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Limited differentiation in microsatellite DNA variation among northern populations of the yellow warbler: evidence for male-biased gene flow?

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Abstract

Comparisons of the patterns of differentiation among genetic markers with different modes of inheritance can provide insights into patterns of sex-biased dispersal and gene flow. Here, we compare the patterns of differentiation in six microsatellite loci among eight northern breeding populations of the yellow warbler (*Dendroica petechia*) with results obtained with mitochondrial DNA. Significant but low levels of differentiation (overall F_{ST} = 0.014; overall R_{ST} = 0.015) were present across all populations. The level of differentiation is substantially less than that observed in the same samples based on mitochondrial DNA control region variation. The presence of low population imbalance index values and significant isolation-by-distance relationships for both F_{ST} and R_{ST} suggests that these populations are at evolutionary equilibrium and that the high degree of similarity between populations may be due to high levels of male-biased gene flow. This suggests that there may be significant but previously unappreciated differences in the long-distance and/or episodic dispersal behaviour of males and females in these birds.

Keywords: *Dendroica petechia*, male-biased gene flow, microsatellite DNA markers, population structure, yellow warblers

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Introduction

Analysis of population structure using multiple genetic markers can identify the contemporary processes that shape patterns of intra-specific variation (Avise 1994). For example, comparison of markers with different modes of inheritance, especially those that are sex-specific (e.g. female-specific mitochondrial DNA (mtDNA) and male-specific Y chromosome variation in humans; Seielstad *et al*. 1998) can be used to provide insights into patterns of sex-biased dispersal and gene flow. This approach has been most effectively used in vertebrate species in which females are philopatric and males disperse. In such animals, female-specific mtDNA variation should show higher levels of genetic structure than bi-parentally inherited autosomal markers, and this pattern has been repeatedly observed (Melnick & Hoelzer 1992; FitzSimmons *et al*. 1997; Ishibashi *et al*. 1997; Patton *et al*. 1997). In contrast, for birds such as red grouse (*Lagopus lagopus*) where behavioural studies indicate female-biased dispersal (e.g. Greenwood & Harvey 1982; Clarke *et al*. 1997), less differentiation has been observed for mtDNA compared to autosomal microsatellite markers even though female-biased gene flow will tend to homogenize both autosomal and mitochondrial variation simultaneously (Piertney *et al*. 1998, 2000). Thus, patterns of differentiation in either type of marker have been found which are consistent with those predicted from different sex-specific patterns of dispersal for a given species.

In North American birds, researchers have used data from a variety of molecular markers to examine structure and test evolutionary models for differentiation (reviewed in Zink 1997). Early results using nuclear markers such as allozymes suggested that, in general, the amount of variation distributed among populations was low (Barrowclough 1983). However, recent results using more variable mtDNA markers have revised this perspective:

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for example, in Zink's (1997) review, about 40% of the 79 species for which mtDNA analyses had been performed showed some evidence for significant genetic structure. This suggests that, contrary to the inferences based on allozymes, many species have experienced restricted gene flow at some point in their history, although the timing and the mechanisms responsible for the observed structure remain controversial (see Klicka & Zink 1997, 1999; Zink 1997; Avise & Walker 1998).

One explanation for the increased genetic structure observed using mtDNA is that the higher mutation rate of mtDNA makes it more suitable for detecting recent isolation between populations (Zink 1997). However, another possibility is that, contrary to behavioural evidence for female-biased dispersal in most birds, especially passerines (Clarke *et al*. 1997), increased structure in mtDNA could indicate previously undetected male-biased gene flow over long distances (see Melnick & Hoelzer 1992). To discriminate between these possibilities, it is necessary to assess structure among broadly distributed populations using nuclear DNA markers with mutation rates that are comparable to those of mtDNA. Under the conventional hypothesis that females are the dispersing sex, these more sensitive bi-parentally inherited markers should show higher levels of structure after genetic differences between marker systems in effective population size and mutation rates are accounted for (e.g. Seielstad *et al*. 1998). In contrast, less structure in rapidly evolving nuclear markers would force consideration of the possibility that male-biased gene flow may occur more frequently than previously suspected.

Microsatellite DNA loci are especially suitable for such additional analyses (for recent review, see Scribner & Pearce 2000). These hypervariable nuclear DNA markers assay variation in the number of repeats, 1–6 bp in size, at a particular locus (Queller *et al*. 1993), and are being increasingly used to assess population structure in a wide variety of organisms including birds (Gibbs *et al*. 1997; Dawson *et al*. 1997; Munday *et al*. 1997; Smith *et al*. 1997; Goostry *et al*. 1998; Piertney *et al*. 1998; McDonald *et al*. 1999; Gibbs *et al*. 2000). However, to date, no studies have used these markers to evaluate structure in a continentally distributed bird in North America.

Recently, Milot *et al*. (2000) showed that analysis of mtDNA control region variation identifies a major subdivision between eastern (Manitoba to Newfoundland) and western (Alaska and British Columbia) breeding populations of the yellow warbler (*Dendroica petechia*). Some localities within these two regions also differed significantly in their genetic composition, suggesting further subdivision on a local geographical scale. These results provided intra-specific support for a model previously developed to account for warbler speciation in North America which argued for a vicariant origin, possibly during the recent Pleistocene, of these two warbler groups (Mengel 1964). In this study, we re-assessed the pattern of variation among these populations, plus one additional population from Alberta, using six microsatellite DNA loci. Our goals were: (1) to provide the first assessment of the levels and patterns of differentiation in microsatellite loci for a North American passerine bird on a continental scale and to compare these results with those obtained from mtDNA control region variation, and (2) to use these comparisons to make inferences about the role of male-biased gene flow in moulding patterns of intra-specific variation in this passerine bird.

Methods

Sample collection

During the 1994, 1995 and 1996 breeding seasons, we caught 240 yellow warblers from eight populations throughout Canada, and in Alaska (Table 1). Abbreviations used to identify each population are given in Table 1. These populations represent a roughly linear East–West transect through the northern breeding distribution of this species in North America (Sauer *et al*. 1997) (Fig. 1). The breeding status of individuals was confirmed by the observation of territorial behaviour (males) or the presence of a brood patch (females). Blood samples were collected from the brachial vein of the birds and stored in lysis buffer; isolation of total genomic DNA was performed by phenol/chloroform extraction following standard procedures (e.g. Dawson *et al*. 1997).

Microsatellite variation

To assay microsatellite variation, we genotyped all individuals with six microsatellite loci (see Appendix I). Four of these loci (Dpμ 01, 03, 15 and 15) were isolated from a yellow warbler genomic DNA library; primer sequences to amplify these loci are described in Dawson

Table 1 Locations, abbreviations and sample sizes for yellow warbler populations used in this study

Locality (abbreviation)	Latitude, longitude	Sample size
Fairbanks, Alaska (AK)	65° N, 148° W	20
Revelstoke, British Colombia (BC)	51° N, 118° W	33
Lac La Biche, Alberta (AB)	55° N, 112° W	22
Delta Marsh, Manitoba (MB)	50° N, 98° W	34
Chaffeys Lock, Ontario (ON)	45° N, 77° W	29
Trois-Rivieres, Québec (QU)	47° N, 73° W	32
Germantown, New Brunswick (NB)	46° N, 65° W	30
Gros Morne, Newfoundland (NF)	50° N, 58° W	40

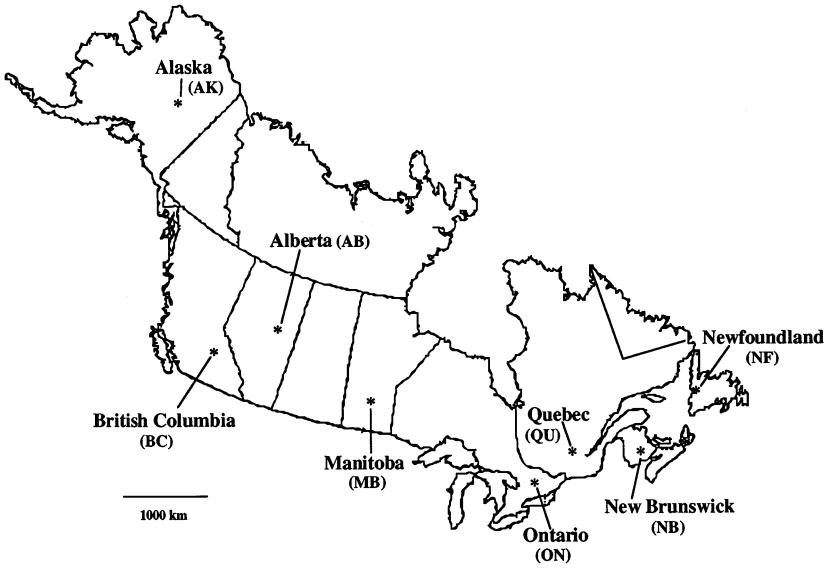

Fig. 1 Sampling sites of the yellow warbler in Canada and Alaska. Additional details about each location and sample sizes are given in Table 1.

et al. (1997). The other two loci (Caμ 28 and Maμ 23) were isolated from a Swainson's thrush (*Catharus ustulatus*) and a brown-headed cowbird (*Molothrus ater*) library, respectively, and primer sequences for these loci are provided in Gibbs *et al*. (1999) and Alderson *et al*. (1999), respectively.

Details of the techniques used for genotyping individuals are given in Dawson *et al*. (1997) and Alderson *et al*. (1999). Briefly, primers for each locus, one of which was end-labelled with ^{33}P, were used to amplify sample DNA using the polymerase chain reaction (PCR) at an optimized annealing temperature. Annealing temperatures for the four yellow warbler loci are given in Dawson *et al*. (1997). The experimentally determined annealing temperature for both Caμ 28 and Maμ 23 was 50 °C. PCR products (3.5 μL) were resolved by electrophoresis on 6% denaturing polyacrylamide gels at 55 W for approximately 2.5 h. Gels were then dried and exposed to X-ray film overnight. Product sizes were determined within and among gels by reference to a sequencing reaction of a known control template and/or a clone of known size for each locus.

Analysis of variation

We estimated the levels of non-random association of alleles within warbler populations by calculating locus-specific and overall values for the inbreeding coefficient (F_{IS}) as described by Weir & Cockerham (1984) using FSTAT (Goudet 1995). We also used the permutation procedure in FSTAT to test whether a particular F_{IS} value was significantly different from zero.

We quantified differentiation among populations by calculating two kinds of fixation indices (F_{ST} and R_{ST}). F_{ST} values were calculated assuming Kimura & Crow's (1964) infinite alleles model (IAM) of mutation. However, microsatellites are believed to evolve in a stepwise fashion via polymerase slippage and the gain or loss of one or a few repeat units. Thus, statistics that assume Ohta & Kimura's (1973) stepwise mutation model (SMM) may be more appropriate for microsatellites. R_{ST} is an analogue of F_{ST} developed by Slatkin (1995) that takes into account variance in allele sizes as in the SMM. Therefore, we present sub-population fixation indices based on both the IAM (F_{ST}; estimated as described by Weir & Cockerham (1984)) and the SMM (R_{ST}; estimated as described by Slatkin (1995)). We used FSTAT to calculate F_{ST} values and RSTCALC (Goodman 1997) to calculate R_{ST} values, and permutation procedures in both programs to test whether particular values were significantly different from zero. Table-wide critical values were adjusted using a sequential Bonferroni correction (Rice 1989) to reduce type I statistical errors. Finally, to directly compare the levels of differentiation in microsatellite versus mtDNA variation (e.g. Gibbs *et al*. 2000), we used AMOVA (Excoffier *et al*. 1992) as implemented in the program Arlequin (Schneider *et al*. 1997) to estimate the overall percentage variation apportioned within and between populations for each type of marker when genetic differences between alleles or haplotypes were taken into account.

A hierarchical analysis of differentiation (Holsinger & Mason-Gamer 1996) was performed to assess patterns of geographical differentiation between populations. This method uses pairwise estimates of differentiation (e.g. in our case, F_{ST} or R_{ST} values) between different combinations of populations to construct a dendrogram, which identifies patterns of hierarchical population structure in the data.

The two localities showing the smallest F_{ST} or R_{ST} value (the least genetic divergence) are grouped together, and pairwise fixation indices are then re-computed between the remaining localities and the new cluster. The analysis proceeds until all locations have been added, and a tree of the relationships among them is constructed. The statistical significance of F_{ST} or R_{ST} values at each node is then tested by creating a null distribution of values generated by random re-sampling and comparing the observed value with this null distribution. This approach allows any patterns to emerge naturally from the data rather than having *a priori* hypotheses about structure imposed prior to the analyses. The topology of the tree can then be interpreted as reflecting patterns of gene flow between sets of populations (Holsinger & Mason-Gamer 1996). Our method differs slightly from the original approach in that the measures of differentiation that we used were adjusted for differences in sample size between populations compared (single and/or pooled).

To assess the demographic history of our sampled populations, we estimated the imbalance index (β) derived by Kimmel *et al*. (1998) for each population. This index is calculated as $\beta = \Theta_V/\Theta_{P_0}$, where Θ_V is twice the observed variance in allele size for a given locus and Θ_{P_0} is estimated as $((1/P_0^2) - 1)/2$ with P_0 estimated as the expected homozygosity for the same locus. Populations which have been stable for long periods of time have β values that are close to 1.0, while populations not in demographic equilibrium have β values either greater or less than 1.0. Specifically, populations that have experienced a bottleneck and recently expanded are predicted to have β values >1.0 because the loss of individual alleles due to increased drift during bottlenecks will result in a disproportionately greater decline in heterozygosity as compared to variance in allele size. In contrast, populations that have expanded from non-bottlenecked populations will show β values <1.0 because, given a relaxation of the effects of drift, heterozygosity will increase more rapidly than variance in allele sizes. To obtain a mean estimate ± SE of β for each population, we used locus-specific values of Θ_V and Θ_{P_0} to calculate β for each locus and then averaged these values across loci.

Results

Levels of variability

The genetic characteristics of the six microsatellite loci in the eight populations of yellow warblers are described in Appendix I. These loci detect between 3 and 23 alleles per locus among the 20–40 individuals surveyed per population. The mean heterozygosity averaged across populations ranged from 0.38 (Dpμ 03) to 0.92 (Dpμ 01) with an overall mean heterozygosity of 0.69. Thus, these loci exhibit high levels of variation at the intra-specific level and should be suitable for analyses of population differentiation.

Table 2 F_{IS} values for each locus–population combination

	Locus					
Population	Caμ 28	Dpμ 01	Dpμ 03	Dpμ 15	Dpμ 16	Maμ 23
AK	0.392	0.080	0.097	0.137	−0.154	0.156
BC	0.430	0.034	0.048	0.082	−0.011	0.010
AB	0.295	−0.003	0.364	0.091	−0.085	0.054
MB	0.196	0.027	−0.097	0.256	0.077	−0.67
ON	0.241	−0.001	−0.107	0.069	0.127	0.045
QU	0.141	0.080	−0.107	0.220	0.015	0.119
NB	0.262	0.022	0.403	0.078	−0.106	−0.083
NF	0.476	−0.104	−0.052	0.018	0.052	−0.072
Overall	0.302*	0.013	0.043	0.120*	0.002	0.020

Overall F_{is} values were tested using the permutation procedures in FSTAT. The asterisks indicate that the overall value is significantly ($P < 0.05$) different from zero.

We calculated inbreeding coefficient values (F_{IS}) for each locus by population combination as a way of assessing the possible presence of null or non-amplifying alleles within populations (Table 2) (see Brookfield 1996 and discussion in Gibbs *et al*. 1998). Most F_{IS} values for four of the loci (Dpμ 01, 03, 16 and Maμ 23) were low and not consistently positive across populations, indicating little evidence for null alleles. However, the overall values for two loci (Caμ 28 and Dpμ 15) were moderately high and positive (0.302 and 0.120); both are significantly different from zero. If we assume that the heterozygote deficiency observed in each population is entirely due to the presence of null alleles, the mean frequency of these alleles per population may be estimated as ranging from 0.13–0.18 for Caμ 28 and 0.05–0.07 for Dpμ 15, based on the formulae presented in Brookfield (1996). Thus, null allele(s) may form a significant component of the alleles present at Caμ 28 in different populations. This result is confirmed by inconsistent patterns of segregation at this locus between parents and offspring within the genetically verified families of yellow warblers studied by Yezerinac *et al*. (1999) (H.L. Gibbs, unpublished data). The effect of null alleles on measures of differentiation is unclear at present. However, because they increase the estimated levels of homozygosity within populations through the Wahlund effect, null alleles may act to inflate measures of differentiation based on fixation indices to an unknown extent (P. Smouse, personal communication).

Patterns of differentiation

Fixation indices indicate significant but limited differentiation among populations. Overall locus-specific F_{ST} values

Table 3 Locus-specific and overall F_{ST} and R_{ST} values for yellow warbler populations based on microsatellite DNA variation

Locus	F_{ST}	R_{ST}
Caμ 28	0.0211*	0.00109
Dpμ 01	0.0087*	−0.00813
Dpμ 03	0.0142*	0.05720
Dpμ 15	0.0091*	0.02465
Dpμ 16	0.0162*	0.00139
Maμ 23	0.0183*	0.01554
Overall	0.0144*	0.01529*

*$P < 0.03$.

were all positive and ranged in magnitude from 0.0211 (Caμ 28) to 0.0087 (Dpμ 01) (Table 3). The overall F_{ST} value (0.0144) indicates that 1.4% of the overall variation was apportioned between populations. R_{ST} values are similar. Locus-specific values range from essentially zero (−0.00813 for Dpμ 01) to 0.02465 (Dpμ 15), with an overall value of 0.0153 (Table 3). Thus, while the majority of variation at these loci is shared among populations, both fixation indices demonstrate that a small but significant amount of differentiation between populations is present.

Pairwise comparisons of F_{ST} and R_{ST} values between populations (Table 3) suggest an isolation-by-distance pattern of differentiation (see Slatkin 1993), as a disproportionately large number of significant F_{ST} (7/12) values involved comparisons between the Alaska or Newfoundland and other populations. Of special note are the relatively high F_{ST} values for comparisons of Newfoundland with other nearby populations (e.g. Québec) (see below). Although none of the pairwise R_{ST} values remained significant after adjusting significance levels, a pattern of the extreme populations generally having higher pairwise R_{ST} values was present (Table 4).

To determine whether isolation-by-distance patterns exist, we tested for a significant relationship between F_{ST} or R_{ST} and the linear distance between all pairs of populations using a Mantel test (ISO subroutine in GENEPOP; Raymond & Rousset 1995). For both F_{ST} ($P = 0.013$) (Fig. 2a) and R_{ST} ($P = 0.002$) (Fig. 2b), a significant positive correlation exists between distance and the magnitude of differentiation between populations. A straight line provides the best fit to the data (F_{ST}: $r^2 = 0.29$; R_{ST}: $r^2 = 0.35$) for both measures of differentiation as compared to all other simple linear relationships [evaluated using the simple linear curves subroutine in Table Curve 2-D (Jandel)]. The presence of significant isolation-by-distance patterns for both F_{ST} and R_{ST} argues that these populations are in equilibrium with respect to levels of gene flow between populations and drift within populations, and that the balance between these processes can account for observed patterns of differentiation (Slatkin 1993; Hutchison & Templeton 1999).

Hierarchical analysis of differentiation

The hierarchical analysis of differentiation (Fig. 3) suggests that populations fall into genetically distinct but weakly differentiated groups, although the composition of these groups depends on the measure of differentiation used. When F_{ST} is used (Fig. 3a), there is significant divergence between a 'western' cluster consisting of populations from British Columbia to Ontario and an 'eastern' cluster consisting of Québec and New Brunswick, while Newfoundland is distinct from both these sets of populations. Roughly similar results were obtained with R_{ST} (Fig. 3b); again there are 'western' (Alaska, British Columbia, Alberta and Manitoba) and 'eastern' (Ontario, Québec, New Brunswick and Newfoundland) sets of populations, while, within the 'eastern' set, groups made up of Ontario/Québec and New Brunswick/Newfoundland are distinct from each other. Contrary to the results for mtDNA (Milot *et al*. 2000), this analysis highlights the distinctiveness of the Maritime populations of these birds, particularly those found in Newfoundland.

Table 4 Pairwise F_{ST} (above diagonal) and R_{ST} (below diagonal) values for yellow warbler populations

	AK	BC	AB	MB	ON	QU	NB	NF
AK	—	0.0060	0.0024	0.0068	0.0045	0.0291*	0.0244*	0.0305*
BC	−0.0116	—	−0.0025	0.0075	0.0079	0.0247*	0.0177*	0.0206*
AB	0.00092	−0.00046	—	0.0063	0.0088	0.0232*	0.0121	0.0148
MB	−0.00968	0.0044	−0.0052	—	0.0027	0.0152*	0.0179*	0.0275*
ON	0.0163	0.0260	0.00478	0.0128	—	0.0056	0.010	0.0173*
QU	0.0254	0.0318	0.00351	0.0170	−0.0095	—	0.0064	0.0259*
NB	0.0283	0.0334	0.00294	0.0172	0.0142	0.0111	—	0.0082
NF	0.0508	0.0432	0.02145	0.0428	0.0136	0.0077	0.0074	—

Asterisks denote F_{ST} and R_{ST} values that were significantly different from zero following table-wide adjustment of significant levels according to Rice (1989).

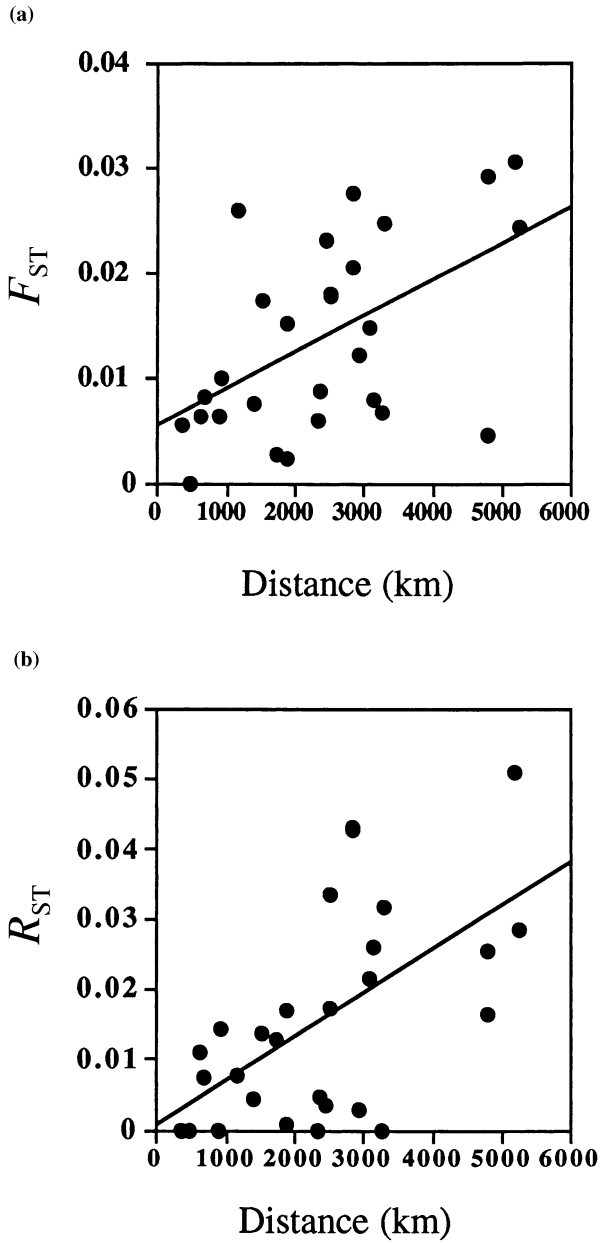

Fig. 2 Isolation-by-distance plots for all pairwise values of (a) F_{ST} and (b) R_{ST}. The best-fit linear equation for F_{ST} versus distance was $F_{ST} = 0.0054 + 0.0000035$ (km); that for R_{ST} versus distance was $R_{ST} = 0.00061 + 0.0000063$ (km).

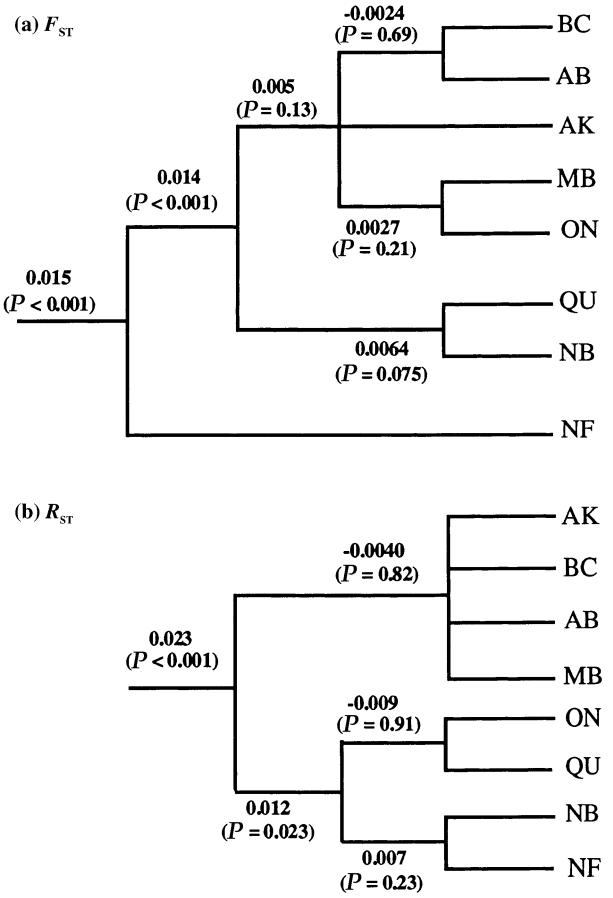

Fig. 3 Phenograms showing the results of hierarchical analyses of differentiation among yellow warbler populations using (a) F_{ST} and (b) R_{ST} as the measures of differentiation. Values at each node represent the level of differentiation between sister clusters. The probability that a given value is significantly different from zero is given in parentheses.

Population imbalance analyses

The mean values for β for each population are all greater than one, ranging in value from 1.28 for Alaska to 1.79 for Québec, with an overall mean value of 1.54 (Table 5). However, all population-specific values have 95% confidence intervals which overlap with an equilibrium value of 1.0 (Table 5). This supports the idea that these yellow warbler populations are close to or at demographic equilibrium.

Table 5 Variance in allele size (Θ_V), homozygosity (Θ_{P_0}) and imbalance (β) index values for microsatellite loci for yellow warbler populations

Population	Θ_V	Θ_{P_0}	β ± SE	95% CI
Alaska	14.04	0.262	1.28 ± 0.47	0.34, 2.22
Bristish Columbia	12.59	0.239	1.46 ± 0.71	0.04, 2.88
Alberta	11.25	0.262	1.64 ± 0.93	−0.22, 3.50
Manitoba	11.52	0.240	1.46 ± 0.81	−0.16, 3.08
Ontario	10.51	0.222	1.66 ± 0.90	−0.14, 3.46
Québec	12.63	0.231	1.79 ± 0.79	0.21, 3.37
New Brunswick	10.45	0.250	1.50 ± 0.62	−0.26, 2.74
Newfoundland	9.29	0.254	1.51 ± 0.52	0.47, 2.55
Mean	11.54	0.245	1.54	

Values are means averaged across six loci. 95% confidence intervals for mean β values for each population were calculated as ±2 SE.

Discussion

Our main result is that, in contrast to results from mtDNA control region variation (Milot *et al*. 2000), continent-wide comparisons of yellow warbler populations show low levels of genetic differentiation in microsatellite variation. Below, we discuss possible reasons for the differences in the levels of differentiation between these two types of rapidly evolving DNA markers.

Comparisons with mtDNA

Comparisons between microsatellite and mtDNA are complicated by the fact that each represents different kinds of genetic data (allelic variation at specific loci versus DNA sequence variation), requiring different measures of differentiation. However, simple measures of differentiation based on haplotype (G_{ST}; Nei 1987) or allele frequencies (F_{ST}) may be roughly comparable: they show that differentiation based on mtDNA is more than nine times greater (mtDNA: overall $G_{ST} = 0.13$ (Milot *et al*. 2000); microsatellites: overall $F_{ST} = 0.014$). When measures that account for genetic differences between individual haplotypes and alleles are compared, the difference is more than 30 times greater (e.g. overall $\hat{g}_{ST}$ value for mtDNA = 0.52; overall R_{ST} for microsatellites = 0.015). Similarly, when we use AMOVA (Excoffier *et al*. 1992) to estimate the overall percentage variation apportioned between populations for each type of marker, the values are 1.5 and 53.0% for microsatellite and mtDNA variation, respectively.

Despite this difference in the magnitude of differentiation, both types of markers appear to detect roughly similar patterns of differentiation. Both show evidence for isolation-by-distance effects (Mantel test for correlation between mtDNA differentiation (g_{ST}) and linear distance between the seven populations analysed by Milot *et al*. (2000); $P < 0.01$; $r^2 = 0.28$), and the relative magnitude of the differentiation in mtDNA and microsatellite variation among populations is significantly correlated for one measure of differentiation in microsatellites (R_{ST}) (Mantel test for correlation between pairwise measures $\hat{g}_{ST}$ and R_{ST}: $P = 0.05$) and almost so for the other (F_{ST}) (Mantel test: $P = 0.13$). Finally, as with mtDNA variation, the microsatellite data show the presence of distinct 'eastern' and 'western' sets of populations, although the location and number of splits between sets of populations differ. We emphasize that isolation-by-distance effects probably play a major role in determining the patterns shown in Fig. 3; populations which are close to each other (e.g. those within the 'eastern' and 'western' clusters) may group together simply because they frequently exchange migrants and not due to the effects of common history. In summary, the key issue that requires explanation is why the mtDNA data suggest that yellow warbler populations are highly structured whereas the microsatellite data do not.

We suggest three possible reasons for the increased structure observed in mtDNA relative to microsatellites: (1) yellow warbler populations are not in evolutionary equilibrium and the high similarity in microsatellites is mainly due to recent common ancestry and not gene flow, (2) differences in the effective population sizes and mutation rate of each type of marker result in mtDNA showing inherently higher levels of differentiation, or (3) high levels of male-biased gene flow have tended to homogenize microsatellite but not female-specific mtDNA variation between populations. We consider each of these possibilities below.

Common ancestry as an explanation for similarity

If populations are not in demographic or evolutionary equibrium, then an alternative explanation for a high level of similarity among populations is that it is due to a high level of retained ancestral polymorphism within populations. It seems possible that the yellow warbler populations studied here may not be in equilibrium as they re-colonized this region of North America following the last glacial retreat, roughly 10 000 years ago (Pielou 1991). However, our analyses provide two pieces of evidence which suggest that these populations are close to or at evolutionary equilibrium and thus that common ancestry is not a viable alternative to gene flow as an explanation for a lack of differentiation. First, the presence of significant linear isolation-by-distance patterns provides direct support for the idea that populations are in equilibrium and that distance-dependent levels of migration can account for differences in the level of differentiation between populations (Hutchison & Templeton 1999). We acknowledge that distance effects explain no more than one-third of the variation in either fixation index, but this low value may be due to errors in estimating levels of inter-population differentiation due to the small number of loci used for such estimates (Ruzzante 1998).

Second, the finding that, in all populations, the confidence interval for the imbalance index includes the expected equilibrium value of 1 argues that all sampled populations are close to or at equilibrium. As discussed by Kimmel *et al*. (1998), key assumptions made in the calculation and interpretation of β include: (1) that the stochastic variance in locus-specific values of Θ_V and Θ_{P0} is low, (2) that mutations at all loci follow a stepwise mutation model or restricted stepwise model, and (3) that mutation rates are similar among loci. Our interpretation requires that these assumptions apply to our data, although we have no direct way of assessing this.

Genetic characteristics of markers

As noted by Seielstad *et al*. (1998), comparisons of levels of differentiation among different classes of genetic makers need to take into account genetic characteristics that could bias such comparisons. Specifically, the fourfold difference in effective population size between organelle and autosomal markers (Birky *et al*. 1989) means that mtDNA may inherently show higher levels of differentiation at equilibrium compared to microsatellites even though mutation rates per gene for microsatellites are roughly an order of magnitude higher (see below).

To assess the possible effect of these genetic differences on levels of differentiation, we used the approach suggested by Seielstad *et al*. (1998) of comparing expected levels of divergence, measured as F_{ST}, based on theory for diploid and haploid markers, with observed levels to see whether the differences in effective population size and mutation rates could alone account for the increased divergence in mtDNA. Theory based on the island model of migration (e.g. Takahata 1983) indicates that F_{ST} is approximately equal to $1/(1 + 4N_e v)$ (eqn 1) for diploid systems (e.g. microsatellites) and $1/(1 + N_e v)$ (eqn 2) for haploid systems (e.g. mtDNA), where N_e is the effective population size and v is equal to $m + u - mu$, where m is the migration rate and u is the mutation rate (Cavalli-Sforza & Bodmer 1971). The best current estimate of the mutation rate for dinucleotide microsatellite loci is 5×10^{-4} per locus per generation (Goldstein *et al*. 1995), whereas Milot *et al*. (2000) estimated the per sequence mutation rate for the 344 bp section of control region mtDNA they studied to be roughly 3×10^{-5} per sequence per generation. We used these values in combination with the overall observed value of F_{ST} (0.014) for the microsatellite data, and an estimated N_e of 20 000 (see Milot *et al*. 2000) to estimate m from eqn 1. We then used this value of m and the parameter values given above to estimate the expected F_{ST} for mtDNA based on eqn 2. This calculation yielded an expected mtDNA value of 0.054, which is almost four times greater than the observed microsatellite-based F_{ST} value of 0.014. However, this increase is much less than the empirically observed 9–30-fold increase observed for yellow warblers. Thus, differences in genetic characteristics of the two types of makers can only account for a very small proportion of the increased differentiation observed for mtDNA in these birds, and another explanation must be sought to explain the majority of the difference.

Sex-biased dispersal

The explanation that we are left with is that the difference in levels of differentiation between these different markers may reflect differences in patterns of dispersal between the sexes. Specifically, because mitochondrial DNA is maternally inherited in birds, then, if females are more philopatric than males, this alone could lead to increased structure in mtDNA compared to bi-parentally inherited microsatellites. This explanation is contrary to the general pattern for passerine birds, based largely on band recovery data, which has shown that both natal and breeding dispersal are either female-biased or that no differences exist between the sexes (Greenwood 1980; Clarke *et al*. 1997). For example, in a recent literature review, Clarke *et al*. (1997) found that, based on data for 53 passerine species, the majority (74%; 39/53 species) showed evidence for a female bias in natal and/or breeding dispersal, while only 11% (6/53 species) provided evidence for male-biased dispersal, and 15% (8/53 species) showed no support for any sex-biased patterns in either form of dispersal.

A lack of sex-specific patterns of natal dispersal in yellow warblers is also implied by data from a long-term study of this species at Delta Marsh, Manitoba (e.g. Sealy 1995), which suggested no differences in natal dispersal distances between male and female yellow warblers (J. Briskie & S.G. Sealy, unpublished data). Specifically, for data collected over a three-year period, distances between the breeding locations and natal nest locations were not significantly different for 28 male yellow warblers (mean distance = 0.81 km) (SD 0.54, range 0–2.5 km) and 15 female yellow warblers (mean distance = 0.88 km) (SD 0.69, range 0.1–2.4 km) ($t = 0.36$; $P = 0.72$).

However, as stressed by Koenig *et al*. (1996), because banding studies have limited geographical and temporal scope, they cannot reliably detect either rare instances of long-distance dispersal and/or rare episodic instances of high dispersal. Because genetic measures of dispersal involve comparisons between widely separated populations and infer dispersal over many generations, they have the potential to incorporate the effects of both types of dispersal which are 'missed' by behavioural estimates. For example, using mtDNA data, Edwards (1994) was able to show evidence for previously undetected migration events over thousands of kilometres in a highly social, co-operatively breeding bird that was previously thought to be highly philopatric. Thus, the genetic results reported here suggest that dispersal by male yellow warblers over long distances or at irregular points in time may be more significant than previously suggested by behavioural data alone, and this is the best explanation available at present for the differences in the magnitude of differentiation between microsatellite and mtDNA variation. Confirming such a pattern with more complete dispersal data, understanding why there should be sex-related differences in the tendency to disperse long distances or episodically, and determining whether such patterns also are present in other passerines are questions that need to be addressed by future genetic and field-based analyses.

Conclusions

We draw the following conclusions from our study. First, our results underscore the need to assess patterns of genetic structure using multiple loci as the patterns detected with mtDNA and microsatellite loci were quite different. However, they also emphasize the insights that can be gained into difficult-to-study behaviours, such as long-distance dispersal, through genetic analyses. Second, they raise the possibility that there may be significant differences in the tendency to disperse long distances by male and female passerine birds that have previously been undetected due to the limitations of behavioural studies but could nonetheless play a significant role in determining the genetic structure of bird populations. This suggests the alternative hypothesis that the previously observed lack of differentiation in allozymes among populations of some North American birds may be as much due to male-biased gene flow as a lack of sensitivity of allozymes to the recent effects of isolation due to their slow rate of evolution (see Zink 1997). This possibility could be further examined by comparing patterns of differentiation in DNA sequence variation in non-coding regions on bi-parentally inherited autosomes (e.g. introns; Friesen *et al*. 1997) with variation in non-coding regions on the female-specific W chromosome.

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This study is the result of a long term collaboration between HLG and KAH whose goal is to use genetic and stable isotopic methods to examine population structure and migratory behaviour in a number of species of Neotropical migrant birds. HLG is an Associate Profoessor of Biology at McMaster University, RJGD was a post-doctoral fellow in the same department, and KAH is a Research Scientist with the Canadian Wildlife Service.

Appendix I Genetic characteristics of microsatellite loci in eight yellow warbler populations

	Locus					
Population	Caμ 28	Dpμ 01	Dpμ 03	Dpμ 15	Dpμ 16	Maμ 23
Alaska						
n	20	21	21	21	21	21
Number of alleles	5	17	4	14	11	4
Frequency	0.35	0.17	0.78	0.29	0.24	0.36
H_E	0.651	0.930	0.368	0.880	0.870	0.730
H_O	0.400	0.857	0.333	0.762	1.0	0.619
British Columbia						
n	33	33	33	33	33	31
Number of alleles	8	23	3	18	12	6
Frequency	0.45	0.15	0.77	0.32	0.18	0.35
H_E	0.740	0.940	0.382	0.850	0.900	0.752
H_O	0.424	0.909	0.364	0.781	0.909	0.677
Alberta						
n	22	22	22	22	22	22
Number of alleles	6	19	3	14	12	5
Frequency	0.57	0.11	0.80	0.41	0.23	0.30
H_E	0.640	0.951	0.354	0.836	0.882	0.767
H_O	0.455	0.955	0.227	0.727	0.955	0.727
Manitoba						
n	34	35	35	35	35	35
Number of alleles	11	21	3	15	16	7
Frequency	0.25	0.11	0.87	0.30	0.14	0.30
H_E	0.837	0.940	0.235	0.842	0.927	0.778
H_O	0.677	0.914	0.257	0.629	0.857	0.829
Ontario						
n	29	30	30	30	26	30
Number of alleles	7	21	7	13	12	6
Frequency	0.29	0.13	0.73	0.33	0.23	0.32
H_E	0.814	0.932	0.453	0.822	0.878	0.767
H_O	0.621	0.933	0.500	0.767	0.769	0.733
Québec						
n	32	36	34	35	34	35
Number of alleles	8	21	8	14	11	6
Frequency	0.33	0.18	0.69	0.46	0.46	0.30
H_E	0.762	0.934	0.506	0.767	0.866	0.777
H_O	0.656	0.861	0.559	0.600	0.853	0.686
New Brunswick						
n	30	30	30	31	27	31
Number of alleles	7	19	4	12	14	5
Frequency	0.37	0.18	0.73	0.53	0.22	0.29
H_E	0.764	0.920	0.444	0.689	0.906	0.775
H_O	0.567	0.900	0.267	0.636	1.0	0.839
Newfoundland						
n	40	38	36	39	38	40
Number of alleles	6	16	7	9	10	5
Frequency	0.45	0.16	0.69	0.32	0.38	0.33
H_E	0.711	0.907	0.502	0.808	0.804	0.747
H_O	0.375	1.0	0.528	0.795	0.737	0.800

n, number of individuals genotyped; number of alleles, number of different size alleles present in population; frequency, frequency of the most common allele; H_E and H_O, expected and observed heterozygosities as calculated using GENEPOP.