

Genetic signatures of intermediate divergence: population history of Old and New World Holarctic ravens (*Corvus corax*)

KEVIN E. OMLAND,* JASON M. BAKER† and JEFFREY L. PETERS*

*Department of Biological Sciences, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, Maryland 21250, USA,

†Department of History and Philosophy of Science & Center for the Integrative Study of Animal Behaviour, Indiana University, Goodbody Hall 130, Bloomington, Indiana 47405, USA

Abstract

Many studies of phylogeography, speciation, and species limits restrict their focus to a narrow issue: gene tree monophyly. However, reciprocal monophyly does not provide an ideal touchstone criterion of any aspect of evolutionary divergence. There is a continuum of divergence stages as isolated populations go from initial allele frequency differences to well-differentiated species. Studying intermediate stages of divergence will increase our understanding of geographical speciation, species limits, and conservation priorities. We develop a conceptual framework and terminology for thinking about the stages of 'intermediate polyphyly'. The Holarctic clade of common ravens (*Corvus corax*), found throughout much of Eurasia and North America, provides a case study of these stages of intermediate divergence. We used coalescent, phylogenetic, and population genetic methods to investigate the history and current status of this Old World–New World distribution using 107 mitochondrial control region sequences. Phylogenetically, New World and Old World samples are intermixed. However, most samples are grouped into small subclades that are restricted to either the New World or the Old World, and only one haplotype is shared between the hemispheres. Analysis of molecular variance (AMOVA) results reflect this low haplotype sharing between hemispheres ($\Phi_{ST} = 0.13$, $P < 0.01$). Isolation with Migration (IM) coalescent results suggest a sustained period of divergence between the hemispheres and low levels of maternal gene flow. Although there has not been sufficient time to evolve reciprocal monophyly and some gene flow may occur, New World and Old World ravens are genetically quite distinct. We use this example to demonstrate these early stages of divergence as populations go from sharing only internal haplotypes, to sharing no haplotypes, to having population specific subclades. Studies of phylogeography, speciation and systematics will benefit from increased attention to these stages of intermediate polyphyly.

Keywords: coalescent methods, *Corvus corax*, intermediate polyphyly, phylogeography, reciprocal monophyly, speciation

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Introduction

Many studies of population isolation focus on a narrow question: are the mitochondrial lineages within the populations reciprocally monophyletic (Zink 2004)? Reciprocal

monophyly has been treated as a touchstone of significant genetic divergence for determining species limits (Donoghue 1985; Zink & McKittrick 1995), conservation priorities (Moritz 1994), and phylogeographical history (Avice 2000). However, reciprocal monophyly develops in mitochondrial DNA (mtDNA) as a result of stochastic processes whose time course generally has little predictability (Neigel & Avice 1986; Hudson & Turelli 2003). Consequently, the achievement of

Correspondence: Kevin E. Omland, Fax: 410-455-3875. E-mail: omland@umbc.edu

gene tree monophyly at the species level may have no particular evolutionary significance (Baker *et al.* 2003; also see Rosenberg & Nordborg 2002). Reciprocal monophyly of mtDNA is simply one stage on the continuum of genetic divergence that two populations undergo following isolation. Each stage of this continuum is informative, and as studies include larger sample sizes and more sophisticated analyses, it is useful to recognize and evaluate all stages in this process. Many studies have pointed out that stochasticity leads to problems if focusing on a single locus (e.g. Hare 2001; Hudson & Turelli 2003). However, stochasticity also means that it is problematic to look for any one pattern such as reciprocal monophyly in gene trees. The recent focus on monophyly has obscured interesting patterns in systems exhibiting intermediate levels of genetic divergence. Indeed, few empirical or theoretical studies have emphasized the many stages along the pathway to reciprocal monophyly.

Studies of population isolation, evolutionary distinctiveness and gene tree monophyly have been especially prominent for widespread species. Understanding widespread distributions is a major focus of avian phylogeography and biogeography (Avice 2000). Over 100 species of birds have Holarctic distributions, many of which are found in much of Eurasia and North America (Del Hoyo *et al.* 2004). Each likely originated in one hemisphere and expanded its range to occupy the other (Simpson 1944; Bremer 1992; Ronquist 1997). Regardless, studying genetic differentiation between such separated populations is important for understanding speciation, population divergence and conservation priorities. Furthermore, once species are present in both hemispheres, it is important to understand how much gene flow occurs (e.g. Zink *et al.* 1995; Wenink *et al.* 1996; Drovetski *et al.* 2004). Determining levels of gene flow has implications for species management and helps illuminate the role of genetic exchange in maintaining species coherence across large geographical ranges (Rieseberg & Burke 2001).

In this study, we reconstruct the population history of Old and New World ravens exhibiting intermediate levels of genetic divergence. Common ravens (*Corvus corax*) are a well-known Holarctic species that show little or no discrete morphological variation throughout their range (Vaurie 1959). Common ravens form two deeply divergent mitochondrial clades, designated the 'Holarctic clade' and the 'California clade' (Omland *et al.* 2000). The Holarctic clade is found in Europe, Asia, and northern and eastern North America. (This clade also includes divergent populations in the Canary Islands, *C. corax tingitanus*, which were excluded from the present analysis; see Baker & Omland 2006.) The California clade is concentrated in southern California but is also found intermixed throughout much of the western United States (Omland *et al.* 2000; unpublished). Our previous work did not address the population

genetics, origins and history of the Holarctic clade. Here we use coalescent, phylogenetic, and population genetic analyses of new and published mitochondrial DNA sequences to address these questions for the Holarctic clade of common ravens.

We also use this Holarctic raven case study to illustrate and define interesting intermediate stages of genetic divergence. We propose a conceptual framework and terminology to help identify and describe these stages of divergence, which we collectively term intermediate polyphyly. This framework will provide additional road marks for a wide range of researchers studying the continuum of divergence involved when one panmictic population divides into two well-differentiated species.

Methods

We obtained mitochondrial control region (CR) sequences from 107 common ravens (*Corvus corax*) in the Holarctic clade, which were collected throughout the taxon's range (85 from the New World, 22 from the Old World; Fig. 1). Fifty-eight of these individuals were sequenced for this study; 49 of the sequences were previously published (Appendix). In addition, we used outgroup CR sequences from a representative of the California clade, Chihuahuan raven (*Corvus cryptoleucus*), pied crow (*Corvus albus*), and American crow (*Corvus brachyrhynchos*). We extracted DNA from new samples using DNeasy Tissue Extraction Kits (QIAGEN), and we amplified and sequenced 314 base pairs of CR with primers corII-LGL2 and cor-H417 following standard protocols (detailed in Omland *et al.* 2000). We deposited new CR sequences in GenBank under accession numbers AY710355–AY710378 and AY710393–AY710426.

We conducted phylogenetic analyses in PAUP* (version 4.0b10; Swofford 2002) using maximum-parsimony (MP) and maximum-likelihood (ML) methods. We performed equally weighted MP searches using the heuristic search algorithm with 100 random additions. Insertions and deletions (present only in the outgroup) were treated as fifth character states. To determine the model of sequence evolution that best fit our data, we performed hierarchical likelihood-ratio tests in MODELTEST (Posada & Crandall 1998). We then performed an ML search in PAUP* using the heuristic search algorithm with an arbitrarily selected starting tree from the MP searches and the model and parameter values estimated by MODELTEST. To assess the statistical support for the topology we found using our original ML search, we performed three constrained ML searches using the same parameter values (but starting with the neighbour-joining tree). The constrained trees forced reciprocal monophyly, New World paraphyly, and Old World paraphyly. (We constrained trees to a pattern of 'simple paraphyly' with the focal hemisphere paraphyletic, and the other hemisphere a nested monophyletic group.) We then

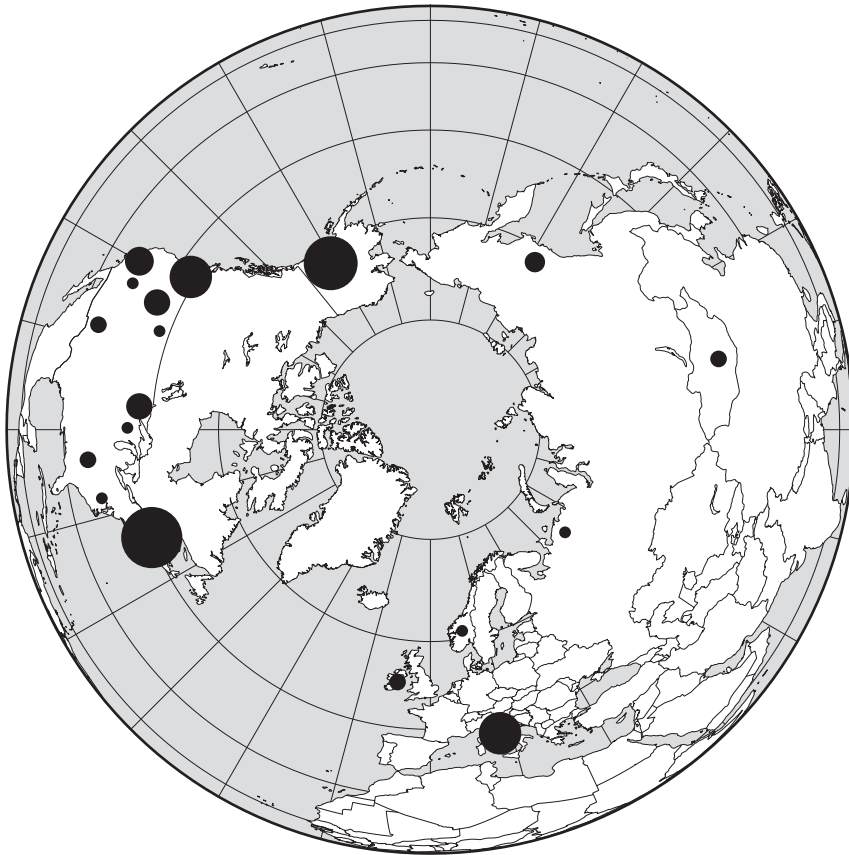


Fig. 1 Polar map of the New and Old World. Approximate sampling locations indicated by black circles. The area of the circles indicates relative sample sizes.

performed a Shimodaira–Hasegawa test (Shimodaira & Hasegawa 1999) to determine whether the likelihood score of the unconstrained ML tree is significantly better than the likelihood scores of any constrained tree. Finally, we used NETWORK 4.111 (Bandelt *et al.* 1999; www.fluxus-engineering.com, MJ algorithm) to construct an unrooted minimum-spanning network.

To test for population structure within the Holarctic clade, we performed hierarchical analyses of molecular variance (AMOVA) (Excoffier *et al.* 1992; Schneider *et al.* 2000). We defined 6 Old World populations: Ireland ($n = 2$), Italy ($n = 13$), Mongolia ($n = 2$), Norway ($n = 1$), Western Russia ($n = 1$), and Siberia ($n = 3$). We defined 12 New World populations: Alaska ($n = 21$), California ($n = 6$), Idaho ($n = 5$), Maine ($n = 27$), Minnesota ($n = 5$), Montana ($n = 1$), Nevada ($n = 1$), New Mexico ($n = 2$), North Carolina ($n = 1$), Tennessee ($n = 2$), Washington ($n = 13$), and Wisconsin ($n = 1$).

To estimate effective population sizes, migration rates, and divergence time for Old and New World ravens, we used the program Isolation with Migration (IM, Hey & Nielsen 2004), which tests for length of genetic isolation and levels of migration. Note that IM assumes selective neutrality, that these two populations are each other's closest relatives, random sampling from a panmictic population,

as well as several other common and unique assumptions (Hey & Nielsen 2004). We simultaneously estimated θ_{OW} (effective number of female ravens in the Old World), θ_{NW} (effective number of female ravens in the New World), θ_{A} (effective number of female ravens in the ancestral population at the time of divergence), t (time of divergence of populations), TMRCA (time to most recent common ancestor), and m (effective number of female migrants per generation; we forced $m_1 = m_2$). [Preliminary analyses indicate that allowing for asymmetrical migration rates did not qualitatively change our findings (unpublished results).] These parameter values are scaled to the mutation rate, u . We estimated u for CR by comparing nucleotide diversity between CR and cytochrome *b* (*cyt b*) (Peters *et al.* 2005) within the Holarctic and California clades of ravens (Omland *et al.* 2000). CR contains about 2.5 times greater nucleotide diversity than *cyt b*, suggesting that it evolves that much faster in ravens. We used a rough mitochondrial coding region calibration of 2% per million years (Shields & Wilson 1989; also see Fleischer *et al.* 1998; Lovette 2004). Hence we estimate that CR diverges at a rate of 5.0% per million years for ravens, and our estimated CR mutation rate (u) is 2.5×10^{-8} substitutions/site/year (but see Lambert *et al.* 2002).

We conducted a total of three runs in IM using a two-step heating increment. The first run was conducted to determine

appropriate priors for subsequent runs; we used unrealistic upper bounds for our priors for this preliminary run. Uninformative priors (i.e. a range that encompassed our entire posterior distribution) were then set for the final two runs. The final runs were conducted using identical conditions but different random number seeds to test that multiple runs gave similar results. We ran approximately 4 million steps for each run so that minimum effective sample sizes were at least 50 (Hey 2005). Because both runs gave similar results, we present data from the longer of the two runs. We report the mode along with the 95% HPDs (highest posterior densities; Hey & Nielsen 2004).

Results

Phylogenetic analyses

Parsimony searches produced 245 equally parsimonious trees. We computed a 50% majority rule consensus tree (not shown), which revealed extensive intermixing of New and Old World haplotypes. However, most haplotypes had other haplotypes from the same hemisphere as their closest relatives. Hierarchical likelihood-ratio tests suggested the HKY + Γ model (Hasegawa *et al.* 1985) with the following parameter values best fit our data: A = 0.3600, C = 0.3185, G = 0.1110, T = 0.2105, ti/tv = 7.7478, α = 0.1801. ML searches produced one tree ($-\ln L = 1070.7443$), which likewise showed the hemispheres to be nonmonophyletic (Fig. 2). The unrooted haplotype network also showed haplotypes from the two hemispheres intermixed (Fig. 3). Using different methods and different models of evolution did change minor details of the topology; however, the pattern of intermixed hemisphere specific clades remained. Indeed, results from the Shimodaira–Hasegawa test strongly favoured nonmonophyly as the most likely topology given our data. We can statistically reject reciprocal monophyly ($P = 0.004$), simple New World paraphyly ($P = 0.014$), and simple Old World paraphyly ($P = 0.008$) as the best explanation for our data.

Nevertheless, some phylogenetic structuring was evident. Of the eight major Holarctic subclades in the ML tree, four are exclusive to the New World and three are exclusive to the Old World. Only one subclade contains a mixture of Old and New World samples (Fig. 2). Moreover, only one haplotype is shared between hemispheres. Those patterns are indicative of shallow (but real) evolutionary divergence.

Population genetic analyses

AMOVA results suggest that Old World and New World ravens are genetically differentiated ($\Phi_{ST} = 0.126$, $P = 0.0019$). The majority of variation within the Holarctic clade, however, is accounted for by within-population divergence (68%, Table 1). The small sample size of several populations

means that our estimates of within-population variation may be unreliable, but increased sampling would be unlikely to dramatically change our qualitative AMOVA results. For example, excluding populations with less than two individuals did not change the between hemisphere results much ($\Phi_{ST} = 0.130$). Similarly, using conventional F_{ST} also showed significant differences between hemispheres ($F_{ST} = 0.077$, $P = 0.0078$, including all populations).

IM results provided strongly unimodal posterior distributions of all parameter estimates (Fig. 4). The smoothed posterior distributions of θ_{NW} peaked at 51.9, θ_{OW} at 63.9, and θ_A at 7.9. The 95% HPDs for the Old and New World parameter distributions overlapped substantially, and both distributions showed evidence of expansion from the ancestral population size. The posterior distributions of m peaked at 0.06 (95% HPD: 0–0.233). Converting this value we estimate 3.4 migrants per generation (95% HPD: 0–44 incorporates the HPDs of both θ and m); importantly we cannot reject the hypothesis that there has been no gene flow between hemispheres following population divergence. The posterior distributions of t peaked at 2.14 [corresponding to 270 000 years before present (BP); 95% HPD: 140 000–440 000 BP] and TMRCA at 3.77 (corresponding to 480 000 BP; 95% HPD: 310 000–760 000 BP). The difference between t and TMRCA indicates that much of the extant genetic diversity of Holarctic ravens was present prior to the divergence between Old and New World ravens. Thus, our IM results strongly suggest a sustained period of isolation between hemispheres, with little or no maternal gene flow (Fig. 4).

Discussion

New World and Old World samples of common ravens are phylogenetically intermixed. No simple pattern of nesting reveals a direction of colonization. At first glance, this intermixing of haplotypes might seem to indicate substantial gene flow or very recent divergence (Avice 2000). However, both IM and AMOVA results demonstrate substantial divergence between hemispheres. Furthermore, closer inspection of the gene trees reveals more complex patterns suggestive of substantial differentiation.

The first pattern that suggests substantial divergence is the very low level of haplotype sharing between the two hemispheres. Although many haplotypes are shared within hemispheres, there is only one haplotype shared between hemispheres (a total of six individuals from Alaska, Italy, Maine, and Russia). This haplotype is thus relatively common, and it is at an internal position in the network (Fig. 3), suggesting that it is a retained ancestral haplotype (Castelloe & Templeton 1994). That this haplotype is found in several distant locations in both the Old and New World further discounts the possibility that this shared haplotype is the result of recent or ongoing gene

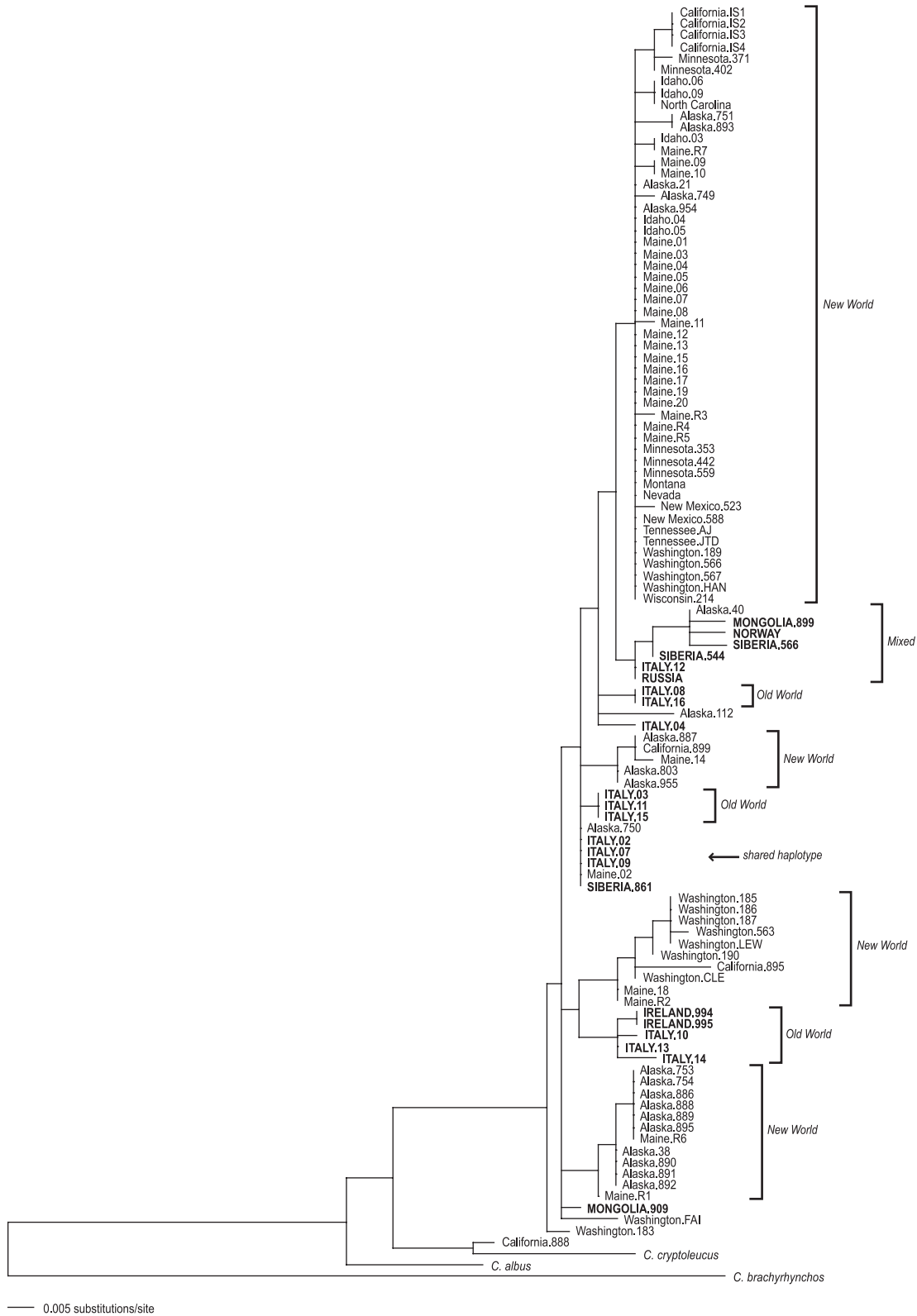


Fig. 2 Maximum-likelihood phylogram of *Corvus corax* (Holarctic clade) and close relatives. Of the eight labelled subclades within the Holarctic clade, seven are exclusive to either the Old World or the New World. Note also that only one haplotype is shared between the hemispheres. (The parsimony strict consensus tree contains seven of these subclades. However, the large New World subclade at the top is found in only 81% of the most parsimonious trees.)

Source of variation	d.f.	Sum squares	Variance components	% variation	<i>P</i> value
Between hemispheres	1	17.8	0.316	12.6	0.0098
Among populations	16	67.2	0.489	19.4	< 0.00001
Within populations	89	152.0	1.708	68.0	< 0.00001
Total	106	237.0	2.513		

Table 1 AMOVA results for Old World vs. New World samples. Results show significant structure at all hierarchical levels

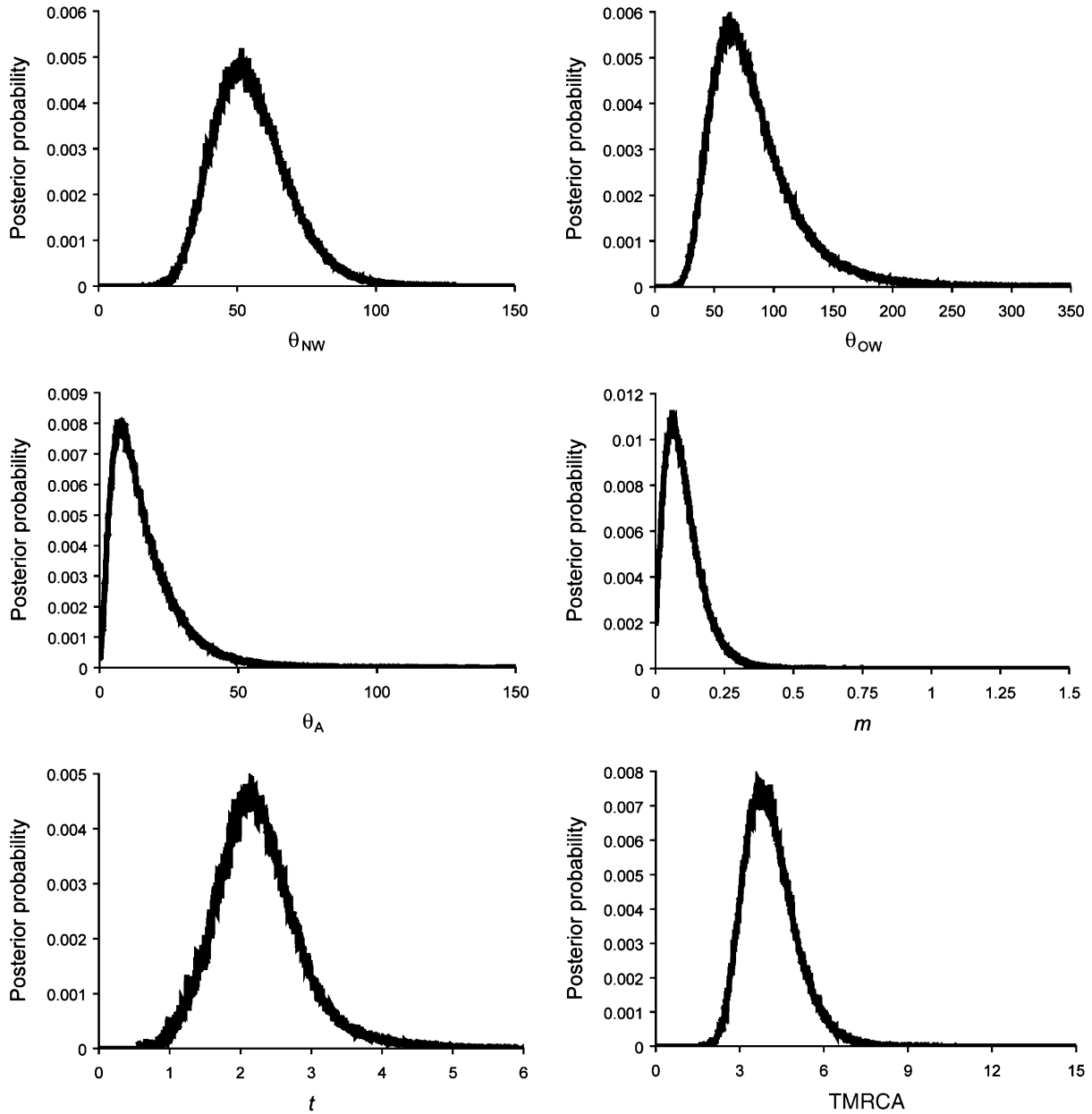


Fig. 4 Posterior distributions of parameter estimates from the IM program scaled to the mutation rate, u . θ_{NW} , θ_{OW} , and θ_A are effective population sizes of the Old World, New World, and ancestral populations; m is the migration rate; t is the time since population divergence, and TMRCA is the time to most recent common ancestor of the haplotypes. (All curves are shown on the scale of the prior probability distributions used in the analyses.)

Table 2 Typical stages of population divergence (for one locus, e.g. mtDNA). These stages assume divergence caused when a single population is divided by a vicariant event or when a large number of founders disperse across a barrier* (see Fig. 5)

Rampant polyphyly (haplotypes completely intermixed)

- A. Panmixia – initially both populations have indistinguishable haplotype frequencies
- B. Frequency differences – haplotype frequency differences, many haplotypes shared

Intermediate polyphyly (haplotypes start to sort)

Stage 1: Neotypy (novel mutation) – only common ancestral haplotypes are shared; new haplotypes are created by mutations and are not shared by populations† (e.g. Baker *et al.* 2003; this study).

Stage 2: Allotypy (local fixation) – no haplotypes shared between populations (e.g. Fig. 4a in Hare *et al.* 2002; Peters *et al.* 2005)

Stage 3: Allophyly (deeper fixation) – populations from two groups generally in monophyletic subclades, although samples from the two populations are still intermixed across the tree (e.g. McCracken *et al.* 2001; Fig. 4b in Hare *et al.* 2002; also see this study).

Simple paraphyly (one group a monophyletic subset of the other) –

One population forms a monophyletic group that is genetically nested within the other group.‡

Reciprocal monophyly (haplotypes completely sorted)

- A. Sample monophyly – all individuals sampled within each population share a more recent common ancestor with individuals in their own population.§
- B. Group monophyly – verifying reciprocal monophyly of the entire two populations would require sequencing *all* individuals in each of the two populations. Thus this yes/no criterion probably has seldom been verified for populations or species.¶

*More research is needed on whether all diverging populations go through all of these stages and to confirm that the stages are hierarchically nested in this order. (These stages may also apply to other scenarios of divergence including sympatric speciation.)

†Some new haplotypes may be shared due to homoplasy.

‡Molecular systematists beginning with Neigel & Avise (1986) have generally referred to these stages as ‘paraphyly’, without explicit regard for the original definition which emphasizes ancestral nodes that are included with named extant taxa (Wiley 1981). Simple paraphyly resulting from lineage sorting can be deep (for example in two large allopatric groups that have been diverging for a long time), or shallow [generally when there is recent divergence initiated by a very small number of founders (e.g. one pair, Harrison 1991; Avise 2004)].

§The inference of monophyly is actually statistical and highly dependent on the sample size used. Having a high level of confidence that a low frequency haplotype is absent requires huge sample sizes (Wiens & Servedio 2000). This statistical perspective applies to all stages along this continuum of genetic divergence.

¶Furthermore, occasional gene flow and hybridization makes it likely that complete group monophyly may only come after millions of years of isolation and that reciprocal monophyly can be reversed (Funk & Omland 2003). (This stage could also be termed ‘population monophyly’, although this term has been used in several other contexts.)

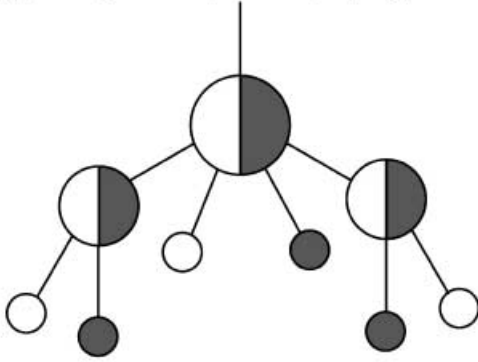
an intermediate stage in the pathway to reciprocal monophyly. At present, we have no terms to describe such intermediate stages: it is not rampant polyphyly but neither is it simple paraphyly (with one hemisphere a monophyletic nested group). A new terminology of lineage divergence is needed to describe these intermediate levels of divergence.

Coalescence theory predicts that all isolated populations will eventually achieve reciprocal monophyly (Hudson 1990; incomplete lineage sorting, Neigel & Avise 1986). Not all diverging populations follow the same path or time course through these stages, although diverging populations generally go through a predictable series of stages of divergence (Table 2). Immediately at the time of isolation, the two populations will have very similar haplotypes and haplotype frequencies (except in the case of a founder event). Shortly thereafter, the two populations will develop haplotype frequency differences. Researchers frequently test for such early frequency differences in haplotypes (or alleles), for example using F_{ST} , Φ_{ST} , and AMOVA.

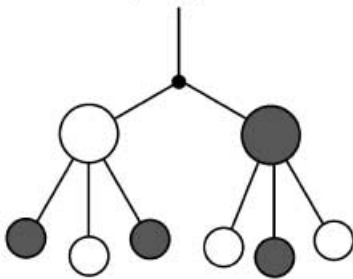
However, there are a number of intermediate stages that are seldom explored. We label these as ‘intermediate

polyphyly’, and we discuss three separate stages below. First, new mutations begin to occur in each of the populations, and these will generally not be shared. Common ancestral haplotypes at internal nodes in networks will be shared, but novel haplotypes will generally not be (Castelloe & Templeton 1994). Second, after enough time, genetic drift will result in the loss of the ancestral haplotypes in one, and then eventually both, of the populations such that no haplotypes are shared between the populations. Note that at this stage, haplotypes in a given population may have as their closest relative a haplotype in the other population, so the haplotypes can still be completely intermixed (also see Davis 1996; Fig. 3). Third, more and more haplotypes will drift to extinction, so that most haplotypes will have as their closest relatives other haplotypes from the same population. Phylogenetically, these haplotype groups can still be largely intermixed between the two populations. Like any pattern controlled by stochastic lineage sorting, the order of these stages may sometimes be different, they may not be mutually exclusive, and their durations will not be precisely predictable. We describe

(a) neotypy – only internal (ancestral) haplotypes shared



(b) allotypy – no shared haplotypes



(c) allophyly – monophyletic subclades

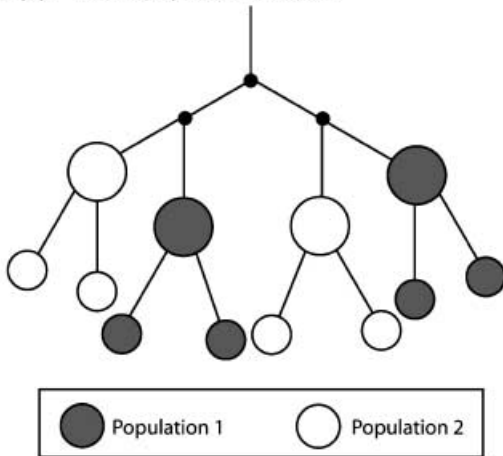


Fig. 5 Haplotype networks showing the temporal progression in the stages of intermediate polyphyly. These stages occur as lineage sorting proceeds in two populations from panmixia to reciprocal monophyly (see Table 2).

and name these three stages of intermediate polyphyly in Table 2 and Fig. 5.

Several other recent studies provide examples of each of these three stages of intermediate polyphyly. Baker *et al.* (2003) provided an example of very recent divergence in the orchard oriole complex (*Icterus spurius* and *Icterus fuertesi*); only internal haplotypes were shared between these two recently diverged taxa ('neotypy', Stage 1, Table 2). Peters

et al. (2005) showed a likely intermediate stage of divergence between eastern and western populations of wood ducks (*Aix sponsa*) in North America. In that study haplotypes from both sides of the continent were closely related, but no haplotypes were shared ('allotypy', Stage 2, Table 2) (also see Fig. 4a in Hare *et al.* 2002). Finally, McCracken *et al.* (2001) provided an example of likely deeper divergence in eastern (Florida) and western (Texas and Louisiana) populations of the mottled duck (*Anas fulvigula*). Although haplotypes from the eastern part of the range are broadly intermixed with haplotypes from the western part of the range, all the eastern haplotypes are in four distinct clades. There are no eastern individuals that have as their closest relatives western individuals nor vice versa, thus providing a dramatic example of the likely last stages of polyphyly ('allophyly', Stage 3, Table 2) (also see Encalada *et al.* 1996; Fig. 4b in Hare *et al.* 2002). New World vs. Old World ravens strongly display the pattern of 'neotypy', with only one internal ancestral haplotype shared between hemispheres. These Holarctic ravens also seem to be approaching the stages of 'allotypy' where no haplotypes will be shared and 'allophyly' where, although intermixed, all the clades will be hemisphere specific.

Coalescent methods enable researchers to study this progression of intermediate paraphyly in its entirety (Knowles 2004). Nested clade analysis also utilizes some aspects of this process (Templeton 1998). Nevertheless, there remain many unexplored opportunities to describe and assess these additional patterns apparent in gene trees and haplotype networks. Focusing on each of these intermediate stages may also lead to new uses of or additions to existing coalescent approaches. We assume that fixation during this progression is driven largely by genetic drift [researchers are generally using noncoding regions (e.g. control region, introns) or third position changes (e.g. mitochondrial coding genes) for these intraspecific studies]. However selection, especially at linked loci, likely also plays a role (Ballard & Kreitman 1995; Rand 2001).

Our findings have important implications for our understanding of divergence and ongoing speciation in the common ravens (*Corvus corax*) and other closely related *Corvus* species. Omland *et al.* (2000) found a deep mitochondrial split in some common ravens from the western United States. This 'California clade' is over 4% divergent from other common ravens in mitochondrial cytochrome *b* (Omland *et al.* 2000). However, this split is not reflected by any other known character sets including plumage, shape, size, calls, behaviour, ecology, etc. Furthermore, nearly all locations sampled in the western United States contain a mixture of the California and Holarctic clades, and several lines of evidence suggest the likelihood of random gene flow between the two clades (K. E. Omland *et al.*, unpublished). It is possible that this wide contact zone in the western United States reflects a fairly recent expansion of

the Holarctic clade, perhaps out of the Old World. However, our current phylogenetic and coalescent results do not provide any evidence on the direction of colonization for the Holarctic clade. Future study of both the Holarctic and California clades using coalescent methods and multiple loci may shed light on the divergence and possible reemerging that have occurred in common ravens. More sampling in the Old World, especially along the southern parts of the range, will likely reveal more details (Baker & Omland 2006). In addition, several other named species are very closely related to these common raven clades, including the Chihuahuan raven (*Corvus cryptoleucus*) and the African pied crow (*Corvus albus*), both of which are nested within common ravens in mitochondrial trees (Omland *et al.* 2000; Feldman & Omland 2005). Thus, this entire raven species complex presents many remaining challenges and opportunities for understanding intermediate stages of divergence, species limits and speciation.

Conclusions

In this study, we have used ravens as a case study of intermediate polyphyly. Coalescent, population genetic, and phylogenetic analyses all support intermediate levels of population divergence between Old and New World ravens. We can reject simple paraphyly and reciprocal monophyly as better explanations for our data, and yet IM results suggest sustained genetic isolation (several hundred thousand years) and low levels of gene flow. According to the terminology of genetic divergence that we propose, the Holarctic clade exhibits Stage 1 polyphyly ('neotypy'), but is nearing Stages 2 and 3 (with few haplotypes shared, and intermixed hemisphere-specific clades). This case study illustrates the coalescent process at an interesting, but little studied stage. Examining systems that exhibit similar patterns of nonmonophyly and intermediate divergence will lead to important insights in many areas.

Reciprocal monophyly of single locus gene trees (especially mtDNA) has been emphasized as a criterion for evaluating species limits (e.g. Zink & McKittrick 1995; Zink & Blackwell-Rago 2000), determining conservation priorities (Moritz 1994), studying biogeographical history (Avice 2004), and identifying new taxa (Hebert *et al.* 2004). Focusing on monophyly has clarified evolutionary thinking at higher taxonomic levels (Hennig 1966). However, at the lowest taxonomic levels, the emphasis on reciprocal monophyly of gene trees has often obscured clear understanding of evolutionary processes and speciation. Reciprocal monophyly of individual gene trees should neither be a necessary nor sufficient criterion for any category of evolutionary divergence. Increasingly, researchers are incorporating processes long understood by population

geneticists, especially the stochastic nature of lineage sorting, and explicitly incorporating statistical studies of coalescence into all of these areas (Rosenberg & Nordborg 2002; Knowles 2004). Given the complex and continuous nature of evolution, divergence and speciation will be better understood by (i) evaluating multiple criteria (de Queiroz 1998), (ii) using a variety of approaches (Wiens 2004), and (iii) looking for many different patterns (this study). For a wide range of basic and applied evolutionary questions at and below the species level, examining the patterns typical of intermediate divergence can be as interesting and informative as focusing exclusively on reciprocal monophyly.

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Kevin Omland is a faculty member at UMBC whose laboratory focuses on speciation, character evolution and sexual selection in birds. He is especially interested in recently derived groups at the interface between population-level divergence and species-level phylogenies. Jason Baker is a PhD student in the history and philosophy of science, focusing on speciation and species limits. Jeff Peters is completing his PhD work at UMBC on another Holarctic group, the gadwall (*Anas strepera*). His postdoctoral research at the University of Alaska will apply coalescent methods and multiple loci to test for a colonization bias across Holarctic waterfowl.

Appendix

Species*	Individual	Locality	Accession no.
<i>C. corax</i> (Hol.)	Alaska.112	USA, Alaska, Fairbanks County	AY710355
<i>C. corax</i> (Hol.)	Alaska.21	USA, Alaska, Fairbanks County	AY710356
<i>C. corax</i> (Hol.)	Alaska.38	USA, Alaska, Fairbanks County	AY710357
<i>C. corax</i> (Hol.)	Alaska.40	USA, Alaska, Fairbanks County	AY710358
<i>C. corax</i> (Hol.)	Alaska.749	USA, Alaska, Fairbanks County	AY710359
<i>C. corax</i> (Hol.)	Alaska.750	USA, Alaska, Fairbanks County	AY710360
<i>C. corax</i> (Hol.)	Alaska.751	USA, Alaska, Fairbanks County	AY710361
<i>C. corax</i> (Hol.)	Alaska.753	USA, Alaska, Fairbanks County	AY710362
<i>C. corax</i> (Hol.)	Alaska.754	USA, Alaska, Fairbanks County	AY710363
<i>C. corax</i> (Hol.)	Alaska.803	USA, Alaska, Fairbanks County	AY710364
<i>C. corax</i> (Hol.)	Alaska.886	USA, Alaska, Fairbanks County	AY710365
<i>C. corax</i> (Hol.)	Alaska.887	USA, Alaska, Fairbanks County	AY710366
<i>C. corax</i> (Hol.)	Alaska.888	USA, Alaska, Fairbanks County	AY710367
<i>C. corax</i> (Hol.)	Alaska.889	USA, Alaska, Fairbanks County	AY710368
<i>C. corax</i> (Hol.)	Alaska.890	USA, Alaska, Fairbanks County	AY710369
<i>C. corax</i> (Hol.)	Alaska.891	USA, Alaska, Fairbanks County	AY710370
<i>C. corax</i> (Hol.)	Alaska.892	USA, Alaska, Fairbanks County	AY710371
<i>C. corax</i> (Hol.)	Alaska.893	USA, Alaska, Fairbanks County	AY710372
<i>C. corax</i> (Hol.)	Alaska.895	USA, Alaska, Fairbanks County	AY710373
<i>C. corax</i> (Hol.)	Alaska.954	USA, Alaska, Fairbanks County	AY005895+
<i>C. corax</i> (Hol.)	Alaska.955	USA, Alaska, Fairbanks County	AY005896+
<i>C. corax</i> (Hol.)	California.895	USA, California, San Bernardino County	AY005897+
<i>C. corax</i> (Hol.)	California.899	USA, California, San Bernardino County	AY710374
<i>C. corax</i> (Hol.)	California.is1	USA, California, San Diego County	AY710375
<i>C. corax</i> (Hol.)	California.is2	USA, California, San Diego County	AY710376
<i>C. corax</i> (Hol.)	California.is3	USA, California, San Diego County	AY710377
<i>C. corax</i> (Hol.)	California.is4	USA, California, San Diego County	AY710378
<i>C. corax</i> (Hol.)	Idaho.03	USA, Idaho, Ada County	AY005898+
<i>C. corax</i> (Hol.)	Idaho.04	USA, Idaho, Ada County	AY005899+
<i>C. corax</i> (Hol.)	Idaho.05	USA, Idaho, Ada County	AY005900+
<i>C. corax</i> (Hol.)	Idaho.06	USA, Idaho, Ada County	AY005901+
<i>C. corax</i> (Hol.)	Idaho.09	USA, Idaho, Ada County	AY005902+
<i>C. corax</i> (Hol.)	Ireland.994	Ireland, Cork County	AY710339§
<i>C. corax</i> (Hol.)	Ireland.995	Ireland, Cork County	AY710340§
<i>C. corax</i> (Hol.)	Italy.02	Italy, Friuli-Venezia Giulia region	AY710341§
<i>C. corax</i> (Hol.)	Italy.03	Italy, Friuli-Venezia Giulia region	AY710342§
<i>C. corax</i> (Hol.)	Italy.04	Italy, Friuli-Venezia Giulia region	AY710343§
<i>C. corax</i> (Hol.)	Italy.07	Italy, Friuli-Venezia Giulia region	AY710344§
<i>C. corax</i> (Hol.)	Italy.08	Italy, Friuli-Venezia Giulia region	AY710345§
<i>C. corax</i> (Hol.)	Italy.09	Italy, Friuli-Venezia Giulia region	AY710346§
<i>C. corax</i> (Hol.)	Italy.10	Italy, Friuli-Venezia Giulia region	AY710347§
<i>C. corax</i> (Hol.)	Italy.11	Italy, Friuli-Venezia Giulia region	AY710348§
<i>C. corax</i> (Hol.)	Italy.12	Italy, Friuli-Venezia Giulia region	AY710349§
<i>C. corax</i> (Hol.)	Italy.13	Italy, Friuli-Venezia Giulia region	AY710350§
<i>C. corax</i> (Hol.)	Italy.14	Italy, Friuli-Venezia Giulia region	AY710351§
<i>C. corax</i> (Hol.)	Italy.15	Italy, Friuli-Venezia Giulia region	AY710352§
<i>C. corax</i> (Hol.)	Italy.16	Italy, Friuli-Venezia Giulia region	AY710353§
<i>C. corax</i> (Hol.)	Maine.01	USA, Maine, Franklin County	AF115305+
<i>C. corax</i> (Hol.)	Maine.02	USA, Maine, Franklin County	AF115306+
<i>C. corax</i> (Hol.)	Maine.03	USA, Maine, Franklin County	AF115307+
<i>C. corax</i> (Hol.)	Maine.04	USA, Maine, Franklin County	AF115308+
<i>C. corax</i> (Hol.)	Maine.05	USA, Maine, Franklin County	AF115309+
<i>C. corax</i> (Hol.)	Maine.06	USA, Maine, Franklin County	AY710427+
<i>C. corax</i> (Hol.)	Maine.07	USA, Maine, Franklin County	AY710428+
<i>C. corax</i> (Hol.)	Maine.08	USA, Maine, Franklin County	AY710393
<i>C. corax</i> (Hol.)	Maine.09	USA, Maine, Franklin County	AY710394
<i>C. corax</i> (Hol.)	Maine.10	USA, Maine, Franklin County	AY710395
<i>C. corax</i> (Hol.)	Maine.11	USA, Maine, Franklin County	AY710396

Appendix Continued

Species*	Individual	Locality	Accession no.
<i>C. corax</i> (Hol.)	Maine.12	USA, Maine, Franklin County	AY710397
<i>C. corax</i> (Hol.)	Maine.13	USA, Maine, Franklin County	AY710398
<i>C. corax</i> (Hol.)	Maine.14	USA, Maine, Franklin County	AY710399
<i>C. corax</i> (Hol.)	Maine.15	USA, Maine, Franklin County	AY710400
<i>C. corax</i> (Hol.)	Maine.16	USA, Maine, Franklin County	AY710401
<i>C. corax</i> (Hol.)	Maine.17	USA, Maine, Franklin County	AY710402
<i>C. corax</i> (Hol.)	Maine.18	USA, Maine, Franklin County	AY710403
<i>C. corax</i> (Hol.)	Maine.19	USA, Maine, Franklin County	AY710404
<i>C. corax</i> (Hol.)	Maine.20	USA, Maine, Franklin County	AY710405
<i>C. corax</i> (Hol.)	Maine.r1	USA, Maine, Franklin County	AY710406
<i>C. corax</i> (Hol.)	Maine.r2	USA, Maine, Franklin County	AY710407
<i>C. corax</i> (Hol.)	Maine.r3	USA, Maine, Franklin County	AY710408
<i>C. corax</i> (Hol.)	Maine.r4	USA, Maine, Franklin County	AY710409
<i>C. corax</i> (Hol.)	Maine.r5	USA, Maine, Franklin County	AY710410
<i>C. corax</i> (Hol.)	Maine.r6	USA, Maine, Franklin County	AY710411
<i>C. corax</i> (Hol.)	Maine.r7	USA, Maine, Franklin County	AY710412
<i>C. corax</i> (Hol.)	Minnesota.353	USA, Minnesota, St. Louis County	AY005904†
<i>C. corax</i> (Hol.)	Minnesota.371	USA, Minnesota, Pine County	AY005905†
<i>C. corax</i> (Hol.)	Minnesota.402	USA, Minnesota, St. Louis County	AY005907†
<i>C. corax</i> (Hol.)	Minnesota.442	USA, Minnesota, St. Louis County	AY005906†
<i>C. corax</i> (Hol.)	Minnesota.559	USA, Minnesota (county unknown)	AY710413
<i>C. corax</i> (Hol.)	Mongolia.899	Mongolia, Arhangay province	AY005908†
<i>C. corax</i> (Hol.)	Mongolia.909	Mongolia, Övörhangay province	AY005909†
<i>C. corax</i> (Hol.)	Montana	USA, Montana, Meagher County	AY710414
<i>C. corax</i> (Hol.)	Nevada	USA, Nevada, Nye County	AY710415
<i>C. corax</i> (Hol.)	New Mexico.523	USA, New Mexico, Santa Fe County	AY005910†
<i>C. corax</i> (Hol.)	New Mexico.588	USA, New Mexico, Taos County	AY710416
<i>C. corax</i> (Hol.)	North Carolina	USA, North Carolina, Avery County	AY710417
<i>C. corax</i> (Hol.)	Norway	Norway, Nordland County	AY710354§
<i>C. corax</i> (Hol.)	Russia	Russia, Krasnodarskiy territory	AY005914†
<i>C. corax</i> (Hol.)	Siberia.544	Siberia, Tyumenskaya province	AY005912†
<i>C. corax</i> (Hol.)	Siberia.566	Siberia, Yamalo-Nenetskiy district	AY005913†
<i>C. corax</i> (Hol.)	Siberia.861	Siberia, Magadanskaya province	AY005911†
<i>C. corax</i> (Hol.)	Tennessee.aj	USA, Tennessee, Unicoi County	AY710418
<i>C. corax</i> (Hol.)	Tennessee.jtd	USA, Tennessee, Carter County	AY710419
<i>C. corax</i> (Hol.)	Washington.183	USA, Washington, Jefferson County	AY710420
<i>C. corax</i> (Hol.)	Washington.185	USA, Washington, Clallam County	AY710421
<i>C. corax</i> (Hol.)	Washington.186	USA, Washington, Jefferson County	AY710422
<i>C. corax</i> (Hol.)	Washington.187	USA, Washington, Clallam County	AY710423
<i>C. corax</i> (Hol.)	Washington.189	USA, Washington, Clallam County	AY710424
<i>C. corax</i> (Hol.)	Washington.190	USA, Washington, Clallam County	AY710425
<i>C. corax</i> (Hol.)	Washington.563	USA, Washington, Jefferson County	AY005917†
<i>C. corax</i> (Hol.)	Washington.566	USA, Washington, Grant County	AY005915†
<i>C. corax</i> (Hol.)	Washington.567	USA, Washington, Grant County	AY005916†
<i>C. corax</i> (Hol.)	Washington.cle	USA, Washington, Kittitas County	AY005918†
<i>C. corax</i> (Hol.)	Washington.fai	USA, Washington, Clallam County	AY005919†
<i>C. corax</i> (Hol.)	Washington.han	USA, Washington, Benton County	AY005920†
<i>C. corax</i> (Hol.)	Washington.lew	USA, Washington, Lewis County	AY710426
<i>C. corax</i> (Hol.)	Wisconsin.214	USA, Wisconsin (county unknown)	AY005921†
<i>C. corax</i> (Calif.)	California.888	USA, California, San Bernardino County	AY005875†
<i>C. albus</i>		Ghana, central region	AY527252‡
<i>C. brachyrhynchus</i>		USA, Maryland, Montgomery County	AY005869†
<i>C. cryptoleucus</i>		USA, New Mexico, Lea County	AY005922†

*Hol., Holarctic clade; Calif., California clade (Omland *et al.* 2000).

†Previously published (Omland *et al.* 2000).

‡Previously published (Feldman & Omland 2005).

§Previously published (Baker & Omland 2006).