817	0.813	0.576
	wbwc-28	131	19	0.791	0.893	**0.014**
	wbwc-58	131	13	0.824	0.824	0.064

APPENDIX B

P-values for genotypic disequilibrium between all pairs of loci within each population and over all populations. *P*-values were based on 1200 permutations and were not significant after correction for multiple comparisons with an adjusted 5% nominal level of 0.00083.

Pair of loci	**Fray Jorge**	**Santa Inés**	**Parga**	**Chaihuín**	**Chiloé-AL**	**Chiloé-NP**	**All**
Man 1 X Man 3	1.000	1.000	1.000	0.636	0.208	0.188	0.209
Man 1 X Man 13	0.960	0.537	1.000	0.623	0.193	0.832	0.395
Man 1 X wbwc 28	1.000	1.000	0.483	1.000	0.674	1.000	0.834
Man 1 X wbwc 58	1.000	1.000	1.000	1.000	0.606	0.984	0.88
Man 3 X Man 13	1.000	0.305	1.000	0.255	0.385	0.698	0.337
Man 3 X wbwc 28	1.000	0.125	1.000	1.000	0.038	0.688	0.042
Man 3 X wbwc 58	1.000	0.195	1.000	1.000	0.032	0.949	0.100
Man 13 X wbwc 28	0.909	0.385	0.369	1.000	0.104	0.042	0.025
Man 13 X wbwc 58	0.674	0.573	1.000	1.000	0.802	0.882	0.855
wbwc 28 X wbwc 58	0.220	0.285	0.353	1.000	0.467	0.823	0.263

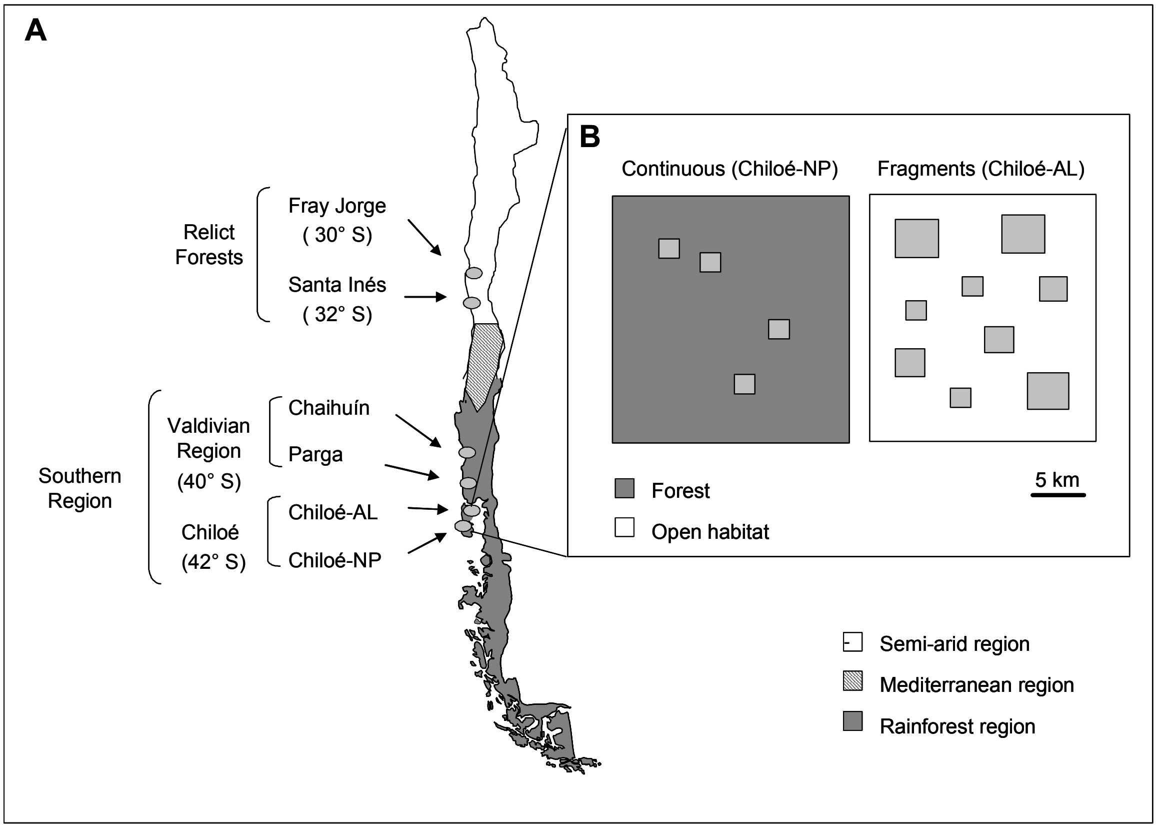

Figure 1. (A) Six sample sites across central-southern Chile located in the northern semiarid region and southern temperate forest region (B) Populations in Chiloé were subdivided into four sites in the continuous forest (Chiloe-NP) and into nine forest fragments in the agricultural landscape (Chiloé-AL).

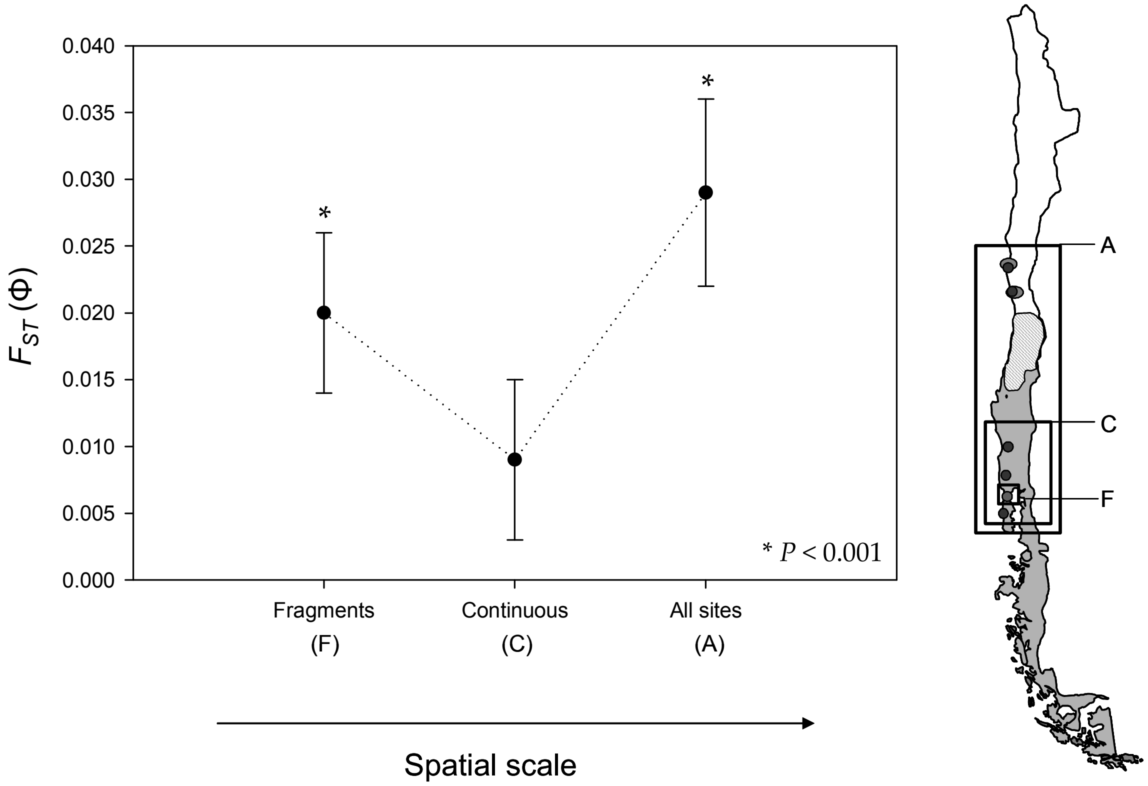

Figure 2. Global population differentiation estimates (Weir & Cockerham's F_{ST} (Φ) ± SE), measured among populations at three different spatial scales: small scale comparison among 9 human-caused fragments in an agricultural landscape (F), meso-scale comparison among 3 populations located in continuous forests (C), and large scale comparison among 6 populations including relict, continuous and fragmented forest populations (A). Asterisks indicated $P < 0.001$.

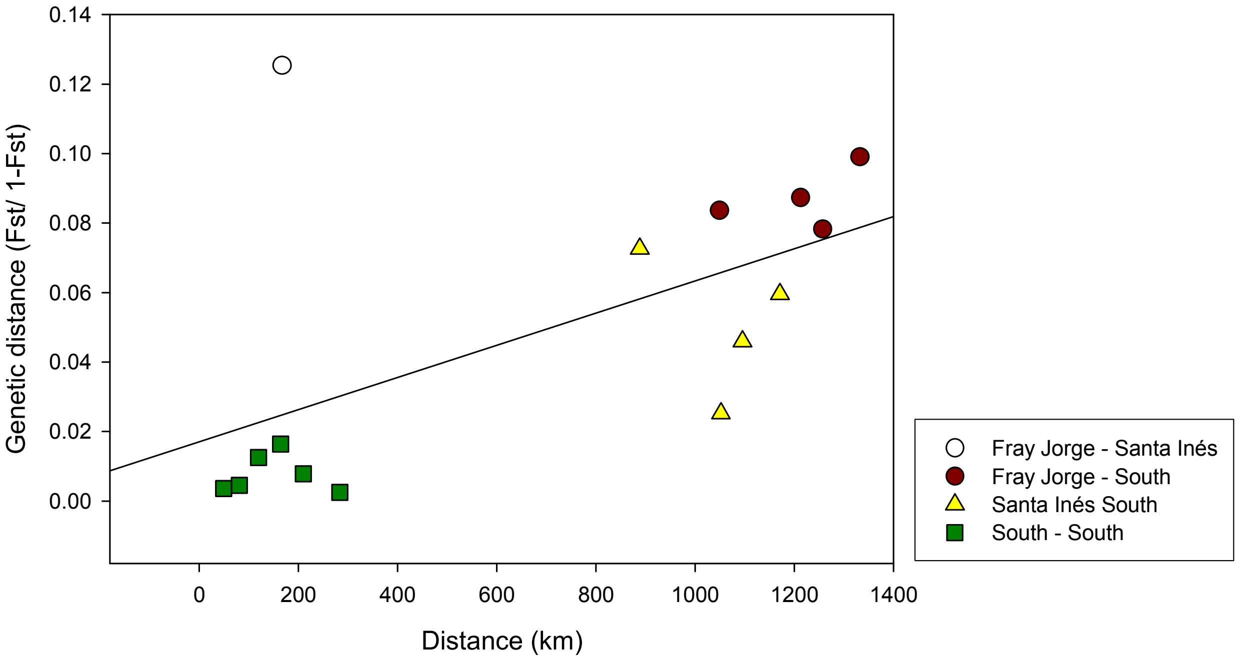

Figure 3. Relationship between pairwise geographic distance and genetic distance among the six populations studied at the large spatial scale (Mantel test $R^2 = 0.34$, $P = 0.023$).

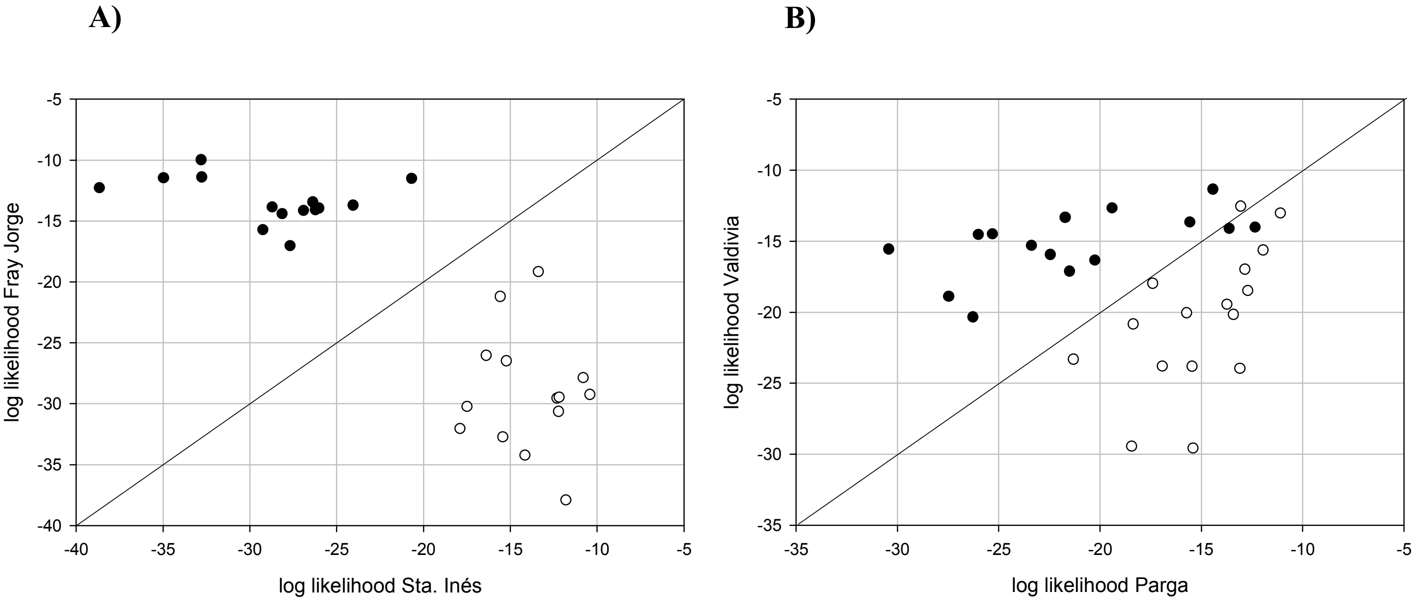

Figure 4. (A) Assignment test between individuals sampled in Fray Jorge and Santa Inés, two relict forests separated by 165 km of semiarid scrub and (B) assignment test between individuals sampled in Chaihuín and Parga located in a continuous forest in the southern region separated by 165 km of forest.

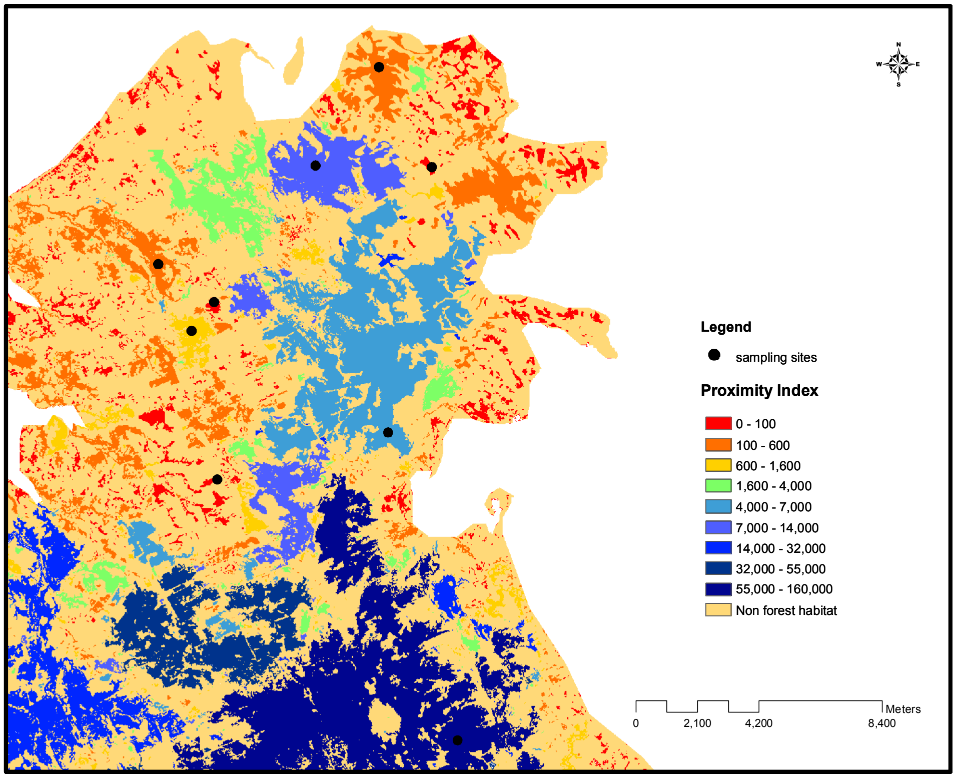

Figure 5. Agricultural landscape in north-eastern Chiloé in which *A. spinicauda* was sampled in nine forest fragments. Fragments are classified according to their isolation using a modified version of Gustafson and Parker (1992) proximity index (PX) calculated in FRAGSTAT with a 1 km buffer from each focal patch. Low values of PX indicate high levels of isolation (i.e. fewer and smaller neighboring fragments) whereas larger values indicate less isolation (more and larger neighboring fragments).

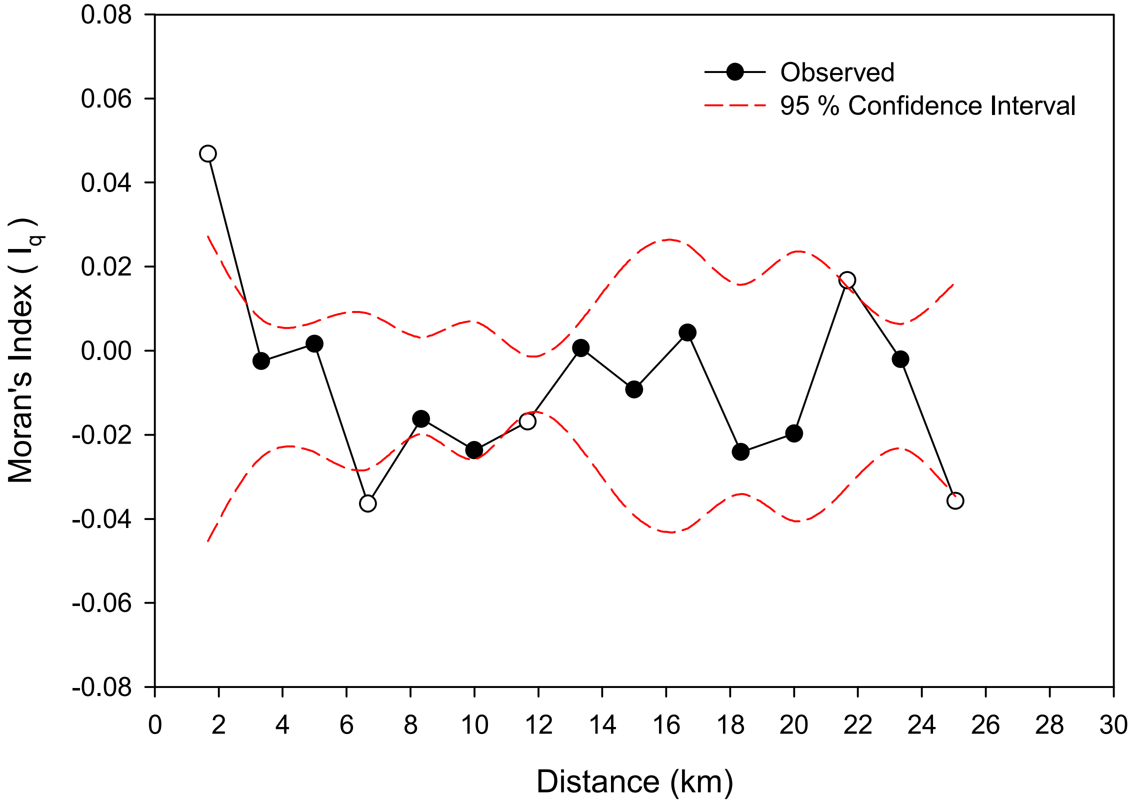

Figure 6. Moran's I (Iq) correlogram indicating the pattern of spatial autocorrelation of individual genotypes. Open circles indicate values of relatedness that are higher or lower than expected by chance ($P < 0.05$).

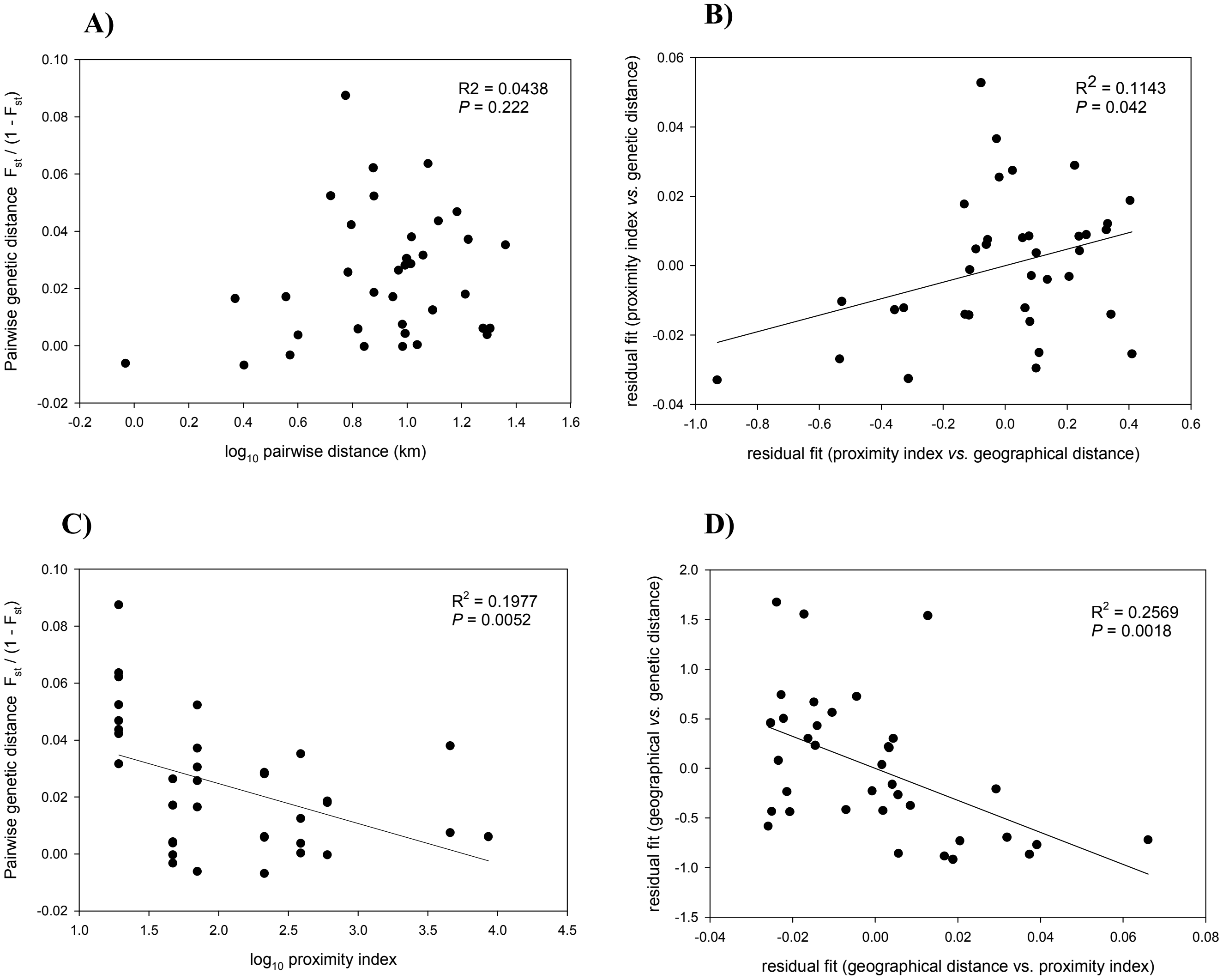

Figure 7. (A) Relationship between geographic and genetic distance among subpopulations in the nine forest fragments in the agricultural landscape in Chiloé and (B) controlling for patch proximity as measured by the index PX. (C) Relationship between patch proximity (a measure of fragment isolation) and genetic distance and (D) controlling for geographic distance among fragments. Significance was obtained after 10,000 permutations using Mantel and partial-Mantel tests.

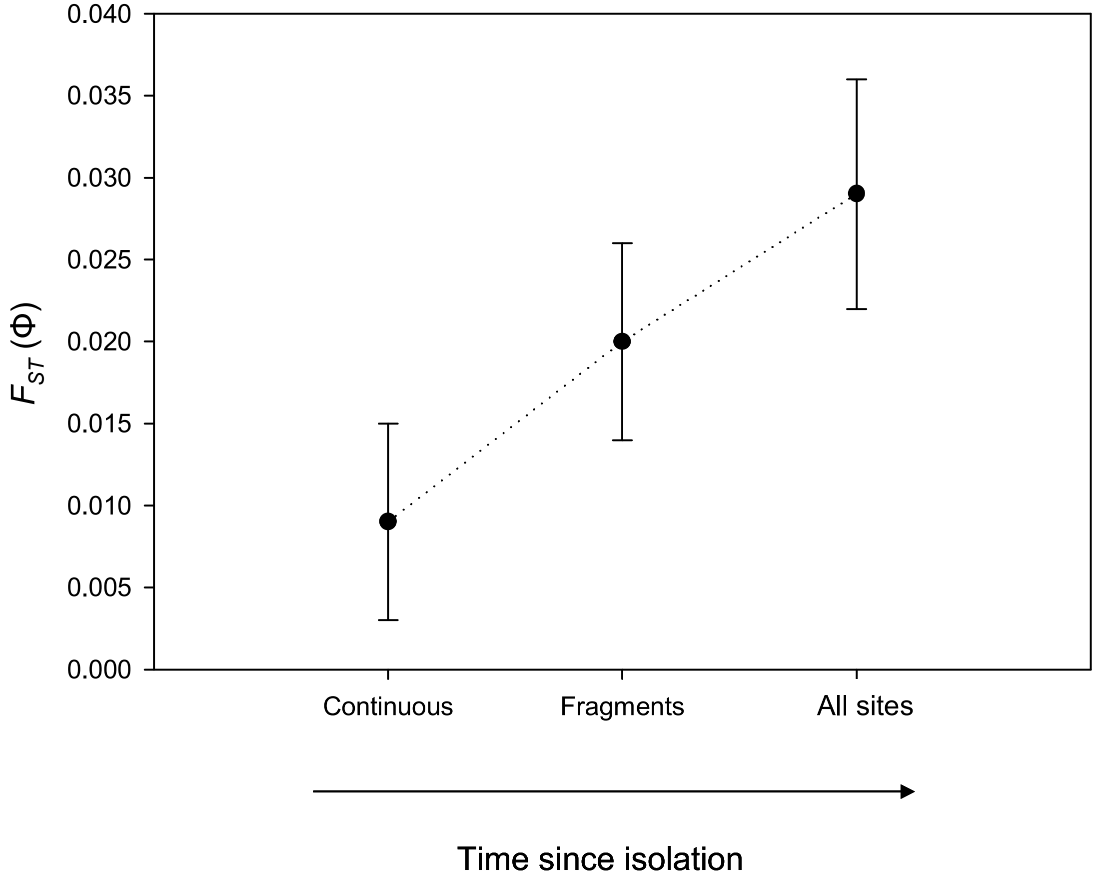

Figure 8. Global population differentiation estimates based in Weir & Cockerham's F_{ST} (Φ) measured among populations but re-ordered along a time-since-isolation scale.

CHAPTER II

Separating ecological effects of forest structure and habitat fragmentation on breeding birds: an experimental study with a secondary cavity-nesting bird

Abstract -. Human-driven landscape modifications usually entail habitat degradation and habitat fragmentation. In many cases, however, these two processes occur simultaneously making it difficult to disentangle their effects. The goal of this study is to separate the ecological effects of changes in forest structure caused by selective logging from those triggered by habitat fragmentation on breeding birds. I studied populations of a secondary cavity-nester (*Aphrastura spinicauda*, Furnariidae) in the South American temperate rainforest during three breeding seasons (2003-2005). In 2003, I evaluated nest-site availability, density and nest success of *A. spinicauda* in three habitat treatments each replicated in two 10-ha plots. The habitat treatments were old-growth forest (OGF), selectively-logged forest (SLF; large enough to test for habitat degradation *per se*), and selectively-logged forest fragments (LFR; small and isolated fragments to test for additional effects of fragmentation). To test for nest-site limitation, a nest-site supplementation experiment was carried out in SLF and LFR plots. Nest boxes were placed after the 2003 breeding season and populations were followed during two subsequent years. This experiment allowed me to assess the roles of selective logging and habitat fragmentation in determining nest-site availability. Overall, there were more potential natural-nest sites in OGF than in SLF and LFR. In 2003, before nest box addition, density of *A. spinicauda* in OGF was higher than in SLF, but did not differ from density in LFR. In 2004, density in OGF remained unchanged, but density increased in SLF after nest boxes were added, suggesting nest-site limitation in selectively-logged forests. Density in LFR did not increase after nest box addition as expected, but nest box use was higher than in SLF suggesting nest-site limitation in fragments as well. Patterns were similar in 2005 except for an overall decrease in density attributed to high mortality during the previous winter. Daily nest survival was not different among the three habitat treatments, but nests in snags had higher nest success than nests in live trees, regardless of forest type. Results suggest that nest site availability, which is affected by changes in forest structure, is an important factor limiting densities of *A. spinicauda*, but that landscape-level processes triggered by habitat fragmentation can also influence population responses to nest-site limitation. Individual birds in fragments may face a nest-site selection trade-off with respect to the cost of dispersing across open habitats or staying in fragments with reduced availability of cavities.

Key words: Aphrastura spinicauda, cavity-nester, Chile, fragmentation, habitat degradation, nest-site limitation, south-temperate rainforest.

Introduction

Human-driven landscape modifications, such as selective logging and habitat fragmentation, may influence the distribution, abundance and fitness of species, especially if those species rely on resources that are strongly affected by habitat alteration. Secondary cavity-nesting birds are non-excavators that typically nest in old trees or snags, where most cavities are formed (Newton 1994). If cavities are limited, competition can force some individuals to use lower quality cavities which may lead to population limitation (Li & Martin 1991; Martin & Pingjung 1992; Newton 1994; Holt & Martin 1997; Martin et al. 2004; but see Wiebe et al. 2006). Selective logging often leads to removal of large trees and, as a consequence, can reduce the availability of cavities and thereby decrease habitat quality for secondary-cavity nesters.

Landscape modifications that also involve habitat fragmentation can trigger processes that have additional detrimental consequences on populations. In general, habitat fragmentation involves two types of processes that influence populations, those associated with size and isolation of patches (e.g. metapopulation dynamics) and those associated with reduction in habitat quality. In most human-modified landscapes, however, these processes are not independent (Armstrong 2005). In some cases, habitat quality is correlated with fragment size, because habitat degradation is associated with edge effects that have a greater influence in small patches (e.g. increased predation rates or physical microclimatic changes on edges; Murcia 1995). In other cases, habitat deterioration may be intensified by effects of human encroachment (e.g. fire, selective logging and cattle) that generally extend well beyond the usual "edge effects" (Laurance 2000). In this case, the overall habitat quality within fragments may be reduced more than expected simply on the basis of area, invalidating the use of fragment size as a surrogate for habitat quality. Therefore, the explicit and independent consideration of habitat fragmentation and reduction of habitat quality is essential for a complete understanding of population responses to habitat loss (Harrison & Bruna 1999) because even large forests or well-connected fragments cannot ensure the persistence of populations if habitat is not adequate for successful breeding.

In fragmented landscapes, reproductive success of secondary cavity-nesting birds should, therefore, not only depend on the presence of key structures, such as old trees and snags, but also on landscape-level processes such as those triggered by habitat fragmentation. These processes can be manifested as population changes of nest predators or changes in microclimatic conditions that can modify the quality of available nesting cavities. In temperate forests of the northern hemisphere, increased nest predation has been identified as one of the main negative effects of habitat fragmentation on bird populations (Robinson et al. 1995; Lampila et al. 2005).

Similarly, in the temperate rainforest of South America, nest predation is also an important factor both for open-cup and cavity-nesting birds in fragmented landscapes (Willson et al. 2001; De Santo et al. 2002; Vergara & Simonetti 2003).

Other landscape-level processes, such as those related to dispersal capabilities and connectivity of the landscape, are important because they determine whether individual birds will be able to disperse if cavities are limited. Most studies of secondary cavity-nesting birds have been carried out in undisturbed forests (Aitken et al 2002; Brightsmith 2005) or in managed forests (Brawn & Balda 1988; Newton 1994; Holt & Martin 1997) where nest sites can be limited. No studies have attempted, however, to experimentally separate the effects of reduction in nest-site availability (e.g., through selective logging) from the effects due to reduction in landscape connectivity (e.g., through habitat fragmentation). Yet, consequences of nest-site limitation, at the population and individual level, may depend on the landscape context in which nest-site limitation occurs. The goal of this study is to separate the effects of reduction in habitat quality due to selective logging from those triggered by habitat fragmentation on populations of a secondary cavity-nesting bird. For this I established a nest-site supplementation experiment in study plots located in forests that differ in quality and degree of isolation.

Study layout and predictions

Secondary cavity-nesting birds are a good model system because an important component of fitness (i.e. reproduction) relies on the presence of easy-to-quantify structures, such as large trees and snags where cavities are formed. Habitat quality is often defined in relation to population density and resource availability, but population density should not be used as the only indicator of habitat quality (Van Horne 1983; Brawn & Robinson 1996). Habitat selection models (Fretwell & Lucas 1970; Rosenzweig 1991) predict that habitats of higher quality, which maximize fitness, will be preferred over poor-quality sites, resulting in an “ideal-free distribution” (Fretwell & Lucas 1970), hereafter IDF. Under this scenario, better-quality habitats (e.g. old-growth forest) are occupied first, until their carrying capacity is reached or until the quality has been reduced to the point where it equals that of unoccupied lower-quality habitats (e.g. selectively-logged forest). At that point, use of “suboptimal” habitats might be more beneficial than staying in a “good” habitat in which high densities have decreased suitability. Thus, reproductive success should be similar in lower-density, poor-quality habitats when compared to high-density, good-quality habitats (Fretwell & Lucas 1970).

Based on the “ideal-free distribution” model this study aims to test two predictions. First, I expect that density of secondary cavity-nesting birds should follow an IFD, in which density should decrease along a gradient of habitat quality (old-growth forest > logged forest > logged fragments) as measured by reduction in availability and quality of cavities. Secondly, I do not expect reproductive success to remain constant along the habitat-quality gradient as the IFD model would predict. Such a distribution assumes that individuals are “free” to enter and leave any habitat on an equal basis, which may not be the case if the connectivity of the landscape is reduced. As populations increase in density, density-dependent processes may become important, resulting in decreased reproduction, decreased survival or increased emigration (Turchin 1999). Thus, under a scenario of reduced connectivity, I predict that individuals are forced to stay in habitat fragments and use cavities of lower quality causing a decline in the average reproductive success in fragments.

To test these two predictions, I compared density and nesting success of a secondary cavity-nesting bird among three habitat treatments. The first two habitat treatments (large and connected old-growth forests and large and connected selectively-logged forests), allowed me to determine the consequences of habitat degradation independent of habitat fragmentation. The third habitat treatment (selectively-logged forest fragments) allowed me to contrast the effects of habitat degradation with the added effect of habitat fragmentation. Then, to measure whether cavities were a limiting resource in selectively-logged forests, I conducted a nest-site supplementation experiment. Finally, I determined if the nest-site limitation process was modified because of habitat fragmentation.

Methods

Study system

The Thorn-tailed Rayadito (*Aphrastura spinicauda*, Furnariidae) is an insectivorous, year-round resident and endemic bird of the temperate rainforest of southern South America. This species is a secondary-cavity nester that nests mostly in natural cavities in large trees and snags (Chapter 3), it is an ideal species for this study because it occurs in different forest types at varying densities (Estades & Temple 1999; Diaz et al. 2005) and has been identified as a large-tree user (Willson et al. 1994; Diaz et al. 2005; Tomasevic & Estades 2006). Non-raptor cavity-nesting birds in the south-temperate forest include four species considered good to weak excavators and eight non-excavator species, four of which are strictly understory birds (Rhinocryptidae). *Aphrastura spinicauda* is found in the canopy and sub-canopy, and may interact or eventually compete for suitable cavities with three other non-excavator birds (two parrot and one swallow species) and a few excavator species such as another furnarid (*P. albogularis*) and three species of woodpeckers. Although no studies have specifically investigated interactions among cavity nesters in this system with low species richness, it is very likely that interactions are less complex than in other more diverse temperate (Martin et al. 2004) and tropical forests (Brightsmith 2005).

This study was conducted in the temperate rainforest region on Isla Grande de Chiloé, southern Chile (41°55'S, 73°35'W), during each spring and early summer (September-January) of 2003 through 2005. The forest is a broad-leafed evergreen rainforest of the Valdivian and North Patagonian types with mixed dominance of *Nothofagus*, *Drymis*, *Eucryphia*, and *Podocarpus* trees, several trees and shrubs from the family Myrtaceae, and a dense bamboo (*Chusquea* spp.) understory. Large and old trees usually are covered with vines and epiphytes such as ferns, mosses, and bromeliads (Muñoz et al. 2003). The climate is wet temperate with a strong oceanic influence (2,000-2,500 mm rainfall/year; mean annual temperature of 12°C; Di Castri & Hajek 1976). The study area is in an approximately 25 x 25-km agricultural landscape in the vicinity of Senda Darwin Biological Station, northern Chiloé (Fig. 1a). The landscape is characterized by flat lands and hills (50 to 100 m elevation) that are covered by woodlands and forest fragments dispersed in a matrix of pastures, cultivated fields, and scrublands. Major forms of human-caused habitat degradation have been widespread use of fire to clear land for pastures, forest encroachment by cattle, selective logging of valuable timber trees, and a significant amount of logging for domestic fire use. Logging is carried out usually by local landowners with no defined silvicultural system. These activities, which have mostly occurred over the past 100 years, have markedly modified the landscape by increasing forest fragmentation and habitat degradation (Willson & Armesto 1996, Castellón & Sieving 2006b). The island was covered by forest in the late 1800s but current forest cover in the study area is about 35% (Castellón & Sieving 2006a). Human practices have, as a result, generated a landscape mosaic with forests that exhibit a broad range of successional stages and degrees of degradation, from recently disturbed to a few protected old-growth forests (Aravena et al. 2002). Forest remnants differ in their structure and presence of habitat features that are important for birds (Willson et al. 1994; Reid et al. 2004; Diaz et al. 2005).

Experimental design

I established three habitat treatments, old-growth forest (OGF), selectively-logged forest (SLF), and selectively-logged forest fragment (LFR), to evaluate the effects of habitat degradation *per se* and the added effects of habitat fragmentation. Each treatment was replicated in two distinct 10-ha study plots, for a total of six study plots (Fig. 1b). In each plot, I established an array of three to eight transects, with a total transect length per plot of 1,600 to 1,800 m. Transects were systematically placed every 50 m with a random starting point. Bird, nest, and vegetation surveys (see below) were conducted along these transects. To test for effects of forest structure *per se*, OGF and SLF plots were located in sufficiently large (> 1000 ha) and not isolated forests (see Chapter 1) where processes associated with habitat fragmentation were assumed to be unimportant. In contrast, LFR plots were established in isolated forest fragments (see Chapter 1) of about the same size as the study plots (10-12 ha), to test for the added effects of habitat fragmentation. My intent was not to identify effects of different processes associated with habitat fragmentation (i.e. related to size, size-edge ratio, and isolation). Instead, the goal was to test for the combined effects of these processes in two fragments of similar size, degree of isolation (both are surrounded by open pastures and similarly isolated, Chapter 1), and size-edge ratios. Moreover, because I controlled for effects of sample area by studying plots of the same size but in different habitat contexts, I was able to quantify the effects of forest structure and fragmentation apart from any confounding effects associated with sample area.

Forest structure and nest site availability

The diameter at breast height (dbh) of all live trees and snags (> 40 cm dbh and >15 cm dbh, respectively) were counted and measured within 10-m radius (314 m^2) circular plots located every 100 m along transects within each study plot (18 vegetation plots/10 ha). Cut-off diameters for live trees was an arbitrary value below the lower boundary of the confidence interval for nest trees found in 2003 (n = 12, mean = 77.1 cm, 95% CI of 55.9 - 98.2 cm). Cut-off diameter for snags was as low as the minimum value recorded because snags are likely to have cavities independent of their size (n = 17, mean = 68.8 cm, 95% CI of 45.3 - 92.2 cm). To compare the number of large trees and snags among study plots the mean of three circular plots along a 300 m transect was calculated and a nested ANOVA was conducted, with study plots nested within each forest type and with the mean number of trees or snags per transect as the dependent variable. Numbers of trees and snags / transect were transformed using a Box-Cox transformation to attain conditions of normality.

The number of large trees and snags was used as a surrogate for nest-site availability. In general, *A. spinicauda* nests more than 15 m from the ground in small, hidden cavities or crevices in trunks or in hollow, broken branches (Chapter 3). Because many cavities were concealed by epiphytes, it was impossible to quantify the actual availability of cavities in this forest. However, forest structure, in terms of number of large, old trees (i.e. that usually form cavities) and number of snags, can serve as a good surrogate for availability of potential nest sites for secondary-cavity nesters (Newton 1994). This is especially true if birds rely on natural cavities rather than cavities made by other birds, which is the case for *A. spinicauda* (Chapter 3).

Density estimates and habitat specific detection functions

I estimated density of *A. spinicauda* in each plot using line-transect surveys and the distance sampling method (Buckland et al. 2001). Birds were counted after pairs established their territories but before young fledged (October – November) in three consecutive breeding seasons (2003-2005). Each plot was surveyed weekly four or five times per season on non-rainy days between dawn and 10:00 hr. The focal species was recorded while walking along transects and perpendicular distances to each detection (aural or visual) were estimated with the aid of previously measured marks placed along transects. Surveys were performed by three observers who had been trained to estimate distances in this habitat. Two adjacent transects were never sampled simultaneously when more than one observer surveyed a plot to avoid movement of birds between transects because of observers (Buckland et al. 2001).

The mean and variance of *A. spinicauda* density were estimated using DISTANCE 5.0 (Thomas et al. 2005). I modeled detectability functions for each habitat type (i.e. OGF, SLF and LFR) to account for the effect of differences in forest structure on estimates of density. Habitat specific detection functions were based on data pooled over the three years of study to increase robustness of the model. Because habitat structure did not change over the study period, there was no reason to expect detection functions to change from year to year. To increase model fit and to minimize effects of errors in distance estimations in the field, distance data were truncated at 30 m and placed into five categories for OGF and into six categories for SLF and LFR (Buckland et al. 2001). I tested half-normal and hazard-rate key functions with different combinations of cosine, simple-polynomial and hermite-polynomial series expansions. Competing models of habitat-specific detection functions were compared based on Akaike's Information Criteria (AIC, Akaike 1973). The model that best fit grouped data was selected according to the lowest AIC*c* (second-order AIC for small samples) among candidate models; χ^2 goodness-of-fit tests were examined among best candidate models.

The detection function for each habitat type (Table 1) was a joint detection function based on detections from the two forest plots within each habitat category. This method was selected in opposition to a specific detection function for each plot, based on minimum AIC*c*. In all three cases, the sum of AIC*c* values across individual study plots within a habitat category was very similar to the AIC*c* value from the pooled analysis (ΔAIC_C ranged from 0.78 – 2.28), which suggests that detection functions were similar between the two replicate study plots and, thus, that a global-detection function for each habitat type was warranted (Buckland et al. 2001).

Density estimates of *A. spinicauda* from plots within each habitat type (i.e. OGF, SLF and LFR) were compared with two-tailed *t*-tests (or *Z*-tests if df >30). I used a correction for lack-of-independence for *t*- and *Z*-tests because densities were estimated based on the same detection function (Buckland et al. 2001). Densities did not differ between the two replicate plots for any habitat type in any year of the study. Therefore, I report mean density (± SE) based on data pooled across the two study plot replicates for each year and for each habitat type using the corresponding detection function for each habitat type.

Densities estimated with the same detection function, however, are not truly independent estimates and, thus, violate the assumption of independence for statistical analyses (Buckland et al. 2001). Therefore, when comparing densities among forest types using plots as replicates with ANOVA's (see below), I used density estimates for each study plot based on plot-specific detection functions. Density estimates from plot-specific detection functions were qualitatively and quantitatively similar to density estimates from habitat-specific detection functions.

Nest site supplementation experiment

I supplemented natural-cavity availability with nest boxes to test for nest-site limitation in logged forests. Nest boxes were added in the two SLF and the two LFR plots during January and February 2004, after surveys and nest monitoring were finished for the 2003 breeding season. This allowed me to have before-and-after manipulation data on the same study plots. OGF plots did not receive nest boxes and served as controls to account for natural changes in density between years (i.e. as a result of environmental variation). Moreover, it was assumed that nest-sites were not limited in old-growth forest (Wiebe et al. 2006; Newton 1998) and that other factors, such as actual carrying capacity of the system or territoriality of birds, rather than nest site availability, limit maximum density.

A total of 432 nest boxes were placed across the four study plots, with clusters of three nest boxes every 50 m along transects in each 10-ha plot (i.e. 36 clusters of 3 nest boxes, or 108 boxes per study plot). Nest boxes were made of wood following Tomasevic & Estades (2006) and were attached to trees 3 to 4 m from the ground. This was sufficiently high for *A. spinicauda* (Tomasevic & Estades 2006) and sufficient to ensure that other small understory cavity-nesting species would not use nest boxes. The total number of nest boxes added per study plot was based on the maximum density of *A. spinicauda* observed in old-growth forest in 2003 (3.9 ± 0.28 ind./ha), corresponding to approximately 20 breeding pairs in 10 ha. The goal was to supply enough potential nest sites so that density could increase to a point where other factors, such as territoriality and / or carrying capacity, rather than nest sites, would limit density. Clusters of three boxes located 15 m from each other were used to provide birds with multiple options when choosing nest-sites within a potential territory (Tomasevic & Estades 2006).

Response to the supplementation experiment was measured by change in density from before (2003) to after (2004 and 2005) nest-box addition and by comparison of number of nest-boxes occupied between habitat types. Densities were compared with one-way ANOVA's with habitat type (OGF, LFO, LFR) as a fixed factor for each year separately. Density estimates were normally distributed ($W = 0.974$, $P = 0.92$). The number of occupied nest boxes was compared between habitat types using χ^2. I also used a repeated-measure ANOVA with years as within-subject factor and habitat type as between-subject factor to compare density of *A. spinicauda* over the three-year period among the three forest types. Sphericity assumption was met for the rmANOVA (Mauchly's test $P = 0.061$).

Nest success and reproductive output

Systematically searches for nests in natural cavities were conducted along transects in each plot, following standard protocols (Martin & Geupel 1993), during the three breeding seasons. Mist-net captures were also conducted within plots targeting *A. spinicauda* by using play-backs to attract birds to nets. Adult birds were marked with uniquely numbered aluminum bands and a unique color combination to aid nest-searching, territory determination and nest monitoring. Between 50 and 80 % of individuals were color banded in each study plot. Behavioral cues were used, such as birds carrying nesting material or food, to help find nests; searches were not restricted to specific nest substrates to minimize potential for bias of where nests were found (Rodewald 2004). Once a nest was found, the tree was marked and then visited every three to four days to determine nest status. Nests were observed from the ground with binoculars for 20-30 min and the number of times birds entered the cavity was combined with observations of adults carrying material, food or fecal sacs to determine the stage of each nest (i.e. constructing, incubating, or feeding nestlings; Martin & Geupel 1993). A nest was classified as successful either if no activity was observed at the nest after 21 days of feeding, the typical length of the nestling stage for this species (Moreno et al. 2005), or if a family group was found that could be attributed to that particular nest (i.e. based on color bands of adults). If the nest was found prior to the feeding stage and if no activity was observed during two consecutive observation periods before 21 days of feeding had elapsed, the nest was considered to have failed. Nests found during the feeding stage and to which no family group could be assigned, were classified as undetermined unless evidence of failure was observed (e.g. destroyed cavity). Contents of nests in natural cavities could not be determined because cavities were inaccessible to observers; causes of failure were not, therefore, always possible to determine.

The mean and variance of daily nest-survival (DNS) rate were estimated with a maximum-likelihood approach based on the Mayfield method (Mayfield 1975) and implemented with Program MARK (White 2000) using the nest-survival procedure (Dinsmore et al. 2002). I used AIC*c* to evaluate different hypotheses about the source of variation in nest survival for *A. spinicauda* in our study area. I used biologically-meaningful pre-defined hypotheses to develop eight specific models to explain variation in nest success (Table 2). I examined the effects of forest type (OGF, SLF, LFR), year (2003, 2004, 2005), and nest-tree type (live tree, snag). Factors in models were incorporated as covariables and competing models were compared against the null-hypothesis model $S_{(.)}$ of constant DNS. I used a sine-link function for the constant DNS model and a logit-link function for models that incorporated covariates (Dinsmore et al. 2002). This method, however, does not allow incorporation of nests with uncertain fate into the model, which has been shown to bias DNS estimates (Manolis et al. 2000). In my data set, 12.7 % of nests (13 out of 102) had uncertain fate. Therefore, I also conducted the traditional DNS Mayfield calculation including nests with uncertain fate (Manolis et al. 2000) to determine if our DNS estimates from program MARK were upward-biased. Nesting success was assessed as the probability of surviving the entire nesting cycle, from the egg-laying period through the nestling period until fledgling, which was considered to be 45 days for *A. spinicauda* based on Moreno *et al*. (2005) and our field observations. Nest success and its associated variance were estimated by raising DNS rate to the exponent of the duration of the nestling cycle (Rotella 2005).

Nest-boxes also were monitored every 3 to 4 days in 2004 and 2005 to determine their content. In this study, I only report nest success in natural nests because nest success in nest-boxes was lower than in natural cavities (unpublished data), corroborating the bias that nest boxes can sometimes introduce in ecological field studies (Moore & Robinson 2004). Data on clutch size and number of nestlings fledged/brood from nest boxes were used, however, to compare these parameters between the two habitats where nest boxes were placed (i.e., SLF and LFR). Clutch size and number of nestlings between the two forest types with nest-boxes were compared with a *t*-test. These parameters were not recorded in OGF because contents of natural nests could not be monitored.

Results

Forest structure and nest site availability

Density of live large trees differed among the three forest habitats ($F_{2,34} = 30.41$, $P < 0.0001$), with more large trees in OGF than in SLF or LFR ($P < 0.001$, both cases) but no difference between SLF and LFR. In contrast, there was no significant difference in density of snags among the three forest habitats ($F_{2,34} = 2.59$, $P = 0.09$; Fig. 2). In addition, there was no effect of forest plot within each forest habitat for snags ($F_{3,34} = 2.57$, $P = 0.07$), but there was an effect for large trees ($F_{3,34} = 10.82$, $P < 0.0001$). Differences in density of large trees between the two SLF plots

(P <0.0001) and between the two LFR plots ($P = 0.009$) probably reflect differences in logging intensity between study plots. In contrast, density of large trees did not differ between the two OGF plots ($P = 0.63$) where no logging has occurred.

Density and nest site supplementation

Density of *A. spinicauda* was lower in SLF than in OGF and LFR in 2003 before nest-site supplementation ($F_{2,6} = 15.12$, $P = 0.027$; Fig. 3a). In 2004, there was no difference in density among the three forest types ($F_{2,6} = 0.31$, $P = 0.75$) because of an increase in density in SLF after nest-site supplementation ($t_{18} = 2.59$, $P = 0.018$; Fig. 3b). Although a net increase in density was not observed in LFR after nest boxes were added ($t_{24} = 1.28$, $P = 0.21$), the number of nest boxes used over the two year period was higher in LFR (47 out of 216 boxes) than in SLF (26 out of 216 boxes; $\chi2 = 6.67$, $P = 0.007$). There was a general decrease in density from 2004 to 2005 (significant only for LFR; $t_{24} = 5.43$, $P < 0.0001$) but, as in 2004, there was no difference in density among forest types ($F_{2,6} = 4.73$, $P = 0.118$; Fig. 3c).

Density of *A. spinicauda* varied across years (rmANOVA, $F_{2,18} = 44.71$, $P < 0.001$), with a significant overall increase in density from 2003 to 2004 after nest site supplementation (LSD test, $P = 0.04$) and a significant decrease between 2004 and 2005 ($P < 0.001$) and between 2003 and 2005 ($P = 0.016$). A significant interaction between year and forest type ($F_{4,18} = 5.72$, $P = 0.03$) indicated that the patterns of differences in density of *A. spinicauda* among forests (OGF, SLF, LFR) changed among years (Fig. 4). Over the three years there was no effect of forest type ($F_{2,18} = 1.03$, $P = 0.46$) on density of *A. spinicauda*, which was expected as a result of nest site supplementation in 2004 and 2005 (because nest site addition increased densities eliminating the forest-type effect present in 2003).

Nest success and reproductive output

A total of 102 nests of *A. spinicauda* were found in natural cavities located in both snags and live trees. Fifteen nests were not included in the analysis because they were abandoned during nest construction and were not used for reproduction. The best DNS model (see Table 2 for candidate models) only incorporated type of tree (live tree or snag), suggesting that DNS and, hence, nest success was influenced by the type of tree where the nest cavity was found but not by forest type (OGF, SLF, LFR) or year. Based on DNS estimates from this model, the overall nesting success (±SE) was 74.8% ± 9 in snags (n = 36) and 46.9 % ± 10 in live trees (n = 38). The best model and three other candidate models, however, had ΔAIC_c values that ranged only between 0 – 1.93 and model weights (w_i), the probability that model i is the actual expected best model for the sample situation considered, ranged between 0.30 – 0.11 (Table 2). Among these equally supported models (with $\Delta AIC < 2$), however, the best-supported model was also the simplest (i.e. with the fewest parameters), supporting the conclusion that the main source of variation for nest survival was given by the tree type in which the nest-cavity was found. The general and simplest model assuming no source of variation in nest survival was poorly supported (Table 2).

Nest success (± SE) based on DNS estimates from MARK was not different among forests; 61% ± 9 in OGF (n = 33), 61% ± 8 in SLF (n = 27) and 60% ± 14 in LFR (n = 14). When DNS was calculated using the Mayfield method including nests with uncertain fate, results were similar (Fig. 5), indicating that DNS estimates based on MARK were not upward biased. Data from nest boxes in SLF and LFR showed that mean clutch size (± SE) was 4.3 ± 0.12 (range 3-6, n = 46) and, considering successful nests only, mean number of nestlings fledged/brood was 4.1 ± 0.21 (range 2-5, n = 19). There was no difference in clutch size or number of nestlings fledged/brood between the two forest habitats in which nest boxes were added (SLF and LFR) ($t_{24} = 0.12$, $P = 0.9$, and $t_{24} = 0.77$, $P = 0.47$ respectively).

Discussion

In most human-modified landscapes, habitat degradation and habitat fragmentation occur simultaneously (Armstrong 2005; Harrison & Bruna 1999). Despite this, most studies on habitat fragmentation have focused on landscape-level processes, such as the spatial configuration of patches and dispersal patterns of organisms, and have given less attention to more basic but essential questions related to habitat degradation within fragments (Harrison & Bruna 1999). This study is the first to use an experimental approach to compare the consequences of nest-site limitation due to habitat degradation *per se* with those due to habitat fragmentation for populations of a secondary cavity-nesting bird. Here, I discuss potential mechanisms responsible for the differences in population responses to nest-site limitation.

Forest structure, habitat fragmentation and density of A. spinicauda

Selective logging in the study area has reduced the number of large trees and, hence, availability of cavities, but has had no effect on the number of snags. Previous studies in the south-temperate rainforest have shown that abundances of birds that rely on large trees for nesting or foraging were considerably less in pine plantations and secondary forests, and these birds were almost absent from early-successional forests where large trees and snags were lacking suggesting nest-site limitation (Estades & Temple 1999; Diaz et al. 2005; Tomasevic & Estades 2006). Yet, abundances of most large-tree users, including *A. spinicauda,* were not lower in small forest patches (Willson et al. 1994), although this study did not control for the effect of forest structure.

Results from the present study show that density of *A. spinicauda* was lower in forests with selective logging (SLF) than in old-growth forest (OGF), but that density was not lower in logged-fragments (LFR) as predicted, despite the reduction of cavity availability, and thereby presumed reduction in habitat quality in fragments. Therefore, it is likely that mechanisms other than those related to selective logging, are responsible for the observed pattern in fragments. A widespread pattern observed in fragmented systems in many taxa, including insects and birds, is a positive relationship between area and population density (Connor et al. 2000). In other cases, however, confounding factors associated with the matrix surrounding habitat fragments or the development of ecological traps (Estades 2001; Ewers & Didham 2006), source sink dynamics (Brawn & Robinson 1996), stochastic processes and predator release in small fragments (Terborgh et al. 1997) may result in a departure from the expected positive relationship between abundance of forest birds and size of fragments. Similarly, in my study site, some of these mechanisms may be responsible for the observed higher density of *A. spinicauda* in logged-forest fragments.

Nest-site limitation

Density of *A. spinicauda* increased in selectively-logged forest (SLF) plots after nest box addition, supporting the nest-site limitation hypothesis. Density in selectively-logged fragments (LFR), however, did not increase after supplementation. SLF plots were located in well-connected and large forests where birds can leave the forest plot when nest-sites are limited and then re colonize from surrounding areas when nest sites increase in availability. In logged-fragments (LFR), however, birds may be less likely to disperse if nest sites are limiting. In fact, density of *A. spinicauda* was higher than predicted by the availability of cavities (as indexed by abundance of large trees and snags) and there was a higher proportion of nest box use than in logged-forest (SLF) plots. Therefore, even though density did not increase after nest site addition, nest sites are probably also limited in fragments. Moreover, genetic data on populations of *A. spinicauda* in this same fragmented landscape suggest a reduction of movement of individuals among forest fragments (Chapter 1).

That the increase in density of *A. spinicauda* from 2003 to 2004 in logged-forest plots (SLF) was a consequence of nest-box additions rather than a response to environmental variation, is supported by two lines of evidence. First, while density in SLF increased from 2003 to 2004, density in old-growth forest (OGF), where nest boxes were not added, remained unchanged during this period. Second, the relationship between density of *A. spinicauda* among the three forest types was influenced by year (i.e. whether nest boxes were added or not) as shown by a significant year × forest type interaction. Although this interaction may also be influenced by natural environmental changes, such as the overall decrease in density observed in 2005, results suggest that the nest-site supplementation experiment did influence density relationships. In 2003, there was a lower density of birds in SLF than in OFG but this difference was not detected in the following two years when nest boxes were present. Thus, the relationship between SLF and OGF was maintained after nest site addition (i.e. they remained with similar densities) even in 2005 when all densities decreased because of changes in overall environmental conditions (Fig. 4).

The overall decrease in density of *A. spinicauda* populations in 2005 was probably a result of low survival during the previous winter. Total rainfall in winter 2005 (May – August) was 1,501 mm, while rainfall in 2003 and 2004 winters was only 927 mm and 867 mm, respectively (data from Senda Darwin Biological Station weather station). The fact that density in logged fragments (LFR) decreased in 2005 to a greater extent than density in large and connected old-growth forest (OGF) and logged forest (SLF), is probably also a consequence of isolation of populations in forest fragments. Populations in fragments may have a smaller chance to recover from high mortality events through re colonization from surrounding areas (Davies et al 2001). Furthermore, density in fragments was lower than in the other two forest types in 2005. Although this difference was not statistically different, it may provide evidence for the combined negative effects of changes in forest structure and fragmentation on density that was predicted initially. Finally, this study illustrates the dynamic nature of ecological systems and the importance of long-term monitoring programs. In only three years of monitoring populations of *A. spinicauda*, it was possible to describe the influence of natural environmental variation on density patterns. It is clear that if the study had been conducted only in 2005 (after the overall population decline presumably due to low survival during a rainy winter), conclusions would have been very different (e.g. reduced density of *A. spinicauda* in forest fragments). Therefore, long-term monitoring programs should be incorporated whenever possible in research agendas in the southern rainforests and other human-modified landscapes.

Nest success among forests

Daily nest survival (DNS) rate and, hence, nesting success of *A. spinicauda* did not differ among the three forest types (OGF, SLF, LFR); nest success varied from 53% to 63%. Whereas density was lower in forests with reduced nest-site numbers [i.e. higher in old-growth forest (OGF) than in logged-forests (SLF)], there was no decrease in nest success, implying that habitat degradation *per se* caused by selective logging produced a pattern according to an Ideal Free Distribution (Fretwell & Lucas 1970). Contrary to my predictions, however, nest success in logged-fragment (LFR) plots was not lower than in OGF and SLF plots. Although the lack of difference in nest success might be due to the small sample size of natural nests in LFR plots (n = 15), this may also indicate that there were fewer pairs nesting in forest fragments given that nest-search effort was constant across all study plots. Therefore, there are two possible nonexclusive hypotheses that can explain the lack of a reduced nest success in logged-fragments.

The first is that some birds in fragments were not reproducing because of nest-site limitation and, therefore, nest success rates were only given by pairs reproducing in available good-quality cavities. Movement of forest bird species (Rhinocryptidae and Furnariidae) can be reduced or modified because of habitat fragmentation in the agricultural landscape of Chiloé (Sieving et al. 1996; Willson 2004; Castellón & Sieving 2006a,b; Diaz et al. 2006; Chapter 1). As a consequence, individuals of *A. spinicauda* may face a nest-site selection trade-off in fragments with respect to the cost of dispersing across open habitats or staying in fragments with reduced availability nest sites. Under this scenario, some birds would stay in fragments but would not nest. Some bird species living in extremely patchy habitat may preclude reproduction because of inability to disperse and stay as helpers in parents territories (e.g. Florida scrub-jay; Woolfenden & Fitzpatrick 1984). Although this is a less likely scenario for *A. spinicauda*, this hypothesis could at least in part explain the larger proportion of nest boxes used in fragments than in large and connected logged-forests (SLF).

The second hypothesis, and the most likely, is that quality of cavities is not lower in forest fragments as I had initially predicted. Quality of cavities may change because of environmental changes caused by fragmentation, such as microclimatic changes triggered by edge effects or changes in predator populations. In the present study area, there is evidence that small mammals are the main predators of forest bird nests (Willson et al. 2001). Although abundance of small rodents may increase in forest fragments (Saavedra & Simonetti 2005), these are probably poor predators of nests in cavities located high in trees because most show poor climbing abilities (Gallardo-Santis et al. 2005). The best candidate predator in the study area is a forest and arboreal marsupial (*Dromiciops gliroides*) that frequently placed nests in nest-boxes on top of *A. spinicauda* nests, after eating their eggs (pers. obs.). There is little information about the biology and sensitivity of this marsupial to habitat fragmentation, but it has been reported to be absent from forest fragments in an area north of Chiloé (Saavedra & Simonetti 2005). In Chiloé this marsupial was observed depredating nest boxes in fragments, but there is no information available about their densities in isolated forests. Several arboreal marsupials, however, have shown to be affected by habitat fragmentation in other forest systems (Lindenmayer et al. 1999). Thus, the lack of a reduction in nest success for *A. spinicauda* in logged-fragments is possibly related to reduced nest predation rates. As a result, cavities that are usually of low quality because of higher predation rates in old-growth or logged-forest are no longer of low quality in logged-fragments. Therefore, even if cavity availability is quantitatively the same in logged-forests and logged-fragments, in fragments cavities are actually of higher quality and, thus, functionally there are more cavities available in logged-fragments than in large and connected logged forests.

Although the relative importance of these two proposed mechanisms can not be quantified, results of this study suggest that both mechanism (i.e. reduced dispersal and predator changes) are likely responsible for observed patterns. I conclude that selective logging results in nest-site limitation for *A. spinicauda*, that population responses to nest-site limitation vary because of landscape level processes (e.g. changes in predator densities in fragments) and that habitat fragmentation may impose a trade-off situation in nest site selection.

Selective logging and nest success

Logging activities in the area of this study affected cavity availability mostly by reducing the number of large live trees. Results of this study suggest that pairs of *A. spinicauda* using live-tree cavities had lower nest success than pairs nesting in snag cavities, regardless of the forest type in which they were found. Thus, cavities in snags seem to be safer nesting sites than cavities in live trees. Snags used as nest-sites were usually bare trunks with little or no epiphyte cover (Chapter 3), which may make potential nest predators, such as the arboreal marsupial, more exposed to their own predators when attempting to reach nests. In contrast, trunks of large live trees in the study area, especially those of *Nothofagus* trees, are usually covered by a dense vine and epiphyte layer (Muñoz et al. 2003). Other studies have also shown that failed nests of secondary cavity-nesting birds were generally more concealed by foliage than successful nests (Li & Martin 1991; Nilsson 1984).

Large *Nothofagus* trees, however, provide habitat on their foliage and dense epiphyte cover for a rich and abundant arthropod assemblage (Sapagarino et al. 2001), and therefore, can be important structures in determining the quality of territories in terms of food availability. Food availability was not directly evaluated in this study, but it is well known that changes in availability of food resources affect reproductive parameters such as clutch size and number of fledglings/brood among others (Martin 1987; Newton 1998). I did not find differences in clutch size or in the number of nestlings fledged/pair between logged forests (SLF) and logged fragment (LFR) plots, suggesting no differences in resource supply among these forests. Although in some cases small fragments have reduced food resources for insectivorous birds (Zanette et al. 2000; Burke & Nol 1998), other studies have shown no such reductions (Şekercioğlu et al. 2002). My data on reproductive output were based on nest-boxes placed in logged-fragment and logged-forest plots but these types of data could not be recorded from nests in old-growth forests where only natural nests were monitored. Therefore, further studies are needed to determine the role of food availability for insectivorous birds in these rainforests, which is especially important in fragmented landscapes (Zanette et al. 2000), and specifically for

A. spinicauda which is one of the most abundant insectivorous birds in the south-temperate rainforest ecosystem.

Concluding remarks

This study shows the ecological effects of habitat degradation due to selective logging, and the additional effects of habitat fragmentation on populations of a secondary cavity-nesting bird. Here, I show that the main factor limiting densities of a secondary cavity-nesting bird in a fragmented landscape is nest-site availability induced by changes in forest structure, rather than differences in nest success among forests. But processes related to changes in landscape connectivity also influenced the outcome of nest-site limitation. Thus, the availability of nest sites depends not only on the presence of key structures such as snags and large trees, but also on changes at the landscape level that are triggered by forest fragmentation, such as changes in dispersal patterns of birds or populations of nest-predators.

Temperate rainforests in South America continue to be transformed by human activities causing rapid loss, degradation and fragmentation of this ecosystem (Etcheverria et al. 2006). Other cavity-nesting species also have shown evidence of nest-site limitation (Tomasevic & Estades 2006; Diaz et al. 2005; De Santo et al. 2002) and, thus, are also likely to be affected by processes similar to those described in this study. In this region, management guidelines and forest legislation should explicitly consider forest fragmentation and habitat degradation (in terms of changes in forest structure and presence of key structures) to develop better forest management strategies, at both large and local scales of logging and forest use.

Literature Cited

Aitken K.E.H., Wiebe K.L., Martin K. (2002) Nest-site reuse patterns for a cavity-nesting bird community in interior British Columbia. *Auk* 119: 391-402

Akaike H. (1973) Information theory and an extension of the maximum likelihood principle, p. 267-281. In: F. Csaki (ed.), Second International Symposium on Information Theory. Akamemiai Kiado, Budapest, Hungary.

Aravena J.C., Carmona M.R., Perez C., Armesto J.J. (2002) Changes in tree species richness, stand structure and soil properties in a successional chronosequence in northern Chiloe, Chile. *Revista Chilena de Historia* Natural 75: 339-369

Armstrong D.P. (2005) Integrating the metapopulation and habitat paradigms for understanding broad-scale declines of species. *Conservation Biology* 19: 1402-1410

Brawn J., Balda R. (1988) Population biology of cavity nesters in northern Arizona: do nest sites limit breeding densities? *The Condor* 90: 61-71

Brawn J., Robinson S. (1996) Source-sink population dynamics may complicate the interpretation of long-term census data. *Ecology* 77: 3-12

Brightsmith D.J. (2005) Competition, predation and nest shifts among tropical cavity nesters: ecological evidence. *Journal of Avian Biology* 36: 64-73

Buckland S.T., Anderson D.R., Burnham K.P., Laake J.L., Borchers D.L., Thomas L. (2001) Introduction to Distance sampling: estimating abundance of biological populations. Oxford University Press, New York

Burke D.M., Nol E. (1998) Influence of food abundance, nest-site, and forest fragmentation on breeding Ovenbirds. *Auk* 115: 96-104

Castellón T.D., Sieving K.E. (2006a) An experimental test of matrix permeability and corridor use by an endemic understory bird. *Conservation Biology* 20: 135-145

Castellon, T. D. & Sieving, K. E. (2006b) Landscape history, fragmentation, and patch occupancy: models for a forest bird with limited dispersal. *Ecological Applications* 16: 2233-2234.

Connor E.F., Courtney A.C., Yoder J.M. (2000) Individuals-area relationships: the relationship between population density and area. *Ecology* 81: 734-748

Davies K.F., Gascon C., Margules C.R. (2001) Habitat fragmentation, pp. 81-98. In: *Conservation Biology: research priorities for the next decade* (Soule M.E., Orians G.H., eds.). Island Press Washington, DC,

De Santo T.L., Willson M.F., Sieving K.E., Armesto J.J. (2002) Nesting biology of tapaculos (Rhinocryptidae) in fragmented south-temperate rainforests of Chile. *Condor* 104: 482-495

Di Castri F., Hajek E. (1976) Di Castri F., Hajek E. (1976) Bioclimatología de Chile. Ediciones Universidad Catolica de Chile, Santiago, Chile

Diaz I.A., Armesto J.J., Reid S., Sieving K.E., Willson M.F. (2005) Linking forest structure and composition: avian diversity in successional forests of Chiloé Island, Chile. *Biological Conservation* 123: 91-101

Diaz I.A., Armesto J.J., Willson M.F. (2006) Mating success of the endemic Des Murs' Wiretail (*Sylviorthorhynchus desmursii*, Furnariidae) in fragmented Chilean rainforests. *Austral Ecology* 31: 13-21

Dinsmore S.J., White G.M., Knopf F.L. (2002) Advance techniques for modeling avian nest survival. *Ecology* 83: 3476-3488

Estades C.F. (2001) The effect of breeding-habitat patch size on bird population density. *Landscape Ecology* 16: 161-173

Estades C.F., Temple S. (1999) Deciduous-forest bird communities in a fragmented landscape dominated by exotic pine plantations. *Ecological Applications* 9: 573-585

Ewers R.M., Didham R.K. (2006) Confounding factors in the detection of species responses to habitat fragmentation. *Biological Reviews* 81: 117-142

Fretwell S., Lucas H.J. (1970) On territorial behavior and other factors influencing habitat distribution in birds. *Acta Biotheoretica* 19: 16-36

Gallardo-Santis A., Simonetti J.A., Vasquez R.A. (2005) Influence of tree diameter on climbing ability of small mammals. *Journal of Mammalogy* 86:.969-973

Harrison S., Bruna E. (1999) Habitat fragmentation and large-scale conservation: what do we know for sure? *Ecography* 22: 225-232

Holt R.F., Martin K. (1997) Landscape modification and patch selection: The demography of two secondary cavity nesters colonizing clear cuts. *Auk* 114: 443-455

Lampila P., Monkkonen M., Desrochers A. (2005) Demographic responses by birds to forest fragmentation. *Conservation Biology* 19: 1537-1546

Laurance W.F. (2000) Edge effects and ecological processes: are they on the same scale? *Trends in Ecology & Evolution* 15: 373

Lindenmayer D.B., Cunningham R.B., Pope M.L., Donnelly C.F. (1999) The response of arboreal marsupials to landscape context: a large-scale fragmentation study. *Ecological Applications* 9: 594-611

Li P., Martin T.E. (1991) Nest-site selection and nesting success of cavity-nesting birds in high elevation forest drainages. *Auk* 108: 405-418

Manolis J.C., Andersen D.E., Cuthbert F.J. (2000) Uncertain nest fates in songbird studies and variation in Mayfield estimation. *Auk* 117: 615-626

Martin K., Aitken K.E.H., Wiebe K.L. (2004) Nest sites and nest webs for cavity-nesting communities in interior British Columbia, Canada: Nest characteristics and niche partitioning. *Condor* 106:5-19

Martin T.E., Geupel G.R. (1993) Nest-monitoring plots: methods for locating nests and monitoring success. *Journal of Field Ornithology* 64: 507-519

Martin T.E. (1987) Food as a limit on breeding birds: a life-history perspective. *Annual Review of Ecology and Systematics* 18: 453 – 487

Martin T.E., Li, P. (1992) Life history traits of open- vs. cavity-nesting birds. *Ecology* 72: 579-592

Mayfield H. (1975) Suggestions for calculating nest success. *Wilson Bulletin* 87: 456-466

Moore R.P., Robinson W.D. (2004) Artificial bird nests, external validity, and bias in ecological field studies. *Ecology* 85: 1562-1567

Moreno J., Merino S., Vásquez R.A., Armesto J.J. (2005) Breeding Biology of the Thorn-tailed Rayadito (Furnariidae) in south-temperate rainforests of Chile. *The Condor* 107: 69-77

Muñoz A., Chacon P., Perez F., Barnert E.S., Armesto J.J. (2003) Diversity and host tree preferences of vascular epiphytes and vines in a temperate rainforest in southern Chile. *Australian Journal of Botany* 51: 381-391

Murcia C. (1995) Edge effects in fragmented forests: Implications for conservation. *Trends in Ecology and Evolution* 10: 58-62

Newton I. (1994) The role of nest-sites in limiting the numbers of hole-nesting birds: a review. *Biological Conservation* 70: 265-276

Newton I. (1998) Population limitation in birds. Academic Press Limited, San Diego, CA.

Nilsson S.G. (1984) The evolution of nest-site selection among hole-nesting birds: the importance of nest predation and competition. *Ornis Scandinavica* 15: 165-175

Reid S., Diaz I.A., Armesto J.J., Willson M.F. (2004) Importance of native bamboo for understory birds in Chilean temperate forests. *Auk* 121:515-525

Robinson S., Thompson F., Donovan N.S., Whitehead D., Faaborg J. (1995) Regional forest fragmentation and the nesting success of migratory birds. *Science* 267: 1987-1990

Rodewald A.D. (2004) Nest-searching cues and studies of nest-site selection and nesting success. *Journal of Field Ornithology* 75:31-39

Rosenzweig M. (1991) Habitat selection and population interactions: the search for mechanisms. *American Naturalist* 137: S5-S28

Rotella, J. 2005. Nest Survival Models, pp. 122-125. In: *Program MARK "A gentle introduction"* (Cooch, E., and White, G., eds.). 5th edition.

Saavedra B., Simonetti J.A. (2005) Small mammals of Maulino forest remnants, a vanishing ecosystem of south-central Chile. *Mammalia* 69: 337-348

Sapagarino C., Martinez Pastur G., Peri P.L. (2001) Changes in *Nothofagus pumilio* forest biodiversity during the forest management cycle. 1. Insects. *Biodiversity and Conservation* 10: 2077-2092

Şekercioğlu C.H., Ehrlich P., Daily G., Aygen D., Goehring D., Sandi R.F. (2002) Disappearance of insectivorous birds from tropical forest fragments. Proceedings *of the National Academy of Science USA* 99: 263-267

Sieving K.E., Karr J.R. (1997) Avian extinction and persistence mechanisms in lowland Panama, pp 156-170. In: *Tropical Forest Remnants* (Laurance W.F., Bierregaard R., eds) University of Chicago Press, Chicago, IL.

Sieving K.E., Willson M.F., De Santo T.L. (1996) Habitat barrier movement of understory birds in fragmented south-temperate rainforest. *Auk* 113: 944-949

Terborgh J., Lopez L., Tello J. (1997) Bird communities in transitions: The Lago Guri islands. *Ecology* 78:1494-1501

Thomas L., Laake J.L., Strindberg S., Marques F.F.C., Buckland S.T., Borchers D.L., Anderson D.R., Burnham K.P., Hedley S.L., Pollard J.H., Bishop J.R.B., Marques T.A.

(2005) Distance. [5.0 Beta 4]. Research Unit for Wildlife Population Assessment, University of St. Andrews, UK.

Tomasevic J.A., Estades C.F. (2006) Stand attributes and the abundance of secondary cavity-nesting birds in southern beech (*Nothofagus*) forests in south-central Chile. *Ornitología Neotropical* 17: 1-14

Turchin P. (1999) Population regulation: a synthetic review. *Oikos* 84: 153-159

Van Horne B. (1983) Density as a misleading indicator of habitat quality. *Journal of Wildlife Management* 47: 893-901

Vergara P.M., Simonetti J.A. (2003) Forest fragmentation and rhinocryptid nest predation in central Chile. *Acta Ornithologica* 24: 285-288

White G.M. (2000) MARK: Mark and Recapture Survival rate Estimation. [5.1]. 2000. Fort Collins, CO, Department of Fishery and Wildlife, Colorado State University.

Wiebe K.L., Koenig W.D., Martin K. (2006) Evolution of clutch size in cavity-excavating birds: the nest site limitation hypothesis revisited. *American Naturalist* 167: 343-353

Willson M.F. (2004) Loss of habitat connectivity hinders pair formation and juvenile dispersal of Chucao Tapaculos in Chilean rainforest. *Condor* 106: 166-171

Willson M.F., Armesto J.J. (1996) The Natural History of Chiloé: on Darwin's trail. *Revista Chilena de Historia Natural* 69: 149-161

Willson M.F., De Santo T.L., Sabag C., Armesto J.J. (1994) Avian communities of fragmented south-temperate rainforests in Chile. *Conservation Biology* 8: 508-520

Willson M.F., Morrison J., Sieving K.E., De Santo T.L., Santisteban L., Diaz I. (2001) Patterns of predation risk and survival of bird nests in a Chilean agricultural landscape. *Conservation Biology* 15: 447-456

Woolfenden G.E., Fitzpatrick J.W. (1984) The Florida Scrub Jay: demography of a cooperative-breeding bird. Princeton University press, Princeton, New Jersey, USA.

Zanette L., Doyle P., Tremont S.M. (2000) Food shortage in small fragments: Evidence from an area- sensitive passerine. *Ecology* 81: 1654-1666

Table 1. Summary of habitat-specific detection functions (selected among candidate models based on smallest AIC*c*) used to estimate density of *A. spinicauda* in the south-temperate rainforest in Chiloé (2003 – 2005). N = number of observations used to model the detection function, f(0) = probability detection function at 0 m, *cv* = coefficient of variation for f(0), P = probability of detection up to 30 m.

Forest Type	**N**	**Key Function**	**f(0)**	***cv* (%)**	**P**	***P*-value***
Old-growth	722	Hazard Rate	0.0421	2.7	0.79	0.6
Logged-forest	682	Hazard Rate	0.0418	3.2	0.80	0.8
Logged-fragments	473	Half-normal	0.0593	3.9	0.56	0.4

* Goodness of fit test for grouped data

Table 2. Summary of model selection results using MARK for the nest survival of *A. spinicauda* in the south-temperate rainforest in Chiloé (2003-2005) based on 73 known-fate nests in natural cavities. Models are ranked by ascending ΔAIC*c; w_i* is normalized model weight and *K* number of parameters.

Model	***K***	**AIC*c***	**ΔAIC*c***	w_i
S (tree type)	1	151.622	0.00	0.300
S (tree type + year)	3	151.970	0.35	0.252
S (forest type + tree type)	3	153.528	1.91	0.116
S (forest type + year + tree type)	4	153.548	1.93	0.114
S (.)	1	153.742	2.12	0.104
S (year)	2	154.989	3.37	0.056
S (forest type)	2	155.746	4.12	0.038
S (forest type + year)	3	156.971	5.35	0.021

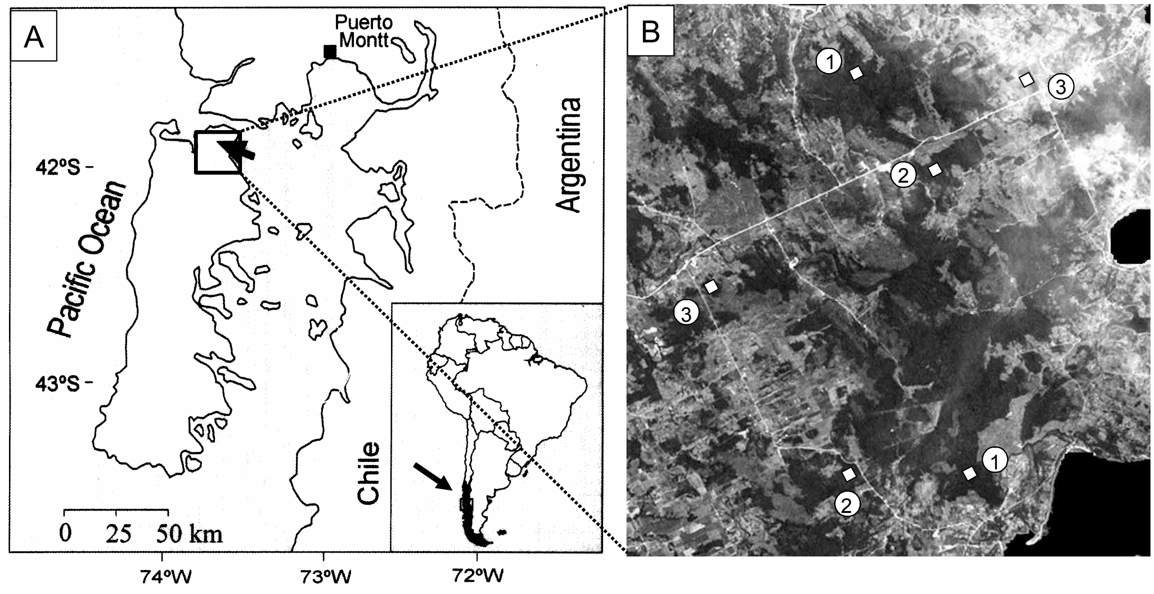

Figure 1. (A) Study region and (B) study site in Isla Grande de Chiloé, southern Chile. The satellite image (Landsat 5-T 2001) shows the spatial pattern of forest fragment distribution in the study area. Squares represent 10-ha plots in each of three habitat treatments [1 = old-growth forest (OGF), 2 = selectively-logged forest (SLF), 3 = selectively-logged forest fragments (LFR)].

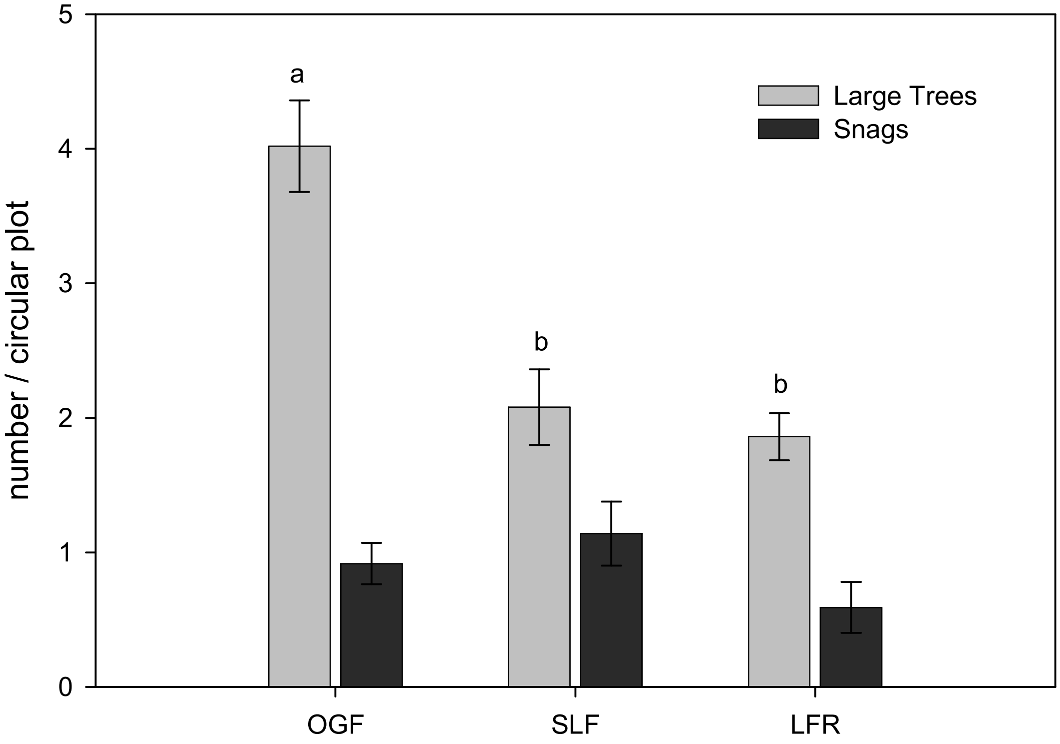

Figure 2. Mean number (± SE) of large trees and snags per circular vegetation plot (340 m^2) among the three habitat treatments; OGF = old-growth forest, SLF = selectively-logged forest, LFR = selectively-logged fragments. Different letters indicate $P < 0.001$ from Tukey post-hoc test for comparison of live trees among habitat treatments.

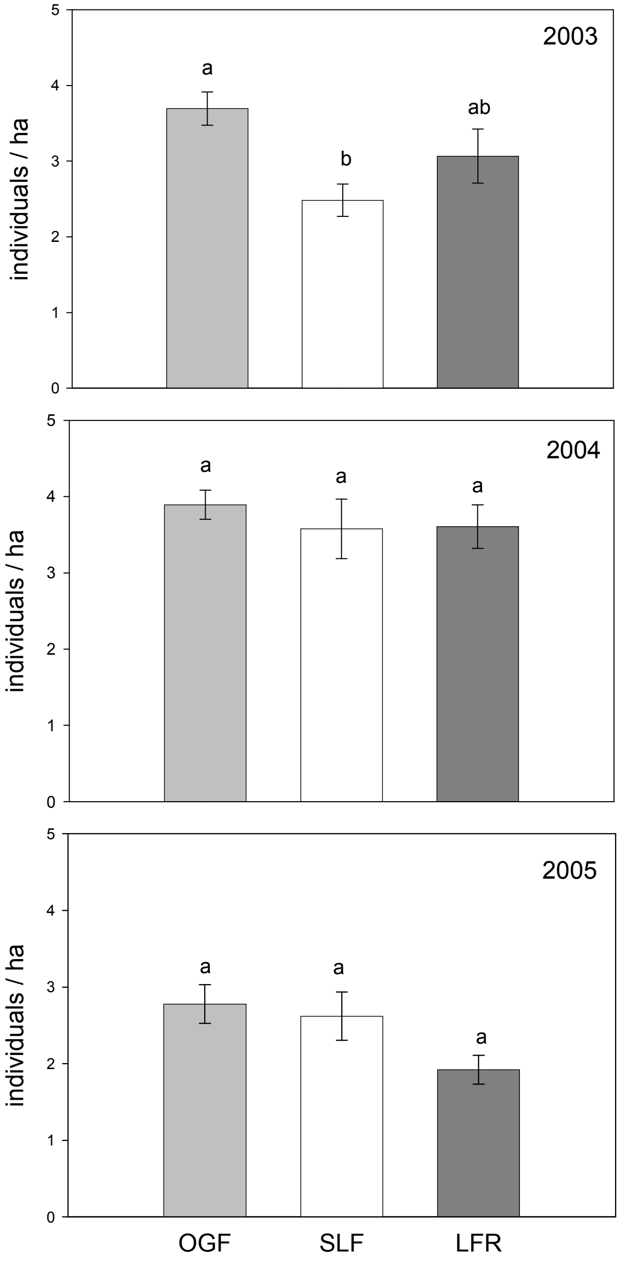

Figure 3. Density of *Aphrastura spinicauda* (± SE) estimated with habitat-specific detection functions among the three forest habitats before nest-site supplementation (2003), and after nest-site supplementation (2004 and 2005). Different letters indicate $P<$ 0.05 from Tukey post-hoc test within each year (OGF = old-growth forest, SLF = selectively-logged forest, LFR = selectively-logged fragments).

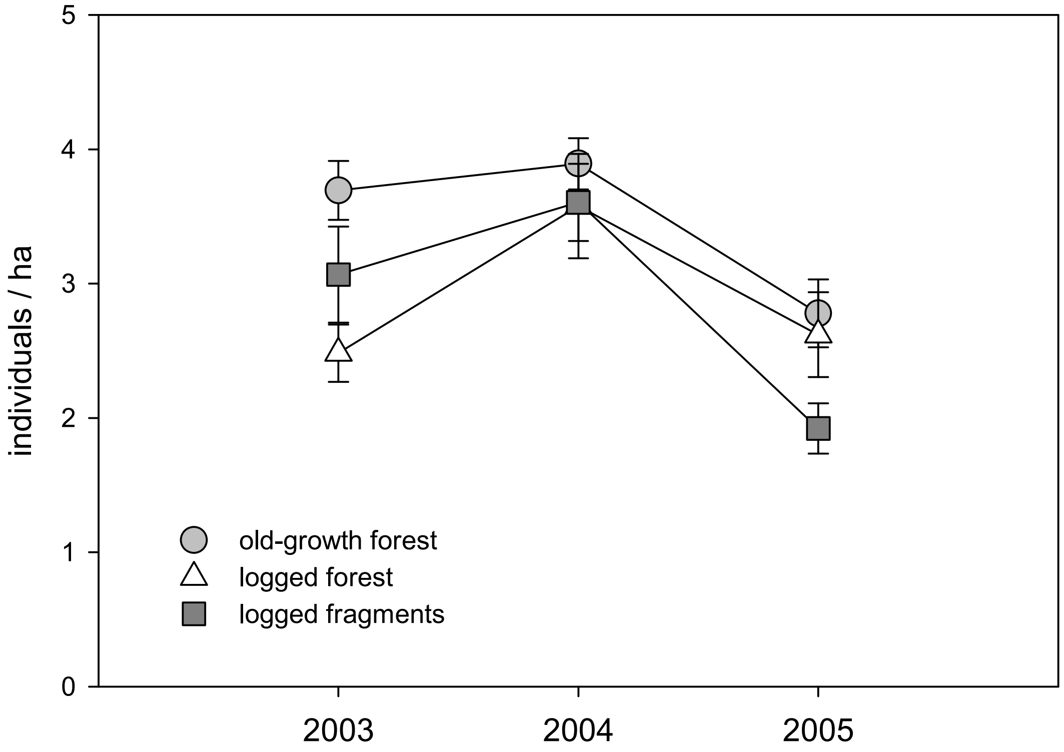

Figure 4. Comparison among years of *A. spinicauda* density (± SE) based on habitat-specific detection functions for the three forest habitats.

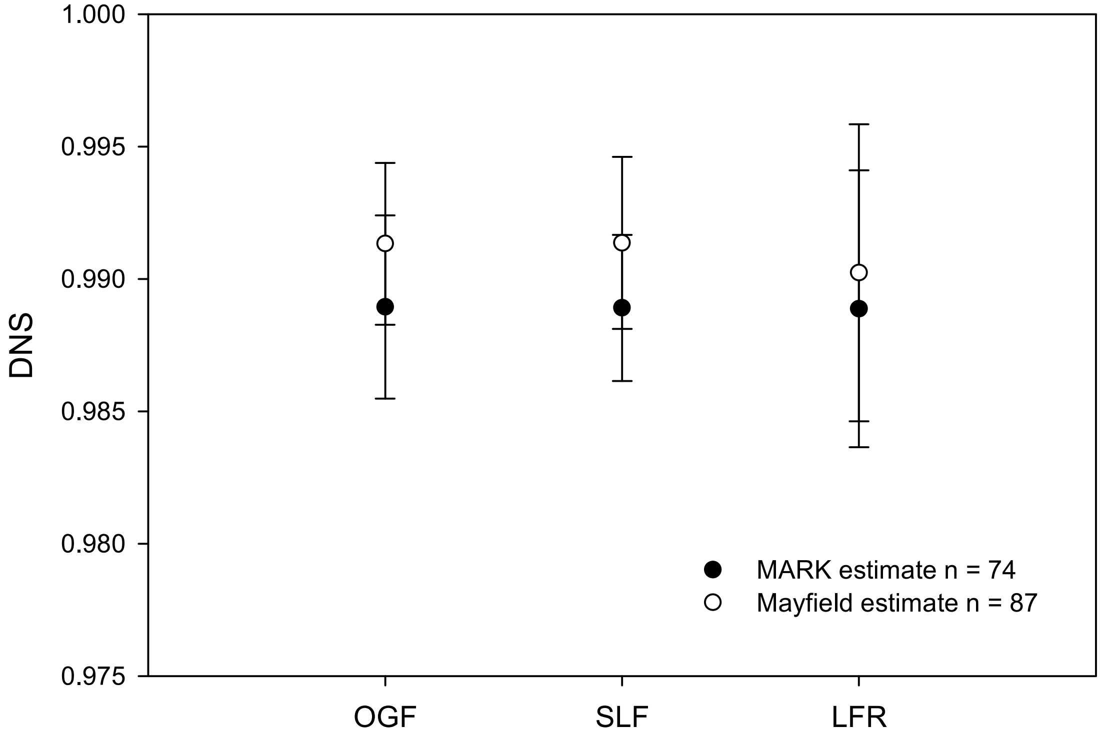

Figure 5. Daily Nest Survival (DNS ± SE) for *A. spinicauda* in each forest type, estimated with MARK including known-fate nests only, and estimated with the Mayfield method including nests with uncertain fate (OGF = old-growth forest, SLF = selectively-logged forest, LFR = selectively-logged fragments).

CHAPTER III

Nest-Site and Territory Selection by a Secondary Cavity-Nesting Bird Breeding in Forests with Different Nest-Site Availabilities

Abstract. - Nest site choice can have important consequences on fitness and, as a result, characteristics of nest sites that influence nesting success should be important in determining nest site preferences. Characteristics of successful *vs.* unsuccessful nest sites, however, are likely to vary over time and space in response to habitat changes that influence nest-site quality. As a consequence, nest-site selection patterns should also vary across the landscape. The goal of this study was to determine if nest-tree selection by a secondary cavity-nesting bird, and associated consequences on fitness, vary spatially in response to human-driven landscape modifications such as changes in forest structure and connectivity. In this study, I characterized territories and nest trees used by *Aphrastura spinicauda* (Furnariidae) in the temperate rainforest of southern South America among three forest types that differed in nest-site availability and levels of connectivity (old-growth forest, logged forest, and logged-forest fragments). Results indicated that landscape variation in nest-site selection was given by differences in connectivity and not by differences in forest structure among study plots. *Aphrastura spinicauda* used smaller trees and different tree species in fragments than in large and connected forests (logged and unlogged), although this differential nest-tree use had no consequences on fitness. Overall, nest success decreased with epiphyte cover of trees. Live trees had more epiphyte cover than snags, and nests in snags had a higher success

(74.8% ± 9) than nests in live trees (46.9 % ± 10). In large and connected forests (logged and unlogged), snags were used in a higher proportion than based on their availability suggesting an adaptive nest-site choice; among live trees, *Nothofagus nitida* was used more than expected based on their availability. In logged fragments, however, use of nest trees was proportional to availability. Territories were similar among forest types and were characterized by forest-gap conditions with open canopy, dense understory and with more large trees and snags than unused areas. Results of this study showed evidence for an adaptive nest-tree preference by *A. spinicauda* and also that nest-site choice is spatially variable in response to ecological gradients produced by human activities.

Key words: Aphrastura spinicauda, cavity-nester, Chile, habitat fragmentation, nest-site selection, south-temperate rainforest.

Introduction

Selection of a nest site can have important consequences on fitness and, as a result, nest-site preferences are usually under strong selection in birds (Martin 1998; Clark & Shutler 1999). The choice of a particular nest site is based on the behavior of individuals which, in many cases, is genetically based (Jaenike & Holt 1991), supporting the idea of an adaptive value of nest-site selection. Therefore, factors that influence nest site choice should be related to processes that affect nesting success (i.e., the probability of fledging at least one young). For example, nest predation is the primary cause of nest failure in birds (Ricklefs 1969; Martin 1993) and, therefore, an important selective force for most bird species, including cavity-nesting species (Nilsson 1984; Martin & Li 1992; Martin 1993, Fontaine & Martin 2006).

The chance that an individual bird will find a suitable nest site depends on the availability of the substrate that is preferred for nesting. Availability, however, is influenced not only by the abundance of the preferred substrate but also by access to that substrate (Jones 2001). Forest birds that nest in cavities typically rely on old trees or snags, where most cavities are formed (Newton 1994), and in many cases their populations can be limited by the availability of suitable nesting sites (Martin & Li 1992; Newton 1994; 1998; Wiebe et al. 2006). Limitation of nesting sites, however, is more likely for secondary cavity-nesting species (i.e., non-excavators) because they rely on the presence of existing cavities (Newton 1994). As a consequence, nest-site availability for secondary-cavity nesters also is influenced by the presence of primary-cavity nesters that create cavities (Martin et al. 2004), and by the number of individuals seeking or competing for suitable nesting sites. Therefore, if nest sites are limited, some individuals may be forced to use lower quality cavities, suggesting that competition may be another important factor in nest-site choice (Nilsson 1984; Li & Martin 1991).

Distribution of resources important for breeding can vary naturally across the landscape. Human activities, however, such as selective logging or clearing of forest for pastures, can influence the distribution, abundance and availability of resources, especially those important for cavity-nesting birds (i.e., large trees and snags). For example, logging practices typically target large trees and can, therefore, reduce the abundance of cavities, leading in many cases to population limitation (e.g, Holt & Martin 1997, Chapter 2). On the other hand, habitat fragmentation can reduce the number of potential nest sites available to birds by reducing landscape connectivity. Connectivity refers to the degree to which a landscape facilitates or impedes ecological flow, such as the movement of organisms among habitat patches (Turner & Gardner 1990). Changes in landscape connectivity, such as those resulting from habitat fragmentation, may interfere with the dispersal of birds (Chapter 1), reducing the "functional connectivity" of the landscape (Belisle 2005). As a consequence, not all nest sites may be truly available for birds in areas with reduced connectivity. Therefore, if nest sites are limited (e.g., because of selective logging) birds may either disperse to other areas in search of suitable nesting sites or, if connectivity is reduced, stay in fragments and not reproduce or use cavities that otherwise would not be considered suitable.

Besides reducing landscape connectivity, habitat fragmentation can trigger changes in microclimatic conditions or changes in predator populations (Andren 1994), that also can influence the quality of nesting cavities in forest fragments. For instance, abundance of nest predators such as small mammals may either increase or decrease in small fragments, depending on the idiosyncrasies of each system (reviewed in Chalfoun et al. 2002). Although several studies have shown that nesting success for secondary-cavity nesters is not reduced in fragments (e.g., Matthysen & Adriansen 1998; Walters et al. 1999) other studies have reported increased nest predation in fragments (e.g., because of edge effects, Deng & Gao 2005). Therefore, because characteristics of successful *vs.* unsuccessful nest sites are likely to vary over time and space (Wiens 1985), nest site selection patterns should vary across the landscape as well.

Aphrastura spinicauda (Furnariidae) is a small and common secondary cavity-nesting bird in the temperate rainforest region of southern South America. It is most abundant in old-growth forests, but it does occur at lower densities in secondary forests (Diaz et al. 2005), forests with selective logging (Chapter 2), and exotic pine plantations

(Estades & Temple 1999). A nest-site supplementation experiment indicated that population densities of *A. spinicauda* were limited by nest-site availability in forests where selective logging had reduced the number of large trees (Chapter 2). Density and population responses to nest-site supplementation, however, varied not only in relation to the number of large trees and snags, but also in relation to connectivity (Chapter 2). These results suggested that nest-site availability, influenced by changes in forest structure, was an important factor limiting densities of *A. spinicauda*, but that other processes related to habitat fragmentation were also important in determining nest-site availability.

The goal of this study was to determine if nest-site selection by *A. spinicauda,* and associated consequences on fitness, vary spatially in response to forest structure (i.e. nest-site availability) and connectivity. Three aspects of nest-site selection were addressed (following Clark & Shutler 1999). First, characteristics of used and non-used territories and nest-trees were described across the landscape; differences in such characteristics, if they exist, are thought to provide evidence consistent with long-term natural selection. Second, characteristics of successful and unsuccessful nest-trees were compared by determining the source of variation in nest survival; such a comparison can provide evidence consistent with ongoing natural selection. Third, to show evidence for adaptive nest-site preferences, fidelity to nest-trees and the increased use of specific nest-trees were evaluated in relation to fitness.

Methods

Study site

This study was conducted in the temperate rainforest region on Isla Grande de Chiloé, southern Chile (41°55’S, 73°35’W), during three breeding seasons from 2003 through 2005. Climate in this region is wet temperate with a strong oceanic influence (2,000-2,500 mm rainfall/year; mean annual temperature of 12°C; Di Castri & Hajek 1976). The forest is a broad-leafed, evergreen rainforest of the Valdivian and North Patagonian types, with mixed dominance of *Nothofagus*, *Drymis*, *Eucryphia*, and *Podocarpus* trees, several trees and shrubs from the family Myrtaceae, and a dense bamboo (*Chusquea* spp.) understory. Large trees usually are covered with vines and epiphytes such as ferns, mosses, and bromeliads (Muñoz et al. 2003). The study area is in an approximately 25 x 25-km agricultural landscape in the vicinity of Senda Darwin Biological Station, northern Chiloé (Fig. 1a). The landscape is characterized by flat lands and hills (50 to 100 m elevation) that are covered by woodlands and forest fragments dispersed in a matrix of pastures, cultivated fields, and scrublands. Major forms of human-caused habitat degradation include habitat fragmentation caused by widespread use of fire to clear land for pastures, forest encroachment by cattle, and selective logging by local landowners for domestic and commercial use. As a consequence, human practices have generated a landscape mosaic with forest remnants that differ in their structure and presence of habitat features that are important for birds (Willson et al. 1994; Reid et al. 2004; Diaz et al. 2005).

Study design

Nest-site selection was compared among three forest habitats: old-growth forest, selectively-logged forest, and selectively-logged forest fragment (old-growth, logged-forest, and logged-fragments hereafter). Each habitat treatment was replicated in two distinct 10-ha study plots, for a total of six study plots (Fig. 1b). Old-growth and logged-forest plots were located in large (> 1,000 ha) and non-isolated forest stands (see Chapter 1) where processes associated with habitat fragmentation were assumed to be unimportant. In contrast, logged-fragment plots were established in forest fragments of about the same size as the study plots (10-12 ha); both fragments were isolated from other large forest fragments (see Chapter 1) and completely surrounded by open pastures with distances to the nearest forest ranging from 100 to 300 m (Fig. 1b). An array of six 300-m transects, encompassing a total of 1,800 m, was established in each 10-ha plot to facilitate nest searches and habitat measurements. In fragments, however, the number and length of transects was adjusted to fit the form of fragments but still covering a 10-ha area with a total transect length of 1,800 m in one fragment and 1,250 m in the other fragment.

Nest-site availability (as indexed by density of trees larger than 40 cm dbh and snags) was higher in old-growth forests than in logged-forest and logged-fragments (Chapter 2). Density of *A. spinicauda* was higher in old-growth forest than in logged-forest but not different from density in logged fragments. Consequently, density of birds in logged-fragments was higher than expected based on nest site availability. For details on *A. spinicauda* density estimations see Chapter 2. Further, a nest-site supplementation experiment provided evidence for nest-site limitation in both logged-forests and logged-fragments (see Chapter 2).

Nest-tree and territory characteristics

Nests in natural cavities were located during the three breeding seasons by systematically searching along transects in each plot, following standard nest-searching protocols (Martin & Geupel 1993). Behavioral cues, such as birds carrying nesting material or food, were used to help find nests; searches were not restricted to specific nest substrates to minimize potential for bias in where nests were found (Rodewald 2004). I used mist nets and play-backs to capture *A. spinicauda* individuals; birds were marked with numbered aluminum bands and a unique color combination to allow identification of birds associated with particular nests. Between 50% and 80% of individuals were color banded in each study plot. Each identified nest tree was monitored to determine its status (see below); nest-tree and cavity characteristics were recorded only for nests that were used for reproduction. Nest-tree characteristics recorded were tree species, tree condition (live or snag), diameter at breast height (dbh), height, and epiphyte cover (estimated in 5 cover classes 0: 0%, 1: 1%-25%, 2: 26% - 50%, 3: 51% - 75%, 4: 76% - 100%). Cavities were characterized by height from the ground, cavity type (1: in main trunk top, 2: in main trunk, 3: in secondary branch, 4: in broken branch end), origin of cavity (1: hole-fissure, 2: bird-made), diameter class (< 3 cm, 3-6 cm, > 6 cm), and concealment (percentage of vegetation, estimated in classes of 10% increments, in a 1-m radius and 1-m tall imaginary cylinder around the branch or trunk where the cavity was located). To determine characteristics of available trees and snags in each forest type, 10-m radius (314 m^2) circular plots were systematically placed every 100 m along transects within each study plot (18 vegetation plots/10 ha). In each circular plot, all large live trees and snags (> 40 cm dbh and >15 cm dbh, respectively) were counted with height, dbh and species recorded. All variables were tested for normality using a Shapiro-Wilk test and transformed to attain normality when possible. Nest-tree, cavity and available tree characteristics were compared using one- or two-way ANOVAs with forest type (old-growth, logged-forest, logged-fragments) and status (used and not used) as factors; Mann-Whitney or Kruskal-Wallis tests were used when data did not fit assumptions of parametric tests.

Nest boxes were systematically added along transects (108 boxes / 10 ha) in 2004 to conduct a nest-site-supplementation experiment in logged-forest and logged-fragment plots; details about nest boxes and their distribution within study plots are described in Chapter 2. Territory characteristics were recorded within the area surrounding natural nests (n = 30) and used nest boxes (n = 24). Habitat characteristics were measured in each territory along two 40-m perpendicular transects centered at the nest. All snags and live trees found in 2-m strips on each side of each transect were identified to the species level, counted, and assigned to a size class (dbh < 40 cm, 40 – 80 cm, and > 80 cm). Canopy cover, canopy height, and understory volume were recorded in five 2-m radius circular plots, one at each end of a transect and one centered on the nest. Canopy cover was estimated using four cover classes (1: 0-25%, 2: 26%-50%, 3: 51%-75%, 4: 76%-100); understory volume was determined by measuring understory height (h) and ground cover (c) estimated in 10%-increment cover classes within the 2-m radius (r) circle. Volume was then calculated as $h*c*\pi r^2$ following Reid et al. (2004). Mean values across the five circular plots were used in further analyses. To compare territory characteristics of used *vs.* unused territories, habitat measurements were recorded following the same methods but with transects centered on non-used nest boxes (n = 16). Non-used territories were defined as the area surrounding a nest box that was unoccupied during any season and had neither a natural nest nor a territory ever recorded within a radius of 50 m. Non-used territories were measured in logged-forest plots (n = 8) and in logged-fragment plots (n = 8).

Habitat variables were used to discriminate among the three different territory types evaluated in this study (i.e., natural nest, used nest box, and non-used territory) and among the three forest types using a discriminant function analysis. Covariance matrices were tested for homogeneity using Box's M criterion. The matrices showed non-significant heteroscedasticity ($P > 0.05$) so within-group covariance matrices were used. Wilk's lambda was used for separation of groups. Prior probabilities were computed from group sizes to account for differences in sample sizes among groups. Results from a MANOVA were also used to describe significant differences among habitat variables that determined group memberships.

Nest-success and nest-tree characteristics

Nest trees were visited every three to four days to determine nest status. Actual content of nests could not be assessed because cavities were too high and not accessible to the observers. Therefore, parent activity was observed from the ground with binoculars for 20-30 min. This observation period allowed recording at least one incubation exchange and several feeding visits (unpublished data). The number of times birds entered the cavity combined with observations of adults carrying material, food or fecal sacs was used to determine the stage of each nest (i.e. constructing, incubating, or feeding nestlings) (Martin & Geupel 1993). A nest was classified as successful either if no activity was observed at the nest after 21 days of feeding, the typical length of the nestling stage for this species (Moreno et al. 2005), or if a family group was found that could be attributed to that particular nest (i.e. based on color bands of adults). If the nest was found prior to the feeding stage and if no activity was observed during two consecutive observation periods before 21 days of feeding had elapsed, the nest was considered to have failed. Nests found during the feeding stage and to which no family group could be assigned, were classified as undetermined unless evidence of failure was observed (e.g. destroyed cavity).

The mean and variance of daily nest survival (DNS) rate were estimated with a maximum-likelihood approach based on the Mayfield method (Mayfield 1975) using Program MARK (White 2000) and the nest-survival procedure (Dinsmore et al. 2002). Akaike's information criterion corrected for small sample size (AIC*c*) was used to evaluate different hypotheses about the source of variation in nest survival for *A. spinicauda* in the study area. Nests in boxes had lower nest success than natural nests in this study area (unpublished data), so only natural nests were used to estimate nest success (i.e., to minimize potential biases from nests in boxes), with similar number of nests in each habitat treatment (see results). Daily nest survival rate was examined in relation to nest-tree characteristics (tree type, height, dbh and epiphyte cover), cavity characteristics (cavity height, type, concealment and diameter), year, and forest type. The relative importance of the eight tree and cavity variables in explaining variation in DNS was examined by first running a series of models that incorporated one variable at a time and second by comparing these models against the null-hypothesis model $S_{(.)}$ of constant

DNS. Of these models, only two models (that included the variables tree type or epiphyte cover) had more support than the S $_{(.)}$ model. Therefore, only the variables tree type (snag, live tree) and epiphyte cover (1-5) were used in addition to forest type (old-growth, logged-forest, logged-fragments) and year (2003, 2004, 2005) to develop a set of 11 *a priori* defined models (Table 1). Factors in models were incorporated as covariables and competing models were compared against the null-hypothesis model $S_{(.)}$ of constant DNS. A sine-link function for the constant-DNS model was used and a logit-link function for models that incorporated covariates (Dinsmore et al. 2002). These analyses only included natural nests with known fate (n = 74) because nests with uncertain fate can not be included into these models. Although this may bias DNS estimates (Manolis et al. 2000), a previous analysis with the same data set showed that DNS estimates from program MARK were not downward-biased when nests with uncertain fate were excluded (Chapter 2). Nesting success was assessed as the probability of surviving the entire nesting cycle, from the egg-laying period through the nestling period until fledging, which was considered to be 45 days for *A. spinicauda* based on Moreno et al. (2005) and field observations. Nest success and its associated variance were estimated by raising DNS rate to the exponent of the duration of the nestling cycle (Rotella 2005)

Nest-tree preferences and reuse patterns

Nest-tree preferences were evaluated by comparing the number of trees used for nesting with the number of trees available within predefined categories using Likelihood Ratio tests (*G*) and their associated *P*-values. To determine if use of nest-trees was adaptive, categories compared were defined by characteristics of successful and unsuccessful nests. First, use and availability were compared between snags and live trees without considering the identity of species within categories. Second, use and availability were compared among species of trees to evaluate preference for or avoidance of particular tree species.

As a second line of evidence for an adaptive value of nest-site preference, fidelity to nest-sites was examined in relation to nest success. A cavity was considered to be reused if at least one banded bird (i.e., an individually identifiable bird) used the same cavity in two or more consecutive breeding attempts within or among seasons. Because not all birds were banded, it was not always possible to determine the identity of birds reusing a particular cavity. From 39 reused nests, 12 had known-identity birds (both individuals banded n = 7; one individual banded n = 5). In all 12 cases, at least one individual reused a nest cavity in subsequent nesting attempts. Thus, in this study I assume that most if not all reuse events involved at least one bird from the previous nesting attempt. Moreover, all active nests in a breeding season were checked for reuse in subsequent seasons; therefore, patterns observed represented true reuse frequencies.

If fidelity to nest-sites is more frequent for nests in which fitness was previously high, as expected if nest-site choice is adaptive, then nests that are reused in subsequent seasons should have an overall higher nest success than nests that are not reused. To determine this, DNS was evaluated in relation to reuse of nest-trees. Daily nest survival was also evaluated in relation to tree type, forest type and year to determine if reuse varied across space and time. The reuse variable was determined by classifying each nest as either used or not used in the subsequent season. Consequently, only 2003 and 2004 nests (n = 56) were included in this analysis because 2005 nests were not checked for reuse in the following season. A set of predefined models was developed and candidate models were evaluated based on minimum AIC*c* values (Table 2). Again, factors in models were incorporated as covariables and competing models were compared against the null-hypothesis model $S_{(.)}$ of constant DNS following the same procedures described above.

Results

Nest-tree and territory use patterns

A total of 80 different nest trees (corresponding to 102 nesting attempts) were identified during the course of three breeding seasons. Of these, 15 were not used for reproduction and were excluded from analyses. Nest trees were either large canopy trees (n = 35) or snags (n = 35); size (dbh) of nest trees and snags did not differ ($F_{1,69} = 2.13$, $P = 0.13$). Mean size (± SE) of nest trees and snags combined (85.1 ± 3.5 cm dbh) was higher than the mean size of available trees and snags (57.3 cm ± 1.8 cm; $F_{1,341} = 27.3$, $P < 0.001$; Fig. 2). When used and available trees were compared among forests (two-way ANOVA), there was a significant effect of size of trees among forests ($F_{2,341} = 6.72$, $P = 0.001$) and a significant difference between size of trees used for nesting and those available ($F_{1,341} = 27.3$, $P < 0.001$) with a significant interaction ($F_{2,341} = 4.76$, $P = 0.009$) between forest and tree status (i.e. used *vs.* available). Thus, in terms of diameter, trees used for nesting in fragments were, overall, smaller and more similar to trees available than in old-growth and logged-forest plots where trees used for nesting were larger than available trees (Fig. 3).

In live trees, most cavities used for nesting were in large canopy *Nothofagus nitida* (27 out of 35 nests in live trees). Other tree species used were *Eucryphia cordifolia*, *Laureliopsis philippiana,* and three species of the family Myrtaceae. Most snags used for nesting were dead *Nothofagus nitida* trees (24 out of 35 nests in snags); remaining snags were mostly *Weinmania trichosperma* or undetermined species: a few were *Saxe-gothaea conspicua*, *Laureliopsis philipiana,* and *Eucryphia cordifolia*. For further analyses, all snags were grouped into a single category regardless of species. Proportional use of live tree species and snags differed among forest types ($G = 26.33$, df = 12, $P = 0.01$). In old-growth and logged-forest plots, most nest cavities were in *Nothofagus* trees or in snags, whereas nests were found in a greater variety of tree species in fragments (Fig. 4). For 73 active-nest cavities, tree-height /cavity-height ratio did not differ among forest types ($F_{2,72} = 1.9$, $P = 0.16$). Cavities used for nesting were usually in tall canopy trees at a mean height (± SE) of 15.5 ± 0.7 m; two nest cavities found in forest fragments were, however, less than 1 m from the ground. Types of cavities used for nesting included small crevices or fissures in trunks (47.9%) or in secondary branches (26%), within broken branch ends (15.1%), or in trunks with broken tops (4.1%); only 6.8 % were in cavities made by other birds. Proportional use of cavity types did not differ among forest types ($G = 9.15$, df = 8, $P = 0.33$). Most cavities were small with diameters smaller than 3 cm (46.8 %) or of 3 – 6 cm (51.1 %); only one had an entrance diameter larger than 6 cm. Most cavities had no or very little concealing vegetation (70.1% of cavities had 0% or 10% cover; Fig. 5).

Habitat characteristics varied among territories that surrounded natural nests, nest-boxes, and non-used nest boxes (MANOVA, $F_{2,12} = 3.51$, $P < 0.0001$, Fig. 6). The first axis of the discriminant function analysis was highly significant ($W = 0.557$, $P < 0.001$) and explained 75.1% of the variance; the second axis accounted for the remaining 24.9% and was only marginally significant ($W = 0.851$, $P = 0.065$). The structure coefficients

(SC) of the correlations between discriminating variables and the first standardized canonical discriminant function showed a high positive relationship for canopy cover (SC = 0.92), number of small live trees (SC = 0.427), and canopy height (SC = 0.304) and a negative correlation for understory volume (SC = -0.426). Number of large live trees (SC = -0.195) and number of snags (SC = -0.127) had low loadings in the first axis, but had high positive loadings in the second axis (SC = 0.729 and SC = 0.446, respectively; Fig. 7). Based on these two axes, territories of *A. spinicauda* surrounding natural nests and nest boxes were characterized by an open canopy with a dense understory with a few large trees and snags whereas unused territories had a closed canopy, scant understory and many small trees.

The canonical axes correctly classified 60% of territories. This success rate appears rather low because used nest-box and natural-nest territories were interchangeably classified with each other. Of 24 nest-box territories, 41.7% were classified correctly, whereas 45.8% were classified as a natural-nest territory. Similarly, of 30 natural-nest territories, 66.7% were classified correctly with 20% classified as a a used nest-box territory. The remaining natural-nest and nest-box territories were classified as non-used territories (13.3% and 12.5%, respectively). Non-used territories (i.e. the area that surrounds an unused nest box) had the greatest classification accuracy, with 75% (12 of 16) of non-territories classified correctly. When the analysis was repeated after pooling natural-nest and used nest-box territories into one category, there was a highly significant separation ($W = 0.655$, $P < 0.001$) between used and non-used territories. Structure coefficients remained qualitatively and quantitatively similar but classification accuracy increased substantially for nest site categories, with 90.7% of used territories (i.e., either nest-boxes or natural nests) classified correctly. Classification success did not change for non-territories. Finally, characteristics of territories around nests (natural nests and nest-box territories combined) were similar among the three forest types, with no significant separation among forests ($W = 0.666$, $P = 0.082$).

Nest success and tree characteristics

Characteristics of 74 known-fate nests in old-growth ($n = 33$), logged-forest ($n=27$) and logged-fragments ($n = 14$) were used to evaluate the effects of nest-tree type (snag or live tree), tree epiphyte cover, forest type and year on DNS, using 11 *a priori* models (Table 1). The first six models were equally supported with ΔAIC*c* values < 2; of these models, $S_{\text{(tree)}}$ and $S_{\text{(epiphyte)}}$ had the fewest parameters and had similar model weights and likelihood (Table 1). Thus, there were no differences in nest success among the three forest types; differences were only given by the characteristics of trees in which nests were placed. This suggests that nest success was influenced more by tree characteristics than by forest type or year. Based on estimates from the $S_{\text{(tree)}}$ model, nest success (± SE) was higher in cavities in snags (74.8% ± 9) than in cavities in live trees (46.9 % ± 10). Based on the $S_{\text{(epiphyte)}}$ model, DNS decreased with epiphyte cover (Fig. 8). Because epiphyte cover was correlated with type of tree, snags had less epiphyte cover than live trees ($U = 640.0$, $P = 0.034$), the underlying variable that links tree type with DNS is very likely the amount of epiphyte cover on trees. This is also supported by the model with lowest AIC*c* that included both tree type and epiphyte cover as explanatory variables (Table 1). Moreover, among nest trees, *Nothofagus* was intermediate in epiphyte cover relative to snags (least cover) and the remaining tree species (most cover); Kruskal-Wallis test; $\chi^2 = 9.05$, $df = 2$, $P = 0.011$).

Nest-site selection and reuse patterns

The frequency of use of different tree types was compared to the frequency of available tree types to identify potential nest-site preferences. These preferences were also evaluated among forests to determine if nest-site selection varied over space and between successful and unsuccessful nests to determine if nest site selection is adaptive. Overall, snags were used as nest sites more often than expected based on their availability ($G = 7.67$, df = 1, $P = 0.006$) compared to when all live trees were combined (Fig. 9). This result, however, was not consistent among forests. In old-growth forest and logged-forest plots, snags and live *Nothofagus* trees were used in a higher proportion than expected based on their availability whereas all other tree species combined were used less than expected ($G = 20.56$, df = 2, $P < 0.001$ and $G = 36.63$, df = 2, $P < 0.001$, respectively). In contrast, in logged fragments, the use of snags, live *Nothofagus* trees, and all other tree species combined did not differ from expected based on their availability ($G = 2.79$, df = 2, $P = 0.248$; Fig. 9).

To examine nest-tree fidelity patterns, nest success was evaluated in relation to reuse (i.e., reused or not), tree type (snag or live tree), forest type, and year. The first two models $S_{(reuse + tree)}$ and $S_{(reuse)}$ had more support than the constant DNS model $S_{(.)}$, but the first model had almost twice the model likelihood and weight as the second (Table 2). Thus, based on the model with most support, $S_{(reuse + tree)}$, nest success (± SE) of *A. spinicauda* in trees that were reused was higher (89.7 % ± 9.7 %, n = 16) than in trees that were not reused (56 % ± 9.8 %, n = 40). Moreover, nests in snags, which have a higher probability of survival, also were more likely to be reused than nests in live trees.

Models that included the variables reuse and forest type or year were ranked low (Table 2), suggesting that reuse patterns were similar among forest types and years.

Discussion

Spatial differences in nest-site selection by *Aphrastura spinicauda* were mostly given by patterns observed in fragments relative to patterns observed in large and connected forests regardless of nest-site availability (i.e. with or without logging). Here, I discuss possible mechanisms that may drive the observed spatial differences in nest-site characteristics and nest-site selection.

Nest trees and territories used: evidence for long-term selection

Most trees used for nesting by *A. spinicauda* were large canopy trees or large snags, presumably because these trees are more likely to form cavities than are younger and smaller trees (Newton 1994). The majority of cavities used for nesting were natural cavities that developed where branches became detached, because of fungal decay or because of trunk wounds, knots or crevice formation; less than 7% of used cavities were made by other birds. Although primary-cavity nesters have an important role in determining cavity availability for small secondary-cavity nesters in northern forests (Martin et al. 2004), in the south-temperate forest, primary-cavity nesters seem not to play an important role for small cavity nesters like *Aphrastura*.

In old-growth and logged-forest plots*, Aphrastura spinicauda* selected large snags and large live *Nothofagus nitida* trees. In fragments, however, nest-trees used were not different in size from available trees and nests were found in several different tree species, including several species of the family Myrtaceae, which were not used as nesting sites in old-growth or logged-forest plots despite their presence in these forests.

This different pattern of nest-tree use could be attributed to differences in forest structure. However, logged-fragments and logged-forest plots had similar forest structure, in terms of density of snags and large trees (Chapter 2), but had different nest-tree use patterns. On the other hand, nest-site use patterns were similar between old-growth and logged-forest plots despite their differences in forest structure. Therefore, the observed nest-tree use pattern in fragments is more likely a consequence of birds having to stay in fragments because of isolation (i.e. reduced connectivity), and not a consequence of differences in forest structure.

Contrary to the pattern observed for nest-trees, characteristics of territories were similar among the different forest types. In general, *A. spinicauda* territories were characterized by areas with canopy gaps, with fewer small trees and more large trees and snags, and with a dense understory. Several lines of evidence in this study suggest that territory selection is relatively conserved. Nest-boxes used for nesting were in areas with habitat characteristics similar to those surrounding natural nests. Second, territory characteristics were similar regardless of the level of habitat degradation and connectivity of the forests (i.e., territory characteristics did not vary among the three forest types examined). Third, characteristics of habitat surrounding nest-boxes that were not used for nesting were very different, characterized by a secondary-forest type, with a closed canopy and very little understory vegetation. Territory characteristics are important for survival of adults and for success of breeding attempts by providing shelter and food resources. Insect biomass is usually higher in canopy gaps than in closed-canopy areas within forests in this study area (Chacón & Armesto 2006) and an open canopy also allows a dense understory to develop. Understory vegetation, which in this forest region is characterized by the presence of a dense bamboo (*Chusquea* spp*)* layer*,* provides foraging substrate and shelter that is especially important for the post-fledgling period (as observed for other bird species in this region, Reid et al. 2004). A less variable territory selection pattern across the landscape may be due to the fact that selective logging and fragmentation (e.g., by producing more edge habitat) may actually provide open-gap conditions for *A. spinicauda* similar to those found in natural tree-fall gaps in old-growth forests.

These results suggest that certain territory characteristics are required and, therefore, the presence of a good cavity by itself does not necessarily imply a suitable nesting site. As suggested by nest-tree and territory-use patterns in this study, the behavior for territory selection seems less variable than the behavior for nest-tree selection (i.e., with a higher level of plasticity). Moreover, whereas nest-tree selection is likely explained by a "predation-avoidance" model (e.g., Nilsson 1984), territory selection is more likely explained by a "food-based" model (e.g., Burke & Nol 1998). Studies that specifically test these hypotheses have not been carried out yet, and would be important to determine the relative importance of food and predators as limiting factors (Martin 1995).

Successful and unsuccessful nest-sites: evidence for ongoing selection

Nests in snags had a higher probability of survival than nests in live trees in the study area. Moreover, trees with little epiphyte cover were also safer nesting sites than trees covered by epiphytes. These two variables, however, are correlated because snags usually have little or no epiphyte cover, whereas live trees usually have a dense epiphyte cover (Muñoz et al. 2003). Small mammals are important nest predators in the south-temperate rainforest (Willson et al. 2001) but no information is available about the specific identity of nest predators for *Aphrastura*. A small arboreal marsupial (*Dromicipos gliroides*), however, is among the most probable predators for *Aphrastura spinicauda* nests in the study region because other small rodents have poor climbing abilities (Gallardo-Santis et al. 2005) and snakes do not usually enter moist forest habitats in this region (Greene & Jaksic 1992). Therefore, it is likely that a bare trunk provides a safer nest-site because it exposes nest-predators, like the small opossum, to their own predators. Nest-cavities also had very low vegetation concealment regardless of the tree type in which they were found, supporting this hypothesis. Other studies also have shown that successful nests of cavity nesters are usually those with low vegetation concealment because attending parents have a better view of predators approaching nests (Li & Martin 1991). Finally, nest success was not different among the three forest types studied, despite the fact that patterns of nest-site use were different in fragments as manifested by the use of smaller trees and a different set of tree species. This differential use, however, did not have consequences for nesting success, as initially predicted.

Adaptive nest-tree preferences

Support for an adaptive nest-site choice (Clark & Shutler 1999) in this study comes from two lines of evidence. First, nest trees in which nest success was high (i.e., snags) were used in a higher proportion than expected based on their availability and, secondly, fidelity was high in nest trees in which fitness was previously high.

In old-growth and logged-forest plots, located in large and connected forests, snags were used more often than expected based on their availability suggesting that nest-site choice is adaptive. Among live trees, *A. spinicauda* nested more often than expected in *Nothofagus nitida* trees, based on their availability. *Nothofagus* are likely better trees for nesting because they have intermediate levels of epiphyte cover when compared to snags and other live trees. Other tree species, however, were used less than expected based on their availability. Although this also may be related to differences in epiphyte cover, it may reflect differences in tree-structure that influence the propensity to form cavities rather than an active avoidance of other trees.

In fragments, where nest-site availability was reduced by selective logging and density of birds was higher than expected (Chapter 2), birds used nest-trees in direct proportion to their availability in relation to both size and species. This result could be interpreted as a lack of nest-site selection in fragments. On the other hand, an adaptive nest-site selection implies that fitness is higher in nest-sites preferred for nesting (Jones 2001). Therefore, in old-growth forest, nest-sites not used for nesting are expected to be avoided because of the negative effects on fitness they may entail. In fragments, however, the use of nest-trees that were usually not used in old-growth forests had no consequences on nest success. Given that there was no cost to using different nest trees in fragments, at least at the level of nest success, it is possible that cavities are of higher quality in fragments.

If processes triggered by habitat fragmentation influence quality of available cavities (e.g. through changes in predator densities, interacting species or microclimatic conditions), and if birds are able to assess these changes, observed nest-site use patterns could reflect behavioral adaptive plasticity in nest site selection (Forstmeier & Weiss 2004). A few studies have suggested that birds are capable of assessing changes in habitat quality and adjust their nest-site choice accordingly (Forstmeier & Weiss 2004; Haemig

1999; Schmidt et al. 2006; Fontaine & Martin 2006*b*). Therefore, if fragmentation affects populations of nest-predators, then cavities that are usually of low quality in old-growth forest are safer nesting sites in fragments. For instance, the strength of top-down forces can vary across the landscape depending on the ecological responses of prey and predator populations to landscape changes (Patten & Bolger 2003). In fact, populations of the small arboreal marsupial have been shown to be affected by fragmentation in an area north of the site where this study was conducted (Saavedra & Simonetti 2005).

Finally, re-use patterns of nest-sites were consistent with an adaptive response of nest-site choice. Nests that were reused had an overall higher daily nest survival than nests that were not reused. This suggests that successful nests were more likely to be reused than nests that failed in a previous nesting attempt. Furthermore, nests in snags had a higher nest success than nests in live trees and snags were reused more often than live trees. Nest reuse patterns were not influenced by nest-site availability or connectivity, showing a consistent pattern of reuse across the landscape.

Concluding remarks

Nest-site preferences are considered to be adaptive if habitat characteristics of sites used for nesting are different from available habitat, if variation in characteristics of successful and unsuccessful nests exists, and, finally, if an adaptive response of nest-site preference is demonstrated (Clark & Shutler 1999; Jones 2001). Results of this study not only show evidence for an adaptive nest-site preference in *A. spinicauda* but also show that nest-site choices are spatially variable in response to ecological gradients produced by human-driven landscape changes.

In logged-fragments, individual birds are likely faced with a nest-site selection trade-off between dispersing to other sites in search of suitable cavities and nesting in a forest with reduced nest-site availability. Given that there was no cost to using different nest trees in fragments, an alternative explanation is that birds are able to assess changes in habitat quality (Forstmeier & Weiss 2004). As a consequence, birds may stay in fragments as long as suitable territories are available and not because of a cost associated with dispersal over open habitats. On the other hand, genetic data on *A. spinicauda* populations in this same fragmented landscape (Chapter 1) show that gene flow is reduced to a certain degree among fragments, and that small fragments account for most of the observed genetic structure among populations. Therefore, it is likely that nest-site selection patterns in fragments are a combination of a dispersal trade-off and the capability of individual birds to assess changes in habitat quality. In this human-dominated south-temperate forest system, populations of the cavity-nesting bird *A. spinicauda*, as also shown for ground cavity-nesting species of the family Rhinocryptidae (Willson et al. 2001; De Santo et al. 2002), are more likely to be limited by nest-site availability and the access to these nest sites than by nesting success.

Literature Cited

Andren H. (1994) Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. *Oikos* 71: 355-366

Belisle M. (2005) Measuring landscape connectivity: The challenge of behavioral landscape ecology. *Ecology* 86:1988-1995

Burke D.M., Nol E. (1998) Influence of food abundance, nest-site, and forest fragmentation on breeding Ovenbirds. *Auk* 115: 96-104

Chacón P., Armesto J.J. (2006) Do carbon-based defenses reduce foliar damage? Habitat-related effects on tree seedling performance in a temperate rainforest of Chiloé Island, Chile. *Oecologia* 146: 555-565

Chalfoun A.D., Thompson F.R., Ratnaswamy M.J. (2002) Nest predators and fragmentation: a review and meta-analysis. *Conservation Biology* 16: 306-318

Clark R.G., Shutler D. (1999) Avian Habitat Selection: patterns from process in nest-site use by ducks? *Ecology* 80: 272-287

De Santo T.L., Willson M.F., Sieving K.E., Armesto J.J. (2002) Nesting biology of tapaculos (Rhinocryptidae) in fragmented south-temperate rainforests of Chile. *Condor* 104: 482-495

Deng W.H., Gao W. (2005) Edge effects on nesting success of cavity-nesting birds in fragmented forests. *Biological Conservation* 126: 363-370

Di Castri F., Hajek E. (1976) Bioclimatología de Chile. Ediciones Universidad Católica de Chile, Santiago, Chile

Diaz I.A., Armesto J.J., Reid S., Sieving K.E., Willson M.F. (2005) Linking forest structure and composition: avian diversity in successional forests of Chiloé Island, Chile. *Biological Conservation* 123: 91-101

Dinsmore S.J., White G.M., Knopf F.L. (2002) Advance techniques for modeling avian nest survival. *Ecology* 83: 3476-3488

Estades C.F., Temple S. (1999) Deciduous-forest bird communities in a fragmented landscape dominated by exotic pine plantations. *Ecological Applications* 9: 573-585

Fontaine J.J., Martin T.E. (2006) Parent birds assess nest predation risk and adjust their reproductive strategies. *Ecology Letters* 9: 428-434

Fontaine J.J., Martin T.E. (2006b) Habitat selection responses of parents to offspring predation risk: and experimental test. *American Naturalist* 168: 811-818.

Forstmeier W., Weiss I. (2004) Adaptive plasticity in nest-site selection in response to changing predation risk. *Oikos* 104:487-499

Gallardo-Santis A., Simonetti J.A., Vasquez R.A. (2005) Influence of tree diameter on climbing ability of small mammals. *Journal of Mammalogy* 86: 969-973

Greene H.W., Jaksic F.M. (1992) The feeding-behavior and natural history of two Chilean snakes, *Philodrias chamissonis* and *Tachymenis chilensis* (Colubridae). Revista *Chilena de Historia Natural* 65: 485-493

Haemig P.D. (1999) Predation risk alters interactions among species: competition and facilitation between ants and nesting birds in a boreal forest. *Ecology Letters* 2:178-184

Holt R.F., Martin K. (1997) Landscape modification and patch selection: The demography of two secondary cavity nesters colonizing clear cuts. *Auk* 114: 443-455

Jaenike J., Holt R.D. (1991) Genetic variation for habitat preference: evidence and explanations. *American Naturalist* 137: S67-S90

Jones J. (2001) Habitat selection studies in avian ecology: A critical review. *Auk* 118:557-562

Li P., Martin T.E. (1991) Nest-site selection and nesting success of cavity-nesting birds in high elevation forest drainages. *Auk* 108: 405-418

Manolis J.C., Andersen D.E., Cuthbert F.J. (2000) Uncertain nest fates in songbird studies and variation in Mayfield estimation. *Auk* 117: 615-626

Martin K., Aitken K.E.H., Wiebe K.L. (2004) Nest sites and nest webs for cavity-nesting communities in interior British Columbia, Canada: Nest characteristics and niche partitioning. *Condor* 106:5-19

Martin T.E. (1993) Nest predation and nest sites - new perspectives on old patterns. *BioScience* 43: 523-532

Martin T.E. (1995) Avian life-history evolution in relation to nest sites, nest predation, and food. *Ecological Monographs* 65: 101-127

Martin T.E. (1998). Are microhabitat preferences of coexisting species under selection and adaptive? *Ecology* 79: 656-670.

Martin T.E., Geupel G.R. (1993) Nest-monitoring plots: methods for locating nests and monitoring success. *Journal of Field Ornithology* 64: 507-519

Martin T.E., Li P. (1992) Life history traits of open- vs. cavity-nesting birds. *Ecology* 72: 579-592

Matthysen E., Adriansen F. (1998) Forest size and isolation have no effect on reproductive success of Eurasian Nuthatches (*Sitta europea*). *Auk* 115:955-963

Mayfield H. (1975) Suggestions for calculating nest success. *Wilson Bulletin* 87:456-466

Moreno J., Merino S., Vásquez R.A., Armesto J.J. (2005) Breeding Biology of the Thorn-tailed Rayadito (Furnariidae) in south-temperate rainforests of Chile. *The Condor* 107: 69-77

Muñoz A., Chacon P., Perez F., Barnert E.S., Armesto J.J. (2003) Diversity and host tree preferences of vascular epiphytes and vines in a temperate rainforest in southern Chile. *Australian Journal of Botany* 51:381-391

Newton I. (1994) The role of nest-sites in limiting the numbers of hole-nesting birds: a review. *Biological Conservation* 70: 265-276

Newton I. (1998) Population limitation in birds. Academic Press Limited, San Diego, CA.

Nilsson S.G. (1984) The evolution of nest-site selection among hole-nesting birds: the importance of nest predation and competition. *Ornis Scandinavica* 15: 165-175

Patten M.A., Bolger D.T. (2003) Variation in top-down control of avian reproductive success across a fragmentation gradient. *Oikos* 101: 479-488

Reid S., Diaz I.A., Armesto J.J., Willson M.F. (2004) Importance of native bamboo for understory birds in Chilean temperate forests. *Auk* 121: 515-525

Ricklefs R. (1969) An analysis of nesting mortality in birds. *Smithsonian Contributions in Zoology* 9: 1-48.

Rodewald A.D. (2004) Nest-searching cues and studies of nest-site selection and nesting success. *Journal of Field Ornithology* 75:31-39

Rotella J.J. (2005) Nest survival models, pp. 122-124. In: *Program MARK "A gentle introduction"* (Cooch E., White G.M., eds), 5th edition.

Saavedra B., Simonetti J.A. (2005) Small mammals of Maulino forest remnants, a vanishing ecosystem of south-central Chile. *Mammalia* 69: 337-348

Schmidt K.A., Ostfeld R.S., Smyth K.N. (2006) Spatial heterogeneity in predator activity, nest survivorship, and nest-site selection in two forest thrushes. *Oecologia* 148: 22-29

Turner M., Gardner R. (1990) Quantitative methods in landscape ecology. Springer-Verlag, New York

Walters J.R., Ford H.A., Cooper C.B. (1999) The ecological basis of sensitivity of brown treecreepers to habitat fragmentation: a preliminary assessment. *Biological Conservation* 90: 13-20

White G.M. . MARK: Mark and Recapture Survival rate Estimation. [5.1] 2000, Department of Fishery and Wildlife, Colorado State University, Fort Collins, CO

Wiebe K.L., Koenig W.D., Martin K. (2006) Evolution of clutch size in cavity-excavating birds: the nest site limitation hypothesis revisited. *American Naturalist* 167: 343-353

Wiens J.A. (1985) Habitat selection in variable environments: shrub-steppe birds, pp. 227-251. In: *Habitat selection in birds* (Cody M., ed.). Academic Press, Toronto, Canada.

Willson M.F., De Santo T.L., Sabag C., Armesto J.J. (1994) Avian communities of fragmented south-temperate rainforests in Chile. *Conservation Biology* 8: 508-520

Willson M.F., Morrison J., Sieving K.E., De Santo T.L., Santisteban L., Diaz I. (2001) Patterns of predation risk and survival of bird nests in a Chilean agricultural landscape. *Conservation Biology* 15: 447-456

Table 1. Summary of model selection to evaluate the effects of nest-tree characteristics, forest type and year on nest survival of *A. spinicauda* in the south-temperate rainforest in Chiloé (2003-2005) based on 74 known-fate nests in natural cavities. K = number of parameters; models are ranked by ascending ΔAIC*c*; w_i is normalized model weight.

Model	K	**AIC*c***	**ΔAIC*c***	w_i	**Model Likelihood**
$S_{\text{(tree + epiphyte cover)}}$	3	151.241	0.00	0.176	1.0000
$S_{\text{(tree + epiphyte cover + year)}}$	4	151.277	0.04	0.173	0.982
$S_{\text{(tree)}}$	2	151.622	0.38	0.146	0.827
$S_{\text{(tree + year)}}$	3	151.970	0.73	0.123	0.695
$S_{\text{(epiphyte cover)}}$	2	152.239	1.00	0.107	0.607
$S_{\text{(tree + epiphyte cover + forest)}}$	4	153.176	1.94	0.067	0.380
$S_{\text{(tree +forest)}}$	3	153.528	2.29	0.056	0.319
$S_{\text{(tree + forest + year)}}$	4	153.548	2.31	0.056	0.316
$S_{(.)}$	1	153.742	2.50	0.051	0.286
$S_{\text{(year)}}$	2	154.989	3.75	0.027	0.154
$S_{\text{(forest)}}$	2	155.746	4.51	0.019	0.105

Table 2. Summary of model selection to evaluate reuse patterns in relation to nest-tree type, forest type and year on nest survival of *A. spinicauda* in the south-temperate rainforest in Chiloé (2003-2005) based on 56 known-fate nests in natural cavities. *K* = number of parameters; models are ranked by ascending ΔAIC*c; w_i* is normalized model weight.

Model	***K***	**AIC*c***	**ΔAIC*c***	w_i	**Model Likelihood**
$S_{\text{(reuse + tree)}}$	3	96.676	0.00	0.26023	1.0000
$S_{\text{(reuse)}}$	2	97.983	1.31	0.13537	0.5202
$S_{\text{(reuse + tree + year)}}$	4	98.229	1.55	0.11966	0.4598
$S_{\text{(tree)}}$	2	98.304	1.63	0.11526	0.4429
$S_{\text{(reuse + tree + forest)}}$	4	98.331	1.66	0.11375	0.4371
$S_{\text{(reuse + forest)}}$	3	98.851	2.18	0.08768	0.3369
$S_{\text{(reuse + year)}}$	3	99.988	3.31	0.04966	0.1908
$S_{(.)}$	1	100.029	3.35	0.04867	0.1870
$S_{\text{(reuse + year + forest)}}$	4	100.859	4.18	0.03213	0.1235
$S_{\text{(forest)}}$	2	101.844	5.17	0.01964	0.0755
$S_{\text{(yeart)}}$	2	102.023	5.35	0.01796	0.0690

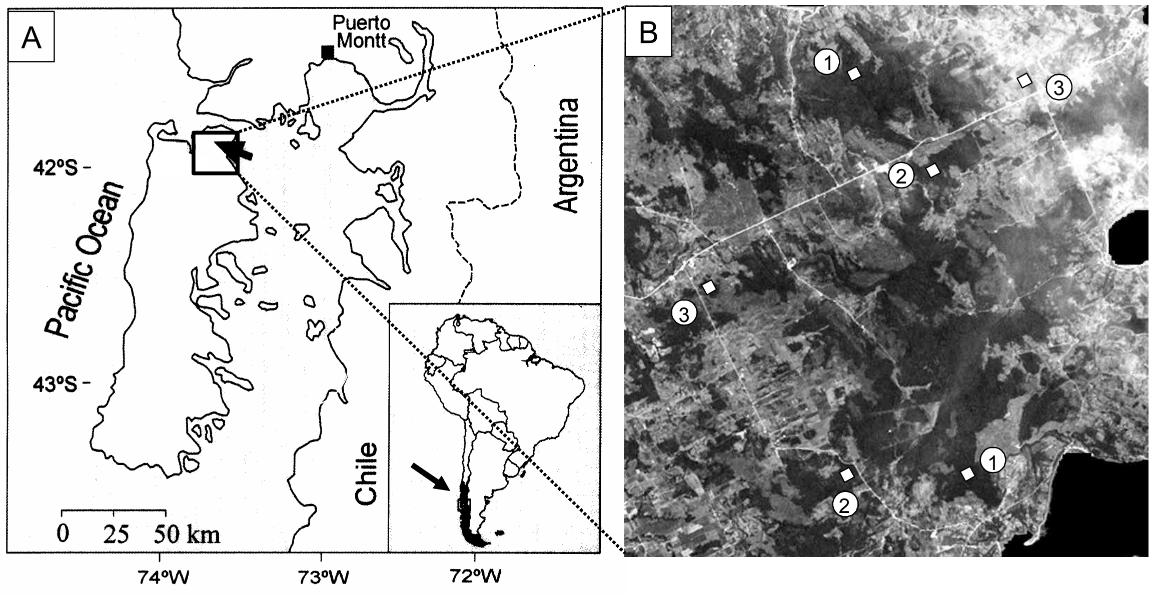

Figure 1. Study region (A) and study sites (B) in Isla Grande de Chiloé, southern Chile. The satellite image (Landsat 5-T 2001) shows the spatial pattern of forest fragments in the study area. Squares represent 10-ha study plots in each of three forest types (1 = old-growth forest, 2 = logged-forest, 3 = logged-fragments)

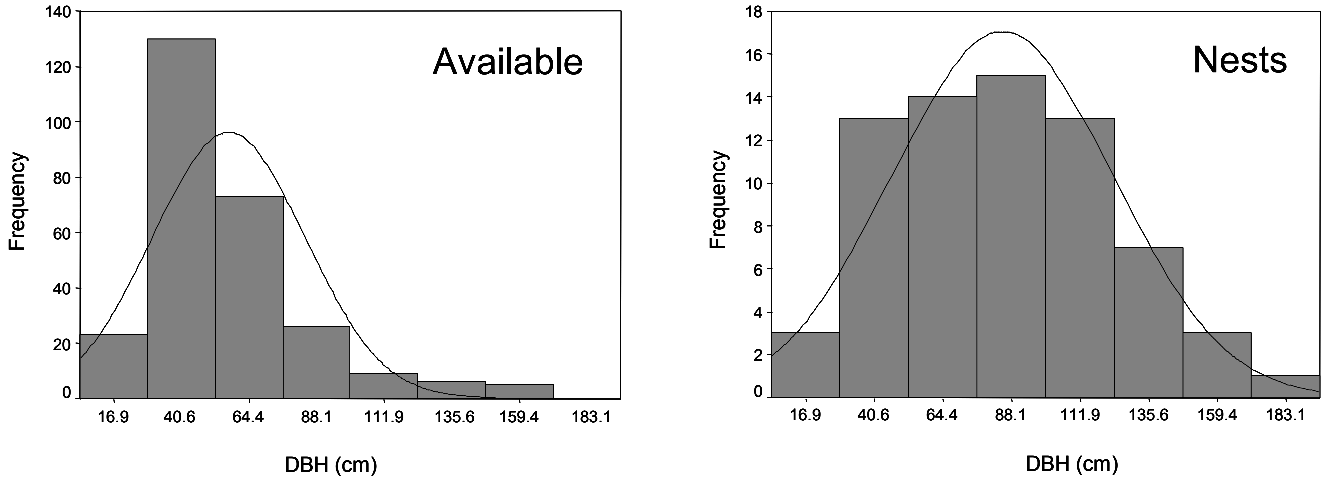

Figure 2. Size distribution and normal curve, as measured by diameter at breast height (dbh), of available trees and trees used for nesting by *Aphrastura spinicauda* in Chiloé.

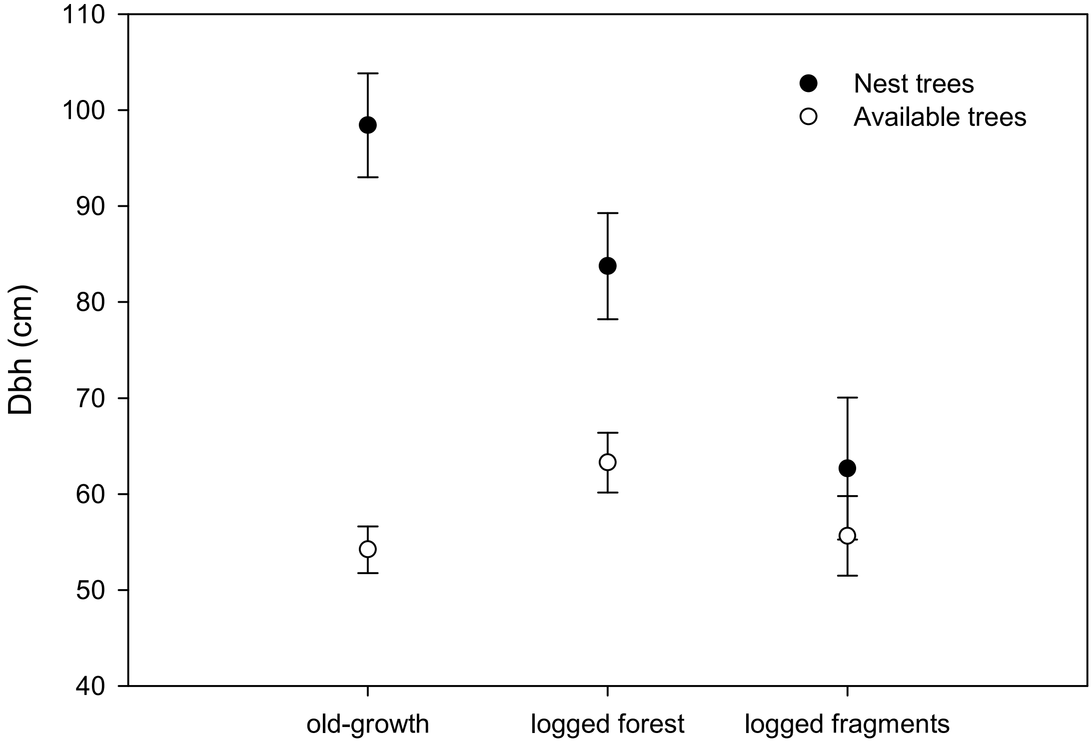

Figure 3. Mean size (± SE) as measured by diameter at breast height (dbh), of nest trees used by *Aphrastura spinicauda* and trees available in the three forest types in Chiloé.

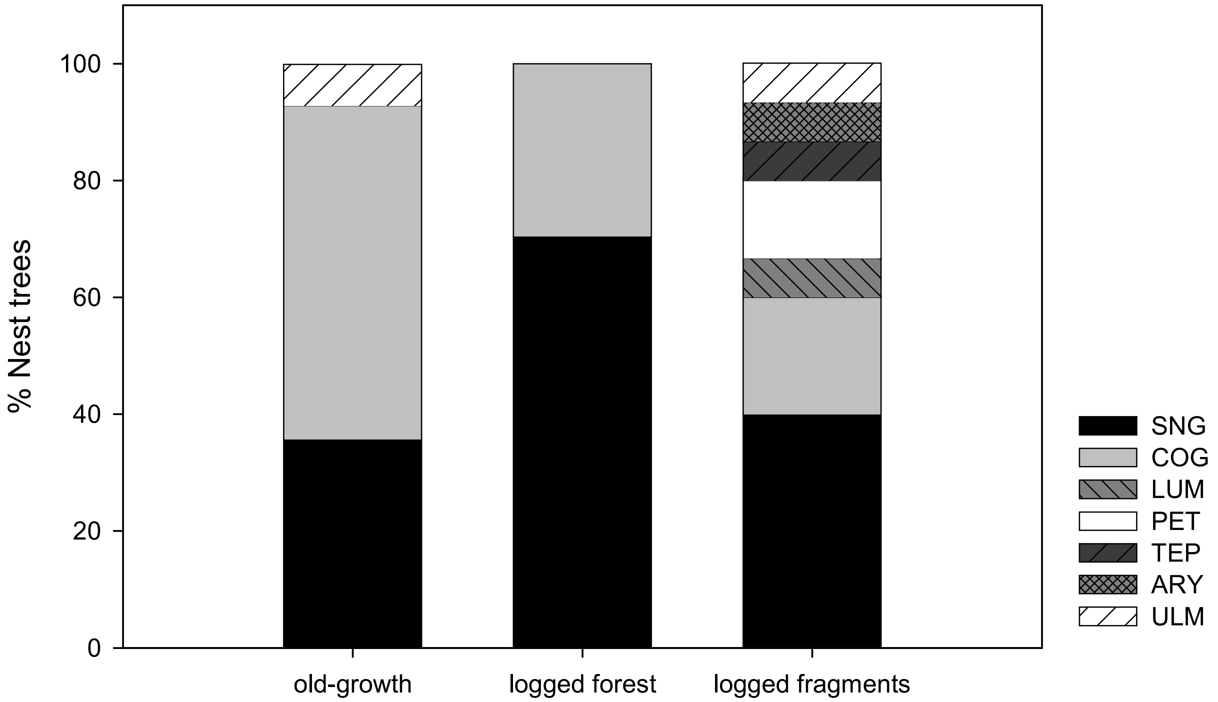

Figure 4. Percentage of tree species and snags used for nesting by *Aphrastura spinicauda* in the three forest types in Chiloé. SNG = snag, COG = Coigüe (*Nothofagus nitida),* LUM = Luma (*Amomyrtus luma*), PET = Peta (*Myrceugenia exsucca*), TEP = Tepa (*Laureliopsis philippiana*), ARY = Arrayán (*Luma apiculata*)*,* ULM = Ulmo (*Eucryphia cordifolia*).

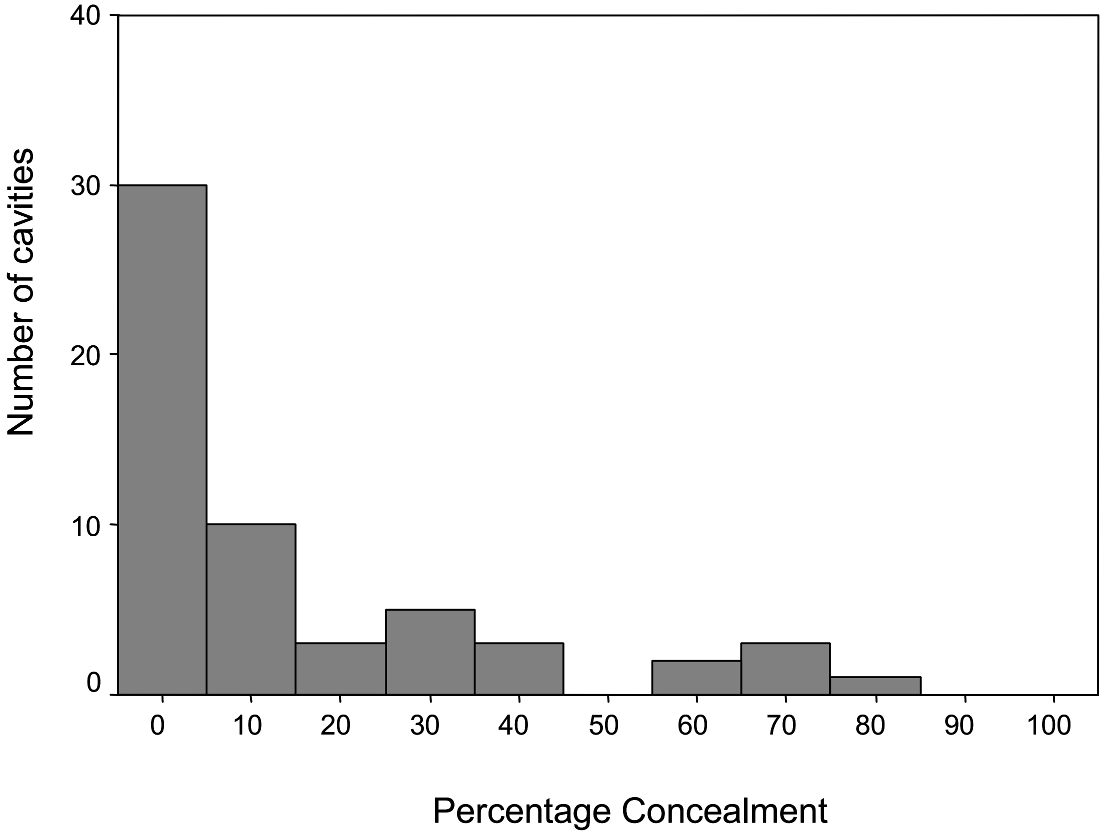

Figure 5. Frequency of nest cavities with different levels of vegetation concealment in Chiloé forests based on 57 active nests.

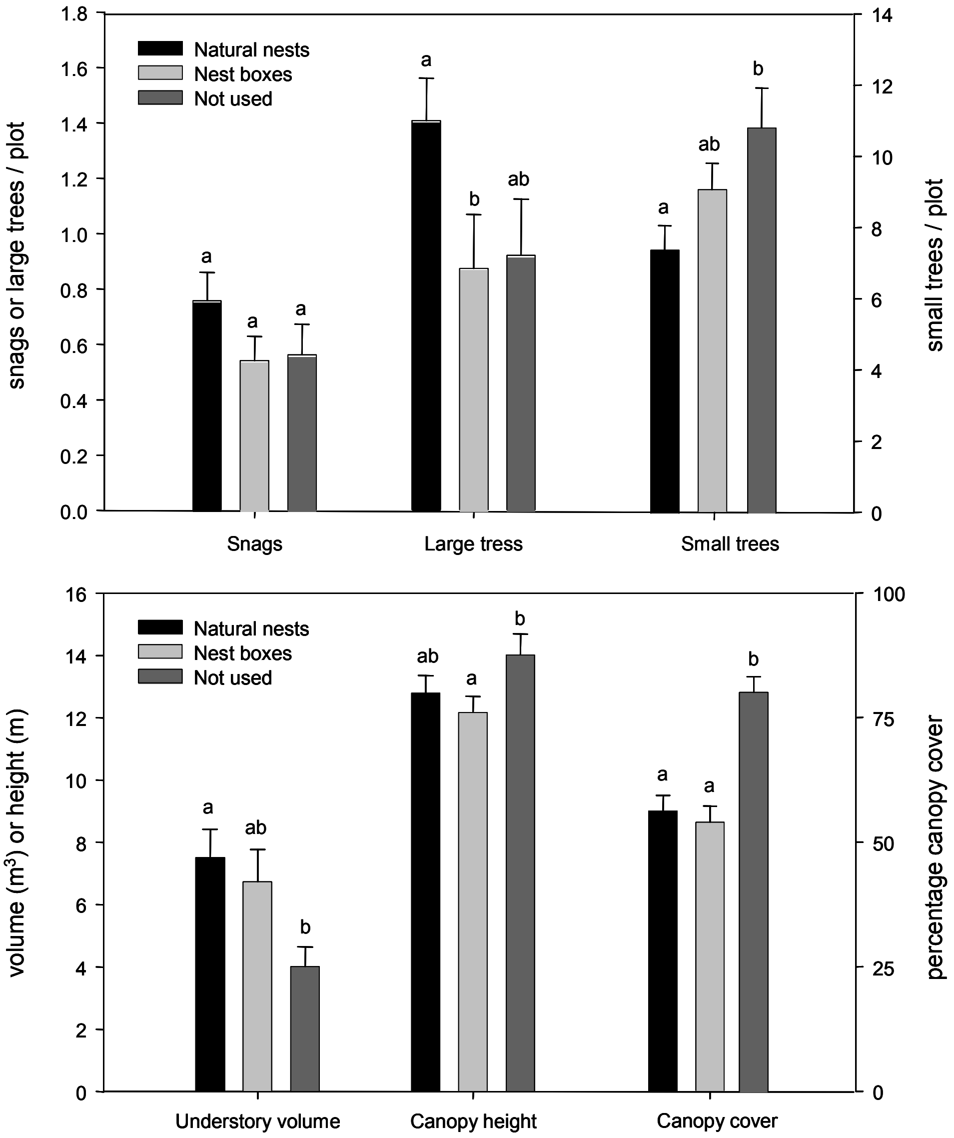

Figure 6. Vegetation characteristics within a 20-m radius plot centered on a natural nest, a used nest box, and a non-used nest box (i.e. unused territory). Different letters denote significant differences at the $P < 0.05$ level within each vegetation variable (Tukey post-hoc test for multiple comparisons).

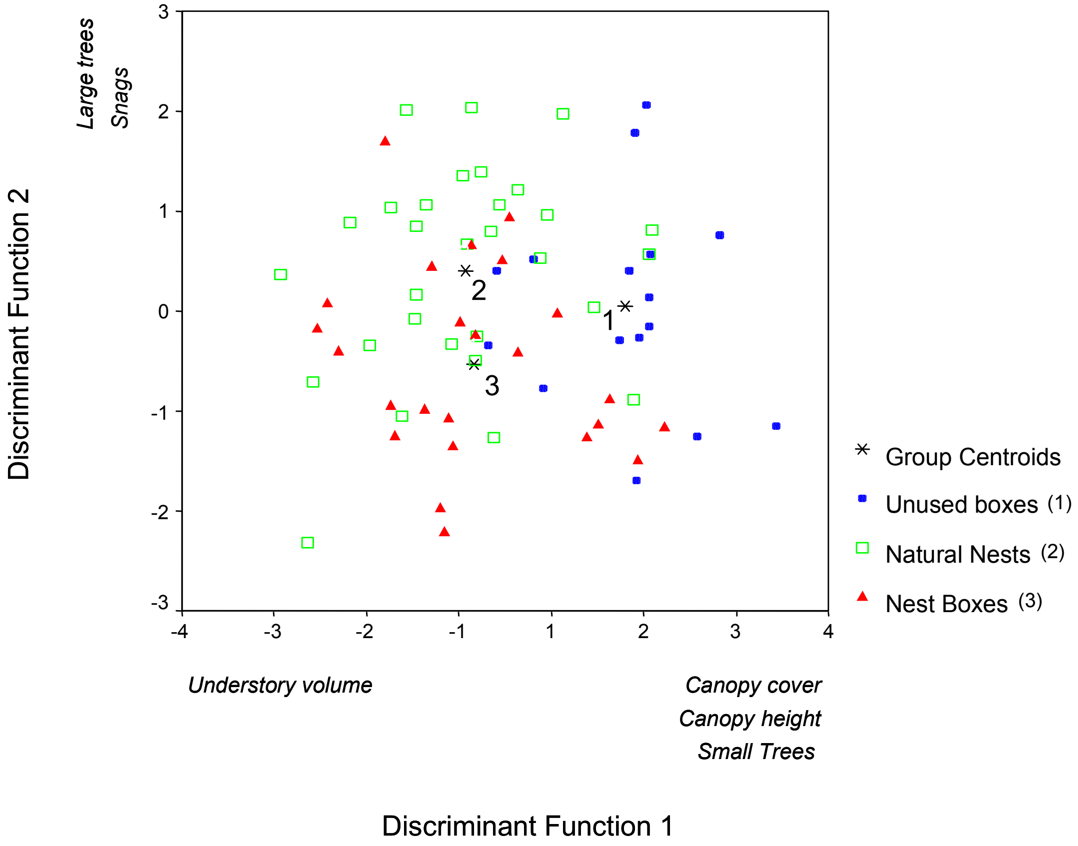

Figure 7. Canonical discriminant function of habitat characteristics around natural nests, used nest boxes, and unused nest boxes of *Aphrastura spinicauda* in Chiloé.

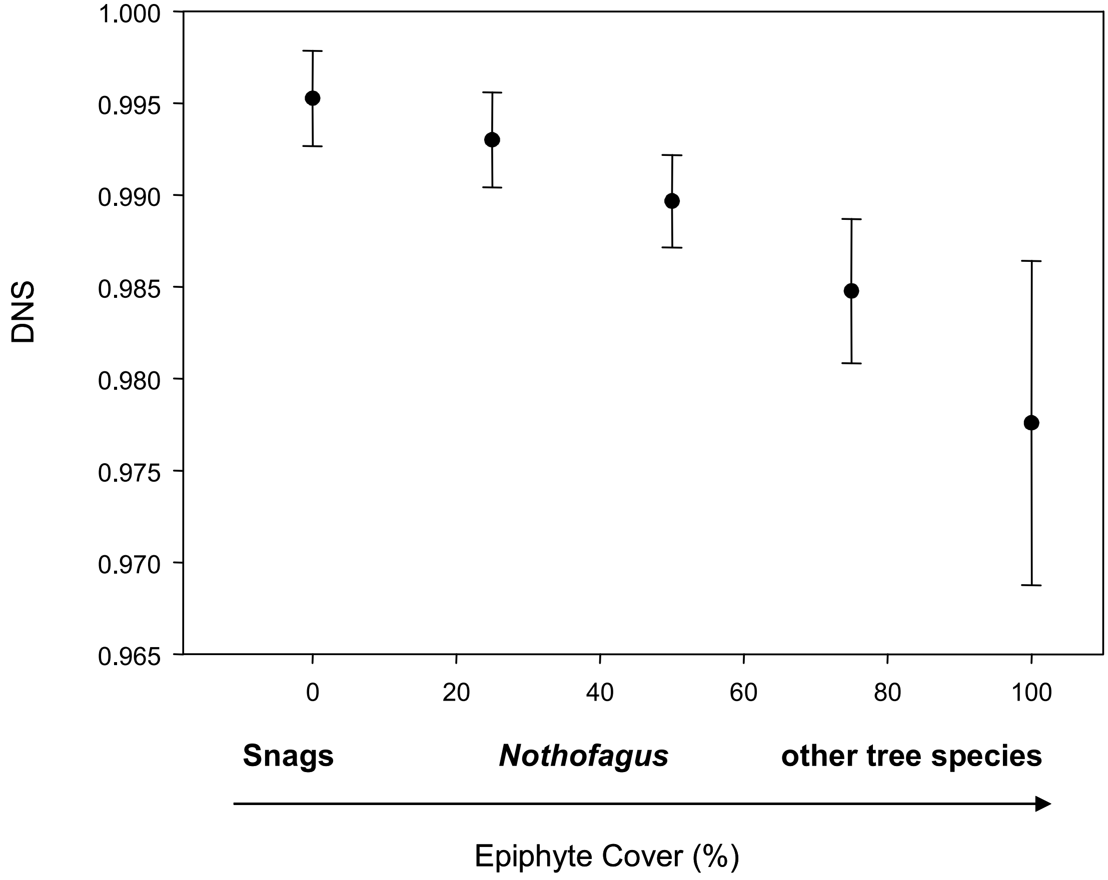

Figure 8. Daily nest survival (DNS) for *Aphrastura spinicauda* related to epiphyte cover of trees, based on 74 known-fate models of three breeding seasons (2003-2004) in Chiloé.

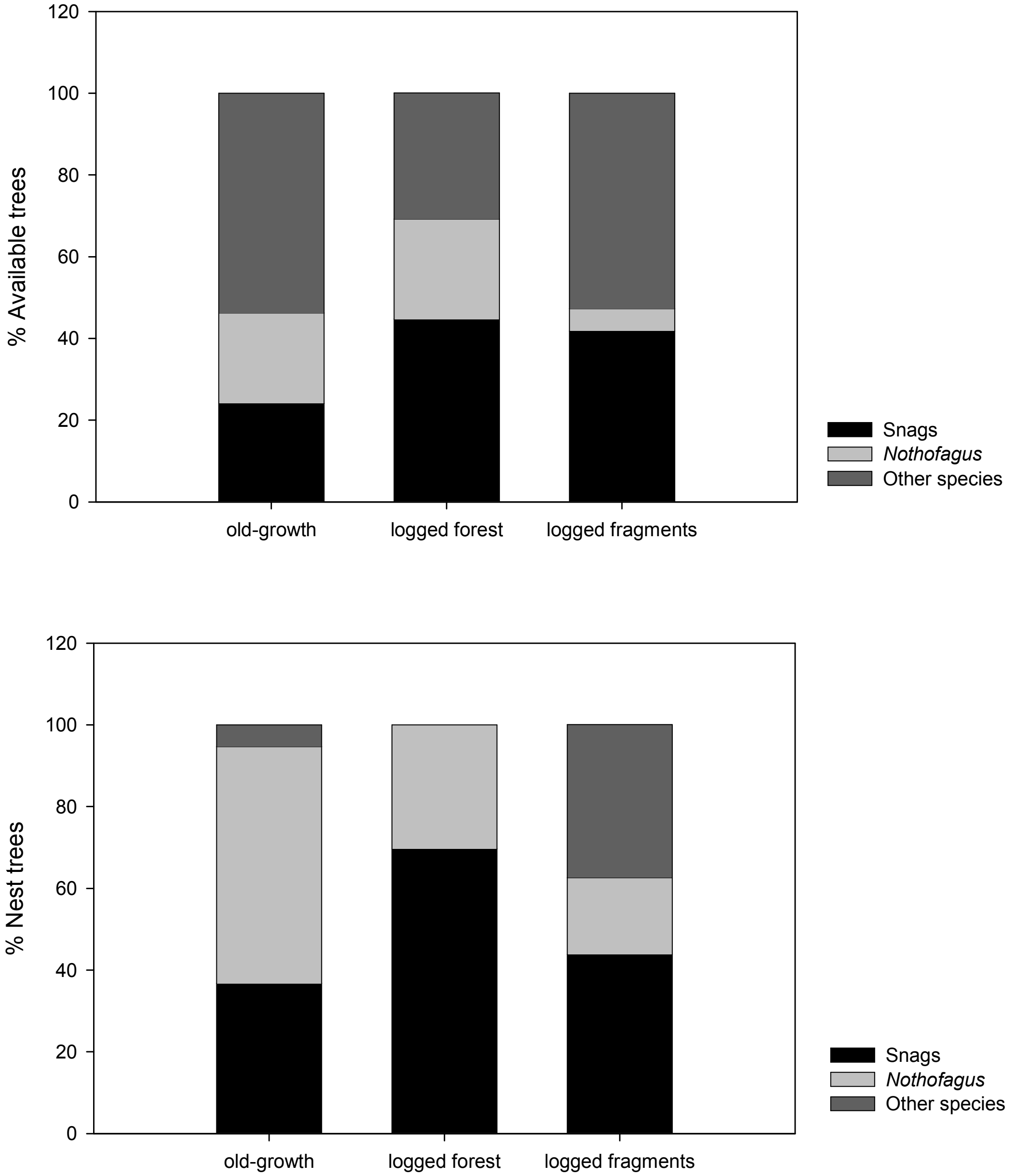

Figure 9. Comparison of proportions of trees available and trees used for nesting by *Aphrastura spinicauda* within three categories (snags, *Nothofagus nitida* trees and other tree species) among the three forest types in Chiloé.