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The Genetics of Seasonal Migration and Plumage Color

Highlights

- Variation in migratory route and plumage color is explained by genomic variation

- A region on chromosome 4 is strongly and additively associated with orientation

- This region includes circadian, nervous system, and cell signaling genes

- Recurrent selective sweeps have shaped variation in this region

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In Brief

The Swainson's thrush includes two subspecies groups that differ in their migratory routes and plumage color. Delmore et al. integrate data from light-level geolocators and next-generation sequencing to identify candidate genomic regions associated with both traits and link them to the maintenance of reproductive isolation between the two groups.

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Report

The Genetics of Seasonal Migration and Plumage Color

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SUMMARY

Details on the genetics of behavioral and quantitative phenotypes remain limited to a few organisms. Such information is central to understanding both adaptation and speciation, as many of these phenotypes reduce gene flow between taxa [1–3]. Hybrid zones provide an ideal arena for studying this topic, as they consist of recombinant genotypes that allow genetic mapping of traits distinguishing natural populations [4]. We examined the genetic basis of migratory orientation and plumage color, both of which may contribute to speciation, in a hybrid zone between two groups of Swainson's thrushes that differ in these traits. We identified a cluster of SNPs on chromosome 4 strongly associated with migratory orientation. Genes involved with the circadian clock, nervous system, and cell signaling were located here and included candidates implicated in smaller-scale studies of migration in different animal groups, supporting previous suggestions that there is a common gene package for migration [5]. Plumage color was more polygenic than migratory orientation but showed strong associations on the Z chromosome that included SNPs linked to *TYRP1*, a gene involved in the production of eumelanin. We integrated these results with genomic data from pure populations and found that regions associated with both phenotypes co-localized with regions of elevated relative differentiation between the groups. This finding relates to the literature on islands of differentiation [6–8] by implicating divergent selection in generating these peaks. Together, our results identify specific genomic regions involved in both the regulation of complex phenotypes across animal groups and speciation [9].

RESULTS AND DISCUSSION

The Swainson's thrush (*Catharus ustulatus*) includes two subspecies groups (coastal and inland) that hybridize along the Coast Mountains of North America [10]. We genotyped 190 birds from the center of this zone using 59,485 SNPs. Estimates of ancestry and interspecific heterozygosity show that this hybrid zone is composed mostly of backcrosses and later-generation hybrids (Figure S1) [4]. The two groups differ in migratory orientation and plumage color: coastal birds have russet back feathers and migrate southward to Mexico and Central America, whereas inland birds have olive back feathers and migrate southeastward to South America (Figure 1A) [11]. We began by quantifying variation in these traits in our hybrid populations and examining their relationship with ancestry.

Relationship between Phenotypes and Ancestry

We quantified orientation using two relatively new technologies, light-level geolocators (n = 21; Figure 1A) [13] and video-based orientation cages (n = 169) [14]. Geolocator data were originally published in [12] and are summarized as the longitudes at which birds wintered and crossed 30°N on fall and spring migration (Figure 1A). The former study found a relationship between inland ancestry (estimated using three intronic markers) and more easterly migration. We found the same result here using our genome-wide dataset (Figure 1B) and a similar relationship for the orientation of birds in cages (Figure S1). This is the first time data from birds tracked over the entire year have been combined with genomic data to show that migratory routes are genetically determined; previous support came mostly from displacement [12, 14, 15] and laboratory crossbreeding experiments [16, 17].

Using spectrophotometrically quantified plumage color of hybrids, we estimated the relative stimulation of each of the four cones in the avian visual system [15]. Three of the four cones had relationships with ancestry (UV, short, and long wavelengths; Figure S1), showing that there is a genetic basis to color differences between the two groups.

Genetic Architecture of Phenotypes

We used multiple-SNP Bayesian models to investigate the genetic basis of migratory orientation and plumage color. These models consider all SNPs together and account for factors that influence phenotypes and are correlated with genotypes (e.g., population structure and ancestry [16]). Results showed that the proportion of variance explained (PVE) by SNPs was high for all traits (Table 1). These estimates were higher for migratory traits, which also had higher estimates of PGE (the proportion of genetic variance explained by sparse effects), suggesting that a
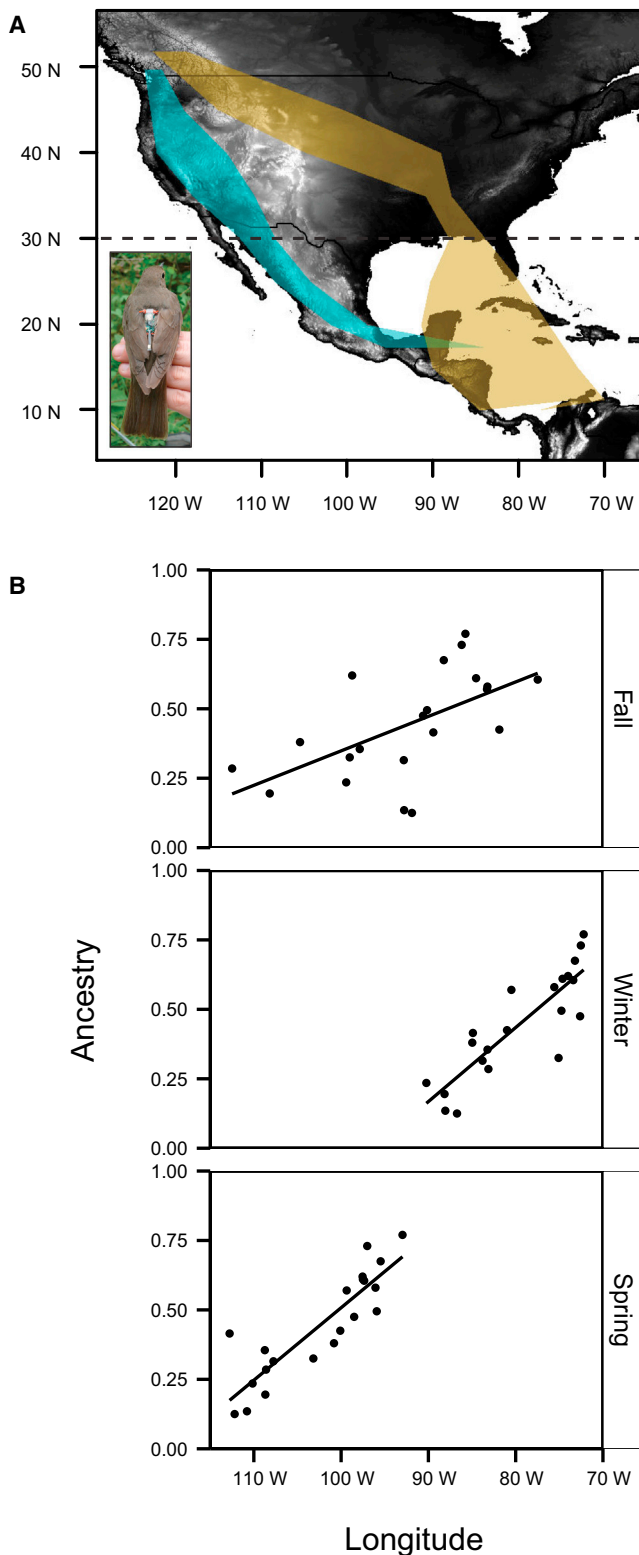
few genes of large effect explain much of the variation in this trait. This finding supports Helbig [17], who observed that second-generation lab-bred hybrid European blackcaps exhibited greater variation in orientation than both first-generation hybrids and pure forms.

Lower values of PVE and PGE for color suggest that variation in this trait may be less genetically based than migration, and/or that the genetic component is highly polygenic. This finding contrasts with many previous studies of color in vertebrates [18–21], which have found genes of major effect. Such studies often focus on discrete traits; the transition from russet to olive in thrushes is more subtle and thus may be influenced proportionally more by environmental variation and/or small-effect genes [20].

Isolated Effects of SNPs on Phenotypes

Because genetic variation explains a substantial proportion of variation in both migratory orientation and plumage color, we investigated the effects of specific genomic regions on each trait using single-SNP linear models that also account for factors that influence phenotypes and are correlated with genotypes [22] (Figures 2 and 3; Table 1). A cluster of SNPs on chromosome 4 is strongly associated with all three geolocator traits (Figure 2). The effect sizes were remarkably high (Figure 2B), and the probability of finding a cluster of this size by chance is low (permutation test, $p = 0.001$). Previous work in this system suggested that migratory orientation could be inherited additively, as hybrids take intermediate routes on migration [12]. We computed the ratio of dominant to additive effects [24] for SNPs in the former cluster showing the strongest associations with wintering longitude (seven SNPs; Figure 2C). The average estimate was -0.14, suggesting alleles at these SNPs are indeed additively inherited (expected value for additive alleles is 0; Figures 2D and S3). Patterns for cage orientation were less clear, but some SNPs showed similar patterns in cage and geolocator datasets. Interestingly, one of the SNPs showing a strong association with cage orientation is in the same linkage block as one of the seven SNPs strongly associated with wintering longitude in the cluster on chromosome 4. The chance of the same linkage block being randomly associated with migration quantified using these independent datasets is extremely small ($p = 0.000018$ for winter and 0.00079 for cages; joint $p = 0.000000014$), and several candidate genes for migration are located in this block (see below).

Turning to color, we found two peaks of association on chromosome Z, a sex chromosome, for both short and long wavelengths (Figure 3). The chance of this clustering occurring randomly is likewise low (permutation test, $p = 0.001$).

Selective History of Regions Associated with Phenotypes

We continued by integrating results from genetic mapping with genomic data from pure populations to examine the selective

Figure 1. Migratory Routes and Their Relationship to Ancestry Quantified Using Light-Level Geolocators and Ancestry

(A) Polygons show routes taken by pure allopatric coastal (blue, n = 5) and inland (yellow, n = 4) thrushes tracked on fall migration (±1.6 SD longitude every 5°N; [11]). The dotted line marks the longitude at which migration was quantified.

(B) Tracking data from thrushes breeding in the hybrid zone [12]. Ancestry refers to the proportion of SNP alleles from the inland group. All relationships are significant (fall $R^2 = 0.34$, $F_{1,19} = 9.98$, $p < 0.005$; winter $R^2 = 0.73$, $F_{1,19} = 52.42$, $p < 0.0001$; spring $R^2 = 0.77$, $F_{1,19} = 64.45$, $p < 0.0001$). See also Figures S1 and S2.

Table 1. Results from Bayesian Sparse Linear Mixed Models and Linear Mixed-Effects Models Examining the Genetic Architecture of Phenotypic Traits

	PVE (SD)	PGE (SD)	SNPs (SD)	#SNPs (#Chr)[a]		F_{ST} Perm Test	
				$\alpha = 0.0001$	$\alpha = 0.001$	$\alpha = 0.0001$	$\alpha = 0.001$
Fall	0.76 (0.29)	0.45 (0.32)	22 (70)	0 (0)	44 (20)	0.02	0.13**
Winter	0.92 (0.28)	0.57 (0.33)	20 (73)	8 (2)†	52 (14)	0.27**	0.16**
Spring	0.91 (0.29)	0.52 (0.33)	19 (65)	3 (3)	27 (11)	0.19	0.16**
Cages	0.77 (0.23)	0.45 (0.21)	45 (73)	5 (5)	74 (24)†	0.08	0.08
UV	0.56 (0.27)	0.24 (0.28)	34 (76)	9 (6)†	79 (27)†	0.09	0.09
Short	0.67 (0.27)	0.27 (0.28)	35 (78)	30 (8)†	114 (21)†	0.34**	0.23**
Medium	0.51 (0.28)	0.27 (0.28)	33 (80)	7 (7)†	71 (26)†	0.09	0.07
Long	0.61 (0.27)	0.25 (0.28)	30 (77)	10 (8)†	79 (24)†	0.1	0.13*

Results from Bayesian sparse linear mixed models (first three columns) include the proportion of variance explained by all SNPs (PVE), proportion of variance explained by sparse effects (PGE), and posterior estimates for the number of SNPs in the models. Results from linear mixed-effects models (next two columns) include the number of SNPs with significant associations with each phenotype and the number of chromosomes on which these SNPs reside. F_{ST} between coastal and inland thrushes in regions associated with each trait is shown in the final two columns (statistical significance: **p < 0.0001; *p < 0.001). See also Figure S2.

[a]At $\alpha = 0.0001$ we expect 5.9 false positives, and at $\alpha = 0.001$ we expect 59 (59,485 SNPs × 0.0001 or 0.001). The dagger symbol (†) indicates that the number of SNPs exceeds the expected rate of false positives.

history of regions associated with each trait and their connection with speciation. Previous work suggests that Swainson's thrushes are partially reproductively isolated [10, 12]. Differences in migration and color likely contribute to this isolation: hybrids take intermediate routes over arid and mountainous regions that are likely unsuitable for refueling and difficult to navigate, and narrow clines in color have been documented in this hybrid zone [10].

Previously, whole-genome resequencing data from pure populations of inland and coastal thrushes were used to map variation in relative nucleotide differentiation (F_{ST}) [23]. If differences in migration and color are subject to selection and cause reproductive isolation, genomic regions underlying these traits should show elevated F_{ST} [9]. As predicted, permutation tests showed that SNPs associated with all geolocator traits, as well as coloration in short and long wavelengths, have significantly higher F_{ST} than background levels (Table 1; Figure 2C). There is considerable interest in the hypothesis that migratory differences could promote speciation [17, 25–27]; combined with tracking work in the Swainson's thrush [12], this association provides the strongest support for this hypothesis to date. Note also that SNPs showing the strongest associations with orientation are fixed or nearly fixed in pure populations, with the allele conferring a more southeastern orientation at highest frequency in the inland population (Figure S2).

We can use data from [23] to further elucidate the selective history of these regions. The former study noted that regions of elevated F_{ST} coincide with reductions in d_{xy}, π, and Tajima's D. This pattern was interpreted as evidence of recurrent selective sweeps followed by divergent selection. As d_{xy} is a measure of absolute differentiation, reductions in this parameter are indicative of more recent mean coalescence times. More recent coalescence times could arise if a globally favorable allele arose somewhere in the range of the ancestral species and swept to fixation throughout the range [9]. Tajima's D and π are measures of within-population variation, and lower values in these parameters are indicative of more local, population-specific selective

sweeps [9]. Finally, the fact that F_{ST} is elevated in these regions suggests that these recent sweeps fixed alternate alleles despite the presence of a hybrid zone between the groups [9]. Figure S3 demonstrates the connection between these patterns and genetic mapping results for the orientation-associated cluster on chromosome 4.

Gene Content and Ontology of Regions Associated with Phenotypes

We now have a set of regions associated with migration and color that have likely experienced strong selection and contribute to reproductive isolation. Data S1 lists all genes within regions of particularly strong linkage (hereafter "linkage blocks") around these SNPs; we used these lists to focus on potentially causal variants. First, we looked for enrichment of specific gene ontologies (GOs). Genes associated with color were not enriched for any GOs, although the gene TYRP1 is in a linkage block on the Z chromosome associated with both short and long wavelengths (Figure 3). TYRP1 is involved in the synthesis of eumelanin and has been associated with plumage color in the Japanese quail [28] and Ficedula flycatchers [29]; genes for color tend to be conserved across taxa and are often involved in the synthesis of melanin [18–20]. Our findings of phenotypic associations on the Z (Figure 2) and higher F_{ST} on the Z [23] reinforce the role the sex chromosomes likely play in speciation; theory suggests that genes associated with plumage traits, species recognition, and hybrid incompatibilities in birds could preferentially be located on the Z chromosome [30–32].

Turning to migration, genes associated with cage orientation were enriched for two GOs: DNA binding and sequence-specific DNA binding (p = 0.04 and 0.05 [adjusted for multiple comparisons]; Data S1). Many of these genes are involved in embryo development (e.g., homeobox genes for brain development). Genes associated with winter and spring longitudes were enriched for the same GO, endopeptidase inhibitor activity (p = 0.00002 and 0.006; Data S1). Endopeptidases regulate circadian locomotor behaviors [33]. Zhu et al. [34] used a microarray to

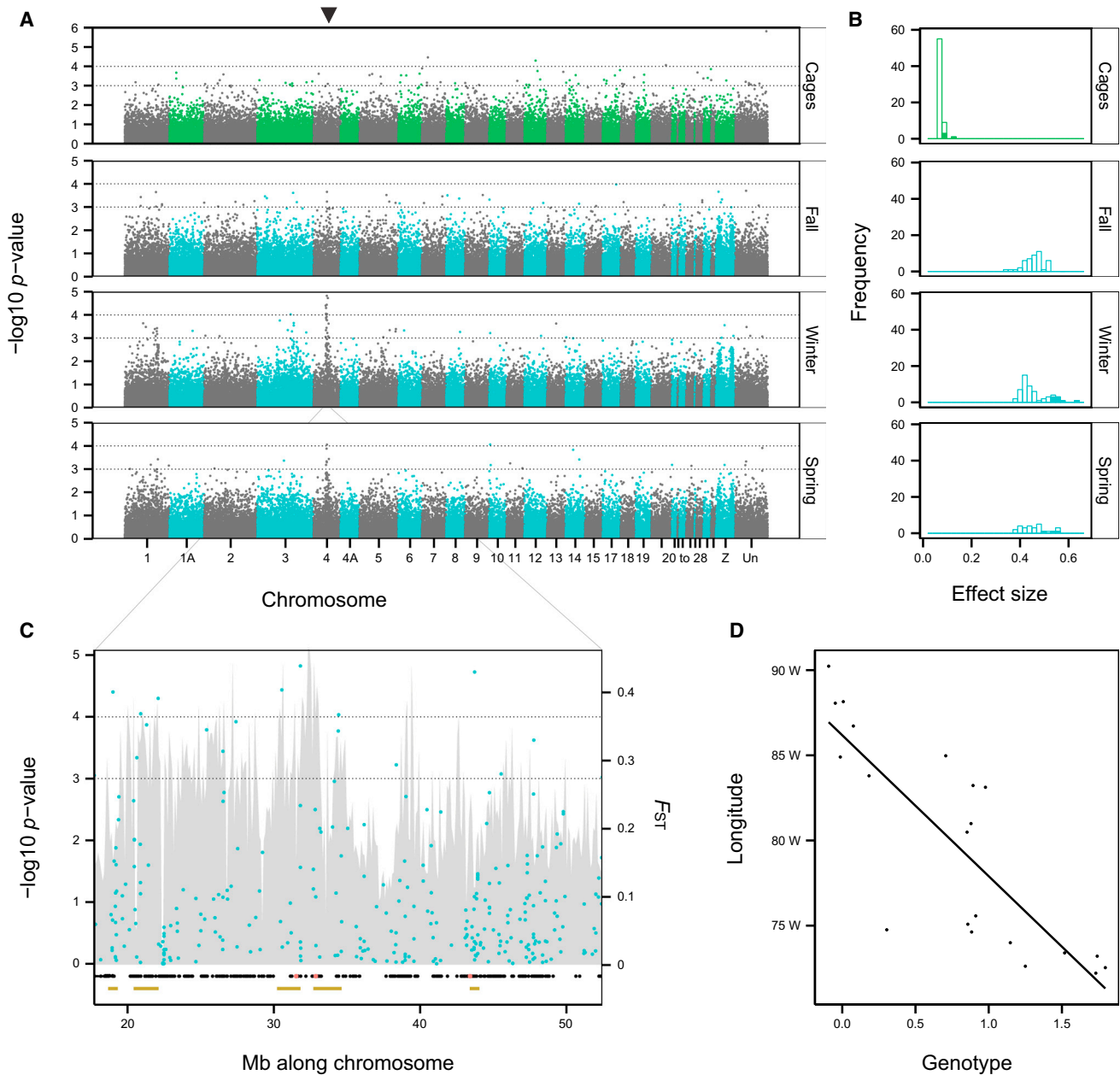**Figure 2. Association between Migratory Orientation and SNPs, as Estimated by Single-SNP Linear Mixed-Effects Models**

(A) Statistical associations, with dotted lines indicating two significance thresholds ($\alpha = 0.0001$ [$-\log10$ value = 4] and $\alpha = 0.001$ [$-\log10$ value = 3]).

(B) Effect sizes for SNPs above the two significance thresholds (filled for those above the more stringent threshold).

(C) Detailed section of chromosome 4 where a cluster of SNPs shows strong associations with winter longitude (seven SNPs above 4, indicated by the black triangle in A). F_{ST} from [23] is included in this plot (gray shading). Yellow lines represent linkage blocks around these SNPs. Black dots show the location of genes; pink dots show the location of candidate genes discussed in the text (from left to right: *TMEM192*, *PALLD*, and *CLOCK*). Protein kinases are found in the last two linkage blocks, the last of which was also strongly associated with cage orientation.

(D) The average genotype of individuals at SNPs showing the strongest associations with winter longitude (0 = homozygous for reference allele, 2 = homozygous for alternate allele) is plotted against winter longitude.

See also Figures S2, S3, and S4.

identify 40 differentially expressed genes between migrant and resident monarch butterflies; these genes were enriched for two of the same GOs, supporting previous suggestions that taxonomically distant groups employ similar functional pathways to migrate [5].

Prompted by the former finding, we compared our results to those of Lundberg et al. [35] and Baerwald et al. [36], Lundberg et al. identified 39 genes with SNPs that differentiated populations of willow warblers with different migratory orientations; Baerwald et al. identified 27 genes in genomic regions that
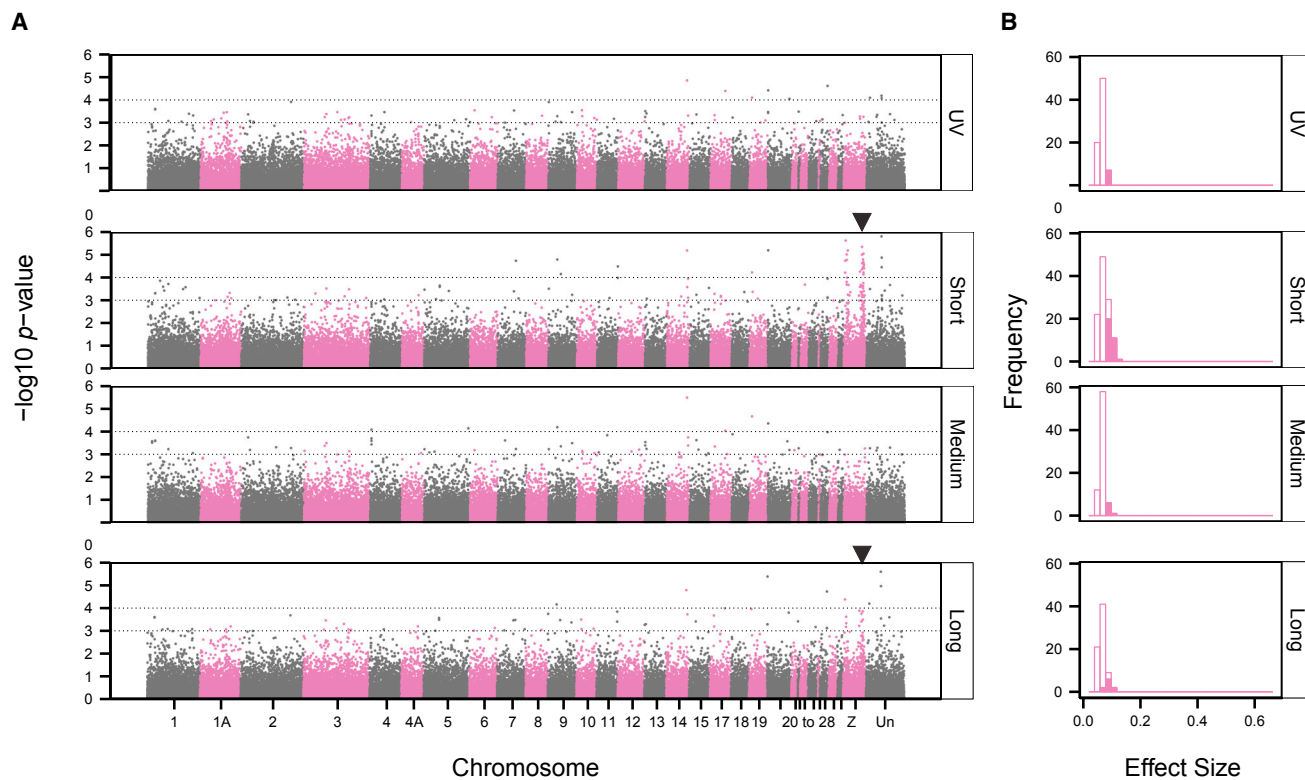
Figure 3. Association between Plumage Color and SNPs

(A) Statistical associations, with dotted lines indicating two significance thresholds ($\alpha = 0.0001$ [$-$log10 value = 4] and $\alpha = 0.001$ [$-$log10 value = 3]). The location of *TYRP1* is indicated by the black triangle.

(B) Effect sizes for SNPs above the two significance thresholds (filled for those above the more stringent threshold).

were differentially methylated between migrant and resident salmonids. Of 60 genes in linkage blocks on chromosome 4 showing the strongest associations with winter longitude in our study, two were identified in the former studies: *TMEM192* (Lundberg et al.), which encodes a transmembrane protein with no known function, and *PALLD* (Baerwald et al.), which encodes a cytoskeletal protein involved in organizing the actin cytoskeleton (Figure 2C).

TMEM192 and *PALLD* are not the only candidates in this cluster; one of the linkage blocks includes *CLOCK* and several protein kinases (Figure 2C). This is the linkage block described above that also shows an association with cage orientation. *CLOCK* encodes a transcription factor in the circadian clock and is associated with variation in migratory timing [5]. Protein kinases are involved in phosphorylation, which helps generate circadian rhythms; both Zhu et al. [34] and Baerwald et al. [36] identified protein kinases in their analyses.

The co-localization of genes associated with the circadian clock (e.g., *CLOCK*), nervous system (e.g., endopeptidases), and cell signaling (e.g., *PALLD*) that are associated with migratory traits in other animal groups is intriguing. Specifically, migration is taxonomically widespread and integrates a suite of adaptive traits (e.g., fat deposition, migratory restlessness, orientation, and timing [37]). It has been argued that these traits may be inherited as a common gene package: they show a correlated response to selection, can evolve rapidly, and make

use of existing pathways [5, 38, 39]. The co-localization of genes on chromosome 4 supports this suggestion and is the first time a potential region for this gene package has been identified. Future work with larger sample sizes and additional species will be needed to narrow in on a smaller region for this potential gene package.

Conclusion

It is remarkable that a complex behavior like migratory orientation has a strong genetic basis; although all Swainson's thrushes have the machinery to orient on migration, we have shown that the decision to go south or southeast has a genetic basis, and we have localized a large-effect region for this behavior to chromosome 4. We previously documented several regions of elevated genomic differentiation between inland and coastal thrushes [23] (often referred to as "islands of differentiation"). The results presented here show that migratory orientation and plumage color map to three of these islands, implicating divergent selection in their generation. Many studies have documented islands of differentiation between closely related populations, and there is considerable interest in determining the relative importance of various features of the genome as well as divergent or background selection in generating these islands [6–8]. By integrating results from genetic mapping, estimates of genomic divergence, and rigorous quantification of phenotypic traits, we suggest that our results are consistent with divergent

selection generating at least some of the peaks between thrushes. We hope that our approach will guide future studies in this field, expanding beyond trait mapping to inform our understanding of evolution and speciation.

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