Reduced Genetic Diversity and Increased Dispersal in Guigna (*Leopardus guigna*) in Chilean Fragmented Landscapes

Constanza Napolitano, Diego Díaz, Jim Sanderson, Warren E. Johnson, Kermit Ritland, Carol E. Ritland, and Elie Poulin

From the Laboratorio de Ecología Molecular, Instituto de Ecología y Biodiversidad, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Santiago, Chile (Napolitano and Poulin); Departamento de Ciencias Ambientales y Recursos Naturales, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, Chile (Díaz); Small Wild Cat Conservation Foundation, Campbell, CA (Sanderson); Smithsonian Conservation Biology Institute, Front Royal, VA (Johnson); and Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, BC, Canada (K. Ritland and CE. Ritland).

Address correspondence to Constanza Napolitano at the address above, or e-mail: cnapolit@uchile.cl.

Data deposited at Dryad: [http://dx.doi.org/doi:10.5061/dryad.65pm3](http://dx.doi.org/doi:10.5061/dryad.65pm3)

Received July 31, 2014; First decision December 14, 2014; Accepted March 27, 2015.

Corresponding editor: Antonio Solé-Cava

Abstract

Landscape fragmentation is often a major cause of species extinction as it can affect a wide variety of ecological processes. The impact of fragmentation varies among species depending on many factors, including their life-history traits and dispersal abilities. Felids are one of the groups most threatened by fragmented landscapes because of their large home ranges, territorial behavior, and low population densities. Here, we model the impacts of habitat fragmentation on patterns of genetic diversity in the guigna (*Leopardus guigna*), a small felid that is closely associated with the heavily human-impacted temperate rainforests of southern South America. We assessed genetic variation in 1798 base pairs of mitochondrial DNA sequences, 15 microsatellite loci, and 2 sex chromosome genes and estimated genetic diversity, kinship, inbreeding, and dispersal in 38 individuals from landscapes with differing degrees of fragmentation on Chiloé Island in southern Chile. Increased fragmentation was associated with reduced genetic diversity, but not with increased kinship or inbreeding. However, in fragmented landscapes, there was a weaker negative correlation between pairwise kinship and geographic distance, suggesting increased dispersal distances. These results highlight the importance of biological corridors to maximize connectivity in fragmented landscapes and contribute to our understanding of the broader genetic consequences of habitat fragmentation, especially for forest-specialist carnivores.

Resumen

La fragmentación del paisaje es una de las principales causas de extinción de especies debido a que afecta una amplia variedad de procesos ecológicos. El impacto de la fragmentación varía en las distintas especies dependiendo de diversos factores, como las características de historia de vida y la capacidad de dispersión. Los felinos son uno de los grupos más amenazados por la fragmentación del paisaje debido a sus extensos ámbitos de hogar, conducta territorial y baja densidad poblacional. Estudiemos los impactos de la fragmentación del hábitat sobre los patrones de diversidad genética...
de la güiña (Leopardus guigna), un pequeño felino estrechamente asociado a los bosques templados lluviosos del sur de Sudamérica, fuertemente impactado por actividades antrópicas. Evaluamos la variación genética en 1798 pb de secuencias del ADN mitocondrial, 15 loci microsatélites y 2 genes del cromosoma sexual y estimamos la diversidad genética, parentesco, endogamia y dispersión en 38 individuos pertenecientes a paisajes con distintos grados de fragmentación en la isla de Chiloé en el sur de Chile. Un mayor grado de fragmentación estuvo asociado con una menor diversidad genética, pero no con mayor parentesco o endogamia. Sin embargo, en paisajes con mayor grado de fragmentación se encontró una correlación negativa débil entre parentesco entre pares de individuos y distancia geográfica, sugiriendo un aumento en las distancias de dispersión. Estos resultados resaltan la importancia de los corredores biológicos para maximizar la conectividad en paisajes fragmentados y contribuyen al conocimiento de las amplias consecuencias genéticas de la fragmentación del hábitat, especialmente para carnívoros especialistas de bosque.

**Subject areas:** Conservation genetics and biodiversity

**Key words:** conservation genetics; habitat fragmentation; inbreeding; kinship; South America; temperate rainforests
been the least affected historically, retaining a substantial portion of its original native vegetation (Willson and Armesto 1996). However, continuation of current trends in deforestation would lead to substantial loss and fragmentation of the remaining forest fragments over the next decades (Echeverría et al. 2008).

The guigna (Leopardus guigna; Carnivora: Felidae) is a small felid closely associated with temperate rainforests of southern South America (Acosta-Jamett and Simonetti 2004). It has the most restricted distribution of all the New World cat species, inhabiting only about 300 000 km² of Chile (30°–48° S) from sea level up to 2500 m and a narrow strip of southwestern Argentina (39°–46° S, 70° W; Napolitano et al. 2014). Considered to be one of the two most threatened wild cat species in South America, together with the Andean cat (Leopardus jacobita; Napolitano et al. 2008), guignas are classified by the IUCN Red List as vulnerable with a decreasing population trend (Napolitano et al. 2015). Current threats for guignas include severe habitat loss and fragmentation and direct persecution (Napolitano et al. 2015; Gálvez et al. 2013). Recent evidence of feline immunodeficiency virus and feline leukemia virus infection in free-ranging guignas from human perturbed landscapes on Chiloé Island (possibly transmitted from domestic cats) may pose a further challenge to guigna populations (Mora et al. 2015). Guigna abundance estimates suggest total effective population size may be fewer than 10 000 mature breeding individuals and no subpopulation having an effective population size larger than 1000 mature breeding individuals (Napolitano et al. 2015). A recent population genetics and phylogenetics study revealed that guigna populations inhabiting Chiloé Island are genetically isolated from mainland populations, but do not show genetic structure within the island (Napolitano et al. 2014).

In the highly modified human agricultural landscapes of northern Chiloé Island, guignas exclusively use vegetation corridors (as small as 3 m wide) to move among forest fragments, avoiding open areas (pastures with vegetation <0.4 m high; Sanderson et al. 2002). As with most felids, vegetation cover is an important ecological requirement for guignas, used for dispersion, stalking prey, and reproduction (Palomares et al. 2000). Although roads do not seem to be a barrier for guigna movement (Sanderson et al. 2002), they are relevant in terms of mortality, given that road kills are not infrequent on Chiloé Island (Napolitano 2012). Home range sizes and maximum dispersal distances of guignas in the highly modified, fragmented landscape of northern Chiloé Island were 1.3–22.4 km² and 13.9 km (mean = 5.5 ± 4.9), respectively (Sanderson et al. 2002), whereas in 2 pristine protected areas in the Aysén Region (Laguna San Rafael and Queulat National Parks), home range sizes and maximum dispersal distances were 0.3–2.2 km² and 1.83 km (mean = 1.49 ± 0.25), respectively (Dunstone et al. 2002). In fragmented landscapes, home ranges of males are exclusive of other males and likewise for females (females can be found within the range of males; Sanderson et al. 2002), whereas in 2 pristine protected areas in Aysén Region, extensive overlap of home ranges and core areas was observed (Dunstone et al. 2002). These patterns suggest facultative dispersal ability and spatial overlap of guignas in relation to landscape features (Napolitano 2012).

Landscape modification and habitat fragmentation are central research topics of conservation biology, recently leading to important analytical approaches and insights on how ecological and microevolutionary processes can be affected in fragmented landscapes. This research has resulted in novel pattern-oriented and species-oriented research approaches (Fischer and Lindenmayer 2007), and the increasing integration of new genetic and ecological techniques to document and predict the impacts of landscape modification on demographic and genetic patterns of key species (Keyghobadi 2007).

The objective of this study was to investigate the genetic consequences of anthropogenic landscape fragmentation on patterns of genetic diversity, kinship and inbreeding levels, and dispersal ability of L. guigna inhabiting landscapes with differing degrees of fragmentation on Chiloé Island. Our hypotheses considered that L. guigna exclusively uses areas with significant vegetation cover as habitat (i.e., forest), other vegetation cover for migration only (i.e., shrubland), avoids open areas (i.e., pastures), and in fragmented landscapes is subjected to other disturbing elements, which increase isolation among subpopulations and migration-related mortality risk (i.e., roads). Our hypotheses were based on the theoretical genetic consequences associated with small, isolated populations inhabiting fragmented landscapes. Reduced population size would lead to a loss of genetic diversity due to the increased influence of genetic drift and local extinctions. Gene flow disruption between small, isolated subpopulations would lead to increased levels of kinship and inbreeding, due to a higher probability that 2 mating individuals will share recent common ancestry. Based on these theoretical expectations and our understanding of L. guigna ecology, we predicted a priori that increasing degrees of landscape fragmentation would be associated with 1) low carrying capacity, patch isolation, local extinctions, and higher mortality rates during dispersal (road kills), driving L. guigna populations to small effective sizes and consequently decreased genetic diversity, 2) increased kinship and inbreeding levels due to a higher prevalence of matings among related individuals in these small, isolated populations, and 3) increased dispersal to seek for available resources (i.e., habitat, food, etc.) in response to the gaps in habitat. We tested our hypotheses by using landscape genetic, quantifying land cover with biological meaning for L. guigna and comparing genetic patterns of populations inhabiting landscapes with differing degrees of fragmentation on Chiloé Island, allowing us to test for differences among groups.

Methods

Sample Collection

Blood samples from captured free-ranging guignas, fecal samples, and tissue samples from recent road kills and recent retaliatory killings belonging to 38 guigna individuals were collected from 2008 to 2010 in 22 localities from 3 study areas, Northern, Central, and Southern Chiloé Island (NCI, CCI, and SCI; Figure 1, see Supplementary Table S1 online). The study areas share similar climatic and biogeographic characteristics (Luebert and Pliswof 2006); however, according to previous records (Willson and Armesto 1996), they differ in their degree of landscape fragmentation, which is greatest in the northern part of the island and decreases moving southward. Thus, the definition of NCI, CCI, and SCI groups was based on these landscape differences, allowing us to test for differences among groups in relation to our hypothesis.

Live captures were carried out using 40 Tomahawk live traps, baited with chicken and fish and checked twice a day. Total trapping effort was 2875 trap-nights, using sampling grids and placing traps 1 km apart in similar proportions in NCI, CCI, and SCI. Captured guignas were anesthetized intramuscularly with ketamine hydrochloride (Ketamina 100, Chemie, 15 mg/kg), blood samples obtained by cephalic venipuncture, and released at the same capture site once completely recovered. Small passive microchips (8.5 x x2.12 mm) were implanted subcutaneously to individualize captured guignas. Guigna capture and tissue collection were carried out with permission from the Agriculture and Livestock Service (SAG), following handling and supervision protocols within bioethical and animal welfare frameworks (National Research Council 2011).
We have deposited the primary data underlying these analyses with Dryad following data archiving guidelines (Baker 2013).

**Cartographic Database Analysis**

Geographic positions for each collected sample were located spatially on a map, considering for each of them a circle of 10-km radius as buffer area. The extent of buffer areas was defined as a conservative criterion following estimations of maximum linear movement for the species on northern Chiloé Island (13.9 km, Sanderson et al. 2002). Three polygons (A, B, and C) including sample collection sites and their respective buffer areas were drawn for each of the 3 study areas (Figure 1 II). Each of the 3 polygons encompassed approximately 125 000 hectares, allowing for further analysis to be based on comparable amounts of landscape area. Within each polygon, land cover was derived from the database Catastro y Evaluación de Recursos Vegetacionales Nativos de Chile (CONAF-CONAMA-BIRF 1997), whereas roads were derived from cartography of the Dirección General de Aguas, Ministerio de Obras Públicas de Chile (DGA) at a scale of 1:50 000. The database recognizes 8 different main land cover types (Figure 1), from which we selected a suite with biological meaning with respect to their influence on guigna spatial use and migration through a landscape: 1) forest, including old-growth and young temperate rainforests (guigna habitat); 2) shrubland (used for migration only); 3) pastures, including agricultural and natural grassland or herbaceous cover (avoided matrix); 4) roads, including paved, gravel, and dirt roads (potentially increasing fragmentation and migration-related mortality risk). We analyzed the selected land cover types within each of the 3 polygons to compare the degree of landscape fragmentation among study areas.

**Laboratory Procedures**

Genomic DNA was extracted from blood, tissue, or fecal samples using commercially available kits (DNeasy Blood and Tissue kit, QIAamp DNA Stool Mini Kit; Qiagen) following the manufacturer’s suggested protocols. Nucleotide sequences of 5 mitochondrial DNA (mtDNA) gene segments encompassing 1798 base pairs (bp) were obtained by PCR amplification (Saiki et al. 1983): 1) NADH dehydrogenase subunit 5 (NADH-5, 720bp) using primers ND5-DF1 and ND5-DR1 (Trigo et al. 2008); 2) 16S rDNA gene (364 bp) as in Hoelzel and Green (1992) and Johnson et al. (1998); 3) adenine triphosphate (ATP-8); 4) part of the ATP-6 gene (275 bp) using primers ATP8-DF1 and ATP6-DR1 (Trigo et al. 2008); and 5) the 5ʹ portion of the control region (CR) containing the First Hypervariable Segment (HVS-I; 439 bp) using primers CHF3 and CHR3 (Freeman et al. 2001). PCR reactions were performed in a 25-µL volume containing 1.5 µL of PCR buffer, 1.5–2.0 mm of MgCl2, 0.2 mm each dNTP, 0.5 U of Taq DNA polymerase (Invitrogen), and 0.2 µm of each primer. Thermocycling parameters consisted of an initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 53–63 °C for 30 s (depending on the primer set), 72 °C for 30 s, and a final extension of 72 °C for 10 min. PCR products were checked using ethidium bromide-stained 1.5% agarose gels. Forward and reverse strands were sequenced using an ABI 3730XL analyzer by Macrogen Inc.

Fifteen nuclear DNA microsatellite loci (12 tetranucleotide repeat loci [FCA441, F124, F41, FCA424, F141, F146, FCA391, FCA453, F42, F98, F164, and F27] and 3 dinucleotide repeat loci [FCA008, FCA176, FCA698]), developed originally for the domestic cat (Menotti-Raymond et al. 1999), were amplified separately by PCR in a 15-µL volume containing 1.5 µL of PCR buffer, 1.5–2.0 mm of MgCl2, 0.2 mm each dNTP, 0.5 U of Taq DNA polymerase (Invitrogen), and 0.2 µm of each primer. Thermocycling parameters consisted of an initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 53–55 °C for 1 min (depending on the primer set), 72 °C for 1 min, and a final extension of 72 °C for 10 min. PCR products were checked using ethidium bromide-stained 1.5% agarose gels. Forward and reverse strands were sequenced using an ABI 3730XL analyzer by Macrogen Inc.

Fifteen nuclear DNA microsatellite loci (12 tetranucleotide repeat loci [FCA441, F124, F41, FCA424, F141, F146, FCA391, FCA453, F42, F98, F164, and F27] and 3 dinucleotide repeat loci [FCA008, FCA176, FCA698]), developed originally for the domestic cat (Menotti-Raymond et al. 1999), were amplified separately by PCR in a 15-µL volume containing 1.5 µL of PCR buffer, 1.5–2.0 mm of MgCl2, 0.2 mm each dNTP, 0.5 U of Taq DNA polymerase (Invitrogen), and 0.2 µm of each primer. Thermocycling parameters consisted of an initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 53–55 °C for 1 min (depending on the primer set), 72 °C for 1 min, and a final extension of 72 °C for 10 min. PCR products were checked using ethidium bromide-stained 1.5% agarose gels. Forward and reverse strands were sequenced using an ABI 3730XL analyzer by Macrogen Inc.

Fifteen nuclear DNA microsatellite loci (12 tetranucleotide repeat loci [FCA441, F124, F41, FCA424, F141, F146, FCA391, FCA453, F42, F98, F164, and F27] and 3 dinucleotide repeat loci [FCA008, FCA176, FCA698]), developed originally for the domestic cat (Menotti-Raymond et al. 1999), were amplified separately by PCR in a 15-µL volume containing 1.5 µL of PCR buffer, 1.5–2.0 mm of MgCl2, 0.2 mm each dNTP, 0.5 U of Taq DNA polymerase (Invitrogen), and 0.2 µm of each primer. Thermocycling parameters consisted of an initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 53–55 °C for 1 min (depending on the primer set), 72 °C for 1 min, and a final extension of 72 °C for 10 min. PCR products were checked using ethidium bromide-stained 1.5% agarose gels. Forward and reverse strands were sequenced using an ABI 3730XL analyzer by Macrogen Inc.
3) the program Micro-Checker 2.2.3 (Van Oosterhout et al. 2004) to identify genotyping errors, null alleles, and allele dropouts in the data. PCR products were checked on an ethidium bromide-stained 1.5% agarose gel and sent for direct fragment analysis using an ABI Prism 3730xl DNA Analyzer at the University of Illinois DNA Core Sequencing Facility.

Two sex chromosome genes, Zinc-finger and Amelogenin, using primers specifically designed for felds (Pilgrim et al. 2005; Bhagavatula and Singh 2006) were amplified separately using the same PCR conditions used for microsatellite loci, checked, and sent for fragment analysis.

Data Analysis
MtDNA sequences (forward and reverse) were aligned against reference sequences using ProSeq 2.91 (Filatov 2002) and checked by eye. MtDNA gene segments were concatenated (Huenenbeck et al. 1996), and the number of haplotypes and polymorphic sites, gene diversity, differences between pairs of sequences (II) and nucleotide diversity (π) per nucleotide site were estimated with Arlequin 3.5.1.2 (Excoffier and Lischer 2010).

Microsatellite and sex chromosome genotypes were scored with GeneScan 3.7 (ABI) and Peak Scanner 1.0 (ABI) to calibrate allele sizes precisely. Microsatellite data were analyzed with Genepop 4.0.10 (Raymond and Rousset 1995; Rousset 2008) for general diversity estimates including heterozygosity and number of alleles per locus, and to test for deviations from Hardy–Weinberg equilibrium and linkage disequilibrium. For microsatellite loci, the probability of identity P(ID) between sibs (P(ID)sibs) and with low sample size correction (P(ID)unbiased) was estimated with GIMLET v.1.3.2 (Valiere 2002), whereas observed P(ID) was calculated with APICALC v.1.0 (Ayres and Overall 2004).

We explored fine scale signatures of genetic subdivision within Chiloé Island with the Bayesian individual clustering approach in Structure 2.3.3 (Pritchard et al. 2000). We used 100,000 iterations, 100,000 MCMC, and an admixed ancestry model.

To assess pairwise-kinship coefficients (r) and inbreeding coefficients (F), we used microsatellite data to calculate maximum likelihood estimates for triadic identical by descent (IBD) coefficients (Wang 2007) with the software Coancestry 1.0 (Wang 2011). Maximum likelihood calculations account for inbred individuals, thus giving non-biased estimates of relatedness (Wang 2011). Mean kinship and mean inbreeding were estimated for each study area, and tests for significant differences between groups were carried out using bootstrap methods with Coancestry 1.0.

To infer dispersal, we assessed the spatial distribution of pairwise kinship by performing correlation analysis between pairwise-kinship coefficients (r) and geographic distance (km) among sampled individuals for each study area.

Rarefaction analysis with PAST (Hammer et al. 2001) was used when possible to adjust for unequal samples sizes for comparisons among groups, on both mtDNA and microsatellite data. For those data, which cannot be adjusted for unequal samples sizes using rarefaction analysis, we performed the Welch t-test for unequal variances to test for statistical significance of comparisons among study areas (Ruxton 2006).

Results
Landscape Fragmentation
Polygons from NCI, CCI, and SCI differed in degree of landscape fragmentation depending on land cover type (Figure 2, see Supplementary Table S2 online). Total forest cover (TF; guigna habitat) increased and pasture cover (PA; avoided matrix) decreased from north to south. NCI had the highest degree of habitat loss (50.8% TF; 31.8% PA) followed by CCI (53.6% TF; 24.0% PA), and lastly SCI (74.01% TF; 8.8% PA). Regarding shrubland area (used for migration only), NCI (14.7%) and SCI (15.3%) had similar coverage areas but were lower compared with CCI (20.4%). For roads (associated with fragmentation and migration-related mortality), NCI (445 km) and SCI (499 km) had similar road extents, greater than SCI (351 km).

Table 2.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>TF (ha)</th>
<th>PA (ha)</th>
<th>North</th>
<th>Center</th>
<th>South</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>CCI</td>
<td>750</td>
<td>750</td>
<td>750</td>
<td>750</td>
<td>750</td>
</tr>
<tr>
<td>SCI</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
</tbody>
</table>

Figure 2. Land cover types with biological meaning for guigna spatial use on Chiloé Island study areas.
with heterozygote deficiency, but as this was not observed in all populations we included them in our analyses. None of the populations revealed heterozygote excess. Micro-Checker 2.2.3 did not identify genotyping errors, null alleles, or allele dropout in our data and there was no evidence of linkage disequilibrium among loci. All 38 individuals included in the analysis had >50% locus data, most of them displaying 80–100%. The Bayesian clustering approach conducted in Structure 2.3.3 found no genetic substructure within Chiloé Island (K = 1). Observed heterozygosity (HObs) was low in all 3 areas (Figure 3, Table 1): NCI (H = 0.505 ± 0.143; n = 21), CCI (H = 0.477 ± 0.164; n = 9), and SCI (H = 0.459 ± 0.265; n = 8), with no significant differences among them. In contrast, statistically significant differences were found in allelic richness indices following rarefaction analysis (A*), but only between NCI and CCI (t-test; NCI × CCI [P = 0.013]), whereas NCI × SCI (P = 0.312) and CCI × SCI (P = 0.809) were not significant. Richness was lowest in NCI (A* = 2.691 ± 0.520; n = 8*), followed by SCI (A* = 3.2308 ± 1.36; n = 8*) and CCI (A* = 3.3565 ± 0.41; n = 8*; Figure 3, Table 1).

**Kinship, Inbreeding, and Dispersal**

For the 13 microsatellite loci analyzed, cumulative observed P(ID) was 0.0001 with the 5 most informative loci (see Supplementary Figure S1 online), whereas P(ID)/sibs was 0.00028 (1/3.571), significantly lower than the standard P(ID) = 0.005 used for kinship analysis.

Mean pairwise kinship (r) was highest for SCI (mean r = 0.374 ± 0.334; n = 28 pairwise comparisons) and was significantly different from NCI (mean r = 0.143 ± 0.172; n = 210 pairwise comparisons) and CCI (mean r = 0.091 ± 0.108; n = 36 pairwise comparisons; Welch’s t-test; NCI × SCI [P = 0.001], CCI × SCI [P = 0.0002], NCI × CCI [P = 0.082]; Figure 4). Average inbreeding coefficients (F) were also highest for SCI (mean F = 0.292 ± 0.239; n = 8), followed

---

**Figure 3.** Measures of genetic diversity on (a) mtDNA and (b) microsatellite loci for guigna on Chiloé Island study areas. Mean, error bars show ± standard deviation (SD). Different letters indicate statistically significant differences (P < 0.05). Number in parentheses indicates sample size. * indicates sample size after rarefaction analysis. Number of haplotypes (K*), number of alleles per locus (A*) following rarefaction analysis.
Table 1. Measures of genetic diversity for mtDNA and microsatellite loci for guigna on Chiloé Island study areas

<table>
<thead>
<tr>
<th>Study area</th>
<th>Number of mtDNA sequences</th>
<th>Number of microsatellite loci</th>
<th>Number of polymorphic sites (S)</th>
<th>Number of haplotypes (K)</th>
<th>Rarefaction*: average number of haplotype diversity (H)</th>
<th>Average number of haplotypes per locus (K*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>38</td>
<td>20</td>
<td>5</td>
<td>5</td>
<td>0.539 ±0.15</td>
<td>2.54 ±0.16</td>
</tr>
<tr>
<td>North Chiloé Island</td>
<td>21</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>0.539 ±0.15</td>
<td>2.54 ±0.16</td>
</tr>
<tr>
<td>Center Chiloé Island</td>
<td>8</td>
<td>17</td>
<td>5</td>
<td>5</td>
<td>0.539 ±0.15</td>
<td>2.54 ±0.16</td>
</tr>
<tr>
<td>South Chiloé Island</td>
<td>8</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>0.539 ±0.15</td>
<td>2.54 ±0.16</td>
</tr>
</tbody>
</table>

*Rarefaction curves to compare the average number of alleles per locus in geographic groups with different sample sizes. ± corresponds to standard deviation (SD).

<table>
<thead>
<tr>
<th>Study area</th>
<th>Number of mtDNA sequences</th>
<th>Number of microsatellite loci</th>
<th>Number of polymorphic sites (S)</th>
<th>Number of haplotypes (K)</th>
<th>Rarefaction*: average number of haplotype diversity (H)</th>
<th>Average number of haplotypes per locus (K*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>38</td>
<td>20</td>
<td>5</td>
<td>5</td>
<td>0.539 ±0.15</td>
<td>2.54 ±0.16</td>
</tr>
<tr>
<td>North Chiloé Island</td>
<td>21</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>0.539 ±0.15</td>
<td>2.54 ±0.16</td>
</tr>
<tr>
<td>Center Chiloé Island</td>
<td>8</td>
<td>17</td>
<td>5</td>
<td>5</td>
<td>0.539 ±0.15</td>
<td>2.54 ±0.16</td>
</tr>
<tr>
<td>South Chiloé Island</td>
<td>8</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>0.539 ±0.15</td>
<td>2.54 ±0.16</td>
</tr>
</tbody>
</table>

*Rarefaction curves to compare the average number of alleles per locus in geographic groups with different sample sizes. ± corresponds to standard deviation (SD).

Discussion

Phylogeographic Context

In a phylogeographic context across their whole distribution range (32.5–46.5° S; DR; n = 87), guignas display higher mtDNA genetic diversity compared with Chiloé Island group (CI, this study; n = 37), in number of haplotypes (DR = 45; CI = 16), polymorphic sites (DR = 55; CI = 20), haplotype diversity (DR = 0.94 ± 0.02; CI = 0.76 ± 0.08), and average number of nucleotide differences between pairs of sequences (DR = 7.01; CI = 4.20; Napolitano et al. 2014). For microsatellites, guignas across the whole distribution range (n = 102) display similar average heterozygosity compared with Chiloé Island group (this study; n = 38; DR = 0.49 ± 0.10; CI = 0.49 ± 0.10), but higher average number of alleles per locus (DR = 6.54 ± 2.0; CI = 4.54 ± 1.61; Napolitano et al. 2014).

Using categories of population distinctiveness (Crandall et al. 2000), Management Units for guigna conservation were defined based on genetic and ecological exchangeability and for both recent and historical time frames (Napolitano et al. 2014). One of the two proposed Management Units for guignas includes Chiloé Island, Lake District, Argentinian and San Rafael Lake groups (Napolitano et al. 2014).

The Chiloé Island group, genetically diverged since being separated from the mainland 7000 years BP, harbors a unique genetic identity. The Chacao channel is a recent effective barrier to gene flow for guignas, but was not a historical effective barrier to gene flow in the past, where connectivity across the land bridge between Chiloé Island and mainland populations occurred (Napolitano et al. 2014).

Genetic Diversity

The larger amount of fragmentation observed in NCI relative to CCI and SCI was linked with significantly reduced mitochondrial haplotype genetic diversity in guigna populations and with lower,
but not significant measures of microsatellite allelic richness. This was in spite of the relatively large sample size from the NCI guigna population compared with the other 2 populations (NCI: \(n = 21\); CCI: \(n = 9\); SCI: \(n = 8\)).

This trend supports theoretical predictions and our hypothesis that small, isolated populations would have relatively less genetic diversity due to the increased influence of genetic drift and local extinctions. For microsatellite diversity, as observed here, allelic richness will theoretically decrease more rapidly than heterozygosity as population size decreases, given that the former is more sensitive to the loss of low-frequency or rare alleles (Allendorf 1986; Spencer et al. 2000). For guigna inhabiting fragmented landscapes, small effective population size is probably the major force driving the decrease in genetic diversity (e.g., low carrying capacity, local extinctions, road kills). Chiloé Island group has been previously described to display an overall pattern of \(N_e \gg N\), suggesting it may be going through a current population size reduction (Napolitano et al. 2014).

Lower genetic diversity in fragmented populations has been described in a wide range of taxonomic groups including rodents (Tallmon et al. 2002; Hirota et al. 2004), reptiles (Cunningham and Moritz 1998; Sumner et al. 2004), amphibians (Wahbe et al. 2005), marsupials (Banks et al. 2005; Lancaster et al. 2011), different carnivores such as ursids (Dixon et al. 2007), canids (Leigh et al. 2012), mustelids (Kyle and Strobeck 2001; Dallas et al. 2002), and several felid species such as jaguar (\(Panthera onca\); Haag et al. 2010), ocelot (\(Leopardus pardalis\); Janecka et al. 2011), Amur leopard (\(Panthera

Figure 4. (a) Pairwise kinship (\(r\)) and inbreeding coefficients (\(F\)) for microsatellite loci of guigna on Chiloé Island study areas. (b) Intrasexual measures of pairwise kinship (\(r\)) for microsatellite loci of guigna on Chiloé Island study areas. Mean, error bars show ± standard deviation (SD). Different letters indicate statistically significant differences (\(P < 0.05\)). Sample size and number of pairwise comparisons for kinship estimates are given in parentheses.
pardus orientalis; Uphyrkina et al. 2001), Iberian lynx (Lynx pardinus; Johnson et al. 2004), European lynx (Lynx lynx; Schmidt et al. 2011), and puma (Puma concolor; Ernest et al. 2003).

Kinship, Inbreeding, and Dispersal

Inbreeding is the mating of related individuals (i.e., who share common ancestry) and the level of inbreeding in a population reflects the
prevalence of such matings, as well as the degree of shared ancestry of mating pairs (Keyghobadi 2007). Theoretically, the level of inbreeding would be inversely related to population size because in small populations, there should be an increased probability that 2 mating individuals will share recent common ancestry (Frankham et al. 2005). Moreover, theory predicts that gene flow disruption between small, isolated subpopulations in fragmented landscapes would lead to increased levels of kinship and inbreeding that may potentially result in reduced fitness associated with inbreeding depression (Keller and Waller 2002; Frankham et al. 2005; Reed 2005; Keyghobadi 2007).

Contrary to theoretical expectations, increasing degrees of landscape fragmentation were not associated with increased kinship or inbreeding in guigna populations. Pairwise kinship ($r$) and inbreeding coefficients ($F$) were significantly greater in SCI ($r = 0.374 \pm 0.334$; $F = 0.292 \pm 0.239$) relative to NCI ($r = 0.143 \pm 0.172$; $F = 0.152 \pm 0.119$) and CCI ($r = 0.091 \pm 0.108$; $F = 0.182 \pm 0.123$).

In SCI, pairwise kinship strongly decreased as a function of distance, indicating low guigna dispersal range in these pristine landscapes. Low dispersal may cause a higher prevalence of matings between related individuals, explaining why this group displays relatively high kinship and inbreeding levels. This pattern coincides with a radiotelemetry study in nonperturbed protected areas, which showed small and overlapping home ranges, high local densities, and low dispersal distances for guignas (Dunstone et al. 2002). Low dispersal rates and lack of territoriality in pristine areas suggest that food resources are either too abundant to require defense or too clumped to be effectively defended (Sandell 1989; Dunstone et al. 2002) and may be associated with a hypothesis regarding low costs of tolerating relatives when resources are abundant (Randall et al. 2007). By contrast, pairwise kinship did not decrease substantially as a function of distance in NCI, suggesting that there were increased levels of guigna dispersal in the more fragmented landscapes. Higher dispersal rates in NCI may lead to a reduced probability of mating with related individuals, resulting in lower kinship and inbreeding levels. This pattern coincides with a radiotelemetry study in highly perturbed landscapes on NCI, which showed low population density, exclusive home ranges, and high dispersal distances for guignas (Sanderson et al. 2002).

The costs and benefits of dispersal predict that evolutionary changes in dispersal traits may take place in response to habitat fragmentation (Hanski and Gilpin 1997; Young and Clarke 2000). Variability observed in dispersal patterns is likely due to changing environmental conditions that alter the costs and benefits of dispersal (Janecka et al. 2007). Dispersal may reduce competition over poor quality and scarce resources in highly fragmented landscapes with low carrying capacity. Costs associated with dispersal through fragmented landscapes include increased energy expenditure and mortality risk.

Heterogeneity in a landscape (at scales that are relevant to components of individual fitness) will impose selective pressures on organisms to adapt to spatial patchiness (Spong and Creel 2001; Didham 2010). In a metapopulation scenario with moderately isolated populations, one might expect that selection would favor increased dispersal distances in response to the gaps in habitat (Fahrig and Merriam 1994; Matthysen et al. 1995). Theoretical modeling by Ewers and Didham (2006) predicts that species capable of low and/or high dispersal distances would have lower susceptibility to extinction because the former tends to stay within 1 fragment, while the latter is capable of moving freely between fragments.

However, species with intermediate dispersal capabilities would be more sensitive to patch isolation because they are less able to reach the next fragment when dispersing and would have higher mortality rates during dispersal (Thomas 2000). Thus, increased fragmentation is expected to favor dispersal only for individuals that are well-adapted to disperse (Roff and Fairbairn 2001; Van Dyck and Baguette 2005).

Evolutionary responses to landscape fragmentation and dispersal have been described in diverse studies. In the silver-spotted skipper butterfly (Hesperia comma), higher migration rates and thorax-mass (mostly flight muscle) investment were correlated with habitat area and isolation (i.e., landscape fragmentation; Thomas et al. 1998; Hill et al. 1999). In caribid beetles (Pogonos chalcus and Dicheirotrichus gustavi), there was a decrease in the mobility of populations inhabiting fragments for a long period of time (Desender et al. 1998). In both butterflies (Baguette et al. 2011) and fish (Haugen et al. 2006), landscape fragmentation (i.e., low-quality habitat) led to increased dispersal. However, in ocelots (L. pardalis) from southern Texas (Janecka et al. 2011) and Cunningham’s skinks (Egermia cunnighamii; Stow et al. 2001), dispersal in fragmented landscapes decreased. Different responses to habitat fragmentation highlight the species and context dependency of dispersal patterns (Ricketts 2001).

The degree of plasticity in dispersal behavior largely determines if a species can adjust to profound environmental changes (or only to minor short-term changes) and if this adjustment is sufficient to prevent extinction (Candolin and Wong 2012). For guigna, differential dispersal distances in landscapes with differing degrees of fragmentation suggest adaptive dispersal plasticity that might be correlated with landscape features. In highly fragmented landscapes with saturated habitats (i.e., unavailable free breeding territories), most dispersers may not be successful, but when they are they can have a rapid and significant effect in these small and isolated populations, counteracting to some degree the negative effects of isolation and thus reducing extinction rates in small fragments (de Vries et al. 1996; Haag et al. 2010). This behavioral flexibility may be relevant to the long-term survival of guignas in an increasingly anthropogenically modified environment.

We recognize that additional sampling, especially in SCI, would likely strengthen the inferences derived from the genetic patterns described here. In SCI, 2 samples from Lelbún (2 males) and 3 samples from Colonia Yungay (1 male and 2 females) may be close relatives (parent-cub or full-sib relationships), possibly leading to the overestimation of mean pairwise kinship (SCI, $r = 0.374 \pm 0.334$). Nevertheless, similar sample sizes for CCI (2 samples from Cucao [2 females] and 2 samples from Rahue [2 males]) did not result in higher pairwise-kinship estimates (CCI, $r = 0.091 \pm 0.108$).

Obtaining large samples is a common constrain for conservation geneticists working on rare and elusive threatened species, where small sample sizes are often unavoidable. Threatened, fragmented populations are expected to be small (Ewers and Didham 2006), and researchers seeking to detect genetic signals in fragmented landscapes therefore need to accept and overcome the fact that there will be few individuals to sample (Struebig et al. 2012).

Small sample sizes might affect the interpretation of results. However, small sample sizes would generally cause population differences to be statistically nonsignificant (Waples 1998), so the bias would tend to underestimate differences. In contrast, in this study, we did find reduced mtDNA in NCI where we had the largest sample size ($n = 21$). Rare alleles may be difficult to detect with small sample sizes (Struebig et al. 2012). Nonetheless, this problem is most acute...
when samples are small relative to the size of the population (Belkhir et al. 2006), an unlikely scenario in these threatened, fragmented populations. Moreover, fewer rare alleles are expected in fragmented populations because of the effects of passive sampling and genetic drift (Allendorf 1986).

Additionally, our sampling effort was distributed throughout several localities within the 3 studied groups (number of localities NCI = 10, CCI = 7, SCI = 5) and not concentrated in just 1 location, minimizing the potential for false genetic diversity bias. This bias should be even lower for microsatellites because the ability to reliably detect the signal is enhanced by amplifying multiple, independent loci and hence sampling more alleles (Waples 1998; Landguth et al. 2012).

**Sex-Biased Dispersal**

The adaptive advantages of dispersal have been linked not only with lowered competition for resources and mates, but also with inbreeding avoidance (Pusey and Wolf 1999; Perrin and Mazarov 2000). For mammals, inbreeding avoidance may represent the most important adaptive advantage of sex-biased dispersal (Pusey and Wolf 1996; Bick et al. 2006). Most solitary mammals exhibit differential dispersal patterns for both sexes, where females are philopatric and males disperse from their natal area and establish permanent residence in a new home range to reproduce (Waser and Jones 1983; Pragnolle and de Meeus 2002; Ratnayeke et al. 2002; Moyer et al. 2006). Sex-biased dispersal is often hypothesized to be a means of avoiding inbreeding (Wolff 1993; Costello et al. 2008). From an evolutionary perspective, sex-biased dispersal in mammals occurs due to different reproductive strategies between the sexes. Females have higher energy requirements and investment when providing parental care, thus they benefit more than males from familiarity with food resources (i.e., philopatry; Greenwood 1980).

In NCI, we observed male sex-biased dispersal in guignas; females were philopatric, with higher intrasexual kinship levels than males. Sex-biased dispersal in NCI may act as a mechanism for inbreeding avoidance, thus contributing to low inbreeding levels in this group.

**The Importance of Corridors for Connectivity in Fragmented Landscapes**

Given that guignas exclusively use areas with significant cover and avoid open areas (i.e., pastures), the increased dispersal rates for guignas in fragmented landscapes highlight the importance of preserving vegetation corridors to facilitate connectivity between forest fragments or larger forested areas, as has been suggested in other studies (Dunstone et al. 2002; Sanderson et al. 2002; Gálvez et al. 2013). The conservation of vegetation corridors is essential to maintain viable populations in fragmented landscapes, by contributing to gene flow, reducing negative demographic and genetic consequences of small isolated populations, and decreasing local extinctions (Reed 2004; Gilbert-Norton et al. 2010; Haag et al. 2010; Rabinowitz and Zeller 2010; Janecka et al. 2011; Sharma et al. 2013; Yumnam et al. 2014).

Habitats used for dispersal need to provide only the basic ecological resources while individuals are moving between high-quality patches (Palomares et al. 2000). For guignas, corridors with vegetation cover >0.4 m high and as small as 3 m wide are adequate for guigna to move across a fragmented landscape (Sanderson et al. 2002). Along with vegetation corridors, safe road crossing elements such as culverts, overpasses, and underpasses are also important to favor guigna connectivity in fragmented landscapes (Sanderson et al. 2002; Haines et al. 2006). The lack of corridors or safe passages within a fragmented landscape increases the costs of dispersal (i.e., energy, time, mortality risk) and reduces the net benefit of leaving a patch in search for another (Candolin and Wong 2012). Mortality by road kills is a major death cause for many felids and may be a significant cost of dispersal (Nielsen and Woof 2002; Ferreras et al. 2004; Haines et al. 2005). Road kills accounted for 29% of the 38 total samples collected during this 3-year study in Chiloé Island.

Conservation policy needs to shift the focus from protected area centered preservation to landscape scale conservation (Yumnam et al. 2014). Incorporating private lands outside protected areas to function as corridors is crucial for guigna conservation (Simonetti and Acosta-Jamett 2002; Acosta-Jamett et al. 2003; Haines et al. 2006; Simonetti 2006; Gálvez et al. 2013). This depends heavily on positive perceptions and attitudes of land owners and rural people towards guignas and other wildlife (Sillero-Zubiri and Laurson 2001; Silva-Rodríguez et al. 2007; Herrmann et al. 2013). Human–felid conflicts are one of the most urgent conservation issues for the protection and management of wild cats worldwide, affecting >75% of the world’s felid species (Woodroffe et al. 2005; Inskip and Zimmermann 2009). In rural landscapes of central and southern Chile, most people have negative attitudes toward guignas and illegal killing as retaliation for poultry predation is frequent (Sanderson et al. 2002; Silva-Rodríguez et al. 2007; Zorondo-Rodríguez et al. 2014). Retaliatory killings for poultry depredation accounted for 39.4% of the 38 samples collected during this 3-year study. Given this scenario, long-term conservation of guigna populations outside protected areas should include increased local participation and education to reduce conflict in areas where guignas are considered to be pests.

**Future Directions**

Human population and deforestation are both increasing rapidly in the Chilean temperate rainforest (Wilson et al. 2005; Echeverría et al. 2008). In addition, climate change and emerging pathogens may be underappreciated threats (Malcolm et al. 2006; Mora et al. 2015). The long-term survival of guignas in these remaining and increasingly fragmented landscapes will depend on their plasticity and ability to adapt to a network of vegetation corridors (mostly across private land). This will also require the positive attitudes of local people and an integrated interdisciplinary approach.

Landscape fragmentation in a given ecosystem is likely to produce similar impacts on species with comparable natural history and ecological requirements, thus our results will likely be applicable to other species requiring significant amount of vegetative-cover, including the Darwin’s fox (Pseudalopex fulvipes; Vilá et al. 2004; Jiménez 2007) and the southern pudu deer (Pudu pudu; Silva-Rodríguez et al. 2009). Future carnivore studies would benefit from the comparison of behavioral and genetic patterns among different contexts of habitat deterioration, landscape fragmentation, intraguild competition and prey scenarios, facilitating further comparative hypotheses for much-needed research efforts.

Obtaining large samples remains desirable for conservation genetics studies. The use of noninvasive genetic techniques to increase sample size is suitable when investigating fine scale patterns of genetic diversity and the functionality of corridors (Yumnam et al. 2014). A pilot study at the beginning of this research assessed the possibility of working with fecal samples. Finding feces was rare throughout Chiloé Island, due to low guigna density and the remoteness of the terrain. Moreover, given high annual precipitation on the island (3000–5000 mm spread throughout the year; most rainfall May–August), feces were usually found disintegrated, washed away by heavy rain. These constraints hindered successful,
high-quality amplification of DNA from fecal samples in this study area (Goossens and Salgado-Lynn 2013). However, the use of noninvasive genetic techniques continues to be an excellent methodology in less humid geographic areas.

Conservation geneticists need to be wary of sampling limitations. However, these warnings should not detract from undertaking research, and in particular, working on threatened species—those most sensitive to the effects of habitat modification, susceptible to population declines and of most conservation concern (Struik et al. 2012).

In the current global scenario of ever-increasing human landscape perturbation, this research enhances our understanding of the genetic consequences of anthropogenic landscape fragmentation across different guigna populations and provides insights on how it may affect genetic and behavioral patterns of this rare and elusive felid. This study highlights the importance of vegetation corridors for maintaining guigna metapopulation structure within perturbed landscapes in the unique temperate rainforests of southern Chile. Our results will assist informed decision making for conservation planning of guignas on Chiloé Island and other perturbed landscapes within their range, providing a sound basis to formulate conservation policy.

Supplementary Material
Supplementary material can be found at http://www.jhered.oxfordjournals.org/

Funding
Instituto de Ecología y Biodiversidad (IEB, Facultad de Ciencias, Universidad de Chile). Doctoral fellowship ICM P05-002 from IEB, the Emerging Leaders in The Americas Program (ELAP, Canadian Bureau for International Education, Government of Canada), Becas Chile para pasantías doctorales 2010 (CONICYT, Chile), Panthera Kaplan Awards Program (Panthera Foundation, NY, USA), Scott Neotropical Fund Award (Cleveland Metroparks Zoo and the Cleveland Zoological Society, Cleveland, OH, USA), and the Eric York Scholarship (Felidae Conservation Fund, CA, USA) to C.N.

Acknowledgments
Guigna captures and tissue collection were carried out with permission from SAG, capture permit numbers 814/13 February 2008; 109/9 January 2009; 1220/22 February 2010; 1708/26 March 2010. We thank L. Eaton and an anonymous reviewer for helpful comments, which improved our manuscript. We thank J. Cabello (Chiloé Silvestre), J. Valenzuela (CECPAN), B. Silva, L. Quezada, J. Lélis (Fundación Sendha Darwin), E. Elgueta, M. Valdés, A. Bravo (Universidad Austral), and F. Ovidio Castro (Universidad de Concepción) for generous collaboration with samples. We also thank M. Bennett, S. Faugeron, J. Vianna, J. Heitz, M. Mora, V. Sánchez, T. Vaskovic, V. Sóli, R. Palou, A. Charrèze, Y. Zúñiga, and J. Vidal. We thank Parque Ahuenco, especially C. Villarroel; Parque Tantauco, especially A. Bannister; Senda Darwin Foundation; Inés Hanning from Caulín, and many other local land owners in Chiloé Island for their support and for kindly letting us work in their land.

References

Access this journal article online at http://jhered.oxfordjournals.org/

Supplementary material can be found at http://www.jhered.oxfordjournals.org/


Didham RK. 2010. Ecological consequences of habitat fragmentation. Encyclopedia of Life Sciences—Wichelay, Chichester, UK; John Wiley & Sons Ltd.


Page dimensions: 614.9x802.0


