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SYMPOSIUM

Patterns of DNA Methylation Throughout a Range Expansion of an Introduced Songbird

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Synopsis The spread of invasive species presents a genetic paradox: how do individuals overcome the genetic barriers associated with introductions (e.g., bottlenecks and founder effects) to become adapted to the new environment? In addition to genetic diversity, epigenetic variation also contributes to phenotypic variation and could influence the spread of an introduced species in novel environments. This may occur through two different (non-mutually exclusive) mechanisms. Individuals may benefit from existing (and heritable) epigenetic diversity or de novo epigenetic marks may increase in response to the new environment; both mechanisms might increase flexibility in new environments. Although epigenetic changes in invasive plants have been described, no data yet exist on the epigenetic changes throughout a range expansion of a vertebrate. Here, we used methylation sensitive-amplified fragment length polymorphism to explore genome-wide patterns of methylation in an expanding population of house sparrows (*Passer domesticus*). House sparrows were introduced to Kenya in the 1950s and have significant phenotypic variation dependent on the time since colonization. We found that Kenyan house sparrows had high levels of variation in methylation across the genome. Interestingly, there was a significant, potentially compensatory relationship between epigenetic and genetic diversity: epigenetic diversity was negatively correlated with genetic diversity and positively correlated with inbreeding across the range expansion. Thus, methylation may increase phenotypic variation and/or plasticity in response to new environments and therefore be an important source of inter-individual variation for adaptation in these environments, particularly over the short timescales over which invasions occur.

Introduction

Introduced species offer an opportunity to study the evolution of small populations in novel, or changing environments. The expansion of newly introduced populations, which often are small and presumably not adapted to their new habitat, is somewhat of a genetic paradox (Allendorf and Lundquist 2003). The reduction of genetic diversity in small populations can limit population growth and the ability to evolve in novel environments, as is often observed in conservation biology (Allendorf and Lundquist 2003). However, many introduced species are successful in their new environments despite few initial colonists, bottlenecks, and founder effects. Epigenetic mechanisms, such as DNA methylation, may contribute to the success of introduced species in novel habitats (Perez et al. 2006; Rando and Verstrepen 2007; Herrera et al. 2012; Richards et al. 2012; Schrey et al. 2012).

Research in both plants and animals (Dolinoy et al. 2007; Kucharski et al. 2008; Bossdorf et al. 2010; Zhang et al. 2013) suggests that epigenetic processes partially mediate environmentally induced phenotypic variation (Vogt et al. 2008; Angers et al. 2010; Gao et al. 2010; Richards et al. 2010). Epigenetic marks can be induced or removed in response to environmental cues throughout the lifetime of an individual (Angers et al. 2010; Verhoeven et al. 2010). Changes in methylation not only influence mean values of traits but also the

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plasticity of certain traits (Bossdorf et al. 2010; Zhang et al. 2013). Increased plasticity and the fact that epigenetic changes can occur within the lifetime of an individual indicate that epigenetic mechanisms may mediate changes on a finer timescale than genetically-based evolution; this ability to finely-tune a phenotype in response to environmental cues might be especially important in novel and/or changing habitats. Given the rapid response of epigenetic changes, they may increase the fitness of local populations (Herrera and Bazaga 2011). Additionally, variation in methylation can be greater than (Richards et al. 2012; Schrey et al. 2012) and independent of genetic variation (Herrera and Bazaga 2011), indicating that changes in epigenetic diversity may occur despite reductions in genetic diversity (e.g., as might be expected following a bottleneck). There have been few methylation sensitive-amplified fragment length polymorphism (MS-AFLP) studies focusing on differences in methylation in introduced species (but see Chwedorzewska and Bednarek 2012; Richards et al. 2012; Schrey et al. 2012) and few of natural variation in MS-AFLP among vertebrates (but see Massicotte et al. 2011; Massicotte and Angers 2012; Schrey et al. 2012), but these studies indicate that DNA methylation may play an important role in the adaptation of introduced vertebrates in novel environments.

House sparrows (*Passer domesticus*) are one of the world's most broadly distributed vertebrate species and have been introduced to much of their range (Anderson 2006). Phenotypic differences among populations indicate they are able to circumvent the loss of genetic diversity associated with an introduction (e.g., Johnston and Selander 1971; Martin and Fitzgerald 2005; Martin et al. 2005). Epigenetically, Schrey et al. (2012) revealed greater methylation at two loci in a recently introduced population of house sparrows compared with one that had colonized earlier; this may indicate that methylation is an important facet of house sparrow invasion and range expansion.

One of the most recently introduced house sparrow populations was to Mombasa (MO), Kenya, in the 1950s (Anderson 2006); from MO, despite a small founding population (Anderson 2006), house sparrows have expanded to most major cities in Kenya and, compared with other populations, Kenyan house sparrows have reduced genetic diversity (Schrey et al. 2011). Within Kenya, microsatellite-based genetic data indicate that the main expansion of house sparrows occurred along the major highway in southern Kenya (connecting MO to Nairobi [NA] and then west toward Uganda) with genetic admixture (A. Schrey et al., in review). Despite low genetic diversity, genetic admixture among cities, and a brief existence in Kenya, house sparrows exhibit phenotypic differentiation in a pattern consistent with the length of time since colonization (Liebl and Martin 2012, 2013; L. B. Martin et al., in review). If phenotypic differentiation in Kenyan house sparrows was solely dependent on underlying genetic differentiation, we would expect no clear pattern of phenotypic variation to emerge; this outcome is, however, contrary to what we find.

Here, we screened natural variation in DNA methylation among cities throughout the range expansion of house sparrows in Kenya. We described epigenetic diversity and differentiation among sites in relation to the initial point of introduction, MO. Our goal was to determine whether variation and differentiation exist in genome-wide DNA methylation among individuals and/or cities across Kenya. By further extending the scope of our analysis by comparing our results with that of a microsatellite-based genetic study of the same individuals (A. Schrey et al., in review), we could determine whether epigenetic and genetic variation are related in Kenyan house sparrows. Thus, we were able to test the hypothesis that epigenetic diversity might act as a compensatory mechanism for reduced heterozygosity and increased inbreeding, as is often seen after an introduction.

Materials and Methods

Collection of data on epigenesis

We screened epigenetic variation in 43 individuals from seven cities across Kenya (Table 1 and Fig. 1): MO, Malindi/Watamu (MA), Garsen (GA), NA, Nyeri (NY), Nanyuki (NN), and Kakamega (KA). Individuals were bled at capture and blood was preserved in RNAlater (Qiagen) at room temperature for up to 3 months before being frozen (−20°C). DNA was extracted from 50 µl of sample mixture (i.e., RNAlater plus blood) using a standard phenol:chloroform protocol for DNA extraction (Russell and Sambrook 2001).

We performed MS-AFLP (Reyna-Lopez et al. 1997) with the protocol described by Schrey et al. (2012), which modified an AFLP protocol by substituting methylation-sensitive isoschizomeric enzymes *MspI* and *HpaII* for *MseI*. *MspI* and *HpaII* have different sensitivities to cytosine methylation. Thus, by performing the protocol in parallel for each enzyme for every individual, we could identify the state of methylation at each restriction site. We used one primer combination for selective PCR (Schrey et al. 2012) at a final volume of 10 µl; the

Table 1 Kenyan cities where house sparrows were collected and screened for variation in DNA methylation (with abbreviations, Abb.)

City	Abb.	dfM	*N*	M/F/I	*h*	%*P*	H_O	H_E	F_{IS}
Mombasa	MO	0	5	1/2/2	0.28	58.06	0.82	0.75	−0.24
Malindi	MA	120	6	4/2/0	0.37	83.87	0.69	0.82	0.06
Garsen	GA	230	4	3/1/0	0.44	80.65	0.64	0.82	0.10
Nairobi	NA	500	8	3/2/3	0.34	77.42	0.68	0.71	−0.03
Nyeri	NY	630	5	2/3/0	0.32	61.29	0.80	0.71	−0.26
Nanyuki	NN	675	9	4/5/0	0.41	90.32	0.71	0.75	−0.01
Kakamega	KA	885	6	4/2/0	0.39	80.65	0.69	0.80	0.01
Total			43	21/17/5	0.36	76.04	0.72	0.77	−0.05

The distance (km) from Mombasa (dfM), number of individuals screened for variation in DNA methylation and microsatellite loci (*N*), individual information (i.e., number of males, females, and immature individuals (M/F/I)), epigenetic diversity (as haplotype diversity, *h* and percentage of polymorphic loci, %*P*; determined using GENALEX-6), along with a summary of data from microsatellite-based genetic data (observed heterozygosity, H_O, expected heterozygosity, H_E, and the inbreeding coefficient, F_{IS}; A. Schrey et al., in review) are provided for each city.

thermal cycle was 95°C for 2 m, 95°C for 30 s, 53°C for 30 s, 72°C for 30 s, and 70°C for 5 m, repeated 40 times. We used PEAKSCANNER v 1.0 (Applied Biosystems) to analyze resultant gel files and define fragment sizes. We duplicated the entire protocol for at least two individuals from each city to identify bands that consistently occurred and eliminated bands that inconsistently amplified or occurred at highly variable intensities. We pooled data into two categories: methylated (Type II and Type III) or not methylated (Type I) (Salmon et al. 2008). Type IV epigenetic variation was not included in the analysis, as it can be generated either by an epigenetic modification or a change in DNA sequence at the restriction site.

Analysis of epigenetic data

All analyses were conducted using a binary haplotype-binding pattern (above; 1 for methylated and 0 for not methylated) for a total of 31 banding sites between 50 and 500 bp. Due to low sample sizes of either sex from each city, we pooled all individuals for all analyses. We calculated haplotype diversity (*h*) and the proportion of polymorphic loci (%*P*) with GENALEX-6 (Peakall and Smouse 2006) to characterize epigenetic diversity. These estimates were compared with distance from MO (dfM), the site of initial introduction, as a proxy of time since colonization (Table 1) (Liebl and Martin 2012). We also calculated Φ_{ST} among sites using the AMOVA framework of GENALEX-6 to estimate the amount of epigenetic differentiation among cities. Φ_{ST} was calculated over all loci and locus-by-locus; statistical significance was estimated after 9999 permutations. We used a sequential Bonferroni correction of $\alpha = 0.05$ for multiple tests (Rice 1989).

Contrasting epigenetic and genetic characteristics

As the genetic and epigenetic estimates of diversity and differentiation are fundamentally different, we could not directly compare them; therefore, we compared the pattern of change in these estimates throughout the range expansion. To determine whether epigenetic and genetic variation and differentiation were similar, we compared the MS-AFLP-based diversity (*h* and %*P*) and differentiation (Φ_{ST}) with genetic characteristics of microsatellite loci of the same individuals, described elsewhere (Table 1) (A. Schrey et al., in review). Specifically, we compared the MS-AFLP results with observed heterozygosity (H_O; which increased with dfM), expected heterozygosity (H_E; which tended to decrease with dfM), inbreeding (F_{IS}; which decreased with dfM), and F_{ST} (which detected significant genetic differentiation among all seven cities, yet no relationship with geographic distance) (A. Schrey et al., in review). We used a sequential Bonferroni correction of $\alpha = 0.05$ for multiple tests.

To address the possibility of Type II error in the Φ_{ST} estimates of differentiation at MS-AFLP loci, we performed a power analysis following Cohen's (1988) proportion of variance method for *F* statistics. We determined the power of our MS-AFLP data to detect differentiation among Kenyan house sparrows from the effect size corresponding to the amount of genetic differentiation detected with microsatellites ($\Phi_{ST} = 0.127$). We then estimated power from the effect size, with degrees of freedom = 6, $n = 42$ and 44 (bracketing the actual $n = 43$), and $\alpha = 0.05$.

Results

Observed epigenetic diversity and differentiation

There was a great deal of variation in DNA methylation among individuals, such that all individuals

Fig. 1 Map of Kenya. House sparrows were introduced to MO, Kenya, in the1950s and have subsequently spread across Kenya and into other countries in East Africa. In this study, house sparrows were captured from seven cities (Mombasa, Malindi, Garsen, Nairobi, Nyeri, Nanyuki, and Kakamega) indicated by blue squares on the map. Figure was adapted from the USA CIA's open database.

had unique epigenotypes. Among cities, h ranged from 0.28 to 0.44 and $\%P$ ranged from 0.58 to 0.90 (Table 1). There was no relationship between h and dfM, $\%P$ and dfM, nor was there epigenetic differentiation among any of the cities; over all loci $\Phi_{ST} = 0.004$, $P = 0.41$, and locus-by-locus Φ_{ST} ranged from −0.14 to 0.19. No locus-by-locus estimate of Φ_{ST} was significant. The power analysis indicated that the MS-AFLP data had power >0.995 to detect a similar level of differentiation as that detected among cites with microsatellites.

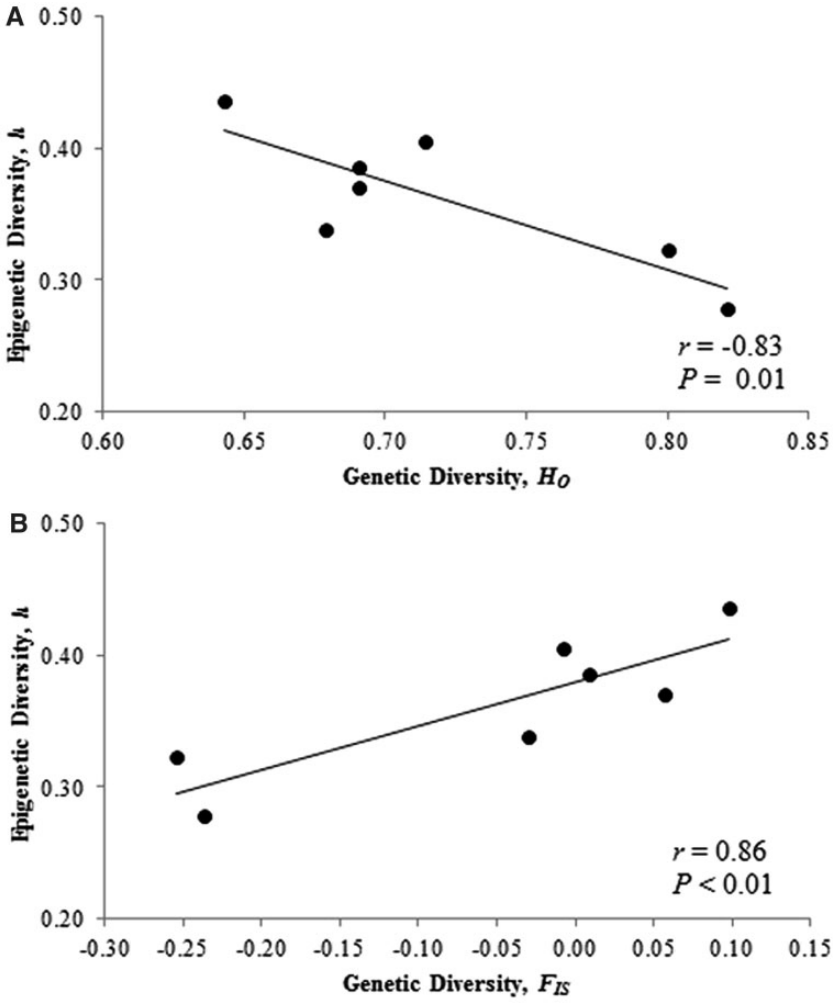

Fig. 2 Epigenetic diversity is related to genetic diversity among house sparrows from seven Kenyan cities screened at both MS-AFLP and microsatellite loci. (**A**) Epigenetic diversity was negatively correlated with observed heterozygosity (H_O): as H_O increased, both haplotype diversity (h) and the proportion of polymorphic loci (%P) decreased ($r = -0.83$, $P = 0.01$ and $r = -0.82$, $P = 0.01$ respectively). (**B**) Epigenetic diversity was positively correlated with the inbreeding coefficient (F_{IS}): as F_{IS} increased, both h and %P increased ($r = 0.86$, $P = 0.007$ and $r = 0.89$, $P = 0.004$, respectively). Only h is shown here due to the high correlations between h and %P.

Contrasting epigenetic and genetic characteristics

We detected a significant relationship between epigenetic and genetic diversity (Fig. 2). Epigenetic diversity (both h and %P) was negatively correlated with H_O ($r = -0.83$, $P = 0.01$; and $r = -0.82$, $P = 0.01$, respectively); further, epigenetic diversity was positively correlated with F_{IS} (h and F_{IS}: $r = 0.86$, $P = 0.007$; and %P and F_{IS}: $r = 0.89$, $P = 0.004$). There was only a marginal relationship between epigenetic diversity and H_E (h and H_E: $r = 0.64$, $P = 0.06$; %P and H_E: $r = 0.50$, $P = 0.13$).

Discussion

Epigenetic mechanisms likely impact the evolutionary potential of wild populations (Jablonka and Raz 2009; Bossdorf et al. 2010); however, this possibility has rarely been tested in wild vertebrates (but see Massicotte et al. 2011; Morán and Pérez-Figueroa 2011; Liu et al. 2012; Schrey et al. 2012). Epigenetic variation could be particularly important in introduced populations, which must adjust to novel habitats with relatively low levels of genetic variation (Richards et al. 2012). We have already shown that Kenyan house sparrows have lower levels of genetic diversity than native and longer established populations (Schrey et al. 2011) and also that they have higher levels of methylation at some loci compared with another, longer established population (Schrey et al. 2012). Although we did not find a pattern of epigenetic differentiation throughout the range in Kenya, we did find a great deal of variation in methylation among individuals. Additionally, we detected a significant, negative

relationship between epigenetic and genetic diversity. Our results suggest that following introduction to a novel habitat, epigenetic diversity may increase in areas where genetic diversity is low and inbreeding occurs.

Considerable phenotypic diversity exists among Kenyan house sparrows. Individuals on the edge of the range are more exploratory (Liebl and Martin 2012), have a greater corticosterone response to stressors (Liebl and Martin 2012, 2013), and regulate immune responses differently (L. B. Martin et al., in review) than birds from the oldest Kenyan sites. However, reduced genetic diversity and overall genetic admixture throughout the expanded range suggest that selection on genetic polymorphisms alone cannot explain these patterns. Although we did not detect epigenetic differentiation among cities throughout the expanded range (contrary to genetic differentiation) (A. Schrey et al., in review), power analyses indicate that if such variation existed (at least to the same degree as with microsatellites), we would have detected it. The lack of differentiation and overall high variability among individuals suggest that individuals maintain high levels of epigenetic variability or preserve the ability to change epigenetic marks in response to the environment.

Interestingly, we detected a significant, potentially compensatory, relationship between epigenetic diversity and genetic diversity: epigenetic diversity increased as observed heterozygosity decreased and the inbreeding coefficient increased. Epigenetic variation likely contributes to existing genomic variation. Further, when genomic variation is low, epigenetic marks may be an especially important source of phenotypic variation (Geoghegan and Spencer 2012). In fact, epimutations have been implicated as a faster source of adaptation than genetic mutations (Jablonka and Lamb 1989, 1998); organisms may use the additional variation afforded by epigenetic mechanisms as a bet-hedging strategy in unknown environments (Jablonka and Lamb 1989, 1998; Pál and Miklós 1999). We predict that when genetic diversity is low (through the loss of allelic diversity or inbreeding), greater epigenetic diversity may rescue phenotypes through increased phenotypic variation and/or plasticity in response to new environments (but see Vergeer and Ouborg 2012).

Although our design cannot discriminate the three kinds of epigenetic variation described by Richards (2006): obligatory (dependent on genetic variation), facilitated (directed by genetic variation), and pure (generated by environmental stimuli), we predict all three may play a role in range expansions. Pure epigenetic variation may be particularly important when induced in direct response to the environment, which would be adaptive when environments are unpredictable and/or changing rapidly. Regardless of the type, epigenetic effects can be generated de novo in response to environmental cues that occur within the lifetime of an individual (Herrera et al. 2012) or be stably inherited from a parent (Richards 2006; Verhoeven et al. 2010). Although inherited epigenetic marks may play a role in expansions of geographic ranges, particularly if those marks were determined during a generation following the initial expansion, marks generated de novo might be more impactful as they are likely tailored to each individual's unique developmental and environmental experience. In this respect, methylation serves as a mechanism of phenotypic plasticity (Richards et al. 2010; Herrera et al. 2012). If adaptive, these environmentally dependent effects may eventually lead to the fixation of certain traits (i.e. through genetic assimilation), but if the trait's adaptive value is contingent on environmental context, then selection should maintain methylation that is responsive to environmental stimuli (West Eberhard 2003). Therefore, if changes in the environment occur too rapidly, or too often for inherited traits to produce adaptive outcomes, epigenetic changes may facilitate the persistence of populations (Price et al. 2003; Bonduriansky and Day 2009). Even labile epigenetic marks can alter evolution (Day and Bonduriansky 2011). In the absence of environmentally induced epigenetic variation, allele frequency could increase and eventually spread to fixation; in a constant environment, an allele influenced by environmentally induced methylation could also increase in frequency, but, if the environment changed, only the allele influenced by environmentally induced methylation would have the ability to change, releasing further variation of that allele (Day and Bonduriansky 2011). In rapidly changing environments, selection should favor mechanisms that allow a wide variety of traits or lability in traits.

Both environmental and genomic stress stimulate epigenetic "repatterning," which increases phenotypic variation and could lead to novel phenotypes subject to natural selection (Rapp and Wendel 2005). Individuals presumably undergo both environmental stressors (e.g., novel environment with novel resources, predators, and parasites) and genomic stressors (e.g., bottlenecks, founder effects, and inbreeding) during introduction into a new area and subsequent range expansion. Epigenetic mechanisms increase variation and affect adaptation and divergence of stable variants without underlying genetic variation (Kalisz and Purugganan 2004; Rapp and

Wendel 2005; Jablonka and Raz 2009) and are therefore likely an important mechanism in the success of many introduced species.

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