Divergence in mating signals correlates with ecological variation in the migratory songbird, Swainson's thrush (*Catharus ustulatus*)

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Abstract

Song divergence among populations of passerine birds is recognized as a potentially important premating isolation mechanism and may represent the first step in speciation. Because song divergence may be influenced by an array of acoustic, ecological, and genetic factors, the study of its origin requires a multifaceted approach. Here we describe the relationship between acoustic, neutral genetic and ecological variation in five populations of the Swainson's thrush: two from coastal temperate rainforest habitat representing the 'russet-backed' subspecies group, two from inland coniferous forest habitat representing the 'olive-backed' subspecies group, and one mixed locality that resides within a contact zone between the two groups. Song in the five populations is analysed using a multivariate analysis of spectral and temporal measurements, population genetic structure is assessed using an analysis of five microsatellite loci and ecological differences between populations are quantified using an analysis of climatic parameters. Matrix correspondence tests are used to distinguish between the potential for drift and selection in driving song divergence. No significant correlation was found between acoustic and genetic distance suggesting that song divergence cannot be explained by drift alone. A significant correlation between ecological and acoustic distance after accounting for genetic distance, suggests a potential role for ecological selection on divergence in spectral and temporal components of Swainson's thrush song.

Introduction

Divergence in mating signals is important to the evolution of premating isolation in animals and is often the first step toward speciation (reviewed in Mayr 1963; Coyne & Orr 2004). In passerine birds, song is a key trait used in both mate choice and species recognition (Marler 1960; Searcy & Andersson 1986; Catchpole 1987; Grant & Grant 1996). Considerable controversy surrounds the extent to which local song dialects represent a barrier to gene flow. Some authors suggest females prefer males that sing local song dialects (reviewed in Searcy & Yasukawa 1996), and positive assortative mating based on song characteristics may help maintain population boundaries and/or lead to further differentiation between groups (Baker 1982; MacDougall-Shackleton & MacDougall-Shackleton 2001; Patten *et al.* 2004). Others contend that in systems with vocal learning such as songbirds, local song dialects are rarely associated with biologically meaningful levels of genetic divergence (Zink & Barrowclough 1984; Soha *et al.* 2004; Wright *et al.* 2005). Regardless of the role that local song dialects play in limiting gene flow between populations, there remains a great deal to be learned about the origins of vocal divergence.

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Divergence in acoustic features of learned song is generally believed to evolve through drift, selection or both (see Ellers & Slabbekoorn 2003; reviewed in Slabbekoorn & Smith 2002a; Podos *et al.* 2004). Under a neutral mechanism of drift, the accumulation of copying errors or cultural 'mutations' may occur as the song is passed on to the next generation (Payne 1996). Under a drift model of song evolution, song divergence will be negatively correlated with gene flow, so that acoustic divergence will be greatest between populations that have been isolated for the longest amount of time. Selection for song types suited to different habitats or ecological conditions may also contribute to song divergence (Slabbekoorn & Smith 2002b; Podos & Nowicki 2004; Podos *et al.* 2004). Under a selection model of song evolution, song divergence may be weakly correlated with gene flow, but strongly correlated with differences in ecology. Distinguishing between the influence of drift and selection on song evolution requires an integrative approach combining analyses of genetics and ecology with song variation.

We investigate evolutionary forces shaping acoustic variation in the Swainson's thrush (*Catharus ustulatus*), a long distance migratory passerine. Within the Swainson's thrush, there are two morphologically distinct groups, namely: (i) the coastal, 'russet-backed' group that breeds along the Pacific coast of North America, migrates along a coastal flyway and winters in southern Mexico and Central America, and (ii) the inland, 'olive-backed' group that breeds in interior boreal and coniferous forests, migrates along an eastern route and winters from Panama to the northern tip of Argentina (Mack 2000; Ruegg & Smith 2002). A survey of mitochondrial DNA (mtDNA) variation across the breeding grounds suggests that in addition to having divergent migratory pathways and overwintering locations, coastal and inland groups are also genetically distinct (Ruegg & Smith 2002). Despite its widespread distribution, very little is known about acoustic variation in the Swainson's thrush. The song has generally been described as a series of complex syllables increasing in frequency with significant individual variation in syllable type and order (Dobson & Lemon 1977); however, information on geographical variation in acoustic characters is limited (Mack 2000).

To investigate the determinants of song variation we combine acoustic, neutral genetic and ecological data sampled from five localities: two coastal populations from temperate rainforest habitat, representing the coastal group, two inland populations from coniferous forest habitat, representing the inland group and one mixed site that has previously been determined to be a contact zone between the two groups based on mtDNA (Ruegg & Smith 2002). We measure spectral and temporal components of the song to determine acoustic similarity, examine variation at five microsatellite markers to infer genetic divergence and assess environmental similarity using climatic data. By comparing these data, we assess the relative roles of drift, selection and ecology in song divergence. Specifically, we address the following four hypotheses: (i) If processes of random drift affect patterns of acoustic variation, then we expect a strong correlation between acoustic and genetic distance. (ii) Alternatively, if patterns of acoustic variation are more strongly affected by selection, we expect a weak correlation between acoustic and genetic distance. (iii) If acoustic variation is affected by ecological selection, then we expect a strong correlation between acoustic and ecological distance. (iv) Alternatively, a weak correlation between ecology and song would suggest minor role for environmental selection and a more important role for cultural drift or other forms of selection.

Methods

Sampling and screening of mtDNA

We sampled 119 individuals for genetic analysis from five locations across the Pacific Northwest during the 1999 breeding season: (i) two populations, Squamish and Siulsaw, from the coastal group; (ii) two populations, Revelstoke and Umatilla, from the inland group; and (iii) one population, Pemberton, from a contact zone between the two groups (Fig. 1; Table 1). Birds were captured in mist nets and the two outer tail retrices were collected (for subsequent genetic and isotopic analysis) before they were banded and released. Acoustic recordings were made from 45 individuals at the same 5 sites during the breeding season of 2000 (Fig. 1; Table 1). In order to minimize spatial autocorrelation in our analyses of population genetic structure, ecological and acoustic similarity, populations sampled within groups were located farther from each other than populations sampled between groups.

All of the genetic samples screened in the current study were also screened for mtDNA and the result was reported in Ruegg & Smith (2002). In the present study, 'pure' populations have greater than 90% coastal or inland haplotypes according to Ruegg & Smith (2002) and fall within the boundaries of morphologically distinct 'olive-backed' and 'russet-backed' subspecies ranges (Mack 2000). Alternatively, the 'mixed' population from Pemberton, BC has 60% inland and 40% coastal haplotypes and falls on the border of the two subspecies ranges (Mack 2000).

Acoustic variation

Song recordings were made with a Sennheiser ME67 directional microphone and a Sony TCM-5000EV tape recorder with TDK SA90 tapes to record 10–20 min of song for each individual. We selected high quality recordings (low background noise, high signal to noise ratio, and songs without other loud noises or birds singing) and analysed
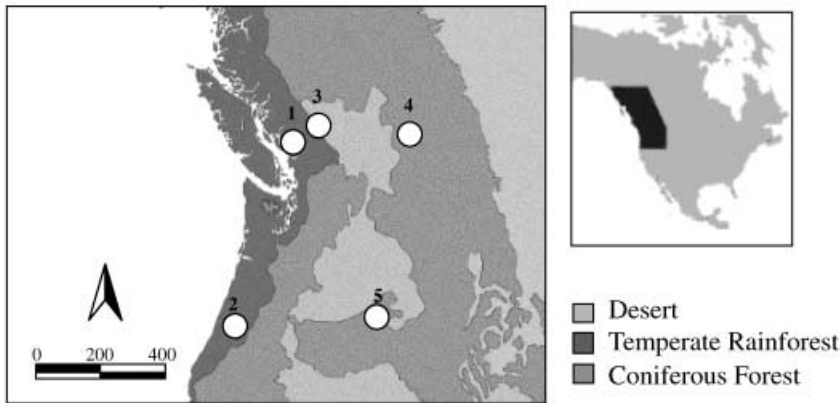
Fig. 1 Location of sampling sites in relation to temperate rainforest and interior coniferous forest ecological regions. Ecological boundaries adapted from a map by Canadian Council on Ecological Areas, Ecological Regions of North America Level 1 (*www.cec.org/pubs_info_resources/publications/ enviro_conserv/zipdown.cfm?varlan = english*). Numbers correspond to site names listed in Table 1.

Table 1 Localities, mtDNA group according to Ruegg & Smith (2002) and numbers of individuals sampled. Location numbers refer to population numbers in Fig. 1

Location	mtDNA group	Latitude/longitude	Song (*n*)	Msat (*n*)
1. Squamish, BC	Coastal	49°45′00″N, 123°08′00″W	10	34
2. Siulsaw NF, Oregon	Coastal	44°03′32″N, 123°48′56″W	9	19
3. Pemberton Valley, BC	Mixed	50°30′00″N, 123°08′00″W	7	20
4. Revelstoke, BC	Inland	50°59′00″N, 118°12′00″W	10	24
5. Umatilla NF, OR	Inland	44°03′32″N, 123°48′56″W	9	22

462 songs from 38 individuals (Table 1). Acoustic characteristics of songs were quantified by sonagraphic measurements (Beeman 1996). Song recordings were digitized at a sampling rate of 25 kHz. We used Hanning windows and applied a Fast Fourier Transformation size of 512, with a time interval between FFT's (T-Inc.) of 10 ms. These settings led to a frequency resolution of 49 Hz and a temporal resolution of 20.5 ms. The measurements used for statistical analyses were individual means, based on the measurements of a set of more than 12 song replicates on average. Consequently, the resolution of these individual means is about 3.5 times higher than the original variance divided by the square root of the number of samples (Sokal & Rohlf 1995).

To assess geographical variation in birdsongs, we measured the following set of spectral and temporal characteristics: Fmin, the lowest frequency on the sonagram; Fmax, the highest frequency on the sonagram; Fint, the frequency with the highest amplitude within the often very short and relatively faint introductory note; F1-5, the frequency with the highest amplitude measured for five equally sized time frames from the start to the end of the song; and DUR, the duration of the song. All sonagrams were created using the same standard settings in signal in order to guarantee that the grey-scale of the sonagram is automatically normalized based on the peak amplitude in the song across all individuals. Song onset, song offset, Fmin and Fmax were determined onscreen by cursor placement. In order to reduce the potential for observer bias, all other measurements were calculated using a programmed routine in SIGNAL that automatically determined five equally sized time frames between the start and the end of each song and wrote the highest amplitude in each time frame directly into a data file.

To determine whether the five populations differed significantly in the nine song characters, we performed a multivariate analysis of variance (MANOVA). To determine whether populations can be distinguished on the basis of acoustic characters as well as to identify the combination of acoustic characters most important to group membership, we performed a discriminant functions analysis in which each population was a separate group. All variables were tested for co-correlation, and in all but the correlation between F5 and Fmax, $r < 0.7$. Removing F5 and/or Fmax from the analysis did not affect the overall pattern of relationships between populations or the variables identified as most important to group membership; therefore, all variables were included in the final analysis. Post-hoc Bonferroni tests were used to determine which populations differed significantly in the characters identified as important to group membership by the MANOVA and the DFA. Acoustic distance between populations was calculated as the Euclidean distance between group centroids for the first two discriminant functions. All statistical analyses were preformed using SPSS for Windows version 10 (SPSS 1999).

Table 2 Locus information

Locus	Allele size range	No. of alleles	H_O	H_E
Cu02	132–164	16	0.88	0.9
Cu04	116–156	20	0.74	0.91
Cu10	130–162	14	0.42	0.71
Cu28	133–225	23	0.73	0.86
Cu32	122–138	9	0.71	0.79

H_O, observed heterozygosity; H_E, expected heterozygosity.

Genetic variation

Population genetic structure was assessed using five micro-satellite loci developed from a Swainson's thrush library (Gibbs *et al.* 1999) (Table 2). The forward primer in each set was fluorescently labelled (Table 2). The following reagents were combined in a 12.5 μL reaction: ~50 ng genomic DNA, 0.16 mM dNTP's, one-tenth volume of PCR buffer (10 mM Tris-HCl pH 8.3, 500 mM KCl), 1.5 mM MgCl$_2$, 50 ng/μL BSA, 4% DMSO, 0.1 μM each primer and 0.5 U *Taq* DNA polymerase (Ampli*taq*, Applied Biosystems). For each sample, 2 μL of PCR product, 2.5 μL formamide loading dye and 0.2 μL of a 350 bp ROX-labelled fragments size standard (Applied Biosystems) were mixed and electrophoresed on a 5.8% acrylamide gel on an Applied Biosystems 377 automated system. Allele sizes were scored using GENESCAN software (Applied Biosystems) after calibration with the internal size standard.

Assumptions of independence of loci and Hardy–Weinberg equilibrium (HWE) of loci and populations were tested using GENEPOP version 3.1c (Rousset & Raymond 1995). For the former, the combined probability of linkage disequilibria between each locus pair in each population was calculated. To determine if any violation of HWE was due to null alleles, a specific test for heterozygote deficit was conducted for each locus and each population. Significance of heterozygote deficit was corrected for multiple comparisons across five loci (adjusted $P = 0.01$) and five populations for each locus (adjusted $P = 0.006$). Genetic structure was quantified using the software GENEPOP (Rousset & Raymond 1995) as pairwise F_{ST} measures that summarize within vs. among population variation (Weir & Cockerham 1984). In addition to F_{ST}, we estimated genetic distance using D_S (Nei 1972) and obtained similar results irrespective of the method. Most measures of genetic distance estimate some function of F_{ST} (Hartl 2000) and we choose to report Weir & Cockerham's (1984) measure of F_{ST} because it is widely used, easy to interpret across species and provides a coherent estimation framework. Significance values for pairwise F_{ST} were assessed with 1000 permutations using the software package ARLEQUIN (Schneider *et al.* 2000).

Ecological variation

We used climatic variables to describe environmental variation across our five sampling localities. The two coastal populations are located in temperate rainforest where precipitation is high and mild winters allow for year round growth of vegetation (Barbour & Billings 1988). The inland and mixed populations are located in drier, inland coniferous forest habitats dominated by species that can withstand hot summers and cold winters such as Ponderosa pine (*Pinus ponderosa*) and Douglas fir (*Pseudotsuga menziesii*) (Barbour & Billings 1988). Other studies equating ecological variation with acoustic differences generally focus on one or two aspects of the environment potentially important to sound transmission (Slabbekoorn & Smith 2002b). However, sound transmission across the environment is affected by a multitude of factors such as daily fluctuations in air temperature and humidity, precipitation, proximity to water, vegetation type and vegetation density (Wiley & Richards 1982; Slabbekoorn 2004), and is therefore not easily summarized by one or two habitat variables. The use of climatic variables allows for a gross level approximation of environmental variation that potentially encompasses more than selecting one or two habitat variables that may or may not be related to sound transmission. To assess ecological similarity between the five sampling locations, we calculated a dissimilarity matrix using seven climatic variables related to seasonality and precipitation (temperature seasonality, temperature annual range, annual precipitation, precipitation of the wettest month, precipitation seasonality, precipitation of the wettest quarter and precipitation of the coldest quarter). While the climate data used in our analyses also covered periods when the Swainson's thrush are not on the breeding grounds, our assumption is that climate during all seasons will affect vegetation structure and influence variables important to sound transmission during the breeding season. Climate data (summarized as means for the years 1950–2000) was extracted from Worldclim, a global climate database with a spatial resolution of ~1 km (Hijmans *et al.* 2005), using the software DIVA-GIS (Hijmans *et al.* 2004). Climatic variables were scaled to values between 0 and 1 and a dissimilarity matrix was calculated using SPSS version 10 for Windows (SPSS 1999).

Comparison between acoustic, ecological and genetic distance

To examine the relationship between acoustic, genetic, and ecological distances, we performed matrix correspondence analysis or a series of pairwise and partial Mantel tests using XLSTAT (Addinsoft 2005). We compared two matrices, acoustic distance (Euclidean distance) vs. genetic distance (F_{ST}) and acoustic distance vs. ecological distance (Euclidean distance). Significance of association between matrices was

assessed using 10 000 randomizations and significance levels were adjusted using a Bonferroni correction for multiple comparisons. To address whether acoustic distance is correlated with ecological distance after accounting for genetic similarity, we conducted a partial Mantel test. Assessing the significance of partial Mantel tests using randomizations methods may be problematic when spatial autocorrelations of the dependent variables are important (Raufaste & Rousset 2001; Castellano & Balletto 2002; Rousset 2002); however, our sampling design in which the geographical distance between most closely related populations is larger than the distance between more distantly related populations decreases the possibility of spatial autocorrelation in the data.

To visualize the relationships between acoustic, genetic, and ecological distance, we constructed a neighbour-joining network from the distance matrices using the programs NEIGHBOUR and DRAWTREE within the software package PHYLIP 3.65 (Felsenstein 2005). The program NEIGHBOUR uses the UPGMA method of clustering to construct an unrooted tree representing relationships between groups.

Results

Acoustic variation

Overall song structure was consistent with previous descriptions of a series of syllables increasing in frequency and decreasing in amplitude towards the end of the song (Dobson & Lemon 1977). While a quantitative description of song structure was not the focus of the current study, visual inspection of sonagrams revealed that each individual has multiple song types with significant individual variation in syllable structure and order. Recognizable song types were often consistent between individuals within groups; the most common song type from two representative individuals in each population is depicted in Fig. 3.

Multivariate analysis of variances indicates that overall populations differed significantly in acoustic characteristics (Wilks' lambda = 0.002, $F_{25,9}$ = 1699, $P < 0.001$). An analysis of the between subjects effects reveals that duration ($F_{271384,4}$ = 7.307, $P < 0.001$), frequency in regions 1–3 (F1: $F_{189461,4}$ = 5.267, $P < 0.001$; F2: $F_{198116,4}$ = 5.180; $P < 0.05$; and F3: $F_{146315,4}$ = 3.133, $P < 0.05$) differ significantly between populations. Discriminant functions analysis of acoustic variables indicates that the combination of duration and frequency in the first one-fifth of the song (F1) can be used to discriminate coastal populations from inland populations along canonical variate 1 (CV_1 correlation coefficients: duration = 0.362, F1 = –0.189, F2 = –0.108, F3 = 0.130, F4 = 0.104, F5 = –0.066, Fint = –0.100, Fmin = 0.155, Fmax = –0.155). The first and second canonical variates (CV) accounted for 78% and 12% of the variance, respectively (Fig. 2). Visual inspection of the first and second canonical variate plot suggests that of the seven individuals from
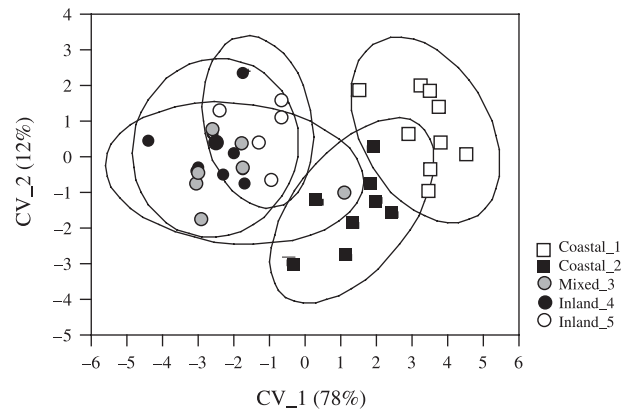

Fig. 2 Plot of canonical discriminant functions 1 and 2 describing song variation. Inland populations are represented by white and black circles, coastal populations are represented by white and black squares and the mixed population is represented by grey circles. Coastal and inland groups are bounded by 95% confidence ellipses. The plot illustrates separation of coastal and inland populations along function 1.

the mixed population six individuals had songs that were more similar to inland populations and one individual had a song more similar to coastal populations.

Post-hoc Bonferroni pairwise comparisons of the variables that were most important to separation along canonical variate 1 indicate that Coastal_1 songs (mean ± SD: 1710 ms ± 229) are significantly longer in duration then Inland_4 (1244 ms ± 114; $P < 0.001$), Inland_5 (1363 ms ± 185; $P < 0.05$), and Mixed_3 songs (1293 ms ± 195; $P < 0.001$), but not significantly longer then Coastal_2 songs (1434 ms ± 193; $P > 0.05$). In addition, post-hoc comparisons also revealed that Coastal_2 songs (2091 kHz ± 169) are significantly lower in frequency in the first one-fifth of the song (F1) than Inland_4 (2452 kHz ± 202; $P < 0.05$) and Inland_5 songs (2540 kHz ± 189; $P < 0.05$), but not significantly lower in F1 frequency from Coastal_1 (2272 kHz ± 124; $P > 0.05$) and Mixed_3 songs (2281 kHz ± 227; $P > 0.05$).

Overall, the multivariate analyses suggest that a combination of spectral and temporal components can be used to discriminate coastal populations from inland populations, and that the mixed population contains songs that are similar to those of either subspecies group, rather than intermediate between the two groups. The pairwise Euclidean distances between group centroids confirmed that songs within coastal and inland subspecies groups are more similar than songs between groups and that the majority of songs from the mixed population were more similar to inland than to coastal songs (Table 3a).

Genetic variation

Linkage disequilibrium was not detected for any locus comparison ($P > 0.3$ in all cases); therefore, loci can be
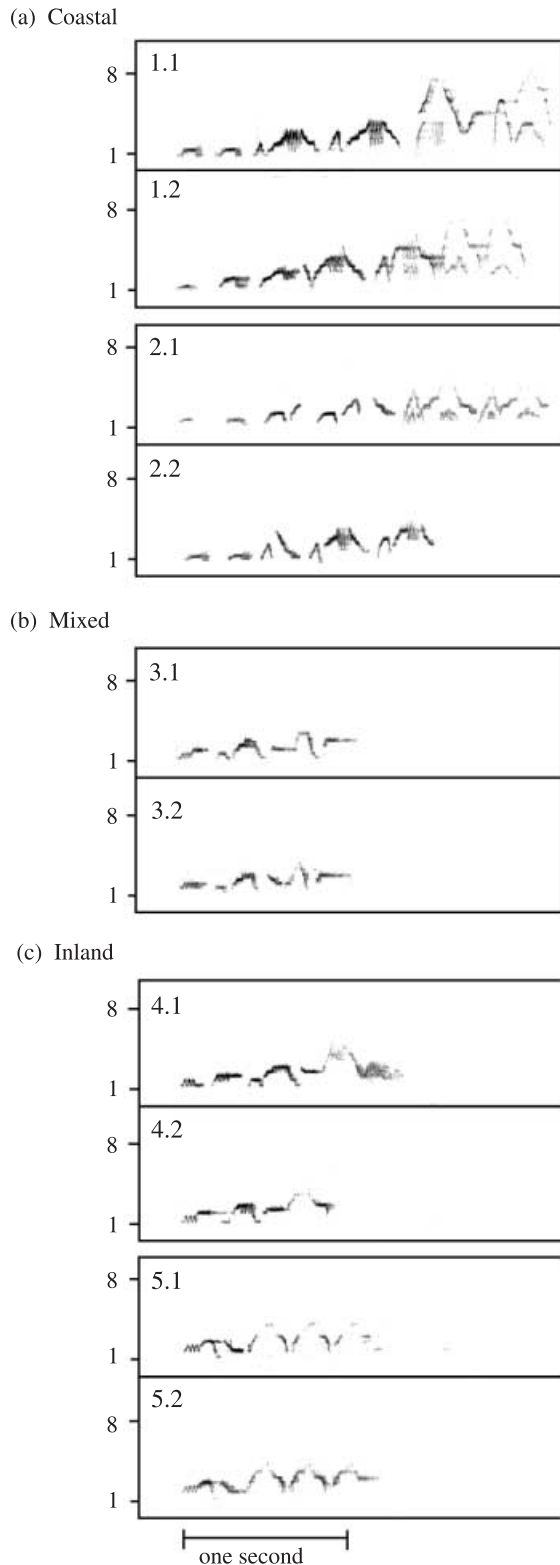
(a) Coastal

(b) Mixed

(c) Inland

Fig. 3 Sonagrams from two individuals in each population (numbers in the upper left represent the population number followed by the individual number). While each individual had multiple song types, the figure illustrates the most common song type in the population. For details on locations see Table 1 and Fig. 1.

Table 3 Distance matrices. All comparisons between pure populations are in dark grey and all between mixed and pure population comparisons are in light grey. (a) Acoustic distance measured as Euclidean distance between group centroids. (b) Genetic distance measured as pairwise population F_{ST}. (c) Ecological distance measured as dissimilarity between climatic variables

(a) Acoustic

	Coastal_1	Coastal_2	Mixed_3	Inland_4	Inland_5
Coastal_1	0				
Coastal_2	3.053	0			
Mixed_3	5.498	3.474	0		
Inland_4	5.91	4.226	0.89	0	
Inland_5	4.693	3.621	1.584	1.399	0

(b) Genetic

	Coastal_1	Coastal_2	Mixed_3	Inland_4	Inland_5
Coastal_1	0				
Coastal_2	0.011	0			
Mixed_3	−0.002	0.009	0		
Inland_4	0.018*	0.043*	0.015*	0	
Inland_5	0.026*	0.043*	0.025*	0.007	0

(c) Ecological

	Coastal_1	Coastal_2	Mixed_3	Inland_4	Inland_5
Coastal_1	0				
Coastal_2	2.336	0			
Mixed_3	3.641	5.013	0		
Inland_4	4.003	5.651	1.425	0	
Inland_5	4.911	5.701	2.064	2.299	0

*Indicates F_{ST} significantly different from zero.

considered independently. There were some cases where a significant heterozygote deficit was found in a particular population at a particular locus; however, no population showed a consistent heterozygote deficit across all loci ($P > 0.01$ in most cases), and no locus had a significant heterozygote deficit across all or most populations ($P > 0.006$ in most cases), indicating that samples have not been taken from substructured populations and no evidence of null alleles. Observed and expected heterozygosities for each locus are shown in Table 2. The population pairwise F_{ST} values (Table 3b) indicate that most significant distances are in comparisons between coastal and inland sites and that the mixed population is genetically more similar to the coastal population.

Ecological variation

The dissimilarity matrix of seven climate variables from each sampling locality revealed that coastal and inland

Table 4 Climatic variables by population

Population name	Temperature seasonality (STD*100)	Temperature range (Max-min)	Annual precip. (mm)	Precip. of wettest month (mm)	Precip. seasonality (CV)	Precip. wettest quarter (mm)
Coastal_1	662.84	27.8	1301	196	151	563
Coastal_2	537.53	26.3	1187	200	76	576
Mixed_3	722.53	31.4	876	127	134	363
Inland_4	816.39	32.8	959	131	174	355
Inland_5	727.81	34.7	714	100	77	286
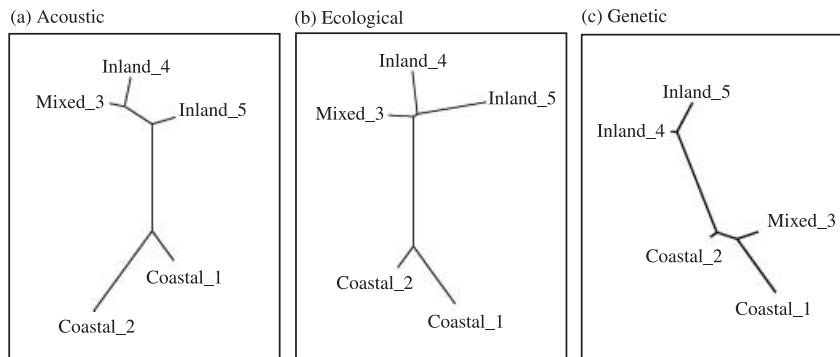
Fig. 4 Neighbour-joining trees representing the relationship between the five populations in: (a) acoustic, (b) ecological, and (c) genetic variation.

populations across our study area occupy distinct climatic regions (Table 3c). Differences in climate between the regions may in part explain the variation in the vegetation communities of the coastal temperate rainforest and the inland coniferous forest. Coastal habitats have more precipitation overall and less seasonal variation in temperature and precipitation than inland habitats (Table 4). The mixed population habitat was more similar climatically to inland habitat than coastal habitat (Tables 3c and 4).

Comparison between acoustic, ecological and genetic distance

The Mantel tests revealed a trend towards a correlation between ecological distance and acoustic distance ($r = 0.612$; $P < 0.07$) and a weak and nonsignificant correlation between acoustic distance and genetic distance ($r = 0.176$; $P < 0.57$). Because the weak correlation between acoustic distance and genetic distance may confound the relationship between acoustic distance and ecological distance, we used a partial Mantel test to statistically remove the portion of the variation in acoustic distance due to genetics. This resulted in a significant relationship between acoustic distance and ecological distance ($r = 0.623$; $P < 0.05$).

The three neighbour-joining (NJ) trees provide a visual representation of the relationships between the five populations in regards to acoustic, genetic and ecological characters (Fig. 4a, b, c). Visual inspection of the trees reveals a close relationship between the Mixed_3, Inland_4 and Inland_5 populations in acoustic and ecological characters, contrasting strongly with the close relationship between the Mixed_3, Coastal_1 and Coastal_2 populations in genetic characters. The discrepancy between the three trees with regards to the relationship of the mixed population to the four pure populations further supports the results from the matrix correspondence analysis, suggesting no significant relationship between acoustic and genetic distance, but a significant relationship between ecological and acoustic distance.

Discussion

A comparison of acoustic, genetic and ecological divergence in five populations of the Swainson's thrush (two coastal, two inland and one mixed population) suggests a potential role for ecological selection in the evolution of vocal divergence in this species. Our two main findings in support of this hypothesis are: (i) the absence of a significant correlation between genetic and acoustic distance suggesting random drift alone cannot explain observed patterns of acoustic variation, and (ii) the correlation between acoustic and ecological distance after accounting for genetic distance, suggesting ecological factors may help drive divergence in spectral and temporal components of Swainson's thrush song. A factor complicating the interpretation of our results is that the correlation between ecology and song divergence could be an artefact of the relationship between acoustic divergence and subspecies boundaries. In other words, the subspecies may differ in a variety of characters important to acoustic divergence, resulting in a spurious correlation between ecology and song. The key piece of evidence against

this alternative interpretation is that the mixed population is genetically more coastal but ecologically and acoustically inland. The ecological and acoustic similarity of the mixed population to inland populations despite apparent on going gene flow with the coastal group suggests convergent evolution in acoustic characters as a result of ecological selection. While the patterns of acoustic, genetic and ecological divergence are suggestive, more extensive sampling throughout the breeding range as well as cross-species comparisons are required before our results can be broadly interpreted.

The effects of environmental variation on acoustic signals have been documented for both suboscines (Seddon 2005) and oscines (Irwin 2000; Slabbekoorn & Smith 2002b; Slabbekoorn & Peet 2003) as well as many nonavian species (reviewed in Coyne & Orr 2004). Differences between coastal and inland populations in specific aspects of climate such as air temperature, humidity and rainfall may directly affect transmission of acoustic signals. In general, attenuation or the reduction in intensity of a sound increases with increasing temperature and decreasing humidity. High frequency sounds attenuate faster than low frequency sounds, and therefore, low frequency sounds generally transmit more efficiently, especially in hot, dry climates (Wiley & Richards 1982; Slabbekoorn 2004). Additionally, the frequency spectrum of noise generated by rainfall often overlaps broadly with the frequency spectrum of acoustic signals used by birds (Lengagne & Slater 2002). In order to compensate for high background noise, individuals may increase signal duration as demonstrated by humpback whales in response to sonar noise (Miller *et al.* 2000) or alter the spectral composition of their acoustic signal as demonstrated by European great tits that increase the minimum frequency of their song in response to urban noise pollution (Slabbekoorn & Peet 2003). Alternatively, climate can indirectly affect vegetation density and vegetation type and influence sound transmission in ways that run counter to the effects of humidity, temperature and rainfall (Marten & Marler 1977; Marten *et al.* 1977). Typically, high frequency, short, syllables characterize songs in open habitat, while low frequency, long syllables characterize songs in dense habitat (Morton 1975; Ryan & Brenowitz 1985).

An analysis of climatic variables across our study region indicates high annual precipitation and low annual variation in precipitation and temperature characterizes coastal temperate rainforest habitat, while seasonal variation in precipitation and temperature characterizes inland coniferous forest habitat. Based upon our analysis of climate variables, one hypothesis that may explain patterns of acoustic variation in the Swainson's thrush is that sound transmission is more difficult in temperate rainforest habitat where background noise generated by rainfall during the breeding season is abundant and mild winters allow for year-round growth of vegetation (Barbour &

Billings 1988). In contrast, sound might travel more easily in inland coniferous forest habitat where summers are drier and understorey vegetation is more sparse (Barbour & Billings 1988). We find that the combination of longer duration and lower initial frequency separates coastal from inland songs — a pattern similar to what we might expect if coastal birds are singing longer in order to compensate for background noise generated by rainfall and at lower frequencies in response to high density vegetation. Future analyses combining climate data with measures of background noise and sound transmission through vegetation may lend insight into the relative importance of direct and indirect effects of climate on spectral and temporal components of Swainson's thrush song.

Previous research suggests that coastal and inland forms of the Swainson's thrush are distinct at the level of mtDNA (Ruegg & Smith 2002), have divergent migratory pathways and overwintering locations (Ruegg & Smith 2002), and occupy distinct ecological regions on the breeding grounds (Ruegg *et al.* in press). Here we show that differences in ecology may help explain patterns of acoustic variation between the five populations in our study. The degree to which differences in song help maintain or promote further divergence between coastal and inland forms of the Swainson's thrush remains an open question and depends in part upon the mode of song-learning in this species. In some songbird species, males learn their entire song repertoire during a sensitive period early in life — this period may be predispersal (Marler 1987; Grant & Grant 1996) or postdispersal (Baptista & Petrinovich 1984; McGregor & Krebs 1989). In other species, learning is open-ended and males continue to change their repertoire throughout their life (Nottebohm 1978; Payne 1981). Theoretical models suggest song divergence in predispersal learners is more likely to lead to genetic subdivision than song divergence in open-ended learners (Ellers & Slabbekoorn 2003). Future research focusing on the mode of song learning in the Swainson's thrush will reveal the potential for acoustic variation to maintain or promote further divergence in this species.

Many questions remain regarding the evolution of vocal divergence and its potential role in speciation. Among the most interesting questions are the interplay between indirect and direct effects of climatic variation on vocal divergence and the balance between song learning and acoustic variation in the evolution of genetic subdivision. Tackling such questions will require continued integration of multiple sources of information. Here we integrate ecological, acoustic and genetic data in a migratory passerine, the Swainson's thrush and find a potential role for ecological selection in acoustic divergence. Our results lend further support to the growing body of research suggesting that ecological variation is important to mating signal divergence across a variety of taxa (Endler 1992; Endler & Basolo 1998;

Irwin 2000; Schluter 2000; Podos 2001; Slabbekoorn & Smith 2002b; Seddon 2005).

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