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# Genetic signature of population fragmentation varies with mobility in seven bird species of a fragmented Kenyan cloud forest

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## Abstract

**Habitat fragmentation can restrict geneflow, reduce neighbourhood effective population size, and increase genetic drift and inbreeding in small, isolated habitat remnants. The extent to which habitat fragmentation leads to population fragmentation, however, differs among landscapes and taxa. Commonly, researchers use information on the current status of a species to predict population effects of habitat fragmentation. Such methods, however, do not convey information on species-specific responses to fragmentation. Here, we compare levels of past population differentiation, estimated from microsatellite genotypes, with contemporary dispersal rates, estimated from multi-strata capture–recapture models, to infer changes in mobility over time in seven sympatric, forest-dependent bird species of a Kenyan cloud forest archipelago. Overall, populations of sedentary species were more strongly differentiated and clustered compared to those of vagile ones, while geographical patterning suggested an important role of landscape structure in shaping genetic variation. However, five of seven species with broadly similar levels of genetic differentiation nevertheless differed substantially in their current dispersal rates. We conclude that post-fragmentation levels of vagility, without reference to past population connectivity, may not be the best predictor of how forest fragmentation affects the life history of forest-dependent species. As effective conservation strategies often hinge on accurate prediction of shifts in ecological and genetic relationships among populations, conservation practices based solely upon current population abundances or movements may, in the long term, prove to be inadequate.**

*Keywords*: Afrotropical, birds, dispersal, genetic clustering, genetic differentiation, landscape connectivity, Taita Hills

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## Introduction

Habitat loss and fragmentation are considered key drivers of biodiversity loss (Turner 1996), in particular in historically stable, species-rich ecosystems such as tropical rainforests (Laurance *et al.* 2002; Waltert *et al.* 2005; Kirika *et al.* 2008). While habitat fragmentation may affect population dynamics in diverse and complex ways (Lewis 2006), long-term viability of species often hinges on their genetic population structure (Frankham *et al.* 2002). Habitat fragmentation can affect the genetic structure of populations both directly and indirectly, i.e. by restricting gene flow, reducing neighbourhood effective population sizes and/or increasing the levels of genetic drift and inbreeding in small, isolated habitat

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remnants (reviewed in Frankham *et al.* 2002). The extent to which habitat fragmentation leads to population fragmentation, however, depends on properties of the landscapes in which suitable habitat patches are embedded and of the taxa that depend on these patches for their survival or reproduction (Caizergues *et al.* 2003). Along these lines, levels of genetic differentiation are generally lower in more connective landscapes, defined as the degree to which landscape elements facilitate movements between resource patches (Taylor *et al.* 1993), and in taxa that can cross hostile habitats more easily, such as birds (Crochet 2000; Ehrich *et al.* 2001; Ehrich & Stenseth 2001; Goossens *et al.* 2001). Because birds are considered a highly vagile group able to transverse wide spans of unsuitable habitat, their degree of within-species genetic structuring has been underappreciated in the past, especially so in montane species and tropical forest specialists (e.g. Brown *et al.* 2004; Moore *et al.* 2008 and references therein). However, a number of avian genetic studies did show high levels of population differentiation over small spatial scales, either as a result of severe philopatry, narrow habitat requirements or a ground-dwelling lifestyle with restricted flight ability (Avise 1996; Brown *et al.* 2004).

When key habitat of sedentary or specialist species becomes progressively fragmented, dispersal among remnant subpopulations may become disrupted and, in turn, affect population viability and rates of local adaptation (Hanski & Gilpin 1991). Dispersal and gene flow therefore comprise key processes underlying the regulation, persistence and adaptive evolution of spatially structured populations in heterogenous landscapes (Slatkin 1987; Hanski & Gilpin 1991; Frankham 1997; Hanski 1998; Whitlock & McCauley 1999). However, not all species appear to be equally sensitive to fragmentation (Van Houtan *et al.* 2006, 2007; Sekercioglu 2007), so it is of high importance to determine which species are more prone to extinction to improve future conservation actions. Commonly, researchers use information on the current status of a species (e.g. patch occupancy, current dispersal; Lens *et al.* 2002), but these methods do not imply any information on the species-specific response to fragmentation. For example, a species can have built up an 'extinction debt', which can result into a higher vulnerability for future extinctions (Tilman *et al.* 1994; Hanski & Ovaskainen 2002; Bulman *et al.* 2007), while other species might be more buffered from the loss of genetic diversity caused by fragmentation (Howeth *et al.* 2008). An alternative approach, therefore, is to infer species-specific responses to habitat fragmentation from genetic data which can provide information on historical processes prior to the date of sampling (Wilmer & Wilcox 2007; Lada *et al.* 2008; Howeth *et al.* 2008; Oddou-Muratorio & Klein 2008; Pavlacky *et al.* 2009). Genetic differentiation among populations is traditionally measured by Wright's (1931) $F_{ST}$ or its derivates $G_{ST}$, $G'_{ST}$, and $D_{est}$ (Hedrick 2005b; Hedgecock *et al.* 2007; Jost 2008; Waples *et al.* 2008). These estimates are considered to reflect historical rates of geneflow integrated over multiple generations (Allendorf & Luikart 2007), although geneflow estimates can be biased in case of mutation-drift (Hedrick 2005b) or migration-drift (Whitlock & McCauley 1999; Lowe & Allendorf 2010) disequilibrium. Genetic estimates that (indirectly) reflect past population connectivity may be particularly informative when combined with direct estimates of contemporary dispersal rates among a restricted number of focal populations (Koenig *et al.* 1996; Whitlock & McCauley 1999; Faubet *et al.* 2007), thereby allowing an evaluation of changes in mobility over time (Howeth *et al.* 2008; Lowe & Allendorf 2010).

Here, we report on a study of the genetic signature of population fragmentation in seven sympatric, forest-dependent bird species of a Kenyan cloud forest archipelago that were earlier shown to differ in their response to forest fragmentation, as inferred from post-fragmentation levels of mobility and patch occupancy (Lens *et al.* 2002). A diverse set of genetic parameters/tests were used to infer signals of bottlenecks (deviation from mutation/drift equilibrium) and changes in gene flow over time (migration/drift equilibrium). We compare species-specific levels of genetic population differentiation using $D_{est}$ (Jost 2008), representing historic dispersal rates averaged over time (Bohonak 1999; Allendorf & Luikart 2007; Palsboll *et al.* 2007), and compare these with contemporary dispersal rates based on multi-strata mark-recapture analysis (data from Lens *et al.* 2002). Ultimately we assess whether the magnitude of genetic effects is correlated with loss of mobility.

## Materials and methods

### *Study area and species*

The Taita Hills (SE Kenya, 03°20'S, 38°15'E) represent the northernmost part of the Eastern Arc Mountains biodiversity hotspot of Kenya and Tanzania (Lovett & Wasser 1993). They cover an area of *c.* 250 km$^2$ and are isolated from other highlands by over 80 km of semi-arid plains in either direction (Lovett 1985). During the last 200 years, indigenous forest cover in the Taita Hills decreased by *c.* 98%, and forest remnants are mainly located at hilltops and ridges and isolated by small holder cultivation plots and exotic plantation forests (Lens *et al.* 1999; Adriaensen *et al.* 2006; Pellikka *et al.* 2009). Three larger forest fragments (86–220 ha) and

eight small ones (2–8 ha) are located on two mountain isolates (Dabida: 9 fragments; Mbololo: 2 fragments) separated by a low-altitude valley (Fig. 1; Brooks *et al.* 1998; Pellikka *et al.* 2009). Smaller fragments are most heavily disturbed, mainly as a result of logging, pole cutting and cattle grazing (Chege & Bytebier 2005). Among the three largest fragments, Chawia forest (CH, 86 ha; Dabida isolate) is most heavily degraded, Ngangao forest (NG, 120 ha; Dabida isolate) is intermediately degraded, and Mbololo forest (MB; 220 ha; Mbololo isolate) is most pristine (Brooks *et al.* 1998; Lens *et al.* 1999). The three largest fragments are inhabited by all seven study species, whereas the cluster of smaller fragments hosts subsets of these species only (based on breeding evidence during 1996–2001 in Lens *et al.* 2002), and analyses in this study are restricted to breeding populations in fragments CH, NG and MB.

Between 1996 and 2009, a total of 5002 individuals of seven forest-dependent species were trapped (no use of tape luring or artificial feeders), marked, measured and blood-sampled in fragments CH, NG and MB: Stripe-cheeked greenbul (*Andropadus milanjensis striifacies*; 626 ind), Cabanis's greenbul (*Phyllastrephus cabanisi placidus*; 679 ind), Taita thrush (*Turdus helleri*; 491 ind),

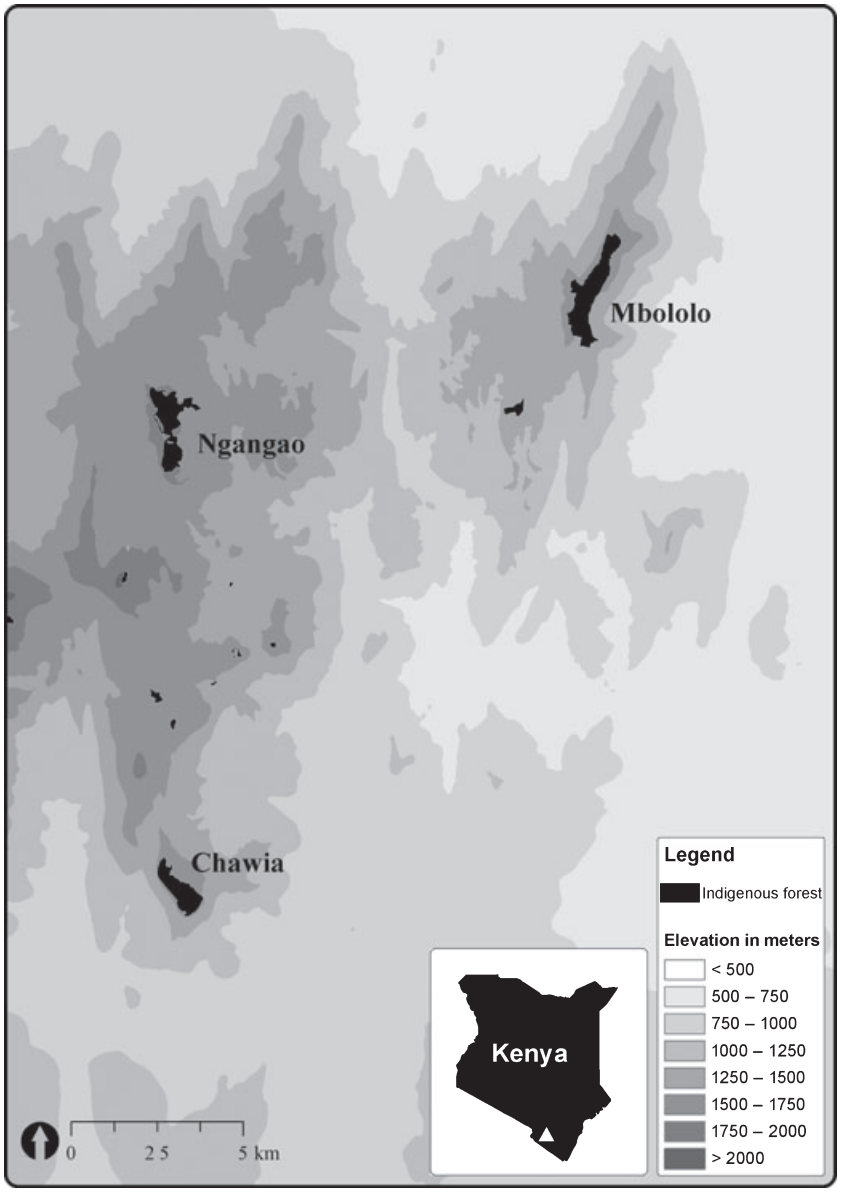


**Fig. 1** Map of the study area indicating the geographical position in Kenya and the location of three large indigenous forest fragments (Mbololo, Chawia, Ngangao) and eight small ones (fragment Sagala falls outside the figure boundary).

White-starred robin (*Pogonocichla stellata helleri*; 2262 ind), Taita white-eye (*Zosterops (poliogaster) silvanus*; 323 ind), Olive sunbird (*Nectarinia olivacea changamwensis*; 493 ind), and Yellow-throated wood-warbler (*Phylloscopus ruficapilla minullus*; 128 ind). *P. ruficapilla*, *T. helleri*, *A. milanjensis*, *P. cabanisi* and *N. olivacea* are presumed forest specialists, while *Z. silvanus* and *P. stellata* are presumed forest generalists (Bennun *et al.* 1996).

### *DNA extraction and genotyping*

Upon capture of an individual, we collected 2–3 μL of blood from its brachial vein and stored it in 95% ethanol or DMSO. DNA was isolated either by boiling in a 5% Chelex solution (Biorad) after an incubation period of 90 min at 55 °C in the presence of 100 μg proteinase K (ethanol storage) (Walsh *et al.* 1991) or by a normal phenol–chloroform extraction in the presence of 100 μg proteinase K (DMSO storage) (details in Galbusera *et al.* 2000). PCR amplification was executed in a 10-μL reaction volume containing approximately 100 ng DNA, 1× buffer (75 mM Tris–HCl pH 9.0, 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20), 0.5 U Taq polymerase (Eurogentec), 200 μM dNTPs (GIBCO), 1.0–3.0 mM $MgCl_2$ (product size ranges and optimal reaction conditions in Appendix 1) and 250–500 nM of each primer from 4 to 10 variable microsatellite markers. Starting from the original PCR conditions, variable $MgCl_2$, template DNA concentrations and annealing temperatures were tested in a gradient PCR device (PC-960G Gradient Thermal Cycler; Labotechnic). Genotypes were scored on a 6% acrylamide gel in an automatic sequencer (ALF express; Pharmacia Biotech) or on an ABI 3130 Genetic Analyzer (Applied Biosystems), and microsatellite lengths were determined with GeneMapper Software v4.1. Microsatellite data for all species have been deposited in the Dryad data repository (doi: 10.5061/dryad.8054).

### *Genetic analysis*

Presence of null alleles was tested with program MICRO-CHECKER 2.2.3 by running 10 000 Monte Carlo simulations and calculating 95% confidence intervals (Van Oosterhout *et al.* 2006). Deviation from Hardy–Weinberg equilibrium and linkage disequilibrium per locus were tested with program GENEPOP 4.0 (Raymond & Rousset 1995; Rousset 2008). Apart from the following marker*population combinations, all loci were consistent with Hardy–Weinberg equilibrium (all $P > 0.05$ after Bonferonni correction, Rice 1989): *P. stellata*: WBSW9 ($P = 0.0016$) and Pat14 in population CH ($P = 0.0019$); GF5B in population MB and NG ($P < 0.002$). Apart from WBSW9 and GF5B in *P. stellata*, none of the loci showed null alleles (removing both loci did not change our results; see also Galbusera *et al.* 2004). No linkage disequilibrium was detected between any pair of loci after correction for multiple testing (Rice 1989).

Observed and expected levels of heterozygosity were calculated with GENALEX 6.4 (Peakall & Smouse 2006), while allelic richness corrected for sample size was calculated with FSTAT 2.9.3.2 (Goudet 1995). To infer levels of population genetic differentiation, we calculated overall and pairwise $D_{est}$ values across all loci with SMOGD 1.2.5 (Crawford 2009). This estimate is increasingly considered more accurate than traditional ones such as $F_{ST}$ (Wright 1951, 1965) and $G_{ST}$ (Nei 1973; Nei & Chesser 1983) in accounting for differences in allelic diversity, especially for highly polymorphic microsatellite markers and in cases where assumptions for traditional calculations (e.g. migration/drift equilibrium) are violated (Jost 2008). For the sake of comparison, we also presented $F_{ST}$ values (Wright 1951, 1965) calculated with GENEPOP 4.0 using parameter θ (Weir & Cockerham 1984; Raymond & Rousset 1995; Rousset 2008). To test whether relationships between genetic population differentiation and contemporary dispersal rates differed between fragments located on the same mountain isolate (NG–CH) or two different isolates separated by a dry valley (MB–NG, MB–CH), we performed an analysis of covariance in SAS 9.2 (SAS Institute, 2002–2008).

The genetic population structure of each species was inferred from a Bayesian admixture model implemented in TESS 2.3. This procedure earlier proved to be more powerful than nonspatial algorithms, especially in weakly differentiated populations (Chen *et al.* 2007; Durand *et al.* 2009). To properly assess the genetic population structure of each species, we explored a wide range of values for the number of genetic clusters, K (varying from 1 to 9), and assessed the fit of the model to the data for each value (Francois & Durand 2010). A total of 100 independent iterations (each 50 000 sweeps long and discarding the first 30 000 sweeps) were run for each value of K. Model fits were compared with the Deviance Information Criterion (DIC, Spiegelhalter *et al.* 2002), a penalized measure of fit accounting for model complexity (models with lower DIC values fit data better). DIC values averaged over 100 independent iterations were plotted against K, and K-values for which DIC values first reached a plateau were selected (procedure similar to 'logarithm of evidence' in STRUCTURE; Evanno *et al.* 2005). The 10 runs with lowest DIC values for the selected K-value were retained and their admixture estimates were averaged using CLUMPP version 1.1.1 (Jakobsson & Rosenberg 2007), applying the greedy algorithm with random input order and 1000 permutations to align the runs and calculate G' statistics. Results were visualized using DISTRUCT 1.1 (Rosenberg 2004).

Deviation from mutation/drift equilibrium was tested by comparing levels of heterozygosity calculated from observed allele frequencies (*sensu* Nei *et al.* 1975; Nei 1987) with those expected under equilibrium with program Bottleneck 1.2.02 (Piry *et al.* 1999). As the mutation model underlying our microsatellite markers was unknown, data were analysed under two different model assumptions: two-phase model (TPM) and stepwise mutation model (SMM; Luikart & Cornuet 1998; Di Rienzo *et al.* 1994; Jarne & Lagoda 1996; Piry *et al.* 1999). When modelling TPM models, combinations of 95% single-step mutations and 5% multistep mutations were used, with a variance of 30 among multiple-step mutations ($10^4$ replications; Piry *et al.* 1999).

Deviation from migration/drift equilibrium was tested by comparing the relative likelihoods of 'geneflow/drift' and 'drift only' models with the program 2MOD, using a MCMC procedure with $10^5$ iterations and a burn-in of $10^4$ (Ciofi *et al.* 1999). Time intervals between population founding and population sampling were assumed sufficiently short such that effects of mutations were negligible (drift only), while mutation rates were assumed much smaller than migration rates (geneflow/drift). Data were analysed under both models, and Bayes factors were calculated to infer decisive power estimates of the most likely models (Jeffreys 1961; Goodman 1999).

### *Contemporary dispersal rates*

Contemporary dispersal rates were available for each of the seven study species from Lens *et al.* (2002), based on capture–recapture histories of 3089 individuals trapped and individually marked in the large fragments CH, NG and MB between 1996 and 2002. Individual recapture histories were generated using time intervals of 1 month. A total of 889 individuals were captured–recaptured in two or more months, and among these, 47 individuals were captured–recaptured in two or more fragments. Monthly probabilities of between-fragment dispersal were estimated from multistrata mark-recapture recapture models in program MARK (White & Burnham 1999). Based on Akaike's Information Criteria (AIC), the most parsimonious model was selected from a candidate set in which monthly probabilities of survival, recapture and dispersal were modelled simultaneously. Because distances differed between pairs of fragments (MB–NG, 11.3 km; MB–CH, 19.4 km; NG–CH, 10.9 km) and estimates of dispersal might be biased by differences in abundance between fragments for the different species, multistrata models were constructed that estimated monthly dispersal probabilities between each pair of fragments irrespective of the direction of dispersal. In a final step, we regressed pairwise dispersal probabilities against pairwise distances between the three forest fragments for each species, then used the intercept of each regression equation as the species-specific measure of contemporary dispersal (see Lens *et al.* 2002 for details on candidate models). Contemporary dispersal rates were positively correlated with contemporary geneflow rates estimated with BAYESASS 1.3 (Wilson & Rannala 2003) ($F_{1,5} = 9.93$, $P = 0.025$). Species-specific estimates were based on 3 000 000 iterations, a burn-in of 999 999, a sampling frequency of 2000, and a delta–value of 0.15. Because of the large confidence intervals (probably resulting from the low number of individuals and markers compared to those recommended by Wilson & Rannala 2003) and the fact that geneflow estimates are difficult to interpret when levels of genetic differentiation are low (Faubet *et al.* 2007), contemporary mobility was inferred from rates of dispersal, rather than geneflow, that were based on larger data sets, multiple recapture events and multiple years.

## Results

### *Genetic population structure*

Genetic differentiation was highly variable among the seven study species, as shown by strong differences in both pairwise (between populations CH, NG and MB) and overall estimates of $D_{est}$ (Table 1, see $F_{ST}$ values for comparison). $D_{est}$ estimates were very low (close to 0) in *N. olivacea*, markedly high (0.2–0.4) in *T. helleri*, and intermediate in the remaining species. Figure 2 depicts the level of genetic clustering for each study species with barplots per individual visualizing the inferred admixture proportions within each fragment. Individuals of *T. helleri*, *P. ruficapilla* and *P. cabanisi* were assigned to three genetic clusters corresponding to the three forest fragments (see Appendix 2 for details on model choice). Cluster differentiation was strong for *T. helleri* and *P. ruficapilla* but lower for *P. cabanisi* (Fig. 2). Individuals of *A. milanjenis*, *P. stellata* and *Z. silvanus* were assigned to two genetic clusters, in each case with one cluster consisting of populations CH and NG, and the other consisting of population MB, with the highest differentiation in *A. milanjensis* and the lowest differentiation in *P. stellata* (Fig. 2). Individuals of *N. olivacea* were assigned to a single panmictic cluster. Overall, strong genetic clustering corresponded with strong genetic differentiation (high $D_{est}$) and *vice versa* (Fig. 2, Table 1). In all species except for *P. cabanisi*, pairwise genetic differentiation between populations CH–NG, located on the same isolate, was consistently lower than between populations CH–MB and NG–MB,

**Table 1** Levels of genetic differentiation across loci between populations Chawia (CH), Mbololo (MB) and Ngangao (NG) as inferred from overall $D_{est}$ and $F_{ST}$ values (bold font) and harmonic means of the pairwise values (regular font), for seven bird species of the Taita Hills (SE Kenya)

| | $D_{est}$ | | | $F_{ST}$ | | |
|---|---|---|---|---|---|---|
| | CH | MB | Overall | CH | MB | Overall |
| *Nectarinia olivacea* | | | | | | |
| MB | 0.002 | | | 0.009 | | |
| NG | 0.00003 | 0.0007 | **0.001** | 0.001 | 0.006 | **0.006** |
| *Pogonocichla stellata* | | | | | | |
| MB | 0.029 | | | 0.025 | | |
| NG | 0.015 | 0.054 | **0.034** | 0.008 | 0.039 | **0.024** |
| *Zosterops silvanus* | | | | | | |
| MB | 0.057 | | | 0.049 | | |
| NG | 0.022 | 0.086 | **0.064** | 0.019 | 0.065 | **0.044** |
| *Andropadus milanjensis* | | | | | | |
| MB | 0.049 | | | 0.114 | | |
| NG | 0.002 | 0.055 | **0.036** | 0.010 | 0.096 | **0.076** |
| *Phyllastrephus cabanisi* | | | | | | |
| MB | 0.054 | | | 0.040 | | |
| NG | 0.044 | 0.041 | **0.058** | 0.043 | 0.040 | **0.042** |
| *Phylloscopus ruficapilla* | | | | | | |
| MB | 0.048 | | | 0.083 | | |
| NG | 0.021 | 0.062 | **0.058** | 0.066 | 0.133 | **0.095** |
| *Turdus helleri* | | | | | | |
| MB | 0.422 | | | 0.250 | | |
| NG | 0.204 | 0.362 | **0.356** | 0.113 | 0.207 | **0.207** |

located on two different isolates ($F_{1,11.1} = 7.48$; $P = 0.019$; Fig. 1, Table 1).

### *Genetic equilibria and bottlenecks*

Levels of allelic richness and observed and expected heterozygosity did not significantly differ among populations (Nonparametric Friedman test: all Fr between 1.556 and 4.571, all $P > 0.05$; see values in Table 2). Values of allelic richness and $H_O$ in populations MB and NG of *P. ruficapilla* were low compared to all other species. However, because of large variability in number and type of microsatellite markers among species, this difference could not be tested statistically. Levels of heterozygosity significantly exceeded those expected under mutation-drift equilibrium (Table 3) in population CH of species *T. helleri* (Wilcoxon test; TPM: $P = 0.008$; SMM: $P = 0.016$), populations MB and NG of *P. ruficapilla* (MB: TPM and SMM: $P = 0.02$; NG: TPM and SMM: $P = 0.008$) and population NG of *Z. silvanus* (TPM: $P = 0.03$; not significant after clustering with population CH; Appendix 3). None of the other populations or clusters showed evidence of heterozygosity excess. In *P. ruficapilla*, 'drift only' models were 16.8 times more likely than 'migration/drift equilibrium' models (Table 4). In all other species, 'migration/drift' models were more likely than pure drift models, whereby evidence for migration/drift equilibrium ranged from decisive to substantial in *T. helleri*, *P. stellata*, *A. milanjensis*, *N. olivacea*, but was weak in *Z. silvanus* and *P. cabanisi*.

### *Change in mobility over time*

Figure 3 relates species-specific levels of genetic population differentiation and patch occupancy to contemporary dispersal rates. *N. olivacea* showed the lowest level of genetic differentiation, the highest levels of patch occupancy and contemporary dispersal, and a single genetic cluster. *T. helleri*, in contrast, showed very strong genetic differentiation, very low levels of patch occupancy and contemporary dispersal, and three genetic clusters. The other five species were distinguished by broadly similar levels of genetic differentiation and two or three genetic clusters, but varied strongly in contemporary dispersal (ranging from zero in *P. ruficapilla* to relatively high in *Z. silvanus*) and patch occupancy (3–8 forest fragments occupied). Relationships between genetic differentiation and contemporary dispersal did not differ when fragments were located on the same or different mountain isolates ($F_{1,11.2} = 1.04$, $P = 0.3286$).

## Discussion

Loss of gene flow because of reduced dispersal, and reduction in effective population size because of genetic drift, can re-distribute genetic variability among spatially structured populations over the course of a few generations only (Harrison & Hastings 1996). Within the isolated Taita Hills of South-East Kenya, severe fragmentation of the original indigenous forest cover resulted in varying levels of population subdivision among seven sympatric, ecologically related forest bird species. There was strong consensus among species in the geographical patterning of genetic variation, reflecting the important role of landscape structure in genetic clustering and population differentiation (Storfer *et al.* 2007). For instance, in all three species that comprise two genetic clusters, cluster MB was consistently separated from cluster NG–CH and between-cluster genetic differentiation was always higher than within-cluster differentiation. The strong isolation of populations in fragment MB from those in fragments CH and NG is likely due to the presence of a low-altitude valley that reduced gene-flow levels even prior to human-induced deforestation (Brooks *et al.* 1998; Pellikka *et al.* 2009). When combining information on past and current mobility, however, the level of correspondence between

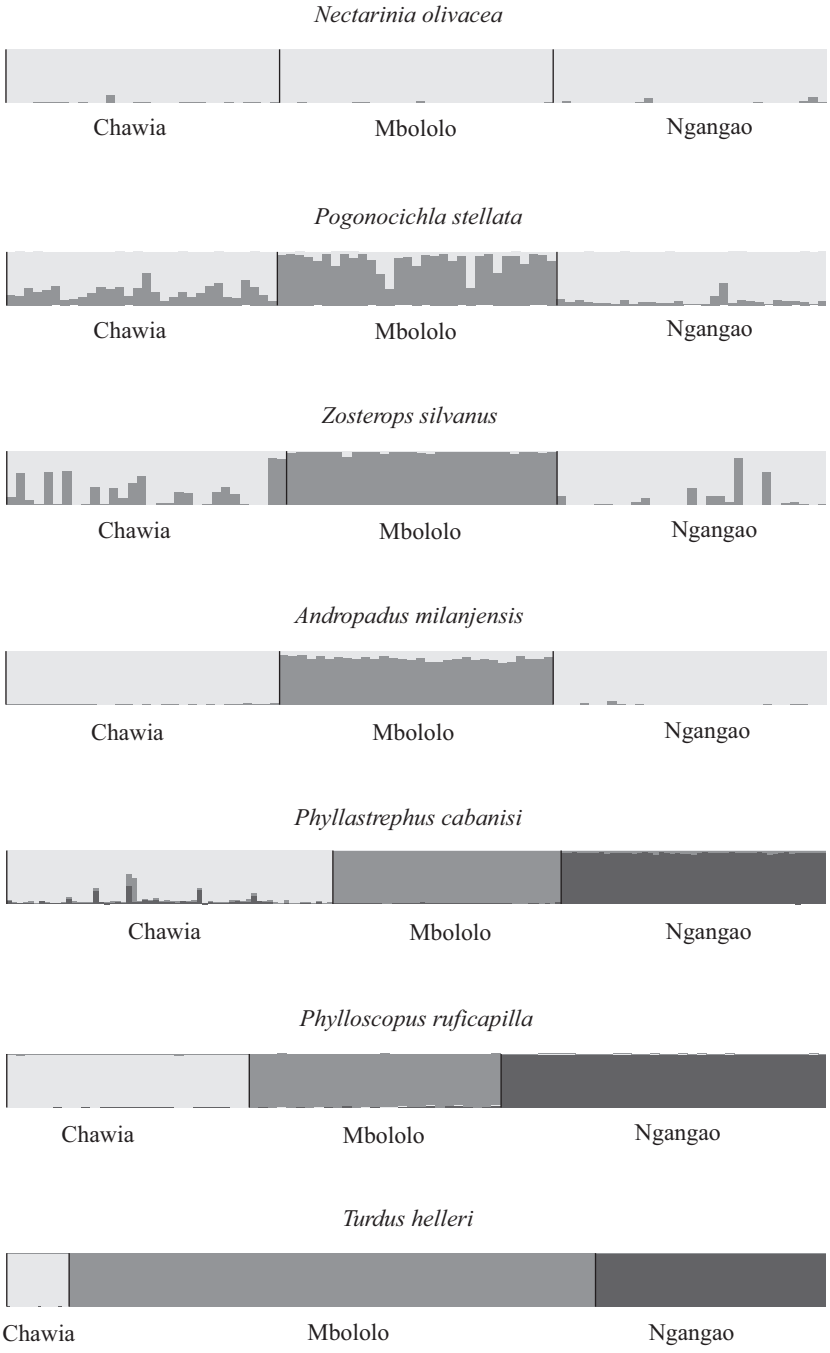


**Fig 2.** Admixture proportions for seven bird species of the Taita Hills (SE Kenya) as inferred from Bayesian genetic clustering. Each bar represents an individual sample with colour codes reflecting the likelihood of belonging to one of the inferred genetic clusters.

both sources of information was only weak. While two species clearly stood out as having either low or high mobility according to both historic and current estimates (*T. helleri* and *N. olivacea*, respectively), five other species showed highly variable current dispersal rates despite broadly similar levels of genetic population differentiation.

The most contrasting species pair in terms of current and historic mobility was *P. ruficapilla* and *T. helleri*, both highly sedentary species with (near) zero current dispersal but relatively low and very high levels of genetic differentiation, respectively. While genetic differentiation, reflecting geneflow over many generations, cannot be compared in absolute terms with dispersal

**Table 2** Population-level allelic richness and heterozygosity in seven bird species of the Taita Hills (SE Kenya) with *N* = number of original samples; loci = number of loci; $A_R$ = allelic richness corrected for sample size (number of samples between brackets); $H_O$ = observed heterozygosity; $H_E$ = expected heterozygosity

| Population | Species | *N* | Loci | $A_R$ | $H_O$ | $H_E$ |
|---|---|---|---|---|---|---|
| Chawia | *Nectarinia olivacea* | 30 | 8 | 4.1 (30) | 0.48 | 0.50 |
| | *Pogonocichla stellata* | 30 | 7 | 8.7 (30) | 0.65 | 0.71 |
| | *Zosterops silvanus* | 30 | 4 | 5.5 (29) | 0.53 | 0.59 |
| | *Andropadus milanjensis* | 30 | 7 | 3.1 (30) | 0.41 | 0.39 |
| | *Phyllastrephus cabanisi* | 60 | 10 | 6.0 (42) | 0.64 | 0.61 |
| | *Phylloscopus ruficapilla* | 26 | 7 | 3.3 (26) | 0.56 | 0.52 |
| | *Turdus helleri* | 18 | 6 | 4.0 (18) | 0.68 | 0.62 |
| Mbololo | *Nectarinia olivacea* | 30 | 8 | 4.0 (30) | 0.51 | 0.49 |
| | *Pogonocichla stellata* | 31 | 7 | 7.2 (30) | 0.57 | 0.64 |
| | *Zosterops silvanus* | 29 | 4 | 4.0 (29) | 0.49 | 0.51 |
| | *Andropadus milanjensis* | 30 | 7 | 3.3 (30) | 0.45 | 0.44 |
| | *Phyllastrephus cabanisi* | 42 | 10 | 5.4 (42) | 0.63 | 0.62 |
| | *Phylloscopus ruficapilla* | 27 | 7 | 2.6 (26) | 0.44 | 0.44 |
| | *Turdus helleri* | 152 | 6 | 4.5 (18) | 0.59 | 0.59 |
| Ngangao | *Nectarinia olivacea* | 30 | 8 | 4.2 (30) | 0.49 | 0.47 |
| | *Pogonocichla stellata* | 30 | 7 | 7.4 (30) | 0.60 | 0.67 |
| | *Zosterops silvanus* | 29 | 4 | 6.0 (29) | 0.66 | 0.65 |
| | *Andropadus milanjensis* | 30 | 7 | 3.4 (30) | 0.46 | 0.44 |
| | *Phyllastrephus cabanisi* | 49 | 10 | 6.4 (42) | 0.61 | 0.58 |
| | *Phylloscopus ruficapilla* | 35 | 7 | 2.3 (26) | 0.45 | 0.42 |
| | *Turdus helleri* | 67 | 6 | 5.4 (18) | 0.67 | 0.66 |

**Table 3** Population-level excess of heterozygote genotypes in three populations of seven bird species of the Taita Hills (SE Kenya) with CH = Chawia; MB = Mbololo; NG = Ngangao. Wilcoxon-based levels of significance are shown for different mutation models ($P < 0.05$ indicated in bold)

| Species | Population | TPM* | SMM† |
|---|---|---|---|
| *Nectarinia olivacea* | CH | 0.680 | 0.844 |
| | MB | 0.629 | 0.727 |
| | NG | 0.809 | 0.902 |
| *Pogonocichla stellata* | CH | 0.594 | 0.973 |
| | MB | 0.992 | 1.000 |
| | NG | 0.766 | 0.973 |
| *Zosterops silvanus* | CH | 0.438 | 0.938 |
| | MB | 0.156 | 0.156 |
| | NG | **0.031** | 0.563 |
| *Andropadus milanjensis* | CH | 0.711 | 0.766 |
| | MB | 0.594 | 0.656 |
| | NG | 0.656 | 0.711 |
| *Phyllastrephus cabanisi* | CH | 0.754 | 0.862 |
| | MB | 0.246 | 0.313 |
| | NG | 0.862 | 0.947 |
| *Phylloscopus ruficapilla* | CH | 0.148 | 0.148 |
| | MB | **0.020** | **0.020** |
| | NG | **0.008** | **0.008** |
| *Turdus helleri* | CH | **0.008** | **0.016** |
| | MB | 0.781 | 0.977 |
| | NG | 0.578 | 0.922 |

*Two-Phase Model.
†Stepwise Mutation Model.

**Table 4** Species-level likelihood of 'migration/drift equilibrium' and 'drift only' models. Bayes factors are calculated as ratios of most to least likely models and translated into levels of decisive power following Jeffreys (1961). The single species that shows strong evidence for migration-drift disequilibrium is indicated in bold

| Species | Migration-drift equilibrium model | Drift only model | Bayes factor |
|---|---|---|---|
| ***Phylloscopus ruficapilla*** | **1123** | **18 877** | **16.8 (Strong)** |
| *Turdus helleri* | 19 945 | 55 | 362.6 (Decisive) |
| *Pogonocichla stellata* | 19 446 | 554 | 35.7 (Very strong) |
| *Andropadus milanjensis* | 17 514 | 2486 | 7.0 (Substantial) |
| *Nectarinia olivacea* | 15 101 | 4899 | 3.1 (Substantial) |
| *Zosterops silvanus* | 13 311 | 6689 | 2.0 (Weak) |
| *Phyllastrephus cabanisi* | 11 490 | 8510 | 1.4 (Weak) |

events that may not result in reproduction (Slatkin 1987; Koenig *et al.* 1996; Thompson & Goodman 1997; Whitlock & McCauley 1999; Hedrick 2005a; Holsinger & Weir 2009; Lowe & Allendorf 2010), contrasting both estimates suggests that *P. ruficapilla* suffered a severe relative loss of mobility over time. While both species showed evidence of a genetic bottleneck in one or more populations, *P. ruficapilla* was the only species that also showed evidence of migration/drift disequilibrium, hence confirming our interpretation of a recent decrease in gene flow among its remnant populations. Lack of evidence for a similar decrease in gene flow in *T. helleri* suggests that the extremely low mobility of this critically endangered species is not of recent origin. Earlier, radio-telemetric data showed that individuals forage and move strictly within indigenous forest boundaries, with no evidence of excursions into the landscape matrix (Lehouck *et al.* 2009). Such a strong dependence of *T. helleri* on prime indigenous forest matches with its exceptionally high level of stress sensitivity inferred from spatio-temporal patterns in tarsus asymmetry, a presumed proxy of environmental stress (Lens *et al.* 1999). Stress-sensitive forest specialists such as *T. helleri* can be expected to have become locked in very early in the fragmentation process, which is thought to have been ongoing for several hundred years in the Taita Hills (Pellikka *et al.* 2009). In contrast, more generalist low-mobility species, such as *P. ruficapilla*, may have been able to persist longer in degraded forest patches, allowing gene flow to continue for a longer time during

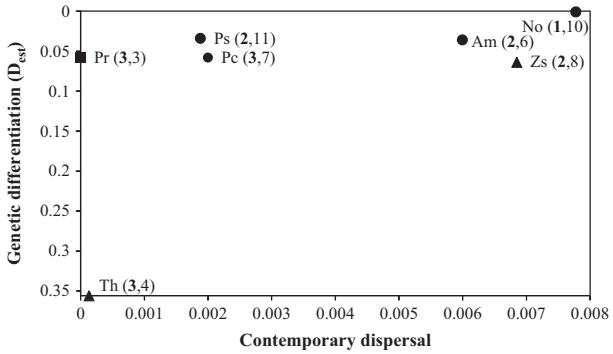


**Fig 3.** Genetic population differentiation versus contemporary dispersal in seven bird species of the Taita Hills (SE Kenya). Species abbreviations are No, *Nectarinia olivacea*; Ps, *Pogonocichla stellata*; Zs, *Zosterops silvanus*; Am, *Andropadus milanjensis*; Pc, *Phyllastrephus cabanisi*; Pr, *Phylloscopus ruficapilla*; Th, *Turdus helleri*. High $D_{est}$ values correspond to low levels of historic geneflow. Values in brackets indicate numbers of genetic clusters (bold font) and forest patches occupied (regular font); ● no mutation/drift or migration/drift disequilibrium; ▲ mutation/drift disequilibrium only; ■ mutation/drift and migration/drift disequilibrium. Contemporary dispersal rates (ψ–values corrected for species-specific recapture/survival probabilities and inter-fragment distances) and patch occupancy data from Lens *et al.* (2002).

the fragmentation process. However, progressing deterioration of these small stepping-stone patches (an estimated 50% of indigenous forest cover was lost to agriculture and admixture with secondary growth and exotic plantations between 1955 and 2004; Pellikka *et al.* 2009) may have resulted in a strong and recent reduction in mobility, explaining the migration/drift disequilibrium of *P. ruficapilla* with evidence for a recent bottleneck in fragment MB, the largest, most pristine, but also most isolated fragment of the Taita archipelago.

Two other species with intermediate levels of genetic differentiation, *P. stellata* and *P. cabanisi*, showed low to moderate dispersal rates, suggesting (moderate) losses in mobility over time, however without evidence for migration/drift disequilibrium. While individuals of *P. cabanisi* were assigned to three genetic clusters, *P. stellata* showed only two clusters. Such a difference in genetic clustering among two species with comparable contemporary dispersal rates may result from variation in sensitivity to habitat disturbance. Unlike all other study species, *P. cabanisi* forages and breeds in small family groups (T. Callens, unpublished data). Group living species are thought to exceed the carrying capacity of small and disturbed habitat remnants more easily and pay higher costs of biotic interactions near habitat edges and in the landscape matrix than pair living species (Van Houtan *et al.* 2006). This may explain why *P. cabanisi* is currently absent from nearly half of the Taita forest remnants (Lens *et al.* 2002). In contrast, *P. stellata* breeds in most of the small forest patches, including highly degraded forest remnants as small as 0.2 ha (Spanhove *et al.* 2009). Given such tolerance to degraded habitat, *P. stellata* dispersers from large, intact forest fragments may regularly settle in small, degraded remnants scattered across the landscape, resulting in step-wise gene flow between the larger fragments. Alternatively, discrepancies between historic and current estimates of mobility might result from a downward bias in dispersal estimates, e.g. because fledglings are only captured post-dispersal (see Van Treuren *et al.* 1999). In *P. cabanisi*, 127 nestlings (ringed 2007–2009) yielded 26 retraps, none of which originated from another fragment, while in *P. stellata*, 282 nestlings (ringed 2002–2005) yielded 50 retraps, 5 of which originated from another fragment (T. Callens & T. Spanhove, unpublished data). While these limited data sets prevent us from formally testing variation in timing of natal dispersal between both species, they do suggest that the accuracy of dispersal rates estimated from post-fledgling mark-recapture data may differ among species (see Desante 1995; Thomson *et al.* 1999). Yet, given that dispersal estimates used to infer current mobility rates in this study were statistically corrected for between-species heterogeneity in survival and recapture rates and did not comprise recapture events of ringed nestlings (Lens *et al.* 2002), they are still considered valid for comparison of relative mobility rates.

Results from this study hence confirm the conclusion from other multi-species studies that sympatric forest birds may differ strongly in genetic signature of forest fragmentation (Bates 2002; Brown *et al.* 2004; Burney & Brumfield 2009). More importantly, our study shows that such differences may also appear among species with broadly similar levels of current mobility. In line with this, Van Houtan *et al.* (2006, 2007) hypothesized that post-fragmentation levels of vagility may not be the best predictor of how forest fragmentation affects the life history of forest-dependent species. Rather, species which range more widely pre-fragmentation, e.g. those that track unpredictable food resources or frequently join (mixed-species) flocks, may be more vulnerable to post-fragmentation extinction compared to species that do not cross gaps as often. Earlier, we proposed that conservation tactics in the fragmented Taita ecosystem may fail unless they include action both within sites, to minimize habitat deterioration, and across sites within the landscape, to maximize dispersal (Lens *et al.* 2002). As part of a series of conservation initiatives funded by Conservation International, over 150 000 indigenous tree seedlings have been raised in community-owned tree nurseries since 2006.

To address within-site issues, initial efforts are being undertaken to restore disturbed sections within the indigenous forest fragments. For between-site action, priority areas for reforestation within the matrix were selected based on a combination of least-cost modelling analysis (where we quantified landscape connectivity and identified likely dispersal corridors linking the indigenous fragments; Adriaensen *et al.* 2006), forest ecology (silvicultural) characteristics and sociological aspects (Githiru & Lens 2007). Findings from the current study provide evidence that habitat isolation results in reduced genetic connectivity, but most importantly show that not all species are equally sensitive. Combining landscape, demographic and behavioural data with population genetic data, such as presented here, therefore helps to determine which species may be most prone to extinction, and hence, to rationalize conservation action at species level (Pavlacky *et al.* 2009; Lowe & Allendorf 2010). For instance, while conservation action for *P. ruficapilla* should primarily focus on the restoration of landscape connectivity through creation of small (stepping-stone) forest patches, such action may not suffice for *T. helleri* unless combined with habitat-restoration programmes in all occupied forest fragments. For *P. stellata* and *P. cabanisi*, the apparent loss in current versus historic mobility and different patterns of genetic clustering merely act as an early warning system against future losses in gene flow and genetic variation.

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This study is part of the PhD research of T.C. in Luc Lens's laboratory. T.C. is currently working on the demography and genetics of Afrotropical passerines in the highly-fragmented cloud forests of the Taita Hills in south-east Kenya. P.G. studies the conservation genetics of intensively managed populations, in the wild as well as in captivity, of a wide range of animal species. E.M. studies behavioural and ecological aspects of dispersal in different animal groups, with a particular interest in birds. E.D's research focuses on developing mathematical and computational methods in population genetics, with a particular interest in the study of admixed populations. M.G. works at the research-policy-conservation interface, undertaking biodiversity assessments, cost-benefit analyses, and identifying threats and biodiversity values for informing management plans for landscape-scale conservation. J.H's research focuses on the application of statistical methods for understanding human genetic variation, and its contribution to phenotypic variation and common complex disease susceptibility. L.L. is interested in the evolutionary ecology and conservation genetics of fragmented populations.

## Appendix 1

Microsatellite DNA specifications and PCR conditions for seven bird species of the Taita Hills, SE Kenya.

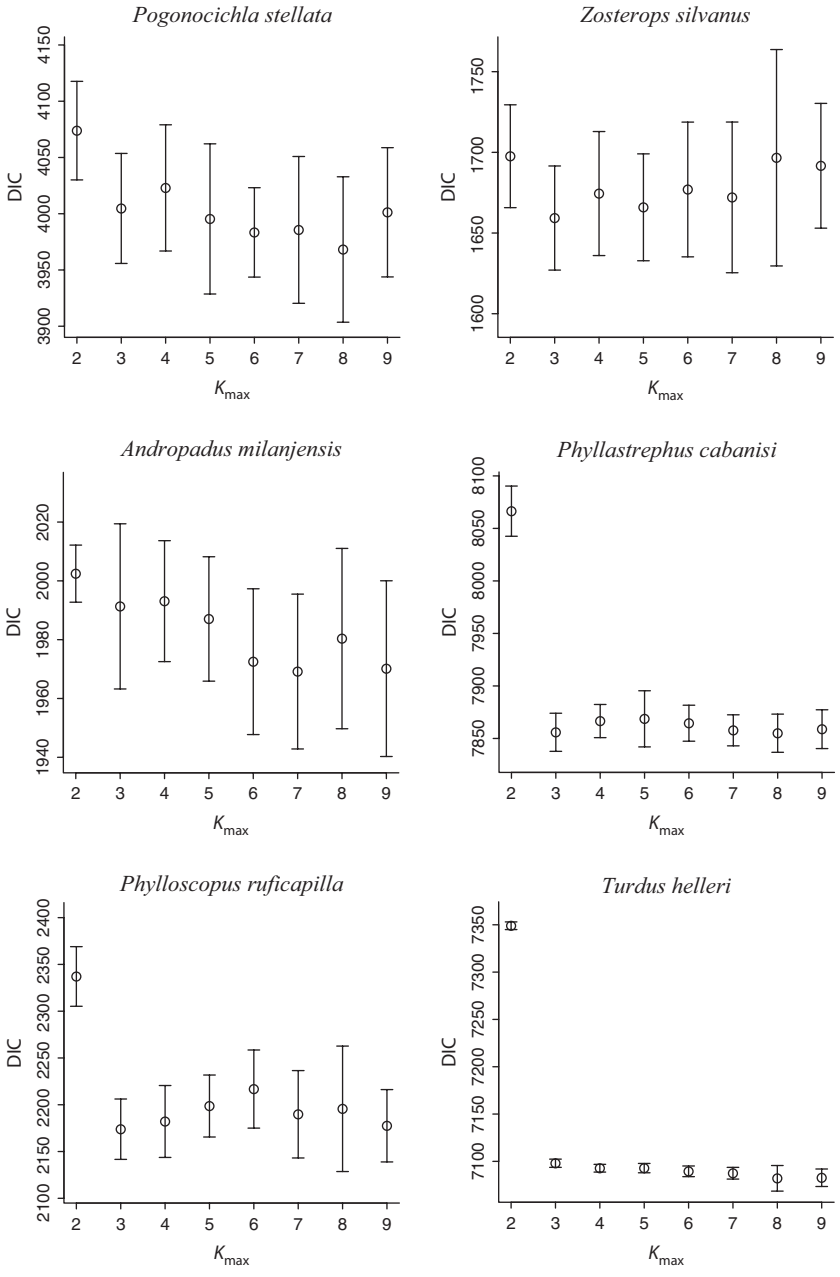
| Locus | Species* | Reference | Product size (bp) | Hybridisation temp. (°C) | Concentration $MgCl_2$ (mM) |
|---|---|---|---|---|---|
| *Nectarinia olivacea* | | | | | |
| Pc3 | *Parus caeruleus* | Dawson *et al.* 2000 | 141–155 | 50 | 1.0 |
| Pocc8 | *Phylloscopus occipitalis* | Bensch *et al.* 1997 | 220–222 | 55 | 2.0 |
| Ppi2 | *Pica pica* | Martinez *et al.* 1999 | 229–243 | 55 | 2.5 |
| Gf6 | *Geospiza fortis* | Petren 1998 | 166–180 | 56 | 2.5 |
| Pc9 | *Parus caeruleus* | Dawson *et al.* 2000 | 143–165 | 55 | 1.5 |
| Pat14 | *Parus atricapillus* | Otter *et al.* 1998 | 126–130 | 50 | 1.0 |
| Ls1 | *Lanius ludovicianus* | Mundy & Woodruff 1996 | 190–214 | 50 | 1.5 |
| Pdo1 | *Passer domesticus* | Neumann & Wetton 1996 | 162–170 | 50 | 2.0 |

| | | | | | |
|---|---|---|---|---|---|
| *Pogonocichla stellata* | | | | | |
| Pat14 | *Parus atricapillus* | Otter *et al.* 1998 | 143–173 | 50 | 1.5 |
| Mcyμ4 | *Malurus cyaneuss* | Double *et al.* 1997 | 132–152 | 55 | 1.0 |
| Ltmr6 | *Chiroxiphia linearis* | McDonald & Potts 1994 | 190–198 | 54 | 2.0 |
| Gf6 | *Geospiza fortis* | Petren 1998 | 138–154 | 56 | 2.5 |
| WBSW2 | *Plocepasser mahali* | McRae & Amos 1999 | 125–131 | 54 | 1.5 |
| WBSW9 | *Plocepasser mahali* | McRae & Amos 1999 | 102–122 | 54 | 1.5 |
| GF5B | *Geospiza fortis* | Petren 1998 | 199–227 | 57 | 1.5 |
| *Zosterops silvanus* | | | | | |
| Mcyμ4 | *Malurus cyaneus* | Double *et al.* 1997 | 137–161 | 55 | 2.5 |
| Cu28 | *Catharus ustulatus* | Gibbs *et al.* 1999 | 166–168 | 60–51 ('touchdown') | 2.5 |
| Zl12 | *Zosterops lateralis* | Degnan *et al.* 1999 | 110–120 | 57 | 1.5 |
| Pocc1 | *Phylloscopus occipitalis* | Bensch *et al.* 1997 | 222–246 | 55 | 2.0 |
| *Andropadus milanjensis* | | | | | |
| Pdo1 | *Passer domesticus* | Neumann & Wetton 1996 | 164–168 | 50 | 2.0 |
| WBSW2 | *Plocepasser mahali* | McRae & Amos 1999 | 226–234 | 45 | 1.0 |
| Pc3 | *Parus caeruleus* | Dawson *et al.* 2000 | 165–171 | 50 | 1.0 |
| Mcyμ4 | *Malurus cyaneus* | Double *et al.* 1997 | 134–138 | 55 | 2.5 |
| WBSW11 | *Plocepasser mahali* | McRae & Amos 1999 | 162–176 | 46 | 1.0 |
| Pat14 | *Parus atricapillus* | Otter *et al.* 1998 | 136–138 | 50 | 1.0 |
| Dpu16 | *Dendroica petechia* | Dawson *et al.* 1997 | 151–157 | 52 | 1.0 |
| *Phyllastrephus cabanisi* | | | | | |
| Ase18 | *Acrocephalus sechellensis* | Richardson *et al.* 2000 | 236–353 | 57 | 2.0 |
| Indigo41 | *Vidua chalybeata* | Sefc *et al.* 2001 | 276–312 | 57 | 2.0 |
| Ls1 | *Lanius ludovicianus* | Mundy & Woodruff 1996 | 164–220 | 50 | 1.5 |
| Ls2 | *Lanius ludovicianus* | Mundy & Woodruff 1996 | 191–200 | 50 | 1.5 |
| Mcyμ4 | *Malurus cyaneus* | Double *et al.* 1997 | 131–159 | 55 | 1.5 |
| Pc3 | *Parus caeruleus* | Dawson *et al.* 2000 | 157–169 | 53 | 1.0 |
| Pc4 | *Parus caeruleus* | Dawson *et al.* 2000 | 152–164 | 53 | 2.0 |
| Pfi04 | *Phyllastrephus cabanisi* | R. C. K. Bowie, unpublished data | 139–201 | 57 | 1.5 |
| Pfl54 | *Phyllastrephus cabanisi* | R. C. K. Bowie, unpublished data | 222–251 | 57 | 2.0 |
| WBSW2 | *Plocepasser mahali* | McRae & Amos 1999 | 212–230 | 45 | 1.0 |
| *Phylloscopus ruficapilla* | | | | | |
| Cu02 | *Catharus ustulatus* | Gibbs *et al.* 1999 | 154–162 | 60–48 ('touchdown') | 2.5 |
| Pat43 | *Parus atricapillus* | Otter *et al.* 1998 | 117–125 | 55 | 1.0 |
| Zl18 | *Zosterops lateralis* | Degnan *et al.* 1999 | 177–191 | 55 | 1.5 |
| Mslp4 | *Locustella pryeri* | Ishibashi *et al.* 2000 | 136–140 | 55 | 1.5 |
| Pocc1 | *Phylloscopus occipitalis* | Bensch *et al.* 1997 | 229–233 | 52 | 1.0 |
| Pocc8 | *Phylloscopus occipitalis* | Bensch *et al.* 1997 | 214–218 | 55 | 2.0 |
| Dpu16 | *Dendroica petechia* | Dawson *et al.* 1997 | 152–154 | 50 | 1.5 |
| *Turdus helleri* | | | | | |
| Ltmr6 | *Chiroxiphia linearis* | McDonald & Potts 1994 | 214–226 | 55 | 2.0 |
| Pc3 | *Parus caeruleus* | Dawson *et al.* 2000 | 115–125 | 52 | 2.0 |
| Pat 43 | *Parus atricapillus* | Otter *et al.* 1998 | 141–171 | 54 | 1.5 |
| GF5B | *Geospiza fortis* | Petren 1998 | 199–227 | 57 | 1.5 |
| Mjg1Te | *Aphelocoma ultramarina* | Li *et al.* 1997 | 100–172 | 55 | 1.5 |
| Pdo5 | *Passer domesticus* | Griffith *et al.* 1999 | 266–274 | 57 | 2.5 |

*Species for which the primer was originally developed.

## Appendix 2

Bayesian admixture model selection in TESS 2.3. For each study species, DIC values are plotted against K-values (maximal number of clusters). Values corresponding to DIC values that first level off, are selected.



## Appendix 3

Cluster-level excess of heterozygote genotypes in seven bird species of the Taita Hills (SE Kenya) with CH = Chawia, MB = Mbololo and NG = Ngangao. Wilcoxon-based levels of significance are shown for different mutation models (all $P > 0.05$).

| Species | Population Cluster | TPM* | SMM† |
|---|---|---|---|
| *Nectarinia olivacea* | CH–MB–NG | 0.770 | 0.809 |
| *Pogonocichla stellata* | CH–NG | 0.656 | 0.980 |
| | MB | 0.992 | 1.000 |
| *Zosterops silvanus* | CH–NG | 0.563 | 0.906 |
| | MB | 0.156 | 0.156 |
| *Andropadus milanjensis* | CH–NG | 0.656 | 0.766 |
| | MB | 0.531 | 0.656 |

*Two-Phase Model.
†Stepwise Mutation Model.