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POPULATION GENETICS OF A RECENT TRANSCONTINENTAL COLONIZATION OF SOUTH AMERICA BY BREEDING BARN SWALLOWS (*HIRUNDO RUSTICA*)

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Abstract.—The natural range extension and colonization of a new continent by a bird species is rare, and even more rarely is it observed and documented. In 1980, six pairs of Barn Swallows were found breeding in Argentina within the species' historical wintering range, and this South American population has since grown to thousands of pairs. We explored the genetic context and consequences of this natural trans-hemispheric colonization event via comparisons among the South American population and two North American populations. We tested for evidence of a genetic founder event by assessing allelic diversity at eight microsatellite loci and haplotype diversity of mitochondrial ND2 sequences. Contrary to our expectations, the recently established South American breeding population showed no evidence of a founder effect, with no difference in heterozygosity, allelic diversity, haplotype diversity, or population differentiation in comparison to the large North American populations. The genetic similarity of these populations suggests that this long-distance colonization event was not associated with a strong demographic bottleneck, perhaps because the South American population has been augmented by ongoing immigration from North America. *Received 3 November 2010, accepted 3 May 2011.*

Key words: Barn Swallow, colonization, founder event, *Hirundo rustica*, microsatellite, population bottleneck.

Genética Poblacional de una Colonización Transcontinental Reciente de Sur América por una Población Reproductiva de *Hirundo rustica*

Resumen.—La extensión natural de la distribución geográfica y la colonización de un nuevo continente por parte de una especie de ave son eventos raros, que rara vez son observados y documentados. En 1980, seis parejas de *Hirundo rustica* se encontraron reproduciéndose en Argentina, dentro del área de distribución invernal histórica de la especie. Desde entonces, esta población suramericana ha crecido hasta alcanzar miles de parejas. Desde un punto de vista genético, exploramos el contexto y las consecuencias de esta colonización natural trans-hemisférica mediante comparaciones entre la población suramericana y dos poblaciones norteamericanas. Evaluamos la evidencia genética de un evento fundador examinando la diversidad alélica en ocho loci microsatélites y la diversidad de haplotipos en secuencias del gen mitocondrial ND2. De modo contrario a lo esperado, la población reproductiva recientemente establecida en Sur América no presentó evidencias de un evento fundador, pues no existieron diferencias en heterocigocidad, diversidad alélica ni diversidad haplotípica, ni diferenciación poblacional en relación con las poblaciones norteamericanas grandes. La similitud genética de las poblaciones sugiere que este evento de colonización a gran distancia no estuvo asociado con un cuello de botella demográfico fuerte, quizás porque la población suramericana ha sido suplementada por inmigración continuada desde Norte América.

Long-distance colonization events, although rare, can have important consequences for the distribution and diversification of populations and species (Carson and Templeton 1984, Grant et al. 2001). In most cases, new populations founded via long-distance colonization are small and may therefore be subject to substantial genetic drift. These types of founding events have a venerable intellectual place in evolutionary theory. For example, as part of his articulation of the Modern Synthesis, Mayr (1954) suggested that a founding population, when sufficiently small, may undergo a cascade of genetic changes that foster the population's rapid differentiation. In addition to rapid differentiation, genetic drift associated with founder events could lead to a reduction in genetic variability, as well as an accumulation of inbreeding effects and increased levels of homozygosity in the colonizing population (Templeton 1980, Barton and Charlesworth 1984, Carson and Templeton 1984).

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Because patterns of genetic variation can change rapidly during and just after a founder event (Templeton 1980), understanding how colonization events affect patterns of genetic variation requires studies of ongoing or recent colonization events. Perhaps because the earliest stages of population colonization have only rarely been fully documented and sampled, there are surprisingly few studies of how natural colonizations affect genetic variation in animals (e.g., Tarr et al. 1998, Rasner et al. 2004, Hawley et al. 2006, Pruett et al. 2005, Baker et al. 2008). One of the most comprehensive examples involves birds of the Silvereye species complex (*Zosterops lateralis*) that have colonized multiple islands off Australia and New Zealand (Clegg et al. 2002, Estoup and Clegg 2003). In that Silvereye system, genetic bottlenecks at microsatellite loci are most evident after the sequential stepping-stone colonization of multiple islands. By contrast, single-step founder events have a much smaller effect on allelic diversity. This pattern has suggested that colonization events that result from single founder events may have little effect on the diversity and divergence of neutral genetic markers (Clegg et al. 2002). The same effects of sequential colonizations have also been observed in reintroduced populations of the endangered New Zealand Saddleback (*Philesturnus carunculatus rufusater*), where populations have been successively introduced to new islands (Lambert et al. 2005).

In another example of the effects of sequential founder effects, Abdelkrim et al. (2005) studied Ship Rats (*Rattus rattus*) on the Guadeloupe Islands of the West Indies. Historical eradication attempts had caused a well-documented population bottleneck, which was detectable using microsatellite markers. Two distinct bottlenecks were discernible in this Ship Rat population: an older population bottleneck, which could be attributed to the original colonization event in the 1700s, and a more recent bottleneck attributed to the eradication attempts that occurred around 2001.

Larger single-event founder effects have been documented in other avian systems. For example, a small number of captive House Finches (*Carpodacus mexicanus*) were introduced to the eastern United States in the 1940s, and the population subsequently expanded rapidly. These birds became abundant throughout much of eastern North America by 1990 (Hawley et al. 2006). Despite its large census population size, the introduced House Finch population now has substantially lower diversity than the native population at both microsatellite and mitochondrial DNA (mtDNA) loci, probably as a result of the small and highly male-biased founding population (Hawley et al. 2006, 2008). Other studies of founder events and population bottlenecks in avian populations have addressed reintroduced populations of endangered species (Tarr et al. 1998, Lambert et al. 2005). The Laysan Finch (*Telespiza cantans*) has been introduced to several small islands as part of a recovery effort, and Tarr et al. (1998) found that those populations with the smallest number of founders and the slowest post-introduction population growth had significantly reduced allelic diversity and heterozygosity when compared with the source population.

In general, the effect of a founder event on levels of allelic diversity will depend on the size and composition of the founding population and the duration of the associated demographic bottleneck (Nei et al. 1975, Hoelzel 1999, Eales et al. 2008). In addition, the genetic signature of a founder event may change after the initial event because of subsequent gene flow between the original and the new population. For example, a population of Purple Martins (*Progne subis*) in British Columbia has retained high genetic diversity despite severe population declines, likely (at least in part) because of the immigration of individuals from larger populations (Baker et al. 2008). Similarly, a recent study of a Caribbean *Anolis* lizard has illustrated how limited genetic structuring due to high gene flow increases the likelihood that even a small founding population will retain a relatively high degree of genetic diversity (Eales et al. 2008).

Here, we explore patterns of genetic variation in North and South American breeding populations of Barn Swallows (*Hirundo rustica erythrogaster*). Most Barn Swallows that breed in North America migrate to South America during the northern winter–austral summer, but this species did not historically breed south of northern Mexico (Brown and Brown 1999). In 1980, however, six pairs were discovered breeding in Argentina (Martínez 1983) (Fig. 1). We are confident that the Barn Swallow population had not been established in this area much earlier than 1980, given the highly visible nature of Barn Swallow colonies, the fact that they primarily build nests on human-constructed structures, and the active ornithological community in the area that it colonized. This austral breeding population has grown dramatically, and our observations put the current population size in the thousands of breeding pairs. In addition, the breeding sites in Argentina are separated from any currently known wintering population of Barn Swallows from North America. Phenotypic and genetic evidence indicate that the South American breeding population was derived from the North American population rather than from populations in Eurasia, providing us with the opportunity to compare the newly founded South American population with its source, and thereby explore the early genetic consequences of a natural long-distance colonization event by a migratory bird in concert with its associated behavioral and ecological shifts.

We used both microsatellite and mitochondrial DNA (mtDNA) markers to compare patterns of genetic diversity between North and South American breeding Barn Swallow populations. With an observed founding population of only a few pairs in the earliest documented breeding seasons (Martínez 1983), we expected to find evidence for a genetic founder event in the South American population, as evidenced by substantially reduced allelic diversity and reduced heterozygosities (Nei et al. 1975) at microsatellite loci, and similarly reduced haplotype diversities in mtDNA. However, the South American breeding population appears to have increased very rapidly after its founding, and there could also be ongoing gene flow in the form of recruitment of North American birds into the breeding South American population, both of which would act to reduce the genetic signature of a population bottleneck.

Methods

Sampling

We obtained genetic samples from the newly founded South American population in Buenos Aires Province, Argentina (Fig. 1), and from populations in central California and New York state. We chose these North American sampling sites because they span

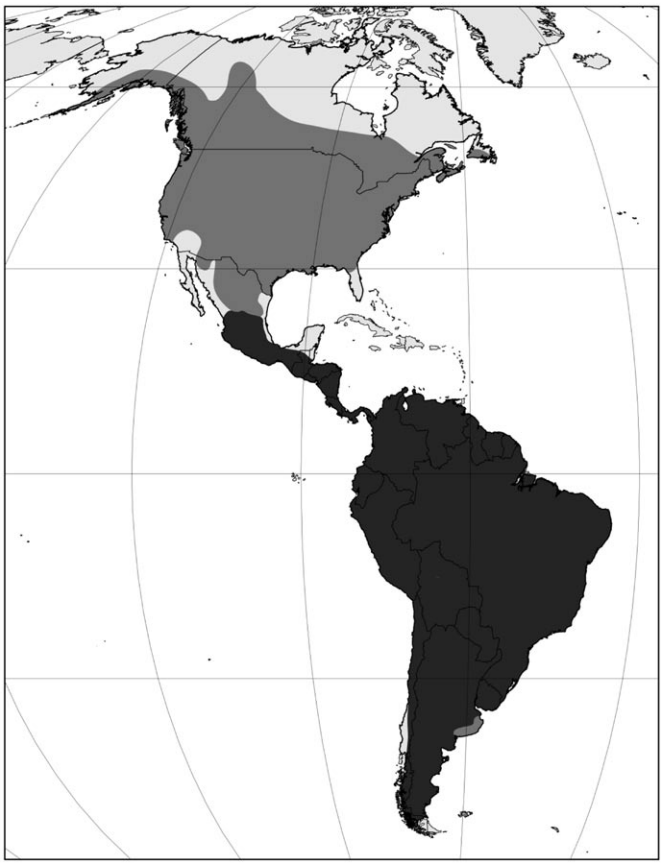

Fig. 1. Map of North America and South America, showing the complete range of Barn Swallows. Breeding ranges in North America and Argentina are shown in pale gray, and the non-breeding range in Mexico, Central America, and South America in dark gray. Map adapted from InfoNatura (NatureServe 2007).

the North American breeding range of this species and because the New York population has been previously monitored and investigated (Safran and McGraw 2004, Safran et al. 2005). Blood was collected from the brachial vein and immediately stored in Queens lysis buffer (Seutin et al. 1991) prior to processing for DNA extraction. All samples were collected during the breeding season, May–July in North America and November–January in Argentina. Although some samples were collected from nestlings, care was taken not to include siblings, or parents and offspring, in the analyses.

DNA Extraction and Amplification

Genomic DNA was extracted from blood samples using Perfect gDNA Blood Mini kits (Eppendorf) and stored at –80°C until needed. To survey allelic diversity, we genotyped each individual at eight polymorphic microsatellite loci (Table 1), five originally isolated from Barn Swallows (Tsyusko et al. 2007) and three isolated from other species (Hanotte et al. 1994, McDonald and Potts 1994, Bensch et al. 1997, Kleven et al. 2005). All eight loci were amplified via polymerase chain reaction (PCR) using a cycling protocol that included an initial denaturation step at 95°C for 5 min, followed by 34 cycles of 30 s at 95°C, a locus-specific annealing temperature (Table 1), and 30 s at 72°C, followed by a final extension step of 72°C for 4.5 min. Each 10-uL reaction contained 10–100 ng of genomic DNA, 10 mM Tris-HCl (ph 8), 50 mM KCl, 0.12uM forward and reverse primers, one of which was fluorescently labeled at the 5' end with PET, 6-FAM, VIC, or NED (Applied Biosystems, Foster City, California), 3.25 mM $MgCl_2$, 0.2 mM of each nucleotide (Invitrogen), and 0.025 U of Taq Jumpstart DNA polymerase (Sigma-Aldrich, St. Louis, Missouri). Labeled PCR products were electrophoresed on an ABI 3100 Genetic Analyzer (Applied Biosystems), and allele sizes were estimated using GENEMAPPER, version 3.0 (Applied Biosystems).

Table 1. Primer sequences for eight polymorphic microsatellite markers in Barn Swallows, with their respective annealing temperatures (T_a), observed heterozygosity (H_O), expected heterozygosity (H_E), total number of alleles (K), and the allelic richness found among all populations.

			H_0			H_E				Allelic richness		
Locus	Primer sequence (5'→3')	T_a	NY	CA	ARG	NY	CA	ARG	K	NY	CA	ARG
Escμ6[1]	F: CATAGTGATGCCCTGCTAGG R:GCAAGTGCTCCTTAATATTTGG	50	0.887	0.913	0.811	0.877	0.897	0.87	16	11.29	13	10.13
Ltr6[2]	F: GCCATGCCACAGGAGTGAGTC R: AGTCATCTCCATCAAGGGCAT	50	0.528	0.565	0.676	0.589	0.654	0.660	7	5.09	5	5.29
POCC6[3]	F:TCACCCTCAAAAACACACACA R:ACTTCTCTCTGAAAAGGGGAGC	50	0.808	0.696	0.851	0.862	0.874	0.846	14	10.43	9	10.77
Hir 6[4]	F: GACGGCCTGGGGGTAGA R: AAGAGCATGACCACCAGAGAT	50	0.84	0.87	0.781	0.833	0.882	0.818	14	8.07	10	9.04
Hir 11[4]	F: AACACCTGAAAACCTACAC R: CTTTGAGCAAAATGAGTG	58	0.673	0.826	0.581	0.822	0.829	0.784	9	8.05	8	7.27
Hir 17[4]	F: ATGCCATGCTTCAGAT R: CTGTCATGCCTAAGTATCA	58	0.510	0.913	0.722	0.879	0.906	0.907	18	11.91	14	12.29
Hir 19[4]	F: GCTCACAACCAGCTAGAC R: ATAGCCACAGGGAAAGTCT	58	0.679	0.609	0.824	0.839	0.840	0.887	14	9.63	10	11.37
Hir 20[4]	F: GAAGTTGGAGAAAGATTAG R: TTATTGCTCTGGGTATGT	58	0.865	0.652	0.824	0.836	0.790	0.866	14	8.49	7	10

References for primers: [1]Hanotte et al. 1994, [2]McDonald and Potts 1994, [3]Bensch et al. 1997, [4]Tsyusko et al. 2007.

To assess mitochondrial haplotype variation, we sequenced the NADH dehyodrogenase subunit II gene (ND2; 1,023 base pairs). Each 10-uL PCR reaction contained 10–50 ng of genomic DNA, 10 mM Tris-HCl (pH 8), 50 mM KCl, 2.5 mM $MgCl_2$, 0.25 mM of each nucleotide, 0.25 mM of forward and reverse primers METB and TRPC (Eberhard and Bermingham 2004), and 0.025 U of Taq Jumpstart polymerase (Sigma-Aldrich). We followed lab protocols for sequencing reactions using BigDye Terminator Ready Reaction Cycle Sequencing (Applied Biosystems) as in Lovette and Rubenstein (2007) and obtained sequences using the Cornell University Life Sciences Core Laboratories Center facility. We used SEQUENCHER, version 4.5, to trim and align sequences. We also checked ND2 sequences for premature stop codons and other indications of nuclear pseudogene copies of this mitochondrial gene. Sequences were deposited in GenBank (accession nos. HQ333550–HQ333663).

Data Analysis

Measuring population genetic structure.—A combination of markers and analyses was applied to assess differences in population genetic structure among the three sampled populations of Barn Swallows. First, we used GENEPOP, version 1.2 (Raymond and Rousset 1995), to estimate population differentiation at the microsatellite loci, and to test for Hardy-Weinberg Equilibrium within and among populations. We used FSTAT, version 2.9.3.2 (Goudet 1995), to estimate pairwise population differentiation, population pairwise F_{ST} values, allelic richness, and the number of alleles found in each population per locus. We used GENETIX (Belkhir et al. 2004) to calculate the *P* values for the population pairwise F_{ST} values. We used GENALEX, version 6 (Peakall and Smouse 2006), to determine the number of private alleles in each of the three sampled populations. We used DNASP, version 5 (Librado and Rozas 2009), to determine basic nucleotide diversity and divergence within our mitochondrial sequence data as well as two measures of selection, Tajima's *D* and Fu's *Fs*.

To test for population structure between North and South American Barn Swallows, we used STRUCTURE, version 2.1 (Pritchard et al. 2000), employing a burn-in period of 100,000 with 500,000 Markov Chain Monte Carlo replicates after burn-in. We used an admixture model, with an initial ALPHA value of 1.0. We assumed that allele frequencies are correlated among populations, where different values were assumed for F_{ST}. The value of lambda was set at 1.0 and we tested values of *K* from 1 through 5, with 1 representing no population structure and 5 being the highest likely number of subpopulations we might expect to observe. Although a *K* of three populations might be expected given the three main areas we sampled, five is the maximum number based on the collection sites in South America, where we included three distinct collection localities separated by nearly 100 km. If Barn Swallows were not distributed evenly across the landscape in Argentina, it may be that population substructure could have developed there.

Testing for a founder event.—To determine whether the South American Barn Swallow breeding population had experienced a pronounced genetic founder event, we used BOTTLENECK, version 1.2.02 (Luikart and Cornuet 1999, Piry et al. 1999), to test for heterozygosity excess. In founder events, rare alleles are lost from the population more quickly than heterozygosity declines and, thus, populations that have recently experienced a bottleneck will tend to show an apparent heterozygosity excess (Nei et al. 1975, Luikart and Cornuet 1999, Piry et al. 1999). We ran the two-phase mutation model (TPM) in BOTTLENECK using two parameter sets: (1) a 95% stepwise mutation model (SMM) with a variance of 12 (Piry et al. 1999) and (2) a 0% SMM with a variance of 0.36. We also tested for a heterozygote excess under the IAM (independent alleles model) and the SMM because we could make no *a priori* assumptions as to the mutation model of our microsatellite markers. It has been shown that the IAM may better represent the mutation model of microsatellite loci with dimeric repeat motifs and those with imperfect repeat motifs (Hawley et al. 2006).

We used the program M (Garza and Williamson 2001) to calculate the ratio (*M*) of the number of alleles in each population to the total range in allele size for the microsatellite data. Rare alleles are lost randomly with respect to allele size during founder events, so the *M* ratio decreases with both the strength and duration of a population bottleneck (Garza and Williamson 2001). We used the standard protocols suggested by Garza and Williamson (2001), with a 90% proportion of one-step mutations, and an average size of non-one-step mutations of 3.5 bases. We assumed a large prebottleneck population size in the two North American populations (N_e = 5,000 and N_e = 10,000), which corresponds to values ($4N_e\mu$) of 10 and 20, respectively. We also ran the same tests with an 80% proportion of one-step mutations, because we thought that this value might better reflect the mutation mode of our microsatellite loci, given the distribution of allele sizes. The program runs 10,000 simulations to estimate the *M* ratio.

Relationships among mitochondrial haplotypes were estimated using the statistical parsimony approach in the program TCS, version 1.21 (Clement et al. 2000). We did not employ the nested-clade approach (Templeton et al. 1995) because of uneven sampling across populations and because no geographic clustering of haplotypes was found (Templeton 1998). We used ARLEQUIN, version 3.11 (Excoffier 2005), to calculate mismatch distributions among populations. We used the general settings of demographic and spatial expansion (Excoffier 2005), with an epsilon value of $1x10^{-7}$, a deletion, transition, and transversion weight each of 1, and 10,000 bootstrap replicates.

RESULTS

Measuring population genetic structure.—In total, we sampled 73 Barn Swallows from the Argentine population, 23 from California, and 53 from New York. Of these, all individuals were genotyped, and a total of 114 individuals (77% of those genotyped) were sequenced at the mitochondrial ND2 gene.

All eight microsatellite loci were polymorphic, with between 7 and 18 alleles per locus. Heterozygosity varied among loci but did not differ among populations (Table 1). Allelic richness also did not vary among populations (Fig. 2). Five of eight loci were in Hardy-Weinberg equilibrium (HWE) in all populations when corrected for multiple comparisons using a sequential Bonferroni procedure (Rice 1989). Deviations from HWE involved locus Hir11 in one subpopulation in Argentina, Hir19 in California, and Hir17 and Hir19 in New York. No single locus was consistently out of HWE, which indicates that these deviations are unlikely to have resulted from the presence of null alleles.

All three populations had private alleles. The Argentine population had the highest number, with 21 private alleles. The

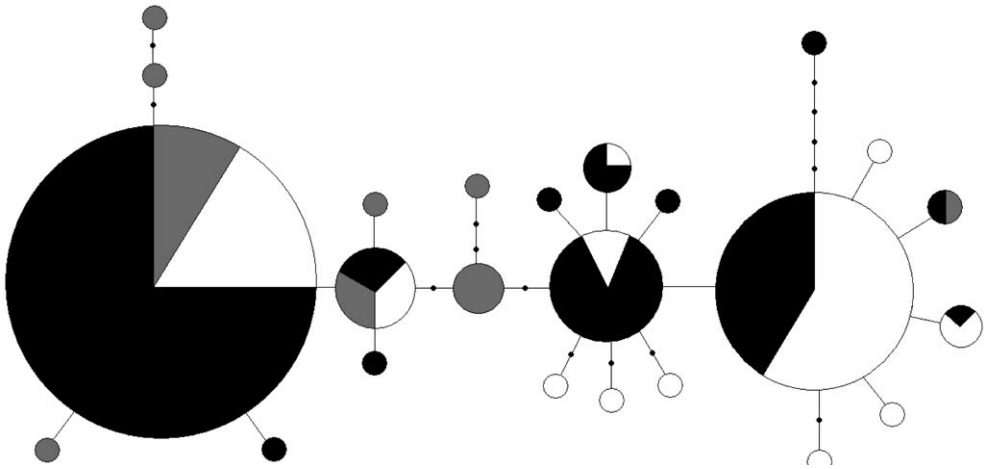

FIG. 2. Haplotype network for ND2 in three populations of Barn Swallows. Sizes of circles correspond to the numbers of individual birds with each haplotype (Argentina black, *n* = 63; California gray, *n* = 19; New York white, *n* = 32). Dots between haplotypes represent inferred (unsampled or ancestral) haplotypes.

TABLE 2. F_{ST} results among the three sampled populations for microsatellite data. Fu's *Fs* and Tajima's *D* are given for mitochondrial sequence data (asterisks indicate significance at $P \leq 0.05$).

	Argentina			California			Ithaca		
	F_{ST}	Tajima's *D*	Fu's *Fs*	F_{ST}	Tajima's *D*	Fu's *Fs*	F_{ST}	Tajima's *D*	Fu's *Fs*
Argentina		—		0.018*	−0.23	−0.69	0.011*	−0.23	−0.69
California		—			—		0.012*	−0.96	−2.99
Ithaca		—			—			—	

California population had 10, and the New York population of breeding Barn Swallows had 14 private alleles.

Between-population analyses revealed significant, but very low, genetic differentiation, with F_{ST} values for the microsatellite data ranging between 0.011 and 0.018 (Table 2). Tajima's *D* and Fu's *Fs* values for the mitochondrial sequence data were not significant (Table 2). Individuals within populations did not generally cluster together when assigned to populations using STRUCTURE, again showing very little population differentiation. Analyses using STRUCTURE identified that the model in which *K* = 1 was best supported, with a probability of 0.99, and were hence unable to detect any population structure among the three sampled groups. Models in which *K* was equal to 2 through 5 received support values of 0.01 or lower.

Testing for a founder event.—Within-population analyses using the program BOTTLENECK showed no evidence for a founder effect in any of the three populations, including the recently founded Argentine population, under either the stepwise mutation model (SMM) or the two-phase model (TPM) (Table 3). However, under the independent alleles model (IAM), all three pop ulations showed a significant heterozygote excess (Table 3). *M* ratio patterns were similar to those of the SMM and TPM results of BOTTLENECK, in that the Argentine breeding population (*M* ratio = 0.84) had a value intermediate between those of New York (0.90) and California (0.83). All of the *M* ratio values were significant at $P = 0.05$ when the effective population size (N_e) was assumed to be 10,000 and when the proportion of single-step mutations was 80%. In addition, *M* ratio values fell within expected values for stable populations (Garza and Williamson 2001) (Table 3).

TABLE 3. The *M* ratios and BOTTLENECK results of the three sampled populations of Barn Swallows when run with different parameters (see text), showing the one-tailed probability for a heterozygosity excess for each for the independent alleles model (IAM), the stepwise mutation model (SMM), and the two-phase model (TPM), using the parameters of 0% proportion SMM and 0.36 variance for the TPM. Values significant at $P \leq 0.05$ are reported, and "NS" denotes values that were not significant. $\theta = 4N_e\mu$, where N_e = effective population size, and μ = mutation rate.

		Argentina	California	New York
90% single-step mutations	$\theta = 10$	NS	NS	NS
	$\theta = 20$	NS	0.825	0.895
80% single-step mutations	$\theta = 10$	NS	0.825	0.895
	$\theta = 20$	0.840	0.825	0.895
IAM		0.002	0.002	0.002
SMM		NS	NS	NS
TPM		NS	NS	NS

We reconstructed the haplotype network of the complete ND2 gene using TCS. In total, we found 24 distinct haplotypes across all 3 sampled populations, with 4 haplotypes representing the majority of 106 individuals sampled. Five haplotypes were

Table 4. Results from ARLEQUIN, version 3.11 (Exoffier 2005), for mismatch distribution of the three populations of Barn Swallows sampled, and from DNASP (Librado and Rozas 2009) for haplotype diversity (H_d) and nucleotide diversity (P_i). Given here are the sum of the squared deviations and the Harpendings raggedness index; no values were significant at $P = 0.05$.

Population	Sum of the squared deviations	H_d	P_i	Harpendings raggedness index
Argentina	0.07554381	0.73169	0.00373	0.10691839
California	0.00477155	0.85965	0.00321	0.01824458
New York	0.03048844	0.81653	0.00341	0.05458995

unique to Argentina, six haplotypes were unique to New York, and six haplotypes were unique to California (Fig. 2). These unique haplotypes represent a small proportion of those within each population, considering that 26 individuals shared a haplotype with individuals from another population.

On the basis of haplotype mismatch distributions, we were unable to reject the null hypothesis of population growth in any of the three populations of Barn Swallows. Neither the sum of the squared deviations nor the Harpendings raggedness index had significant value at $P = 0.05$ (Table 4).

Discussion

Our study of the South American breeding population of Barn Swallows represents the first genetic investigation of this founding population in Argentina since its formal description in 1983 (Martínez 1983). Populations like this represent unique opportunities to study the genetic effects of natural colonization events. Contrary to our *a priori* expectations, our analyses of microsatellite and mtDNA data revealed no evidence for a founder effect or population bottleneck in the South American breeding population. There are several potential, non-exclusive reasons for this absence of a genetic signature of a bottleneck in this population.

Theory predicts that genetic drift may be minimal when a recently colonized population grows rapidly (Nei et al. 1975, Hoelzel 1999). For example, Eales et al. (2008) suggested that rapid population growth in a recently founded population of a Caribbean *Anolis* was one of several factors that caused that population to maintain high levels of genetic diversity. This could similarly be true for the austral-breeding population of Barn Swallows, which went from six breeding pairs observed in 1980 (Martínez 1983) to the thousands of pairs found today.

Alternatively, any early genetic signatures of a bottleneck in South America could have been erased by ongoing gene flow from the North American population. This scenario is supported by our observations in November 2008 and 2010 (G. H. Huber and D. W. Winkler unpubl. data) of several hatch-year birds in full body molt at the breeding colonies in Argentina. Because the Argentine Barn Swallows had not yet fledged any young at that time, and because Barn Swallows typically molt on the nonbreeding grounds, these molting young birds were almost certainly hatched in North America earlier that year. Given that most North American birds appear to winter at least 1,000 km north of the Argentine breeding population (G. H. Huber pers. obs.), these birds likely represent migrants that overshot their destination and settled into the breeding colonies. Because Barn Swallows are colonial breeders, the presence of many breeding birds may stimulate these migrant overshoots to come into breeding condition sooner or to adopt an austral breeding cycle and, hence, contribute to intercontinental gene flow. This pattern may also explain the presence of Cliff Swallow (*Petrochelidon pyrrhonota*) nests at several Barn Swallow colonies in Argentina (Petracci and Delhey 2004). Like the Barn Swallow, the Cliff Swallow is a colonial species that breeds in North America and migrates as far south as Chile and Argentina but is not otherwise known to breed in the southern hemisphere.

To further investigate the potential for ongoing gene flow from North America to Argentina, we also analyzed our mtDNA data using Isolation with Migration (IMa). However, we were unable to let the program run long enough so that independent runs using different random starting seeds converged. Despite the fact that these values did not converge, they were consistent in showing that there was much higher migration from the two North American populations to the Argentina population than from Argentina to either North American population. This is highly suggestive that there is gene flow (ongoing or recent historical) from North American Barn Swallows to South American Barn Swallows.

Microsatellite markers have long been one of the primary tools for research on the genetic structure of wild populations of organisms, specifically for looking at recent demographic changes in populations (e.g., Tarr et al. 1998, Spencer et al. 2000, Clegg et al. 2002, Abdelkrim et al. 2005, Lambert et al. 2005, Hawley et al. 2006, Eales et al. 2008). However, other markers, including mtDNA sequences, can be useful for looking at population genetic patterns (Baker et al. 2008, Hawley et al. 2008). Sequences from mtDNA may be particularly powerful for detecting recent population bottlenecks because of its four-fold smaller effective population size in relation to autosomal nuclear markers. For example, Hawley et al. (2008) found that the demographic bottleneck they observed with mtDNA sequence data was nearly twice that observed in the microsatellite data for the same House Finch populations. By contrast, in the Argentine breeding population of Barn Swallows, both microsatellite and mtDNA sequence data showed a similar lack of evidence for any reduction in genetic diversity that might have been related to a founder event. Comparisons of divergence using microsatellite data and mitochondrial sequence data were different because values for the mtDNA were not significant. However, this discordance between marker classes could be due to differences in dispersal rates between males and females. Because mtDNA is maternally inherited, the expected higher dispersal rates in females (Clarke et al. 1997, Winkler et al. 2005) would lead to a much weaker signal of divergence in the ND2 sequences compared to the microsatellite loci.

The mitochondrial haplotype data can provide some further insights into the minimum size of the founder Barn Swallow population. If the founder population was indeed the six pairs observed in 1980, then there should be no more than six mitochondrial haplotypes (and probably fewer, given the high frequency of the most common haplotypes in the North American samples). However, we found a total of 12 haplotypes in the South American breeding population, and with further sampling would likely have discovered more. Additionally, we found that the haplotypes most common in North America were also most common in Argentina,

which suggests that gene flow has kept these populations generally similar in their haplotype frequencies. Considered together, these patterns suggest that substantially more than 12 immigrant females contributed to the South American population during or after its founding.

Although the BOTTLENECK results are in conflict depending on the mutation model used, we are confident that they provide no robust evidence of a population bottleneck. A founder event was supported only in the analyses that used the IAM, but it has been shown that this model does not properly characterize the nature of microsatellite mutation (Hawley et al. 2006) and that it will identify bottlenecks in populations that have not experienced them (Luikart and Cornuet 1998, Eales et al. 2008).

An alternative explanation for the absence of any population bottleneck could be poor detectability of the Argentine Barn Swallows. It is possible, though unlikely, that Barn Swallows were breeding in Argentina well before 1980 and that they had a much larger founding population that went unobserved. This scenario is unlikely for this highly visible and human-associated species, which nests beneath major roadways in the most densely settled and intensely observed regions of Argentina, where there has been a very active community of field ornithologists for more than five decades.

Information on private alleles also provides evidence against a population bottleneck. Given that rare alleles are lost during a founder event, the presence of a high number of private alleles in the Argentina population compared with both North American populations argues against strong genetic drift in the south.

In summary, there are several potential explanations for the lack of any observable genetic bottleneck in the South American Barn Swallow population. It seems likely that this population has received, and may continue to receive, substantial gene flow from birds of North American origin. This past and potentially continuing gene flow may have had the strongest effect on the population genetics of the Argentina Barn Swallows, but other factors, such as rapid population growth, may have also been important in this population. Thus, we conclude that these immigrants have repeatedly shifted their breeding and molt cycles by 6 months to adopt an austral breeding pattern. The behavioral and ecological plasticity exhibited by these Barn Swallows echoes their flexibility in migratory behavior (Winkler 2006), and it is intriguing in the context of other systems (Sutherland 1998) in which long-distance colonization events have required substantial shifts in the timing of reproduction or other behavioral changes in response to the different environmental conditions at the new breeding site.

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