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GENETIC DIVERSITY AND RELATEDNESS WITHIN AND AMONG NORTHERN BOBWHITE COVEYS IN SOUTH TEXAS

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ABSTRACT

Although biologists have studied northern bobwhite (*Colinus virginianus*) genetic diversity and population structure, there is little known about the genetic diversity, structure, and relatedness of their winter coveys. Both flexible mating strategies and the fall shuffle may have implications for inbreeding and genetic diversity in northern bobwhite populations. Our goal was to determine genetic diversity and relatedness for coveys on a private ranch in Jim Hogg County in South Texas. During the 2010–2011 and 2011–2012 hunting seasons, 96 northern bobwhites were sampled from 29 coveys. We analyzed 11 northern bobwhite microsatellite DNA loci, measured genetic structure with an Analysis of Molecular Variance and F_{ST} , and determined an inbreeding coefficient (F_i). We determined Queller and Goodnight's coefficients of relatedness (R) and then used a maximum-likelihood algorithm in COLONY to assign relationships (parent–offspring, full siblings, and half siblings). Most genetic variation (92%) was within coveys. The overall F_{ST} was 0.073, indicating moderate genetic structure among coveys. Relationship coefficients ranged from -0.82 to 1.00 but most of the bird pairs were unrelated ($R = -0.004 \pm 0.002$ SE). In 2010–2011, COLONY assigned 130–149 half sibling, 1–4 full sibling, and 0–3 parent–offspring pairs ($n = 2,887$, 5 trials, probability >0.99). Thirteen coveys (56%) had related individuals ($n = 20$ pairs). In 2011–2012, COLONY assigned 5–10 half sibling and 1 full sibling pairs ($n = 161$). Two coveys (33%) had related individuals (2 pairs). The occurrence of relatives in different coveys suggests that the fall shuffle is effective at mixing families and the high half sibling count among coveys may suggest polygamy. These strategies may help northern bobwhite populations maintain moderate genetic diversity.

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Key words: *Colinus virginianus*, covey dynamics, full sibling, half sibling, northern bobwhite, relatedness, South Texas

Flexible mating strategies and the fall shuffle are understood for northern bobwhite (*Colinus virginianus*). However, little is known of their effects to genetic diversity, covey structure, and relatedness of coveys. The northern bobwhite employs polyandry (Burger et al. 1995, Faircloth 2008) and male incubation of nests (Stoddard 1931, Lehmann 1984, Curtis et al. 1993). As nesting tapers off, family units will gradually form heterogeneous groups, or coveys. In late September, northern bobwhite

individuals often move among coveys and the exchange of covey members is termed the “fall shuffle” (Lehmann 1984:36).

Repeated observations of color-banded birds and molecular analyses have shown that polygamy is a fairly common occurrence in many bird species. For males, having multiple mates is advantageous because of the opportunities to have more offspring and pass on genes (Freeland 2005:202; Pearson et al. 2006). Females may benefit from mating with multiple males to ensure fertilization and fitness where male quality varies (Griffith et al. 2002). Females also may benefit from the assistance of males in tending offspring, which raises the offsprings' survival chances and thus the female's reproductive fitness (Freeland 2005:202). In *r*-selected species, male

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incubation of the nest may allow the female to produce a second clutch, again improving her fitness (Freeland 2005:204).

Lack (1968:103) characterized the New World quail (Odontophoridae) as monogamous. However, subsequent studies have revealed the group to be polygamous, where male incubation and brood care are common (Sibley 2001). For instance, Burger et al. (1995) found that 71% of northern bobwhite females engaged in polyandrous mating at some point during the breeding season. Polyandry may be facilitated by male incubation of nests: if the male incubates the nest, the female is then able to have a second nest with a different male. Male incubation of nests was documented for the northern bobwhite in early natural history studies (Stoddard 1931:30, Lehmann 1984:87) and occurs in 20–30% of nests (Brennan et al. 2014). Curtis et al. (1993) noted that a female may renest with a different male if the first male incubates the eggs or tends the brood, sometimes within days after laying her initial clutch. These strategies may result in a large number of siblings in a given year.

The fall shuffle affects the distribution of the related northern bobwhites within and among coveys. According to Lehmann (1984:19), the fall shuffles in South Texas finish by November, at which point most northern bobwhites have sorted themselves into winter coveys composed of 6–16 individuals. Northern bobwhites have a greater survival rate when the covey size is 11–12 birds (Williams et al. 2003). This optimal covey size is maintained over the winter as large coveys lose members and small coveys gain members (Lehmann 1984:appendix E). Living in coveys is advantageous for locating food and evading predators (Williams et al. 2003). During cold periods, northern bobwhites huddle together in the covey to conserve heat (Case 1973, Lehmann 1984:11, Roseberry and Klimstra 1984). Finally, winter coveys often have fairly even sex ratios and provide access to potential mates in the spring (Lehmann 1984:50).

Promiscuity and shuffling of individuals among coveys may allow northern bobwhite populations to retain genetic variation despite loss of habitat and an overall decline in census size throughout their range (Berkman 2012, Evans et al. 2013, Williford et al. 2014). Promiscuous mating can result in a greater effective population size than monogamous mating systems (Sugg and Chesser 1994), whereas the fall shuffle should increase gene dynamics within a population and reduce the chances of a local inbreeding (Leopold 1931). Inbreeding avoidance is crucially important to a species that exhibits short-distance movements, such as the northern bobwhite (Stoddard 1931:182, Leopold 1933:75). For example, Agee (1957) found that during fall males tended to join the coveys closest to their home range. A combination of promiscuous mating and social group structure might contribute to the retention of genetic diversity and perhaps to gene flow. To date, there is little quantitative information on the genetic composition of northern bobwhite social groups.

Our overall objective was to determine genetic diversity, genetic structure, and relatedness within and among northern bobwhite coveys on a private ranch in

Jim Hogg County in southern Texas. We developed 3 research hypotheses focused on the potential patterns of genetic relatedness within and among coveys. First, we hypothesized that families remaining intact through the shuffle and into the winter should result in coveys with full siblings and parents, but if families were separated during the fall shuffle, full siblings and half siblings would be distributed among coveys rather than within coveys. Secondly, we expected a gradient of relatedness over time. At the beginning of the winter, coveys should consist of related individuals, but due to the transfers among coveys and the effects of hunting and predation, related individuals should be dispersed among coveys by the end of winter. Finally, we hypothesized that the degree of promiscuity should be reflected in the ratio of full siblings to half siblings in the sample. For example, if northern bobwhite females are monogamous during their first nesting attempt and there are 10 females with nests and the clutch size = 10 eggs in each nest, 9% (450) of the pairs should be full siblings (Fig. 1). If 30% of the females then lay second nests with different males, we would expect 7% (585) full sibling pairs and 3.6% (300) half sibling pairs. This is an estimated 2 : 1 ratio of full to half siblings (Fig. 1).

STUDY AREA

We conducted the study on a 3,558-ha private ranch near Hebronville, Jim Hogg County, Texas (Fig. 2). Temperatures vary from 20° C (68° F) in winter to 36° C (97° F) in summer. Average annual rainfall is 60 cm (24 in; 1980–2010, <http://www.ncdc.noaa.gov/>). Most of the land can be classified as sandy mesquite (*Prosopis glandulosa*) savanna, sandy mesquite woodland, and deep sand grassland (Elliott 2011). These plant communities are characterized by native (gulfdune paspalum [*Paspalum monostachyum*], red lovegrass [*Eragrostis secundiflora*], tanglehead [*Heteropogon contortus*], and seacoast bluestem [*Schizachyrium scoparium* var. *littorale*]) and nonnative grass species (Coastal bermudagrass [*Cynodon dactylon*], Lehmann lovegrass [*Eragrostis lehmanniana*], and buffelgrass [*Pennisetum ciliare*]). Forbs include camphor weed (*Heterotheca subaxillaris*), camphor daisy (*Rayjacksonia phyllocephala*), and partridge pea (*Chamaecrista fasciculata*). Elliott (2011) also defined these areas as having patchy overstories of woody species (honey mesquite, huisache [*Acacia farnesiana*], and blackbrush [*Acacia rigidula*]). Prickly pear (*Opuntia engelmannii* var. *lindheimeri*) can be found throughout the area. Shallow scrubland, row crops, disturbed grasslands, and mesquite woodland are also present (Elliott 2011, Texas Ecological Land Classification Project).

Management of the ranch was targeted at habitat for northern bobwhite and white-tailed deer (*Odocoileus virginianus*) hunting operations. Habitat management efforts included roller chopping, herbicide treatments for invasive grass, disking, and rotational grazing of cattle ranch manager, personal communication).

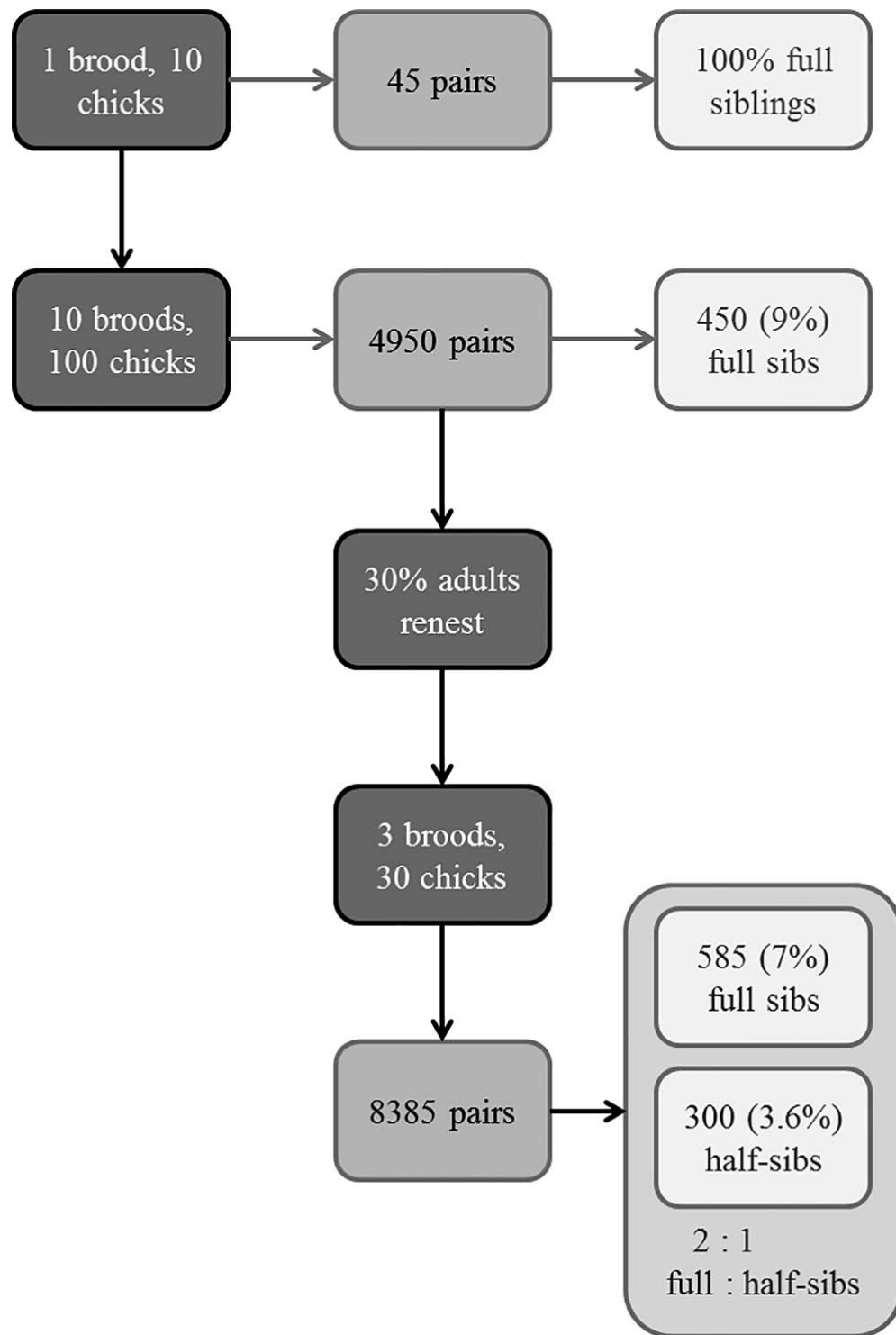


Fig. 1. A hypothetical model where northern bobwhites (*Colinus virginianus*) are monogamous during their first mating, and each clutch produces 10 offspring, and there are 10 initial nests. If 3 females (30%) then lay second broods fertilized by a different male, these would be half siblings to the full siblings of the first brood.

METHODS

Data Collection and DNA Amplification

We sampled coveys via hunter-harvest during 2 hunting seasons (Dec–Feb of 2010–2011 and 2011–2012) on the ranch. Hunters recorded the time and date of

harvest, sex of the birds, and whether birds were taken from the same covey. We extracted DNA from all individuals representing coveys where ≥ 3 birds were harvested. This consisted of 3–5 samples/covey. We classified individuals as juvenile or adult based on presence or absence of buffy tips on the primary coverts

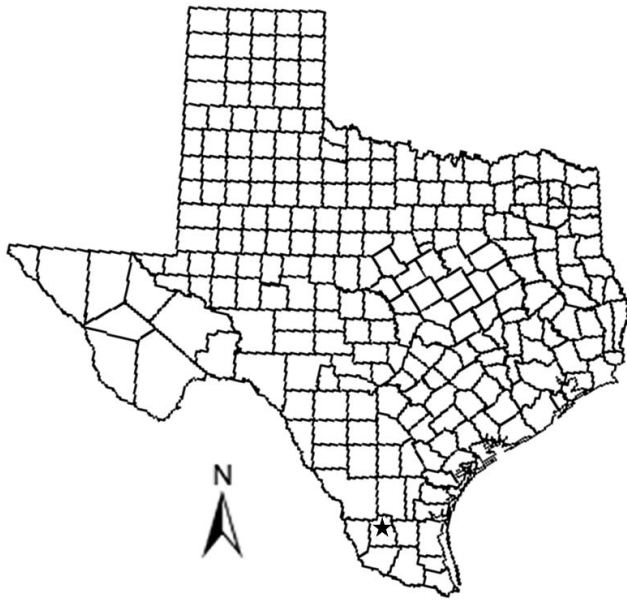


Fig. 2. The ranch, denoted with a star, located in Jim Hogg County, South Texas. Northern bobwhite (*Colinus virginianus*) samples were collected in the winters of 2010–2011 and 2011–2012 to examine covey genetic relatedness.

(Rosene 1969:54). We removed a portion of tissue from each wing and extracted DNA with a commercial kit (DNeasy, Qiagen, Inc., Valencia, CA, USA).

We amplified 13 microsatellite DNA loci (Schable et al. 2004, Faircloth et al. 2009). We amplified the DNA using polymerase chain reaction (PCR) in 10- μ L volumes containing 1.5 μ L DNA extract, 5.0 μ L AmpliTaq Gold PCR Master Mix (Applied Biosystems, Carlsbad, CA), 0.5 μ M of primer (forward and reverse), and 2.5 mM $MgCl_2$. For Schable et al.'s (2004) microsatellites, all PCR protocols started with an initial denaturation step of 94° C for 10 min. We amplified loci Quail 10, Quail 21, Quail 22, Quail 24, and Quail 26 with 21 cycles of 94° C for 20 sec, an annealing temperature of 59° C for 30 sec, and elongation at 72° C for 1 min (Schable et al. 2004). The protocol for Quail 23 differed only in the annealing temperature: 54.4° C. For Quail 14 and Quail 32, we used a touchdown protocol with 2 steps. The first step consisted of 21 cycles of 94° C for 20 sec, a starting annealing temperature of 60° C that decreased 0.5° C with each cycle for 30 sec, and elongation at 72° C for 1 min. The second step consisted of 10 cycles of 94° C for 30 sec, 50° C for 30 sec, and elongation at 72° C for 1 min. The PCR protocols for all of Schable et al.'s (2004) loci included a final extension at 72° C for 7 min.

For microsatellite loci in Faircloth et al. (2009), all PCR protocols included an initial denaturation step at 95° C for 10 min. For CV-PBA4 and CV-PCF5, we used a 2-step touchdown protocol. The first step consisted of 20 cycles of 95° C for 20 sec, a starting annealing temperature of 65° C that decreased 0.5° C each cycle for 30 sec, and elongation at 72° C for 90 sec. The second step consisted of 20 cycles of 95° C for 20 sec, 60° C for 30 sec, and elongation at 72° C for 90 sec (Faircloth et al.

2009). For CV-PBA7, CV-PC1F2, and CV-PC1F3, we used a similar touchdown protocol with an initial annealing temperature of 60° C in the first step and 50° C in the second step. We included a final extension at 72° C for 10 min for all loci (Faircloth et al. 2009). We amplified all microsatellites on ABI 2720 (Applied Biosystems, Foster City, CA, USA) and MyCycler (Bio-Rad, Hercules, CA, USA) thermal cyclers. We combined PCR products with denaturing formamide and ROX™ size standard for separation and detection of fragments, and loaded the samples on an ABI Prism 3130 Genetic Analyzer. We genotyped samples with Gene Mapper 4.0 (Hitachi, Applied Biosystems). We determined the genotyping error rate by reamplifying and regenotyping 30 randomly selected samples.

Data Analysis

We estimated the rate of null alleles with ML-NullFreq (Kalinowski and Taper 2006). We quantified genetic diversity with allelic richness (HP-Rare v. 1.1; Kalinowski 2005) and heterozygosity (ARLEQUIN 3.5; Excoffier and Lischer 2010). We tested for significance of departure from Hardy–Weinberg expectations with 10,000 random permutations of alleles among individuals. We partitioned genetic structure within and among coveys with an Analysis of Molecular Variance (Weir and Cockerham 1984) and F_{ST} . We tested for significant departure of global F_{IS} and F_{ST} from 0 by jackknifing over loci. These analyses were performed in ARLEQUIN 3.5 (Excoffier and Lischer 2010). We determined an individual inbreeding coefficient (F_i , the probability of identity-in-state between genes within individuals; Ritland 1996, Hardy 2003) for each covey with SPAGeDi v. 1.4 (Hardy and Vekemans 2002). Here, F_i is determined by the probability of identity-in-state between genes within individuals and the probability of identity-in-state between random genes from a reference population (Hardy 2003).

We used multiple approaches to determine genetic relatedness among individuals and among coveys. This is because the sampling variance of the relatedness estimator often results in a departure of the estimate from expected values of identity by descent (DeWoody 2005). First, we determined pairwise coefficients of relatedness (R , Queller and Goodnight 1989) with SPAGeDi v. 1.4 (Hardy and Vekemans 2002) and determined parent–offspring or full siblings (expected $R = 0.50$) and half siblings (expected $R = 0.25$). We used a 99% confidence interval to establish the lower and upper bounds of what we considered a parent–offspring, full sibling, or half sibling relationship. We then determined full sibling, half sibling, and parent–offspring pairs using a full pedigree maximum-likelihood algorithm in COLONY v. 2.0.4.5 (Wang 2004). We treated the mating system as polygamous for both sexes, with no inbreeding, and no prior knowledge of sibship size. We ran the maximum likelihood algorithm 5 times, varying length of run (short vs. medium) and used different random number seeds.

Finally, we generated R -values for all pairs with COANCESTRY v. 1.0 (Wang 2011). COANCESTRY

Table 1. Number of alleles (A), allelic richness (A_R ; $k = 2$ genes), observed (H_O) and expected heterozygosity (H_E), and individual inbreeding coefficient (F_i) for northern bobwhite (*Colinus virginianus*) on a private ranch in South Texas (2010–2012). Ninety-six birds in 29 coveys were sampled.

	A	A_R	H_O	H_E	F_{IS}	F_i	$P(F_i)$	Reference
Quail 10	12	1.77	0.50	0.72	0.37	0.40	0	Schable et al. 2004
Quail 21	11	1.74	0.46	0.65	0.32	0.48	0	Schable et al. 2004
Quail 22	17	1.90	0.87	0.87	−0.04	0.04	0.195	Schable et al. 2004
Quail 23	17	1.86	0.93	0.84	−0.13	−0.07	0.079	Schable et al. 2004
Quail 24	8	1.60	0.59	0.58	−0.10	0.04	0.584	Schable et al. 2004
Quail 32	9	1.54	0.62	0.57	−0.10	0.00	0.937	Schable et al. 2004
CV–P1A7	8	1.71	0.75	0.72	−0.06	−0.04	0.472	Faircloth et al. 2009
CV–PBA4	10	1.70	0.61	0.69	0.09	0.20	0.002	Faircloth et al. 2009
CV–PCF5	17	1.81	0.63	0.74	0.17	0.22	0	Faircloth et al. 2009
CV–PIF2	11	1.86	0.64	0.80	0.30	0.37	0	Faircloth et al. 2009
CV–PIF3	12	1.79	0.72	0.73	−0.03	0.07	0.158	Faircloth et al. 2009

estimates R with 5 moment estimators (Queller and Goodnight 1989, Li et al. 1993, Ritland 1996, Lynch and Ritland 1999, Wang 2002) and 2 likelihood algorithms (Milligan 2003, Wang 2007). We allowed the TrioML likelihood method to account for inbreeding. We compared these different estimators with Pearson's correlations. Results are presented as $\pm$ SE unless otherwise noted.

RESULTS

Over the course of the study period, we sampled 96 birds representing 29 coveys. The winter (Dec–Feb) of 2010–2011 followed a moist year (Palmer Modified Drought Index, <http://www.ncdc.noaa.gov>) and northern bobwhite population numbers were high. During this season, we collected 77 birds representing 23 coveys. The following summer, South Texas experienced a severe drought and the ranch reduced its harvest. As a result, we only collected 19 birds from 6 coveys. We pooled the samples from 2011 to 2012 with the 2010–2011 data for

analysis. We calculated an error rate of 0.049, averaged over loci, and used the genotyping error rate in COLONY runs. In 2010–2011, most birds were juveniles (40 M, 30 F) rather than adults (4 males, 3 female). In 2011–2012, all birds were adults (9 M, 10 F). Average number of samples per covey was 3.27 ± 0.12 birds.

Two microsatellite loci were not included in the analyses. Quail 14 was removed from the set because of a very low heterozygosity and low polymorphism and Quail 26 was removed because of the amount of missing data from nonamplification. Out of the 11 remaining loci, MLNull-Freq identified 2 markers as having potential null alleles, Quail 10 and Quail 21. These were included in subsequent analyses to provide additional genetic information for parentage analyses.

Allelic richness ranged from 1.54 to 1.90 and heterozygosity ranged from 0.46 to 0.93, over 96 samples (Table 1). F_{IS} , averaged over all loci, was 0.117. The inbreeding coefficient (F_i) ranged from −0.017 to 0.48; therefore, there was a deficiency of heterozygotes for Quail 10, Quail 21, CV–PBA4, CV–PCF5, and CV–PIF2 (Table 1). The overall F_{ST} was = 0.073, $P < 0.001$, indicating moderate structure among coveys. Most variation was within the coveys (Table 2).

Pairwise relationship coefficients (R) ranged from −0.82 to 1.00, average $R = -0.004 \pm 0.002$. Relatedness within coveys was greater in 2010–2011 (0.10 ± 0.002 , $n = 121$ pairs) than 2011–2012 ($0.02 \pm SE = 0.05$, $n = 26$ pairs; Fig. 3). Among coveys, average relatedness was low in both 2010–2011 (-0.003 ± 0.003 , $n = 4,815$ pairs) and 2011–2012 (-0.006 ± 0.012 , $n = 272$ pairs). Relatedness of juvenile males and females within coveys was lower than relatedness of juvenile or adult birds among coveys (Fig. 4). Average relatedness of females within coveys (0.01 ± 0.06 , $n = 17$ pairs) was lower than average relatedness of males within coveys (0.13 ± 0.04 , $n = 34$ pairs). Average relatedness of females or males among coveys suggested most birds were not related ($F = -0.02 \pm 0.007$, $n = 745$ pairs, $M = -0.007 \pm 0.006$, $n = 1,396$ pairs; Fig. 4).

The 5 COLONY run results were similar in the number of assignments made for half siblings, full siblings, and parent–offspring (Table 3). COLONY

Table 2. Analysis of Molecular Variance for northern bobwhite (*Colinus virginianus*) coveys on a private ranch in South Texas, 2010–2012.

Source of variation	df	Sum of squares	Variance components	% of variation
2010–2011				
Among coveys	22	128.17	0.31 Va	7.49
Within coveys	131	477.84	3.84 Vb	92.51
Total	153	606.01	4.15	
		$F_{ST} = 0.075$	$P < 0.001$	
2011–2012				
Among coveys	5	24.73	0.22 Va	5.37
Within coveys	32	108.79	3.90 Vb	94.63
Total	37		4.12	
		$F_{ST} = 0.054$	$P < 0.001$	
Over all years				
Among coveys	28	160.43	0.30 Va	7.30
Within coveys	163	586.63	3.85 Vb	92.70
Total	191	747.07	4.16	
		$F_{ST} = 0.073$	$P < 0.001$	

Table 3. Comparison of 5 COLONY runs and Queller and Goodnight's *R* to determine northern bobwhite (*Colinus virginianus*) relatedness in coveys of 2010–2011, 2011–2012, and across years. Probability of relatedness in COLONY $P > 0.99$. For Queller and Goodnight's *R*, parent–offspring / full siblings = 0.50, 99% CI = 0.492–0.508, and half siblings = 0.25, 99% CI = 0.247–0.253).

Relationship	Random number and length of run					Queller and Goodnight's <i>R</i>
	1,234 Short	1,234 Medium	1,367 Short	1,810 Short	1,810 Medium	
Within 2010–2011						
No. of pairs	2,887	2,887	2,887	2,887	2,887	2,887
Full siblings	2	4	1	1	4	12
Half siblings	149	139	137	136	130	111
Parent–offspring	0	3	0	1	0	0
No relationship	2,736	2,741	2,749	2,749	2,753	2,764
Frequency (%)						
Full siblings	0.07	0.14	0.03	0.03	0.14	0.42
Half siblings	5.16	4.81	4.75	4.71	4.50	3.84
Parent–offspring	0.00	0.10	0.00	0.03	0.00	0.00
No relationship	94.77	94.94	95.22	95.22	95.36	95.74
Full sibling : Half sibling	1 : 74	1 : 34	1 : 137	1 : 136	1 : 32	1 : 9
Within 2011 Season						
No. of pairs	161	161	161	161	161	161
Full siblings	1	0	0	0	1	3
Half siblings	5	8	9	10	7	10
Parent–offspring	0	0	0	0	0	0
No relationship	155	153	152	151	153	148
Frequency (%)						
Full siblings	0.62	0	0	0	0.621	1.86
Half siblings	3.10	4.97	5.59	6.21	4.35	6.21
Parent–offspring	0	0	0	0	0	0
No relationship	96.27	95.03	94.41	93.80	95.03	91.93
Full sibling : Half sibling	1 : 5				1 : 7	1 : 3
Among seasons						
No. of pairs	1,463	1,463	1,463	1,463	1,463	14,63
Full siblings or Parent–offspring	2	1	0	0	1	5
Half siblings	43	57	54	41	26	47
No relationship	1,418	1,405	1,409	1,422	1,436	1,411
Frequency (%)						
Full siblings or Parent–offspring	0.14	0.07	0	0	0.07	0.34
Half siblings	2.94	3.90	3.69	2.80	1.78	3.21
No relationship	96.92	96.03	96.31	97.20	98.15	96.45

assignments with probability >0.99 indicated relatives within 13 coveys (56.5%) in 2010–2011 (Fig. 5). From 2,887 pairwise comparisons of individuals, 130–149 pairs were half siblings, 1–4 pairs were full siblings, and 0–3 were parent–offspring pairs. Queller and Goodnight's *R* revealed a high ratio of half siblings ($R = 0.25$, 99% CI = 0.247–0.253) to full siblings ($R = 0.50$, 99% CI = 0.492–0.508), though the disparity was not as extreme (111 half siblings to 12 full siblings; Table 3). Most pairs (94–95%) were not related. Relatedness within coveys did not decrease over time ($n = 4$ coveys in Dec, 4 in Jan, and 5 in Feb). In 2011–2012, 161 pairwise comparisons resulted in 5–10 half siblings and 0–1 full sibling pairs (Fig. 6). The remaining pairs (94–96%) were not related. The high half sibling to full sibling ratio was supported again by Queller and Goodnight's *R* (10 half siblings, 3 full siblings; Table 3). Out of 1,463 pairwise comparisons among the years, 26–57 were half siblings, and 1–2 were either full siblings (1 from 2010–2011 and 1 from 2011–2012) or parent–offspring pairs where the juvenile was from 2010–2011

and the adult survived until 2011–2012 (Fig. 7). The remaining pairs (96–98%) were not related (Fig. 7).

We found that the methods used to generate relatedness estimates in COANCESTRY provided full: half sib ratios from 1 : 2 (TrioML) to 1 : 55 (DyadML) in 2010. In the 2011–2012 season, full:half sib ratio ranged from 1 : 1 (Rit) to 1 : 2 (DyadML). COLONY estimates were weakly correlated to the other relatedness estimators; however, all other relatedness estimators showed moderate to strong positive correlations (Table 4).

DISCUSSION

We detected low genetic diversity and fine-scale (2–12 km) genetic structure among coveys on our study site, potentially due to the presence of male relatives within coveys. However, genetic diversity and structure are strongly influenced by the sample size and sampling method. Hunters harvested 3–5 birds from a covey and

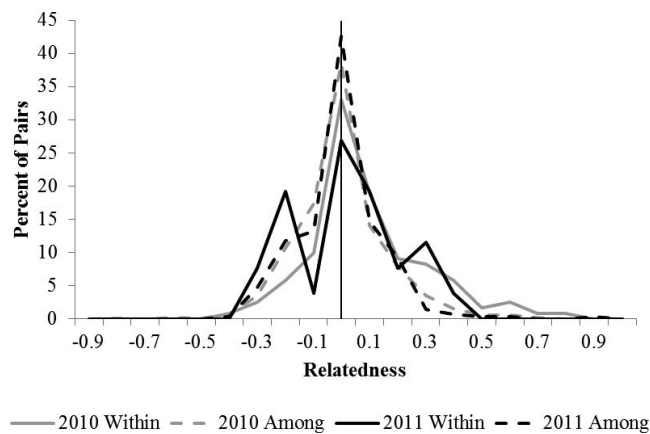


Fig. 3. Frequency of relationship coefficients (Queller and Goodnight's F) within and among northern bobwhite (*Colinus virginianus*) winter coveys, South Texas, 2010–2011 and 2011–2012. Average relatedness within and among coveys the first winter and among coveys the second year was low. Relatedness within coveys the second year should be interpreted with caution; this was the smallest group of pairwise comparisons (26 pairs).

then moved on to the next covey; live-trapping and sampling a full covey of ~ 12 birds would have yielded a more accurate estimate of genetic diversity than nonrandom sampling. Moderate fine-scale structure may indicate that the genetic diversity, already low, has some spatial pattern within the context of our study. Further, the F_i is determined in part by the probability of identity-in-state between random genes from a reference population (Hardy 2003) and our sampling method did not provide a true reference population.

We expected that northern bobwhite coveys would consist of related individuals, at least at the start of winter. However, related individuals were distributed among coveys throughout the winter. Further, we expected to find a gradient of relatedness over time for coveys, if northern bobwhites stayed in family groups through the fall shuffle and then proceeded to lose members over the course of the winter. Instead, relatedness within and among coveys did not change over the season. The moderate genetic structure of this population and the pattern of siblings (both half and full) spread throughout the coveys throughout the winter suggest that the fall shuffle may be distributing family members among coveys and that coveys continue to gain and lose members through the winter. We also expected that if northern bobwhite females were monogamous with respect to each clutch, one would expect an approximate ratio of 2 : 1 full to half siblings, even after accounting for second broods. In contrast, the ratio in 2010–2011 was 1 : 9–137 and in 2011–2012 it was 1 : 3–5. This high ratio of half siblings to full siblings suggests that promiscuity was common in this population.

Fall Shuffle and Mixing of Coveys

From the pattern of related individuals throughout coveys, it appears that the fall shuffle is an effective

means of distributing northern bobwhites among coveys. This mixing among northern bobwhite families can start as early as late summer, if a male and his young from the first nest join the female with young from her second nest. Although the formation of and mixing of coveys is likely driven by the optimal number of individuals for survival (Lehmann 1984, Williams et al. 2003), if unrelated young are amalgamated into the group to further brood survival, as in Faircloth et al. (2005), this also effectively creates a mixed group. For example, Faircloth et al. (2005) noted most broods of 3–7-day-old chicks contained related young, but that for broods of 10–12-day-old chicks, brood amalgamation increased to 20–22%.

Covey size in northern bobwhite coveys usually ranges from 6–25 birds (Rosene 1969:91) but the optimal covey size is 11–12 birds (Lehmann 1984:23, Williams et al. 2003). Smaller coveys had lower individual survival and more movement as the covey sought to join with another covey. Larger coveys had lower individual survival and a decrease in individual mass (Williams et al. 2003). Lehmann (1984:23) found that in South Texas only 10% of coveys had <6 or >16 birds. Therefore, if the covey tries to hold its size at approximately 12 members, it likely will lose and gain individuals in the process (Lehmann 1984:43, Williams et al. 2003). Yoho and Dimmick (1972) documented this exchange to be around 1 bird every 3 days. Both Ellis et al. (1969) and Robel and Klopfenstein (1985) documented that northern bobwhite coveys would lose and gain personnel through the winter.

The fall shuffle is usually over by the end of November (Lehmann 1984) and, although hunting season is open, most hunters and ranch managers rarely hunt before December in South Texas. Hunting parties may cause individuals of a covey to scatter and the birds that survive the hunt may reform their covey or move to other coveys. This adds to the already dynamic status of winter coveys (Lehmann 1984). Therefore, although related birds may be within a covey at the beginning of the winter, they may not be in the same covey by the end of the winter.

Northern Bobwhite Promiscuity

The high ratio of half to full siblings in our study suggests that northern bobwhites are promiscuous, and perhaps more so than previously documented. One possible reason for promiscuity may be linked to double-brooding, which may evolve through 2 methods (Blomqvist and Johansson 1994). In monogamous species where the male begins to incubate, the female is “liberated” to develop a second nest. In species where one or both sexes are polygamous, a hen may have 2 nests from 2 separate males (Blomqvist and Johansson 1994). The high ratio of half siblings to full siblings observed in this study far exceeds the expected ratio produced under scenarios of multiple broods, unless the number of broods per female was far greater than ever documented. For such a high half sibling to full sibling ratio, northern bobwhites must display a high degree of promiscuity, which has been suggested from behavioral studies (Burger et al. 2005, Brennan et al. 2014) and documented by

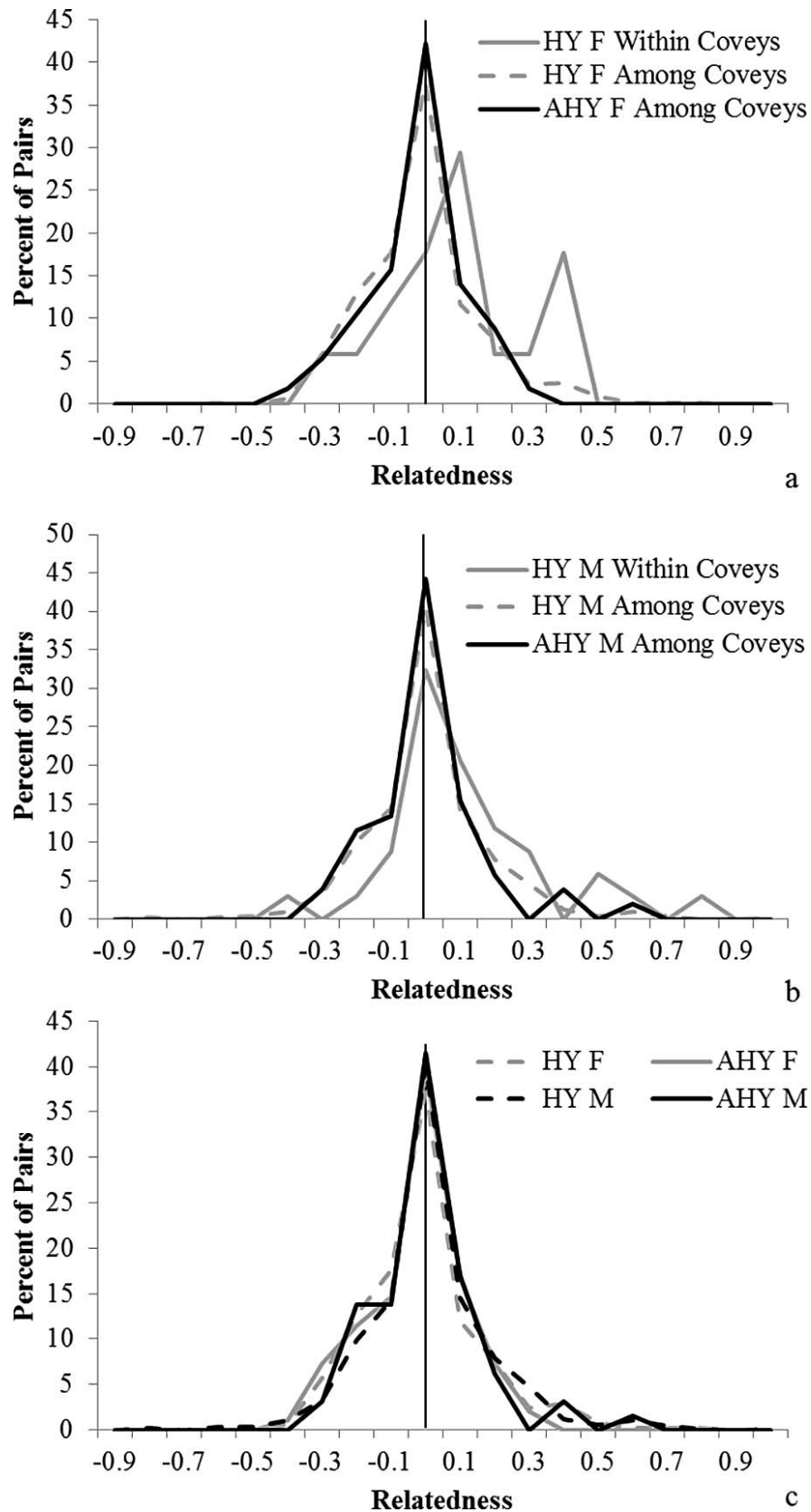


Fig. 4. Frequency of relationship coefficients (Queller and Goodnight's R) within and among northern bobwhite (*Colinus virginianus*) winter coveys. Frequency is shown for (a) juvenile (HY) and adult (AHY) females (F), (b) HY and AHY males (M), and (c) across age and gender groups.

Table 4. Comparisons of relatedness in northern bobwhite (*Colinus virginianus*) coveys: Queller and Goodnight relatedness computed with SPAGeDi, relatedness from COLONY, and 5 point estimators (Queller and Goodnight 1989, Li et al. 1993, Ritland 1996, Lynch and Ritland 1999, Wang 2002) and 2 likelihood ratios (Milligan 2003, Wang 2007) from COANCESTRY. Values represent Pearson's r , tests were significant at $\alpha = 0.05$, $df = 4559$.

	QG	COLONY	TrioML	Wang	LynchLI	LynchRD	Rit	QG	DyadML
QG	—	0.284	0.723	0.833	0.904	0.725	0.717	0.999	0.739
COLONY		—	0.384	0.274	0.263	0.378	0.362	0.287	0.383
TrioML			—	0.672	0.658	0.744	0.707	0.722	0.979
Wang				—	0.926	0.686	0.641	0.836	0.697
LynchLI					—	0.658	0.662	0.908	0.682
LynchRD						—	0.905	0.723	0.768
Rit							—	0.716	0.731
QG								—	0.738
DyadML									—

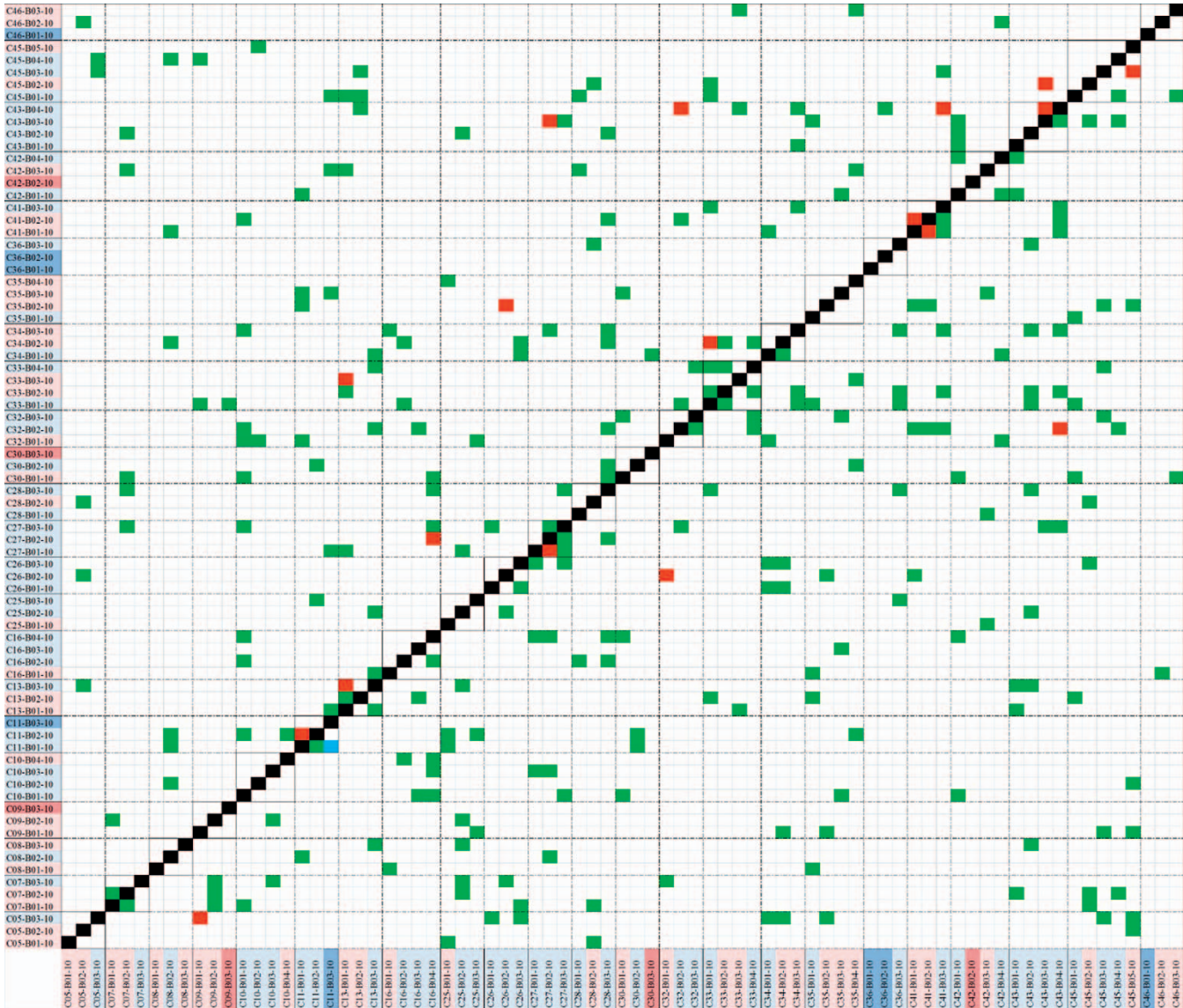


Fig. 5. Covey genetic relatedness for northern bobwhite (*Colinus virginianus*) in 2010–2011, South Texas. Probability of relatedness ≥ 0.99 from COLONY v. 2.0.4.5 (Wang 2004) is shown in the lower right of the matrix. Queller and Goodnight's R relatedness is shown above in the upper left of the matrix. For Queller and Goodnight's R , parent–offspring / full siblings = 0.50, 99% CI = 0.492–0.508, and half siblings = 0.25, 99% CI = 0.247–0.253). Red: full siblings, green: half siblings, blue: parent–offspring. Males are shown in blue (light blue: juvenile, dark blue: adult). Females are shown in pink (light pink: juvenile, dark pink: adult).

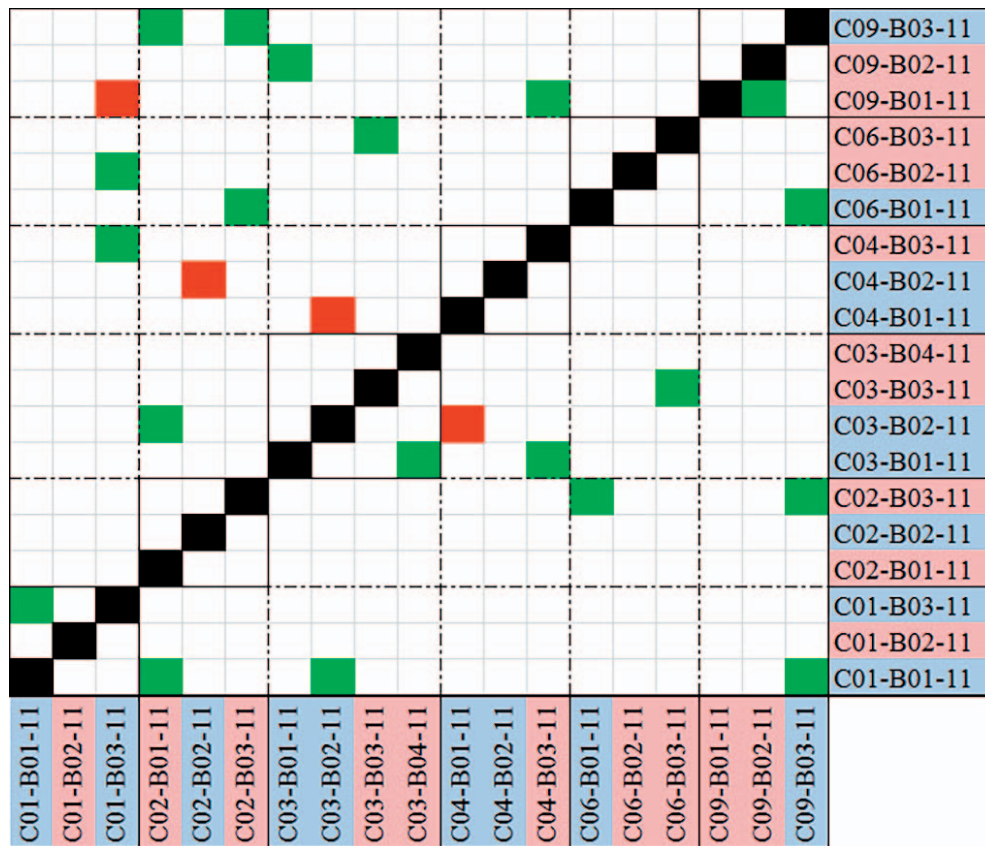


Fig. 6. Covey genetic relatedness for northern bobwhite (*Colinus virginianus*) within 2011–2012, South Texas. Probability of relatedness ≥ 0.99 from COLONY v. 2.0.4.5 (Wang 2004) is shown in the lower right of the matrix. Queller and Goodnight's R relatedness is shown above in the upper left of the matrix. For Queller and Goodnight's R , parent–offspring / full siblings = 0.50, 99% CI = 0.492–0.508, and half siblings = 0.25, 99% CI = 0.247–0.253). Red: full siblings, green: half siblings. Males are shown in blue, and females are shown in pink.

Faircloth (2008). Faircloth (2008) found evidence of extra-pair fertilizations to result in 1.8 ± 0.4 and 1.4 ± 0.6 chicks/wild and incubated broods, respectively, and extra pair fertilization rates of 10–20%/brood.

Genetic markers have revealed that promiscuity and extra-pair fertilizations are common in avian taxa (Griffith et al. 2002), including socially monogamous species (Carvalho et al. 2006, Ballenger et al. 2009). However, marker-based estimates of relatedness are not precise and may be error-prone or biased if assumptions are not met (DeWoody 2005, Jones and Wang 2010). Nevertheless, multiple methods used here produced similar results and it appears that the most likely explanation is promiscuous mating. An estimate of the extent and frequency of promiscuous mating would require genotyping the presumed parents and all chicks from each northern bobwhite nest.

Male and Female Relatedness

We did not expect to find a difference in male and female relatedness within the coveys. Average male relatedness (0.13) was at the level of first cousins (expected $R = 0.125$). Female relatedness within coveys (0.01) was essentially 0, close to the average relatedness of females (–0.02) and males (–0.007) among coveys

(Fig. 4). This suggests that for families that stay together into the winter covey, the males either are staying closer to home than females or that male survival until the fall hunting may be slightly greater than female survival.

The disparity in relatedness for males and females is not readily apparent from studies of northern bobwhite movements. Both sexes may move similar distances at local scales (Stoddard 1931; Miller et al. 2017). Data from the Encino Division of the King Ranch, which is approximately 65 km southeast of our study area, indicates that males had longer average daily movements than adult females but only slightly longer than juvenile females. Maximum distance moved was shortest in adult females and longest for juvenile males (Miller et al. 2017; Fig. 1). Maximum distance moved was different among seasons for males and females. Adult males moved farther ($583 \text{ m} \pm 121 \text{ SE}$) than juvenile males ($531 \text{ m} \pm 115 \text{ SE}$), juvenile females ($425 \text{ m} \pm 36 \text{ SE}$), and adult females ($418 \text{ m} \pm 62 \text{ SE}$, Miller et al. 2017).

Data submitted to the South Texas Quail Associates Program (L. A. Brennan, Caesar Kleberg Wildlife Research Institute, unpublished data) shows that winter harvest male:female ratios from the study area ranged from 1.03 to 1.24, 2008–2013. The male:female ratio ($\sim 1 : 1$) seems largely unaffected by annual rainfall,

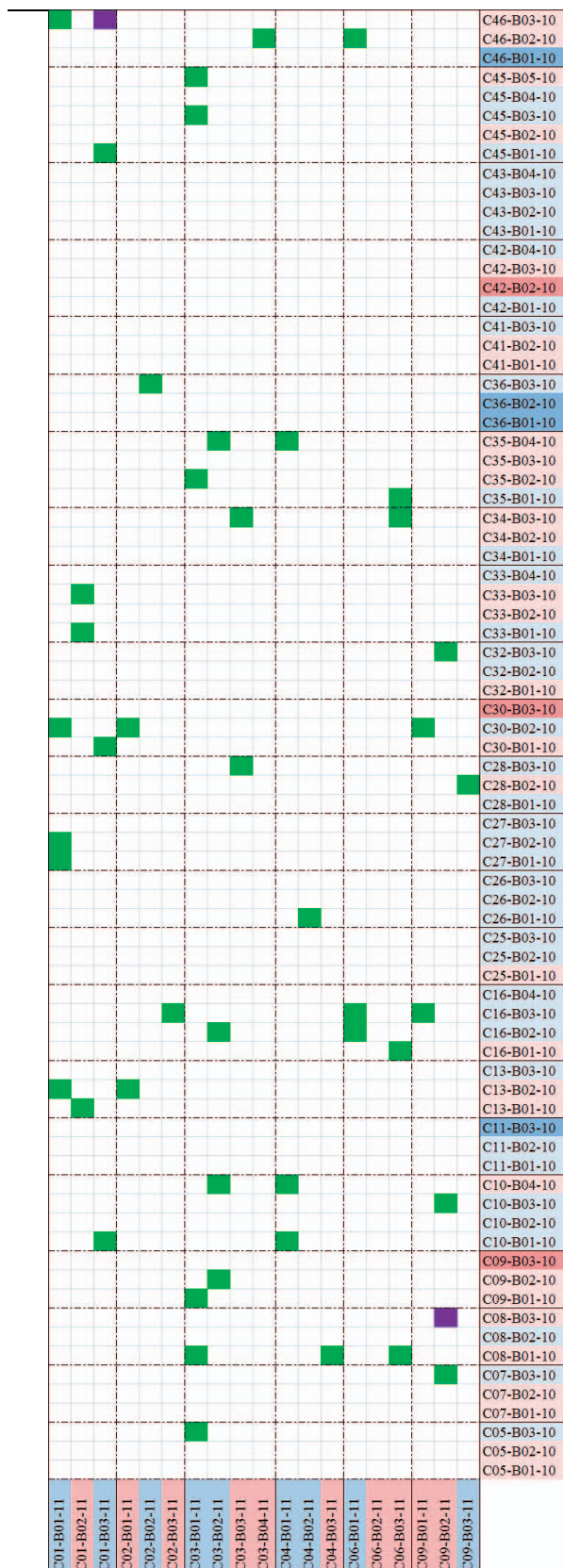


Fig. 7. Covey genetic relatedness for northern bobwhite (*Colinus virginianus*) among years. Probability of relatedness ≥ 0.99 , from COLONY v. 2.0.4.5 (Wang 2004). Green: half siblings, purple: full siblings or parent-offspring (either the adult

unlike juvenile:adult ratios, which are strongly affected by rainfall from April through August. It would seem the high degree of relatedness in males at the study area is not due to a skewed sex ratio in the winter but may be due to dispersal by females in the families. Tri et al. (2013) found a strong correlation between harvest juvenile:adult ratios and preceding summer rainfall totals but no correlation of male:female ratios to rainfall. This supports Lehmann's (1984:45) findings that winter coveys tended to have a fairly even ratio of males to females, regardless of weather.

This study provides a snapshot of covey relatedness in a defined area over a short time period. Analysis of entire coveys through live-trapping might give a more definitive overall distribution of siblings and, particularly after dry summers, might provide valuable insights into the mechanisms that maintain genetic diversity during periods of low census numbers. Further investigation of full:half sib relationships may require genotyping a subsample of these birds with a more extensive panel of markers and sampling stratified to areas of high northern bobwhite density and hunting pressure and areas of low density and low hunting pressure.

MANAGEMENT IMPLICATIONS

Multiple mating, the joining of larger social groups, and the fall shuffle may all play a role in maintaining genetic diversity for northern bobwhite populations. Northern bobwhite individuals appear to be moving among coveys enough to sufficiently disperse related individuals into other groups. This shuffling of genes may offset the "bust" years when populations drop drastically. Additionally, some gene flow among pastures and nearby ranches may be driven by the few northern bobwhites that move relatively longer distances (≥ 1 km). Populations isolated by distance may rely on these short distance movements, covey dynamics, and breeding strategies to sustain genetic diversity within their population. Further studies incorporating parentage analyses, movements, and covey dynamics will help biologists understand how this hunting may impact the genetic diversity of northern bobwhites.

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← harvested in 2011–2012 was the parent to the young harvested in 2010–2011 or the birds are full siblings harvested in 2 separate years). Males are shown in blue (light blue: juvenile, dark blue: adult). Females are shown in pink (light pink: juvenile, dark pink: adult).

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