Recent invasive population of the European starling Sturnus vulgaris has lower genetic diversity and higher fluctuating asymmetry than primary invasive and native populations

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Research Article

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RECENT INVASIVE POPULATION OF THE EUROPEAN STARLING *STURNUS VULGARIS* HAS LOWER GENETIC DIVERSITY AND HIGHER FLUCTUATING ASYMMETRY THAN PRIMARY INVASIVE AND NATIVE POPULATIONS

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Abstract

Fluctuating asymmetries (FA) are small stress-induced random deviations from perfect symmetry that arise during the development of bilaterally symmetrical traits. One of the factors that can reduce developmental stability of the individuals and cause FA at a population level is the loss of genetic variation. Populations of founding colonists frequently have lower genetic variation than their ancestral populations that could be reflected in a higher level of FA. The European starling (*Sturnus vulgaris*) is native to Eurasia and has been introduced successfully in USA in 1890 and Argentina in 1983. In this study, we documented the genetic diversity and FA of starlings from England (ancestral population), USA (primary introduction) and Argentina (secondary introduction). We predicted the Argentinean starlings to have the highest level of FA and lowest genetic diversity of the three populations. We captured wild adult European starlings in England, USA, and Argentina and allowed them to molt under standardized conditions, to evaluate their FA of primary feathers and their mtDNA diversity. For genetic analyses, we extracted DNA from blood samples of individuals from Argentina and USA and from feather samples from individuals from England and sequenced the mitochondrial control region. Argentinean starlings showed the higher composite FA and exhibited the lowest haplotype and nucleotide diversity from all populations studied. USA population showed a level of FA and genetic diversity similar to England population. Therefore, the level of asymmetry and genetic diversity found among these populations was consistent with our predictions based on their invasion history.

Keywords: exotic bird species, fluctuating asymmetry, genetic variability, *Sturnus vulgaris*

Introduction

Fluctuating asymmetries are small stress-induced random deviations from perfect symmetry that arise during the development of bilaterally symmetrical traits (Ludwig 1932). Factors that cause fluctuating asymmetry (FA) can be either genetic or environmental in origin (Møller and Swaddle 1997). Developmental stability is the production of a phenotype, such as bilateral symmetry, under a given set of specified environmental and genetic conditions (Møller and Swaddle 1997). One of the factors that can reduce developmental stability of the individuals and cause FA at a population level is the loss of genetic variation (Parsons 1990). Populations that have experienced a bottleneck or small populations of founding colonists frequently have lower genetic variation than their ancestral populations (Barret and Kohn 1991, Nei *et al.* 1975, Sakai *et al.* 2001). In part, this is because uncommon haplotypes are unlikely to be represented in founding, invasive populations (Futuyma 1997).

Reduced genetic variation appears to destabilize developmental processes and increases FA in a wide range of taxa (Møller and Swadlle 1997). For example, populations of cheetah (*Acynoxis jubatus*), a species that has experienced a considerable population bottleneck followed by intense levels of inbreeding, have elevated levels of FA in cranial morphology compared with a similar species of wild cat that have not experienced demographic declines (Wayne *et al.* 1986). Similarly, populations of British doer deer (*Capreolus capreolus*) established from a small number of introduced founder individuals have a lower genetic diversity and higher FA than relatively less disturbed populations (Baker and Hoelzel 2013).

Increased FA can also result from a large number of environmental factors, such as abnormal ambient temperatures, nutritional stress, parasitic infection, and habitat

69 fragmentation (Møller and Swaddle 1997, Anciães and Marini 2000, Gebremichael et al.
70 2019). When an organism is exposed to a novel set of environmental conditions, or even
71 to a novel element within the same environment, developmental processes become
72 disrupted resulting in increased individual expression of FA. For example, Møller (1992a)
73 found that feather FA of male barn swallows (*Hirundo rustica*) increased with ectoparasitic
74 load. Anciães and Marini (2000) recorded greater wing and tarsus FA within bird species
75 that occupied fragmented forest habitats compared with those in contiguous forests.

76 The European starling, *Sturnus vulgaris* (hereafter starling) is native to Eurasia and
77 the northern-most part of North Africa and has been introduced successfully in many
78 countries, including the United States of America (USA), New Zealand, Australia, South
79 Africa, and some Pacific and Caribbean islands (Blackburn et al. 2009, Craig 2020, Feare
80 1984, Rollins et al. 2009). The USA invasion started with the release of a small number of
81 individuals from England in Central Park, New York (60 in 1890 and 40 in 1891, Feare
82 1984). The starling population in the USA has subsequently grown to at least 140 million
83 individuals (Jernelov 2017). Starlings' invasion into Argentina came about through escapes
84 from the pet trade near Buenos Aires in 1983, with birds imported from the USA (Navas
85 2002). As in the USA, the invasion of Argentina has been rapid and prolific, with starling
86 populations booming in urban areas (Di Giácomo et al. 1993, Jensen 2008). Just 20 years
87 after introduction, estimates of the relative density of starlings in urban and natural areas
88 varied between 2.21 and 0.22 individuals ha^{-1}, respectively (Palacio et al. 2016, Rebolo
89 and Fiorini 2010) and an estimate of the number of starlings in urban parks of Buenos
90 Aires in 2010 suggests approximately 4,600 individuals occupy just those habitats (Rebolo
91 and Fiorini 2010).
92 Consistent with the arguments discussed above, genetic diversity in sequences of
93 mitochondrial DNA (mtDNA) within starling populations is lowest in the most recently

94 colonized areas of Western Australia (Rollins et al. 2011). In this study, we sought to
95 document the genetic diversity and FA of starlings from ancestral populations in Europe
96 with introduced populations in the USA and Argentina. Because the Argentinean
97 population was founded by starlings from a primary introduction population in the USA and
98 is more recent, we predicted Argentinean starlings to have the highest level of FA and
99 lowest genetic diversity of the three geographies.
100 The aim of this study was to test if starlings from a recent invasive population
101 (Argentina) have higher FA and lower mtDNA diversity than those from an older invasive
102 population (USA) and from the large original ancestral population (England). We captured
103 wild adult starlings in Argentina, USA, and England and maintained them under
104 standardized experimental conditions during the feather molt after which, we compared the
105 FA of their primary feathers (left feather length minus right feather length). Feathers are a
106 morphological trait that grow *de novo* each year and feather growth is affected by
107 environmental and genetic factors (Gill 2001). Therefore, by controlling the environmental
108 factors that individuals were exposed to during the molting, we evaluated how genetic
109 variation was associated with feather growth and FA. We predicted the populations
110 established from a small number of founders (i.e. Argentina, where starlings were
111 introduced in 1981 from the USA; and USA, where starlings were introduced in 1891 from
112 England; Figure 1) to have higher levels of FA and lower genetic variability than a
113 population from the ancestral range of the species (i.e. England). In addition, we also
114 predicted that there would be higher FA and lower mtDNA diversity in the starlings from
115 the Argentinean population than in those from the USA population, as genetic diversity
116 may have recovered somewhat in the USA population since their introduction in the 19th
117 century.
118

Methods

Fluctuating Asymmetry

We conducted experiments on wild-caught adult European starlings of undetermined sex (as FA is not expected to vary between sexes) to evaluate FA of primary feathers under standardized conditions. The number of experimental individuals was 20 from Bristol, England (51° 27′ 0″ N, 2° 35′ 0″ W), 20 from Williamsburg, Virginia, USA (37° 16′ 15″ N, 76° 42′ 25″ W), and 17 from Bernal, Buenos Aires, Argentina (34°42′0″ S, 58°17′0″ W). The individuals were captured during summer, at the end of their breeding season, and before they started molting. In England they were captured during June 1993, in United States during June 2008, and in Argentina during November 2008. All the individuals from the same country were housed in one group in an indoor aviary of approximately 3.0 x 2.0 x 2.5 m (length x width x height). We decided to use one large free-flight room with lots of perches instead of individual small cages because in small cages the birds experience excessive feather wear and damage. They were housed at a constant temperature of approximately 20 °C, illuminated with a regular overhead fluorescent tube lighting, fed with *ad libitum* chick starter crumbs (with same nutritional characteristics in the three countries), and with drinking and bathing water available. The birds were maintained on a short day (8L:16D) photoperiod to induce feather molt (Witter and Swaddle 1994). They remained on this photoperiod throughout the experiment.

We captured and examined the birds to check if molt of the primary feathers was completed. When the feathers completed their growth (12 weeks on average), we measured the length of the primaries 3, 5 and 7 with Vernier calipers (± 0.1 mm). To minimize measurement error, we measured each feather on both wings three times (Swaddle and Witter 1998). We always checked carefully the tips of the primaries and when any damage or wear was noted those values were excluded from the analyses (see

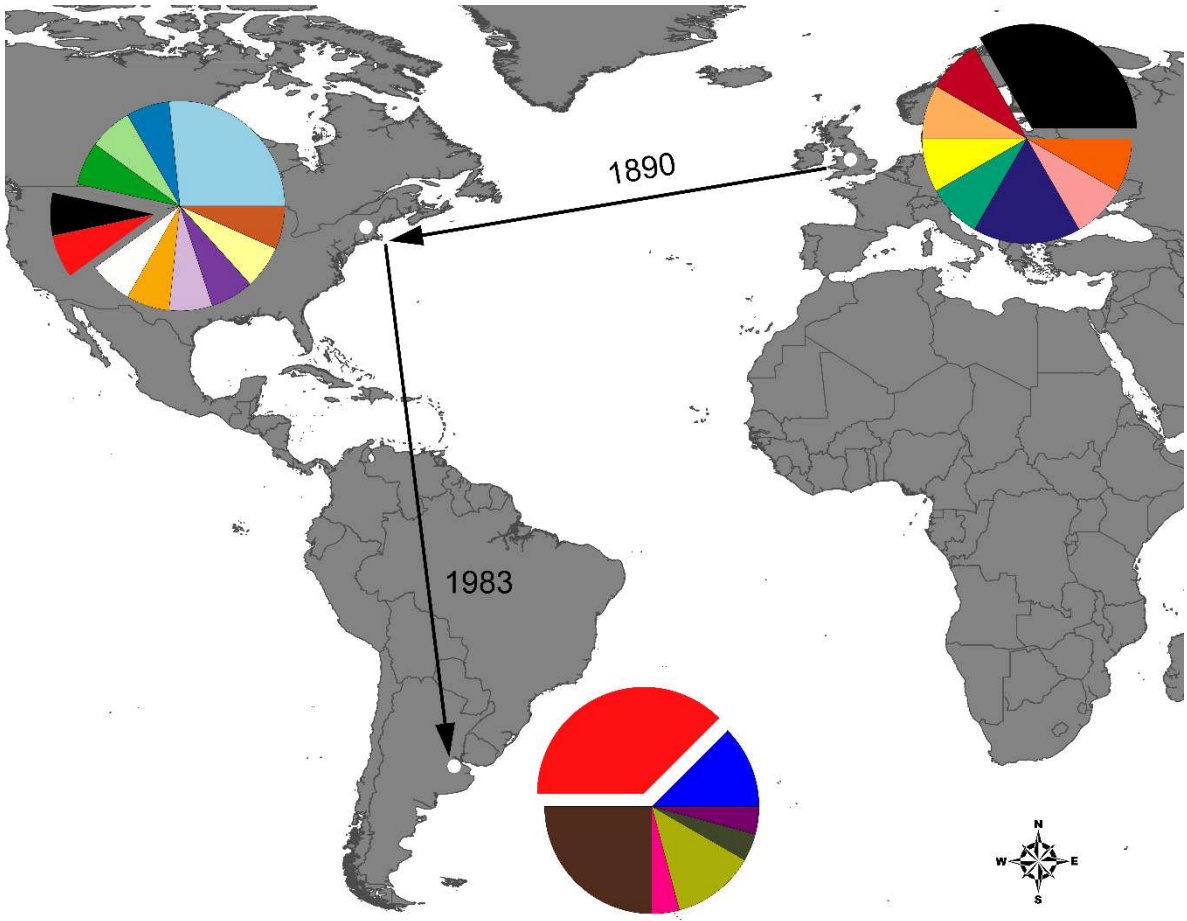
144 Cuthill et al. 1993). Only one individual that experienced extensive feather damage and
145 one other that did not complete molt (both from the Argentine dataset) had to be excluded.

146 As each feather was measured three times, we obtained three values of
147 asymmetry (left feather minus right feather length). These repeats were averaged to obtain
148 a signed asymmetry for primary feathers 3, 5, and 7. Then, we standardized for size
149 differences among the feathers by dividing the signed asymmetry by feather length
150 (relative signed asymmetry) and then obtained the relative FA (absolute value of the
151 relative signed asymmetry). After that, we calculated the composite asymmetry as the
152 average of the relative signed asymmetry of the three feathers and the relative FA
153 (absolute composite fluctuating asymmetry value) for each individual. As the distribution of
154 composite asymmetry values closely approximated a normal distribution (visual
155 assessment of normal probability plot) and did not differ significantly from a mean of zero
156 (t_{54} = -0.97, p = 0.34), the asymmetries measured were considered as fluctuating
157 asymmetries (Swaddle *et al.* 1994). Previously, we have demonstrated that our
158 measurements of primary asymmetry are highly repeatable for each of the three feathers
159 within each country (*F* range: 4.5 - 51.4, *P* < 0.0001**)** (Swaddle and Witter 1994). We then
160 used the absolute composite FA of each individual to compare levels of plumage
161 asymmetry among countries. Because of the particular “half normal” frequency distribution
162 of absolute composite FA, we used a Box-Cox transformation (with λ_1 = 0.3 and to λ_2 =
163 0.008) to normalize the data (Swaddle et al. 1994), and performed a one-way ANOVA to
164 explore if there were differences among countries and Tukey contrasts for pairwise
165 comparisons. Statistical analyses were performed using R software (R Core Team 2019).
166 All tests were two tailed, values are reported as means ± SE and differences were
167 considered significant at *P* < 0.05.

168

Genetic Analyses

We analyzed blood samples from 15 of the 20 experimental individuals from Williamsburg, USA (the samples of the other five, could not be amplified) and 24 individuals from Bernal, Argentina (17 experimental and 7 non-experimental individuals captured in the same place). We obtained feathers from nine starlings captured in Oxford, England. Given there is constant gene flow among European starling populations in England (Neves et al. 2010) and that Oxford and Bristol are only 117 km apart, we assumed these starlings came from the same genetic pool. Mitochondrial DNA was extracted from both blood and feather samples using an ethanol-salting out protocol. In order to sequence the mitochondrial control region we used the pair of primers svCRL2 and svPheH3 developed by Rollins et al. (2011). We performed polymerase chain reaction (PCR) amplifications in a total volume of 25 µl with 50–100 ng of total genomic DNA template, 0.2 µM of both forward and reverse primers, 0.2 mM of each dNTP, 1X PCR buffer (Invitrogen), 2.5 mM $MgCl_2$, and 0.1 U Taq DNA Polymerase. PCR conditions were 5 min of hot start at 94 °C; followed by 30 cycles of denaturation for 30 s at 94 °C, 15 s at 53 °C annealing temperature, and 30 s at 72 °C, and a final extension for 10 min at 72 °C. Amplification products were purified with the ExoSAP method and sequenced in an ABI 3130 XL (Applied Biosystems, Foster City, CA, USA) sequencer using ABI Big Dye™ Terminator Chemistry.



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189

190 **Figure 1.** Map showing the time and direction of common starlings (*Sturnus vulgaris*)
191 introductions into America. White circles denote sampling localities. Pie charts show each
192 population's haplotypes and their frequencies. Shared haplotypes (H2 and H12) are
193 separated from main pie charts. For Argentina chart: H1 *blue*, H2 *red*, H3 *brown*, H4
194 *fucsia*, H5 *olive*, H6 *mosque*, H7 *purple*. For USA chart: H8 *light blue*, H9 *darker light blue*,
195 H10 *soft green*, H11 *dark lime green*, H12 *black*, H2 *red*, H13 *white*, H14 *light orange*, H15
196 *light violet*, H16 *violet*, H17 *light yellow*, H24 *light brown*. For England chart: H12 *black*, H2
197 red, H19 *soft orange*, H20 *yellow*, H18 *carmine*, H21 *dark blue*, H22 *pink*, H23 *orange*.

198

199 Our mtDNA control region sequences were edited and aligned using BioEdit 7.2.5
200 (Hall 1999). Identification of the sequences as part of mtDNA noncoding control region D-

loop highly variable domains I-III was confirmed by aligning our sequences with other populations of starlings obtained from the GenBank database. Population parameters of genetic diversity were defined as the total number of haplotypes, number of polymorphic sites, common and private haplotypes, haplotype diversity (probability that two randomly sampled haplotypes are different) and nucleotide diversity (π, average number of nucleotide differences per site in pairwise comparisons among sequences) and were all estimated in DNAsp 5.0 (Librado and Rozas 2009) for each population. Population structure was assessed using exact tests (Raymond and Rousset 1995) implemented in Arlequin v3.5.1 (Excoffier et al. 2010).

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Results

Fluctuating Asymmetry

We observed significant differences in final asymmetry of the primary feathers among starlings from Argentina, USA, and England (ANOVA, $F_{2,52} = 30.09$, $P < 0.0001$, Figure 2). Levels of composite feather FA were higher in the Argentinean population compared with the ones from USA (Tukey HSD: mean difference 0.048 ± 0.009, P < 0.001) and England (Tukey HSD: mean difference 0.066 ± 0.009, P < 0.001). Starlings from USA also exhibited higher levels of asymmetry than those from England, although the difference did not reach statistical significance (Tukey HSD: mean difference 0.018 ± 0.008, P = 0.067).

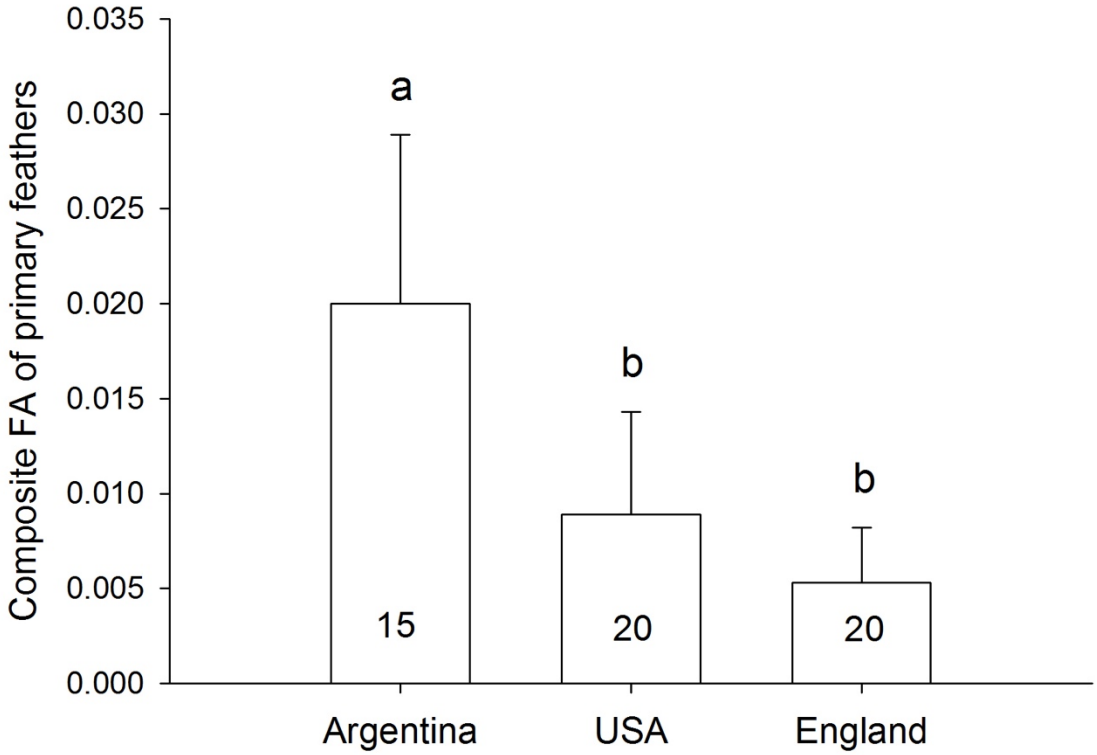

Figure 2. Mean ± SE levels of Composite Fluctuating Asymmetry (FA) of the primary feathers 3, 5, and 7, for three populations of European starlings that molted in aviaries under similar conditions. Number inside each bar show sample sizes. Different letters indicate significant differences ($P < 0.05$) in Tukey contrasts.

Genetic Analysis

Sequencing of the 942bp of mtDNA control region in 48 starlings revealed 24 haplotypes consisting of 31 polymorphic sites among all samples (Figure 1 and Table 1). All polymorphic sites have 2 variants, except from one site with 4 variants (polymorphic site

11, Table 1). The average nucleotide composition of these control region sequences was: 28.13% A, 26.65% T, 32.16% C, 13.05% G, with a bias against G. Among these haplotypes, six had already been described by Rollins et al. (2011) in Australian/UK populations: HapA, HapE, HapF, UKA, UKC, UKK (GenBank accession numbers FJ542126, FJ542133, FJ542128, HQ263631, HQ263633 and HQ263641, respectively). New haplotypes have been deposited in GenBank under accession numbers MW513733-MW513750.

Table 1. Variable sites in the mitochondrial DNA control region for 24 haplotypes found in samples from *Sturnus vulgaris* collected in Argentina, USA and England. Haplotypes in parenthesis were also found in starlings from Australia and UK by Rollins et al. 2011.

	1 19 23 24 37 38 47 69 88 93 125 139 143 190 215 216 274 325 374 519 623 647 663 667 679 850 881 898 902 926 941
H1	T T C C A T A A G C A A A G T A C C A T A A T C C C T C A G C
H2(HapF)	. . . . . T . A .
H3	C . . T . C . . . . . G . C G . . . C . . . . . T . A .
H4	C . . . G C . . . . . T . A .
H5	C . . . G C . . . . . T . C T . A .
H6	. C . . . . . C . . . . . T . A .
H7	C . . . . C . . . . . T . C T . A .
H8	. . . . . G . . . . . T . A .
H9	C . . T . C . G A . C . . A . . T . . C . . . . . T . A .
H10	C . . T . C . . . . . G G . . . . . G . . G . . . . . T . A T
H11	. . . . . G . . . . . T . A .
H12(UKC)	C . . T . C . . . . . G . . . . . C . . . . . T . A .
H13(HapE)	. . . . . C . . . . . T . A .
H14	. . . . . C G . . . G . . . . . C . . . . . T . A .
H15	C . T . . C . . . . . T . A .
H16(UKA)	C . . T . C . . . . . G G . . . . . G C . G . . . . . T . A T
H17	C C . T . C . . . . . T . C . . . T . . . T . A .
H18(HapA)	C . . . . C . . . G . . G . . . . . C . . . . . T . T . A .
H19	C . . T . C . . . . . G G . . . . T . C . . . . . T . A T
H20	. . . . . G . . . . . T . A .
H21	. . . . . T G A .
H22	C . T . . C . . . . . T . . T . A .
H23(UKK)	. . . . . T . . . . . C . . . . T . A .
H24	. . . T . C . . . . . T . A .

Several haplotypes were unique to populations in Argentina (n = 8), USA (n = 11) or England (n = 6) (Figure 1, Table 2). Two frequent haplotypes (H2, 38%; H3, 25%) were found in Argentina, one in USA (H8, 27%), and one in England (H21, 22%). Interestingly, one of the six previously described haplotypes is the most frequent one in Argentina (called H2 in this study, HapF in Rollins et al. 2011). Most haplotypes were found in only one population with low frequencies.

Diversity indices varied among geographic regions (Table 2). Birds sampled from Argentina showed the lowest haplotype and nucleotide diversity of all populations studied. While we found evidence of genetic differentiation between Argentina and the other populations (pairwise exact test p-value $_{\text{Argentina-USA}}$=0.00001; pairwise exact test p-value $_{\text{Argentina-England}}$=0.00064) no genetic separation was suggested for starlings from USA and England populations (pairwise exact test p-value$_{\text{England-USA}}$=0.18604).

Table 2. Mitochondrial DNA control region polymorphisms for each population of *Sturnus vulgaris*

	***N*[a]**	**Hap[b]**	**Poly Sites[c]**	**Haplotype Diversity**	**π[d] (%)**	**Common haplotypes**	**Private Haplotypes**
Argentina	24	7	13	0.793±0.003	4.72	H2 (38%) H3 (25%)	8
USA	15	12	20	0.943±0.003	5.65	H7 (27%)	11
England	9	8	15	0.972±0.004	5.40	H20 (22%)	6

[a] number of individuals, [b] number of haplotypes, [c] number of polymorphic sites, [d] nucleotide diversity.

Discussion

Levels of FA and genetic diversity differ among populations of European starlings from England, USA, and Argentina in ways that are largely predicted by their history of invasions into different continents through human interventions. Because the relevant conditions under which the starlings molted were controlled and similar in the three sites, these morphological differences cannot be attributed to differences in environmental conditions at the time of molting or extent of local adaptation to them during molt, but could be explained by the level of genetic diversity in the populations of starlings in the three areas. The level of asymmetry and genetic diversity found among these populations was consistent with our predictions as starlings in Argentina, which derived from a primary invasive population (USA), have the highest FA and lowest genetic variation. To our knowledge, this is the first study in which the FA of two exotic and one native conspecific populations were studied under controlled ambient conditions minimizing the potential effects of environment or genotype-environment interactions (e.g. local adaptation) on FA (Kristoffersen and Magoulas 2009). Previous studies based on allozymes variation have shown that genetic variability of different populations of starlings widely distributed in the USA (Cabe 1998) is reduced compared to the population of origin in England (Ross 1983). Cabe (1998) found that the level of heterozygosity of the population in the USA is comparable to the one of England (Ross 1983), but the former lost 42% of the alleles at variable loci. This decrease in allelic diversity in the bottleneck population would be the remaining signature of the loss of rare alleles of the source population (Cabe 1998). Unlike the findings of Cabe (1998), our work did not show significant differences in genetic variability between England and USA. One possibility to explain this fact is that our samples were more recent and over time the population of USA recovered diversity values

314 and it is no longer different from UK. Another possibility might be due to the use of different
315 genetic markers that follow different evolutionary paths with different mutation rates.
316 Our results suggest that starlings from the population in Argentina display the
317 lowest genetic diversity of the populations analysed in this study, based on a lower number
318 of haplotypes (despite the larger sample size), inferior number of polymorphic sites, and
319 minor nucleotide diversity. The genetic differentiation found could be due to geographic
320 differences in evolutionary forces (selection, genetic drift, gene flow caused by spatial
321 sorting) acting over time in different regions.
322 In recent years, a reference genome and a liver transcriptome for this species
323 were sequenced and became available (Richardson et al. 2017). These resources could
324 be used in the future to better analyze genetic variability allowing the development of new
325 genetic markers that could be added in genetic studies.
326 Similarly to our results, Lovatt and Hoelzel (2011) found that FA and morphological
327 variation were higher in bottlenecked populations of reindeers (*Rangifer tarandus*)
328 comparing to the source populations. Also Zachos et al. (2007) found a negative
329 relationship between FA and genetic variability in the roe deer (*Capreolus capreolus*).
330 We expect the differences in primary feather FA among the starling populations
331 we studied to be also present in other free-living starlings in these same locations. It is
332 possible that local adaptation might result in lower expression of FA in England and the
333 USA than in Argentina. As Argentinian starlings have been in that location for only a few
334 decades, it is less likely that those birds have adapted to local conditions to the same
335 degree as the birds in England and the USA. It could also be that environmental factors in
336 Argentina, USA, and England vary to produce alternate patterns in the feather FA of free-
337 living birds. Feather FA might be a sensitive indicator of many forms of environmental
338 pollution and adverse environmental conditions during feather molt and regrowth (Møller
339 and Swaddle 1997). Therefore, it would be interesting to capture and measure free-living

340 starlings just after they molt in their natural environments at the same sites our
341 experimental birds were captured, to compare feather FA of free-living and experimental
342 birds. A low difference between these values might give insight into a how well genomes
343 are adapted to current environmental conditions.
344 As the aim of our study was to isolate genetic effects and minimize the influence
345 of environmental factors on the production of FA, we placed our experimental birds in
346 indoor aviaries in which variables were controlled throughout the study. Nevertheless, if
347 molting is affected by the conditions to which starlings were exposed to before they were
348 captured, it could be that we did not sufficiently control environmental variation in our
349 experiment. However, because plumage molt is expensive in terms of molecular and
350 energetic resources (Cornelius et al. 2011; Hoye and Buttemer 2011) and our captive birds
351 were fed unrestricted amounts of high quality food, it would be expected that the
352 environmental factors present during molting have a much higher effect on the feather
353 structure than the ones the individuals experienced before this period.

354 Within populations, at an individual level, there is evidence of negative
355 relationships between FA and reproductive rates (Møller 1992b). Additionally, the primary
356 feather FA we report here might have functional consequences for flight and therefore
357 negatively impact daily energetic budgets (Swaddle 1997). Therefore, we could predict
358 that populations with greater FA might have overall fitness deficits relative to populations
359 with lower FA. However, the reality is that the measures report here deliberately down-
360 weight the influence of environmental factors on the expression of FA. Free-living
361 populations of starlings in Argentina, USA, and England might not differ in FA as much as
362 we report here, depending on their degree of local adaptation (as discussed above). It is
363 more likely that any observations of population performance differences across these
364 localities is driven by demographic effects of initially small populations sizes (i.e. more

likely in Argentina), the extent of local adaptation, and direct effects of environmental factors (e.g., temperature, pollution, parasitic infections) on individual performance. It is possible that FA relates to these factors but it would be extremely surprising if FA were a driver of any performance-related differences among populations.

In conclusion, FA of primary feathers of starlings molted under controlled conditions was higher in Argentina than in USA and England. In line with the predicted effects of genetic variability on FA, the genetic diversity (mitochondrial marker) of starling populations was lower in Argentina, where the invasion was more recent, than in USA, where the invasion is oldest, and England, within the starling original range. These results contribute new evidence to support the relationship between genetic variability and the expression of symmetry in morphological characters.

Declarations

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Conflicts of interest/Competing interests

This manuscript is not being considered elsewhere and all co−authors have agreed to this submission. We have no conflicts of interest to disclose

Availability of data and material

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable

Ethics approval

Experiment protocol have been established in compliance with the ethical standards, ensuring that all necessary precautions have been taken and the welfare of the birds has been respected.

The capture and housing of starlings in the UK was permitted by the UK Home Office. The capture of starlings in the US was permitted by the Virginia Department of Game and Inland Fisheries and all animal procedures were approved by the William & Mary Institutional Animal Care and Use Committee. In Argentina the work complied with the Argentinean Law for the Conservation of Wild Fauna (22421/81).

Consent to participate

All coauthors gave their approval for the submission

Consent for publication

All coauthors gave their approval for publication

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Figures

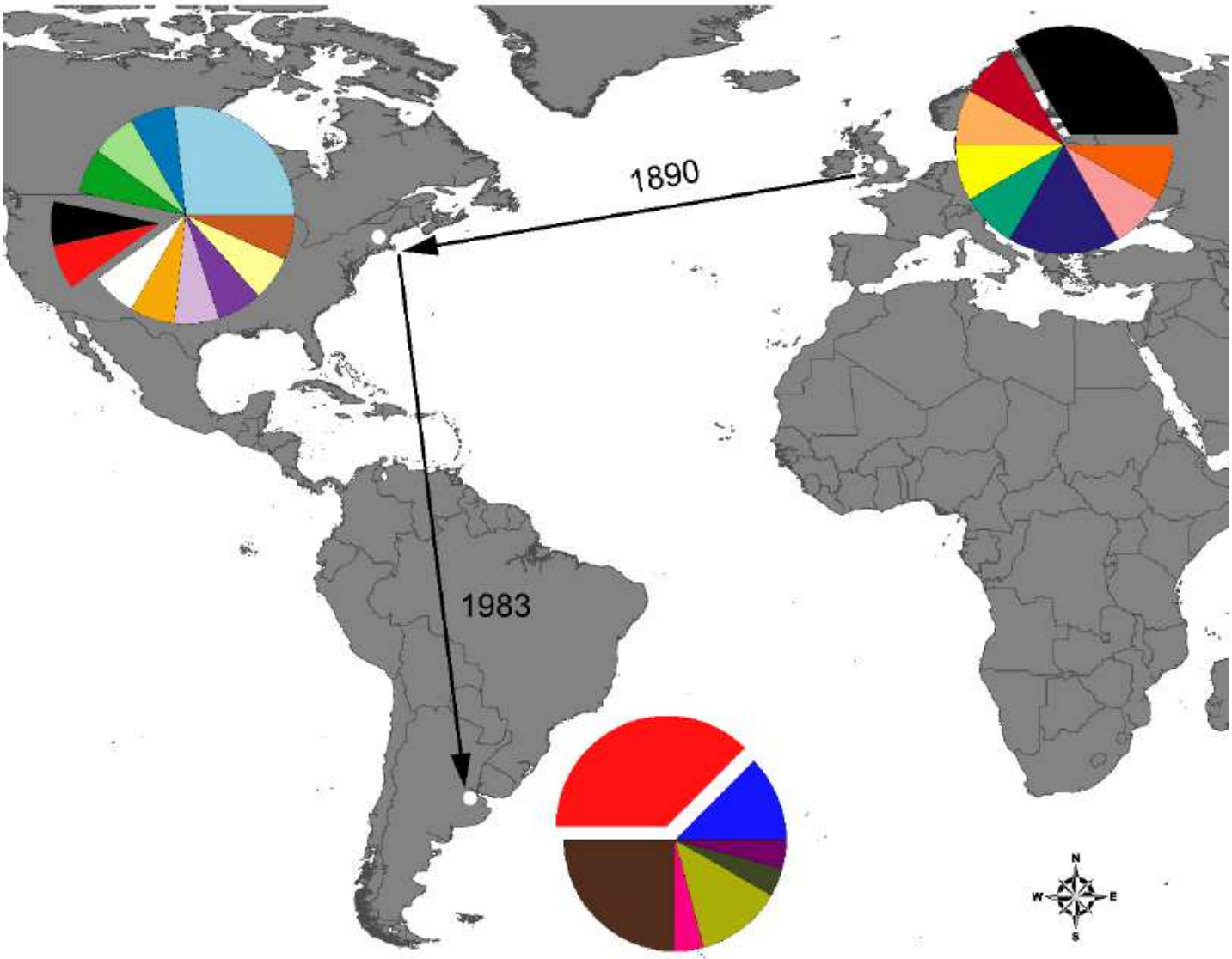

Figure 1

Map showing the time and direction of common starlings (Sturnus vulgaris) introductions into America. White circles denote sampling localities. Pie charts show each population's haplotypes and their frequencies. Shared haplotypes (H2 and H12) are separated from main pie charts. For Argentina chart: H1 blue, H2 red, H3 brown, H4 fucsia, H5 olive, H6 mosque, H7 purple. For USA chart: H8 light blue, H9 darker light blue, H10 soft green, H11 dark lime green, H12 black, H2 red, H13 white, H14 light orange, H15 light violet, H16 violet, H17 light yellow, H24 light brown. For England chart: H12 black, H2 red, H19 soft orange, H20 yellow, H18 carmine, H21 dark blue, H22 pink, H23 orange. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its

authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

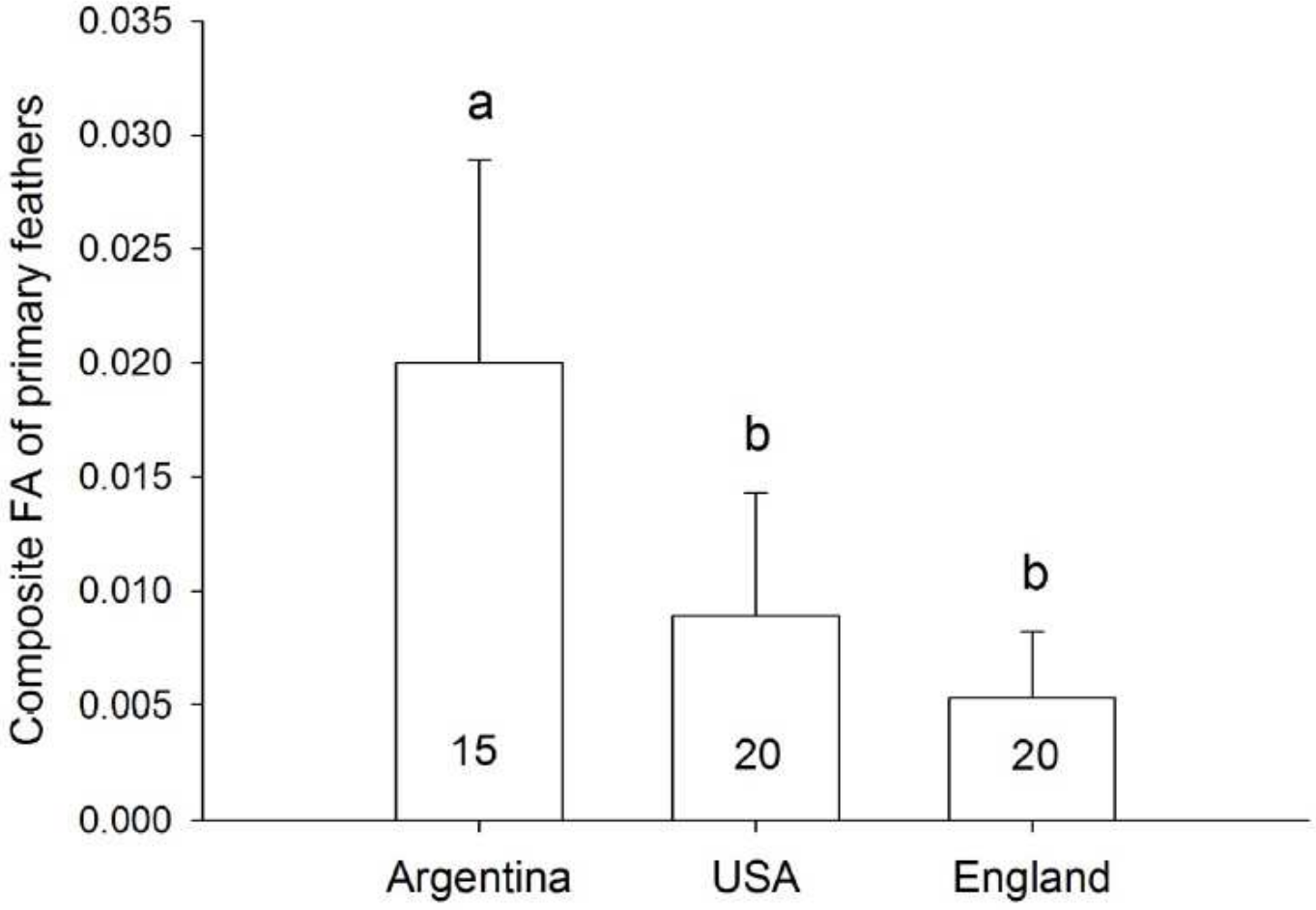

Figure 2

Mean ± SE levels of Composite Fluctuating Asymmetry (FA) of the primary feathers 3, 5, and 7, for three populations of European starlings that molted in aviaries under similar conditions. Number inside each bar show sample sizes. Different letters indicate significant differences ($P < 0.05$) in Tukey contrasts.