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Biome and migratory behaviour significantly influence vertebrate genetic diversity

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Genetic diversity (GD) is largely determined by effective population size, which may vary dramatically for species that differ in key aspects of their biology such as vagility. To identify evolutionary patterns associated with animal distributions and movements, we examined relationships among GD (i.e. microsatellite heterozygosity and allelic richness), taxonomic class, biome and migratory behaviour. Linear regression revealed that migratory mammals, reptiles, amphibians and fishes had less GD compared to nonmigratory species, whereas migratory birds had more GD than their nonmigratory counterparts. We also found that the biome a species inhabits influences the GD of migratory and nonmigratory species differently. For example, migratory amphibians at low latitudes are more genetically diverse than migratory amphibians at higher latitudes. However, we found the reverse relationship (i.e. decreased GD in low-latitude migratory species compared to higher latitude migratory species) in mammals and fishes and no influence of biome on reptile GD. We suggest that these differences are a result of differences in vagility, the extent of philopatry among the classes and perhaps differential selection between terrestrial and aquatic species. We argue that these categorical disparities in GD reflect changes in effective population size driven at least partly by differences in habitat.

ADDITIONAL KEYWORDS: allelic richness – effective population size – habitat – heterozygosity – microsatellite.

INTRODUCTION

The rate at which genetic diversity (GD) is lost can be predicted using population genetics theory. In particular, effective population size (N_e) is a useful concept for understanding genetic drift and provides a mathematical framework for predicting the loss of neutral GD (Crow & Kimura, 1970; Allendorf & Luikart, 2007). N_e is the size of an idealized population with the same amount of standing GD as the focal population (Wright, 1939), where populations with a smaller N_e lose diversity more rapidly than populations with a larger N_e. Theory and empirical data indicate that managing populations to maintain a constant population size (Vilà *et al.*, 2003), equitable sex ratio (Melampy & Howe, 1977), nonoverlapping generations (Crow & Denniston, 1988) and equal family sizes (Frankham *et al.*, 2000) will maximize N_e and slow the loss of GD. Although the natural history of most species violates many of these idealized assumptions, population managers often act to retain or increase N_e in an effort to retain neutral GD.

Declines in GD can reduce evolutionary fitness, initiating a negative feedback loop that leads to smaller population sizes, increased drift and additional inbreeding (Gilpin & Soulé, 1986) that can result in an increased risk of population extinction (Saccheri *et al.*, 1998), reduced population growth rates (Hanski & Saccheri, 2006) and reduced potential for response to environmental change (Waples, 1991). For example, evolutionary fitness often decreases as homozygosity increases (Coltman & Slate, 2003), and a negative

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feedback loop leads to further decreases in fitness in subsequent generations. Fortunately, the negative effects of low GD can often be mitigated by increasing gene flow through the enhancement of population connectivity or by increasing the standing GD within a population (e.g. via stocking or reintroductions).

The movement of individuals across a landscape facilitates allelic exchange, and thus, population genetic theory implies that migratory behaviour is associated with higher N_e and levels of standing GD. However, migrating species require at least two distinct habitats, meaning that these species are more likely to be impacted by ecological changes (e.g. competition, predation and pathogens) compared to species that require only a single habitat (Rappole, 1995). Additionally, GD in vertebrates is often associated with the extent and continuity of habitat (Ward, Woodward & Skibinski, 1994; DeWoody & Avise, 2000; Doyle *et al.*, 2015). Because habitat fragmentation is not equally distributed among biomes (Wade *et al.*, 2003), some species may be more impacted by fragmentation because of their habitat needs (Debinski & Holt, 1999). Species in areas with less anthropogenic disturbance may have a higher mean N_e and, therefore, higher mean GD compared with species that occur in areas more impacted by humans. If so, then, it follows that because migratory species require multiple habitats, these species should be more likely than nonmigratory species to have been impacted by fragmentation and to have suffered population declines that lead to less GD in migratory compared to nonmigratory species.

In addition to allelic exchange between populations, factors relating to species' habitat characteristics may also influence the standing GD (Epps *et al.*, 2005; Keyghobadi, 2007; Dixo *et al.*, 2009). Herein, we consider global patterns of habitat characteristics in terms of biomes, which have a substantial latitudinal gradient (see Olson *et al.*, 2001). Geographic area varies with latitude (biomes are larger in tropical regions compared to polar regions; Terborgh, 1973), and because geographic areas are positively related to population size (Gaston & Blackburn, 1996) and population size is related to N_e and GD (Frankham, 1996), latitude may also be related to GD. Furthermore, climatic stability varies globally such that lower latitude habitats tend to be more climatically stable than higher latitude habitats (Stevens, 1989). Over many generations, perturbations in climate result in habitat changes and variation in population sizes (Lande & Barrowclough, 1987), reducing N_e and GD in high latitudes more so than in low latitudes. Together, these findings (see also Gaston, 2000) suggest that the biome in which species reside may influence standing levels of GD.

Herein, we explicitly test the hypothesis that migratory vertebrates harbour less standing GD than nonmigratory species. Because similarities in life history characteristics (e.g. typically high fecundity in fishes) or other traits (e.g. ability to fly in birds) within particular clades are likely to result in lineage-specific trends in the response to migratory behaviours, we analyze our data with respect to taxonomic classes. We expect that migratory species are likely more impacted by fragmentation than nonmigratory species and will have lower GD because migratory species require multiple habitats or, at the very least, multiple locations. Furthermore, we consider the effects of species' biome on standing GD because geographic area and climatic stability, which vary with biome, influence N_e and GD. We expect that species that reside in larger, more stable biomes will have more GD (and larger N_e) compared to species in smaller, more variable biomes. However, we hypothesize that migratory behaviour will influence standing GD such that the relationship between biome and GD will differ between migratory and nonmigratory species. Our results reveal patterns that vary among vertebrate classes, and our proposed explanations attempt to identify the biological processes underlying these patterns.

METHODS

As a measure of GD, we relied on heterozygosity and allelic richness estimates previously gathered from a systematic survey of the literature (see Willoughby *et al.*, 2015). Briefly, ISI (Institute for Scientific Information) Web of Science was used to identify thousands of microsatellite studies published on wild vertebrate populations between 1990 and February 2014 using the topic search: microsatellite and (amphibian or avian or bird or fish or mammal or reptile). These records were manually curated for applicability and scope – we required all studies to contain data for a wild vertebrate population, use at least five microsatellite loci and genotype at least ten individuals – and the means and standard deviations (across loci) were gathered for observed heterozygosity and allelic richness. We filtered our data set to include only one record per species; in cases where we obtained multiple GD estimates for a particular species, we selected the estimate that was generated from the largest sample size, which we defined as the product of the number of individuals genotyped and the number of loci surveyed. The microsatellite data we collected were not correlated with the phylogenetic history of the species and did not show systematic evidence of bias relating to the source (heterologous vs. species-specific) of primer sequences used or the number of loci and individuals genotyped (Willoughby *et al.*,

2015). We previously investigated the potential impact of these effects using phylogenetic eigenvector regression (PVR), implemented in the R package PVR (Santos *et al*., 2013), as nuisance variables such as sample size and primer specificity may not be phylogenetically correlated; so methods such as independent contrasts and phylogenetic least squares regression were inappropriate. To avoid potential confounding effects of migratory behaviour and conservation status on GD, we used a subset of the Willoughby *et al*.'s (2015) data for our current analysis: those species identified as either *least concern* or *near threatened* by the International Union for the Conservation of Nature (IUCN, 2013).

To investigate the impact of migration on GD, we scored species based on migratory behaviours. We define migration as the predictable mass movement of individuals which is not limited to specific sexes or age classes, occurs in the same direction or towards a particular common habitat and is farther in distance travelled than the typical daily movements of individuals for a particular species (Begon *et al*., 2006; e.g. anadromous salmon, amphibians that require ephemeral ponds, peregrine falcons, elephants and sea turtles). We used a variety of approaches to score a species as migratory or nonmigratory. For mammals, we relied upon formal species accounts published by the American Society of Mammalogists as well as species descriptions given in Mammals of the World (Walker, Nowak & Paradiso, 1983; Nowak, 1999). Avian migration status was determined using the online Birds of the World database (del Hoyo et al., 2014), and FishBase (Froese & Pauly, 2014) was used to score the fishes included in our data set. If a species was not represented in one of these databases, we relied on the primary literature associated with that species to score migratory behaviour. This was the case with all amphibians and reptiles used in our analyses and for many fishes.

Because habitat characteristics often influence population GD (Epps *et al*., 2005; Keyghobadi, 2007; Dixo *et al*., 2009), we also quantified the ranges for each species in terms of biome composition. We determined the biome composition of each species range using IUCN species-specific range maps, when available. First, we transformed all maps into the cylindrical equal area projection using ArcMap10 (Environmental Systems Research Institute, 2010). We then estimated the spatial contribution of terrestrial biomes, each with distinct biotas and ecological conditions, within each species range using the World Wildlife Fund terrestrial biomes shapefile (Olson *et al*., 2001).

Statistical analyses

To meet assumptions of normality, we transformed our heterozygosity and allelic richness estimates for each species. We calculated the log odds of heterozygosity (*loh*) using the equation

$$loh = \log\left(\frac{H_O}{1 - H_O}\right)$$

where H_O is the observed heterozygosity for a particular population. We interpreted the calculated odds ratio (before logarithm transformation) as the odds of a randomly drawn individual being homozygous at any particular locus. After the log transformation, larger *loh* values represent species with higher heterozygosity values compared to species with smaller *loh* estimates. After square root transformations of allelic richness to correct for normality (Doyle *et al*., 2015), we removed statistical outliers, which we defined as data points that were more extreme than 1.5, the interquartile range for each taxonomic class.

We evaluated the impact of migratory behaviour on GD using linear models. In our first set of models, we used log odds of heterozygosity and the square root of the number of alleles as our response variables. We used class, migratory behaviour (nonmigratory = 0, migratory = 1) and the interaction between class and migratory behaviour as predictors. We fit our models with different intercept estimates for both predictors to facilitate comparisons of the mean *loh* across migratory and nonmigratory groups between classes. In order to account for variation in GD resulting from the choice of molecular markers in each study and the sampling strategy used, we employed a bootstrap approach that was based on the variability in heterozygosity and alleles reported by each study. Specifically, we pulled a response variable estimate (i.e. heterozygosity or number of alleles) from a normal distribution with mean and standard error equal to the mean and corresponding standard error computed across loci for each study, respectively. We then regressed the random pulls against the predictor variables. Whenever a random pull for a study resulted in a heterozygosity estimate outside the range of 0–1 or resulted in number of alleles < 0, we dropped the study from that particular regression. We repeated our sampling and computation of regression coefficients 1000 times. To assess significance, we estimated the mean class-specific coefficient estimate for migratory and nonmigratory species across all 1000 bootstrapped coefficient estimates and compared standard error confidence intervals (CIs) estimates around each mean ($\alpha = 0.05$; Payton, Greenstone & Schenker, 2003).

We also evaluated the interaction of biome with migratory behaviour for the subset of species for which these data were available. First, we used nonmetric multidimensional scaling (NMDS) to reduce the dimensionality of the biome data. Ordinations

were computed with three axes from one dissimilarity matrix generated using the area of each biome occurring in each species range estimate and were run using the metaMDS function within the vegan package (Oksanen *et al.*, 2014) using a Bray–Curtis transformation; we determined the number of dimensions to use based on the stress–dimensionality plot. In order to interpret the NMDS vectors, we compared the NMDS vectors with our original biome values using a Spearman correlation and examined the variable-specific correlation coefficients. We selected the top biome vector to use in our later models. Next, we again ran linear models and used either the log odds of heterozygosity or the square root of allelic richness as our dependent variable and the top NMDS axis with an interaction with migratory behaviour as the independent variable. We again required separate intercept estimates for both parameters and bootstrapped our coefficient estimates using the method described previously (n = 1000). However, fitting coefficients for migratory behaviour, classes, interaction between migratory behaviour and class, NMDS axis and the interaction between NMDS axis and migratory behaviour in a single model resulted in overparameterization. To avoid overparameterizing our models, we subdivided our data by class and ran separate regressions predicting GD as a function of the interaction between NMDS Axis 1 and migratory behaviour for each class. We assessed significance by comparing the 95% CI of the model slope to 0 (α = 0.05) and compared the 95% CI of the slopes between the migratory and nonmigratory groups within each taxonomic class.

All analyses and data manipulation were conducted in R (R Development Core Team, 2013). In our linear models, we used a means parameterization, instead of an effects parameterization, to fit independent slope and intercept estimates for each class. (The necessary R coding followed Kéry, 2010.) We chose a means parameterization approach because it results in the same interpretation as effects parameterization but has the advantage of providing a usable standard error estimate for the estimated model coefficients; with effects parameterization, we could appropriately sum intercept and slope estimates to obtain the coefficient estimate, but combining the standard error estimates is not trivial in that they cannot be directly summed. All of our analysis code is available in the online Supporting Information. Figure 1 provides a heuristic overview of our workflow.

RESULTS

Our microsatellite database is more fully described in Willoughby *et al.* (2015), but it consists of 5916 vertebrate populations surveyed at a total of 17 988 loci (Table 1; Table S1). We scored migratory behaviour for most species in our microsatellite database, and all but a small proportion could be confidently scored

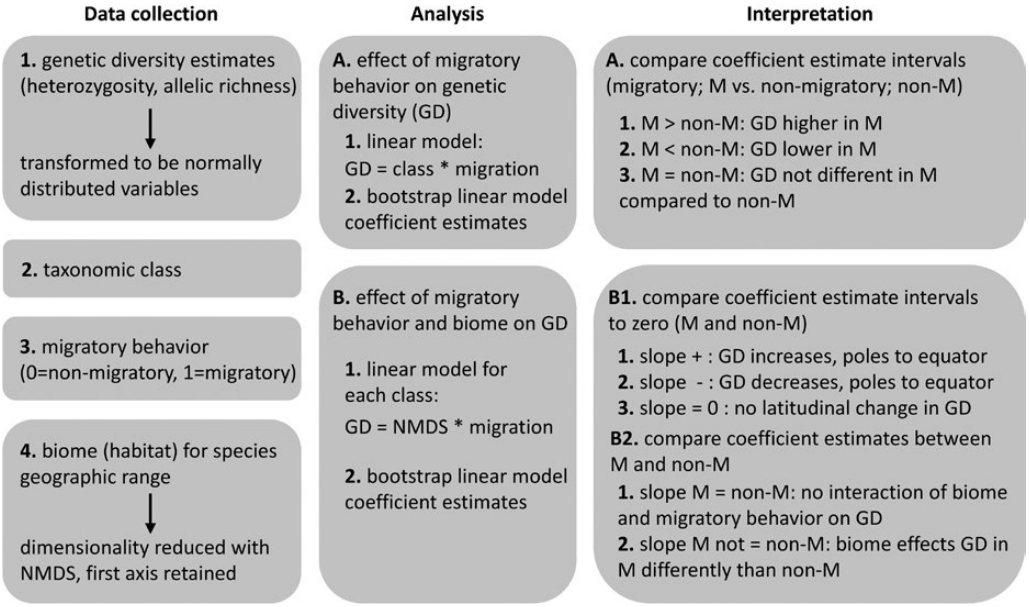

Figure 1. Heuristic overview of data collection, analysis and interpretation. We utilized four sources of data (i.e. genetic diversity, taxonomy, migratory behaviour and biome) to evaluate differences in genetic diversity between migratory and nonmigratory species (Analysis A) and the interaction between migratory behaviour and species biome (Analysis B). In both the analyses, we assessed significance by bootstrapping the linear model coefficient estimates and comparing the coefficient estimates.

Table 1. Number of data points gathered within each class

	Genetic	Biome	Migration
Fish	157	48	48 (m = 68, nm = 53)
Amphibian	71	57	57 (m = 36, nm = 31)
Bird	222	212	212 (m = 65, nm = 156)
Mammal	246	87	87 (m = 29, nm = 196)
Reptile	54	42	42 (m = 29, nm = 19)

Note: Each data point represents a unique species. Migratory behaviours are further divided by species exhibiting migratory (m) and nonmigratory (nm) behaviours.

(percentage scored: fishes = 80%, amphibians = 95%, birds = 98%, mammals = 94% and reptiles = 87%; Table S2; Figs S1 and S2). We also determined terrestrial biome composition for those species where IUCN species range maps were available (n = 573; Table S3).

We tested for a significant impact of migratory behaviours within each class by comparing the coefficient estimates based on the bootstrapped linear regressions. We found significant differences in standing GD among the classes we analyzed [Fig. 2; heterozygosity F = 62.12, degrees of freedom (d.f.) = 10 and 672, R^2 = 0.49, P < 0.0001; allelic richness F = 1234, d.f. = 10 and 672, R^2 = 0.94, P < 0.0001]. We also observed significantly less GD in migratory fishes, amphibians, mammals and reptiles compared to nonmigratory species (Fig. 2). However, migratory birds had a significantly higher GD compared to nonmigratory birds (Fig. 2b).

To better understand how migratory behaviours interact with various biomes, we collected and analyzed biome data relative to GD. We first reduced the dimensionality of our range-wide biome data using NMDS (NMDS axes values for each species are available in Table S4). The stress of our final NMDS model (three axes, stress = 0.137) indicated a quality model. Based on the weights of the variables used to create the first NMDS axis (NMDS1) and the correlation of the biome variables with the NMDS values, we interpreted the primary NMDS axis as the trade-off between tropical biomes (positively weighted variables) and polar biomes (negatively weighted variables) that are associated with the habitat changes that occur when moving from high to low latitude (Table 2). We found mixed relationships among class, migratory behaviour and biome (Fig. 3). Overall, the relationship between NMDS1 and GD was not consistent between classes and did not support the geographic area or climatic stability hypotheses. Furthermore, we found that migratory and nonmigratory species differed in the relationship between NMDS1 and GD; migratory amphibians had significantly more GD at higher NMDS1 values (low-latitude biomes closer to the

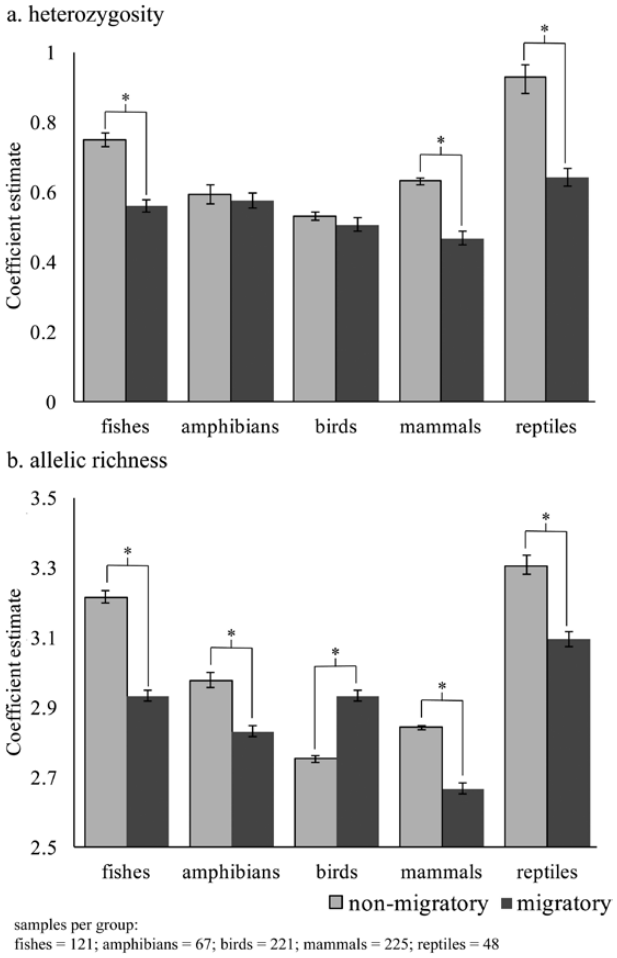

Figure 2. Linear regression model results for the interaction between class and migratory behaviour predicting microsatellite genetic diversity (heterozygosity, Panel a; allelic richness, Panel b). Error bars depict standard error estimates, calculated from bootstrapped estimates of 1000 linear models where the response variable was randomly pulled from a normal distribution with study mean and variance for the response variable under consideration. Significant differences between migratory and nonmigratory species within each class are depicted with an asterisk.

equator) relative to migratory amphibians with lower NMDS1 values (high-latitude biomes closer to the poles) and to nonmigratory amphibians higher NMDS1 values. We also found that migratory fishes and mammals had less GD at higher NMDS1 values relative to nonmigratory fishes and mammals with large NMDS1 values. In fishes, this was driven by a significant positive relationship between GD and NMDS1 in nonmigratory species, whereas in mammals this was a result of a significant negative relationship between GD and NMDS1 in migratory species (Tables 3 and 4). In both amphibians and fishes, these trends were found in the heterozygosity and allelic richness models, although

Table 2. Composition of nonmetric multidimensional scaling (NMDS) Axis 1

Biome	Weight	Correlation coefficient
Tundra	−0.775	−0.345
Rocks and ice	−0.669	−0.194
Boreal forests/taiga	−0.641	−0.403
Temperate coniferous forests	−0.502	−0.451
Temperate broadleaf and mixed forests, northern	−0.497	−0.503
Temperate broadleaf and mixed forests, eastern	−0.409	−0.415
Lakes	−0.407	−0.153
Mediterranean forests, woodlands, and scrubs	−0.281	−0.289
Montane grasslands and shrublands	−0.137	0.112
Deserts and xeric shrublands	−0.124	−0.029
Tropical and subtropical coniferous forests	−0.032	0.104
Flooded grasslands and savannas	0.189	0.122
Tropical and subtropical dry broadleaf forests	0.321	0.373
Mangroves	0.341	0.297
Tropical and subtropical grasslands, savannas and shrublands	0.437	0.344
Tropical and subtropical moist broadleaf forests	0.613	0.586

Note: Weights are from the NMDS procedure and indicate the weight of each variable on Axis 1. The correlation coefficients are from a Spearman correlation between the variables and the NMDS axis. Based on the weights and correlation coefficients, we interpreted NMDS Axis 1 as representing the changes across biomes associated with the changes in latitude.

significant differences in the mammals were only found in the allelic richness model (Table 4). Finally, we found no significant difference between migratory groups and biome in birds or reptiles.

DISCUSSION

We used thousands of microsatellite loci surveyed in hundreds of species to test the hypothesis that migratory vertebrate species harbour less GD than nonmigratory species, based on the premise that migratory species would be more susceptible to the effects of fragmentation because of their need for multiple habitats relative to nonmigratory species. We found that the effects of migration differ among taxonomic classes: migratory fishes, amphibians, mammals and reptiles harbour less GD than nonmigratory fishes, amphibians and reptiles (Fig. 2). In contrast, migratory birds harbour more GD compared to their nonmigratory counterparts (Fig. 2). Additionally, we found that the relationship between biome and GD is not predicted by increasing geographic area or climatic stability expected at decreasing latitude. Furthermore, the effects of biome influence GD differently in migratory fishes, amphibians and mammals compared to nonmigratory fishes, amphibians and mammals (Tables 3 and 4); these differences in GD between species with differing migratory behaviours suggest that class-specific biological characteristics (e.g. life history traits) influence migration or that different taxonomic groups of organisms respond differently to migration.

The differences in GD between migratory and nonmigratory species suggest that, compared to the nonmigratory species in each class, N_e is generally smaller for migratory fishes, amphibians, mammals and reptiles, but higher for migratory birds. One possible explanation for this pattern is that, as we hypothesized, fragmentation in multiple environments leads to decreased N_e in migratory fishes, amphibians, mammals and reptiles, but not in migratory birds. However, we did not find significant relationships between GD and biome data (potentially representing differences in fragmentation) for all the classes we examined (Tables 3 and 4), suggesting that this is not a primary cause of the observed trends.

An alternative explanation is that population sizes and risk associated with migration vary among classes. Many migratory birds have huge population sizes (e.g. various geese, Fox *et al.*, 2010), making these species more robust to environmental perturbations during migration. Smaller population sizes can lead to greater risk associated with migration: harsh abiotic conditions during migration can lead to decreased survival (Stokke *et al.*, 2005), resulting in reduced population sizes in already-small populations. While the association between population size and relative risk of migration is also true for fishes, more stable aquatic habitats may provide a buffer against many environmental perturbations. We suggest that population sizes for migratory species may be larger in terrestrial species to counter the risk associated with migration and that, because fishes often have relatively stable environments (e.g. the high specific heat of water leads to a more predictable and consistent temperature pattern; Vannote & Sweeney, 1980), a relatively smaller risk is imposed on migrating individuals. In other words, harsh environmental conditions that are likely to disrupt migration and lead to heavy losses (e.g. hurricanes, droughts and cold fronts) may be more common in terrestrial biomes compared to aquatic biomes. If it is true, this should promote large population sizes in terrestrial migratory species but would not impose a sufficiently

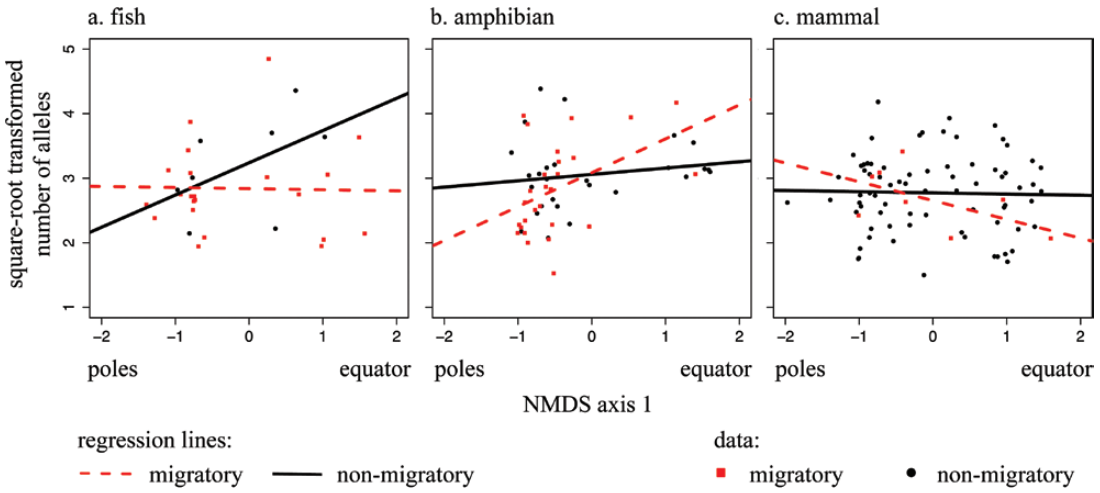

Figure 3. Linear models comparing the relationship between biome and genetic diversity among migratory behaviour groups. We assessed biome over a reduced dimensionality vector (nonmetric multidimensional scaling Axis 1), which we interpreted as the change in biome characteristics that occurs from the high to low latitude (poles towards equator). Between migratory and nonmigratory species, we compared the change in genetic diversity (represented by the number of alleles) across this biome gradient using a bootstrapped regression (plotted points represent the mean, transformed number of alleles).

strong selection gradient to impact aquatic migratory species' population sizes because the risk associated with migration is smaller.

Another potential cause for the differences in effect of migration between classes is the inherent movement ability of fishes, amphibians, mammals and reptiles compared to birds. Vagility is known to significantly influence GD via limitations on dispersal in many vertebrates (Hillman *et al.*, 2014). Because increased vagility means that migratory birds can move farther to more effectively mix alleles, this explanation may help explain an increased N_e in birds. Increased vagility also means increased ability to explore new areas in the face of environmental changes such as fragmentation (Diamond & Mayr, 1976). In contrast, reduced vagility in non-avian species could lead to a concomitant reduction in N_e and GD due to increased mortality, similar to the effects observed during long-distance dispersal events (e.g. increased predation and establishment of new territories; Waser, Creel & Lucas, 1994). Although highly vagile species can explore new areas in the face of fragmentation, less vagile species may be more impacted by fragmentation of their habitat, especially for migratory species that inhabit more than one habitat during their lifetime.

Difference in the incidence of philopatry, or returning to breed at the location of an individual's birth, among taxonomic groups is another potential explanation for the different impacts of migration on GD. For example, the N_e of most migrating birds may be larger than that of typical amphibians. In birds, first-year breeders do not typically return to their hatching site (Weatherhead & Forbes, 1994), and natal dispersal of individuals (and their genes) occurs in many birds and mammals (Sutherland *et al.*, 2000). Additionally, nest site relocation in adult birds is not uncommon (e.g. Shields, 1984; Gavin & Bollinger, 1988; Haas, 1998) and can maximize lifetime fitness (Hoover, 2003). Together, these behaviours should result in a more panmictic population, which means a larger N_e in birds compared to species with more structured populations. In contrast, many fishes return to their birthplace for spawning (Stabell, 1983; Dittman & Quinn, 1996; Thorrold *et al.*, 2001), as do many amphibians where juvenile dispersal is the primary mode of population connectivity (reviewed in Cushman, 2006). This pattern of returning to breed in the same location over a lifetime limits allelic exchange by restricting the pool of potential mates (Anderson, Rhymer & Rohwer, 1992; Matthiopoulos, Harwood & Thomas, 2005). Therefore, differences in philopatric behaviours may lead to disparities in the N_e values among breeding migratory species meaning that migration may lead to greater mixing of alleles in birds but actually restrict breeding pools in other taxa.

We also found that the biome in which the species resides impacts GD, although not typically in the direction predicted by the increasing geographical area or climatic stability hypotheses (both hypotheses suggest that GD in tropical biomes is greater than GD in polar biome; Tables 3 and 4). Furthermore, the relationship between GD and NMDS1 (biome) was not consistent within classes. Globally, this suggests that, compared to geographic area or climatic stability, other forces more profoundly influence GD. For example, the mutation

Table 3. Linear regression model results where heterozygosity (log odds of heterozygosity) was predicted from the interaction between the migratory behaviour variable (nonmigratory = 0, migratory = 1) and nonmetric multidimensional scaling Axis 1 (NMDS1), used to reduce dimensionality of biome variables for each species range

Class	Model/predictor	Sample size	Intercept (95% CI)	Slope (95% CI)	*P*-value	*F*	d.f.	R^2
Fishes	Full model	31	–	–	<0.001	9.114	4 and 27	0.57
	Nonmigratory	8	0.35, 0.77	**0.36, 0.95**	–	–	–	–
	Migratory	23	0.34, 0.66	–0.31, 0.01	–	–	–	–
Amphibians	Full model	54	–	–	<0.001	12.39	4 and 50	0.5
	Nonmigratory	27	0.53, 0.77	–0.10, 0.18	–	–	–	–
	Migratory	27	0.60, 0.90	**0.23, 0.64**	–	–	–	–
Birds	Full model	211	–	–	<0.001	36.61	4 and 207	0.41
	Nonmigratory	150	0.48, 0.57	–0.09, 0.03	–	–	–	–
	Migratory	61	0.49, 0.70	**0.03, 0.49**	–	–	–	–
Mammals	Full model	82	–	–	<0.001	23.36	4 and 78	0.54
	Nonmigratory	74	0.53, 0.64	**–0.16, -0.01**	–	–	–	–
	Migratory	8	0.52, 0.81	–0.05, 0.25	–	–	–	–
Reptiles	Full model	37	–	–	<0.001	16.43	4 and 33	0.67
	Nonmigratory	15	0.53, 1.05	**–0.75, –0.10**	–	–	–	–
	Migratory	22	0.56, 0.81	–0.28, 0.07	–	–	–	–

Note: NMDS1 was interpreted as the change in biome characteristics that occurs from the equator to the poles. Data were separated into classes and run as mean parameterization models, and coefficient estimates were bootstrapped using 1000 replicate linear models with response variable estimates drawn from a normal distribution (mean and variance equal to the study mean variance of log odds heterozygosity). Slope estimates of biome and migration interactions that were significantly different from zero are noted in bold. Main model statistics are also shown (*F*, *F*-statistic; CI, confidence interval; d.f., degrees of freedom; *P*-value; R^2).

rate is higher in low latitudes relative to high latitudes (Gillman *et al.*, 2009) and is also higher in endotherms compared to ectotherms (Gillooly *et al.*, 2005). Thus, complex forces that act over scales not accounted for herein are likely to contribute significantly to the overall pattern of GD we observed (Fig. 3).

The relationship between GD and biome was different in migratory species compared to nonmigratory species within some classes (Fig. 3; Tables 3 and 4). Specifically, we found that nonmigratory, low-latitude (high NMDS1 values) fishes had a higher GD compared to nonmigratory fishes at higher latitudes (low NMDS1 values) and to low-latitude, migratory fishes (Fig. 3a). Although these patterns may be due to biologic or habitat constraints (e.g. reduced dispersal ability between waterbodies or increased fragmentation in temperate climates), we cannot eliminate the effect of artificial stocking as a cause of the observed patterns of GD. Stocking is common worldwide (Watanabe *et al.*, 1990; Martinez *et al.*, 1993; Hengsawat & Ward, 1997) and can increase N_e as well as result in the movement of alleles that would not otherwise occur (Martinez *et al.*, 1993). Additionally, many hatcheries use inbred populations that, when released, swamp the native gene pools (Waples, 1991). In these ways, stocking may have altered the pattern of GD observed in fish populations in areas where stocking has been common for over a century (e.g. portions of the US; Edwards & Nickum, 1993) compared to other biomes. If so, this would lead to a signal of increased N_e in equatorial, nonmigratory species and would reduce the effect of the biome in migratory fishes.

We also found significant relationships between biome and GD in the migratory and nonmigratory amphibians and mammals (Fig. 3b and c). Migratory, low-latitude (equatorial) amphibians had more GD than migratory amphibian species found at higher latitudes (nearer the poles). Nonmigratory mammals had higher GD than migratory mammals in low latitudes and lower GD than migratory mammals at higher latitudes. As differences in the population densities between the lower and higher latitudes could have led to the observed pattern, so too could differences in contemporary fragmentation patterns within biomes. Human-caused fragmentation is greater in temperate biomes; the percentage of total fragmentation attributed to anthropogenic causes is 10% higher in temperate biomes compared to tropical rainforest biomes (Wade *et al.*, 2003). If the fragmentation led to reduced connectivity in the higher latitude biomes compared to the lower latitude biomes (Crooks *et al.*, 2011), we would expect the higher latitude species to have reduced N_e compared to species with the less fragmented, lower latitude biome (although habitat use patterns may somewhat mitigate this effect). Migratory species travel more than nonmigratory species, increasing the likely impact of fragmentation. Therefore, increased fragmentation in temperate biomes may lead to reduced N_e and decreased GD in

Table 4. Linear regression model results where allelic richness (square root of the number of alleles) was predicted from the interaction between migratory behaviour variable (nonmigratory = 0, migratory = 1) and nonmetric multidimensional scaling Axis 1 (NMDS1) used to reduce dimensionality of biome variables for each species range

Class	Model/predictor	Sample size	Intercept (95% CI)	Slope (95% CI)	*P*-value	*F*	d.f.	R^2
Fishes	Full model	31	–	–	<0.001	133.4	4 and 27	0.95
	Nonmigratory	8	**3.06, 3.39**	**0.27, 0.73**	–	–	–	–
	Migratory	23	**2.68, 2.95**	–0.14, 0.09	–	–	–	–
Amphibians	Full model	54	–	–	<0.001	321.5	4 and 50	0.96
	Nonmigratory	27	2.96, 3.14	–0.02, 0.20	–	–	–	–
	Migratory	27	2.97, 3.17	**0.42, 0.63**	–	–	–	–
Birds	Full model	211	–	–	<0.001	968.6	4 and 207	0.95
	Nonmigratory	150	**2.71, 2.78**	**0.03, 0.13**	–	–	–	–
	Migratory	61	**2.88, 3.06**	**0.01, 0.35**	–	–	–	–
Mammals	Full model	82	–	–	<0.001	448.8	4 and 78	0.96
	Nonmigratory	74	2.72, 2.81	–0.07, 0.03	–	–	–	–
	Migratory	8	2.56, 2.74	**–0.40, –0.18**	–	–	–	–
Reptiles	Full model	37	–	–	<0.001	153.7	4 and 33	0.95
	Nonmigratory	15	3.05, 3.40	–0.33, 0.05	–	–	–	–
	Migratory	22	3.10, 3.33	–0.33, 0.02	–	–	–	–

Note: NMDS1 was interpreted as the change in biome characteristics that occurs from the equator to the poles. Data were separated into classes and run as mean parameterization models, and coefficient estimates were bootstrapped using 1000 replicate linear models with response variable estimates drawn from a normal distribution (mean and variance equal to the study mean variance of square root of the number of alleles). Slope estimates of biome and migration interactions that were significantly different from zero are noted in bold. Main model statistics are also shown (*F*, *F*-statistic; CI, confidence interval; d.f., degrees of freedom; *P*-value; R^2).

temperate-dwelling amphibians compared to species at more tropical latitudes. Our comparison between amphibian and mammalian migratory species suggests that mammals may be better able to utilize corridors (e.g. due to differences in vagility and/or cognition) to overcome the fragmentation present in the temperate regions. Because we did not detect the same pattern in migratory fishes, birds or reptiles, and we detected no relationship in the less mobile, nonmigratory amphibians, we suggest that amphibians may be more sensitive to fragmentation relative to other vertebrates (Cushman, 2006).

Caveats

Although we identified statistically significant relationships between GD and migratory behaviours, our analysis has obvious limitations. We note that our data lack the population replicates necessary to evaluate trends across taxa (e.g. individual species) and thus represent average effects across taxonomic class. We are also limited with regard to the scope of the available data. For example, Figure 3 shows a paucity of data for amphibians that live near the equator, which likely reflects a lack of studies that genetically characterize tropical amphibian populations and not a lack of tropical amphibian species. Similarly, we have used microsatellite GD as a proxy for N_e to better understand the effects of migration, even though the relationship between the two may be compromised by other factors (e.g. overlapping generations and unequal sex ratios). Furthermore, historical events during colonization after the Quaternary ice ages and very recent population declines are likely to add additional noise to our analysis, as this will be reflected in the GD estimates. Finally, we have assumed that a lower mean heterozygosity or allelic richness, relative to the class average, represents a significant loss in GD. However, at least in theory, the lower GD could represent species-specific strategies that result in lower GD but that do not result in negative consequences for that species. Thus, the effect of fragmentation on GD needs to be investigated, class by class, and further documentation is required to fully understand and vet the mechanisms we propose.

CONCLUSION

Our analyses indicate the effect of migratory behaviour on GD varies between vertebrate classes. The migratory behaviours of fishes, amphibians, birds, mammals and reptiles appear to impact N_e differently, which in turn impacts GD, potentially due to the different risks associated with migrating through terrestrial or aquatic biomes, species vagility and/or the incidence of philopatry. Importantly, the biome in which the species occurs impacts migratory fishes, amphibians

and mammals differently than nonmigratory fishes, amphibians and mammals.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Boxplot comparing heterozygosity (transformed to log-odds heterozygosity) in non-migratory and migratory species.

Figure S2. Boxplot comparing allelic richness (transformed by square root) in non-migratory and migratory species.

Table S1. Results of literature reivew for >5000 manuscripts, as presented in Willoughby et al (Biological Conservation, in press). Column titles: reference, reference number; year, publication year; journal, publication journal; relevant, y=meets study requirments n=does not meet study requirements; initials, reviewer/author initials; G_s species name in original paper; hetM, mean heterozygosity; hetSD, heterozygosity standard deviation; allelesM, mean number of alleles; allelesSD standard deviation around the number of alleles; numIndv, number of individuals genotyped; numLoci, number of loci genotyped; primer, y=heterologous primer, n=species specific primer, m=both hterologous and species specific primer; MERdb, Molecular Ecology Resources database number for entry if applicable.

Table S2. Migration scoring details. We scored each species as non migratory (0) or migratory (1) in the 'migration' column, using the sources listed in the 'citation' column. Also included in this table is the original species from the primary manuscript, the species for which the migration behavior was scored (for cases when taxonomy changed) and other pertinant notes.

Table S3. Biologic realm composition for species ranges. Column headings stand or different realms: B0-unclassified; B1-tropical and subtropical moist broadleaf forest; B2- tropical and subtropical dry broadleaf forest; B3-tropical and subtropical coniferous forest; B4-temperate broadleaf mixed forests (north America); B5-temperate coniferous forest; B6-boreal forest/taiga; B7 tropical and subtropical grassland, savanna and shrubland; B8-temperatue broadleaf mixed forest (eastern); B9-flooded grasslands and savanna; B10-montane grassland and shrubland; B11-tundra; B12-mediteranean forest, woodland and scrub; B13-dester and xeric shrubland; B14-mangroves; B98-lakes; B99-rock and ice.. We excluded unclassified (B0) from our NMDS analysis.

Table S4. Nonmetric multidimensional scaling results from biologic realm data. The axes are labled NMDS1, NMDS2 and NMDS3.