The Painted Bunting *Passerina ciris* is a Neotropical songbird which breeds primarily in the USA during the summer and migrates to Mexico, Central America, southern Florida and the Caribbean over the winter. Male Painted Buntings are brightly coloured, which makes them highly sought after as pets, particularly in Mexico, Central America and Europe. We used short sequence repeats (microsatellite DNA) to investigate the population genetic structure of the Painted Bunting and its implications in conservation management of migratory populations. We found a detectable level of population differentiation as revealed by pairwise $F_{ST}$ and $R_{ST}$ comparisons and Bayesian clustering analyses, with strong support for differentiation between eastern and western Painted Buntings (e.g. Oklahoma and Georgia $F_{ST} = 0.1; P = 0.005; R_{ST} = 0.18; P = 0.04$) in accordance with previous mitochondrial DNA analysis. We recovered additional support for two subgroups within the western clade. While linking migrant songbirds captured outside of the USA to their breeding populations remains a challenge, we show that natural levels of population genetic differentiation can be detected via microsatellite DNA markers and exploited in migratory connectivity studies. We also demonstrate the potential utility of our low-cost markers for population identification of birds recovered from the pet trade by screening a small subset of samples ($n = 5$) collected as part of wildlife tracking. We discuss the implications of our results for future efforts to understand patterns of population decline in Painted Buntings more generally, as well as how we might expand this methodology to combat illegal pet-trade activity in this and other songbird species.

**Keywords:** Convention on International Trade in Endangered Species of Wild Fauna and Flora, forensics, international pet trade, migration, population genetics.

Over half of the Neotropical migrant bird species found breeding in North America have shown marked declines in abundance over the last several decades (Robbins et al. 1989, Sauer et al. 2013). Population declines are thought to relate to stressors encountered by migrants at each stage in the annual cycle, including habitat loss, predation by house cats, exposure to disease, illegal poaching and global climate change (Jonzén et al. 2006,
Altizer et al. 2011, Loss et al. 2013). For migrants, ecologists have long recognized that conservation measures on the breeding grounds are most effective when wintering and migratory ecology are understood, and winter and migratory stopover habitat protected (Sherry & Holmes 1993, Marra et al. 1998, Norris & Taylor 2006), but such information is often difficult to attain.

The Painted Bunting *Passerina ciris* is a neotropical migratory songbird that is estimated to have declined by ~ 55% in some parts of its range over the last 30 years (Sauer et al. 2003, 2013). This species breeds in a variety of habitats across the southern USA and northern Mexico, wintering in parts of Mexico, Central America, the Florida Keys and Florida Peninsula, and the Bahamas (Rappole & Warner 1980, Howell & Webb 1995, Lowther et al. 1999, Mlodinow & Hamilton 2005, Bridge et al. 2011). While early taxonomic studies identified two subspecies within Painted Buntings (*P. c. pallidior* and *P. c. ciris*) separated by a zone of introgression in the central USA (Storer 1951), phylogenetic analysis using mitochondrial DNA supports the idea that the major split between the two subspecies is defined by a 550-km gap between eastern Mississippi and western Georgia (Herr et al. 2011, see also Thompson 1991a,b, Shipley et al. 2013) (Fig. 1). Breeding Bird Survey data suggest that the eastern population in particular is declining and that several populations from the south-central and eastern United States and northeastern Mexico are now locally extirpated from parts of their former range (Sauer et al. 2003, NAS 2004a,b, USFWS 2004). As a result,
the Painted Bunting has been listed as Near Threatened on the IUCN Red List, as a species of conservation concern by both the U.S. Fish and Wildlife Service and Partners in Flight, and as Near Threatened by Birdlife International (Sauer et al. 1997, Sykes & Holzman 2005, Sykes et al. 2007, USFWS 2008). Habitat loss (both at wintering and at stopover sites), habitat fragmentation and brood parasitism by the Brown-headed Cowbird *Molothrus ater* may all contribute to declines in Painted Buntings, but it has also been suggested that capture for the international cage bird trade is also a particularly serious threat (Hamilton 2001, Iñigo-Elias et al. 2002, Lopez Medellín & Iñigo-Elias 2002, Arizmendi Arriaga & Ramos Rivera 2017). According to some historical estimates, nearly 15,000 Painted Buntings were collected and exported every year from Mexico from the mid-1970s up until the early 1980s (Iñigo-Elias 1986). In 1982, Mexico banned wildlife exports, but over a decade later, Mexico resumed the international trade of wild animals, mostly to South America, northern Europe and Malaysia (Ramos 1982, Iñigo-Elias et al. 2002). Since 1999, Mexico has utilized capture quotas and management unit areas (UMAs) to promote and monitor sustainable harvesting of Painted Buntings and other wildlife, but precise control of these quotas is difficult to enforce (SEMARNAJT 2013). In 2004, the Painted Bunting was proposed for inclusion in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, Appendix II), but it did not meet listing criteria (under Resolution Conf. 9.24) because of the official annual harvested and exported stock from Mexico was < 0.3% of the estimated global population size (IUCN and TRAFFIC 2004). The lack of CITES protection makes it extremely difficult to quantify legal and illegal captures, as well as international exports of this threatened species (Cruz-Romo & de Oliveras Ita 2011).

One option for understanding the role that pet trafficking and other anthropogenic stressors might play in population declines in the Painted Bunting is to use genetic markers to identify samples collected anywhere along the migratory pathway back to their breeding population of origin. Modern molecular markers such as mitochondrial DNA, short sequence repeats (microsatellites) and single nucleotide polymorphisms (SNPs) are useful intrinsic markers for determining population structures as well as for tracking large-scale movements of migratory individuals (Avise 2004, 2008, Rundel et al. 2013, Ruegg et al. 2014, Contina et al. 2017). The same sorts of genetic tools have been employed in a forensic DNA framework to identify individuals that have been confiscated as a result of illegal wildlife trafficking (Iyengar 2014). While these forensic approaches have been used effectively on several species such as baleen whales, fish, macaws and lizards (Baker & Palumbi 1994, Ogden et al. 2009, Galimberti et al. 2013, Welton et al. 2013a, Presti et al. 2015), to date the efficacy of such techniques has not been tested in an exploited neotropical migratory bird system.

Here we investigate patterns of population structure in Painted Buntings from across the species breeding and wintering range using microsatellite DNA markers. We begin with an assessment of population structure across the breeding range and discuss the utility of our markers for migratory connectivity analysis more generally. We then take the opportunity to test the utility of our markers on a small subset of samples from the pet trade and discuss the implications of our results in the Painted Bunting as well as the use of genetic markers for combating wildlife tracking more broadly.

**METHODS**

**Natural population dataset and pet-trade examples**

Our genetic sampling comprised 131 Painted Buntings sampled at 15 sites across the species breeding and wintering geographical range (Fig. 1). Our dataset included DNA aliquots for 50 birds previously sequenced by Herr et al. (2011) and purified genomic DNA for 81 adult birds using blood, muscle and feather samples from museum collections and mist-netting operations (Supporting Information Table S1).

We included samples from two distant populations in Louisiana – Johnson Bayou and Bayou Cocodrie – which are about 245 km apart. However, our field site at Bayou Cocodrie was practically contiguous to the Mississippi state border; therefore, we refer to the samples collected in Bayou Cocodrie as from a 'Mississippi population' for a better geographical characterization and to distinguish them from the samples collected at Johnson Bayou in southwestern Louisiana. At the wintering grounds in Costa Rica, eight birds were...
captured at sites < 40 km apart (Tamarindo and Guardia), so we treated them as if they were obtained from a single population.

To provide an example of an applied pet-trade forensic investigation, we acquired five Painted Bunting samples from the pet market in central Mexico and Europe, and performed genetic assignment tests. In Mexico, between October and December 2013, we visited eight markets in Mexico City that were referred to us for trading in birds. We found Painted Buntlings for sale in three markets for about 10 USD each, and tail feathers were taken from four individuals: one adult male was acquired from Mercado La Argentina; one bird of unknown sex and age was acquired from Mercado de Xochimilco; and one adult male together with one bird of unknown sex and age were acquired at the Catedral de Texcoco, a town about 20 km northeast of Mexico City. We relied on plumage characteristics to age and sex each bird according to Pyle (1997). In Italy, we acquired one male Painted Bunting from a large distributor of exotic birds located near Pavia for 700 USD.

DNA extraction

We stored ~30 μL of whole blood from the brachial vein in 0.4 mL of Queen’s lysis buffer at 4 °C (Seutin et al. 1991). Muscle tissues from Sinaloa birds were frozen in liquid nitrogen and stored at −20 °C. We stored primary and tail feathers (first or ninth primary feather or first and second rectrices) at room temperature in paper envelopes to absorb humidity and allow the feather calamus to dry, thus limiting DNA degradation. We purified DNA from blood and muscle samples using the Qiagen DNA extraction kit (Qiagen Inc., Valencia, CA, USA) according to the protocols supplied by the manufacturer, whereas DNA from feather samples was purified following a modified feather digestion procedure (8 h at 57 °C) using a DNA extraction mixture without dithiothreitol (modified from De Volo et al. 2008).

Microsatellite genotyping

We made use of 13 microsatellite primer pairs developed by Contina et al. (2016a,b) (Supporting Information Table S2) to PCR-amplify loci, and visualized the electropherograms and scored allele sizes in PEAKSCANNER 2.0 (Applied Biosystems, Carlsbad, CA, USA). We implemented a microsatellite genotyping method following the M13 hybrid primer technique to reduce the costs of molecular laboratory work (Schuelke 2000). We analysed each DNA sequence using an ABI 3130XL sequencer with an internal size standard (Genescan LIZ-600; Applied Biosystems). We performed PCR following thermocycler conditions detailed in Contina et al. (2016a). We used MICRO-CHECKER (Van Oosterhout et al. 2004) to detect the presence of null alleles, large allele dropout and scoring errors. We did not rescore individuals to estimate an error rate.

Population genetic diversity and differentiation

We used ARLEQUIN 3.5.1.3 (Excoffier & Lischer 2010) to compute several population genetic tests implementing the Markov chain Monte Carlo (MCMC) algorithm using 10^6 iterations, 10^5 dememorization steps and significance level α < 0.05. First, we computed the expected and observed heterozygosity for each locus within each population and tested for deviation from Hardy–Weinberg equilibrium (HWE) (Guo & Thompson 1992) and linkage disequilibrium (LD) (Slatkin 1995). We used the program FSTAT (Goudet 2001), which corrects for variation in the number of sampled individuals, to calculate the number of alleles and allelic richness per locus and population to make comparisons across the species range (Nei 1988, Petit et al. 1998). We tested the isolation-by-distance (IBD) model (Wright 1943) in GENEPOP through the ISOLDE option, which runs a regression of FST/(1 − FST) estimates for population pairs on the logarithm of their geographical distances (Raymond & Rousset 1997).

Population genetic structure

We ran the Bayesian clustering algorithm implemented in the program STRUCTURE 2.3.2.1 (Pritchard et al. 2000) to investigate the potential for population structure across the breeding grounds with the full set of 13 Painted Bunting loci developed by Contina et al. (2016a). We ran STRUCTURE using the correlated allele
frequencies prior and both with and without the location prior function (LOCPRIOR). When using the LOCPRIOR, individuals sampled from the same area are assumed to have their admixture proportions (Q values) drawn from the same Dirichlet distribution (Hubisz et al. 2009). We tested the number of clusters \( K = 1 - 6 \) using 10 replicate simulations of \( 10^5 \) MCMC repetitions each and a burn-in of \( 10^5 \) iterations. We determined the most likely value of \( K \) by following the \( \Delta K \) method (Evanno et al. 2005) implemented in STRUCTURE HARVESTER (Earl & vonHoldt 2012). We merged the results for the best model with the highest likelihood assignment scores (François & Durand 2010) with CLUMPP (Jakobsson & Rosenberg 2007), and converted the output into a postscript file with DISTRUCT (Rosenberg 2004). We visualized the postscript file in Adobe ILLUSTRATOR CC (Adobe Systems, San Jose, CA, USA).

Assignment of wintering and pet-trade individuals

To assign birds of unknown origin back to their breeding population we used the USEPOPINFO function within a population admixture framework in STRUCTURE. We used the result of our population structure analysis from above to inform the separation of the breeding grounds into three separate groups: (1) western breeders (Central TX and OK), (2) central breeders (Western TX, Eastern TX, AR, LA, MS) and (3) eastern breeders (GA and NC). Samples from the wintering grounds (Sin, Oax, Yuc, GU, NI, CR) and our five pet-trade individuals were coded as ‘migrants from unknown breeding populations’ and assigned to one of the three breeding populations using 10 replicate simulations of \( 10^5 \) MCMC repetitions each and a burn-in of \( 10^5 \) iterations, and we used the default parameters GENSBACK = 2 and MIGRPRIOR = 0.05.

We also ran a genotype assignment test based on allele frequencies for samples collected in Oklahoma, a representative western breeding population, as well as samples collected in North Carolina, a representative eastern breeding population, and used the pairwise log-likelihood assignment probabilities calculated in ARLEQUIN to visualize the extent of differentiation between these populations following Paetkau et al. (1997) and Waser and Strobeck (1998).

RESULTS

Population genetic diversity and structure

We provide insight on the genetic diversity of Painted Bunting populations across most of the species breeding and wintering range in the USA and Central America through PCR-amplification of 3406 alleles across 13 microsatellite loci among 131 individuals (Dryad Digital Repository: https://doi.org/10.5061/dryad.mg4nc21). A detailed account of the number of alleles sampled and allelic richness per locus and population across the species distribution range is presented in Table 1. No sample failed to PCR-amplify at any locus and we did not find evidence for large allele dropout or scoring error due to stuttering.

We found significant pairwise \( F_{ST} \) and \( R_{ST} \) distances in 71.4% and 24.7% of all comparisons across 15 populations, respectively, with larger values generally observed between populations of more distant sites (Table 2, Supporting Information Table S6). The test for isolation-by-distance (IBD) recovered a moderate but significant correlation between genetic differentiation \( (F_{ST}) \) and geographical distance \( (R^2 = 0.46; \ P = 0.005) \). \( F_{ST} \) values ranged from 0.008 to 0.12 whereas \( R_{ST} \) values ranged from 0.001 to 0.31 across all the populations from the breeding and wintering grounds. The highest significant \( F_{ST} \) value was between Georgia and Costa Rica \( (F_{ST} = 0.12, \ P = 0.02) \) and the lowest was between Arkansas and eastern Texas \( (F_{ST} = 0.008, \ P = 0.48) \). The highest significant \( R_{ST} \) value was between Oklahoma and western Texas \( (R_{ST} = 0.31, \ P < 0.0001) \) and the lowest, but significant, value was between Oklahoma and Sinaloa \( (R_{ST} = 0.04, \ P = 0.02) \). We also recovered a very low \( F_{ST} \) value between Oklahoma and Sinaloa \( (F_{ST} = 0.01, \ P = 0.07) \), the latter of which is known to host birds migrating from Oklahoma in the autumn (Contina et al. 2013). We found that locus 11 (Pb11 in Table S2) had the highest allelic richness value (ARV) across populations (ARV locus 11 = 3.6). Some populations showed loci deviating from Hardy–Weinberg equilibrium (HWE) and evidence of linkage disequilibrium (Supporting Information Tables S3 & S4). As results for HWE and LD were not consistent among populations, we use all the loci in the analyses reported below.

STRUCTURE suggested differentiation among samples from the western, central and eastern
Table 1. (a) Number of alleles sampled per locus and population. (b) Allelic richness per locus and population. Population sample size is provided in parentheses.

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(a) Number of alleles

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Locus 2 | 3 | 2.689 | 2.624 | 2.624 | 2.48 | 2.81 | 2.497 | 1 | 2.276 | 3.016 | 3.384 | 2.697 | 2.859 | 3.104 | 2.789
Locus 4 | 4 | 2.985 | 3.051 | 2.495 | 2.699 | 3.283 | 2.97 | 1 | 2.44 | 2.67 | 3.56 | 3.18 | 3.485 | 3.419 | 2.846
Locus 8 | 2 | 1.974 | 1.909 | 2.495 | 2.81 | 2.142 | 2.791 | 3 | 2.924 | 1.971 | 1.791 | 2.434 | 2.445 | 1.975 | 1.76
Locus 10 | 3 | 2.609 | 2.182 | 2.402 | 2.312 | 1.816 | 2.97 | 1 | 3.356 | 2.474 | 2.712 | 2.404 | 3.031 | 2.147 | 2.383
Locus 12 | 4 | 2.645 | 2.917 | 3.145 | 2.699 | 2.115 | 2.92 | 4 | 2.775 | 3.364 | 2.591 | 2.311 | 2.81 | 2.845 | 2.838
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breeding populations (Fig. 2, Supporting Information Fig. S2). We found that the most likely hierarchical group ($K$) was equal to two for the admixture model according to the Evanno et al. (2005) $\Delta K$ method and three when we included the location information as a prior (LOCPRIOR; Supporting Information Fig. S1). The Bayesian population structure analysis based on either the admixture or the admixture + LOCPRIOR model suggested a similar sub-division within the species range for higher values of $K$ (e.g. $K \geq 3$; Fig. S2).

Overall, the population subdivision at the breeding grounds supported evidence for three main groups: a western cluster covering Oklahoma and central Texas, a central cluster formed by eastern and western Texas, Arkansas, Louisiana and Mississippi, and an eastern group that includes the two breeding populations along the Atlantic coast of the USA (Fig. 2 and Fig. S2). STRUCTURE identified some individuals, possibly migrants from the central cluster, within the western and eastern breeding populations as indicated by the red $Q$-bars in Figure 2 (see also Fig. 3 USEPINFO results for further details). The breeding populations of the central cluster shared some genetic similarity with individuals sampled from the eastern cluster (Georgia and North Carolina; Fig. 2).

**Origin of migrants wintering in Central America**

Although we could not determine the breeding origin of all migrants, many birds sampled on the wintering grounds were confidently assigned to one of the three putative breeding region clusters (western, central, eastern; Fig. 3). The lack of assignment power might be due to admixed birds breeding between our two sampled breeding source populations or, alternatively, to the occurrence of unsampled genetic variation on the breeding grounds, which, if included, would improve assignments of wintering birds. About 60% of migrants that passed through Sinaloa and to some extent Oaxaca could be assigned to western breeders with confidence ($Q > 0.8$). Migrants wintering in Guatemala, Nicaragua, Costa Rica and the Yucatan Peninsula showed a mixture of assignment probabilities to one of the three breeding groups (e.g. individuals 98, 107, 116 and 125 in Fig. 3 and Supporting Information Table S5), perhaps reflecting gaps in our sampling.

![Figure 2. Individual $Q$ matrix calculated in STRUCTURE. The graph shows the assignment probabilities ($Q$) of Painted Bunting clusters across the species breeding distribution range. Each area within two vertical black lines represents a population and the area width is proportional to the number of individuals sampled. The probability ($Q$) of each individual to be assigned to one or more clusters is shown on the vertical axis and the clusters are represented by different colours. The figure shows results for $K = 3$ based on the admixture (ADMX) and admixture + LOCPRIOR (ADMX + LOCPRIOR) models, respectively. The two models yielded similar results with slightly less evident population structure when LOCPRIOR was not implemented. [Colour figure can be viewed at wileyonlinelibrary.com](#)
effort across the breeding grounds. A few migrants wintering in the Yucatan Peninsula could be assigned to eastern breeders ($Q > 0.6$; e.g. individuals 118 and 122 in Fig. 3 and Supporting Information Table S5), whereas pet-trade samples were consistently associated ($Q$ scores ranged from 0.6 to 0.77) with the breeding birds of the central clade (Fig. 3, Table S5).

To illustrate the resolving power of the genotype analysis based on population allele frequencies computed in ARLEQUIN, we plotted the pairwise log-likelihood assignment probabilities of individuals from Oklahoma (western breeding population) against the log-likelihood assignment probabilities of individuals from North Carolina (eastern breeding populations). This plot shows marked differences in assignment probabilities between the representative samples from the western and eastern population, although no apparent plumage or other morphological differences are evident (Fig. 4).

**DISCUSSION**

Our research demonstrates that Painted Buntings can be separated into three main groups on the breeding grounds using microsatellite markers – a western, central and eastern group. By taking advantage of the genetic distinctiveness on the breeding grounds, we demonstrate how microsatellites can serve as a low-cost method (about 10 USD per sample) for identifying the population of origin of birds captured from anywhere along their migratory cycle, as well as sold in the international avian pet market. Below we outline the implications of our findings for our understanding of migratory connectivity in the Painted Bunting, as well as the potential use of microsatellite markers for forensic analysis in this species.

Overall, we recovered $F_{ST}$ and $R_{ST}$ distances between pairwise comparisons of populations that followed a pattern of differentiation in agreement with the STRUCTURE results, with greater differentiation between more distant sampling sites. However, in the STRUCTURE results we also recovered a signature of eastern alleles occurring within the central clade, whereas the western clade appeared separated from the central group despite geographical continuity (Figs 1 & 2). Storer (1951) studied Painted Bunting morphology and plumage coloration and claimed that the
two sub-species met along a zone of introgression running through the middle of the western range, providing an expanded range to ‘P. c. ciris’ to the west and ignoring the species’ discontinuous geographical breeding distribution. The concept of two breeding populations of Painted Bunting connected through a zone of introgression running across parts of Oklahoma and Texas was supported by the results from STRUCTURE analyses without the LOCPRIOR function implemented (e.g. \( K = 2 \) in Fig. S2). Furthermore, Battey et al. (2017) investigated the possibility of a migratory divide in the Painted Bunting using single nucleotide polymorphisms (SNPs) and showed evidence of a zone of introgression running through the western part of the species breeding range. Our microsatellite DNA results also support the occurrence of genetic differentiation among populations within the western breeding range (Fig. 2 and Fig. S2). The biogeographical underpinning of this pattern remains difficult to explain even in light of the results provided by Battey et al. (2017). We speculate that differences in moult and migration strategy have evolved across the species range in response to a variety of environmental conditions. Determining where the zone of introgression occurs between the western and central breeding clades will improve our understanding of which factors contributed to the population differentiation patterns described here and elsewhere.

Furthermore, when we implemented the LOC PRIOR function in STRUCTURE we found clear support for three populations on the breeding grounds (Fig. 2 and Fig. S1). Because one of the objectives of the present study was to test the utility of microsatellite markers for population assignment of non-breeding birds to breeding locations at as fine a spatial scale resolution as possible, we used \( K = 3 \) as the number of source populations on the breeding grounds and added usable information (i.e. LOCPRIOR) to our assignment tests. Our results suggest that microsatellite DNA can be successfully adopted in the Painted Bunting to study population genetic structure, assign breeding origin of migrants or pet-trade birds, and in some cases investigate sequence repeats in candidate genes related to migration (Contina et al. 2016b, 2018). On the other hand, the full potential of adopting an SNP approach might emerge once genomic variant screening and selection are conducted through samples collected from multiple breeding populations in proximity of the zone of introgression, where an informative sub-set of variants is developed through reproducible molecular assays, and novel candidate genes under selection
are discovered throughout the genome (DePristo et al. 2011, Jonker et al. 2012, Bay et al. 2018).

Through our analysis of assignment posterior probabilities using the program STRUCTURE, we found that birds wintering in Guatemala, Nicaragua, Costa Rica and the Yucatan Peninsula were most likely from the central and eastern breeding population, whereas birds sampled from Sinaloa and, to some extent, Oaxaca (southern Mexico) were most likely from the western clade. However, our assignment probabilities for 22 wintering samples (about 35% of our migratory bird dataset) did not resolve their breeding origin with confidence (grey colours in Table S5). This may result from unsampled genetic variation on the breeding grounds or because the central population represents a transition zone between the western and eastern group (see also Battey et al. 2017).

Our assignment results for the Sinaloa and about half of the Oaxaca samples are consistent with the pattern for the western population recently inferred by Contina et al. (2013). Contina et al. (2013) implemented an ultra-light geolocator design to track Painted Buntings from a breeding population in Oklahoma and revealed a moult-migration strategy: birds moving westward at the end of the summer toward Sinaloa (northwest Mexico) stopped to moult before continuing along the west coast of Mexico and then returned to the breeding sites in the spring along the east coast of Mexico. It is thought that this moult-migration strategy may allow Painted Buntings to exploit higher biological productivity resulting from monsoonal rains in Mexico (Rohwer et al. 2005, Pyle et al. 2009, Bridge et al. 2016). Laws for the conservation of migratory species take into account spatial and temporal challenges of protecting populations that move across natural landscapes and geopolitical borders (Ellison 2014) and we recommend that conservation action plans should specifically address the migratory behaviour occurring across the entire annual cycle (Marra et al. 2015). Therefore, we propose that Sinaloa should be considered a target area for prioritizing conservation efforts for the Painted Bunting and potentially other migrant species.

Although wintering population contact was previously considered unlikely on the basis of an earlier study which examined differences in wing-length across wintering locations and used such morphological differences to assign birds to breeding populations of origin (Thompson 1991a), our results suggest possible contacts between individuals from the western and eastern populations on the wintering grounds (e.g. Yucatan Peninsula). Our results are thus consistent with banding records that indicate that at least some eastern Painted Buntings migrate through Cuba and the Caribbean (Sykes et al. 2007). Our findings are important in light of conservation management of separate breeding populations of Painted Bunting, including the small and declining eastern bunting population, as wintering locations hosting a mixture of western and eastern buntings should be closely monitored as potential sites for conservation efforts.

In addition to using our markers to identify patterns of migratory connectivity across the range, we were also able to identify the population of origin of a small sub-sample of birds sold in the international avian pet market (both Mexico and Europe). This exploratory examination showed that the pet-market birds are most likely from the central breeding population of Painted Buntings (Fig. 3, Table S5). Due to our small sample size we do not exclude the possibility that many buntings sold as pets worldwide may have been sourced from populations breeding along the Atlantic coast of the USA and wintering in Central America and the Caribbean. We simply note that our low-cost molecular approach to assign pet-market samples to natural populations is a valid method with great investigative potential in forensic genetic analyses of the Painted Bunting and other songbirds exploited in the international pet-trade industry, such as Indigo Bunting Passerina cyanea, Lazuli Bunting Passerina amoena, Blue Grosbeak Passerina caerulea and Northern Cardinal Cardinalis cardinalis. We note that microsatellite analyses presently offer a cheaper and easier option compared with many genomic approaches, as they can be easily implemented on degraded and low concentrations of DNA and are easier to add new samples to than, for example, ddRAD approaches such as those used by Battey et al. (2017) for the Painted Bunting.

**CONCLUSIONS**

Our findings highlight the importance of songbird migration stopover sites and wintering areas in relation to conservation plans for multiple breeding populations coming into contact during specific phases of their annual cycle. The genetic
distinctiveness and negative population trend of the eastern Painted Bunting populations (e.g. Georgia, North Carolina) supports the idea that this population represents an evolutionarily significant unit (ESU, sensu Moritz 1994) in agreement with Herr et al. (2011). The demonstrated utility of our makers for connectivity and forensic analysis suggests that microsatellites may function as a low-cost alternative for population identification in the Painted Bunting and other closely related migratory species.

We thank Connie Herr, Paul Sykes and John Klicka for providing 50 Painted Bunting DNA aliquots used in this study and Sievert Rohwer for collecting tissue samples in Sinaloa. We thank Ann Harris and the Core Molecular Biology Laboratory at the University of Oklahoma for helping with laboratory work. Larry Weider, Michael Patten, Bruce Hoagland, Tom Smith, two reviewers, Jon Martin Collinson and Rauri Bowie provided valuable comments on earlier drafts of the manuscript. We are grateful to Tyler Michels, Alice Andolfatto and the many field assistants who helped collect samples across the USA and Europe. We thank The Institute for Bird Populations together with MAPS and MoSI banding station managers, and UCLA – Center for Tropical Research, for providing feather samples. We thank the Wichita Mountains Wildlife Refuge in Oklahoma and St. Catherine Creek National Wildlife Refuge in Mississippi for allowing us to work in special use areas. We thank Frank Moore, Kristen Covino and the Migratory Bird Research Group of the Department of Biological Sciences at The University of Southern Mississippi in Hattiesburg for collecting bunting samples at Johnson Bayou, Louisiana. All work with animals was performed with relevant state and federal permits (#23215) and was approved by the Institutional Animal Care and Use Committee of the University of Oklahoma. This research was funded by the National Science Foundation (IDBR 1014891, DGE 1545261 and DEB 0946685) and by the United States Department of Agriculture (NIFA-AFRI-003536). Additional funding for covering the costs of ‘open access’ publication was provided by the University Strategic Organization in ‘Applied Aerocology’ at the University of Oklahoma.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** STRUCTURE HARVESTER output. The K-values of 2 and 3 are the most likely number of clusters across the Painted Bunting breeding range based on (A) admixture (ADMX) and (B) admixture + LOCPRIOR (ADMX + LOCPRIOR) models, respectively. Model runs were averaged with 10 replicates for each value of K.

**Figure S2.** (A) Admixture (ADMX) model. (B) Admixture + LOCPRIOR (ADMX + LOCPRIOR) model. Individual Q matrix calculated in STRUCTURE. Each area within two vertical black lines represents a population and the area width is proportional to the number of individuals sampled. The probability (Q) of each individual to be assigned to a single or more clusters is shown on the vertical axis and the clusters are represented by different colours. Model runs averaged with 10 replicates for each value of K.

**Table S1.** List of sampling latitude and longitude coordinates and population cluster considered for genetic analyses. Sample ID numbers indicated in bold are from Herr *et al.* (2011).

**Table S2.** List of loci used in the present study and their corresponding code from Contina *et al.* (2016a) and the National Center for Biotechnology Information (NCBI) accession numbers.

**Table S3.** Expected and observed heterozygosity for each locus for each population and Hardy–Weinberg equilibrium results (significant P-values are highlighted in bold).

**Table S4.** Schematic table showing linkage disequilibrium results in each population. The + symbol indicates possible occurrence of linked loci.

**Table S5.** STRUCTURE assignment probabilities for migratory birds collected at the wintering grounds. The probability (Q) of each individual to be assigned to a single clade is shown in the assignment probability columns and ranges from 0 to 1 (with Q > 0.6 considered a robust clade assignment value). Assignment probabilities for sample numbers 92, 102, 105 and 106 were rounded to 0.6. The western, central and eastern clades are represented by different colours, and assignments that yielded uncertain results (Q < 0.6) are shown in grey.

**Table S6.** $R_{ST}$ values for pairwise comparisons of populations calculated in ARLEQUIN. Significant values ($P < 0.05$) are highlighted in bold.