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Spatial genetic structure of hatchlings of *Cyclura ricordii* (Reptilia: Iguanidae) in Pedernales province, Dominican Republic is inconsistent with natal philopatry

By

Rosanna Carreras De León

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Biological Sciences (Zoology)
in the Department of Biological Sciences

Mississippi State, Mississippi

May 2015

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Spatial genetic structure of hatchlings of *Cyclura ricordii* (Reptilia: Iguanidae) in
Pedernales province, Dominican Republic is inconsistent with natal philopatry

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Pages in Study: 58

Candidate for Degree of Master of Science

Cyclura ricordii is an endemic iguana from Hispaniola and is listed as Critically Endangered on the IUCN. The main threats are predation by introduced mammals, habitat destruction and hunting. The present study focused on two nesting areas in Pedernales in the Dominican Republic. The hypothesis tested is that natal philopatry influences dispersal and nest site selection. Monitoring and sampling took place during 2012-2013. Polymorphic markers were used to evaluate whether natal philopatry limits dispersal at multiple spatial scales. Ripley's K , revealed that nests were significantly clustered. Hierarchical AMOVA revealed that nest site aggregations did not explain a significant portion of genetic variation. However, Mantel's tests revealed significant positive correlations between genetic and geographic distance. These results indicate that natal philopatry limits dispersal at a coarse spatial scale, but does not influence nest site selection at a fine spatial scale.

Key words: *Cyclura ricordii*, natal philopatry, nest site fidelity, Ripley's K , edge thinning technique, Mantel test.

ACKNOWLEDGEMENTS

First of all I would like to thank my academic adviser, Dr. Mark E. Welch, who welcomed me to his laboratory as a Master's student and gave continual guidance throughout my degree program. Also, I would like to thank the members of my thesis committee, Dr. Christopher P. Brooks, Dr. Lisa Wallace and Dr. Glenn Gerber, for giving me valuable insights into my research. A special acknowledgement is expressed to postdoctoral fellow Dr. Stesha A. Pasachnik from the San Diego Zoo for her support with the funding and academic guidance. Others to thank are Grupo Jaragua iguana specialists, Ernst Rupp, M.S. and Dr. Yolanda León. Ernst Rupp introduced me to iguana research and monitored my involvement during my first years in the field. He provided crucial data for the accomplishment of this study. The funding agencies that supported this project include the Fulbright program that supported me while I studied at Mississippi State University (MSU), the International Iguana Foundation (IIF), the Durrell Conservation Trust, Conservation International, Instituto Tecnológico de Santo Domingo (INTEC university) and the Biology Faculty Fund maintained by the Department of Biological Sciences at MSU. Giuliano Colosimo, Arned Rasberry and Jessica Martin deserve special thanks for their assistance with laboratory work and data analysis. I would also like to thank Madison Buras, an undergraduate researcher under my direction, for her assistance with laboratory work required for this study in its final phases.

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CHAPTER I

INTRODUCTION

‘Philopatry’ is a behavior defined by Mayr (1963) as a general tendency of some animals to return to or stay in their home area or natal location. This behavior has been observed in many mobile animal species (Bowen *et al.*, 2004; Hueter *et al.*, 2005; Freedberg *et al.*, 2005; Brown and Shine, 2007). Philopatric behavior may influence nest site selection in females. Hendrickson (1958) proposed that ‘natal homing’, or natal philopatry, explains the fidelity that marine turtles show toward their nesting locations. Hence, natal philopatry refers to the tendency of a female to return to nest at the site where she hatched. Natal philopatry can be contrasted with ‘nest site fidelity’ that refers to the tendency of iteroparous females to return to a specific location to nest. Nest site fidelity does not necessarily imply natal philopatry. Among reptiles, the taxon for which natal philopatry is best supported by genetic and behavioral evidence include sea turtles and giant river turtles. Both migrate hundreds to thousands of kilometers from feeding grounds to their natal nesting grounds (Bowen and Karl, 1996; Valenzuela, 2001).

The occurrence and extent of philopatry has important implications for the understating of animal dispersal (Bock *et al.*, 1985; Dittman and Quinn, 1996; Ruusila *et al.*, 2001; Bolker *et al.*, 2007; Chilvers *et al.*, 2008), their nesting behavior (Bock *et al.*, 1985; Ruusila *et al.*, 2001; Bowen *et al.*, 2004; Freedberg *et al.*, 2005; Brown and Shine, 2007; Knapp and Owens, 2008), the identification of important fisheries stocks (Dittman

and Quinn, 1996; Hueter *et al.*, 2005; Pawson *et al.*, 2008), and the management and conservation of endangered species (Hueter *et al.*, 2005; Knapp and Owens, 2008; Chilvers *et al.*, 2008; Salinas-Melgoza *et al.*, 2009). The continued visitation to the same location to nest that many animals show may bring some advantages for these species persistence as well as disadvantages if these areas were threatened in some way. These locations may be important refugia for the maintenance of these species and for successful hatching of future generations. Also, the elimination of these locations may increase energetic cost to females because of the necessity to disperse in search of new locations to nest, and in some cases a new location may not yield the same hatching success enjoyed at the preferred site.

In this study I investigated competing hypotheses designed to explain dispersal and nest site selection in the endangered Ricord's iguana, *Cyclura ricordii*, to better inform conservation management planning for this species. Individuals of *Cyclura* species often nest communally (Iverson *et al.*, 2004), and characterizing key aspects of the animal's life history including its dispersal tendencies, nest and forage site selection, and nesting behavior may enhance conservation strategies by identifying critical habitat and potential sites to incorporate into yearly monitoring programs. I used neutral molecular markers to study aspects of the nesting behavior that are difficult to characterize through observation alone.

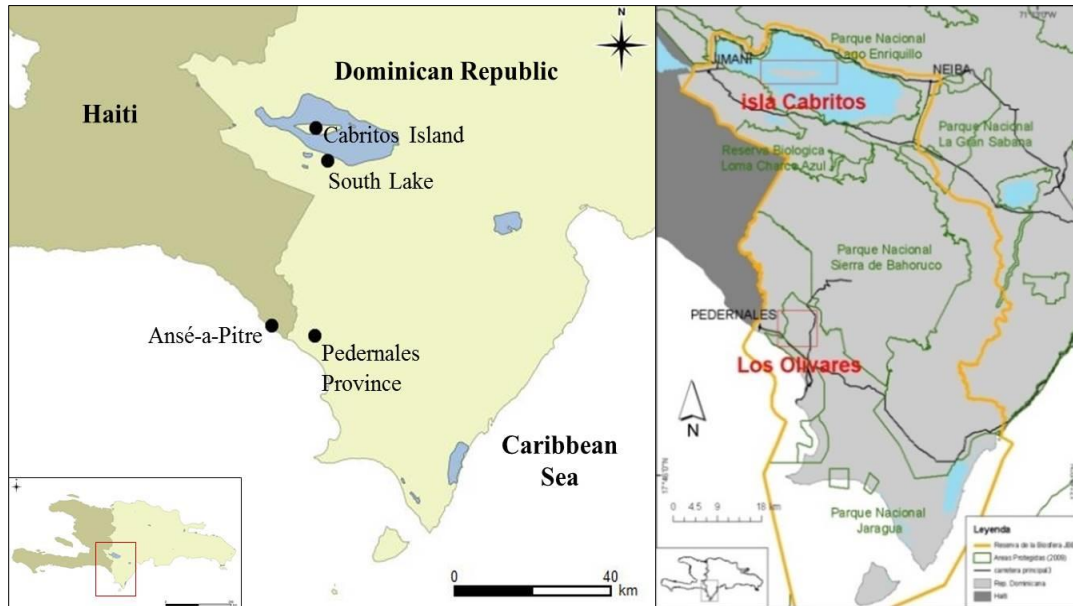


Figure 1.1 Maps of the southwestern region of the Dominican Republic.

Left image: black dots indicate known locations of Ricord's iguanas. Right image: green lines depict National Park boundaries, and the orange line is the boundary for the Jaragua-Bahoruco-Enriquillo Biosphere Reserve (map credit Yolanda León).

Cyclura ricordii is an endemic vertebrate on Hispaniola, the only island in the Caribbean where two species of *Cyclura* (*C. ricordii* and *C. cornuta*) can be found in sympatry. These species are the largest native herbivores on the island, and they fulfill a crucial ecological role in their dry forest ecosystems as seed dispersers, and by contributing to nutrient cycling through foliage grazing (Iverson, 1985; Hartley *et al.*, 2000; ISG/IUCN, 2002). *Cyclura ricordii* is critically endangered according to the IUCN Red List (2014), and only three natural populations are known in the southwestern areas of the Dominican Republic. Additional nesting areas are found across the border near Anse a Pitre, Haiti (Ottenswalder, 1996; Rupp *et al.*, 2009; Figure 1.1). The most recent assessment of population size estimated that 2,000 to 4,000 individuals remain in the wild (Ottenswalder, 2000). These numbers have likely dropped due to habitat loss associated

with infrastructure construction and agricultural development (see Figure 1.2 for agricultural development in Los Olivares nesting habitat).

For the past 10 years a local non-governmental organization (NGO), Grupo Jaragua (www.grupojaragua.org.do), has continually monitored *C. ricordii* at all localities except the one on Cabritos Island. The main population under study is in the southwestern region of the Dominican Republic near Los Olivares in Pedernales Province (Rupp, 2010). Los Olivares is the only area where nesting activity is known (Figure 1.2). Nesting at Los Olivares occurs primarily in bottomlands called “fondos” where the iguanas excavate their retreats (Arias *et al.*, 2004). Fondos are characterized by fine, red, argillic soils with scrub-like vegetation, and an open canopy (Arias *et al.*, 2004). Four major fondos are monitored by Grupo Jaragua at Los Olivares: Tierra (10.3 ha), Malagueta (47.1 ha), Robinson (25.1 ha) and Jinagosa (0.6 ha) (Rupp *et al.*, 2009; Figure 1.2). The distinctive soil and nesting ground dimensions are unique, and it is the only area where two species of iguana nest sympatrically.

In this study, dispersal and nest site selection will be evaluated in Fondo de la Tierra and Fondo de la Malagueta, which are the two most active sites with over 100 nests per year at each location since 2004 (Rupp, 2010). These sites differ greatly in their nesting dynamics largely due to differences in land use and consequent disturbance. To protect and preserve these unique areas, they were both declared as Municipal Protected Areas (EMP, for its Spanish name *Espacio Municipal Protegido*) in the resolution 05-2005 of the Pedernales Province City Hall (Rupp *et al.*, 2009). Attempts to develop these fondos for agricultural purposes were made in 2006 and 2007 (Rupp *et al.*, 2009). Moreover, both fondos are heavily impacted by invasive feral cats, dogs and cows. The

feral dogs are of special concern given that at least 30 iguanas were killed by dogs in the 2012 and 2013 nesting seasons combined (José Luis Castillo, pers. comm.). Also, hunting pressure for the illegal pet trade has caused these fondos to be hunting “hot spots” because of the vulnerability of iguanas during the time of oviposition.

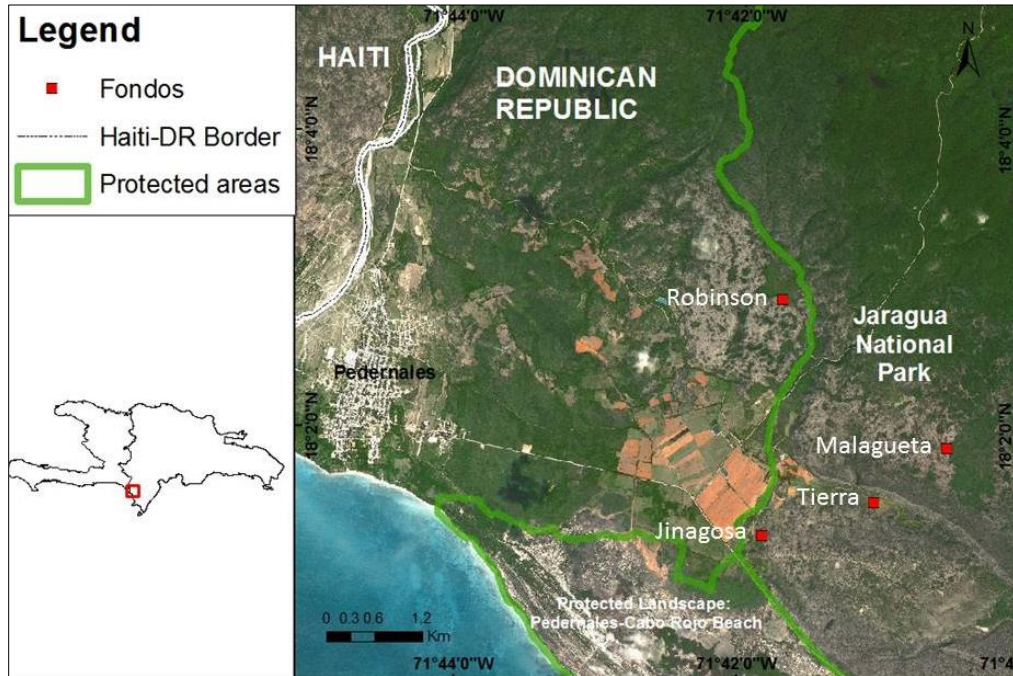


Figure 1.2 Map of Los Olivares in Pedernales Province.

The figure above shows the locations of the four bottomlands or ‘fondos’ (red squares) monitored by Grupo Jaragua. Green lines represent the limits of the protected areas (map credit Yolanda León).

While there are limited data regarding natal philopatry for most iguanids (Bock *et al.*, 1985; Rauch, 1988), there is ample information regarding nest site fidelity of *Cyclura* in the Caribbean (Iverson *et al.*, 2004; Knapp & Owens, 2008). Wiewandt (1982) was one of the first to anticipate natal philopatry in iguanas. There are no prior records of natal philopatry for either species of *Cyclura* from Hispaniola, but my preliminary data

for *C. ricordii* indicated a high return rate of iguanas to the specified fondos, and this is consistent with at least a limited degree of nest site fidelity. Field ecologists have inferred that some areas within fondos have greater nest density. In some of these putative clusters of nests, multiple females have constructed multiple nest chambers from the same entrance tunnel (Ernst Rupp, pers. comm.). Using camera traps, these researchers have recorded opportunistic females excavating burrows dug by other females to construct their nests. Similar behavior was reported by Rauch (1988) for marine iguanas. The presence of these putative clusters may be related to natal philopatric behavior where females are nesting in the same nest site from where they hatched.

While ecological studies are crucial, it is difficult to assess relatedness among animals in these studies (Bock *et al.*, 1985; Rauch, 1988; Iverson *et al.*, 2004; Knapp & Owens, 2008). Conservation plans for endangered reptiles have benefitted from the use of molecular techniques (Bowen *et al.*, 2004; Lee *et al.*, 2007). Molecular analyses can be used to assess genetic variation, population structure and infer the genetic health of endangered species. Little is known about the status of natural populations of *C. ricordii*, and there are no studies that assess the genetic structure of these populations. To inform conservation management planning, current monitoring programs need to be enhanced. Implementation of molecular techniques focused on genetic screening and variability of these populations are needed if further population declines and chances of extinction are to be mitigated. Several conservation efforts have been implemented to help recover the species since 2002, when a 5-year Recovery Plan was created (ZOODOM *et al.*, 2002), and land in these fondos was purchased to limit further agricultural development in 2002 (ISG/IUCN, 2002) and 2012.

The hypothesis addressed is that philopatry influences dispersal and nest site selection for *Cyclura ricordii* in the Dominican Republic. To test this hypothesis, three spatial scales were considered. At the broadest spatial scale natal philopatry could limit dispersal between distinct geographic regions, Cabritos Island and Pedernales populations. If true, we anticipate that genetic differences between animals located in these two geographic regions will be statistically significant. Two other spatial scales were assessed to further characterize potential effects of natal philopatry on the species population genetic structure. Fondo de la Tierra and Malagueta, the two fondos under study, are separated by as little as 1 km, and there are no physical barriers to dispersal between them. Any genetic structuring observed between them likely reflects limited migration. If philopatric behavior exists at this coarse spatial scale, then significant genetic differentiation may be present due to restricted gene flow between fondos. Natal philopatry may also influence population dynamics at a finer scale. Nest sites appear to occur in aggregations within fondos. Aggregations or clustering of nests may reflect the distribution of appropriate nesting habitat. However, it may also result from related females returning to nest at or near that site where they themselves emerged. If true, hatchlings emerging from nests in the same cluster should be more closely related to each other than hatchlings emerging from nests in other clusters. My objectives were to determine whether genetic structure occurs on a coarse or a fine spatial scale, and whether the observed structure is consistent with natal philopatric behavior.

CHAPTER II

METHODS

2.1 Study System

The genus *Cyclura*, the West Indian rock iguana, inhabits tropical dry forest in the Bahamas and Greater Antilles (Alberts, 2000). Species in this genus are among the world's most endangered lizards, primarily as a result of habitat degradation and the presence of exotic species (Henderson, 1992). Rock iguanas are the largest native herbivores on many of these islands. Within the Iguanidae, 36% are known to nest communally (Doody *et al.*, 2009). Iguanas in the genus *Cyclura* are iteroparous, and univoltine (Alberts, 2000). However, Iverson *et al.* (2004) found for *Cyclura cychlura inornata* that only one in three females nested every year. A typical nesting sequence for rock iguanas includes digging an entrance tunnel and chamber, laying eggs, back filling the tunnel and defense of the nest (Figure 2.1; Wiewandt, 1982). The nesting season of *Cyclura ricordii* varies slightly from year to year; it has been reported to start as early as 8 March and continue as late as 16 June (Rupp *et al.*, 2007; Ottenwalder, 2000). The incubation period is reported to be 95 to 100 days (Ottenwalder, 2000), and emergence has been reported between 10 June and 19 September (Rupp *et al.*, 2007). The average clutch size is 12.5 (range 1-23) eggs per nest (Rupp *et al.*, 2007). Females are reported to take two to three years to reach sexual maturity and have a mean snout-vent-length (SVL)

of 34 cm (Ottenwalder, 2000). Hatchling mean mass is about 30 g (Ottenwalder, 2000), and hatching success can be high (highest value recorded is 95.8%; Rupp, 2010).

Some studies for other *Cyclura* (Knapp and Owens, 2008) suggest that iguanas require well-drained soil and warm terrain for oviposition. When areas flood periodically, as can happen during the hurricane season in the Caribbean (1 June through 30 November), these requirements likely limit the use of the soil for nest excavation because nests would get too wet and mortality would increase (Iverson *et al.*, 2004). West Indian iguanas, including *C. ricordii*, inhabit areas of karst limestone with limited areas of sand and soil accumulation, and nest site selection can be constrained by the availability of appropriate habitat (Knapp & Owens, 2008). Males are highly territorial and the most dominant individuals establish home ranges that monopolize the highest quality habitat with the best forage and greatest density of females during the nesting season (Pérez-Buitrago *et al.*, 2010).



Figure 2.1 Pictures show *Cyclura ricordii* during nesting season.

(A) An adult *Cyclura ricordii* foraging among cacti, (B) a nesting female preyed upon by a feral dog, (C) a nesting female resting while covering the nest, and (D) a nesting female completing the covering of the nest and guarding it.

2.2 Sampling

Daily systematic surveys were conducted during the 2012 and 2013 nesting and hatching season across Fondos de la Tierra and Malagueta in Los Olivares, Pedernales Province in southwestern Dominican Republic. These surveys consisted of daily transects through the fondos to detect and tag new nests with a number and date (Figure 2.2). Each nest was characterized in terms of species, and dates of egg deposition and hatchling emergence. The position of each nest was recorded with a Garmin GPS (model VISTA HCx - etrex) using the UTM projection in the WGS84 system. Hatchlings were captured at emergence with enclosures built from metal flashing around each nest. Daily transects

around the fondos were done for opportunistic capture of hatchlings where enclosures were not present. Hatchlings were tagged by toe clipping (following Ferner, 1979 and modified by Iverson *et al.*, 2006), and the toe clips provided genetic samples that were stored in 95% ethanol at ambient temperature. Carcasses from unsuccessful hatching were also sampled.

Adults were sampled from multiple populations, Cabritos Island and Pedernales, during field seasons in 2012 and 2013. Tomahawk traps positioned in the areas surrounding the fondos allowed for the collection of additional genetic samples from adults. Trapping of adults also occurred on Cabritos Island; additional sampling techniques were used (e.g., nooses and nets). Blood was drawn from the caudal vein of adults, and tissue samples were collected from adult females that were killed by feral dogs at Pedernales fondos. Blood was stored in the field at ambient temperature in SDS lysis buffer (0.1M Tris-HCl pH 8.0, 0.1M EDTA, 0.01NaCl, SDS 2%; Longmire *et al.*, 1997). All individuals captured and sampled were released at the site of capture. A total of 102 individuals were sampled in 2012, 18 adults and 84 hatchlings. In 2013, a total of 367 individuals were sampled, including 30 adults and 337 hatchlings. Six additional samples of adults from Cabritos Island from 2010 were included (all adults sampled are listed in Appendix B).

2.3 Laboratory Work

Genomic DNA was extracted from whole blood and tissue samples with a Maxwell[®] 16 Tissue DNA Purification Kit in a Maxwell[®] 16 MDx Research Instrument (Promega, Mannheim, Germany). Anonymous microsatellites were used to infer patterns of population structure and rates of gene flow. Over seventy microsatellite markers were

screened and characterized for this species with the 2012 samples of hatchlings and adults (all microsatellite markers designed by Rosas *et al.*, 2008; Welch *et al.* 2011; Junghwa *et al.* 2004; and Lau *et al.* 2009 were included in the screening). Amplifications were conducted with a 2720 Thermal Cycler (AB Applied Biosystems) following standardized 3-primer PCR amplification according to Schuelke (2000) and modified by Welch *et al.* (2011) in a total volume of 10 μ l (i.e., 7.3 μ l of ddH₂O, 1.2 μ l of master mix (10 μ M of each dNTPs, 10x Tricine Taq Buffer and ddH₂O), 0.04 μ l forward primer, 0.2 μ l reverse primer, 0.2 μ l fluorescent tagged primer, 0.4 U taq polymerase and 1.0 μ l of DNA template). Fragment analysis was carried out at Arizona State University, and alleles were manually annotated with Peak ScannerTM Software v1.0 (Applied Biosystems).

Attempts were also made to develop mtDNA markers because these would be especially useful for inferring sex specific patterns of dispersal since the mtDNA is strictly maternally inherited. For this study I surveyed a region of the mtDNA bounded by ND4 and tRNA LEU for polymorphisms. Samples from Pedernales (16 individuals) and Cabritos Island (10 individuals) were sequenced according to Malone *et al.* (2000) using primers ND4 and LEU (Arévalo *et al.*, 1994). There was no additional variation beyond that reported by Malone *et al.* (2000), both haplotypes were observed in both populations, and there was insufficient variation within and among populations at this locus for a meaningful analysis of fine scale population genetic structure. Hence, this locus was dropped from the study.

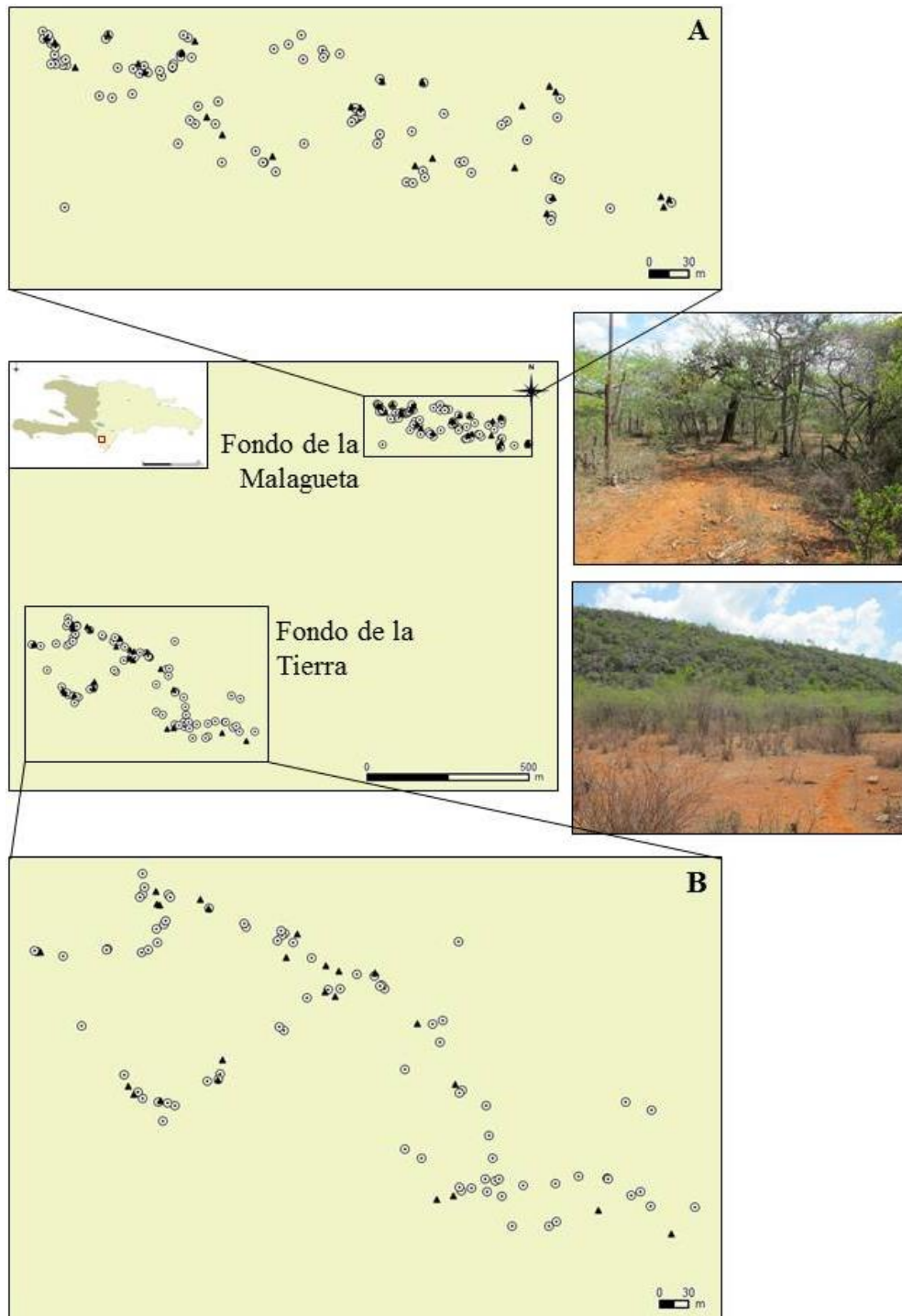


Figure 2.2 Los Olivares nesting habitat in Pedernales.

Black triangles represent sampled nests and circles un-sampled nests. (A) Fondo de la Malagueta nests for 2013 and (B) Fondo de la Tierra nests for 2013. Images to the right depict the general habitat features at these locations.

2.4 Genetic analysis

Genepop v. 4.2 was used to detect null alleles for the polymorphic microsatellites (Raymond and Rousset, 1995; Rousset, 2008). Loci with null allele frequencies > 0.20 were removed from fine scale genetic analysis (Dakin and Avise, 2004). I used the private allele method (Barton and Slatkin, 1986), corrected for sample size, to estimate gene flow (N_m) between the two major fondos. N_m was estimated using the Population Genetic Analysis software, Genepop on the web v. 4.2 (Raymond and Rousset, 1995; Rousset, 2008). Descriptive statistics for each locus in both adults and hatchlings from the Pedernales sites and for adults from Cabritos Island were also calculated. These include the number of alleles (N_a) per locus, expected and observed heterozygosity (H_E and H_O , respectively) for each locus (Guo and Thompson, 1992), and the inbreeding coefficient for each locus (F_{IS} ; Weir and Cockerham, 1984; Excoffier *et al.*, 1992). Population specific F_{IS} indices using 1023 permutations were also performed. Mean values were calculated for H_E , H_O and F_{IS} locus by locus results and, an additional value using a composite value for all of the data at once per population was estimated. All descriptive statistics were calculated with Arlequin v. 3.5.1.3 (Excoffier and Lischer, 2010). For the adjustment of significance thresholds a sequential Bonferroni correction was implemented (Holm, 1979).

2.5 Dispersal between and within geographic regions

Other F-statistics were calculated at multiple spatial scales with Analysis of Molecular Variance (AMOVA) to assess genetic differentiation. The F_{ST} between geographic regions, Pedernales and Cabritos Island, was estimated (Figure 1.1), and tested for statistical significance to evaluate if there is limited dispersal between them.

F_{ST} between fondos de la Tierra and Malagueta within Pedernales was also estimated and its significance assessed to determine if natal philopatry limits dispersal at the coarse spatial scale, between fondos, where intra-population genetic differentiation ought to be expected. All AMOVA were conducted with Arlequin v. 3.5.1.3 (Weir and Cockerham, 1984; Excoffier *et al.*, 1992).

2.6 Spatial structure of nests

Spatial analysis tested the null hypothesis that nests are distributed randomly within each fondo. An alternative hypothesis of non-random distribution was also considered. To test these hypotheses spatial point data was obtained from the monitoring program of Grupo Jaragua NGO. They provided spatial coordinates of nest positions across the years 2008–2013 for Tierra and Malagueta fondos. These analyses were made with the ‘spatstat’ guide in R created by Baddeley and Turner (2014). Ripley’s K was estimated and tested for significance to determine whether nest distribution within the fondos is overdispersed, randomly dispersed or clustered (Ripley, 1977). The function $K(r)$ was tested for significant departures from a homogeneous Poisson process, assuming *complete spatial randomness* (CSR) for our dataset (Dixon, 2002). The $K(r)$ function is:

$$K(r) = \lambda^{-1} E \quad (2.1)$$

where $K(r)$ refers to two dimensional spatial data, λ is the density (number per unit area) of events, and E refers to the number of extra events within distance r of a randomly chosen event (Ripley, 1976; Ripley, 1977). When a Poisson process is assumed to represent CSR, the function can be written in closed-form as (Dixon, 2002):

$$K(r) = \pi r^2 \quad (2.2)$$

Isotropy or uniformity is a key assumption of this model. For example, longitudinal and latitudinal distances should be equally correlated with density (Dixon, 2002). The function $K(r)$ (Equation 2.2) can also be interpreted as nonstationary given that the function is defined in terms of choosing an event randomly (Dixon, 2002). The boundaries of the study area are usually arbitrary and ignoring the influence of edge effects may produce a $K(r)$ estimator that biases the results (Dixon, 2002). According to Dixon (2002) it is best to use the corresponding $L(r)$ function (Equation 2.3) (Doguwa and Upton, 1989) because its variance is approximately constant under CSR (Dixon, 2002) and the function is:

$$L(r) = \sqrt{\frac{K(r)}{\pi}} \quad (.3)$$

assuming CSR, $L(r) = r$.

Significant departures between $L(r) - r$ can indicate two distinct deviances from a random distribution. If $L(r) - r < 0$, then spatial data points are regularly distributed, or overdispersed. If $L(r) - r > 0$, then points are underdispersed, or show evidence of clustering (Dixon, 2002). Because this is inherently a two-tailed test, the significance threshold was set appropriately ($\alpha = 0.975$; Dixon, 2002). To determine statistical significance most authors employ the Monte Carlo method (Haase, 1995). To compute statistical significance the ‘envelope’ command from the ‘spatstat’ guide was used. The envelope command computed 95 simulation envelopes of the summary ‘Kest’ (K estimate) to assess the goodness-of-fit of a point process model to the point pattern data (Baddeley *et al.*, 2014). The lowest and highest values of $K(r)$ (Equation 2.2) defined a lower, $K_{lo}(r)$, and upper, $K_{hi}(r)$, boundaries of a 95% confidence envelope (Haase, 1995).

Significant departures from these confidence envelopes indicate that a nonrandom distribution of nests may be biologically relevant.

To further evaluate the scale of aggregation or clustering, I applied the edge thinning technique (Keitt *et al.*, 1997) with a modified R script (Appendix A) from Brooks (2006). I was able to assign nodes, that refers to the spatial data points (nests), to specific aggregations or clusters and estimate the diameter (distance, r) in which these nodes were connected. The value of r is iteratively increased until the entire system forms part of a single cluster (Brooks, 2006). Edge thinning uses the distribution of pairwise distances between nodes, to infer the average distance between nodes that can be considered part of a single cluster. The plots will have ‘plateaus’ that represent distances where little or no change in the spatial pattern occurs. The minimum distance at which each of these plateaus occurs is where the spatial structure will be minimally connected (Brooks, 2006). These minimum distances will be referred to as ‘threshold distances’ from this point forward.

If significant clustering is present, then there should be an over representation of short and long edges. There should also be an under-representation of edges with intermediate length. Each year was evaluated individually to study time-space structuring pattern. With the spatial coordinates from 2013, edge thinning technique and molecular data were joined to further evaluate spatial structuring within each fondo. If significant clustering is observed within each fondo, then the presence of natal philopatry could be one of the factors causing this pattern. Other influences on nest site selection such as habitat quality could also generate a similar pattern. To test the natal philopatry hypothesis, I determined whether genetic differentiation among nests within sites of nest

aggregations was smaller than between nests in different aggregation sites within each fondo.

2.7 Spatial genetic structure of nests

A hierarchical AMOVA was performed to detect differentiation at a finer spatial scale within each fondo. The proportion of genetic variance attributable to clusters within fondos was estimated and tested for significance. The variance components were defined in four hierarchic levels: (i) among clusters (F_{CT}), (ii) among nests within clusters (F_{SC}), (iii) among individuals within nests (F_{IS}), and (iv) within individuals (F_{IT}). These variance components were assessed with Arlequin v. 3.5.1.3 (Excoffier and Lischer, 2010). Arlequin implements Wright's fixation index (F_{ST}) to describe the amount of genetic variation for each hierarchic level according to Weir and Cockerham (1984).

2.8 Testing the isolation-by-distance model

A second approach tested the natal philopatry hypothesis with an Isolation-by-Distance model (Wright, 1943). Mantel's test estimates the significance of correlation between distance matrices (Mantel, 1967). Here the two distance matrices were genetic and geographic distances between individuals. A predictor matrix of pairwise linear distances in meters and a dependent matrix of individual pairwise genetic distances were created with GenAlEx 6.5 (Peakall and Smouse, 2006 and 2012). To create the pairwise genetic distance matrix, Euclidean genetic distance was estimated in GenAlEx through the option of Genetic Distance (Codom-Genotypic) (Smouse and Peakall, 1999). To create a geographic distance matrix, a pairwise, linear geographic distance matrix was created from longitude and latitude coordinates for each sampled individual. The null

hypothesis was that the two distance matrices are independent. The alternative hypothesis is that of a positive association between the two distance matrices, suggesting the presence of isolation-by-distance (Smouse *et al.*, 1986). Two assumptions were made. First, that the conditions influencing hatchling fitness (nest quality) are stable at each fondo. Second, that the presence of nest aggregations is a consequence of the preference of daughters to nest in the same or similar locations as their mothers.

CHAPTER III

RESULTS

3.1 Sampling

Two of the three populations of *C. ricordii* in the Dominican Republic were successfully sampled, Cabritos Island and Pedernales. The Pedernales population is spread across a large area, and identifying areas with high densities of individuals has been challenging. Of the 53 adults sampled (Appendix B), 15 (28.3%) were animals killed by dogs, and 12 (22.6%) were captured with Tomahawk traps. On Cabritos Island, I collected samples from animals found dead from natural causes (7.5%). The rest of the wild captures were made with nooses and nets (41.5%). The sampling success reflects the high level of threat these iguanas face, given that 35.8% of the individuals sampled were carcasses. Tomahawk traps proved to be a very successful method of capturing wild adults on Cabritos Island and in the Pedernales population.

A total of 26 nests from Malagueta and 23 from Tierra were sampled during 2013, and the number of individuals captured per nest ranged from 1 to 23. Some individuals were removed from the dataset because of poor DNA quality (i.e., these individuals usually came from carcasses of hatchlings found while opening the nest and from hatchlings that died before hatching). Appendix B presents the number of hatchlings from 2013 that were successfully genotyped and included in the analyses. The number of individuals per nest ranged from 1 to 15. The 84 hatchlings sampled on 2012 were used

for the screening of molecular markers and excluded for further analysis, because they only represented a very small area of Fondo de la Tierra and sampling effort could not be compared with the 2013 sampling.

3.2 Microsatellite data

Samples were genotyped at 14 microsatellite loci found to be variable for *C. ricordii*. The polymorphic markers used were: Ccste05 (Rosas *et al.*, 2008); CIDK135, CIDK144 and CIDK184 (Welch *et al.* 2011); Z106, Z148, Z154 and Z494 (Junghwa *et al.* 2004); and D1, D11 D101, D110, D111 and D140 (Lau *et al.* 2009). Only CIDK135 (Welch *et al.*, 2011) was eliminated from further analysis because of the null allele frequency $p = 0.48$ (Dakin and Avise, 2004; Table 3.1). Thirteen polymorphic molecular markers were used in the rest of the analyses.

Table 3.1 Polymorphic microsatellite markers used in this study.

No.	Locus	Null allele frequency (p)	No.	Locus	Null allele frequency (p)
1	Ccste05	0.06	8	Z494	0.08
2	CIDK135	0.48	9	D1	0.06
3	CIDK144	0.08	10	D11	0.05
4	CIDK184	0.13	11	D101	0.03
5	Z106	0.13	12	D110	0.11
6	Z148	0.07	13	D111	0.12
7	Z154	0.08	14	D140	0.13

Identifier (No.), locus name (Locus) and null allele frequency (p). In bold, CIDK135 with a null allele frequency > 0.20 .

3.3 Genetic variation between geographic regions and between fondos

The numbers of migrants (N_m) were evaluated between geographic regions and between fondos. Gene flow between Cabritos and Pedernales populations was below the

value of one ($N_m = 0.99$, Table 3.2). For the estimation of gene flow between Malagueta and Tierra the value exceeded one ($N_m = 8.38$), which signifies that local levels of gene flow between fondos is present (Table 3.2). The level of gene flow between fondos indicates that nesting females may nest interchangeably between fondos. Number of alleles per locus (N_a) ranged from 4 to 16 for the thirteen markers (Table 3.3). F_{IS} population specific indices obtained from Arlequin were significant for both populations when all individuals were included (adults and hatchlings), and when only adults were considered (Table 3.4). For both populations a significant excess of homozygotes was evidenced. The degree of homozygosity for Pedernales decreases greatly when hatchlings were included. Pedernales hatchling data were divided into each of the fondos. F_{IS} indices per fondo suggested also an excess of homozygotes for hatchlings from both fondos, but was only significant for Fondo de la Tierra (Table 3.4).

Table 3.2 Output from Genepop on the web v4.2 – Option 4 N_m estimates (private allele method).

PED vs. CAB: Adults	MAL vs. TIE: Hatchlings
Mean frequency of $P_a = 0.09$	Mean frequency of $P_a = 0.01$
$N_m = 0.99$	$N_m = 8.38$

Pedernales (PED), Cabritos Island (CAB), Malagueta (MAL) and Tierra (TIE). Private alleles (P_a).

Table 3.3 Genetic variation by locus in sampled adults and hatchlings from Cabritos Island and Pedernales populations

		Cabritos Island Adults					Pedernales Adults				
Locus	N _a	H _E	H _O	P	S.D.	F _{IS}	H _E	H _O	P	S.D.	F _{IS}
Ceste05	10	0.795	0.526	0.002	0.000	0.344	0.807	0.632	0.102	0.000	0.222
CIDK144	4	0.440	0.400	0.614	0.001	0.093	0.579	0.316	0.023	0.000	0.461
CIDK184	6	0.782	0.750	0.904	0.000	0.042	0.772	0.579	0.115	0.000	0.256
Z106	6	0.737	0.550	0.041	0.000	0.259	0.756	0.750	0.080	0.000	0.009
Z148	6	0.565	0.579	0.005	0.000	-0.026	0.737	0.650	0.092	0.000	0.121
Z154	9	0.677	0.350	0.000	0.000	0.489	0.603	0.412	0.003	0.000	0.323
Z494	7	0.682	0.524	0.025	0.000	0.236	0.659	0.550	0.270	0.000	0.169
D1	6	0.512	0.500	0.076	0.000	0.023	0.779	0.600	0.056	0.000	0.235
D11	14	0.765	0.789	0.009	0.000	-0.033	0.865	0.947	0.259	0.000	-0.098
D101	8	0.449	0.450	0.378	0.001	-0.003	0.687	0.556	0.037	0.000	0.196
D110	10	0.586	0.526	0.905	0.000	0.105	0.587	0.526	0.501	0.001	0.107
D111	12	0.822	0.526	0.000	0.000	0.366	0.541	0.278	0.001	0.000	0.494
D140	16	0.798	0.842	0.567	0.000	-0.057	0.819	0.737	0.253	0.000	0.103
Mean		0.662	0.563			0.141	0.707	0.579			0.200
		Fondo de la Tierra Hatchlings					Fondo de la Malagueta Hatchlings				
Locus	N _a	H _E	H _O	P	S.D.	F _{IS}	H _E	H _O	P	S.D.	F _{IS}
Ceste05	10	0.698	0.660	0.005	0.000	0.055	0.702	0.706	0.002	0.000	-0.006
CIDK144	4	0.613	0.464	0.000	0.000	0.245	0.603	0.610	0.000	0.000	-0.011
CIDK184	6	0.749	0.667	0.002	0.000	0.111	0.788	0.757	0.006	0.000	0.040
Z106	6	0.726	0.725	0.000	0.000	0.001	0.682	0.630	0.000	0.000	0.077
Z148	6	0.653	0.567	0.002	0.000	0.132	0.698	0.750	0.003	0.000	-0.075
Z154	9	0.560	0.490	0.001	0.000	0.125	0.569	0.517	0.007	0.000	0.090
Z494	7	0.563	0.544	0.016	0.000	0.034	0.594	0.610	0.006	0.000	-0.027
D1	6	0.751	0.767	0.348	0.000	-0.021	0.744	0.767	0.045	0.000	-0.031
D11	14	0.838	0.796	0.000	0.000	0.050	0.798	0.826	0.000	0.000	-0.035
D101	8	0.708	0.647	0.000	0.000	0.086	0.728	0.718	0.000	0.000	0.013
D110	10	0.482	0.362	0.012	0.000	0.250	0.570	0.407	0.000	0.000	0.286
D111	12	0.657	0.451	0.000	0.000	0.315	0.257	0.150	0.000	0.000	0.417
D140	16	0.800	0.830	0.014	0.000	-0.037	0.773	0.669	0.000	0.000	0.135
Mean		0.677	0.613			0.103	0.654	0.624			0.067

Locus name (Locus). Number of alleles (N_a). Expected and observed heterozygosity (H_E and H_O, respectively), and their respective P value and Standard Deviation (S.D.) (Guo and Thompson, 1992). Coefficient of inbreeding (F_{IS}) according to Weir and Cockerham (1984). In bold significant P values.

Table 3.4 Population specific F_{IS} indices.

	F_{IS}	P (Rand $F_{IS} \geq$ Obs F_{IS})
Adults:		
CAB	0.091	0.028
PED	0.141	0.000
Adults + Hatchlings:		
PED	0.056	0.000
Hatchlings:		
MAL	0.019	0.151
TIE	0.067	0.002

Output from Arlequin v. 3.5.1.3. F_{IS} indices with 1023 permutations. Pedernales (PED), Cabritos Island (CAB), Malagueta (MAL) and Tierra (TIE). In bold significant P values.

3.4 Dispersal between and within geographic regions

An Analysis of Molecular Variance between Cabritos (CAB) and Pedernales (PED) adults revealed that a significant portion of the differences in the genetic makeup of these two areas can be explained by relative isolation and lack of gene flow ($F_{ST} = 0.132$, $p \ll 0.01$; Table 3.5). A significant, although less pronounced, degree of isolation was also found with an AMOVA apportioning genetic variance between Malagueta (MAL) and Tierra (TIE) fondos ($F_{ST} = 0.020$, $p \ll 0.01$; Table 3.5). When adults and hatchlings were combined in the third AMOVA design, the degree of differentiation between CAB and PED increased to 15.4% ($F_{ST} = 0.154$, $p \ll 0.01$; Table 3.5).

Table 3.5 Pairwise F_{ST} estimates.

	F_{ST}	$P \pm S.E.$
CAB vs. PED (Adults)	0.132	0.000 \pm 0.000
MAL vs. TIE (Hatchlings)	0.020	0.000 \pm 0.000
CAB vs. PED (Adults + Hatchlings)	0.154	0.000 \pm 0.000

Pedernales (PED), Cabritos Island (CAB), Malagueta (MAL) and Tierra (TIE). Significant P values in bold. Standard Error (S.E.).

3.5 Spatial structure of nests

3.5.1 Between fondos: coarse spatial scale

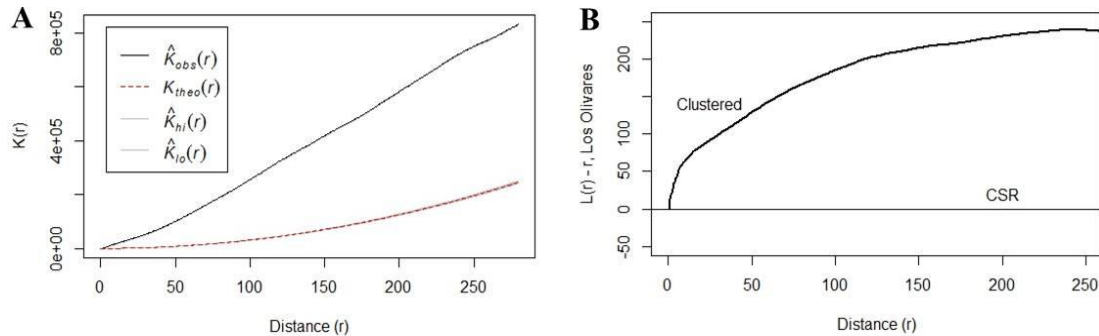


Figure 3.1 Plots of $K(r)$ vs. distance (r) and corresponding function $L(r) - r$ plotted against distance (r) for Los Olivares.

(A) Plot of $K(r)$ vs. distance (r) for Los Olivares (MAL and TIE) using the envelope function. $K_{obs}(r)$ represents the observed value of $K(r)$ for the data, $K_{theo}(r)$ is the theoretical value of $K(r)$ assuming a random distribution (CSR under a poisson model), $K_{hi}(r)$ and $K_{lo}(r)$ represent the upper and lower boundaries for the curve from 95 simulations which closely overlap with $K_{theo}(r)$. Significance level of the Monte Carlo test: $2/96 = 0.0208$. (B) Ripley's K corresponding function $L(r) - r$ plotted against distance (r) .

Ripley's K was estimated at a coarse scale including data from both fondos in Los Olivares. A total of 1,166 spatial points (nests) from 2008-2013 occurred within a 1,100 x 1,500 m rectangular plot. $K(r)$ was estimated for distances up to 250 m. After this cut-off distance value, the spatial pattern of nests became a random process. Observed variation in node density for all $K(r)$ estimates, as measured by $K_{obs}(r)$, exceeded the expected variance that assumed a random distribution of points, $K_{theo}(r)$. This indicates that nests are significantly clustered when evaluated at a coarse scale (Figure 3.1), and within a radius of 250 m, the clustering pattern is clearly evident.

3.5.2 Within fondos: fine spatial scale

Ripley's K was estimated at a finer scale where each fondo was evaluated individually. For Malagueta fondo a total of 573 spatial points (nests) from 2008-2013 that were within a 170 x 476 m rectangular plot were measured. $K(r)$ was estimated for distances up to 40 m, the threshold distance at which nest density approaches that expected under the null model (Figure 3.2 A). Fondo de la Tierra included 593 spatial points in a 454 x 695 m rectangular plot. $K(r)$ was estimated for distances of up to 100 m, the threshold for the null model (Figure 3.2 B). Observed variation in node density for all $K(r)$ estimates, as measured by $K_{\text{obs}}(r)$, exceeded the expected variance assuming a random distribution of points, $K_{\text{theo}}(r)$. This suggests a similar pattern as the one for Los Olivares, where nests are significantly clustered even when the scale under evaluation decreases (Figure 3.2). This indicates that for Malagueta fondo within a radius of 40 m, nests are significantly clustered and for Tierra the radius where significant clustering is present is 100 m. The corresponding function, $L(r) - r$, also suggests that the degree of clustering in relation to distance (r) varies between sites. Because of the pre-existing clustering pattern of the fondos at the Pedernales site and due to the significant degree of clustering observed for each fondo individually, both fondos are analyzed separately from this point forward.

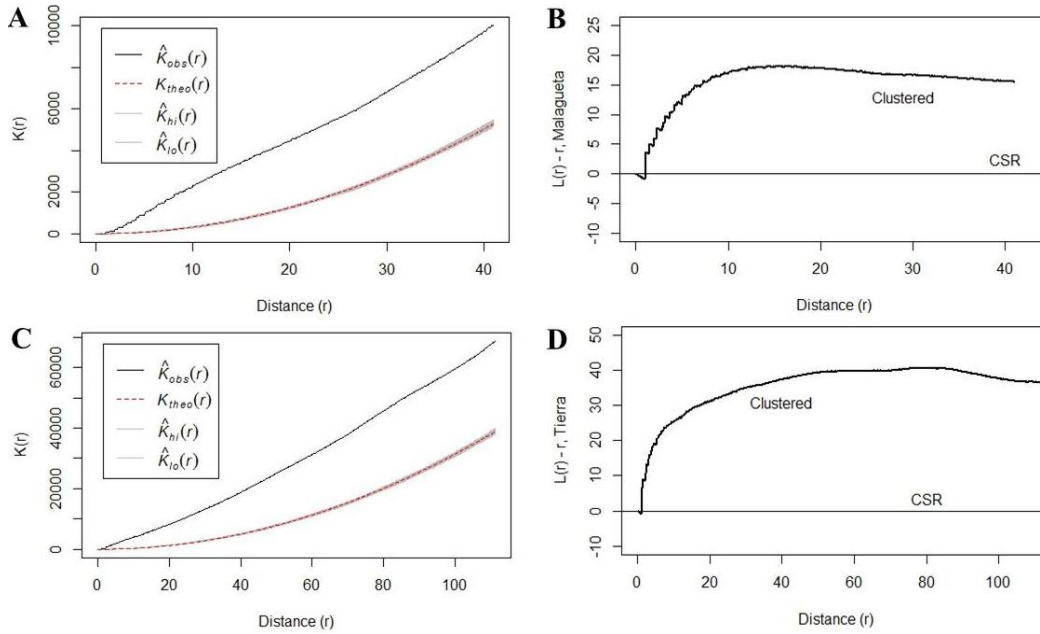


Figure 3.2 Plots of $K(r)$ vs. distance (r) and corresponding function $L(r) - r$ plotted against distance (r) for Malagueta and Tierra.

(A and C) Plots of $K(r)$ vs. distance (r) for (A) Malagueta and (C) Tierra using the envelope function. $K_{\text{obs}}(r)$ represents the observed value of $K(r)$ for the data, $K_{\text{theo}}(r)$ is the theoretical value of $K(r)$ assuming a random distribution (CSR with a Poisson model), $K_{\text{hi}}(r)$ and $K_{\text{lo}}(r)$ represent the upper and lower boundaries for the curve from 95 simulations which closely overlap with $K_{\text{theo}}(r)$. Significance level of the Monte Carlo test for Malagueta and Tierra were: $2/96 = 0.0208$. (B and D) Ripley's K corresponding function $L(r) - r$ plotted against distance (r) for (B) Malagueta and (D) Tierra.

3.5.3 Spatial structure of nests by year

Spatial points for each fondo for every year from 2008-2013 were plotted to evaluate the pattern of clustering suggested by Ripley's K at a finer scale and to evaluate if the clustering pattern observed changes over time. Dotted lines on Figures 3.3 and 3.4 indicate distances that were underrepresented, or where plateaus occur, and supplementary information for these plateaus are provided in Appendix C. Results suggests that the scale of aggregation varies across years as well as the distances between aggregations. The ranges of distances at which clustering patterns were detected for

Tierra ranged from 50-70 m and for Malagueta these values ranged from 45-70 m (Appendix C). If circles with radii of 50-70 m at Tierra or 45-70 at Malagueta are considered, clustering of nests is observed over the years. For 2013 no pattern of spatial aggregation was found. All nests were considered to be part of one cluster when the edge thinning technique was used. This suggests that nests were more dispersed across fondos for 2013. Other cases of this sort of pattern include Tierra in 2009 (Figure 3.3) and Malagueta in 2008 (Figure 3.9).

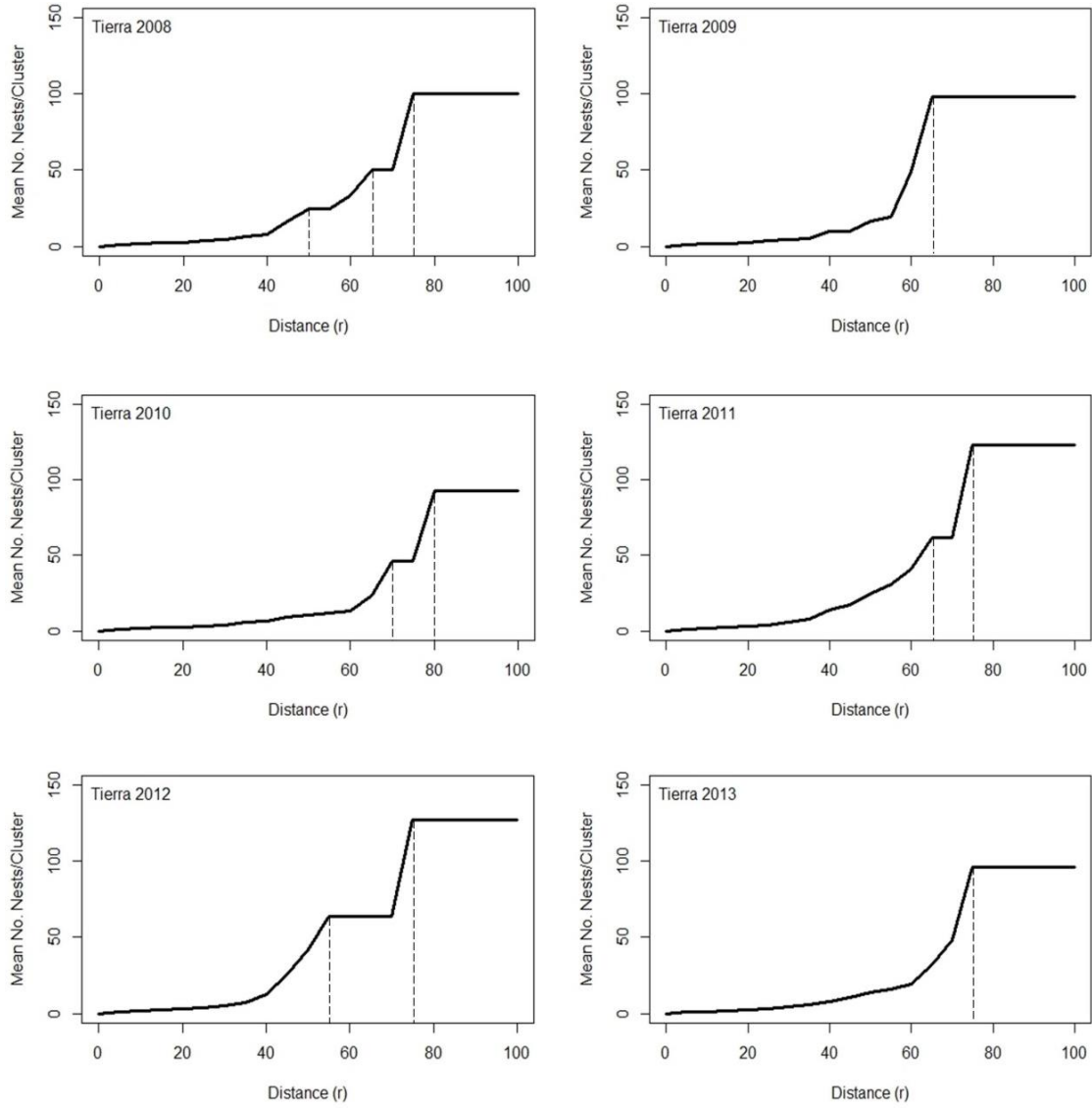


Figure 3.3 Edge thinning technique applied for Fondo de la Tierra by year.

Plotted in increments of 5 m (step command = 5 in R). Plots represent data from the $L(r) - r$ function for $K(r)$. Distance (r) is in meters. Dotted lines represent the minimal distance at which plateaus are formed. Each graph represents a different year.

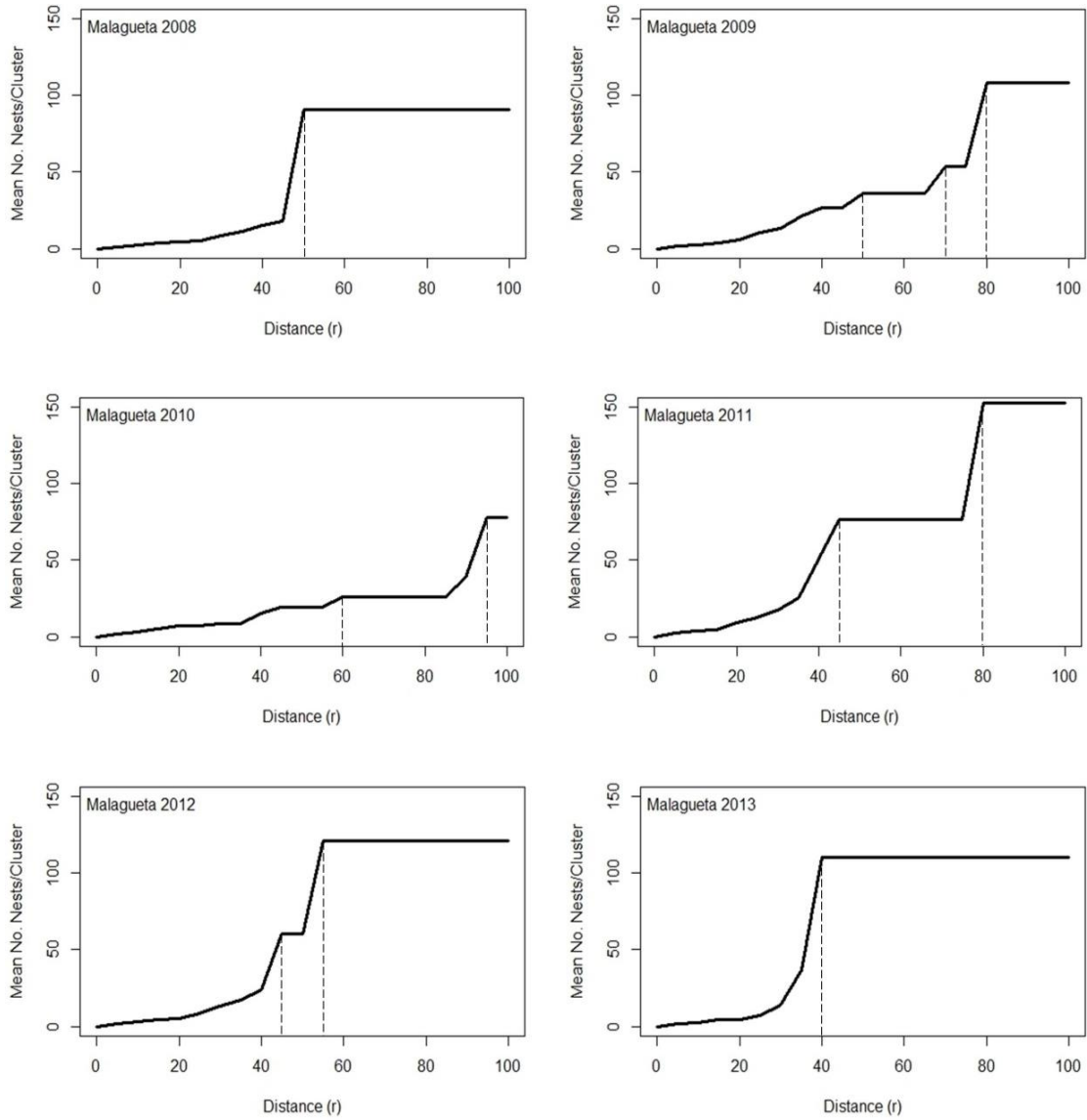


Figure 3.4 Edge thinning technique applied for Fondo de la Malagueta by year.

Plotted in increments of 5 m (step command = 5 in R). Plots represent data from the $L(r) - r$ function for $K(r)$. Distance (r) is in meters. Dotted lines represent the minimal distance at which plateaus are formed. Each graph represents a different year.

3.6 Spatial genetic structure of nests

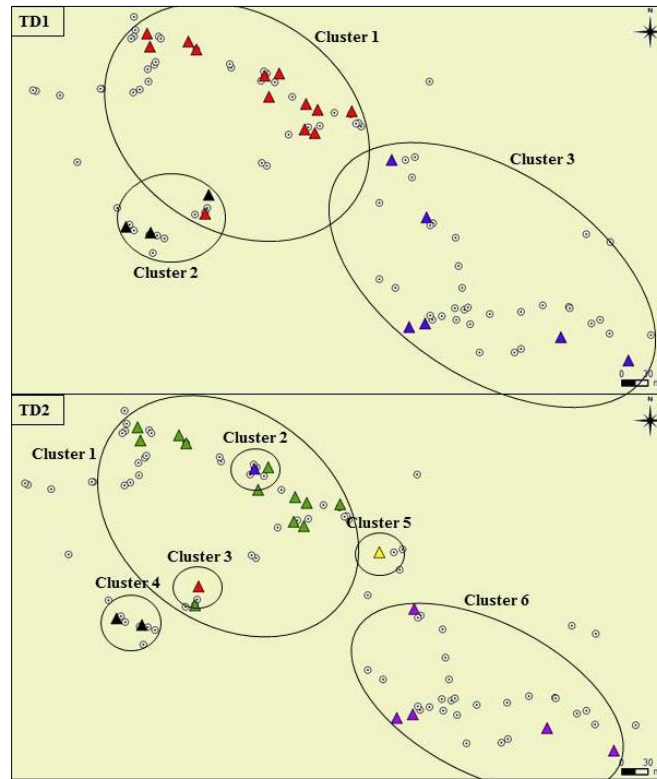


Figure 3.5 Fondo de la Tierra clustering pattern from edge thinning technique.

TD1 implements a distance radius of 60 m and TD2 of 40 m to detect clusters. Each cluster is sequentially numbered. Circles represent un-sampled nests from 2013 in Fondo de la Tierra and colored triangles are sampled nests. Triangles that belong to a same cluster are represented with the same color.

Distances below the minimal plateau distance for the data points from 2013 that were obtained from the edge thinning technique were combined with molecular data. Even if plateaus were not clearly defined in the results of the 2013 edge thinning procedures and only one pattern of aggregation was observed, multiple distances were tested. The females within each putative cluster labeled on Figures 3.5 and 3.6 should be more closely related when compared to females from other clusters. The distances used to subdivide each fondo into clusters were based on the result of the edge thinning analysis.

Figure 3.5 presents two different threshold distances (TD) used for Tierra and the clustering pattern at those distances. Figure 3.6 shows the TD used for Malagueta fondo.

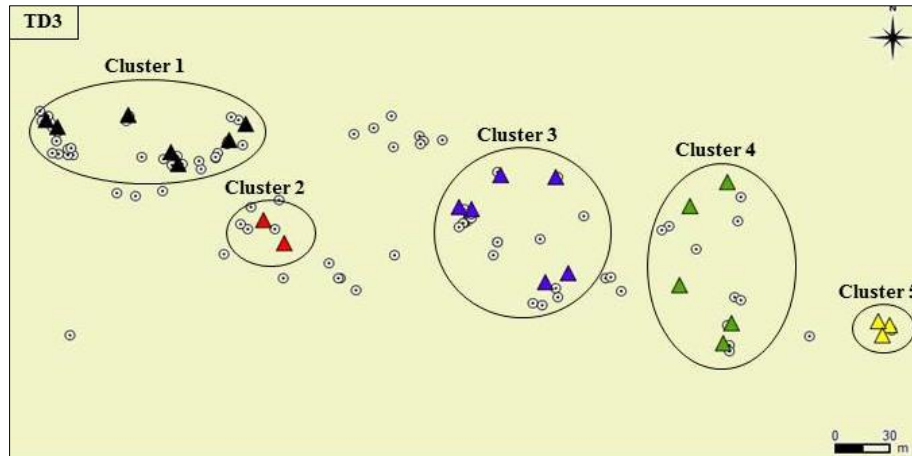


Figure 3.6 Fondo de la Malagueta clustering pattern from edge thinning technique.

TD3 implements a radius of 30 meters to detect clusters. Each cluster is sequentially numbered and represented with a different color. Circles represent un-sampled nests from 2013 in Fondo de la Malagueta and colored triangles are sampled nests. Triangles that belong to a same cluster are represented with the same color.

F-statistics for each of the clustering patterns tested are shown in Table 3.6. Genetic structuring for the hierarchical levels (i) among clusters within fondo (F_{CT}) and (iii) among individuals within nests (F_{IS}) was not significant. This indicates that the spatial genetic structuring suggested in Figures 3.5 and 3.6 was not supported when the genetic structuring involved clusters and individuals within nests. Smaller distances were tested and no significant differences in the AMOVA were detected. To further decrease the radii of distances used, a higher amount of nests should be included in this type of analysis. The most significant level of differentiation was (ii) among nests within clusters (F_{SC}). This result indicates that 20% of the variation in Tierra and 17.5% of the variation in Malagueta can be explained among the nests within clusters. This suggests that nests

within a single cluster are not more closely related than nests from other clusters. The hierarchical level related to the degree of differentiation (iv) within individuals (F_{IT}) was moderately significant for Tierra, and for Malagueta it was not significant. This suggests that there is a significant deviation of alleles from Hardy-Weinberg expectations within individuals relative to the entire fondo (Holsinger and Weir, 2009), which is consistent with the population specific F_{IS} indices estimated for fondo de la Tierra (Table 3.4).

Table 3.6 F-statistics to evaluate the clustering patterns depicted in Figures 9 and 10.

Hierarchical level (i – iv)	Fondo de la Tierra		Fondo de Malagueta
	TD1: 60 m	TD2: 40 m	TD3: 30 m
$F_{CT} =$	-0.015	-0.003	0.001
$P \pm S.E. =$	0.887 ± 0.009	0.531 ± 0.005	0.552 ± 0.012
$F_{SC} =$	0.204	0.200	0.175
$P \pm S.E. =$	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
$F_{IS} =$	-0.158	-0.158	-0.173
$P \pm S.E. =$	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
$F_{IT} =$	0.064	0.070	0.033
$P \pm S.E. =$	0.003 ± 0.002	0.003 ± 0.001	0.129 ± 0.010

Hierarchic levels on table: (i) among clusters (F_{CT}), (ii) among nests within clusters (F_{SC}), (iii) among individuals within nests (F_{IS}), and (iv) within individuals (F_{IT}). Significant P values in bold.

3.7 Testing the isolation-by-distance model

To evaluate natal philopatry for both Tierra and Malagueta fondos, a Mantel's test was performed using 13 microsatellite markers for 22 nests from Tierra (109 samples in total) and 23 nests from Malagueta (148 samples in total). There was a positive R^2 value for all Mantel's tests performed, and the R^2 values shown in Table 3.7 for all correlations are very small. These results suggest that only 0.02% and 0.80% of the variation in Tierra

and Malagueta, respectively, can be explained by the isolation-by-distance model (Wright, 1943). This was only significant for Malagueta. When both fondos were evaluated together, a significant positive correlation was still present but very little of the variation can be explained with this model (only 0.7%). In this respect, the alternative hypothesis of a positive association between matrices was supported. However, very little of the genetic variation could be explained by the model, which is consistent with some degree of fine scale philopatry.

Table 3.7 Paired Mantel Tests for hatchlings from Malagueta and Tierra.

Genetic distance	Sample	Linear fit	R	R ²	P
vs. Geographic distance	All individuals from 45 nests (Tierra+Malagueta)	$y=0.0007x+19.52$	0.083	0.0069	0.010
vs. Geographic distance	All individuals from 22 nests (Tierra)	$y=0.0006x+20.502$	0.015	0.0002	0.410
vs. Geographic distance	All individuals from 23 nests (Malagueta)	$y=0.0042x+18.483$	0.089	0.0078	0.020

Tests performed between a pairwise geographic distance matrix (GGD) and a pairwise genetic distance matrix (GD) for hatchlings emerging from nests in Tierra and Malagueta Fondos, combined and separately. Significant P values in bold.

CHAPTER IV

DISCUSSION

In this study I investigated overall genetic structure for the species based on individuals sampled from two locations, Pedernales and Cabritos Island. I found significant differences between these populations suggesting restricted gene flow between sites. I also analyzed the nesting behavior of *C. ricordii* with neutral molecular markers to test the hypothesis of natal philopatry. I focused on two fondos, or nesting areas, in Pedernales province: Fondo de la Tierra and Fondo de la Malagueta. I found support for high levels of spatial clustering of nest sites within fondos consistent with a high return rate of females to nest in specific areas within these communal sites. The hypothesis of ‘natal philopatry’, however, was not supported because females nesting in the same cluster were no more closely related to each other than other females in these fondos. Further, the relationship between geographic distance and genetic distance among hatchlings within fondos was not strong enough to have a marked effect on fine scale genetic structure.

4.1 Population genetic structure

Descriptive statistics for adults collected from Pedernales and Cabritos Island showed a significant excess of homozygotes in both populations. When the F_{IS} indices were generated for both hatchlings and adults in Pedernales, they were lower indicating

that homozygote excess is lower in hatchlings than adults. However, the excess homozygosity in adults may reflect sampling (i.e. small sample size, Pedernales = 20 and Cabritos = 21). Hatchlings were collected from discrete nesting sites (fondos), and the assumption that these animals belong to the same population seems appropriate. Collection of adults was more haphazard. Many of the adults sampled were deceased as a result of predation by feral dogs. Because the precise origins of adults sampled are unknown there is a distinct probability that these belong to a broader geographic range, and do not meet the assumption that they belong to a single panmictic population. Hence, the apparent elevated observed homozygosity in adults may reflect genetic differences among populations at a broader geographic scale than was assessed for hatchlings.

The significant degree of differentiation observed between allele frequencies in Pedernales and Cabritos Island populations ($F_{ST} = 0.132$, $p < 0.01$) is suggestive of nearly complete genetic isolation between these populations. Estimating number of migrants between these populations based on private alleles also supports limited gene flow between geographic regions ($N_m = 0.99$). According to Slatkin (1987), $N_m < 1$ cannot counteract the effects of genetic drift, and is suggestive of nearly complete isolation between these populations. The pattern of genetic structure was not entirely surprising because of the magnitude of the geographic barriers between sites. Sierra de Bahoruco (a mountain system on Hispaniola), which reaches almost 2,000 m asl and Enriquillo Lake are important barriers for dispersal between Pedernales and Cabritos Island populations. Many studies have demonstrated how these types of barriers influence genetic divergence of other species of *Cyclura* (Colosimo *et al.*, 2014) and other species from Hispaniola (Gifford *et al.*, 2004; Sly *et al.*, 2010; Brace *et al.*, 2012). Colosimo *et al.* (2014) found

that as much as 27% of the total genetic variance in the Andros Island Rock Iguana (*Cyclura cychlura cychlura*) was explained by differences among populations. These authors concluded that iguanas may only rarely disperse over wider water channels that separate the major landmasses that compose Andros Island, or that dispersal rarely results in successful migration over these water channels. Gifford *et al.* (2004) conducted an extensive evaluation of a small reptile, *Ameiva chrysolema*, and found the isolating effects of the Cordillera Central and Sierra de Batoruco mountains. Gifford *et al.* (2004) found a 14% mitochondrial sequence divergence between the northern and southern populations. Similar results were also found for a small rodent, *Plagiodontia aedium*, which also exhibits sequence divergence of almost 3% between populations separated by these geographic barriers (Brace *et al.*, 2012). Sly *et al.* (2010) found mitochondrial sequence divergence of 5% between the northern and southern populations of Palm-Tanagers (*Phaenicophilus*) sampled from multiple mountain systems of Hispaniola. Overall, these studies provide clear evidence that the landscape of Hispaniola can limit the dispersal for many terrestrial vertebrates.

4.2 Natal philopatry

Genotypic data for hatchlings collected from Tierra and Malagueta revealed an excess of homozygotes for both fondos, though it was only significant for Tierra. The homozygosity observed in hatchlings from Pedernales, along with elevated levels of homozygosity for adults suggests that positive assortative mating may occur in the Pedernales population, and that limited dispersal is present between fondos within Pedernales. This may indicate that the breeding population from Pedernales is small. Natal philopatry in a small breeding population may reinforce patterns of inbreeding

among the adult population. Within fondos, fine-scale genetic structuring observed for iguanas indicates that random mating is not present ($F_{ST} = 0.020$, $p \ll 0.01$). This also suggests that some behavior related to the females' ability to choose a site to nest that increases her fitness ought to be expected. Given the threats that the Pedernales population faces, it is possible that the absence of an excess of heterozygotes in the hatchling data set may be an artifact caused by a small population size where nesting female relatedness will be high, and because of low recruitment among hatchlings.

Fine scale genetic structuring, within distances of 0.1 to 2 km, has been detected in a number of mobile animal species such as rattlesnakes (Gibbs *et al.*, 1997; Clark *et al.*, 2008), carabid beetles (Brouat *et al.*, 2003), ungulates (Coltman *et al.*, 2003) and bush rats (Peakall *et al.*, 2003). Moore *et al.* (2008) studied a long-lived reptile (tuatara, *Sphenodon punctatus*) and found an overall genetic differentiation of 1.2% among subpopulations that were only 400 m apart ($R_{ST} = 0.012$, $p = 0.025$). When the authors expanded their analysis to include a wider spatial range (750 m) the pattern disappeared (Moore *et al.*, 2008). Moore *et al.* (2008) found that tuatara lack a philopatric behavior and concluded that long-lived animals may present high genetic variation at a small scale without the presence of a complex social system. Female philopatry can enforce fine scale genetic structuring, and it has been observed in several marine and terrestrial animals (Hueter *et al.*, 2005; Nussey *et al.*, 2005; Frantz *et al.*, 2008; Browne *et al.*, 2010). With Ricord's iguana in Pedernales, very little is known about the species social system. Given the sedentary nature of these lizards, the highly fragmented habitat in Pedernales and the constant threats that the species faces (Rupp, 2010), fine scale genetic structure may be a product of all these factors. Similar results were found by Moore *et al.*

(2008) for tuatara. The social system at the Pedernales nesting habitat may present complex intra- and interspecies interactions between males that are known to present home range philopatry during the mating season, and females that tend to be philopatric towards a communal nesting area (Pérez-Buitrago *et al.* 2010). Sex-biased dispersal is a common pattern for many lizards (Gardner *et al.*, 2001; Stow *et al.*, 2001; Valenzuela and Janzen, 2001), and to study these sex specific traits other types of molecular markers need to be evaluated. The use of nuclear molecular markers may not provide the necessary resolution to fully discriminate sex specific differences. However, R^2 values for the Mantel's test were positive. This highlights the importance of using appropriate markers that reflect dispersal patterns for both sexes if the complex nature of nesting in this genus is to be better understood. Because of the lack of variation observed for the mitochondrial markers tested (Arévalo *et al.*, 1994), additional mitochondrial markers will have to be developed for this species before mtDNA sequence variation can be used as an effective tool for studying female biased dispersal patterns.

When spatial statistics were leveraged to test for natal philopatric behavior, a significant level of spatial clustering of nest sites at coarse and at fine spatial scales was supported through the years (2008-2013). This degree of clustering between fondos, coarse spatial scale, was expected due to the pre-existing clustering nature of these communal nesting areas (fondos) in Pedernales. However, when combined, spatial and genetic data do not support the return of related females to a specific nest aggregation. Although, a strong fidelity towards specific areas, from females of unknown origin, within these fondos was supported with spatial data. Ripley's K and results from edge thinning techniques supports an alternative hypothesis. The "by-product" hypothesis

states that communal nesting results from a scarcity of appropriate nesting sites or other factors that cause coincidental aggregations of nesting mothers (Vitt, 1993; Doody *et al.*, 2009). The pattern detected using spatial statistic tools could hence be explained by the overall shortage of nest sites in Pedernales province. However, an “adaptive” hypothesis may be soon considered, which states that fitness benefits to mothers, eggs, and hatchlings drives communal nesting (Doody *et al.*, 2009). Where a suitable habitat for reproduction might be scarce and restricted to small areas, natural selection has favored individuals that return to the same natal areas to reproduce. Many reproductive advantages have been attributed to philopatric behaviors (Robinson & Bider, 1988; Eckrich & Owens, 1995; Giraldeau, 1997; Galef & Giraldeau, 2001; Giraldeau *et al.*, 2002; Doody *et al.*, 2009).

A significant proportion of the genetic variation detected within fondos distinguishes among nests within clusters ($F_{sc} = 0.204$, $p < 0.01$ for Tierra and $F_{sc} = 0.175$, $p < 0.01$ for Malagueta). This suggests that the patterns of aggregation observed with Ripley’s K estimate are independent of the genetic structuring observed within each fondo. These results are inconsistent with natal philopatry limiting dispersal for nest site selection within these fondos. Nests within each of the putative clusters are not more related to each other than the ones from other clusters. If natal philopatry were present, nests grouped within a single cluster should be more related to each other than nests from other clusters and variation among clusters should be significant. Nevertheless, unrelated females may have selected the same nest site, and elucidating these differences in relatedness may benefit from the gathering of ecological and behavioral data at the individual level for the Pedernales population.

Besides all of the points discussed previously, imprinting is a concept that has been discussed for salmon and sea turtles (Lohmann *et al.*, 2008). Hatchlings may imprint on environmental parameters such as the type of soil and vegetation when they hatch. The southern nesting areas for *C. ricordii* are characterized by low-lying geological formations covered by fine, red argillic soil (Arias *et al.*, 2004) and a rather uniform vegetation type where *Acacia* is predominant along with cacti. Given that significant gene flow between fondos was evident ($N_m = 8.38$), this indicates that natal philopatric behavior, if present, may not be strong or exclusive to finer spatial scales. Or this evidence could be overcome by a more general “homing” behavior driven by other ecological factors like imprinting on habitat features. The significant 2% of genetic differentiation between fondos within Pedernales shows how even with a long-lived reptile, very fine scale (< 1km) genetic structuring can be present and limited dispersal may be an outcome of natal philopatry for the Pedernales population. Changes in patterns of aggregations by year indicated that the observed patterns are not constant through space and time. Multiple factors may influence these yearly changes in the patterns of nest sites aggregations. First, is the possibility that females are not nesting every year (Iverson *et al.*, 2004) and second, many environmental factors may be stimulating females to choose a different location every year. Tropical storms have a negative influence on hatching success (Iverson *et al.*, 2004), and anthropomorphic disturbances influence females when they are choosing locations to build nests and lay their eggs. These factors may bias female decision making, and nests may appear more spatially scattered. This might have been the case for the 2013 edge-thinning results, where nests were more dispersed across both fondos than in previous years (Figure 3.3 and 3.4).

4.3 Conservation

The lack of philopatric behavior may have important consequences for future management decisions for *C. ricordii*. Multiple factors, such as quality of the nesting habitat, vegetation and lack of good nesting patches, might influence the recurrent visit to a specific site within the nesting areas by the same female (Bock *et al.* 1985; Knapp and Owens, 2008). Philopatric behavior may not be discounted given that a very slight but positive trend following an isolation-by-distance model was observed with the Mantel's test. Further exploration of the species' social system may be needed and highly variable sex-specific markers should be developed to better test for natal philopatry at these sites. More conservation actions ought to be considered to improve the genetic health of these populations. Extensions of the nesting habitats and translocation techniques have been discussed, and even if they are not the immediate plans for the conservation of the species, we may need to revisit them in the future. Both of these techniques are not likely to be successful if animals reject their new homes. This would likely happen if this species reveals evidence of natal philopatry.

Understanding nesting dynamics may be helpful to detect the most important nesting requirements for this critical endangered species. Several conservation actions have been taken in hopes of preventing further population declines for the species since 2002 and discussions regarding possible solutions in the future have started. Grupo Jaragua NGO implemented the monitoring program for the species nesting's areas in Pedernales Province, and continues to actively work on habitat restoration and protection in Pedernales and the South of the Lake populations. These actions have ensured the maintenance of these populations for the past 10 years.

The success and maintenance of these actions depends heavily on how much information we can gather about the nesting behavior of the species. Ecological parameters and intra- and interspecies interactions are extremely important if we want to translocate a population to a new environment or simply restore historic nesting grounds. Individuals confiscated from the illegal pet trade and from hunters may benefit from an understanding of the genetic structure of their populations. Hence, better informed management decisions for the re-introduction of these individuals to their populations of origin ought to be accomplished, avoiding outbreeding among populations. Given the high degree of differentiation observed between Cabritos Island and Pedernales populations, and the lack of gene flow between them, it may be prudent to maintain both of these populations as independent units for conservation purposes.

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APPENDIX A

R SCRIPT USED FOR THE RIPLEY'S K ESTIMATION AND THE EDGE
THINNING TECHNIQUE MODIFIED FROM BROOKS (2006)

A.1 Ripley's K

```
> mydata1<-read.csv("datasheet.csv")
> head(mydata1)
> mypattern1<-
ppp(mydata1[,2],mydata1[,3],c(215689,216390),c(1994551,1994
996))
> duplicated(mypattern1)
> mypattern2<-unique.ppp(mypattern1)
> plot(unique(mypattern2))
> plot(mypattern2)
> summary(mypattern2)
> plot(Kest(mypattern2))
> summary(Kest(mypattern2))
> plot(envelope(mypattern2,Kest,nsim=95))
> summary(envelope(mypattern2,Kest,nsim=95))
> plot(density(mypattern2))
> summary(density(mypattern2))
> fondol<-Kest(mypattern2)
> str(fondol)
> sqrt(fondol$theo/pi)
> fondol
> plot(sqrt(fondol$theo/pi)- fondol$r~ fondol$r,type='l')
> plot(sqrt(fondol$iso/pi)- fondol$r~ fondol$r,type='l')
> plot(sqrt(fondol$border/pi)- fondol$r~ fondol$r,type='l')
> plot(sqrt(fondol$trans/pi)- fondol$r~ fondol$r,type='l')
> plot(sqrt(fondol$iso/pi)-fondol$r~fondol$r,type='l',
      lwd='3', xlim=c(0,110), xlab='Distance (r)', ylim=c(-
      10,50), ylab='Fondol : L(r) - r'))
> text(40, 31, "Clustered", col='black', lwd='12')
> abline(h=0, col='black', lwd='1')
> text(80, 3.5,"CSR", col="black")
```

A.2 Edge Thinning Technique

```
> dataXY = read.csv('datasheet.csv',header=TRUE)
> head(dataXY)
> distances = as.matrix(dist(dataXY))
> edge.thin <- function(distances, min = 0, max, step) {
```

```

et.clusters = list()

for (i in 1:((1+(max-min)/step))) {

    d = min+(i-1)*step

    M = ifelse(distances < td & distances != 0, 1, 0)
    G = graph.adjacency(M)
    C = clusters(G)
    if(i==1) et.clusters = list(td,C)
    else et.clusters[[i]] = list(td,C)
}
et.clusters
}
> plot.edge.thin = function(x) {
    td = numeric(length(x))
    sz = numeric(length(x))
    for (i in 2:length(x)) {
        td[i] = x[[i]][[1]]
        sz[i] = sum(x[[i]][[2]]$csize)/x[[i]][[2]]$no
    }
    plot(td, sz, type = 'l', lwd = 3, xlim = c(0,150),
xlab
        = 'Distance (r)', ylim = c(0,150), ylab =
        'Mean No. Nests/Cluster, Fondo 2013')
}
> Q = edge.thin(distances,max=max(distances),step=100)
> str(Q)
> plot.edge.thin(Q)
> Q2 = edge.thin(distances,max=100,step=5)
> plot.edge.thin(Q2)
> str(Q2)

```


APPENDIX B
SAMPLING SUCCESS DURING 2012 AND 2013 FIELD SEASONS FOR CYCLURA
RICORDII POPULATIONS

Table B.1 Sampling success for hatchlings and adults.

No.	Malagueta Hatchlings		Tierra Hatchlings		Pedernales Adults		Cabritos Adults	
	NestID (2013)	Qt.	NestID (2013)	Qt.	Year	Qt.	Year	Qt.
1	N02	1	E01	8			2010	5
2	N03	1	E02	10	2012	5	2012	13
3	N08	12	E05	1	2013	21	2013	9
4	N09	11	E06	9				
5	N10	12	E08	3				
6	N11	1	E12	1				
7	N12	15	E13	2				
8	N13	5	E14	15				
9	N15	13	E16	12				
10	N17	1	E18	3				
11	N19	10	E19	1				
12	N20	6	E23	4				
13	N21	4	E26	3				
14	N26	1	E34	4				
15	N27	1	E37	1				
16	N28	1	E40	6				
17	N32	5	E45	8				
18	N36	10	E56	1				
19	N3613	7	E58	1				
20	N43	1	E70	8				
21	N49	2	E73	1				
22	N56	13	E77	3				
23	N77	15						
		148		105		26		27

APPENDIX C

EDGE THINNING TECHNIQUE RESULTS FOR $L(r) - r$ FUNCTION FROM
RIPLEY'S K BY YEAR (2008-2013) FOR EACH FONDO (TIERRA AND
MALAGUETA)

Table C.1 Summary spatial pattern data from edge thinning analysis for Fondo de la Tierra and Malagueta across years 2008-2013.

Year	Tierra Fondo				Malagueta Fondo			
	Plateau ID.	Mean No. Nests/Cluster	No. Clusters	Distance	Plateau ID.	Mean No. Nests/Cluster	No. Clusters	Distance
2008	08-I	25	4	50	08-I	91	1	50
	08-II	50	2	65				
	08-III	100	1	75				
2009	09-I	65	1	65	09-I	36	3	50
					09-II	54	2	70
					09-III	108	1	80
2010	10-I	46.5	2	70	10-I	26	3	60
	10-II	93	1	80	10-II	78	1	95
2011	11-I	61.5	2	65	11-I	76.5	2	45
	11-II	123	1	75	11-II	153	1	80
2012	12-I	63.5	2	55	12-I	76.5	2	45
	12-II	127	1	75	12-II	76.5	2	55
2013	13-I	96	1	75	13-I	110	1	40