Distribution of Genetic Diversity within a Population of *Liolaemus xanthoviridis* and an Assessment of its Mating System, as Inferred with Microsatellite Markers

Author(s): Paula C. Escudero, Derek B. Tucker , Luciano J. Avila, Jack W. Sites Jr., Mariana Morando
Source: South American Journal of Herpetology, 12(3):183-192.
Published By: Brazilian Society of Herpetology
https://doi.org/10.2994/SAJH-D-16-00037.1
URL: http://www.bioone.org/doi/full/10.2994/SAJH-D-16-00037.1

South American Journal of Herpetology, 12(3), 2017, 183–192

Distribution of Genetic Diversity within a Population of *Liolaemus xanthoviridis* and an Assessment of its Mating System, as inferred with Microsatellite Markers

Paula C. Escudero[1,*], Derek B. Tucker [2], Luciano J. Avila[1], Jack W. Sites Jr.[2], Mariana Morando[1]

[1] *Grupo de Herpetología Patagónica. Instituto Patagónico para el Estudio de los Ecosistemas Continentales. Centro Nacional Patagónico- Consejo Nacional de Investigaciones Científicas. Puerto Madryn, Argentina.*
[2] *Department of Biology 4102 LSB, Brigham Young University, Provo, USA.*
[*] *Corresponding author. Email: paulaescudero2@gmail.com*

Abstract. Microsatellites are useful markers to address questions of recent gene flow, given that they are relatively neutral to natural selection and show high levels of variability. To date, only one study has used these markers to answer ecological questions in the species-rich lizard genus *Liolaemus*. Here, we use microsatellite loci to estimate population structure, paternity, and effective size of a population of *L. xanthoviridis*. The study took place in Isla Escondida Bay, Chubut (Argentina), during four spring–summer seasons (2012–2015). We marked and sexed 227 captured individuals and transported 10 gravid females to our laboratory. Digits of marked lizards were used for molecular work, and we resolved eight microsatellite loci to characterize genetic diversity, paternity, and population structure. We found no evidence of multiple paternity, and our samples constitute a single genetic population of *L. xanthoviridis*. Our results show that genetic diversity is higher in *L. xanthoviridis* than in many other species of lizards we found in the literature. Such high genetic diversity is important given the restricted geographic distribution of this species.

Keywords. Genetic diversity; Paternity; Population structure; Rawson lizard.

Resumen. Los microsatélites son marcadores útiles para responder cuestiones en escala de tiempo ecológico, dado que son relativamente neutrales a la selección natural y muestran altos niveles de variabilidad. Hay un único estudio previo que utiliza estos marcadores moleculares para responder cuestiones ecológicas en un género tan ampliamente extendido y rico en número de especies como es *Liolaemus*. En este trabajo usamos estos marcadores para estimar la estructura poblacional, la paternidad de puestas de huevos y el tamaño efectivo de una población de *L. xanthoviridis*. Este estudio se llevó a cabo en la Bahía Isla Escondida, Chubut (Argentina), durante cuatro temporadas primavera-verano (2012-2015). Marcamos y sexamos 227 individuos y transportamos diez hembras grávidas a nuestro laboratorio. Los dígitos extraídos durante el marcado de los individuos fueron usados para los estudios moleculares, donde amplificamos ocho loci microsatélites para caracterizar la diversidad genética, paternidad y estructura poblacional. Los análisis de paternidad revelaron ausencia de paternidad múltiple y que a lo largo de todo el área de estudio hay una sola población genética de *L. xanthoviridis*. Nuestros resultados mostraron que la diversidad genética era más alta que para otras especies de lagartijas que revisamos en la literatura. Esta alta diversidad genética es importante dado la restringida distribución geográfica de esta especie.

INTRODUCTION

The genetic structure of a population is determined by its demography (which is established by all processes associated with birth, death and dispersal) and genetic processes such as selection, genetic drift, migration, recombination, and mutation (Slatkin, 1994). To infer the genetic structure of any species, it is necessary to characterize the pattern of genetic variation within that species.

While the mating system of a population contributes to its genetic structure, studies of mating strategies in nature are challenging because it is usually difficult to observe matings in natural populations. Even when this is feasible, the number of matings may not match estimates of reproductive success as inferred from molecular markers (Hughes, 1998). In many apparently monogamous species, genetic analyses often show extra-pair copulations (Westneat and Webster, 1994). Hence, observed social organization in a population does not necessarily correspond to a specific mating system. Many studies provide evidence for both high (Pemberton et al., 1992; Altmann et al., 1996; Abell, 1997; Gullberg et al., 1997) and low (Amos et al., 1993; Coltman et al., 1999) correlation between reproductive success measured by behavioral observations and inferred from genetic markers. Coltman et al. (1999) suggest that in species with stable dominance hierarchies, or those in which males guard females, the social organization is more likely to match the mating system. In contrast, in species where male–female associations are brief and transient, or in species characterized by frequent shifts in dominance rank, there may be little or no correlation between mating system and social organization.

Genetic data can make important contributions to species conservation, especially if combined with ecological, ethological, and biogeographic studies. These data allow estimation of patterns of hybridization, gene flow, mating systems, effective population size, and population

Submitted: 01 August 2016
Accepted: 04 April 2017
Handling Editor: Ana Carolina Carnaval
doi: 10.2994/SAJH-D-16-00037.1

viability, among others (DeYoung and Honeycutt, 2005). Microsatellite markers characterized by high within-population polymorphism are especially suitable for population-level studies (Zane et al., 2002). This high degree of polymorphism is due to a high mutation rate (from 10^{-6} to 10^{-2} per generation per site, Schlötterer, 2000). These high levels of variability make microsatellites useful for answering questions at very fine (ecological) time scales (Maudet et al., 2002). For example, one can estimate variability within and between populations, consanguinity, mating systems, parenthood relationships and reproductive success, gene flow and dispersal of individuals, and, for populations and metapopulations, levels of differentiation and hybridization (Schlötterer and Pemberton, 1998).

Microsatellites have been widely used for reptile studies in the last decade (e.g., Cooper et al., 1997; Stow et al., 2001; Laloi et al., 2004; Johansson et al., 2008), but only one study (Ariani et al., 2013) has used microsatellites to answer ecological questions within a single species of the widespread and species-rich lizard genus *Liolaemus* Wiegmann, 1834. A recent study characterized 10 polymorphic microsatellite loci for *L. fitzingerii* Duméril and Bibron 1837, and their cross-amplification in *L. chehuachekenk* Avila et al. 2008 (Hanna et al., 2012). Among the oviparous species of *Liolaemus,* no records exist of a taxon being able to incubate and hatch eggs in captivity. These eggs are extremely fragile and, until now, no protocol has been optimized to allow the hatching of *Liolaemus* eggs in the laboratory. The present study is the second to use microsatellites to estimate the genetic structure in a single species of *Liolaemus* and the first to estimate paternity within a natural population of this genus.

Here, we focus on *Liolaemus xanthoviridis* Cei and Scolaro 1980, an arenicolous species restricted to an area of approximately 50 × 200 km around the Montemayor Plateau (Rawson, Gaiman and Florentino Ameghino Departments) in Chubut Province, Argentina. This species is sexually dimorphic in size; males are larger and more robust than females (adult male mean snout vent length = 82.34 ± 7.75 mm, females = 78.39 ± 6.48 mm; Escudero, 2016). In both sexes, individuals are polymorphic in dorsal coloration (yellow-green to orange, Escudero, 2016) and in ventral melanism (Escudero et al., 2016). The activity period of these lizards begins in late September and early October and continues until mid-late March, depending on environmental temperature (Escudero, 2016). A behavioral study showed that males of *L. xanthoviridis* are not territorial, yet they perform "custody behavior" of females (Escudero, 2016). In this paper, we describe the distribution of genetic diversity within a well-defined region of occurrence of this species, estimate paternity of several clutches of eggs and the effective size of the sampled population of *L. xanthoviridis,* and briefly discuss the correlation between the microsatellite-based results here obtained and the social organization of the species, as inferred from behavioral studies.

MATERIALS AND METHODS

Study area

The study area is located in the Patagonian Steppe ecoregion (Burkart et al., 1999), a homogeneous plant community dominated by low shrubs. The climate is cold and dry; mean annual precipitation is ~ 250 mm, winds are strong, and mean annual temperatures range between 5–14°C. In the spring (October–December), temperatures range between 10–20°C; in the summer (January–March), they can reach 38°C. The focal population of this study occupies the coastline of Isla Escondida Bay, a marine coastal dune environment located 50 km south of Playa Unión village, Rawson Department (43°40′38.49′S; 65°20′26.54′W, Datum WGS84, 6 m above sea level), Chubut province, Argentina.

Field methodology

We used a system of capture-mark-recapture on a grid, and collected data during four sampling seasons (January–March 2012, November 2012–February 2013, October 2013–March 2014, and October 2014–March 2015), which correspond to periods of lizard activity. We demarcated a grid of 100 × 100 m^2, captured lizards, marked them with colored beads as per Fisher and Muth (1989) and toe-clipping as per Ferner (1979), and released them at their capture points. We determined sex through hemipenial eversion, by comparing the relative width at the base of the tail, or by the presence of precloacal pores. These animal handling procedures do not require permission from the provincial fauna authority and are in agreement with regulations detailed in Argentinian National law Nº 14346.

Along the Isla Escondida Bay and around the demarcated grid, we also captured adult *Liolaemus xanthoviridis* (including ten gravid females), and transported them to our laboratory at the Instituto Patagónico para el Estudio de Ecosistemas Continentales (IPEEC)–Centro Nacional Patagónico. We introduced each lizard into a terrarium with enough sand to enable females to dig small burrows, simulating natural conditions. Once females laid their eggs, we carefully extracted each clutch and placed them in sterilized plastic containers with sand substrate (taken from the burrow built by the female), along with sterilized vermiculite and distilled water. We recorded the length and width of each egg for the complete clutch of each female. All containers were incubated at a temperature of 25°C. Hydration levels of the clutch substrates

were maintained according to the condition of the eggs. Once females completed oviposition, they were returned to their capture points in good health.

Laboratory work

We marked lizards by removing digits, which were stored in 96% ethanol and used to extract genomic DNA with the Qiagen Genomic Tissue Kit DNeasy® 96 following the manufacturer protocol. We extracted DNA from 227 individuals (148 from the grid and 80 from nearby areas maintained in terrariums), of which 214 amplified microsatellites (145 from the grid and 69 from the terrariums). Of the 214, 16 were embryos from three clutches of eggs.

Microsatellite loci were amplified with the M13-tailed protocol of Schuelke (2000), where each forward primer is 5′-augmented with the same M13 forward sequence (5′–CACGACGTTGTAAAACGAC–3′). This tailed primer is then used in combination with a 6-FAM fluorescently labeled M13 primer. With this protocol, the polymerase chain reactions contain three primers: a forward M13 fluorescent primer, a 5'-augmented microsatellite forward primer, and an unmodified microsatellite reverse primer. We originally attempted to amplify all 10 loci developed by Hanna et al. (2012), but two of them lacked polymorphism and were discarded prior to analysis, so we amplified the following eight loci: DI-7938, DI-159, TET-3500, DI-1570, TET-2216, DI-3138, TET-1177, TET-1501. Reactions consisted of 1.5 μL of DNA, 0.04 μL of the 10 μM forward M13 tailed primer, 0.45 μL of the 10 μM reverse primer, 0.45 μL of 10 μM fluorescently labeled M13 forward primer, 1.25 μL of 10X $MgCl_2$, 1.25 μL of 5X PCR buffer, 0.21 μL of a 10 μM dNTP mixture, 0.1 μL of taq, and 7.75 μL of water for a total volume of 13.0 μL. The thermal profile was set to 94°C for 2 min, followed by 19 cycles of 94°C for 30 s, followed by a primer-specific annealing temperature (see Hanna et al., 2012 for details) for 30 s and 68°C for 30 s, and a final 3 min extension at 68°C. A second round of 10 cycles was run with an annealing temperature of 53°C to ensure proper annealing of the fluorescently labeled primer. Gel electrophoresis was used to confirm the presence of appropriate sized DNA fragments. No clean-up step was necessary and the microsatellites were genotyped on an ABI 3730xl DNA Analyzer at the Brigham Young University DNA Sequencing Center. Fragment lengths were called using Geneious software v6.1.8 (Kearse et al., 2012).

Data analysis

Population genetic diversity

Using the program Cervus 3.0.7 (Marshall et al., 1998), we estimated the following genetic diversity indices: number of alleles per locus, observed and expected heterozygosity, polymorphic information content (PIC), conformance to Hardy-Weinberg expectations, inbreeding coefficient (Fis), and probability of null alleles.

Table 1. Characteristics of clutches of 10 females brought into the lab during this study; tissues providing DNA for paternity analyses were available for three of these.

ID	Eggs laid	Clutch size	DNA extracted
H269	Yes	6	*n* = 3, remainder damaged
H272	Yes	9	*n* = 7, remainder damaged
H263	No	—	—
H267	Yes	6	damaged
H270	No	—	—
H271	Yes	8	damaged
H273	Yes	8	damaged
H274	Yes	5	damaged
H275	Yes	7	*n* = 6, remainder damaged
H276	No	—	—

Paternity analysis

With Cervus 3.0.7 (Marshall et al., 1998), we performed a parentage analysis of the three clutches of eggs that were obtained in the laboratory. Cervus uses maximum likelihood to estimate relationships between parental candidates and the group of descendants (Wang, 2012). The algorithm calculates a logarithm of odds (LOD) score, which reports the probability that an individual (or pair of individuals, Trio LOD score) is the father (or parent) of a particular descendant, divided by the probability that those individuals are not related. The offspring is assigned to the parent with the highest LOD score. The analysis consists of three steps: (1) calculate allele frequencies, (2) a simulate kinship probabilities, and (3) with these estimates, perform parentage analysis.

Of the 10 apparently gravid females collected in October 2014, seven oviposited between 7–25 November. Unfortunately, none of these eggs hatched; most dehydrated or developed fungus. All eggs (*n* = 49) of the seven females that oviposited were preserved in 96% ethanol and dissected, and three of the seven clutches had sufficiently developed embryos for DNA extraction (Table 1).

Population structure

We used a Bayesian clustering method to infer the population structure of *Liolaemus xanthoviridis* at Isla Escondida Bay (Fig. 1). The landscape at this site is not completely homogeneous and, in general, the patches occupied by the lizards are widely separated from each other. Therefore, we tested whether all sampled individuals belong to the same population or if, within that environment, there were genetically structured smaller groups. STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al.,

2003) can assign individuals (probabilistically) to different groups without *a priori* specification of the units and/or population limits. We used a mixed model (admixture), which considers that individuals might have multiple ancestry (calculating the proportion of membership in each group), correlated with a pattern of frequencies (Falush et al., 2003), which implies that the allele frequencies in different populations might be very similar (probably by migration or shared ancestry). The number of possible groups/populations (K) analyzed ranged from one to eight, with eight replicates performed per run to verify the consistency of the results between different analyses for each K. The length of Monte Carlo Markov chain (MCMC) operations and the process of burn-in were set at 25,000 and 10,000, respectively. The optimal value of K was selected from two methods: (1) the logarithm of the maximum likelihood, where the best value of K to explain population structure is the one with maximum value of the logarithm of the posterior probability of the data [Ln Pr (X|K)] and the lowest standard deviation

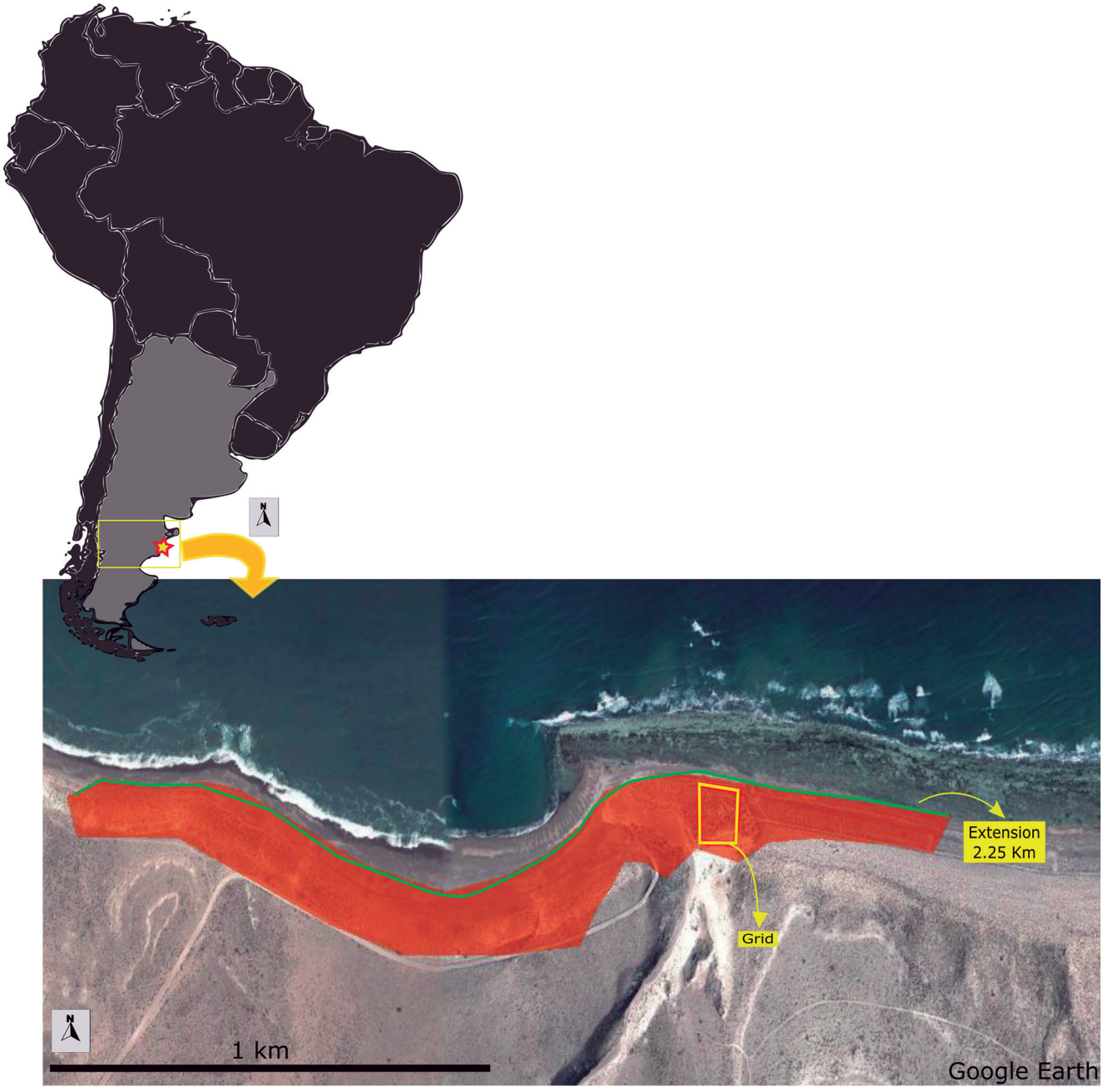

Figure 1. Map of South America showing the location of the sampling area, Isla Escondida Bay in the Province of Chubut, Argentina. Orange shaded area around the collection of individuals, comprising an area of 2.25 km and within that grid study area is shown.

Table 2. Estimation of genetic diversity per locus. k: number of alleles, N: number of individuals analyzed, HO: Observed heterozygosity, HE: Expected heterozygosity, PIC: polymorphic information content, NE-1P: Average non-exclusion probability for one candidate parent., NE-2P: Average non-exclusion probability for one candidate parent given the genotype of a known parent of the opposite sex., NE-PP: Average non-exclusion probability for a candidate parent pair., NE-I: Average non-exclusion probability for identity of two unrelated individuals., NE-SI: Average non-exclusion probability for identity of two siblings., HW: Hardy-Weinberg equilibrium, F(Null): Frequency of null alleles , NS: non-significant, ***: significant.

Locus	k	N	HO	HE	PIC	NE-1P	NE-2P	NE-PP	NE-I	NE-SI	HW	F(Null)
ADI	11	186	0.457	0.856	0.838	0.451	0.289	0.121	0.037	0.332	***	0.303
BDI	13	208	0.615	0.646	0.623	0.736	0.546	0.329	0.148	0.465	NS	0.033
CTET	23	212	0.792	0.885	0.873	0.375	0.231	0.08	0.024	0.314	NS	0.054
DDI	6	212	0.59	0.678	0.622	0.74	0.575	0.396	0.159	0.452	NS	0.069
ETET	26	210	0.781	0.932	0.926	0.247	0.141	0.033	0.009	0.287	NS	0.087
FDI	16	207	0.845	0.823	0.805	0.501	0.329	0.142	0.048	0.352	NS	-0.019
HTET	11	206	0.845	0.848	0.829	0.468	0.303	0.132	0.041	0.337	NS	0.001
ITET	11	205	0.712	0.796	0.765	0.574	0.397	0.213	0.071	0.371	NS	0.057
Mean	14.625	205.75	0.705	0.808	0.785	0.512	0.351	0.181	0.067	0.363		0.073

(Pritchard et al., 2000), and (2) ΔK, determined following the Evanno method (Evanno et al., 2005) whereby ΔK is based on the rate of change of the logarithm of the data probability on successive values of K. Both methods were evaluated using Structure Harvester v0.6.94 (Earl and vonHoldt, 2012). As a starting point, we considered that all individuals in the area belonged to the same population (i.e., we did not assign possible groups *a priori*).

Although these two methods are alternative ways to estimate K, Structure provides other parameters to analyze the congruence of these estimates. For each individual, STRUCTURE 2.3.3 assigns probabilities that it belongs to a certain population. When these probabilities are very similar, individuals are assigned symmetrically to the inferred populations (e.g., an individual would get a 33% probability of belonging to population 1, 33% probability of belonging to population 2, 33% probability of belonging to population 3). In these cases, it is likely that these populations are not structured and are actually a single population (Pritchard et al., 2010). Another useful parameter estimated by STRUCTURE 2.3.3 is α, the level of relative mixing between populations. When populations are structured, this parameter will normally stabilize to be relatively constant (often with a range of 0.2 or less).

Effective population size (*Ne*)

We determined effective population size using the linkage disequilibrium (LD) method of Waples and Do (2008), as implemented in NeEstimator v.2 (Do et al., 2014). This method is based on the assumption that, in a reproductive system in which gametes are randomly distributed among a small number of zygotes, deviations from expected gametic and genotypic frequencies occur and LD is affected only by drift, which can be used to estimate *Ne* (Hill, 1981; Waples, 2006; Waples and Do, 2010).

RESULTS

Population genetic diversity

We recorded high genetic diversity in this population of *Liolaemus xanthoviridis*. Table 2 summarizes estimates of genetic diversity per locus for this population of *L. xanthoviridis*. Considering all loci, the average number of alleles per locus was 14.6, the average observed heterozygosity was 0.705, and the average expected heterozygosity was 0.808. Given that these values are above 0.5, they have high genetic variability and are useful for paternity analysis (Sosa et al., 2002). The average polymorphic information content (PIC) was 0.785, providing similar information as expected heterozygosity (Botstein et al., 1980). As shown in Table 2, the ADI locus is the only one that is not in Hardy-Weinberg equilibrium and segregates the greatest proportion of null alleles. The degree of inbreeding (F_{IS}) was 0.127 (considering the eight loci) and 0.076 (excluding the ADI locus), indicating a slight deficit of heterozygotes. The probability of parental exclusion was 0.003 for the first father, < 0.001 for the second parent, < 0.001 for the parental pair, and 2.62^{E-11} for the combined probability of exclusion for non-parental identity.

Paternity analysis

The majority of clutches had only one male that fathered the embryos within each clutch. The three embryos of female 269 were assigned to male 268, with a strict confidence level of 95% (Table 3). Cervus 3.0.7 also estimates the probability of both female 269 and male 268 being parents of the three embryos (trio confidence), and for this case the level of confidence assigned to these candidate parents was 95% (Table 3, trio confidence). Five of the offspring of female 272 were assigned to male 264,

Table 3. Results of the paternity test in Cervus 3.0.7 (Marshall et al., 1998). (*: 95% confidence, +: 80% confidence, –: father most likely, empty cell: no father was named as the most likely).

ID Off-spring	NE-1P	NE-2P	ID Mother	ID Father candidate	Par confi-dence	Trio confi-dence
269-1	4.24^{E}-05	4.70^{E}-13	269	268	*	*
269-2	1.89^{E}-04	2.12^{E}-10	269	268	*	*
269-3	9.66^{E}-05	7.48^{E}-12	269	268	*	*
272-1	6.78^{E}-03	7.35^{E}-07	272	264	*	*
272-2	4.26^{E}-03	1.68^{E}-07	272	264	*	*
272-3	7.24^{E}-03	7.64^{E}-08	272	264	*	*
272-4	2.57^{E}-03	1.55^{E}-08	272	264	*	*
272-5	3.46^{E}-02	2.80^{E}-04	272	264		
272-5	3.46^{E}-02	2.80^{E}-04	272	279		
272-6	1.38^{E}-03	1.65^{E}-08	272	264	+	*
275-1	3.71^{E}-04	9.17^{E}-11	275	229	*	*
275-2	4.98^{E}-04	7.45^{E}-11	275	229		–
275-3	2.03^{E}-04	2.32^{E}-10	275	229		–
275-4	2.66^{E}-04	1.54^{E}-10	275	229	+	*
275-5	5.09^{E}-04	1.95^{E}-10	275	264		–
275-6	4.76^{E}-04	3.08^{E}-09	275	229	+	*

four with 95% confidence and one with 80% confidence (Table 3). Offspring 272–275 only amplified for three loci, and the most likely parents were males 264 and 279 (no confidence level); as in the previous case, female 272 and male 264 were assigned with 95% confidence as the most likely parents of all offspring except for 272–275, whose data are not sufficient to obtain an accurate result. For female 275, three offspring were assigned to male 229, one with a 95% confidence level and two with 80% (Table 3). Of the three remaining offspring, two were assigned to male 229 and one to male 264, but without any confidence level (Table 3). Of the first three embryos

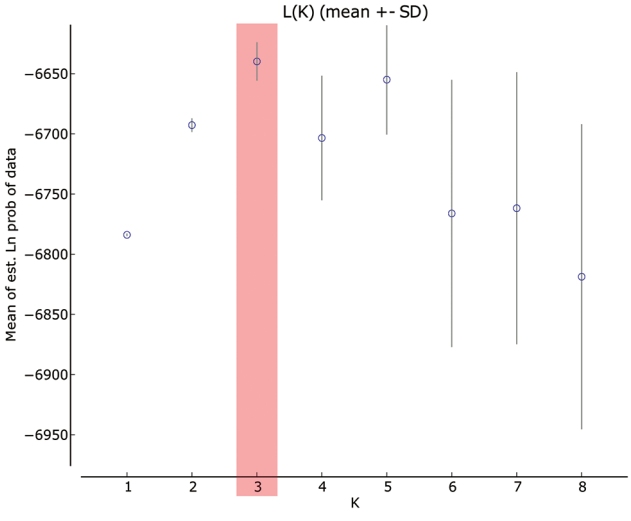

Figure 2. Mean values of LnP(D) (maximum likelihood) and the standard deviation obtained depending on the different values of K. The red rectangle shows the highest likelihood value with the smallest deviation occurs when K = 3.

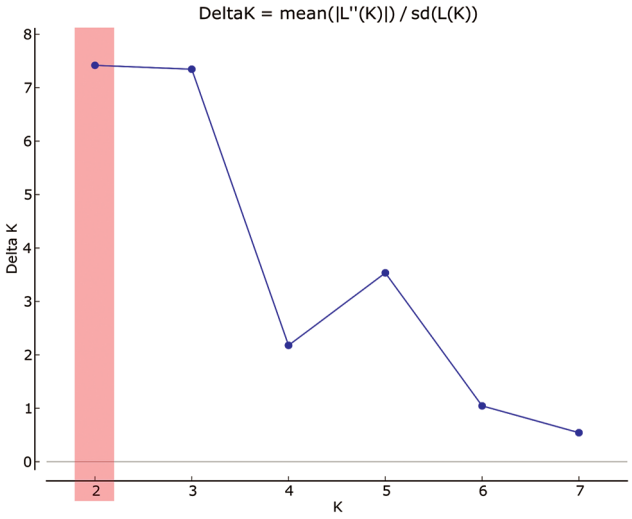

Figure 3. Exchange rate LnP(D) (ΔK) depending on the different values of K. The red box shows that the highest value of ΔK occurs when K = 2.

that have an assigned father (male 229) with some level of confidence, female 275 and male 229 were assigned as the most likely parents with 95% confidence, while for the other three offspring those same parents were the most likely in two cases but without a confidence level.

Population structure

The Bayesian clustering method, under the approximation of the value of maximum likelihood, estimates three populations (K = 3) on Isla Escondida Bay (Fig. 2). The ΔK method estimates only two populations (Fig. 3), although this algorithm cannot estimate a single population, and therefore fails to identify K when the real structure comprises a panmictic population (Evanno et al., 2005). *Liolaemus xanthoviridis* population structure estimates are characterized by symmetric probability assignments of individuals and a highly variable α value during the course of the analysis (Pritchard et al., 2010). This is reflected in the bar graph of probabilities (Fig. 4), in which every individual is a bar and every color is a population; the majority of individuals have similar probabilities of belonging to any population. The effective population size (*Ne*) estimated from eight loci is 196.5 (CI 158.4–252.1). Excluding the ADI locus, it is 210.4 (CI 166.1–277.7).

DISCUSSION

Genetic diversity is one of the most important attributes of populations because high levels of genetic variation increase their potential to respond to environmental change (Garrigós Esquer, 2008). In fact, there is abundant evidence that genetic diversity enhances features related to fitness. For example, heterozygosity levels have been

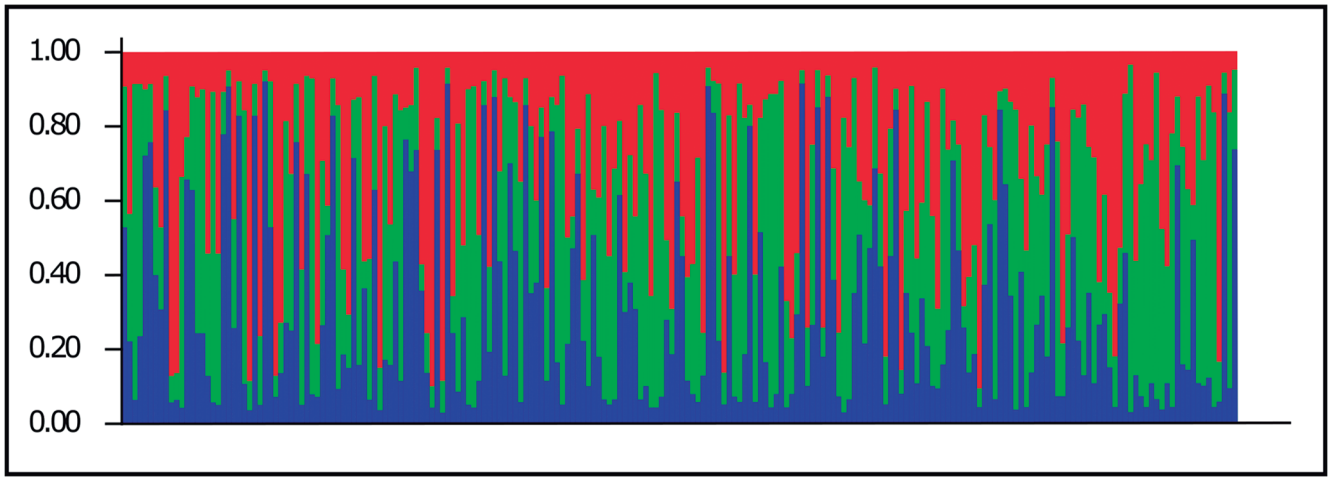

Figure 4. Bar graph of the probability (0–1) of each individual belonging to each of three populations (blue, green and red).

associated with increases in morphological variation, individual performance, and developmental homeostasis (Mitton and Grant, 1984; Allendorf and Leary, 1986; Mitton, 1993). In contrast, low levels of genetic diversity are associated with reduced survival and reproductive success of individuals and, as a consequence, compromise population survival (Freeland, 2005).

A parameter that describes the genetic variability of a population is its effective size, defined as the number of reproductive individuals that contribute to the next generation in terms of demographics and especially genetic variability (Wright, 1969; Hedrick, 2000). It is expected that a large effective population size will reduce rate of loss of genetic variation by genetic drift, and maintain diversity for many generations (Cruzan, 2001; Kawata, 2001). In this population of *Liolaemus xanthoviridis,* we found genetic diversity to be higher than previously reported for *L. lutzae* Mertens, 1938 (Ariani et al., 2013) and other lizard species (Gullberg et al., 1997; Rowe et al., 2002; Garrigós Esquer, 2008). This is shown by the highest average number of alleles per locus, high observed and expected heterozygosities (greater than 0.7), polymorphism information content (PIC), and low level of inbreeding (F_{IS}). Ariani et al. (2013) recorded a very low effective population size in *L. lutzae,* finding evidence of a bottleneck event, as observed when populations experience a loss of rare (low frequency) alleles and reductions in heterozygosity, as well as in total population size. Small effective population sizes were also reported by Rowe et al. (2002) and Garrigós Esquer (2008) for the island lizards *Cnemidophorus vanzoi* Baskin and Williams, 1966 (Maria Major Island) and *Aspidoscelis hyperythra* Cope, 1864 (Baja California Sur, from Loreto to Los Cabos and several islands), respectively. Those authors suggested that the low levels of genetic variation were due to small effective population sizes since the studied populations were thought to have had originated from few individuals. Not surprisingly, in our study of a continental species that retains a large population size, *L. xanthoviridis,* we find the opposite pattern. The high levels of total genetic diversity inferred from the neutrally evolving microsatellites and effective population size suggest the adaptive potential of these populations may be sizable.

The genetic structure of a species refers to the geographic distribution of allele frequencies among its populations and can be influenced by the interaction between environmental factors and life histories (e.g., mating systems), random forces (e.g., genetic drift, mutation), spatial (isolation by distance, Balloux and Lugon-Moulin, 2002) and deterministic factors (e.g., selection; Hartl and Clark, 1997; Freeland, 2005). In this study we found evidence consistent with the interpretation of a single panmictic population of *Liolaemus xanthoviridis* over the entire sampling area. We found no evidence of population structure among samples; however, it remains to be seen whether structure exists outside the sampled area. Although the total linear distance between both extremes of our sampling region was small (2 km), the Sotomayor Plateau isolates some of our sampling sites. Our data, nonetheless, demonstrate that the plateau is not sufficient to lead to genetic structure across sampling sites.

The information provided by the microsatellite loci (i.e., high genetic diversity and low probability of a parent misassignment, no parental exclusion) reports on the reliability of paternity analysis in *Liolaemus xanthoviridis*. The paternity tests revealed that the clutches of two females were each sired by a unique and distinct male. The third clutch, belonging to female 275, which was not captured near a male, was also assigned to a single male as the most likely father of the offspring (with variable levels of confidence).

Although our sample size is limited to three clutches (n = 3), we suggest that this species does not exhibit multiple paternity. We are aware of our small sample size, but we consider these results to be very relevant in providing new information for this species-rich genus, for which

very few ecological data are available given that the eggs are extremely fragile: after years of failed attempts, this was the first time we (or anyone else working with this genus) obtained eggs sufficiently developed to recover embryonic tissue. Multiple paternity is usually associated with competition between males for female access and has been observed in several other species of lizards (Olsson et al., 1996; Abell, 1997; Laloi et al., 2004; Fitze et al., 2005; Calsbeek et al., 2007). It often occurs in territorial species (e.g., Abell, 1997; Lebas, 2001; Morrison et al., 2002).

In non-territorial species like *Liolaemus xanthoviridis* (Escudero, 2016), multiple paternity mating systems are rare, and in some species males have "custodial" care of females (Olsson, 1993; Olsson et al., 1996; Cooper and Vitt, 1997; Bull and Pamula, 1998; Cuadrado, 1998; Martín and López, 1999). In a typical custody behavior, the male chases the female a short distance and does not defend a territory, but he defends an exclusive area around the female that varies in space and time, according to the position that she occupies. This behavior has been recorded in *L. xanthoviridis* (Escudero, 2016). In this species, the custody behavior of the male towards the female after copulation might occur while the female remains responsive, thereby preventing another male from mating with her. Although this behavior would be costly for the male (lost mating opportunities with other females), the absence of multiple paternity would ensure the paternity of all offspring with the female in custody without the need to establish a territory and allocate resources to its defense. It has been widely accepted that a male's fitness depends on the number of females with which he mates (Bateman, 1948), and polygyny is the more widespread mating system among lizards, (e.g., Blanc and Carpenter, 1969; Stamps, 1977; Schoener and Schoener, 1980; Fox and Shipman, 2003). Nevertheless, there is considerable variation in its degree, from monogamous species (Tinkle, 1967; Jenssen, 1970; Milstead, 1970) to others having harems of two or three females per male (Harris, 1964; Rand, 1967; Ruby, 1981).

There is no general scientific agreement if the female's fitness depends on the number of males with which she mates. Unlike males, females produce a limited number of eggs per litter and/or season. Different authors (Thornhill and Alcock, 1983; Uller and Olsson, 2004; Wolff and Macdonald, 2004) have suggested that females could benefit directly and indirectly from multiple matings by obtaining food resources from their partners, improving the genetic quality or diversity of their descendants, and protecting themselves against male infertility or genetically defected partners. However, Sheldon (1993) suggests that mating with multiple males can be costly for the female, for example due to increased risk of sexually transmitted diseases, higher amounts of time and energy used to mate, or increased predation risk. For female *Liolaemus xanthoviridis,* for which we did not detect multiple paternity, one can envision multiple scenarios: on one hand, the costs of multiple matings might outweigh the benefits; alternatively, the custodial behavior of males might prevent them from mating with multiple partners. Future studies of higher numbers of litters may clarify this question.

ACKNOWLEDGMENTS

We thank M.A. Gonzales Marín, R. Blum, C.D. Medina, M. Olave, I. Minoli, F. Feliciani, A. Morales, J.C. Rua and L.J. Natali for field assistance. Financial support was provided by CONICET graduate fellowships (PCE, IM, MAGM) and the following ANPCyT-FONCYT and CONICET grants PICT-2011-0784 (issued to LJA), PICT-2011-1397 (issued to MM) and PIP 0336/13. The National Science Foundation Emerging Frontiers award (EF-1241885) supported collaborative research on lizard biodiversity, eco-physiology, population biology, and climate-driven extinctions to the following institutions (listed alphabetically): Brigham Young University, Ohio University, University of California Santa Cruz, and Villanova University, and partner institutions in Latin America, including the IPEEC-CENPAT. The research was conducted with the approval of the Dirección de Fauna y Flora Silvestre de la Provincia de Chubut (collecting permits #37/2012 [Exp.-02304/12]-MP and #17/2015 [Exp. 0425/15]-MDTySP, issued by Law XI (#10), Dec. Regl. 868/90 y Disp. #48/08, DFyFS-SSRN-MIAyG]). Animal care procedures follow the guidelines approved by COSELABI-CENPAT and CONICET under the Argentinean National Law #14346. To our knowledge, all our study followed the advices presented in the document ASIH-HL-SSAR (Beaupre 2004). We also thank the Associate Editor helpful comments that improved the English grammar of the last version.

REFERENCES

Abell A.J. 1997. Estimating paternity with spatial behaviour and DNA fingerprinting in the striped plateau lizard, *Sceloporus virgatus* (*Phrynosomatidae*). *Behavioral Ecology and Sociobiology* 41:217–226.

Allendorf F.W., Leary R.F. 1986. Heterozygosity and fitness in natural populations of animals. Pp. 57–76, in Soulé M.E. (Ed.), Conservation Biology: The Science of Scarcity and Diversity. Sinauer Associates, Massachusetts.

Altmann J., Alberts S., Haines S.A. 1996. Behavior predicts genetic structure in a wild primate group. *Proceedings of the National Academy of Sciences of the USA* 93:5797–5801.

Amos W., Twiss S., Pomeroy P., Anderson S. 1993. Male mating success and paternity in the grey seal, *Halichoerus grypus,* a study using DNA fingerprinting. *Proceedings of the Royal Society of London, Series B* 252:199–207. DOI

Ariani C.V., Pickles R.S.A., Jordan W.C., Lobo-Hajdu G., Rocha C.F.D. 2013. Mitochondrial DNA and microsatellite loci data

supporting a management plan for a critically endangered lizard from Brazil. *Conservation Genetics* 14:943–951. DOI

Avila L.J., Morando M., Sites J.W. Jr. 2008. New species of the iguanian lizard genus *Liolaemus* (Squamata, Iguania, Liolaemini) from Central Patagonia, Argentina. *Journal of Herpetology* 42:186–196. DOI

Balloux F., Lugon-Moulin N. 2002. The estimation of population differentiation with microsatellite markers. *Molecular Ecology* 11:155–165. DOI

Baskin J.N., Williams E. E. 1966. The lesser Antillean *Ameiva* (Sauria,Teiidae). Re-evaluation, zoogeography, and the effects of predation. *Studies Fauna Curazao Caribe Sl.* 23:144–176.

Bateman A.J. 1948. Intrasexual selection in *Drosophila*. *Heredity* 2:349–368.

Blanc C.P., Carpenter C.C. 1969. Studies on the Iguanidae of Madagascar. III. Social and reproductive behavior of *Chalarodon madagascariensis*. *Journal of Herpetology* 3:125–134.

Botstein D., White R.L., Skolmick H., Davis R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisn. *The American Journal of Human Genetics* 32:314–331.

Bull C.M., Pamula Y. 1998. Enhanced vigilance in monogamous pairs of the lizard, *Tiliqua rugosa*. *Behaviour Ecology* 9:452–455. DOI

Burkart R., Bárbaro N.O., Sánchez R.O., Gómez D.A. 1999. Ecorregiones de la Argentina. *Administración de Parques Nacionales, Buenos Aires*.

Calsbeek R., Bonneaud C., Prabhu S., Manoukis N., Smith T.B. 2007. Multiple paternity and sperm storage lead to increased genetic diversity in the Cuban anole, *Anolis sagrei*. *Evolutionary Ecology Research* 9:495–503.

Cei J.M., Scolaro J.A. 1980. Two new subspecies of the *Liolaemus fitzingeri* complex from Argentina. *Journal of Herpetology* 14:37–43.

Coltman D.W., Bancroft D.R., Robertson A., Smith J.A., Clutton-Brock T.H., Pemberton J.M. 1999. Male reproductive success in a promiscuous mammal: Behavioural estimates compared with genetic paternity. *Molecular Ecology* 8:1199–1209. DOI

Cooper S.J., Bull C.M., Gardner M.G. 1997. Characterization of microsatellite loci from the socially monogamous lizard *Tiliqua rugosa* using a PCR-based isolation technique. *Molecular Ecology* 6:793–795.

Cooper W.E., Vitt L.J. 1997. Maximizing male reproductive success in the broad-headed skink (*Eumeces laticeps*): preliminary evidence for mate guarding, size-assortative pairing, and opportunistic extra-pair mating. *Amphibia Reptilia* 18:59–73. DOI

Cope E.D. 1864. Descriptions of new American Squamata in the Museum of the Smithsonian Institution. *Proceedings of the Academy of Natural Sciences of Philadelphia* 15:100–106.

Cruzan M.B. 2001. Population size and fragmentation thresholds for the maintenance of genetic diversity in the herbaceous endemic *Scutellaria montana* (Lamiaceae). *Evolution* 55:1569–1580. DOI

Cuadrado M. 1998. The influence of female size on the extent and intensity of mate guarding by males in *Chamaeleo chamaeleon*. *Journal of Zoology* 246:351–358. DOI

DeYoung R.W., Honeycutt R.L. 2005. Molecular toolbox: genetic techniques in wildlife ecology and management. *Journal of Wildlife Management* 69:1362–1384. DOI

Do C., Waples R.S., Peel D., Macbeth G.M., Tillett B.J., Ovenden J.R. 2014. NeEstimator v2: reimplementation of software for the estimation of contemporary effective population size (*Ne*) from genetic data. *Molecular Ecology Resources* 14:209–214. DOI

Duméril AMC., Bibron G. 1837. Erpétologie Générale ou Histoire Naturelle Complète des Reptiles. Tome Quatrième. Librarie Encyclopédique Roret, Paris. DOI

Earl D.A., vonHoldt B.M. 2012. Structure Harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4:359–361. DOI

Escudero P.C. 2016. Polimorfismo de coloración, melanismo y estrategias reproductivas en una población de lagartijas Patagónicas del grupo *Liolaemus fitzingerii*. Ph.D. thesis, Universidad Nacional de Córdoba, Argentina.

Escudero P.C., Minoli I., Gonzalez Marín M.A., Morando M., Avila L.J. 2016. Melanism and ontogeny: a case study in lizards of the *Liolaemus fitzingerii* group (Squamata: Liolaemini). *Canadian Journal of Zoology* 94:199–206. DOI

Evanno G., Regnaut S., Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14:2611–2620. DOI

Falush D., Stephens M., Pritchard J.K. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164:1567–1587.

Ferner J.W. 1979. A review of marking techniques for amphibians and reptiles. *Herpetological Circular* 9:42.

Fisher M., Muth A. 1989. A technique for permanently marking lizards. *Herpetologica Review* 20:45–46.

Fitze, P.S., Le Galliard J.F., Federici P., Richard M., Clobert J. 2005. Conflict over multiple-partner mating between males and females of the polygynandrous common lizards. *Evolution* 59:2451–2459. DOI

Fox S.F., Shipman P.A. 2003. Social behavior at high and low elevations: environmental release and phylogenetic effects in *Liolaemus*. Pp. 310–354, in Fox S.F., McCoy J.K., Baird T.A. (Eds.), Lizard Social Behavior. The John Hopkins University Press, Baltimore.

Freeland, J.R. 2005. Molecular Ecology. Wiley, Canada.

Garrigós Esquer Y.S. 2008. Variación y estructura genética en la Lagartija de Cola Rayada *Aspidoscelis hyperythra* (Sauria: Teiidae) mediante en el uso de marcadores microsatélites. M. S. thesis, Centro de Investigaciones Biológicas del Noroeste, México.

Gullberg A., Tegelstrom H., Olsson M. 1997. Microsatellites in the Sand Lizard (*Lacerta agilis*): description, variation, inheritance, and applicability. *Biochemical Genetics* 35:281–295. DOI

Hanna N., Brown D., Avila L.J., Sites J.M. Jr., Morando M., Fontanella F.M. 2012. Characterization of 10 polymorphic microsatellite loci in the South American lizard *Liolaemus fitzingerii* with cross-amplification in *L. chehuachekenk*. *Conservation Genetics Resources* 4:105–107. DOI

Harris V.A. 1964. The Life of the Rainbow Lizard. Hutchison, Bristol.

Hartl D.L., Clark A.G. 1997. Principles of Population Genetics. Sinauer Associates, Massachusetts.

Hedrick P.W. 2000. Genetics of populations. Jones & Bartlett Publishers, Boston.

Hill W.G. 1981. Estimation of effective population size from data on linkage disequilibrium. *Genetical Research Cambridge* 38:209–216.

Hughes C. 1998. Integrating molecular techniques with field methods in studies of social behaviour: a revolution results. *Ecology* 79:383–399. DOI

Jenssen T.A. 1970. Female response to filmed displays of *Anolis nebulosus* (Sauria, Iguanidae). *Animal Behaviour* 18:640–647.

Johansson H., Surget-Groba Y., Thorpe R.S. 2008. Microsatellite data show evidence for male-biased dispersal in the Caribbean lizard *Anolis roquet*. *Molecular Ecology* 17:4425–4432. DOI

Kawata M. 2001. The influence of neighborgood size and habitat shape on the accumulation of deleterious mutations. *Journal of Theoretical Biology* 211:187–199. DOI

Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., ... Drummond A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649. DOI

Laloi D., Richard M., Lecomte J., Massot M., Clobert J. 2004. Multiple paternity in clutches of Common Lizard *Lacerta vivipara:* data from microsatellite markers. *Molecular Ecology* 13:719–723. DOI

Lebas N.R. 2001. Microsatellite determination of male reproductive success in a natural population of the territorial Ornate Dragon Lizard, *Ctenophorus ornatus*. *Molecular Ecology* 10:193–203. DOI

Marshall T.C., Slate J., Kruuk L.E.B., Pemberton J.M. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7:639–655. DOI

Martin J., Lopez P. 1999. An experimental test of the costs of antipredatory refuge use in the Wall Lizard, *Podarcis muralis*. *Oikos* 84:499–505. DOI

Maudet C., Miller C., Bassano B., Breitenmoser-Würsten C., Gauthier D., Obexer-Ruff G., ... Luikart G. 2002. Microsatellite DNA and recent statistical methods in wildlife conservation management: applications in *Alpine ibex* [*Capra ibex* (*ibex*)]. *Molecular Ecology* 11:421–436. DOI

Mertens R. 1938. Bemerkungen über die brasilianischen Arten der *Gattung Liolaemus*. *Zoologischer Anzeiger* 123:220–222

Milstead W.W. 1970. Late summer behavior of the lizards *Sceloporus merriami* and *Urosaurus ornatus*. *Herpetologica* 26:343–354.

Mitton J.B. 1993. Theory and data pertinent to the relationship between heterozygosity and fitness. Pp. 17–41, in Tornhill N.W. (Ed.), The Natural History of Inbreeding and Outbreeding. University of Chicago Press, Chicago.

Mitton J.B., Grant M.C. 1984. Associations among protein heterozygosity, growth rate, and developmental homeostasis. *Annual Review of Ecology and Systematics*15:479–499.

Morrison M.L., Kuenzi A.J., Brown C.F., Swann D.E. 2002. Habitat use and abundance trends of rodents in southeastern Arizona. *The Southwestern Naturalist Journal* 47:519–526. DOI

Olsson M. 1993. Contest success and mate guarding in male Sand Lizards, *Lacerta agilis*. *Animal Behaviour* 46:408–409.

Olsson M., Gullberg A., Tegelström H. 1996. Mate guarding in male Sand Lizards (*Lacerta agilis*). *Behaviour* 133:367–386.

Pemberton J.M., Albon S.D., Guinness F.E., Clutton-Brock T.H., Dover G.A. 1992. Behavioral estimates of male mating success tested by DNA fingerprinting in a polygynous mammal. *Behavioral Ecology* 3:66–75.

Pritchard J.K., Stephens M., Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945–959.

Pritchard J.K., Wen X., Falush D. 2010. Documentation for Structure software: Version 2.3.

Rand A.S. 1967. Ecology and social organization in the iguanid lizard *Anolis lineatopus*. *Proceedings of the United States National Museum* 122:1–79.

Rowe G., Dickinson H., Gibson R., Funk S.M., Fa J.E. 2002. St Lucia Whiptail Lizard *Cnemidophorus vanzoi* (Sauria: Teiidae) microsatellite primers. *Molecular Ecology Notes* 2:124–126.

Ruby D.E. 1981. Phenotypic correlates of male reproductive success in the lizard, *Sceloporus jarrovi*. Pp. 96–107, in Alexander R.D., Tinkle D.W. (Eds.), Natural Selection and Social Behavior: Recent Research and New Theory. Chiron Press, New York.

Schlötterer C. 2000. Evolutionary dynamics of microsatellite DNA. *Chromosoma* 109:365–371. DOI

Schoener T.W., Schoener A. 1980. Ecological and demographic correlates of injury rates in some Bahamian *Anolis* lizards. *Copeia* 1980:839–850.

Schötterer C., Pemberton J. 1998. The use of microsatellites for genetic analysis of natural populations a critical review. Pp. 71–87, in DeSalle R., Schierwater B. (Eds). Molecular Approaches to Ecology and Evolution. Birkhäuser Verlag, Basel.

Schuelke M. 2000. An economic method for the fluorescent labelling of PCR fragments. *Nature Biotechnology* 18:233–234.

Sheldon B.C. 1993. Sexually transmitted disease in birds: occurrence and evolutionary significance. *Philosophical Transactions: Biological Sciences* 339:491–497.

Slatkin M. 1994. Gene flow and population structure. Pp. 3–17, in Real L. (Ed), Ecological Genetics. Princeton University Press, Princeton.

Sosa P., Batista F., González M.A., Bouza N. 2002. La conservación genética de las especies amenazadas. Pp. 133–160, in Bañares Á. (Ed), Biología de la Conservación de Plantas Amenazadas: Técnicas de diagnóstico del estado de conservación. Ministerio de Medio Ambiente, Madrid.

Stamps J.A. 1977. The relationship between resource competition, risk, and aggression in a tropical territorial lizard. *Ecology* 58:349–358. DOI

Stow J., Sunnucks P., Briscoe D., Gardner M.G. 2001. The impact of habitat fragmentation on dispersal of Cunningham's skink (*Egernia cunninghami*): Evidence from allelic and genotypic analyses of microsatellites. *Molecular Ecology* 10:867–878. DOI

Thornhill R., Alcock J. 1983. The Evolution of Insect Mating Systems. Harvard University Press, Cambridge.

Tinkle D.W. 1967. The life and demography of the Side-blotched Lizard, *Uta stansburiana*. *Miscellaneous Publications of the Museum of Zoology* 132:1–182.

Uller T., Olsson M. 2004. Multiple copulations in natural populations of lizards: evidence for the fertility assurance hypothesis. *Behaviour* 142:45–56. DOI

Wang J. 2012. Computationally efficient sibship and parentage assignment from multilocus marker data. *Genetics* 191:183–194.

Waples R.S. 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. *Conservation Genetics* 7:167–184. DOI

Waples R.S., Do C. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources* 8:753–756. DOI

Waples R.S., Do C. 2010. Linkage disequilibrium estimates of contemporary *Ne* using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evolutionary Applications* 3:244–262. DOI

Westneat D.F., Webster M.F. 1994. Molecular analysis of kinship in birds: Interesting questions and useful techniques. Pp. 91–128, in Schierwater B., Streit B., Wagner G.P., DeSalle R., (Eds.). Molecular Ecology and Evolution: Approaches and Applications. Birkhäuser Verlag, Basel.

Wiegmann A.F.A. 1834. Systematis saurorum prodromus, e specimene herpetologiae mexicanae primo seorsim editus. C. G. Lüderitz, Berolini.

Wolff J.O., Macdonald D.W. 2004. Promiscuous females protect their offspring. *Trends in Ecology and Evolution* 19:127–134. DOI

Wright S.1969. Evolution and the genetics of populations. Vol. 2: The theory of gene frequencies. University of Chicago Press, Chicago.

Zane L., Bargelloni L., Patarnello T. 2002. Strategies for microsatellite isolation: a review. *Molecular Ecology* 11:1–16. DOI