

# Release of Pen-reared Bobwhites: Potential Consequences to the Genetic Integrity of Resident Wild Populations

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**In response to low encounter rates with wild northern bobwhite (*Colinus virginianus*; hereafter bobwhite) during bird dog field trials at Ames Plantation in Tennessee, a large-scale release program of pen-reared bobwhites was implemented in fall 2002. To evaluate genetic effects of pen-reared releases on wild populations, we monitored survival of pen-reared and wild bobwhites from fall release of pen-reared bobwhites through the breeding season and collected feather samples from wild, pen-reared, and free-ranging juvenile bobwhites following the first breeding season after the initial release. We used genotypes from 6 polymorphic microsatellite loci to measure genetic diversity and conduct population assignment tests. Wild bobwhites experienced greater fall-spring and annual survival than pen-reared bobwhites; however, pen-reared bobwhites experienced greater fall-spring and annual survival than reported in most other studies. Genetic diversity, number of alleles, and allelic richness were greatest in the wild, intermediate in the F1 generation, and lowest in the pen-reared populations. Likelihood analysis and cluster analysis indicated 20.4% and 33.6%, respectively, of juveniles captured after the first breeding season following release were ambiguous in population assignment; suggesting successful reproduction between wild and pen-reared individuals. These results suggest that large-scale releases of pen-reared bobwhite may result in negative impacts on genetic integrity of resident wild populations.**

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## Introduction

As northern bobwhite (*Colinus virginianus*; hereafter bobwhite) populations continue to decline throughout most of their range, the use of commercially produced bobwhites in lieu of wild bobwhites for bird dog training and field trials has become increasingly common (Hurst et al. 1993, Kozicky 1993, DeVos and Speake 1995, Dailey 2002). Pen-reared bobwhites are also used to augment existing wild populations to meet harvest demands far greater than sustainable by wild populations. Despite previous research (Baumgartner 1944, Buechner 1950, Sexson and Norman 1972) documenting the inability of pen-reared bobwhites to contribute substantially (i.e., survive and reproduce successfully) to wild

populations, the release of pen-reared bobwhites remains a common practice used to meet short-term population goals. Whereas most releases of pen-reared bobwhites by private landowners have been for recreational purposes (i.e., shooting, dog training) and consist of relatively few birds; large-scale releases consisting of up to several thousand birds are becoming commonplace, especially at shooting preserves and field trial areas (Kozicky 1993, Sisson et al. 2000).

Large-scale releases of pen-reared bobwhites present potential for unforeseen ecological consequences. Disease transmission, displacement of wild bobwhites, and increased mortality of wild bobwhites due to numerical or functional predator

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responses to pen-reared bird releases may pose short to intermediate term risks (Hurst et al. 1993, Sisson et al. 2000, Hutchins and Hernandez 2003). However, longer term, less easily recognizable risks such as reduction in genetic variability of resident populations of wild bobwhites or introgression of maladaptive alleles is less well understood (Sexson and Norman 1972, Landers et al. 1991, DeVos and Speake 1995, Sisson et al. 2000, Hutchins and Hernandez 2003). Gutierrez (1993) suggested that if wild bobwhites exist in isolation at low densities and have adapted to local environmental conditions, large-scale release of pen-reared individuals may be detrimental to the genetic integrity of the population through dilution of locally adapted genepools. As such, a concern among most land managers and researchers is the likelihood of decreased natural genetic variability of wild populations or introgression of maladaptive genes following pen-reared release efforts (Wooten 1991, Hurst et al. 1993, Nedbal et al. 1997). However, these concerns are currently unsubstantiated because no research has investigated the effects of pen-reared bobwhite releases on the genetic structure of wild resident populations of bobwhites.

Ellsworth et al. (1988) reported less genetic variation in pen-reared than wild bobwhites. Breeding in captivity can produce extremely skewed reproduction and unintended selection which may reduce genetic variability (Roseberry et al. 1987, Ellsworth et al. 1988, Kozicky 1993) and facilitate the inadvertent selection of traits that may be maladaptive in the wild. Crossing of pen-reared with wild bobwhites has been suggested as a means to mediate loss of genetic diversity; however, backcrossing is *prima facie* evidence acknowledging genetic differentiation and directional selection in pen-reared populations.

Transference of pen-reared genes to wild populations necessitates that pen-reared bobwhites develop pair bonds, copulate, and successfully produce viable offspring with wild bobwhites. DeVos and Speake (1995) reported pen-reared bobwhites integrated into 72% of resident wild coveys; however, observations of pair bonds and reproduction

of pen-reared and wild bobwhites was sparse. Confirmatory, genetic-based information of pen-reared and wild bobwhite production is non-existent. Secondly, pen-reared bobwhites must survive until the breeding season. Given the relatively low survival of pen-reared bobwhites (Fies et al. 2000, Oakley et al. 2002, Perez et al. 2002), releases conducted during the fall may not pose a threat to native gene pools because, in most instances, pen-reared bobwhites do not survive to the breeding season and thus do not participate in reproduction. However, Frye (1942) reported up to 58% fall-spring survival for pen-reared bobwhites released in Florida. Given this fall-spring survival, pen-reared bobwhites released in the fall may survive to the breeding season, compete for mates, and subsequently reproduce with wild bobwhites; thereby contributing to local gene pools.

Our objectives were to estimate fall-spring and annual survival of pen-reared and resident wild bobwhites at Ames Plantation in southwest Tennessee and to evaluate the genetic consequences of pen-reared bobwhite releases on the genetic structure of the local wild bobwhite population during the first breeding season following initiation of a large-scale release program. We hypothesized that the release of pen-reared bobwhites would result in the introgression of pen-reared alleles in the F1 generation.

## Study Area

Our study was conducted at Ames Plantation in Hardeman and Fayette counties, Tennessee (89° 11' W, 35° 8' N). Owned and operated by the Hobart Ames Foundation, Ames Plantation is home to the National Bird Dog Championship and also serves as a branch of the University of Tennessee Agricultural Experiment Station system. Of the 7,552 ha plantation, approximately 2,429 ha were used to host field trials and was managed intensively for wild bobwhites. Land cover on the field trial courses consisted predominantly of corn (*Zea mays*) and soybean (*Glycine max*) row crop fields interspersed with idle and perennial grass fields and woodlands. Pre-

scribed burning, disking, rotational agriculture, and selective herbicide applications were used to maintain early succession plant communities within open lands and pine woodlands. Sorghum (*Sorghum vulgare*), soybean, and wheat (*Triticum aestivum*) food plots were planted in small (<1 ha) patches. For a more complete study area description, see Seckinger (2004).

Despite the success of habitat management efforts to elevate and maintain relatively high densities of wild bobwhites; encounter rates with bobwhite during field trials still remained below desired levels. Consequently, Ames Plantation instituted a pen-reared bobwhite release program in fall 2002 to elevate bobwhite densities to desired levels (1 bird/0.5 ha) for conducting field trials. Approximately 3,200 pen-reared bobwhites were released each fall (1 October) from 2002-2004.

## Methods

### *Capture, Marking, And Releasing*

Pen-reared bobwhites were purchased from a commercial producer (Clear Creek Farms, Lamar, Mississippi, USA) and held on site for 95-105 days prior to release in 2 holding pens. Each holding pen consisted of a 4.6-m × 6.1-m enclosed brooding area with a 3.7-m × 6.1-m × 45.7-m flight pen. Commercial feed (28% crude protein, medicated with BMD and a coccidiostat) and water were provided *ad libitum*.

Prior to release (4-14 days), we sexed, weighed, banded with a #8 aluminum leg band, and fitted a 5-6 g pendant style radio transmitter (American Wildlife Enterprises, Tallahassee, Florida, USA) to a sample (2002,  $n = 191$ ; 2003,  $n = 216$ ) of these pen-reared bobwhites. On the evening prior to the release (1 October each year), 1-2 radiomarked birds were placed into each of 160 release boxes containing 18-19 non-radiomarked pen-reared bobwhites. All bobwhites within each box were then released the following morning at 1 of 160 release sites distributed over the study area. We released 3,200 pen-reared bobwhites each fall, with an additional 200 pen-reared bobwhites released during January 2003.

Release sites were selected to provide cover in close proximity to food resources with most release sites situated in dense food plots of sorghum or corn or a natural herbaceous community. Food (7.6 L of sorghum) and water (1.9 L) dispensers were located at each of the release sites.

Wild resident bobwhites were captured during the fall and winter of each year from 2000-2004 with baited walk-in funnel traps (Stoddard 1931) or by night netting (Truitt and Dailey 2000). We also captured periodically additional bobwhites during the breeding season using call-back traps and by night-netting. Captured wild bobwhites were identified and radiomarked in a similar fashion as the pen-reared bobwhites, except wild bobwhites were released at the capture site immediately after radiomarking. Capture, handling, tagging, and radiomarking procedures were consistent with the American Ornithologist's Union Report of Committee on the Use of Wild Birds in Research (American Ornithologists' Union 1988).

We used a programmable scanning receiver with a 3-element Yagi antennae to monitor radiomarked pen-reared and wild bobwhites  $\geq 5$  days/week from 1 October 2002-30 September 2004. Radio transmitters operated on 148.000-151.000 MHz wavelengths and were equipped with a 12-hr motion sensitive mortality switch. When a mortality signal was detected, we located the transmitter and determined fate of the radiomarked bird using evidence at the recovery site (i.e., bird remains, scat, tracks, white-wash) and transmitter damage (Dumke and Pils 1973). Intact birds for which no apparent cause of mortality could be determined readily were considered to have died due to exposure.

### *Survival Analysis*

We used Cox's partial likelihood regression (Cox 1975) in PROC PHREG (Allison 1995) to estimate survival and test hypotheses of no difference in proportional hazard between pen-reared and wild bobwhites and sex. We calculated survival for 2 post-release time intervals (fall-spring, 183 days; annual, 365 days) for each year (2002-2003 and 2003-2004)

beginning on the release date of pen-reared bobwhites (1 October). Wild bobwhites radiomarked prior to the release of pen-reared bobwhites entered the survival analyses on the release date of the pen-reared bobwhites for each year (i.e., survival estimates of wild bobwhites began on the same day as pen-reared bobwhites). We right-censored birds due to transmitter failure, suspected emigration from the study site, or trap-related mortality on the last date a signal was recorded. Right-censoring accounts for incomplete data that is not a result of a failure to survive during the study period and is therefore "censored" during analysis (Martinussen and Sheike 2006). Wild bobwhites that were marked in one year and survived to the next were right-censored on 30 September and introduced as new independent observations on 1 October. Pen-reared bobwhites surviving >365 days ( $n = 5$ ) were not included in the subsequent year's estimate because we desired to measure only post-release survival of pen-reared bobwhites up to 1 year. We assumed sexes were sampled randomly, individual survival times were independent, the censoring mechanism was random, and capturing, handling, and radiomarking did not affect survival (Pollock et al. 1989). Results were considered significant at  $\alpha = 0.05$ . Because variation in annual survival of bobwhites has been well documented (Rosene 1969, Burger et al. 1995), we analyzed each year independently and did not test for year effects.

### *Genetic Analysis*

*Feather Samples.*- We collected feather samples from wild, pen-reared, and F1 generation bobwhites during both years of study; however, because funding for the genetic analyses was limited, we chose only to analyze the 2002-2003 feather samples because pen-reared bobwhite survival was greatest for this time interval and would likely represent the "worst case" scenario of pen-reared bobwhite contribution to production. We collected 5-10 body feathers from the ventral tract of each of approximately 200 wild bobwhites captured from January-August 2002, 900 randomly selected pen-reared bob-

whites released in the fall 2002, and from all pen-reared bobwhites released in January 2003. From September 2003 to May 2004 we captured and collected feather samples from approximately 200 juvenile bobwhites (F1 generation) from multiple coveys within the study area using baited walk-in funnel traps. To avoid cross-contamination, feather samples from each individual were stored separately in dry envelopes. Bird handling and feather sampling were conducted under the auspices of the Mississippi State University Institutional Animal Care and Use Committee (permit #01-051).

We selected randomly 50 feather samples from each of the wild, pen-reared, and F1 generation groups. DNA was extracted from feather tips using a Qiagen DNeasy Tissue Extraction Kit (Qiagen Inc., Valencia, Ca) combined with Dithiothreitol (DTT) to aid in the breakdown of the keratinized feather shaft. Six di- and tetra-nucleotide microsatellite markers (K. W. Fok, University of Georgia, unpublished data, Fok and Parkin 2003, Schable et al. 2004) were amplified in 10  $\mu$ l polymerase chain reactions (PCR) containing DNA template, Takara Ex-Taq DNA polymerase, 10X PCR buffer (containing 20 mM  $Mg^{+2}$ ), 2.5 mM each dNTP (pH 7~9), and 1  $\mu$ M each fluorescent-labeled primer (Prologo LLC, Boulder, Co). PCR reactions were conducted with an initial denaturation of 5 min at 95°C, followed by 40 cycles of 95° C for 30 sec, 30 sec at the locus-specific annealing temperature (Table 1), and 72° C for 30 sec. Cycling was followed by a final extension period of 20 min at 72° C. Following amplification, products were identified and sized by capillary electrophoresis on a DNA Sequencer (CEQ 8000XL, Beckman-Coulter Inc., Fullerton, Ca). Fragments representing pairs of alleles at each locus (i.e., genotypes) were generated for each individual in a population and binning analysis of alleles at each locus was conducted to ensure accurate scoring of fragment sizes and alleles.

*Genetic Differentiation.*- Deviations from Hardy-Weinberg (HW) and linkage equilibrium were calculated using Program GENEPOP 3.3 (Raymond and Rousset 1995). To reduce the probability of Type I er-

Table 1: Locus identity, annealing temperatures ( $^{\circ}\text{C}$ ), and accession numbers for each locus used to examine introgression of pen-reared individuals with wild bobwhite populations on Ames Plantation, Tennessee, 2002–2003.

Locus	Annealing Temp.	Accession No.
LEI-142	68	X83257
LEI-160	66	X85523
LEI-70	63	X82869
LEI-197	63	Z83776
NBGP-9	57	AY522966
NBGP-11	57	AY522968

ror due to multiple testing, we used sequential Bonferroni to adjust nominal significance levels (Rice 1989). Allele frequencies, gene diversity, number of alleles, allelic richness, and inbreeding coefficients ( $F_{IS}$ ) were calculated for each population (wild, pen-reared, F1 generation) using Program FSTAT 2.9.3 (Goudet 2001).

Degree of introgression of pen-reared and wild individuals was analyzed using assignment test procedures of Paetkau et al. (1995). Individuals were first assigned to likely source populations using Program WHICHRUN 4.1 (Banks and Eichert 2000), designating the pen-reared bobwhite group as the critical population (stringency = 2). A second analysis was conducted designating the wild bobwhite group as the critical population (stringency = 2). Likelihood values were calculated for each individual and the  $\log_{10}$  of the quotient of the critical population's likelihood divided by the most likely population's likelihood was calculated to generate a LOD score. Individuals possessing LOD values greater than stringency values belonged to the defined critical population. Most likely population probabilities were also calculated and the probability ( $P$ ) an individual belonging to the most likely (ML 1) population divided by the probability of the individual belonging to the second most likely population (ML 2) was calculated. Values  $<3.00$  were characterized as ambiguous in population assignment.

Bayesian analysis of allele frequencies was con-

ducted to evaluate admixture in the F1 generation using Program STRUCTURE 2.0 (Pritchard et al. 2000). Posterior probabilities of  $K$  (number of populations) were used to assign individuals to populations and using a prior population model ( $K = 3$ , Burnin = 10,000, MCMC Reps = 10,000) and correlated allele frequencies.

## Results

### Survival

We used 409 pen-reared and 316 wild bobwhites to estimate survival. We right-censored 10 pen-reared bobwhites due to suspected emigration from the study site, 4 due to transmitter failure or transmitter related mortality, and 5 due to trap related mortality. We right-censored 12 wild bobwhites due to suspected emigration, 27 to transmitter failure or transmitter related mortality, and 4 to trap related mortality.

Fall-spring survival (183 day) did not differ between sexes in 2002 ( $\chi^2 = 1.09$ ,  $P = 0.296$ ) or 2003 ( $\chi^2 = 0.03$ ,  $P = 0.873$ ). Wild bobwhites experienced greater fall-spring survival than pen-reared bobwhites in 2002 ( $\chi^2 = 3.98$ ,  $P = 0.046$ ) and 2003 ( $\chi^2 = 8.82$ ,  $P = 0.003$ ; Table 2). Annual survival was similar between sexes in 2002 ( $\chi^2 = 0.02$ ,  $P = 0.882$ ) and 2003 ( $\chi^2 = 1.56$ ,  $P = 0.211$ ). Wild bobwhites had greater annual survival in 2002 ( $\chi^2 = 5.83$ ,  $P = 0.016$ ) and 2003 ( $\chi^2 = 17.90$ ,  $P < 0.001$ ; Table 2).

Table 2: Survival (S) of pen-reared and wild northern bobwhite for 183 and 365 days following release (1 October) of pen-reared northern bobwhite at Ames Plantation, Tennessee, 2002–2004.

Period	Year	Pen-reared			Wild			P-value
		<i>n</i>	S	SE	<i>n</i>	S	SE	
183-days	2002–2003	190	29.8	0.03	124	44.6	0.05	0.046
	2003–2004	219	12.2	0.02	137	32.9	0.05	0.003
365-days	2002–2003	190	3.2	0.01	150	8.4	0.02	0.016
	2003–2004	219	0.5	0.00	166	4.5	0.01	<0.001

### Genetics

Four individuals from the wild, 3 from the pen-reared, and 1 from the F1 generation groups were removed prior to analysis due to missing data at 3 or more loci. We found no evidence of HW or linkage disequilibrium; indicating a random union of gametes and independence of loci within each group (wild, pen-reared, F1 generation).

Wild and pen-reared birds shared 44 common alleles across all loci (Figure 1). Allele frequency analysis indicated 4 instances where alleles were specific to pen-reared and F1 generation populations but not found in the wild population (Locus LEI-97 [133, 153, 155], Locus LEI-142 [128]; Figure 1). There were 14 instances of private alleles: 11 specific to wild birds, 2 specific to pen-reared birds, and 1 specific to F1 generation birds (Figure 1). However, only 2 alleles from the wild population (LEI 142 [106], NBP 9 [194]) exceeded the threshold frequency of 0.05 required to ensure that the alleles are a product of population differences and not random sampling (Beaumont et al. 2001).

Gene diversity, number of alleles, and allelic richness averaged across all loci were greatest in the wild population, intermediate in the F1 generation population, and lowest in the pen-reared population (Table 3). Overall, genetic diversity estimates were high for all three populations (range = 0.790–0.841; Table 3). Relative to the wild population, the F1 generation population exhibited less genetic diversity, possibly due to the introduction of pen-reared birds

(Table 3).

Likelihood ratio analysis of the 49 F1 generation birds indicated that 30 individuals (61.2%) were most likely sired from two pen-reared adults ( $P_{(ML1/ML2)} > 3.00$ ), and 13 of those 30 individuals were assigned to the pen-reared population when LOD values were compared to a stringency value of 2 (<1/100 chance of error). Nine individuals (18.4%) most likely were sired from two wild adults ( $P_{(ML1/ML2)} > 3.00$ ), but only 1 individual was significantly assigned to the wild population when LOD values were compared to a stringency value of 2. Ten individuals (20.4%) were ambiguous in population assignment ( $P_{(ML1/ML2)} < 3.00$ ; Figure 2). This ambiguity may reflect possible hybrid offspring that resulted from the cross of wild and pen-reared adults.

Estimation of the proportion of membership of individuals into clusters was successful for individuals in the wild and pen-reared population. Cluster 1 grouped wild individuals with a high proportion of membership ( $q_1 = 0.985$ ) whereas cluster 2 grouped the pen-reared individuals with a high proportion of membership ( $q_2 = 0.980$ ). However, members of the F1 generation population were derived from the wild population cluster ( $q_1 = 0.311$ ), the pen-reared population cluster ( $q_2 = 0.353$ ), and from its own F1 generation cluster ( $q_3 = 0.336$ ); suggesting that 33.6% of individuals in the F1 generation cluster were possible hybrids that could not be placed into either the wild or pen-reared populations due to an

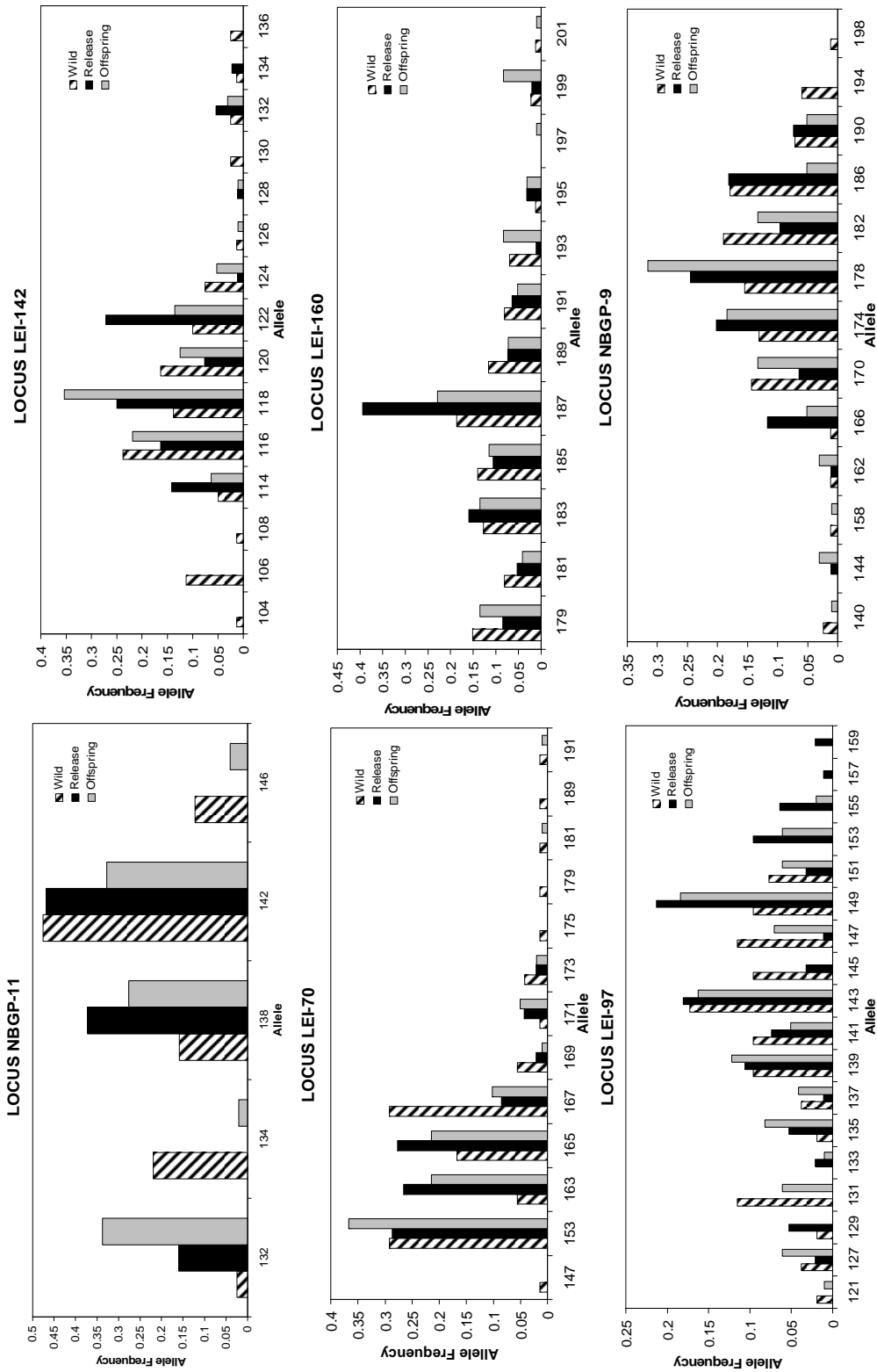


Figure 1: Allele frequencies per locus per population of wild, pen-reared, and F1 generation bobwhite at Ames Plantation, 2002.

Table 3: Gene diversity (H), number of alleles (N), allelic richness (RS), and inbreeding coefficient (FIS) averaged over all loci for wild, pen-reared, and F1 generation bobwhites at Ames Plantation, Tennessee in 2002.

Population	H	N	RS	FIS
Wild	0.841	11.300	10.250	0.035
Pen-reared	0.790	9.000	8.116	-0.029
F1 Offspring	0.814	10.000	8.920	0.032

admixture of alleles.

## Discussion

Our survival results were consistent with those of other studies (DeVos and Speake 1995, Fies et al. 2000, Perez et al. 2002) in that wild bobwhites experienced greater survival than pen-reared bobwhites. However, survival of pen-reared bobwhites in our study (12.2-29.8% fall-spring, 0.5-3.2% annual) was substantially greater than survival reported in most other studies of pen-reared bobwhites, except for Frye (1942). Oakley et al. (2002) reported 0-11% fall-spring survival for pen-reared bobwhites in Maryland whereas Roseberry et al. (1987) attained only 15% recovery of pen-reared bobwhites in Illinois. DeVos and Speake (1995) reported winter-spring (154-day interval) survival of approximately 18% for pen-reared bobwhites in Alabama. All game-farm birds in Fies et al. (2000) and Perez et al. (2002) died within 3 and 12 weeks, respectively, of release. Fall-spring survival of wild bobwhites in our study were similar to that on intensively managed plantations in Georgia (47.2%, 10-48.2%; Burger et al. 1998, Sisson et al. 2000, respectively) but greater than that reported for un-managed farmlands in Missouri (15.9%, Burger et al. 1995).

Guthery and Lusk (2004) suggested inherent negative bias in bobwhite survival from telemetry studies due to effects of radiomarking. However, Corteville (1998) reported similar survival for wild bobwhites fitted with mock transmitters as those with leg bands only. Although no studies of transmitter effects on released pen-reared bobwhites have

been conducted, we assumed that if transmitters negatively biased survival in wild bobwhites, similar biases would occur with pen-reared bobwhites. Secondly, pen-reared bobwhites were radiomarked and then released without an "acclimation" period whereas some wild bobwhites were radiomarked prior to the monitoring period and thus had greater time to adjust to radiomarking. Insofar as the above sources of bias may have influenced survival, our survival estimates likely reflect the lower bounds of pen-reared bobwhite survival. Regardless of potential telemetry induced bias, survival of pen-reared birds in our study was substantially greater than that reported in most other radio-telemetry studies; with several pen-reared bobwhites surviving to the breeding season.

Hybridization of genetic stocks has often been associated with beneficial results such as increased genetic diversity (Roy et al. 1994, Randi and Bernard-Laurent 1999) and greater survival and seasonal production (Niewoonder et al. 1998). However, several instances have been reported where purposeful or incidental re-stocking of species has led to hybridization, introgression of captive alleles, and eventual detrimental effects on native populations (Templeton 1986, Rhymer and Simberloff 1996).

As such, a common, although previously unsubstantiated, concern with bobwhite release programs is that pen-reared individuals may not be adapted to the local environment and may hybridize with wild individuals; thereby decreasing overall fitness of the local resident population (Rhymer and Sim-



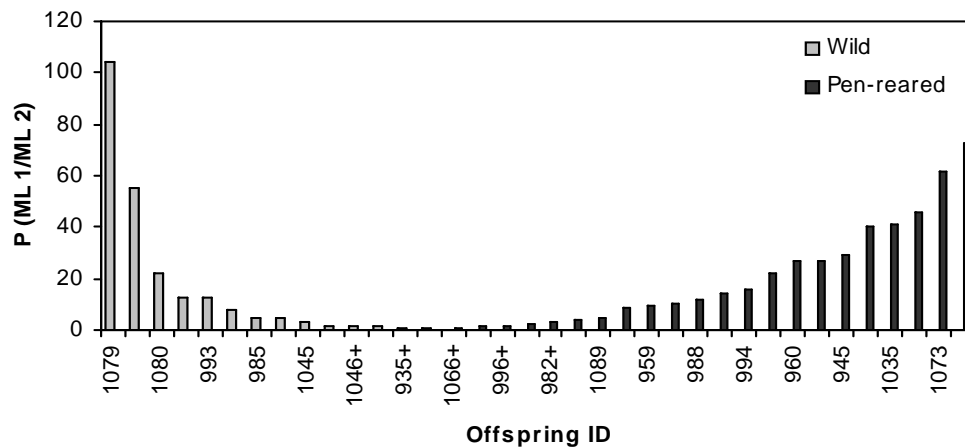


Figure 2: Probability of F1 generation bobwhites belonging to the most likely population (ML 1) divided by the probability of belonging to the second most likely population (ML 2) at Ames Plantation, 2002. +Individuals with a probability ratio approaching 1.00 represent ambiguous population assignments and are therefore probable hybrids. Excludes individuals that significantly belonged to the critical population.

berloff 1996). Nedbal et al. (1997) reported that wild bobwhites originating from south Texas did not contribute to reproduction when transplanted to east Texas due to differences between subspecies. Pen-reared and wild individuals shared several alleles across all 6 loci. However, we observed greater than five times as many private alleles in the wild population than in the pen-reared population (although only 2 possessed frequencies  $>0.05$ ); suggesting that the wild population possessed greater genetic variability at these particular loci. Similarly, Ellsworth et al. (1988) reported lower percentage of polymorphic loci in game farm than wild bobwhites.

Of greater concern was the subsequent lack of genetic diversity, number of alleles, and allelic richness observed in the F1 generation relative to the wild population. Cross-breeding of pen-reared and wild individuals was likely responsible for the reduced genetic variability we observed in the F1 sample. Less genetic variability in pen-reared populations is plausible given that most captive breeding systems expose birds to artificial selective forces (Roseberry et al. 1987, Ellsworth et al. 1988, Kozicky 1993) and transferring low genetic variability would occur when pen-reared and wild individuals cross-breed.

Pen-reared individuals released during the fall

of 2002 survived to and, as evidenced by our genetic analysis, reproduced successfully during the 2003 breeding season. Assignment tests demonstrated that pairs of pen-reared adults bred, pairs of wild adults bred, and some pen-reared adults may have bred with wild adults. We could not assign 20.4-33.6% of F1 generation individuals to either the wild or pen-reared population. Results from cluster analysis (33.6%) were greater than the estimate provided from the likelihood ratio analysis (20.4%). However, because the threshold value of 3.00 (by which  $P_{(ML1/ML2)}$  was compared) was a user-defined value, it may have produced more stringent results when compared to cluster analysis. There is no specified value of  $P_{(ML1/ML2)}$  to indicate a hybrid, only that as the value approaches 1.00 there is ambiguity in population assignment (Banks and Eichert 2000).

This ambiguity in population assignment for several F1 generation individuals was likely due to the high proportion of shared alleles between the wild and pen-reared individuals. However, our assignment test procedures utilized allele frequencies and not allele identity to classify individuals into populations. Future studies could use parentage analysis on a larger sample of individuals and loci to

determine with greater accuracy if these potentially hybrid individuals were truly hybrids.

Differential capture probabilities between F1 generation and wild bobwhites may have influenced substantially our results. Although pen-reared bobwhites have a greater tendency for recapture after release than wild bobwhites (Roseberry et al. 1987, L. W. Burger, Mississippi State University, personal communication); biases in capture probability of pen-reared offspring and offspring of pen-reared and wild hybrids is unknown. Similarly, capture probability of wild or hybrid offspring coveys containing pen-reared individuals is unknown. If offspring of two pen-reared birds or hybrid offspring exhibit greater capture probability than wild bobwhites, our results likely overestimated the introgression of pen-reared genetic material into wild populations. Therefore, within the limitations of our study, we recommend that our results be considered only as a cursory examination of determining the effects of pen-reared bobwhites on the genetic structure of local wild populations.

## Management Implications

Reduction of genetic variability in wild bobwhite populations has been a point of concern for several decades and only recently have researchers been able to feasibly study genetic structure and variability of wild populations. Observing that 73% of resident wild coveys contained pen-reared bobwhites on areas where pen-reared releases occurred, DeVos and Speake (1995) speculated that cross-breeding may produce biologically inferior offspring. However, no studies have yet examined the survival of F1 generation hybrids raised *in situ* by wild bobwhites. Given the 29.8% fall-spring survival of 3,200 pen-reared bobwhites released in 2002, a conservative estimate of 954 pen-reared bobwhites were alive at the beginning of the 2003 breeding season (1 April). Wild bobwhite density on the field trial course at Ames Plantation was estimated to be approximately 1 bird/0.6 ha (3,981 birds) during fall 2002 with an expected 2003 breeding population of 1,776 birds, assuming 44.6% survival. Pen-reared birds repre-

sented approximately 35% of the total 2003 breeding population. Therefore, we believe our estimates that 20.4-33.6% of the F1 generation birds captured during the fall of 2003 may have been pen-reared-wild hybrids are plausible; suggesting that cross-breeding of pen-reared and wild bobwhites likely occurred.

Given the relatively high fall-spring survival of pen-reared bobwhites combined with our observations of the genetic diversity of the F1 generation, we recommend that managers and researchers consider the potential effects of large-scale releases of pen-reared bobwhites on the genetic integrity of wild bobwhite populations. Additionally, we suggest that future research focus on genetic analysis of populations for multiple generations in areas where releases of pen-reared birds occur. Because we were only able to examine genetic variability for one generation following release of pen-reared bobwhites, we view this research as a precursor in determining the effects of pen-reared bobwhite releases on resident wild populations. We suggest future studies should incorporate a greater number of loci and larger sample sizes of individuals over multiple generations before definitive conclusions regarding the effects pen-reared bobwhite releases on the genetic variability of local wild bobwhite populations can be determined.

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