doi:10.1111/j.1558-5646.2007.00263.x

GENETIC, MORPHOLOGICAL, AND ECOLOGICAL CHARACTERIZATION OF A HYBRID ZONE THAT SPANS A MIGRATORY DIVIDE

Kristen Ruegg

Museum of Vertebrate Zoology, Department of Integrative Biology, University of California, Berkeley, California 94720

E-mail: kruegg@stanford.edu

Received October 4, 2006
Accepted September 10, 2007

This study characterizes a hybrid zone that spans a migratory divide between subspecies of the Swainson's thrush (*Catharus ustulatus*), a long distance migratory songbird, in the Coast Mountains of British Columbia. To assess the potential for a barrier to gene flow between the subspecies, I: (1) analyzed the shape and width of genetic and morphological clines relative to estimates of dispersal distance, (2) assessed the ratio of parental to hybrid genotypes across the hybrid zone, (3) estimated population density across the hybrid zone, and (4) compared the spatial relationship between the hybrid zone and an existing environmental gradient. The results indicate that the hybrid zone is characterized by mostly concordant character clines that are narrow relative to dispersal, the absence of a hybrid swarm, and low population density at the center of the zone. This hybrid zone and additional regions of contact between these subspecies are found on the border between coastal and interior climatic regions throughout the Pacific Northwest. An identified shift in the location, but not the width, of the mtDNA cline relative to the nuclear clines is consistent with asymmetrical hybridization. Neutral diffusion of populations following secondary contact and hybrid superiority within an ecotone are insufficient explanations for the observed patterns. The hypothesis that best fits the data is that the Swainson's thrush hybrid zone is a tension zone maintained by dispersal and ecologically mediated barriers to gene flow.

KEY WORDS: **AFLP, cline, hybridization, ecological selection, environmental gradient, gene flow, migratory divide, tension zone.**

Hybrid zones, narrow regions in which genetically distinct populations meet, mate, and produce offspring, provide a natural laboratory for investigating some of the most challenging questions in evolutionary biology (Hewitt 1988; Harrison 1993). Specifically, understanding the dynamics of gene flow between closely related forms within a hybrid zone may provide insight into the process of speciation. Many hybrid zones are positioned on environmental gradients, and reproductive isolation is thought to evolve as a byproduct of ecological selection (Harrison and Rand 1989; Arnold 1997). In migratory birds, hybrid zones are sometimes correlated with migratory divides (Ticehurst 1938; Hedenstrom and Pettersson 1987; Helbig 1991; Ruegg and Smith 2002; Irwin and Irwin 2004), defined as narrow regions of contact between populations with divergent migratory directions. The link between hybrid zones and migratory divides is especially interesting in light of theoretical and empirical research suggesting that differences in migration-related traits may promote premating and/or postmating reproductive isolation (Rohwer and Manning 1990; Helbig 1991; Bensch et al. 1999; Webster et al. 2002; Irwin and Irwin 2004; Webster and Marra 2005; Bearhop et al. 2005). A first step toward defining the role that migration-related traits may play in speciation is to characterize the dynamics of gene flow across migratory divides.

Ruegg and Smith (2002) used mtDNA and banding recapture data to identify a hybrid zone that spans a migratory divide in the Swainson's thrush (*Catharus ustulatus*) in southern British Columbia. Within the Swainson's thrush, there are two morphologically and genetically distinct subspecies: (1) the coastal, russet-backed group (*C. ustulatus ustulatus*), which breeds in riparian areas west of the Coast, Cascade and Sierra Nevada mountain ranges in North America, migrates along a western flyway, and winters in southern Mexico and Central America; and (2) the inland, olive-backed group (*C. ustulatus swainsoni*), which breeds in boreal and coniferous forests east and north of the Coast, Cascade and Sierra Nevada mountain ranges, migrates along an eastern route, and winters from Panama to the northern tip of Argentina (Rappole and Warner 1980; Evans Mack and Yong 2000; Ruegg and Smith 2002; Fig. 1A). Coastal and inland subspecies are separated by five diagnostic mutations in the mtDNA control region (Ruegg and Smith 2002) and low, but statistically significant differentiation at microsatellite loci (pairwise population F_{ST}s between subspecies range: 0.018–0.043; Ruegg et al. 2006b). Genetic analyses in combination with climatic models of the distribution of populations at the last glacial maximum suggest that divergence likely occurred sometime during the late Pleistocene, and that a subsequent postglacial range expansion led to secondary contact between coastal and inland groups in the Coast Mountains (Ruegg and Smith 2002; Ruegg et al. 2006a). The focus of the present study is to determine whether there is evidence for a barrier to gene flow across the region of secondary contact that spans a migratory divide.

Hybrid zone theory provides a basis for inferring the strength of barriers to gene flow through an analysis of the shape and width of genetic and morphological character clines (Endler 1977; Moore 1977; Barton and Hewitt 1985). Three hypotheses are proposed to explain the existence of steep character clines: the neutral diffusion hypothesis (Endler 1977; Barton and Gale 1993), the bounded hybrid superiority hypothesis (Moore 1977; Moore and Buchanan 1985), and the tension zone hypothesis (Barton and Hewitt 1985). The neutral diffusion hypothesis predicts that in the absence of a barrier to gene flow, steep character clines will decay over time, resulting in clines that are wide relative to root-mean-square (RMS) dispersal distance. Alternatively, the bounded hybrid superiority model states that hybrid zones fall on ecotones and are maintained by ecological selection for hybrids and against parentals within the hybrid zone. Under this model, the width of the hybrid zone varies with the width of the environmental transition (Moore and Price 1993). In contrast, the tension zone hypothesis asserts that hybrid zones are a balance

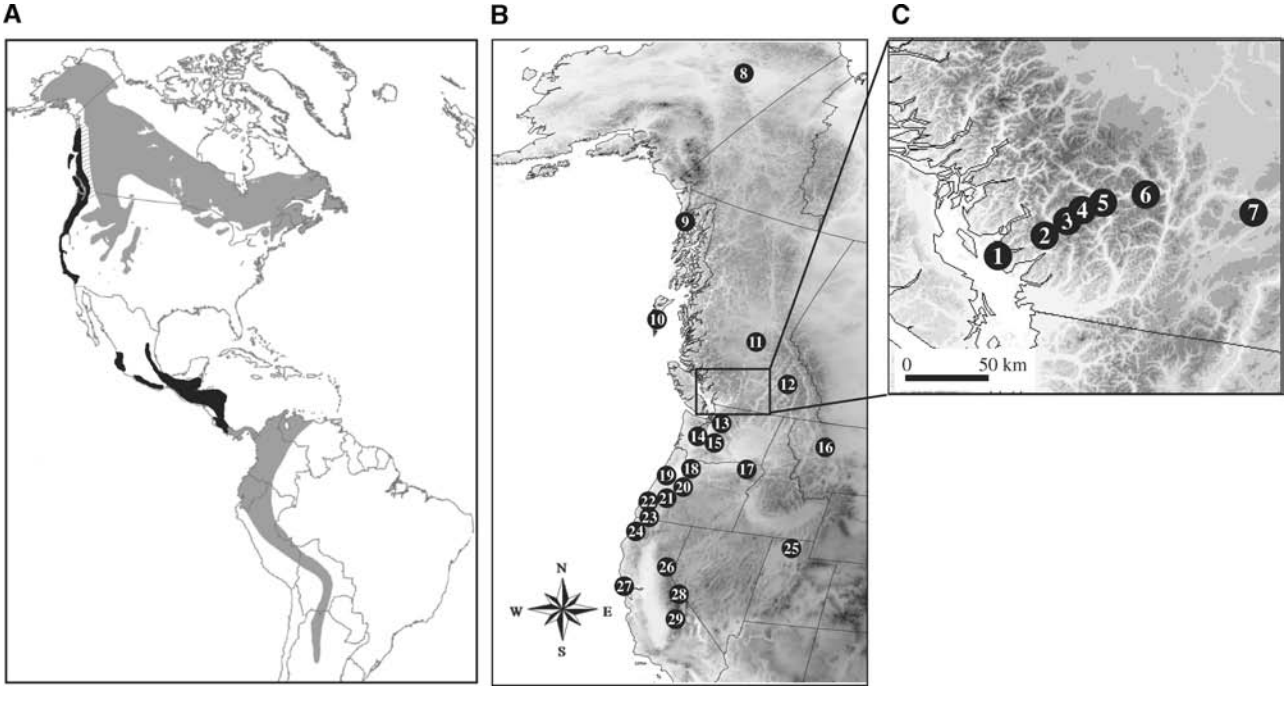

Figure 1. **Range map and sampling localities. (A) Range map of the Swainson's thrush, modified from map created by Cornell Laboratory of Ornithology, Nature Serve (2002). Gray indicates the distribution of the inland form and black indicates the distribution of the coastal form (based on morphologically described subspecies distributions). Dashed line indicates regions of potential secondary contact. (B) Sampling localities across western North America. (C) Sampling localities within the hybrid zone in British Columbia. Numbers refer to location names and details listed in Table 1.**

between dispersal of parentals into the center of the zone and selection against hybrids (Barton and Hewitt 1985). Tension zones are characterized by multiple coincident character clines that are narrow relative to RMS dispersal, and by regions of low population density in the center of the hybrid zone (Barton and Hewitt 1985; Barton and Gale 1993). This latter model has been applied in analyses of intrinsic (e.g., hybrid breakdown) and extrinsic (e.g., ecological) selection against hybrids (Bridle et al. 2001; Phillips et al. 2004; Alexandrino et al. 2005).

This article has two main goals: (1) to determine whether there is evidence for a barrier to gene flow between Swainson's thrush subspecies representing alternate migratory forms; and (2) to place the hybrid zone in a broader geographic and ecological context to determine the potential role of ecological selection in the maintenance of the subspecies boundary. To accomplish the first goal, I calculated genetic, morphological, and plumage cline positions and widths relative to estimates of RMS dispersal distance for the Swainson's thrush; estimated the ratio of parental to hybrid genotypes across the hybrid zone; and assessed population density across the hybrid zone. To accomplish the second goal, I compared the geographic location of the hybrid zone with an environmental gradient and assessed the distribution of mtDNA clades across Western North America relative to the transition between coastal and interior climatic regions.

Methods

BROAD SCALE SAMPLING

The two major groups within the Swainson's thrush have been referred to previously as coastal and inland (Ruegg and Smith 2002; Kelly et al. 2005), russet- and olive-backed (Evans Mack and Yong 2000), and the *ustulatus* and *swainsoni* subspecies (Evans Mack and Yong 2000) on the basis of plumage color (Bond 1963; Phillips 1991) and genetic differences (Ruegg and Smith 2002; Ruegg et al. 2006b). Throughout this article I use the terms coastal and inland to refer to the subspecies. To examine the broad scale distribution of coastal and inland mtDNA clades, I combined my sampling efforts with those of 19 bird banding stations to sample 460 total individuals (Table 1; Fig. 1B). Genetic samples consisted of the calamus of one rectrix or approximately 100 μl of blood collected by brachial vein puncture and preserved in lysis buffer (Seutin et al. 1991).

MORPHOLOGY AND PLUMAGE ACROSS THE HYBRID ZONE

To characterize the hybrid zone, I established a southwest-to-northeast transect through a narrow valley transecting the Coast Mountains in southern British Columbia (Fig. 1C). This is essentially perpendicular to the direction of contact between the subspecies as defined morphologically (Fig. 1A), and for the purpose of cline analysis, it was assumed to represent the steepest transition between the two forms. Fieldwork was conducted during the month of June in the years 2000–2005. At each of seven locations (locations 1–7, Table 1; Fig. 1C), I captured 20–44 individuals using mist nets (Ralph et al. 1993). Each individual was measured, banded, and sampled for genetic analysis. Photographs of wing shape and plumage color for a subset of individuals from across the hybrid zone can be found in the University of California Museum of Vertebrate Zoology's digital collection.

To exclude the effects of sexual size dimorphism and to limit the possibility of inadvertently including migrants or hatch year birds, only adult breeding males known to be singing on their territories were used in the morphological analysis. I measured six morphological characters following the methods of Baldwin et al. (1931): wing cord (length of unflattened wing from bend of wing to longest primary); tail (point of insertion of central rectrices to tip of longest rectrix); tarsus length (the length between the intertarsal joint and the distal end of the last leg scale before the toes emerge); culmen length (from the anterior edge of the nare to the tip of the bill); bill depth (at its base); and bill width (at its base).

I quantified plumage coloration in live birds using an X-Rite digital swatchbook spectrophotometer (X-Rite Inc., Grandville, MI). The digital swatchbook provides an unbiased, repeatable method of quantifying plumage coloration in the field (Hill 1998). Plumage color was measured as the average of four spectral readings taken from the central back region. Each spectral reading consisted of the percent transmission at 10-nm intervals from 390 nm to 700 nm. I used Principle Components Analysis (PCA; SPSS Inc., Chicago, IL) to analyze the morphological and plumage color data.

GENETIC ANALYSIS

Previous phylogenetic analysis of mtDNA control region sequences identified two reciprocally monophyletic haplotype groups corresponding to coastal and inland clades (Ruegg and Smith 2002). To assign individuals from across the Pacific Northwest to coastal and inland groups, including those from the hybrid zone transect, I screened 455 individuals using a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method (see Ruegg and Smith 2002).

To assess the degree of hybridization between coastal and inland forms within the hybrid zone, I screened amplified fragment length polymorphisms (AFLPs) following a modified version of the methods used in Vos et al. (1995). To limit the possibility of false negatives, only high quality extractions (260/280 ratio between 1.8 and 2.0) with a starting concentration greater than 20 ng/μL were used in the AFLP analysis. Approximately 250 ng of total genomic DNA was digested with 1 unit of *MseI* and 5 units of *EcoRI* and ligated with 60 Weiss units of T4

Table 1. Locations of sampling localities and numbers of individuals belonging to the coastal and inland mtDNA clades.

Code	Location name (km along transect)	State / Prov.	Latitude	Longitude	mtDNA assignment	
					Coastal (*N*)	Inland (*N*)
1	Sunshine Coast (0)	BC	49.50	−123.75	34	2
2	*Squamish (33)	BC	49.91	−123.29	37	7
3	Whistler (51)	BC	50.09	−123.04	8	12
4	Shadow Lake (63)	BC	50.22	−122.88	12	14
5	Pemberton (70)	BC	50.31	−122.80	9	31
6	Lillooet (116)	BC	50.53	−122.13	0	31
7	Kamloops (215)	BC	50.50	−120.36	1	19
8	*Yukon Flats	AK	66.38	−148.10	0	15
9	*Tongass National Forest	AK	58.42	−136.55	5	5
10	*Queen Charlotte Island	BC	53.00	−132.00	20	0
11	*Quesnel	BC	53.00	−122.50	0	20
12	*Revelstoke	BC	50.89	−118.20	0	20
13	Mt. Baker National Forest	WA	48.05	−121.50	10	0
14	Pierce County, Fort Lewis	WA	47.06	−122.58	7	0
15	Wenatchee National Forest	WA	46.95	−121.31	5	4
16	*Flathead National Forest	MT	47.93	−113.00	0	20
17	*Umatilla National Forest	OR	45.83	−117.95	0	20
18	Colton	OR	45.09	−122.27	10	0
19	*Siuslaw National Forest	OR	44.33	−123.00	20	0
20	Willamette National Forest #1	OR	44.20	−122.00	12	0
21	Willamette National Forest #2	OR	43.32	−122.85	2	1
22	Camas Valley	OR	42.94	−123.69	8	0
23	Siskyou National Forest	OR	42.15	−123.42	11	1
24	Humboldt	CA	41.25	−123.70	9	0
25	*Migratory Bird Refuge	UT	41.50	−112.37	0	11
26	Tahoe National Forest	CA	39.62	−120.53	0	2
27	*PRBO	CA	37.92	−122.75	20	0
28	Mono Lake	CA	37.97	−119.11	0	1
29	Sequoia National Park	CA	36.78	−118.58	2	0

*Indicates samples that were included in Ruegg and Smith (2002).

DNA ligase (New England Biolabs Inc., Ipswich, MA), 1.0 μL of 50 μM E-adaptor (5′-CTCGTAGACTGCGTACC-3′ and 3′-CATCTGACGCATGGTTAA-3′), and 1.0 μL of 5 μM M-adaptor (5′-GACGATGACTCCTGAG-3′ and 3′-TACTCAGGACTCAT-5′) (Vos et al. 1995). The restriction-ligation reaction was incubated at room temperature (25°C) for 12–18 h and subsequently diluted with H_2O to a volume of 100 μL, for a final DNA concentration of 2.5 ng/μL. Products were stored at −20°C for up to six months. The preselective amplification followed the methods of Bensch et al. (2002). In short, 10 μL of the diluted ligation-digestion product was combined with 1.9 μL of H_20, 0.06 μL of 100 μM E-primer with an additional T (5′-GACTGCG TACCAATTCT-3′), 0.06 μL of 100 μM M-primer with an additional C (5′-GATGAGTCCTGAGTAAC-3′), 4.0 μL of 1 mM dNTPs, 1.9 μL of 25 mM $MgCl_2$, 2.0 μL of 10× PCR buffer, and 0.08 μL of 5 u/μL Taq DNA polymerase (Roche Inc., Nutley, NJ). The thermocycle for preamplification was as follows: 94°C for 2 min, followed by 20 cycles of 94°C for 30 sec, 56°C for 30 sec, and 72°C for 60 sec, and a final extension at 72°C for 10 min. Eight microliter of product was visualized on a 1% agarose gel and the remaining 12 μL was diluted in 100 μL of H_2O and stored at −20°C. For selective amplification, 2.5 μL of diluted preamplification product was added to 2.2 μL of H_20, 0.6 μL of 10 μM fluorescently labeled selective amplification E-primer (FAM, blue), 0.6 μL of 10 μM selective amplification M-primer, 2 μl of 1 mM dNTPs, 0.6 μL of 25 mM $MgCl_2$, 1 μL of 10× PCR buffer, and 0.08 μl of 5 u/μL Taq DNA polymerase. The selective amplification reactions were incubated according to the following protocol: 2 min denaturation at 94°C, followed by 12 cycles of 94°C for 30 sec, 65°C for 30 sec (increasing by 0.7 with each cycle), 72°C for 60 sec, 23 cycles of 94°C for 30 sec, 56°C for 30 sec, and 72°C for 60 sec, and a final extension at 72°C. Selective amplification products were diluted with two parts TE buffer, combined with one volume formamide, 0.25 μL blue dextran, and

0.50 μl GeneScan-500 (Perkin Elmer Inc., Waltham, MA) ROX-labeled (red) size standard, and run on a 5% polyacrylamide gel. Fluorescently labeled products were visualized by running on an ABI 377 for 2.5 h.

To identify repeatable and polymorphic loci, I prescreened 15 selective primer combinations in 20 individuals (10 coastal, 10 inland), with one individual from each of these groups run five times. The initial screening panel was composed of individuals from a diversity of coastal and inland locations (coastal populations 8 and 1, inland populations 7, 9, and 10; Table 1; Fig. 1) to ensure that the markers were informative on a broader geographic scale. The ABI Gene Scan files were visualized using Genographer software (http://hordeum.oscs.montana.edu/genographer/). All visible bands from 50 to 350 base pairs were scored in the initial screening of primer combinations. Most AFLP bands were monomorphic or present at low frequency. To select robust and repeatable polymorphic markers for screening in all individuals across the hybrid zone, I considered only AFLP bands that occurred in 25–75% of individuals and were repeatable within an individual. Of the 15 primer combinations screened, nine primer pairs yielded polymorphic markers meeting the above criteria, resulting in a total of 15 markers that were scored in 154 individuals across the hybrid zone.

To determine the frequency of coastal, inland, and hybrid individuals across the hybrid zone, AFLP data were scored as present or absent for each band size and the data were analyzed using NewHybrids software, version 1.1 beta 3 (Anderson and Thompson 2002). Using a Markov chain Monte Carlo (MCMC) scheme incorporating allele frequency uncertainty, NewHybrids computes, for each individual, the posterior probability of belonging to pure coastal, pure inland, F_1, F_2, coastal backcross, and inland backcross categories. These categories are defined in terms of the expected proportions (E_0, E_1, and E_2) of loci at which an individual carries 0, 1, or 2 gene copies of coastal origin. For example, a coastal backcross bird has $E_0 = 0$, $E_1 = 0.5$, and $E_2 = 0.5$; an F_1 has $E_0 = 0$, $E_1 = 1.0$, and $E_2 = 0.0$. The model underlying NewHybrids includes the population of origin of each gene copy as a latent variable, allowing inference even with markers having nondiagnostic allele frequency differences. An AFLP marker is modeled as a diallelic locus with alleles, *a* and *A*, that are unobserved latent variables that get integrated out during MCMC. The observed datum at each AFLP locus is the presence or absence of a band, which is determined by the alleles carried at the locus: that is, *aA* and *AA* yield bands, whereas *aa* does not (Eric C. Anderson, NOAA Fisheries, pers. comm.).

Numerous, randomly started MCMC runs confirmed consistent convergence. Final results were obtained with 10,000 sweeps of burn-in and 50,000 sweeps of sample collection. Summing $E_1 + 2E_2$ over all hybrid and parental categories, weighted by the average posterior probability for each category across all individuals at each sampling site, yielded an estimate of the average proportion of genes of coastal origin at each sampling site. This proportion was used in the following cline analysis to estimate the change in nuclear genetic frequency across the hybrid zone. It should be noted that given the number of divergent AFLP markers available, second- or third-generation (or more advanced) backcrosses may have high posterior probability for the "parental" categories. This does not, however, greatly affect the identification of recent hybrids (F_1s, F_2s, and first-generation backcrosses) or the estimation of the proportion of coastal alleles in each population. Therefore throughout the remainder of the article, the term "parental" refers to anything greater than or equal to a second-generation backcross, whereas the term "hybrid" refers to F_1s, F_2s, and first-generation backcrosses.

CLINE COINCIDENCE AND WIDTH

I fitted maximum-likelihood (ML) clines for morphological characters (body size estimated as PC1 from the morphological analysis, and plumage color estimated as PC2 from the analysis of spectral data) and molecular characters (proportion of coastal mtDNA and proportion of coastal alleles based on the AFLP analyses) using the tanh and exponential cline fitting model of Szymura and Barton (1986) as implemented in the ANALYSE software package (Barton and Baird 1996). A tanh cline is defined as $y = (1 + \tanh(2(x-c)/w))/2$, where x is the distance from the center of the cline, c is the position of the center of the cline, and w is the width of the cline, defined as the inverse of the maximum slope (Szymura and Barton 1986). I assumed a simple single locus tanh cline and did not explore the more complex stepped cline due to limitations in my sampling at the tails of the clines (Barton and Gale 1993). The tanh cline one-dimensional model was first developed for one locus with two alleles ranging in frequency from 0 to 1, but has subsequently been applied to assignment scores for nondiagnostic loci and morphological traits (Bridle et al. 2001; Phillips et al. 2004; Takami and Suzuki 2005). To apply the model to quantitative characters, morphology and plumage population means were scaled to values between 0 and 1 following the methods of Takami and Suzuki (2005). P_{max} and P_{min} (the maximum and minimum gene frequency values in the tail ends of a cline) were set at 0 and 1 and not allowed to vary. The distribution of sampling localities was most appropriate for a one-dimensional cline analysis—sampling started at the edge of the Pacific Coast of British Columbia and continued in a northeasterly through a narrow valley within the Coast Mountains (Fig. 1C). Based on the broad scale assessment of the distribution of coastal and inland groups (see Results), this orientation represents a good approximation of the direction of steepest character change.

I evaluated cline center coincidence and cline width concordance using the ML cline fitting procedures described by Phillips et al. (2004). In short, the likelihood surface of each character

was explored stepwise along the axes for both center position c and width w simultaneously. The ML confidence intervals were estimated at the points where the log likelihood dropped two units below the maximum. Cline center coincidence and cline width concordance were assessed using a likelihood ratio test, in which values of the ML cline center and width calculated assuming coincidence and concordance were compared with values of ML centers and widths calculated assuming no coincidence and no concordance (Phillips et al. 2004). A significant difference between the ML values of the consensus cline width and center and the ML values generated for each individual cline suggests clines are not concordant.

ESTIMATION OF DISPERSAL DISTANCE

The RMS dispersal is the distance along a single dimension from where an individual was born to where it lays its first clutch (reviewed in Moore and Dolbeer 1989). I estimated RMS dispersal distance using U.S. Fish and Wildlife Service (USFWS) recovery records for Swainson's thrushes and the methods described by Moore and Dolbeer (1989). Bird banding localities are recorded in 10-min latitude–longitude blocks and therefore any movement within the 10-min block was considered no dispersal. Due to the fact that short-distance movements factor less into the overall calculation of RMS dispersal than do long-distance movements (Moore and Dolbeer 1989), the potential bias toward underestimating dispersal distance was considered negligible. Only nestling and hatch year birds banded and recaptured in the months of June, July, and August, when Swainson's thrushes are most likely near the nesting site and least likely to be migrating (Evans Mack and Yong 2000), were considered in the analysis. An estimate of RMS dispersal was calculated as the square root of the sum of the individual dispersal distances squared, divided by the number of observations (Moore and Buchanan 1985).

ENVIRONMENTAL GRADIENT AND DENSITY ANALYSIS

To compare the position of the hybrid zone to variation in climate, I used DIVA-GIS software (Hijmans et al. 2004). I extracted 10 climate variables (summarized as means for the years 1950–2000) from each sampling point based on an interpolated climate surface created by Worldclim, a global climate database with a spatial resolution of $\sim$ 1 km (Hijmans et al. 2005). Ten variables were selected from a group of 16 environmental variables because they were the least correlated ($r < 0.80$): (1) seasonal variation in temperature (coefficient of variation across all months); (2) mean temperature of the driest quarter; (3) annual precipitation; (4) seasonal variation in precipitation (coefficient of variation across all months); (5) precipitation of the coldest quarter; (6) precipitation of the warmest quarter; (7) precipitation of the driest quarter; (8) annual mean temperature; (9) mean temperature of the wettest quarter; and (10) mean temperature of the warmest quarter. The data were reduced into two vectors using PCA, and the PC1 score for each population was plotted to illustrate overall climatic variation across the hybrid zone.

To estimate density across the hybrid zone, I conducted transects following the methods of Ralph and Scott (1981). In short, density was approximated as the number of birds seen or heard singing or calling while walking a 100-m transect in 10 min. To reduce the potential for variation in the data given weather, time of day, and time of year, all transects were conducted on clear weather days, at peak singing h (6:30–9:00 p.m.), and during the beginning of the breeding season in British Columbia (June 1–June 29) (Campbell et al. 1997). All transects were conducted at sampling locations where Swainson's thrushes are known to occur and care was taken to conduct transects in the highest quality habitat available (usually near a lake or a stream within designated Forest Service land). A total of 13 transects were completed. In some locations, especially in the center of the hybrid zone in which personal observation suggested that density was low, two or three replicate transects were completed at multiple sites within a sampling locality on the same evening and the data were pooled to attain a mean density with standard errors. Coastal transects were conducted approximately two to three weeks before inland transects to account for variation in the start of the breeding season within coastal and inland habitat types (Campbell et al. 1997).

Results

BROAD SCALE DISTRIBUTION OF MTDNA CLADES

PCR-RFLP screening of individuals from across western North America indicates that coastal and inland mtDNA clades are roughly concordant with the distributions of the previously described olive- and russet-backed subspecies (Evans Mack and Yong 2000) (Figs. 1A, 3A). The coastal mtDNA clade is restricted to the Pacific Coast, west of the Sierra, Cascade, and Coast Mountain ranges, and as far north as Tongass National Forest near Juneau, Alaska, whereas inland populations are found throughout the remainder of the breeding range (Fig. 3A; Table 1). The PCR-RFLP screen also revealed admixture between coastal and inland populations and thus potential hybridization in five separate locations: (1) the Coast Mountains of southern British Columbia (populations 1–7); (2) Wenatchee National Forest, Washington (population 15); (3) Siskyou National Forest, Oregon (population 23); (4) Willamette National Forest, Oregon (population 20); and (5) Tongass National Forest, Alaska (population 9; Figs. 1B, 3A; Table 1). All regions of contact are within the Coast and Cascade Mountain ranges. Differences in mtDNA from populations on either side of the Sierra Crest in California are concordant with expectations from the described distribution of subspecies—inland populations are to the east and coastal populations are to

the west of the Sierra Crest (Fig. 3A). Sample sizes in the Sierras were low, however, and additional sampling may reveal admixed populations in this region.

MORPHOLOGY AND PLUMAGE

The PCA of morphological traits and the univariate analysis of morphological measurements indicate that coastal birds are larger than inland birds (Appendix 1; Fig. 2A). PC1 accounted for 37% of the total variation in the data, whereas PC2 accounted for 23%. All morphological traits included in the analysis loaded positively along PC1 (component matrix: tail = 0.335, tarsus = 0.622, culmen = 0.751, depth = 0.712, width = 0.530), suggesting that PC1 represents differences in body size.

The PCA of plumage measurements indicates that coastal and inland populations differ in plumage color (Appendix 1; Fig. 2B). PC1 accounted for 70% of the variation in the data, whereas PC2 accounted for 28%. Coastal and inland populations separated along PC2 (Fig. 2B), an axis that is generally thought to represent hue and saturation components of color (Grill and Rush 2000), but did not separate along PC1, which represents brightness or color intensity. These observed differences reflect the fact that coastal populations have significantly more russet-colored plumage whereas inland populations have olive-colored plumage.

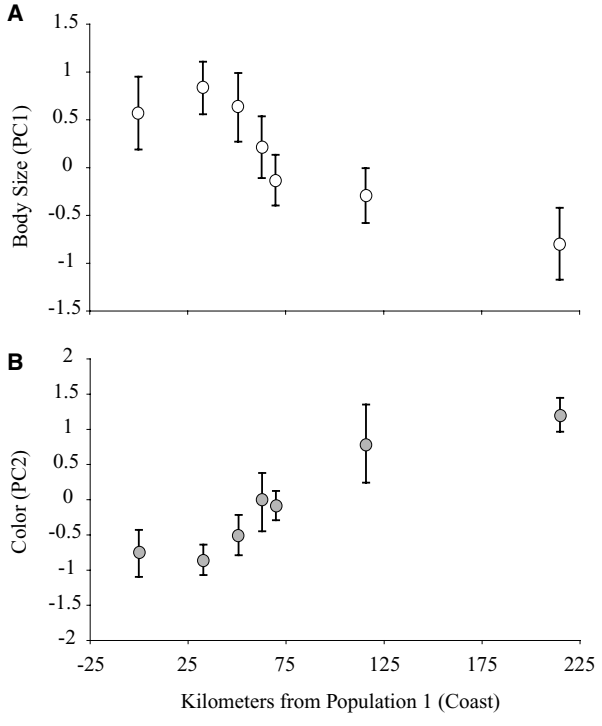

Figure 2. Morphology and plumage color population means across the hybrid zone (±2 standard error). (A) Mean PC1 from the morphological analysis, demonstrating larger body size in coastal birds. (B) Mean PC2 from the plumage color analysis, indicating that coastal and inland birds have differently colored plumage.

GENETICS OF HYBRID ZONE POPULATIONS

The PCR-RFLP analysis of mtDNA indicates a sharp transition (<115 km) between coastal and inland forms across the British Columbia transect (populations 1–7; Fig. 3B, C). Ninety-four percent of individuals in population 1 have coastal mtDNA (Fig. 3B) and the percentage of individuals with coastal mtDNA becomes incrementally less in successive populations with distance from the coast, until population 7 in which 95% of individuals have inland mtDNA (Fig. 3B, Table 1).

The AFLP analysis revealed that of the 15 polymorphic loci screened, eight showed significant frequency shifts across the hybrid zone, and four of those eight were fixed for alternative alleles at opposite ends of the hybrid zone (Appendix 2). The assignment of individuals to coastal, inland, and hybrid categories using 15 AFLP markers and the NewHybrids software reveals a sharp transition between coastal and inland forms across the hybrid zone (Fig. 3C; Table 2). Parental individuals occur throughout the hybrid zone, with 27% of the individuals in the centermost population (population 5) inferred to be parentals, 40% inferred to be hybrids, and 33% were of uncertain assignment (Fig. 3C; Table 2). All of the individuals in populations 1 and 2 were classified as coastal, and the percentage of individuals classified as coastal becomes incrementally less in successive populations until population 7, in which 100% of the individuals were classified as inland. Twelve of the individuals could not be classified with greater than 90% posterior probability to any one of the three categories (Table 2).

CLINE COINCIDENCE AND WIDTH

Overall the analysis of cline width and center indicates that clines for body size, mtDNA, plumage color, and AFLP assignment are concordant in width, but that the center of the mtDNA cline is shifted significantly toward the coast (Fig. 4). The likelihood-ratio test indicated no significant difference in likelihood scores when the clines were considered separately versus when they were considered together ($G_{Wsame–Wdiff} = 4.7$, df = 3, $P > 0.05$), suggesting that all four clines are similar in width. The combined cline width was approximately 80 km (CI = 66–97). Constraining all clines to a common center resulted in a significant decrease in likelihood ($G_{Csame–Cdiff} = 11.6$, df = 3, $P < 0.05$). Visual inspection of the log-likelihood scores indicates that the cline center for mtDNA is shifted approximately 25 km southwest (Table 3). Removing mtDNA and constraining the remaining clines to a common center is consistent with cline center coincidence ($G_{Csame–Cdiff} = 0.03$, df = 2, $P > 0.05$). Without mtDNA, the combined cline center was 79 km (CI = 73–86) northeast of population 1, located near population 5.

ESTIMATION OF DISPERSAL DISTANCE

Of the 251 recovery records in the USFWS database for the Swainson's thrush, only 10 nestling or hatchling year birds were banded

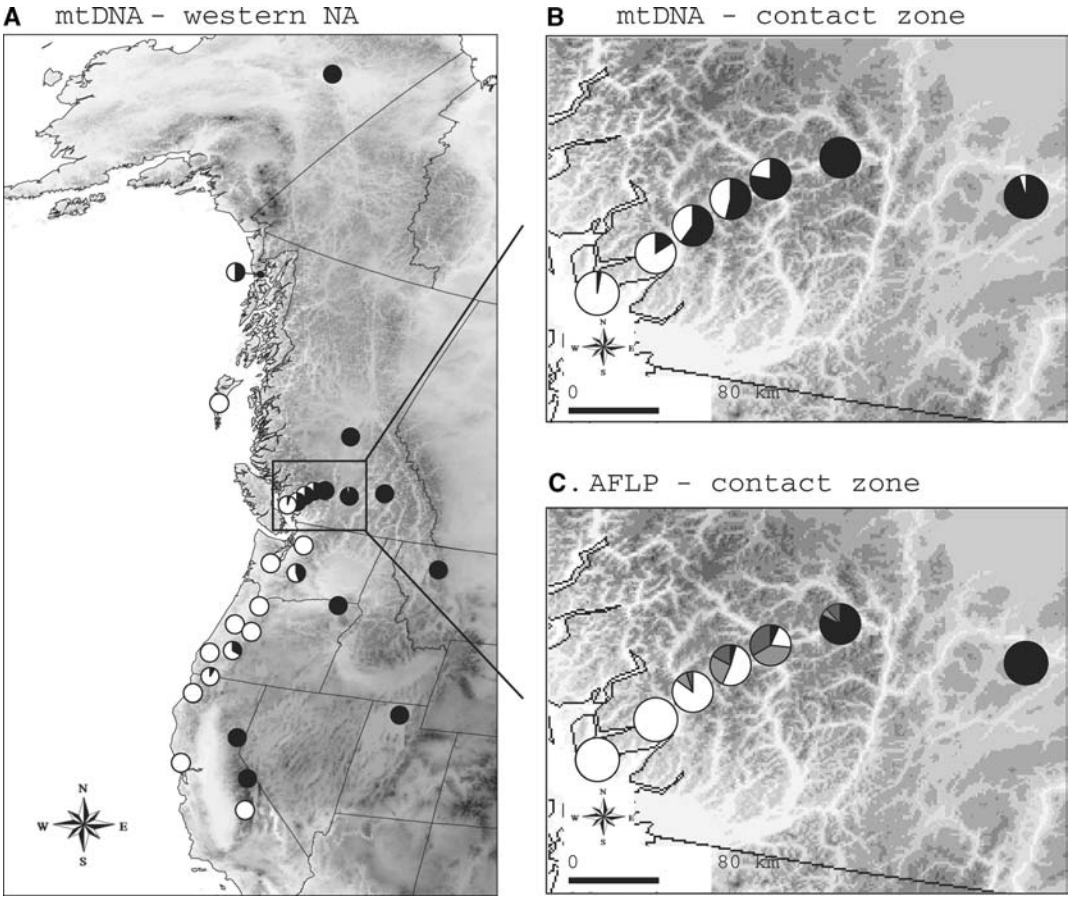

Figure 3. Results of PCR-RFLP screening for mtDNA clade membership across (A) western North America and (B) the hybrid zone. Pie diagrams indicate the proportion of individuals belonging to coastal (in white) and inland (in black) mtDNA clades. For location details and numbers of individuals belonging to each clade, see Table 1. (C) Results of AFLP screening within the hybrid zone. White, black, light gray, and dark gray indicate the proportion of individuals assigned to coastal, inland, hybrid, and unknown categories, respectively. For numbers of individuals assigned to each category, see Table 2.

and then recaptured in subsequent years during the months of June, July, and August. The calculated dispersal distance was 0 for six individuals not captured outside the original 10-min latitude–longitude block in which they were banded, and 476 km, 8 km, 11 km, and 11 km, for the four individuals captured outside of the 10-min latitude–longitude block, yielding an estimate of RMS dispersal of 150 km.

CLIMATIC GRADIENT AND DENSITY ANALYSIS

The analysis of climatic variables revealed that the hybrid zone is situated on a steep environmental gradient (Fig. 5A). PC1 accounted for 75% of the variation in the data and is explained by differences between coastal and inland climatic regions in seasonal variation in temperature and precipitation: seasonal variation in temperature loaded strongly negatively (−0.927), whereas

Table 2. Proportion of individuals per population with a >90% posterior probability of belonging to coastal, hybrid, inland categories based on their AFLP phenotype. Individuals with <90% posterior probability of belonging to one of the three categories are classified as unknown.

	Location	Coastal (*N*)	Inland (*N*)	Hybrid (*N*)	Unknown (*N*)
1	Sunshine Coast	1.00 (29)	0 (0)	0 (0)	0
2	Squamish	1.00 (7)	0 (0)	0 (0)	0
3	Whistler	0.85 (17)	0 (0)	0.10 (2)	0.05 (1)
4	Shadow Lake	0.52 (12)	0.05 (1)	0.26 (6)	0.17 (4)
5	Pemberton	0.20 (3)	0.07 (1)	0.40 (6)	0.33 (5)
6	Lillooet	0 (0)	0.82 (14)	0.06 (1)	0.12 (2)
7	Kamloops	0 (0)	1.00 (19)	0 (0)	0

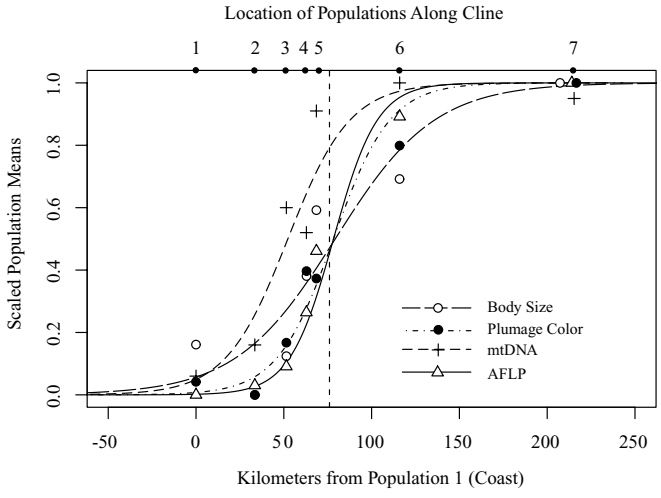

Figure 4. ML-fitted sigmoid clines generated using ANALYSE software. Vertical dashed line represents the consensus cline center. Values on the lower x-axis are scaled relative to the distance from population 1. Population numbers along the upper x-axis refer to localities listed in Table 1 and Figure 1.

annual precipitation (0.947), seasonal variation in precipitation (0.972), precipitation of the driest quarter (0.923), precipitation of the warmest quarter (0.968), and precipitation of the coldest quarter (0.999) loaded strongly positively. The PC1 score per population suggests that the climate becomes drier and more seasonal as one moves northeast across the hybrid zone transect (Fig. 5A).

The number of birds heard singing along a 100-m transect, a proxy for overall density of breeding individuals, was lowest in the three populations near the center of the hybrid zone (Fig. 5B). The census data suggest that the hybrid zone is correlated with a region of low population density. Over the course of the six years of this study, the census data remain consistent with the trapping effort at each sampling locality, with birds being more difficult to locate and capture in the center of the hybrid zone (K. Ruegg, unpubl. data). The region of lowest population density was found near the center of the hybrid zone and the center of the transition between the coastal and inland climatic regions (Fig. 5A, B).

Discussion

Although avian hybrid zones have been studied extensively (reviewed in Barton and Hewitt 1985; Harrison 1993), this is one of just a few studies that has characterized a hybrid zone that spans a migratory divide (but see Rohwer and Manning 1990; Bensch et al. 1999). To clarify the dynamics of gene flow between subspecies of the Swainson's thrush that also represent alternate migratory forms, the following discussion will be separated into two parts: (1) placing the hybrid zone in a broader geographic and ecological context; and (2) characterizing the hybrid zone in relation to the neutral diffusion model, the bounded hybrid superiority model, and the tension zone model.

BROAD SCALE DISTRIBUTION OF MTDNA

The broad scale analysis of mtDNA in Swainson's thrush populations from across western North America indicates that all regions of potential hybridization fall on environmental gradients. The analyses highlight five locations within the Coast and Cascade mountain ranges where both coastal and inland mtDNA types occur (Fig. 3A). The crest of the Coast and Cascade Mountains and the Sierra Nevada Crest impose a significant precipitation shadow resulting in a continent-wide shift from moist, coastal coniferous forest to the cool-dry forests of interior Western North America. A recent study of climatic space occupied by the Swainson's thrush supports the idea that coastal and interior populations occupy distinct climatic regions (Ruegg et al. 2006a). The position of mixed populations at the transition between coastal and interior climatic regions raises the possibility that differences in migration-related traits are a byproduct of environmental differences. If differences in migration-related traits subsequently result in premating or postmating reproductive isolation then this may help explain the location of the Swainson's thrush migratory divide and corresponding hybrid zone on an environmental gradient. A similar scenario has been proposed to explain the existence of the Baltimore and Bulluck's oriole hybrid zone in North America. Rohwer and Manning (1990) suggest that differences between Baltimore and Bullok's orioles in molting times are an adaptation to mesic and xeric climates and that hybrids with an

Table 3. Cline widths and centers for morphological and genetic characters (±2 maximum-likelihood scores are shown in parentheses).

Character	Width	Center (km)	Log likelihood (km from pop. 1)
Morphology			
Body size (mean PC1)	113 (81,165)	79 (68, 92)	−13.039
Plumage color (mean PC2)	64 (42, 108)	78 (69, 94)	−3.862
Genetic characters			
AFLP (% coastal alleles)	50 (31, 81)	78 (71, 86)	−1.211
mtDNA	72 (50, 105)	52 (44, 61)	−10.716

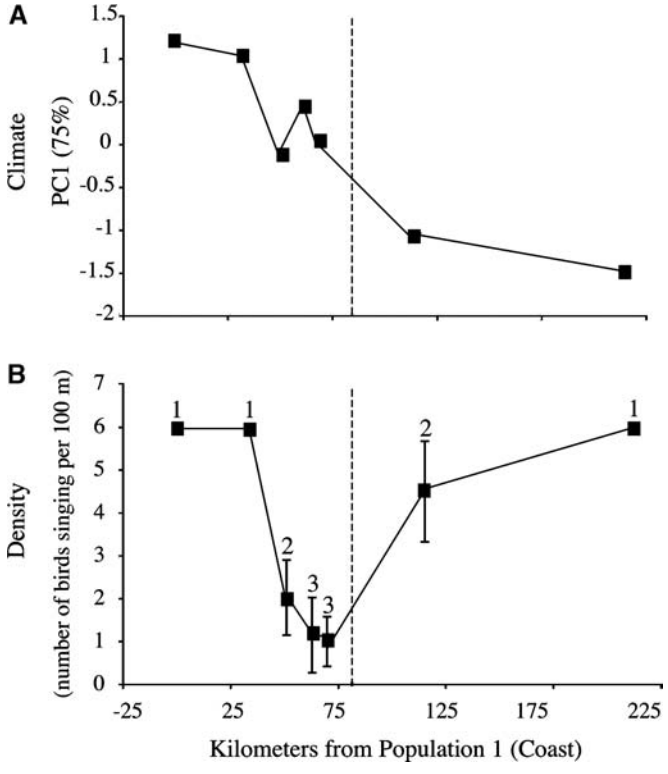

Figure 5. Climate and density in the Swainson's thrush hybrid zone. The dotted line represents the approximate consensus cline center from the cline analysis. (A) Climate variation summarized as the mean PC1. (B) The number of birds singing along a 100-m transect as a proxy for density. Numbers above the mean density represent the number of transects completed in each locality.

intermediate molting time may be selected against. The coastal-to-interior climate shift is correlated with hypothesized regions of contact between numerous sister species and subspecies of birds (Johnson 1978; Cicero 2004) and the role that migration-related traits may play in the maintenance of species and/or subspecies boundaries provides an interesting area for future research.

CHARACTERIZATION OF THE BRITISH COLUMBIA HYBRID ZONE

Neutral diffusion following secondary contact

Hybrid zones are complex entities and understanding the factors responsible for their existence requires consideration of multiple alternatives. One possibility is that the Swainson's thrush hybrid zone is a region of neutral diffusion following secondary contact (e.g., Barrowclough 1980). The neutral diffusion hypothesis proposes that in the absence of selection, the cline width (w) will be proportional to the RMS dispersal per generation (σ) and the number of generations since contact (t) ($w \approx \sigma t^{0.5}$; Endler 1977; Barton and Gale 1993).

Solid estimates of RMS dispersal are notoriously difficult to attain (Barton and Gale 1993), especially for migratory passerines (Barrowclough 1978). Direct estimates from mark–recapture studies tend to underestimate dispersal as a result of limitations imposed by the size of the study area (e.g., 1 km for Audubon warblers, Barrowclough 1980; 33 km for Great Reed warblers, Hansson et al. 2002), whereas estimates based on banding recapture data may be inflated by the possible inclusion of nonbreeding birds (e.g., 111.4 km for Common Grackles, 94.6 km for Red-winged Blackbird, Moore and Dolbeer 1989). Indirect estimates based on morphological data (e.g., 31 km for Hermit/Townsend warblers, Rohwer and Wood 1998) are also prone to wide confidence intervals. I calculated an estimate of 150 km for the Swainson's thrush using banding recapture data. Although there are many reasons to believe that RMS dispersal is large for migratory birds (Moore and Dolbeer 1989), the low sample size and the possible inclusion of nonbreeding birds suggests that caution should be used when interpreting this estimate of RMS dispersal for the Swainson's thrush.

Given an estimate of RMS dispersal and a probable amount of time since contact, the expected cline width can be compared with the actual cline width to determine the likelihood that the observed clines are a result of neutral diffusion. Genetic evidence and climatic models of change in population and range size support the idea that coastal and inland populations underwent postglacial range expansions (Ruegg et al. 2006a) and have likely been in contact for approximately 6000 years. Assuming 6000 years since contact (generation time $\approx$ 2 years) and RMS dispersal of 150 km, the expected cline width under neutral diffusion would be more than 8000 km greater than the observed cline width of 80 km. For the observed cline width to be consistent with the neutral diffusion model, dispersal would have to be less than or equal to 1.46 km, which would be among the smallest estimates of dispersal for a migratory passerine.

It is also possible that although suitable habitat has been present for 6000 years, habitat differences between the subspecies prevented contact until anthropogenic disturbances broke down ecological barriers (Gill 1980; Gill and Lanyon 1997; Lamont et al. 2003). Under this scenario, the time since contact would likely date back to the early 1900s when the Great Pacific Railway was built, transecting populations 2–7, and logging cleared much of the old-growth forest from the lower valley (Decker et al. 1977; Turner 1990; Petersen et al. 1995). The earliest record of a hybrid zone in this region comes from Phillips (1991), who confirms that specimens collected in August of 1945 from Alta Lake, near population 5 (Fig. 1), included both coastal and inland forms. Using 1945 (61 years ago) as a highly conservative estimate of time since contact, the predicted cline width under neutral diffusion would still be more than 700 km greater than the observed cline width of 80 km. For the cline width to be the result of neutral diffusion, the time since contact would have to be no greater than 61 years and the estimate of RMS dispersal would have to be 14.5 km or less. Thus, even if contact were very recent, it is

unlikely that neutral diffusion following secondary contact provides an adequate explanation for the observed steep clines in genetics, morphology, and plumage color in the Swainson's thrush.

Bounded hybrid superiority

An alternative to neutral diffusion is the bounded hybrid superiority model, which predicts that hybrid zones form on ecotones and are maintained by environmental selection favoring hybrid phenotypes within transitional habitat, and parental phenotypes outside of the hybrid zone (Anderson 1948; Moore 1977). This model is in keeping with the idea that hybrids thrive within ecotones and that cline widths vary with the width of the ecological gradient (Anderson 1948; Moore 1977), as is thought to be the case in the Red-shafted and Yellow-shafted flicker hybrid zone across the Great Plains region in North America (Moore and Price 1993). Under a hybrid superiority model, ecological selection should favor particular hybrid genotypes, resulting in a lack of concordance between multiple character clines (Barton and Hewitt 1985; Hewitt 1988). Although the Swainson's thrush hybrid zone does track an ecological gradient (Fig. 5A), bounded hybrid superiority does not provide a sufficient explanation for the narrow, concordant clines (Fig. 4) and the low population density in the center of the hybrid zone (Fig. 5B). Additional tests of this hypothesis would be to directly compare the fitness of hybrids to that of parentals at the center of the hybrid zone (Good et al. 2000), and to compare the widths of additional hybrid zone transects in which the environmental gradient is more gradual.

Tension zone

The hypothesis that best fits the data is that the Swainson's thrush hybrid zone is a tension zone maintained by a balance between dispersal and ecologically mediated barriers to gene flow. Three lines of evidence in support of this hypothesis are: (1) clines for body size, plumage color, and AFLP assignment are concordant in width and coincident in position, suggesting the presence of a genome-wide barrier to gene flow (Barton and Hewitt 1985); (2) low population density and the occurrence of both parental and hybrid genotypes at the center of the hybrid zone are consistent with a hybrid sink, defined as a region in which genes flow in from either side but are eliminated in the maladapted individuals at the center (Barton and Hewitt 1985); and (3) cline widths that are narrow relative to the estimate of RMS dispersal, indicating that a barrier to gene flow is preventing the clines from becoming wider.

The extent to which premating or postmating reproductive isolation is helping to maintain the subspecies boundary depends to a large extent upon the dispersal rate into the hybrid zone. If dispersal into the hybrid zone were low, then the center of the hybrid zone would be a complete hybrid swarm in fewer then three generations unless there were some premating barriers to gene flow. Alternatively, if dispersal is high and mating is random, than some form of selection must be acting to explain why the clines remain narrow. Of particular interest within the context of a migratory divide is the potential for premating isolation as a result of differences in arrival times on the breeding grounds (Bearhop et al. 2005) and/or selection against hybrids as a result of intermediate orientation behavior (Helbig 1991). The frequency of hybridization at the center of this hybrid zone indicates that if premating isolation is occurring, it is far from complete. In addition, the significant proportion of second year hybrid birds at the center of the hybrid zone indicates that hybrids are returning to the breeding grounds despite the potential for selection against individuals with intermediate orientation. The position of the hybrid zone on a steep environmental gradient provides a potential role for ecological selection, but further investigation is needed. Studies of hybrid fitness and assortative mating within the hybrid zone would provide additional tests of the tension zone model.

Additional considerations

One striking result from the hybrid zone analysis was the position of the mtDNA cline relative to the three other clines in body size, plumage color, and AFLP assignment. Lack of concordance between clines resulting from biparentally inherited nuclear markers and maternally inherited mtDNA markers is a common feature in many hybrid zones and may arise for a variety of reasons (reviewed in Wirtz 1999; Chan and Levin 2005). A shift in position, but not width of the mtDNA cline is likely a result of asymmetrical hybridization in which coastal males hybridize more frequently with inland females than vice versa. Asymmetrical hybridization in the Swainson's thrush may be facilitated by the fact that coastal males in British Columbia are known to arrive more than two weeks ahead of inland populations outside of the hybrid zone (Campbell et al. 1997), potentially giving them a significant advantage in territory selection and establishment. If differences in arrival times also occur within the hybrid zone and coastal males are able to maintain higher quality territories, then inland and hybrid females may mate more frequently with coastal males. The differences in arrival times between the subspecies may be a byproduct of ecological selection within distinct climatic regions, helping to explain the position of the hybrid zone on an environmental gradient.

A similar scenario has been suggested to help explain the existence of Hermit Warbler mtDNA in Townsend's Warbler populations from across the Pacific Northwest. Extensive behavioral research has shown that Townsend's Warblers are competitively superior to Hermit Warblers (Pearson 2000; Pearson and Rohwer 2000), and Rohwer et al. (2001) hypothesized that differences in competitive abilities have caused the Townsend/Hermit warbler hybrid zone to move southward, leaving Hermit Warbler mtDNA in its wake. The difference between hybrid zones of the

Townsend/Hermit warbler and the Swainson's thrush is that the former zone appears to move freely, whereas movement of the latter zone appears to be restricted by an environmental gradient.

Conclusions

The data presented here are consistent with a barrier to gene flow across a hybrid zone that spans a migratory divide in the Swainson's thrush. All regions of contact between the subspecies are positioned on the transition between coastal and interior climatic regions, providing a potential role for ecological selection in the maintenance of the subspecies boundary. Barriers to gene flow in this system may be influenced by a variety of pre- and postzygotic isolating mechanisms and exploring these alternatives requires further investigation. The characteristics of this hybrid zone indicate that the potential interaction between ecological selection, the timing of arrival on the breeding grounds, and mate choice provides an interesting area for future work.

ACKNOWLEDGMENTS

I thank E. Anderson for his statistical expertise and help with the construction of Figure 4. I also thank S. Baird and J. Alexandrino for their help with the cline analyses, and C. Moritz and J. Patton for comments on early drafts of this manuscript. I thank T. Smith and the Center for Tropical Research at UCLA, as well as the Institute for Bird Populations and the MAPS (Monitoring Avian Productivity and Survivorship) program for their contribution of genetic samples. The field component of this research was funded by student research grants awarded to KR from the Society of Integrative and Comparative Biology, the American Ornithologists' Union, and the Museum of Vertebrate Zoology at the University of California. NSF Dissertation Improvement Grant (NSF0208862) awarded to KR funded the laboratory work.

LITERATURE CITED

Alexandrino, J., S. J. Baird, L. Lawson, J. R. Macey, C. Moritz, and D. B. Wake. 2005. Strong selection against hybrids at a hybrid zone in the *Ensatina* ring species complex and its evolutionary implications. Evolution 59:1334–1347.

Anderson, E. 1948. Hybridization of the habitat. Evolution 2:1–9.

Anderson, E. C., and E. A. Thompson. 2002. A model-based method for identifying species hybrids using multilocus genetic data. Genetics 160:1217–1229.

Arnold, M. L. 1997. Natural hybridization and evolution. Oxford Univ. Press, New York.

Baldwin, S. P., H. C. Oberholser, and L. G. Worley. 1931. Measurements of birds. Scientific Publications of the Cleveland Museum of Natural History, Cleveland, OH.

Barrowclough, G. F. 1978. Sampling bias in dispersal studies based on finite area. Bird-Banding 49:333–341.

———. 1980. Genetic and phenotypic differentiation in a wood warbler (genus *Dendroica*) hybrid zone. Auk 97:655–668.

Barton, N. H., and S. J. E. Baird. 1996. ANALYSE 1.30 PPC. Available at www.biology.ed.uk/research/institutes/evolution/software/Mac/Analyse/index.html.

Barton, N. H., and K. S. Gale. 1993. Genetic analysis of hybrid zones. Pp. 13–45 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, New York.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. Ann. Rev. Ecol. Syst. 16:113–148.

Bearhop, S., W. Fiedler, R. W. Furness, S. C. Votier, S. Waldron, J. Newton, G. J. Bowen, P. Berthold, and K. Farnsworth. 2005. Assortative mating as a mechanism for rapid evolution of a migratory divide. Science 310: 502–504.

Bensch, S., T. Andersson, and S. Akesson. 1999. Morphological and molecular variation across a migratory divide in Willow Warblers, *Phylloscopus trochilus*. Evolution 53:1925–1935.

Bensch, S., A. J. Helbig, M. Salomon, and I. Seibold. 2002. Amplified fragment length polymorphism analysis identifies hybrids between two subspecies of warblers. Mol. Ecol. 11:473–481.

Bond, G. M. 1963. Geographic variation in the thrush, *Hylocichla ustulata*. Proc. U. S. Natl. Mus. 114:373–387.

Bridle, J. R., S. J. Baird, and R. K. Butlin. 2001. Spatial structure and habitat variation in a grasshopper hybrid zone. Evolution 55:1832–1843.

Campbell, R. W., N. K. Dawe, I. McTaggart-Cowan, J. M. Cooper, G. W. Kaiser, M. C. E. McNall, and G. E. J. Smith. 1997. The birds of British Columbia. Vol. 3, Passerines: flycatchers through vireos. Univ. of British Columbia Press, Vancouver, BC.

Chan, K. J., and S. A. Levin. 2005. Leaky prezygotic isolation and porous genomes: rapid introgression of maternally inherited DNA. Evolution 59:720–729.

Cicero, C. 2004. Barriers to sympatry between avian sibling species (Paridae: Baeolophus) in local secondary contact. Evolution 58:1573–1587.

Decker, F., M. Fougberg, and M. Ronayne. 1977. Pemberton: the history of a settlement. Hemlock Printing Ltd., Burnaby, BC.

Endler, J. A. 1977. Geographic variation, speciation, and clines. Princeton Univ. Press, Princeton, NJ.

Evans Mack, D., and W. Yong. 2000. Swainson's thrush (*Catharus ustulatus*). Pp. 1–19 *in* A. Poole and F. Gill, eds. The birds of North America, no. 540. Birds of North America Inc., Philadelphia, PA.

Gill, F. B. 1980. Historical aspects of hybridization between Blue-winged and Golden-winged warblers. Auk 97:1–18.

Gill, F. B., and W. E. Lanyon. 1997. Local cytonuclear extinction of the Golden-winged Warbler. Evolution 51:519–525.

Good, T. P., J. C. Ellis, C. A. Annett, and R. Pierotti. 2000. Bounded hybrid superiority in an avian hybrid zone: effects of mate, diet, and habitat choice. Evolution 54:1774–1783.

Grill, C. P., and V. N. Rush. 2000. Analysing spectral data: comparison and application of two techniques. Biol. J. Linn. Soc. 69:121–138.

Hansson, B., S. Bensch, D. Hasselquist, and B. Nielsen. 2002. Restricted dispersal in a long-distance migrant bird with patchy distribution, the Great Reed Warbler. Oecologia (Berl.) 130:536–542.

Harrison, R. G. 1993. Hybrid zones and the evolutionary process. Oxford Univ. Press, NY.

Harrison, R. G., and D. M. Rand. 1989. Mosaic hybrid zones and the nature of species boundaries. Pp. 111–133 *in* D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer Press, Sunderland, MA.

Hedenstrom, A., and J. Pettersson. 1987. Migration routes and wintering areas of Willow Warblers *Phylloscopus trochilus* (L.) ringed in Fennoscandia. Ornis Fenn. 64:137–143.

Helbig, A. J. 1991. SE- and SW-migrating Blackcap (*Sylvia atricapilla*) populations in Central Europe: orientation of birds in the hybrid zone. J. Evol. Biol. 4:657–670.

Hewitt, G. M. 1988. Hybrid zones—natural laboratories for evolutionary studies. Trends Ecol. Evol. 3:158–167.

Hijmans, R. J., L. Guarino, C. Bussink, I. Barrantes, and E. Rojas. 2004. DIVA-GIS: a geographic information system for the analysis of biodiversity data. Available at www.diva-gis.org.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25:1965–1978.

Hill, G. E. 1998. An easy, inexpensive means to quantify plumage coloration. J. Field Ornithol. 69:353–363.

Irwin, D. E., and J. H. Irwin. 2004. Siberian migratory divides: the role of seasonal migration in speciation. Pp. 27–40 *in* R. Greenberg and P. P. Marra, eds. Birds of two worlds: the ecology and evolution of migratory birds. Johns Hopkins Univ. Press, Baltimore, MD.

Johnson, N. K. 1978. Patterns of avian geography and speciation in the intermountain Region. Great Basin Nat. 2:137–159.

Kelly, J. F., K. C. Ruegg, and T. B. Smith. 2005. Combining isotopic and genetic markers to identify breeding origins of migrant birds. Ecol. Appl. 15:1487–1494.

Lamont, B. B., T. He, N. J. Enright, S. L. Knauss, and B. P. Miller. 2003. Anthropogenic disturbance promotes hybridization between *Banksia* species by altering their biology. J. Evol. Biol. 16:551–557.

Moore, W. S. 1977. An evaluation of narrow hybrid zones in vertebrates. Q. Rev. Biol. 52:263–277.

Moore, W. S., and D. B. Buchanan. 1985. Stability of the Northern Flicker hybrid zone in historical times: implications for adaptive speciation theory. Evolution 39:134–151.

Moore, W. S., and R. A. Dolbeer. 1989. The use of banding recovery data to estimate dispersal rates and gene flow in avian species: case studies in the Red-winged Blackbird and Common Grackle. Condor 91:242–253.

Moore, W. S., and J. T. Price. 1993. Nature of selection in the Northern Flicker hybrid zone and its implications for speciation theory. Pp. 196–225 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, New York.

Pearson, S. F. 2000. Behavioral asymmetries in a moving hybrid zone. Behav. Ecol. 11:84–92.

Pearson, S. F., and S. Rohwer. 2000. Asymmetries in male aggression across an avian hybrid zone. Behav. Ecol. 11:93–101.

Petersen, F., S. Mitchell, and J. L. Morrison. 1995. Whistler reflections. Terra Bella Publishers, West Vancouver, BC.

Phillips, A. R. 1991. The known birds of North and Middle America, Part II. A. R. Phillips, Denver, CO.

Phillips, B., S. J. E. Baird, and C. Moritz. 2004. When vicars meet: a narrow hybrid zone between morphologically cryptic phylogeographic lineages of the rainforest Skink, *Carlia rubrigularis*. Evolution 58:1536–1548.

Ralph, C. J., and J. M. Scott. 1981. Estimating numbers of terrestrial birds. Studies in avian biology no. 6. Cooper Ornithological Society, Lawrence, KS.

Ralph, C. J., G. R. Geupel, P. Pyle, T. E. Martin, and D. F. DeSante. 1993. Handbook of field methods for monitoring landbirds. USDA Pacific Southwest Research Station, Albany, CA.

Rappole, J. H., and D. H. Warner. 1980. Ecological aspects of avian migrant behavior in Veracruz, Mexico. Pp. 353–393 *in* A. Keast and E. S. Morton, eds. Migrant birds in the neotropics: ecology, behavior, distribution, and conservation. Smithsonian Institution Press, Washington, DC.

Rohwer, S., and J. Manning. 1990. Differences in timing and number of molts for Baltimore and Bullock's orioles: implications to hybrid fitness and theories of delayed plumage maturation. Condor 92:125–140.

Rohwer, S., and C. Wood. 1998. Three hybrid zones between Hermit and Townsend's warblers in Washington and Oregon. Auk 115:284–310.

Rohwer, S., E. Bermingham, and C. Wood. 2001. Plumage and mitochondrial DNA haplotype variation across a moving hybrid zone. Evolution 55:405–422.

Ruegg, K. C., and T. B. Smith. 2002. Not as the crow flies: a historical explanation for circuitous migration in Swainson's thrush (*Catharus ustulatus*). Proc. R. Soc. Biol. Sci. Ser. B 269:1375–1381.

Ruegg, K. C., R. J. Hijmans, and C. Moritz. 2006a. Climate change and the origin of migratory pathways in the Swainson's thrush (*Catharus ustulatus*). J. Biogeogr. 33:1172–1182.

Ruegg, K. C., H. Slabbekoorn, S. Clegg, and T. B. Smith. 2006b. Divergence in mating signals correlates with ecological variation in the migratory songbird, the Swainson's thrush (*Catharus ustulatus*). Mol. Ecol. 15:3147–3156.

Seutin, G., B. N. White, and P. T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analyses. Can. J. Zool. 69:82–90.

Szymura, J. M., and N. H. Barton. 1986. Genetic analysis of a hybrid zone between the Fire-bellied Toads, *Bombina bombina* and *B. variegata*, near Cracow in southern Poland. Evolution 40:1141–1159.

Takami, Y., and H. Suzuki. 2005. Morphological, genetic and behavioral analyses of a hybrid zone between the Ground Beetles *Carabus lewisianus* and *C. albrechti* (Coleoptera, Carabidae): asymmetrical introgression caused by movement of the zone? Biol. J. Linn. Soc. 86:79–94.

Ticehurst, C. B. 1938. A systematic review of the genus *Phylloscopus*. British Museum, London.

Turner, R. D. 1990. Logging by rail: the British Columbia story. Sono Nis Press, Winlaw, BC.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, et al. 1995. AFLP—a new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407–4414.

Webster, M. S., and P. P. Marra. 2005. The importance of understanding migratory connectivity and seasonal interactions. Pp. 199–209 *in* R. Greenberg and P. P. Marra, eds. Birds of two worlds: the ecology and evolution of migration. Johns Hopkins Univ. Press, Baltimore, MD.

Webster, M. S., P. P. Marra, S. M. Haig, S. Bensch, and R. T. Holmes. 2002. Links between worlds: unraveling migratory connectivity. Trends Ecol. Evol. 17:76–83.

Wirtz, P. 1999. Mother species-father species: unidirectional hybridization in animals with female choice. Anim. Behav. 58:1–12.

Associate Editor: M. Sorenson

Appendix 1. **Summary of morphological measurements that differ across the hybrid zone. Number of individuals (*N*), mean, and standard deviation (SD). See Figure 1 and Table 1 for location information (site code).**

Site code	Distance (km)	Body size_PC1			Color_PC2			Culmen (mm)			Tarsus (mm)		
		N	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD
1	0	21	0.6342	0.8	11	−0.77	0.56	24	9.73	0.49	25	29.1	0.88
2	33	35	0.8281	0.82	40	−0.86	0.67	35	9.82	0.45	35	29.33	1.05
3	51	20	0.6087	0.81	20	−0.51	0.75	20	9.73	0.5	20	28.94	0.63
4	63	28	0.14975	0.88	16	−0.04	0.83	29	9.45	0.4	29	28.58	0.85
5	70	45	−0.10341	0.88	26	−0.09	0.52	45	9.36	0.51	45	28.23	1.06
6	116	27	−0.32852	0.77	8	0.79	0.79	27	9.3	0.42	27	27.89	0.72
7	215	20	−0.86568	0.87	18	1.2	0.51	20	9.25	0.5	20	27.67	0.78

Site code	Wing (mm)			Bill depth (mm)			Bill width (mm)			Tail (mm)		
	N	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD
1	25	98.44	2.06	25	4.12	0.27	25	4.51	0.24	24	72.68	2.95
2	35	99.54	2.64	35	4.23	0.17	35	4.47	0.26	35	73.79	2.8
3	20	99.14	2.35	20	4.22	0.22	20	4.52	0.18	20	72.75	3.16
4	28	99.76	3.11	28	4.19	0.18	28	4.5	0.24	28	72.01	3.14
5	45	101.11	3.08	45	4.13	0.16	45	4.47	0.21	45	72.91	2.53
6	27	100.26	2.28	27	4.16	0.17	27	4.43	0.17	27	72.03	2.58
7	20	100.77	3.02	20	3.98	0.13	20	4.45	0.23	20	69.99	2.7

Appendix 2. Frequency of AFLP phenotypes in seven populations across the hybrid zone. Each column represents a different locus and locus names are formed with the last two base pairs of each selective amplification primer combination, followed by the base pair. A frequency of one signifies the marker was present in all individuals.

Population	ct/aa_248	ct/gt_222	ct/gg_220	ga/ac_319	ct/aa_112	ga/ga_76	ct/ga_68	ga/ac_331	ga/tc_240	ga/aa_295	ct/ga_185	ga/ga_162	ga/ga_91	ga/tc_371	ct/ga_323
1. Sunshine Coast	0	1	1	0	0.93	0.93	0.23	0.93	0.11	0.52	0.86	0.41	1	0.71	0.93
2. Squamish	0	0.67	1	0	1	1	0	1	0.14	0.2	1	0.29	0.86	0.71	0.71
3. Whistler	0.11	0.95	1	0.1	0.95	0.88	0.45	1	0.35	0.6	0.9	0.35	0.94	0.47	0.9
4. Shadow Lake	0.38	0.81	0.88	0.29	0.91	0.77	0.57	1	0.45	0.45	0.77	0.5	0.91	0.35	0.87
5. Pemberton	0.42	0.36	0.64	0.5	0.54	0.79	0.46	0.69	0.36	0.38	0.69	0.6	0.87	0.27	0.75
6. Lillooet	1	0	0.21	1	0	0.5	0.63	0.57	0.82	0.12	0.69	0.63	0.81	0.12	0.69
7. Kamloops	1	0	0	1	0	0	0.88	0.47	0.75	0.37	0.61	0.32	1	0.63	0.95