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Evolutionary dynamics across selected African taxa and their policy implications

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Evolutionary dynamics across selected African taxa and their policy implications

A thesis submitted in partial satisfaction of the

requirements for the degree of Master of Science

in Biology

by

Jessica Arriens

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ABSTRACT OF THE THESIS

Evolutionary dynamics across selected African taxa and their policy implications

by

Jessica Arriens

Master of Science in Biology

University of California, Los Angeles, 2018

Professor Thomas Bates Smith, Chair

Unraveling the unique ways anthropogenic effects impact the genomes of populations, and applying such genomic knowledge to improve conservation policy, is paramount for effective preservation and management of biodiversity in our changing world. The central African country of Cameroon, widely recognized as a global conservation priority, is a prime landscape to study these issues. Here, I explore genetic and epigenetic variation between natural and human-altered habitats in the Cameroonian sunbird *Cyanomitra olivacea*. I use SNP data and methylation data to contrast patterns of genetic and epigenetic variation, finding that methylation frequency levels shift with changes in environment, and are higher overall in human-altered habitats. Then, I develop a decision-making framework to enable more effective research-to-policy translation, and apply it to a particular case study: preserving adaptive genetic variation in Cameroon. This study is one of the first to explore genetic and epigenetic variation in concert, and reveals the potential ways organisms adapt to anthropogenic change. It is also an attempt to bridge the much-maligned science-policy gap and develop policy tools to bolster conservation.

The thesis of Jessica Arriens is approved.

Kirk Edward Lohmueller

Gregory F. Grether

Thomas Bates Smith, Committee Chair

University of California, Los Angeles

2018

DEDICATION

To Martin, for your love and contestation.

TABLE OF CONTENTS

Chapter 1: Genetic and epigenetic variation between natural and human-altered habitats in an African rainforest sunbird

Chapter 2: A case study in science-to-policy translation: Preserving Cameroon's ecotones

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGMENTS

Genetic and epigenetic variation between natural and human-altered habitats in an African rainforest sunbird

Introduction

The rainforests of Cameroon are one of the world's biodiversity hotspots. Nearly half of the country is covered in forest (de Wasseige et al., 2009), which is home to nearly 8,000 species of plants, 250 mammals, 848 birds and more than 500 reptiles and amphibians (Fomete et al., 1998). Cameroon is widely recognized as a global conservation priority, but it is also one under siege, facing increasing anthropogenic threats. Annual average deforestation rates in Cameroon have risen steadily since the 1980s (FAO, 2010), fueled by commercial logging, road building, mining and industrial-scale agriculture. Cameroon has the second-highest deforestation rate of the Congo Basin countries, and approximately 75% of the country's forests are subject to intense pressure (Dkamela, 2010). Extensive deforestation and forest fragmentation have already left their mark on the region's biodiversity (Mallon et al., 2015).

Deforestation and related anthropogenic effects also leave marks on the genetics of wild populations, impacting overall genetic diversity, gene flow and levels of selection (Bouétard et al., 2014; Kalkvik et al., 2012). Unraveling the effects of anthropogenic pressures on the population genetics of species has important implications for evolution and conservation, and has been widely investigated in many taxa (Smith & Bernatchez, 2008). Yet how the epigenome – all the chemical compounds that regulate gene expression – responds in human-altered environments has not been as fully explored.

Epigenetics can play a unique role in driving the diversity and adaptive potential of populations. Epigenetic variation helps explain heritable phenotypic variation observed in natural

1

populations, variation that cannot be explained by DNA sequence variation alone (Cervera et al., 2002; Cubas et al., 1999; Keyte et al., 2006; Vaughn et al., 2007). Epigenetic traits can also have adaptive value, especially in response to abiotic stressors or environmental changes (Bossdorf et al., 2008). In addition, epigenetic modifications may play a role in speciation (Pál & Miklós, 1999). Exploring epigenetic responses to anthropogenic effects enables a more nuanced understanding of how populations may respond to these changes, as well as the intertwining roles of epigenetics and genetics in the evolutionary dynamics of a species.

In this study, I explore how epigenetic and genetic variation in olive sunbirds is shaped by anthropogenic effects across natural and human-modified habitats in Cameroon. Previous research on olive sunbird populations in the country found evidence for divergence between different habitat types, including significant phenotypic difference between populations across the forest-savanna ecotone and different rates of disease prevalence between populations in disturbed and undisturbed forests (Chasar et al., 2009; Smith et al., 2011). Here, I use genomic and epigenomic data: single nucleotide polymorphism (SNP) data generated from restriction site-associated DNA (RAD) sequencing, and methylation data generated from high-throughput bisulfite sequencing.

Specific objectives of this study were to: 1) contrast patterns of genetic and epigenetic variation in olive sunbirds in Cameroon, 2) model how methylation frequency levels shift with changes in environment, particularly with respect to habitat type and climate, and 3) explore how human-altered environments might alter the epigenome of olive sunbirds.

Methods

Study species and sampling

Olive sunbirds are commonly found across Central Africa, in primary and secondary forests as well as larger forest fragments in the ecotone between rainforest and savanna. They feed on insects and nectar and are one of the most common forest sunbirds (Cheke et al., 2001; Fry et al., 1988; Mackworth-Praed & Grant, 1973). Adult olive sunbirds were captured from 14 different sampling sites in Cameroon. Sampling sites encompassed four geographically distinct habitat types – mature forest, secondary forest, ecotone and island – which were previously described and characterized using remote sensing data (Smith et al., 2005; Smith et al., 2011; Smith et al., 2013).

Genomic data collection

Blood samples from adult olive sunbirds in Cameroon were collected and stored in Queen's Lysis Buffer. DNA extraction, RADseq library construction and RADseq bioinformatics processing followed the previously developed protocols of Zhen et al. (2017). RADseq library preparation followed traditional methods as described in Ali et al. (2016), with some modification as described in Baird et al. (2008). RADseq reads were processed using the *Stacks* pipeline, version 1.32 (Catchen et al., 2011; Catchen et al., 2013) in the manner described by Zhen et al. (2017). The final set of high-quality RAD loci were identified by filtering out loci present in less than 80% of all samples, and filtering out loci with more than 40 SNPs along an 80-base-pair RAD loci sequence. A total of 59,807 RAD loci were included in the final consensus set. Genotypes for the final consensus set were exported in VCF format using *Stacks* populations program (only biallelic SNPs). Additional filters based on genotype calls were performed in *vcftools* or using custom scripts, which included filtering sites that had a genotyping rate less than 80% of all samples.

The 183 samples were collected from 14 sampling sites, representing 14 populations (*Figure 1*). Each population was represented by 5-16 individuals, with a mean of 13 sampled per site. This included nine forest populations ($N = 113$), a single population on the island of Bioko, Equatorial Guinea ($N = 10$) and four ecotone populations ($N = 44$).

Methylation sequence data collection and processing

DNA was extracted from whole blood collected from 95 sunbirds sampled from seven sites in Cameroon. All sampling sites overlapped with sampling sites for the genomic analysis. Each population was represented by 10-25 samples, with a mean of 13.6 sampled per site. This included two mature forest populations ($N = 37$), two secondary populations ($N = 36$) and two ecotone populations ($N = 24$). Methylation analysis was not conducted on the island population. Of the 95 individuals used in the methylation analysis, 67 were also genotyped using the RADseq protocol described above.

The pipeline *BS-Seeker2* was used to map converted DNA sequence reads to the reference genome with *Bowtie2*, and then subsequently infer the level of methylation at each cytosine sequenced (Guo et al., 2013). The olive sunbird sequence reads were mapped to the zebra finch (*Taenopygia guttata*) reference genome (*taegut2*). Methylation levels were represented as methylation frequency (MF) values and calculated following Janowitz Koch et al. (2016). MF is the ratio of reads that sequenced the methylated nucleotide (mC) to the total number of reads at that cytosine. Consequently, MF ranges from MF~0, for sites that are never methylated across all reads that align to it, to MF~1 for cytosines that are always sequenced as mC. The data was filtered to retain cytosines that had a minimum of 10x sequence coverage and

4

used a confidence interval threshold value of 0.33 to retain those with little variation across reads per cytosine. This works to exclude cytosines with high variance in MF, which may represent error or unknown biological processes. Methylation at CG motifs only (excluding CHH and CHG) was analyzed. After filtering and retaining cytosines that were sequenced across all 95 olive sunbirds using *Methylkit* (Alkalin et al., 2012), we retained 27,646 cytosines for methylation analysis.

Statistical analyses: Genetic data

To visualize the genetic data and detect any underlying population structure, I performed Principal Components Analysis (PCA) using the bioconductor package *SNPrelate* (Zheng et al., 2012). PCA, a dimensionality reduction method, is widely used to explore genetic data and relatedness between populations. After filtering for a minimum minor allele frequency of 2%, there were a total of 58,094 SNPs used in the PCA. The first six PCAs were examined visually to identify any clustering and patterns of differentiation.

Statistical analyses: Methylation sequence data

I first compared average methylation values for each bird across habitat type (mature forest, secondary forest and ecotone) to identify any significant differences in methylation frequency. I also performed a PCA on the methylation data across the 27,646 cytosines using the *stats* and *factoextra* packages in R to detect and visualize epigenetic structure.

To more closely explore overall methylation frequency differences across habitats, and identify cytosines that may be driving those differences, I identified outlier cytosines. These were cytosines that were either hypermethylated or hypomethylated between a pairwise

comparison of habitats. I first separated the data into birds from natural habitats and human-altered habitats (mature forest populations versus secondary forest and ecotone populations). I then estimated the absolute difference per cytosine in that pairwise comparison and created a distribution of methylation differences. Taking the absolute difference ensured the outliers included both hypermethylated and hypomethylated cytosines. Cytosines in the 99th percentile of the distribution of absolute differences were considered outliers. All outlier cytosines were associated to an annotated gene in the reference genome via a custom Python script. Finally, I used g:Profiler, a web-based toolkit for functional exploration of gene lists, to further determine if gene(s) associated with an outlier cytosine(s) were functionally enriched for ontological categories, or other biological databases. g:Profiler takes a gene list and returns a set of enriched functional terms from Gene Ontology and other biological databases, with terms ranked by their statistical significance (Reimand et al., 2007).

Genetic and methylation structure

To identify structure within the genetic and methylation data, I used the program *Admixture*, which calculates model-based estimation of ancestry in unrelated individuals (Alexander et al., 2009). I analyzed structure assuming one to 14 genetic groups (K) for the SNP genotype data, using the cross-validation procedure to identify the most likely number of K genetic groups. To analyze the methylation data in parallel, I first converted MF data into single methylation polymorphisms (SMPs) following Janowitz Koch et al. (2016): Cytosines with high or fixed methylation (MF > 0.66) were classified as BB genotypes. Cytosines with little or no methylation (MF < 0.33) were classified as AA genotypes, and intermediate MF values (0.33 $\geq$ MF $\leq$ 0.66) were classified as AB. This data retained some features of the methylation patterns

yet were represented as allelic data, and could be formatted using *PLINK* (Purcell et al., 2007) and analyzed in *Admixture*. I assumed one to seven groups, corresponding to the seven sampling sites, and again used the cross-validation procedure to estimate the most likely value of *K*.

Environmental modeling

To explore associations between methylation frequencies and environment, I used *gradientForests* (Ellis et al., 2012). Gradient forests are an extension of random forests (Breiman, 2001), a nonparametric, machine-learning regression tree approach to community-level modeling. Gradient forests provide summary statistics indicating the overall association of genomic variation to different environmental variables, which can then be mapped onto geographic space. I used SMPs as the program's response variables. I ran *gradientForests* with all SMPs and with outlier SMPs, in two separate analyses. The outlier SMPs were the same outlier cytosines identified by the analysis detailed earlier, just converted from raw MF values into SMP format as described above. Predictor variables were a suite of 19 environmental variables, capturing both biotic and abiotic processes. Bioclim variables, including measures of temperature and precipitation, were downloaded from the Worldclim database. NASA's MODerate-resolution Imaging Spectroradiometer (MODIS) provided tree cover and vegetation measurements, and surface moisture estimate measures came from the Quick Scatterometer (QuikSCAT). I also used the Euclidean distance measures of latitude and longitude as predictor variables in all model runs, to account for any associations arising from spatial processes alone. Gradient Forests were run with these settings: number of SMPs included in each bin = 500, number of trees run for each environmental variable = 500.

7

Results

Contrasting genetic and methylation variation

Principal Component Analysis performed with all 183 samples (58,094 SNPs with a minor allele frequency of 2%) shows all populations clustering along PC1, with essentially no separation (*Figure 2*). Removing outliers and running the PCA again on 172 samples (59,092 SNPs with a minor allele frequency of 2% – applying the minor allele frequency cutoff to a subset of samples enabled more SNPs to pass through) reveals a similar clustering pattern, though samples from Bioko – the sole island habitat – separate out along PC1 (*Figure 3*). Principal Components three through six show generally the same pattern, with samples from all habitat types largely clumping together. Principal Component Analysis with the MF data shows samples from all three habitat types in loose, overlapping clusters. A confidence ellipse drawn around each group's mean point does reveal subtle structuring: Mature forest populations show slightly more constraint along axes PC1 and PC2 when compared to secondary forest and ecotone populations (*Figure 4*). A total of 279 cytosines were considered outliers in my analysis. A PCA with only methylation outlier cytosines shows a similar pattern to the previous PCA, but with more visible separation of the mature forest populations (*Figure 4*). Secondary forest populations segregate along PC1 and overlap with ecotone populations. Once again, mature forest populations show the most constraint along both axes of variation.

The *Admixture* analysis identified no population structure in olive sunbirds. The best fit was a single group ($K = 1$) based on genotypes derived from both the SNP and SMP datasets. A visualization of three groups, consistent with the three habitat types, does show weak structuring, though SNPs and SMPs show a very similar pattern (*Figure 5*).

Environmental associations with methylation frequency

A total of 102 cytosines, or 0.369% of the total number of cytosines, had R^2 values above zero when testing for a correlation between environmental variables and methylation (mean R^2 = 0.0391) via *gradientForests*. The normalized difference vegetation index (NDVI), which measures greenness of vegetation on the Earth, was the top predictor variable, followed by temperature seasonality and precipitation of the driest month. Purely geographic variables, in the form of latitude and longitude, ranked relatively poorly as predictors of methylation frequency variation (*Figure 6*).

In the *gradientForests* outlier analysis, a total of 43 outlier cytosines, or 15.4% of the total number of outlier cytosines, had R^2 values above zero (mean R^2 = 0.0204). Temperature seasonality and precipitation seasonality were the top two predictor variables, followed by predictors related to vegetation and elevation (NDVI and elevation standard deviation). Latitude and longitude continued to rank poorly as predictor variables. Mapping the outlying MF variation onto geographic space shows a pattern of MF turnover across Cameroon (*Figure 7*). There is distinct turnover in the country's rainforest-savanna transition zone, in the northern region of Cameroon.

Methylation frequency levels in human-altered habitats

Olive sunbirds from mature forest sites had significantly lower average methylation frequency values as compared to other regions. Birds from secondary forest and ecotone sites had very similar methylation frequency values (*Figure 8*). The average methylation frequency values for each habitat type were ecotone = 0.0597, *SD* = 0.0046; mature = 0.0557, *SD* 0.0032; secondary = 0.0594, *SD* 0.0047.

The outlier analysis identified 279 cytosines as outliers. Of these, 154 were annotated with Ensembl gene names and were analyzed for enriched functional terms with g:Profiler. Ranking g:Profiler's results by the FDR-adjusted *p*-value shows a significant cluster of enriched functional terms related to cellular and macromolecule biosynthetic processes, and their regulation, as the most significant (*Table 1*). Sequence-specific DNA binding also ranked as a highly significant functional term. Genes related to metabolism, including nucleic acid metabolic processes and the regulation of RNA metabolic process, were also identified in the analysis.

Discussion

In this study, I explored genetic and epigenetic variation in olive sunbird populations across different habitats, both natural and human-modified, in Cameroon. Recent work on another rainforest passerine, the little greenbul (*Andropadus virens*), found strong evidence for genetic structure in Cameroon populations, especially across the ecotone formed by the rainforest-savanna transition zone (Zhen et al., 2017). In contrast, no genetic structure was found in sunbirds, via PCA or *Admixture*, despite greenbuls and sunbirds sharing similar habitats across Cameroon. Olive sunbirds are known to be a highly mobile species, however, with high levels of gene flow (Lens et al., 2002). A study on olive sunbird phylogeny found no apparent genetic structure in populations from Ghana to northwest Kenya (Bowie et al., 2004).

In contrast to a total lack of genetic structure, epigenetic structuring was detected in sunbirds. Principal Components Analysis with MF data showed a pattern of differentiation between mature forest populations and other populations (secondary forest and ecotone). This pattern was even stronger in the PCA results with outlier MF sites. The *Admixture* analysis, however, did not reveal any epigenetic structure in sunbirds. This may be because the epigenetic

structuring is limited, and the method used to convert MF into SMPs grouped the data in ways not consistent with its distribution. The methylome is also predicated to evolve faster than the genome (Jablonka & Raz, 2009), suggesting it may be possible to "see" greater epigenetic structure and variation before genetic structure.

This pattern of epigenetic variation without corresponding genetic variation has been identified in other birds, as well as multiple plant species (Cubas et al., 1999; Cervera et al., 2002; Keyte et al., 2006; Massicotte & Angers, 2012; Schrey et al., 2012; Vaughn et al., 2007). It has been suggested this pattern represents the role of epigenetics in driving phenotypic plasticity. Methylation in particular can increase phenotypic variation in response to new environments (Liebl et al., 2013). Epigenetic variation, therefore, may be driving the significant morphological differences previously identified between sunbird populations in different habitats in Cameroon (Smith et al., 2011).

Environmental variation can also spur DNA methylation polymorphisms at the population level (Massicotte et al., 2011). Using *gradientForests*, I found that environmental variables related to seasonal changes in temperature, precipitation and forest cover are the most important variables driving turnover in methylation frequencies. These environmental variables also vary greatly between forest and savanna habitats, and between natural and human-altered habitats. This, combined with the fact that purely geographic variables did a poor job explaining the pattern of methylation variation according to these models, provides further support for the role of habitat-associated environmental differences in driving epigenetic variation.

Finally, I identified a significant difference in average methylation level between human-altered and natural habitats, with lower overall methylation seen in mature forest populations. The increased levels of methylation may be enabling olive sunbirds to adapt to human-modified

11

habitats in some way. Most of the genes identified in the gene enrichment analysis of the outlying methylation sites were tied to core cellular processes, such as those modulating rates of chemical reactions, the formation of macromolecules and sequence-specific DNA binding. Some genes were also found to be involved in metabolic processes. Metabolic rate in birds is associated with habitat temperature and precipitation; it is possible these enriched regions may reflect the methylome responding differently to different habitats (White et al., 2007). Other enriched functions identified via g:Profiler may be more reflective of the mechanism of methylation itself.

The relationship between environment and epigenetics is still being explored. There may be more cryptic reasons for the higher methylation levels in sunbird populations from secondary forests and ecotone. Human-altered habitats may harbor some additional biotic or abiotic factors driving higher methylation frequencies. Common garden experiments are one avenue for future research, and would allow for a more controlled exploration of the causes behind epigenetic variation. No methylation data has been collected or analyzed from little greenbuls, so it is possible they show a similar pattern of increased epigenetic variation between habitat types.

Researchers are just beginning to explore the ways epigenetic variation responds to different environments and anthropogenic impacts (Foust et al., 2016; McNew et al., 2017). This study is, to my knowledge, one of the first to explore epigenetic and genetic variation, in concert, between natural and human-altered habitats. Overall, it shows the importance of looking at both the genetics and epigenetics of wild populations. Analyzing these changes together provides a more nuanced view of variation and adaptive potential, and raises interesting questions about how organisms can adapt in the face of anthropogenic change.
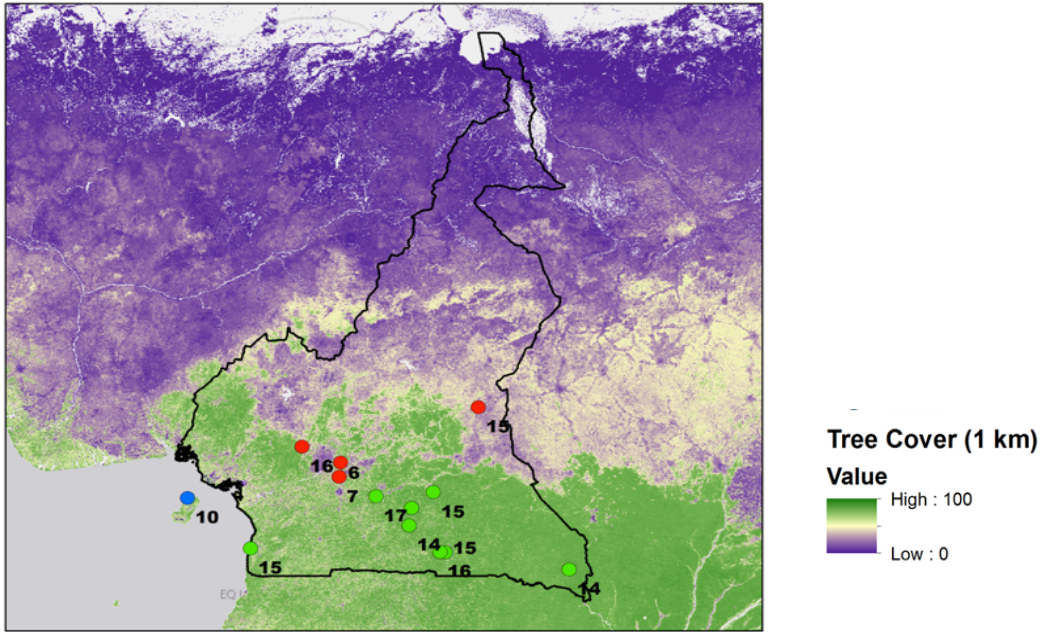
Figure 1. Tree cover of Cameroon, based on MODIS data, and olive sunbird sampling locations. Green dots represent forest sampling sites, red dots represent ecotone sampling sites and the blue dot represents the island sampling site. Numbers correspond to the sample size.
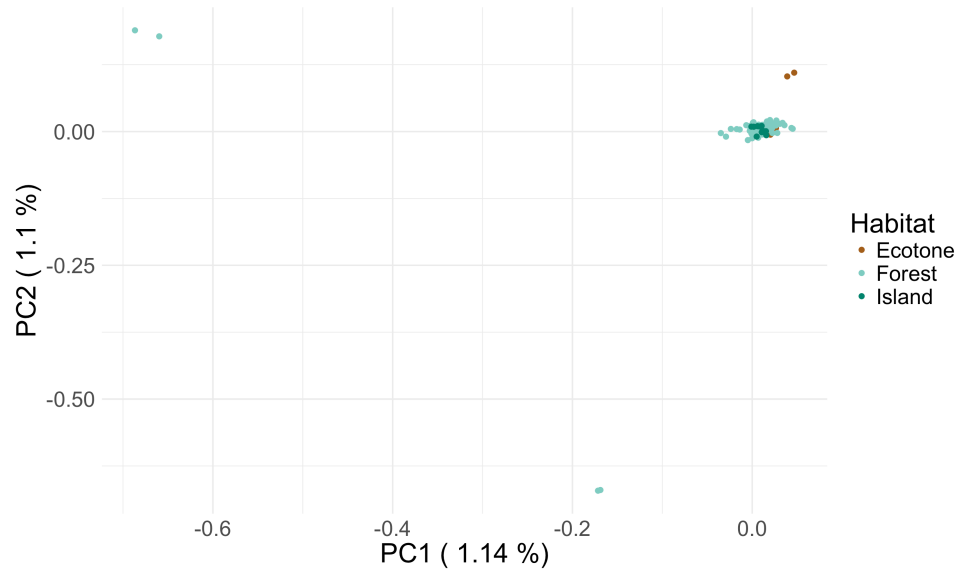
Figure 2. Principal Component Analysis (PCA) showing sunbirds by habitat. Populations from ecotone, forest and island cluster together along PC1 with little separation. A total of 58,094 SNPs with a minor allele frequency of 2% across 183 sunbirds were used in the PCA.
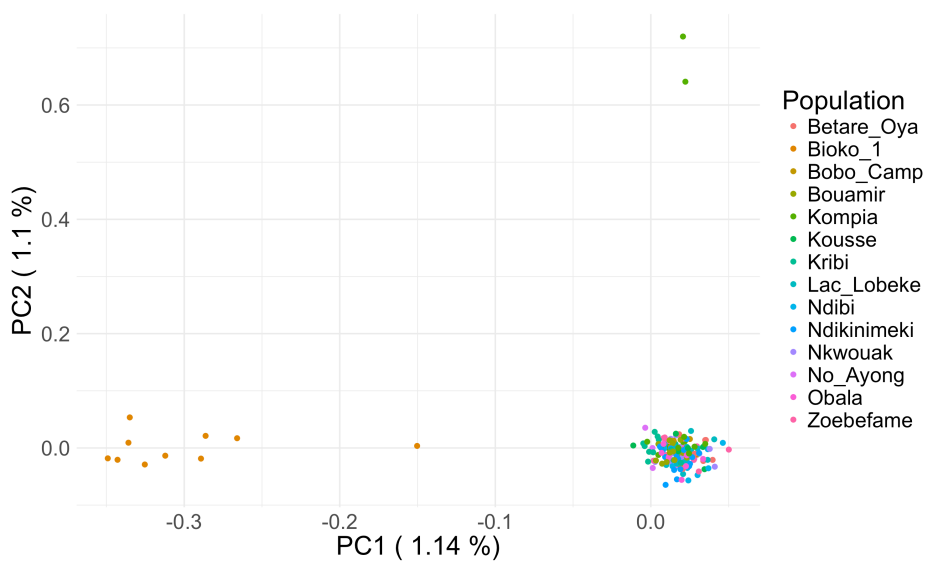
Figure 3. Principal Components Analysis (PCA) showing sunbirds by population, with some outliers shown in Figure 1 removed. This PCA used 172 samples and 59,092 SNPs (minor allele frequency at 2%). No discernable population structure is shown, similar to Figure 1, though samples from Bioko – the sole island habitat – separate out along PC1, away from the larger clump.

14
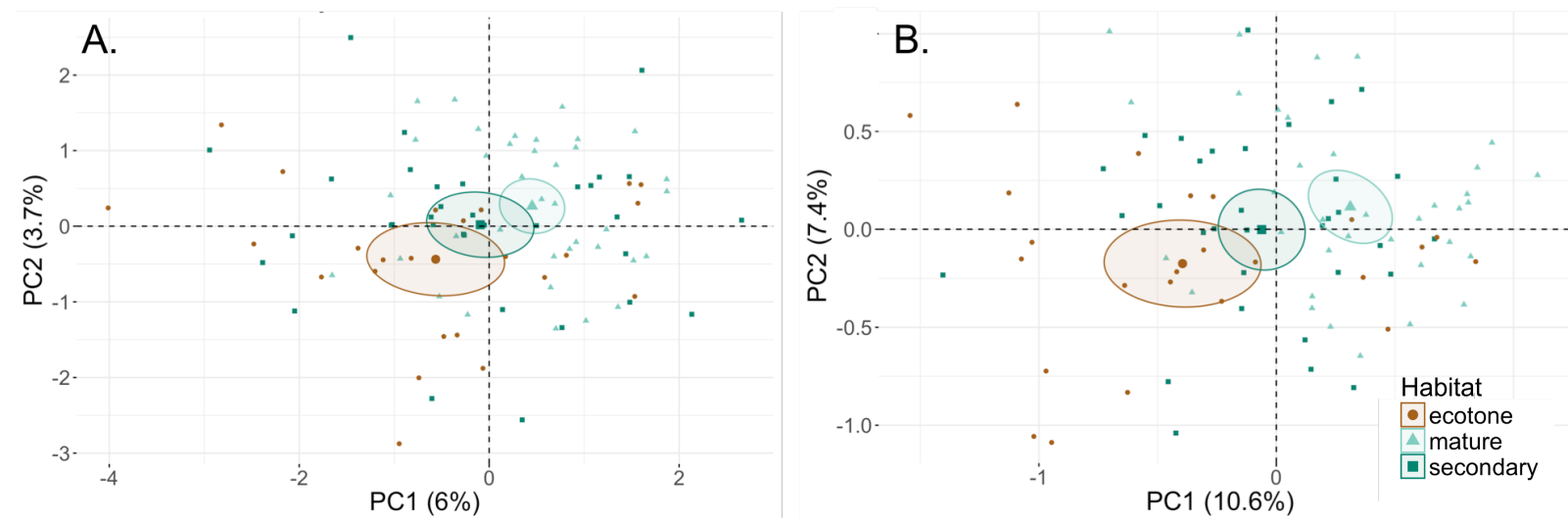
Figure 4. PCAs with methylation frequency sites. All 27,646 MF sites from 95 sunbirds are shown in panel A. Panel B shows 279 outlier cytosine sites from 95 sunbirds. Brown circles and ellipses denote samples from ecotone habitats. Dark green squares and ellipses denote samples from secondary forest habitats. Light green ellipses and triangles are samples from mature forest habitats. Group mean points are in the center of the ellipses, which are drawn with 95% confidence intervals.
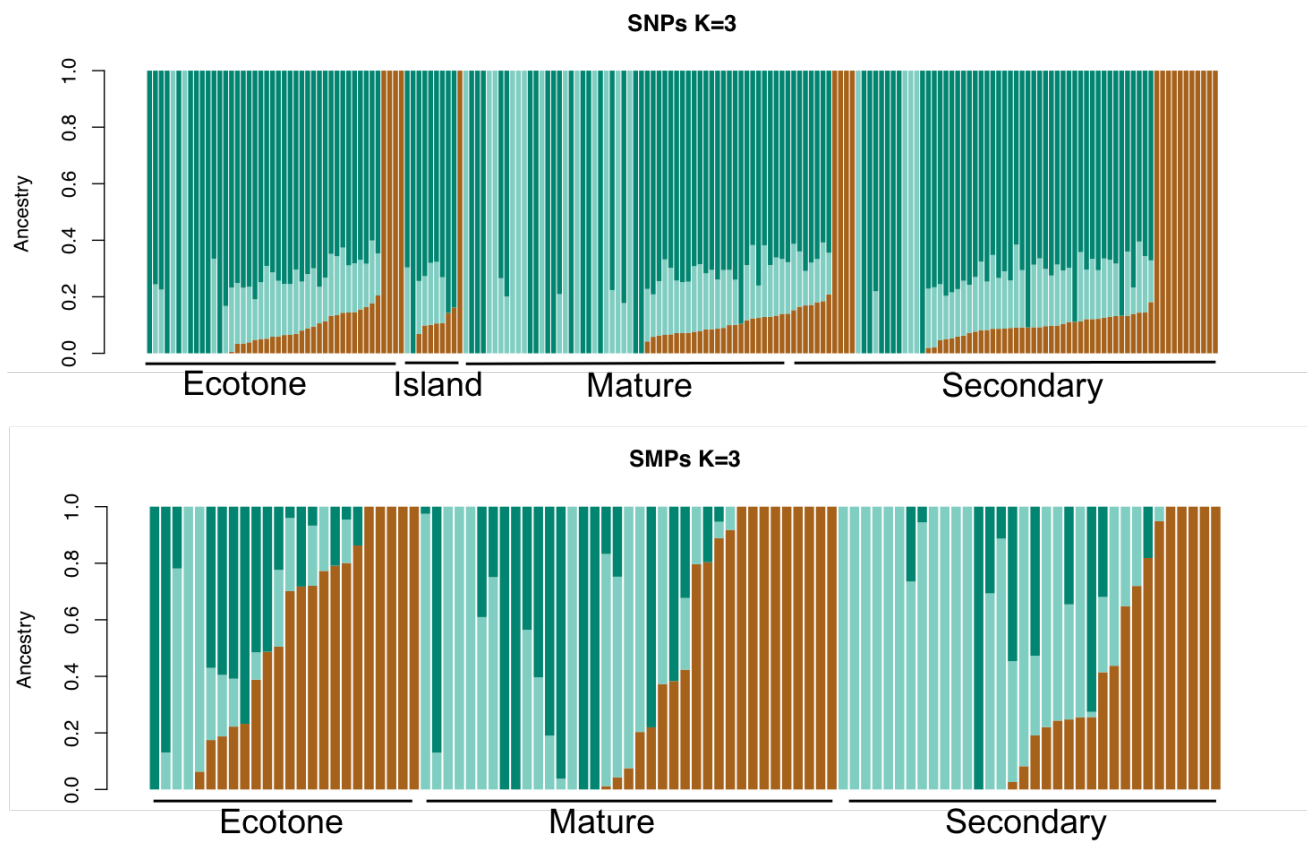
Figure 5. Admixture results showing population structure with SNP genotype data (top figure) and SMP data (bottom figure) for *K*=3.
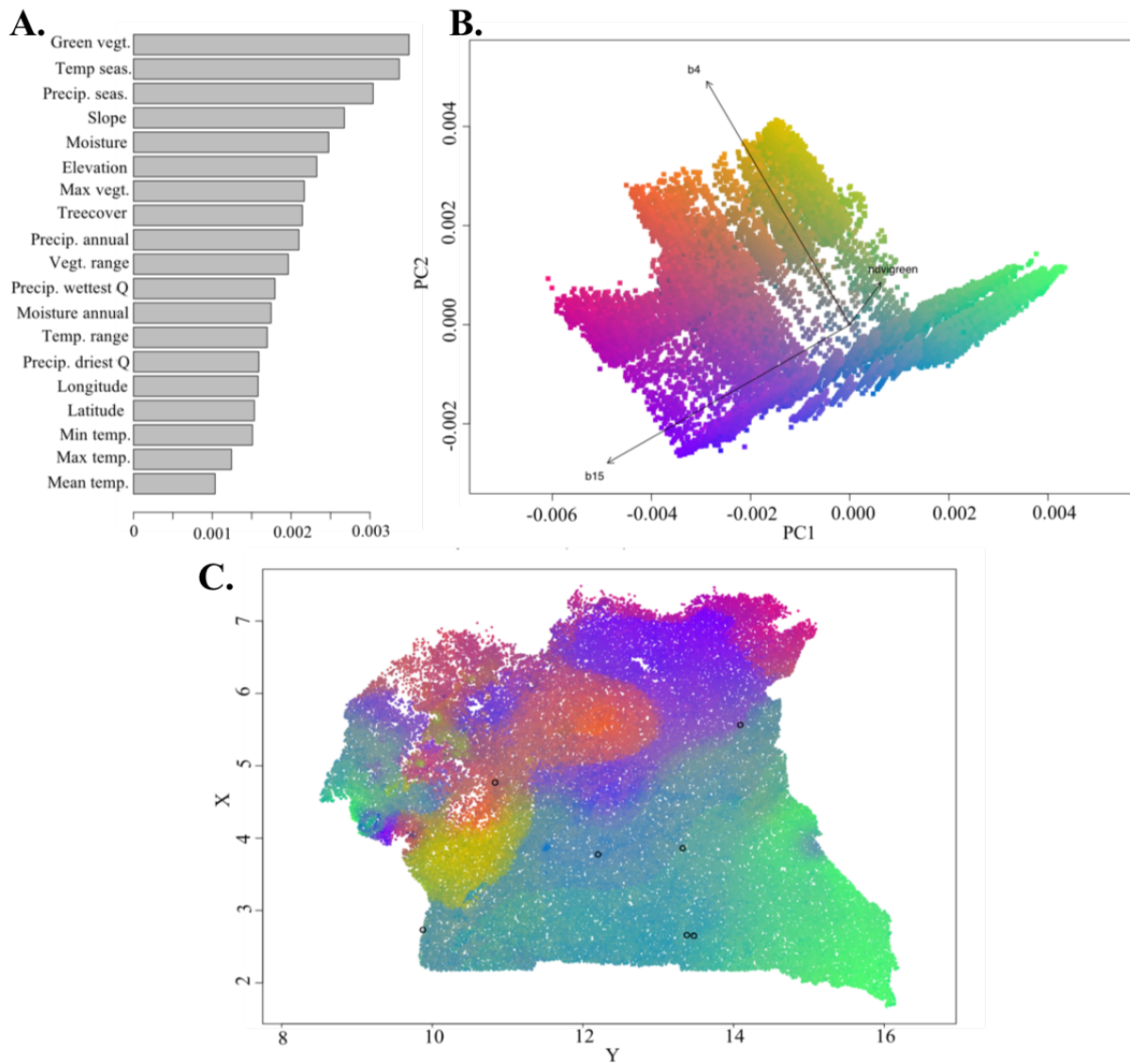
Figure 6. Environmental drivers of methylation frequency turnover for all SMPs (N = 27,647). Environmental variables are shown ranked by their predictive importance in panel A. The top three are green vegetation, temperature seasonality and precipitation seasonality. Panel B shows a PC plot indicating the contributions of those environmental variables to methylation frequency turnover (b4 corresponds to temperature seasonality, ndvigreen is green vegetation and b15 is precipitation seasonality). The top three environmental variables from panel A are shown as vectors on the PC. In panel C, a map of Cameroon (limited to the range of the olive sunbird) is shown with the methylation frequency turnover. The greater the difference in color equates to a greater turnover in methylation frequency. Black circles represent sampling sites.
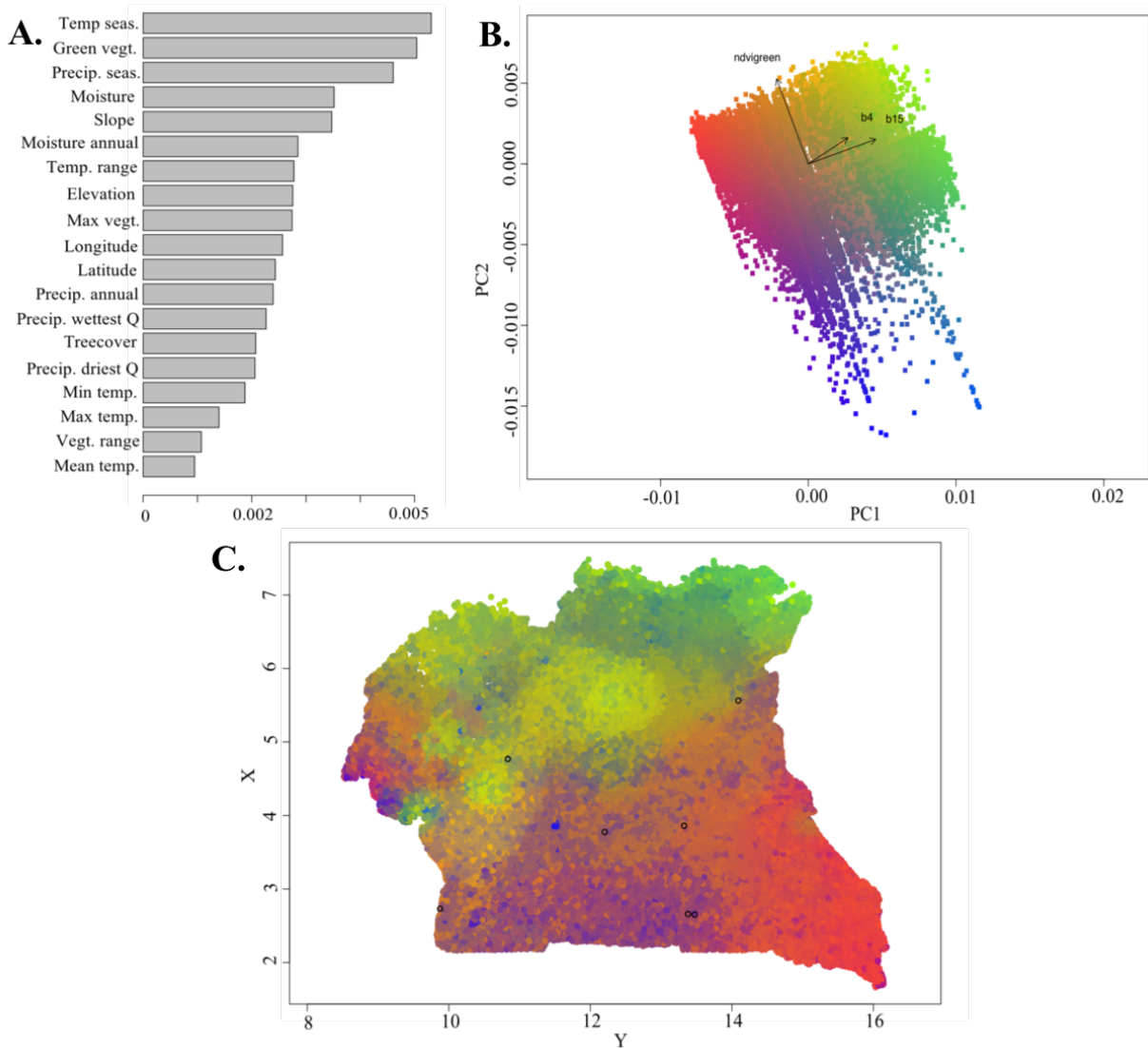
Figure 7. Environmental drivers of methylation frequency turnover for outlier SMPs (N = 279). Panel A shows environmental variables ranked by their predictive importance. The top three are temperature seasonality, green vegetation and precipitation seasonality. Panel B shows a PC plot indicating the contributions of those environmental variables to methylation frequency turnover. The top three environmental variables from panel A are shown as vectors, indicating their direction and magnitude, on the PC. In panel C, a map of Cameroon (limited to the range of the olive sunbird) is shown with the methylation frequency turnover. The greater the difference in color equates to a greater turnover in methylation frequency. Black circles represent sampling sites.
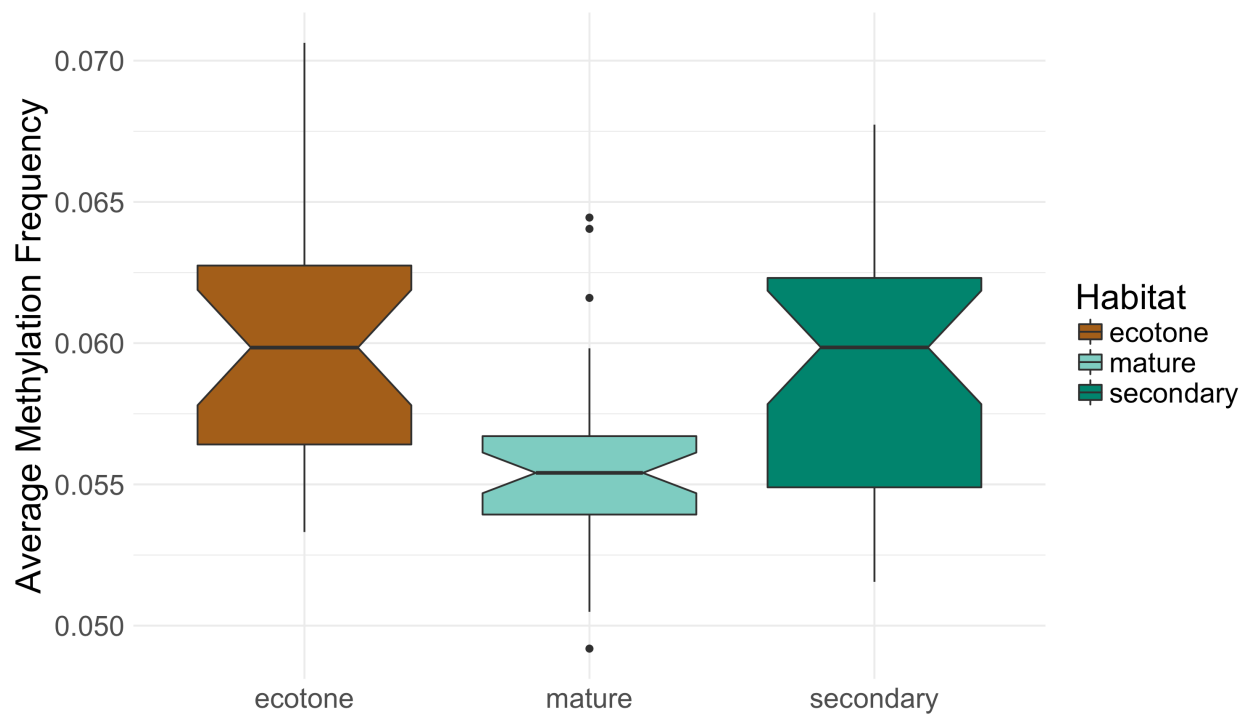
Figure 8. Average methylation frequency values (27,646 sites; 95 birds) for olive sunbirds from each habitat type.

Gene Ontology ID	Description	*p*-value
GO:0010556	regulation of macromolecule biosynthetic process	5.30E-06
GO:0043565	sequence-specific DNA binding	5.30E-06
GO:0044249	cellular biosynthetic process	8.83E-06
GO:0009889	regulation of biosynthetic process	8.83E-06
GO:0031326	regulation of cellular biosynthetic process	8.83E-06
GO:2000112	regulation of cellular macromolecule biosynthetic process	8.83E-06
GO:0019219	regulation of nucleobase-containing compound metabolic process	8.83E-06
GO:0005634	nucleus	8.83E-06
GO:0048513	animal organ development	9.42E-06
GO:0090304	nucleic acid metabolic process	9.42E-06
GO:0051252	regulation of RNA metabolic process	9.42E-06
GO:2001141	regulation of RNA biosynthetic process	9.42E-06

Table 1. Top g:Profiler results

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A case study in science-to-policy translation: Preserving Cameroon's ecotones

Moving research from the confines of literature and academia into effective, tangible public policies is perhaps the preeminent challenge for the field of conservation. It is also a particularly pressing one, as we confront vast biological destruction, pervasive anthropogenic influence on ecosystems and creeping climatic changes (Millenn. Ecosys. Assess., 2005; Scheffers et al., 2016). Translating research into policy, and making research relevant for decision-makers, has long been recognized as both exceedingly important and exceedingly difficult (Jacobs et al., 2005).

The field of conservation biology has no shortage of clarion calls about the importance of connecting researchers with conservation practitioners, and no shortage of suggestions on how to apply research insights, particularly related to genomics and evolutionary biology, to improve conservation policy (Carroll et al., 2014; Crandall et al., 2000; Hoban et al., 2013; Lankau et al., 2011; Laurance et al., 2012; Mace & Purvis, 2008; Mortiz, 2002; Shafer et al., 2015; Smith et al., 2005, Smith & Grether, 2008). In fact, scientists have advocated for "evolutionary responsible" thinking in conservation for decades (Frankel, 1974). This has led to some policy innovations – such as the use of evolutionary significance units to preserve adaptive variation – and there are successful examples of conservation genomic insights applied to policy (Balmford, 2003; Garner et al., 2016; U.S. Fish and Wildlife Service, 2008).

What's lacking from these discussions is not a recognition of the importance of connecting scientists and policy-makers – or even the challenges of doing so – but practical guidance and examples of how it might be done. Here, I attempt to bridge this science-policy gap through the use of a decision-making framework applied to a specific conservation goal:

incorporating adaptive genetic variation into conservation planning in Cameroon. My goal is to show how research from the fields of evolutionary biology, public policy and decision science can braid together to inform conservation policy. The framework is as follows:

1. Identify valuable land for conservation

2. Assess land use profile (people and land)

3. Choose most appropriate policy tool

This framework is simple yet robust. It explicitly incorporates information on land use and decision-makers operating in the land use sphere. It recognizes that conservation decisions are not made in a vacuum, absent from existing land uses, policies and people. Though I focus here on Cameroon, this framework could be applied to other countries or regions.

Frameworks are not new in conservation policy (Margules & Pressey, 2000; Myers et al., 2000). Most existing frameworks, however, are about deciding where to place protected areas (PAs). PAs are a historic keystone of conservation and have a strong legacy of support because they can reduce ongoing biodiversity losses. Yet biodiversity and habitat loss continues around the world. In Central Africa in particular, PAs suffer from numerous problems, such as ecological isolation, high surrounding human pressure and insufficient enforcement (Struhsaker et al., 2004). There is an urgent need to both enhance PA management and develop policies outside the formal PA system (Clarke et al., 2013). The policy tools highlighted here do not include new PAs for this reason. This is not meant to discount their role in preserving biodiversity, but an attempt to think outside the PA box.

Overview of framework

Step 1: Identify valuable land for conservation. This stage should identify a specific conservation goal. Ideally one developed via an inclusionary process in conjunction with relevant stakeholders. "Valuable" means different things to different people – biologists, community members and politicians likely all have different ideas of where and what is valuable to conserve. Recognizing these differences, and working to accommodate them via a decision-making framework, is an important part of responsible conservation policy development. Scientific agencies have long recognized the need for public engagement in environmental decision making, as it can ensure policies are more effective and democratic (National Research Council, 2008). Public engagement is perhaps of additional importance for western-funded conservation practitioners operating in the developing world, where legacies of paternalistic policies have clouded the reputation of conservation as a whole (Garland, 2008; Neumann, 1997).

Step 2: Assess land use profile. This stage should identify the complete land use profile. Not only land types in the area, but all landowners and relevant decision-makers. This will determine what policy tools are available and feasible, and help identify the people and organizations that should be involved any conservation decision-making discussions.

Step 3: Choose the most appropriate policy tool. Once there are agreed-upon goals and an understanding of the land use sphere, a policy tool(s) can be selected. Who selects depends on the land owners, land users and decision-makers identified in step 2. This framework includes a policy toolkit instead of a single recommendation. A toolkit serves as a diverse menu of policy instruments, and is appropriate here because I have not included any value weighting or community feedback in the case study. By including multiple options, I hope to acknowledge

27

these shortcomings. The tools listed here were selected with a particular eye towards what might be most appropriate and feasible in Cameroon.

There are various mechanisms to engage stakeholders at each step, from the initial discussion of values to the final decision about solutions. Potential mechanisms include focus groups, advisory committees and public forums. A particularly effective and inclusionary method is Structured Decision Making (SDM), a flexible decision-making framework that combines human behavioral insights and analytical methods from decision analysis and applied ecology (Failing et al., 2012). SDM approaches enable decision-makers, and anyone participating in the decision-making process, to incorporate values, uncertainty, and practical needs. The method has been used successfully to build consensus among diverse groups (Failing et al., 2012).

Each engagement mechanism has pros and cons. The most appropriate mechanism for the job depends on the particulars of the conservation issue – the region, the expertise of the individuals involved, the time and money that can be devoted to the project. It is beyond the scope of this paper to compare each mechanism or advocate for one over another. Instead, I aim to acknowledge the importance of engagement, especially in conservation settings.

Case study: Preserving Adaptive Genetic Variation in Cameroon

In this case study, I focus on incorporating evolutionary processes into conservation policies. Traditional approaches to conservation emphasize protecting species hotspots: areas of exceptional concentrations of endemic species that are also experiencing exceptional levels of habitat loss (Myers, 1988). This method accounts for the fact that endemic species are particularly vulnerable to extinction when their habitats are degraded or destroyed, and goes a

long way to protecting endemic species (Myers et al., 2000). The hotspot approach is also a succinct policy that can be pitched to conservation planners and funding agencies worldwide.

Conservation plans based solely on endemic richness and threat, however, do not always capture biodiversity's multifaceted nature. These plans ignore the ecological and evolutionary processes that produce and maintain species; the processes that promote adaptive potential and viability in populations (Smith et al., 1993). If conservation plans account for evolutionary processes, by focusing on preserving regions where adaptive genetic variation is maximized, it should be possible to protect the evolutionary potential of populations, and thus preserve their ability to respond to climate or other human-induced changes (Smith & Grether, 2008).

Step 1: Identify valuable land for conservation

First, some background on Cameroon. Home to about 25 million people, sitting at the hinge of west and central Africa, Cameroon is oft-called "Africa in miniature" because of its rich ethnic, geographic and biological diversity. The country has a presidential republic government and is divided into 10 administrative regions. In Cameroon, areas of genetic richness largely correspond to ecotones, transitional areas between two different biomes. Cameroon's ecotone region bridges the rainforest to the south and savanna to the north. Typified by savanna interspersed with gallery forests, ecotones exhibit different ecological characteristics than their neighboring environments: smaller amounts of rainfall, less tree cover and greater annual fluctuation in environmental variables overall (Longman & Jenik, 1992). These abiotic differences translate into different selective pressures. It has long been hypothesized that ecotones may play a role in speciation, because they are contact zones between species and

subspecies, and can harbor greater numbers of recently evolved species (Chapin, 1932; Fjeldsa, 1994).

Research suggests the unique selective pressures of ecotones are important in fostering diversification across many taxa. For little greenbuls, a common rainforest passerine, morphological and genetic differentiation occurs along the forest-ecotone boundary (Smith et al., 1997; Smith et al., 2005). Recent work on this species suggests populations along the rainforest-savanna gradient are diverging genomically (Zhen et al., 2017). This pattern is echoed in other taxa in Cameron (Freedman et al., 2010; Mitchell et al., 2011; Slabbekoorn & Smith 2002) as well as other parts of the world (Kark et al., 2002; Ogden & Thorpe 2002).

Cameroon's ecotones are especially crucial to conserve given what we expect climate change to do in the region. Climate models predict that by the 2080s, Cameroon will see precipitation decrease by 40%, and seasonal temperature increase by 3-5℃ (Fotso-Nguemo et al., 2017). Increasing temperatures will likely spur shifts in biome distributions, severely impacting species (IPCC, 2014). In the face of these impending environmental shifts, it is even more crucial to preserve adaptive genetic variation.

Step 2: Assess current land use profile: Land types

Major land uses in the ecotone region include mining, logging, community forests and protected areas (*Figure 9*). The two major protected areas are Mbam Djerem National Park and Deng Deng National Park. Logging is permitted in the handful of Forest Management Units (FMU) which are allocated to logging companies via a bidding process. National parks and FMUs are considered Permanent Forest Estate, a specific legal designation in Cameroon signifying the land is to remain permanently forested and managed sustainably. Community

forests are considered Non-Permanent Forests. They are forests of no more than 5,000 hectares

managed by and for village communities. There are strict restrictions on gazetting a community

forest and how the community can actually use the land (use of heavy machinery to log is

prohibited, for example).

The ecotone region also includes a cluster of mining permits. Land designated for mining

falls outside the overall forest estate. Though Cameroon has abundant mineral resources, there

are a limited number of large-scale mining operations in the country, or at least a limited number

of successful operations (Bakia, 2014; Freudenthal, 2016). Small-scale mining – issued via

artisanal permits, which only Cameroonian nationals can receive – likely constitutes most of the

mining that actually occurs in the country (Bakia, 2014). While up to 20% of Cameroon's

territory is technically subject to valid mineral research licenses, mining exploration activities are

likely happening in about 4% of the country's territory (Schwartz et al., 2012). It is important to

note that many of the land uses in the ecotone region overlap with each other in complicated and

conflicting ways, as can be seen in Figure 9. Mining permits have been issued for sections of

protected areas as well as forestry concessions (Dkamela, 2010).

Step 2: Assess current land use profile: Decision-makers

A mix of government agencies and officials, international organizations and conservation

NGOs comprise the ecotone region's decision-makers. The most prominent influencer at the

national government level is the Ministry of Forestry and Wildlife (MINFOF). This agency

develops and implements forestry and wildlife policy, and is charged with managing the

country's forests. MINFOF approves FMUs and Community Forest management plans, oversees

protected areas and liaises with conservation organizations. Another assortment of federal

ministries oversee mining operations in Cameroon, most notably the Ministry of Industries, Mines and Technical Development. Prominent government decision-makers at the local level include *communes*, or local councils. *Communes* are in charge of managing any community forest in its borders. There are more than 370 *communes* in Cameroon.

At the international level there are prominent donor groups and NGOs which have long operated in Cameroon and the Congo Basin region, including the African Development Bank, European Union and western development agencies such as U.S. AID, the Canadian International Development Agency and Germany's GIZ. The World Bank has also been involved in land-use policies in the country for decades. Global forces have influenced most of Cameroon's forest-related laws and reforms (Dkamela et al., 2014).

There are two major conservation NGOs operating in Cameroon, both with deep roots in the country: World Wildlife Fund (WWF) and the Wildlife Conservation Society (WCS). WCS is MINFOF's principal partner in conservation work; the organization has worked with MINFOF on priority-setting, plays a critical role in training MINFOF employees and produced Mbam Djerem National Park's inaugural management plan. WWF works with MINFOF on protected area management, has extensive field operations in the country and is Cameroon's largest donor for protected areas (Singer, 2008). Foreign organizations like WWF and WCS essentially act as sponsors for national parks in Cameroon. Though MINFOF has legal authority to develop and manage protected areas, in reality these international groups drive conservation policies and management decisions on the ground (Singer, 2008). Other international conservation organizations working in Cameroon include the African Wildlife Foundation and the International Union for the Conservation of Nature.

National NGOs play a role in land-use and conservation decisions as well, particularly ones that advocate for indigenous people's rights and work to support community governance. Examples include OKANI and CEFAID (*Centre pour l'Education, La Formation et L'Appui aux Initiatives de Developpement au Cameroun*).

Finally, private industry has ties to the ecotone region, especially in the mining and timber sectors. Foreign enterprises are responsible for most of the timber production in Cameroon (Atyi, 1998). International interest in mining in Africa is surging in general, with tens of billions of dollars in foreign investment flowing into the continent (Edwards et al., 2014). Cameroon is no exception. Revisions to Cameroon's mining code, made in the early 2000s, will likely make the country more attractive to foreign mining investment (Anselme Kamga et al., 2018).

Step 3: Choose most appropriate policy tool

Cameroon's ecotone region is a complex space, harboring a mosaic of land-use types governed by multiple actors at multiple scales of influence. To be effective in this space, policy tools need to be targeted to a particular land-use and influencer. The policy toolkit (*Table 1*) highlights some tools with examples of how they have been, or could be, used. Here I elaborate on one example from each sector.

Regulatory: Include consideration of adaptive genetic variation in land management plans. Taking advantage of Cameroon's existing regulatory frameworks, and editing them to include explicit consideration of adaptive genetic variation, could be done at various regulatory levels. For example: Community forests and FMUs both require MINFOF-approved management plans to ensure sustainability of forest resources, including biodiversity. MINFOF

policies could be edited to require inclusion of protecting adaptive genetic variation in either of these plans. This would be a top-down method of inserting evolutionary responsible thinking into policies governing land use in the ecotone region. In Cameroon, Cerutti et al. (2017) found these plans, on average, reduce overall timber volume and carbon emissions from forest operations. This success suggests they can be an important policy tool in sustainable forest management.

Modifying existing regulations has certain advantages, as it does not entail the oftentimes lengthy or politically fraught process of instituting a novel law or reform. Effectively enforcing this new requirement, however, requires more than just new policy language. Clear guidance on how to assess adaptive genetic variation is needed, as well as buy-in from MINFOF and conservation NGOs. The former could be based on existing guidance from the conservation genomics literature (Ashley et al., 2003; Mace & Purvis, 2008; Schwartz et al. 2007). The Genetic Monitoring for Managers website, hosted by the U.S. Fish and Wildlife Conservation Genetics Laboratory, Alaska Region and the Conservation Genetic Community of Practice, also offers guidance on genetic monitoring tools and techniques.

Economic: Payment for ecosystem services. Payments for ecosystem services (PES) have grown in popularity over the last decade as a policy solution to ecosystem degradation. They are agreements between "sellers" and "buyers" over an environmental service, often a specific land type. PES schemes explicitly recognize the tradeoffs inherent in many conservation projects – the different values of landowners, local land users and external actors – and aim to bridge these conflicting interests through compensation (Wunder, 2006). Conservation International's Conservation Stewards program is one example. In return for specific conservation actions by communities – everything from stopping illegal hunting to reforesting land – the program provides benefit packages, which can include direct payments or investments in social services

34

like health and education. The "environmental service" PES schemes usually protect are specific habitats or ecosystems. Implementing a PES scheme in the ecotone region, and thus expanding the concept of an environmental service worth protecting to include environments that generate and maintain genetic diversity, is an economic tool to conserving the ecotone. This tool could be targeted at the conservation organizations working in Cameroon as well as the country's national NGOs focused on community land rights. The success of the Conservation Stewards program shows that PES schemes can be used effectively by international NGOs. National NGOs may be interested because PES schemes can be designed to build bridges between traditional conservation actors and local land-users, and offer an opportunity to incorporate community values into conservation planning.

Information and communication: Conservation genetics information sharing. Communication among conservation geneticists and policy-makers would help highlight the importance of preserving adaptive genetic variation, and ideally generate creative ideas about how it could be done. This would require education on both sides. Decision-makers likely need explanations of core genetics concepts and a high-level review of conservation research. Scientists likely need lessons on the policy-making process and its economic and social constraints (Hoban et al., 2009). Information-sharing could occur through formal working groups or informal networks, and would be an opportunity for conservation NGOs operating in the ecotone region to connect with academic researchers in Cameroon and abroad. The European Union's ConGRESS project is one example of a virtual network designed to connect policy-makers and conservation managers with accessible genetic expertise and research. The Congo Basin Institute (CBI), a multi-institution environmental research center with facilities in Cameroon, is a potential hub for information-sharing in the region, as CBI already works across

a diverse range of organizations, including academia, industry, government and development groups.

Conclusion

There is no one-size-fits-all approach in conservation planning. The effectiveness of plans and policies is constrained by the realities of politics and budgets, culture and history. More than one policy tool may need to be used to achieve a conservation goal. The tool may evolve over time.

The solutions proposed above are by no means perfect. They are focused specifically on conservation genomics, though it should be noted there are other relevant fields of study besides – such as community ecology and biogeography – that can benefit decision-makers. The solutions presented here are an attempt to place insights from conservation genomics and evolutionary biology in the context of actual land uses and decision-makers. This complicated real-life setting is the space conservation planners – and conservation policies – perform in. Understanding the setting, and exploring how research insights can work within it, is one way to bridge the science-policy gap.

Integrating scientists with policy-makers and local communities at the beginning of research projects, and continually engaging with them throughout the project, is another way to bridge that gap. This can ensure research outputs are highly relevant and useful for policy-makers and communities, and can allow groups to tackle a range of complex issues (Mace & Purvis, 2008; Reid et al., 2016). Both of these methods highlight the importance of collaborating and communicating across disciplines, and with local communities directly impacted by conservation plans. Though the policy tools listed here were not developed with engagement

from local decision-makers, or communities in the ecotone region, this framework aims to acknowledge the importance of that engagement, and recommends mechanisms for achieving it.

The science-policy gap has been discussed and fussed over for decades, by scientists and policy-makers alike. In light of current threats to biodiversity and ecosystems on all corners of the Earth, it is more important than ever to close that gap, and commit the necessary political, financial and social resources to translate research into policy.
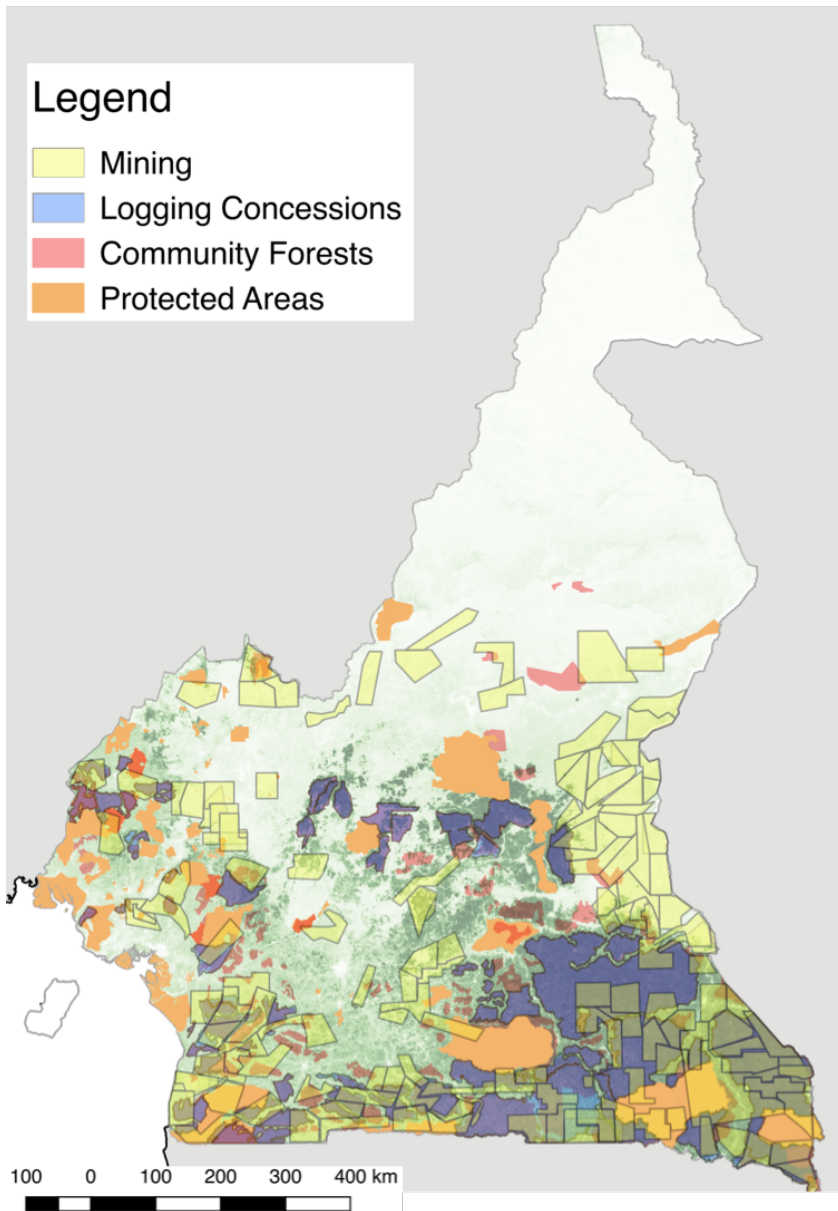
Figure 9. Map showing major land uses in Cameroon. Many of the land uses overlap in complicated and conflicting ways, such as mining leases within protected areas. Purple polygons show where logging concessions and community forests overlap. The ecotone region is the middle swath of Cameroon where the country narrows (going from south to north). The background of the map shows tree layer, derived from MODIS data. Information on mining concessions, protected areas, community forests and logging concessions comes from the World Resources Institute's Forest Atlas of Cameroon.

Table 2. Policy toolkit.

Policy Sector	Tool	Details	References
Regulatory	Environmental Impact Assessments (EIA)	EIAs are legally required in Cameroon for any new project that may affect the environment. EIAs could be expanded to include consideration of adaptive genetic variation.	Appiah-Opoku, 2001 Glasson et al., 2005
	Conservation corridors	Contiguous natural areas that allow otherwise disconnected populations to move between habitats. Corridors could be established between the ecotone's existing national parks, or between community forests.	Rouget et al., 2016
	Forest Management Plans	Community Forests and Forest Management Units require these plans, meant to ensure sustainability of forest resources. Policies could be edited to require inclusion of protecting adaptive genetic variation in these management plans.	Hess & Fischer, 2001
Economic	Conservation concessions (CCs)	Contracts between a landowner and conservation-minded buyer. CCs could be an opportunity for conservation NGOs to protect ecotone land.	Ellison, 2008 Schleicher, 2018
	Payments for ecosystem services (PES)	Agreement between buyers and sellers of an ecosystem service. PES scheme could consider adaptive	Wunder, 2006 Börer et al., 2017

		genetic variation as an ecosystem service.	
	Transfer of development rights	Market-based instruments governments can use to promote transfer of development rights from ecologically sensitive areas to areas with higher development potential.	Barrows & Prenguber, 1975 Panayotou, 1994
	Development of alternative livelihoods	Promotion of ecotourism, agro-forestry approaches and other methods to discourage ecologically damaging activities and encourage conservation.	Krüger, 2005 Brandon, 1996 McNeely & Schroth, 2006
	Valuation of genetic diversity	Evaluating the economic and ecological value of adaptive genetic variation, genetic diversity and the services it provides to society. This would bolster arguments for its consideration in conservation planning and policies.	TEEB, 2010
Information & Communication	Information-sharing tools	Designed to improve information sharing, speed the science-to-policy transfer and communicate scientific and policy information in clear, accessible ways. Can take the form of synthesis groups or online networks.	Hoban et al., 2013
	Framework for stakeholder dialogue	Methods of encouraging greater collaboration among those involved in conservation decisions; can occur formally or informally.	Laurance et al., 2012
	Community Forest support	Support communities in ecotone region to create new community	Smith et al., 2009 Rodríguez et al., 2007

		forests or manage existing ones, with management plans that recognize the importance of adaptive genetic diversity. This would be a method of engaging and bolstering local communities and local institutions in conservation actions.	

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