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INVASION GENETICS: THE BAKER AND STEBBINS LEGACY

Shared genetic diversity across the global invasive range of the monk parakeet suggests a common restricted geographic origin and the possibility of convergent selection

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Abstract

While genetic diversity is hypothesized to be an important factor explaining invasion success, there is no consensus yet on how variation in source populations or demographic processes affects invasiveness. We used mitochondrial DNA haplotypic and microsatellite genotypic data to investigate levels of genetic variation and reconstruct the history of replicate invasions on three continents in a globally invasive bird, the monk parakeet (*Myiopsitta monachus*). We evaluated whether genetic diversity at invasive sites could be explained by (i) the native source populations from which they were derived and (ii) demographic bottlenecks during introduction. Genetic data indicated a localized source area for most sampled invasive populations, with limited evidence for admixing of native source populations. This pattern largely coincides with historical data on pet trade exports. However, the invasive populations are genetically more similar than predicted from the export data alone. The extent of bottleneck effects varied among invasive populations. The observed low genetic diversity, evidence of demographic contraction and restricted source area do not support the hypothesis that invasion is favoured by the mixing and recombining of genetic variation from multiple source populations. Instead, they suggest that reduced genetic variation through random processes may not inhibit successful establishment and invasion in this species. However, convergent selection across invasive sites could also explain the observed patterns of reduction and similarity in genetic variation and/or the restricted source area. In general, the alternative explanation of intraspecific variation in invasive potential among genotypes or geographic areas is neglected, but warrants more attention as it could inform comparative studies and management of biological invaders.

Keywords: bottleneck, founder effect, invasion genetics, native origin, population genetic structure, selection

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Introduction

Biological invasions are a major component of global change, with potentially large detrimental effects on public health, agriculture and biodiversity (Mack *et al.* 2000; Sakai *et al.* 2001; Simberloff *et al.* 2013). Identifying the biological attributes of successful invaders is among the most pressing questions still to be answered (Kolar & Lodge 2001; Lockwood *et al.* 2007). Some research has focused on the genetic variability of initial founder populations as a key predictor of invasion success. High genetic variability could increase the establishment success if it increased the likelihood that some individuals possessed genetic variants more suited to the new environment (Lee 2002; Kolbe *et al.* 2004; Facon *et al.* 2006, 2008; Lavergne & Molofsky 2007; Roman & Darling 2007; Suarez & Tsutsui 2008). Invasive populations may have high genetic variability if a large number of individuals are introduced or if individuals stem from multiple genetically differentiated native source populations.

Yet, previous studies have uncovered a broad range of patterns regarding the relationship between genetic diversity and invasion success: invasive populations can stem from both single and multiple native sources and can have higher or lower genetic diversity relative to native populations (reviewed in Novak & Mack 2005; Wares *et al.* 2005; Roman & Darling 2007). Because of this lack of consistency, there is no consensus on whether invaders stemming from multiple native origins are more successful than those from single populations or whether demographic bottlenecks may limit a species' invasion success.

Understanding the historical context of an invasion could provide important insights into the role of genetic variability in invasion success. By comparing genetic variability in native and invasive populations, it is possible to deduce the demographic and evolutionary changes (including genetic drift and selection) that shaped the introduced population (Fonseca *et al.* 2000; Dlugosch & Parker 2008). However, inferring processes underlying successful invasion remains analytically challenging, largely because of a lack of information about invasion history (Estoup & Guillemaud 2010). This lack of historical context could lead to errors in the identification of the sources of invasive populations, which are expected to be more likely when populations are minimally structured in their native range or if sampling in the native area has been incomplete or inappropriate. Furthermore, genetic divergence between native and invasive populations may occur rapidly during the invasion process (e.g. through drift or selection) such that divergence might confound inference of the source population(s) (Estoup & Guillemaud 2010). To understand the interaction between genetic diversity and invasive potential, it is critical to obtain information on population genetic structure and composition from both native and invasive ranges, and with a sufficient geographic coverage to track most of the genetic diversity potentially sampled during the invasion process.

Birds probably constitute the best studied taxa to identify life history traits associated with invasion success, given the well-recorded and deliberate worldwide introductions of hundreds of species (e.g. Blackburn *et al.* 2009; Sol *et al.* 2012). However, very little is known regarding the genetic processes linked to successful establishment of exotic bird species (Blackburn *et al.* 2009). One of the most notorious and widespread orders of invasive birds are parrots (Psittaciformes; Blackburn *et al.* 2009). We focus here on the monk parakeet (*Myiopsitta monachus*), a successful invader with a native range restricted to southern South America and with invasive populations occurring worldwide (Lever 2005; Fig. 1). In contrast to past deliberate introductions, these invasions were formed as an unintentional by-product of the pet trade. Millions of wild-caught parakeets have been transported from their native range to pet shops and homes across the globe, and a number of mostly accidental escapes or small-scale releases resulted in the establishment of new populations (Carrete & Tella 2008; Russello *et al.* 2008).

Previous studies have focused on determining the geographic origins and source populations for invasive monk parakeets. An analysis comparing mitochondrial DNA (mtDNA) control region sequences between invasive populations in the United States of America (USA) and native populations in South America concluded that the source for USA invasive populations is likely in the northern region of Argentina, but that unsampled populations may have also contributed to the invasion (Russello *et al.* 2008). Although mtDNA is useful in detecting the historical origin(s) of an invasion in cases where there is sufficient geographic structure in the native range, it provides limited power to infer demographic and genetic processes during and after invasion. A subsequent study based on hypervariable microsatellite loci revealed that high propagule pressure and long-range dispersal in the invasive range probably contributed to monk parakeet invasion success in the USA (Gonçalves da Silva *et al.* 2010). It remains unknown whether inferences from the USA populations apply to invasive populations elsewhere in the world, or, alternatively, whether these invasive populations have distinct invasion histories.

In this study, we aim to unravel the global invasion history of the monk parakeet, in terms of both geographic origins and demographic processes. We combined the mtDNA haplotype and nuclear microsatellite

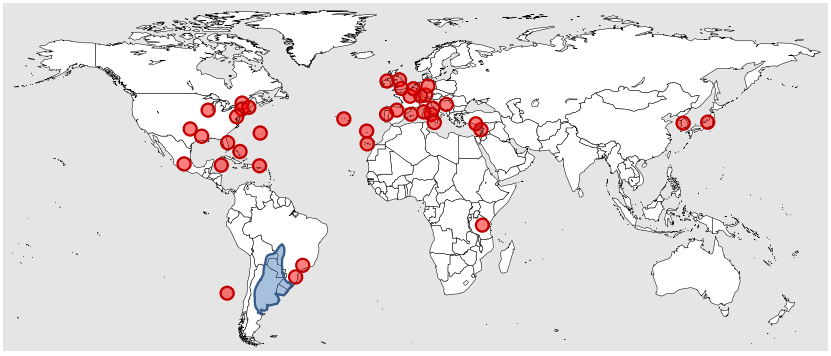

Fig. 1 Native range (blue, approximate) and established invasive populations (red, nonexhaustive, including some oceanic islands) of the monk parakeet.

data previously collected from populations in the native range in South America and the invasive range in the USA (Russello *et al.* 2008; Gonçalves da Silva *et al.* 2010) with newly collected data from a broadly expanded sampling of the native range (including the previously unsampled southern portion) and that of invasive populations from two other continents (Europe and Africa). Our goal was to evaluate whether genetic variation observed in established invasive populations could be explained by (i) the number, identity and characteristics of native source populations from which invasive populations were derived or by (ii) effects of demographic bottlenecks during the introduction. We also explore whether invasion histories differ between North America and Europe. Additionally, we compare the results obtained by our genetic approach with detailed spatiotemporal historical records on the monk parakeet pet trade. We place our results in the context of the role that genetic diversity may play in promoting invasion success. Finally, we discuss the extent to which natural selection might have influenced genetic variation and patterns in our putative neutral markers, and the potential importance of selection within the context of invasive species biology.

Materials and methods

The first published records of escaped monk parakeets in Spain are from 1975, when the species established in Barcelona (Batllori & Nos 1985), followed by the establishment on Canary Islands (Tenerife) in 1980, Madrid in 1985, Mallorca in 1986 and Zaragoza in 1991 (M. Carrete, J.D. Anadon, J.L. Tella, unpubl. data). In the USA, the first records of established populations are from the 1960s, with separate populations becoming established in Florida in 1969 (Owre 1973), New Jersey in 1970 (Neidermeyer & Hickey 1977) and Connecticut in 1973 (Olivieri & Pearson 1992). However, the data from the long-term annual Audubon Christmas Bird Count (CBC, http://netapp.audubon.org/cbcobservation/) indicate that initial populations in New Jersey and Connecticut may have gone extinct or nearly so and were subsequently augmented or reestablished in the late 1980s/early 1990s. All these dates of establishment should be viewed in the context of the life history of the species: we estimate life expectancy of full-grown parakeets to be about five years based on survival rates (Conroy & Senar 2009), whereas young birds are nearly two years old when they first reproduce (Martín & Bucher 1993). Historical records suggest that all of these introductions were independent of each other, although all had their original source in animals moved from South America by the pet trade. Likewise, there are no indications of exchange or transfer among different sites within either Spain or the USA, or between continents as reported by the CITES Trade Data Base (www.cites.org).

Sampling

Samples were collected at 22 sites: 14 in the native range in South America, four in the invasive range in Europe (Spain), one from an African island and three in the invasive range in North America (USA) (Table 1). In Spain, we also sampled recently imported wild-caught birds provided by three pet shops/pet owners (Pet Shops). This sample can be considered a rare sampling of an invader during the transport stage of the invasion process, prior to potential introduction into the novel range. Sampling locations are further specified in Table 1 and Fig. 2, and additional information on the USA samples and several South American samples can be found in Russello *et al.* (2008) and Gonçalves da Silva *et al.* (2010). Newly collected blood samples from wild individuals were collected by venipuncture and preserved in ethanol before extraction. DNA isolation followed standard phenol–chloroform extraction protocols (Sambrook *et al.* 1989) or Qiaquick DNEasy DNA extraction kits (Qiagen). For museum samples from Boquerón, Paraguay (collection of Estación Biológica de Doñana-CSIC, Spain, collected in the 1960s), DNA isolation was carried out in a laboratory free from PCR

Table 1 Overview of populations (full name, country and abbreviation) sampled from the invasive and native ranges

		Nuclear microsatellites			MtDNA haplotypes		
Population	Abbreviation	*n*	H_E	AR	*n*	H_D	Year
Connecticut (USA)	CNCT	19	0.58	2.93	9	0.00	1973/1985?
New Jersey (USA)	NWJY	*NA*	*NA*	*NA*	11	0.55	1970/1990s?
Florida (USA)	FLRD	91	0.63	3.26	43	0.54	1969
Zaragoza (Spain)	ZRGZ	21	0.51	2.55	20	0.00	1991
Madrid (Spain)	MADR	23	0.64	3.47	28	0.27	1985
Barcelona (Spain)	BARC	102	0.61	3.16	91	0.31	1975
Mallorca (Spain)	MALL	40	0.63	3.25	9	0.42	1986
Canary Islands (Spain)	CANR	28	0.65	3.53	21	0.66	1980
Pet Shops (Spain)	PETS	8	0.58	3.20	8	0.71	—
Mato Grosso (Brazil)	MTGS	*NA*	*NA*	*NA*	5	0.90	—
Tucumán (Argentina)	TUCU	*NA*	*NA*	*NA*	5	0.00	—
Concepción (Paraguay)	CCEP	*NA*	*NA*	*NA*	11	0.55	—
Santiago del Estero (Argentina)	SEST	*NA*	*NA*	*NA*	5	1.00	—
Boquerón (Paraguay)	BOQR	7	0.69	4.24	9	0.69	—
Corrientes (Argentina)	CRRT	*NA*	*NA*	*NA*	13	0.73	—
Entre Ríos (Argentina)	ENRS	49	0.70	3.80	37	0.83	—
Rio Grande do Sul (Brazil)	RGSL	*NA*	*NA*	*NA*	6	0.53	—
Algarrobo (Argentina)	ALGA	20	0.57	3.08	10	0.47	—
General San MartÍn (Argentina)	SMRT	11	0.56	3.23	12	0.41	—
Buenos Aires (Argentina)	BAIR	19	0.57	3.22	12	0.30	—
Parque Luro (Argentina)	LURO	43	0.58	3.09	9	0.50	—
General Rondeau (Argentina)	RDEA	19	0.57	3.16	10	0.53	—
Mayor Buratovich (Argentina)	BURT	9	0.62	3.53	10	0.47	—

n, number of individuals sampled; H_E, unbiased expected heterozygosity; AR, rarefied allelic richness; H_D, haplotype diversity; Year, approximate year of introduction based on published observations of first continued presence of monk parakeets at the locality.

products and especially designated for museum samples. For these last samples, four independent PCR replicates were performed for both mitochondrial and microsatellite markers.

Mitochondrial DNA

We amplified and sequenced a 439-bp fragment of the control region for all 23 populations following Russello *et al.* (2008) and Eberhard *et al.* (2001). Polymerase chain reaction (PCR) amplification and cycling conditions were as follows: denaturation for 2 min at 94 °C, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s and an extension at 72 °C for 90 s. PCRs consisted of 4 μL of DNA extract (40–60 ng of DNA) in a final volume of 20 μL, containing 1.5 mM MgCl2, 0.25 mM dNTPs, 2 pmol each primer and 0.5 unit of *Taq* polymerase (Bioline). Amplified products were sequenced on an automated sequencer (ABI 3100, Applied Biosystems, Foster City, CA). Sequence data were edited and aligned in SEQUENCHER 4.5 (Gene Codes Corporation, Ann Arbor, MI) and Bioedit (Hall 1999) and manually checked. Sequences were aligned with previously published sequences in GenBank (Russello *et al.* 2008) to determine haplotype identity. Haplotype diversity (H_D) was calculated after Nei & Tajima (1981).

Nuclear microsatellites

A total of seven microsatellite markers developed by Russello *et al.* (2007) were used in this study and analysed in 16 populations (Table 1). PCRs were carried out in 25 μL using 12.5 μL of QIAGEN multiplex PCR master mix, 6 μL of RNase-free water (provided with the QIAGEN master mix), 2.5 μL of primers mix (4 μL of each primer at a final concentration of 2 μM) and 4 μL of DNA template (40–60 ng of DNA). Cycling parameters were as follows: 5 min at 95 °C and 30 s at 95 °C, 90 s at 55 °C, 30 s at 72 °C repeated 32 times followed by 30 min at 60 °C. PCR products were run on 1.5% agarose gels and a posteriori on an ABI3100 DNA analyser to determine DNA sizes. GENEMAPPER v1.90 (SoftGenetics LLC®) was used to score alleles and genotypes. Allele assignments were calibrated using samples of one population analysed in both laboratories.

Departures from linkage equilibrium and Hardy–Weinberg equilibrium (HWE) were tested using exact

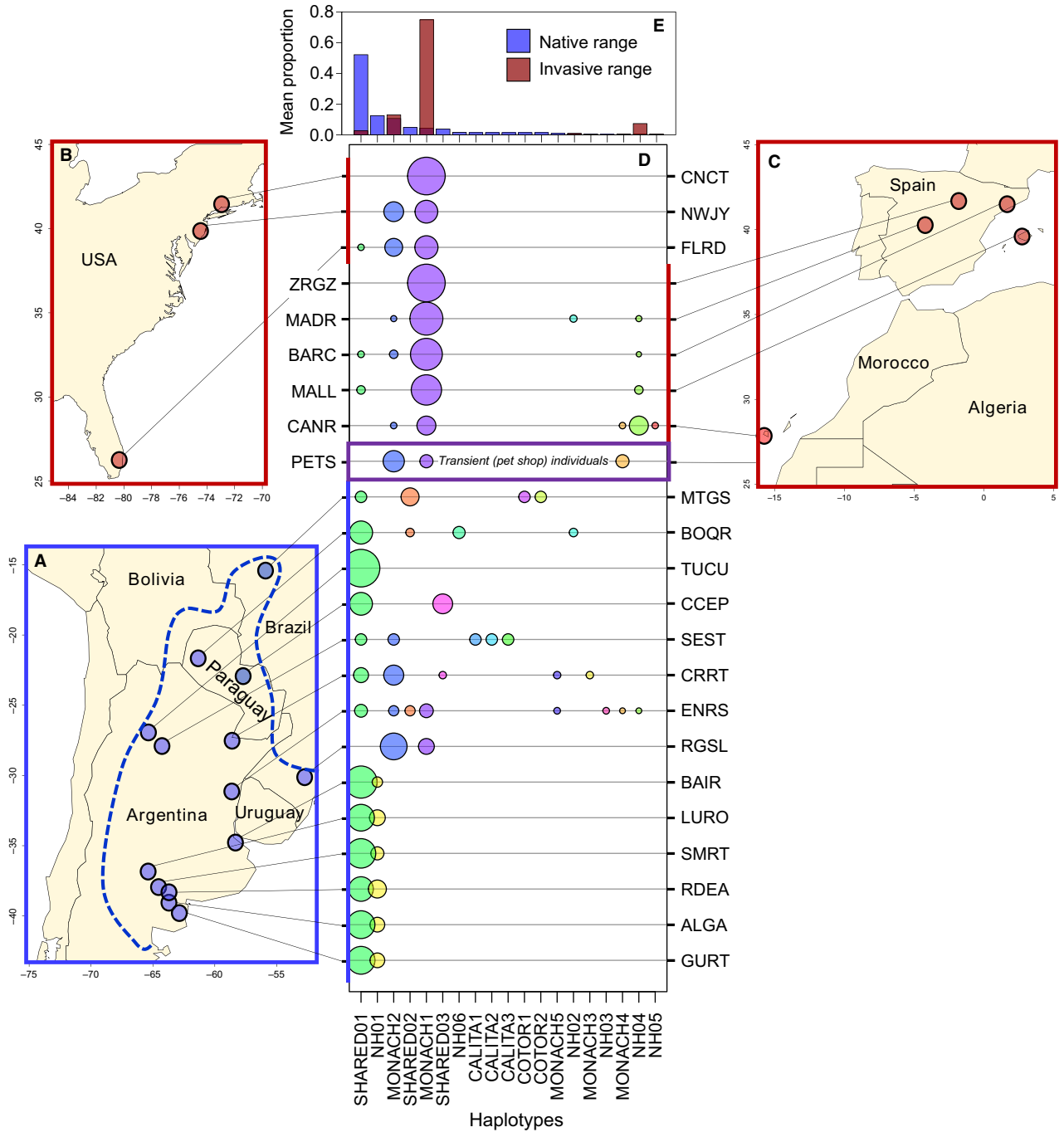

Fig. 2 Overview of mtDNA variation across the native and invasive range of *Myiopsitta monachus*. (A). Distribution of sampled populations across the entire native range (indicated by the dotted line). (B). Location of sampled populations in the USA. (C). Location of sampled populations in Spain. Wild-caught birds sampled in Pet Shops (in between uptake and potential introduction) do not have a location. (D). Haplotype frequencies in each population. The names at the bottom indicate each haplotype, whereas the size of the bubble is proportional to the number of individuals with this haplotype. (Ordering or similarity in colour does not refer to haplotype relatedness.) (E). Proportions of each haplotype across the native range (blue bars, ordered from highest to lowest) and invasive range (red bars). The full names of abbreviated sampling sites are given in Table 1.

tests based on Markov chains (10 000 de-memorizations, 1000 batches, 5000 iterations per batch), as implemented in GENEPOP on the Web (Raymond & Rousset 1995; Rousset 2008). The inbreeding coefficient (F_{IS}) and unbiased expected heterozygosity (H_E) were estimated using GENETIX v.4.03 (Belkhir *et al.* 2004). Allelic richness

corrected for sample size was determined using HP-RARE (Kalinowski 2005).

Population structure analyses

The partitioning of the total genotypic variation into different genetic clusters was assessed by two methods. First, we performed a factorial component analysis (FCA) with default settings in GENETIX, which determines the axes of genetic variation that best differentiate among predefined populations based on population allelic frequencies. We then plotted the individuals in this genetic space to evaluate population overlap. Second, we employed the model-based clustering method implemented in STRUCTURE version 2.3.4 (Pritchard *et al.* 2000), which assigns individuals to clusters that are derived without information on population membership. We ran STRUCTURE for 10 replicate runs each for $K = 1$–16 using the default parameters for an admixture model, no sampling site information, correlated allele frequencies between populations, a burn-in chain length = 100 000 and a Markov chain Monte Carlo length = 100 000. We used STRUCTURE HARVESTER (Earl & vonHoldt 2012) to determine the most likely K following the Evanno method (Evanno *et al.* 2005). The individual population assignment graphs for the 10 replicate runs for the most likely K were compiled using CLUMP 1.1.2 (Jakobsson & Rosenberg 2007) and default parameters for the Greedy algorithm. The composite assignments were graphically displayed using DISTRUCT 1.1 (Rosenberg 2004).

Results

MtDNA haplotypes

We found 19 haplotypes across our 23 population samples (Fig. 2). Six of these haplotypes (32%, haplotypes NH01-6: GenBank Accession nos KP873200–KP873205) had not been previously reported. Of these, haplotypes NH04 and NH05 showed well-defined polymorphisms (overlapping fluorescence peaks of equal heights) which were maintained even after repeated sequencing of the same individuals. As duplication of the control region does not occur in this species (Schirtzinger *et al.* 2012), these polymorphisms probably indicate the presence of heteroplasmy in the mitochondrial genome.

Within the native range, populations were diverse and differentiated, and frequencies of haplotypes varied considerably over relatively short distances (Fig. 2). An exception to this pattern was a cluster of populations at the southern end of the native range, which were composed of only two haplotypes (Shared01 and NH01, the last one unique to this cluster). These two haplotypes were found in similar proportions, even at relatively distant sites (Fig. 2).

Only seven of the 19 haplotypes (37%) were found in samples from the invasive ranges. All established populations from both the European and North American invasive range were dominated by the same haplotype (Monach1), which occurred in low frequencies in just two native populations (Entre Ríos and Rio Grande do Sul; Fig. 2). The population from Canary Islands differed somewhat in that Monach1 was less dominant and haplotype diversity was higher. Haplotype NH05 was unique to the invasive range and was not documented in any of our samples from the native range (Fig. 2).

Wild-caught birds sampled in Spanish Pet Shops (i.e. before their potential introduction into the invasive range) were more diverse than invasive populations (Table 1). Interestingly, Monach1 was not the dominant haplotype in the Pet Shops; thus, this sampling more closely resembled some of the native populations rather than the invasive populations in Spain (Fig. 2). Overall, transient birds (Pet Shops) and invasive populations showed the greatest similarity in haplotype composition with populations from Entre Ríos on the border of Argentina and Uruguay, and Rio Grande do Sul (Brazil) (Fig. 2).

Nuclear microsatellites

Across the 16 populations analysed (Table 1), expected heterozygosities of the seven loci ranged between 0.51 and 0.70, while rarefied allelic richness ($n = 8$ individuals) varied between 2.55 and 4.24 (Table 1). Global multilocus Hardy–Weinberg exact tests detected deviations from equilibrium expectations for only two of the 16 populations (one invasive and one native). Absolute F_{IS} values averaged across loci were low in all populations (<0.10; significant, and negative, in only one population), with an average across populations of −0.0096. Loci appeared unlinked as only one comparison in one population remained significant following sequential Bonferroni correction (data not shown).

Genetic diversity was highest in the native range, but decreased towards the southern end (Table 1). Invasive populations were overall less diverse, but levels of diversity did vary among populations, with the Canary Islands population being the most diverse (Table 1). The factorial correspondence analysis uncovered structuring of genotypic variation among populations (Fig. 3). The first three axes described 47%, 23% and 17% (88% in total) of the total among-population variation. Invasive populations from the USA clustered together with invasive populations from mainland Spain and birds from the Pet Shops. Populations from

the southern end of the native range formed another distinct cluster. The remaining populations in the native range also showed similarity, while the population from Canary Islands was distinct but most resembled the northern populations of the native range (Fig. 3).

The most likely number of clusters inferred from the STRUCTURE analysis was $K = 3$ ($\Delta K = 20$, more than twice as large as any other ΔK). The graphical output of individual population memberships for $K = 3$ (Fig. 4) showed that a first cluster was formed by individuals that were almost exclusively encountered in the populations from the southern end of the native range. A second cluster was formed by individuals mostly found in populations from the northern end of the native range, from Canary Islands, from the Pet Shops and, to a lesser extent, from the invasive USA populations (especially Connecticut) and the Madrid population from Europe. A third cluster was formed by individuals mostly found in populations from both the continental European and the North-American invasive range and, to a lesser extent, from the Pet Shops.

Relationship between nuclear and mitochondrial variation

Overall, nuclear and mitochondrial genetic diversities appear correlated across populations in both the native and invasive range (Fig. 5). Populations from the southern part of the native range have a lower diversity than those from the north for both marker types (Table 1, Fig. 5). Similarly, populations from the invasive range generally have lower diversity than those from the native range for both marker types: some populations are even fixed for a single mtDNA haplotype. In contrast, the birds from the Spanish Pet Shops have relatively high mitochondrial diversity (Table 1, Fig. 5).

Discussion

We used patterns of variation at mtDNA control region sequences and nuclear microsatellites to reconstruct the history of replicate invasions by the South American monk parakeet on three continents. Our goal was to evaluate whether genetic variation observed in established invasive populations could be explained by (i) the native source populations from which invasive populations were derived and (ii) genetic effects of demographic bottlenecks during the introduction. Nuclear microsatellite and mtDNA haplotypes both exhibited strong and consistent patterns of geographic structuring. Genetic diversity was highest in the northern parts of the native range. This northern area was identified as the most likely native source for invasive populations, and genetic analyses provide evidence for a single native source for virtually all sampled invasive populations. Nonetheless, genetic diversity varied among invasive populations and was overall lower than that in native populations. Although these patterns indicate that genetic bottlenecks probably reduced the diversity of invasive populations compared to the native source, many of these invasive populations are thriving. The low genetic diversity, evidence for bottleneck effects and the restricted area of native source populations that we observed in this highly successful invader do not support the hypothesis that high genetic variation inherently favours biological invasion or that invasion is favoured by the combining or mixing of genetic variation from multiple source populations. Below, we

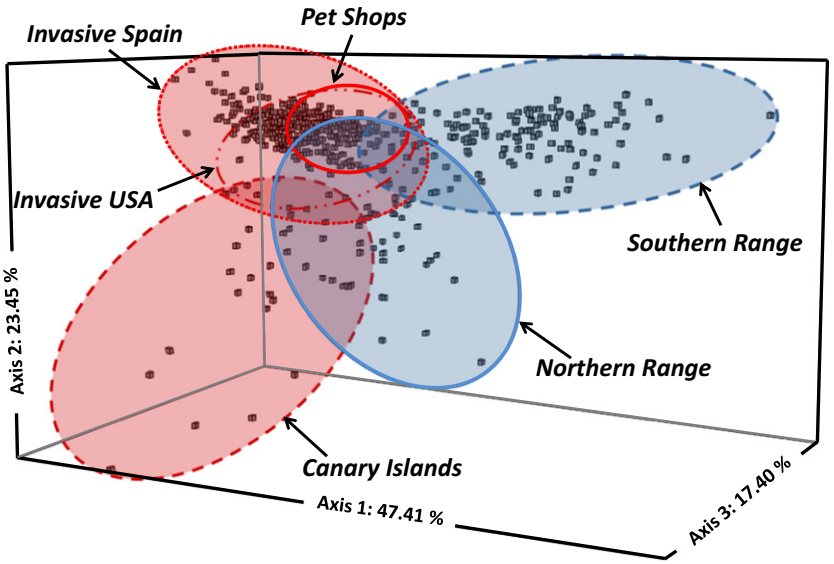

Fig. 3 Microsatellite divergence across the native and invasive range, as determined by factorial correspondence analysis. Plotted are individual genotypes along the three axes that best differentiate the genetic divergence among populations. Coloured ellipses indicate the approximate ranges of a priori and a posteriori determined groups (blue for native groups, red for invasive groups).

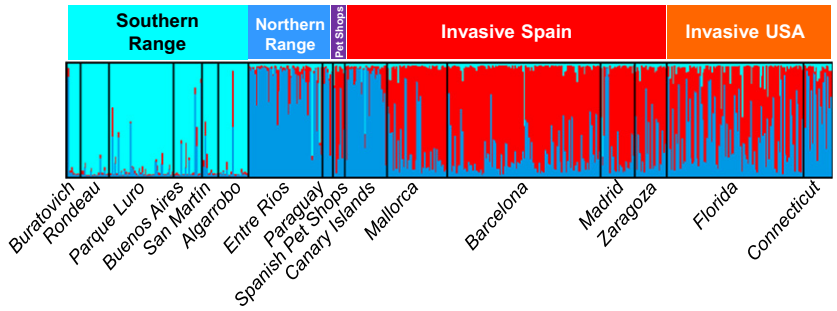

Fig. 4 Individual population membership coefficients estimated by the program STRUCTURE for $K = 3$ as the most likely number of clusters. Bottom labels refer to each sampled location. Top labels indicate a priori population groupings (pale and dark blues for native populations; red for invasive populations; purple for intermediate captive wild birds in Pet Shops). Note that the three clusters uncovered by STRUCTURE correspond well to our a priori population groupings, with Canary Islands as the largest exception.

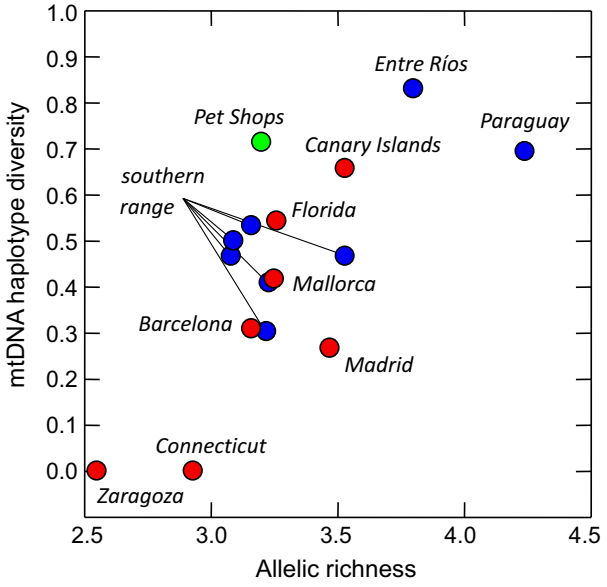

Fig. 5 Nuclear (microsatellite) and mitochondrial (control region) genetic diversity in native (blue dots) and invasive populations (red dots), showing how correlated reductions in diversity occur going from native to invasive populations. The sample of birds from the Spanish Pet Shops (representing the transport phase of invasion) is indicated separately in green.

discuss these results in more detail and relate them to known historical patterns of transport of birds via the global pet trade.

Spatial genetic structuring in native range

We found evidence for strong spatial structuring of genetic diversity. In the native range, genetic diversity decreased along a north–south axis in the native range (Figs 2 and 5, Table 1). The high genetic diversity and structuring at the northern end of the native range suggest that populations are relatively stable here and that dispersal is relatively restricted in this species. Short dispersal distances for this species have been reported in the native range based on mark–recapture methods (Martín & Bucher 1993), although genetic evidence has suggested longer dispersal events may occur in invasive populations (Gonçalves da Silva *et al.* 2010). In contrast, there is less structuring in the southern end of the native range. There is no evidence that this is due to a difference in dispersal rates. Instead, lack of geographic structure can occur as the result of a recent expansion of the range (Avise 2004). Indeed, such an expansion (filling up a gap in the distribution) has been well-documented for the Pampas region of Argentina (Bucher & Aramburú 2014). Interestingly, the southern populations we sampled lie on opposite sides of this recently invaded area yet are genetically very similar, suggesting that they may be part of a larger expansion that predated the 20th-century expansion into the Pampas documented by Bucher & Aramburú (2014). Further sampling is necessary to confirm and clarify this pattern.

When native populations are strongly structured in neutral genetic markers, this typically indicates reduced dispersal among populations. Reduced dispersal generally increases the potential for local adaptation to emerge (Lenormand 2002). In that case, it therefore becomes more important to establish which areas or populations have acted as sources. At the same time, stronger spatial structuring allows for more accurate identification of the origin of invasive populations. However, our results may act as a warning that the degree of population structuring can itself be heterogeneous: structuring is much stronger among northern than among southern native populations (Figs 2 and 3). Local results on population structuring may therefore not generalize rangewide. We therefore recommend that (in the absence of any other information) studies directed towards inferring source populations start with a very broad but coarse sampling and then iteratively

sample areas at a finer scale that might contain putative source localities.

Inferring source populations

The strong structuring of native populations allows insight into the invasion pathways of the monk parakeets. Most sampled native populations can be discounted as potential source localities as the general haplotype composition of invasive populations differed substantially from those in the native range. There are, however, relatively close fits to the haplotype compositions for the native populations of Entre Ríos and Rio Grande do Sul (Fig. 2D). This is especially clear for the Monach1 haplotype, which is dominant in all sampled invasive populations but virtually absent in all sampled native populations except for Entre Ríos and Rio Grande do Sul. However, even in these two native populations, the Monach1 haplotype is not dominant. This pattern suggests that the source populations could be even more spatially restricted than what our current sampling can resolve and might lie between the two putative native source populations in Uruguay. Such a restricted source area is also indicated by the microsatellite data, because the sampled invasive populations are genetically quite similar, suggesting they share a similar origin, but are distinct from anything we have sampled in the native range.

Comparison with historical geographical data on transports

Another approach to deduce source areas of biological invasions is the use of historical records on the movements of organisms, if available (Blackburn *et al.* 2009; Estoup & Guillemaud 2010). For the monk parakeet, natural overseas dispersal events are highly unlikely as this species, like most parrots, is nonmigratory (Forshaw 1989). We also find it highly unlikely that this bird would be accidentally transported (e.g. stowaway in a plane). In contrast, close to 1 000 000 wild-caught individuals have been exported across the world to be sold as pets (CITES Trade Data Base, www.cites.org). While the numbers obtained from CITES are only approximate, our summary of the database indicates that Uruguay has been the main exporter of monk parakeets in the world from 1980 onwards (Fig. 6). This observation corroborates our conclusion based on the genetic data. This conclusion is further supported by the mtDNA haplotype obtained from a single Uruguayan sample (Russello *et al.* 2008). This individual had the Monach2 haplotype, which is the second-most common haplotype across the invasive populations but spatially restricted in the native range (Fig. 2D,E). Hence, the historical transport data appear to corroborate our genetic assessment that there is a single main source for most invasive populations and that it is likely located in Uruguay.

However, the relative proportions of monk parakeets imported from Uruguay vs. Argentina differ considerably between Spain and the USA, and among years (Fig. 6). Moreover, data collected by the US Fish and Wildlife Service (Form 3-177 reports) indicate that before 1980 (when at least the invasive Florida population established in the USA), Paraguay was the principal source. Together, these data would predict variation in genetic composition among invasive populations of

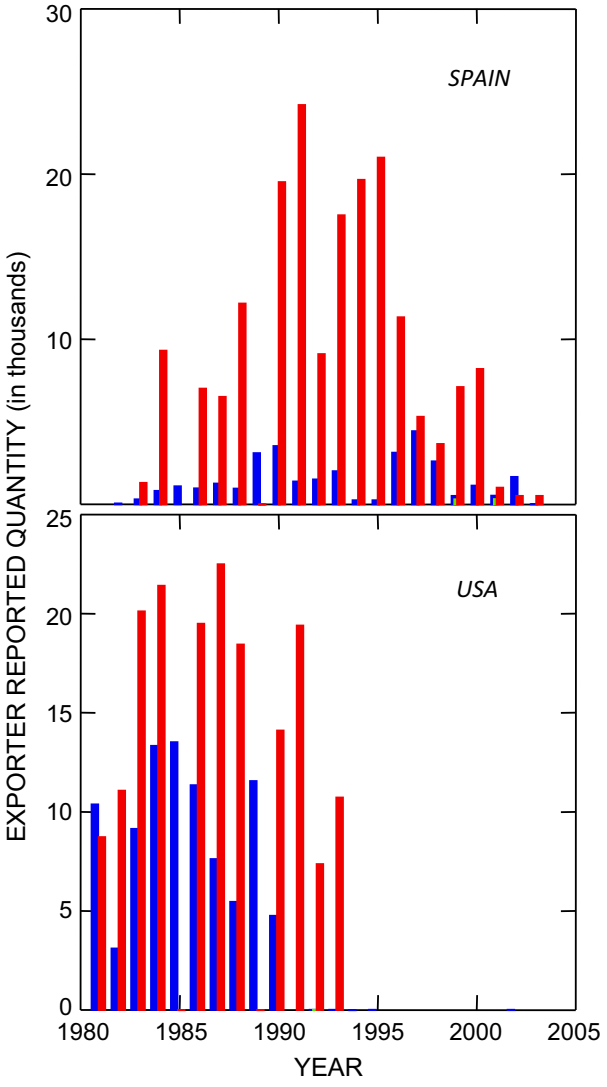

Fig. 6 Difference in number of monk parakeets exported from potential invasion source areas to Spain and the USA (red: Uruguay, blue: Argentina, green: Paraguay – just a few around the year 2000; CITES Trade Data Base, www.cites.org). Note that export data are missing from Uruguay in 1985 and 1989 and that exports to the USA largely stopped in 1994.

monk parakeets, as these became established during a wide temporal window (1969–1991) and in different countries. This prediction contrasts with our observation of high genetic similarity among invasive populations, suggesting a similar origin. We therefore conclude that well-sampled genetic data provide a more comprehensive picture of which native populations actually contributed to invasive populations as it integrates over individuals that may have been transported in different years or from different sources and held in captivity for some time before founding or joining invasive populations. Furthermore, the genetic approach is the only option available for many invasive species for which no historical trade or transport data are available.

Reduced genetic diversity in invasive populations

One striking pattern we recovered was the lower mitochondrial haplotype diversity and microsatellite allelic richness in the invasive populations. This lower genetic variation in invasive populations probably stems from two effects. First, reduced genetic diversity may be a characteristic of the native source population. The strong genetic similarity among invasive populations suggests that their resemblance is due to a common origin; if this source area had low genetic variation to begin with, subsequent invasive populations would also exhibit low genetic variability. Our samples from the native range show that genetic diversity does vary considerably among native populations (Table 1). However, because we do not have population genetic samples that exactly correspond to the inferred native source, this hypothesis cannot yet be tested directly. Second, genetic diversity in both markers is especially low for some populations such as Connecticut and Zaragoza (Fig. 5), which may be indicative of a demographic bottleneck. In contrast, the Canary Islands population has the highest genetic diversity of all invasive populations (Table 1) and, to the best of our knowledge, is the only deliberately introduced invasive monk parakeet population involving dozens of released and supplementary-fed individuals (R. Riera, pers. comm.). However, it is worth pointing out that the Canary Islands has a different microsatellite composition and, alternatively, may have been founded from a source population with more genetic diversity.

Invasive success vs. genetic diversity

The low genetic diversity, evidence for bottleneck effects and the restricted area of native source populations that we observed in this highly successful invader do not support the hypothesis that high genetic variation inherently favours biological invasion or that invasion is favoured by the combining of genetic variation from multiple source populations (Lee 2002; Kolbe *et al*. 2004; Facon *et al*. 2006, 2008; Lavergne & Molofsky 2007; Roman & Darling 2007; Suarez & Tsutsui 2008; Blackburn *et al*. 2009). Instead, we find that a single, spatially restricted source area likely has given rise to virtually all successful invasive populations across different continents, with little evidence for admixture of multiple native source populations. Furthermore, our results suggest that this restricted native source population most likely had reduced genetic variability to begin with and that bottlenecks during invasion reduced this variation even more. Nonetheless, the invasive populations are viable and have high initial population growth rates. As an extreme example, the Zaragoza population from Spain is thought to have been established by perhaps as little as two or three individuals in 1991, is fixed for a single haplotype and has the lowest nuclear heterozygosity and allelic richness that we detected across our sampling. Yet, this population grew to a size of over 1000 in 15 years, which means an average population growth rate of nearly 50% per year (M. Carrete, J.D. Anadon, J.L. Tella, unpubl. data). Even if the number of founders was higher, a growth rate of >20% was probably experienced. Hence, we can conclude that high genetic diversity per se is not critical for successful establishment in this species. Instead, there might be particular traits that are characteristic for this species that make it such a successful invader. These may include the capacity to build its own nest instead of relying on cavities for breeding, tolerance of human disturbance and dietary flexibility (Strubbe & Matthysen 2009; Carrete & Tella 2011; Bucher & Aramburú 2014). Nonetheless, high propagule pressure (close to 1 million individuals exported) will have also facilitated invasion.

Might selection explain observed genetic patterns?

The dominance of a single haplotype (Monach1) in all independently established continental invasive populations compared to the low frequency of this haplotype in native populations (Fig. 2) is striking. In addition, it has a higher frequency in invasive populations than in the transient (pre-establishment) Pet Shops sample (Fig. 2). Similarly, it is predominant in the populations from Connecticut and Canary Islands (Fig. 2) even though these populations are distinct from other invasive populations with regard to microsatellite variation (especially Canary Islands; Figs 3 and 4). These observations could be interpreted as a signature that natural selection favours this haplotype within invasive populations, putatively linked to specific variants within non-recombining mitogenomic coding regions. If convergent

selection is acting on invasive populations, what are the underlying drivers? Climates and associated vegetations vary greatly across the invasive range, with an average winter temperature of 18 °C on the subtropical Canary Islands vs. −3 °C in cold-temperate Connecticut, suggesting that such factors are not driving convergent selection. (As an aside, it does appear as if populations exposed to lower average winter temperatures (Connecticut, New Jersey, Zaragoza) have lost more genetic diversity than populations with higher temperatures (Florida, Canary Islands, Mallorca; Table 1, Fig. 5). One interpretation could be that colder climates have caused greater demographic bottlenecks, for example due to mortality related to cold spells. An independent set of populations would be needed to properly test this suggestive pattern.)

One aspect that all invasive populations do share is that they occur in urban environments, which have been shown to exert selection on genes related to behaviour in other avian populations (Mueller *et al.* 2013). Future comparisons of invasive and native populations that sample more widely across the genome may help detect whether specific genes have responded to selection (e.g. Puzey & Vallejo-Marín 2014) imposed by the novel urban settings and whether any of these are functionally linked to the Monach1 haplotype.

Alternatively, the haplotype Monach1 might be dominant in the invasive range because it is already dominant in a restricted but unsampled source area that we inferred using both marker types. It is notable that the historic trade data document that exports originated from a broad area involving several countries (Paraguay, Argentina, Uruguay), yet we do not see a genetic signal of such diverse origins in the invasive populations. This disparity indicates that monk parakeets from some source areas (e.g. Paraguay) failed to establish. It further suggests that there might be some characteristics particular to monk parakeets from a restricted subset of the native range from which exports originated that is favoured by selection in the novel range, for example a certain (potentially behavioural) urban phenotype. In general, this scenario suggests that having propagules originate from more areas would increase the likelihood that some suitable individuals have been introduced, favouring establishment and subsequent invasion.

Even though our data do not currently permit strong inferences regarding selection and its potential contributions towards shaping observed patterns, we do feel that it provides an alternative explanation that warrant future testing with new genomic approaches. At present, the role of selection in invasion success is often neglected. A limited number of intraspecific studies have shown that invasive potential may differ considerably between introduced populations from the same species (e.g. Kelly *et al.* 2006; Kang *et al.* 2007; Ciosi *et al.* 2008). We argue (see also Carrete *et al.* 2012) that taking into account intraspecific variation in invasive potential may yield further insights, additional options for effective management of biological invasions and improved prediction of the potential range limits of invaders (e.g. when based on climatic niche modelling).

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Data accessibility

Control region DNA sequences: GenBank Accession nos KP873200-KP873205 (this study) and EU545521-EU545537 (Russello *et al.* 2008). Haplotypes of all individuals, haplotype frequencies per population, haplotype alignment and sampling locations and microsatellite genotypes: Dryad doi: http://dx.doi.org/10.5061/dryad.5pr61.