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Journal

G3: Genes, Genomes, Genetics, 8(12)

ISSN

2160-1836

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Publication Date

2018-12-01

DOI

10.1534/g3.118.200754

Peer reviewed

G3 Genes | Genomes | Genetics

INVESTIGATION

Whole-Genome Analysis of Introgression Between the Spotted Owl and Barred Owl (*Strix occidentalis* and *Strix varia*, Respectively; Aves: Strigidae) in Western North America

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ABSTRACT As the barred owl (*Strix varia*; Aves: Strigiformes: Strigidae) expands throughout western North America, hybridization between barred and spotted owls (*Strix varia* and *S. occidentalis*, respectively), if abundant, may lead to genetic swamping of the endangered spotted owl. We analyzed low-coverage, whole-genome sequence data from fifty-one barred and spotted owls to investigate recent introgression between these two species. Although we obtained genomic confirmation that these species can and do hybridize and backcross, we found no evidence of widespread introgression. Plumage characteristics of western *S. varia* that suggested admixture with *S. occidentalis* appear unrelated to *S. occidentalis* ancestry and may instead reflect local selection.

KEYWORDS
bird
avian
hybridization
raptor
population genomics
admixture

Over the past century, humans have introduced several non-native vertebrate species in western North America into the native range of closely related species and generated moving hybrid swarms. For example, in California, genes of the non-native barred tiger salamander (*Ambystoma tigrinum*) are spreading into the range of the California tiger salamander (*A. californiense*) (Fitzpatrick *et al.* 2009, 2010). In the Flathead River system of Montana and British Columbia, the non-native rainbow trout (*Oncorhynchus mykiss*) is rapidly hybridizing with the native westslope cutthroat trout (*O. clarkii lewisi*) (Muhlfeld *et al.* 2014).

doi: https://doi.org/10.1534/g3.118.200754
Manuscript received September 20, 2018; accepted for publication October 19, 2018; published Early Online October 24, 2018.

Supplemental material available at Figshare: https://figshare.com/s/fecd4ae5b6514a66f29d.
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In addition to hybridization resulting from intentional introductions of non-native species, changing global climatic conditions and the documented movement of species ranges have led many species to invade novel geographic regions (Parmesan *et al.* 1999; Parmesan 2006) and establish broad contact with related taxa (Rieseberg *et al.* 2007).

The spotted owl (*Strix occidentalis*) is a large wood owl inhabitant of western North American forests. The U.S. Fish and Wildlife Service listed the northern spotted owl (*S. o. caurina*) as "threatened" under the Endangered Species Act (ESA) in 1990 (Thomas *et al.* 1990) and the species remains protected due to continuing population declines (Dugger *et al.* 2015; Davis *et al.* 2016). While researchers considered habitat loss the primary threat to the northern spotted owl in 1990 (Forsman *et al.* 1984; Anderson and Burnham 1992), recent research has confirmed a second major threat to its persistence: the invasion of the congeneric barred owl (*S. varia*) into western North American forests (Dugger *et al.* 2015; Diller *et al.* 2016). Previously inhabiting areas east of the Rocky Mountains and Great Plains (Mazur and James 2000), the barred owl has expanded its range to western North America over the last

50-100 years (Dark *et al.* 1998; Livezey 2009a, 2009b). At present, sympatric populations of spotted and barred owls exist from British Columbia to southern California (Taylor and Forsman 1976; Haig *et al.* 2004; Livezey 2009a).

Strix occidentalis and *S. varia* are approximately 13.9% divergent in the mitochondrial control region (Haig *et al.* 2004), 10.74% divergent in non-tRNA mitochondrial genes (Hanna *et al.* 2017a), and 0.7% divergent across the nuclear genome (Hanna *et al.* 2017c). *Strix occidentalis* and *S. varia* hybridize and backcross (Haig *et al.* 2004; Kelly and Forsman 2004; Funk *et al.* 2007), with heterospecific matings and F_1 hybrids commonly reported in areas where *S. varia* is rare and *S. occidentalis* is common (Kelly and Forsman 2004). Recently collected adult western *S. varia* specimens in museum collections display striking morphological variation. *Strix varia* from the eastern Klamath Mountains in Siskiyou County, California, have darker plumage overall, more spotting on the belly, and are smaller than barred owls from the Coast Range (Figure 1 and Figure S1). These differences suggest either local selection for this phenotype or possible introgression of spotted owl genes. Hybridization of these species creates a potential for a loss of biodiversity in western North America due either to replacement of the spotted owl by the barred owl or to collapse of the boundaries of the two species (Huxel 1999).

For this study, we obtained fifty-one low-coverage whole-genome sequences (median 0.723X coverage) from *Strix varia* and *S. occidentalis* sampled outside and across their contact zone in western North America (Figure 2). Existing methods available for detecting and quantifying admixture in genomic data require either specific genotype calls or accurate genotype likelihood estimates (*e.g.*, Skotte *et al.* 2013; Wall *et al.* 2016; Corbett-Detig and Nielsen 2017; Meisner and Albrechtsen 2018). For most individuals, the vast majority of variants had a depth of coverage of 0 or 1. In this context, genotype likelihoods or called genotypes are not meaningful and we were unable to utilize existing methods that rely on them. This necessitated that we develop a new method for quantifying admixture that only requires single reads at various sites. We utilized available medium and high-coverage whole-genome sequences from an eastern *S. varia* (15.549X coverage) and a pre-contact *S. occidentalis* (60.815X coverage) to identify variant sites potentially fixed between *S. varia* and *S. occidentalis*. For each low-coverage individual, we determined the genome-wide average ancestry and searched for windows of ancestry that were outliers from the average to detect rare, introgressed regions. We used these data to identify the extent of introgression between *S. varia* and *S. occidentalis* in western North America.

Figure 1 Comparison of eastern barred owl, Siskiyou County barred owl, and northern spotted owl plumages. This image displays the darker ventral plumage of a *Strix varia* collected in Siskiyou County, California compared with that of typical *S. varia* and *S. occidentalis caurina* individuals. On the left is the ventral plumage of a *Strix varia* from eastern North America. In the center is a *S. varia* from Siskiyou County, California. On the right is a *S. occidentalis caurina* from northern California. Author Z.R.H. took this photograph.

METHODS

Samples

We obtained fifty-one samples from museum collections that included eleven *Strix occidentalis* samples (two samples predated contact with *S. varia*), thirty-eight *S. varia* samples (including five from eastern North America), and two samples identified by other researchers as probable hybrid *S. varia* x *occidentalis* individuals (Tables S1-S2). We mapped the samples using QGIS version 2.18.2 (Quantum GIS Development Team 2017) with raster and vector files from Natural Earth (http://www.naturalearthdata.com) (File S1 section 1.1).

Sequence data

Hybridization of *Strix varia* and *S. occidentalis* has previously been investigated using a set of four microsatellite (Funk *et al.* 2007) and fourteen amplified fragment-length polymorphism (Haig *et al.* 2004) markers, which the authors found useful for diagnosing F_1 and F_2 hybrids (Haig *et al.* 2004; Funk *et al.* 2007). We utilized a whole-genome sequencing approach to enable us to detect any introgression that has taken place over the last 50-70 years that *S. varia* and *S. occidentalis* have been in contact in western North America (Taylor and Forsman 1976; Livezey 2009a).

We utilized whole genome sequencing data from a previous study (Hanna *et al.* 2017c) for our reference pre-contact *Strix occidentalis* and eastern *S. varia* samples (NCBI Sequence Read Archive (SRA) run accessions SRR4011595, SRR4011596, SRR4011597, SRR4011614, SRR4011615, SRR4011616, SRR4011617, SRR4011618, SRR4011619, and SRR4011620 for *S. occidentalis* sample CAS:ORN:98821; SRR5428115, SRR5428116, and SRR5428117 for *S. varia* sample CNHM < USA-OH>: ORNITH:B41533, hereafter referred to as CNHMB41533). We prepared whole genome libraries for fifty-one additional (*i.e.*, non-reference) *Strix* samples using a Nextera DNA Sample Preparation Kit (Illumina) and obtained paired-end sequences from a HiSeq 2500 (Illumina) (File S1 section 1.2) resulting in coverage ranging from 0.02-6.41X after filtering.

Alignment and filtering

For the sequence data of the reference samples *Strix occidentalis* CAS:ORN:98821 and *S. varia* CNHMB41533, which Hanna *et al.* (2017c) generated for their study, we followed the sequence processing methods described in Hanna *et al.* (2017c) to trim and filter the genomic sequence data. For all other samples we used Trimmomatic version 0.32 (Bolger *et al.* 2014) to remove adapter sequences and perform quality

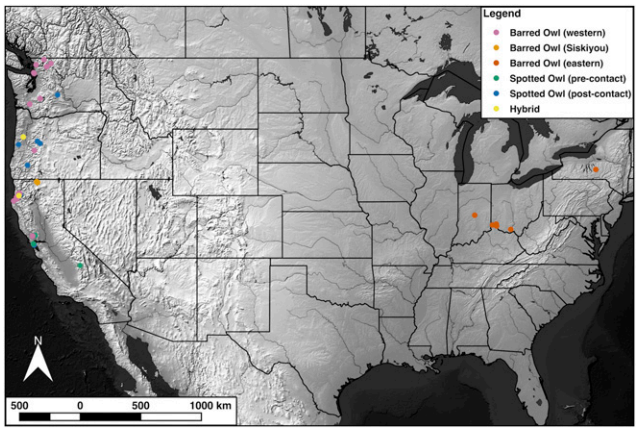

Figure 2 Sample map. This map displays the sampling locations of all of the *Strix* specimens included in this study.

trimming of all of the low-coverage, short-read data (File S1 section 1.3). We used BWA-MEM version 0.7.12-r1044 (Li 2013) to align the processed reference and low-coverage sequences to the repeat-masked *S. o. caurina* genome "StrOccCau_1.0_nuc_masked" (Hanna *et al.* 2017d, 2017c). We merged the alignments, sorted the alignments, and marked duplicate sequences using Picard version 1.104 (http://broadinstitute.github.io/picard; accessed 2017 Oct 1) (File S1 section 1.4.1-1.4.2). We filtered the alignment files to only retain alignments of high quality using the Genome Analysis Toolkit (GATK) version 3.4-46 PrintReads tool (McKenna *et al.* 2010; DePristo *et al.* 2011; Van der Auwera *et al.* 2013; GATK Dev Team 2017) (File S1 section 1.4.3).

Variant calling and filtering

We called variants using the GATK version 3.4-46 UnifiedGenotyper tool (McKenna *et al.* 2010; DePristo *et al.* 2011; Van der Auwera *et al.* 2013) with the alignment files for all samples included as simultaneous inputs (File S1 section 1.5.1). We used the vcf_qual_filter.sh script from SPOW-BDOW-introgression-scripts version 1.1.1 (Hanna *et al.* 2017b) to exclude indels and low genotyping quality sites while retaining only biallelic sites where CAS:ORN:98821 (the source of the StrOccCau_1.0_nuc_masked reference genome) was homozygous for the reference allele and CNHMB41533, the *Strix varia* reference sample, was homozygous for the alternative allele (File S1 section 1.6.1). Of the remaining variable sites, we excluded those with excessively high coverage [greater than the mean plus five times the standard deviation (σ), as recommended by the GATK documentation (https://software.broadinstitute.org/gatk/guide/article?id=3225; accessed 2017 Oct 1)] (File S1 section 1.6.2). We used DP_means_std_dev.sh from SPOW-BDOW-introgression-scripts version 1.1.1 to calculate the mean and standard deviation (σ) of the depth of coverage for each sample across the final set of variant sites.

Ancestry and diversity analyses

For each sample at each of the final variant sites, we calculated a percentage spotted owl ancestry, which was the percentage of the coverage that supported the CAS:ORN:98821 (the *Strix occidentalis* reference sequence) allele. We calculated the mean and standard deviation of the spotted owl ancestry of each sample across all variant sites (File S1 section 1.6.3). We tested for significant differences between the mean spotted owl ancestries in populations using Welch's *t*-test (Welch 1947) as the populations had unequal numbers of samples and then applied a Bonferroni adjustment (Dunn 1961) when we evaluated significance (File S1 section 1.6.4).

We estimated the probabilities of observing an introgressed region greater than 50,000 nt, 100,000 nt, or 150,000 nt in length if *Strix varia* and *S. occidentalis* hybridized in 1945, approximately the earliest date of their potential contact (Livezey 2009a), using the formula from Racimo *et al.* (2015). For the recombination rate, we used 1.5 centimorgans/million nucleotides (cM/Mnt), which Backström *et al.* (2010) estimated for the zebra finch (*Taeniopygia guttata*). For the number of generations since the earliest potential date of hybridization, we assumed a generation time of two years (Gutiérrez *et al.* 1995; Mazur and James 2000) even though *S. o. caurina* is able to breed in its first year and others have used ten years as the generation time for *S. o. caurina* (Noon and Biles 1990; USDA Forest Service 1992). With that generation time, approximately thirty-five generations have potentially elapsed since the two species first contacted in 1945 and 2014, the date of our most recent sample.

In order to probe further for evidence of introgression in the samples that did not appear as hybrids from their genome-wide average spotted owl ancestry, we attempted to identify regions that were outliers from the genome-wide ancestry average by conducting a sliding window analysis. We examined adjacent windows of 50,000 nucleotides (nt) where a sample had data for at least ten variant sites within that window and calculated the average spotted owl ancestry for the window. We assumed that, if a region was introgressed from the other species, the average should be close to 0.5. Thus, in samples with an average genome-wide ancestry close to 0, we called a window an outlier if the average spotted owl ancestry was $>= 0.4$. Inversely, in samples with an average genome-wide ancestry close to 1, we called a window an outlier if the average spotted owl ancestry was $<= 0.6$ (File S1 sections 1.6.5-1.6.6).

In order to estimate the genome-wide diversity harbored by *Strix varia* and *S. occidentalis* populations, we considered all biallelic variant sites (not just those fixed between our *S. varia* and *S. occidentalis* references) and calculated π_{Within}, the number of nucleotide differences within populations, and $\pi_{Between}$, the number of nucleotide differences between populations using the countFstPi script from SPOW-BADO-introgression-scripts (Hanna *et al.* 2017b). We also used countFstPi to calculate the fixation index (F_{ST}) (Hudson *et al.* 1992) in order to estimate the differentiation of *S. varia* and *S. occidentalis* populations (File S1 section 1.6.7).

Data availability

Raw whole genome sequences are available from the NCBI Sequence Read Archive (SRA) run accessions SRR4011595-SRR4011597, SRR4011614-SRR4011620, SRR5428115-SRR5428117, SRR6026668, SRR6032894-SRR6032902, SRR6032904-SRR6032907, and SRR6032910-SRR6033014. See Table 1 for the specific accessions corresponding with each sample. We uploaded supplementary material (Figures S1-S3, Tables S1-S5, and File S1) to figshare. Supplemental material available at Figshare: https://figshare.com/s/fecd4ae5b6514a66f29d.

RESULTS

After filtering, the final set of variable sites fixed between the *Strix varia* and *S. occidentalis* reference individuals included 5,816,692 sites. The median genome coverage per individual was 0.723X (Table S3). Except for the two putative hybrid samples that we included as a test of our methodology, the genome-wide average spotted owl ancestry for all samples was close to either 0 or 1, indicating that they were either pure *S. varia* or *S. occidentalis*, respectively (Figure 3 and Table S3). A genome-wide average

Table 1 Genomic sequence data details for each sample

Voucher Specimen Identifier	Other Sample Identifier	Sample Set	SRA ACCN
CAS:ORN:98821	Sequoia	N/A	SRR4011595, SRR4011596, SRR4011597, SRR4011614, SRR4011615, SRR4011616, SRR4011617, SRR4011618, SRR4011619, SRR4011620,
CNHM < USA-OH>:ORNITH:B41533	CMCB41533	N/A	SRR5428115, SRR5428116, SRR5428117
CAS:ORN:87569	CAS87569	1	SRR6032959
CAS:ORN:92982	ASG007	1	SRR6032957
CAS:ORN:95475	MK994	1	SRR6032939
CAS:ORN:95789	JMR920	1	SRR6032933
CAS:ORN:95790	ASG037	1	SRR6032960
CAS:ORN:95964	MEF457	1	SRR6026668
CAS:ORN:97181	MK1020	1	SRR6032934
CNHM < USA-OH>:ORNITH:B40819	CMCB40819	1	SRR6032951
CNHM < USA-OH>:ORNITH:B40824	CMC40824	1	SRR6032952
CNHM < USA-OH>:ORNITH:B41566	CMCB41566	1	SRR6032935
CUMV:Bird:51478	CU51478	1	SRR6032936
MVZ:Bird:189508	ZRH455	1	SRR6032920
UWBM:Bird:62061	UWBM62061	1	SRR6032940
UWBM:Bird:76815	UWBM76815	1	SRR6032937
UWBM:Bird:91379	UWBM91379	1	SRR6032938
UWBM:Bird:91382	UWBM91382	1	SRR6032931
UWBM:Bird:91408	UWBM91408	1	SRR6032932
CAS:ORN:92979	MK968	2	SRR6032898, SRR6032899, SRR6032916
CAS:ORN:92980	MK987	2	SRR6032914, SRR6032915, SRR6032917
CAS:ORN:92981	MEF404	2	SRR6032941, SRR6032945, SRR6032946
CAS:ORN:95476	MK998	2	SRR6032910, SRR6032912, SRR6032913
CAS:ORN:95477	ASG017	2	SRR6032902, SRR6032904, SRR6032905
CAS:ORN:97049	LCW491	2	SRR6032943, SRR6032944, SRR6032950
CAS:ORN:97052	LCW443	2	SRR6032947, SRR6032948, SRR6032949
CAS:ORN:97174	MEF432	2	SRR6032894, SRR6032895, SRR6032942
CAS:ORN:97175	MK1012	2	SRR6033011, SRR6033013, SRR6033014
CAS:ORN:97176	JPD386	2	SRR6032926, SRR6032927, SRR6032928
CAS:ORN:97177	MEF435	2	SRR6032896, SRR6032897, SRR6033012
CAS:ORN:97201	LCW405	2	SRR6032925, SRR6032929, SRR6032930
CAS:ORN:97815	Hoopa20005	2	SRR6032900, SRR6032906, SRR6032907
CAS:ORN:97816	Hoopa20018	2	SRR6032924, SRR6032961, SRR6032962
CAS:ORN:97818	Hoopa20011	2	SRR6032901, SRR6032965, SRR6032966
CAS:ORN:97819	Hoopa20019	2	SRR6032921, SRR6032922, SRR6032923
CAS:ORN:97820	Hoopa20017	2	SRR6032967, SRR6032968, SRR6032970
CAS:ORN:97822	Hoopa20014	2	SRR6032963, SRR6032964, SRR6032969
CAS:ORN:98171	ZRH962	2	SRR6032955, SRR6032956, SRR6032958
CAS:ORN:98198	ZRH602	2	SRR6032992, SRR6032997, SRR6032998
CAS:ORN:99315	ZRH604	2	SRR6032995, SRR6032996, SRR6032999
CAS:ORN:99320	ZRH607	2	SRR6032953, SRR6032954, SRR6033000
CAS:ORN:99423	NSO138799040	2	SRR6032911, SRR6032918, SRR6032919
CAS:ORN:99425	NSO168709365	2	SRR6032988, SRR6032989, SRR6032990
UWBM:Bird:53433	UWBM53433	2	SRR6032985, SRR6032986, SRR6032987
UWBM:Bird:65055	UWBM65055	2	SRR6032982, SRR6032983, SRR6032984
UWBM:Bird:67015	UWBM67015	2	SRR6032981, SRR6033003, SRR6033004
UWBM:Bird:74078	UWBM74078	2	SRR6033005, SRR6033006, SRR6033007
UWBM:Bird:79007	UWBM79007	2	SRR6033008, SRR6033009, SRR6033010
UWBM:Bird:79049	UWBM79049	2	SRR6032972, SRR6033001, SRR6033002
UWBM:Bird:79141	UWBM79141	2	SRR6032971, SRR6032973, SRR6032974
UWBM:Bird:91380	UWBM91380	2	SRR6032975, SRR6032976, SRR6032978
UWBM:Bird:91392	UWBM91392	2	SRR6032977, SRR6032979, SRR6032980
UWBM:Bird:91393	UWBM91393	2	SRR6032991, SRR6032993, SRR6032994

The "Specimen Identifier" column provides the voucher specimen codes. The "Other Sample Identifier" column provides an abbreviated sample code. Column "Sample Set" refers to the round of sequencing that produced the sequence data for a given sample. The main and supplemental methodology sections provide details of the production of these two sets of sequence data. Column "SRA ACCN" provides NCBI Sequence Read Archive (SRA) run accessions in which the raw sequences for each sample are archived.

spotted owl ancestry of 0.538 confirmed the F_1 hybrid (*S. varia* x *occidentalis*) identity of a sample from Humboldt County, California. We calculated a spotted owl ancestry of 0.359 for the second hybrid sample from Benton County, Oregon, which suggested that this individual was likely a F_2 hybrid (F_1 x *S. varia* backcross).

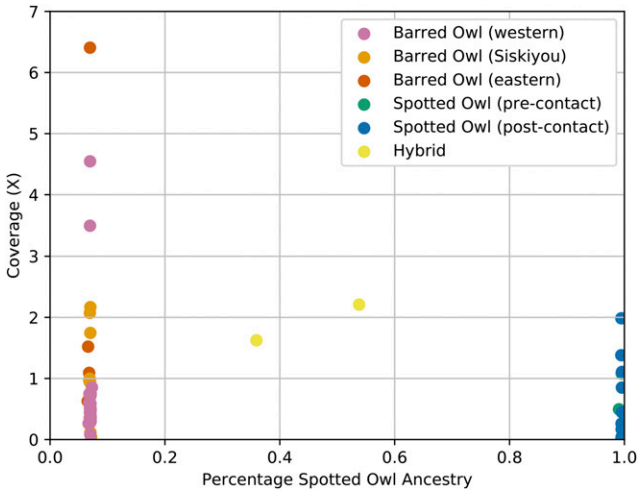

Figure 3 Plot of coverage vs. genome-wide average spotted owl ancestry. The average spotted owl (*Strix occidentalis*) ancestry of all of the samples for which we collected low-coverage, whole-genome sequence data. We plotted DNA sequence coverage on the y-axis to display that the average percentage of spotted owl ancestry was independent of the amount of coverage for a given sample.

The mean genome-wide spotted owl ancestry of the Siskiyou County *Strix varia* population was 0.0696 whereas the mean was 0.0699 for the rest of the western *S. varia* (Table S4). There was no significant difference in spotted owl ancestry between these two populations (Table S5). When we combined all *S. varia* from western North America together (0.0698 mean spotted owl ancestry) and compared their spotted owl ancestry with that of the eastern *S. varia* (0.0676 mean spotted owl ancestry), we found no significant difference in ancestry between the western and eastern *S. varia* after applying a Bonferroni adjustment (Tables S4 and S5). There was also no significant difference in spotted owl ancestry between *S. occidentalis* individuals sampled from populations not in contact with *S. varia* and those from populations already in contact with *S. varia* (mean ancestries of 0.9930 and 0.9952, respectively) (Tables S4 and S5).

The average spotted owl ancestry in the *Strix varia* samples ranged from approximately 6.55–7.28% greater than the 0% value at which our methodology set the reference *S. varia* (Table S3). The *S. occidentalis* samples ranged from approximately 0.43–0.94% less than the 100% value for the reference *S. occidentalis*. The standard deviation in the *S. varia* samples was consistently more than two times greater than the standard deviation in the *S. occidentalis* samples. The genome-wide average spotted owl ancestry values for the *Strix varia* individuals deviated more from those of the reference *S. varia* than did the *S. occidentalis* individuals from the *S. occidentalis* reference due to the greater amount of genetic variation within *S. varia* (Hanna *et al.* 2017c). The sites fixed between our reference *S. varia* and *S. occidentalis* samples were not fixed across all of the low-coverage *S. varia* and *S. occidentalis* individuals. Further high-coverage sequencing of whole-genomes for both species will help to more clearly identify the fixed genetic differences between the two species.

Based upon an estimate of thirty-five generations as the maximum number of generations since contact of *Strix varia* and *S. occidentalis* (Gutiérrez *et al.* 1995; Mazur and James 2000; Livezey 2009a) and the recombination rate of *Taeniopygia guttata* (Backström *et al.* 2010), we estimated that the probability of observing a tract $>$ 50,000 nt resulting from hybridization during the initial contact of *S. varia* and *S. occidentalis* was 97.41%, the probability of observing a track $>$ 100,000 nt was 94.89%, and the probability of observing a track $>$ 150,000 nt was 92.43%.

Of the forty-nine samples for which we conducted an outlier window analysis, we detected outlier windows in thirty-nine samples (79.6%). Across all samples, we detected 316 outlier windows of length 50,000 nt, forty-one of length 100,000 nt, and only three of length 150,000 nt and none exceeded this length (Figure S2). In all samples the outlier windows represented $<$ 1.01% of the analyzed windows. For thirty-six of the thirty-nine samples with outliers, the number of outlier windows was $<$ 0.08% of the analyzed windows. There were three samples for which the outlier windows represented between 0.1% and 1.01% of the analyzed windows. However, the increased proportion of outlier windows in these samples appeared to be related to exceptionally low sequence coverage as these three *Strix varia* samples had the lowest coverage (0.036-0.118X) and, consequently, the fewest number of analyzed windows of any of the samples in which we detected outlier windows (Figure S3). A *S. occidentalis* sample with 0.017X coverage was the only sample with lower coverage than those three, but our analyses did not recover any outlier windows for it.

We found little evidence of differentiation between the Siskiyou *Strix varia* and the other western *S. varia*, recovering a low F_{ST} (0.008) and very similar levels of nucleotide diversity in the two populations (Table 2). Similar levels of nucleotide diversity also exist in the *S. varia* populations from western and eastern North America. We additionally estimated a low F_{ST} value (0.051) between western and eastern *S. varia*, which suggests a low level of differentiation between these populations. *Strix occidentalis* populations pre and post-contact with *S. varia* exhibited similar levels of nucleotide diversity and appeared weakly differentiated (F_{ST} = 0.022). We estimated approximately 14X greater nucleotide diversity in *S. varia* than *S. occidentalis* and a high level of divergence (F_{ST} = 0.833) between the species.

DISCUSSION

Our genome-wide average spotted owl ancestry analysis confirmed that our two positive control hybrids from Humboldt County, California, and Benton County, Oregon, were an F_1 and F_2 (F_1 x *Strix varia*) backcross, respectively. Apart from those hybrids, our genome-wide average spotted owl ancestry analysis indicated that all individuals were either pure *S. occidentalis* or pure *S. varia* (Figure 3 and Table S3). Our global analysis found no evidence for admixture, but we cannot rule out low (*e.g.*, $<$ 2%) levels of admixture. We also implemented a sliding window approach to determine whether any such regions existed in our data. Scanning for ancestry windows that were outliers from a given individual's genome-wide average ancestry using a sliding window approach corroborated the genome-wide average results and provided no evidence of introgression between *Strix varia* and *S. occidentalis* within the past 50-70 years of their contact in western North America (Taylor and Forsman 1976; Livezey 2009a). Although our test found short windows of outlier ancestry, these represented a small proportion of the total windows analyzed for each individual. Thus, we can confidently exclude the possibility of introgression within the past ten generations. Hybridization that has occurred in the last thirty-five generations (assuming a generation time of two years for both species and erring conservatively on the side of overestimating the maximum number of generations of contact) should have yielded much longer outlier blocks than we found. Even with this conservative estimate, there is a $>$ 97% probability of introgressed regions being larger than the 50,000 nt windows that we used to check for potential introgression and a $>$ 92% probability of the introgressed regions being larger than the 150,000 nt length of the longest outlier window that we detected with our sliding window analysis.

Table 2 Nucleotide diversity and fixation index statistics calculated for various population comparisons

Population 1	Population 2	π_{Within} Pop 1	π_{Within} Pop 2	$\pi_{Between}$	F_{ST}
Western Barred Owls	Siskiyou Barred Owls	2.097E-03	2.068E-03	2.100E-03	0.008
Western Barred Owls	Eastern Barred Owls	2.119E-03	2.228E-03	2.291E-03	0.051
Siskiyou Barred Owls	Eastern Barred Owls	2.066E-03	2.203E-03	2.259E-03	0.055
All Western Barred Owls	Eastern Barred Owls	2.128E-03	2.242E-03	2.301E-03	0.051
All Barred Owls	All Spotted Owls	2.202E-03	1.572E-04	7.052E-03	0.833
Spotted Owls (pre-contact)	Spotted Owls (post)	1.073E-04	9.998E-05	1.060E-04	0.022

The π_{Within} statistic signifies the average number of pairwise differences between two individuals sampled from the same population. The $\pi_{Between}$ statistic denotes the average number of pairwise differences between two individuals sampled from different populations (Populations 1 and 2). "Pop 1" and "Pop 2" refer to Population 1 or 2 from columns 1 and 2, respectively. The "All Western Barred Owls" population is a combination of the "Western Barred Owls" and "Siskiyou Barred Owls" populations. The "Spotted Owls (pre-contact)" and "Spotted Owls (post)" populations indicate *Strix occidentalis* from populations not in contact or in contact with *S. varia*, respectively.

Since *Strix varia*'s zone of contact with *S. occidentalis* in western North America began in British Columbia and expanded southward to the southern Sierra Nevada, California (Taylor and Forsman 1976; Haig *et al.* 2004; Livezey 2009a), we expected *S. varia* individuals in the southern portion of the zone of sympatry to have the highest chance of being admixed. With this prediction in mind, we focused our sampling on *S. varia* populations in California (Figure 1) and targeted our sampling to include the morphologically anomalous western *S. varia* population in Siskiyou County, California. It is notable that we found no evidence of admixture even though these populations visually appeared intermediate in plumage between *S. varia* and *S. occidentalis*. Range expansion simulations suggest that we should predict asymmetric introgression into *S. varia* even when the hybridization rate is less than 2% (Currat and Excoffier 2011). Coupled with these predictions, our findings suggest that, although hybridization between *S. varia* and *S. occidentalis* occurs, it has either been vanishingly rare on the edge of the *S. varia* expansion wave or other processes, such as selection or migration, are effectively removing introgressed genetic material from *S. varia* and *S. occidentalis* populations.

We estimated that *Strix varia* has more than ten times greater nucleotide diversity than *S. occidentalis* and we calculated a high F_{ST} between the species (Table 2), closely matching results from high-coverage genomes of the two species (Hanna *et al.* 2017c). We estimated similar levels of nucleotide diversity in the Siskiyou *S. varia* population and the population comprised of other western *S. varia*, which was consistent with our having found no difference in spotted owl ancestry between these populations (Tables S4 and S5). Similarly, *S. occidentalis* populations pre and post-contact with *S. varia* exhibited similar levels of nucleotide diversity, appeared weakly differentiated, and did not differ in spotted owl ancestry.

We were surprised to find similar levels of nucleotide diversity in western and eastern North American *Strix varia* populations. We expected western *S. varia* populations to harbor lower genetic diversity than the eastern *S. varia* after having been subjected to successive founder effects and corresponding reductions in nucleotide diversity (Austerlitz *et al.* 1997). Simulations have suggested that long-distance dispersal by individuals of a species undergoing a range expansion can inhibit the loss of genetic diversity in the newly formed populations on the edge of the range (Ray and Excoffier 2010). Engler *et al.* (2015) suggested that this explains why some populations retained genetic diversity in an Old World warbler, *Hippolais polyglotta*, experiencing a range expansion. Recent simulations have also suggested that long-distance dispersal in an invading taxon can counteract introgression of local genetic material into the invader by inhibiting the "surfing" of introgressed genetic regions (Amorim *et al.* 2017). Livezey (2009b) reported the mean natal dispersal distance of *Strix varia* as 41.3 km, but mentioned that some individuals have dispersed as far as 488.1 km. Even if long-distance dispersal has only been occurring at low levels during the *S. varia* range expansion, this could account both the lack of reduction in genetic diversity in western *S. varia* and for the lack of large-scale introgression of *S. occidentalis* genetic material into western *S. varia* populations (Ray and Excoffier 2010; Amorim *et al.* 2017). Long-distance dispersal would have been especially capable of countering introgression of *S. occidentalis* material if non-introgressed *S. varia* were dispersing to the front of the expansion wave (Amorim *et al.* 2017). Long-distance dispersal may also lead to high rates of intraspecific gene flow in western *S. varia*, which could both maintain *S. varia* genetic diversity and counter introgression of *S. occidentalis* genetic material (Ray *et al.* 2003; Currat *et al.* 2008; Petit and Excoffier 2009).

Although our results provide genomic confirmation that hybridization and backcrossing does occur, we found no evidence of widespread admixture between *Strix varia* and *S. occidentalis* in western North America. The distinctive plumage of the *S. varia* individuals collected in Siskiyou County, California, (Figure 1 and Figure S1) does not appear to be a result of hybridization with *S. occidentalis*. We conclude that some plumage characteristics that appear intermediate between *S. varia* and *S. occidentalis* do not in fact indicate hybridization. Previous investigators have issued similar cautionary statements after their genetic studies of hybridization in these taxa (Haig *et al.* 2004; Funk *et al.* 2007). The lack of spotted owl ancestry in these oddly plumaged western *S. varia* suggests that some western *S. varia* may be undergoing drift or local selection, which has affected plumage and size. Coupled with demographic studies (Kelly *et al.* 2003; Dugger *et al.* 2015; Diller *et al.* 2016), our results indicate that the expansion of *S. varia* into the range of *S. occidentalis* in western North America is following a pattern of pure replacement, rather than inducing extinction through hybridization and introgression (Rhymer and Simberloff 1996). It seems unlikely that even introgressed remnants of the *S. occidentalis* genome will remain in areas in contact with *S. varia* if *S. occidentalis* is not able to persist.

ACKNOWLEDGMENTS

For access to specimens and genetic samples, we thank Lowell Diller; J. Mark Higley and Aaron Pole of the Hoopa Valley Indian Reservation Tribal Forestry department; Susan Haig, Tom Mullins, and Mark Miller of the USGS Forest and Rangeland Ecosystem Science Center; Mary Estes and Morgan of the Chintimini Wildlife Center, Corvallis; Jami Ostby-Marsh and Oroville of the West Valley Outdoor Learning Center, Spokane Valley; Melanie Piazza and WildCare, San Rafael; Sharon Birks and the Burke Museum; Herman L. Mays, Jr., Jane MacKnight, Lauren Hancock, and the Cincinnati

Museum Center; Irby Lovette and the Cornell University Museum of Vertebrates; Maureen Flannery, Laura Wilkinson, and the California Academy of Sciences; and Carla Cicero, Theresa Barclay, Shelby Medina, Elizabeth Wommack, and the Museum of Vertebrate Zoology. We thank Anna Sellas for assistance with laboratory work. We generated genomic libraries at the Center for Comparative Genomics, California Academy of Sciences.

LITERATURE CITED

Amorim, C. E. G., T. Hofer, N. Ray, M. Foll, A. Ruiz-Linares *et al.*, 2017 Long-distance dispersal suppresses introgression of local alleles during range expansions. Heredity 118: 135–142. https://doi.org/10.1038/hdy.2016.68

Anderson, D. R., and K. P. Burnham, 1992 Demographic analysis of Northern Spotted Owl populations. In *Final Draft Recovery Plan for the Northern Spotted Owl*, Volume 2. USDI Fish and Wildlife Service, Region 1, Portland, OR. p. 66–75.

Austerlitz, F., B. Jung-Muller, B. Godelle, and P.-H. Gouyon, 1997 Evolution of Coalescence Times, Genetic Diversity and Structure during Colonization. Theor. Popul. Biol. 51: 148–164. https://doi.org/10.1006/tpbi.1997.1302

Backström, N., W. Forstmeier, H. Schielzeth, H. Mellenius, K. Nam *et al.*, 2010 The recombination landscape of the zebra finch *Taeniopygia guttata* genome. Genome Res. 20: 485–495. https://doi.org/10.1101/gr.101410.109

Bolger, A. M., M. Lohse, and B. Usadel, 2014 Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30: 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Corbett-Detig, R., and R. Nielsen, 2017 A hidden Markov model approach for simultaneously estimating local ancestry and admixture time using next generation sequence data in samples of arbitrary ploidy. PLoS Genet. 13: e1006529. https://doi.org/10.1371/journal.pgen.1006529

Currat, M., and L. Excoffier, 2011 Strong reproductive isolation between humans and Neanderthals inferred from observed patterns of introgression. Proc. Natl. Acad. Sci. USA 108: 15129–15134. https://doi.org/10.1073/pnas.1107450108

Currat, M., M. Ruedi, R. J. Petit, and L. Excoffier, 2008 The hidden side of invasions: massive introgression by local genes. Evolution 62: 1908–1920. https://doi.org/10.1111/j.1558-5646.2008.00413.x

Dark, S. J., R. J. Gutiérrez, and G. I. Gould, Jr., 1998 The Barred Owl (*Strix varia*) Invasion in California. Auk 115: 50–56. https://doi.org/10.2307/4089110

Davis, R. J., B. Hollen, J. Hobson, J. E. Gower, and D. Keenum, 2016 Northwest Forest Plan—the first 20 years (1994–2013): status and trends of northern spotted owl habitats. Available at: http://www.treesearch.fs.fed.us/pubs/50567. Accessed: October 7, 2016.

DePristo, M. A., E. Banks, R. Poplin, K. V. Garimella, J. R. Maguire *et al.*, 2011 A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat. Genet. 43: 491–498. https://doi.org/10.1038/ng.806

Diller, L. V., K. A. Hamm, D. A. Early, D. W. Lamphear, K. M. Dugger *et al.*, 2016 Demographic response of northern spotted owls to barred owl removal. J. Wildl. Manage. 80: 691–707. https://doi.org/10.1002/jwmg.1046

Dugger, K. M., E. D. Forsman, A. B. Franklin, R. J. Davis, G. C. White *et al.*, 2015 The effects of habitat, climate, and Barred Owls on long-term demography of Northern Spotted Owls. Condor 118: 57–116. https://doi.org/10.1650/CONDOR-15-24.1

Dunn, O. J., 1961 Multiple Comparisons among Means. J. Am. Stat. Assoc. 56: 52–64. https://doi.org/10.1080/01621459.1961.10482090

Engler, J. O., J. Secondi, D. A. Dawson, O. Elle, and A. Hochkirch, 2015 Range expansion and retraction along a moving contact zone has no effect on the genetic diversity of two passerine birds. Ecography 39: 884–893. https://doi.org/10.1111/ecog.01520

Fitzpatrick, B. M., J. R. Johnson, D. K. Kump, H. B. Shaffer, J. J. Smith *et al.*, 2009 Rapid fixation of non-native alleles revealed by genome-wide SNP analysis of hybrid tiger salamanders. BMC Evol. Biol. 9: 176. https://doi.org/10.1186/1471-2148-9-176

Fitzpatrick, B. M., J. R. Johnson, D. K. Kump, J. J. Smith, S. R. Voss *et al.*, 2010 Rapid spread of invasive genes into a threatened native species. Proc. Natl. Acad. Sci. USA 107: 3606–3610. https://doi.org/10.1073/pnas.0911802107

Forsman, E. D., E. C. Meslow, and H. M. Wight, 1984 Distribution and Biology of the Spotted Owl in Oregon. Wildl. Monogr. 48: 1–64.

Funk, W. C., T. D. Mullins, E. D. Forsman, and S. M. Haig, 2007 Microsatellite loci for distinguishing spotted owls (*Strix occidentalis*), barred owls (*Strix varia*), and their hybrids. Mol. Ecol. Notes 7: 284–286. https://doi.org/10.1111/j.1471-8286.2006.01581.x

GATK Dev Team, 2017 GATK Tool Documentation. Available at: https://software.broadinstitute.org/gatk/documentation/. Accessed: October 3, 2017.

Gutiérrez, R. J., A. B. Franklin, and W. S. Lahaye, 1995 Spotted Owl (*Strix occidentalis*). *The Birds of North America Online* (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology. Retrieved from the Birds of North America Online: Available at: https://birdsna.org/Species-Account/bna/species/spoowl. Accessed: October 1, 2016.

Haig, S. M., T. D. Mullins, E. D. Forsman, P. W. Trail, and L. Wennerberg, 2004 Genetic identification of spotted owls, barred owls, and their hybrids: legal implications of hybrid identity. Conserv. Biol. 18: 1347–1357. https://doi.org/10.1111/j.1523-1739.2004.00206.x

Hanna, Z. R., J. B. Henderson, A. B. Sellas, J. Fuchs, R. C. K. Bowie *et al.*, 2017a Complete mitochondrial genome sequences of the northern spotted owl (*Strix occidentalis caurina*) and the barred owl (*Strix varia*; Aves: Strigiformes: Strigidae) confirm the presence of a duplicated control region. PeerJ 5: e3901. https://doi.org/10.7717/peerj.3901

Hanna, Z. R., J. B. Henderson, and J. D. Wall, 2017b *SPOW-BDOW-introgression-scripts. Version 1.1.1*, Zenodo. Available at: https://zenodo.org

Hanna, Z. R., J. B. Henderson, J. D. Wall, C. A. Emerling, J. Fuchs *et al.*, 2017c Northern Spotted Owl (*Strix occidentalis caurina*) Genome: Divergence with the Barred Owl (*Strix varia*) and Characterization of Light-Associated Genes. Genome Biol. Evol. 9: 2522–2545. https://doi.org/10.1093/gbe/evx158

Hanna, Z. R., J. B. Henderson, J. D. Wall, C. A. Emerling, J. Fuchs *et al.*, 2017d Supplemental dataset for Northern Spotted Owl (*Strix occidentalis caurina*) genome assembly version 1.0. Zenodo.

Hudson, R. R., M. Slatkin, and W. P. Maddison, 1992 Estimation of Levels of Gene Flow from DNA Sequence Data. Genetics 132: 583–589.

Huxel, G. R., 1999 Rapid displacement of native species by invasive species: effects of hybridization. Biol. Conserv. 89: 143–152. https://doi.org/10.1016/S0006-3207(98)00153-0

Kelly, E. G., and E. D. Forsman, 2004 Recent Records of Hybridization Between Barred Owls (*Strix varia*) and Northern Spotted Owls (*S. occidentalis caurina*). Auk 121: 806–810. https://doi.org/10.1642/0004-8038(2004)121[0806:RROHBB]2.0.CO;2

Kelly, E. G., E. D. Forsman, and R. G. Anthony, 2003 Are barred owls displacing spotted owls? Condor 105: 45–53. https://doi.org/10.1650/0010-5422(2003)105[45:ABODSO]2.0.CO;2

Li, H., 2013 Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. ArXiv:1303.3997 Q-Bio. Accessed: February 16, 2016.

Livezey, K. B., 2009a Range Expansion of Barred Owls, Part I: Chronology and Distribution. Am. Midl. Nat. 161: 49–56. https://doi.org/10.1674/0003-0031-161.1.49

Livezey, K. B., 2009b Range Expansion of Barred Owls, Part II: Facilitating Ecological Changes. Am. Midl. Nat. 161: 323–349. https://doi.org/10.1674/0003-0031-161.2.323

Mazur, K. M., and P. C. James, 2000 Barred Owl (*Strix varia*). *The Birds of North America Online* (A. Poole, Ed.) Ithaca: Cornell Lab of Ornithology. Retrieved from the Birds of North America Online: https://birdsna.org/Species-Account/bna/species/brdowl. Accessed: October 1, 2016.

McKenna, A., M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis *et al.*, 2010 The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20: 1297–1303. https://doi.org/10.1101/gr.107524.110

Meisner, J., and A. Albrechtsen, 2018 Inferring population structure and admixture proportions in low-depth NGS data. Genetics 210: 719–731. https://doi.org/10.1534/genetics.118.301336

Muhlfeld, C. C., R. P. Kovach, L. A. Jones, R. Al-Chokhachy, M. C. Boyer *et al.*, 2014 Invasive hybridization in a threatened species is accelerated by climate change. Nat. Clim. Chang. 4: 620–624. https://doi.org/10.1038/nclimate2252

Noon, B. R., and C. M. Biles, 1990 Mathematical Demography of Spotted Owls in the Pacific Northwest. J. Wildl. Manage. 54: 18–27. https://doi.org/10.2307/3808895

Parmesan, C., 2006 Ecological and Evolutionary Responses to Recent Climate Change. Annu. Rev. Ecol. Evol. Syst. 37: 637–669. https://doi.org/10.1146/annurev.ecolsys.37.091305.110100

Parmesan, C., N. Ryrholm, C. Stefanescu, J. K. Hill, C. D. Thomas *et al.*, 1999 Poleward shifts in geographical ranges of butterfly species associated with regional warming. Nature 399: 579–583. https://doi.org/10.1038/21181

Petit, R. J., and L. Excoffier, 2009 Gene flow and species delimitation. Trends Ecol. Evol. 24: 386–393. https://doi.org/10.1016/j.tree.2009.02.011

Quantum GIS Development Team, 2017 Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. Available at: http://qgis.org. Accessed: September 16, 2017.

Racimo, F., S. Sankararaman, R. Nielsen, and E. Huerta-Sánchez, 2015 Evidence for archaic adaptive introgression in humans. Nat. Rev. Genet. 16: 359–371. https://doi.org/10.1038/nrg3936

Ray, N., M. Currat, and L. Excoffier, 2003 Intra-Deme Molecular Diversity in Spatially Expanding Populations. Mol. Biol. Evol. 20: 76–86. https://doi.org/10.1093/molbev/msg009

Ray, N., and L. Excoffier, 2010 A first step towards inferring levels of long-distance dispersal during past expansions. Mol. Ecol. Resour. 10: 902–914. https://doi.org/10.1111/j.1755-0998.2010.02881.x

Rhymer, J. M., and D. Simberloff, 1996 Extinction by Hybridization and Introgression. Annu. Rev. Ecol. Syst. 27: 83–109. https://doi.org/10.1146/annurev.ecolsys.27.1.83

Rieseberg, L. H., S.-C. Kim, R. A. Randell, K. D. Whitney, B. L. Gross *et al.*, 2007 Hybridization and the colonization of novel habitats by annual sunflowers. Genetica 129: 149–165. https://doi.org/10.1007/s10709-006-9011-y

Skotte, L., T. S. Korneliussen, and A. Albrechtsen, 2013 Estimating individual admixture proportions from next generation sequencing data. Genetics 195: 693–702. https://doi.org/10.1534/genetics.113.154138

Taylor, A. L., and E. D. Forsman, 1976 Recent range extensions of the Barred Owl in western North America, including the first records for Oregon. Condor 78: 560–561. https://doi.org/10.2307/1367110

Thomas, J. W., E. D. Forsman, J. B. Lint, E. C. Meslow, B. R. Noon *et al.*, 1990 *A conservation strategy for the northern spotted owl: report of the Interagency Scientific Committee to address the conservation of the northern spotted owl. USDA Forest Service*, USDI Bureau of Land Management, Fish and Wildlife Service, National Park Service, Portland, Oregon.

USDA Forest Service, 1992 Final Environmental Impact Statement on Management for the Northern Spotted Owl in the National Forests. USDA Forest Service, National Forest System: Portland, Oregon. Vol. 2.

Van der Auwera, G. A., M. O. Carneiro, C. Hartl, R. Poplin, G. del Angel *et al.*, 2013 From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Curr. Protoc. Bioinforma. 11: 11.10.1–11.10.33.

Wall, J. D., S. A. Schlebusch, S. C. Alberts, L. A. Cox, N. Snyder-Mackler *et al.*, 2016 Genomewide ancestry and divergence patterns from low-coverage sequencing data reveal a complex history of admixture in wild baboons. Mol. Ecol. 25: 3469–3483. https://doi.org/10.1111/mec.13684

Welch, B. L., 1947 The Generalization of "Student's" Problem When Several Different Population Variances Are Involved. Biometrika 34: 28–35.

Communicating editor: J. Fay