Evolutionary Ecology Research, 2007, **9**: 21–39

Genetic differentiation after founder events: an evaluation of F_{ST} estimators with empirical and simulated data

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ABSTRACT

Question: Given a model of speciation by host shift, how do different marker types and measures of genetic differentiation compare in detecting reproductive isolation between a small, recently founded population and its large source population?

Data incorporated: We used empirical data from brood parasitic indigobirds (*Vidua* spp.) as well as simulated mitochondrial and microsatellite data for a founding event with immediate cessation of gene flow and subsequent population growth.

Method of analysis: We evaluated the performance of different estimators (θ_{ST}, R_{ST}, and Φ_{ST}) in detecting population differentiation and compared our simulation results with previously collected empirical data.

Conclusions: With much greater variance, R_{ST} was less reliable than θ_{ST} in detecting incipient differentiation in microsatellite data. Negative R_{ST} values for individual loci in both the empirical data and up to 20% of simulation replicates occurred when genetic drift changed allele frequencies but not allele size variance. Both Φ_{ST} and θ_{ST} reliably detected genetic isolation from simulated sequence data. As bottlenecked populations regained variability, however, θ_{ST} values decreased due to a negative correlation with polymorphism, an effect also observed in the empirical data. Given this effect, the standardization of differentiation estimates relative to population heterozygosity is recommended for both types of markers, although this approach does not correct for the reduction of θ_{ST} due to allele size homoplasy in microsatellites. In contrast, Φ_{ST} values gradually increased over time as novel mutations increased the divergence between haplotypes. Our analyses suggest that for microsatellite data, θ_{ST} outperforms R_{ST} in detecting recently established reproductive isolation, whereas Φ_{ST} is preferable for sequence data regardless of divergence time.

Keywords: bottleneck, F_{ST} estimators, microsatellites, population growth, standardized genetic differentiation measure, *Vidua*.

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INTRODUCTION

The detection and quantification of genetic isolation and divergence of populations has long been a focal point of population genetics. The original estimators of population differentiation in the framework of *F*-statistics (Wright, 1951) have been modified to account for small numbers of sampled populations and uneven sample sizes [θ_{ST} (Weir and Cockerham, 1984)], and new estimators have been formulated to exploit the information content of specific marker types such as microsatellites [R_{ST} (Slatkin, 1995)] and molecular sequence data [Φ_{ST} (Excoffier *et al.*, 1992)]. Different F_{ST} analogues, however, can yield substantially different estimates of population differentiation for the same data set (e.g. Gaggiotti *et al.*, 1999; Balloux and Goudet, 2002). In the most extreme cases, highly significant differentiation may be indicated by one estimator, while an alternative algorithm yields results consistent with panmixis. The sources of such discrepancies lie in the different treatment of marker characteristics [e.g. consideration of mutation models (Excoffier *et al.*, 1992; Slatkin, 1995)], in the deviation of actual evolutionary processes from model assumptions (Balloux and Lugon-Moulin, 2002), and in the mathematical properties of individual algorithms [e.g. maximum F_{ST} estimates limited by heterozygosity (Hedrick, 1999)]. Despite these pitfalls and the recent development of alternative, although computationally intensive, coalescent methods, most published population genetic studies still base their conclusions in part or exclusively on estimates of population differentiation as measured by F_{ST} analogues.

In a study of brood parasitic indigobird populations (*Vidua* spp.) from several African localities (Sefc *et al.*, 2005), the magnitude and statistical significance of different measures of population differentiation varied considerably, making choice of the most appropriate and informative algorithm crucial to interpretation of the data. The model of indigobird speciation by host shift, as developed from field studies and behavioural experiments (e.g. Payne and Payne, 1994; Payne *et al.*, 1998, 2000), suggests a small initial population size for a novel host race and immediate cessation of gene exchange with the source population because of assortative mating among associates of the same host species (Payne *et al.*, 2000, 2002). Genetic data for indigobird species and populations are generally consistent with this model, and further indicate that indigobird species are of very recent origin (Klein and Payne, 1998; Sorenson *et al.*, 2003; Sefc *et al.*, 2005). Differentiation among indigobird populations is therefore strongly influenced by ancestral polymorphism, variable demographic histories of individual populations, likely including bottlenecks and population expansion, and the stochasticity of genetic drift at individual loci; and may therefore deviate from expectations developed for long-term isolation following a vicariant event (Jin and Chakraborty, 1995).

We seek here to evaluate the low level of genetic differentiation between indigobird species (Sorenson *et al.*, 2003; Sefc *et al.*, 2005) in relation to expectations developed from a demographic scenario that more closely matches the proposed model of speciation in this group. The empirical data consist of genetic differentiation estimates (θ_{ST}, Φ_{ST}, and R_{ST}) obtained from microsatellite genotypes and mitochondrial sequences from populations of indigobirds (*Vidua* spp.) across sub-Saharan Africa. We combine detailed examination of the empirical data with simulation studies to elucidate the causes for the observed incongruence between alternative estimates and marker types. Computer simulations have proved useful for systems too complex for analytical approaches or when assumptions underlying analytically derived estimators of population parameters are violated (e.g. Le Corre *et al.*, 1997; Bohonak *et al.*, 2001; Ibrahim, 2001; Lee and Hastings, 2006). We simulated the trajectories of microsatellite and mitochondrial differentiation estimates following a founding event derived from a large

source population and assuming subsequent growth of the novel population. Based on these simulations, estimators of population differentiation were compared with regard to their variance and sensitivity to recently established reproductive isolation under different demographic parameters. We show that incongruence between differentiation estimates obtained from empirical data originates in part from lineage sorting patterns associated with a founder effect, and in part from the mathematical properties of the various estimators. Our analysis is also relevant to the interpretation of differentiation estimates in other scenarios involving recent population splits and bottlenecks, including the evaluation of founder-effect models (Clegg *et al.*, 2002) and speciation by host switch in phytophagous insects (Berlocher and Feder, 2002; Svensson *et al.*, 2005).

MATERIALS AND METHODS

The present study is motivated by analyses of microsatellite and mtDNA differentiation among indigobird populations as described in detail by Sefc *et al.* (2005). Briefly, microsatellite genotypes for nine loci and mitochondrial sequences (1100 bp comprising most of the ND6 gene, tRNA glutamine, and the 5′ half of the control region) were obtained for populations of indigobirds (*Vidua* spp.) across sub-Saharan Africa. Genetic differentiation between species and populations in nuclear and mitochondrial markers was estimated with θ_{ST} (Weir and Cockerham, 1984), Φ_{ST} (Excoffier *et al.*, 1992), and R_{ST} (Slatkin, 1995), using the programs Arlequin (Schneider *et al.*, 2000) and RstCalc (Goodman, 1997). To compare the trajectories of θ_{ST} and R_{ST} estimators of microsatellite differentiation over time, we simulated the evolution of microsatellite allele frequencies in populations after a founding event intended to approximate the indigobird model of speciation by host shift (Payne *et al.*, 2002; Sorenson *et al.*, 2003). Source and founder populations were drawn from the observed allele frequency distribution at microsatellite locus INDIGO 38 (12 alleles at frequencies 0.100, 0.065, 0.157, 0.170, 0.143, 0.096, 0.096, 0.061, 0.057, 0.039, 0.013, 0.004). Simulations based on empirical allele frequency distributions found at other loci yielded similar results.

A large source population was assumed to be unaffected by genetic drift over the simulated period of 1000 generations, while the founder population started with size $N_0 = 100$ gene copies, and expanded according to a logistic growth curve with rate r (we used values of r ranging from 0.005 to 0.025) up to a carrying capacity $K = 10{,}000$ genes, after which it remained at constant size. According to the model of indigobird speciation by host shift and supported by previous genetic data (Payne *et al.*, 2002), novel populations are founded by a small number of individuals sampled from a large source population. Based on coalescent analyses of mitochondrial and nuclear data, the effective population size of southern African indigobirds is on the order of 10^6 (M.D. Sorenson *et al.*, unpublished results). To simplify the simulations and reduce computation time, allele frequencies in the source population were therefore held constant, approximating the minimal effect of drift in the large source population. Additional simulations showed that simulating drift in a source population of $N = 10{,}000$ slightly increased the differentiation estimates, on average, but did not alter the conclusions presented here.

In the newly founded population, microsatellite gene copies were drawn randomly from the prior generation to simulate the effects of random drift, and mutations were introduced at rate μ ($\mu = 10^{-5}$, 10^{-4}, or 10^{-3}) following a strict single-step mutation model, such that allele sizes were equally likely to increase or decrease by one repeat unit. The allele size range was constrained to 32 alleles (10 repeat units above and below the initial size range),

but these limits were never reached in our simulations. Samples of 100 genes were taken at intervals of five generations from both populations to monitor gene diversity in the new population and differentiation from the source population over time. Gene diversity H_e was calculated as $1 - \Sigma p_i^2$, where p_i are the allele frequencies (Nei, 1987). Population differentiation was estimated using R_{ST} (Slatkin, 1995) and θ_{ST} according to equation (6) in Weir and Cockerham (1984).

We simulated drift and mutation in mitochondrial haplotypes following a similar procedure. Source and founder populations were drawn from the observed haplotype frequency distribution for 232 southern African indigobirds (73 haplotypes; the most common with a frequency of 0.116; 43 unique haplotypes with a frequency of 0.004). As in the microsatellite simulations, starting with different underlying haplotype distributions with comparable genetic diversity had little effect on the results. Several combinations of founder population size ($N_0 = 25$, 50, and 100 genes), logistic growth rates ($r = 0.0075$ and 0.02), and carrying capacities ($K = 10{,}000$ and 2500 genes) were tested; the values $N_0 = 25$ and $K = 2500$ reflect the smaller effective population size of mtDNA and correspond to the values used in the microsatellite simulations ($N_0 = 100$, $K = 10{,}000$). Each generation consisted of randomly drawn genes from the previous generation with new mutations introduced at rate μ ($\mu = 0$, 10^{-5}, or 10^{-4} in a 1100 bp sequence) following a finite sites model. Pair-wise haplotype distances were recorded as the number of nucleotide differences for subsequent calculation of Φ_{ST}. Samples of 100 genes were taken at intervals of 10 generations from both populations to monitor changes in gene diversity and population differentiation over a period of 1000 generations. In simulations where the novel population started with 25 gene copies, the entire population was sampled until population size exceeded 100. Population differentiation was estimated using θ_{ST} according to equation (6) in Weir and Cockerham (1984) and Φ_{ST} according to Excoffier (2001). Some combinations of parameters were not sampled for the mitochondrial analysis because simulations with $\mu > 10^{-5}$ required considerable computation time associated with keeping track of pair-wise distances.

Because we were specifically interested in monitoring the time course or trajectory of genetic differentiation and diversity after population foundation, we employed a forward simulation procedure rather than a coalescent approach. To our knowledge, no forward simulation program is currently available that would allow us to set demographic and evolutionary parameters according to the indigobird model. Therefore, demographic simulations and the calculation of population differentiation estimates from the simulated data were programmed in perl v5.8.2 (included in the cygwin package, and run under Windows NT). We checked the accuracy of our programmed calculations by comparing the results for a small sample of simulated data sets with those obtained from Arlequin (Schneider *et al.*, 2000). We also compared the results of our forward simulations (gene diversity and population differentiation at the end of the simulated period) with coalescent simulations run in SIMCOAL (Laval and Excoffier, 2004) for selected parameter values. Exact replication of results using the two simulation programs was not possible as it would require that the pattern and level of genetic variation in the source population at the start of the forward simulation were matched at the point of population splitting in the coalescent simulation. Nonetheless, the general trends observed in the results of our forward simulations were corroborated by results from SIMCOAL.

RESULTS AND DISCUSSION

Contrasting θ_{ST} and R_{ST} estimates from empirical microsatellite data

As reported in Sefc *et al.* (2005), a population of *V. codringtoni* in Chipinge, Zimbabwe, showed significant mitochondrial differentiation from all other indigobird populations in southern Africa. In agreement with the mitochondrial data, microsatellite differentiation was significant when measured by θ_{ST}, but lower and not significant when measured by R_{ST}. An explanation for this discrepancy between differentiation estimates can be found in the observed distributions of allele sizes in different populations. After a founding event, the new population will likely retain only a subset of the alleles in the source population and these will be nested within the range of allele sizes in the source population. Allele frequencies, however, may diverge between the populations due to genetic drift. Under these conditions, allele frequency differences are detected by θ_{ST}, but the similar allele size ranges in the two populations result in similar variance in allele size and R_{ST} values close to zero. Allele frequency distributions resembling this pattern were found for *V. codringtoni* in comparison with other populations at three loci with relatively high and significant θ_{ST} values (INDIGO 7: $\theta_{ST} = 0.115$; INDIGO 8: $\theta_{ST} = 0.064$; and INDIGO 38: $\theta_{ST} = 0.071$), all of which produced small or negative R_{ST} values (Fig. 1A).

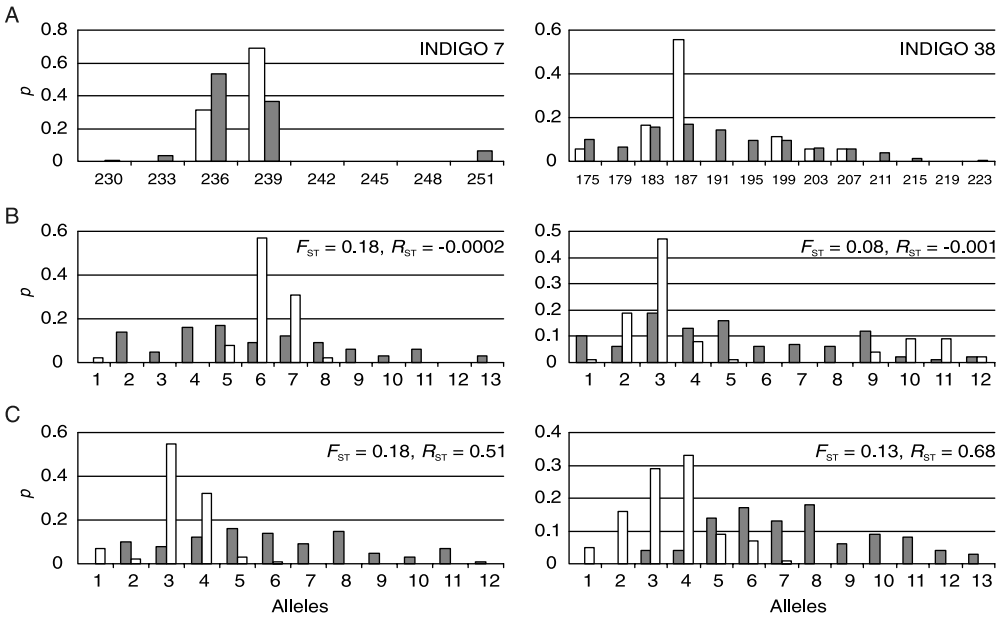

Fig. 1. Allele frequency distributions giving rise to disparate R_{ST} and F_{ST} estimates. (A) Observed allele frequencies in *V. codringtoni* from Chipinge, Zimbabwe (white bars) and in the combined sample of indigobirds from southern Africa (grey bars) at two microsatellite loci with negative R_{ST} values and significantly positive θ_{ST} estimates. The low differentiation among southern African indigobird populations (except Chipinge *V. codringtoni*) makes allele frequencies in a pooled sample representative for those of individual populations (see Sefc *et al.*, 2005). (B, C) Allele frequencies resulting from replicate simulations of a population bottleneck. Selected examples giving rise to negative R_{ST} and positive F_{ST} values (B), and substantially higher R_{ST} than F_{ST} values (C), are shown.

Under the assumption of increases or decreases in allele size by one repeat unit per mutation (i.e. SSM), even a high mutation rate in the newly founded population may not generate the differences in allele size that make R_{ST} a potentially superior measure of differentiation (Slatkin, 1995). Given that the most frequent alleles are likely to be sampled in a founding event, stepwise mutations will often restore to the new population low-frequency alleles also present in the source population. More generally, the likelihood that most mutations will produce identical-in-state microsatellite alleles will increase the time required – especially for small populations – to accumulate the mutations needed for differentiation to be detected by R_{ST}.

Simulation of microsatellite differentiation after a population founder event

We used simulations of population founding events to track the post-bottleneck trajectories of θ_{ST} and R_{ST} between a source population and a newly established population. Genetic drift is the dominant evolutionary force in the bottlenecked population, but with strictly stepwise mutations and no gene flow, conditions are presumably optimal for the performance of the R_{ST} estimator (Slatkin, 1995; Balloux *et al.*, 2000; Balloux and Goudet, 2002). Simulations were carried out over a range of mutation rates and post-bottleneck growth rates (see 'Materials and methods') to identify parameter combinations under which R_{ST} estimates, for example, are consistent with high gene flow or panmixis, whereas θ_{ST} correctly detects differentiation between the completely isolated populations. We do not evaluate quantitative estimates of gene flow or divergence time under this non-equilibrium situation, but rather are concerned with the relative frequency at which the two measures fail to detect existing reproductive isolation.

Representative graphs of θ_{ST} and R_{ST} values over time are shown in Fig. 2, and a summary of the simulation results is presented in Fig. 3. Under most parameter combinations, there is a gradual increase in median θ_{ST} or R_{ST} values over the first 200–300 generations, followed by relatively flat curves thereafter (Fig. 2). As is typically found in vicariant models of population differentiation, the variance of R_{ST} is much larger than that of θ_{ST} (Fig. 3B) (e.g. Balloux and Lugon-Moulin, 2002). Indeed, the range of R_{ST} values encompasses zero across the entire simulated period of 1000 generations for every combination of parameters tested, with 10–30% of the replicates yielding negative R_{ST} values at any given generation (Fig. 3C). Given moderate mutation rates ($\mu = 10^{-5}$ and 10^{-4}), θ_{ST} values consistently indicate some degree of differentiation both after severe and less severe bottlenecks. In contrast, R_{ST} values are generally lower than the corresponding F_{ST} estimates, and the R_{ST} estimator fails to detect differentiation in a considerable proportion of simulation replicates (Fig. 2A, B; Fig. 3). With a higher mutation rate ($\mu = 10^{-3}$), the lower 25th percentiles for R_{ST} are similar to those for $\mu = 10^{-4}$ over the range of simulated generations, as is the proportion of negative R_{ST} values (Fig. 2C, D; Fig. 3C). The distributions of the θ_{ST} and R_{ST} estimates at the simulation endpoints summarized in Fig. 3 differ significantly from each other (Kolmogorov-Smirnov test, $P < 0.01$ in each of the 15 parameter settings). Similarly, the distributions of θ_{ST} and R_{ST} estimates taken at intervals of 100 generations from the simulations in Fig. 2 were significantly different from each other in all comparisons ($P < 0.02$). Clearly, θ_{ST} performs better than R_{ST} in detecting recently established genetic isolation from single loci under the conditions tested in our simulation. The range of R_{ST} estimates, however, includes high values in all parameter combinations, sometimes exceeding the corresponding θ_{ST} values by more than 100% (Fig. 2). Using multiple loci to

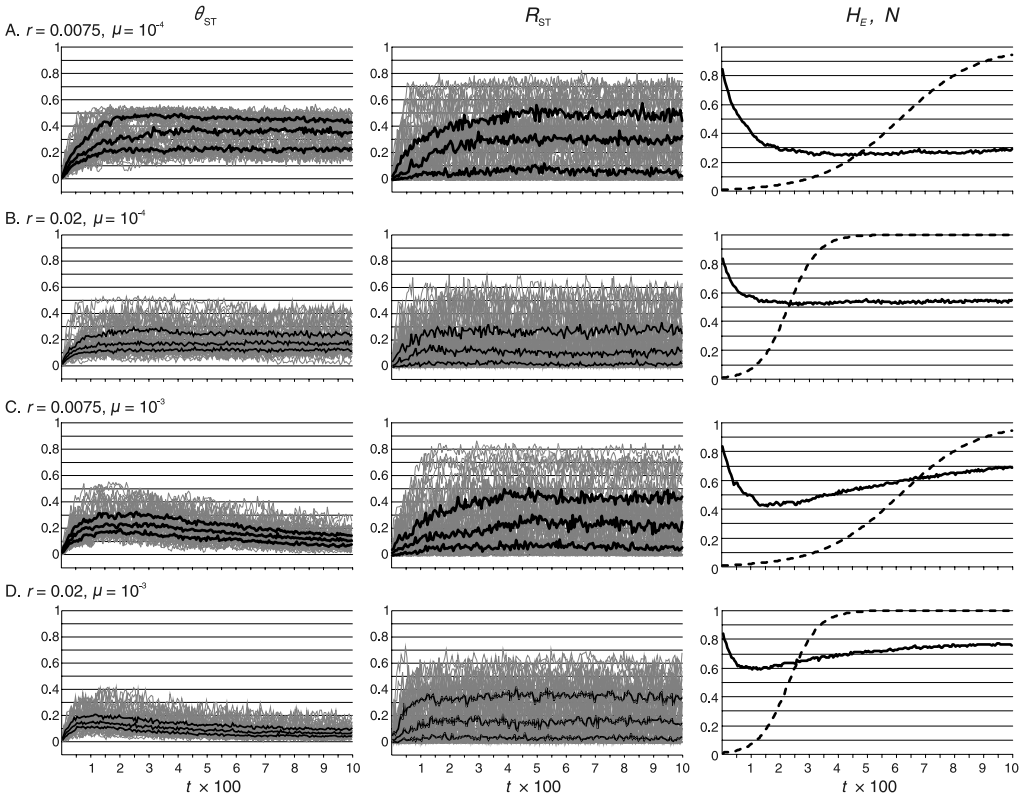

Fig. 2. Trajectories of θ_{ST} and R_{ST} estimates in simulations of microsatellite loci over 1000 generations after a population founder event, and plots of gene diversity (H_e, black lines) and population size (N, broken lines) in the novel, bottlenecked population, under varying growth (r) and mutation rates (μ). Grey lines for θ_{ST} and R_{ST} estimates represent results from 50 replicate runs; the median, 25%, and 75% quartiles are indicated by bold black lines. $N_0 = 100$; $K = 10{,}000$.

estimate R_{ST} and θ_{ST} reduces the variance of both estimators, and reduces the downward bias of R_{ST} estimates as compared to an estimate obtained from a 500-locus data set (Table 1) (see also Gaggiotti *et al.*, 1999; Balloux *et al.*, 2000). Less than 0.5% of R_{ST} estimates based on 5 loci and none of the estimates based on 10 loci were negative, such that a failure to detect existing differentiation is significantly less likely with multi-locus R_{ST} estimates. Even with 10-loci data sets, however, the variance of R_{ST} estimates is 5–30 times higher than that of θ_{ST} estimates (Table 1). The averages of θ_{ST} estimates across replicates are almost identical regardless of how many loci (1, 5, 10, or 500) are combined, and negative estimates are unlikely even if only one locus is considered. In simulations of 12, 24, and 96 loci data sets, Balloux and Goudet (2002) observed that increasing the number of loci above 12 only slightly improves R_{ST} estimates, and provides almost no benefit to θ_{ST} estimates. Our analyses of 1, 5, and 10 loci show that, for θ_{ST}, the reduction in variance is greater when moving from 1 to 5 loci than from 5 to 10 loci, whereas a substantial reduction in the variance of R_{ST} is still achieved between 5 and 10 loci. Our simulations support the usual recommendation to use multiple microsatellite loci to average out the effects of stochasticity in lineage sorting, but

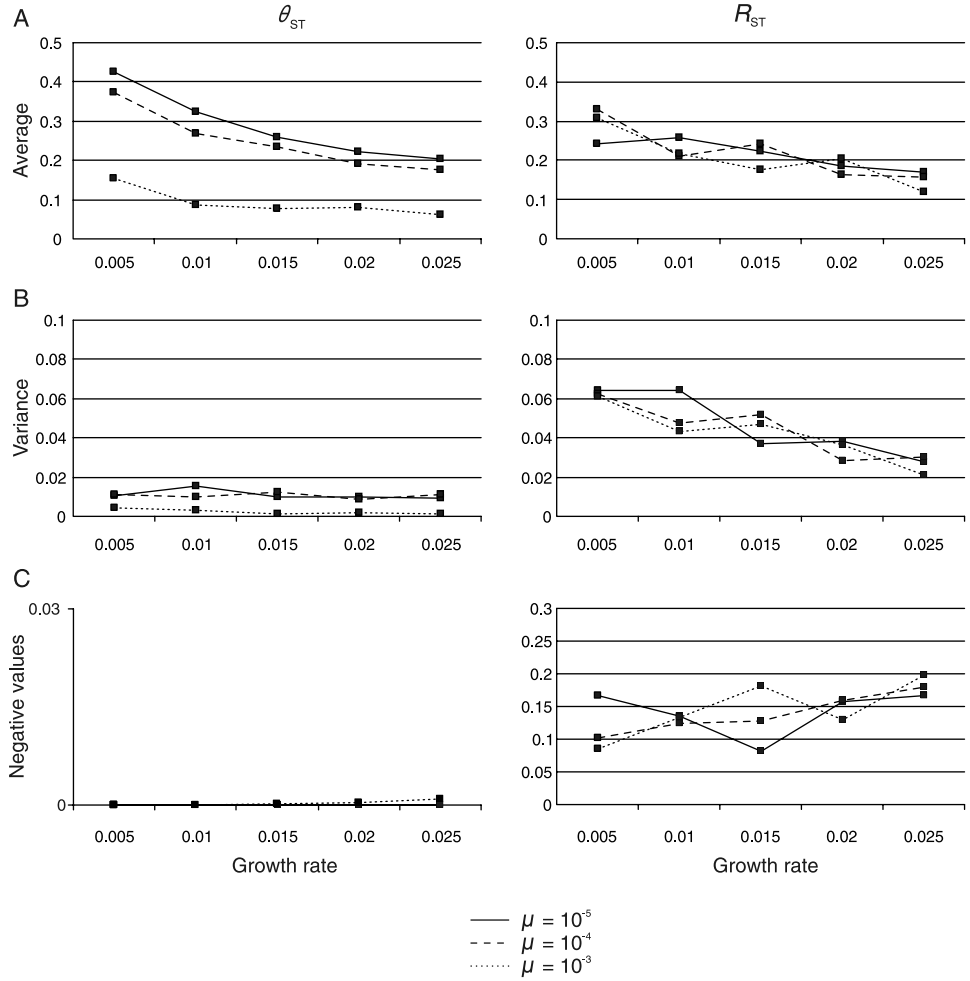

Fig. 3. Results of simulated microsatellite drift under different growth and mutation rates. $r = 0.005$, 0.01, 0.015, 0.02, 0.025; $\mu = 10^{-5}$, 10^{-4}, 10^{-3}; $N_0 = 100$; $K = 10{,}000$. (A) Average and (B) variance of θ_{ST} and R_{ST} estimates from 50 replicate runs at the simulation endpoint (1000 generations). (C) Proportion of negative θ_{ST} and R_{ST} estimates averaged across generations 200 through 1000 in 50 replicate runs. Note the 10-fold difference in scale of the y-axis between the graphs for θ_{ST} and R_{ST} in (C).

if data are limited to a few loci, θ_{ST} is more sensitive to the early stages of population differentiation.

The performance of θ_{ST} estimates under a high mutation rate increases with the severity of the population bottleneck, such that differentiation is most reliably detected by θ_{ST} when population growth in the new population is slow (Figs. 2, 3). Instead of steadily increasing over time, however, θ_{ST} values begin to decline as the novel population grows. This decline is associated with increasing heterozygosity as mutations accumulate in an expanding population. Increases in heterozygosity reduce the theoretical upper limit of θ_{ST} (Hedrick, 1999, 2005). The counterintuitive consequence of this effect is a decrease in differentiation

Table 1. Single-locus and multi-locus estimates of θ_{ST} and R_{ST}

		θ_{ST}				R_{ST}			
		500-loci ($n = 1$)	1-locus ($n = 500$)	5-loci ($n = 100$)	10-loci ($n = 50$)	500-loci ($n = 1$)	1-locus ($n = 500$)	5-loci ($n = 100$)	10-loci ($n = 50$)
Fig. 2A	average	0.319	0.318	0.319	0.319	0.378	0.281	0.353	0.363
	variance		0.0141	0.0026	0.0016		0.0588	0.0201	0.0129
	% neg.		0	0	0		10.055	0.025	0
Fig. 2B	average	0.187	0.186	0.187	0.187	0.228	0.179	0.213	0.219
	variance		0.0081	0.0017	0.0011		0.0349	0.0122	0.0068
	% neg.		0	0	0		15.345	0	0
Fig. 2C	average	0.117	0.116	0.117	0.117	0.361	0.266	0.332	0.347
	variance		0.0031	0.0006	0.0003		0.0578	0.0211	0.0111
	% neg.		0	0	0		10.086	0.025	0
Fig. 2D	average	0.067	0.066	0.067	0.067	0.207	0.169	0.196	0.202
	variance		0.0013	0.0003	0.0002		0.0303	0.0104	0.0053
	% neg.		0.045	0	0		15.040	0.425	0

Note: Average and variance of θ_{ST} and R_{ST} estimates from n replicate runs at the simulation endpoint (1000 generations); and proportion of negative θ_{ST} and R_{ST} estimates averaged across generations 200 through 1000 in n replicate runs (% neg.). Simulation conditions are as in Fig. 2: $N_0 = 100$, $K = 10{,}000$, $r = 0.0075$, $\mu = 10^{-4}$ (Fig. 2A); $N_0 = 100$, $K = 10{,}000$, $r = 0.02$, $\mu = 10^{-4}$ (Fig. 2B); $N_0 = 100$, $K = 10{,}000$, $r = 0.0075$, $\mu = 10^{-3}$ (Fig. 2C); $N_0 = 100$, $K = 10{,}000$, $r = 0.02$, $\mu = 10^{-3}$ (Fig. 2D).

Estimates obtained from the 500-loci data set differ from the averages across 500 single-locus estimates, because the ratio of the sum of variance components does not equal the sum of the ratios (Balloux and Goudet, 2002).

estimates with increasing time of separation, even though no migrants are being exchanged between the two populations (Fig. 2).

Our examination of empirical data (see above) suggested that incongruent θ_{ST} and R_{ST} estimates depend on a particular pattern of allele size distributions, one in which the allele size range of one population is nested within the size range of a second population. We observed a similar pattern of allele frequency distributions in those simulation runs with strongly divergent F_{ST} and R_{ST} estimates. In cases with negative R_{ST} values and moderate, significant θ_{ST} values, the alleles of the bottlenecked population were indeed a subset of those found in the source population, but the frequencies of the common alleles differed between populations (Fig. 1B). In contrast, R_{ST} estimates considerably exceeded corresponding θ_{ST} values when the bottlenecked population developed a partly non-overlapping allele size distribution (Fig. 1C).

Our simulations suggest that for analyses of microsatellite loci the frequency-based estimator θ_{ST} detects incipient population differentiation more reliably than R_{ST}, the larger variance of which outweighs the benefit of incorporating information about allele size differences accumulated over only a short divergence period. Our results are consistent with previous simulations of less complex demographic models. Large variance in R_{ST} has been reported in all studies comparing θ_{ST} and R_{ST} (e.g. Slatkin, 1995; Gaggiotti *et al.*, 1999; Balloux *et al.*, 2000), although R_{ST} provides a more accurate estimate of differentiation when between-population

coalescence times are large relative to within-population coalescence times – that is, when populations have been separated for a long time, gene flow has been low, and/or the mutation rate is high (Slatkin, 1995; Lugon-Moulin *et al.*, 1999). Estimates of R_{ST} are biased downwards, however, by small sample sizes and low numbers of scored loci (Gaggiotti *et al.*, 1999; Balloux *et al.*, 2000), small population sizes (Gaggiotti *et al.*, 1999), deviations from the stepwise mutation model (Balloux *et al.*, 2000), constraints on allele size, and allele size homoplasy (Gaggiotti *et al.*, 1999; Estoup *et al.*, 2002). Balloux and Goudet (2002) also report a striking effect of the number of populations sampled on the variance of single-locus θ_{ST} and R_{ST} estimates, and a downward bias of R_{ST} values when only two populations are compared. Although empirical studies typically encounter the above constraints, all of which bias R_{ST} downwards, R_{ST} values often exceed θ_{ST} values derived from the same data (e.g. Collevatti *et al.*, 2001; Feldheim *et al.*, 2001; Lemes *et al.*, 2003; Hoffmann *et al.*, 2005). Rather than indicating congruence with model assumptions, higher R_{ST} than θ_{ST} values perhaps most often reflect the limits to θ_{ST} values imposed by the high within-population heterozygosity typical of microsatellite loci (see above) (Charlesworth, 1998; Hedrick, 1999; Hoffmann *et al.*, 2005). In contrast, R_{ST} does not decline with increasing heterozygosity, because – constraints on allele size notwithstanding – allele size variance can increase indefinitely. The usual recommendation (Slatkin, 1995; Lugon-Moulin *et al.*, 1999) to employ θ_{ST} in recent population splits where differentiation is dominated by drift and R_{ST} when the split is sufficiently deep that mutations contribute to population differentiation is well-justified, but this approach does not eliminate biases affecting θ_{ST} and R_{ST} due to marker polymorphism and estimator variance, respectively. Careful examination of the underlying data is essential for a judicious interpretation of the numerical results.

Simulation of haplotype differentiation after a population founder event

As with analyses of microsatellite data, mitochondrial DNA sequences can be tested for population differentiation based on haplotype frequency differences (θ_{ST}) or by additionally integrating sequence divergence into an analysis of molecular variance (Φ_{ST}). In contrast to microsatellite loci, point mutations most often generate novel haplotypes, such that homoplasy among the closely related set of haplotypes within a species is typically low. Evolutionary distances are therefore more accurately estimated from sequence divergence than from microsatellite allele size differences, and incorporation of genetic distance (as a proxy for coalescence time) should be more straightforward. To compare a frequency-based analysis of mitochondrial data with an analysis of molecular variance (Φ_{ST}) in a scenario of recent differentiation dominated by drift, we extended our simulations of post-bottleneck population growth and differentiation under the finite sites model and a transition : transversion ratio of 10 : 1. Simulations of different mutation rates and bottleneck sizes revealed two main patterns (Fig. 4). With infrequent mutations ($\mu = 0$ and 10^{-5} per 1100 bp sequence), the 25%, 50%, and 75% medians of θ_{ST} and Φ_{ST} over a period of 1000 generations are similar (Fig. 4A) and the distributions of θ_{ST} and Φ_{ST} estimates do not differ significantly from each other ($P = 0.15$). In contrast, when the mutation rate and population size are sufficiently high to restore some variation to the bottlenecked population after the initial loss of heterozygosity ($\mu = 10^{-4}$ per 1100 bp sequence, growth at $r = 0.02$ from 25 to 10,000 gene copies), θ_{ST} estimates decrease in response to increased within-population polymorphism (Fig. 4B), and the distributions of θ_{ST} and Φ_{ST} estimates differ significantly from each other ($P < 0.005$). The variance of Φ_{ST} is larger than that of θ_{ST} (between generations 100 and 1000 with $\mu = 10^{-5}$ and 10^{-4}, respectively, average variance of

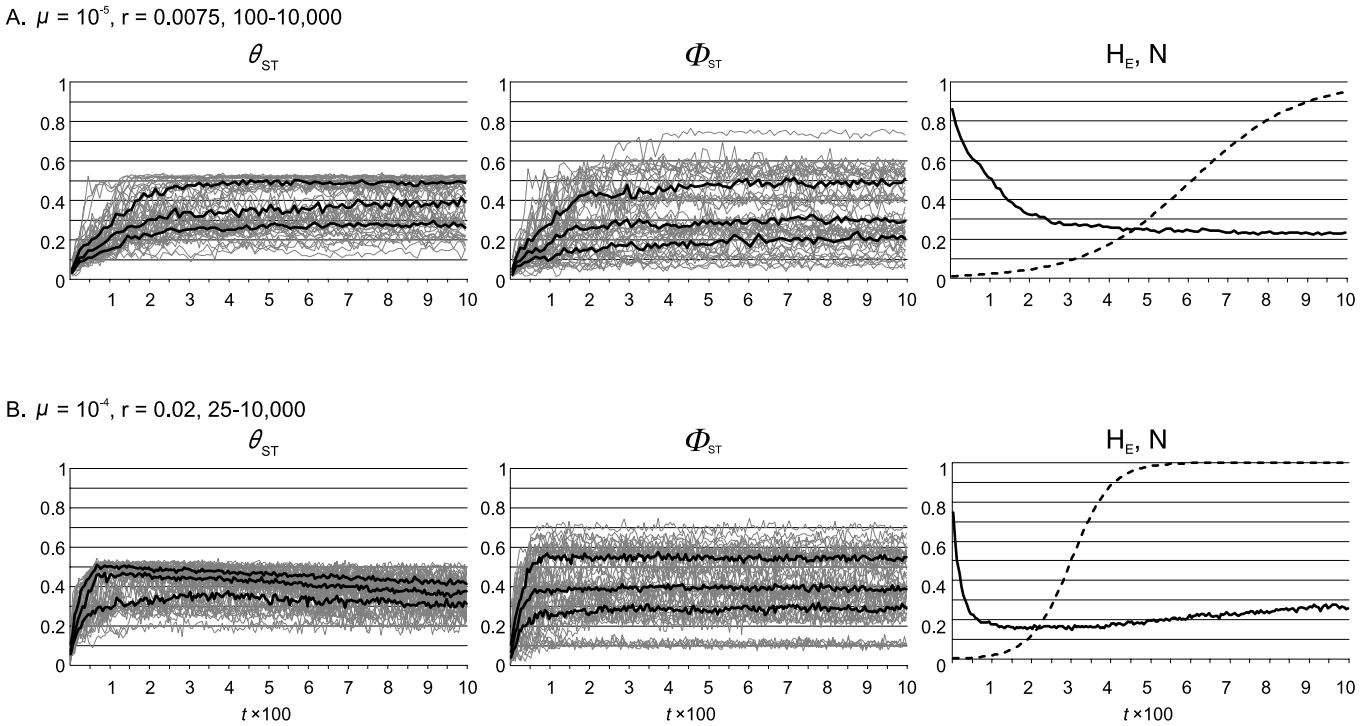

Fig. 4. Trajectories of θ_{ST} and Φ_{ST} estimates in simulations of mtDNA sequence data over 1000 generations after a population founder event, and plots of gene diversity (H_e, black lines) and population size (N, broken lines) in the novel, bottlenecked population. Grey lines for θ_{ST} and Φ_{ST} estimates represent results from 50 replicate runs; the median, 25%, and 75% quartiles are indicated by bold black lines. (A) $N_0 = 100$, $K = 10{,}000$, $\mu = 10^{-5}$, $r = 0.0075$. (B) $N_0 = 25$, $K = 10{,}000$, $\mu = 10^{-4}$, $r = 0.02$.

$\Phi_{ST} = 0.029$ and 0.027, whereas average variance of $\theta_{ST} = 0.014$ and 0.008; data shown in Fig. 4), but in contrast to the microsatellite R_{ST} statistic, Φ_{ST} values in the absence of gene flow exceed zero in all replicates. Under the conditions considered in this study, the incorporation of haplotype divergence in the analysis of DNA sequence variation did not reduce the power to detect population differentiation after recent isolation. Moreover, as population heterozygosity imposes no limits on Φ_{ST} values, Φ_{ST} may reflect reproductive isolation and differentiation more accurately than the corresponding θ_{ST} estimates. Whereas R_{ST} is less reliable than θ_{ST} in detecting incipient differentiation by drift from microsatellite data, there seems to be no compelling reason to favour θ_{ST} over Φ_{ST} for the analysis of DNA sequence data, even over time spans too short for new mutations to contribute to the differentiation of populations.

Influence of gene diversity on F_{ST} estimates

Negative correlations between the level of polymorphism and F_{ST} estimates have previously been reported in studies employing microsatellite markers (e.g. Olsen *et al.*, 2004; O'Reilly *et al.*, 2004; Hoffmann *et al.*, 2005). The above simulations indicate that the bias introduced by high levels of genetic diversity is not limited to microsatellite loci, but can also lead to difficulties in comparing pair-wise mitochondrial differentiation estimates. Our mitochondrial data set for indigobirds revealed cases where θ_{ST} estimates were low between highly variable populations with few shared haplotypes. As previously reported (Sorenson *et al.*, 2003; Sefc *et al.*, 2005), mitochondrial haplotypes of indigobirds from Nigeria and Cameroon fall into two divergent clades. Individuals of all species and from almost all locations share a set of closely related haplotypes, whereas *V. chalybeata* samples from Garoua, Cameroon, fall within a distinct cluster approximately ten base substitutions divergent from the main clade (Fig. 5). The genetic distance between these clades leads to high Φ_{ST} estimates (0.6–0.8) for pair-wise population comparisons between the Garoua *chalybeata* population and other *Vidua* species, whereas the corresponding θ_{ST} estimates were low (mostly < 0.1; Table 2). Due to the effect of heterozygosity, θ_{ST} estimates for populations with shared haplotypes but low haplotypic diversity can be higher than those for highly polymorphic populations with non-overlapping sets of haplotypes. For example, haplotypic diversity was comparatively low in two *V. nigeriae* populations ($H_e = 0.533$ and 0.722), and although each share a portion of their haplotypes with a population of *V. raricola* ($H_e = 0.904$), the θ_{ST} values between the two *V. nigeriae* populations and *V. raricola* are relatively high (0.151 and 0.102, respectively; $P < 0.005$). The corresponding Φ_{ST} estimates for these population pairs were 0.204 and 0.167, respectively, consistent with some genetic differentiation but also incomplete lineage sorting and/or gene flow as indicated by the haplotype genealogy (Fig. 5) (see Sorenson *et al.*, 2003).

In the above examples, θ_{ST} estimates, taken at face value, implied greater differentiation between populations sharing haplotypes than between populations that lack common haplotypes, whereas Φ_{ST} reflected the relative levels of differentiation among populations with varying degrees of haplotypic diversity more accurately (as compared to inferences based on the haplotype genealogy). Recently, Hedrick (2005) suggested that differentiation estimates be standardized by expressing the observed values in relation to their theoretical maxima as determined by the level of genetic variation. The standardized differentiation measure G'_{ST} is defined as the ratio of G_{ST} (Nei, 1987), calculated as $(H_T - H_S)/H_T$, and the maximum value $G_{ST(max)}$, given by $(1 - H_S)/(1 + H_S)$ for two equal-sized populations. The

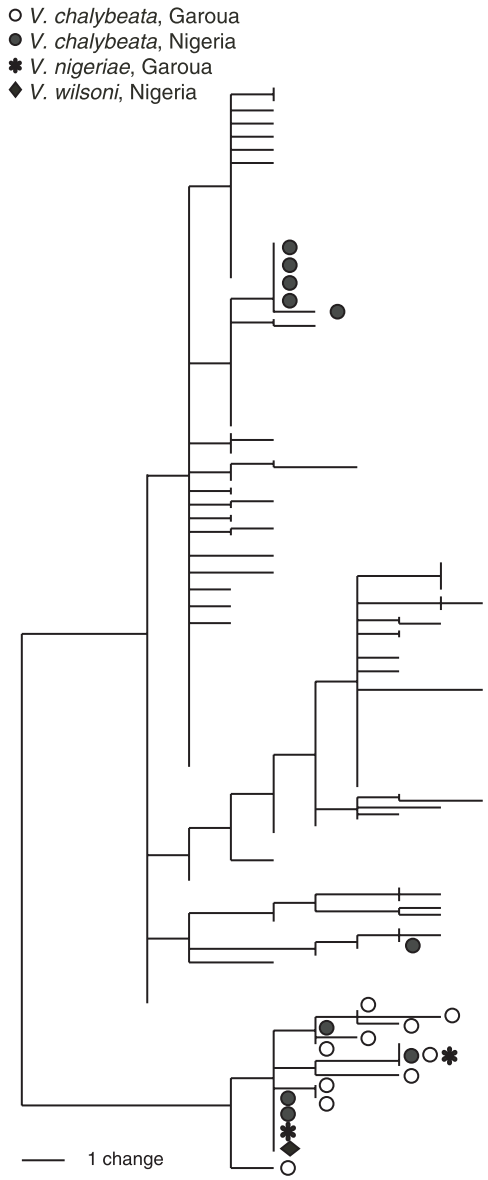

Fig. 5. Phylogenetic relationships of mitochondrial haplotypes found in *Vidua chalybeata* and other West African indigobird species. One of several most parsimonious trees is shown. Open and solid circles mark *V. chalybeata* haplotypes from Garoua and Nigeria, respectively. Three haplotypes of *V. nigeriae* and *V. wilsoni* that fall in the separate clade are also identified, but population labels have been removed from the main clade. The tree includes a total of 183 individuals.

theoretical maxima of differentiation estimates such as θ_{ST} correspond to θ_{ST} values obtained from populations with the observed levels of within-population heterozygosity, but having non-overlapping sets of alleles or haplotypes. Standardizing θ_{ST} estimates by their theoretical maxima yields θ'_{ST} estimates that are consistent with the observed levels

Table 2. Differentiation estimates in relation to haplotype diversity

Population comparison	% *PS*	θ_{ST}	(*P*)	Φ_{ST}	(*P*)	H_S	$\theta_{ST(max)}$	$\theta_{ST}/\theta_{ST(max)}$
V. raricola								
– *V. nigeriae*, Garoua	24.3	0.102	(0.0048)	0.167	(0.0018)	0.813	0.187	0.545
– *V. nigeriae*, Nigeria	14.3	0.151	(0.0002)	0.204	(0.0004)	0.718	0.226	0.668
V. chalybeata, Garoua								
– *V. chalybeata*, Nigeria	10.0	0.068	(0.0682)	0.363	(0.0062)	0.911	0.078	0.872
– *V. camerunensis*-1	0	0.038	(0.0274)	0.725	(<0.0001)	0.969	0.038	1
– *V. larvaticola*	0	0.041	(0.0456)	0.715	(<0.0001)	0.960	0.041	1
– *V. raricola*	0	0.055	(0.0125)	0.782	(<0.0001)	0.941	0.055	1
– *V. camerunensis*-2	0	0.098	(0.0113)	0.772	(<0.0001)	0.894	0.098	1

Note: θ_{ST} and Φ_{ST} values with corresponding *P*-values, the theoretical upper limit of θ_{ST} ($\theta_{ST(max)}$), and θ_{ST} as a proportion of $\theta_{ST(max)}$ are presented for selected population pairs with overlapping and non-overlapping haplotype distributions. Due to different levels of haplotype diversity between populations, the θ_{ST} estimates are not correlated with the proportion of shared haplotypes [% *PS* (Bowcock *et al.*, 1994)]. In contrast, values of Φ_{ST}, $\theta_{ST(max)}$, and $\theta_{ST}/\theta_{ST(max)}$ reflect haplotype sharing and reciprocal monophyly between populations. The populations included in this table are described in detail in Sefc *et al.* (2005). *V. camerunensis*-1 and *V. camerunensis*-2 refer to song mimics of *Lagonosticta rubricata* and *L. rara*, respectively (see Payne *et al.*, 2005; Balakrishnan and Sorenson, 2006).

of haplotype sharing (Table 2). Uncorrected θ_{ST} estimates between pairs of populations sharing 24, 14, or 10% of their haplotypes were 0.102, 0.151, and 0.068, respectively, whereas pairs of populations without shared haplotypes had values ranging from 0.038 to 0.098. In contrast, the standardized measure θ'_{ST} increased with decreasing haplotype sharing (Table 2).

We also applied the standardized differentiation measure to the simulated microsatellite data shown in Fig. 2C ($\mu = 10^{-3}$, $r = 0.0075$), which showed a distinct decrease in θ_{ST} values with time as the bottlenecked population regained variability after approximately 150 generations (Fig. 6A). Increasing H_S caused a decline in $\theta_{ST(max)}$. However, θ_{ST} values dropped at a faster rate than their theoretical maxima, such that θ'_{ST} also assumed a negative slope after the initial bottleneck period because the difference between $\theta_{ST(max)}$ and θ_{ST} increased (Fig. 6A). The convergence of allele frequencies in the two drifting populations, suggested by the decline in θ'_{ST} and the increase in proportions of shared alleles, is likely caused by the high degree of homoplasy inherent in the single-step mutation model (Estoup *et al.*, 2002).

Eliminating the effects of homoplasy by simulating an infinite sites model but with the same parameters as above led to θ_{ST} values that slowly approached their theoretical maxima as drift progressed and allele sharing proportions decreased (Fig. 6B). Nevertheless, the ratio of θ_{ST} and $\theta_{ST(max)}$ ($= \theta'_{ST}$) declined slightly after both $\theta_{ST(max)}$ and θ_{ST} had dropped below 0.2, although the absolute difference between θ_{ST} and $\theta_{ST(max)}$ declined as well and θ_{ST} moved towards $\theta_{ST(max)}$. Despite this shortcoming in reflecting the increasing genetic differences between populations with time, θ'_{ST} provides a more robust measure of population differentiation than θ_{ST} when genetic variation within populations is high.

As found by Kalinowski (2002) in simulations of two diverging populations of identical constant size, θ_{ST} values did not differ in our simulations of the SSM and infinite sites model (Fig. 6). This does not necessarily imply that θ_{ST} is unaffected by the mutational

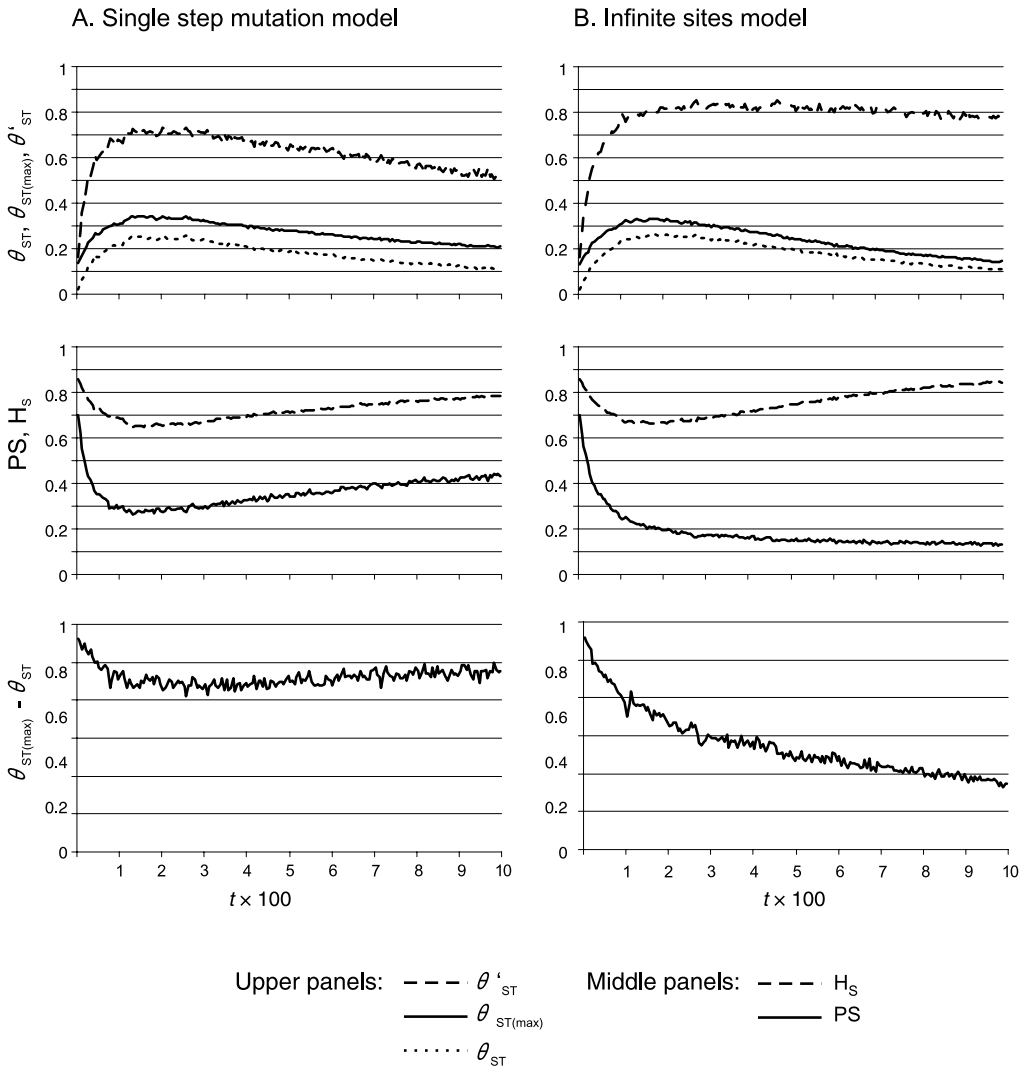

Fig. 6. Trajectories of the uncorrected and standardized differentiation estimates θ_{ST} and θ'_{ST} over 1000 generations after a population founder event under the SSM model (A) and infinite sites model (B). The upper panels compare θ_{ST} with its theoretical upper limit ($\theta_{ST(max)}$) and the standardized measure of differentiation θ'_{ST} ($\theta_{ST}/\theta_{ST(max)}$). In the middle panels, the proportion of shared alleles between populations (PS) and the average within-population heterozygosity (H_S) are shown. The lower panels illustrate the trajectories of the difference between θ_{ST} and θ'_{ST} ($\theta'_{ST} - \theta_{ST}$). The lines represent means of 50 replicate runs. $N_0 = 100$, $K = 10{,}000$, $\mu = 10^{-3}$, $r = 0.0075$.

model, but rather that different effects of the two models produce similar end results. Under the SSM, size homoplasy restricts the increase in population heterozygosity, raising the upper boundary of differentiation estimates in comparison to the infinite sites model, but size homoplasy also reduces allelic differences between populations. In contrast, allele-sharing between populations declines more quickly under the infinite sites model, but θ_{ST} values are biased downward by higher population heterozygosity.

CONCLUSIONS

Based on empirical observations and simulations, our study extends existing tests of the performance of two alternative estimates of differentiation, R_{ST} and θ_{ST}, by considering a model of divergence by founder event and subsequent population growth. This demographic model is relevant not only to indigobirds but also to speciation in peripheral isolates or following dispersal to a new area. In addition, we present comparisons between measures of mitochondrial differentiation based on haplotype frequencies (θ_{ST} estimates) and pair-wise sequence differences (Φ_{ST}) under the same demographic scenario. Finally, we show that variance in haplotype diversity among populations influences mitochondrial θ_{ST} estimates in much the same way as previously described for analyses of microsatellite markers.

At least for the demographic scenario analysed here, θ_{ST} was more sensitive to incipient population differentiation than R_{ST} in the analysis of microsatellite data, whereas Φ_{ST} appears preferable to θ_{ST} for mtDNA sequence data. Careful scrutiny of empirical data and computer simulations of population differentiation under various parameters support this conclusion. A serious drawback of θ_{ST} is its negative correlation with polymorphism, which affects the analysis of both microsatellite and haplotype data and complicates the quantitative comparison or interpretation of differentiation estimates among samples with varying levels of polymorphism. R_{ST} is unaffected by the level of polymorphism, but failed to detect newly established reproductive isolation in a considerable proportion of simulations because of its large variance. In contrast, simulations of haplotype evolution showed that estimates of population structure based on sequence differences (Φ_{ST}) reflected the progressive increase of genetic differences between two isolated populations, and were not reduced by increasing polymorphism. The variance of Φ_{ST} was higher than that of θ_{ST}, but less than that of R_{ST}, and even the lowest Φ_{ST} values at the end of the simulated period were significantly different from zero. Therefore, estimating differentiation with Φ_{ST} from mitochondrial sequence data proved the most successful approach to detecting population isolation in the present study. These contrasting results for microsatellite and sequence data are ultimately the result of different mutation mechanisms and in turn different levels of homoplasy for these different kinds of genetic markers. When variation in polymorphism among populations confounds comparisons of pair-wise θ_{ST} values, the standardization of differentiation estimates relative to their upper limits as determined by population heterozygosity appears highly useful, although this approach does not correct for the additional reduction of θ_{ST} by allele size homoplasy.

Finally, our analyses indicate that low, and at times non-significant, estimates of genetic differentiation among indigobird species are potentially consistent with reproductive isolation under a model of recent speciation by host shift. It remains to be tested whether more recently developed coalescent methods (e.g. Nielsen and Wakeley, 2001; Hey and Nielsen, 2004) will allow more robust inferences in examples of recent population divergence.

ACKNOWLEDGEMENTS

We thank Phil Hedrick for advice on calculations of the standardized differentiation estimates, and François Balloux and two anonymous reviewers for valuable suggestions on the analyses and the manuscript. Field work and the collection of empirical data was made possible by permits provided by the governments of Cameroon, Malawi, Nigeria, Zambia, and Zimbabwe; and support from the Institute of Agricultural Research for Development and the Ministry of the Environment and Forests,

Cameroon; the University of Jos, Nigeria; the National Museums of Malawi; the Department of National Parks and Wildlife, Zambia; and the National Museum of Zimbabwe. For help in the field we thank Chris Balakrishnan, Clive Barlow, Bob Dowsett, Mark Hopkins, Kit Hustler, Nedra Klein, Kevin Njabo, Martin Nhlane, Laura Payne, and Bob Stjernstedt. Janet Hinshaw helped organize the genetic materials. Funding was provided by US National Science Foundation grants to R.B.P. and M.D.S., the Austrian Science Fund (Erwin Schrödinger Fellowship and P17380-B06 awarded to K.M.S.), Boston University, and the University of Michigan Museum of Zoology. Support of the Dr. Heinrich-Jörg-Stiftung, Karl-Franzens-Universität Graz, Austria, is also acknowledged.

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