



## Retention of neutral genetic diversity and connectivity within the metapopulation of Florida Scrub-Jay at Kennedy Space Center

### Retención de la diversidad genética neutral y la conectividad dentro de la metapoblación de la Chara de la Florida (*Aphelocoma coerulescens*) en el Centro Espacial Kennedy

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**ABSTRACT.** Habitat specialists, impacted by anthropogenic land modification, are often of great conservation concern. Florida Scrub-Jays (*Aphelocoma coerulescens*) are threatened, scrub-endemic birds primarily found in small, isolated patches where they have a high probability of extirpation. One of the last large tracts of scrub habitat is found at Kennedy Space Center on the Atlantic coast of Florida, where almost 300 breeding pairs of scrub-jays occur. The majority of breeders are found among four subpopulations separated by suboptimal habitat matrix that could become barriers to movement. Neutral genetic diversity and connectivity among patches were investigated using 15 microsatellite loci. Globally and within study sites, heterozygosity ( $H_e$  0.69–0.74) was comparable to values from similar studies using the same microsatellite loci in Florida Scrub-Jays. In addition, birds with territories in lower quality habitats had genetic diversity comparable to those in higher quality habitats. Three genetic clusters were identified; however, one of the clusters included individuals that were closely related but had dispersed to neighboring territories. Genetic relatedness was correlated with geographic distance, but not with habitat resistance, or with the size of the gap between suitable habitats. These results suggest that geographic distance, rather than the presence of suboptimal habitats or the size of habitat gaps, could limit dispersal among subpopulations at Kennedy Space Center. Territories found in higher quality habitats contained individuals with a higher probability of being first-generation migrants, possibly showing a preference for movement into high-quality areas. Continuing management for high quality scrub habitats is needed to maintain current levels of genetic diversity and connectivity among subpopulations at Kennedy Space Center.

**RESUMEN.** Las especies especialistas en hábitats específicos, afectadas por la modificación del suelo causada por las actividades humanas, suelen ser motivo de gran preocupación para la conservación. La Chara de la Florida (*Aphelocoma coerulescens*) es un ave amenazada, endémica de los matorrales, que se encuentra principalmente en pequeños fragmentos aislados donde tiene una alta probabilidad de desaparecer. Una de las extensiones más grandes de matorrales se encuentra en el Centro Espacial Kennedy en la costa Atlántica de Florida, donde habitan casi 300 parejas reproductoras de charas. La mayoría de las parejas reproductoras se distribuyen entre cuatro subpoblaciones separadas por áreas de hábitat menos adecuado, lo que podría convertirse en barreras para su movimiento. Se investigó la diversidad genética neutral y la conectividad entre estos parches utilizando 15 loci microsatélites. A nivel general y dentro de los sitios de estudio, la heterocigosis ( $H_e$  0,69-0,74) fue comparable a los valores de estudios similares que utilizaron los mismos loci microsatélites en la Chara de la Florida. Además, las aves con territorios en hábitats de menor calidad tenían una diversidad genética comparable a las de hábitats de mayor calidad. Se identificaron tres grupos genéticos; sin embargo, uno de los grupos incluía individuos estrechamente relacionados que se habían dispersado a territorios vecinos. La relación genética se correlacionó con la distancia geográfica, pero no con la resistencia del hábitat ni con el tamaño de la separación entre hábitats adecuados. Estos resultados sugieren que la distancia geográfica, más que la presencia de hábitats subóptimos o el tamaño de las brechas entre hábitats, podría limitar la dispersión entre subpoblaciones en el Centro Espacial Kennedy. Los territorios ubicados en hábitats de mayor calidad contenían individuos con mayor probabilidad de ser migrantes de primera generación, lo que posiblemente muestra una preferencia por el movimiento hacia áreas de alta calidad. Es necesario continuar con el manejo de los matorrales de alta calidad para mantener los niveles actuales de diversidad genética y conectividad entre las subpoblaciones del Centro Espacial Kennedy.

**Key Words:** *Aphelocoma coerulescens*; fragmentation; gene flow; habitat management; microsatellite

#### INTRODUCTION

Current extinction rates have been estimated at ~1000 times higher than natural background levels, based on the fossil record (de Vos et al. 2015). Humankind's negative interactions with the environment are closely tied to climate change (Benito-Garzón et al. 2013) and habitat alteration (Otto 2018). Anthropogenic actions have led to the reduction and isolation of many species throughout their ranges, with an estimated net loss of 3 billion

birds since the 1970s (Rosenberg et al. 2019). These influences are compounded for species that are endemic, habitat specialists such as Florida Scrub-Jays (*Aphelocoma coerulescens*) (Woolfenden and Fitzpatrick 1985, Clavel et al. 2011). In addition, habitat fragmentation and small population size are linked with lower genetic connectivity among habitat fragments, less genetic diversity, and an increased extinction risk (Evans and Sheldon 2008, Coulon et al. 2010, 2012, Frankham et al. 2010).

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Florida Scrub-Jays are found in scrub habitats in Florida and are a species of conservation concern that were listed as threatened in 1987 under the Endangered Species Act (U.S. Fish and Wildlife 1987) and continue to remain threatened decades later (U.S. Fish and Wildlife 2019). The IUCN Red List (BirdLife International 2020) designates Florida Scrub-Jays' conservation status to be vulnerable and decreasing. Declining population sizes are linked to anthropogenic exploitation of scrub habitat (Woolfenden and Fitzpatrick 1985). Scrub is now restricted to scattered pockets throughout Florida on ridge systems (McCoy and Mushinsky 1999) and must be maintained by periodic fires to limit encroachment of native and invasive plant species (Myers and Ewel 1990). Areas that are overgrown typically have low recruitment of scrub-jays and could constitute population sinks (Breininger et al. 2009).

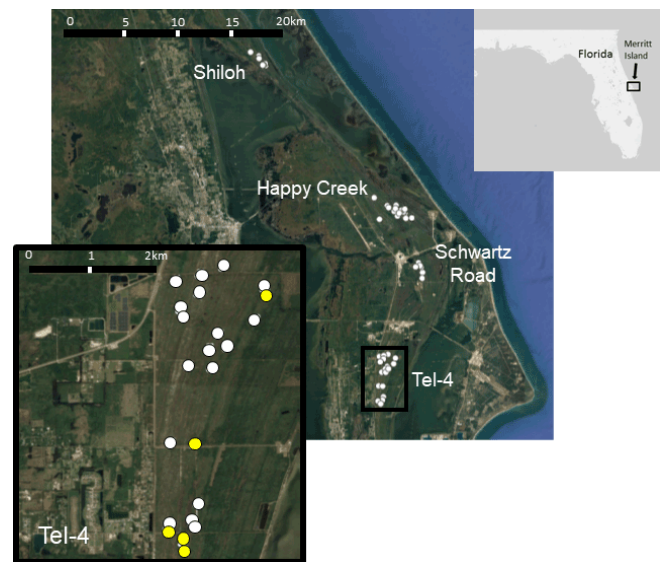
Florida Scrub-Jays are cooperative breeding birds. This form of breeding typically involves a breeding pair and several helpers that are generally offspring from previous years, although unrelated individuals can join family groups (Woolfenden and Fitzpatrick 1985). Helpers aid in rearing young, watching for predators, and territory defense (Woolfenden and Fitzpatrick 1985, Mumme 1992, Breininger et al. 2006, Mumme et al. 2015, Fitzpatrick and Bowman 2016), and are associated with higher fecundity within territories (Breininger et al. 2023). Territory size is greatly influenced by quality of habitat, which has a direct effect on reproductive success and family size (Woolfenden and Fitzpatrick 1985, Fitzpatrick et al. 1991, Breininger and Oddy 2004). Higher quality scrub increases the chances breeding pairs will successfully produce offspring and acquire larger territories (Woolfenden and Fitzpatrick 1985, Breininger et al. 2009).

Florida Scrub-Jays generally disperse short distances after leaving their natal territories (~2.8 km for females and 1.6 km for males in fragmented habitats; Fitzpatrick et al. 1999, Breininger et al. 2006), and thus connectivity among habitat fragments is limited (Coulon et al. 2012). Dispersal among suitable habitats is key to minimizing the risk of inbreeding and inbreeding depression in scrub-jays (Chen et al. 2016). Males may spend their entire lives in one territory, if they are successful enough to "bud" off from their parents and establish a border territory. However, females may traverse several territories to become a helper or (eventual) breeder once a vacancy opens (Woolfenden and Fitzpatrick 1985, Suh et al. 2020). Secondary habitats, such as southeastern pine savannas, have been used as corridors to enable jays to reach fragments of scrub, and thus jays can move through suboptimal habitats (Woolfenden and Fitzpatrick 1985, Breininger et al. 1991). Here we use the definitions of suboptimal habitat outlined in Breininger (1999) and Duncan et al. (1999) as those with tall, overgrown shrubs and few openings, and optimal habitats as those with intermediate height shrubs with sandy openings (also see Breininger et al. 2023, Carter et al. 2023).

During the mid-1990s, efforts were made to identify remaining groups of scrub-jays, and assess risks of extinction throughout Florida. Stith et al. (1996) surveyed populations of Florida Scrub-Jays across the state and identified three distinct metapopulations that had more than 100 breeding pairs. One of these metapopulations encompassed federal lands of Kennedy Space Center (KSC) on Merritt Island. The total population at KSC was approximately 275 families in 2009 and distributed north to

south in four subpopulations (Breininger et al. 2009), Shiloh, Happy Creek, Schwartz Road, and Tel-4 (Fig. 1) with respective subpopulation sizes of 34, 102, 45, and 94 families. The subpopulations are separated by suboptimal habitat including creeks, marshes, forests, abandoned citrus groves, and industrial areas (Fig. 1). Breininger et al. (1996a) identified a number of corridors that may possibly connect the four study sites using suboptimal habitat.

**Fig. 1.** Location of study sites for population genetic analyses of Florida Scrub-Jay (*Aphelocoma coerulescens*) at Kennedy Space Center, Brevard County, FL. Birds in Tel-4 site inset map that are indicated with yellow circles are in a separate genetic cluster (Fig. 2) than other birds and are closely related to one another.



Management for scrub-jays at KSC has focused on restoring and maintaining scrub habitat. To meet management goals, a compound strategy has been implemented. These goals include adequate prescribed fire programs to increase optimal scrub habitat (described below), prevent loss of scrub habitat on protected lands, increase connectivity among sites, and perform translocations of scrub-jays in isolated areas to increase genetic diversity (U.S. Fish and Wildlife 2019). In the absence of fire, scrub can become overgrown, which is unfavorable for scrub-jays and has been linked to poor recruitment (Breininger and Carter 2003). In addition, overgrown scrub may become fire-resistant and therefore mechanical treatments may be necessary to return oak stands to manageable sizes (Schmalzer and Adrian 2001). To aid in management goals, scrub-jays are actively monitored using colored bands to identify breeders and offspring (Breininger et al. 2023). However, genetic data are also useful in understanding cryptic population dynamics.

Previous studies of the genetic diversity of Florida Scrub-Jays that included individuals from KSC have focused on comparisons with closely related species (McDonald et al. 1999), or within species comparisons at broad spatial scales (Coulon et al. 2008). Coulon et al. (2008) performed a state-wide study of Florida Scrub-Jay population genetics and concluded that 10–12 genetic

groups existed within Florida. Genetic data supported observational data that scrub-jays are likely to use corridors of suboptimal habitat (Coulon et al. 2008). One study of Florida Scrub-Jay population genetics was conducted at KSC and surrounding mainland areas between 2004 and 2007 (Khodadad 2008). Khodadad (2008) found lower heterozygosity in subpopulations with poor habitat quality based on microsatellite data. However, the study did not assess connectivity among habitat fragments or overall population structure. The studies by Coulon et al. (2008) and Khodadad (2008) are useful for understanding population genetics at a coarse grain scale. The study sites at KSC make up a network of scrub habitat (Breininger et al. 1996a); therefore, to understand how and if these sites function as a network, a genetic study is needed at a finer spatial scale. Aguillon et al. (2017) found fine-scale (within 10 km) genetic structure for a population of Florida Scrub-Jays in central Florida suggesting that this pattern could also occur at KSC.

To provide an understanding of the effects of habitat fragmentation and management of habitats on Florida Scrub-Jay genetic diversity, population structure, and connectivity at KSC, the following questions were examined: (1) What is the genetic diversity of each subpopulation and do sites with smaller numbers of families (e.g., Schwartz Road) have lower diversity? (2) Is suboptimal habitat among study sites sufficient to maintain connectivity among locations as suggested by Coulon et al. (2008) and Breininger et al. (1996a)? (3) Do birds living in territories with lower habitat quality have lower genetic diversity as suggested by Khodadad (2008)? (4) Are birds more likely to move into habitats that are of higher or lower quality? These questions were addressed using 15 microsatellite loci.

## MATERIALS AND METHODS

Feathers were collected from 71 uniquely banded Florida Scrub-Jays at KSC, Brevard County, FL between 2013 and 2015 at four study sites (Fig. 1, Table 1). Sites represent long-term study areas for Florida Scrub-Jays at KSC. Birds were captured using baited Potter traps and drop traps (Breininger et al. 2009). Body contour feathers (or in one instance a dropped flight feather) were removed and stored in paper envelopes until DNA could be extracted. The use of feathers for the purpose of DNA analyses is only moderately invasive when compared to venipuncture, especially considering the handling of threatened or endangered species (Bush et al. 2005). The calamus was clipped from at least three feathers for each individual and then prepared following QIAGEN's tissue extraction protocol (Qiagen Inc., Valencia, California, USA), along with the addition of 30  $\mu$ L 1M dithiothreitol (DTT) to aid in keratin digestion during the initial incubation step (De Volo et al. 2008). After extractions were completed, polymerase chain reaction (PCR) was used to amplify 19 microsatellite loci (Stenzler and Fitzpatrick 2002). PCR amplifications were performed using a Bio-Rad MyCycler Thermocycler. Thermocycler settings followed the method in Coulon et al. (2008). Amplifications were genotyped at the University of Florida genomics facility (ICBR) on an ABI 3130XL sequencer (Applied Biosystems, Foster City, California, USA). Ten percent of extracts were randomly reamplified to insure data reproducibility. Alleles were scored manually using Genemapper (ABI, Foster City, California, USA).

**Table 1.** Study sites, number of individuals sampled (N), average observed heterozygosity ( $H_o$ ), average expected heterozygosity ( $H_e$ ), and allelic richness ( $A_r$ ) of Florida Scrub-Jay (*Aphelocoma coerulescens*) at Kennedy Space Center. Estimated percentage of sampled individuals found with territories in high-quality scrub habitat (% HQ) are also given. Standard deviations are provided in parentheses.

Site	N	$H_o$	$H_e$	$A_r$	% HQ
Shiloh	9	0.70 (0.18)	0.71 (0.17)	5.09 (1.19)	100
Happy Creek	20	0.74 (0.15)	0.72 (0.10)	5.18 (1.74)	90
Schwartz Road	11	0.68 (0.19)	0.69 (0.21)	5.27 (1.40)	100
Tel-4	24	0.69 (0.16)	0.74 (0.12)	5.32 (1.31)	17

To minimize errors in PCR and sequencing, MICRO-CHECKER was used to detect null alleles, stutter bands, and large-allele drop out (Van Oosterhout et al. 2004, Barson et al. 2009). ARLEQUIN 3.5 (Excoffier and Lischer 2010) was used to test for Hardy-Weinberg equilibrium and linkage disequilibrium. Benjamini and Hochberg (1995) adjustments were made for multiple tests to reduce the false discovery rate. Any loci that were out of Hardy-Weinberg equilibrium across subpopulations, showed linkage disequilibrium across subpopulations, or showed evidence of null alleles were removed from subsequent analyses. Maintaining loci that are out of Hardy-Weinberg and have null alleles would violate the assumptions of genetic tests (Carlsson 2008).

The program POWSIM 4.1 (Ryman and Palm 2006) was used to perform a power analysis to determine if per subpopulation sample sizes and number of loci were sufficient to detect genetic structure among subpopulations. POWSIM simulates populations using a provided number of alleles and allele frequencies so that a given  $F_{ST}$  value occurs among populations. The power to detect the given  $F_{ST}$  value was determined by calculating the proportion of 1000 simulations that showed a significant difference among populations (Ryman and Palm 2006). Arlequin was also used to compute observed heterozygosity ( $H_o$ ) and pairwise differentiation ( $F_{ST}$ ) among sites. Permutations (10,100) were performed to determine if  $F_{ST}$  values were significantly different from zero. Benjamini and Hochberg (1995) adjustments were performed to correct for false discovery rates. Allelic richness and average expected heterozygosity (gene diversity) were calculated using the software FSTAT (Goudet 1995).

Eight loci in this study were also used in an earlier study of Florida Scrub-Jays at KSC (Khodadad 2008). Average observed heterozygosity values were compared for these loci between Khodadad (2008) and the present study for the Happy Creek and Tel-4 locations. Schwartz Road and Shiloh were not examined in the earlier study. Wilcoxon rank-sum tests were used for comparisons using R (R Core Team 2022).

The program COANCESTRY (Wang 2011) was used to identify relatedness ( $r$ ) of individuals within the same territory. This was accomplished by calculating the maximum likelihood of relatedness between pairs of individuals. COANCESTRY calculates the likelihood of relatedness by identifying shared alleles through descent. Wang (2011) offers seven different estimators. Three estimators were chosen to evaluate relatedness

of scrub jays at KSC: (1) *TrioML* was chosen because it accounts for inbreeding and uses triads of individuals to calculate relatedness, (2) *Wang and LynchLi* was chosen because it provides an unbiased estimation of populations with small sample sizes, and (3) “Account for inbreeding” was also selected in the parameters (Wang 2011). If two individuals had an  $r$  value of 0.5 or greater they could have a parent-offspring or sibling-sibling relationship (Blouin 2003). If individuals were closely related and within the same territory, one of the individuals was removed from analysis to reduce pseudo-replication.

STRUCTURE 2.3.4 (Pritchard et al. 2000, Hubisz et al. 2009) was used to assign individuals to “genetic clusters” that are in Hardy-Weinberg and linkage equilibrium. A priori geographical information was used in the analysis (Pritchard et al. 2000). STRUCTURE uses a Markov Chain Monte Carlo (MCMC) approach to assign individuals to genetic groups. The admixture model was used, as scrub habitat was historically contiguous at KSC (Breininger et al. 1996a). Ten independent STRUCTURE runs for each possible number of genetic clusters ( $K = 1 - 6$ ) with 10,000 initial burn-in and 20,000 subsequent iterations were performed. Longer STRUCTURE runs (50,000 iterations) and runs without prior geographical information were also evaluated, but this had no effect on the number of clusters identified. STRUCTURE outputs were examined using StructureHarvester (Earl and von Holdt 2012) to obtain graphical representations of the likelihood for each  $K$  value. These outputs were loaded into CLUMPP to assemble replicates into a single output using the Greedy method (Jakobsson and Rosenberg 2007). The Evanno et al. (2005) method implemented in StructureHarvester was used to identify the number of genetic clusters within the data.

To test for recent movement among study sites, GeneClass2 was used to identify first generation migrants. GeneClass2 uses an MCMC method to identify genotype likelihoods for populations and then determines the likelihood an individual would belong to each subpopulation (Piry et al. 2004). The program simulated populations of 10,000 individuals to create probability thresholds for each study site. To determine the probability that an individual was a recent migrant (Paetkau et al. 2004, Piry et al. 2004), scrub-jays were ranked using the test statistic  $L\text{-home}/L\text{-max}$  (Paetkau et al. 2004). This test statistic is the ratio of the  $-\log$  likelihood of being from the location where the bird was sampled (home) over the maximum  $-\log$  likelihood of being from another location (including the home location). Thus, the higher the  $L\text{-home}/L\text{-max}$  value the more likely the bird is a recent immigrant (Piry et al. 2004).

GeneClass2 was also used to determine how well individuals assigned to their respective location by using the methods of Rannala and Mountain (1997) and Paetkau et al. (2004). If the probability of assignment was below 95% ( $P$  or  $\alpha \leq 0.05$  based on 10,000 simulated individuals), that individual was considered to have come from another location. Individuals not assigned to their subpopulation of origin were loosely assigned to another location if the probability of assignment was greater than 0.25. If an individual did not meet the minimum probability for any of the tested locations, it was assumed to come from an unsampled location because there is scrub habitat and scrub-jays outside the study areas (Breininger and Carter 2003).

Observed heterozygosity was calculated for each individual bird to compare genetic diversity of birds found in different habitats. Habitat quality within scrub-jay territories was previously assessed based on soils and scrub oak cover (IHA Environmental Services Branch 2014). High-quality habitats had well drained soils on oak scrub ridges; conversely lower quality habitats had soils that were poor or moderately drained and limited scrub oak cover. A Wilcoxon rank-sum test was used to compare observed heterozygosity between high- and low-quality habitats. In addition, Spearman correlation coefficients were used to test if observed heterozygosity and log transformed size of territory (ha) were associated.  $L\text{-home}/L\text{-max}$  values from GeneClass2 (see above) were also compared among habitats using a Wilcoxon rank-sum test. Statistical analyses were performed in R (R Core Team 2022).

Geographic distance can have a significant impact on genetic diversity and gene flow (Zimmerman et al. 2015). Here we define geographic distance as a straight line between each subpopulation center or between each individual. Along with geographic distance, gap size is a term used by Coulon et al. (2012) to refer to “areas of unsuitable habitat” or the “distance between patches of suitable habitat.” Isolation-by-distance (IBD) at the individual level was determined by calculating the pairwise proportion of shared alleles (DPS; Bowcock et al. 1994) using the adegenet package (Jombart 2008) in R (R Core Team 2022) and comparing these values with log transformed geographic distance between each pair of birds. QGIS 3.16 (QGIS Development Team 2020) was used to determine distances between birds. IBD was assessed at two-levels: across all individuals at KSC and within each subpopulation. The method of Somers and Jackson (2022) was used to perform Mantel tests by comparing simple differences instead of Euclidean or squared Euclidean distance between matrices. This method resolved problems with Mantel tests (Somers and Jackson 2022), including poor agreement between correlation coefficients for Mantel and Pearson correlations and inflated Type I and Type II error rates (Legendre et al. 2015). Calculations of simple differences were determined using the ecodist package (Goslee and Urban 2007) within R (R Core Team 2022).

The difference in habitat quality among subpopulations could affect individual scrub-jay movements (Graves et al. 2014). Through the use of Geographic Information Systems (GIS), habitats can be ranked based on an organism’s ability to traverse the environment. Spear et al. (2010:3577) defines habitat resistance as “... spatial layers that assign a value to each landscape or environmental feature that represents the degree to which that feature impedes or facilitates connectivity for an organism of interest.” The pairwise proportions of shared alleles between individuals were compared with an estimate of habitat resistance between individuals using the same Mantel test method as used for assessing IBD (Somers and Jackson 2022). Monitoring efforts at KSC use a grid cell approach to prioritize habitat management and to assess territory quality. Grid cells are 10 ha, based on the approximate size of scrub-jay territories at KSC, and are assigned a habitat quality (Breininger et al. 2010). Habitat resistance measures were built on this grid framework by counting the number of cells of each habitat type that were found between each pair of birds based on straight-line distance. Each grid cell was

weighted based on habitat quality. Grid cell quality scores included 0 resistance for primary habitats that were well-drained oak woodlands on large scrub ridges, resistance of 1 for secondary habitats that were poorly drained oak on small scrub ridges, a resistance of 2 for small scrub ridges primarily made up of pine woodlands, resistance of 3 for dense vegetation that are unsuitable for scrub-jays, and cells made up of water with a resistance of 4. The weighting scheme was based on studies that have examined scrub-jay movement (Stith 1999, Breininger and Carter 2003, Breininger et al. 2009). QGIS 3.16 (QGIS Development Team 2020) was used to determine the number of each grid cell habitat type.

Coulon et al. (2012) found that genetic distance among scrub-jays increased with the size of habitat gaps. Coulon et al. (2012) defines habitat gaps as regions or lengths of unsuitable habitat for scrub-jays. This idea was evaluated at KSC by randomly choosing 18 pairs of individuals with each individual in the pair being used only once and coming from a separate subpopulation (9 individuals from each subpopulation were included). DPS between individual pairs was used as the response variable with log-transformed Euclidean distance and log-transformed size of habitat gap as predictor variables in a multiple regression analysis using R (R Core Team 2022). Size of habitat gaps were determined by measuring the Euclidean distance of suboptimal habitat (pine forest and water) between individual birds in each pair using QGIS 3.16 (QGIS Development Team 2020). Multicollinearity was assessed for the two predictor variables using the Farrar and Glauber (1967) test in the “mctest” package (Imdadullah et al. 2016, Imdad and Aslam 2020) in R (R Core Team 2022).

## RESULTS

DNA was successfully extracted from all birds. Seven individuals were removed from analyses based on relatedness within the same territory and a single individual was removed from habitat, territory, and IBD analyses because location information was unavailable. Sixty-four individuals were used in the majority of analyses (Table 1).

### Genetic diversity among study sites

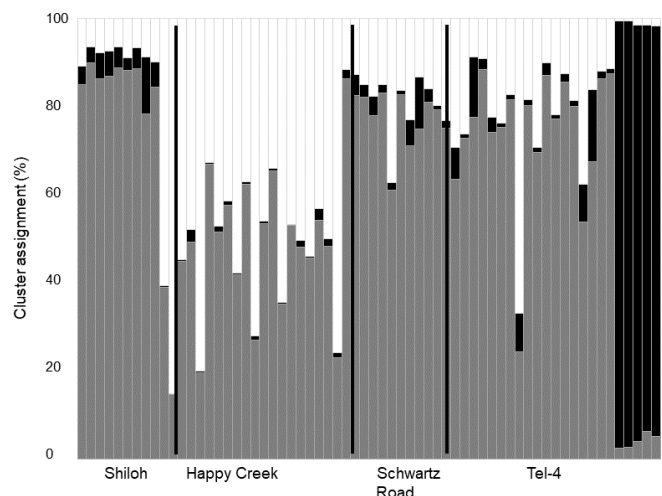
Four loci (ApCo 22, 23, 29, and 55; Stenzler and Fitzpatrick 2002) were removed from analyses because they were not in Hardy-Weinberg equilibrium at several study sites and showed evidence of null alleles. All loci were in linkage equilibrium after correction for multiple tests except for ApCo 36 and 88 in the Tel-4 subpopulation and only for the less conservative Benjamini and Hochberg (1995) false discovery method (adjusted  $P = 0.04$ ). Given that only one instance of linkage disequilibrium was detected in a single subpopulation, these loci were maintained in subsequent analyses. Based on POWSIM simulations, there was a 99% probability of detecting an  $F_{ST}$  of 0.015, and an 89% probability of detecting an  $F_{ST}$  of 0.010 among subpopulations, given the sample sizes and 15 loci used in the study. Average observed heterozygosity ( $H_o$ ), average expected heterozygosity ( $H_e$ ), and allelic richness ( $A_p$ ) values were similar across sites ( $t = 0.58$ ,  $P = 0.570$ ,  $df = 12.5$ ; Table 1). Khodadad's (2008) estimates of average  $H_o$  values of  $0.64 \pm 0.24$  and  $0.57 \pm 0.27$  for Happy Creek ( $n = 48$ ) and Tel-4 ( $n = 72$ ), respectively; did not differ significantly from the present study (Tel-4  $H_o$ :  $0.57 \pm 0.25$ ;  $W = 29$ ,  $P = 0.79$ ; Happy Creek  $H_o$ :  $0.69 \pm 0.27$ ;  $W = 32$ ,  $P = 0.99$ ). Results from Coancestry indicated that no individuals within the same territory had a relatedness above 0.5 (Appendix 1).

The Happy Creek study site had pairwise  $F_{ST}$  values that differed significantly from zero after Benjamini and Hochberg (1995) adjusted in comparison to the other subpopulations; Shiloh ( $F_{ST} = 0.027$ ,  $P = 0.011$ ), Schwarz Road ( $F_{ST} = 0.018$ ,  $P = 0.034$ ), and Tel-4 ( $F_{ST} = 0.0201$ ;  $P = 0.010$ ). All other comparisons did not show differences among subpopulations (all nonsignificant tests;  $F_{ST} < 0.003$ ,  $P > 0.402$ ).

### Connectivity among locations

STRUCTURE identified  $K = 3$  as the most probable number of genetic clusters in the dataset (Appendix 2). The majority of individuals sampled from territories at Shiloh, Schwarz Road, and Tel-4 assigned most strongly to one genetic cluster. Scrub-jays from Happy Creek and a single bird from Tel-4 had the highest proportion of membership in a second genetic cluster (Fig. 2). Birds in the third genetic cluster were in different territories but had a high proportion of shared alleles with one another (mean DPS  $0.63 \pm 0.09$ ), much higher than among all other scrub-jays (mean DPS =  $0.38 \pm 0.09$ ). A bootstrap analysis of 10,000 replicates in R (R Core Team 2022), based on drawing five random individuals from the dataset, calculating DPS for each pair of birds, and taking the mean of these values, showed that birds in the third genetic cluster were more closely related than expected based on the overall dataset (DPS 95% CI: 0.3240–0.4328).

**Fig. 2.** Cluster assignments for individual Florida Scrub-Jay (*Aphelocoma coerulescens*) from four sites at Kennedy Space Center, Brevard County, FL. Different colors indicate genetic clusters ( $K = 3$ ) identified using STRUCTURE. Five individuals in Tel-4 in a separate genetic cluster (black bars) are identified in Fig. 1 with yellow circles.



Results from GeneClass2 indicate that four individuals were likely to be first-generation migrants showing a probability of assignment to any subpopulation below 99% ( $P < 0.01$ ). One individual from Happy Creek likely came from Tel-4 ( $P = 0.003$ ), one bird from Schwarz Road likely originated from Happy Creek ( $P = 0.002$ ), and two birds from Tel-4 probably moved from Shiloh and Happy Creek ( $P = 0.004$ ,  $P = 0.007$ ), respectively. None of these birds are within the third genetic cluster of birds at Tel-4 (Figs. 1, 2). Results from the assignment tests indicate that three individuals (none were identified as first-generation migrants

above) did not assign strongly to any single site and most likely originated from an unsampled area. This includes two birds sampled from Shiloh and one from Happy Creek.

#### **Habitat quality, genetic diversity, and connectivity**

Heterozygosity between habitat types did not differ between high- and low-quality habitats ( $W = 464.5$ ;  $P = 0.733$ ). In addition, no association was found between observed heterozygosity per individual bird and the size of the territory where the bird was found ( $r = -0.059$ ;  $P = 0.646$ ). However, L-home/L-max ratios were higher in higher quality habitats than lower quality habitats ( $W = 598.0$ ;  $P = 0.016$ ).

A signal of IBD among all individuals was supported ( $r = -0.076$ ;  $P = 0.001$ ). IBD was detected within the Shiloh ( $r = -0.355$ ,  $P = 0.017$ ), Happy Creek ( $r = -0.176$ ,  $P = 0.006$ ), and Tel-4 ( $r = -0.200$ ,  $P = 0.001$ ) subpopulations, but not within the Schwartz Road ( $r = -0.084$ ,  $P = 0.271$ ) subpopulation. Log-transformed habitat resistance was not associated with the proportion of shared alleles between birds ( $r = -0.033$ ,  $P = 0.073$ ); non-transformed resistance was also not associated with DPS ( $r = -0.005$ ,  $P = 0.409$ ).

Geographic distance, gap size, and the interaction between these two variables were analyzed through multiple regression analyses, revealing no association between DPS and any of the variables or the possible interaction between geographic distance and gap size ( $r^2 = 0.196$ ,  $df(1, 13) = 1.06$ ,  $P = 0.401$ ). However, the two-predictor variables were shown to be strongly correlated ( $\chi^2 = 13.5$ ), thus limiting the usefulness of multiple regression (Mansfield and Helms 1982). Therefore, geographic distance was removed from the regression analysis leaving a comparison between gap size and DPS. However, DPS and gap size were not associated ( $r^2 = 0.077$ ,  $df(1, 15) = 1.25$ ,  $P = 0.282$ ).

## **DISCUSSION**

### **Genetic diversity among study sites**

Kennedy Space Center contains one of the largest populations of Florida Scrub-Jays and thus is of great importance in the conservation of the species. However, birds do not breed across a continuous optimal habitat type but are found in four geographically separated locations with suboptimal habitats in between. Poor habitat permeability could limit gene flow and cause subpopulations to lose genetic diversity and be at risk of inbreeding depression (Chen et al. 2016). However, the subpopulations of Florida Scrub-Jays at KSC appear to have retained genetic diversity possibly by dispersing through these suboptimal habitats.

Each study site had similar genetic diversity values (Table 1) and these values were comparable to those found in other studies of Florida Scrub-Jays using microsatellite loci (Coulon et al. 2008, Khodadad 2008). With the present study and Khodadad (2008) sharing two study sites (Happy Creek and Tel-4), observed heterozygosity values of shared loci did not differ between studies. These findings suggest that genetic diversity has been retained over the last two generations for the Tel-4 and Happy Creek subpopulations.

The number of families present at the study site did not affect diversity measures. Although Happy Creek had almost five times as many families as at Schwartz Road, sites had similar expected heterozygosity and allelic richness values. These findings suggest

that active management for scrub-jays has aided in maintaining genetic diversity across the area as a whole and it is likely that ongoing dispersal has allowed the subpopulations to maintain connectivity. At least two mosquito-borne arbovirus epidemics caused statewide die-offs of scrub-jays in 1979 and 1997 (Breininger et al. 2009). At Shiloh, the breeding subpopulation suffered substantial declines, due to predation by migratory hawks in 2014–2015 (Geoff Carter, Ecologist at KSC, *personal communication*). However, no loss of diversity was detected; this suggests that the core population at KSC was able to maintain a sufficient size to at least temporarily avoid a loss of genetic diversity.

### **Connectivity among locations**

Florida Scrub-Jays typically disperse to areas within 2 km of their natal areas (Breininger et al. 2006) and become sedentary after establishing territories (Woelfenden and Fitzpatrick 1985), although longer-distance movements ( $> 30$  km) have been detected in northern Florida (Miller et al. 2021). A weak signal of IBD was found among all genotyped individuals but habitat resistance was not associated with genetic relatedness. These findings suggest that geographic distance, rather than the type of habitat that must be traversed, plays a stronger role in shaping movement of scrub-jays at KSC. In addition, based on first-generation migrants and assignment tests, the subpopulations at KSC are connected to some extent by dispersal across suboptimal habitats. Shiloh and Schwartz Road subpopulations have limited sample sizes (Table 1), which might limit the ability to detect small differences among subpopulations and suggest connectivity among populations that does not exist. However, results of a power analysis showed a 99% probability of detecting an  $F_{ST}$  difference as small as 0.015 suggesting that the data set has the ability to detect weak signals of isolation among subpopulations.

Structure and pairwise  $F_{ST}$  values support low rates of gene flow between Happy Creek and other study sites. Given that Happy Creek has the highest density of territories at KSC, it is possible that individuals moving into the area are less likely to become breeders because of competition for territory space, or we were unable to fully detect individuals from Happy Creek moving to other study sites. This idea could be supported by Coulon et al. (2010), who found that scrub-jays that dispersed longer distances were less likely to successfully breed than those that dispersed short distances.

Dispersal data suggest that small and fragmented populations of Florida Scrub-Jays have greater dispersal than large connected populations (Breininger 1999, Fitzpatrick et al. 1999). Based on this information, IBD might be expected to occur within subpopulations found in unfragmented areas with large numbers of territories such as at Happy Creek or Tel-4 and this result was found. However, Shiloh and Schwartz Road subpopulations exhibited IBD, but are found in areas with very limited amounts of suitable habitat. A possible reason for these patterns is the decreased number of suitable territories at Shiloh and Schwartz Road and distance to other study sites could limit dispersal.

Research on scrub-jays in central Florida (Coulon et al. 2012) found that the size of unsuitable habitat gaps was positively correlated with genetic differentiation. However, this signal was not apparent at KSC, suggesting that birds are able to move across

habitat gaps. Habitat gap size was similar across both studies: up to 25 km for this study and 22 km for Coulon et al. (2012). The primary difference between the present study and the central Florida study (Coulon et al. 2012) is that genetic distances between groups of birds were used. This possibly provided an increased resolution of genetic relatedness rather than the proportion of shared alleles between individual birds. In addition, marginal habitats at KSC may be less difficult for scrub-jays to traverse than the gaps assessed by Coulon et al. (2012).

Florida Scrub-Jays could be moving into study sites from unsampled areas that are only marginally appropriate habitats for reproduction (Breininger and Carter 2003) and these individuals could have a substantial effect on allele frequencies (Chen et al. 2018). Two individuals from Shiloh did not assign strongly to any subpopulation. It is possible that these birds moved from natal areas within marginal habitats into areas with higher habitat quality. Recent movement among sites and from unsampled locations supports the likelihood of connectivity via suboptimal habitat corridors. This is encouraging, considering that it is possible for a small number of individuals to restore much of the genetic diversity lost because of small population size (Ingvarsson 2001). In addition, habitat resistance did not appear to play a role in genetic relatedness among individuals, further supporting the use of suboptimal habitats as movement corridors at KSC. However, additional samples from geographically distant locations might provide additional power to detect habitat resistance if at a minimal scale.

One group of five birds sampled from the Tel-4 site assigned strongly to a separate genetic cluster (Fig. 2), suggesting previously unobserved subpopulation structure in the southern portion of KSC. However, these individuals had a high proportion of shared alleles, much higher than would be expected of five randomly chosen birds. None of the five birds were identified as having originated from an unsampled location, suggesting that recent movement from a genetically differentiated unsampled subpopulation is unlikely. It is also possible that the third genetic cluster is driven by the relatedness among the five birds, but additional samples from Tel-4 are needed to further evaluate this idea.

#### **Habitat quality, genetic diversity, and connectivity**

Khodadad (2008) found lower genetic diversity in habitats that were of lower quality. However, we found no difference in comparisons of the heterozygosity of individuals found in high-quality habitats, with well drained soils and optimal scrub cover, versus the heterozygosity of individuals in lower quality habitats, with poor or moderately drained soil and limited scrub cover. In addition, comparisons across larger spatial scales showed that genetic diversity did not differ among subpopulations even though some subpopulations had more high-quality habitats than others (Table 1). These findings suggest that ongoing habitat management for scrub-jay and/or gene flow from individuals in territories in optimal habitats into suboptimal habitats has enabled the maintenance of genetic diversity in poor quality areas with poor reproductive fitness. In addition, individuals found in larger territories did not have higher (or lower) heterozygosity than individuals in smaller territories. A lack of association between territory quality (and size) and genetic diversity suggests that genetic diversity is likely to be currently maintained by gene flow.

Habitat selection is very important to individual fitness (Rodenhouse et al. 1997). Birds preferentially move into higher quality habitats within breeding seasons (Betts et al. 2008) and between breeding seasons (Rodenhouse et al. 1997). However, Florida Scrub-Jays are non-migratory cooperative breeders who, when they attain the status of breeder, typically remain on the same territory throughout their life with the same mate (Woolfenden and Fitzpatrick 1985). It should also be mentioned that although jays may stay at one location for large portions of their lives, territory boundaries may change because of habitat alternations (i.e., fire) or breeding pair dynamics (i.e., death of a mate or divorce; Breininger 1999, Breininger and Carter 2003). Movement of helpers or individuals that had lost their mates into new areas (Breininger 1999) is the likely driver of gene flow in scrub-jays. Habitat quality did appear to have an effect on movement behavior of Florida Scrub-Jays. Birds occupying high quality territories also had higher probabilities of being first generation migrants (assessed using L-home/L-max values). This is an unexpected result for Florida Scrub-Jay as previous research has shown that optimal territories serve as exporters of birds into suboptimal, sink territories (Breininger and Carter 2003); however dispersal events also occur in suburban locations (Thaxton and Hingtgen 1996). Ongoing habitat restoration and management could provide an explanation for this result; as optimal habitat has increased, so has movement into these types of habitats by unmated individuals. Whether or not these individuals breed in high quality territories is unknown and thus their effect on genetic diversity and connectivity might be limited (Coulon et al. 2010). In addition, as stated previously, high quality habitats can become temporarily unsuitable and unoccupied after a fire causing territories to shift; possibly providing an opportunity for new birds to occupy habitats that will eventually become optimal.

#### **CONCLUSIONS**

Previous and ongoing management efforts appear to have maintained the genetic diversity of the core population at KSC, at least based on an examination of neutral markers. Future studies that examine loci that are under selection could provide even more valuable information about the diversity of scrub-jays at KSC. Extensive efforts have been made to use and implement management plans at the refuge since the early 1990s (Schmalzer et al. 1994). The KSC scrub-jay population appears to have levels of gene flow high enough to maintain neutral genetic diversity, though only time will tell whether land management will remain consistent and continue the same prescribed fire regime in the years to come. However, the current state-wide population size is decreasing, which could have an impact on genetic diversity in the future (Birdlife International 2020). In the midst of global climate change (Frankham et al. 2010), it is uncertain how scrub-jays will respond to the current threats. Merritt Island is at most 3 m above sea level (Breininger et al. 1996b), which could potentially reduce available habitat as sea levels increase. Corridors must be maintained and created, especially around barriers such as industrial complexes and bodies of water. These corridors have the potential to increase or maintain gene flow among small subpopulations that may be impacted by predation or poor habitat.

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### Data Availability:

Data for this study is available at <https://doi.org/10.5281/zenodo.11375118>.

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**Appendix 1.** Table showing Coancestry Dyad pairings to determine relatedness of individuals found within the same territory.

*[Please click here to download file 'appendix1.xlsx'.](#)*

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**Appendix 2.** Estimation of optimal number of genetic clusters (K) using the delta K method of Evanno et al. (2005). The highest delta K values show the most probable number of genetic clusters in the dataset.

