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Source: The Auk, 135(4) : 821-833

Published By: American Ornithological Society

URL: https://doi.org/10.1642/AUK-18-1.1

The Auk
Ornithological Advances
AmericanOrnithology.org

Volume 135, 2018, pp. 821–833
DOI: 10.1642/AUK-18-1.1

RESEARCH ARTICLE

Variation in inbreeding rates across the range of Northern Spotted Owls (*Strix occidentalis caurina*): Insights from over 30 years of monitoring data

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Submitted January 2, 2018; Accepted April 20, 2018; Published July 3, 2018

ABSTRACT

Inbreeding has been difficult to quantify in wild populations because of incomplete parentage information. We applied and extended a recently developed framework for addressing this problem to infer inbreeding rates in Northern Spotted Owls (*Strix occidentalis caurina*) across the Pacific Northwest, USA. Using pedigrees from 14,187 Northern Spotted Owls, we inferred inbreeding rates for 14 types of matings among relatives that produce pedigree inbreeding coefficients of $F = 0.25$ or $F = 0.125$. Inbreeding was most common in the Washington Cascades, where an estimated 15% of individuals are inbred. Inbreeding was lowest in western Oregon (3.5%) and northern California (2.7%), and intermediate for the Olympic Peninsula of Washington (6.1%). Estimates from the Olympic Peninsula were likely underestimates because of small sample sizes and the presence of few pedigrees capable of resolving inbreeding events. Most inbreeding resulted from matings between full siblings or half siblings, although a high rate of inbreeding from mother–son pairs was identified in the Olympic Peninsula. Geographic variation in inbreeding rates may reflect population declines and bottlenecks that have been detected in prior investigations. We show that there is strong selection against inbred birds. Only 3 of 44 inbred birds were later identified as parents (6.8%), whereas 2,823 of 10,380 birds that represented a comparable cross section of the data were later seen as reproducing parents (27.2%). Habitat loss and competition with Barred Owls (*S. varia*) remain primary threats to Northern Spotted Owls. However, given the negative consequences of inbreeding, Spotted Owl populations in Washington with suitable habitat and manageable numbers of Barred Owls may benefit from translocations of individuals from Oregon and California to introduce new genetic variation and reduce future inbreeding events.

Keywords: estimation, inbreeding, incomplete pedigree, missing data, Northern Spotted Owl, pedigree

Variación en las tasas de endogamia a través del rango de *Strix occidentalis caurina*: aprendizajes a partir de más de 30 años de datos de monitoreo

RESUMEN

La endogamia ha sido difícil de cuantificar en las poblaciones silvestres debido a la falta de información sobre los parentescos. Aplicamos y extendimos un marco conceptual recientemente desarrollado para encarar el problema de inferir las tasas de endogamia en *Strix occidentalis caurina* a través del noroeste del Pacífico, EEUU. Usando los pedigríes provenientes de 14187 individuos, inferimos las tasas de endogamia para 14 tipos de apareamiento entre parientes que producen coeficientes de endogamia de pedigrí de $F = 0.25$ o $F = 0.125$. La endogamia fue más común en las Cascadas de Washington, donde se estima que 15% de los individuos son endogámicos. La endogamia fue menor en el oeste de Oregón (3.5%) y el norte de California (2.7%), e intermedia en la Península Olímpica de Washington (6.1%). Las estimaciones de la Península Olímpica fueron probablemente subestimadas debido a los pequeños tamaños de muestreo y a la presencia de pocos pedigríes capaces de resolver los eventos de endogamia.

La mayoría de la endogamia resultó de los apareamientos entre hermanos completos o medios hermanos, aunque se identificó una alta tasa de endogamia en parejas madre/hijo en la Península Olímpica. La variación geográfica en las tasas de endogamia puede reflejar disminuciones poblacionales y cuellos de botella que han sido detectados en investigaciones previas. Mostramos que hay una fuerte selección contra las aves endogámicas. Solo tres de 44 aves endogámicas fueron más tarde identificadas como progenitores (6.8%), mientras que 2823 de 10380 aves que representaron una sección transversal comparable de datos fueron vistas más tarde como progenitores reproductivos (27.2%). La pérdida de hábitat y la competencia con *Strix varia* sigue siendo la principal amenaza para *S. o. caurina*. Sin embargo, dadas las consecuencias negativas de la endogamia, las poblaciones de *S. occidentalis* en Washington con hábitat adecuado y números manejables de *Strix varia* pueden beneficiarse de traslocaciones de individuos de Oregón y California para introducir nueva variación genética y reducir futuros eventos de endogamia.

Palabras clave: datos faltantes, endogamia, estimación, pedigrí, pedigrí incompleto, *Strix occidentalis caurina*

INTRODUCTION

The repercussions of inbreeding are well established (Darwin 1876, Ralls et al. 1979, Crnokrak and Roff 1999, Hedrick and Kalinowski 2000, Frankham 2005, Frankham et al. 2017). Progeny of related individuals are often at a selective disadvantage due to their greater propensity to inherit recessive deleterious mutations (Ralls et al. 1988, Charlesworth and Willis 2009). The reduced fitness of inbred individuals has led to the evolution of inbreeding avoidance mechanisms to minimize its occurrence (Pusey 1987, Blouin and Blouin 1988). Despite the existence of such mechanisms, inbreeding may be inevitable in small, isolated populations and play a role in determining the probability of local extinction events (Frankham and Ralls 1998, Frankham 2005, O'Grady et al. 2006).

Despite its central relevance to numerous facets of ecological and evolutionary theory, inbreeding rates have long been recognized as difficult to calculate in large natural populations because of the challenges associated with reconstructing individual pedigrees. Although individual cases of inbreeding may be identified in the wild, the true extent of inbreeding is difficult to infer because parentage information may be missing or unknown for many individuals in the population under investigation (Howard 1949, Bulmer 1973, Van Noordwijk and Scharloo 1981, Marshall et al. 2002). To address this issue, Miller et al. (2017) developed an estimation framework that incorporates information on the probability of resolving various pedigree configurations, given the extent of unknown parentage information that often exists for empirical datasets. This framework provides bias corrections to an approach proposed by Marshall et al. (2002), which involved calculating inbreeding rates for each of 3 types of inbreeding associated with $F = 0.25$ and 11 types of inbreeding associated with $F = 0.125$ (Table 1), where F is the inbreeding coefficient that reflects the probability that an individual inherits alleles at a locus that are identical by descent and quantifies the severity of an inbreeding event (i.e. "pedigree inbreeding"; Ballou 1983, Keller and Waller 2002). For inbreeding category i, Marshall et al. (2002) calculated the inbreeding rate f_i as $f_i = o_i/c_i$, where o_i is the observed number of individuals demonstrating type i inbreeding and c_i is the number of individuals possessing pedigrees capable of detecting a type i inbreeding event. Miller et al. (2017) showed that this estimator is biased for all inbreeding categories because the probability of resolving an inbred pedigree is greater than the probability of resolving a non-inbred pedigree in datasets where only a fraction of parents are known for all individuals. Differences in the probabilities of resolving inbred vs. non-inbred pedigrees ultimately stem from the fact that the identities of more individuals are required to resolve the pedigree of a non-inbred individual in relation to that of an inbred individual (Figure 1; see also Miller et al. 2017: suppl. fig. 1). Bias corrections provided in Miller et al. (2017) were derived using expressions that quantify $\Pr(I|k_m, k_f)$: the probability of resolving pedigree I conditional on the overall probability of knowing the identity of male (k_m) and female (k_f) parents in the population being investigated.

Northern Spotted Owls (*Strix occidentalis caurina*) have had a substantial influence on management practices for federal lands throughout the Pacific Northwest, USA, where ~100,000 km^2 of land is managed under the Northwest Forest Plan to protect habitat for owls and other species associated with old-growth forests (USDA Forest Service and USDI Bureau of Land Management 1994). Decades of monitoring have documented demographic trends and continued population declines of Northern Spotted Owls across their range, with possible mechanisms for declines identified as habitat availability, climate variability, and the occurrence of the invasive Barred Owl (*Strix varia*) (Franklin et al. 1999, Anthony et al. 2006, Davis et al. 2011, Forsman et al. 2011, Wiens et al. 2014, Dugger et al. 2016). Despite extensive data on the status and habits of the subspecies, many aspects of its biology remain unknown. In particular, a more refined understanding of dispersal patterns, inbreeding, and their joint implications for genetic structure of the subspecies remain important information needs.

TABLE 1. Equations defining the probability of resolving specific inbred (a) and non-inbred (b) pedigrees for 14 different forms of inbreeding associated with $F = 0.25$ (categories 1–3) or $F = 0.125$ (categories 4–14) in Northern Spotted Owls in the Pacific Northwest, USA. Categories correspond to Figure 1. The general notation (k_j^y) refers to the probability of knowing parent y of individual j (e.g., k_1^m refers to the probability of knowing the male parent of individual 1) as determined by the logistic regression analysis described in the text. Values of j in expressions correspond to numbers assigned to specific individuals as outlined in Figure 1. In some expressions, the minimum of 2 probabilities ("min") is used in calculations.

1. Father–daughter	a. $\Pr(ped1) = k_1^m \times k_1^f$
	b. $\Pr(ped1) = k_1^m \times k_1^f$
2. Mother–son	a. $\Pr(ped1) = k_m$
	b. $\Pr(ped1) = k_1^m \times k_1^f$
3. Full siblings	a. $\Pr(ped1) = k_1^m \times k_1^f \times \min(k_2^m, k_3^m) \times \min(k_2^f, k_3^f)$
	b. $\Pr(ped1) = k_1^m \times k_1^f \times k_2^m \times k_2^f \times k_3^m \times k_3^f$
4. Paternal half siblings	a. $\Pr(ped1) = k_1^m \times k_1^f \times \min(k_2^m, k_3^m)$
	b. $\Pr(ped1) = k_1^m \times k_1^f \times k_2^m \times k_3^m$
5. Maternal half siblings	a. $\Pr(ped1) = k_1^m \times k_1^f \times \min(k_2^f, k_3^f)$
	b. $\Pr(ped1) = k_1^m \times k_1^f \times k_2^m \times k_3^f$
6. Paternal grandmother–grandson	a. $\Pr(ped1) = k_1^m \times k_2^m \times k_3^f$
	b. $\Pr(ped1) = k_1^m \times k_1^f \times k_2^m \times k_3^f$
7. Maternal grandmother–grandson	a. $\Pr(ped1) = k_1^m \times k_2^f \times k_3^f$
	b. $\Pr(ped1) = k_1^m \times k_1^f \times k_2^f \times k_3^f$
8. Paternal grandfather–granddaughter	a. $\Pr(ped1) = k_1^f \times k_2^m \times k_3^m$
	b. $\Pr(ped1) = k_1^m \times k_1^f \times k_2^m \times k_3^m$
9. Maternal grandfather–granddaughter	a. $\Pr(ped1) = k_1^f \times k_2^f \times k_3^m$
	b. $\Pr(ped1) = k_1^m \times k_1^f \times k_2^f \times k_3^m$
10. Full paternal uncle–niece	a. $\Pr(ped1) = k_1^m \times k_1^f \times k_3^m \times \min(k_2^m, k_4^m) \times \min(k_2^f, k_4^f)$
	b. $\Pr(ped1) = k_1^m \times k_1^f \times k_2^m \times k_2^f \times k_3^m \times k_4^m \times k_4^f$
11. Full maternal uncle–niece	a. $\Pr(ped1) = k_1^m \times k_1^f \times k_3^f \times \min(k_2^m, k_4^m) \times \min(k_2^f, k_4^f)$
	b. $\Pr(ped1) = k_1^m \times k_1^f \times k_2^m \times k_2^f \times k_3^f \times k_4^m \times k_4^f$
12. Full paternal aunt–nephew	a. $\Pr(ped1) = k_1^m \times k_1^f \times k_3^m \times \min(k_2^m, k_4^m) \times \min(k_2^f, k_4^f)$
	b. $\Pr(ped1) = k_1^m \times k_1^f \times k_2^m \times k_2^f \times k_3^m \times k_4^m \times k_4^f$
13. Full maternal aunt–nephew	a. $\Pr(ped1) = k_1^m \times k_1^f \times k_3^f \times \min(k_2^m, k_4^m) \times \min(k_2^f, k_4^f)$
	b. $\Pr(ped1) = k_1^m \times k_1^f \times k_2^m \times k_2^f \times k_3^f \times k_4^m \times k_4^f$
14. Double first cousins	a. $\Pr(ped1) = k_1^m \times k_1^f \times k_2^m \times k_2^f \times k_3^m \times k_3^f \times \min(k_5^m, k_6^m) \times \min(k_5^f, k_6^f) \times \min(k_4^m, k_7^m) \times \min(k_4^f, k_7^f)$
	b. $\Pr(ped1) = k_1^m \times k_1^f \times k_2^m \times k_2^f \times k_3^m \times k_3^f \times k_4^m \times k_4^f \times k_5^m \times k_5^f \times k_6^m \times k_6^f \times k_7^m \times k_7^f$

In the present study, we applied the general framework of Miller et al. (2017) to infer inbreeding rates in Northern Spotted Owls using data derived from >30 yr of Northern Spotted Owl monitoring. This framework assumed that the probability of knowing the male and female parents of an individual (k_m and k_f) stays constant over time (Miller et al. 2017). This assumption is likely invalid in studies of natural populations, where parentage information may be lacking at the beginning of a study and then increase over time as data are amassed. We therefore further extended the estimation framework to account for temporal variability in the extent of unknown parentage information that may exist in studies of natural populations. Use of this framework allowed us to examine pedigrees for 14,187 individuals, estimate inbreeding rates across the range of Northern Spotted Owls, and identify the most common forms of inbreeding that occur in the taxon. We likewise illustrate that selection against inbreeding has occurred. Our work provides new insights about the status of Northern Spotted Owls and highlights previously undocumented factors that may have negative consequences for demographic processes in this subspecies.

METHODS

We assembled a large dataset of Northern Spotted Owl reproduction events in California, Oregon, and Washington, USA, between 1983 and 2016 (Figure 2). Northern Spotted Owls on their breeding territories have been extensively surveyed throughout their range as part of an ongoing multi-agency monitoring program designed to help discern demographic status and trends (Anderson and Burnham 1992, Burnham et al. 1996, Franklin et al. 1999, Anthony et al. 2006, Forsman et al. 2011, Dugger et al. 2016). Territorial owls were detected with acoustic or live-lured surveys during the breeding season in each year (Reid et al. 1999). Breeding activity was monitored and efforts were made to capture all fledglings produced on each study area each year, as well as any other unbanded subadults or adults. Captured owls were marked with U.S. Geological Survey numbered bands and a separate color band to facilitate reidentification of individuals without recapture (Forsman et al. 1996). When owlets were banded, a concerted effort was made to also band each parent or to confirm the identity of previously banded

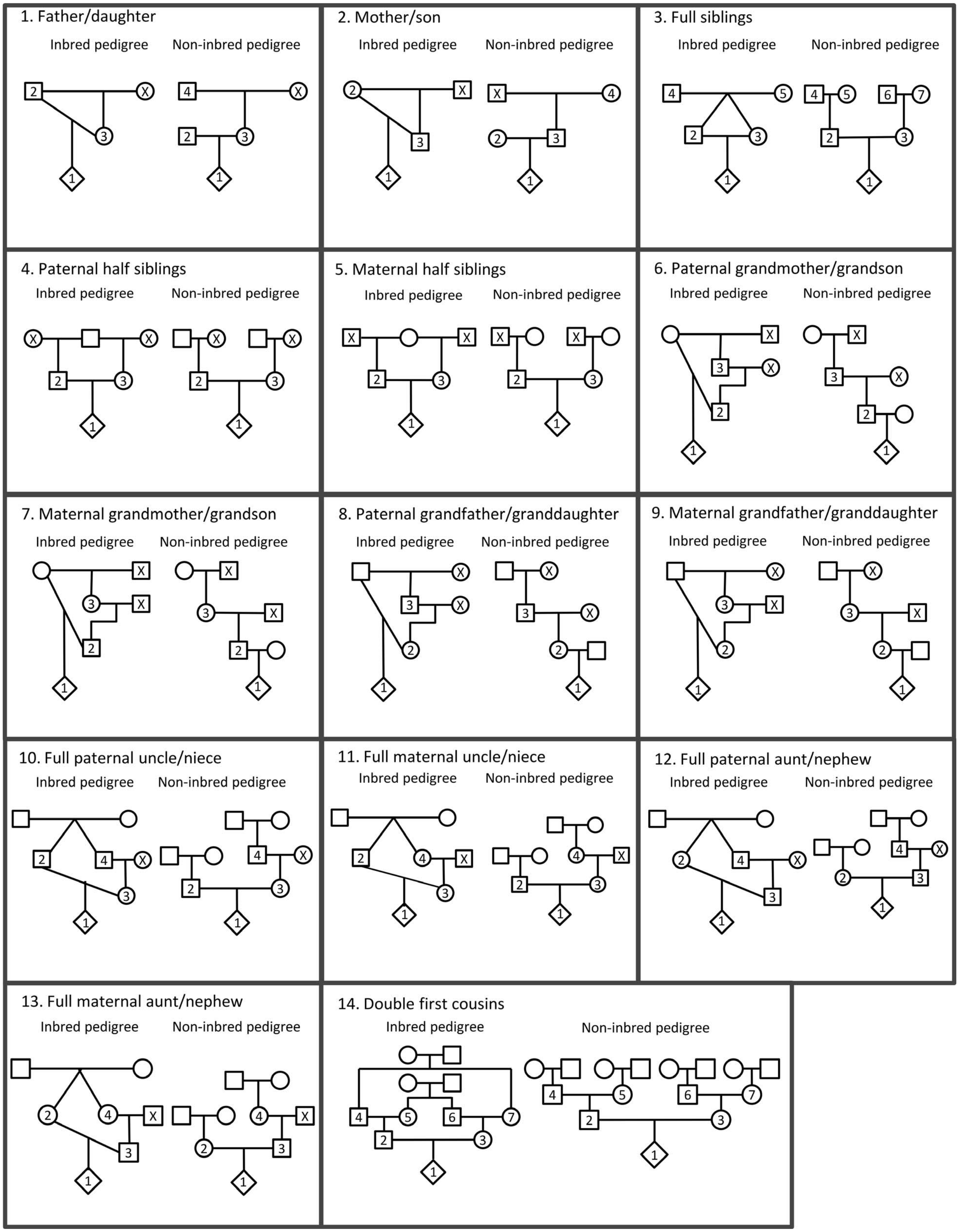

FIGURE 1. Illustrations of inbred and non-inbred Northern Spotted Owl pedigrees associated with 14 types of pairings that can lead to inbreeding coefficients of $F = 0.25$ (pedigrees 1–3) or $F = 0.125$ (pedigrees 4–14). Circles represent females, squares represent males, and diamonds indicate individuals whose inbreeding status is being assessed. Numbers on the pedigrees identify individuals

parental birds. In total, the complete dataset represented banding records for 14,187 individuals (Oregon: $n = 7{,}778$; Washington Cascades: $n = 2{,}170$; Olympic Peninsula: $n = 1{,}007$; California $n = 3{,}232$).

The spatial extent of our study area was large (Figure 2), and the breeding events that formed the basis of the pedigree spanned 34 yr. Consequently, the origin and relationships among all individuals were frequently unknown, and the depth of pedigree information for each individual was highly variable. For example, an individual may have been banded first as a breeding adult, either because it was undetected as a juvenile or because it was a recent migrant into the study region. In this case, no information on the individual's parents or relationships to other individuals in the population could be determined. Likewise, future breeding events were undetected for many individuals first banded as nestlings, either because of natal dispersal outside of our focal area or because of unobserved mortality events.

Given the sparse and variable information contained within the pedigree of each individual, inbreeding rates for Northern Spotted Owls could not be calculated directly from the data as simple proportions (i.e. observed number of inbred individuals divided by the total number of individuals examined). We therefore used an extension of the analysis framework outlined in Miller et al. (2017) to obtain empirical estimates of inbreeding rates in Northern Spotted Owls. Rather than relying on simple estimates of the overall probability of knowing the identities of male (k_m) and female (k_f) parents in the population, we instead account for temporal variation that exists by using logistic regression to infer $k_j^m$and k_j^f: the probabilities of knowing the male or female parents of individual j as a function of time. These probabilities were inferred by regressing the binary variable (father known–unknown or mother known–unknown) against the year that an individual was originally banded as an owlet. The regression model took the form of

$$k_j^m \mathrm{or} k_j^f = \frac{a}{1 + e^{-(x_j - x_0)/b}}$$

where x_j is the banding year of individual j, b describes the steepness of the regression curve, x_0 is a location parameter, and a is an inferred asymptote. In some instances, the banding of an individual may not have occurred at the juvenile stage; in those cases, we assumed that an individual's hatching year was 3 yr earlier than the hatch year for its oldest progeny, reflecting the typical 3 yr to first reproduction that has been observed in Northern Spotted Owls (Forsman et al. 2011). Thus, rather than having static probabilities associated with each pedigree configuration based on the average values of k_m and k_f derived for an entire dataset, our use of k_j^m and k_j^f in calculations allows the probability of each individual's pedigree to vary according to the hatch years of the specific combination of individuals associated with a given pedigree.

On the basis of this framework, the probability of resolving any specific pedigree can be obtained as the product of values of k_j^m and $k_j^f$for specific sets of individuals and their ancestors. Explicit expressions that define the probabilities of resolving 14 different pedigree configurations that are relevant to this study are presented in Table 1. Note that a resolved inbred pedigree for some configurations will include the shared parent of full or half siblings. In these scenarios, we used the smaller probability associated with banding of the older individual (Table 1), if relevant, because resolution of the pedigree was dependent on knowing the identity of an individual's parent at an earlier vs. later point in time.

Using this revised approach, we estimated inbreeding rates separately for each of the 4 discrete geographic regions that were naturally defined by our samples (Figure 2). As recommended by Miller et al. (2017), we recorded o_i and c_i for the purposes of reporting the actual number of observations that were used to obtain estimates from each of the 14 categories and generated 95% confidence limits for inbreeding-rate estimates using the asymmetric Clopper-Pearson approach (Clopper and Pearson 1934, Newcombe 1998). All pedigree analyses were implemented in a Python computer program written by MPM that also derived estimates of k_j^m and k_j^f for each individual. Logistic regression parameters for these calculations were inferred using the "curve_fit" function of the "optimize" library of the SciPy Python package (http://www.scipy.org). We likewise calculated pseudo-r^2 values (Nagelkerke 1991) for each nonlinear regression using R 3.3.2 (R Core Team 2016), the "nagelkerke" function from the "rcompanion" package (Mangiafico 2015), and the "nls2" package for nonlinear regressions (Grothendieck 2013).

We performed a simple test to determine whether there was selection against the inbred birds identified in our analysis. We first recorded the fraction of inbred individuals that were later identified as parents in our dataset. This value served as a surrogate for the approximate proportion of inbred individuals that were able to survive and contribute progeny in future generations of Northern Spotted Owls. For comparison, we recorded the same

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referred to in expressions (Table 1) that quantify the probability of resolving a given pedigree. An "X" over an individual indicates that its identity is not needed to assess the given inbreeding scenario but is included here to facilitate presentation of results.

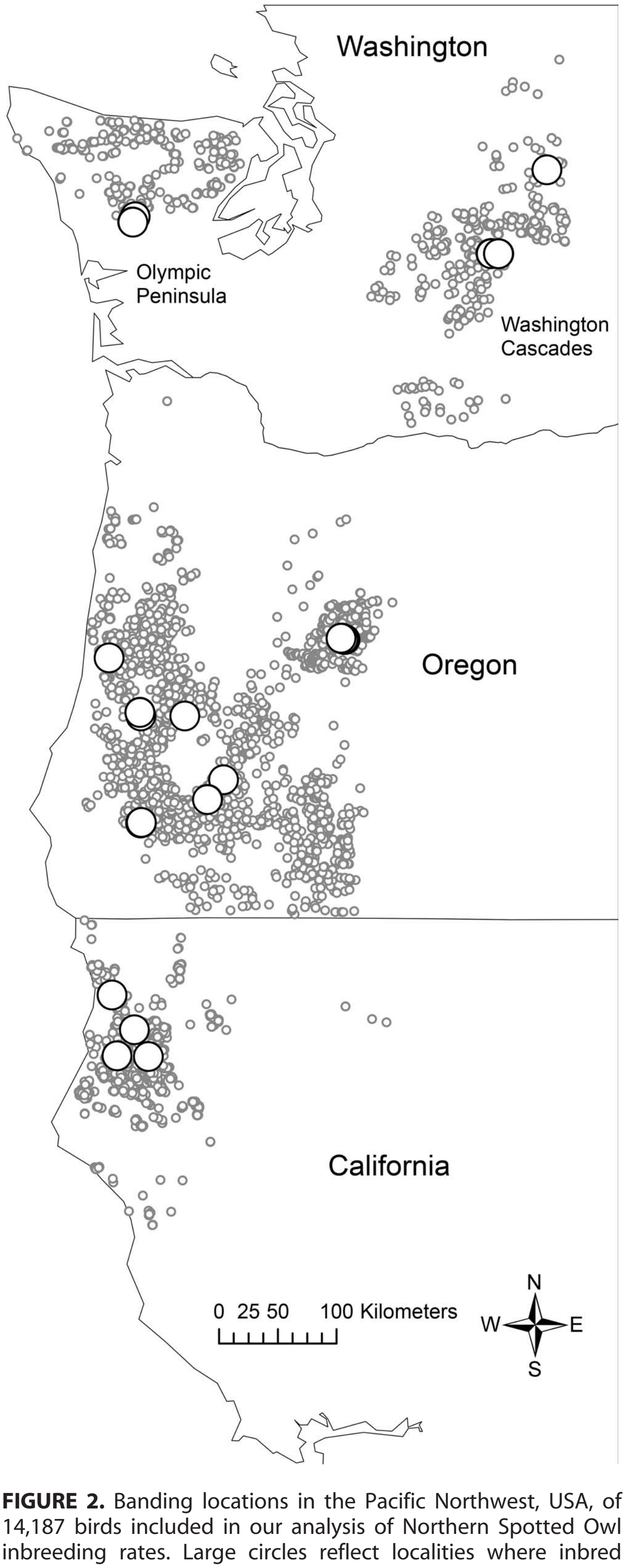

FIGURE 2. Banding locations in the Pacific Northwest, USA, of 14,187 birds included in our analysis of Northern Spotted Owl inbreeding rates. Large circles reflect localities where inbred individuals associated with one of the 14 categories listed in Table 1 were identified.

quantity across the set of remaining birds that were banded after 1991, which reflected the average probability that a randomly selected bird was later identified as a parent. We used 1991 as a cutoff because the first inbred bird in our dataset was identified in 1992, thereby restricting comparisons of groups to the same periods. Note that this latter quantity reflected survival and reproduction of non-inbred birds along with the set of inbred individuals that were not identified because parentage information was incomplete. If natural selection reduced the probability that inbred individuals survive and reproduce, then we would expect the former value to be less than the latter. A test for differences between groups was formed by constructing and testing a 2 × 2 contingency table and using the "fisher.test" function in R 3.3.2.

RESULTS

The observed fraction of known male and female parents (k_m and k_f) varied among regions (Washington Cascades: $k_m = 0.714$, $k_f = 0.702$; Olympic Peninsula: $k_m = 0.586$, $k_f = 0.604$; Oregon: $k_m = 0.657$, $k_f = 0.641$; northern California: $k_m = 0.608$, $k_f = 0.607$), highlighting the importance of accounting for the degree of missing parentage information in our analyses. However, logistic regression models were a good descriptor of changes in the probability of knowing an individual's male and female parents over time (Figure 3; only male results are shown, but results for female parents are similar), which suggests that our refinements to the methods of Miller et al. (2017) were warranted. In particular, inbred individuals were generally detected in later years following the initiation of monitoring in each region at a point in time when parentage information, on average, was more readily available (Figure 3). This pattern indicates that the probabilities of resolving pedigrees is greater in later years than in earlier years in our study.

Of the 14,187 individuals analyzed, we identified 44 inbred Northern Spotted Owls whose pedigrees revealed an inbreeding coefficient of $F = 0.25$ or $F = 0.125$ (Table 2; California: 6 individuals; Oregon: 24 individuals; Olympic Peninsula: 3 individuals; Washington Cascades: 11 individuals). Inbreeding rates associated with $F = 0.25$ were greatest in the Washington Cascades (12.3%) and lowest in Oregon (0.6%) and northern California (1.2%) (Table 2). Inbreeding at this level among individuals from the Olympic Peninsula was intermediate to these values (5.3%). Although variation existed among geographic regions, inbreeding associated with all 3 categories that result in $F = 0.25$ were detected, with inbreeding from full sibling pairs more common than inbreeding arising from parent–offspring pairings (father–daughter, mother–son, and full sibling pairings; Table 2). The Olympic Peninsula

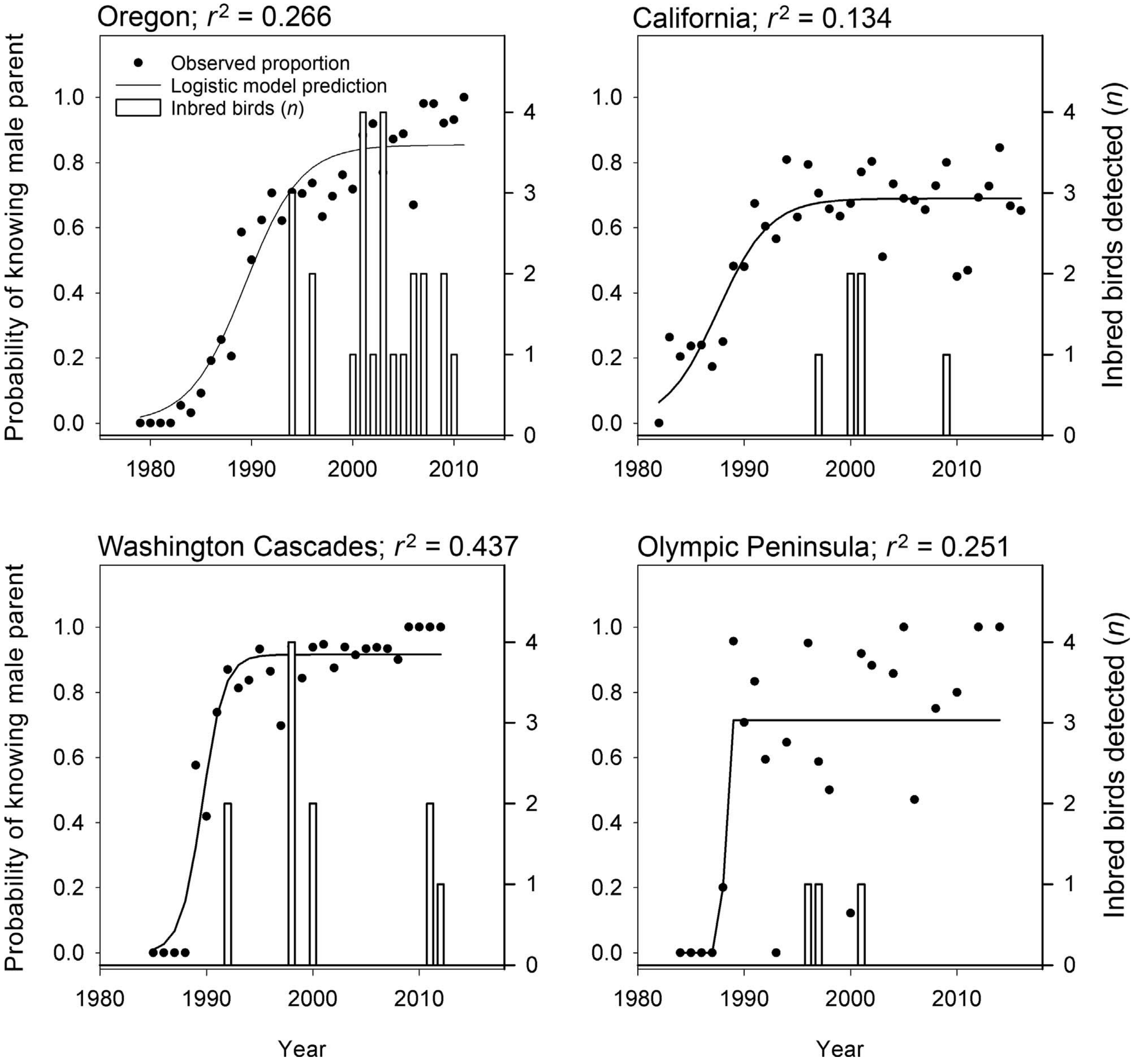

FIGURE 3. Logistic regression results for each geographic region within the Pacific Northwest, USA, illustrating the model predictions for the probability of knowing an individual Northern Spotted Owl's father. The observed proportion of known fathers for each year is plotted as a reference. The number of inbred birds detected each year is also plotted (right *y*-axes) to illustrate that inbreeding events are generally not detected until several years of parentage data have been assembled. Similar results were observed in analyses of female parents (not shown; $r^2 = 0.277$, 0.140, 0.414, and 0.284 for regressions of female data for Oregon, California, Washington Cascades, and the Olympic Peninsula, respectively).

was an outlier to this general pattern, where mother–son pairings were the most common inbreeding form identified (Table 2).

Inbreeding events associated with $F = 0.125$ were primarily observed in cases of mating between half siblings, although 2 cases of progeny from a grandparent–grandchild were detected in Oregon (Table 2). Progeny of paternal half siblings accounted for the majority of inbred individuals at this level (Table 2), which resulted in the highest inbreeding-rate estimate for the Washington Cascades. No inbred individuals associated with $F = 0.125$ were detected among Olympic Peninsula individuals, which likely reflects the relatively small number of individuals with pedigrees of sufficient depth to detect an inbreeding event (as evidenced by the low values of c_i recorded for the region; Table 2).

Of the 44 inbred birds detected in our study, only 3 (6.8%) were later detected in a parental role for a

TABLE 2. Inbreeding-rate estimates for Northern Spotted Owls in 4 geographic regions, for each of 14 categories where relationships between an individual's ancestors can lead to inbreeding coefficients of $F = 0.25$ (categories 1–3) or $F = 0.125$ (categories 4–14); o_i and c_i represent the observed numbers of individuals associated with each category possessing pedigrees that either demonstrate (o_i) or are capable of demonstrating (c_i) each inbreeding type.

	Northern California ($n = 3{,}232$)			Oregon ($n = 7{,}778$)			Washington (Olympic) ($n = 1{,}007$)			Washington (Cascades) ($n = 2{,}170$)		
Parental relationship	o_i/c_i	Estimate (%)	95% CL	o_i/c_i	Estimate (%)	95% CL	o_i/c_i	Estimate (%)	95% CL	o_i/c_i	Estimate (%)	95% CL
1. Father–daughter	0/356	0	0.00–0.30	2/1,144	0.13	0.04–0.32	0/43	0	0.00–2.67	0/266	0	0.00–0.56
2. Mother–son	0/394	0	0.00–0.21	0/1,280	0	0.00–0.11	3/37	5.34	2.01–11.18	2/248	0.68	0.12–1.81
3. Full siblings	3/98	1.18	0.67–1.92	10/494	0.44	0.28–0.66	0/10	0	0.00–2.62	6/69	11.79	9.09–14.95
Category total ($F = 0.25$)	–	1.18	0.67–2.42	–	0.57	0.32–1.09	–	5.34	2.01–15.82	–	12.32	9.19–16.97
4. Paternal half siblings	3/119	1.49	0.66–2.86	4/534	1.69	1.15–2.40	0/10	0	0.00–6.67	3/69	2.71	0.87–6.27
5. Maternal half siblings	0/101	0	0.00–0.74	6/511	0.65	0.34–1.13	0/10	0	0.00–6.74	0/69	0	0.00–1.96
6. Grandson–paternal grandmother	0/71	0	0.00–0.90	2/315	0.59	0.24–1.18	0/0	–	–	0/18	0	0.00–9.79
7. Grandson–maternal grandmother	0/87	0	0.00–0.89	0/265	0	0.00–0.29	0/5	0	0.00–16.36	0/32	0	0.00–4.06
8. Paternal grandfather–granddaughter	0/83	0	0.00–0.77	0/247	0	0.00–0.45	0/0	–	–	0/31	0	0.00–4.95
9. Maternal grandfather–granddaughter	0/40	0	0.00–2.04	0/253	0	0.00–0.31	0/5	0	0.00–13.39	0/69	0	0.00–1.07
10. Paternal uncle–niece	0/40	0	0.00–0.43	0/152	0	0.00–0.23	0/0	–	–	0/19	0	0.00–3.33
11. Maternal uncle–niece	0/16	0	0.00–1.50	0/156	0	0.00–0.09	0/3	0	0.00–3.87	0/17	0	0.00–2.01
12. Nephew–paternal aunt	0/23	0	0.00–1.34	0/171	0	0.00–0.19	0/0	–	–	0/6	0	0.00–17.68
13. Nephew–maternal aunt	0/21	0	0.00–1.04	0/143	0	0.00–0.14	0/0	–	–	0/19	0	0.00–3.94
14. Double first cousins	0/0	–	–	0/16	0	0.00–0.02	0/0	–	–	0/0	–	–
Category total ($F = 0.125$)	–	1.49	0.66–11.84	–	2.90	1.72–6.27	–	0	0.00–39.39	–	2.71	0.87–43.96
Total detected (%)		2.67			3.47			5.34			15.03	

successive generation. By contrast, 2,823 of 10,380 remaining individuals used for comparison (27.2%) were subsequently identified as producing progeny in a future generation. Frequency differences between groups were highly significant ($P < 0.0011$), which suggests selection against inbred Northern Spotted Owls.

DISCUSSION

Documented cases of inbreeding and inbreeding depression are known in some species (for recent summaries, see Neaves et al. 2015, Frankham et al. 2017), but the actual frequency of inbreeding is difficult to measure in wild populations of plants and animals when it requires long-term pedigree data (Haig and Ballou 2002, Marshall et al. 2002). However, new advances in wild pedigree analyses, demonstrated in the present study, will lead to greater ease of carrying out analyses of inbreeding from incomplete pedigrees. Results of analyses based on our approach ultimately enable a more quantitative and realistic evaluation of inbreeding in the wild, which can, in turn, promote development of more effective recovery strategies for small wild populations.

We detected only 44 cases of inbreeding among ~14,000 pedigrees, which could superficially suggest that inbreeding is uncommon in Northern Spotted Owls. However, not all cases of inbreeding were detected, because parentage information was available for only ~65% of the individuals in our dataset (reflected by observed values of k_m and k_f). Under these circumstances, pedigrees may not be known in sufficient depth to resolve all instances of inbreeding, thereby requiring the corrections outlined in Miller et al. (2017) that account for differences in the probability of resolving inbred vs. non-inbred pedigrees in a population when parentage information is incomplete. Miller et al. (2017) also used computer simulations and analytical expressions to define the behavior of the estimator and illustrate practical considerations for inferring inbreeding from incomplete pedigrees. Of primary importance is the concept that the resolution of any given pedigree will be a probabilistic event that is conditional on (1) the degree of unknown parentage in a dataset and (2) the number of known ancestors required to resolve that pedigree. Thus, researchers should be aware that they may, in some instances, be unable to quantify inbreeding for some categories, especially those that require comparatively large numbers of ancestors to document. For smaller datasets, inferences may be restricted estimates for parent–offspring or half sibling pairs, whereas detection of pedigrees associated with aunt–nephew, uncle–niece, or double first-cousin categories could be problematic. For similar reasons, it may not be feasible to extend this approach to infer inbreeding rates for the vast number of complex pedigrees that could give rise to inbreeding coefficients <0.125. Reporting raw values of o_i and c_i (Table 2) is therefore advisable to understand whether a given dataset can reasonably provide estimates for individual inbreeding categories (Miller et al. 2017).

The original estimation framework of Miller et al. (2017) required the assumption that the level of unknown parentage information remains unchanged over time. In the present study, we refined the estimation framework by relaxing this assumption and allowing for unknown parentage information to vary over time. This revised framework appears to be justified by analyses of our dataset, which indicated that the probability of knowing an individual's male or female parent was low at the onset of this monitoring study but tended to increase over time as additional data were amassed (Figure 3). Because this revised framework is based on logistic regression, future investigations in Northern Spotted Owls or other taxa could include additional factors (spatial information, habitat, field conditions, search effort, etc.) in regression models if researchers believe that they may potentially influence the likelihood of knowing the identity of an individual's parents. Use of regression models that include these additional parameters could provide even more refined estimates of $\Pr(m)$ and $\Pr(f)$ for each individual and, therefore, also possibly provide subtle refinements to the inbreeding-rate estimates produced with our analytical framework.

Our results indicate that inbreeding rates vary substantially across the range of the Northern Spotted Owl. In particular, inbreeding appears to be less common in Oregon and northern California but is relatively frequent in the Washington Cascades and Olympic Peninsula (Table 2). Our estimates for the Olympic Peninsula are likely underestimates, given the low values of c_i reported for most inbreeding categories in that dataset (Table 1). It appears that the sample of $n = 1{,}007$ individuals and pedigrees from the Olympic Peninsula was sufficient only to resolve pedigrees capable of identifying inbreeding from parent–offspring pairings (Table 2, categories 1 and 2): the pedigree categories that require the fewest numbers of ancestors to resolve (see Miller et al. 2017: table 2 and appendix 1). Indeed, the inbreeding-rate estimates for many categories were undefined for the Olympic Peninsula dataset (indicated by values of 0 for c_i in Table 2). This pattern highlights the inability of the dataset to provide insights regarding inbreeding for many categories and the likelihood that inbreeding rates are actually higher than estimated for the region.

The greater incidence of inbreeding in the Washington Cascades may reflect patterns detected in other analyses of Northern Spotted Owl genetics and demography. For example, the Cle Elum study area in the Washington Cascades, which comprises a substantial portion of our data for the region, has experienced an average population size reduction of 8.4% yr^{-1} between 1985 and 2013, which exceeds the declines recorded at any other Northern Spotted Owl study area (Dugger et al. 2016). This means that only 23% of the original Spotted Owl population remains on Cle Elum, compared to 45% remaining on the Olympic Peninsula, 32–69% remaining in Oregon, and 45–68% remaining in northern California (Dugger et al. 2016). These declines have been linked to the presence of Barred Owls, with the strongest effects observed from north to south, increasing over time as Barred Owls have expanded their range (Anthony et al. 2006, Forsman et al. 2011, Dugger et al. 2016). Likewise, analyses of nuclear microsatellite genetic data for Northern Spotted Owls have revealed evidence of genetic bottlenecks across its range, and the strongest signal of bottlenecks originated in the Washington Cascades (Funk et al. 2010). Genetic bottlenecks are the result of population size reductions that may increase the likelihood of inbreeding, given that there are fewer potential mates in small populations than in large populations. Among the 4 regions examined, inbreeding was also higher on the Olympic Peninsula. This area has long been recognized as isolated habitat for Northern Spotted Owls and showed some of the earliest evidence of population declines and invasion by Barred Owls. Thus, the higher incidence of inbreeding estimated for the Olympic Peninsula is also highly consistent with results of prior independent studies (Bart et al. 1992). Despite examining >14,000 individual pedigrees in our study, we detected relatively few inbreeding events, which prevented us from determining whether inbreeding rates have changed over time (Table 2 and Figure 3). However, Northern Spotted Owl populations continue to decline across their entire range (Dugger et al. 2016). In light of declining populations, it remains possible that inbreeding rates are increasing across their range and accelerating more rapidly in areas where the largest population declines have occurred.

Under ideal conditions, DNA samples from each juvenile and its putative parents would be available for analysis to confirm parent–offspring relationships (Gullberg et al. 1992, Bird et al. 2013) and ensure that extrapair paternities or misidentified parents were not confounding the pedigrees that were reconstructed for each individual. In our study, blood or tissue samples were not collected to allow for formal evaluation of this phenomenon using molecular genetic techniques. However, multiple lines of evidence indicate that extrapair paternities are uncommon in Northern Spotted Owls. For example, paired Northern Spotted Owls copulate daily over a period of weeks until several days after the last egg has been laid (Forsman et al. 1984). This strategy should minimize the opportunity for extrapair paternities. Also, almost all owls, including *Strix* species, have similar mating systems (i.e. monogamous, territorial, males do all resource provisioning in early reproductive stage) and show low rates (Saladin et al. 2007) or no evidence (Marks et al. 1999, Arsenault et al. 2002, Koopman et al. 2007) of extrapair paternity. Even high frequencies of extrapair copulations may not actually lead to extrapair fertilization events in owls (Hsu et al. 2006). Finally, actual opportunities for extrapair copulation are probably rare in Northern Spotted Owls. Of ~5,300 field records examined, only 5 instances of a nonresident male roosting in the proximity of a nest site have been observed (C. E. McCafferty and J. Reid personal communication), which suggests that the likelihood of parental misidentification is low in this system. In study organisms with higher likelihoods of extrapair paternities, molecular genetics may be needed to confirm observed parentage in a sample of parent–offspring triads to ensure that observational information used for pedigree reconstruction has minimal errors. Computer simulations (e.g., Miller et al. 2017) may be useful to estimate bias due to incorrect parentage assignments on inbreeding-rate estimates when parentage errors cannot be assessed.

Most instances of inbreeding originated from pairings between full siblings or half siblings (Table 2). Nonetheless, variation existed among geographic regions, with instances of mother–son pairings the only inbreeding category recorded in the Olympic Peninsula (Table 2). No instances of aunt–nephew, uncle–niece, or double first-cousin pairings were detected in any region (categories 10–14 in Table 2). The Oregon dataset, in particular, contained abundant pedigrees capable of detecting these forms of inbreeding; thus, their absence indicates that inbreeding as a consequence of these pairing types does not occur or is very rare.

In general, female juvenile Northern Spotted Owls disperse farther than males, which normally settle only 1 or 2 territories away from the natal site (Forsman et al. 2002). On the basis of these findings, we expected that mother–son pairings should occur more frequently than father–daughter pairings. Our data show that parent–offspring pairings are extremely uncommon in general, with the exception of a high rate of inbreeding from mother–son pairings that was observed on the Olympic Peninsula (Table 2). The specific basis for this outlier pattern on the Olympic Peninsula in relation to the other 3 areas is unclear but suggests that the behavioral or environmental basis for inbreeding varies across the Northern Spotted Owl's range. Full sibling and half sibling pairings were more prevalent in the other 3 regions, with the paternal half sibling category accounting for most inbreeding events (Table 2). Inbreeding by paternal half siblings reflects pairings between individuals with the same fathers but different mothers. This finding may therefore be consistent with female-biased dispersal and indicate situations where females disperse into territories occupied by an unpaired male during natal dispersal events.

At this time, there are no other studies of avian taxa that have used comparable methods to those described in here; thus, direct comparisons to published inbreeding rates are not possible. We note, however, that many studies of birds (e.g., Keller et al. 2002, Kruuk et al. 2002, Jamieson et al. 2007) have used what can essentially be described as a variant of the approach of Marshall et al. (2002), which is known to overestimate inbreeding rates in most cases (Miller et al. 2017). Revisiting published pedigree datasets with the approach described here will help establish baseline inbreeding rates in birds and other taxa and provide new insights about levels of inbreeding that exist in wild populations of animals.

In addition to quantifying inbreeding rates, we also found evidence of natural selection against inbred Northern Spotted Owls. In studies of avian species, direct comparisons of known inbred vs. known non-inbred individuals frequently identified varying levels of selection against a broad range of life stages (Keller and Waller 2002, Keller et al. 2002, O'Grady et al. 2006, Jamieson et al. 2007). In our analyses, inbred juveniles were later detected as breeding adults approximately 4× less often compared to random expectations (~7% vs. ~27%). However, we are unable to determine the specific postbanding life stage where selection is having the greatest impact. These stages include postfledging, juvenile dispersal, subadult prospecting, and adult stages (territory and mate acquisition) as the likely periods when inbred birds are less successful than their non-inbred counterparts. Tracking and observation of individual birds across their complete life cycle will ultimately be required to determine the precise mechanisms that differentially affect inbred and non-inbred Northern Spotted Owls.

When population sizes become low as a result of external factors such as habitat loss, disease, and invasive species, the probability of inbreeding and inbreeding depression will increase—and further reduce population sizes through what is known as the "extinction vortex" (Gilpin and Soulé 1986, Fagan and Holmes 2006). To date, conservation efforts for Northern Spotted Owls have primarily focused on maintaining habitat (USDA Forest Service and USDI Bureau of Land Management 1994) and, more recently, exclusion of the invasive Barred Owl (Buchanan et al. 2007, Gutiérrez et al. 2007, Diller et al. 2014, Wiens et al. 2014, 2016) to help minimize the likelihood of further population declines. Our analyses also suggested that inbreeding has negative consequences for future reproduction in Northern Spotted Owls and that inbreeding rates are high in some parts of the Northern Spotted Owl's range. These findings suggest that inbreeding may be contributing to population declines and reiterate the importance of considering the ramifications of inbreeding for species of management concern (Amos and Balmford 2001). Habitat loss and Barred Owls remain primary threats contributing to population declines in Northern Spotted Owls. Our results point to the potential benefit of translocations or other tools that would facilitate genetic rescue of populations (Tallmon et al. 2004, Trinkel et al. 2008, Hedrick and Fredrickson 2010, Frankham et al. 2017). Issues associated with this process would need to be resolved prior to performing translocations, particularly with respect to identifying suitable source and recipient populations, ensuring habitat quality and availability for Northern Spotted Owls in Washington, and minimizing the effects of Barred Owls that could negate the potential benefits of this genetic intervention. As Caughley (1994) suggested, it may be more important to ensure that factors leading to declining populations (e.g., habitat loss and Barred Owls) are addressed prior to addressing those that primarily affect small populations (inbreeding) to ensure that translocated individuals are not perpetually introduced into a demographic sink.

ACKNOWLEDGMENTS

This paper would not have been possible without the help of many dedicated field biologists who have worked to collect Spotted Owl data in Washington, Oregon, and California. In particular, we thank J. Reid, S. Andrews, B. Biswell, J. Swingle, S. Ackers, R. Horn, P. Carlson, J. Rockwell, A. Rex, and C. McCafferty for their commendable efforts over the years. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Funding statement: Funding for this project was provided by Sierra Pacific Industries, Inc., the USDI USGS Forest and Rangeland Ecosystem Science Center, the USDA Forest Service, USDI Bureau of Land Management, Weyerhaeuser Company, Hancock Forest Management, and USDI National Park Service. None of the funders had any influence on the content of the submitted or published manuscript, and none of the funders required approval of the final manuscript to be published.

Ethics statement: Data were collected under relevant state and federal permits that were acquired and maintained during the duration of data collection. Institutional protocols were approved under permits overseen by animal care and use committees at Oregon State University and Colorado State University.

Author contributions: M.P.M., S.M.H., and E.D.F. conceived the research. M.P.M. developed methods and performed the analyses. All authors provided substantial materials (data, resources, or funding) and contributed to manuscript content.

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