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Ecological niche differentiation in the *Aphelocoma* jays: a phylogenetic perspective

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The *Aphelocoma* jays have become an important touchstone in behavioural ecology and biogeography – the corpus of studies of this genus makes it an important point of reference. *Aphelocoma* evolutionary history, nevertheless, has been the subject of two papers reaching opposite conclusions, even though they were based on the same allozyme data set. Herein, we present a second molecular data set – 500 bases of the ND2 gene – and analyse it cladistically to arrive at a new hypothesis of phylogenetic relationships. Recent hypotheses by other investigators of a hybrid origin of *Aphelocoma* populations are strongly contradicted. The ecological context within which these evolutionary processes are taking place is characterized using new tools for modelling ecological niches of species along a spectrum from humid tropical to dry temperate habitats. Evolutionary patterns of ecological niches are shown to consist of drastic departures from rate-uniformity and ecological niche conservatism. © 2003 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2003, **80**, 369–383.

ADDITIONAL KEYWORDS: ecological niche – geographical distribution – niche evolution – phylogeny.

INTRODUCTION

SOCIAL AND ECOLOGICAL VARIATION IN *APHELOCOMA*

The *Aphelocoma* jays provide a fascinating scenario of dramatic geographical variation, incipient speciation, and variation in ecology and social biology. Consequently, *Aphelocoma* populations have been analysed in terms of geographical variation (Pitelka, 1951; Peterson, 1991a; Peterson, 1991b), genetic variation (Peterson, 1990; Peterson, 1992a), phylogeny (Peterson, 1992b), and social systems (Woolfenden & Fitzpatrick, 1984; Peterson & Burt, 1992; Burt & Peterson, 1993). This combination of studies provides a rich basis for integrative analyses that cross traditional boundaries among fields of inquiry, and that illuminate evolutionary, ecological, and behavioural processes in manners not often possible.

Social and ecological variation among species in this genus is impressive (Pitelka, 1951). Most populations are cooperative-breeding, with extra individuals helping to varying degrees with raising young (Woolfenden & Fitzpatrick, 1984; Peterson & Burt, 1992) – this variation in social behaviour has complex interactions with phylogeny and ecology (Peterson & Burt, 1992). Ecologically, populations live in habitats ranging from tall, closed cloud forest and pine-oak forest through a variety of woodlands and scrubs to deserts and even mangrove swamps (Peterson & Vargas-Barajas, 1993). Morphological adaptations have tracked some of the more dramatic of these habitat shifts, particularly with respect to food types (acorns vs. pine nuts) available in particular habitat types (Peterson, 1993; Bardwell, Benkman & Gould, 2001). Although previous surveys (Peterson & Vargas-Barajas, 1993) presented broad summaries, they were limited in their ability to analyse in detail, and pick out the particulars of how this ecological diversity has evolved over the phylogenetic history of the clade.

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Phylogenetic hypotheses for *Aphelocoma*

Questions of social and ecological evolution demand a detailed understanding of the group under study, in particular a detailed and robust phylogenetic hypothesis. A monumental monographic treatment by Pitelka (1951) laid the foundation for an intimate understanding of morphological and ecological variation in the genus. Although Pitelka did not employ phylogenetic methodologies – which had not yet been developed – his scenarios for the evolution of the genus were quite well-founded. Phylogenetic treatment of the genus had to wait 40 years, when a broad survey of allozyme variation in the genus formed the basis for a first phylogenetic hypothesis (Peterson, 1992b).

Brown & Li (1995) revisited Peterson's work, adding four characters to the data matrix and presenting new analyses and interpretations. Although their presentation was handicapped by introduction of new (and inappropriate) names for population groups (e.g. 'Orientalis' for the *potosina* group, and 'Occidentalis' for the *wollweberi* group within the traditional *A. ultramarina*), their cladistic analyses appear to contradict the original analysis of much the same data. Their revised hypotheses, if credible, would change many subsequent conclusions (Peterson & Burt, 1992), as well as much of the view of the evolutionary history of the genus.

Aim

The purpose of the present contribution was to examine the issue of *Aphelocoma* ecological diversity in the light of a robust phylogenetic estimate. First, we present and explore the implications of new data (sequences of the ND2 gene of the mitochondrial genome) for the understanding of the phylogeny of the genus, and discuss aspects of Brown & Li's (1995) reanalysis of the allozyme data. Second, we explore the evolution of ecological characteristics of *Aphelocoma* species; we bring to bear on the question a new suite of tools for describing ecological niches quantitatively. This approach, termed ecological niche modelling, provides a quantitative picture of species' ecological niches, and permits a wide variety of analyses that focus on the dimensions of these niches (Peterson, Stockwell & Kluza, 2002b). Analyses of parameters of these ecological niche models in a phylogenetic context provides a new perspective on ecological processes in the group.

METHODS

Sampling for DNA sequencing

Peterson (1990, 1992a) sampled 35 populations of *Aphelocoma* jays. Voucher specimens and frozen tissue samples deposited at the Field Museum of Natural History and the Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México were provided to us for study (Table 1). All of the ten phylogenetic species in the genus except *A. guerrerensis* were included in this study, each represented by two or three geographically disparate populations (Table 1). A diverse outgroup, consisting of *Corvus brachyrhynchus*, *Cyanolyca cucullata*, *Cyanocorax yncas*, *Gymnorhinus cyanocephalus* and *Cyanocitta stelleri*, was included to assist in polarizing characters and rooting the trees. Throughout, we refer to the taxa under study as full species, which is merited under the phylogenetic/evolutionary species concepts, and in at least several cases under the biological species concept as well (Peterson, 1990).

DNA sequencing

Genomic DNA was extracted from each sample using Qiamp tissue extraction kits available from Qiagen. A 522-bp portion of the ND2 gene was amplified using conventional thermal-cycling techniques, with a thermal profile of denaturing at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 70°C for 90 s (Kocher *et al*., 1989). Extension time was lengthened by 4 s each cycle for 35 cycles. ND2 primers (H-6313: 5′-CTCTTATTTAAGGCTTTGAAGGC-3′ and L-5757: 5′-GGCTGAATRGGMCTNAAYCARAC-3′) were developed by M. Sorenson (pers. comm.; H and L refer to heavy and light strands, respectively; numbers indicate relative position of primers on reference chicken sequence (Desjardins & Morais, 1990)). Amplified product was purified on a low-melting point (1%) NuSieve GTG agarose (FMC BioProducts) gel electrophoresed for 45 min at 85–95 V; bands containing target products were excised from the gel, and DNA recovered using Qiaquick spin columns (Qiagen). Finally, purified product was amplified using one primer (heavy or light), and sequenced with an ABI Prism Automated Sequencer (Model 310). The thermal profile for both primer systems was denaturing at 96°C for 10 s, annealing at 50°C for 5 s, and extension at 60°C for 4 min, repeated for 25 cycles. Negative controls were used at each step to test for reagent contamination.

Phylogenetic analysis

Numbers of variable and phylogenetically informative molecular characters, as well as numbers and classes of transitions and transversions, were calculated using MEGA 1.01 (Kumar, Tamura & Nei, 1993). Several recent studies have found a bias against guanine in avian DNA sequences (e.g. Espinosa de los Monteros & Cracraft, 1997; Table 2). The result may

Table 1. Details of *Aphelocoma* jays represented in this study

Phylogenetic species	AOU (1993)	Peterson (1992a)	Brown & Li (1995)	Subspecies	Present study	Collection	Tissue no.	GenBank accession no.
californica	*coerulescens*	COCAL	California scrub jay	*caurina*	*	FMNH	AMY87-121	AY389363
				immanis				
				oocleptica				
				californica				
				obscura				
				cana?				
				hypoleuca	*	FMNH	4429	AY389367
insularis	*coerulescens*	ACINS	—	*insularis*	*	FMNH	AMY87-112	AY389365
						FMNH	AMY87-1116	AY389366
woodhouseii	*coerulescens*	COWOO,	Western US scrub jay,	*woodhouseii*				
		COMEX	Northern Mexico scrub jay	*nevadae*	*	FMNH	5540	AY389370
				texana	*	FMNH	AMY87-016	AY389369
				cyanotis				
				grisea	*	FMNH	4968	AY389372
sumichrasti	*coerulescens*	COSUM	Southern Mexico scrub jay	*sumichrasti*	*	FMNH	4831	AY389371
				remota	*	FMNH	4874	AY389368
coerulescens	*coerulescens*	ACCO2	Florida scrub jay	*coerulescens*	*	FMNH	FSJ-002	AY389364
wollweberi	*ultramarina*	ULWOL	Occidentalis	*arizonae*	*	FMNH	4430	AY389373
				wollweberi				
				gracilis	*	FMNH	4848	AY389374
potosina	*ultramarina*	ULPOT	Orientalis	*couchii*	*	FMNH	4841	AY389376
				potosina	*	FMNH	4846	AY389375
ultramarina	*ultramarina*	ULULT	Transvolcanic	*ultramarina*	*	FMNH	4422	AY389378
				colimae	*	FMNH	4969	AY389377
unicolor	*unicolor*	UNE	Unicolored jay	*concolor*	*	UNAM	uncat.	AY389380
				oaxacae	*	FMNH	1883	AY389379
				unicolor?				
				griscomi?				
guerrerensis	*unicolor*	ANGUE	Unicolored jay	*guerrerensis*				

'Names' used by Brown & Li (1995) are included both to avoid confusion regarding population references, and to illustrate the odd mix of common and supposedly scientific names used. Tissue number for the *Gymnorhinus cyanocephala* sample was FMNH #1667 (GenBank number AY389362). GenBank numbers for outgroup taxa are: *Corvus brachyrhynchus* (AY389381), *Cyanocorax yncas* (AY389382), *Cyanositta stelleri* (AY389383), *Cyanolyca cucullata* (AY389384).

be a bias against certain classes of transitions and transversions that may obfuscate true saturation measures. Rather than using percent sequence divergence as the distance measure to assess saturation, we used the Tamura–Nei distance, which considers percent base composition for each individual, making this distance measurement more robust than sequence divergence measures (Kumar *et al.*, 1993). All sequences were deposited in GenBank (Table 1).

Phylogenetic trees were estimated based on sequence data using the branch-and-bound procedure of PAUP (version 3.1.1, Swofford, 1993), an approach guaranteed to identify the optimal tree. Support for particular branches in resulting hypotheses was assessed using the branch-and-bound character bootstrapping algorithms in PAUP with 500 replicate searches, branch decay indices (Bremer, 1988, 1994; Sorenson, 1996), and counts of unreversed synapomorphies. MacClade (Maddison & Maddison, 1996) was used to assess tree lengths of alternative hypotheses. For further analysis and interpretation, *A. guerrerensis*, which was not included in the sequence analysis, was added to the tree as sister to *A. unicolor*, a reasonable assumption given many likely synapomorphies in plumage coloration and pattern (Pitelka, 1951).

Point-occurrence information for ecological niche modelling

Distributional data (681 unique species × locality records) for *Aphelocoma* jay 'subspecies groups' (Peterson, 1990; Peterson, 1992b) were assembled via computerization of the lists of collection localities in the most complete monographic treatment of the group (Pitelka, 1951). Specimens with nebulous points of origin (e.g. 'Oaxaca, Mexico') were excluded from analysis, as were complex locality descriptors (e.g. '42 road miles NNE of Monterey, California') for groups for which distributional data were otherwise abundant. All occurrence data were georeferenced to the nearest 0.001° using Internet gazetteer resources (http://www.calle.com), and they were organized in Microsoft Excel 2000 spreadsheets for analysis.

Ecological niche modelling

Ecological niches were modelled and potential geographical distributions predicted using the Genetic Algorithm for Rule-set Prediction (GARP) (Stockwell & Noble, 1992; Stockwell & Peters, 1999; Stockwell, 1999). In general, the procedure focuses on modelling ecological niches (the conjunction of ecological conditions within which a species is able to maintain populations without immigration) (Grinnell, 1917). Specifically, GARP relates ecological characteristics of known occurrence points to those of points randomly sampled from the rest of the study region, seeking to develop a series of decision rules that best summarize those factors associated with the species' presence; these decision rules can then be projected back onto the geography to predict the geographical distribution of the species (Stockwell & Peters, 1999).

In GARP, occurrence points are divided evenly into training and test data sets. GARP uses an iterative process of rule selection, evaluation, testing and incorporation or rejection: a method is chosen from a set of possibilities (e.g. logistic regression, bioclimatic rules), it is applied to the training data, and a rule is developed or evolved. Predictive accuracy is then evaluated based on 1250 points resampled from the test data and 1250 points sampled randomly from the study region as a whole. Rules may evolve by a number of means that mimic DNA evolution, such as point mutations, deletions and crossing over. The change in predictive accuracy from one iteration to the next is used to evaluate whether a particular rule should be incorporated into the model, and the algorithm runs either 1000 iterations or until convergence. GARP's predictive abilities have been tested and proven under diverse circumstances (Peterson & Cohoon, 1999; Peterson, Soberon & Sanchez-Cordero, 1999; Peterson & Vieglais, 2001; Anderson, Gomez & Peterson, 2002a; Anderson, Laverde & Peterson, 2002b; Feria & Peterson, 2002; Peterson *et al.*, 2002b; Peterson, Ball & Cohoon, 2002a; Stockwell & Peterson, 2002a, b).

All modelling in this study was carried out on a desktop implementation of GARP now available for public download (http://www.lifemapper.org/desktopgarp). This implementation offers much-improved flexibility in choice of predictive environmental/ecological GIS data: in this case, we used 12 data layers summarizing elevation, slope, aspect (from the US Geological Survey's (http://edcdaac.usgs.gov/gtopo30/hydro) Hydro-1K data set), and features of climate including daily temperature range, frost days, mean annual precipitation, maximum, minimum, and mean annual temperatures, solar radiation, wet days and vapour pressure (annual means 1960–1990, from the Intergovernmental Panel on Climate Change (http://www.ipcc.ch); New, Hulme & Jones, 1997). Grids were resampled to 0.1° pixel resolution, and clipped to a study region that included North America from the US–Canada border to the southern extreme of Nicaragua.

To optimize model performance, we developed 100 replicate models of species' ecological niches, based on random 50% subsets of available occurrence points (i.e. half of the occurrence points were used to build models, and the other half used to test their predictive ability). Unlike previous applications, which either used single models to predict species' distributions

(Peterson *et al*., 1999) or summed multiple models to incorporate model-to-model variation (Peterson, Scachetti-Pereira & Hargrove, in press), we used a new procedure (Anderson, Lew & Peterson, 2003) for choosing best subsets of models. The procedure is based on the observations that (1) models vary in quality, (2) variation among models involves an inverse relationship between errors of omission (leaving out true distributional area) and commission (including areas not actually inhabited), and (3) best models (as judged by experts blind to error statistics in the original derivation of the method) are clustered in a region of minimum omission of independent test points and moderate area predicted (an axis which includes the commission error). The relative position of the cloud of points relative to the two error axes provides an assessment of the relative accuracy of each model. To choose best subsets of models, we (1) eliminated all but models that had no omission error based on the independent test points, (2) calculated the median area predicted present among these zero-omission models (percent of the area analysed), and (3) identified the 10 models that were closest to the overall median extent for each species. The geographical manifestations of these models were summed to provide a best estimate of the potential geographical distribution of the particular group.

Visualizing ecological niches

To permit visualization of these best-subsets ecological niche models for each *Aphelocoma* group, in ArcView (version 3.2), for each species we combined the original geographical prediction with the 12 ecological dimensions on which the prediction was originally based into a composite grid (Peterson *et al.*, 2002b). The attributes table associated with this grid is effectively a list of all unique environmental combinations in the landscape, with the associated prediction (0–10 models predicting presence). To permit direct comparisons among dimensions, and to avoid biases introduced by differences in scale among ecological dimensions, we *z*-standardized each dimension by subtracting the mean and dividing by the standard deviation, producing a standard normal variable (mean = 0, variance = 1); these standardized values were used in all subsequent analyses.

Two distinct sets of ecological distance measures were developed from this data set: interpredictivity distances and centroid distances (Martínez-Meyer, 2002). Interpredictivity measures were based on the ability of the ecological model of one subspecies group to predict the geographical distribution of each other subspecies group, and vice versa (Peterson *et al*., 1999); this approach measured ecological distances as percent of occurrence points not correctly predicted by the ecological niche model of the other taxon. Although the reciprocal predictions were inspected separately for the purpose of interpretation of interpredictivity measures, the two were averaged to produce an overall measure of ecological distance.

Centroid distances were developed in quite a different manner: over the entire study area (North America), we calculated the mean Euclidean distance in *z*-standardized dimensions among all pairs of subspecies groups using the equation:

$$D_{xy} = \sqrt{\sum_1^n (x_i - y_i)^2},$$

where i represents the ith of n ecological dimensions, and x_i and y_i represent the values of subspecies groups x and y for those dimensions. For comparison, geographical distance matrices were developed based on the geographical centroid of the area predicted present by all of the best-subsets models. Genetic distance matrices and phylogenetic estimates were based on the sequence data described above and genetic distances from Peterson (1990, 1992a). Comparisons among distance matrices that evaluate the degree of correspondence of correlation structure were developed via Mantel tests implemented in NTSYS-pc (Rohlf, 2000), with significance tests based on observed correlation values relative to the randomized distribution.

Analyses of ecological characters

Matrices of genetic distances derived from the allozyme data were applied to the branching patterns identified in the phylogenetic analyses using a least-squares fitting procedure implemented in the FITCH module of PHYLIP (http://evolution.gs.washington.edu/phylip.html). This procedure fits branch lengths to a user-defined tree structure, while minimizing the deviation from the original input distance matrix. No assumptions are made regarding molecular clocks, so the branch lengths fitted represent an estimate of total evolutionary change in molecular characters. To take into account some areas of uncertainty in estimation of the phylogenetic hypothesis, we applied this approach to alternative trees that had been published previously (Peterson, 1992b) as well; because all results were qualitatively identical among alternative topologies, we present only those based on the shortest tree.

More specific inspections of individual characters used techniques designed to reconstruct character evolution on phylogenetic trees. Here, characters analysed included the means and standard errors of species' distributions for particular input ecological variables (e.g. annual mean precipitation), as well as overall ecological amplitude (i.e. number of ecological

combinations in which the ecological niche model for the species predicts presence). We used CONTRAST (Martins & Hansen, 1997), a generalized linear model for analysis of comparative data, to reconstruct ancestral states and estimate overall trends in evolution of niche traits over phylogeny. Finally, using MacClade, we tested for phylogenetic inertia in each character's trace over phylogeny by comparing observed numbers of transitions (in a binary abstraction of each ecological dimension, 0 = below the median, 1 = above the median) on the best-supported phylogenetic hypothesis, with numbers of transitions mapped onto 100 random equiprobable trees.

RESULTS

Biochemical patterns

Inspection of DNA sequences indicated no insertions, deletions or sequencing artefacts. Examination of all sites found 140 variable and 91 phylogenetically informative bases. Partitioning by coding position found that 32 first-position bases were variable (18 phylogenetically informative), 9 second-position bases were variable (4 phylogenetically informative), and 99 third-position bases were variable (69 phylogenetically informative). No saturation was indicated in overall analyses; however, partitioning by coding position revealed possible saturation of third-position transitions (Fig. 1).

The actual third-position transition : transversion ratio was approximately 7.5 : 1 based on the MEGA analyses (Table 2). To assess the potential of a misleading signal caused by third-position saturation, we used step-matrices with third positions weighted in various manners. Exploratory analyses with transition : transversion weightings of 0 : 1, 1 : 1, 2 : 1, 7 : 1 and 10 : 1 resulted in congruent results for higher-level taxa; trees differed mainly in the topology of terminal taxa of otherwise well-resolved clades. Hence, the equal weighting scheme was used for subsequent analyses, which allowed us to employ more extensive searches throughout.

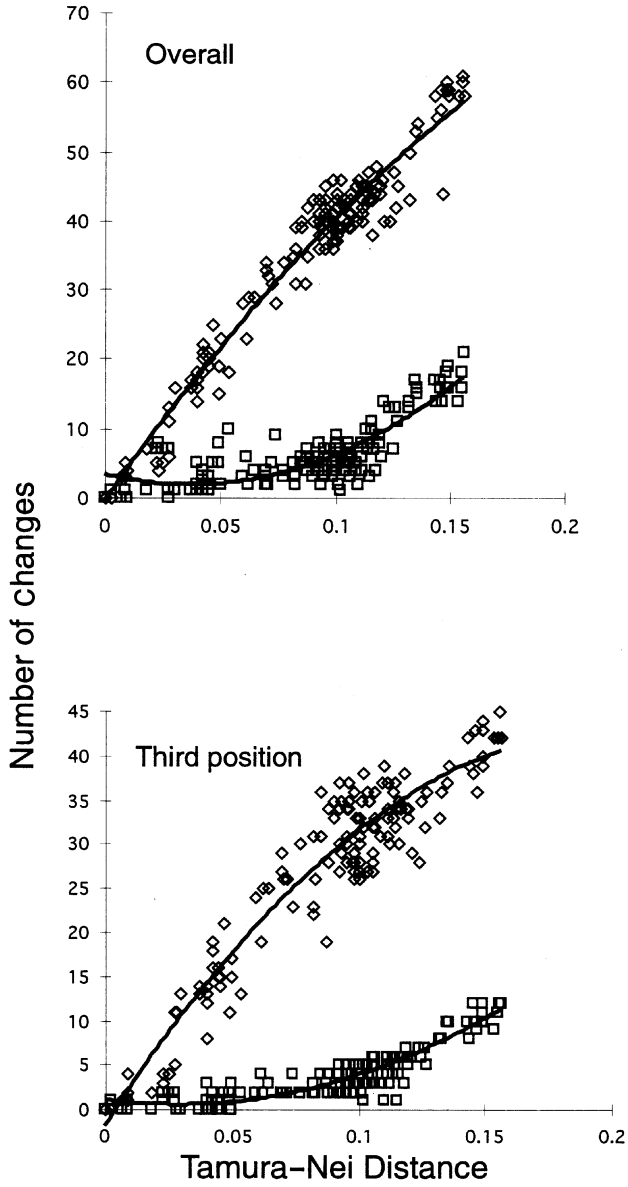

Figure 1. Saturation curves for ND2 sequences. Transitions are depicted with open diamonds, and transversions with open squares.

Table 2. Classes of transitions and transversions for all pairwise comparisons showing the bias against changes to guanine in 5′–3′ sequences from a portion of the ND2 gene

	Transitions			Transversions				
	A–G	T–C	Total	A–T	A–C	T–G	C–G	Total
First	620	930	1050	99	180	22	35	336
Second	150	105	255	18	18	0	34	70
Third	1317	3165	4482	198	329	11	80	618
Overall	2099	3700	5797	315	527	33	149	1024

First, second, and third refer to first, second and third position substitutions, respectively.

PHYLOGENETIC PATTERNS

Preliminary phylogenetic analyses of the data supported monophyly of *Aphelocoma*, with one exception. The sample of *Cyanocitta stelleri* departed consistently from the remaining outgroup species, and entered as the sister taxon to *A. californica*. This relationship was unexpected, and is not supported by any additional characters or evidence; for this reason it was attributed to possible mislabelling of tissue samples on collection; subsequent efforts to sequence *Cyanocitta* for inclusion proved difficult. Otherwise, *Aphelocoma* was monophyletic with regard to all other outgroup taxa, and relationships within the genus were stable regardless of whether close (e.g. *Gymnorhinus*) or distant outgroup taxa (e.g. *Corvus*) were included and of the weighting scheme employed. Hence, to maximize processing speed (and to permit global searches for shortest phylogenetic trees), all further analyses were based on analysis with the outgroup taxon identified in other studies as a close relative (Espinosa de los Monteros & Cracraft, 1997): *Gymnorhinus*.

Parsimony analyses identified a single shortest tree of 244 steps (CI = 0.648, HI = 0.352, RI = 0.753, RC = 0.488; Fig. 2). Ten trees were one step longer, and 49 were two steps longer; the structure of these near-shortest trees was similar to that of the shortest tree, differing mainly in the placement of *A. insularis*, which was variably placed basal to the scrub jay complex. The most parsimonious tree placed *A. unicolor* basally; the weakly supported clade representing the remainder of *Aphelocoma* consisted of three major clades. The first included phylogenetic species from the 'gray-breasted jay' assemblage, with *A. ultramarina* forming the basal lineage and *A. potosina* as the sister of or paraphyletic to *A. wollweberi*. The second major clade included the 'scrub jay' complex. Within this clade, a basal split separated coastal populations (*A. californica*, *A. insularis*) from more eastern populations (*A. coerulescens*, *A. woodhousei*, *A. sumichrasti*). Within the third clade, the Florida populations (*A. coerulescens*) formed the sister lineage of the interior populations. An interesting feature of this hypothesis is that *A. woodhouseii* is paraphyletic with respect to *A. sumichrasti*, and *A. potosina* with respect to *A. wollweberi*; alternative hypotheses of sister-group relationships of two monophyletic species are three and two steps longer, respectively.

Bootstrap analyses indicated generally strong support for nodes, weakest in that uniting all of the taxa

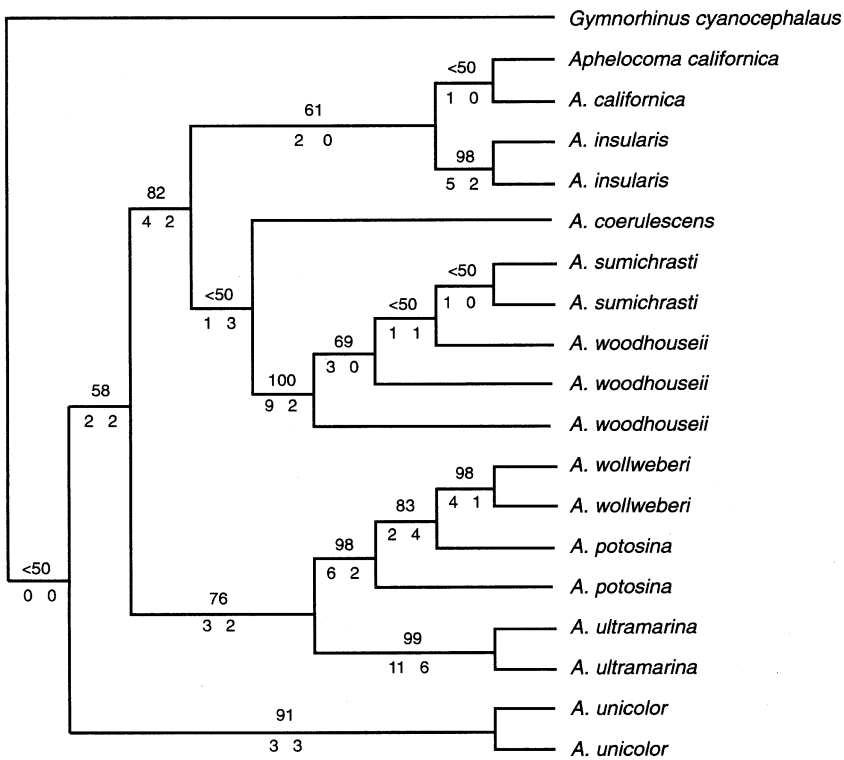

Figure 2. Cladogram showing the most parsimonious tree derived from analyses of the ND2 gene. Numbers above each node relate to bootstrap values, numbers below each node are decay indices (left) and number of unreversed synapomorphies (right) for each clade.

except *A. unicolor* and that uniting *A. californica* and *A. insularis*. Examination of unreversed synapomorphies and decay indices also indicated generally good support, although little clear support existed for either the basal nodes or relationships within *A. sumichrasti* and *A. woodhouseii*. Tree topologies of competing hypotheses (Peterson, 1992a; Brown & Li, 1995) evaluated based on DNA data were substantially longer: our topology was 244 steps long; Peterson & Burt's (1992) topology was 275 steps long; and Brown & Li's (1995) tree required 277 steps. Our tree appears reasonably well supported by available information, is shorter than those of competing hypotheses, and hence warrants more detailed examination.

Ecological niche evolution

Ecological niche models and associated distributional predictions developed for each subspecies group (Fig. 3) were all reasonably accurate depictions of the group's distribution within its general range. The best-subsets models used in this analysis were highly statistically significant, without exception (all $P < 10^{-5}$). As has been observed previously (Anderson *et al.*, 2002a, 2003; Peterson *et al.*, 1999), these models at times overpredicted into other biogeographical regions (e.g. prediction for *A. ultramarina* included the Sierra Madre Occidental), reflecting historical patterns of range limitation of particular groups. The only major area of overprediction at highest confidence adjacent to real distributional areas that appears to constitute true prediction error (i.e. predicting ecological combinations as present when they are in reality not inhabited) would be the eastern fringe of the prediction for the *A. woodhouseii* group, which overextended into the Great Plains of the central United States and north-eastern Mexico.

Viewing these niche models in ecological space provides a view of the distribution of each subspecies group in relation to concrete, tangible ecological parameters. For example, viewing niches in the two-dimensional space defined by annual mean precipitation and annual mean temperature (Fig. 4), ecological differences can easily be appreciated. *A. coerulescens* is distributed in warmer and wetter areas than other scrub-jays, and *A. woodhouseii* is found in the coolest and driest areas. *A. californica* and *A. woodhouseii* have the broadest niches in these dimensions (and overall), whereas other species have more narrow ecological distributions. Similarly, *A. ultramarina* can be seen to inhabit more tropical (warmer and wetter) areas than do *A. potosina* and *A. wollweberi*. In sum, patterns of ecological variation are easily visualized based on the results of the ecological niche modelling analyses.

These differences among *Aphelocoma* species are reflected in the two ecological distance measures that we extracted (Table 3). In the interpredictivity measure, levels of predictivity were in general low in comparison with past studies (Peterson *et al.*, 1999). Curiously, the few high interpredictivity values were not between sister taxa. Centroid distances were similarly extreme, indicating that *Aphelocoma* jay species are well differentiated in ecological space. The two distance measures were significantly similar (Mantel $R^2 = 0.512$, $P << 0.001$), indicating that they measured aspects of the same phenomenon. However, because interpredictivity measures were frequently absolute (i.e. interpredictivity = 0), we used the centroid distances only in all subsequent analyses. Mantel comparisons indicated a close correlation between ecological (centroid) distance and geographical distance (Mantel $R^2 = 0.274$, $P < 0.01$) and no significant association between ecological distance and genetic distance (Mantel $R^2 = 0.033$, $P >> 0.1$) measures.

Reconstructing ancestral character states for the ecological characters on the sequence-based tree indicated – in general – wild variation and abrupt ecological shifts in the history of the *Aphelocoma* jays (Fig. 5). Relatively few clades exhibited clear and unequivocal trends (e.g. the continuously decreasing trend in solar radiation in the scrub-jay clade). Rather, most showed opposite trends (e.g. *A. unicolor* and *A. guerrerensis* trends in wet days) among adjacent branches on the phylogeny. Overall, the most dramatic reversals were in *A. coerulescens*, which almost invariably showed trends opposite to those of the rest of the scrub-jays.

Phylogenetic inertia in ecological characters in *Aphelocoma* jays was negligible. That is, for the four niche dimensions for which both binary character states were present in multiple species (diurnal temperature range, annual mean precipitation, vapour pressure, wet days), all observed numbers of transitions were well within the expected range as compared with transitions on random trees ($P >> 0.05$). Hence, no evidence exists for phylogenetic inertia in ecological characters in *Aphelocoma*. Rather, this clade seems to be quite evolutionarily plastic regarding these ecological parameters.

DISCUSSION

Brown & Li (1995) and *Aphelocoma* phylogeny

Worthy of some comment are the analyses of *Aphelocoma* phylogeny presented by Brown & Li (1995). These discussions were initially intended for a rebuttal, but were reserved for inclusion herein given the availability of new sequence-based information. Brown & Li (1995) presented a rather odd contribution, adding four behavioural characters to an allozyme data set, and reanalysing Peterson's (1992b) work. Their work, however, suffers from three very

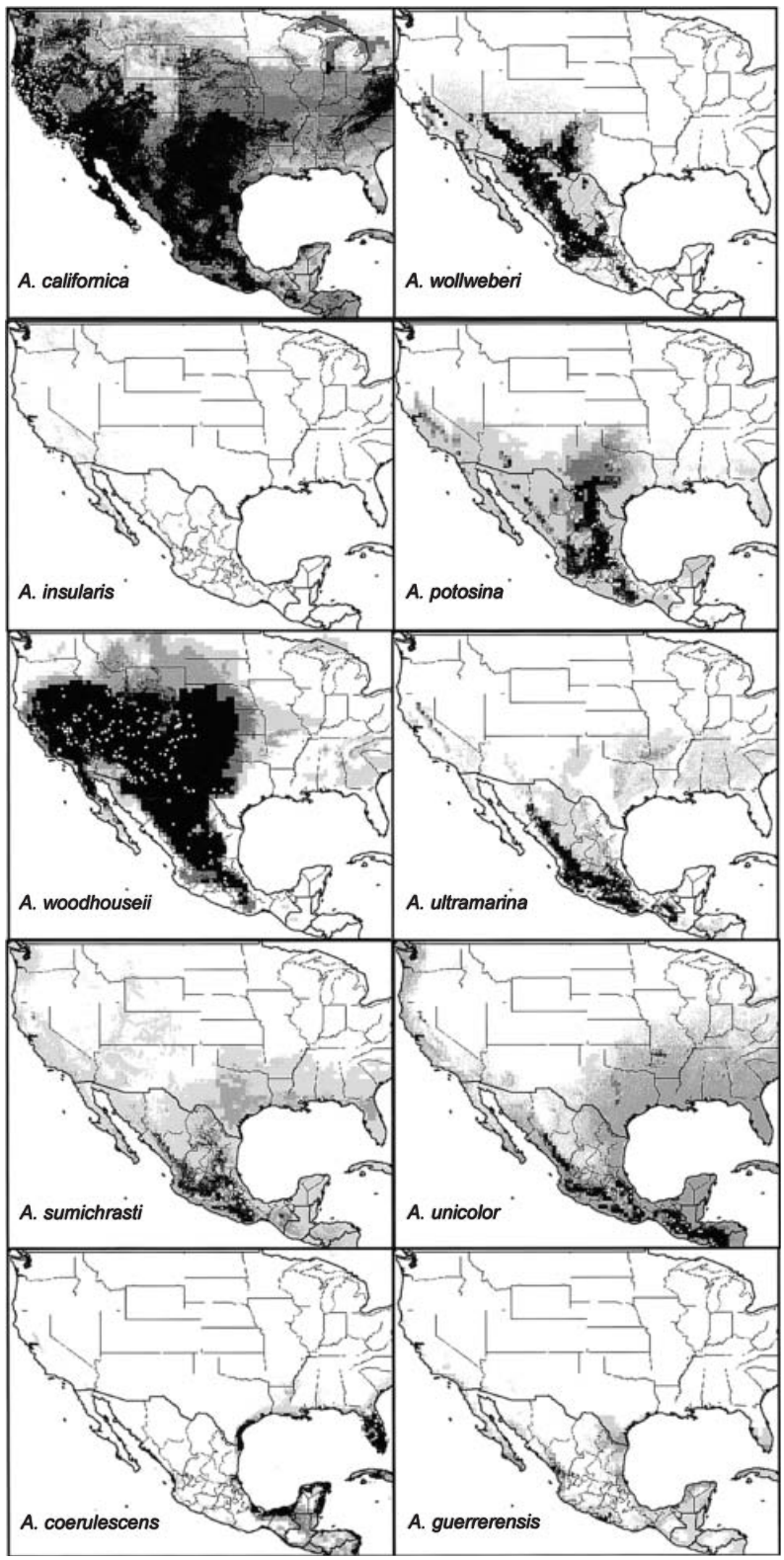

Figure 3. Distributional predictions for each of the subspecies groups of jays in the genus *Aphelocoma*. Increasingly dark shades of grey indicate greater confidence in the prediction of presence, with white indicating absence and black indicating greatest confidence in prediction of presence. White squares indicate occurrence records on which the models were based.

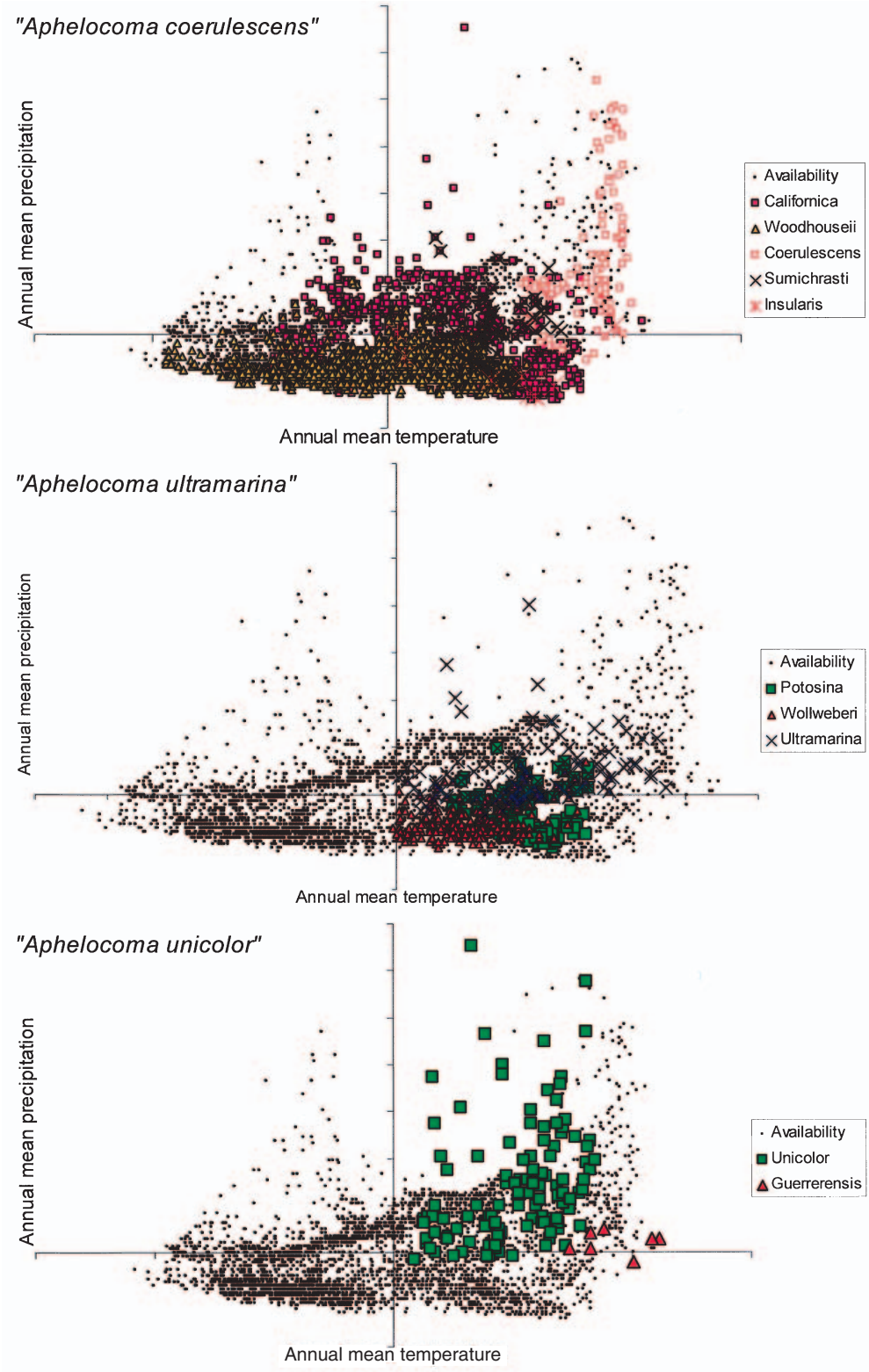

Figure 4. Visualization of ecological niches of *Aphelocoma* jays in two ecological dimensions (annual mean precipitation, annual mean temperature). Small square symbols indicate the distribution of environments available in North America, and other symbols (see legends) indicate the distribution of the jays with respect to that availability. Axes are in standard normal dimensions, and so do not translate directly into values of the original ecological variable.

Table 3. Ecological distance measures developed based on the ecological niche models for *Aphelocoma* jay species: centroid distances (upper right) and mean interpredictivity distances (lower left)

	Accal	Accoe	Acins	Acsum	Acwoo	Augue	Ausor	Auult	Auuni	Auwol
Accal		4.848	3.920	2.144	1.334	4.048	1.362	2.169	3.420	1.273
Accoe	0.076		5.105	4.774	6.019	3.340	4.606	4.582	3.326	5.500
Acins	0.502	0.000		4.111	4.894	3.236	3.883	4.237	4.418	4.585
Acsum	0.202	0.000	0.000		2.896	3.149	1.541	0.432	2.356	1.982
Acwoo	0.510	0.000	0.000	0.156		5.245	2.302	2.937	4.402	1.481
Augue	0.200	0.000	0.000	0.300	0.000		3.418	3.119	2.699	4.371
Ausor	0.475	0.000	0.000	0.247	0.442	0.000		1.690	3.219	1.273
Auult	0.297	0.000	0.000	0.605	0.187	0.200	0.179		2.071	2.043
Auuni	0.072	0.000	0.000	0.279	0.079	0.000	0.029	0.517		3.769
Auwol	0.436	0.000	0.000	0.100	0.579	0.000	0.187	0.063	0.010	

Accal, *A. californica*; Accoe, *A. coerulescens*; Acins, *A. insularis*; Acsum, *A. sumichrasti*; Acwoo, *A. woodhouseii*; Augue, *A. guerrensis*; Ausor, *A. potosina*; Auult, *A. ultramarina*; Auuni, *A. unicolor*; Auwol, *A. wollweberi*.

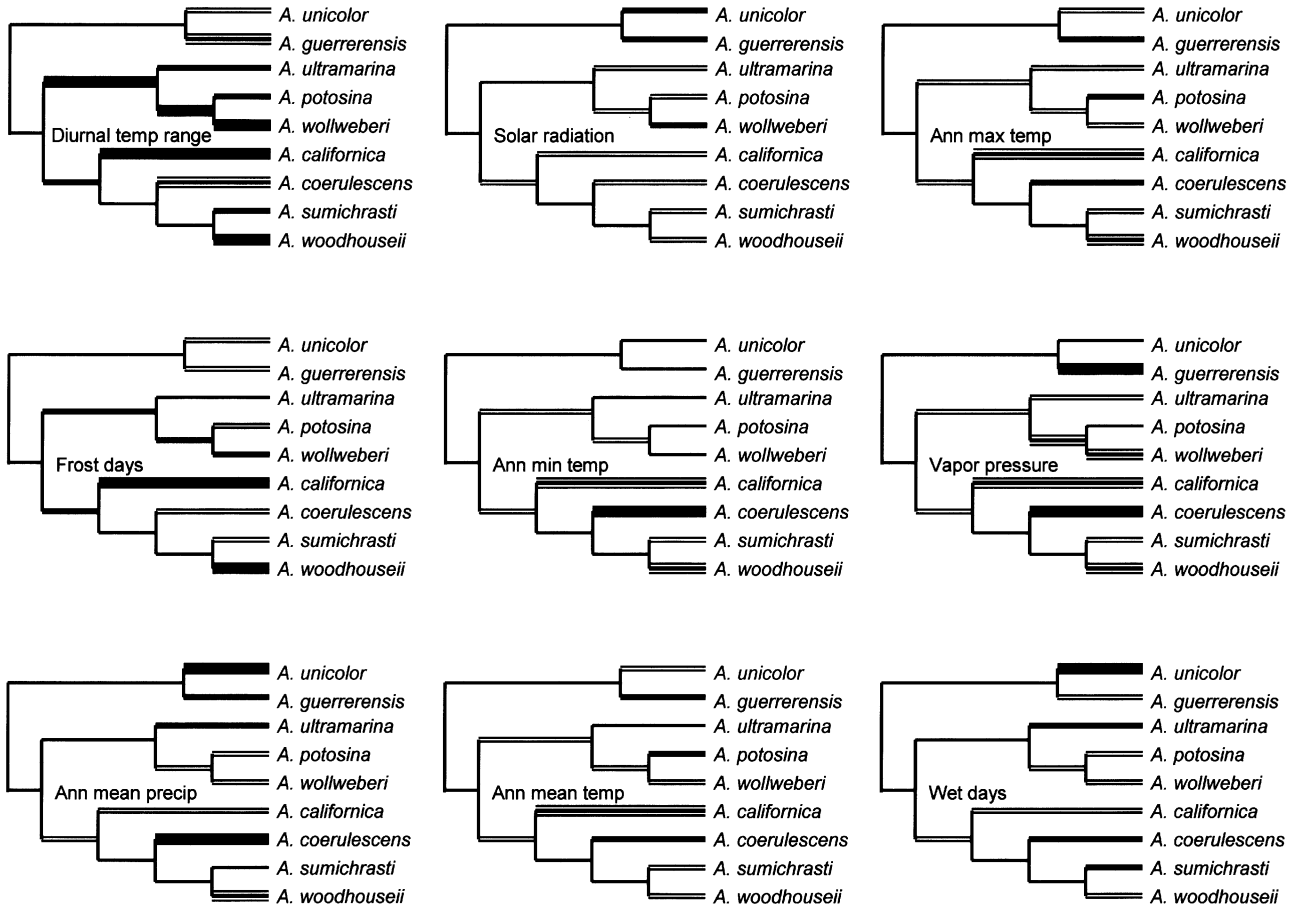

Figure 5. Reconstruction of ancestral character states for ecological niche characters based on the phylogenetic tree derived from mitochondrial DNA sequences. Solid bars indicate increasing tendencies in the character, whereas striped bars indicate decreasing tendencies. Narrow bars indicate subtle changes (10–70%), whereas thick bars indicate dramatic changes (doubling or halving).

distinct failings, which introduce both confusions and biases; these are detailed as follows.

Brown & Li (1995), perhaps unfamiliar with practices customary in systematic circles, introduced names appearing to be valid scientific names to refer to populations of *Aphelocoma* jays. They referred to the two northern Mexican groups (*A. wollweberi* of the Sierra Madre Occidental, *A. potosina* of the Sierra Madre Oriental) of gray-breasted jays (AOU, 1998) as 'Occidentalis' and 'Orientalis', respectively. Although these names are perhaps attractive as labels given their reference to the geographical distributions of the taxa, they have no nomenclatural basis whatsoever (Pitelka, 1951). Use of such terms in publications runs a serious risk of such names becoming established in the scientific literature, which would provoke considerable confusion. Further complicating matters is Brown and Li's insistence that Peterson's (1992b) phylogeny is inconsistent with current taxonomy (Pitelka, 1951): inconsistencies between phylogeny and taxonomy should result in taxonomic revision, rather than criticism of the phylogeny (Wiley, 1981).

Second, Brown & Li (1995) were more than circumspect with regard to the analytical methods employed in their studies. Their methods section stated simply that 'we . . . constructed trees', using PAUP, not specifying whether searches were exhaustive or heuristic, what search algorithms were used, what addition sequences were employed, or any of the numerous other complexities involved in reconstructing phylogenies. They also went on to criticise Peterson's (1992b) discussions of complications arising from character coding and outgroup choice; curiously, however, although these comments were made in their methods section, with respect to these problems, their analyses in no way differed from that of Peterson (1992b)! Hence, Brown & Li's (1995) contribution is at best uncertain, given that their methodology was not documented in any way even approaching the standard for systematic studies.

Most seriously, Brown & Li's (1995) work is compromised by problems of circularity. An important motive for their paper was to address the conclusions of Peterson & Burt (1992) regarding polarity of social system evolution in the genus. Brown & Li's (1995) approach to this issue was to reanalyse Peterson's (1992b) data after adding four characters to the matrix. However, those four characters – presence of a rattle call, delayed soft-part maturation, breeding system and occurrence of helping behaviour – are all characteristics tightly linked to social systems and unlikely to be independent of the character of interest for analysis (Peterson, 1992b). Analyses based on the hypothesis developed herein support the idea of cooperative breeding being primitive in the genus.

What Brown & Li (1995) do not appear to have understood is that inclusion of such characters automatically biases the resulting tree towards their desired conclusions. Although a topic of controversy, and not cited by Brown & Li (1995), inclusion of characters in a phylogenetic analysis aimed at understanding the evolution of the same features clearly tends to group independent derivations into artificial lineages (Kluge, 1989). Brown & Li (1995) introduced such characters into a somewhat unstable hypothesis, and its effect was quite clearly to attract all 'social' lineages into one monophyletic group. Hence, their revised 'conclusions' regarding social evolution in *Aphelocoma* are at best suspect, and most likely are highly biased in favour of the authors' preconceived notions.

Finally, Brown & Li (1995) suggested that phenotypic variation among gray-breasted jay populations resulted from hybridization with scrub jays (*A. woodhouseii*). Our analyses of ND2 sequences nevertheless clearly supported each lineage as monophyletic. In particular, Brown & Li (1995) suggested that features of *A. potosina* resulted from hybridization with *A. woodhouseii*; we have firmly established *A. potosina* as sister taxon of *A. wollweberi*, phylogenetically distant from all scrub jays. Given the distant phylogenetic position of *A. potosina*, and the character variation in the phylogenetic hypothesis, hybridization has clearly not been an important factor in the evolution of the genus.

Implications for ecological niche evolution

Previous theoretical treatments (Holt & Gaines, 1992; Holt, 1996; Holt & Gomulkiewicz, 2003) suggest strongly that ecological niches should be relatively conservative in evolutionary time, and that adaptation in ecological dimensions may take place only under fairly restrictive circumstances. This assertion of conservatism has seen extensive empirical support as well (Huntley, Bartlein & Prentice, 1989; Martínez-Meyer, 2002; E. Martínez-Meyer, A. T. Peterson & W. W. Hargrove, unpubl. data; Peterson *et al.*, 1999; Peterson & Vieglais, 2001). The general picture is one of ecological niches constituting a long-term stable constraint on the distributional potential of species.

Aphelocoma would seem to constitute an excellent counter-example to this conservatism. Sister-species pairs (e.g. *A. unicolor–A. guerrerensis*, *A. potosina–A. wollweberi*, *A. sumichrasti–A. woodhouseii*) do not show any ability to predict each other mutually. Rather, ecological differences among these closely related species pairs appear to prevent any similarity in distributional models. Indeed, no signal of inertia was detectable – the distribution of character states on lineages in the *Aphelocoma* tree was not distinguishable from random patterns.

An even clearer indication of the wild ecological variation in the history of *Aphelocoma* is the lack of significant association between ecological distances and genetic distances. Rather, ecological distances were clearly and significantly related to geographical distances. For this reason, relatively high interpredictivity was observed between species pairs such as *A. potosina* and *A. woodhouseii*, which are not closely related, but are codistributed (Peterson, 1992a). This close relationship of ecological characteristics with geographical context is a clear signal of ecological plasticity.

We considered the possibility that the low (nil) interpredictivity of species pairs such as *A. unicolor* and *A. guerrerensis* might be a result of differentiation in a single ecological dimension, and not in the overall niche. That is, it is possible that differentiation in response to solar radiation (or any of the variables) could be responsible for the lack of interpredictivity. Hence, we developed models for *A. unicolor* and *A. guerrerensis* based on all possible combinations of environmental variables. Surprisingly, the negligible interpredictivity held under all combinations of environmental variables, indicating that the differentiation was broad, and not just focused in a single or a few dimensions.

CONCLUSIONS

The *Aphelocoma* jays present a fascinating scenario of evolutionary change. Previous studies appreciated their wild variation in plumage coloration, size, and shape (Pitelka, 1951), molecular characters (Peterson, 1990, 1992b) and social system (Peterson & Burt, 1992; Burt & Peterson, 1993). A previous ecological survey (Peterson & Vargas-Barajas, 1993) documented great ecological diversity in the species; more detailed analyses of bill morphology suggested morphological adaptations to feeding requirements of food types available in different habitats (Peterson, 1993), which was supported in subsequent analyses (Moyer, Peterson & Clayton, 2002) and experimental tests (Bardwell *et al*., 2001).

Examination of ecological variation in this group based on a robust phylogenetic estimate provides a new appreciation of the rapidity and fluidity of ecological characters in *Aphelocoma*. Unlike many other groups of apparently similar age that have been tested using identical methods (Peterson *et al*., 1999), *Aphelocoma* jays assume the ecological characteristics of the regions where they are distributed, rather than being distributed only where their preferred ecological regimes are represented. This ecological plasticity, apparently genetically based (Peterson, 1993), has opened many fascinating geographical possibilities for the species.

More generally, the combination of phylogenetic information with ecological niche characteristics provides a framework for new insights into the process of ecological niche evolution. The ecological potential of species can be evaluated quantitatively using new techniques drawn from machine-learning applications in quantitative geography, and can be applied to any species or clade for which occurrence data are available (Peterson *et al*., 2002b). Niche evolution in other groups, for which ecological niches have been less plastic over evolutionary time periods, will be understood in much-improved detail using this technique.

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