Random interbreeding between cryptic lineages of the Common Raven: evidence for speciation in reverse

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Abstract

DNA sequence studies frequently reveal evidence of cryptic lineages in morphologically uniform species, many of which turn out to be evolutionarily distinct species. The Common Raven (*Corvus corax*) includes two deeply divergent mtDNA lineages: one lineage seems restricted to western North America and the other is Holarctic in distribution. These deep clades hint of the possibility of cryptic species in the western United States. We tested this hypothesis in a population consisting of an equal proportion of both mtDNA clades, by quantifying mating patterns and associated fitness consequences with respect to mtDNA. We also tested for morphological, behavioural and ecological correlates of sex and mtDNA clade membership. Mate pairings were random with respect to mtDNA clades, and there were no differences in reproductive success between assortatively and nonassortatively mated pairs. We found no differences in survival or resource use between clades. There were no differences in morphological or behavioural characters between mtDNA clades, except one clade trended towards greater mobility. These results suggest there are no barriers to gene flow between mtDNA clades and argue that the mtDNA clades have remerged in this population, likely due to a lack of ecological or signal differentiation between individuals in each lineage. Hence, in Common Ravens, phylogeographic structure in mtDNA is a reflection of likely past isolation rather than currently differentiated species.

Keywords: Common Raven, *Corvus corax*, cryptic species, despeciation, speciation in reverse

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Introduction

Recent genetic studies show that some widespread species with conserved morphology harbour cryptic genetic variation, such as occurs in Blue Tits (*Parus caeruleus*) (Taberlet *et al.* 1992), Snow Geese (*Chen caerulescens*) (Quinn 1992) and Carolina Chickadees (*Poecile carolinensis*) (Gill *et al.* 1993, 1999). These so-called 'cryptic mtDNA clades' differ substantially in their DNA but are not detectable using traditional taxonomic techniques that rely on comparisons of phenotypic characters. However, basing taxonomic decisions on any one type of evidence (e.g., mtDNA monophyly or ability to interbreed) can be problematic, so several authors have argued for the importance of basing species limits on multiple criteria (de Queiroz 1998, 2007; Helbig *et al.* 2002; Sites & Marshall 2004).

The Common Raven (*Corvus corax*) breeds throughout the Northern Hemisphere in a wide variety of habitats (Boarman & Heinrich 1999; Dos Anjos *et al.* 2009), but exhibits very little morphological variation (Vaurie 1959). Several inconsistent subspecies delineations have been described (Willet 1941; Rea 1986; Ratcliffe 1997) based upon slight clinal variation but there is no evidence to suggest substantial population subdivisions. Despite phenotypic homogeneity, phylogeographic surveys show that Common Ravens exhibit substantial genetic variation. Mitochondrial sequence data (mtDNA) reveal two distinct mtDNA clades within the Common Raven designated as the 'Holarctic' and 'California' clades (Omland *et al.* 2000, 2006). These two clades are over 4% divergent in mtDNA coding genes;

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this level of sequence divergence exceeds differences between a number of well-recognized species pairs (Johnson & Cicero 2004). Despite a possibly extended period of isolation, no known character set separates the California and Holarctic clades including plumage, shape, size, calls, behaviour, ecology, etc. (e.g. Willet 1941). Nevertheless, several authors have advocated recognizing the clades as two distinct species based on mitochondrial DNA (Navarro-Sigüenza & Peterson 2004; McKay & Zink 2010).

Common Raven haplotypes exhibit a weak phylogeographical pattern: ravens from Europe, Asia and eastern North America are exclusively Holarctic clade, whereas the California clade is restricted to western North America, but most concentrated in southern California (Omland *et al.* 2000; Fleischer *et al.* 2008). However, the two clades occur sympatrically throughout most of western North America, and representatives of both clades are mixed within the same populations and occur in nearly all locations sampled in the western United States including northern California, Nevada, Idaho and Washington state (Omland *et al.* 2000; Fleischer *et al.* 2008). Although geographical sampling has been limited, it appears that different proportions of mtDNA haplotypes from the two clades occur across the ranges of the three subspecies of Common Ravens in western North America (described in Rea 1986; Ratcliffe 1997). The distribution of the more northern, larger subspecies *Corvus corax principalis* corresponds to populations mostly dominated by the Holarctic clade. The range of the smaller *Corvus corax sinuatus* encompasses the Rocky Mountains and consists of a mixture of both haplotypes, whereas the smallest subspecies, *Corvus corax clarionensis*, occurs from northern California south through Baja California and consists mainly of the California clade (Omland *et al.* 2000; Fleischer *et al.* 2008).

Several alternative explanations could account for species-level paraphyly in the Common Raven (Funk & Omland 2003). For example, the two clades might represent two ecologically distinct taxa. The California clade predominates in arid southern California, and recent work shows this group exhibits extreme human commensalism (Webb *et al.* 2004, 2009; Kristan & Boarman 2007). Perhaps adaptation of the California clade to more xeric or human-dominated landscapes promoted divergence in signal traits, followed by reproductive isolation and divergence from the Holarctic clade. Thus, species-level paraphyly in the Common Raven could represent incorrect taxonomy—when named species fail to capture the limits of separate evolutionary entities (McKay & Zink 2010). Gene trees demonstrating paraphyly provide clues for the existence of 'cryptic species' and elevating cryptic taxa to species rank resolves the complexity posed by these patterns (Omland *et al.* 1999; Voelker 1999; McKay & Zink 2010).

Alternatively, if the two Common Raven clades are randomly interbreeding with respect to mtDNA haplotype over a wide geographical area, then mtDNA differences could represent neutral differences within a single species. Thus, the mtDNA divergence could represent a historical artefact of divergent lineages that have long since remerged. As both clades occur sympatrically across a large geographical area, the potential for interbreeding is substantial. A related explanation would be that the deep mitochondrial lineages had evolved within a single species because large effective population sizes have maintained divergent lineages because of slow lineage sorting. A third possible explanation is that mtDNA introgressed from the Chihuahuan Raven lineage hundreds of thousands of years ago and that mtDNA clade has persisted in the Southwestern populations of Common Ravens. Any of these three explanations predicts current random interbreeding between individuals from the two mitochondrial clades.

Although some instances of paraphyly are the result of incorrect taxonomy (Funk & Omland 2003; McKay & Zink 2010), simply splitting to create monophyletic taxa within the Common Raven would be a hasty decision given the current evidence. While these deep mtDNA divergences suggest a need to evaluate current taxonomy, mtDNA divergence alone is not a sufficient criterion to alter taxonomic designation (Funk & Omland 2003; Moritz & Cicero 2004; Rubinoff & Holland 2005). Genetic divergence in neutral markers reflects time accumulated, rather than divergence in selection pressures that alter adaptations leading to differences in signal traits, reproductive isolation and speciation (Boughman 2002). A more comprehensive approach to assessing whether mtDNA defines evolutionarily distinct lineages is to assess mating patterns between clades, compare the fitness of individuals with different mtDNA haplotypes, assess the reproductive success of 'hybrid' pairs and estimate associations between mtDNA variation and variation in other traits (Crandall *et al.* 2000; Gibbs *et al.* 2006; de Queiroz 2007; Drovetski *et al.* 2009).

On Washington State's Olympic Peninsula, members of both the Holarctic and the California clades of the Common Raven seem to occur in approximately equal proportions (Omland *et al.* 2000). If the clades represent different adaptive lineages in our study area, then barriers to gene flow should exist and genetic divergence should parallel differences in phenotypic characters and individual fitness. Furthermore, differences in mating signals such as calls or behaviour could have accumulated as a result of genetic drift during a period of allopatry that might prevent interbreeding even among

ecologically similar or identical groups (Mendelson & Shaw 2002; Mendelson 2003; Omland & Kondo 2006; Price 2008). In this study, we evaluated evidence for phenotypic character divergence paralleling divergence in mtDNA between Common Ravens with Holarctic and California clade mtDNA. We first tested the hypothesis that Common Ravens pair randomly with respect to mtDNA and then compared the breeding performance of same-clade versus cross-clade pairs. Finally, we compared the variation in mtDNA with three character sets: behavioural differences (movement behaviour), morphology (morphometrics) and ecology (resource use & survival).

Materials and methods

Study site description and raven trapping

Our study took place at low elevations (<300 m) primarily in commercial forestlands of the western side of the Olympic Peninsula of Washington State (Fig. 1; also see Marzluff & Neatherlin 2006; Webb 2010). Between December 2002 and August 2005, we captured 67 ravens including 24 juveniles, 37 adults and 6 subadults using a remote controlled portable net launcher (Coda Enterprises, LTD.). We measured and colour-banded each raven with a unique combination of plastic colour bands and an aluminium USFWS band. We equipped 60 ravens (35 adults, 22 juveniles and 3 subadults) with

Fig. 1 Distribution of the two mitochondrial clades showing sample size and clade proportions for each sampling area. Data mainly from three publications: Omland *et al.* (2000) especially Idaho, Minnesota, Maine and inland Washington; Omland *et al.* (2006) San Clemente Island off California, Alaska, Maine, Tennessee, North Carolina, and Europe; Fleischer *et al.* (2008) Nevada and mainland California. Data from Oregon, Arizona, New Mexico and Greenland, K. E. Omland unpublished; Mexico, S. Rösner unpublished; Olympic Peninsula, Washington, present study. Regions within California and Nevada follow Fleischer *et al.* (2008).
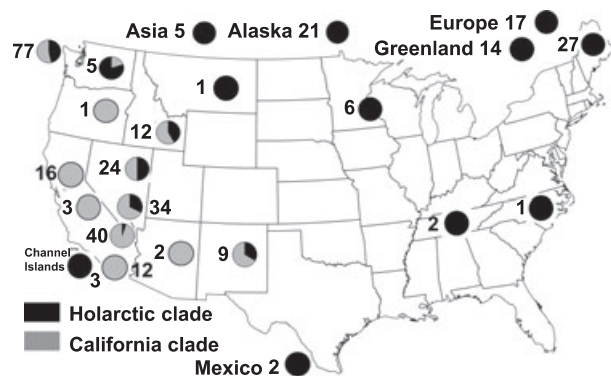
a backpack-mounted radio transmitter (Buehler *et al.* 1995) weighing 27 g (Advanced Telemetry Systems, Isanti, MN, USA) with a projected operational period of 40 months. We used a combination of plumage, mouth lining and breeding behaviour (Heinrich & Marzluff 1992; Pyle *et al.* 1997) to classify the social class of trapped ravens. Juveniles were birds of known age that had fledged from known adult territories during the 2003, 2004 or 2005 breeding season. Juveniles were trapped in their natal territories after fledging, but prior to dispersing. Subadults were unpaired breeding-age individuals, as indicated by black mouth colour (Heinrich & Marzluff 1992).

The following morphological characters were measured on each individual's right side by the lead author: weight, wing chord, tarsus length, tarsus height (measured at the midpoint of the tarsometatarsus), culmen length, culmen width and culmen height. All individuals were weighed with a 2500-g Pesola spring scale (±20 g). Five morphological measurements were taken with Vernier calipers (±0.1 mm): culmen length, culmen depth, culmen width, tarsus length and tarsus height (see Webb 2010 for more details).

Molecular methods

As male and female ravens are morphologically indistinguishable, gender was determined using a PCR that amplifies the CHD genes on the avian W and Z sex chromosomes (*sensu* Griffiths *et al.* 1998). The gender-specific primers are P8 (5′-CTC- CCAAGGATGAGRA AYTG-3′) and P2 (S′-TCTGCATC- GCTAAATCCTTT-3′). These primers simultaneously amplify homologous parts of CHD-W and CHD-Z. The primers anneal to conserved exonic regions but then amplify across an intron in both CHD-W and CHD-Z. Because these introns are noncoding, they are less conserved and their lengths usually differ between the genes. As female birds are heterogametic (WZ) and males are homogametic (ZZ), the PCR products reveal two bands in the female and one band in the male.

We sampled two small growing feathers from each raven trapped on the Olympic Peninsula and used them as a source of tissue for DNA extraction. DNA was extracted using QIAGEN Dneasy Kits (Qiagen, Valencia, CA, USA). PCR amplification for both the clade analysis and the gender analysis was carried out in a total volume of 21 μL. Restriction fragment length polymorphism (RFLP) for the clade analysis was carried out in a total volume of 15 μL. RFLP products were separated by electrophoresis in a 2% agarose gel stained with Cyber Gold (Molecular Probes, Eugene, Oregon). See Webb (2010) for additional details regarding PCR and RFLP methods.

Omland *et al.* (2000) assigned ravens to mtDNA clades using phylogenetic analysis in the program PAUP for previous projects (Omland *et al.* 2000, Feldman & Omland 2005) (Sinauer Associates, Inc.) (*n* = 16) or they were assigned to mtDNA clades based upon RFLPs (*n* = 61) in the current analysis. The RFLP analysis was designed for a 314-bp section of the mitochondrial cytochrome b region sequenced by Omland *et al.* (2000) after inspecting the sequence using BioEdit (Hall 1999) for clade-specific nonvariable base positions. We identified clade-specific restriction sites and then used restriction enzymes to selectively cut fragments prior to gel electrophoresis. We chose the restriction enzymes APO1 (recognition sequence: r'AATT_y) and HINf1 (recognition sequence: G'AnT_C) because APO1 cleaves the California Clade, but not the Holarctic Clade, while HINf1 cleaves the Holarctic Clade but not the California Clade. Coincidentally, both enzymes cleave the sequence at bp208. The primers to amplify the cytochrome b gene region were B1 and B2 from Kocher *et al.* (1989). We used the restriction enzymes to selectively cut fragments prior to gel electrophoresis, and differential PCR products distinguished individuals according to their respective clades. The RFLP method was validated for one bird whose clade was known from sequence data, 10 individuals from the Western Mojave Desert [all California clade (CA), RFLP], 10 individuals from Alaska [all Holarctic clade (HO), RFLP] and 5 individuals from Asia (all HO clade, RFLP).

Behavioural tracking

We tracked ravens continuously from May 2003 to March 2007, mainly by motorized vehicle (Webb 2010). During the spring and summer (March–September), we tracked ravens several times per month and for 1 week per month during the rest of the year until each bird died or its transmitter stopped functioning. The entire study area was visited and searched monthly with the goal of maintaining a uniform effort throughout the study. We used homing techniques (White & Garrot 1990) and recorded raven locations in Universal Transverse Mercator (UTM) coordinates using hand-held global positioning system (GPS) units. We confirmed locations of radio-tagged ravens either visually, using homing techniques (Mech 1983: 60), or by triangulation. Locations of juveniles were plotted on a study site map constructed using GIS (ArcView 3.3, and ArcGIS 9.2, ESRI, Redlands, CA).

To characterize resource use by ravens, we constructed GIS layers combining land cover (vegetation types) and land use (anthropogenic elements). We quantified landscape structure (land cover composition, land use and configuration) using ArcGIS 9.2 (ESRI

2007) and ArcView 3.3 (ESRI 2002). We updated an existing forest cover classification scheme for annual timber harvest and seral development using a combination of our knowledge of the study site, inspection of unfamiliar areas, digital orthophotos and Landsat Thematic Mapper (TM) satellite imagery at a scale of ≥1:3000 m, resulting in a unique map of land cover, land use and distribution for each year between 1991 and 2006. We incorporated several classes of anthropogenic land use categories that we considered prominent features of the landscape (Table 1) into the original data by screen digitizing from orthophotos and Landsat TM satellite imagery. We considered all land use and land cover classes as mutually exclusive in their spatial extent. See Webb (2010) for additional details regarding geospatial data preparation. In addition to the composition of resources within the home range, the configuration of resources may uniquely influence life history parameters (Johnson 2007). Therefore, we quantified landscape structure (composition and configuration) within raven home ranges with Fragstats (v 3.3 build 5) (McGarigal *et al.* 2002) utilizing a moving windows analysis with a circular radius determined by the home range bandwidth (smoothing parameter). We chose the following landscape metrics: patch density (PD); contrast-weighted edge density (CWED); and interspersion and juxtaposition index (IJI). To calculate CWED, we defined edge contrast weights between 0 and 1 for each potential pair of edge types, with increasing weights representing greater edge contrast (Webb 2010). We also calculated two class metrics for the mature forest class; an area-weighted mean for the shape index (SHAPE_AM_1); and total core area (TCA_1). See Webb (2010) for additional details regarding landscape structure analyses. We estimated home ranges based on point locations in ArcGIS 9.2 using the Home Range Tools (HRT) for ArcGIS 9.x (Rodgers *et al.* 2005) by implementing a 99% fixed kernel estimator (Seaman *et al.* 1999). We calculated home ranges for adult ravens with 10 or more locations. We generated individual home ranges for each raven with a user-defined method of selecting the smoothing parameter (Webb 2010). See

Table 1 Sex, clade and mate pairing for adult Common Ravens on the Olympic Peninsula of Washington State

Sex, clade and mate pairing	#
♂ (HO)/♀ (CA)	6
♂ (CA)/♀ (HO)	6
♂ (HO)/♀ (HO)	2
♂ (CA)/♀ (CA)	4

CA, California clade; HO, Holarctic clade.

Webb (2010) for additional details regarding home range analyses.

Statistical methods

All statistical analyses were performed with the statistical software package R (R Development Core Team 2009). We used nonparametric tests after raw data and transformed data both failed to meet parametric assumptions. All tests were two-tailed and considered statistically significant at $P \leq 0.05$. When one of the morphological parameters was missing for an individual, we replaced the missing value by the average value for that mtDNA clade and sex; this had to be performed for less than 2% of values, thus are presumed not to have affected the conclusions. In addition to comparing mtDNA clades, we also included sex as a grouping factor in all relevant analyses because many avian morphometric characters are sexually dimorphic (Gill 1995). We excluded all nonbreeding ravens (juveniles and subadults) from statistical analyses related to behavioural or demographic comparisons because they are known to differ from adults in these respects (Heinrich *et al.* 1994). We excluded behavioural data from one adult female collected after she abandoned her breeding territory subsequent to her mate's death and used the average values across breeding territories for another female that switched breeding territories after her first mate was shot. We excluded three vagrants with insufficient data from morphological comparisons, and we also excluded all juveniles from morphological comparisons given likely age-related differences, but also to avoid pseudoreplication as they were the related offspring of the adults in the study.

We tested the null hypothesis that Common Ravens mate randomly with respect to mtDNA haplotype using the binomial exact test. We compared the observed proportion of same-clade pairs with the proportion obtained if individuals would have mated randomly with any breeding opposite-sex individual in the same population. We also conducted *post hoc* observed power analysis in program R of the detectable effect size (Hoenig & Heisey 2001). We generated confidence intervals and quantified the power associated with a range of hypothetical mating preferences given the observed sample size of genotyped mated pairs [i.e. power vs. $(\mu_0 - \mu_1)$], (R package 'binom'; Dorai-Raj 2009).

We used the resource utilization function (RUF) (Marzluff *et al.* 2004) using the RUFFIT package in R to quantify raven resource use at the third-order, or within home range, scale (Johnson 1980). We included in the analysis adult and floater ravens with >10 locations and juvenile ravens with >10 dispersal locations. This approach assumes that space use relates to resource use

and relates variation in the 'height' (i.e. use density) of the home range to spatially defined resources using multiple linear regression, while accounting for spatial autocorrelation in location data introduced by kernel analyses.

We first conducted a principal component analysis (PCA) on the correlation matrix of all morphological variables measured (R function prcomp), which allowed us to summarize variation in Common Raven body dimensions (principal components, PCs) for 34 adults and 3 subadults. We also employed two-way permutational MANOVA (Anderson 2001; McArdle & Anderson 2001) in three separate analyses to evaluate the null hypothesis of morphological (morphometrics), behavioural (movements) and ecological (resource use) similarity between sexes and mtDNA clades. Permutation runs ($n = 5000$) of the observed data were used to generate probability values for the null hypothesis of no difference between groups using the function adonis in the R package 'vegan' (Oksanen *et al.* 2009). Statistical tests of power were not available for function neither prcomp nor function adonis, although permutation techniques increase power for small sample sizes (Oksanen *et al.* 2009). See Webb (2010) for more details regarding statistical analyses.

Demography

We investigated survival differences between clades by testing and comparing models of adult ($n = 34$) Common Raven survival as a function of clade, sex and resource use. We used the R (R Development Core Team 2009) package RMark (Laake & Rexstad 2008) to construct models for the program MARK (White & Burnham 1999) to test mark–recapture models that compared parameters between groups of ravens and related these to standardized RUF resource coefficients.

As survival and recapture rates were our primary parameters of interest, we did not constrain the Burnham joint live recaptures and dead recoveries analysis (Burnham 1993) for recovery rate (r) or site fidelity (F). Mark–recapture analyses were conducted by testing the global models and then constraining parameters according to a priori hypotheses. The models were constructed using monthly recapture intervals corresponding to the 47 consecutive months of radio-tracking. Model notation follows that of Lebreton *et al.* (1992) and Franklin *et al.* (2000). We constrained parameters as functions of sex (sex), social status (social), constancy (∼1) and the standardized RUF resource coefficients as individual covariates (Table 1). Model-weighted parameter estimates and standard errors were derived from the model with the lowest AICc value and all other models with Δ AICc values <10 (Burnham & Anderson 2002:152). See

Webb (2010) for more details regarding the mark–recapture analyses.

We tested for differences in reproduction between same-clade and cross-clade breeding pairs using a Wilcoxon rank-sum test. Although a statistical test of power was not available (R function wilcox.test), the Wicoxon rank sum test has a considerable power advantages over the parametric *t* test (Nanna & Sawilowsky 1998). We were unable to quantify the reproduction for two pairs of ravens genotyped for mtDNA because they did not receive radio transmitters. We monitored reproductive success by observing nests and postfledging family behaviour at adult territories in which at least one member of the adult pair was radio tagged. We returned to each territory at least once per week throughout the breeding season (March–July) and used the maximum number of fledglings observed during that period of time to represent a pair's reproductive success. For breeding pairs monitored for >1 year (*n* = 13), we used the average of the maximum fledgling counts to represent their annual success.

Results

We genotyped 77 ravens from the Olympic Peninsula for sex and mtDNA haplotypes, including 18 mated pairs (35 individuals) and 42 ravens unrelated to the mated pairs. The unrelated ravens represented an independent sample of the frequency of mtDNA clades within the population. From the 42 independent nonpair mtDNA samples, 21 ravens possessed California clade mtDNA and 21 ravens possessed Holarctic clade mtDNA.

Pairing was random with respect to clade: genotyping of the 18 mated pairs for clade revealed 12 mixed-clade pairs and 6 same-clade pairs (Table 1). These results failed to reject the null hypothesis of random mating with respective to clade (Binomial exact test, *P* = 0.2379, 95% CI = 0.1334–0.5901) and clearly do not suggest positive assortative mating by clade (given that mixed-clade pairs are more common rather than less common than predicted by the null hypothesis). For the mixed pairs, the male was California clade on six occasions (Binomial exact test, *P* = 0.5, 95% CI = 0.21–0.79). Given the observed sample size of mated pairs (*n* = 18), a *post hoc* power analysis (R function 'binom.power' in package 'binom', Dorai-Raj (2009) revealed strong statistical power (0.8) to detect mating preferences greater than 53% (equivalent to 76.5% same-clade pairs; Fig. 2).

Following the 50% variance explained criterion, the PCA to examine associations between mtDNA haplotypes, sex and morphological parameters yielded two principal components. PC1 accounted for 37% of the total variation (eigenvalue = 2.59; factor loadings: bird
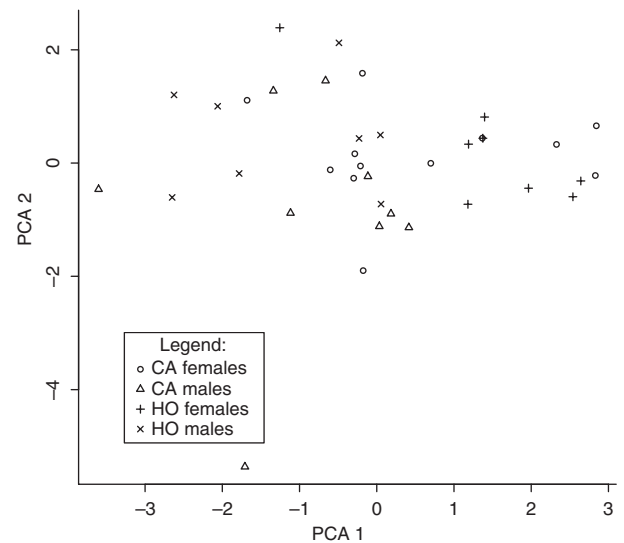
Fig. 2 Results of a principal components analysis (PCA) to examine associations between mtDNA clade, sex and morphological parameters. Data points represent individual scores on the first two principal components. PC1 accounted for 37% of the total variation (eigenvalue = 2.59; factor loadings: bird weight = −0.50, culmen length = −0.44, culmen depth = −043). The PC2 accounted for 24% of the total variation (eigenvalue = 1.7; factor loadings: tarsus length = 0.62, tarsus height = 0.60, and wing cord = −0.33).

weight = −0.50, culmen length = −0.44, culmen depth = −0.43). The PC2 accounted for 24% of the total variation (eigenvalue = 1.7; factor loadings: tarsus length = 0.62, tarsus height = 0.60, and wing cord = −0.33). We used the absolute value of component loadings to interpret the association between component loadings and morphological parameters using the following categories (Tabachnick & Fidell 1996:677): high association (loadings >0.71 and <−0.71), medium (loadings between ±0.55 and ±0.71) low (loadings between ±0.55 and ±0.32) and insignificant (loadings between −0.32 and .32). Overall, PCA failed to clearly separate individuals based upon mtDNA clade or sex (Fig. 3).

As the result of our exploration of the association between morphological parameters with variation in sex and mtDNA haplotypes, morphological parameters did not differ significantly between clades, but the null hypothesis of no differences between sexes was rejected (permutational MANOVA, effect of clades: $F_{1,36} = 0.35$, $P = 0.61$, permutational MANOVA, effect of sex: $F_{1,36} = 9.19$, $P < 0.01$). The interaction between sex and clade was also not significant (permutational MANOVA, clade X sex: $F_{1,36} = 1.32$, $P = 0.24$). *Post hoc* comparisons of group means between sexes were performed using two-tailed Wilcoxon rank sums tests and with the Bonferonni-Holm correction for family-wise error rate. Given the corrected level of significance ($P < 0.01$), wing
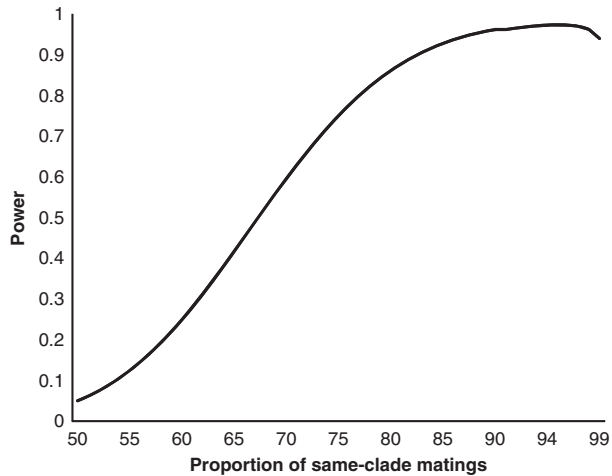
Fig. 3 Graphical presentation of a *post hoc* power analysis (R function 'binom.power' in package 'binom', Dorai-Raj 2009). The curve depicts the relationship between statistical power to detect an actual mating preference under a range of hypothetical departures from random mating, given the observed sample size of mated pairs ($n = 18$). The % mating preference = (((per cent of same-clade pairs) − (50%)) *2)).

parameters within mated pairs revealed that male ravens were larger than their mates in weight ($P < 0.01$), culmen length ($P < 0.01$), culmen depth ($P = 0.02$) and wing cord ($P < 0.01$), but not culmen width ($P = 0.07$), tarsus length ($P = 0 .13$) or tarsus height ($P = 0.35$). The repeatability of the morphological measurements was estimated on a subsample of four adult ravens that were retrapped and remeasured on a second occasion ($R^2 = 0.99$).

Neither clade membership nor sex was useful predictors of ecological variation or movement behaviour. Coefficients of resource use did not differ significantly between clades or sexes (permutational MANOVA, effect of clade: $F_{1,35} = 1.91$ $P = 0.08$; permutational MANOVA, effect of sex: $F_{1,35} = 0.30$, $P = 0.97$; permutational MANOVA, clade X sex: $F_{1,35} = 1.14$, $P = 0.33$). Movement indices did not differ between clades (at the family-wide error rate) or sexes, nor was the interaction significant (permutational MANOVA, effect of clade: $F_{1,32} = 4.43$, $P = 0.04$; permutational MANOVA, effect of sex: $F_{1,32} = .35$, $P = 0.60$; permutational MANOVA, clade X sex: $F_{1,32} = 0.12$, $P = 0.83$). *Post hoc* comparisons of group means between the clades were performed using two-tailed Wilcoxon rank sums tests and Bonferonni-Holm correction for family-wise error rate which resulted in a

cord and culmen length measurements were significantly larger for male adult ravens compared to female adult ravens (Table 2). Additionally, morphometric

Table 2 Summary statistics on morphometric parameters for 34 adult and 3 subadult Common Ravens according to mtDNA Clade (CA, California clade; HO, Holarctic clade) and sex. Values represent means ± standard deviations. We conducted a permutational MANOVA to test for morphometric differences between sexes and clades and *post hoc* pairwise comparisons between the significantly different main effect of sex using Wilcoxon rank sum tests and the Bonferonni-Holm correction for family-wise error rate (P crit = 0.007)

Clade	n	Bird weight (g)	Culmen length (mm)	Culmen width (mm)	Culmen depth (mm)	Wing cord (mm)	Tarsus length (mm)
Males							
CA	9	1187.78 ± 92.00	81.79 ± 3.04	25.19 ± 2.59	27.98 ± 1.10	430.22 ± 8.48	71.12 ± 5.04
HO	8	1251.25 ± 126.91	79.89 ± 2.67	25.73 ± 2.67	27.97 ± 1.26	425.13 ± 10.02	75.73 ± 1.56
Females							
CA	12	1111.83 ± 113.99	77.17 ± 3.35	25.00 ± 2.28	27.11 ± 1.22	417.42 ± 7.42	72.93 ± 2.08
HO	8	1087.88 ± 148.99	76.24 ± 1.91	23.24 ± 1.96	27.08 ± 0.96	408.75 ± 18.13	71.29 ± 3.37
Between sexes							
W_s		257	288.5	214	237	277.5	222.5
P		0.0083	.0003*	0.1838	0.0422	0.0011*	0.1122

Clade	n	Tarsus height (mm)
Males		
CA	9	10.65 ± 0.70
HO	8	10.80 ± 0.52
Females		
CA	12	10.57 ± 0.44
HO	8	10.54 ± 0.37
Between sexes		
W_s		212.5
P		0.1929

corrected level of significance of $P < 0.0125$. At the corrected level of significance, there were no differences revealed by pairwise *post hoc* comparisons of group means between clades for the three movement indices (Table 3).

The predictor variables of mtDNA clade identity and sex were not associated with variation in survival and reproduction. With respect to clade, there was no difference in the number of juveniles fledged between same clade ($n = 6$, $\bar{X} = 1.64$, SD = 1.76) and different clade ($n = 10$, $\bar{X} = 1.40$, SD = 0.95) pairs (Wilcoxon rank-sum test, $W_s = 32.5$, $P = 0.83$). Survival analysis generated a large number of supported models in the candidate model set (i.e., $< \Delta$ AICc < 10), including some models with sex and clade as main effects (Table 4). However, both sexes and clades exhibited similar model-weighted survival rates (monthly survival rates: CA females $S = 0.98$, SE = 0.01, 95% CI (0.94–0.99); CA Males $S = 0.98$, SE = 0.01, 95% CI (0.94–0.99); HO Females $S = 0.98$, SE = 0.01, 95% CI (0.93–0.99); HO Males $S = 0.98$, SE = 0.01, 95% CI (0.93–0.99).

Discussion

The most common phylogenetic pattern in nature is the splitting and diverging of lineages. Remerging of divergent lineages, or speciation in reverse ('despeciation', Turner (2002), is much less common (Seehausen *et al.* 1997; Turner 2002; de 2005; Taylor *et al.* 2006). In this study, we found evidence for remerging between mtDNA lineages within the Common Raven. We tested

the hypothesis that mtDNA haplotypes are associated with adaptive divergence, or cryptic speciation within the Common Raven species as currently circumscribed. We found the focal population to consist of an equal mixture of mtDNA clades, but we found no evidence that ravens mated assortatively by clade; in fact, only 6 of the 18 genotyped pairs were within-clade matings. We tested for evidence that mtDNA clade identity was associated with three phenotypic character sets: morphological, behavioural and ecological. As represented by resource use, we found no differences in ecological characters between mtDNA clades. There were also no significant differences in morphological or behavioural characters between mtDNA clades, except the California clade trended towards greater mobility and males of both mtDNA clades exhibited significantly greater wing cord and culmen length. Finally, there were no significant associations between mtDNA clades and measures of reproduction or survival.

The lack of association observed between mtDNA clades and demographic parameters suggests mtDNA clade identity is unrelated to mate choice decisions, and clade identity is unrelated to any measure of fitness. Moreover, the lack of association between mtDNA clades and differences in most measured character sets suggests a lack of phenotypic adaptive (or neutral) differentiation between individuals in each lineage. These results argue that the California and Holarctic mtDNA clades have remerged in this region (and perhaps over much of the western US), because of a lack of adaptive divergence or barriers to hybridization. Hence, in these birds, mtDNA clades do not identify distinct evolutionary lineages that have undergone adaptive divergence, but rather phylogeographic structure in mtDNA is more likely a reflection of historical isolation. Other hypotheses including long-term retention of deep lineages or ancient introgression will be addressed in future studies.

The ecology of a species may contribute to the likelihood of allopatric speciation. Ecological specialists, or species that evolve specialization, might be less likely to remerge after contact between disjunct lineages is re-reestablished (Schluter 2009; also see Price 2008). Generalist species, on the other hand, owing to wide ecological tolerances might be less likely to evolve unique adaptations in isolation and therefore be more likely to remerge upon secondary contact. If one of the separated lineages evolved ecological specializations, then the two groups would be less likely to remerge after secondary contact. These trends might contribute to explaining why some well-recognized species are separated by very small differences in mtDNA (Johnson & Cicero 2004), while other species harbour relatively large degrees of intraspecific variation in mtDNA. Perhaps

Table 3 Summary statistics on movement indices for 33 adult Common Ravens according to mtDNA clade (CA, California clade; HO, Holarctic clade) and sex. Values represent means ± standard deviation. We conducted a permutational MANOVA to test for differences in movement indices between sexes and clades and post hoc pairwise comparisons between the significantly different main effect of mtDNA clade using Wilcoxon rank sum tests and the Bonferonni-Holm correction for family-wise error rate (P crit. = 0.0125)

Clade	n	Home range area (ha)	Initial movement (m)	Median movement (m)	Maximum movement (m)
Males					
CA	9	111 ± 43	1113 ± 1056	941 ± 262	5430 ± 3808
HO	8	101 ± 40	736 ± 551	922 ± 312	3242 ± 1733
Females					
CA	12	136 ± 50	1504 ± 639	1482 ± 639	6222 ± 3559
HO	8	80 ± 42	1096 ± 1204	767 ± 330	3568 ± 3791
Between sexes					
W_s		180	176	193	198
P		0.1075	0.1448	0.03637	0.02273

Table 4 Ranking of models for adult raven survival ($n = 34$), comparing parameters between sexes and mtDNA clades, and relating to raven resource use as individual covariates. We used the Akaike information criterion (Akaike 1973) corrected for for small sample size (AICc) to rank the models from the list of a priori models. Models with ΔAICc values <10.00 were used for model-weighted parameter estimation. ΔAICc is the numerical difference between each model and the model with the lowest AICc. The likelihood of any model is the AICc Weight for the model of interest divided by the AICc Weight of the best model. The top model predicted survival as a function of adult raven use of prairie land cover. Notation explanation: S, survival; p, recapture; r, recovery rate; F, site fidelity; an, anthropogenic land use; clade, mtDNA clade; cwed, contrast-weighted edge density; iji, interspersion and juxtaposition index; lr, logging roads; mf, mature forest; new, recent clearcuts; old, older clearcuts; pd, patch density; prd, surfaced roads; sa, sapling; shape_am_1, shape index for mature forests, area- weighted mean; tc, towns and cities; tca_1, total core area of mature forest, (~1), constancy. See Webb (2010) for ranked models with AICc weight <0.020)

Model	AICc	Δ AICc	AICc Weight	# Param.
S(~pr)p(~1)r(~1)F(~1	617.690	0.000	0.092	5
S(~cwed + mf + new + old + prd) p(~1)r(~1)F(~1	618.068	0.378	0.076	9
S(~cwed)p(~1)r(~1)F(~1)	618.589	0.899	0.059	5
S(~1)p(~1)r(~1)F(~1	618.878	1.188	0.051	4
S(~tca_1)p(~1)r(~1)F(~1	619.120	1.430	0.045	5
S(~sex)p(~1)r(~1)F(~1	619.190	1.500	0.043	5
S(~oc)p(~1)r(~1)F(~1	619.417	1.727	0.039	5
S(~prd + clade)p(~1)r(~1)F(~1	619.574	1.885	0.036	6
S(~cwed + mf + new + old + prd + clade)p(~1)r(~1)F(~1	619.942	2.253	0.030	10
S(~prd)p(~1)r(~1)F(~1	620.164	2.474	0.027	5
S(~an)p(~1)r(~1)F(~1	620.233	2.543	0.026	5
S(~tca_1 + clade)p(~1)r(~1)F(~1	620.304	2.614	0.025	6
S(~lr)p(~1)r(~1)F(~1	620.329	2.639	0.025	5
S(~mf)p(~1)r(~1)F(~1	620.352	2.662	0.024	5
S(~cwed + clade)p(~1)r(~1)F(~1	620.453	2.763	0.023	6
S(~old)p(~1)r(~1)F(~1	620.461	2.771	0.023	5
S(~clade)p(~1)r(~1)F(~1	620.479	2.789	0.023	5
S(~sa)p(~1)r(~1)F(~1	620.515	2.826	0.022	5
S(~new)p(~1)r(~1)F(~1	620.669	2.980	0.021	5
S(~iji)p(~1)r(~1)F(~1	620.750	3.060	0.020	5

the extreme range of ecological tolerance exhibited by Common Ravens (among the widest of any avian species) makes this species a less likely candidate for allopatric speciation than avian species with narrower ecological tolerances. The deep divergence of the mtDNA clades of the Common Raven suggests that the clades diverged between 3.5 and 1.7 Ma (Feldman & Omland 2005; see Price 2010), but remerging of the lineages suggests that any divergence experienced in isolation was not strong enough to cause substantial adaptive divergence.

Signal traits may also contribute to the likelihood of speciation (Mendelson & Shaw 2002; Omland & Kondo 2006; Mendelson et al. 2007; Price 2008). Both vocalizations and plumage are much conserved across the genus *Corvus* (Madge & Burn 1994), with greater than 50% of the species being monomorphically black. Lack of divergence in such characters during periods of allopatry could facilitate interbreeding upon secondary contact. For example, lack of character divergence is

hypothesized to contribute to interbreeding between the Carolina Chickadee and the Black-capped Chickadee (Robbins et al. 1986; also see Gill et al. 1999).

Movement behaviour, especially juvenile dispersal, has been shown to be heritable in many avian species (Greenwood 1980). Although not statistically significant at the corrected family-wise level, California clade individuals trended towards greater mobility; though, our results suggest that mitochondrial clade may have little or nothing to do with the rest of the genome because of apparent random interbreeding. Although extreme population growth of Common Ravens in areas such as the western Mojave Desert are driven by an expanding human presence, the lack of association of mtDNA clades with ecological characters suggests that population expansion is not driven by different degrees of human commensalism between mtDNA clades. Differences in mobility associated with mtDNA clades might be more detectable during juvenile dispersal, typically when ravens, like other vertebrates, exhibit the greatest

magnitude in movements (Greenwood 1980). Our sample size of genetically independent juveniles (17 juveniles from nine family groups) is insufficient to make statistically valid comparisons of juvenile dispersal distance, defined here as the median movement distance from the natal nest: ($\bar{x} \pm$ SE CA clade, $n = 12$, 13.1 ± 3.3 km; HO clade, $n = 5$, 23.4 ± 10.7 km).

This population of ravens showed evidence of sexual size dimorphism, which is not uncommon for monochromatic avian species, including corvids (Cramp & Perrins 1994; Delestrade 2001; Berzins et al. 2009). Sexual size dimorphism suggests male–male aggressive competition or female choice (Andersson 1994), although multiple factors are likely to influence pair formation in corvids (see Marzluff & Balda 1988). Indirect lines of evidence suggest that mate competition or female choice may influence sexual size dimorphism among mated Common Ravens. For example, nonbreeding ravens congregate at communal food resources ('bonanzas') whereas dominant ravens (typically larger males) gain priority access to carcasses and other food items (Marzluff & Heinrich 1991).

Mate pairing at communal food resources might be influenced by male competition and/or female choice and could have influenced mating between the members of previously separated clades. For example, dominant males may restrict subordinate males from access to females through aggression. However, if female choice operates in pair formation, then dominant males at communal food resources could be viewed as more appealing and gain preferential access to potential mates. It is be likely that some degree of female choice influences pair formation because males provision female during incubation, and male traits associated with preferential resource access, such as larger size in one or more traits, could be favoured by females.

Frequently, in studies of 'DNA barcoding' deep divergence in mtDNA is considered evidence for distinct species (e.g., Kerr et al. 2007). Our work on ravens documents a case in which there is deep divergence in mtDNA, yet no evidence from any other source that there are two distinct species. Joseph & Omland (2009; their table 1) document several other cases where there is deep intraspecific divergence in mtDNA, yet no strong geographical structure. Thus, when using mtDNA barcoding for species discovery, deep mitochondrial divergence alone, even with some evidence of geographical structure, should not be considered strong evidence that there must be multiple distinct species.

Several authors have proposed reclassification of the California and Holarctic clades as distinct species (Navarro-Sigüenza & Peterson 2004; McKay & Zink 2010). Our results show that mtDNA clades within the same population are indistinguishable using morphological parameters such as those used in traditional taxonomy. In addition, there is unrestricted gene flow between mtDNA clades, and the lack of any fitness effects of cross-clade matings argues against any evidence for reproduction isolation. Moreover, current knowledge of the distribution of mtDNA clades is not consistent with any purported geographical distributions of subspecies (Rea 1986; Ratcliffe 1997). Although our sample sizes were relatively small, all of our tests comparing morphology, behaviour and ecology failed to distinguish between clades. These results provide strong evidence that mtDNA clades within the Common Raven are not reproductively isolated, have no diagnosable phenotypic differences, and no simple geographical boundaries, and therefore should not be designated as separate species based on multiple criteria (Helbig et al. 2002; Sites and Marshal 2004). Our study provides evidence of a striking case of the remerging of previously separated lineages. These raven mtDNA lineages much are more divergent than in other reported cases of despeciation or speciation in reverse.

Acknowledgements

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Data accessibility

DNA sequences: GenBank accessions nos for newly reported data which contribute to Fig. 1: JF451127-1136. Data pertaining to mated pairs archived in Dryad: doi:10.5061/dryad.8774.