The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2013, **109**, 78–91. With 4 figures

Morphological adaptation to coastal marshes in spite of limited genetic structure in the Neotropical passerine *Spartonoica maluroides* (Aves: Furnariidae)

DANIEL A. CARDONI[1]*, RUSSELL GREENBERG[2], JESUS E. MALDONADO[3,4] and JUAN P. ISACCH[1]

[1]*Instituto de Investigaciones Marinas y Costeras (IIMyC), Facultad de Ciencias Exactas y Naturales, Consejo Nacional de Investigaciones Científicas y Técnica, Universidad Nacional de Mar del Plata, Funes 3250, (7600) Mar del Plata, Argentina*
[2]*Migratory Bird Center, Smithsonian National Zoological Park, Washington, DC, USA*
[3]*Center for Conservation and Evolutionary Genetics, Smithsonian Conservation Biology Institute, Washington, DC 20008, USA*
[4]*Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20013, USA*

Received 28 August 2012; revised 13 November 2012; accepted for publication 13 November 2012

Tidal marshes present profound adaptive challenges to terrestrial vertebrates. For example, North American sparrows have relatively longer and thinner bills and darker dorsal plumage in coastal saltmarshes than in interior marshes. Bay-capped wren-spinetail (Furnariidae; *Spartonoica maluroides*) show a strong association with South American saltmarshes. We hypothesized that bay-capped wren-spinetail have similar morphological adaptations to North American sparrows to the saltmarsh environment, which would be indicative of the generality of selection on these traits in the coastal saltmarsh ecosystem. We captured individuals of *S. maluroides* from coastal saltmarshes and interior marshes. Populations were compared based on morphology and molecular markers. We found significant phenotypic differences in bill shape and plumage coloration (melanism) between *S. maluroides* populations from coastal and inland marshes. The low levels of genetic variation, weak geographical structure and shallow divergences, based on mitochondrial DNA and microsatellite data, suggest that coastal populations had a recent demographic expansion. Our results are consistent with the pattern of morphological divergence found between North American Emberizids. The possibility of convergent evolutionary adaptations between saltmarsh North American Emberizids and South American Furnariids suggests that there are strong selective pressures associated with saltmarsh environments on the beak, leading to adaptations for food acquisition, and on plumage coloration for better camouflage for predator avoidance (melanism). © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, **109**, 78–91.

ADDITIONAL KEYWORDS: bay-capped wren-spinetail – convergent evolution – genetic – melanism – morphology – phenotypic plasticity – saltmarsh.

INTRODUCTION

Tidal marshes present profound adaptive challenges to the terrestrial vertebrates that attempt to colonize them. The physical flux of tidal cycles and the chemical influence of seawater combine to create a wetland ecosystem where the benthic environment has strong marine characteristics, yet the vegetative structure resembles that of inland marsh habitats (Chan *et al*., 2006). Tidal marshes have been relatively unstable in time and space throughout the Pleistocene (Malamud-Roam *et al*., 2006). Because of this instability of tidal marshes through geological time, colonizing species must adapt rapidly to the environmental challenges posed by tidal marsh life. Birds of North American tidal marshes often show a high degree of local morphological differentiation even

*Corresponding author. E-mail: acardoni@mdp.edu.ar

when divergence is recent and gene flow ongoing (Avise & Zink, 1988; Greenberg *et al*., 1998, 2006; Chan *et al*., 2006). Ten species of birds are either endemic or have well-marked subspecies that are largely restricted to North American tidal marshes (Marshall, 1948; Chan *et al*., 2006; Greenberg & Maldonado, 2006). To date, this emerging picture of relatively rapid adaptation to a dynamic ecosystem among saltmarsh vertebrates is based almost exclusively on work in North American saltmarshes, and primarily on one group of birds, the sparrows (Greenberg & Droege, 1990; Greenberg *et al*., 1998; Grenier & Greenberg, 2005, 2006). One poorly understood fact of tidal marsh biogeography is that very few endemic subspecies or species have been described for coastlines along other continents (Greenberg & Maldonado, 2006). Yet, tidal marshes are widely distributed along the mid- to high-latitude coasts of the major continents (Adam, 1990).

Saltmarshes along the south-eastern coast of South America are less extensive (2133 km^2) than those along the east coast of North America (15 000 km^2; Isacch *et al*., 2006), but with high floristic similarity (Isacch *et al*., 2006). Tidal marshes in both regions are dominated by various species of cordgrass (*Spartina* spp.). Thus, the relatively unstudied fauna of South American marshes provides an excellent opportunity to test adaptive hypotheses for the evolution of saltmarsh specialization developed exclusively for North American taxa (e.g. Greenberg *et al*., 2006).

As in other regions of the world, the saltmarshes of Argentina support a relatively low number of breeding bird species (Isacch *et al*., 2004; Cardoni, Isacch & Iribarne, 2007; Cardoni *et al*., 2011). Two bird species that show the strongest association with South American salt marshes are the bay-capped wren-spinetail (*Spartonoica maluroides* referred to as *Spartonoica* throughout), a member of the predominantly South American Furnariidae, and the spot-winged crake (*Porzana spiloptera*), a small relative of the coot (Isacch *et al*., 2004; Cardoni *et al*., 2007; Isacch & Cardoni, 2011). *Spartonoica* has relatively high densities in saltmarshes (> 5 individuals ha^{-1}; Isacch *et al*., 2004; Cardoni *et al*., 2007; Isacch & Cardoni, 2011), but it can also use other interior tall grass habitats (Canevari *et al*., 1991) with densities always much lower than in saltmarshes (0.2 individuals ha^{-1}; Comparatore *et al*., 1996; Isacch & Martínez, 2001). *Porzana spiloptera* was almost exclusively recorded in saltmarshes, but are far less common than *Spartonoica* (Taylor, 1996; Martínez, Bó & Isacch, 1997; Cardoni, 2011). Therefore, we decided to use *Spartonoica* as the model species for this study.

Spartonoica is a near-threatened species (BirdLife International, 2012) of salt- and brackish marshes along the coast as well as locally in humid grasslands in the interior of Argentina, Uruguay, and southern Brazil (Canevari *et al*., 1991; Isacch & Martínez, 2001; Isacch *et al*., 2004). *Spartonoica* is a partial migrant, with most birds disappearing from breeding sites in central and southern Argentina along with a substantial winter augmentation of populations both in the interior and the coastal marshes in the northern part of its range (Di Giácomo, 2005; D. A. Cardoni & J. P. Isacch, pers. obs.). *Spartonoica* resembles the many species of spinetails, but it is streaked dorsally. This species is found within the same subfamily as the various spinetail genera, but it is not closely related to any extant species and its sister taxon is the genus *Pseudoseisura*, comprising relatively large, jay-like species (Derryberry *et al*., 2011).

In this paper we investigated if *Spartonoica* populations inhabiting coastal and interior marshes have differences at both the morphological and the molecular level. Specifically, first we focus on differences in morphological measurements (bill, wing, tarsus, and tail), plumage coloration and weight. Second, we examine both nuclear and mitochondrial molecular genetic markers in an attempt to determine the genetic structure among habitat. We test if geographical structure is related to morphological and genetic variation. Finally, we test whether *Spartonoica* populations in coastal marsh habitats are morphologically distinct from interior populations in a similar way as coastal Emberizid sparrows have diverged from their interior sister taxa. If *Spartonoica* shares similar morphological characteristics with unrelated taxa under similar selective pressures, this would support the generality of the selective regime of coastal marshes and would reflect convergent evolution.

MATERIALS AND METHODS

Study area

We captured 140 individuals of *Spartonoica* by flushing birds into single mist-nets (12 × 2.5 m, 30-mm mesh) during the summer from six geographically separated populations. The study included coastal saltmarshes, and interior marshes within the Rio de la Plata grasslands (*sensu* Soriano *et al*., 1991). Specific sampling localities were selected from sites described by Bilenca & Miñarro (2004) and Di Giácomo (2005). We captured birds from three main coastal tidal saltmarshes from Argentina (Isacch *et al*., 2006), Bahía Blanca (39°01′S, 56°25′W), Mar Chiquita Lagoon (37°40′S, 57°23′W) and Bahía Samborombón (36°21′S, 57°12′W), and from three inland brackish marshes, La Picasa Lake (34°14′S, 62°50′W), Saladillo river (35°38′S, 59°46′W) and Punta Lara Reserve (34°47′S, 58°00′W) (Fig. 1). We surveyed for birds in two other sites described within the summer

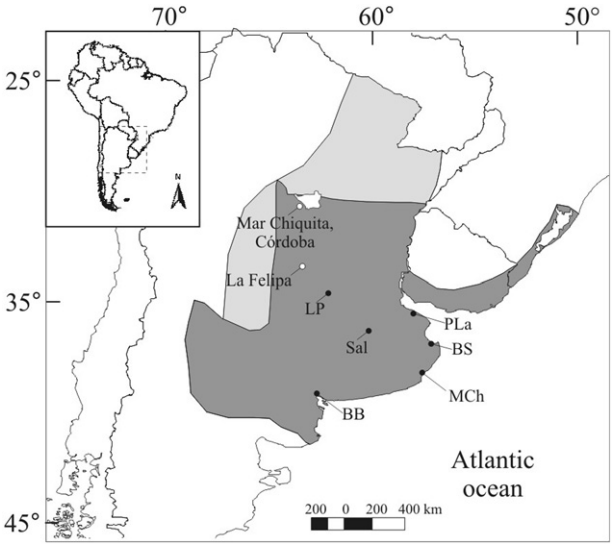

Figure 1. Study area and populations of *Spartanoica* surveyed (BB, Bahía Blanca; MCh, Mar Chiquita Lagoon; BS, Bahía Samborombón; PLa, Punta Lara; Sal, Saladillo river; LP, La Picasa Lagoon; Mar Chiquita lagoon and La Felipa from Córdoba province). Black dots indicated sites where birds were sampled and white dots are sites where few or no birds were found. The map also shows the breeding range of *Spartonoica* (in dark grey) and occasional or winter range (in light grey).

distribution of *Spartonoica* in interior lowland grasslands similar to those used by this species in Saladillo and La Picasa. However, after searching likely sites for 8 field days during the breeding season, we recorded only three individuals in Mar Chiquita Lagoon in Córdoba Province (30°55′S, 62°40′W) and no individuals were recorded in La Felipa Lagoon in Córdoba (33°06′S, 63°33′W) nor from any of the other brackish marshes in the surrounding areas (sites where the species has been recorded in substantial numbers during the winter). Therefore, due to the small sample size, data from individuals from Mar Chiquita (Córdoba) were removed from all of the analyses presented here.

Morphological and plumage variation

We took ten measurements from each individual: bill length, from the anterior point of the nostril to the tip of the bill; bill width, across the base of the bill under the proximal point of the nostrils; bill depth, at the anterior point of the nostrils; length of the reddish cap patch through the mid-line of the crown; length of black on cap, from the end of the reddish cap until to the end of the black cap; tarsus length, from the joint of the tibiotarsus and tarsometatarsus to the distal edge of the most distal unbroken scute overlying the middle toe; tail length, from the base of central rectrices to the end of the longest feather; length of third toe; unflattened wing chord taken from the carpal joint to tip of the longest primary; and body mass. We used a digital calliper (±0.01 mm) for bill, cap and tarsus measurements, a ruler (±1 mm) for tail and wing measurements, and a spring scale (100 g pesola) to record weight. Additionally, we took digital photographs (saved as JPEG format) of every individual that was captured using a Cannon® 35-mm camera. Digital photographs were then analysed using the masking tool of Corel Paint photo editing software version 2003 (Corel Corporation) to estimate the amount of black coloration in the dorsal plumage of each individual (Ballentine & Greenberg, 2010). Sections of digital photographs were sampled from the dorsal part, a roughly rectangular area (from behind the head to the beginning of the rump feathers), and the masking tool was used to quantify the number of black pixels for each section. All photographs used for the plumage analysis were uploaded to morphbank (Collection # 801515, http://www.morphbank.net). The amount of black was estimated as the number of black pixels/total number of pixels of the section. We restricted our morphological analysis to adults, which are easily distinguished from juveniles based on the darker plumage, a well-defined rufous cap, and clearer iris coloration typical of adult *Spartonoica*. Sex was determined using genetic techniques (Cardoni *et al.*, 2009).

DNA extraction

Blood samples were obtained by pricking the basilica vein using 25G $^{5}/_{8}$ needles and immediately stored in lysis buffer (2 M Tris HCl, 0.5 M sodium, 0.01 M NaCl and EDTA, 20% sodium dodecyl sulfate; pH 8.0). DNA was isolated from the blood samples using protocols established in the DNeasy Kit® (Qiagen, Inc., Valencia, CA, USA).

Mitochondrial DNA and microsatellite analyses

We amplified sections of three gene regions of the mtDNA using PCR, performed by a PTC-100 Programmable Thermal Cycler (MJ Research Inc.): (1) control region amplifications were conducted using primers that we developed specifically for this study DLSP754R (5′-GGTTTAGGGGGAAAGAATGG-3′) and DLSP754F (5′-GAAGCCAACCAGTAGAACA-3′); (2) NADH-dehydrogenase subunit 3 (ND3) amplifications were conducted using primers L10755 (5′-GA CTTCCAATCTTTAAAATCTGG-3′) and Hl1151 (5′-GATTTGTTGAGCCGAAATCAAC-3′; Chesser, 1999); and (3) cytochrome-*b* was amplified using primers

2SH (5′-GAATCTACTACGGCTCATAC) and wow (5′-ATGGGTGGAATGGAATTTTGTC-3′; Dumbacher, Pratt & Fleischer, 2003).

Amplification reactions for all primer sets were carried out in a total volume of 25 μL. The final reaction conditions were as follows: 13.8 μL ddH_2O, 0.2 μL of AmpliTaq Gold® DNA polymerase (Applied Biosystems), 1 μL 10 μM of both primers, 2 μL 25 mM of $MgCl_2$, 2.5 μL 10× PCR Gold Buffer (Applied Biosystems), 2.5 μL 2 μM of dNTPs, and 2 μL of BSA. Cycle parameters for ND3 and cytochrome *b* primers were 45 cycles of 1 min at 92 °C, 1 min at 50 °C and 1 min at 72 °C. Cycle parameters for the control region were 35 cycles of 1 min at 92 °C, 1 min at 49 °C, and 1 min at 72 °C. PCR products were then purified using the Qiaquick kit® following the manufacturer's protocol (Qiagen, Inc., Valencia, CA, USA). Sequencing reactions of the PCR products were conducted using the same primers for each mtDNA region and the Big Dye® Terminator v3.1 cycle sequencing kit (Applied Biosystems). These products were sequenced directly in a 3130XL genetic analyser (Applied Biosystems). The resulting sequences were analysed using Sequencher version 4.1® (Genecodes Corp., Ann Arbor, MI, USA). These sequences were cleaned by direct alignment, inspected, and corrected by eye.

As microsatellite primers specific for *Spartonoica* were not available, we screened a total of 49 microsatellite loci that had been proven to be polymorphic in other passerine species and found only three to be polymorphic for this species. We screened 128 individuals for the following primers: *D09* (*Thamnophilus cryptoleucus*; Ágreda *et al*., 2006), *Man3* (*Manacus manacus*; Piertney, Shorey & Höglund, 2002), and *LTMR8* (from McDonald & Potts, 1994).

PCR amplifications were conducted in 10-μL reaction volumes following the conditions described in the original studies. These reactions consisted of an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 94 °C for 20 s, annealing temperature (T_a: 50 °C for D09; 52 °C for MAN3; and 57 °C for LTMR8) for 20 s, 72 °C for 45 s; and a final amplification step of 5 min at 72 °C.

The resulting PCR products were analysed on a 3130XL Genetic Analyzer using GeneScan ROX 500® size standard and genotyped with GeneMapper 4 software (Applied Biosystems). Fragment analysis reactions consisted of 0.5–1 μL PCR product, 0.5 μL size standard, and HiDi Formamide (Applied Biosystems) to 11 μL.

Statistical analysis

We used a nested ANOVA test to determine morphological differentiation between habitats and among sites/population (Zar, 1999). To study the patterns of bill size differentiation, we performed a principal component analysis (PCA) for the three beak dimensions (length, width, depth) and then examined PC1 (PC Bill) for overall differences in beak size. *Spartonoica* shows subtle but statistically significant sexual dimorphism in several morphological characters (Cardoni *et al*., 2009). Therefore, to consider the effects of the individual's sex in the morphological variables, we performed a nested ANOVA using three factors: habitat, population, and sex. If a variable was found to be significantly different between habitats, we performed a Mantel test (Mantel, 1967) with 10 000 permutations to assess the relationship of the morphological distance of this variable with geographical distances among pairwise comparisons. Morphological distance was calculated using the Euclidian distance using the software PRIMER *5.2.9.v* (Clarke & Gorley, 2001).

To estimate the genetic variability within populations for the three mtDNA gene regions, we calculated the haplotype diversity and the nucleotide diversity of each population. In addition, Fu's *Fs* values (Fu, 1997) and the Ramos-Onsins and Rozas's R_2 (Ramos-Onsins & Rozas, 2002) statistics were calculated to verify the null hypothesis of selective neutrality in relation to mtDNA sequences. These statistics were all computed using the software DnaSP 4.10 (Rozas *et al*., 2003). We constructed a haplotype network to infer haplotype relationships with the program TCS v1.21 (Clement, Posada & Crandall, 2000), which uses the principle of parsimony statistics, following the links more parsimonious with a probability equal to or greater than 0.95 (95%), as described by Templeton (1998). We assessed hierarchical levels of genetic structure in the mtDNA data for habitat (inland versus coastal marshes), populations within habitat, and all populations using an analysis of molecular variance (AMOVA) with 10 000 permutations to test the significance of pairwise population comparison (ARLEQUIN 3.11, Excoffier, Laval & Schneider, 2005). We estimated isolation by distance (IBD) by plotting the mean genetic distance (Nei's) versus geographical distance values with a Mantel test (Mantel, 1967) with 10 000 permutations using the program FSTAT 2.9.3.2. (Goudet, 2002). Finally, in order to estimate the age of expansion of *Spartonoica* population we investigated their demographic signature using the mtDNA haplotypes based on coalescence theory. We used a pairwise mismatch distribution to test for population expansion (Rogers & Harpending, 1992). The parameter of demographic expansion (τ) was estimated with a generalized nonlinear least squares approach and approximate confidence intervals were obtained with 10 000 parametric bootstrap replicates in the program

Arlequin (Excoffier, Laval & Schneider, 2005). The goodness-of-fit of the observed data to a simulated model of expansion was tested with Harpending's raggedness index (r) (Harpending, 1994), as implemented in Arlequin. The age of expansion was estimated with the formula $\tau = 2\,\mu tk$, where μ is mutation rate, k is the number of bases analysed, and t is the expansion time in generations.

For microsatellite loci, we estimated the number of alleles per locus (A_N), allelic richness per locus (A_R), a measure of allele number independent of sample size (El Mousadik & Petit, 1996), Nei's gene diversity (H_S) averaged over loci (Nei, 1987), observed heterozygosity (H_O), and the index F_{IS} using FSTAT version 2.9.3.2. (Goudet, 2002). Hardy–Weinberg Equilibrium (HWE) departures over all loci within each population were determined by examining deficiency of heterozygotes (F_{IS}) and generating P values with 800 permutations, after controlling for multiple tests using sequential Bonferroni correction (Rice, 1989). We tested if genetic structure was present within versus among populations with an AMOVA with 10 000 permutations. We then tested our hypothesis of IBD based on geographical locality and also by coastal versus inland habitat. We determined the significance of genetic structure among local and regional population groupings using an AMOVA with Weir & Cockerham, 1984) F_{ST} as the measurement of genetic distance included in ARLEQUIN 3.11 (Excoffier *et al*., 2005). Significance was obtained after 10 000 permutations to determine the probability of a random F_{ST} value being greater than or equal to the observed value (Excoffier *et al*., 2005). The Mantel test (Mantel, 1967) with 10 000 permutations was performed to assess the relationship between geographical and genetic distances using the program FSTAT version 2.9.3.2. (Goudet, 2002). Finally, we performed a rarefaction analysis for allelic richness over the three microsatellite loci and conducted comparisons between the number of individuals and number of molecular markers used in order to determine the reliability of our sampling effort, using the software HP-Rare (Kalinowski, 2005).

RESULTS

Morphological and plumage variation

We found significant differences in morphology and plumage coloration between *Spartonoica* populations from coastal and inland marshes. Both bill width and length were greater for individuals from inland marshes than individuals from coastal marshes. Bill length also showed differences among population within habitat (Table 1). Bill depth, the size of the rufous cap, and the wing chord show differences only

Table 1. Morphological and plumage measurements (mean and standard deviation) for bay-capped wren spinetail individuals that inhabit costal and inland habitats; nested ANOVA statistical values F and P, for habitat, population controlled by habitat [Pop(Habitat)], and sex controlled by population and habitat [Sex(Habitat*Pop)]

	Inland			Coastal			Habitat		Pop(Habitat)		Sex (Habitat*Pop)	
	N	Mean	SD	N	Mean	SD	$F_{1,68}$	P	$F_{4,68}$	P	$F_{6,68}$	P
Morphology												
Bill length (mm)	42	8.40	0.34	38	8.23	0.43	7.22	0.009*	3.26	0.017*	1.36	0.24
Bill width (mm)	42	2.52	0.23	38	2.43	0.26	4.67	0.034*	1.74	0.15	0.78	0.59
Bill depth (mm)	42	3.00	0.16	38	2.94	0.14	2.65	0.11	2.05	0.09	4.14	0.001*
Tarsus length (mm)	42	19.22	0.65	38	19.47	0.44	0.53	0.46	0.89	0.47	1.23	0.29
Wing chord (mm)	42	49.57	1.38	38	49.37	1.28	0.01	0.91	1.89	0.12	3.18	0.008*
Tail length (mm)	41	65.54	6.94	37	63.38	6.19	1.51	0.22	1.33	0.27	0.12	0.99
PC bill (mm)	42	–0.26	0.89	38	0.29	1.05	12.25	0.001*	5.18	0.001*	3.79	0.003*
Plumage												
Dorsal black (%)	34	32.00	12.00	38	43.00	10.00	19.45	0.001*	2.92	0.061	0.90	0.51
Size rufous cap (mm)	42	12.42	2.83	38	11.48	2.41	0.96	0.331	0.88	0.482	2.52	0.03*
Size black cap (mm)	42	6.31	1.87	38	6.44	2.06	0.01	0.931	0.66	0.623	0.21	0.973

*$P < 0.05$.

between sexes (Table 1). The PC Bill shows differences both at the habitat level as well as at the population and sex level (Table 1). For those variables that presented differences only between habitat, the Mantel test showed that geographical distance does not predict differentiation among populations for bill width ($R^2 = 0.20$, $P = 0.09$) nor for tail length measurements ($R^2 = 0.07$, $P = 0.33$). The percentage of dorsal black plumage was significantly higher in coastal populations than in populations from inland marshes (Table 1 and Fig. 2).

Molecular variation

Mitochondrial DNA: We amplified and obtained sequences for three mtDNA regions: a 330-bp fragment of the cytochrome-*b* gene ($N = 124$); a 552-bp fragment of the control region ($N = 107$); and a 332-bp fragment of the ND3 gene ($N = 93$). We determined 12 haplotypes for cytochrome-*b*, five of them restricted to coastal marshes (CM), four restricted to inland marshes (IM), and only one common for both habitats (Fig. 3). The control region had 22 haplotypes, six of them restricted to coastal marsh (CM), nine to interior marsh (IM) and seven for both habitats (Fig. 3). The ND3 region had seven haplotypes, three for CM, one for IM, and two for both habitats (Fig. 3). Haplotype diversity was 0.974, 0.986, and 0.978 for cytochrome-*b*, control region, and ND3, respectively (Table 2).

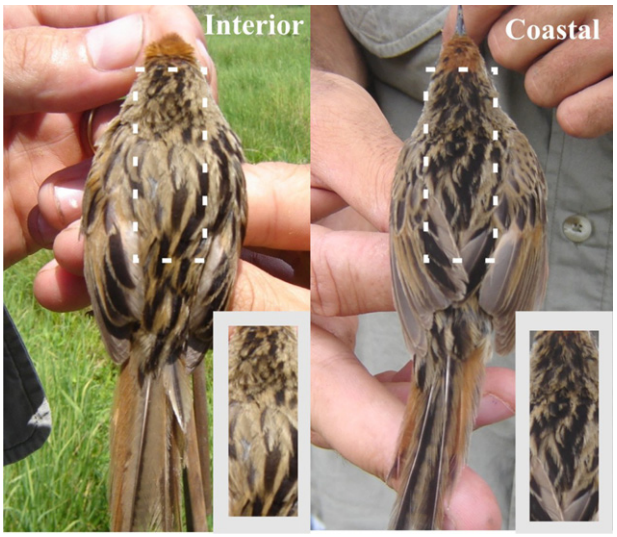

Figure 2. Male *Spartanoica* from interior and costal marshes. Dotted squares denote image section used for the plumage analysis.

The comparison of genetic differentiation between habitats revealed that the CM and IM populations were not genetically differentiated for the three mtDNA regions analysed (Table 3). A higher percentage of genetic variation was recorded among populations (range 95–100%) than between habitats (range 0.11 to 4%; Table 4). Geographical distance did not predict the genetic differentiation among populations (Mantel test; cytochrome-*b*, $R^2 = 0.18$, $P = 0.11$; ND3, $R^2 = 0.14$, $P = 0.17$; control region, $R^2 = 0.13$, $P = 0.19$). The three mitochondrial gene regions showed a star-shaped pattern of haplotype networks with most haplotype pairwise comparisons differing by single substitutions suggesting shallow divergences or recent demographic changes (Fig. 3). In addition, a signature of population expansion was detected for all mitochondrial regions and for the three statistics estimated, *Fs* (Fu, 1997), R_2 (Ramos-Onsins & Rozas, 2002), and Harpending's raggedness index (Harpending, 1994) (Table 2). Following the equation $t = \tau/2\mu k$, the time since population expansion was estimated to be 12 500, 15 000 and 21 000 years before the present for cytochrome-*b*, ND3, and control region, respectively.

Microsatellite loci

The number of alleles ranged from seven to 30 at the three microsatellite loci screened for *Spartonoica* (Table 4). Tests conducted for each locus in each population revealed only three cases of significant deviation from HWE (D09 locus at Punta Lara population, and Man3 locus at Mar Chiquita and La Picasa Lagoon populations). No population departed

Table 2. Number of haplotypes (*N*), haplotype diversity (Hd), nucleotide diversity (Pi), and Fu's (*Fs*), Ramos-Onsins and Rozas's R_2, and Harpending's raggedness index (*r*), estimates of demographic change for each of the mtDNA regions (cytochrome-*b*, ND3, and control region) and for all three regions combined; the parameter τ (tau) used for the age of expansion is also reported

	N	Hd	Pi	*Fs*	R_2	*r*	τ
Cytochrome-*b*	12	0.974	0.0069	0.189***	0.169***	0.305	2.345
ND3	8	0.978	0.006	0.289*	0.210**	0.501	4.582
Control region	22	0.986	0.0057	0.021***	0.130***	0.152	1.967

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

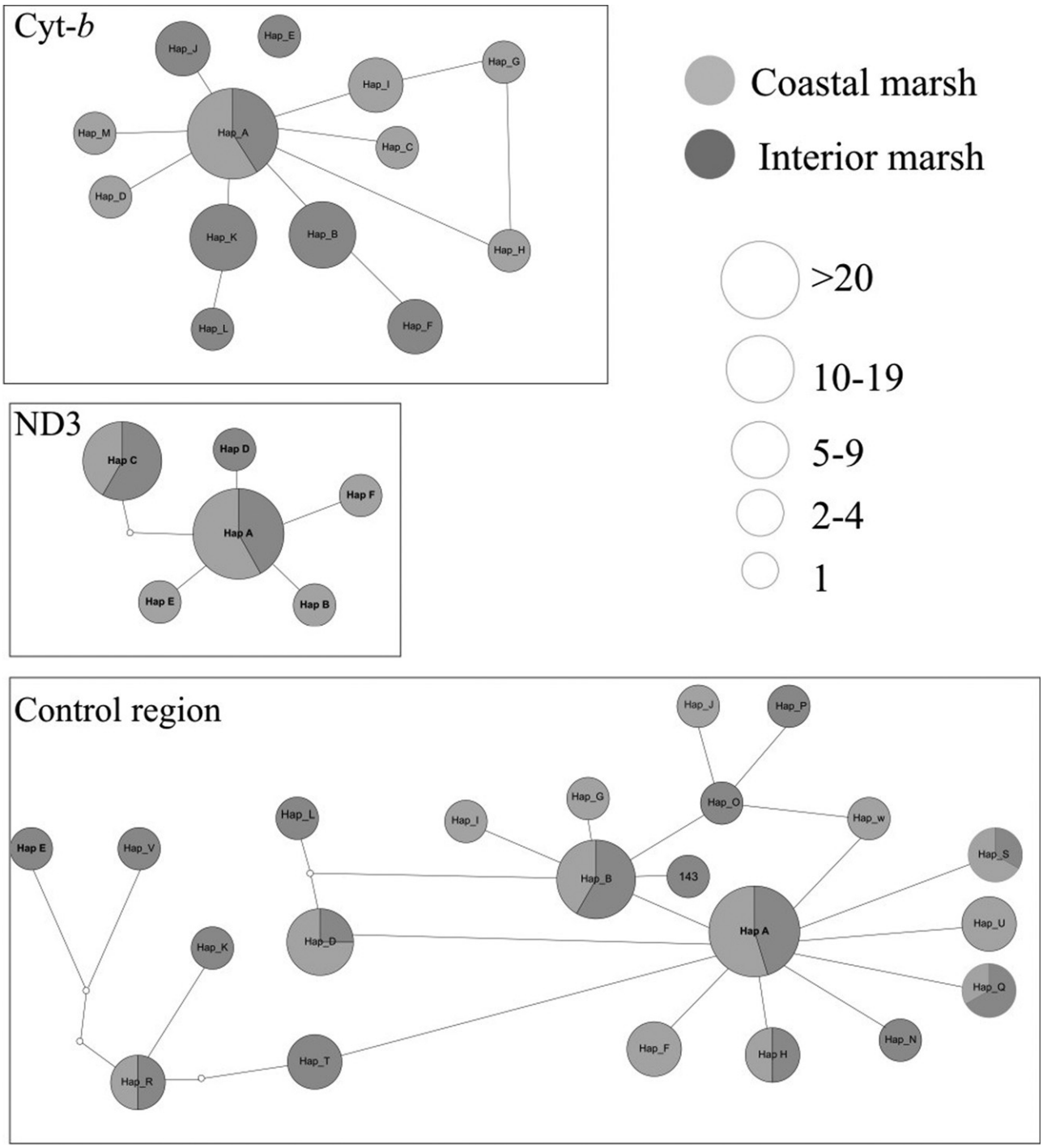

Figure 3. The star-like network represents the haplotype relationships of *Spartonoica* for three mitochondrial DNA markers. Each line between haplotypes was one mutation step. The size of circles indicates the haplotype frequency. TCS software was used in this analysis.

significantly from HWE at more than one locus. Observed heterozygosity (H_O) ranged from 0.72 to 0.97 among the six populations. The gene diversity showed the lowest values in saltmarsh populations (Table 4). Saltmarsh populations were not genetically differentiated from inland populations (AMOVA, $F_{ST} = -0.002$, $P = 0.8$, Table 3). The correlation between the genetic distances based on microsatellites and geographical distances for the six populations also did not show a pattern consistent with isolation by

Table 3. Results from hierarchical AMOVA testing variation between coastal and inland habitats among individuals within habitats and among overall populations using mitochondrial DNA (cytochrome-*b*, ND3, and Control region) and microsattelite markers

	d.f.	Sum of squares	Variance	Percentage variation	Fixation index	*P*
Cytochrome *b*						
Among groups	1	0.61	0.007	4.020	0.04 (F_{CT})	0.06
Among population within groups	5	0.80	–0.001	–0.690	–0.007 (F_{SC})	0.14
Within populations	117	21.04	0.180	96.660	0.033 (F_{ST})	0.7
Total	123	22.45	0.186			
ND3						
Among groups	1	0.51	0.000	–0.110	–0.001 (F_{CT})	0.38
Among population within groups	4	2.00	0.015	5.120	0.051 (F_{SC})	0.08
Within populations	85	23.74	0.279	94.990	0.05 (F_{ST})	0.14
Total	90	26.24	0.294			
Control region						
Among groups	1	1.08	0.007	0.620	0.006 (F_{CT})	0.46
Among population within groups	5	4.21	–0.016	–1.540	–0.016 (F_{SC})	0.6
Within populations	100	105.38	1.054	100.920	–0.009 (F_{ST})	0.59
Microsatellite						
Among groups	1	0.92	–0.003	0.25	–0.002 (F_{CT})	0.8
Among population within groups	4	4.97	0.004	0.37	0.004 (F_{SC})	0.13
Within populations	262	281.71	107.52	99.87	0.001 (F_{ST})	0.15
Total	267	287.60	107.65			

Table 4. Location, habitat, sample size (*N*), and summary of genetic diversity for six bay-capped wren-spinetail populations at three microsatellite loci; measures of genetic diversity include average number of alleles per locus (A_N), allelic richness (A_R), expected heterozygosity (H_E), observed heterozygosity (H_O), and inbreeding coefficient (F_{IS}), and values of significance (*P*) for Hardy–Weinberg equilibrium (HWE) based on the average of three polymorphic loci (LTMR8, Man3, and D09)

Population	Habitat	*N*	A_N	A_R	H_E	H_O	F_{IS}	HWE
Bahía Blanca	Saltmarsh	13	6.33	3.63	0.757	0.728	–0.29	0.0005
Mar Chiquita	Saltmarsh	40	12	3.70	0.748	0.972	0.023	0.7324
Bahía Samborombón	Saltmarsh	19	7.67	3.6	0.723	0.745	0.03	0.7414
Saladillo	Inland	19	8.67	3.83	0.788	0.866	–0.11	0.04
La Picasa	Inland	21	9.33	3.94	0.791	0.73	0.08	0.9457
Punta Lara	Inland	19	9	3.78	0.767	0.894	–0.17	0.0038

distance (Mantel test, $R^2 = 0.047$, $P = 0.81$). A rarefaction analysis of allelic richness over the three microsatellite loci for *Spartonoica* populations corroborated the reliability of our sampling effort (Fig. 4), suggesting that our microsatellite data could be enough to determinate real allelic richness.

DISCUSSION

General patterns

We found significant phenotypic differences in bill and plumage coloration (melanism) between *Spartonoica* populations from coastal and inland marshes. This is the first study to document morphological differentiation in a South American saltmarsh vertebrate taxon. The low levels of genetic variation, weak geographical structure, and shallow divergences, based on both mtDNA and microsatellite data, suggest that this population underwent a recent and rapid demographic expansion. Morphological differentiations without genetic structure between populations have also been reported between coastal marsh and interior populations of other North American birds (e.g. Avise & Zink, 1988; Greenberg *et al*., 1998; Chan *et al*., 2006; Greenberg & Maldonado, 2006) and among populations of migratory birds in general

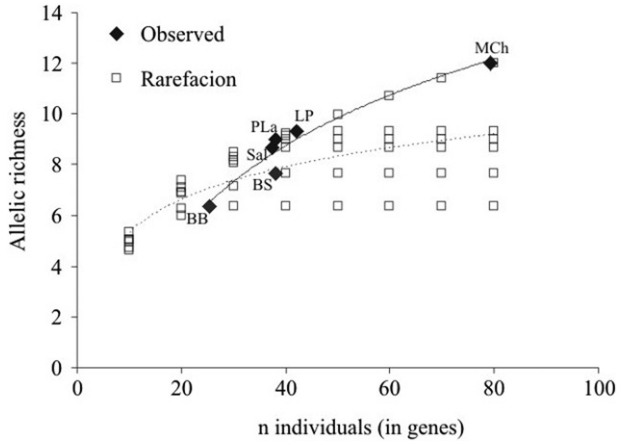

Figure 4. Rarefaction analysis for allelic richness over the three microsatellite loci for *Spartonoica* populations (MCh, Mar Chiquita Lagoon; LP, La Picasa Lagoon; PLa, Punta Lara; Sal, Saladillo river; BS, Bahía Samborombon; BB, Bahía Blanca).

(Milá, Smith & Wayne, 2006). Furthermore, the lack of underlying genetic divergence combined with parallel or convergent adaptations in bill and plumage suggest that similar processes have shaped the evolution of coastal populations of certain North American sparrows and a South American Furnariid.

Bill variation

Bill shape diversity among bird species has long been evidence of the power of natural selection on bill function (Darwin, 1859; Smith, 1987; Grant & Grant, 2002). Population-level or subspecific divergence in bill morphology can reflect local ecological adaptation (Badyaev *et al*., 2008; Ballentine & Greenberg, 2010) and may even contribute to incipient speciation (de León *et al*., 2010). Therefore, bill morphology is an evolutionarily mutable feature in birds that is sensitive to selection following changes in foraging substrate or diet (Grant & Grant, 2002). North American saltmarsh sparrows have longer and narrower bills than their inland counterparts, while *Spartonoica*'s bills are only narrower. However, the fact that we are comparing bird species from different families should be taken into consideration. *Spartonoica* belongs to a typical insectivorous family (Furnariidae) with a narrower and longer bill relative to the typical seed-eating family of North American saltmarsh sparrows (Emberizid). Thus, the overall pattern of saltmarsh birds is to have an acute bill, being narrower and longer in sparrows and narrower in *Spartonoica*. That could indicate that there is a selective pressure on the bill to forage on infaunal invertebrates, as an acute bill could be an advantage to probe into the mud (Greenberg & Droege, 1990). Anecdotally, we observed the presence of a substantial amount of mud on the upper mandible of 13 individuals from coastal marshes (and none from interior sites) suggesting that they may be feeding predominantly on the muddy ground and hence a thinner bill may be advantageous in probing for mud-dwelling invertebrates. Although foraging in *Spartonoica* is difficult to observe, further observation and diet data should be gathered to evaluate this hypothesis.

Saltmarshes are continental ecosystems that seemingly have similar processes to those found on islands (Greenberg & Olsen, 2010), and coastal marsh biotas display many of the features characteristic of true islands, including low species diversity and high population densities. Common features of vertebrate populations on islands have led to the development and refinement of 'the island syndrome', which is characterized by low levels of interspecific competition and predation, high population densities, and ecological niche expansion (Blondel, 2000). *Spartonoica* presents all of the characteristics of an island inhabitant species, such as high population density (Cardoni *et al*., 2007, 2011; Cardoni, 2011), low predation rate (Llambías *et al*., 2009; Cardoni, Isacch & Iribarne, 2012), and low interspecific competition (Cardoni, 2011). The general pattern among passerine birds that inhabit islands versus continental habitats is a trend toward increasing bill size (Scott *et al*., 2003). This pattern was also documented for bird species that inhabit saltmarshes (Greenberg & Olsen, 2010) and mangrove habitats (Luther & Greenberg, 2011), habitats that resemble island ecosystems. Greater bill size of island birds may be associated with a shift toward greater generalization in resource use, but specialization in diet (invertebrates) or foraging behaviour (probing in tidal mud) are legitimate alternatives (Greenberg & Olsen, 2010). For example, North American sparrows show a pattern where populations have relatively longer and thinner bills in coastal saltmarshes than interior habitats. This could be an adaptation for increased consumption of animal foods, particularly marine invertebrates, and a concomitant decrease in eating seeds. Additionally, sparrows in tidal marshes may use their narrower bills to probe the muddy substrate more than their counterparts in inland marshes (Grenier & Greenberg, 2006; but see Greenberg *et al*., 2012). For *Spartonoica*, coastal populations have narrower bills, but not longer, than those found in inland marshes; so the morphological bill pattern was different from that found for North American birds (e.g. Emberizid species), and perhaps the island syndrome, as an explanation for greater generalization in resource use may not apply to *Spartonoica*. Other alternative processes could be influencing the bill morphology in saltmarsh birds. Recently, Greenberg *et al*. (2012)

found that bill size was positively correlated with high summer temperature in saltmarsh sparrows, suggesting that bills may also have a thermoregulatory function.

Plumage variation

Saltmarsh melanism (e.g. a tendency towards a darker dorsal plumage or pelage) has long been documented in North American birds and mammals (Grinnell, 1913; Von Bloeker, 1932; Greenberg & Droege, 1990). Our study confirms a darker dorsal plumage in the saltmarsh population of *Spartonoica* than in inland populations. Furthermore, coastal marsh populations of *Spartonoica* had darker dorsal plumage than inland populations, despite their genetic similarity. This pattern is similar to what is found in North American sparrows and other vertebrates and supports the hypothesis that strong selection favours dark individuals in saltmarshes (Greenberg & Droege, 1990). Greenberg *et al*. (1998) articulated the argument that substrates in tidal marshes contain more iron sulphides than iron oxides and tend to be greyer and darker that the bird would have to match in order to provide for better camouflage. Moreover, the feather is more prone to deterioration by physical abrasion and ultraviolet irradiation in tidal marshes (Bergmann, 1982; Burtt, 1986). Furthermore, bacteria that commonly occur in the soil (*Bacillus licheniformis*; Wood, 1995) are an important biological cause of feather deterioration (Burtt & Ichida, 1999). Peele *et al*. (2009) recently found that populations of the coastal plain swamp sparrow (*Melospiza georgiana nigrescens*), which breed in coastal saltmarshes, have significantly more active feather-degrading bacilli than the swamp sparrows (*Melospiza georgiana georgiana*), which breed in freshwater marshes and fens. Such a difference could be the result of a considerable selection pressure for dark plumage in coastal marsh birds so that they are better able to resist bacterial degradation (Peele *et al*., 2009). However, population phenotypic responses to habitat change could be explained by other processes, such as phenotypic plasticity (Charmantier *et al*., 2008).

Possible causes for lack of concordance in genetic markers and morphology

The lack of concordance between patterns of genetic and morphological variation could simply be an artefact caused by the small number of microsatellite markers screened in this study. However, the rarefaction analysis of the three mircosatellite loci used in this study suggests that they yielded enough allelic diversity to reliably assess genetic diversity of our populations at a gross scale. Furthermore, the low level of differentiation revealed by our mircrosatellite data was also confirmed using three of the fastest evolving mtDNA genes and totalling more than 1000 bp of sequence data. As mtDNA is better suited to study historical patterns of genetic differentiation (Avise & Zink, 1988), we can only infer that there is no signature of historical differentiation between coastal and inland populations. Therefore, it would be very useful to develop a larger suite of highly polymorphic microsatellites that may provide more power to detect patterns of fine-scale genetic structure over the recent past.

On the other hand, phenotypic differences in integument coloration, body size, and other morphological characters may be primarily an environment/genotype interaction during development rather than genetic in origin (Smith & Wayne, 1996; Maldonado, Vilà & Hertel, 2004; but see Ballentine & Greenberg, 2010). Alternatively, evolutionary patterns at neutral loci and loci under selection may differ. With strong selection on quantitative trait loci, phenotypes could diverge rapidly, while neutral loci diverge at a rate proportional to the effective population size. This - established species (Funk & Omland, 2003; Chan *et al*., 2006). Our data suggest that *Spartonoica* underwent a recent and rapid demographic expansion, and due to the geographical proximity of the populations, high levels of gene flow are expected among those populations.

Other explanations than those related to neutral loci could be applied, such as phenotypic plasticity, age-related variation, and intersexual competition. The possibility of phenotypic plasticity, the ability to change and modify phenotype in response to environmental cues (Stearns, 1989), could be the origin of the morphological and plumage differences between coastal and inland *Spartonoica* populations. Invasion into a new environment results in selection pressures favouring divergence from the ancestor, inducing changes in an individual's behaviour, morphology, and physiology. Therefore, if individuals can attain high fitness in the new environment as a consequence of a plastic response, there would then be no adaptive genetic differentiation from the source (Price, Qvarnström & Irwin, 2003). Tidal marsh ecosystems (e.g. *Spartina densiflora* marshes) are relatively new habitats in terms of geological time (Malamud-Roam *et al*., 2006), and such habitats are governed by particular environmental conditions, such as high salinity (Goldstein, 2006), periodic flooding (Reinert, 2006), high temperature (Greenberg *et al*., 2012), substrate colour, and available food (Grenier & Greenberg, 2006). Entry into a new environment results in selection pressures favouring divergence from the ancestor, and could be accompanied by behavioural and

other plastic forms of accommodation, and this will usually be followed by selection in the context of these changes (Price *et al*., 2003). Considering these aspects, it is not surprising that there are differences in phenotypic characters (e.g. plumage melanism, bill morphology) among inland (ancestral) and saltmarsh populations, where plastic responses to environmental pressures are expected for those animals living in saltmarsh habitat.

Convergent pattern between South and North American saltmarsh birds?

The hypothesis of convergence states that under similar environmental conditions, species have become more similar in certain characteristics than their ancestors (Schluter, 2000). Such similarities, e.g. in morphology, are caused by common selection pressures (Grant *et al*., 1976; Futuyma, 1998). Phenotypic convergence among saltmarsh birds was documented for several species inhabiting North American saltmarshes (Grenier & Greenberg, 2006), and such convergences are related to plumage colour (saltmarsh melanism), and longer and thinner bills (Murray, 1969; Greenberg *et al*., 2006). However, to date, no study outside North American saltmarshes had demonstrated phenotypic convergence. South and North American saltmarsh habitats were shaped by similar geological and environmental evolutionary process during the late Pleistocene. Rapid change in the availability of habitat with the receding of Pleistocene glaciers is thought to have driven rapid and extensive expansion of populations, resulting in a lack of genetic structure. At the same time, the rapid availability of novel habitats, such as boreal forest and estuarine tidal marsh, might select for rapid morphological divergence (Milá *et al*., 2006; Ruegg, Hijmans & Moritz, 2006). Although glaciers were less extensive in the Southern Cone, global climatic fluctuations during the Pleistocene produced cyclical advance and retreat of glaciers that caused a marked and concurrent expansion and retraction of arid and humid conditions, particularly in the Pampas region (Isla & Espinosa, 1995; Violante & Parker, 2004). Moreover, South and North American saltmarshes present similar flora (dominated by cordgrass species such as *Spartina* spp.; Isacch *et al*., 2006), and high abundance of invertebrate food items (Mitsch & Gosselink, 2000; Greenberg *et al*., 2006). Saltmarshes are governed by extreme environmental condition, such as high salinity and frequent flooding events. Considering all of these aspects, it is not surprising that phenotypic characters (e.g. plumage melanism, bill) have undergone convergent evolution between South and North American saltmarsh birds, responding in common to the challenges associated with saltmarsh ecosystems.

Morphological convergence was also documented for other habitats among unrelated bird taxa, such as boreal fern habitats (between North America and northern Europe), high-altitude alpine habitats (Landmann & Winding, 1995), and conifer habitats (Korner-Nievergelt & Leisler, 2004). Such examples of habitat-specific convergence demonstrated that selective forces associated with feeding are strong enough to select for particular traits across species. Ecomorphological studies in birds have demonstrated that subtle differences in shape of external morphology can have profound ecological effects (Leisler & Winkler, 1985, 2001). Therefore, the differences that we found in *Spartonoica* could be suggesting an evolutionary process for adaptation related to foraging behaviour (bill) and camouflage (plumage) which would be important for their survival in the saltmarsh environment.

ACKNOWLEDGEMENTS

We thank R. Fleischer, D. Hawley, S. Sonsthagen, B. Ryder, D. Redding, and N. Rotzel for assistance and support in the genetics laboratory at the CCEG. We thank P. Micheluti (Mar Chiquita Cordoba), M. Roda (Salado basin), and J. P. Carricart (Punta Lara) for assistance at captured sites. We thank S. Bachman for help in the field, and C. Azcueta for providing transportation. We thank P. Iriarte and M. Mora for molecular analysis help. Financial support was provided by the Smithsonian Institution restricted endowment funds, Smithsonian graduate student fellowship and a Short Term Visitor grant, CONICET, Universidad Nacional de Mar del Plata, Agencia Nacional de Promoción Científica y Tecnológica (Argentina), Kathleen S. Anderson Award Sigma Xi Grant-in-Aid of Research.

REFERENCES

Adam P. 1990. *Saltmarsh ecology*. Cambridge: Cambridge University Press.

Ágreda A, Lieckfeldt D, Schmidt A, Pitra C. 2006. Characterization of polymorphic microsatellite loci in Castelnau's Antshrike, *Thamnophilus cryptoleucus* (Aves: Thamnophilidae). *Molecular Ecology Notes* **6:** 434–436.

Avise JC, Zink RM. 1988. Molecular genetic divergence between avian sibling species: King and Clapper Rails, Long-billed and Short-billed Dowitchers, Boattailed and Great-tailed Grackles, and Tufted and Black-crested Titmice. *Auk* **105:** 516–528.

Badyaev AV, Young RL, Oh KP, Addison C. 2008. Evolution on a local scale: developmental, functional, and genetic bases of divergence in bill form and associated changes in song structure between adjacent habitats. *Evolution* **62:** 1951–1964.

Ballentine B, Greenberg R. 2010. Common garden experiment reveals genetic control of phenotypic divergence between Swamp Sparrow subspecies that lack divergence in neutral genotypes. *PLoS ONE* **5**(4)**:** e10229. doi:10.1371/journal.pone.0010229.

Bergmann G. 1982. Why are the wings of *Larus f. fuscus* so dark? *Ornis Fennica* **59:** 77–83.

Bilenca D, Miñarro F. 2004. *Identificación de Áreas Valiosas de Pastizal (avps) en las Pampas y Campos de Argentina, Uruguay y sur de Brasil*. Buenos Aires: Fundación Vida Silvestre.

BirdLife International. 2012. Species factsheet: *spartonoica maluroides*. Downloaded from http://www.birdlife.org 2 August 2012.

Blondel J. 2000. Evolution and ecology of birds on islands: trends and prospects. *Vie et Milieu* **50:** 205–220.

Burtt EH Jr. 1986. An analysis of physical, physiologicaland optical aspects of avian coloration with emphasis on wood-warblers. *Ornithological Monograph* **38:** 1–126.

Burtt EH Jr, Ichida JM. 1999. Occurrence of feather degrading bacilli in the plumage of birds. *Auk* **116:** 364–372.

Canevari M, Canevari P, Carrizo GR, Harris G, Rodríguez Mata J, Straneck RJ. 1991. *Nueva guía de las aves argentinas*. Volume 2. Buenos Aires: Fundación Acindar.

Cardoni DA. 2011. Adaptaciones evolutivas y respuestas a la actividad antrópica de aves de marismas del atlántico sudoccidental: un análisis a diferentes escalas temporales. Doctoral Thesis, Universidad Nacional de Mar del Plata, Buenos Aires, Argentina.

Cardoni DA, Isacch JP, Fanjul ME, Escapa M, Iribarne OO. 2011. Relationship between anthropogenic sewage discharge, marsh structure and bird assemblages in a SW Atlantic saltmarsh. *Marine Environmental Research* **71:** 122–130.

Cardoni DA, Isacch JP, Iribarne OO. 2007. Indirect effects of the burrowing crab (*Chasmagnathus granulatus*) in the habitat use of saltmarsh birds. *Estuaries and Coasts* **30:** 382–389.

Cardoni DA, Isacch JP, Iribarne OO. 2012. Effects of cattle grazing and burnings on abundance, habitat selection and nesting success of the Bay-capped Wren-Spinetail (*Spartonoica maluroides*) in coastal saltmarshes of the Pampas region. *Condor* **114:** 803–811.

Cardoni DA, Maldonado JE, Isacch JP, Greenberg R. 2009. Subtle sexual dimorphism in the Bay-capped Wren-Spinetail (*Spartonoica maluroides*; furnariidae) uncovered through molecular sex determination. *Ornitologia Neotropical* **20:** 347–355.

Chan YL, Hill CE, Maldonado JE, Fleischer RC. 2006. Evolution and conservation of tidal marsh vertebrates: molecular approaches. *Studies in Avian Biology* **32:** 54–76.

Charmantier A, McCleery RH, Cole LR, Perrins C, Kruuk LEB, Sheldon BC. 2008. Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science* **320:** 800–803.

Chesser RT. 1999. Molecular systematics of the rhinocryptid genus Pteroptochos. *Condor* **101:** 439–446.

Clarke KR, Gorley RN. 2001. *PRIMER v5: users manual/tutorial*. Plymouth: PRIMER-E Ltd.

Clement M, Posada D, Crandall K. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9:** 1657–1660.

Comparatore VM, Martínez MM, Vasallo AI, Barg M, Isacch JP. 1996. Abundancia y relaciones con el hábitat de aves y mamíferos en pastizales de *Paspalum quadrifarium* (paja colorada) manejados con fuego (Provincia de Buenos Aires, Argentina). *Interciencia* **21:** 228–237.

Darwin C. 1859. *On the origin of species by means of natural selection*. Cambridge, MA: Harvard University Press.

Derryberry EP, Claramunt S, Derryberry G, Chesser RT, Cracraft J, Aleixo A, Pérez-Emán J, Remsen JJV, Brumfield RT. 2011. Lineage diversification and morphological evolution in a large-scale continental radiation: the neotropical ovenbirds and woodcreepers (Aves: Furnariidae). *Evolution* **65:** 2973–2986.

Di Giácomo AS. 2005. Áreas importantes para la conservación de las aves en la Argentina. Sitios prioritarios para la conservación de la biodiversidad. *Temas de Naturaleza y Conservación* **5:** 1–514.

Dumbacher JP, Pratt TK, Fleischer RC. 2003. Phylogeny of the owlet-nightjars (Aves: Aegothelidae) based on mitochondrial DNA sequence. *Molecular Phylogenetics and Evolution* **29:** 540–549.

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

Excoffier L, Laval LG, Schneider S. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analyses. *Evolutionary Bioinformatics* **1:** 47–50.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147:** 915–925.

Funk DJ, Omland KE. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences with insights from animal mitochondrial DNA. *Annual Review of Ecology and Systematic* **34:** 397–423.

Futuyma DJ. 1998. *Evolutionary biology*, 3rd edn. Sunderland, MA: Sinauer.

Goldstein D. 2006. Osmoregulatory biology of saltmarsh passerines. *Studies in Avian Biology* **32:** 110–118.

Goudet J. 2002. FSTAT, a Program to Estimate and Test Gene Diversities and Fixation Indices, Version 2.9.3.2. Available at: http://www2.unil.ch/popgen/softwares/fstat.htm

Grant PR, Grant BR. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. *Science* **296:** 707–711.

Grant PR, Grant BR, Smith JNM, Abbott IJ, Abbott LK. 1976. Darwin's finches: population variation and natural selection. *Proceedings of the National Academy of Sciences of the United States of America* **73:** 257–261.

Greenberg R, Cordero PJ, Droege S, Fleischer RC. 1998. Morphological adaptation with no mitochondrial DNA differentiation in the Coastal Plain Swamp Sparrow. *Auk* **115:** 706–712.

Greenberg R, Danner R, Olsen B, Luther D. 2012. High summer temperature explains bill size variation in salt marsh sparrows. *Ecography* **35:** 146–152.

Greenberg R, Droege S. 1990. Adaptations to tidal marshes in breeding populations of the Swamp Sparrow. *Condor* **92:** 393–404.

Greenberg R, Maldonado J, Droege S, McDonald MV. 2006. Tidal marshes: a global perspective on the evolution and conservation of their terrestrial vertebrates. *BioScience* **56:** 675–685.

Greenberg R, Maldonado JE. 2006. Diversity and endemism in tidal-marsh vertebrate. *Studies in Avian Biology* **32:** 32–53.

Greenberg R, Olsen B. 2010. Bill size and dimorphism in tidal-marsh sparrows: island-like processes in a continental habitat. *Ecology* **91:** 2428–2436.

Grenier JL, Greenberg R. 2005. A biogeographic pattern in sparrow bill morphology: parallel adaptation to tidal marshes. *Evolution* **59:** 1588–1595.

Grenier JL, Greenberg R. 2006. Trophic adaptations in sparrows and other vertebrates of tidal marshes. *Studies in Avian Biology* **32:** 130–140.

Grinnell J. 1913. The species of the mammalian genus *Sorex* of west-central California with a note on the vertebrate palustrine fauna of the region. *University of California Publications in Zoology* **20:** 179–205.

Harpending HC. 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology* **66:** 591–600.

Isacch JP, Cardoni DA. 2011. Different grazing strategies are necessary to conserve endangered grassland birds in the same region: the case of short and tall grasslands from flooding Pampas. *Condor* **113:** 724–734.

Isacch JP, Costa CSB, Rodríguez-Gallego L, Conde D, Escapa M, Gagliardini DA, Iribarne OO. 2006. Distribution of saltmarsh plant communities associated with environmental factors along a latitudinal gradient on the SW Atlantic coast. *Journal of Biogeography* **33:** 888–900.

Isacch JP, Holz S, Ricci L, Martínez M. 2004. Post-fire vegetation change and bird use of a salt marsh in coastal Argentina. *Wetlands* **24:** 235–243.

Isacch JP, Martínez M. 2001. Estacionalidad y relaciones con la estructura del hábitat de la comunidad de aves de pastizales de paja colorada (*Paspalum quadrifarium*) manejados con fuego en la Provincia de Buenos Aires, Argentina. *Ornitología Neotropical* **12:** 345–354.

Isla FI, Espinosa MA. 1995. Coastal environmental changes associated with Holocene sea-level fluctuation: southeastern Buenos Aires, Argentina. *Quaternary International* **26:** 55–60.

Kalinowski ST. 2005. HP-Rare: a computer program for performing rarefaction on measures of allelic diversity. *Molecular Ecology Notes* **5:** 187–189.

Korner-Nievergelt F, Leisler B. 2004. Morphological convergence in conifer-dwelling passerines. *Journal of Ornithology* **145:** 245–255.

Landmann A, Winding N. 1995. Guild organization and morphology of high altitude granivorous and insectivorous birds: convergent evolution in an extreme environment. *Oikos* **73:** 237–250.

Leisler B, Winkler H. 1985. Ecomorphology. *Current Ornithology* **2:** 155–186.

Leisler B, Winkler H. 2001. Morphological convergence in papyrus dwelling passerines. *Ostrich Supplement* **15:** 24–29.

de León LF, Bermingham E, Podos J, Hendry AP. 2010. Divergence with gene flow as facilitated by ecological differences: within-island variation in Darwin's finches. *Philosophical Transactions Royal Society B* **365:** 1041–1052.

Llambías PE, Ferretti V, Cardoni DA, Maldonado JE. 2009. Breeding success and social mating system of the bay-capped wren-spinetail (*Spartonoica maluroides*). *The Wilson Journal of Ornithology* **121:** 803–807.

Luther D, Greenberg R. 2011. The island syndrome in coastal wetland ecosystems: convergent evolution of large bills in mangrove passerines. *Auk* **128:** 201–204.

Malamud-Roam KP, Malamud-Roam FP, Watson EB, Collins JN. 2006. The Quaternary geography and biogeography of tidal saltmarshes. *Studies in Avian Biology* **32:** 11–31.

Maldonado JE, Vilà C, Hertel F. 2004. Discordant patterns of morphological variation in genetically divergent populations of ornate shrews (*Sorex ornatus*). *Journal of Mammalogy* **85:** 886–896.

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

Marshall JTJ. 1948. Ecologic races of Song Sparrows in the San Francisco Bay region. Part I. Habitat and abundance. *Condor* **50:** 193–215.

Martínez MM, Bó MS, Isacch JP. 1997. Hábitat y abundancia de *Porzana spiloptera* y *Coturnicops notata* en Mar Chiquita, Prov. de Buenos Aires, Argentina. *Hornero* **14:** 274–277.

McDonald DB, Potts WK. 1994. Cooperative display and relatedness among males in a lek-mating bird. *Science* **266:** 1030–1032.

Milá B, Smith TB, Wayne RK. 2006. Postglacial population expansion drives the evolution of long-distance migration in a songbird. *Evolution* **60:** 2403–2409.

Mitsch WJ, Gosselink JG. 2000. *Wetlands*. New York, NY: Wiley.

Murray BG Jr. 1969. A comparative study of the Le Conte's and Sharp-tailed sparrows. *Auk* **86:** 199–231.

Nei M. 1987. *Molecular evolutionary genetics*. New York, NY: Columbia University Press.

Peele AM, Burtt JEH, Schroeder MR, Greenberg RS. 2009. Dark color of the Coastal Plain Swamp Sparrow (*Melospiza georgiana nigrescens*) may be an evolutionary response to occurrence and abundance of salt-tolerant feather-degrading bacilli in its plumage. *Auk* **126:** 531–535.

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

Price TD, Qvarnström A, Irwin DE. 2003. The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society B: Biological Sciences* **270:** 1433–1440.

Ramos-Onsins SE, Rozas J. 2002. Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution* **19:** 2092–2100.

Reinert SE. 2006. Avian nesting response to tidalmarsh flooding: literature review and a case for adaptation in the red-winged blackbird. *Studies in Avian Biology* **32:** 77–95.

Rice WR. 1989. Analyzing tables of statistical tests. *Evolution* **43:** 223–225.

Rogers AR, Harpending H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution* **9:** 552–569.

Rozas J, Sánchez-Del Barrio JC, Messeguer X, Rozas R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* **19:** 2496–2497.

Ruegg KC, Hijmans RJ, Moritz C. 2006. Climate change and the origin of migratory pathways in the Swainson's thrush, *Catharus ustulatus*. *Journal of Biogeography* **33:** 1172–1182.

Schluter D. 2000. *The ecology of adaptive radiation*. Oxford: Oxford University Press.

Scott SN, Clegg SM, Blomberg SP, Kikkawa J, Owens IPF. 2003. Morphological shifts in island-dwelling birds: the role of generalist foraging and niche expansion. *Evolution* **57:** 2147–2156.

Smith TB. 1987. Bill size polymorphism and intraspecific niche utilization in an African finch. *Nature* **329:** 717–719.

Smith TB, Wayne RK. 1996. *Molecular genetic approaches in conservation*. New York, NY: Oxford University Press.

Soriano A, León RJC, Sala OE, Lavado RS, Deregibus VA, Cauhépé MA, Scaglia OA, Velázquez CA, Lemcoff JH. 1991. Río de la Plata Grasslands. In: Coupland RT, ed. *Ecosystems of the world, 8A. Natural grasslands, introduction and western hemisphere*. New York, NY: Elsevier, 367–407.

Stearns SC. 1989. The evolutionary significance of phenotypic plasticity. *BioScience* **39:** 436–445.

Taylor PB. 1996. Family Rallidae. In: del Hoyo J, Elliot A, Sargatal J, eds. *Handbook of the birds of the world, Vol 3. Hoatzin to Auks*. Barcelona: Lynx Edicions, 108–209.

Templeton AR. 1998. Nested clade analysis of phylographic data: testing hypothesis about gene flow and population history. *Molecular Ecology* **7:** 381–397.

Violante RA, Parker G. 2004. The post-last glacial maximum transgression in the de la Plata River and adjacent inner continental shelf, Argentina. *Quaternary International* **114:** 167–181.

Von Bloeker JC Jr. 1932. Three new mammal species from salt marsh areas in southern California. *Proceedings of the Biological Society of Washington* **45:** 131–138.

Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38:** 1358–1370.

Wood M. 1995. *Environmental soil biology*. London: Blackie Academic and Professional.

Zar JH. 1999. *Biostatistical analysis*. Englewood Cliff, NJ: Prentice-Hall, Inc.