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Lake level fluctuations and divergence of cichlid fish ecomorphs in Lake Tanganyika

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Abstract

Lake Tanganyika has undergone substantial climate-driven lake level fluctuations that have repeatedly changed the distribution and extent of habitat for endemic fishes. Here we consider whether patterns of population genetic structure and phenotypic divergence within the cichlid fish *Telmatochromis temporalis* have been associated with changing lake levels. The species has a large-bodied rock-living ecomorph and a small-bodied shell-living ecomorph, and both are found in close proximity in littoral habitats. Using mtDNA sequences we found that geographically distant (>50km) populations within the southern lake region diverged approximately 130,000-230,000 years ago, suggesting regional genetic structure persisted through a low stand of over 400 metres ~106,000 years ago that ended with a rise to present levels ~100,000 years ago. We also found signatures of large population expansions since this rise across the study region, suggesting populations positively responded to new habitat as lake levels rose to present levels. Finally, we found geographically adjacent (< 10 km) ecomorphs exhibit both significant genetic differentiation and signatures of gene flow after the lake level rise. The results suggest local ecomorph divergence progressed with gene flow after the last major rise in lake level ~100,000 years ago, potentially facilitated by new ecological opportunities.

Introduction

There is increasing evidence that divergent natural selection operating on ecological traits plays a central role in adaptive radiation (Funk et al., 2006), and that the extent of adaptive radiation may be closely-linked to the availability of niche space. New ecological opportunity has been inferred to be a trigger of rapid radiation in both experimental systems (Rainey & Travisano, 1998), and phylogenetic studies of natural systems, including plants (Hughes & Eastwood, 2006), lizards (Harmon et al., 2008; Mahler et al., 2010), mammals (Tran, 2014) and fishes (Rüber et al., 2003; Salzburger et al., 2005; Siwertsson et al., 2010; Wagner et al., 2012). A role for ecological opportunity in facilitating adaptive radiation is also supported by evidence that the rate of adaptive evolution diminishes when niches are filled (Phillimore & Price, 2008; Price et al., 2014). Thus, the availability of suitable habitat and food resources may be key factors that allow lineages with appropriate genetic variation to undergo rapid speciation and adaptive evolution (Yoder et al., 2010).

Multiple African lakes contain adaptive radiations of cichlid fishes, and macro-evolutionary analyses suggest that the physical dimensions of these lakes are reliable predictors of the presence and extent of adaptive radiations within them (Wagner et al., 2012). Within these radiations species often differ dramatically in their depth distributions, substrate preferences and diet (Konings, 1998; Konings 2007). Moreover, these differences are often intrinsically correlated with differences in morphology (Rüber & Adams, 2001; Muschick et al., 2012), and breeding systems (Sefc, 2011). In an increasing number of cases the functional genes related to these traits have been identified (Sugawara et al., 2002; Gerrard & Meyer, 2007). Together, these patterns are suggestive of a strong role for ecologically-mediated speciation in these lakes, in addition to the role of sexual selection (Wagner et al., 2012).

This study focuses on the Lake Tanganyika cichlid fish *Telmatochromis temporalis* Boulenger 1898, part of the species-rich tribe Lamprologini, which contains over 90 species. Of these, around 80 are endemic to Lake Tanganyika, while the remainder are restricted to the main Congo River system (Schelly et al., 2006). All species are brood-guarding substrate spawners, but species differ substantially in their habitat preferences, depth distributions and dietary preferences (Konings, 1998; Muschick et al., 2012). Our focal species, *T. temporalis*, has a lake-wide distribution and occurs in two distinct ecomorphs each associated with a distinct habitat type. The 'normal' sized rock ecomorph is abundant on the rocky shorelines, whereas the 'dwarf' shell ecomorph occurs on large aggregations of empty shells of the gastropod *Neothauma tanganyicense*, which is endemic Lake Tanganyika (Takahashi, 2004). These shell beds are found throughout the lake, but are patchily distributed and comparatively less common than the rocky habitat (Takahashi et al., 2009). Multiple origins of the shell ecomorph have been suggested based on population genetic evidence from nuclear microsatellite loci and mtDNA sequences (Takahashi et al., 2009). This has been supported by additional population genetic evidence from nuclear AFLPs and mtDNA sequences by Winkelmann et al. (2014), alongside evidence that competition for breeding substrate mediates gene flow between ecomorphs.

In this study we investigate whether the existing population genetic structure and timing of divergence of *Telmatochromis temporalis* ecomorphs has followed lake level rises. The lake has been subject to large lake level changes since formation (Cohen et al., 1997; Scholz et al., 2003), including a major low stand of at least 435 metres below present levels ~106,000 years ago, before a rise to current levels ~100,000 years ago (McGlue et al. 2008), and another less substantial low stand (~260m) during the Last Glacial Maximum 32,000 to

14,000 years ago (McGlue et al., 2008). Population-level genetic studies have shown that these dramatic lake level changes have strongly influenced the population connectivity and demography of multiple Lake Tanganyika cichlids associated with hard substrates (Verheyen et al., 1996; Rüber et al., 1999; Sturmbauer et al., 2001; Duftner et al., 2006; Sefc et al., 2007; Koblmüller et al., 2011; Nevado et al., 2013).

Major lake level changes will have changed the extent and distribution of littoral habitat available for populations of both ecomorphs of *Telmatochomis temporalis*. As water levels rose new rock habitats will have been exposed and colonised, while new *Neothauma* shell habitats will have formed. Additionally, due to strong depth limits of this littoral species (maximum depth 28m, LR and KW pers. obs.), populations will have been lost from former habitats as water levels rose. Thus, we suggest that most recent lake level rises may have generated a new metapopulation structure over local geographic scales, and it is also plausible that ecomorph divergence took place following the rise in water levels to those of the present day.

Here we investigated the spatial and temporal context of *T. temporalis* ecomorph divergence by first quantifying genetic (mtDNA) differences within and between populations. We then investigated if habitat differences or geographic distance were the more reliable predictors of population genetic structuring. Next, we quantified migration between regions, and between ecomorphs within regions. Finally, we estimated the timing of divergence events between regions, and the timing of individual population expansions. Together these data are interpreted in relation to geologically-inferred lake level rises.

Materials and methods

Sampling and laboratory methods

DNA samples analysed for this study were collected from 227 individuals across 16 locations in southern Lake Tanganyika (Fig. 1; Table 1) between 2006 and 2010 and preserved in 95% ethanol. Relevant mtDNA control region sequences were already published from 145 individuals (Winkelmann et al. 2014), while 82 were newly sequenced for this study. Fin clips were collected for samples from locations C and D, all other samples were muscle tissues. Samples were collected from three different habitat types; rock substrate (sites A, B, E-G, K, L and N), shell beds (accumulated from empty gastropod shells of the genus *Neothauma*, sites C, D, I, J and M), and mixed substrate containing both rock and empty shells (sites O and P). Individuals on the mixed substrates were not assigned individually to ecomorphs.

Mitochondrial DNA sequencing

Genomic DNA was extracted using DNeasy Blood & Tissue Kit and a ~900 bp section of the mtDNA control region (D-loop) was amplified using the forward primer 5'-ARA GCR YCG GTC TTG TAA TCC G-'3 and reverse primer 5'- TGG CTA AAT TYA CAC ATG C-'3. Polymerase chain reaction (PCR) were performed in 25.4 µl reactions containing 0.2 µL Taq DNA polymerase (Bioline), 0.5 µL of each primer (10 µM each), 1 µL dNTPs (1 mM each dNTP), 3 µL MgCL (25 mM stock), 5 µL of 5 x PCR reaction buffer, 14 µL double-distilled water and 1.2 µL of the extracted DNA. PCR used the following conditions: 3 min at 94 ºC, then 35 cycles of 1 min at 94 ºC, 1 min at 54 ºC and 1.5 min at 72 ºC, followed by 72 ºC for 5 min. Sequencing was performed using a Big Dye terminator v.1.1 on a 3730xl DNA Analyzer (Applied Biosystems).

Genetic differences among populations

Sequences were aligned using MAFFT 6.814b in Geneious Pro 5.5.6 (Biomatters Ltd., Auckland, New Zealand) using default settings, and the resulting alignment was checked by eye. The number of haplotypes (*H*), haplotype diversity (*He*) and nucleotide diversity (π) for each population were calculated in DNASP (Rozas, 2003). Genetic divergences between populations ($\Phi_{ST.}$) were measured in Arlequin 3.5 (Excoffier & Lischer, 2010), and statistical significance was tested using 10,000 permutations. Analyses of Molecular Variance (AMOVA) in Arlequin 3.5 was used to quantify within and between-population genetic variance. For AMOVA analyses regions were defined as "northern" (populations A-E) and "southern" (populations I-P).

Importance of spatial and environmental variables for genetic variation of populations

To test for dependence of genetic distance (Φ_{ST} between populations) on geographic distance and environmental differences (habitat type, sampling depth), we used distance-based redundancy analysis (dbRDA; Geffen et al. 2004, Legendre & Fortin 2010) using the capscale function in the R package "vegan" (Oksanen et al. 2013). Geographic information was coded as decimal latitude and longitude coordinates. Substrate was coded as 1 rock, 0 for shell, and 0.5 for mixed substrate. Euclidean distances were used within the dbRDA for quantification of environmental and geographic distances. Significance was tested using 100,000 permutations.

Migration estimates

Populations of the same ecomorph in the same region with no significant genetic differences were 'pooled', generating "northern" and "southern" region datasets for each ecomorph.

Migrate-n 3.6.11 (Beerli and Felstenstein 2001) was used to simultaneously estimate the magnitude and direction of historical migration between pairs of the four groups (north rock, north shell, south rock, south shell). For each run we used Bayesian search strategy, and onelong chain. In total we recorded 1 million steps separated by 100 step increments, following a burn-in of 10,000 trees. The Theta uniform prior range was 0 to 0.5, and the M uniform prior range was 0 to 1000. Other search parameters were as default. We conducted three runs allowing bi-directional migration between all combination of sites and ecomorphs. We also conducted one run allowing only migration between sites but not ecomorphs, and one run allowing only migration between ecomorphs but not sites. Relative likelihood of models was compared using Bayes factors.

The timing of migration events was estimated using two mtDNA control region substitution rates previously estimated for African cichlids, namely 0.0324 changes per site per million years (Genner et al. 2010) and 0.057 changes per site per million years (e.g. Koblmüller et al. 2011), and a generation time of either 2 or 3 years, based on estimates from the Tanganyika lamprologine *Neolamprologus modestus* (Hellman et al. 2015).

Timescale of splitting of the northern and southern population groups

Our analyses were consistent with no gene-flow between the northern and southern populations. This enabled us to estimate the timing of the split between the populations using the *BEAST approach (Heled & Drummond 2010) in BEAST 1.8.2. (Drummond et al. 2005). Each analysis was run for 25 million steps using HKY+Γ substitution model, with parameters logged every 1000 generations. A strict molecular clock was employed, again employing the substitution rates 0.0324 and 0.057 changes per site per million years.

Demographic history

To detect historical changes in effective population sizes Bayesian skyline plots were calculated in BEAST (Drummond et al., 2005). Again, each analysis was run for 25 million steps using HKY+Γ substitution model, with parameters logged every 1000 generations. Again a strict molecular clock was employed, alongside the substitution rates of 0.0324 and 0.057 changes per site per million years. A coalescent Bayesian skyline tree prior was used with between 4 and 10 grouped coalescent intervals, and a UPGMA starting tree. All other settings were as the default. Chains convergence and Bayesian skyline plots were both visualised in Tracer 1.5 (http://tree.bio.ed.ac.uk/software/tracer/). We used the Bayesian skyline plots to identify when population expansion towards the present day started, and the time of maximum population growth, following methods in Genner & Turner (2014).

Results

Genetic diversity and population differentiation

The 227 mitochondrial control region sequences produced a 907 base pair long alignment with 153 unique haplotypes (Table 1). Significant genetic divergence was observed between most populations (Table 2). Population pairs without significant genetic differences (Φ_{ST}) were located within the same region (northern or southern). Geographically proximate rock and shell ecomorph populations typically showed significant genetic differentiation.

Genetic variation, geographic distance and environmental distance

When all populations were considered, there was a strong signal of geographic structuring among *T. temporalis* populations, with clear divergence between the "northern" and "southern" populations (Fig. 2). Analysis of molecular variance (AMOVA) showed that

within ecomorphs the largest proportion of genetic variance was found between regions (Table 3). Within regions, most genetic variance was detected within populations. Over the full extent of the study area genetic distance (Φ_{ST}) was significantly associated with geographic variables (latitude and longitude) in the marginal (full) dbRDA of all predictor variables (Table 4). Geographic variables remained significant predictors of genetic distance when substrate and water depth variables were accounted for in a conditional dbRDA. Substrate was additionally significantly associated genetic distance when geographic variables were accounted for in another conditional dbRDA (Table 4). Overall, the results show geographic distance was the most effective predictor of genetic distance over the full extent of the study area, but substrate was also important when geographic variation was accounted for.

Demographic history, population splitting times and gene flow

Bayesian skyline plots using BEAST showed that 14 out of 16 populations experienced an increase in effective female population size over the last 100,000 years (Fig. 3a-b; Supplementary Fig. 1). It was possible to identify timings of the start of population expansion and maximum population growth in 12 of the populations (Table 5). Using the slower mtDNA substitution rate of 0.0324 changes/site/Ma, the start of population growth was often resolved as before the major lake level rise ~106,000 years ago (Fig. 3c-d), but the period of maximum growth was typically after this event (Table 1). Using the faster substitution rate of 0.057 changes/site/Ma, both the start and maximum period of growth were typically after the major lake level rise ~106,000 years ago.

Migrate-n models allowing a full migration matrix (harmonic mean log-likelihood = -3863.0, average of 3 runs) were considerably more likely than the model that allowed migration

between regions but not morphs (harmonic mean log-likelihood = -5365.4, log Bayes Factor = -3004.75), and the model that allowed migration between morphs but not regions (harmonic mean log-likelihood = -4229.80, log Bayes Factor = -733.6).

Migrate-n estimates of effective population sizes of ecomorphs varied between ecomorphs and regions, but shell populations typically had smaller effective population sizes then adjacent rock populations (Table 6). Estimated means of the modal migration rates between the regions were all, except in one case, zero, while more extensive migration was present between ecomorphs within regions (Table 7, Supplementary Fig. 2). In both regions, migration was estimated to be primarily from the shell ecomorph to the rock ecomorph (Table 7, Supplementary Fig. 2). Estimates of the average time of migration events between ecomorphs within regions were between 35,000 and 142,000 years ago, and were highly dependent on the substitution rate and generation time used for calculations. Estimates of the average time of less common migration events between regions were between 40,000 and 283,000 years ago, depending on the substitution rate employed and generation time (Table 8, Supplementary Fig. 3).

Using *BEAST and the substitution rate of 0.0324 change/site/Ma, we estimated that the divergence of the northern and southern populations took place 230,000 years ago (95% Highest Posterior Density intervals 163,000 to 303,000 years). Using the substitution rate of 0.057 change/site/Ma, we estimated the divergence of the northern and southern populations took place 130,700 years ago (95% Highest Posterior Density intervals 92,600 to 172,200 years).

Discussion

Major drivers of population genetic structuring

The results clearly demonstrate a strong signal of geographic structuring, consistent with expectations of limited dispersal among fragmented habitats within both ecomorphs of the species. This general spatial pattern is compatible with previous work on Lake Tanganyika rock cichlids, including representatives of the Tropheini (Wagner and McCune 2009), Eretmodini (Rüber et al., 2001; Taylor et al., 2001; Sefc et al., 2007), Perissodini (Koblmuller et al., 2009) and Lamprologini (Duftner et al., 2006; Koblmüller et al., 2007). Selection presumably favours philopatry in these cichlids due to the benefits of persisting in local known environment relative to the costs of movement across unfamiliar and less structured habitat, such as sand or deep water.

In general, close associations between ecomorphology and breeding habitat can reduce gene flow and facilitate speciation (Edelaar et al. 2008; Webster et al. 2012, Malinsky et al. 2015). Although in *Telmatochromis temporalis* the dominant factor affecting gene flow was geographic proximity, there was also evidence of restricted gene flow between ecomorphs in neighbouring habitats, similar to findings of Takahashi et al. (2009) and Winkelmann et al. (2014). In this species substrate use of individuals appears strongly linked to the availability of shelter and predation threat. Adults of both ecomorphs are believed to be highly vulnerable to multiple predators that characterise hard substrate environments of Lake Tanganyika (Takahashi et al. 2012), including piscivorous fish (catfishes, mormyrids, cichlids, mastacembelid eels), birds (kingfishers and cormorants), mammals (spotted-neck otters) and reptiles (water cobra) (Konings 1998). It has been found that body size matches available shelter size in *T. temporalis*, and that in transplant experiments rock ecomorphs are unable to

make use of empty shells as shelter against predators (Takahashi et al. 2012). Thus, rock females may be unable to use shell habitat, while shell females could in principle use rock habitat, and this may explain the apparent greater migration from shell to rock habitat observed with maternally inherited mtDNA. Laboratory work suggests that competition is important in determining habitat use of this species, with large rock ecomorph cichlids forcing smaller individuals to use less favoured shell habitat (Winkelmann et al. 2014). Taken together, the evidence is consistent with natural selection operating against migrants with non-adapted phenotypes, and at least partially restricting gene flow.

Population divergence after a major lake level rise

On average populations tended to show pulses of maximum growth approximately 43,000 and 75,000 years ago, depending on the rates of molecular evolution employed. These results are consistent with *T. temporalis* ecomorphs undergoing expansions with gene flow after the major lake level rise that would have provided new expanses of littoral habitat for the geographically separate "northern" and "southern" population groups. The results are suggestive of the lake level rise providing the opportunity for the development of a new metapopulation structure and phenotypic divergence between ecomorphs driven by local selective pressure. Notably, the populations do not show clear influence of changes in effective population size during the low stand of approximately 260m during the Last Glacial Maximum 32,000 to 14,000 years ago (McGlue et al. 2008), suggesting genetic diversity was maintained in each region despite environmental changes.

Large lake level changes will have fragmented and reunified rock habitats, and altered the locations and extent of shell habitat. The distributions of this habitat will be dependent upon the locations of suitable substrate for living gastropods, whether hydrodynamic conditions are

favourable for shell aggregation, the extent of bioturbation that maintains shell exposure, and the water chemistry that will influences rates of shell erosion. Individual *Neothauma* shells have been dated up to a maximum of 1,600 years of age (McGlue et al. 2010), however. We know very little of longevity of beds themselves. Nevertheless, it seems plausible that apparent lake level stability for the last 14,000 years, at least, has promoted the generation of a population genetic structure in *T. temporalis* influenced by both geographic proximity of populations and the nature of the substrate present.

In the East African Great Lakes, water level fluctuations have be considered to act as species 'pumps' (Rossiter, 1995; Salzburger, 2009; Danley et al., 2012), with the changes repeatedly splitting populations and promoting phenotypic divergence in allopatry. An opposing view is that such lake level changes may alternatively act as species 'dumps', bringing together formerly separated populations in novel habitat, and leading to 'reverse speciation' (Seehausen 2006; Taylor et al. 2006; Teotonio et al., 2009). The results of this study suggest an alternative perspective on the concept of the species pump. In addition to rising water levels leading to the evolution of new allopatric variants, they may also provide new opportunities for divergence in allopatric, parapatric or sympatric circumstances. Thus, we propose that changes to habitat availability, together with ecological stability over millenial timescales determines whether ecological speciation proceeds.

Associations between genetic and ecological divergence are dependent on spatial scale

Geography was a major predictor of genetic structuring over the spatial scale of the whole study area, while results suggest that habitat plays an additional role for population genetic structuring over more local scales. Therefore, it appears that the ability to detect associations between environmental contrasts and gene flow is strongly influenced by spatial scale in this

339 species, and likely others where parallel evolution of ecomorphs has occurred. A meta-
340 analysis of published studies has demonstrated the ubiquity of isolation-by-ecology in natural
341 systems (Shafer & Wolf, 2013). However, while Shafer & Wolf considered correlations
342 between geographic distance and ecological distances, the changing associations between
343 genetic distances, geographic distances and ecological distances over increasing spatial scales
344 were not explicitly studied. The most important factor governing such patterns is likely to be
345 the dispersal abilities of the studied organism (Sexton et al., 2013). Organisms with large
346 potential dispersal distances, for example birds, may have a strong signal of isolation-by-
347 ecology over the range of hundreds of kilometres (Edelaar et al., 2012). By contrast
348 lamprologine cichlids, which have very limited dispersal abilities, and exhibit clear potential
349 for parallel evolutionary divergence, represent the alternative extreme where isolation-by-
350 ecology must be studied locally.
351
352 In conclusion, our study suggests that metapopulation structure and phenotypic
353 diversification followed changes in lake depth. Thus, in this case, lake level changes may
354 have acted as a facilitator of adaptive diversification and contribute to local reproductive
355 isolation of incipient species. Notably, lake depth is a key predictor of species richness in
356 lacustrine cichlid radiations (Wagner et al., 2012), potentially because deeper lakes contain
357 more ecological niches for species to diversify among. Our results hint at the possibility that
358 lake level changes that characterise deep lakes have repeatedly provided new ecological
359 opportunity in allopatric populations that permits diversifying selection. Evidence from
360 neighbouring Lake Malawi would support this concept, as many phenotypically unique
361 populations of littoral fishes and gastropods have been founded following major level rises
362 over the last 90,000 years (Schultneiss et al., 2009; Genner et al., 2014).
363

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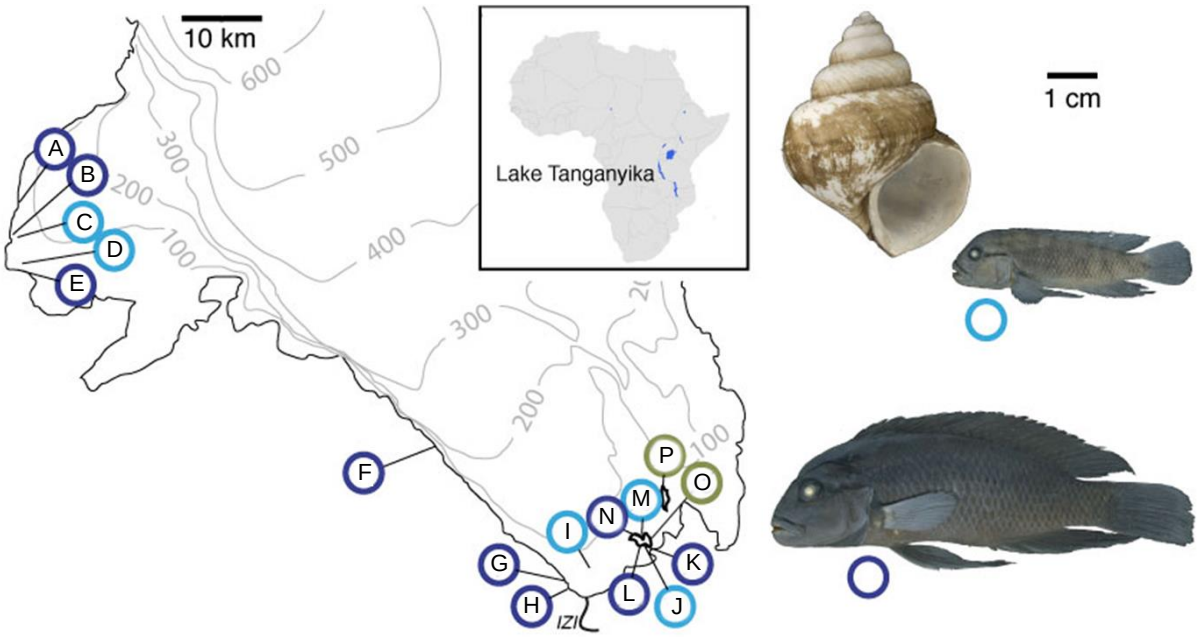
542

Figure Legends

Fig. 1. Locations of the *Telmatochromis temporalis* populations sampled. Dark blue circles are rock ecomorph populations, light blue circles are shell ecomorph populations, and green circles are populations on mixed (rock and shell) substrate. Pictured are adult individuals of both ecomorphs, and a shell of the gastropod *Neothauma tanganyicense* that form the shell beds inhabited by the shell ecomorph.

Fig. 2. (a) Genetic distance (Φ_{ST}) comparisons within and between regions, dark blue = rock, light blue = shell ecomorph. (b) Genetic distance (Φ_{ST}) for all populations in relation to geographic distance. Error bars indicate 95% confidential intervals.

Fig. 3. (a-b) Demographic history of populations reconstructed using a Bayesian skyline approach and mtDNA control region sequences. Dark blue indicates a rock ecomorph population, light blue a shell ecomorph population, and green a mixed substrate population. (c) Lake levels reconstructed from Cohen et al. (1997) and McGlue et al. (2008). (d) Approximate lake level during low stands ~106ka, prior to population expansions. Palaeolake reconstruction from McGlue et al. (2008).



561

562 Fig. 1.

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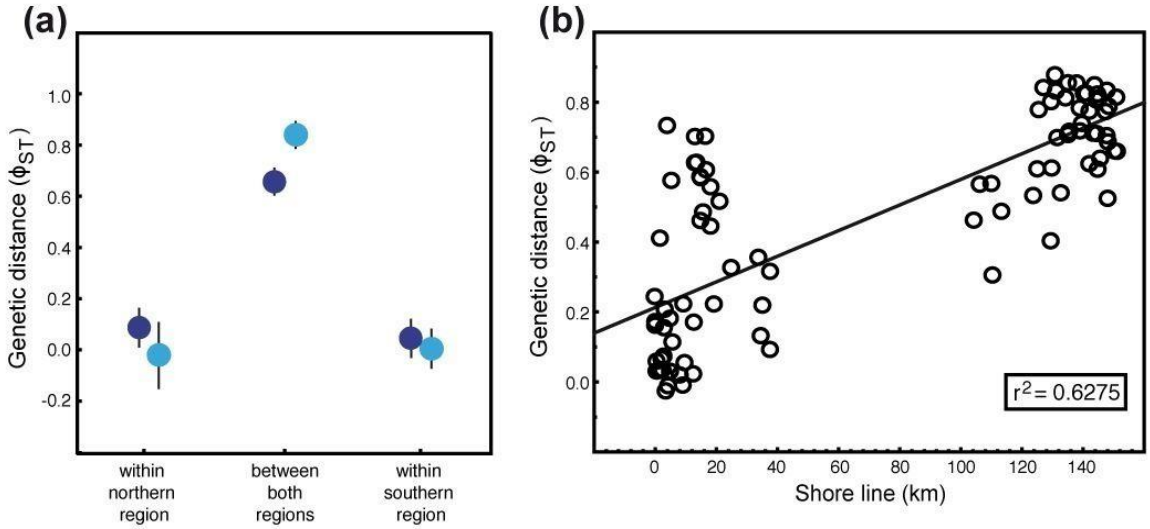

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565 Fig. 2.

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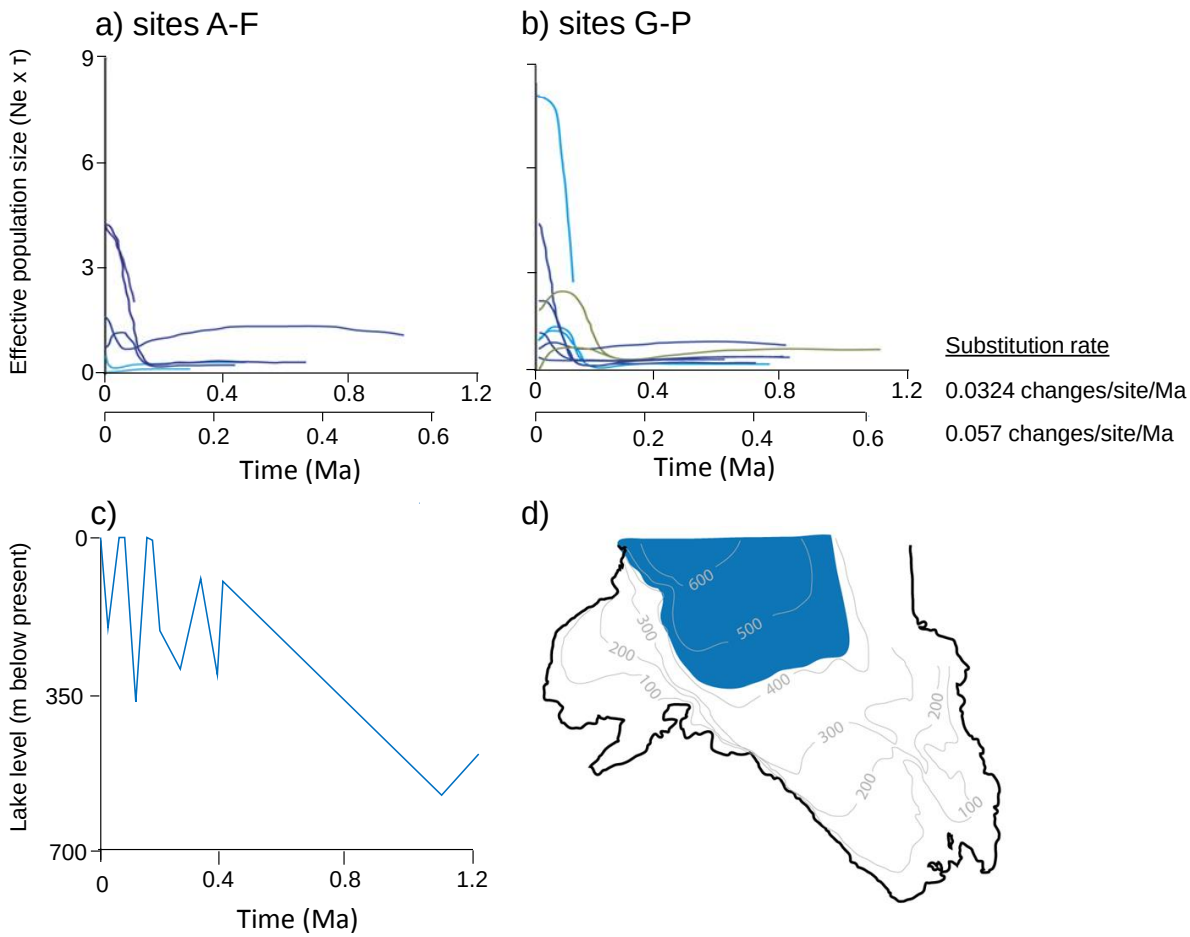

a) sites A-F
b) sites G-P
Effective population size (Ne x τ)
9
6
3
0
0
0.4
0.8
1.2
0
0.2
0.4
0.6
Time (Ma)
Substitution rate
0.0324 changes/site/Ma
0.057 changes/site/Ma
c)
d)
Lake level (m below present)
0
350
700
600
500
400
300
200
100

Fig. 3.

571 **Table 1** Population sample sizes (N) and mtDNA control region diversity. H is the number of haplotypes, H_e is haplotype diversity, π is
572 nucleotide diversity, Neτ is the product of effective population size (N_e) and generation time (τ, in millions of years). For locations, see Fig. 1.
573 *substitution rate of 0.0324 changes per site per Ma, **substitution rate of 0.0570 changes per site per Ma.
574

Code	Location name	Sampling date	Latitude °S	Longitude °E	Substrate	N	H	He	π	Genbank Accessions
A	Kolamo	28/04/2006	8°25'11.9"	30°27'41.2"	Rock	12	11	0.985	0.00694	TBC
B	Chipwensolo (village)	22/05/2010	8°26'31.4"	30°27'09.6"	Rock	11	11	1	0.02059	KJ184465-KJ184475
C	Chipwensolo (offshore)	22/05/2010	8°26'34.1"	30°27'17.6“	Shell	26	14	0.892	0.00781	KJ184436-KJ184501
D	Ndole Bay (offshore)	23/05/2010	8°28'36.7"	30°27'06.8"	Shell	20	9	0.895	0.00464	KJ184446-KJ184489
E	Ntingila	26-30/04/2006	8°28'53.7"	30°27'41.2"	Rock	18	13	0.941	0.01198	KJ184355-KJ184367, TBC(5 seqs)
F	Mupapa	04/05/2006	8°40'41.9"	30°54'02.5"	Rock	11	10	0.982	0.04180	TBC
G	Kombe	21-23/05/2006	8°47'50.8"	31°01'02.7"	Rock	13	11	0.974	0.03966	TBC
H	Katoto (south)	10-11/05/2006	8°48'21.9 "	31°01'34.2"	Rock	14	9	0.912	0.01685	TBC
I	Kapoko (offshore)	10/05/2006	8°47'45.9 "	31°02'44.9 "	Shell	11	9	0.964	0.01169	KJ184384-KJ184394
J	Mbita Island	12/05/2006	8°45'32.8"	31°05'50.4"	Shell	18	15	0.974	0.01477	KJ184395-KJ184412
K	Mbita Island	12/05/2006	8°45'28.1"	31°05'33.4"	Rock	8	8	1	0.01913	TBC
L	Mbita Island	07/05/2006	8°45'28.0"	31°05'33.4"	Rock	10	8	0.956	0.01938	KJ184368-KJ184377
M	Mbita Island	18/05/2010	8°45'05.3"	31°05'44.9"	Shell	12	12	1	0.00985	KJ184415-KJ184505
N	Mbita Island	18/05/2010	8°45'04.8"	31°05'46.5"	Rock	15	11	0.952	0.02492	KJ184413-KJ184503
O	Mbita Island	7-8/05/2006	8°45'05.0"	31°06‘15.9"	Mixed	19	18	0.994	0.01669	TBC
P	Mutondwe Island	29/05/2010	8°41'54.9"	31°07'02.0"	Mixed	9	9	1	0.01885	KJ184437-KJ184445
Mean						14.2	11.1	0.964	0.01785	
SD						4.8	2.8	0.037	0.01050	

575

576 **Table 2** Pairwise population differentiation (Φ_{ST}) between sixteen populations based on mtDNA sequences.

	B - Rock	C - Shell	D - Shell	E - Rock	F - Rock	G - Rock	H - Rock	I - Shell	J - Shell	K - Rock	L - Rock	M - Shell	N - Rock	O - Mixed	P - Mixed
A - Rock	0.155*	0.037	0.031	0.021	0.486***	0.54***	0.808***	0.850***	0.815***	0.775***	0.782***	0.810***	0.657***	0.760***	0.800***
B - Rock		0.163**	0.183**	0.115*	0.305***	0.402***	0.697***	0.716***	0.710***	0.608***	0.637***	0.685***	0.524***	0.650***	0.653***
C - Shell			-0.014	0.028	0.565***	0.613***	0.829***	0.856***	0.831***	0.802***	0.809***	0.831***	0.706***	0.786***	0.824***
D - Shell				0.031*	0.563***	0.610***	0.841***	0.877***	0.843***	0.821***	0.824***	0.845***	0.709***	0.795***	0.841***
E - Rock					0.462***	0.533***	0.777***	0.799***	0.779***	0.721***	0.737***	0.771***	0.623***	0.724***	0.755***
F - Rock						0.222**	0.514***	0.326***	0.356***	0.131	0.219**	0.314***	0.091	0.219**	0.257**
G - Rock							0.410***	0.573***	0.584***	0.461***	0.483***	0.557***	0.447***	0.541***	0.502***
H - Rock								0.730***	0.700***	0.627***	0.625***	0.701***	0.607***	0.660***	0.651***
I - Shell									-0.012	0.224**	0.056	0.019	0.171**	0.116*	-0.013
J - Shell										0.242***	0.060	0.036*	0.209***	0.147***	-0.002
K - Rock											0.032	0.208***	-0.026	-0.035	0.172**
L - Rock												0.070***	0.074	0.019	0.049
M - Shell													0.173***	0.135**	0.014
N - Rock														0.013	0.141*
O - Mixed															0.108*

577 *P < 0.05, **P < 0.01 and *** P< 0.001.

578 **Table 3** Analyses of Molecular Variance (AMOVA) on rock and shell ecomorph populations

579 in northern and southern regions. ns = not significant, ***P < 0.001.

580

Source of variation	Sum of squares	Variance of components	Percentage of variation
Rock ecomorph			
Between northern and southern regions	680	11.755***	40.42
Among populations within regions	591	7.153***	24.59
Within populations	946	10.177***	34.99
Shell ecomorph			
Between northern and southern regions	1099	25.192***	83.13
Among populations within regions	20	0.093^{ns}	0.31
Within populations	412	5.017***	16.56
Northern region			
Between rock and shell ecomorph	11	0.047^{ns}	0.91
Among populations within ecomorphs	35	0.422***	8.19
Within populations	392	4.780***	92.71
Southern region			
Between rock and shell ecomorph	257	3.125***	15.29
Among populations within ecomorphs	575	6.927***	33.89
Within populations	966	10.386***	50.82

581

582 **Table 4** Tests of the association of genetic distance (Φ_{ST}) with geographic variables, substrate
583 type and water depth using distance-based redundancy analysis. The marginal test includes all
584 variables, while the conditional tests account for variation in the selected variables.

Test	Variable predictors	F	P	% variance
Marginal (all variables)	Latitude	45.1819	< 0.001	63.47
	Longitude	10.4643	< 0.001	14.70
	Substrate	3.6152	0.054	5.07
	Depth	0.9281	0.405	1.30
Conditional (latitude and longitude)	Substrate	3.615	0.023	23.25
	Depth	0.928	0.397	5.97
Conditional (depth and substrate)	Latitude	44.540	< 0.001	67.55
	Longitude	10.396	0.001	15.76

585

586

587 **Table 5** Summary of Bayesian skyline plot reconstructions of historic population
588 demography. Neτ is the product of effective population size (N_e) and generation time (τ, in
589 millions of years). For locations, see Fig. 1. *substitution rate of 0.0324 changes per site per
590 Ma, **substitution rate of 0.0570 changes per site per Ma.

591

Location	Mean Neτ	Neτ (Upper 95%)	Neτ (Lower 95%)	Start population expansion (Ma)*	Maximum population growth (Ma)*	Start population expansion (Ma)**	Maximum population growth (Ma)**
A	4.237	20.575	0.396	0.092	0.090	0.052	0.051
B	4.322	18.743	0.517	0.168	0.052	0.095	0.030
C	0.606	3.235	0.026	0.033	0.005	0.019	0.003
D	0.136	0.747	0.003	-	-	-	-
E	0.842	3.998	0.054	0.147	0.093	0.084	0.053
F	1.733	8.879	0.126	0.069	0.030	0.039	0.017
G	2.002	9.444	0.143	0.286	0.183	0.163	0.104
H	0.352	1.899	0.014	-	-	-	-
I	1.172	5.932	0.057	-	-	-	-
J	1.274	5.652	0.095	0.193	0.131	0.110	0.074
K	1.393	5.979	0.183	0.168	0.068	0.095	0.039
L	0.686	2.802	0.088	0.090	0.016	0.051	0.009
M	8.129	36.536	1.211	0.126	0.110	0.072	0.063
N	0.925	4.633	0.051	-	-	-	-
O	4.520	19.468	0.522	0.152	0.042	0.086	0.024
P	2.307	9.769	0.370	0.168	0.080	0.095	0.045
Mean	2.165	-	-	0.141	0.075	0.080	0.043
SD	2.128	-	-	0.066	0.051	0.038	0.029

592

593 **Table 6** Mutation-scaled effective population sizes (Θ), as estimated in three replicate

594 Bayesian runs of Migrate-n with a full migration matrix. Note Θ = Nm x μ, where Nm is the

595 effective population size and μ is the mutation rate per nucleotide per generation.

596

Population	Populations pooled	Total individuals	Θ (average ± standard deviation of modal values from 3 runs)
Rock North	A, E	30	0.1728 (0.0206)
Shell North	C, D	46	0.0081 (0.0004)
Rock South	K, N, L	33	0.0524 (0.0008)
Shell South	I, J	29	0.0371 (0.0017)

597

598

599 **Table 7** Bayesian estimations of mutation-scaled migration rates (M), as estimated in in three
600 replicate runs of Migrate-n with a full migration matrix. [Note that $M = m/\mu$, where m is the
601 effective immigration rate and μ is the mutation rate per nucleotide per generation.

602

Population 1	Population 2	Migration rate (M) population 2 to 1 (average ± standard deviation of modal values from 3 runs)	Migration rate (M) population 2 to 1 (average ± standard deviation of modal values from 3 runs)
Rock North	Shell North	402.9 (148.0)	891.8 (34.9)
Rock South	Shell South	28.0 (2.7)	214.2 (45.1)
Rock North	Rock South	0 (0)	5.3 (4.6)
Rock North	Shell South	0 (0)	0 (0)
Shell North	Rock South	0 (0)	0 (0)
Shell North	Shell South	0 (0)	0 (0)

603

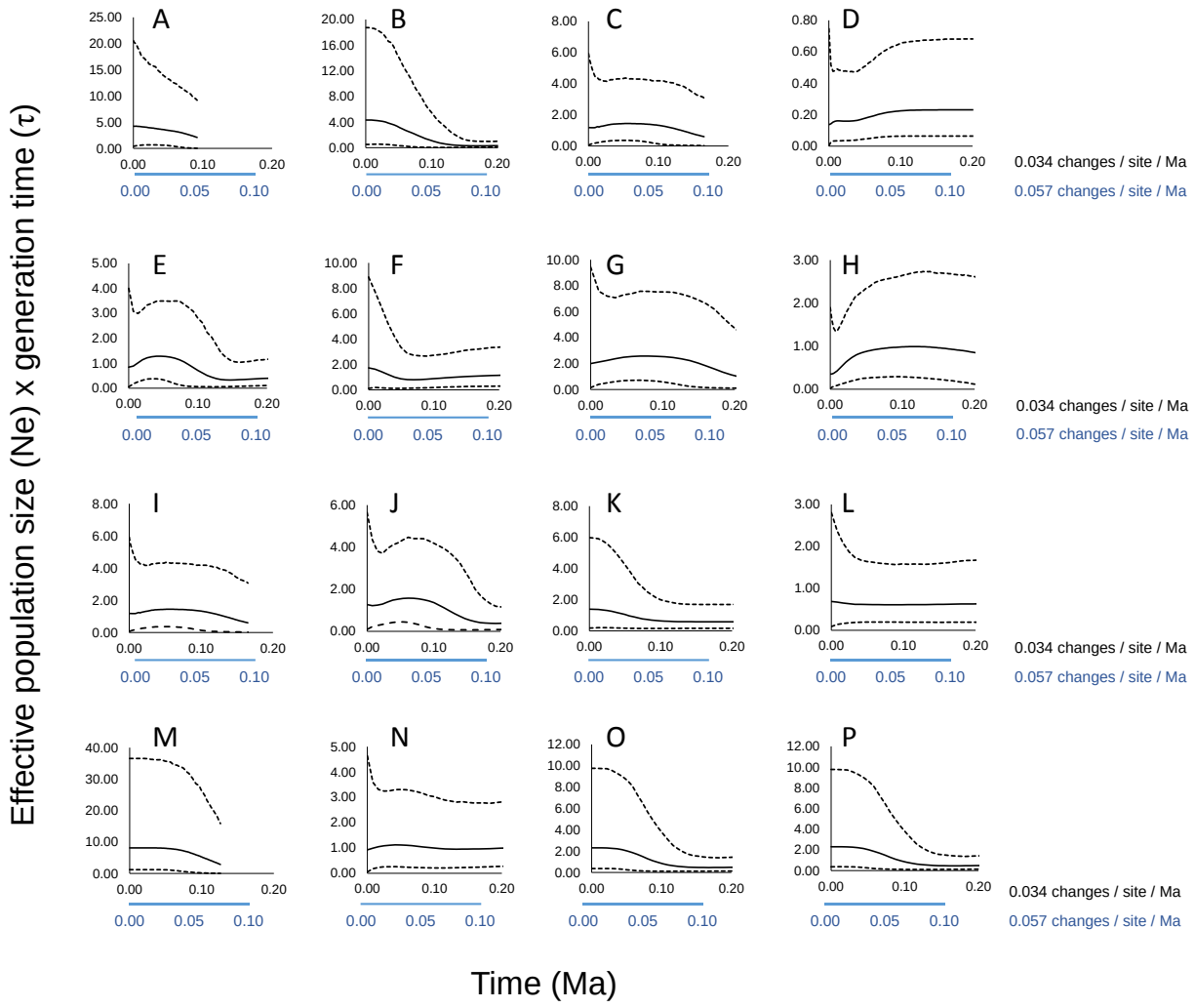
604 **Table 8** Estimates of the average timing of all migration events as estimated in as the average

605 timing of events across three replicate runs of Migrate-n, using the full migration matrix.

606

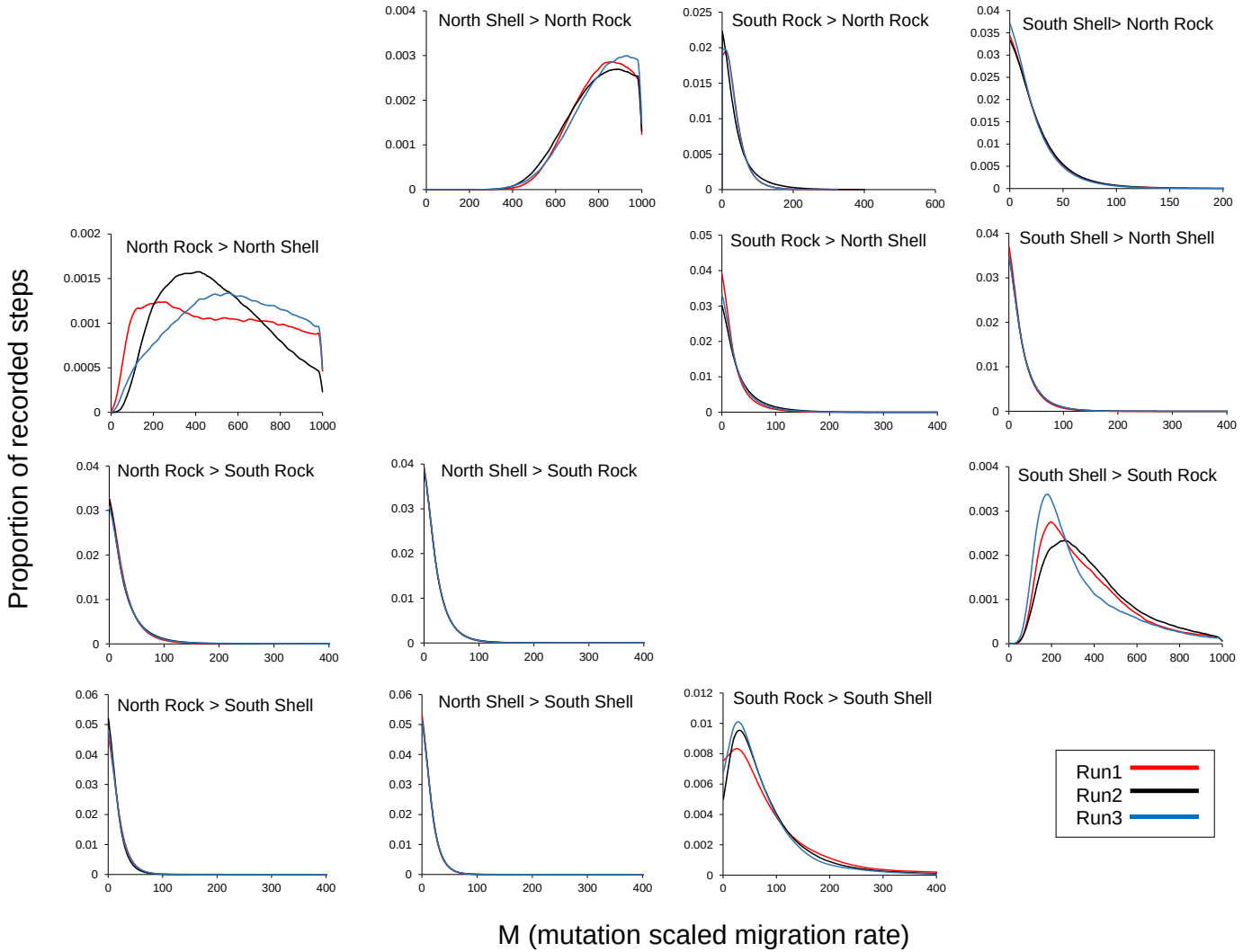
Generation time (years)	2	3	2	3
Substitution rate	0.0324	0.0324	0.0570	0.0570
	Mean time (± standard deviation) of migration from 3 runs (years)			
North Shell > North Rock	100,303 (4,943)	66,869 (3,295)	57,015 (2,809)	38,010 (1,873)
North Rock > North Shell	128,477 (3,183)	85,652 (2,122)	73,029 (1,809)	48,686 (1,206)
South Shell > South Rock	93,431 (799)	62,287 (533)	53,108 (454)	35,405 (303)
South Rock > South Shell	142,099 (3,728)	94,733 (2,485)	80,772 (2,119)	53,848 (1,413)
South Rock > North Rock	106,337 (4,005)	70,892 (2,670)	60,444 (2,276)	40,296 (1,518)
North Rock > South Rock	152,572 (10,867)	101,715 (7,245)	86,725 (6,177)	57,817 (4,118)
South Shell > North Shell	236,379 (4,951)	157,586 (3,300)	134,363 (2,814)	89,575 (1,876)
North Shell > South Shell	283,174 (12,480)	188,783 (8,320)	160,962 (7,094)	107,308 (4,729)
South Shell > North Rock	164,969 (2,526)	109,979 (1,684)	93,772 (1,436)	62,515 (957)
South Rock > North Shell	195,818 (31,288)	130,545 (20,858)	111,307 (17,785)	74,205 (11,856)
North Shell > South Rock	204,727 (10,378)	136,485 (6,985)	116,371 (5,956)	77,581 (3,970)
North Rock > South Shell	242,855 (8,154)	161,903 (5,436)	138,044 (4,635)	92,029 (3,090)

607

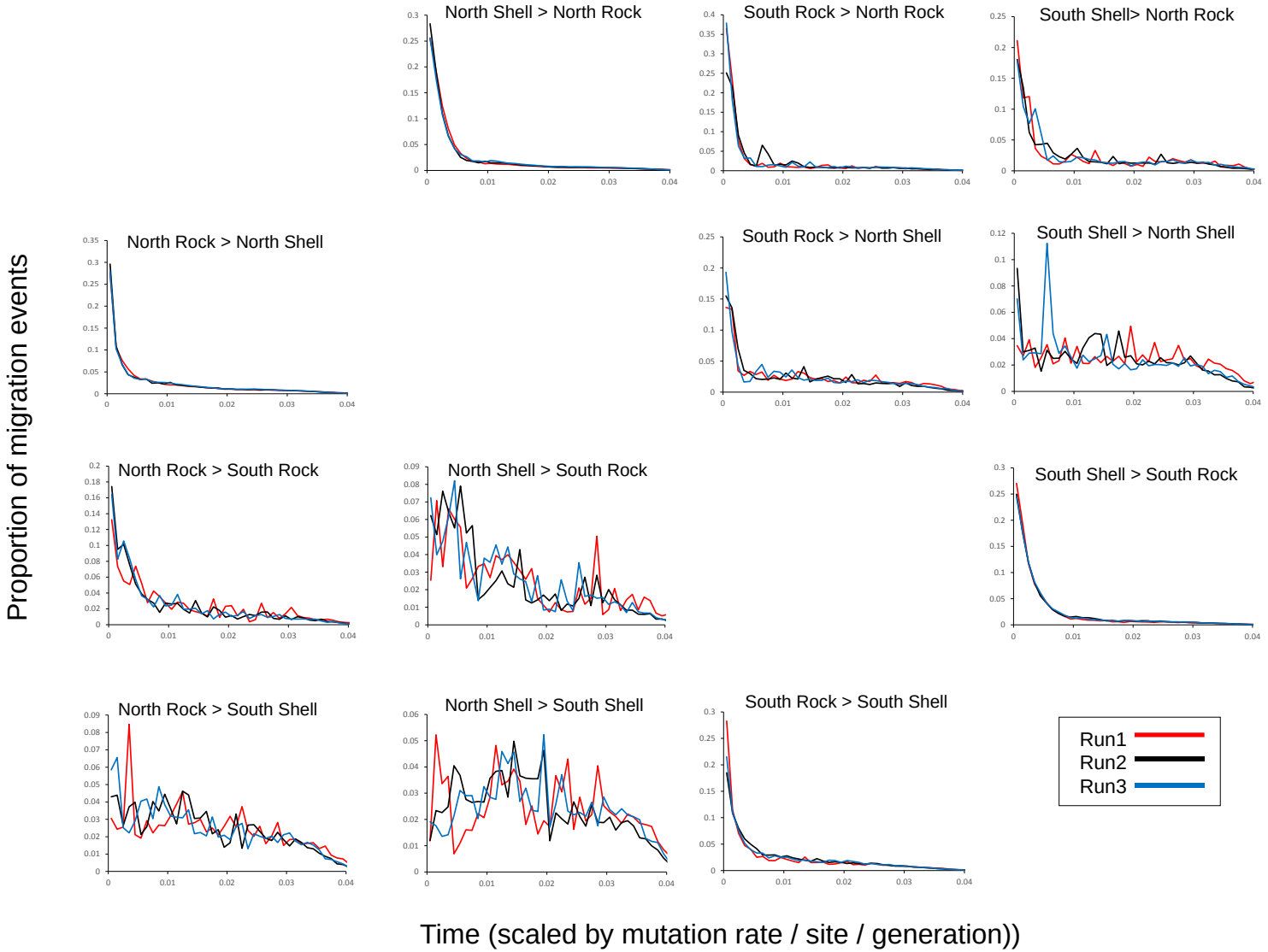


608

609 **Supporting Information Figure 1.** Demographic history of individual populations reconstructed using a Bayesian skyline approach and mtDNA
610 control region sequences. The y-axis values are a product of effective population size (N_e) and generation time (τ, in millions of years). The
611 unbroken line represents the mean value, and the dashed lines represent the upper and lower 95% credibility intervals



Supporting Information Figure 2. Frequency of mutation-scaled migration rates (M), as estimated in in three replicate runs of Migrate-n with a full migration matrix. [Note that $M = m/\mu$, where m is the effective immigration rate (probability that an immigrant is a migrant) and μ is the mutation rate per nucleotide per generation.



Supporting Information Figure 3. Timing of migration events as estimated in in three replicate runs of Migrate-n with a full migration matrix.