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Contrasting genetic structures in sister species of North American scrub-jays

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Threatened Florida scrub-jays (*Aphelocoma coerulescens*) breed communally, are restricted to xeric sandy scrub habitat, generally disperse fewer than three territory diameters. Closely related Western scrub-jays (*A. californica*) do not breed communally, have a broader habitat range, disperse greater distances, and are not threatened. We compared the genetic structure of 445 individuals in 11 populations in Florida with 157 individuals in eight populations of Western scrub-jays. At ten microsatellite loci, Florida had 24 out of 47 total alleles, while Western scrub-jays had 44. The Florida populations were more differentiated ($G_{ST} = 0.048$) than were a set of five California populations ($G_{ST} = 0.015$). A randomization extension of a Mantel test showed a stronger correlation between geographic and Cavalli-Sforza genetic distances among Western than Florida populations. Neighbour-joining trees clustered Florida populations from the same sandy ridge systems, suggesting that habitat continuity is more important than geographic proximity in allowing gene flow and preventing differentiation. For Western populations, isolation by distance appears to be the major determinant of genetic structure. Our results suggest that contrasting genetic structures may arise between closely related species, as a result of differences in ecology and social system. Conserving extant genetic variation in Florida jays will require maintaining viable populations in each of the major sandy ridge systems.

Keywords: genetic structure; microsatellite; conservation; genetic versus geographic distance; scrub-jay; metapopulation

1. INTRODUCTION

The Florida scrub-jay (*Aphelocoma coerulescens*) was recently recognized as a species distinct from the Western scrub-jay (*A. californica*), rather than as a subspecies of the scrub-jay (American Ornithologists' Union 1995). A wide disjunction exists between the geographic ranges of the two species, and along with minor differences in plumage and morphology, they differ in social system and ecology. The Florida scrub-jay, listed as threatened under the US Endangered Species Act, is endemic to peninsular Florida on xeric, sandy ridges characterized by species of scrub oaks (*Quercus*) and sand pine (*Pinus clausa*) (Abrahamson *et al*. 1984). The scrub habitat is patchy, with relatively well-defined boundaries and abrupt transitions to much more mesic habitats (Neill 1957; Laessle 1958).

Florida scrub-jays breed communally, with 55% of breeding pairs having one or more non-breeding group members (Woolfenden & Fitzpatrick 1990, 1996). As a correlate of their social biology, Florida scrub-jays are highly sedentary and habitat specific (Woolfenden & Fitzpatrick 1984). Mean dispersal distance of males ($\bar{x} = 456$ m) is only slightly more than one territory diameter ($\bar{x} = 340$ m), while that of females ($\bar{x} = 1180$ m) is somewhat more than three territory diameters (Woolfenden & Fitzpatrick 1986). Western scrub-jays are widely distributed in western North America, south to southern Mexico. The populations closest to Florida occur in west Texas, approximately 1800 km to the west. Western scrub-jays breed cooperatively only at the southern limits of the range in Mexico (Peterson & Burt 1992; Burt & Peterson 1993). In their first year, Western scrub-jays form loose groups and wander widely. Dispersal distances are long; they have been documented to exceed 50 km (Carmen 1988). The habitat of Western scrub-jays appears to be considerably less patchy and less sharply bounded than that of the Florida scrub-jay and as a result, they occur in a wide range of habitat types (Pitelka 1951; Peterson & Vargas 1993). The difference in mobility between the species may be related to the relatively high frequency of acorn crop failure in California (Carmen 1988), which is virtually unknown in Florida (DeGange *et al*. 1989; Woolfenden & Fitzpatrick 1996).

We studied genetic correlates of differences in ecology and social systems between Western and Florida scrub-jays, using microsatellite loci as genetic markers well suited to such analyses (McDonald & Potts 1997). The comparison affords an unusual opportunity to assess variation in genetic structure as a correlate of major variation in ecology and social system, in two very closely related species (e.g. Peterson 1992*a*). We compared Florida scrub-jay populations to Western scrub-jay populations over a comparable geographic span unbroken by major barriers such as mountain ranges. The western

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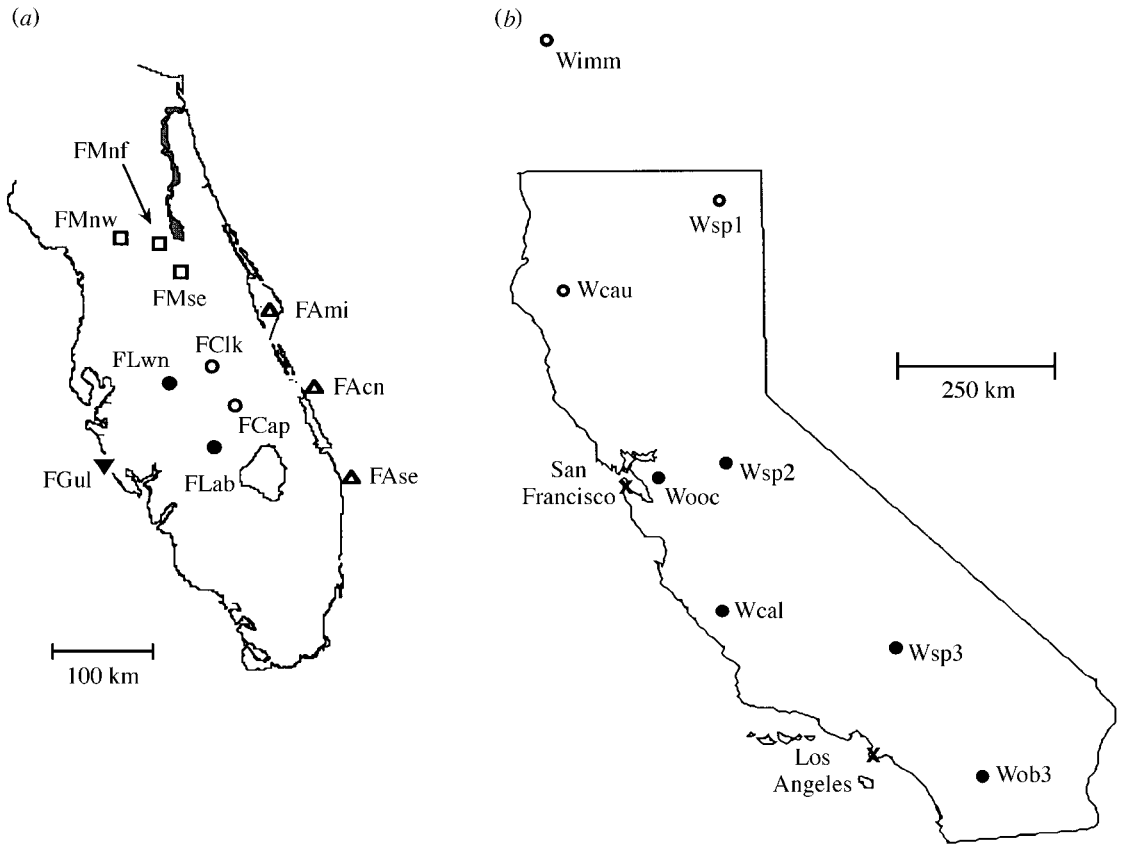

Figure 1. (*a*) Sampling distribution for Florida scrub-jays. Total area spanned 47 000 km^2, with a mean separation between populations of 151 km. The first letter of the four-letter population name denotes the species (F for Florida), and the second letter groups populations from within the same broad ridge system as follows: M for the Mount Dora Ridge in north-central Florida (open squares), L for the Lake Wales Ridge in south-central Florida (filled circles), C for central ridges lying just east of the Lake Wales Ridge (open circles), A for the Atlantic coast (open triangles), and G for the Gulf coast (filled, inverted triangle). Sample sizes were: FAcn 8, FAmi 61, FAse 33, FCap 33, FClk 19, FLab 68, FLwn 8, FMnf 41, FMnw 12, FMse 39 and FGul 45. A random subsample of 68 of the large Archbold Biological Station sample (FLab) was used for most analyses. The three populations with fewer than 15 samples were omitted from certain analyses. (*b*) Sampling distribution for Western scrub-jay. For all eight populations, total area is 234 000 km^2, with mean separations between populations of 539 km. The first letter of the four-letter population names denotes the species (W for Western). The three following letters are based on population names used by Peterson (1992*b*), which refer to subspecies designations in the 1957 AOU checklist (American Ornithologists' Union 1957). For example, our Wcal is from the subspecies *californica*, abbreviated ACCAL by Peterson. Filled circles denote the five 'core' California populations used for certain comparisons to the Florida populations. For these, the total area is 72 305 km^2 and the mean separation between populations is 337 km. Sample sizes were: Wcal 24, Wcau 18, Wimm 16, Wob3 24, Wooc 17, Wsp1 24, Wsp2 14 and Wsp3 20.

region we chose is western California, north to Oregon. Because recent genetic studies (Ball & Avise 1992; Peterson 1990, 1992*b*) have shown that many named subspecies boundaries do not reflect phylogeographic boundaries, we used the allozyme analyses of Peterson (1990, 1992*a*,*b*) and biogeographic considerations as our primary criteria for decisions on the geographic scope of the western region.

Our prediction was that differences in the habitats occupied by Florida and Western scrub-jays have resulted in differences in sedentariness, which in turn have resulted in differences in levels of genetic subdivision. In Florida, habitat acceptable to scrub-jays is patchily distributed (Stith *et al.* 1996), and the jays are extremely sedentary (high philopatry). In western North America, acceptable habitat is relatively continuous (Carmen 1988) and the jays often move greater distances (low philopatry). Florida scrub-jays should therefore show higher levels of genetic subdivision than Western scrub-jays. Our design was a three-level hierarchy: genetic variation within populations in each of the two species; among populations within each species; and between the two species.

2. MATERIALS AND METHODS

(a) *Sample locations, sample sizes, and sample preparation*

We collected samples in Florida by trapping birds in treadle traps, mist-nets or drop traps, individually marking them with US Fish & Wildlife Service bands, and taking 100 µl of blood from the brachial or jugular vein. Each bird was weighed, measured for bill and tarsus length, and released. Most captured birds were later studied for other purposes and no evidence exists of mortality from the blood sampling. Blood was stored in 1 ml lysis buffer until DNA could be extracted in the laboratory. For the Western scrub-jay samples, we extracted DNA from

tissue samples stored at −70 °C at the Field Museum of Natural History that had been collected previously for an allozyme survey (Peterson 1990, 1992*a*).

We analysed 445 samples from 11 Florida populations spanning a minimum convex polygon of 47 000 km^2 (figure 1*a*). Maximum geographic separation between populations was 333 km. From the more than 500 blood samples collected at Archbold Biological Station (FLab) between 1990 and 1994 we randomly selected a subsample of 68 individuals as the population sample. We genotyped an additional 60 Archbold individuals that included family sets as well as a set of breeders versus failed nestlings.

We analysed 157 samples from eight populations of Western scrub-jays (figure 1*b*). Sampling locations for 99 individuals in five 'core' California populations (Wob3, Wsp2, Wsp3, Wcal and Wooc) covered an area of 72 305 km^2, with a maximum separation of 617 km between populations. We used this 'core' California cluster of populations for the majority of comparisons to the Florida populations, supplemented in some analyses by an additional 58 individuals from three populations in northern California (Wspl and Wcau) and Oregon (Wimm). The set of eight western populations encompassed an area of 234 000 km^2, with a maximum separation of 1268 km.

(b) *Development of microsatellite loci*

We isolated ten dinucleotide repeat microsatellite loci. Detailed laboratory procedures are provided in Sambrook *et al.* (1989), Tautz (1989) and Weber & May (1989), summarized briefly as follows: genomic DNA was digested with either *BamH1* and *EcoR1* or *Pst1* and *Sst1*, as was the plasmid vector *Puc19*. Genomic fragments of 200–700 bp were excised from agarose gels and cloned into *Puc19* to create size-selected genomic libraries. The libraries were screened simultaneously with radiolabelled $(CA)_n$ and $(CT)_n$ polynucleotides (Pharmacia). Positive clones were sequenced and polymerase chain reaction (PCR) primers developed for clones containing repeats with greater than seven repeat units. Sequences for two primers (*SJ133* and *LTR6*) that were also used on long-tailed manakins, *Chiroxiphia linearis*, were published in McDonald & Potts (1994).

(c) *Genotyping*

The PCR was performed in 25 µl volumes using the thermostable *Taq*-polymerase under conditions recommended by the supplier (Boehringer-Mannheim), and 100 ng of template DNA. Thirty-five cycles of amplification were performed on a 9600 Perkin Elmer thermocycler for 30 s at 94 °C, 30 s at various annealing temperatures, and 30 s at 72 °C for each cycle. An initial 2 min denaturation at 94 °C and a final extension of 7 min at 72 °C were included. PCR products were mixed with 0.1 volume glycerol dye, resolved on a 7% native acrylamide gel (30 cm × 45 cm), and visualized using ultraviolet illumination and photography of ethidium bromide staining (Potts 1996). Magnesium concentrations, annealing temperatures and primer sequences will be published elsewhere, and are available from the authors upon request.

(d) *Statistical analyses*

For computing genetic distances among all possible population pairs we used PHYLIP's (Felsenstein 1995) GENDIST routine to assess Cavalli-Sforza chord distance (Cavalli-Sforza & Edwards 1967), which makes no biological assumptions and which Takezaki & Nei (1996) showed to perform well with microsatellite data. We tested for Hardy–Weinberg equilibrium, genotypic disequilibrium, and genic and genotypic differentiation among populations using GENEPOP, version 2 (Raymond & Rousset 1995). We also tested population differentiation by the exact test method of Raymond & Rousset (1996). We assessed polymorphism, gene diversity (expected heterozygosity) and Nei's (1978) genetic distances using the program GENESTAT (Lewis & Whitkus 1989). Although Nei's genetic distance is usually applied to interspecific distances, we used it for a coarse-grained intraspecific analysis comparing Florida populations from different ridge systems. We did so because of the considerable divergence among, and limited gene flow between, ridge systems. For the Nei's analysis we therefore pooled samples from within each of four major ridge systems (FL, Lake Wales Ridge; FM, Mount Dora Ridge; FA, Atlantic coast; FG, Gulf coast). We used PHYLIP's NEIGHBOR routine for neighbour-joining tree-building analyses from Nei's and Cavalli-Sforza genetic distances. Because very small population samples tended to cluster due to non-sampling of lower frequency alleles, we omitted samples of less than 15 from the neighbour-joining analyses. To assess tree robustness, we bootstrapped 1000 gene frequency data sets using the PHYLIP's SEQBOOT. We computed consensus trees and branch support using CONSENSE. Allele frequencies for the 19 populations are available on request.

Due to the stepwise mutation process, microsatellites alleles of similar size are presumably phylogenetically related, providing additional information for genetic analyses (Goldstein *et al.* 1995; Shriver *et al.* 1995; Slatkin 1995). We calculated a stepwise measure of differentiation, ϕ_{ST}, using the program WINAMOVA (Michalakis & Excoffier 1996). The underlying mutation process for microsatellites is a subject of current debate (Schlotterer & Tautz 1992; Wright 1994; Garza *et al.* 1995; see references in Amos & Harwood (1998)). Alleles more than a few repeat units larger or smaller than another allele may represent single mutations of large effect rather than a series of stepwise mutations (Sia *et al.* 1997). We therefore ran an additional WINAMOVA analysis in which alleles were coded in rank order of increasing size, rather than by absolute size.

We were particularly interested in the correlation between geographic and genetic distances separating populations. Lack of independence between data points, however, violates the assumptions of regression analysis. We therefore compared the matrix of Cavalli-Sforza genetic distances with the geographic distance matrix using a Mantel test (Manly 1991) with 5000 replicates. An additional randomization procedure compared the strength of the correlations between species. We randomly sampled, with replacement, from the observed pairs of geographic and genetic distances to calculate 5000 matrix correlation coefficients, r, for each species. We then calculated the difference between the 5000 paired r-values from the two species. The proportion of differences that overlapped zero served as a test of significance of interspecific differences. For example, in a hypothetical comparison with 300 of 5000 differences less than zero $p = 0.06$. In summary, a Mantel test assessed whether genetic and geographic distances were correlated within each species; the additional procedure then tested for a difference in strength of correlation between the species. Software for these procedures is available on request.

3. RESULTS

We scored the same ten loci in all 602 samples. Analysis of 24 individuals from four family groups in the

Table 1. *Measures of genetic variation within and among populations and within species for Florida and Western scrub-jays*
(Ap denotes the mean number of alleles per polymorphic locus.)

level of analysis	polymorphism (range)	effective alleles (range)	alleles (range)	Ap (range)	gene diversity (range)
within population					
Florida (11 populations)	48.2% (30–60)	1.22 (1.1–1.4)	1.6 (1.4–2.0)	2.3 (2.0–2.8)	0.141 (0.09–0.19)
'core' western (5 populations)	88% (80–100)	1.94 (1.8–2.1)	3.1 (2.8–3.4)	3.4 (3.2–4.0)	0.339 (0.31–0.37)
within species					
Florida (11 populations)	90%	1.22	2.3	2.4	0.148
'core' Western (5 populations)	100%	2.10	3.7	3.7	0.343

AP denotes mean number of alleles per polymorphic locus.

Archbold population, indicated Mendelian inheritance for all polymorphic loci. The only exception was a female whose genotype at one locus was incompatible with that of three of six of her sampled offspring. For five reasons we tentatively assume that this discrepancy involved a null allele, or possibly a mutation event: (i) promiscuity, and in particular extra-pair maternity, is rare or absent in Florida scrub-jays (Quinn *et al.* 1999); (ii) the excluded mother and offspring showed a single gel band, consistent with a null allele explanation for the exclusion; (iii) probability of complete matching with all six offspring at five other polymorphic loci, coupled with a 50% mismatch at a sixth locus, is low; (iv) all other birds sampled could be excluded as potential parents by genotype, date of birth or date of death (although some possible parents were not genotyped); (v) no behavioural observations suggested anything unusual about the breeding behaviour of the excluded female.

Both within populations and within species, Florida scrub-jays had lower total variation than did the five 'core' Western scrub-jay populations (table 1). The total number of alleles from all 602 samples in the two species (19 populations) was 47. Western scrub-jays had 44 alleles, of which 43 occurred in the five 'core' California populations, compared with 24 alleles in the 11 Florida populations, despite larger sample sizes in Florida. Florida had only three alleles not found in the west, while 26 of the western alleles did not occur in Florida. Two alleles that occurred in all the western populations occurred as rare alleles in just one Florida population. All ten loci surveyed showed at least some overlap in allele sizes between the Florida and western samples. Additional alleles were mostly longer at four loci in the western populations ('non-focal' species *sensu* Amos & Harwood (1998)), shorter at three loci and on both sides at two loci. The final locus had the same sets of alleles in both species.

Both Western scrub-jays and Florida scrub-jays were significantly deficient in heterozygotes (overall p-values 0.0001 and 0.0295, respectively) with significant contributions from loci *SJ4* and *LTMR7*, and loci *SJ6* and *4be5*, respectively. Paired tests of genotypic disequilibrium provided no evidence of non-random associations between any pairs of loci in either species (all pairs $p > 0.05$ using Fisher's method, χ^2 test). Although encompassing a considerably smaller area, the Florida populations ($G_{ST} = 0.048$) showed greater genetic differentiation among populations than did the full eight population western sample ($G_{ST} = 0.034$), or the 'core' California populations ($G_{ST} = 0.015$).

Mean Cavalli-Sforza genetic distance per kilometre of separation between populations was larger in Florida than in the west by a factor of 1.7. Cavalli-Sforza distances ranged from 0.004 to 0.053 for the 11 Florida populations and from 0.033 to 0.053 for the five 'core' Western populations. Interspecific Cavalli-Sforza distances (range 0.2852–0.400) were considerably larger than any intraspecific distance (maximum 0.078). The stepwise measure ϕ_{ST} ranged from 0 to 0.045 among western populations, with a wider range from 0 to 0.172 in Florida. Mean ϕ_{ST} per kilometre of separation was larger in Florida than in the west by a factor of 9.6. Unlike Cavalli-Sforza distances, some intraspecific values of ϕ_{ST} were larger than some interspecific values, although the mean interspecific ϕ_{ST} (0.23) was considerably larger than the mean for either Florida (0.043) or the west (0.015). A test of heteroscadisticity between pairwise populations in the WINAMOVA ϕ_{ST} analysis also indicated greater differentiation in Florida. For the 11 Florida populations, p was less than 0.05 in 23 of 55 possible pair-wise comparisons, a significantly different proportion from one of 28 possible comparisons for the eight western populations ($p < 0.0001$, G-test; Sokal & Rohlf 1981). WINAMOVA analysis using ranked rather than absolute allele sizes did not change the values for ϕ_{ST} within Florida populations, but did slightly change the values among Western scrub-jay populations.

Geographic and Cavalli-Sforza genetic distance matrices were significantly correlated among the eight Western scrub-jay populations (Pearson's $r = 0.38$; Mantel test $p = 0.026$), but were uncorrelated for the 11 Florida populations (Pearson's $r = 0.040$, $p = 0.378$). A randomization procedure used as an extension to the Mantel test indicated significantly weaker correlations for the Florida matrices than for the western matrices ($p = 0.049$). Hence, the degree to which genetic distance represents a simple function of spatial separation is greater in the west.

The topologies of neighbour-joining tree from Cavalli-Sforza distances (figure 2) and ϕ_{ST} were qualitatively similar, with a few branch rearrangements within each species. The Florida groupings on the ϕ_{ST} tree produced a slightly less biogeographically interpretable pattern by, for example, linking the Gulf coast population (FGul) with the further of the Mount Dora ridge populations (FMse), and the central Lake Kissimmee (FClk) population with the Atlantic populations (FA) rather than with other

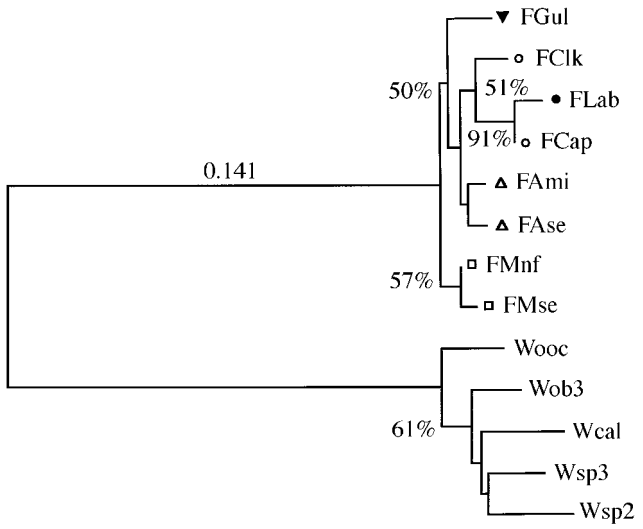

Figure 2. Neighbour-joining tree from Cavalli-Sforza chord distances for eight Florida scrub-jay populations and five Western scrub-jay populations. Symbols preceding Florida population names are those used for major ridge systems in figure 1*a*. Percentage support for nodes that received more than 50% bootstrap support in 1000 replicate trees appears adjacent to the node. The branch length for the midpoint rooted branch separating the species provides a scale.

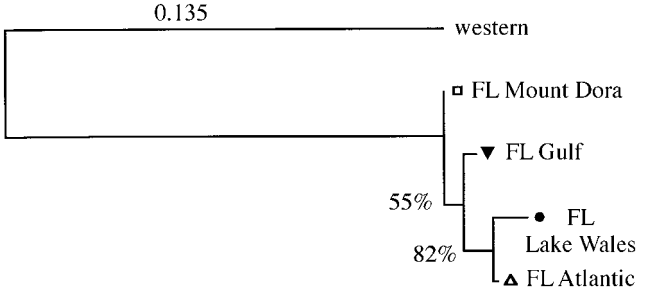

Figure 3. Neighbour-joining tree calculated from Nei's (1978) genetic distance data for four major ridge systems in Florida (Atlantic = FAmi, FAcn, FAse; Gulf = FGul; Mount Dora = FMnf, FMse, FMnw; Lake Wales = FLab, FLwn, FCap, FClk) and the Western scrub-jay samples. The branch length for the mid-point rooted branch separating the species provides a scale. Because the tree from the original Nei's distance data and the consensus of 1000 bootstrapped replicates had the same topology, the tree also shows the support for nodes with >50% bootstrap support.

central ridge populations. Florida branch lengths were generally shorter than those for western populations in the Cavalli-Sforza analysis (figure 2), but were of comparable length in the ϕ_{ST} analysis. The size-rank (as opposed to absolute size) coding scheme made no difference to the topology or branch lengths of the ϕ_{ST} tree. Coding did, however, slightly change the topology of the Western scrub-jay portion of a neighbour-joining tree in an analysis using all 19 populations (not shown).

Nei's (1978) genetic distance analyses provided a coarse-grained comparison of genetic structure among the Western scrub-jay samples, analysed jointly, and four different major ridge systems in Florida. We used the Nei's distances as the basis for a neighbour-joining bootstrapped consensus tree (figure 3). The topology of this tree was identical, at the level of ridges, to that of the Cavalli-Sforza analysis of figure 2. The topology of a neighbour-joining tree for ϕ_{ST} differed in reversing the positions of the Mount Dora and Gulf ridge groups. Regardless of the measure used, genetic differences between species greatly overshadowed differences between populations within species (figures 2 and 3).

4. DISCUSSION

(a) *Phylogeography*

Our results indicate that genetic structure can differ considerably even among two closely related sister taxa (Western and Florida scrub-jays). Western scrub-jays showed more variation within and less differentiation among populations than did Florida scrub-jays, despite a considerably larger geographic span. Geographic and genetic distance were more strongly correlated in the west. These differences suggest that contrasting processes influence the genetic structure of the two species. First, Florida scrub-jays have far fewer alleles, suggesting either intense bottlenecking at the time when the Florida populations were cut off from the rest of the *Aphelocoma* jays, possibly in the early Pleistocene (Neill 1957), or subsequent long-term small and fragmented populations. Second, the now isolated sandy ridge systems in Florida are highly patchy and differ greatly from one another both in age since deposition (resulting from fluctuations in sea level) and in age since any were connected to one another (resulting from fluctuations in aridity across the peninsula). This patchiness means that drift is likely to have played a major role in population differentiation in Florida. In the west, where populations have remained essentially continuously distributed across great distances, isolation by distance, albeit with low total differentiation, may be the dominant mechanism for genetic differentiation. The greater number of alleles also means a relatively greater role for mutation in producing genetic structure within and among populations. Such differences in underlying processes of differentiation underscore the difficulty of using single measures of genetic variation (e.g. stepwise mutation measures assuming predominance of mutation over drift) even at similar spatial scales and among closely related taxa.

A likely explanation for the relative lack of correlation between geographic and genetic distances in Florida is that even a short (<20 km) gap separating patches of scrub habitat may create almost impassable barriers to dispersal for the Florida jays. McDonald & Hamrick (1996) found similar patterns of biogeographic disjunction in the genetic structure of Florida scrub plants. In the west, and particularly in California, no such clear habitat gaps exist, and dispersal distances are greater (Carmen 1988; Peterson & Vargas 1993). As a result, opportunities for extensive genetic divergence of nearby populations are greatly reduced in the west. Despite lower overall differentiation, isolation by distance relationships were significant across the western populations, as indicated by the Mantel-related tests.

The tree-building analyses of figures 2 and 3 have several biogeographic implications for the Florida populations. The Cavalli-Sforza and Nei's analyses agreed in linking the central Lake Wales Ridge (FC and FL) with

the Atlantic coast (FA) and showed the Mount Dora Ridge (FM) as an isolated clade (figures 2 and 3). This concurs with other genetic and biogeographic evidence suggesting (M. Deyrup, personal communication; McDonald & Hamrick 1996) a past connection or stepping stones between the Lake Wales and central ridges and the Atlantic coast. However, the strongest bootstrap support for a clade in the Cavalli-Sforza neighbour-joining tree (figure 2) was for the node uniting the southern Lake Wales Ridge (FLab) and the nearby Avon Park Ridge (FCap), a connection also demonstrated by the movement of one colour-marked jay between these ridges in 1996 (R. Bowman, personal communication). If the ϕ_{ST} result of genetic similarity between the Gulf coast (FGul) and one of the Mount Dora Ridge populations (FMse) represents a true biogeographic connection rather than an artefact, a plausible biogeographic link is the Brooksville Ridge, which lies west of the Mount Dora Ridge and currently contains only a relict jay population (less than ten unsampled individuals). The Brooksville Ridge could have acted as a stepping stone for linking the Mount Dora Ridge to the Gulf coast. No similar stepping stone exists between the Lake Wales Ridge and the Gulf coast, which differed greatly under either the Cavalli-Sforza distance or ϕ_{ST} analyses, and which today are separated by the DeSoto prairie.

(b) *Comparisons with other studies of* Aphelocoma *jays*

Large genetic differences between Florida and western populations corroborate the divergence documented by Peterson (1992*a*) in a broad allozyme survey of the *Aphelocoma* jays. These differences also substantiate the taxonomic splitting of the Western and Florida scrub-jays by the American Ornithologists' Union (1995). Recent discovery of Pliocene fossil scrub-jays in Florida (Emslie 1996) further suggests that divergence times for the two species are ancient, corroborating an earlier suspicion of Neill (1957) based on biogeographic considerations. Our data are consistent with the growing conclusion that isolation of Florida's xeric biota could date at least as far back as the Tertiary.

Considerable detail now exists regarding the interactions between social systems and long-term evolutionary processes in the *Aphelocoma* jays (e.g. Pitelka 1951; Fitzpatrick & Woolfenden 1986; Brown & Horvath 1989; Peterson 1991*a*,*b*; Webber & Brown 1994; Brown *et al.* 1997; de los Monteros & Cracraft 1997; McDonald *et al.* 1996). Peterson (1992*a*) compared isolation-by-distance relationships in Western scrub-jays and gray-breasted jays (*A. ultramarina*), and showed that genetic distance accumulates almost three times faster with geographic distance in the latter, cooperative species than in the former, pair-breeding species. He documented significant isolation by distance relationships among western North American scrub-jays, but only marginal significance among gray-breasted jays, a result that appears to contradict the previous sentence. Although differences in sample size clearly were involved, another factor is the steep slope of the relationship between genetic and geographic distances: in the gray-breasted jays, genetic differentiation accumulated so quickly with geographic distance that its slope was difficult to detect statistically (A. T. Peterson, personal communication). Because the comparison was across a single landscape (northern Mexico), differences in habitat configuration could not have driven the patterns observed. Peterson concluded that social systems with high degrees of philopatry (e.g. gray-breasted jays, Florida scrub-jays) facilitate rapid differentiation over short spatial scales. Hence, our results complement those of Peterson (1992*a*): cooperative-breeding forms accumulate genetic differences over small geographic scales, whereas pair-breeding forms accumulate differentiation over broader geographic scales.

Local structuring of populations in cooperative-breeding forms probably has long-term consequences for evolutionary processes in the jays and other groups showing similar variation in social system (Brown 1987). Peterson (1992*b*) documented faster rates of molecular evolution in at least two cooperative-breeding *Aphelocoma* lineages (including Florida scrub-jays). The locally (Stith *et al.* 1996) and geographically structured nature of these lineages may facilitate rapid 'sweeps' of novel characters through populations and species, such as the bill colour variations documented by Peterson (1993). At broader phylogenetic scales, one can ask whether these microevolutionary inequalities have produced systematic biases in degree of derivation of genomes, fixation of evolutionary novelties and rates of molecular evolution among higher-level clades (e.g. the largely social *Aphelocoma* compared with its non-social sister taxon *Cyanocitta*). These questions raise further questions concerning the factors behind evolutionary 'success' and 'failure', such as whether elevated rates of geographic differentiation translate into more rapid speciation and lineage splitting.

(c) *Microsatellite-specific mutation processes*

Patterns of microsatellite variation may vary when the primers are used in taxa other than those for which they were developed. Forbes *et al.* (1995) found that microsatellite loci were more variable, and had higher mean allele size and higher variance in allele size, in the domestic sheep (*Ovis aries*) for which they were developed than in congeneric Rocky Mountain bighorn sheep (*O. c. canadensis*). Forbes *et al.* (1995) and Ellegren *et al.* (1995) suggested the tendency to select long repeat clones in the screening process will make loci have lower repeat lengths and hence lower polymorphism when used across species. Crawford *et al.* (1998), however, found that ascertainment bias was at most a very minor force in explaining patterns of variability or repeat length in cross-taxon application of 427 primer pairs. In our study, the loci were more polymorphic in the western populations than in the Florida populations from which they were developed. Furthermore, the extra alleles in the more polymorphic western populations were longer in only four out of nine cases.

Nauta & Weissing (1996) suggested that high mutation rates in microsatellites could lead to a Markov chain process that causes allele frequency distributions to converge among populations, regardless of initial conditions. They pointed out that the diversifying pressure of genetic drift can counter the homogenizing effect of mutation pressure, so that a major variable affecting the homogenizing capacity of mutation pressure is population size. Mutation pressure will have the largest effects

in large ($n>1000$) populations, and should be negligible where $n<500$. In the case of the Florida jays, Woolfenden & Fitzpatrick (1984) estimated mean effective population sizes of 298; moreover, mutation pressure alone should lead to similar distributions of relatively small numbers of alleles, but not to either monomorphism or the low but spatially variable diversity that we observed. Likewise, the differences among Florida populations make it unlikely that low genetic variability is due to a metapopulation process of extinction and recolonization (Hedrick & Gilpin 1997), which would tend to create uniformity among populations. Small effective population size in Florida may, however, have contributed to low diversity within populations. Amos & Harwood (1998) presented evidence suggesting that high heterozygosity may increase the mutation rate at microsatellite loci. Because western populations did not show a clear pattern of longer repeats, our results are more consistent with the suggestion of Amos & Harwood that the 'variable get more variable' than with the hypothesis of Schug *et al.* (1997) that shorter repeat lengths *per se* slow the mutation rate.

Two factors could help explain the overall heterozygote deficit in the scrub-jays. The first is the possibility of unamplified 'null' alleles (Callen *et al.* 1993; Paetkau & Strobeck 1994; Pemberton *et al.* 1995) that produce single bands, which are then scored as homozygotes. The effect of such underestimation of heterozygosity would be to overestimate the frequency of common alleles and to underestimate the degree of differentiation between populations or species. High proportions of null alleles may be partly responsible for the surprisingly high degree of monomorphism in the Florida scrub-jay. Null alleles, however, should be more likely in cross-taxon reactions using primers developed for congeners or more distantly related taxa. Our having developed the primers from a Florida scrub-jay library, and having designed the primer sites not to be very close to the repeat itself, make null alleles less likely as a complete explanation. The second possibility, and a likely one for Florida populations, is the Wahlund effect (Weir 1996), which produces excess homozygosity when divergent population samples are pooled, because the alleles required to produce heterozygotes do not co-occur in the same populations.

The neighbour-joining analyses have several implications for the properties of the different measures of genetic differentiation. Although the neighbour-joining trees based on stepwise ϕ_{ST} and Cavalli-Sforza distances (figure 2) grouped together most populations from major ridge systems within Florida, the clearer biogeographic groupings produced by the Cavalli-Sforza distances compared to ϕ_{ST} may reflect the desirable properties of the former compared to those of various microsatellite-specific measures including ϕ_{ST} (Takezaki & Nei 1996). In the ϕ_{ST} analysis, Florida populations with small sample sizes (and hence missing alleles) tended to group together regardless of biogeographic affinity. Additionally, the superiority of the Cavalli-Sforza distance may result from the increased importance of drift in shaping variation in Florida. The inability of ϕ_{ST} to account for the effects of drift may be reflected in the several cases where intraspecific ϕ_{ST} were larger than interspecific ϕ_{ST}.

(d) *Conservation implications*

The Florida scrub-jay is a rapidly declining species, now considered endangered (Woolfenden & Fitzpatrick 1996). Our study adds another complication in efforts to protect this flagship species of the Florida scrub, by showing that preservation of a few sustainable populations would be insufficient to protect the full spectrum of genetic variation in the species. Moreover, recent studies showed demographic differences between coastal and interior populations (Breininger *et al.* 1996), vocal differences among several ridge systems (Woolfenden & Fitzpatrick 1996), and structural and plumage differences between at least two populations (G. E. Woolfenden and J. W. Fitzpatrick, unpublished data). Whether or not these differences are genetic, the results of the present study suggest that different ridges differ genetically as well as phenotypically. We conclude that any translocations of individuals proposed as restoration measures should be within, and not between, ridge systems. Translocations among ridges would risk mixing diverse genomes, perhaps even with fitness consequences. We further suggest that preserving the full range of phenotypic and genetic variation of Florida scrub-jays will require protection of sustainable populations within each of the remaining metapopulations (Stith *et al.* 1996), and that these metapopulations be treated as separate units in recovery plans for the species.

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