The Epigenetic Signature of Colonizing New Environments in *Anolis* Lizards

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Abstract

Founder populations often show rapid divergence from source populations after colonizing new environments. Epigenetic modifications can mediate phenotypic responses to environmental change and may be an important mechanism promoting rapid differentiation in founder populations. Whereas many long-term studies have explored the extent to which divergence between source and founder populations is genetically heritable versus plastic, the role of epigenetic processes during colonization remains unclear. To investigate epigenetic modifications in founding populations, we experimentally colonized eight small Caribbean islands with brown anole lizards (*Anolis sagrei*) from a common source population. We then quantitatively measured genome-wide DNA methylation in liver tissue using reduced representation bisulfite sequencing of individuals transplanted onto islands with high- versus low-habitat quality. We found that lizard sex and habitat quality explained a significant proportion of epigenetic variation. Differentially methylated cytosines mapped to genes that encode proteins with functions likely to be relevant to habitat change (e.g., signal transduction, immune response, circadian rhythm). This study provides experimental evidence of a relationship between epigenetic responses and the earliest stages of colonization of novel environments in nature and suggests that habitat quality influences the nature of these epigenetic modifications.

Key words: **DNA methylation, epigenomics, phenotypic plasticity, *Anolis sagrei*, reduced representation bisulfite sequencing.**

Introduction

Island populations often rapidly diverge from their relatives on nearby islands or mainlands, a phenomenon with profound ecological and evolutionary implications. Such genetic and/or phenotypic differentiation can result from genetic drift occurring in small founding populations, or from island environments differing from each other and from the mainland, which can impose divergent natural selection (Kolbe et al. 2012; Wessel et al. 2013). Previous work has typically focused on the long-term consequences of founder effects and natural selection in colonizing populations over multiple generations, whereas the mechanisms underlying short-term phenotypic response to colonization of new environments remain relatively unexplored (Losos et al. 1994; Schoener et al. 2001; Kolbe et al. 2012).

Recently, both empirical and theoretical studies have demonstrated that epigenetic modifications, particularly DNA methylation, can contribute to rapid phenotypic changes by modulating the gene-regulatory responses to environmental conditions (Feil and Fraga 2012). For example, studies of DNA methylation have revealed associations with several environmentally influenced phenotypes, such as distinct castes in eusocial insects caused by divergent nutritional intake (Kucharski et al. 2008), sex-ratio shifts in turtles related to distinct early developmental temperatures (Matsumoto et al. 2013), and alteration of muscle composition in fish caused by different rearing temperatures (Campos et al. 2013). Recent work has suggested that expansion into new environments can lead to widespread changes in DNA methylation levels (Baerwald et al. 2016; Lea et al. 2016). However, these studies have compared long-established populations (up to 25 generations) with populations from what is thought to be the original source habitat, and thus cannot determine how early in the colonization process these changes may have occurred. Here, we report the results of a replicated whole-ecosystem field experiment in which we transplanted individuals onto small islands of differing habitat quality to quantify the epigenetic modifications accompanying the very earliest (within-generation) stage of colonization of new environments by founder populations.

Due to their experimental tractability and a rich history of studies documenting population dynamics during colonization of small islands, Caribbean *Anolis* lizards are an ideal model system for the study of many ecological and evolutionary phenomena (Losos 2009). For example, there is evidence that populations can undergo relatively rapid differentiation in fitness-related traits (e.g., length of hindlimbs) due to both founder effects and natural selection (Losos et al. 1997; Losos 2009; Kolbe et al. 2012; Jaffe et al. 2016). While these studies have focused on long-term responses of lizards to novel environments, the molecular mechanisms that might underpin immediate responses of lizards to new environments remain unexplored, and no study to date has investigated epigenetic modifications of any kind in *Anolis* lizards. Here, we provide the first single-nucleotide-resolution measurement of DNA methylation in *A. sagrei*, and use experimental introductions of lizards to small islands in the vicinity of a larger source island to evaluate the influence of habitat quality on epigenetic responses at the earliest stage of island colonization. We predicted that the magnitude of epigenetic response in our experiment (measured as differential methylation between the source population and colonizing populations) would increase with the extent of the environmental difference between the source island and newly colonized islands. Furthermore, because it has been suggested that responses to new environments over short time periods may be predictive of large-scale patterns on longer timescales (Losos et al. 1997; Arnold et al. 2001; Simons 2002), we predicted that differential methylation would map to genes associated with functions likely to be important for survival and fitness (e.g., skeletal growth; Kolbe et al. 2012) in novel environments.

Results

We introduced 211 lizards (100 males and 111 females) from the source island, Staniel Cay (Exumas, Bahamas; fig. 1) to eight small islands within 10 km. Prior to the introductions, we surveyed each island to collect vegetation and climatic data, which we used to cluster islands into high- and low-quality groups (fig. 2 and supplementary text S1 and fig. S1, Supplementary Material online), as well as to verify the absence of any existing lizard population. Low-quality islands had an average of 28.4% less-vegetated area (hereafter, "area"; means ± SD: low-quality, 235 ± 200 m^2, high-quality, 328 ± 129 m^2) and 75.6% greater daily temperature fluctuation than did high-quality islands (daily temperature difference between maximum and minimum temperatures during sampling period, mean ± SD: low-quality = 23.7 ± 2.9 °C, high-quality = 13.5 ± 1.1 °C). All experimental islands were much smaller and more thermally variable than the source island: the high-quality experimental islands had an average of 99.9% less area (source = 1,385,637 m^2) and 57.0% greater diel temperature fluctuation (source = 8.6 ± 1.2 °C) than the source island. We expected the distinct patterns of environmental variation between our treatments to cause divergent levels of physiological stress to lizards, and thus to induce different patterns of genome-wide methylation.

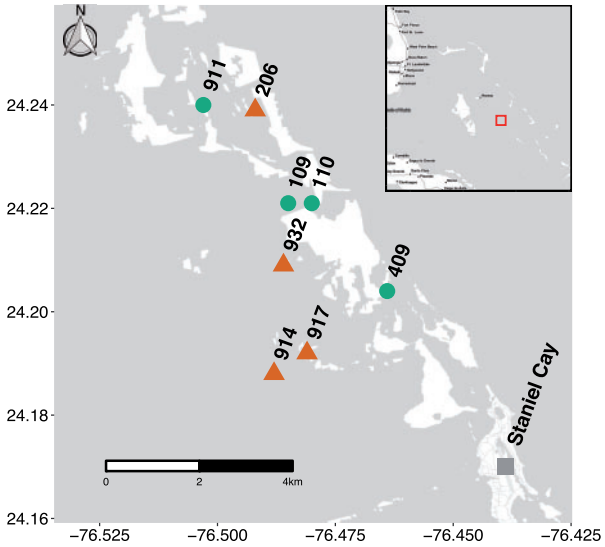

FIG. 1. Map of experimental islands. High-quality islands are shown in circle, low-quality islands are shown in triangle, and the source island is shown in square. The square in the inset shows the location of the experiment in relation to the broader geographic region (the Exuma Chain of the Bahamas).

We recaptured lizards from each experimental island ($n = 16$ from all high-quality islands; $n = 12$ from all low-quality islands) after transplanted lizards had been exposed to the new environment for at least 4 days. We sampled liver tissue from each lizard, from which we extracted DNA for reduced representation bisulfite sequencing (RRBS). We first examined how biological and ecological factors influenced genome-wide methylation levels. Using a tiling-window approach, we quantified the percentage of methylation over 1,000-bp regions throughout the genome for all source and transplanted individuals. We then used a distance-based redundancy analysis (db-RDA) to relate methylation variation to the explanatory variables of bisulfite conversion rate, sex, island, vegetated area, and average daily temperature difference. Both sex and average daily temperature were statistically significant predictors of DNA methylation variation, whereas we detected no significant effect of island, vegetated area, or bisulfite conversion rate. The whole model was significant ($P = 0.001$), with an adjusted R^2 of 0.053 (fig. 3). Partial db-RDAs revealed that the proportion of variance explained by sex was 0.04 (adjusted $R^2 = 0.04$, $F_{(1,30)} = 2.40$, $P < 0.001$), and the proportion of variance explained by average daily temperature difference was 0.013 (adjusted $R^2 = 0.013$, $F_{(1,30)} = 1.40$, $P = 0.026$).

Next, we examined the short-term epigenetic response to colonization at a finer scale by testing for differentially methylated cytosines (DMCs, single sites that vary in methylation levels between groups). We looked for DMCs across three comparisons: lizards from high-quality islands ($n = 14$, with two samples excluded due to small library size) versus lizards from low-quality islands ($n = 12$); lizards from high-quality islands versus lizards from the source island ($n = 7$, with one sample excluded due to small library size); and lizards from low-quality islands ($n = 12$) versus the source island

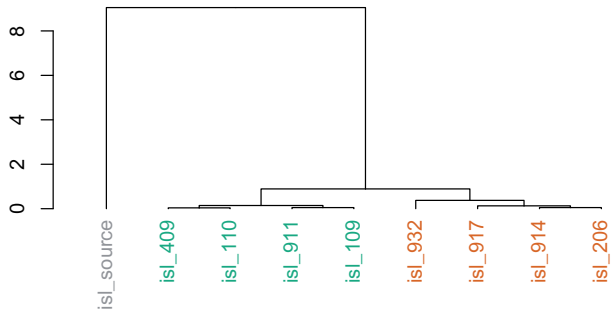

Fig. 2. Dendrogram of island quality for all treatment islands. Height is the Euclidean distance after hierarchical clustering of islands based on scaled vegetated area, which is the vegetated area of each island scaled for the mean vegetated area of all islands, and the scaled temperature difference between maximum and minimum temperatures of each island, which is the average daily mean temperature difference recorded across the sampling period on each island scaled for the average daily temperature difference across all islands. High-quality (109, 110, 409, 911) and low-quality (206, 914, 917, 932) islands are in distinct clusters, which are separated from the source island cluster.

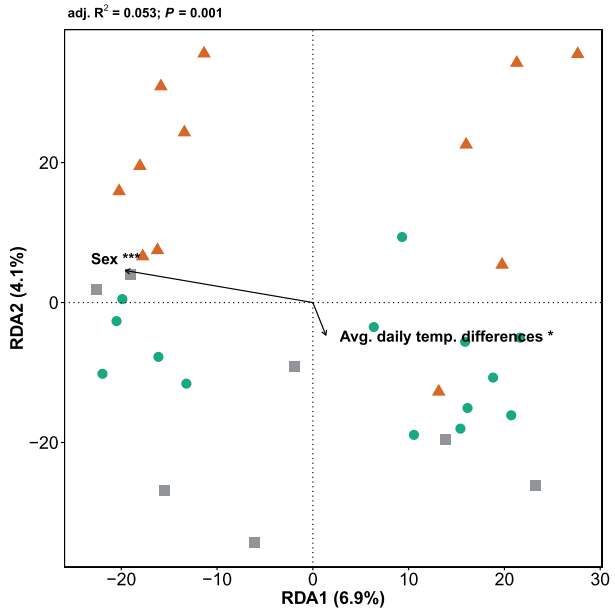

Fig. 3. Distance-base redundancy analysis (db-RDA) performed on the methylation data. Shapes represent island category: circle - high-quality islands; triangle - low-quality islands; square - the source island. The db-RDA was globally significant ($P = 0.001$) and explained 5.3% of all DNA methylation variation (adjusted $R^2 = 0.053$). Sex and average daily temperature difference explained 4.0% and 1.3% of the variation, respectively, after controlling for each other with subsequent partial db-RDAs. ***$P < 0.001$ and *$P < 0.05$.

($n = 7$). After filtering, 471,662 CpG sites met the minimum coverage requirement in all samples. Within our filtered data sets, we identified zero (high- vs. low-quality), 14 (high-quality vs. source), and 29 (low-quality vs. source) DMCs (at a 5% FDR; fig. 4*a*). Permutation analyses (supplementary text S3, Supplementary Material online) revealed that among these comparisons, only the comparison between low-quality islands and the source island had a significantly greater number of DMCs than expected by chance ($P = 0.032$, compared with $P = 0.124$ for high-quality vs. source; fig. 4*b*). Among these significant DMCs between low-quality islands and the source island, we found a significant difference in the number of cytosines that were hypermethylated versus hypomethylated in lizards from low-quality islands relative to the source island, with all DMCs hypermethylated in lizards from low-quality islands (29 vs. 0, $\chi^2 = 29$, df $= 1$, $P < 0.001$; fig. 4*a*). However, we did not detect any differentially methylated regions based on the DMCs, possibly due to their sparse distribution across the genome. These results are consistent with our hypothesis that colonization of new environments can lead to genome-wide changes in DNA methylation, with the magnitude of this response being dependent on the extent of the environmental shift between source and colonized habitats.

When analyzing the proximity of DMCs to CpG islands (CpGi), we found six, one, and one DMCs located within CpGi, CpGi shores (<2 kb away from an island), and CpGi shelves (2–4 kb away from an island), respectively, supporting their potential functional roles in gene expression (supplementary table S4, Supplementary Material online). However, we were not able to test the enrichment of DMCs in regulatory elements (e.g., promoters, gene bodies) or in particular biological pathways due to their small number. For functional annotation, we included the regions within 1-kb upstream and downstream of DMCs and used BLAT (Kent 2002) with default settings to identify the locations and orthologs of all regions in the annotated *A. carolinensis* genome (ENSEMBL version 94). We found 1 and 16 regions overlapping with promoters and gene bodies, respectively, and identified 17 orthologs for the 29 regions. The remaining 12 regions were without significant hits on orthologs. The functional analysis of the 17 orthologs showed that they are possibly associated with functions relevant for responding to environmental change (supplementary table S4, Supplementary Material online, see Discussion below).

Discussion

Phenotypically plastic responses play a central role in facilitating the establishment success of animal populations colonizing new habitats (Lande 2015), which in turn influences key ecological and evolutionary processes such as biological invasion and adaptive radiation. We experimentally simulated a natural colonization scenario on previously unoccupied oceanic islands. We then used a high-resolution technique (RRBS) to quantitatively measure DNA methylation across the *A. sagrei* genome, revealing that colonization can lead to significant changes in methylation, particularly in low-quality environments. Some of the DMCs mapped to genes that are plausibly relevant to survival and fitness in novel environments (see below), suggesting that epigenetic mechanisms may influence the expression of these genes and associated phenotypic plasticity. Our results are consistent with other recent studies demonstrating that habitat quality can affect methylation levels (Huang et al. 2017; Le Luyer et al. 2017; McNew et al. 2017). To our knowledge, this study

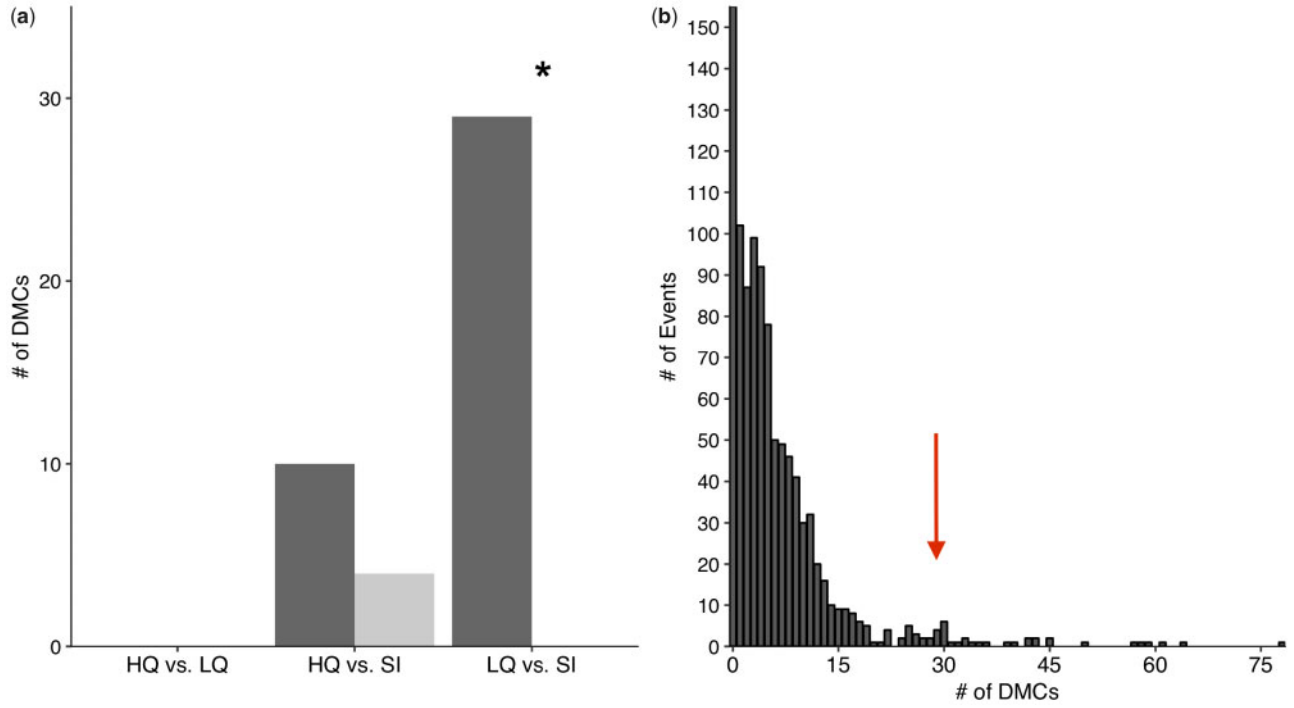

FIG. 4. (*a*) Number of differentially methylated cytosines (DMCs) that are hypermethylated (dark grey) and hypomethylated (light grey) in the three comparisons (high-quality islands, HQ; low-quality islands, LQ; the source island, SI). The y-axis reflects the number of hyper- and hypomethylated DMCs for the former island category relative to the latter island category. For example, in HQ vs. SI, the dark grey and light grey bars reflect the number of hyper- and hypomethylated DMCs for HQ relative to SI. We identified no DMCs in HQ vs. LQ, and no hypomethylated DMCs in LQ vs. SI. Asterisk indicates a significant difference in the number of hypermethylated vs. hypomethylated cytosines in lizards from LQ relative to SI. (*b*) Event histograms of 1000 randomly-generated datasets showing the probability of having the observed number of DMCs in low-quality islands vs. the source island. Three events occur above 80 DMCs and are not shown here. The arrow shows the observed number of DMCs.

represents the first evidence of methylation responses in populations at the earliest stage of colonizing new environments (i.e., within days of colonization).

We found that the number of DMCs between populations on colonized islands and the source island was two times greater when lizards were moved to islands with low- versus high-quality habitat. Indeed, the number of DMCs detected in the high-quality islands versus source comparison was low enough that it could not be distinguished from chance, and we did not detect any significant DMCs between colonized islands. These results suggest that not all environmental change will necessarily result in sufficient stress to trigger an epigenetic response. Our high-quality islands are likely to have represented a modest enough shift in conditions that lizards did not require plastic responses for acclimation (at least in the short term), or the modest level of stress on high-quality islands induced subtle methylation changes that we did not have the statistical power to detect. In contrast, the highly variable temperature and lack of vegetated area encountered by lizards on low-quality islands presented a significant environmental challenge that led to changes in methylation relative to the source population. In addition to immediate effects on gene expression or repression, changes in methylation levels could also lead to accelerated mutation rates over evolutionary time scales due to the positive relationship between the rate of deamination of methylated cytosines and genomic DNA methylation levels (Roberts and Gavery 2012). Thus, it is possible that altered methylation levels in response to short-term environmental change could ultimately result in greater genetic variation, and augment the phenotypic differentiation observed in longer term studies of *A. sagrei* introduced onto new islands (Losos et al. 1997; Kolbe et al. 2012).

There is evidence suggesting that the effects of short-term environmental change (hours to days) on epigenetic variation can affect long-term performance of animals (Kisliouk and Meiri 2009; Kisliouk et al. 2010; Uren Webster et al. 2018). The evolutionary significance of epigenetic variation depends in part on its transgenerational stability and its autonomy from genetic variation (Hu and Barrett 2017). Although we did not test these factors in our study, there is overlap between the functions of DMC-associated genes identified here (e.g., osteoblast differentiation and skeletal growth [*FN1*, Globus et al. 1998] and myogenesis [*TSKU*, Livshits et al. 2016]), and previous long-term studies analyzing genetic and phenotypic change. We also identified genes that should be important under changing environmental conditions, with functions related to cellular signaling pathways, for example, glutamate (*GPRC5B*), neurexin (*NRXN2*), and G protein-coupled receptor (*GPRC5B*). Epigenetic modifications of these signaling pathways have been found in the brains of animals exposed to environmental stress (Hauger et al. 2009; Nasca et al. 2015; Kubota 2016); however, recent studies have also found such modifications in a number of nonneural tissues, such as gills

(Uren Webster et al. 2018), skin (Hu et al. 2018), and fins (Baerwald et al. 2016), suggesting that the these pathways represent an important, wide target of epigenetic regulation in animals responding to environmental change. We also found DMCs associated with genes related to cellular adhesion, including genes related to GTPase activity (*RAB23*), tyrosine phosphorylation (*PTPRS*), tetraspanins (*TSPAN7*), and fibronectins (*FN1*). Cellular adhesion is critical for signal transduction in animals, and epigenetic changes in cellular adhesion genes could play an important role in immune response (Eakin et al. 2016; Uren Webster et al. 2018), structure maintenance and growth (Van Sligtenhorst et al. 2012), and circadian rhythm regulation (Fragoso et al. 2015). This collection of genes may be important for facilitating the colonization of environments with novel sensory, physical, or immune challenges. Recent studies have shown that the selective agents at the early stages of colonization (e.g., novel food resources, temperature regime, and parasites) can trigger physiological responses affecting growth, circadian rhythm, and immune response (Acevedo-Whitehouse and Duffus 2009; Ramirez et al. 2015; Baerwald et al. 2016; Artemov et al. 2017; Uren Webster et al. 2018). At the DMCs identified in the low-quality islands versus the source island comparison, we found a strong bias toward hypermethylation on the low-quality islands. This is consistent with recent reports of genome-wide hypermethylation in animals in stressful environments (Le Luyer et al. 2017; Metzger and Schulte 2017; Hu et al. 2018). Further investigation of DNA methylation patterns across tissues and using more comprehensive sequencing techniques such as whole-genome bisulfite sequencing should allow a more complete understanding of the links between methylation change and potential phenotypic responses.

The genome-wide methylation data presented here provide the first investigation into the relationship between epigenetic variation and responses to short-term environmental change at the very earliest stage of (experimentally controlled) colonization in wild animal populations under natural conditions. It also demonstrates the power of a high-resolution sequencing technique for identifying loci potentially associated with complex cellular responses under natural settings. While the long-term consequences of the individual DMCs in colonizing populations warrant further investigation, our results indicate that consistent changes in DNA methylation occur within just a few days of immersion in a novel environment, and may thus be an important molecular mechanism for regulating responses to environmental stressors during the colonization of novel habitats.

Supplementary Material

Supplementary data are available at *Molecular Biology and Evolution* online.

Authors' Contributions

A.M.A., J.H., and R.D.H.B. conceived and designed the study; A.M.A., J.H., T.J.T., D.A.S., T.M.P., R.M.P., and R.D.H.B. performed experiments; J.H. analyzed the data; R.M.P. and R.D.H.B. obtained funding; J.H. and R.D.H.B. wrote the article with input from A.M.A., T.J.T., and R.M.P. All authors agree to be held accountable for the work and approved the final version.

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