# An integrative study of social and reproductive systems in Northern Bobwhite (*Colinus virginianus*): a non-migratory, avian species bearing precocial young

by

Brant C. Faircloth

(Under the direction of John P. Carroll)

## Abstract

Northern Bobwhite (*Colinus virginianus*) inhabit variable environments. Individual behavior likely arose to address this environmental variability. Specific, resulting actions of bobwhites include group living, brood amalgamation, and breeding. I investigate these behaviors by integrating techniques from field and molecular biology to examine the effects of relatedness on individual cooperation and quantify the breeding behavior of bobwhites. My results suggest that kin selection does not affect bobwhite behavior and bobwhite may adopt behavioral strategies, flexibly expressed in the presence of environmental variation, to maximize individual fitness.

Index words: Northern Bobwhite, *Colinus virginianus*, Microsatellite DNA, Radio telemetry, Mating systems, Social systems, Integrative biology

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## Dedication

For Herbert Stoddard, Henry Beadel, and landowners of the Red Hills.

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Table of Contents

## List of Figures

# List of Tables

# Chapter 1

## Purpose of the Study

Order Galliformes contains species exhibiting varied social and reproductive systems. Social systems in this Order include both solitary (Frith 1959) and group living (Carroll 1994; Collias and Taber 1951; Schmitz-Ornés 1998) during the non-breeding season, mating systems range from purported monogamy to exploded lekking, and parental investment strategies extend from mound incubation with no parental care [e.g. Family Megapodiidae, (Frith 1956)] to bi-parental care extending from birth to sexual maturation [e.g. Gray Partridge (*Perdix perdix*)] (Benson 2002; Potts 1986; Rowden 2001).

Large-scale, contemporary studies of social and reproductive systems of galliformes are scarce. In lieu of research specific to these birds, studies among other avian Orders have been used as "models" for Order Galliformes. Although precocial, similar to members of Order Anseriformes, galliformes are typically non-migratory, removing the impact of this trait on their evolution. However, results of studies among Order Anseriformes are often applied to research and management of galliformes without verification of their applicability. For example, instances of intra-specific brood parasitism among pheasants (*Phasianus colchicus*) and other galliformes are often viewed within the host-parasite framework developed with data gained from waterfowl (Darling 1938; Eadie et al. 1988; Geffen and Yom-Tov 2001; Hill and Robertson 1988; Riedman 1982; Sayler 1992).

I propose that critical differences exist between life history strategies of non-migratory, precocial species and other taxa that preclude these comparisons. Strategies arising from the philopatric, non-migratory, and gregarious nature of galliformes and the potential for relatedness among neighbors across small spatial scales affects the framework within which

to investigate the social and mating system (Brennan 1999; Burger et al. 1995; Jenkins 1961; Potts 1986; Ridley 1983; Stoddard 1931).

Furthermore, characteristics of many galliformes potentially place them at an unstudied point along the continuum between cooperative breeding systems [e.g. Florida Scrub Jay (*Sericornis frontalis*); Red-cockaded Woodpecker (*Picoides borealis*)] and monogamy [e.g. Wilson's Storm-Petrel (*Oceanites oceanicus*)] (Quillfeldt et al. 2000; Walters et al. 1988; Woolfendon and Fitzpatrick 1984). For example, Northern Bobwhite (*Colinus virginianus*) reproduction may incorporate aspects of each of these system: individual mating behavior may be monogamous or polygamous (Baldini et al. 1952; Burger et al. 1995; Curtis et al. 1993; Stettner et al. 1966; Stoddard 1931) and brood rearing often incorporates individual, group, and communal tendencies (Faircloth et al. 2005, Chapter 4).

This dissertation integrates molecular genetic techniques (microsatellite genotyping and genetic sex-determination) with established capture, marking, and radio-telemetry methods to allow objective investigation of social and reproductive systems in a non-migratory galliforme: Northern Bobwhite (*Colinus virginianus*).

## 1.1 Historical Research

Studies of reproductive and social systems among galliformes are often limited to vague or conflicting conclusions arising from small sample sizes or methods of analysis insufficient to yield solid conclusions (Baldini et al. 1952; Burger et al. 1995; Curtis et al. 1993; Potts 1986; Ridley 1983; Stettner et al. 1966; Stoddard 1931). For example, social mate switching among "paired" Northern Bobwhites could not be readily identified until the advent of radio-telemetry and its application to the species of interest. Once social mate switching was identified, means of measuring and quantifying its occurrence became problematic (Burger et al. 1995). As a result, tests of hypotheses derived from social and mating theory are lacking within this group, in contrast to other avian Orders (Eadie et al. 1988; Emlen and Oring 1977; Gowaty 1983, 1996; Webster 1999).

Molecular approaches, while applicable to these questions, have been hindered by the lack of species-specific genetic markers providing the resolution necessary to compute measures of relatedness and parentage (Marshall et al. 1998; Nesje et al. 2000; Wetton and Parkin 1997). As a result, molecular-based studies among Order Galliformes have relied on universally amplifiable, mitochondrial DNA and examined taxonomic and phylogenetic questions arising from management issues (Bellinger et al. 2003; Benedict et al. 2003; Caizergues et al. 2003; Oyler-McCance et al. 1999; Van Den Bussche et al. 2003; Young et al. 2000).

## 1.2 Overcoming Previous Barriers

In order to overcome barriers inhibiting prior study of galliformes, two tasks must be accomplished. First, data sources and techniques from several fields must be integrated to allow objective collection and analysis of data. Second, the use of an abundant, easy to capture, model species is necessary to allow the efficient, non-destructive sampling of individuals and satisfactory sample sizes.

## 1.3 Choice of the Model Species

Within Order Galliformes, a logical species for investigating social and mating systems is the Northern Bobwhite (*Colinus virginianus*). Bobwhites are native, commercially important, habitat generalists with a distribution spanning much of temperate North America (Brennan 1999; Burger et al. 1999; Madge and McGowan 2002). Northern Bobwhites are highly philopatric, gregarious birds, and they are numerous over small spatial scales allowing collection and analysis of sample sizes likely to result in statistically valid inferences (Brennan 1999).

Unlike other North American Galliformes, [e.g. Wild Turkey (*Meleagris gallopavo*); Greater and Lesser Prairie Chicken (*Tympanachus* spp.)] bobwhites have neither been through recent, human-induced genetic bottlenecks, nor have populations been subject to extirpation and subsequent reintroductions (Bellinger et al. 2003; Eaton 1992). Bobwhites

are currently undergoing population declines throughout much of their range (Brennan 1991; Church and Taylor 1992). Fragmentation of suitable habitat within the bobwhite's range also threatens to reduce gene flow among populations, potentially increasing the risk of inbreeding within isolated populations (Brennan 1999). Molecular-based studies have been conducted among this group, but most have examined taxonomic and phylogenetic questions (Nedbal et al. 1997; Wehland et al. In Press).

Prior research provides a large body of basic, species-specific life history data (see Brennan 1999; Church and Taylor 1992, for review), and bobwhites appear to exhibit reproductive strategies consisting of diverse mating behaviors, nesting schemes, and patterns of parental care (Baldini et al. 1952; Burger et al. 1995; Curtis et al. 1993; Stettner et al. 1966; Stoddard 1931). As with similar species, barriers to current research include: quantification of relatedness within social groups, the correlation between family group membership and subsequent reproductive output, accurate identification of social mates, determination of genetic parentage, the relative contribution of reproductive strategies to population growth, and the evolutionary events resulting in observed patterns of behavior (Brennan 1999; Burger et al. 1995; Dimmick 1974; Klimstra 1950; Klimstra and Roseberry 1975; Simpson 1972; Stoddard 1931).

Bobwhite additionally differ from previously studied avian species with respect to kin selection theory as a result of: (1) their shorter lifespans [$<$ 1 year versus $>$ 4 years (Brennan 1999; Westcott 1970)], (2) different mating system [ambisexual polygamy versus cooperative breeding systems with long-term, monogamous pairings (Brennan 1999; Burger et al. 1995; Haig et al. 1993; Jackson 1994; Walters 1990, 1991)], and (3) different levels of parental care (precocial versus altricial offspring). Studies of bobwhite social groups and post-mating behavior suggest that some form of kin selection may be influencing bobwhite behavior (Faircloth et al. 2007). Additionally, some aspects of brood rearing reflect components of cooperative breeding systems with multiple males/females caring for broods amalgamated from several nests (Brooks and Rollins 2007; Faircloth et al. 2005).

Results from this study are applicable to other species within Order Galliformes, of which many are highly social and *r*-selected (Jenkins 1961; Ridley 1983); other avian Orders [e.g. Tinamiformes, (Bump and Bump 1969)]; and similar mammal species (*Microtus leucogaster, Oryctolagus cuniculus, Cynomys ludovicianus*; Hooglund 1996; Stalling 1990; Webb and Hewitt 1995).

## 1.4 Objectives

Current theory has rarely been applied and tested among Galliformes generally and bobwhites, in particular (*see* Charnov and Finerty 1980). Bobwhites exhibit high mortality, seasonal sociality, limited dispersal, high potential for intergroup relatedness, a flexible mating system, and recorded altruistic behavioral events.

Kin selection theory, non-breeding social structure, and post-hatching behavior suggest that Northern Bobwhite social groups exist as extended family groups maintained as a result of inclusive and direct fitness gains to group members (Faircloth et al. 2005; Hamilton 1964*a*,*b*; Stoddard 1931). Banding data show high degrees of relatedness within these groups, indicating parent-offspring group composition (Stoddard 1931, p. 169), which is predicted by the limited dispersal of either sex (Brennan 1999, B. C. Faircloth, unpublished data). However, high, natural mortality rates among bobwhite call into question the the role of kin selection theory when applied to this short-lived, precocial, non-migratory species (Brennan 1999, B. C. Faircloth, unpublished data).

This discrepancy suggests that there may be competing evolutionary processes. My premise is that *r*-selected species subject to unstable environmental resources adopt behavioral strategies, flexibly expressed (e.g. ephemeral social group structures, mating systems) to maximize overall fitness (Gowaty and Hubbell 2005; West-Eberhard 2003).

I investigated these issues by integrating large, individual-specific radio-telemetry, demographic, geographic and molecular data sets (Table 1.1). I collected extensive field data (Table 1.2) and developed a number of polymorphic, microsatellite DNA markers (Faircloth

et al. 2008; Schable et al. 2004) of which I selected a subset to use for molecular analyses (Table 1.4). In order to accomplish our objectives, I:

1. Examined the composition of social groups, the relatedness within and among social groups, and the effects of within-group relatedness on individual survival

2. Investigated the sex ratio of offspring using sex-specific, nuclear DNA markers, and evaluated these data in the context of individual behavior

3. Examined both the rate of post-hatch brood amalgamation in bobwhites using genetic data and the relationship between individuals and offspring within their broods

4. Investigated bobwhite breeding biology by combining individual social and reproductive data including knowledge of social mates, genetic mates, intraspecific nest parasitism, extra-pair fertilization, and the operational sex ratio

## 1.5 Experimental Plan

My research methodology comprised two major components. During 2000-2003, I collected demographic, radio-telemetry, and individual spatial data from a population of Northern Bobwhites on Tall Timbers Research Station (Figure 1.1) using capture, marking, and radio-location techniques described in the following chapters (Table 1.1). I developed species-specific, microsatellite primers for use with bobwhite (Faircloth et al. 2008; Schable et al. 2004), and I used a subset of the developed markers (Table 1.4) to generate our molecular data set. Finally, I integrated these data with the data gained from the field study to complete the stated objectives of this project.

### 1.5.1 Sample Size

Since this work was based on the capture, sampling, and observation of individuals within a *wild* bobwhite population, I did not compute sample size on the basis of an *a priori*

significance level. Our goal was to capture ≥70% of bobwhites present on the study site. I captured, radio-tagged, and took genetic samples from 179, 175, and 267 adult and juvenile bobwhite during 2001-2003 from 15, 19, and 19 unique coveys, respectively (Table 1.2). Given October density estimates of 1.5, 1.7, 2.7 quail per ha during 2000-2002 (W.E. Palmer, *unpublished data*), conservative estimates of percent population captured were 62%, 73%, and 62%, respectively. Jolly-Seber (J-S) (Jolly 1965; Pollock et al. 1990; Seber 1965) and Lincoln-Peterson (L-P) (Lincoln 1930) estimators suggest I captured a slightly lower percentage of the population (Table 1.3). Note the variance of these parameter estimates is high, particularly the Jolly-Seber estimates, largely as a result of low survival during 2001 and 2003. During the breeding seasons of 2001, 2002, and 2003, I captured, sampled, and marked 230, 468, and 143 neonatal bobwhite (Table 1.2). Although I did not capture 100% of the population, the number of individuals across the study area should adequately represent variation in mating and social behavior behavior of individuals in this population.

## 1.6 Justification of Field and Laboratory Methods

### 1.6.1 Selection of a Marker System

Although there are several molecular marker systems available that would facilitate the study of social and reproductive systems in Northern Bobwhite, I chose to use microsatellites throughout the study. Microsatellites (also known as VNTRs, SSRs, etc.) are highly variable regions of "non-coding", nuclear DNA (deoxyribonucleic acid). These regions exist as duplicated or repetitive sequences in the DNA, and microsatellites are generally thought to be selectively neutral (Ellegren 2000). Organisms possess a variable number and density of microsatellites which are composed of 5-40 copies of 1-10 base pairs and, generally, ≤100 total base pairs in size (Selkoe and Toonen 2006). Mutation rates for these regions are $10^{-2}$ to $10^{-5}$ units per locus per generation and significant heterogeneity is seen in these rates at the species, locus, and allelic levels (Ellegren 2000). Neither the function nor the significance of microsatellites is fully understood (Scribner and Pearce 1999).

Microsatellite loci are studied by amplifying the target region of the genome containing these repeats using species-specific primers in combination with the polymerase chain reaction (PCR) (Mullis 1985; Selkoe and Toonen 2006). End-labeling PCR primers with a fluorescent dye and subsequent electrophoresis through a polymer matrix allows visual or automated scoring of alleles that differ in size by as little as 0.5 base pairs (Applied Biosystems 2003; Parker et al. 1998). Binned differences in allelic positions on the gel are used, in conjunction with statistical methods, to determine the degree of relatedness between and parentage of individuals (Hadfield et al. 2006; Marshall et al. 1998; Thompson 1975, 1986).

Given the power of the PCR process, the materials used for acquisition of template DNA are numerous. DNA may be extracted from sources such as hair, plucked and shed feathers, feather pulp, and skin cells (Eguchi and Eguchi 2000; Levy 1999; Marsden and May 1984; Taberlet and Bouvet 1991; Taberlet and Luikart 1999). Use of tissues, such as plucked feathers or skin micro-biopsies, in conjunction with PCR techniques, removes the need for destructive sampling of individuals.

Single-nucleotide polymorphisms (SNPs) are another marker system yielding sufficient resolution to address questions related to bobwhite social and reproductive systems. On a per-locus basis, SNPs are less expensive to genotype and the resulting data simpler to analyze, in part due to a known mutational model (Morin et al. 2004; Weir et al. 2006). However, there is: (1) no current SNP panel available for use with bobwhites, and (2) the discriminatory power of biallelic SNP loci is greatly reduced in comparison to microsatellite loci (see Weir et al. 2006, for review).

### 1.6.2 Social group relatedness

Scant information exists regarding the dynamics and processes by which social groups form in Order Galliformes (Agee 1957; Guthery 2000; Jenkins 1961; Lehman 1984; Stoddard 1931). Banding of individual Gray Partridge (*Perdix perdix*), a member of Order Galliformes, suggest a brood and its respective parents typically form the basic social group with unsuc-

cessful breeders included among family members (Jenkins 1961). Several families may merge, resulting in social groups, similar to those seen among bobwhites, of 5 to 15 individuals with group sizes occasionally >25 members. Both the relationships within and among these social units and the overall function of this mixing are unknown (Jenkins 1961).

Among bobwhites, several studies investigated social group formation during the non-breeding season to determine membership dynamics and the resulting spatial relationship of coveys (Agee 1957; Stoddard 1931). Similar to the research of Jenkins (1961), these studies relied on banding data for a limited number of individuals yielding insufficiently supported conclusions, largely as a result of small numbers of individual observations. During the breeding season, studies using radio-telemetry to investigate the composition of social groups have had limited success in accurately describing their duration or significance (Curtis et al. 1993).

Genetic markers are successfully used to determine relatedness within and among social groups of mammals (Ortega et al. 2003; Ralls et al. 2001; Spong et al. 2002; Valsecchi et al. 2002) and birds (Parker and Lundy 1999; Quinn et al. 1999). Typically, estimates of individual pairwise relatedness and within-group relatedness, in conjunction with data describing group membership, provide the resolution necessary to determine social group composition (Queller and Goodnight 1989; Stone and Bjorklund 2001). Similarly, spatial data, combined with estimates of intra- and inter-group relatedness of individuals allow investigation of the structure of social groups across the landscape (Matocq and Lacey 2004).

Individual radio-location enables observation of temporally-relevant social group membership and the spatial movement of social groups. Microsatellite data allow the estimation of relatedness within and between coveys both spatially and temporally (Queller and Goodnight 1989; Thompson 1975, 1986). The combination of these techniques allows investigation of the effects of relatedness on demographic parameters of individuals within groups and the relationship between groups across the landscape.

### 1.6.3 Sex-ratio of neonates

Sex ratios in non-migratory, precocial neonates have not been investigated using accurate techniques. Similarly, sex ratios among bobwhite neonates are unknown because they have no external markers of sex and are not plumage or size dimorphic as neonates. (Brennan 1999; Petrides and Nestler 1943; Roseberry and Klimstra 1984). Sex ratios in neonatal bobwhites were previously impossible to determine (due to plumage monomorphism) without sacrificing sufficient numbers of neonates, affecting population composition. Similar to techniques used with other precocial species, sex ratios within bobwhite populations were formerly investigated using data gained from counts and/or monitoring of older, dimorphic individuals at various periods during the year. These studies indicated a bias in sex ratio towards females during periods of population increase and towards males during periods of population decline (Roseberry and Klimstra 1984). However, annual survival rates of male bobwhites are greater than females, and juvenile bobwhite are subject to differential survival rates during the fall and winter following hatching (Leopold 1945; Pollock et al. 1989). Therefore, prior results are questionable, and sex ratios should be investigated using genetic techniques with populations at an earlier age.

Furthermore, given the role of operational sex-ratio [OSR, (Emlen and Oring 1977)] in the ambisexually polygamous mating system [one of the potential mating systems ascribed to bobwhite (Burger et al. 1995)], knowledge of both neonatal and juvenile/adult sex ratios is necessary. This knowledge will allow investigation of: (1) the impact of sex ratio skew on subsequent offspring sex ratios and the persistence of the mating system, and (2) the factors affecting operational sex ratio (e.g. does skew in OSR arise from pair bonds, differential mortality, or skew in neonatal sex-ratio).

The impact of maternal condition on offspring sex-ratio and the potential of females in good condition to bias this ratio toward sons (Trivers and Willard 1973) are of additional interest. Adaptive alteration of offspring sex ratios is reported for species both within (Parker

2002) and outside (Albrecht 2000; Ellegren et al. 1996; Ligon and Ligon 1990; Nager et al. 1999) Order Galliformes. Little is known regarding this behavior in Northern Bobwhites.

Molecular sex determination, in combination with capture and radio-location techniques, provides a means to investigate sex ratios of neonatal bobwhite and the factors influencing them (Fridolffson and Ellegren 1999; Griffiths et al. 1996, 1998). We selected the 2550F and 2718R primers developed by Fridolffson and Ellegren (1999) for genetic sex determination. These primers amplify the conserved CHD (chromo-helicase-DNA-binding) gene located on avian sex chromosomes (W and Z). We selected these primers over the P2 and P8 primers of (Griffiths et al. 1996, 1998) because the P2, P8 amplicon of the bobwhite Z chromosome is polymorphic (Casey et al. 2007; Dawson et al. 2001), leading to ambiguity in the molecular assignment of sex.

### 1.6.4 Post-hatch brood amalagamation

Brood amalgamation is largely unstudied in Order Galliformes. Post-hatch brood amalgamation occurs when organisms incubate and hatch their own young then group their offspring with related/unrelated offspring of additional individuals (Afton and Paulus 1993; Eadie et al. 1988). Several studies investigating brood amalgamations conducted among Order Anseriformes resulted in the creation of testable hypotheses (Darling 1938; Eadie et al. 1988; Riedman 1982, Chapter 4). However, few researchers have quantified the costs and benefits of this behavior, and there has been little evaluation of these costs and benefits in light of the hypotheses developed.

Among Galliformes, neither the occurrence nor the frequency of brood amalgamation are well documented, and data suggesting its occurrence is largely anecdotal. Stoddard (1931) first discussed the potential for post-HBA among Northern bobwhite after conducting a series of adoption trials using penned birds. He found 90% of individuals adopted unrelated chicks placed in their pens. Based on capture records collected during fall, he theorized that amalgamation of broods was occurring among wild birds. Lehman (1984) reported lone

females with young were likely to join other females with similarly-aged young, and he noted that, "several bobwhite families were observed with extra adults or young". Three percent of bobwhite broods in Oklahoma exhibited a net gain of chicks from hatching to 20 days old with 25.4% of broods exhibiting net gains from hatch to 29-30 days old (DeMaso et al. 1997). Brooks and Rollins (2007) report several occurrences of "gang-brooding" observed among radio-collared bobwhites in Texas, and anecdotal reports of brood mixing among bobwhites in Missouri are also presented by Burger et al. (1995).

Faircloth et al. (2005) reported that post-hatch brood amalgamation among bobwhite occurs at a high rate (approximately 50%) during years of increased reproductive output. However, their methods of determining the rate of post-HBA may have resulted in an underestimation of its frequency. Additionally, these methods did not provide a means of determining the relationships: (1) among chicks within each brood or (2) between parents associated with a particular brood and the offspring it contained.

In order to evaluate the occurrence, extent, and factors influencing post-HBA among Galliformes, the relationships between brood members and the potential effects on inclusive fitness resulting from the amalgamation of broods must be known. Therefore, the use of parentage inference and relatedness calculations within amalgamated groups is necessary. Additionally, this information is critical to understanding the reproductive system of bobwhite. Knowledge of factors affecting post-hatch brood amalgamation and its degree of occurrence will allow creation, refinement, testing, and refutation of hypotheses explaining its origin and purpose.

### 1.6.5 Mating System of the bobwhite

#### Intraspecific nest parasitism and Extra-pair fertilization

Among non-migratory species bearing precocial young, intraspecific nest parasitism [also know as conspecific brood parasitism (CBP)] is reported for several species (Baskett 1947; Mackie and Buechner 1963; Martin 1984; Redfield 1973). Several reports of INP exist for

the Northern bobwhite, as well (Rosene 1969; Stoddard 1931). However, prior identification of this event used techniques including: recognition of eggs in the nest that did not belong, observation of the parasitic event, identification of abnormally large clutches, or the appearance of eggs out of sequence (Yom-Tov 1993). For example, Rosene (1969) used identification of abnormally shaped or pigmented eggs within clutches to estimate brood parasitism among bobwhite. Unfortunately, among precocial species, high natural variability in these parameters makes the occurrence and degree of INP challenging to estimate (MacWhirter 1989; Stoddard 1931). These techniques cannot be trusted to provide accurate measurements of the occurrence and extent of intra-specific nest parasitism within bobwhites.

Similarly, scant information exists regarding the occurrence and degree of extra-pair paternity (EPP)/fertilization in Galliformes. Non-genetic methods of estimating this behavior in other species include observation of radio-tagged birds or measurements of differences in tarsus length between offspring and their assumed parents (Alatalo et al. 1984; Curtis et al. 1993; Hasselquist et al. 1995; Møller and Birkhead 1993). However, these methods have numerous drawbacks, particularly when studying a large number of individuals. These include: the inability to constantly monitor bird pairs, the assumption that parents contribute an equal proportion of non-genetic influence to the expression of phenotypic traits, natural variability in measurement parameters, and observer-based measurement bias. Given these biases, non-genetic methods often result in an overestimation of the degree of EPP within species (Hasselquist et al. 1995).

Among Order Passeriformes, molecular techniques are commonly used to document the occurrence and quantify the degree of extra-pair fertilization (EPF) and intraspecific nest parasitism (INP) (Gissing et al. 1998; Gowaty and Bridges 1991; Gowaty and Karlin 1984; Hasselquist et al. 1995; Meek et al. 1994; Whittingham et al. 1997). Studies of INP among migratory waterfowl species led to the development of several hypotheses potentially explaining its evolution (Erskine 1990; Joyner 1983; Lokemon 1991; Sayler 1992; Semel et al. 1988). However, these hypotheses may not be generally applicable to other Orders

bearing precocial young. The use of sufficiently polymorphic molecular markers combined with adequate sampling of the population under study allows the use of parentage inference to determine the occurrence and extent of these behaviors within populations (Hughes 1998; Marshall et al. 1998; Slate et al. 2000).

### 1.6.6 Relationships within breeding pairs

Temporal aspects of mate pairing are unknown, due largely to the high annual mortality rate of bobwhite populations (Brennan 1999). Additionally, the equivalence of social mates (as observed using radio-location) with genetic mates is in doubt, and the radio-telemetry studies of Burger et al. (1995) and Curtis et al. (1993) do not provided sufficient resolution to quantify this disparity. This lack of resolution may be a source of the variation in descriptions of the bobwhite mating system. Some pairs are dynamic (e.g. mate switching occurs at various rates) throughout the breeding season (Curtis et al. 1993) whereas others are static (Stoddard 1931), and there is anecdotal information that some breeding pairs are maintained across years (Roseberry and Klimstra 1984, B. C. Faircloth, unpublished data). Additionally, social pairs are often joined by seemingly random male-female pairs or mixed-sex groups for short periods of time during the breeding season. The relationships between these coalescent groups of individuals is unknown, as is the function of their coalescence. Therefore, the mating system of Northern bobwhite has been described as exhibiting aspects of monogamy, rapid multiclutch polygamy, ambisexual polygamy, and promiscuity (Baldini et al. 1952; Burger et al. 1995; Curtis et al. 1993; Stettner et al. 1966; Stoddard 1931). As previously noted, this flexibility potentially extends to parental investment in both clutches and broods (Brennan 1999; DeMaso et al. 1997; Faircloth et al. 2005; Lehman 1984; Stoddard 1931).

The observation of mated-pairs using radio-location, in combination with parentage inference and relatedness inference, allows clarification of the above questions. These data must be combined with analyses of: non-breeding season social group composition, extra-pair fertilization, intraspecific nest parasitism, neonatal sex ratios, and post-hatch brood amalgamation

to determine both the mating behavior of individuals and the social systems of individual bobwhite.

## 1.7 Scientific and Management Impacts

This dissertation addresses questions of sociality and fitness in a gregarious, *r*-selected vertebrate species. It begins to fill a significant gap with respect to the study of kin selection and family group membership among galliformes by investigating the evolution and maintenance of social and breeding behaviors. Additionally, this research helps create the framework for future investigations of social and reproductive behaviors in similar species and establish methods for their quantification. Our data should also aid future, Order-wide comparisons of reproductive behaviors.

With respect to population management, this study provides a better understanding of social and reproductive factors affecting bobwhite populations and aids the construction of population growth and management models. Additionally, this research suggests testable theories and hypotheses while aiding future projects examining reproductive strategies, population subdivision, range-wide genetic health, and the effects of translocation on bobwhite populations. Additionally, these data will allow future genetic comparisons between past and present populations to examine either temporal changes in or spatial distribution of: allele frequencies, population subdivision, and population genetic "health" (Glenn 1996).

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Table 1.1: Social, spatial, demographic, and molecular data sources, collected during 2001-2003 on Tall Timbers Research Station, addressing the objectives of this research.

| | Telemetry | GIS | Molecular | Demographic |
|---|---|---|---|---|
| Age, Sex, Weight, Condition at each capture | | | | ⊗ |
| Social Group Movements | ⊗ | | | |
| Social Group Membership | ⊗ | | ⊗ | |
| Social Group Survival | ⊗ | | | |
| Individual Movements and Mate Associations | ⊗ | | | |
| Mate Identification | ⊗ | | ⊗ | |
| Brood Membership | ⊗ | | ⊗ | |
| Brood Movements | ⊗ | | | |
| Parentage Inference/Brood Relatedness | | | ⊗ | |
| Yearly Study Site Maps | | ⊗ | | |

Table 1.2: Summary data from capture, genetic sampling, and radio-collaring of Northern Bobwhite at Tall Timbers Research Station during 2001-2003. Frequency of telemetry locations gives the weekly frequency during the non-breeding and breeding seasons with which I located all radio-collared birds.

| Year | Captured | | Telemetry Locations | | Brood Capture | | |
|---|---|---|---|---|---|---|---|
| | Females | Males | Non-breeding Frequency | Breeding Frequency | Total Captures | Distinct Broods | Chicks Captured |
| 2001 | 77 | 101 | $\geq$5/week | $\geq$14/week | 29 | 20 | 230 |
| 2002 | 88 | 87 | $\geq$5/week | $\geq$7/week | 77 | 45 | 468 |
| 2003 | 143 | 125 | $\geq$5/week | $\geq$7/week | 24 | 15 | 143 |

Table 1.3: Percentage of the bobwhite population captured during 2001-2003 estimated using Jolly-Seber ($\hat{n}_{J-S}$) and Lincoln-Peterson ($\hat{n}_{L-P}$) estimators(Jolly 1965; Lincoln 1930; Pollock et al. 1990; Seber 1965).

| Year | Captures | $\hat{n}_{J-S}$ ($\pm 95\% CI$) | J-S Percent | $\hat{n}_{L-P}$ ($\pm 95\% CI$) | L-P Percent | Mean Percentage |
|---|---|---|---|---|---|---|
| 2001 | 172 | 353 ($\pm$220.4) | 48.8% | 257 ($\pm$118.7) | 66.9% | 57.9% |
| 2002 | 176 | 209 ($\pm$92.5) | 84.1% | 260 ($\pm$99.8) | 67.7% | 75.9% |
| 2003 | 265 | 477 ($\pm$194.5) | 55.6% | 375 ($\pm$79.6) | 70.7% | 63.1% |

Table 1.4: Characterization of 16 optimal primer pairs amplifying microsatellite loci from Northern Bobwhite compiled from Faircloth et al. (2008); Schable et al. (2004) and tested among adult and juvenile birds ($n = 605$) captured during 2001-2003 on Tall Timbers Research Station.

| Locus | Primer Sequence | Accession Number | Repeats in Cloned Allele | n | Number of Alleles | Size Range |
|---|---|---|---|---|---|---|
| CV-P1A7U | GTTTGTAGCACAGAGATGCTTG | EF687961 | $(GTT)_{10}$ | 592 | 7 | 334-352 |
| CV-P1A7L | CAGTCGGGCGTCATCAGTGCAGATGGATGTCAGCAG | | | | | |
| CV-P1F2U | CAGTCGGGCGTCATCAGTAAACTGCAGATGCAAAC | EF687965 | $(GT)_8$ | 583 | 12 | 232-260 |
| CV-P1F2L | GTTTGTGCCATAGGTCTCCCCTG | | | | | |
| CV-P1F3U | GTTTGTCACCCATCTCCCTGAATA | EF687966 | $(AC)_{12}$ | 591 | 11 | 382-406 |
| CV-P1F3L | CAGTCGGGCGTCATCACTCAGGCTGTATTGACACAAG | | | | | |
| CV-P1H12U | CAGTCGGGCGTCATCACCAATTAGCAAGGTGTTTGCC | EF687969 | $(GTTT)_6$ | 588 | 6 | 315-339 |
| CV-P1H12L | GTTTGAGGACTGTTGCATCCCAG | | | | | |
| CV-P2D7U | GTTTACACTGGCTTGGTGCCTC | EF687983 | $(AAAC)_6$ | 596 | 2 | 265-269 |
| CV-P2D7L | CAGTCGGGCGTCATCACTGCTGCACTTGTGGTCTG | | | | | |
| CV-PA12AU | GTTTGGCTGAACTGATGTATTAG | AY523014 | $(AC)_{23}$ | 597 | 20 | 210-261 |
| CV-PA12AL | CAGTCGGGCGTCATCACCTTCACTCCTGGAGTATTAG | | | | | |
| CV-PA12GU | CAGTCGGGCGTCATCAAACAGTTCAAGTCTTCAAATA | AY523018 | $(AC)_{35}$ | 595 | 24 | 120-177 |
| CV-PA12GL | GTTTGCAGTCTTCGCTTGATAC | | | | | |
| CV-PA1CU | CAGTCGGGCGTCATCAGCATTTGCGTTTAAGTAAG | AY522953 | $(AAAC)_6$ | 600 | 4 | 233-245 |
| CV-PA1CL | GTTTCCAAGCTCAGTGTCTAAGA | | | | | |
| CV-PA1FU | GTTTGTTGCCTTTCTTGTAA | AY522956 | $(AC)_{13}$ | 585 | 20 | 138-186 |
| CV-PA1FL | CAGTCGGGCGTCATCAATAAAACCAAAGATCATTAGTA | | | | | |
| CV-PA3EU | GTTTCATTACCGGGTTCTTATTCTC | AY522966 | $(ACAG)_4$ ...$(AGAT)_{15}$ | 580 | 15 | 357-433 |
| CV-PA3EL | CAGTCGGGCGTCATCAAAAACTGCTTACTACCATACAG | | | | | |
| CV-PA3FU | CAGTCGGGCGTCATCAGATTCACTGCATTTGTTAG | AY522967 | $(AAAG)_{16}$ | 595 | 19 | 228-308 |
| CV-PA3FL | GTTTGGCAGAAACTTATGACAT | | | | | |

Table 1.4 – Continued

| Locus | Primer Sequence | Accession Number | Repeats in Cloned Allele | n | Number of Alleles | Size Range |
|---|---|---|---|---|---|---|
| CV-PA3GU<br>CV-PA3GL | GGAAACAGCTATGACCATGAAATGTGTTGAAGGAAGTAT<br>GTTTCTGTTTGCCTCCAGTC | AY522968 | $(AAAC)_6$ | 600 | 6 | 150-168 |
| CV-PA5FU<br>CV-PA5FL | CAGTCGGGCGTCATCAATAAAACCAGAAATAAACTCA<br>GTTTCGATCCACCTGAAAGTA | AY522978 | $(AC)_{14}$ | 584 | 19 | 276-320 |
| CV-PBA4U<br>CV-PBA4L | CAGTCGGGCGTCATCAATCAGCCCTCTGCTCC<br>GTTTACAACTTTCTGTCAACCTCATCG | EF687986 | $(AAAC)_5$ | 547 | 7 | 402-418 |
| CV-PBH5U<br>CV-PBH5L | GTTTGCCACATTAACAGGAACGGG<br>GGAAACAGCTATGACCATGAGTGAGGCAACATGACAGC | EF687997 | $(AC)_7$ | 598 | 2 | 230-232 |
| CV-PCF5U<br>CV-PCF5L | CAGTCGGGCGTCATCACCTGGCTGCTTTAGACATA<br>GTTTACAGGCTGAAATCATAACAG | EF688003 | $(AC)_{11}$ | 595 | 16 | 193-225 |


Figure 1.1: Tall Timbers Research Station, Tallahassee, Florida. The subdivided area in dark gray was the study area for the entirety of this research.

# Chapter 2

## Group Living in the Absence of Relatedness Effects: Composition, Relatedness, and Demographics of Northern Bobwhite (*Colinus virginianus*) Social Groups [1]

[1]Faircloth, B. C., W. E. Palmer, T. M. Terhune, and J. P. Carroll. To be submitted to *Animal Behaviour*.

## 2.1 Abstract

Northern Bobwhite (*Colinus virginianus*) live in social groups of varying size and composition throughout the non-breeding season, similar to many species within Order Galliformes. Dispersal of individuals from the area inhabited by each group is low; prior research suggests individuals within groups may be relatives; proximate groups interact; individuals within groups may be related; and individuals and groups may experience differential mortality. These observations suggest kin selection may explain the evolution and maintenance of group living in bobwhites. However, avoidance of predation and dilution of predation risk or the accrual of benefits derived from social foraging may alternately explain the evolution and maintenance of bobwhite social groups. In this manuscript, we examine the applicability of kin selection theory to bobwhite social groups by estimating inter- and intra-group relatedness and the effects of relatedness on individual survival. Our results suggest that while some bobwhite social groups are composed of relatives, group relatedness does not effect individual survival, calling into question the applicability of kin selection theory to group formation in this species.

## 2.2 Introduction

Social group formation occurs in many galliformes including members of the families Phasianidae (Collias and Taber 1951; Jenkins 1961; Swenson et al. 1995), Odontophoridae (Brown et al. 1998; Calkins et al. 1999; Carroll 1994; Hale 2006), Numididae (Martínez 1994), and Cracidae (Schmitz-Ornés 1998). The evolution and maintenance of group formation among Galliformes are often explained in the contexts of: predation avoidance (Hamilton 1971; Pulliam 1973; Taylor 1976; Watson et al. 2007), thermoregulation (Case 1973), group foraging (Pulliam and Millikan 1982), and the formation of mating pairs and establishment of dominance hierarchies (Collias 1952; Gee 2003). Social group formation is a persistent feature of

galliformes biology throughout the range of habitat types and climates inhabited by these species.

Northern Bobwhite (*Colinus virginianus*) typify many of these species: they are small, gregarious, ground dwelling, habitat generalists, with a large range consisting of varied habitat types. Bobwhites live in social groups of moderate size, known as coveys, during the non-breeding season (November - April). Individual dispersal is limited in moderate- to high-quality habitat (Brennan 1999; Cook 2004; Stoddard 1931) particularly throughout the southeastern United States (*cf.* Lehmann 1946; Townsend et al. 2003). Similar to its parent Order, the evolution and maintenance of social groups in bobwhites are attributed to advantages arising from increased thermoregulatory efficiency (Case 1973), group vigilance and predation avoidance (Williams et al. 2003), increased foraging efficiency (Case 1973; Williams et al. 2003), and combinations thereof.

Few studies have investigated the the role of Hamilton's rule and kin selection theory (Hamilton 1964*a*,*b*) in the evolution and maintenance of bobwhite social groups. Hamilton's rule provides an explanation for the evolution of altruistic behavior, of which group living may be defined as a subset, and states that altruistic behavior occurring between two individuals is explained as a function of: intra-individual relatedness, costs of the behavior to the altruist, and benefits of the behavior to the recipient. In essence, relatedness between two individuals can offset the effect of behavior costly to the altruist. Group living to avoid predators and/or forage may be explained in this context (West-Eberhard 1975).

By extension, kin selection is the process through which altruistic behaviors are favored; altruistic behavior confers an indirect advantage to one's genes by increasing their persistence in future generations via the survival and reproduction of relatives (Hamilton 1964*a*,*b*; West-Eberhard 1975). While the existence of relatedness between individuals does not, in itself, guarantee that kin selection is the source of a particular behavior (Griffin and West 2002), the absence of relatedness between group members or the lack of an effect of relatedness

on vital rates (fitness) is inconsistent with kin selection as an explanation for particular behavior.

Kin selection predicts that social groups consist of related individuals and that the cooperation of related individuals confers fitness on individuals (directly, indirectly, or inclusively) thus favoring grouping behavior (Hamilton 1964*b*). By extension, one would expect groups composed of closer relatives to exhibit higher fitness relative to groups composed of unrelated individuals. Furthermore, proximate groups, known to interact with one another, should be related, and the relatedness between groups should decrease as a function of inter-group distance. Group membership should also be stable; individuals should rejoin the same kin-groups in successive years, provided they have survived.

Galliformes, in general, and bobwhite, specifically, serve as models with which to investigate the applicability of kin selection theory to social group formation. Stoddard (1931) initially reported social groups comprised unrelated individuals, mated pairs, and offspring from the previous breeding season. Similar studies of the Gray Partridge (*Perdix perdix*) indicate that social groups consist of both related and unrelated individuals (Jenkins 1961). Band returns from juvenile bobwhites tagged as neonates show that surviving offspring belong to the same social groups (B.C. Faircloth, *unpublished data*). Bobwhite social groups, or coveys, interact with one another by feeding in the same areas, issuing alarm calls, and occasionally combining membership (Stoddard 1931). Roseberry and Klimstra (1984) report that group membership carries over during successive years (*cf.* Yoho and Dimmick 1972). Recent research further suggests that individuals within particular social groups experience differential mortality when compared to those is adjacent groups (Faircloth et al. 2005) that may be correlated with the degree of group relatedness.

In this manuscript, we investigate the relatedness structure of a population of wild bobwhites; the degree of intra- and inter-group, individual relatedness; the effect of year, group size, and inter-group distance on intra- and inter-group relatedness; and the effects of intra-group relatedness on group survival, which we use as an index of fitness.

## 2.3 Methods

### 2.3.1 Capture of Adult and Sub-adult Bobwhites

We captured adult and sub-adult bobwhites during October-March 2000-2003 on a 250 ha area of Tall Timbers Research Station, Tallahassee, FL. We identified social group home-ranges within the study area, based on radio-locations of previously captured birds and pointing dog searches of the areas, and we placed $\geq 5$ funnel traps (Stoddard 1931) baited with grain sorghum within these areas. Following capture, we determined weight, sex, and age of each bird, and we applied aluminum leg-bands to all captured birds. We collected 10 body feathers from the flank of each bird captured, and we stored 5 feathers in 70% EtOH for genetic analysis and 5 feathers in paper envelopes as a reserve. We fitted all bobwhites $> 150$ g with a 6 g, necklace-style radio-transmitter (American Wildlife Enterprises, Monticello, FL) and released each within 100 m of their capture site. Radio-tagging bobwhites in this manner does not affect survival (Folk et al. 2007; Palmer and Wellendorf 2007; Terhune et al. 2007). When flush counts of radio-collared individuals within social groups indicated they contained unsampled individuals, we conducted additional trapping, sampling, and radio-tagging until all individuals within a social group were captured.

Beginning 15 January and extending until approximately 1 April, we located all radio-tagged birds 4-5 times per week using the homing method (Kenward 2001; White and Garrott 1990). Using our knowledge of birds captured together in combination with the radio-location of individuals, we determined which birds were present within social groups. We considered all birds located within 10 m of one another during a period of approximately 5 min. to be associates within a social group.

On a geographic information system (GIS) map of the study area, we recorded all radio-frequencies and corresponding band numbers of individuals located within each group, along with the date and time of the location. We determined that individuals: (1) normally a part of the group being located, but (2) located $> 10$ m from other associates comprised a sub-

group. They maintained this status until regrouping with associates, transitioning to a new group, or dying.

We converted all group locations to UTM coordinates and input these, along with date, time, group or sub-group number, and the band numbers of all group associates to a relational database. We attempted to flush all birds that did not appear to be moving or were not located within their typical group or sub-group in order to determine their status. In all cases, we either flushed the bird or located its radio-collar and/or carcass and recorded the individual as a mortality.

### 2.3.2 Microsatellite Genotyping

We extracted DNA from collected feathers using DNeasy kits (Qiagen Inc., Valencia, CA) with a modification to the digestion step, adding 25 $\mu$L of 100 $m$g/$m$L DTT (Dithiothreitol) along with proteinase K. We eluted DNA from the membrane with either: 2 washes of 60 $\mu$L Buffer AE or 1 wash 120 $\mu$L Buffer AE. Prior to amplification, we treated all samples 1:3 with 10% Chelex resin (BioRad Laboratories, Hercules, CA) to remove PCR inhibitors. We performed 96-well PCR amplifications of 16 microsatellite loci (Table 1.4; Faircloth et al. 2008; Schable et al. 2004) in 10 $\mu$L volumes using CAG- or M13R-tagged primers (Glenn and Schable 2005). Reaction concentrations were 0.5 U AmpliTaq Gold (Applied Biosystems, Foster City, CA), 1X Gold Buffer, 1X BSA (New England Biolabs, Ipswich, MA), 1.5 $m$M MgCl, 1.25 $m$M dNTPs, 0.5 $\mu$M untagged primer; 0.05 $\mu$M CAG or M13-reverse tagged primer, 0.45 $\mu$M dye-labelled tag (HEX, FAM, NED + CAG or M13-reverse), 3.3 $\mu$L ddH2O, and 2 $\mu$L DNA template (5-10 $n$g). We included multiple negative controls in each plate of PCR reactions.

We used one of two locus-dependent touchdown thermal cycling profiles (Don et al. 1991), each encompassing a 10 ℃ span of annealing temperatures (ranges: 60 - 50 ℃; 65 - 55 ℃). Cycling parameters included a Taq activation step at 95 ℃ for 5 m followed by 20 cycles at 95 ℃ for 20 s; 60 or 65 ℃ for 30 s minus 0.5 ℃ per annealing cycle; and 72 ℃ for 90 s

followed by 25 cycles at 95 ℃ for 20 s; 50 or 55 ℃, respectively, for 30 s; 72 ℃ for 90 s. We used a final extension period of 10 min at 72 ℃.

We scored fragments using an ABI 3730*xl* sequencer (Applied Biosystems) with ROX500 fluorescent size standard. We sized fragments using GENEMAPPER version 4.0 software (Applied Biosystems) and the Global Southern method (Applied Biosystems 2005). Initial tests indicated that the Global Southern method reduced intra-run variation in inferred fragment length relative to other sizing methods. We binned all fragments using the same binset to ensure allele calls were consistent across years, and we discarded ambiguous genotypes from the data set and re-genotyped plates with failing negative controls.

To assess the rate of genotyping error, we randomly selected a 12% sample from the entire data set, assigned each individual a random identification string, and blindly genotyped, scored, and binned these samples (Hoffman and Amos 2005). We exported records from the GENEMAPPER database, converted each to a useful format using GMCONVERT (Faircloth 2006), and imported data to a separate relational database. Error samples were re-assigned their true identification and compared to the corresponding non-error sample to compute the genotyping error rate on a per-locus and overall basis ($\bar{x} \pm 95\%$ CI).

Using CERVUS (Kalinowski et al. 2007; Marshall et al. 1998), we analyzed the microsatellite data by year to test for Hardy-Weinberg equilibrium. Using procedures outlined in Chapter 4, we tested for linkage using two-point analysis in CRIMAP (Lander and Green 1987) for all pairwise combinations of loci, and we assumed loci were linked when the log-odds for the pairwise-comparison was $> 3.0$.

### 2.3.3 Covey Membership

As a result of mortality or the movement of transient individuals, group membership fluctuates (B. C. Faircloth, *unpublished data*; Williams et al. 2003; Yoho and Dimmick 1972). In order to address the movement of individuals, we used the presence of individuals within each group or sub-group, along with the total number of radio-locations per bird, to determine

to which group each bird belonged. Given that our detection rate was 100%, we considered individuals members of a group when $> 50\%$ of the total number of locations for that individual were within the group. We excluded bird membership when not meeting this criterion, and we excluded all birds that were never assigned to a group. As a result of our observation that groups containing $< 4$ individuals typically disbanded, flush counts of coveys of this size indicating the presence of un-sampled individuals, and the impact of small sample size and/or incomplete sampling on subsequent analyses, we excluded coveys whose membership consisted of fewer than 4 radio-tagged and genotyped birds. To investigate the persistence of covey membership during successive years of the study period, we wrote a Python (Oliphant 2007; van Rossum 2006) program that interfaced with our relational database to: (1) identify individual birds alive during successive years, (2) determine the group members with which we located these individuals during the first year, (3) determine the group members with which we located these individuals during the second year, and (4) determine if any individuals identified in (2) and (3) were identical.

### 2.3.4 Intra-covey Relatedness

Prior to estimating the intra-covey relatedness, we computed estimates of the background level of relatedness in the population using both a pairwise maximum likelihood estimate of relatedness ($r_{MLE}$; Appendix A; Milligan 2003; Thompson 1975; Wagner et al. 2006; Weir et al. 2006) and a regression-based estimate of group relatedness (Queller and Goodnight 1989). We estimated the background level of relatedness as the mean ($\pm$95% CI) of all pairwise relatedness estimates for all sampled and genotyped individuals. Similarly, we determined the mean relatedness of individuals within coveys using $r_{MLE}$ and $r_{QG}$. For the maximum likelihood technique, we adjusted allele frequencies to account for the presence of null alleles using the EM algorithm (Chapuis and Estoup 2007; Dempster et al. 1977) and used a likelihood formula adjusted for their inclusion (Wagner et al. 2006). We estimated pairwise relatedness between all members of each group, and we computed the mean ($\pm$95%

CI) intra-group relatedness across all within-group, pairwise comparisons.For the regression-based estimator ($r_{QG}$), we computed the mean relatedness of each group ($P_y = Group$) relative to that of the sample ($P_x = Sample$), excluding the members of each group from the sample allele frequencies (bias-correction) using Relatedness 5.0.8 (Goodnight 2001; Queller and Goodnight 1989). We estimated confidence intervals ($\pm 95\%$) for each estimate via the bootstrapping procedure implemented by the program. We used GENMOD in SAS 9.1 (SAS Institute 2004) to examine the effects of year, covey size, and year + covey size on estimates of $r_{QG}$ and $r_{MLE}$.

### 2.3.5 Inter-Covey Relatedness

To investigate the relatedness structure among coveys across the landscape, we used methods similar to those outlined above. We computed $r_{MLE}$ between the members of each covey being compared across all combinations of coveys, and we used these estimates to compute the mean ($\pm 95\% CI$) relatedness among all pairwise combinations of coveys during each year of the study. Using the Queller and Goodnight (1989) estimator of relatedness, we computed symmetric relatedness among coveys by examining the relatedness of both groups ($P_x$ = Group 1, Group2) relative to that of the opposite group ($P_y \neq Group1|Group2$). We used bias-corrected allele frequencies for each estimate, and we computed the mean ($\pm 95\%$) relatedness among all pairwise combinations of coveys.

To examine the effect of distance and year on estimates of inter-group relatedness, we created a pairwise matrix of euclidean distance between the central point of each covey's distribution of spatial locations. We then unfolded these distance matrices, along with the corresponding matrices of inter-group relatedness ($r_{MLE}$ and $r_{QG}$), and we analyzed the effect of distance and year on inter-group relatedness using GENMOD in SAS 9.1 (SAS Institute 2004). We ranked and selected models using AICc, and we model-averaged all parameters in the candidate model set ($w_i > 0.0$ Akaike 1974; Burnham and Anderson 2002).

### 2.3.6 Analysis of Covey Survival

We analyzed the effects of the year, group, number of group members, and intra-covey relatedness on survival using the staggered-entry, Kaplan-Meier technique (Kaplan and Meier 1958; White and Burnham 1999). We created survival histories for each individual using 3-day intervals, and we censored individual histories when a bird was lost, present in a subgroup, associated with a covey other than its main covey, or killed by hunters. Other than accounting for staggered entry, we did not left-censor individual histories because prior analyses indicate our capture, handling, and radio-tagging techniques did not affect survival (Faircloth et al. 2006).

Because individuals within coveys may behave as a group, violating assumptions of independence, we adjusted our results for overdispersion using $\hat{c}$ estimated for the global model (Burnham and Anderson 2002). Prior to computing model-averaged parameter estimates, we selected the models containing the relatedness estimate that best fit the data ($r_{QG}$), and excluded models containing $r_{MLE}$ to minimize the effect of the redundant parameter ($r_{MLE}$) on model weights. We then computed model-averaged parameter estimates ($\pm$ 95% CI) (Burnham and Anderson 2002) for each model in the remaining candidate set ($w_i > 0.0$). To avoid overlooking bias introduced by the use of one relatedness estimator over another solely on the basis of model fit, we also computed model-averaged estimates excluding $r_{QG}$ and incorporating $r_{MLE}$ in similar fashion.

## 2.4 Results

During 2001-2003, we captured, banded, sampled, and radio-tagged 621 male and female bobwhites (Table 2.1). Our overall rate of genotyping error was 0.008 ($\pm$ 0.003, $n_{loci} = 16$). Following Bonferroni correction (Rice 1989), CV-PA3E and CV-PA3G deviated from Hardy-Weinberg equilibrium ($P < 0.05$) during 2002 and CV-PA3G and CV-PBA4 deviated from Hardy-Weinberg equilibrium ($P < 0.05$) during 2003. Linkage analysis indicated that 2 loci were linked (CV-PA1F and CV-PA12A) and 1 locus (CV-P1F2) exhibited problems with

Mendelian inheritance, likely due to the presence of null alleles. We dropped CV-PA12A and CV-P1F2 from the microsatellite data set.

Following our criteria for covey membership, we identified 53 distinct social groups (Table 2.2) and the presence of 18, 30, and 32 transient individuals during 2001-2003, respectively. The average number (± 95% CI) of individuals captured and radio-tagged per group by year was 9.0 (± 3.3), 8.6 (± 2.1), and 11.9 (± 2.4), respectively. During 2001, 1 individual was never assigned to a social group. We excluded 4, 3, and 3 groups, respectively, from subsequent analyses because they contained $< 4$ radio-tagged and genotyped members per group.

During 2001-2002, we identified 18 individuals (13%) alive during both the first and second non-breeding seasons. Six individuals (33%; 3 pairs in 3 separate groups) carried over into the same social groups. In one case, 7 individuals occupying the same social group during the first non-breeding season survived the interval, but only 2 were found in identical social groups during the second non-breeding season. During 2002-2003, we identified 24 individuals (15%) alive during both the second and third non-breeding seasons. Four individuals (8%; 2 pairs in 2 separate groups) carried over into the same social group.

For each relatedness estimator, we present the mean intra-group relatedness and the background level of relatedness within the population during each year of the study (Figure 2.1). Four and 5, 7 and 7, and 9 and 14 coveys were related at a level above the background estimates for $r_{QG}$ and $r_{MLE}$ during 2001-2003, respectively (Figure 2.2). We did not find a relationship between year or covey size and intra-group relatedness (95% CI of $\beta_{year,size}$ includes 0.0).

Mean inter-group relatedness was lower than background levels, except during 2001 (Figure 2.1). Year and distance best explained variation in inter-group $r_{QG}$ (Table 2.3). After model-averaging, only distance affected $r_{QG}$ (Figure 2.3; $\beta_{distance} = -0.03 \pm 0.02$). Year effects also explained variation in $r_{MLE}$ (Table 2.4), but the direction of the effect was opposite that for $r_{QG}$ and affected only a single year (Figure 2.3; $\beta_{year_1} = 0.005 \pm 0.003$).

After correcting for overdispersion, we found that year affected survival (Figure 2.4) and was 2.6 and 2.7 times more likely to explain the variation in survival than group size or group relatedness ($r_{QG}$), respectively (Table 2.5). Akaike weights for models containing $r_{QG}$ and $r_{MLE}$ are similar, indicating that neither estimator was vastly better at explaining variation in survival. Although the inclusion of group size and/or group relatedness in several models helped explain some variation in the data, neither parameter affected survival ($\beta_{size} = 0.03 \pm 0.06$; $\beta_{r_{QG}} = 1.65 \pm 3.54$). Model-averaged estimates incorporating $r_{MLE}$ are similar: $r_{MLE}$ did not affect survival ($\beta_{r_{MLE}} = 1.8 \pm 4.3$; Figure 2.5). In contrast to the findings of Faircloth et al. (2005), our results do not indicate an effect of group on covey survival (all group models $w_i = 0.0$).

## 2.5 Discussion

Our results do not support the predictions of kin selection theory. The operation of kin selection is dependent on relatedness within social groups greater than the background relatedness in the population (West-Eberhard 1975). Bobwhite social groups contained related individuals: overall, intra-group relatedness during each year of the study was greater than the background, population relatedness. Mean relatedness of individual groups was also greater than the background in $\geq 40\%$ of coveys, indicating that some coveys contained related individuals while others were largely composed of unrelated members. Group relatedness did not vary as a function of year or covey size, suggesting that the degree of relatedness within coveys was stable and independent of size. Proximate groups were not related during 2002 and 2003: inter-group relatedness was typically lower than the background level of relatedness in the population, except during 2001 - a difference we cannot explain. The effects of distance and year on inter-group relatedness were inconclusive: the effect size was small, the models selected for each estimator were different, and direction of the effects were conflicting.

Covey membership was variable as indicated by the number of transient individuals shifting group affiliation during the study. Shifts in membership may result from individuals

attempting to return to their social group after a disturbance induced by predators, hunters, or other factors causing temporary dispersal. Group membership did not appear stable across successive years with a number of individuals choosing to belong to social groups different than those in which they were originally located.

Finally, intra-group relatedness had no effect on individual survival. Akaike weights ($w_i$; Akaike 1974) indicate that models containing year and size better explain the variation in survival than other parameters. When included, neither relatedness estimate performs substantially better at predicting survival than the other, and the confidence interval for each estimated relatedness parameter includes 0.0. Surprisingly, our results do not demonstrate an effect of particular groups on covey survival. However, prior to adjusting for overdispersion, these models rank at the top of the candidate set. We thought it necessary to adjust for overdispersion as a result of dependence in the data related to the survival of individual in groups, which may have unnecessarily penalized models including group effects (global model $\hat{c} = 2.54$). Without adjusting for overdispersion and averaging over $AIC_c$ versus $QAIC_c$, there remains no effect of $r_{QG}$ or $r_{MLE}$ on individual survival.

In the absence of kin selection, several plausible explanations for the evolution and maintenance of bobwhite social groups exist. Predation may explain the advantages of group formation in the absence of relatedness effects. The formation of groups in time and space reduces the encounter rate with certain types of predators (Hamilton 1971), and groups dilute the individual risk of predation once encountered (Foster and Treherne 1981; Milinski 1984; Wrona and Dixon 1991). Given sufficient predation pressure (Pulliam and Millikan 1982) and the combination of direct fitness benefits arising from grouping behavior, it is possible to explain the evolution of social groups solely in this context (Turner and Pitcher 1986; Wrona and Dixon 1991).

Social foraging theory also may explain the evolution and maintenance of social groups. Groups allow individuals to increase the amount of time spent foraging (Pulliam 1973) while decreasing the variance in (Pulliam and Millikan 1982) and increasing the efficiency of

foraging (Pulliam and Millikan 1982; Thompson et al. 1974). It is also possible that social groups arose as a result of the interaction between the effects of predator avoidance and social foraging, which are not mutually exclusive (Alexander 1974; Giraldeau and Caraco 2000; Pulliam 1973).

The warning behavior exhibited by individuals living in groups proximate to one another may be explained, in the absence of relatedness effects, by reciprocal altruism (Trivers 1972). However, several of Trivers (1972) biological parameters are questionable when applied to bobwhites: individuals may not live sufficiently long to receive the advantages conferred via reciprocal interactions, and the degree of mutual dependence between individuals in different groups is likely low. Dispersal from groups immediately prior to the breeding season tends to re-arrange the structure of individuals across the landscape and may serve to increase both the advantages conferred from reciprocal interactions and the degree of mutual dependence. This is an area that deserves further study.

Generally, it is believed there is a tradeoff between increases in group size and the increase in benefits derived by individual group members. As a result, it is believed social groups may have an inherent, optimal size (Brown 1982) and that the movement of transient individuals or the combination of social groups across the landscape are meant to achieve this optimum (Williams et al. 2003). We failed to identify a relationship between the effect of bobwhite group size and survival, although we did not investigate the influence of a quadratic size effect or the influence of time-varying changes in group size, as a result of the movement or death of individual group members.

It is possible that our results may be explained by the influence of variance in measured parameters. Throughout this manuscript, we have treated our sampling of social groups as complete. We realize this is an impossibility across space and time. We have taken measures to exclude those groups for which we believed we did not have adequate data, but the likely possibility remains that individuals within groups remained unsampled. Our requirements for group membership may have improperly excluded actual group members thus affecting

subsequent estimates of relatedness. However, we believed the quantification of group membership necessary to address the movement of transient individuals between coveys. The exclusion of these individuals potentially change the mean and variance of group relatedness, scaled by the size of the group.

Throughout this manuscript, we employ two fundamentally different estimators of relatedness: the regression-based Queller and Goodnight (Queller and Goodnight 1989) estimator and a maximum-likelihood estimator (Milligan 2003; Thompson 1975; Wagner et al. 2006; Weir et al. 2006). We chose the Queller and Goodnight (1989) estimator because its formulation was derived to address questions associated with Hamilton's rule, and we chose the maximum-likelihood estimator because it tends to estimate certain values of relatedness with less bias and error than other techniques (Milligan 2003).

Regardless of estimator choice, inferring relatedness in the absence of pedigree information is challenging, particularly with a low number ($< 50$) of molecular markers (Blouin 2003; Milligan 2003; Wang 2007). Relatedness estimates are subject to high degrees of variance, particularly for values near the left-tail of the distribution (*i,e.* individuals of low relatedness; Blouin 2003; Milligan 2003). The Queller and Goodnight estimator exhibits higher root-mean square error (RMSE) and bias than the maximum likelihood estimator but is in common use and provides results comparable to similar studies. The maximum likelihood estimator is positively biased when comparing individuals of low relatedness (Figure 2.1 Milligan 2003), but may exhibit less bias and lower RMSE for higher degrees of relatedness than other available estimators (Milligan 2003; Wang 2007).

Finally, the effects of covariate measurement error on logistic models is well known (Stefanski and Carroll 1985). Although we have taken steps to address model selection uncertainty via model ranking and parameter averaging, we cannot prove the lack of relatedness effects on individual survival arise from sources other than measurement error. Particular caution is warranted when analyses involve estimates of relatedness, for reasons given above.

Despite these concerns, we believe that our results remain valid. We can think of no other way to capture and monitor individuals in a natural system that would more accurately and precisely estimate group composition and relatedness. In part, we are limited by the range in natural size of bobwhite social groups (7-30 individuals) (Stoddard 1931; Wing 1941): 50% random sampling of a group of 7 would yield 3 for analysis, potentially increasing variance and bias of the group estimate with moderate numbers of molecular markers (10-25). Estimates of group relatedness may help reduce bias and variance relative to pairwise comparisons (Blouin 2003; Queller and Goodnight 1989). Our remaining concerns do not differ from similar attempts to investigate kin selection in natural systems.

We suggest that future studies focus on additional species within Order Galliformes. Investigation of related species will provide baseline knowledge within the Order, and certain species may free researchers from constraints imposed by small group size (e.g. California Quail, *Callipepla californica*). The range of environmental conditions inhabited by this varied group also offers interesting possibilities for the study of kin selection, predation, and social foraging. Manipulative studies, in addition to that of Williams et al. (2003), investigating the effects of predation, group vigilance, foraging efficiency, and the addition of relatives to or removal of relatives from social groups are also suggested.

## 2.6 Summary

Kin selection theory predicts grouping behavior may have evolved among related individuals as a result of inclusive fitness benefits derived by group members via their interactions with relatives. As such, the theory predicts that groups be composed of related individuals and that increased survival should be correlated with the relatedness of the group. Our results suggest that, while related, the degree of relatedness within bobwhite social groups does not correlate with their survival. Therefore, kin selection theory may not apply to the evolution and maintenance of bobwhite social groups. It is possible that the evolution and maintenance of bobwhite social groups may be explained by direct fitness benefits conferred

via group dilution of predation risk or group-related increases in foraging efficiency. Given the variability in local, regional, and range-wide habitats occupied by Northern Bobwhites, these explanations for the evolution and maintenance of social group formation deserve attention.

## 2.7 Acknowledgments

Computer code used within this manuscript is available for review or use under the terms of the Gnu GPL (v2) from `http://msatsnippets.googlecode.com/`. The authors thank Tall Timbers Research Station and Land Conservancy and its supporters, Daniel B. Warnell School of Forestry and Natural Resources, University of Georgia, University of Georgia Graduate School, Quail Unlimited - Northeast Georgia Chapter, and McIntire-Stennis project GEO-0100-MS and GEO-0136-MS for providing funding for this research. B. Faircloth thanks T. Anderson, A. Butler, C. Croft, K. Crook, J. Gruchy, M. Reasel, B. Shamblin, S. Staller, S. Stapleton, S. Wellendorf, B. Widener, and B. Womack for their invaluable assistance in the field and both T. Brooks and K. Spear for their work in the lab. The authors additionally thank M. Conroy and J. Peterson of the GA Cooperative Fish and Wildlife Research Unit for their statistical advice. Capture and handling of adult, juvenile, and immature bobwhites were approved by the Florida Fish and Wildlife Conservation Committee (permit number: WX01277) and the University of Georgia Institutional Animal Care and Use Committee (approval numbers: A990028M1, A990028C1, and A200310109-0).

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Table 2.1: Northern Bobwhites captured, radio-collared, and sampled for genetic analysis; number of individuals genotyped; number of individuals assigned to a social group; and genotyping success for those assigned individuals during 2000-2003 on Tall Timbers Research Station. We defined a successful genotype as amplification at $\geq 50\%$ of loci ($n_{loci} = 16$).

| Year | Bobwhites Captured | Genotyped (%) | Assigned to Social Group | Assigned to Group and Successfully Genotyped |
|---|---|---|---|---|
| 2001 | 179 | 162 (91%) | 135 (84%) | 123 (91%) |
| 2002 | 175 | 167 (95%) | 164 (98%) | 154 (94%) |
| 2003 | 267 | 266 (100%) | 227 (85%) | 224 (99%) |
| Overall | 621 | 595 (96%) | 526 (88%) | 501 (95%) |

Table 2.2: Number of group members and number of genotyped members per group for all distinct coveys captured on Tall Timbers Research Station during 2001-2003. Asterisks indicate coveys that were excluded from analysis.

| 2001 | | | 2002 | | | 2003 | | |
|---|---|---|---|---|---|---|---|---|
| Group | Members | Genotyped Members | Covey | Members | Genotyped Members | Covey | Members | Genotyped Members |
| 01-01 | 12 | 11 | 02-01 | 5 | 5 | 03-01 | 8 | 8 |
| 01-03 | 6 | 6 | 02-02 | 10 | 10 | 03-02** | 3 | 3 |
| 01-04 | 17 | 15 | 02-03 | 17 | 14 | 03-03 | 21 | 21 |
| 01-05 | 9 | 8 | 02-04 | 8 | 7 | 03-04 | 11 | 11 |
| 01-06** | 1 | 1 | 02-05 | 11 | 11 | 03-05 | 11 | 11 |
| 01-07 | 8 | 5 | 02-06 | 6 | 6 | 03-06 | 11 | 11 |
| 01-08 | 9 | 9 | 02-07** | 4 | 3 | 03-07 | 14 | 14 |
| 01-09 | 23 | 22 | 02-08 | 6 | 6 | 03-08 | 15 | 15 |
| 01-10 | 17 | 16 | 02-09 | 18 | 17 | 03-09 | 15 | 15 |
| 01-11** | 3 | 3 | 02-10 | 14 | 13 | 03-10 | 10 | 10 |
| 01-12** | 3 | 3 | 02-11 | 6 | 5 | 03-11 | 9 | 9 |
| 01-13 | 4 | 4 | 02-12 | 9 | 8 | 03-12 | 19 | 18 |
| 01-14** | 2 | 1 | 02-13 | 13 | 12 | 03-13 | 10 | 10 |
| 01-15 | 7 | 5 | 02-14 | 12 | 12 | 03-14 | 23 | 22 |
| 01-16 | 14 | 14 | 02-15 | 11 | 11 | 03-15** | 1 | 1 |
| . | . | . | 02-16 | 7 | 7 | 03-16 | 8 | 8 |
| . | . | . | 02-17 | 4 | 4 | 03-17 | 17 | 17 |
| . | . | . | 02-18** | 1 | 1 | 03-18** | 1 | 1 |
| . | . | . | 02-19** | 2 | 2 | 03-19 | 20 | 18 |

Table 2.3: Model structure, $AIC_c$, $\Delta AIC_c$, model weight ($w_i$), relative likelihood, and number of parameters for models of inter-covey relatedness measured using $r_{QG}$ on Tall Timbers Research Station during 2001-2003.

| Model | $AIC_c$ | $\Delta AIC_c$ | $w_i$ | Relative $\ell$ | Parameters |
|---|---|---|---|---|---|
| $r_{QG}(.) + year$ | 1099.62 | 0.00 | 0.84 | 1.00 | 2 |
| $r_{QG}(.) + distance$ | 1103.00 | 3.38 | 0.15 | 0.18 | 2 |
| $r_{QG}(.) + distance + year$ | 1108.78 | 9.16 | 0.01 | 0.01 | 3 |

Table 2.4: Model structure, $AIC_c$, $\Delta AIC_c$, model weight ($w_i$), relative likelihood, and number of parameters for models of inter-covey relatedness measured using $r_{MLE}$ on Tall Timbers Research Station during 2001-2003.

| Model | $AIC_c$ | $\Delta AIC_c$ | $w_i$ | Relative $\ell$ | Parameters |
|---|---|---|---|---|---|
| $r_{MLE}(.) + year$ | 1570.50 | 0.00 | 1.00 | 1.00 | 2 |
| $r_{MLE}(.) + distance$ | 1597.38 | 26.88 | 0.00 | 0.00 | 2 |
| $r_{MLE}(.) + distance + year$ | 1610.30 | 39.80 | 0.00 | 0.00 | 3 |

Table 2.5: Model structure, $QAIC_c$, $\Delta QAIC_c$, model weight ($w_i$), relative likelihood, and number of parameters for models of covey survival on Tall Timbers Research Stations during 2001-2003. We estimated and adjusted for overdispersion using $\hat{c}$ from global model of the candidate set (Burnham and Anderson 2002): $S(.) + year + group + size + r_{QG}$. Models in bold ($w_i > 0.0$) indicate those that were used in model-averaging procedures (Burnham and Anderson 2002). We also average over models containing $r_{MLE}$ and excluding $r_{QG}$.

| Model | $QAIC_c$ | $\Delta QAIC_c$ | $w_i$ | Relative $\ell$ | Parameters |
|---|---|---|---|---|---|
| $\mathbf{S(.) + year}$ | 506.692 | 0.00 | 0.30 | 1.00 | 3 |
| $\mathbf{S(.) + year + size}$ | 507.815 | 1.12 | 0.17 | 0.57 | 4 |
| $\mathbf{S(.) + year + r_{QG}}$ | 507.991 | 1.30 | 0.16 | 0.52 | 4 |
| $S(.) + year + r_{MLE}$ | 508.211 | 1.52 | 0.14 | 0.47 | 4 |
| $\mathbf{S(.) + year + size + r_{QG}}$ | 508.691 | 2.00 | 0.11 | 0.37 | 5 |
| $S(.) + year + size + r_{MLE}$ | 508.718 | 2.03 | 0.11 | 0.36 | 5 |
| $S(.)$ | 534.50 | 27.80 | 0.00 | 0.00 | 1 |
| $S(.) + size$ | 535.10 | 28.41 | 0.00 | 0.00 | 2 |
| $S(.) + r_{QG}$ | 536.13 | 29.43 | 0.00 | 0.00 | 2 |
| $S(.) + r_{MLE}$ | 536.43 | 29.75 | 0.00 | 0.00 | 2 |
| $S(.) + group$ | 539.51 | 32.81 | 0.00 | 0.00 | 35 |
| $S(.) + group + size + r_{MLE}$ | 541.41 | 34.71 | 0.00 | 0.00 | 36 |
| $S(.) + group + size$ | 541.41 | 34.71 | 0.00 | 0.00 | 36 |
| $S(.) + group + size + r_{QG}$ | 541.41 | 34.71 | 0.00 | 0.00 | 36 |
| $S(.) + group + r_{QG}$ | 541.41 | 34.71 | 0.00 | 0.00 | 36 |
| $S(.) + group + r_{MLE}$ | 541.41 | 34.71 | 0.00 | 0.00 | 36 |

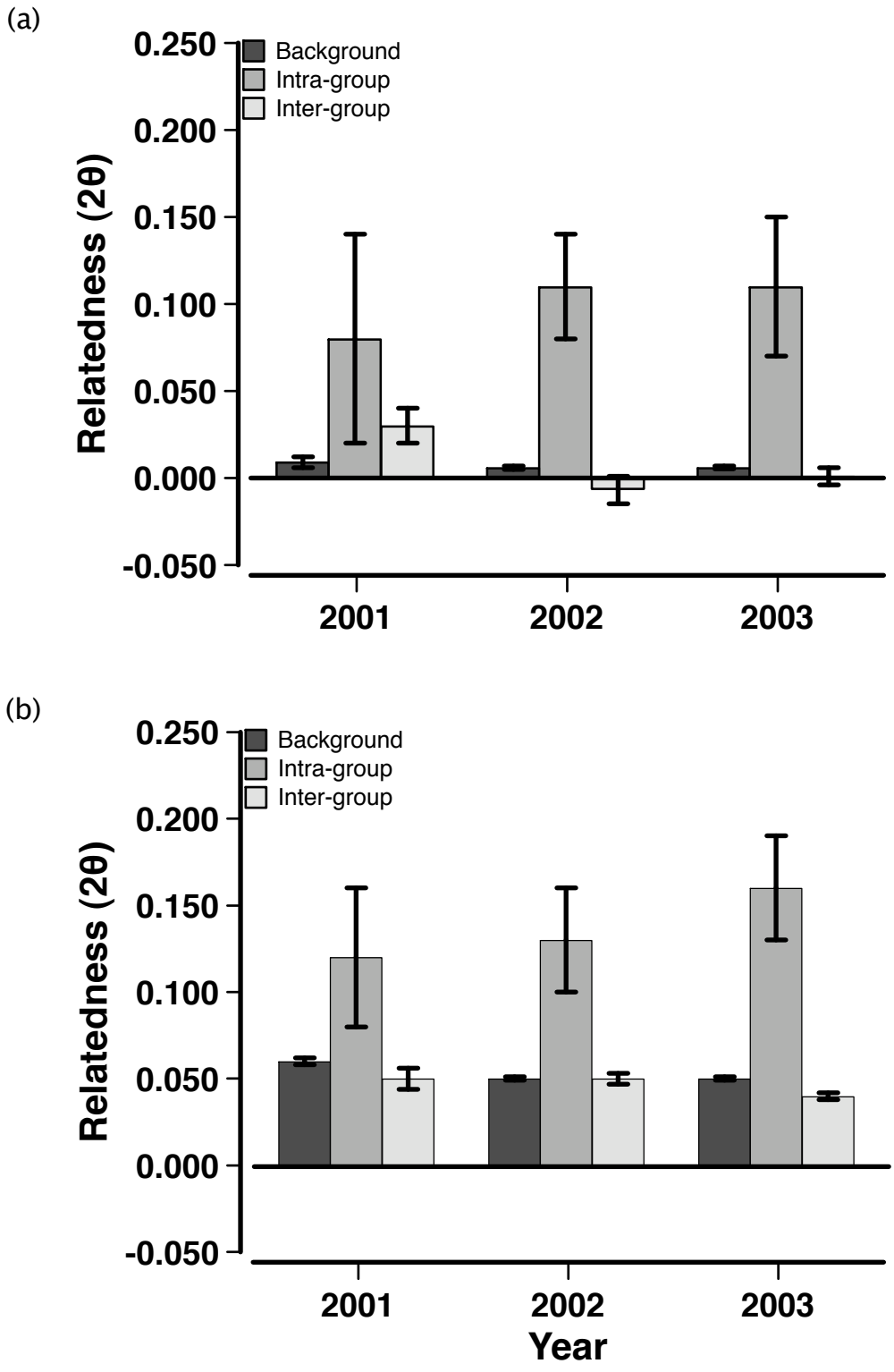


Figure 2.1: Mean background, intra-group, and inter-group relatedness (± 95% CI) estimated using (a) $r_{QG}$ (Queller and Goodnight 1989) and (b) $r_{MLE}$ (Milligan 2003; Thompson 1975; Wagner et al. 2006; Weir et al. 2006) for social groups during 2001-2003 on Tall Timbers Research Station. Note that $r_{QG}$ is not constrained to fall on the interval [0,1].

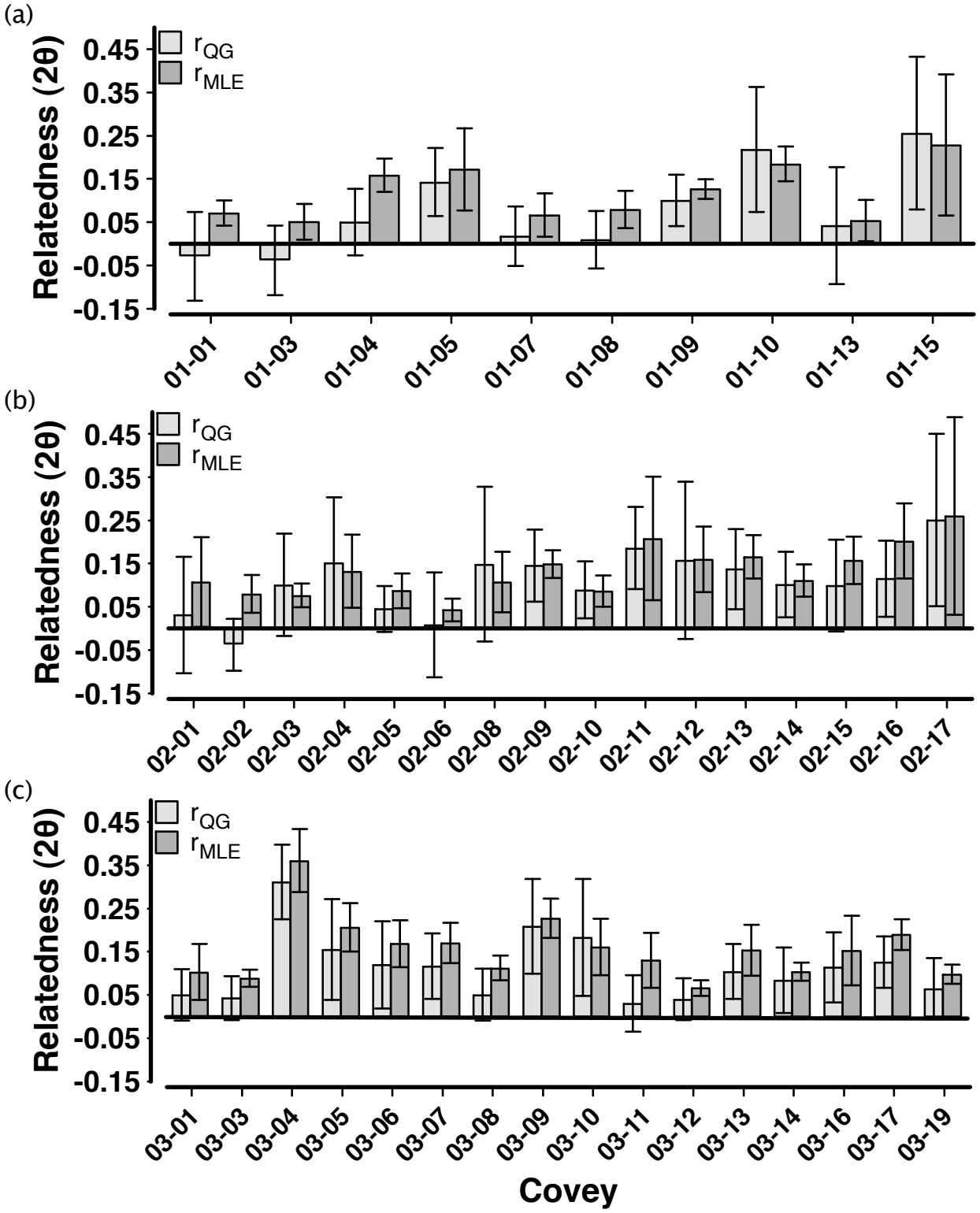


Figure 2.2: Intra-covey relatedness (± 95% CI) estimated by $r_{QG}$ and $r_{MLE}$ for social groups containing $\geq 4$ radio-collared and genotyped individuals captured during (a) 2001, (b) 2002, and (c) 2003 on Tall Timbers Research Station.

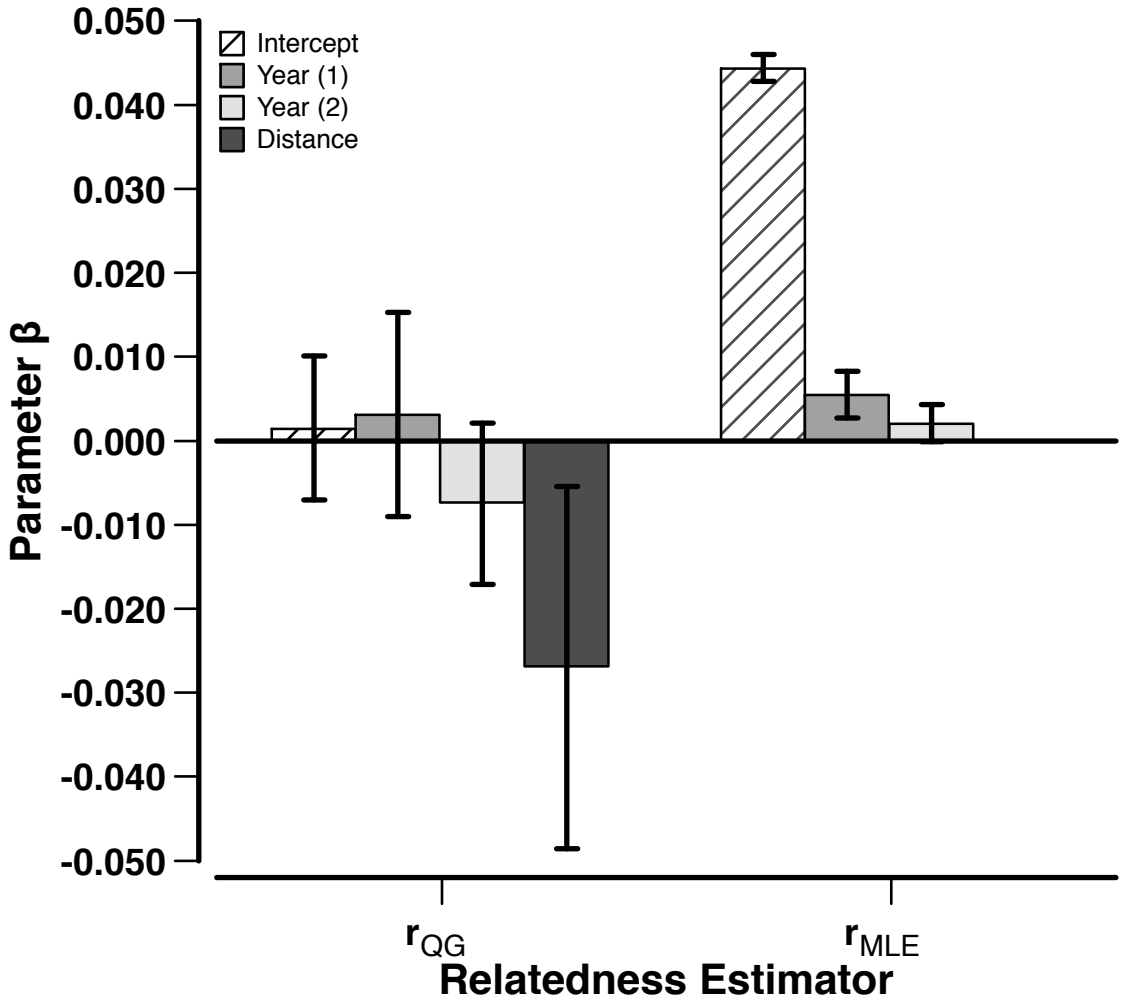


Figure 2.3: Model averaged estimates ($\pm$ 95% CI) for parameters in candidate models ($w_i >$ 0.0) of inter-group relatedness using either $r_{QG}$ and $r_{MLE}$ during 2001-2003.

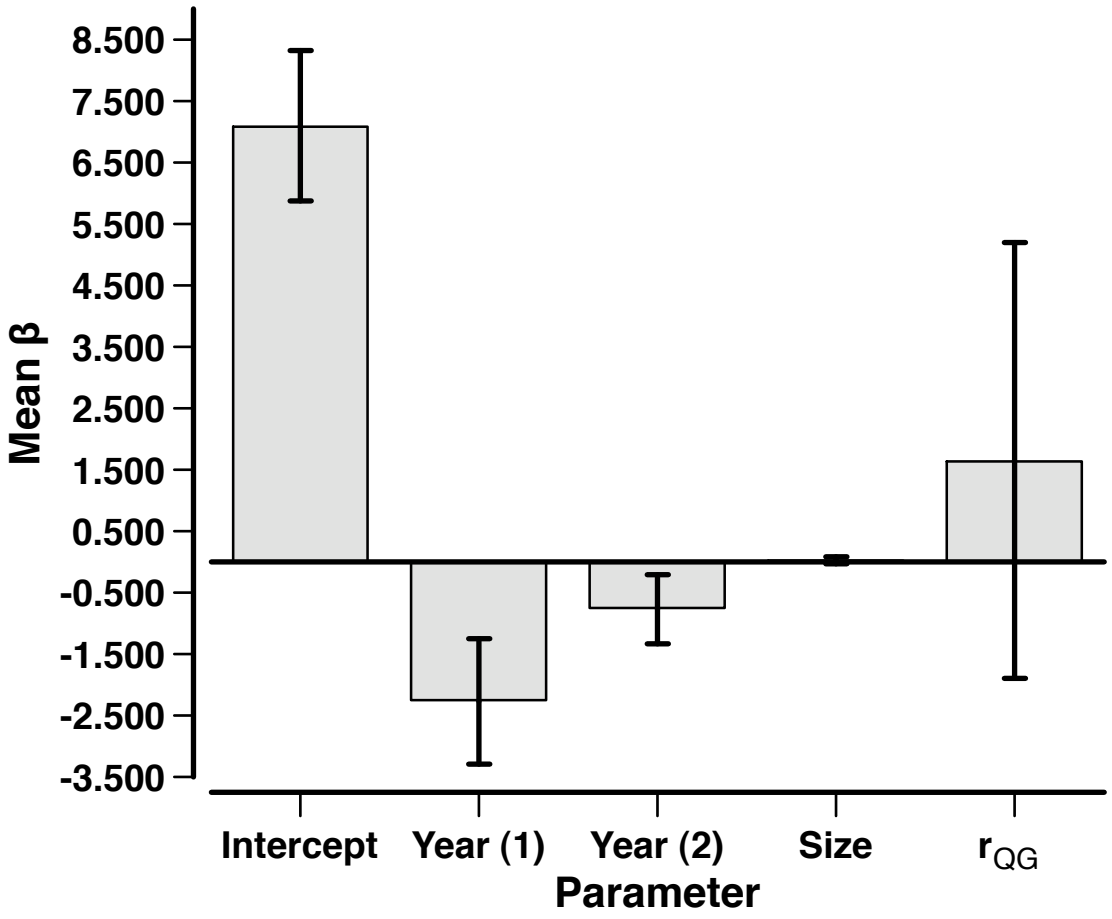


Figure 2.4: Model-averaged parameter estimates (± 95% CI) for models of individual survival on Tall Timbers Research Station during 2001-2003 including $r_{QG}$ ($w_i > 0.0$).

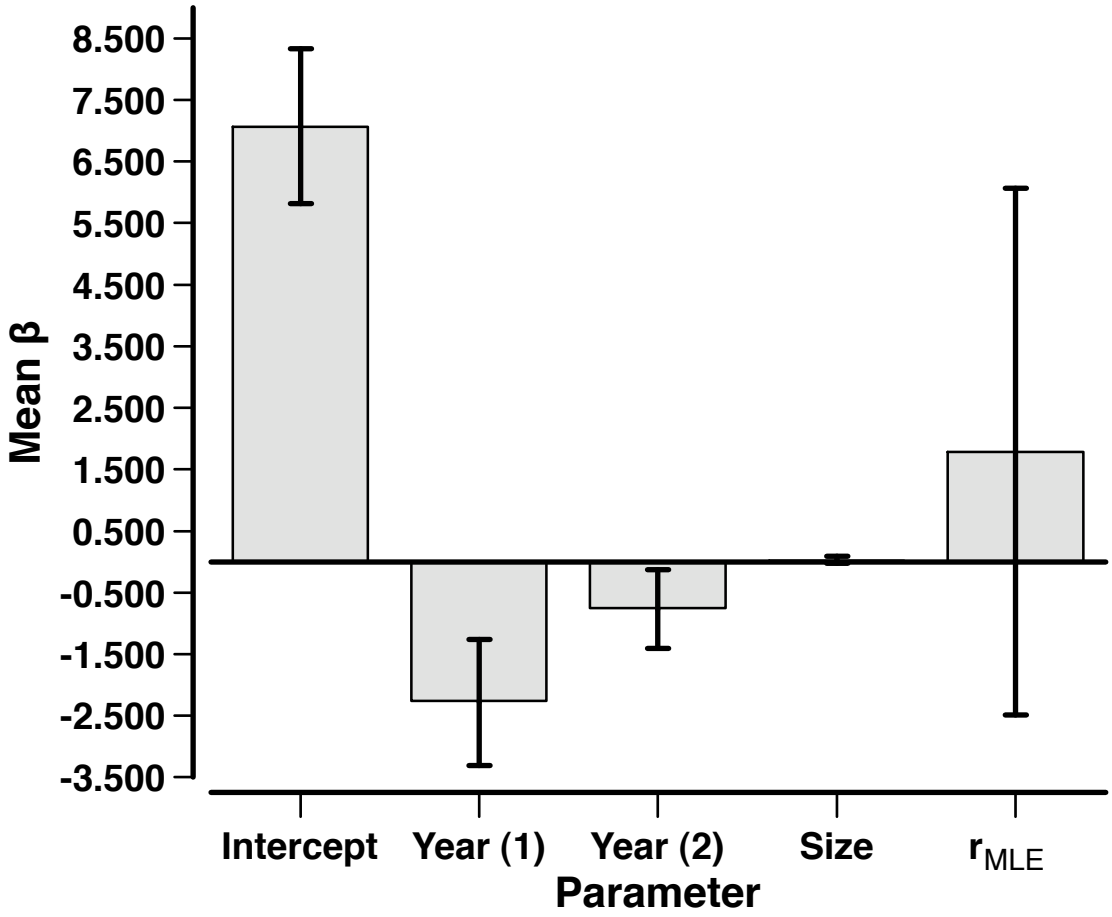


Figure 2.5: Model-averaged parameter estimates ($\pm$ 95% CI) for models of individual survival on Tall Timbers Research Station during 2001-2003 including $r_{MLE}$

# Chapter 3

## Sex-ratio of Neonatal Northern Bobwhites [1]

[1]Faircloth, B. C., W. E. Palmer, J. P. Carroll. To be submitted to *Journal of Avian Biology*.

## 3.1 Abstract

Knowledge of neonatal sex ratio in Northern Bobwhites (*Colinus virginianus*) is minimal. Inferences based on captures of male and female birds suggest that the apparent sex ratio is approximately 1:1. However, prior research indicates that sex ratios may be affected by yearly productivity. In this study, we evaluated sex ratio using 568 chicks collected during 2001-2003 by amplifying introns of the avian CHD gene. We found that bobwhite sex ratio does not differ from 1:1, yearly productivity does not affect neonatal sex ratio, and maternal condition, indexed by weight during the non-breeding season, does not affect sex ratio.

## 3.2 Introduction

Neonatal sex ratio in Northern Bobwhites (*Colinus virginianus*) is unknown (Brennan 1999). Prior to the advent of molecular sex determination, analysis of neonatal sex ratios required the destructive sampling of bobwhite offspring because individuals do not exhibit plumage dimorphism until $>$ 6 weeks of age (Petrides and Nestler 1943). Banding studies of juvenile and adult birds indicate that the population sex ratio is approximately 1:1 (Stoddard 1931), although Roseberry and Klimstra (1984) suggested that sex ratios may be female-biased during periods of population increase and male-biased during periods of population decline. Annual survival rates of male and female bobwhites are variable but generally indicate higher male survival (Pollock et al. 1989), potentially biasing sex ratio inferred using banding data towards males and rendering the inference of offspring sex ratios using these data questionable. Additionally, the bobwhite mating system is believed to be affected by the operational sex ratio (Burger et al. 1995, OSR;), and thus is potentially affected by neonatal sex ratios.

Classical theory (Fisher 1930) predicts that neonatal sex ratios should not deviate from 1:1. However, Trivers and Willard (1973) suggested that sex ratio may be affected by maternal condition, shifting sex ratio on a per-brood or per-clutch basis away from 1:1 and towards males when females are in better condition. Maternal variation in offspring sex ratios was

reported for other members of Order Galliformes (*Gallus gallus* Parker 2002), but effects of maternal condition on sex ratio of many galliformes are unknown.

In this srudy, we investigate offspring sex ratio in a population of bobwhites captured in norther Florida during 2001-2003. We use molecular techniques to examine the overall neonatal sex ratio, yearly effects on sex ratio, and the impact of female condition, as indexed by weight at capture, on variation in the ratio of male to female neonatal bobwhites.

## 3.3 Methods

### 3.3.1 Capture of Adults

We trapped, captured, and radio-collared adult and subadult bobwhite during October-June 2000-2003 on a 250 ha area of Tall Timbers Research Station, Tallahassee, FL according to methods described elsewhere (4). We weighed each bird using an electronic balance and collected 10 body feathers from the flank of each bird captured. We stored 5 feathers in 70% EtOH for genetic analysis and 5 feathers in paper envelopes as a reserve. We located radio-collared birds 4-5 days/week during the non-breeding season (15 October to 15 April) and 7 days/week during the breeding season (15 April to 15 October).

### 3.3.2 Capture of Chicks

We captured bobwhite chicks using brood capture procedures outlined in Smith et al. (2003) and Faircloth et al. (2005). We collected 1.5 $m$m patagial microbiopsies from each chick (Miltex Corp., York, PA), treated the biopsy area with NeoPredef powder (Pfizer Animal Health, New York, NY), and stored each sample in individual 1.5 $m$L microtubes containing a Tris-EDTA buffer (100$m$M Tris pH 8.0, 100$m$M EDTA pH 8.0, 10 $m$M NaCl, 1% SDS). Following capture, we released all chicks within 5 m of the attending adult. We have shown elsewhere that our brood capture procedures typically resulted in complete captures of a brood, released chicks rapidly returned to the attending parent(s), and the collection of

patagial biopsies at 1-4 days of age did not affect neonatal survival (Evans et al. 2008; Faircloth et al. 2005).

### 3.3.3 Sampling of Unhatched Nests

When a radio-tagged bird was killed off-nest, we artificially incubated the contents of each nest for the remainder of the incubation period. Chicks hatching from incubated nests were sampled between 1 and 3 days post-hatch, following procedures outlined above. We pooled these individuals with others collected from successful nests during the study because we had no reason to believe that chicks hatched from eggs within these nests were different from those sampled in the wild.

### 3.3.4 Genetic Sex Determination

We extracted DNA from feathers and patagial microbiopsies using DNeasy kits (Qiagen Inc., Valencia, CA) with a modification to the digestion step, adding 25 $\mu$L of 100 $m$g/$m$L DTT (Dithiothreitol) along with proteinase K. We eluted DNA from the membrane with either: 2 washes of 60 $\mu$L Buffer AE or 1 wash 120 $\mu$L Buffer AE. Prior to amplification, we treated all samples 1:3 (volume:volume) with 10% Chelex resin (BioRad Laboratories, Hercules, CA) to remove PCR inhibitors.

We performed 96-well PCR amplifications of the CHD1Z and CHD1W introns using the 2550F and 2718R (HEX + 2718R) primers identified by Fridolffson and Ellegren (1999). We selected these primers over the widely used P2 and P8 primers (Griffiths et al. 1996, 1998) because the region of the bobwhite Z chromosome amplified by P2 and P8 is polymorphic, leading to ambiguity in the molecular assignment of sex. Polymorphism in this region is not limited to bobwhites (Casey et al. 2007; Dawson et al. 2001).

We conducted all amplifications using 0.5 U AmpliTaq Gold (Applied Biosystems, Foster City, CA), 1X Gold Buffer, 1X BSA (New England Biolabs, Ipswich, MA), 1.5 $m$M MgCl, 1.25 $m$M dNTPs, 0.5 $\mu$M untagged primer; 0.05 $\mu$M CAG or M13-reverse tagged primer,

0.45 $\mu$M dye-labelled tag (HEX, FAM, NED + CAG or M13-reverse), 3.3 $\mu$L ddH2O, and 2 $\mu$L DNA template (5-10 $n$g). We included multiple negative controls, using $ddH_2O$ in place of template DNA, in each plate of PCR reactions.

To amplify CHD introns, we used a touchdown thermal cycling profile (Don et al. 1991), encompassing a 10 ℃ span of annealing temperatures (range: 60 - 50 ℃) similar to that detailed in Fridolffson and Ellegren (1999). Cycling parameters included a Taq activation step at 95 ℃ for 5 min followed by 20 cycles at 95 ℃ for 20 sec; 60 ℃ for 30 sec minus 0.5 ℃ per annealing cycle; and 72 ℃ for 90 sec followed by 25 cycles at 95 ℃ for 20 sec; 50 or 55 ℃, respectively, for 30 sec; 72 ℃ for 90 sec. We used a final extension period of 10 min at 72 ℃.

We scored fragments using an ABI 3730$xl$ sequencer (Applied Biosystems) with ROX500 fluorescent size standard. We sized fragments using GENEMAPPER version 4.0 software (Applied Biosystems) and the Global Southern method (Applied Biosystems 2005). We binned all fragments into an A, B, or AB state, we discarded ambiguous genotypes from the data set, and we re-ran plates with failed negative controls.

To assess the rate of amplification error, we randomly selected a 12% sample from the entire data set, assigned each individual a random identification string, and blindly amplified, scored, and binned each individual (Hoffman and Amos 2005). We exported all samples from the GENEMAPPER database and imported data to a separate relational database. We re-assigned error samples their true identification and compared each to the corresponding non-error sample to compute the rate of amplification error.

To validate the ability of the Fridolffson and Ellegren (1999) primers to determine the sex of bobwhites, we compared the genetic sex of adults and subadults to sex assigned using their phenotype, as determined using plumage dimorphism (Petrides and Nestler 1943).

### 3.3.5 Exclusion of Offspring

Because bobwhites exhibit a high degree of post-hatch brood amalgamation, it was necessary to remove individuals from each brood arising from this behavior. We first excluded all individuals captured at 10-14 days of age (second brood capture) because the rate of amalgamation in broods of this age is high (Faircloth et al. 2005, Chapter 4). We additionally identified individuals within broods of 1-3 days old that resulted from amalgamation using the procedures outlined in Chapter 4.

Similarly, intra-specific nest parasitism occurs within bobwhite broods at a moderate rate (Chapter 5). Using parentage assignment and relatedness inference procedures detailed in Chapter 5, we identified chicks within broods arising as the result of nest-parasitism. Because there existed captured broods for which we could not determine the nest-parasitism status, we also excluded these individuals.

### 3.3.6 Estimation of the Sex Ratio

In theory, a binomial process represents the allocation of sex to offspring: the male sex should be allocated to individuals with a probability of 0.5 (Fisher 1930; Leigh 1970). As such, we estimated the posterior probability of male offspring on a per brood basis using WinBugs (Lunn et al. 2000). We treated each brood as an independent trial, and modeled the data using a binomial distribution, and a non-informative, beta (1,1) prior. For each simulation, we ran 2 chains, 50,000 burn-in iterations, and 50,000 sampling runs which we used to generate the posterior distribution. Following simulations, we examined trace, model deviance, and autocorrelation to ensure convergence and proper sampling of each chain.

We computed the median posterior probability ($\pm$95% Credible Interval) of male offspring for each year of the study and overall for broods with INP-chicks excluded and broods with INP-chicks included. We chose to examine both groups because we were interested in the potential effects of nest-parasitism on sex ratio within broods.

We additionally pooled and analyzed data for only INP chicks to investigate whether the median, estimated sex ratio (±95% Credible Interval) of these offspring differed from that of non-INP offspring.

### Maternal Condition and Sex Ratio

To examine the effect of maternal condition on offspring sex ratio, we computed mean weight (±95% CI), across capture occasions, of females assigned as the mother of broods captured. Using these data, the number of male offspring per brood, and total brood size, we built a binomial, logistic model to estimate the effect of year and maternal condition on the posterior probability of male offspring:

$$P(M \mid \theta) \sim Binomial(p, N) \tag{3.1}$$

where M is the number of males, $N$ = number of offspring in each brood, and:

$$logit(p) = \beta_{intercept} + \beta_{year1} * year_1 + \beta_{year2} * year_2 + \beta_{weight} * weight_{female} \tag{3.2}$$

where $year_1$ and $year_2$ were coded using dummy variables (0 & 1) and the prior distribution for all $\beta$ parameters was normal and non-informative:

$$\beta_{intercept,year1,year2,weight} = Normal(0.0, 1.0 \times 10^{-6}) \tag{3.3}$$

We used WinBugs (Lunn et al. 2000) to run the model (2 chains, 250,000 burn-in iterations, 250,000 sampling iterations, thin = 15), and we examined trace, deviance, and autocorrelation to ensure convergence and proper sampling of both chains. We computed the median (±95% Credible Interval) posterior probability for each value of $\beta$.

## 3.4 Estimation of Survival

To examine patterns of population growth or decline in relation to offspring sex ratios, we estimated population size for each year during the study period. Using records collected

during the capture and radio-collaring of adult and sub-adult birds, we created capture histories for each bird and analyzed these data using the Jolly-Seber (Jolly 1965; Seber 1965) model implemented by program CAPTURE (Pollock et al. 1990). Similarly, using radio-telemetry data, we created weekly capture histories for birds monitored during both the breeding and non-breeding seasons. We estimated the probability of surviving a 28-week period during the non-breeding season and a 27-week period during the breeding season using the Kaplan-Meier model (Kaplan and Meier 1958) implemented in program Mark (White and Burnham 1999). Prior to analysis, we censored individuals from the data set that were lost, killed by hunters, or left the study area. We did not left-censor our data to account for capture and handling effects.

## 3.5 Results

During 2001-2003, we collected 621 adults/subadults and 841 neonatal bobwhites representing 32, 51, and 17 distinct broods, respectively. We amplified the CHD gene in 1,402 samples (96%) and successfully determined the genetic sex of 1,368 individuals (98%). Our rate of amplification error was 0.02. We compared genetic to phenotypic sex using 606 adults, and we identified 9 for which genetic and phenotypic sex disagreed (2%).

Following exclusion of amalgamated chicks, broods containing $< 2$ offspring, or broods for which we could not assign a female parent, we analyzed 123, 332, and 113 offspring sampled from 17, 42, and 13 broods during 2001-2003, respectively.

The median posterior probability of male offspring, excluding parasitic chicks, during the the study was 0.5 ($\pm 0.4$). Neonatal sex ratio did not differ from 1:1 during 2001-2003 in those broods from which parasitic chicks were excluded or those containing parasitic chicks (Figure 3.1). The sex ratio among parasitic chicks did not differ from 1:1 [$P(M \mid \theta) = 0.04 \pm 0.1$]. Neither female condition (Figure 3.2; $\beta_{weight} = -0.0008 \pm 0.01$) nor year ($\beta_{year1} = -0.2 \pm 0.5$; $\beta_{year2} = -0.4 \pm 0.5$) affected sex ratio within broods.

Over-winter survival differed during the study period while breeding-season survival was only different in 2003. Because our estimates of population size were generated using data collected during the non-breeding season, changes in each estimate reflect the productivity of the previous breeding season. While our population estimates exhibit marked variation, the trend of the data suggests that productivity was low during 2001 and high during 2002.

## 3.6 Discussion

Our results demonstrate that sex ratio in bobwhite broods does not differ from 1:1, validating previous inferences (Stoddard 1931). Our results also suggest that bobwhite sex ratios are not skewed in a particular direction in times of population increase, as reported by (Roseberry and Klimstra 1984). Our analyses of survival (Figure 3.3) and estimates of population size (Figure 3.4) suggest that 2002 was a year of high survival and productivity whereas 2001 and 2003 were characterized by lower productivity and/or survival. Although incorporating effects other than survival and productivity, year did not affect bobwhite sex ratios, suggesting that yearly productivity does not directly affect the sex of offspring. Our analyses do not rule out other effects on population sex ratio such as differential mortality of either sex.

Our results also show that maternal condition does not affect offspring sex ratios, as predicted by (Trivers and Willard 1973). An important point to note is that we investigated the effect of maternal condition using the mean of weights measured during the non-breeding season for each of the females assigned as the mother of a brood. Given environmental conditions during this time of year, these values likely underestimate the weight of breeding females, although they may serve as an index of breeding condition and should accurately represent variation in female body weight. We selected this metric because it is challenging to capture females during the breeding season. Additionally, capture-related stress may cause females to desert active nests or abandon nesting attempts, results which would have affected concurrent studies.

We suggest that future studies investigate neonatal sex ratio throughout the range inhabited by bobwhites, which encompasses a variety of habitat types and varied environmental pressures (Brennan 1999). Future studies would also benefit from the use of a standardized index of both female and male condition, incorporating variables including, but not limited to, body weight. Additionally, a better understanding of the relationship between breeding and non-breeding season condition would benefit similar studies and those investigating condition-related effects on bobwhite behavior, both social and reproductive.

## 3.7 Acknowledgments

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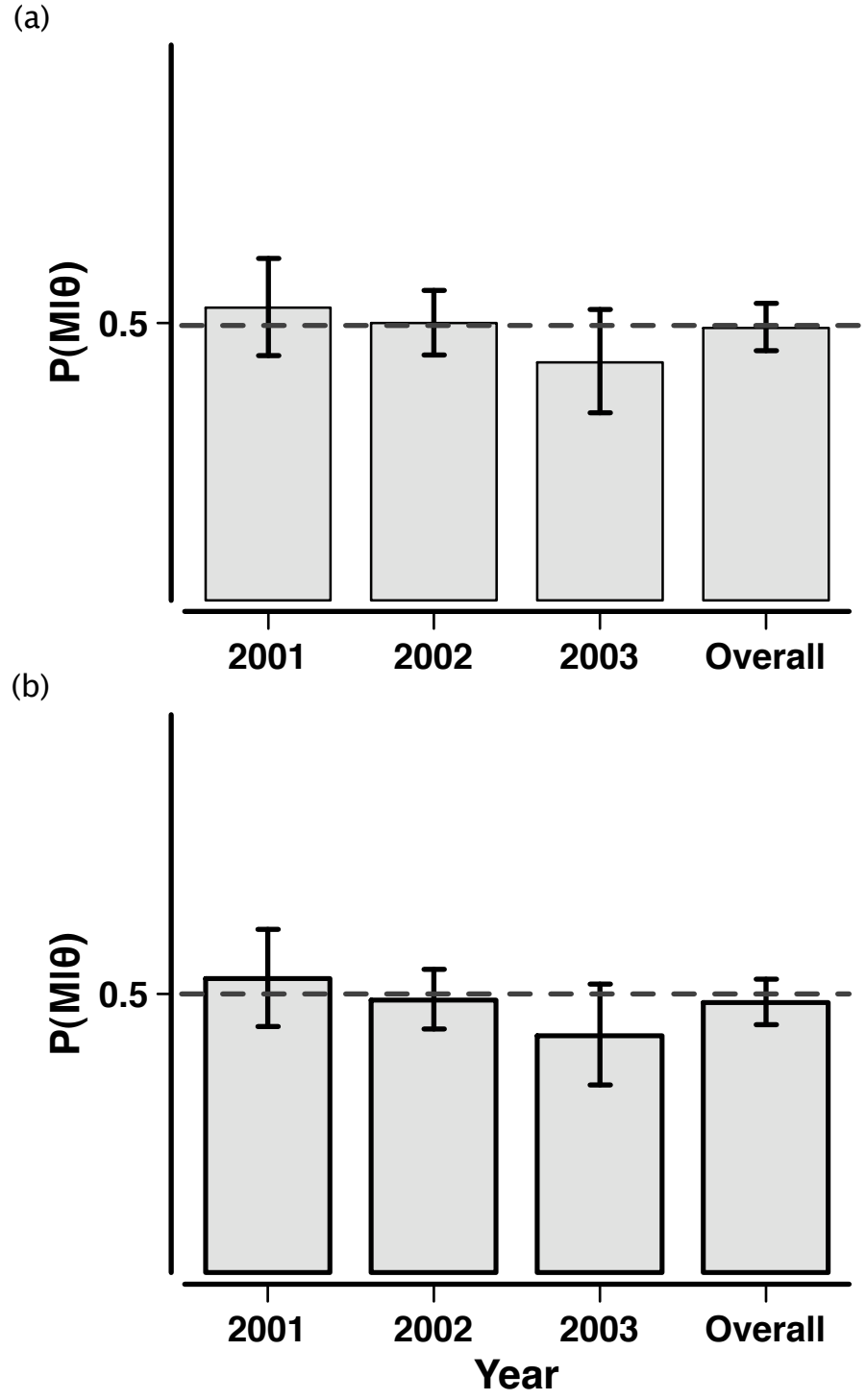


Figure 3.1: Median posterior probability (± 95% CI) of male offspring for broods (a) excluding parasitic chicks and (b) including parasitic chicks captured during 2001-2003 on Tall Timbers Research Station.

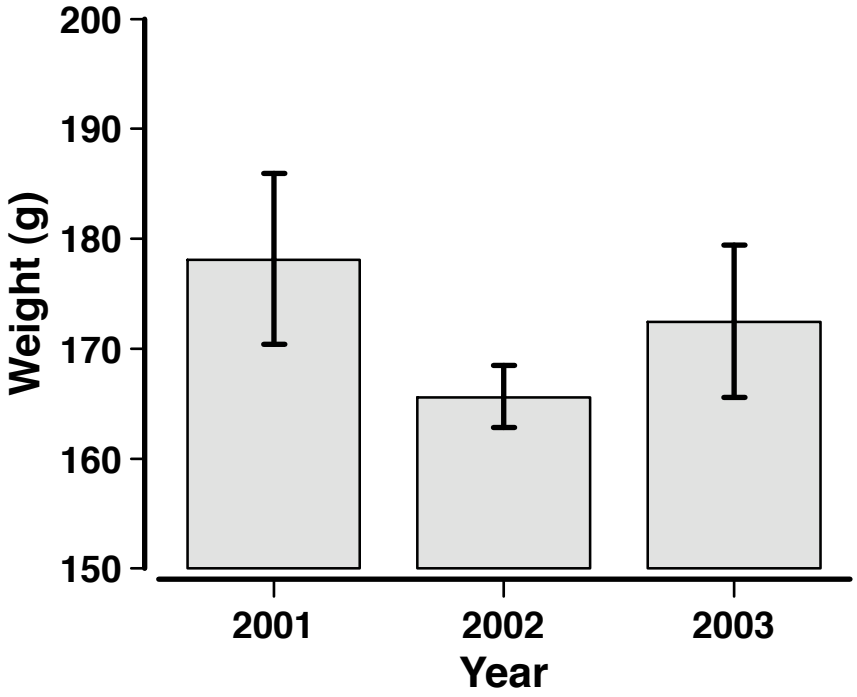


Figure 3.2: Mean weight (± 95% CI) of females captured January-March during 2001-2003 on Tall Timbers Research Station.

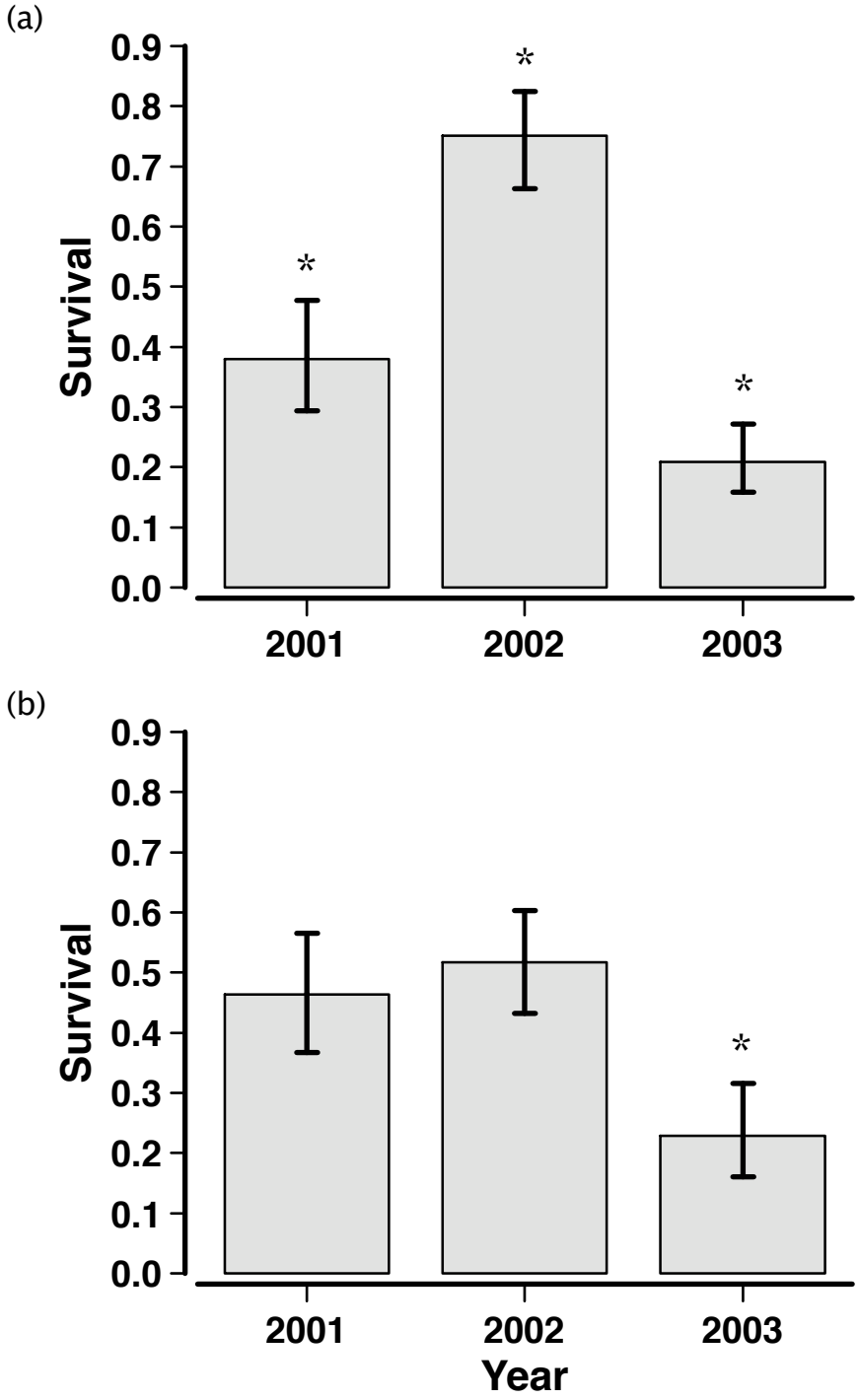


Figure 3.3: Over-winter (a) and breeding season (b) survival (±95% CI) for birds captured on Tall Timbers Research Station during 2000-2003. Asterisks indicate differences.

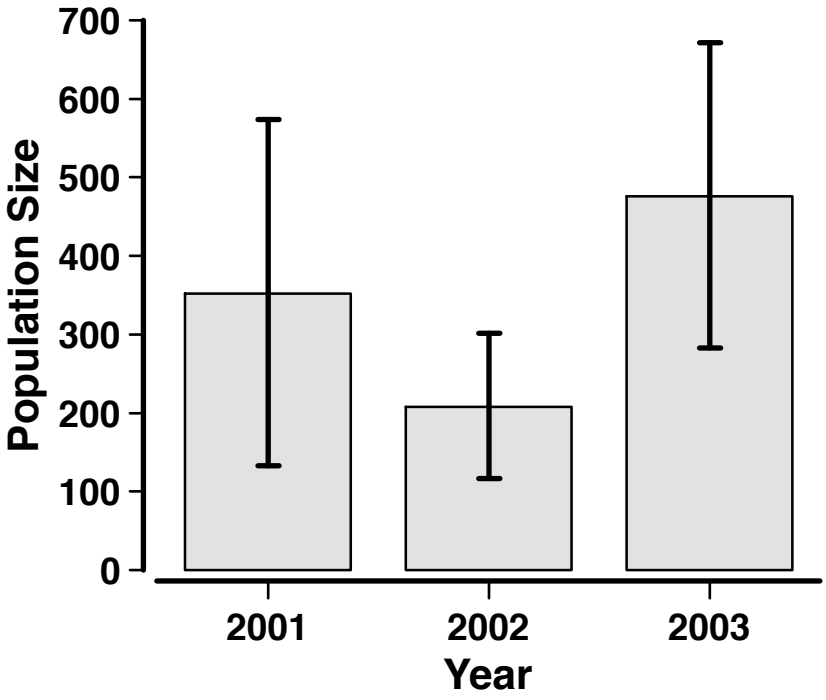


Figure 3.4: Jolly-Seber (Jolly 1965; Pollock et al. 1990; Seber 1965) estimates of population size (±95% CI) during 2000-2003 on Tall Timbers Research Station.

# Chapter 4

## Genetic Analysis of Post-Hatch Brood Amalgamation in Northern Bobwhites (*Colinus virginianus*) [1]

[1] Faircloth, B. C., W. E. Palmer, T. M. Terhune, and J. P. Carroll. To be submitted to *Molecular Ecology*.

## 4.1 Abstract

Post-hatch brood amalgamation occurs in Northern Bobwhites (*Colinus virginianus*). However, identification of amalgamated broods previously relied on the use of mark-recapture or radio-location techniques, neither of which provide objective information regarding the relationship of amalgamated chicks to their adopting brood. Prior to empirically testing hypotheses explaining the evolution and maintenance of brood amalgamation, it may be important to reduce their number given disagreement between observational data and predictions of certain hypotheses. In this manuscript, we use a combination of field and molecular data to refine our estimates of the rate of brood amalgamation in bobwhites, investigate spatio-temporal effects on this rate, and examine the relatedness between amalgamated chicks and their adoptive brood. We find that brood amalgamation occurs at rates similar to that previously estimated, spatio-temporal factors may explain the occurrence of amalgamation, and amalgamated chicks are related to their surrogate parents and brood mates at levels typically below the background relatedness in the population. We additionally develop a method of estimating the rate of brood amalgamation in the absence of genetic data. Our results suggest that several hypotheses explaining amalgamation behavior may not be applicable to bobwhites, and we discuss future, experimental studies suggested by our results.

## 4.2 Introduction

Post-hatch brood amalgamation (PHBA or brood amalgamation) occurs when organisms incubate and hatch their own young then group these offspring (actively, passively, or against their will) with those of other individuals (Eadie et al. 1988; Faircloth et al. 2005). Brood amalgamation encompasses several categories of related behavior, including: (1) adoption, (2) creching, (3) gang-brooding, and (4) kidnapping (Afton and Paulus 1993; Eadie et al. 1988). Several studies report brood amalagamation within the Odontophoridae (Brown et al. 1998; Erwin 1977; Lott and Mastrup 1999), and there are anecdotal reports of both chick adoption

by bobwhites or bobwhite broods containing additional offspring (Brooks and Rollins 2007; Burger et al. 1995; DeMaso et al. 1997; Lehman 1984; Stoddard 1931). Brood amalgamation in bobwhites was verified using mark-recapture techniques by Faircloth et al. (2005) for a population in Florida.

Identification of brood amalgamation is based on a number of techniques that include: radio-location and subsequent observation of multiple broods in the same location, observation of differences in plumage morphology among captured offspring, differences in weights among captured offspring, marking and recapturing marked broods, increase in brood size over multiple observations, and the escape by flight of offspring being captured (brood captures are conducted when chicks are incapable of flight) (Brooks and Rollins 2007; Erwin 1977; Faircloth et al. 2005; Lott and Mastrup 1999). Faircloth et al. (2005) note that all of these techniques have shortfalls (marks can be lost or inaccurately reported, chicks from the same brood have varying weights), and all typically provide information insufficient to investigate amalgamation behavior beyond estimating its rate.

The application of molecular techniques to the study of brood amalgamation allows us to better classify its occurrence and understand its potential causes. However, no single molecular tool specifically addresses the question at hand. Parentage inference may be insufficient to determine the amalgamation status of broods given the possibility for extra-pair paternity and/or intra-specific nest parasitism in many bird species. Similarly, the relatedness between brood mates may insufficiently resolve putative relationships given: (1) the typically low number of loci available for use with avian species; (2) inherent issues associated with the various relatedness estimators, particularly at the left-tail of the distribution of relatedness values (Blouin 2003; Milligan 2003), and (3) the possibility of intra-specific nest parasitism (Rosene 1969; Stoddard 1931) where relatedness between brood mates can mimic the pattern seen for amalgamated broods. In many cases, it is only through the combination of field and molecular techniques with multiple methods of analysis that we can address our questions.

Specifically, our questions relate to explaining the evolution and maintenance of amalgamation behavior. Faircloth et al. (2005) provide a summary of several hypotheses explaining of the "donation" of offspring to surrogate parents as resulting from (numbering of original manuscript used): (1a) grouping of members from different broods following attack or confusion caused by predators or natural factors; (2a) mixing among broods as a consequence of competition for brood habitat; (3a) donation of young to surrogate parents by adults unable or unwilling to provide continued care; (4a) donation of young to related individuals allowing more experienced or fecund individuals to renest, thus increasing inclusive fitness of families; and (5a) donation of offspring to male parents, allowing the female to renest (Eadie et al. 1988; Hamilton 1964*a*,*b*; Munro and Bedard 1977). Those hypotheses explaining the "receipt' of offspring by surrogate parents are no less numerous: (1b) adults may be unable to discriminate among related and unrelated offspring; (2b) adults are unable to resist adoption as a result of residual hormone levels; (3b) costs are low when adopting precocial chicks; (4b) adoption may dilute the risk of predation; (5b) "helpers" may acquire parenting experience; (6b) offspring may be kidnapped to reduce predation risk to the offspring of a dominant pair (Birkhead and Nettleship 1984; Darling 1938; Eadie et al. 1988; Foster and Treherne 1981; Hamilton 1971; Heinsohn 1991; Nastase and Sherry 1997; Riedman 1982; Taylor 1976)

In this manuscript, we expand the data-set evaluated in Faircloth et al. (2005) and re-evaluate the occurrence of brood amalgamation among bobwhites by incorporating molecular data (Faircloth et al. 2008; Schable et al. 2004). We use our combined data to determine the status of captured broods (amalgamated or non-amalgamated). We then use this amalgamation status to: (1) examine non-genetic methods of identifying amalgamated broods, (2) investigate spatio-temporal factors affecting the occurrence of brood amalgamation, and (3) analyze the degree of relatedness between brood mates, parents, and amalgamated chicks. Finally, we address several of the hypotheses explaining the movement of offspring between broods in the context of our results.

## 4.3 Methods

### 4.3.1 Capture of Adults

We trapped, captured, and radio-collared adult and subadult bobwhite during October-June 2000-2003 on a 250 ha area of Tall Timbers Research Station, Tallahassee, FL according to methods described in Faircloth et al. (2005). We collected 10 body feathers from the flank of each bird captured, and we stored 5 feathers in 70% EtOH for genetic analysis and 5 feathers in paper envelopes as a reserve.

During the breeding season (April 15 - October 15), we located all birds by radio-location using the homing method (Kenward 2001; White and Garrott 1990) at least once per day. We recorded the band number of any birds within 5 - 10 m of the bird being located, as determined by radio-location. We identified nests when individuals were found in the same location >1 day. We flagged these areas >3 m from each nest, and we searched each area for the presence of a nest and to determine clutch-size when radio-location indicated the incubating bird was away from the nest. We recorded daily locations of radio-collared adults, nests, and broods on a GIS map of the study area, and we converted these locations to UTM coordinates, which were then input to a relational database.

### 4.3.2 Capture of Offspring

We captured bobwhite chicks using the brood capture procedures outlined in Smith et al. (2003) and Faircloth et al. (2005). During the breeding seasons of 2001-2003, two captures were conducted for broods at 1-4 and 10-13 days after hatching. At the initial capture in 2001, chicks were marked with patagial wing-bands (Carver et al. 1999). Because we suspected chicks banded at 1-4 d might be losing patagial bands, during 2002-2003 we marked chicks at the first capture with permanent markers (Sharpie ®, Oak Brook, IL) on the ventral surface of the throat with a color pattern allowing individual identification upon recapture. We then applied patagial wing bands to chicks at the second capture.

During 2001-2003, all chicks were weighed to the nearest 0.1 g using a 20 g spring-scale (Pesola ®, Baar, Switzerland). We collected 1.5 $m$m patagial microbiopsies from each chick (Miltex Corp., York, PA), treated the biopsy area with NeoPredef powder (Pfizer Animal Health, New York, NY), and stored each sample in individual 1.5 $m$L microtubes containing a Tris-EDTA buffer (100$m$M Tris pH 8.0, 100$m$M EDTA pH 8.0, 10 $m$M NaCl, 1% SDS). We released all chicks $\leq$ 5 m from the attending adult, as determined from radio-telemetry.

We have shown that our brood capture procedures typically result in complete captures of a brood, released chicks rapidly return to the attending parent(s), these procedures do not introduce observer bias by inducing subsequent amalgamation of broods, and the collection of patagial biopsies at 1-4 days of age does not affect neonatal survival (Evans et al. 2008; Faircloth et al. 2005).

### 4.3.3 Microsatellite Genotyping

We extracted DNA from feathers using DNeasy kits (Qiagen Inc., Valencia, CA) with a modification to the digestion step, adding 25 $\mu$L of 100 $m$g/$m$L DTT (Dithiothreitol) along with proteinase K. We eluted DNA with either: 2 washes of 60 $\mu$L Buffer AE or 1 wash 120 $\mu$L Buffer AE. Prior to amplification, we treated all samples 1:3 (volume:volume) with 10% Chelex resin (BioRad Laboratories, Hercules, CA) to remove PCR inhibitors. We performed 96-well PCR amplifications of 16 microsatellite loci (Table 1.4; Faircloth et al. 2008; Schable et al. 2004) in 10 $\mu$L volumes using CAG- or M13R-tagged primers (Glenn and Schable 2005). Reaction concentrations were 0.5 U AmpliTaq Gold (Applied Biosystems, Foster City, CA), 1X Gold Buffer, 1X BSA (New England Biolabs, Ipswich, MA), 1.5 $m$M MgCl, 1.25 $m$M dNTPs, 0.5 $\mu$M untagged primer; 0.05 $\mu$M CAG or M13-reverse tagged primer, 0.45 $\mu$M dye-labelled tag (HEX, FAM, NED + CAG or M13-reverse), 3.3 $\mu$L ddH2O, and 2 $\mu$L DNA template (5-10 $n$g). We included multiple negative controls, using $ddH_2O$ in place of template DNA, in each plate of PCR reactions.

We used one of two locus-dependent touchdown thermal cycling profiles (Don et al. 1991), each encompassing a 10 ℃ span of annealing temperatures (ranges: 60 - 50 ℃; 65 - 55 ℃). Cycling parameters included a Taq activation step at 95 ℃ for 5 min followed by 20 cycles at 95 ℃ for 20 sec; 60 or 65 ℃ for 30 sec minus 0.5 ℃ per annealing cycle; and 72 ℃ for 90 sec followed by 25 cycles at 95 ℃ for 20 sec; 50 or 55 ℃, respectively, for 30 sec; 72 ℃ for 90 sec. We used a final extension period of 10 min at 72 ℃.

We scored fragments using an ABI 3730xl sequencer (Applied Biosystems) with ROX500 fluorescent size standard. We sized fragments using GENEMAPPER version 4.0 software (Applied Biosystems) and the Global Southern method (Applied Biosystems 2005). Initial tests indicated that the Global Southern method resulted in reduced intra-run variation in microsatellite fragment length relative to other sizing methods. We binned all fragments using the same binset to ensure that binned fragments were consistent across years, and we discarded ambiguous genotypes from the data set and re-genotyped plates with failing negative controls.

To assess the rate of genotyping error, we randomly selected a 12% sample from the entire data set, assigned each individual a random identification string, and blindly genotyped, scored, and binned these samples (Hoffman and Amos 2005). We exported all samples from the GENEMAPPER database, converted each to a useful format using GMCONVERT (Faircloth 2006), and imported records to a separate relational database. We then re-assigned error samples their true identification and compared each to the corresponding non-error sample to compute the genotyping error rate on a per-locus and overall basis ($\bar{x} \pm 95\%$ CI).

### 4.3.4 Parentage Assignment and Linkage

Using CERVUS (Kalinowski et al. 2007; Marshall et al. 1998), we analyzed the microsatellite data by year to test for Hardy-Weinberg equilibrium and infer parentage of all offspring. Along with genotypes of chicks captured during a particular year, we included genotypes of parents known to be alive at the start of breeding season. We used the delta approach

(Marshall et al. 1998) to determine significance of all parentage assignments at the 99% confidence level. We ran delta simulations for 100,000 iterations, using yearly estimates of percent population captured (Table 1.3), twice the average number of opposite sex associates for incubating birds determined by radio telemetry ($n = 10$), and our overall estimate of genotyping error.

Following parentage inference, we conducted an additional parentage exclusion step using the parents inferred for a majority of each brood. This ensured that putative parents were not inappropriately excluded for certain parent-offspring combinations as a result of the contribution of the weight of the data to the log-odds computation. We assigned parents to members of a brood when: (1) one of the parents matched the adult with which each brood was captured, (2) the inference/exclusion occurred at the 99% level, and (3) there were fewer than 3 mismatches between parents and offspring at any locus.

Following this exclusion step, we identified 6 large families where parentage of all offspring was attributed to only two parents ($\bar{x}_{offspring} = 8.5$). We used genotypes from these 6 family groups to test for linkage using two-point analysis in CRIMAP (Lander and Green 1987) for all pairwise combinations of loci, and we assumed loci were linked when the log-odds for the pairwise-comparison was $> 3.0$.

### 4.3.5 Genetic Identity Among Samples

Using CERVUS, we analyzed microsatellite data to determine genetic identity among offspring sampled. Genetic identity may result by chance, with few loci; as a result of the presence of identical twins; or due to resampling offspring with marks that were lost or improperly recorded. In all cases, we assumed genetic identity was a result of lost or improperly recorded marks. We assumed samples were identical when genotypes matched exactly at all loci ($\bar{x}_{p(ID_{sibs})} = 4.64 * 10^{-6}$). We dropped duplicate members of any pair from the data set.

### 4.3.6 Determination of Amalagamation Status

In order to determine the amalgamation status of broods at first and second capture, we used a 2-step process to: identify broods that were potentially amalagamated, and verify the relationship status between chicks in amalgamated broods. We identified potential amalgamations when captured broods: (1) contained chicks having marks originating from another brood, (2) contained chicks lacking any marks, (3) had chicks escape by flight from the brood capture ring, and/or (4) exhibited gross differences in plumage morphology. We excluded broods of $\leq 1$ chick, those broods where none of the chicks captured at the first occasion were recaptured at the second, and those broods where all chicks escaped by flight. We assumed all broods not meeting these criteria were not potentially amalgamated.

#### Relationship Status, Verification of Amalgamation, and Brood Amalgamation Rate

Although our capture procedures typically resulted in complete captures of broods at both capture occasions (Faircloth et al. 2005), incomplete captures did occur. In these cases, single chicks often escaped capture and marking, and it was possible to recapture them at future occasions with a marked brood which would then be recorded as potentially amalgamated.

In order to identify cases in which this occurred and verify the amalgamation status of a brood (amalgamated or not-amalgamated), we estimated the relationship between brood mates and potentially amalgamated chicks. We used pairwise relationship estimation (Appendix A; Milligan 2003; Thompson 1975; Wagner et al. 2006; Weir et al. 2006) to avoid the problems associated with pairwise relatedness estimates: the creation of arbitrary cut-points for relationship classes, subsequent "binning" of results into these classes, and the potential for observer bias in assigning relatedness values to relationship classes.

Prior to estimation, we computed allele frequencies, adjusted for the presence of null alleles, using the EM (Expectation Maximization) algorithm (Dempster et al. 1977) as implemented in the software FreeNA (Chapuis and Estoup 2007). We assigned individuals being

compared into common relationship classes (classes considered: parent-offspring, full-siblings, half-siblings, double first-cousins, first-cousins, and unrelated).

We assumed that captures of 1-2, potentially amalgamated chicks sharing a full- or half-sibling relationship with a majority ($\geq$ 50%) of the brood could be the result of incomplete captures. If these individuals were the only "amalgamated" chicks with which a brood was captured, the amalgamation status of the brood was not verified and the brood was not used in subsequent analyses.

Using WinBugs (Lunn et al. 2000), we estimated the posterior probability of the data given a Bernoulli distribution and a non-informative, uniform prior (0,1). We computed the mean and 95% credible interval of the posterior probability of amalgamation (50,000 burn-in iterations; 50,000 simulations). We used an identical process to estimate the posterior probability of males or females being associated with all broods and only amalgamated broods. Given the small, within year sample size for amalgamated broods, we combined data across years for our analyses. Finally, using only broods with verified amalgamation status, we computed mean number of amalgamated chicks ($\pm$ 95% CI) per amalgamated brood.

### 4.3.7 Spatial, Temporal, and Spatio-Temporal Clustering of Broods

We computed the 95% minimum convex polygon (MCP) home range for each brood using the ADEHABITAT package in R (Calenge 2006; R Development Core Team 2006) and the UTM coordinates collected during the brooding period (22 d post-hatch). To ensure a representative number of points were used to generate each MCP, we excluded the home-range of any individual with fewer than the lower 95% CI of the average number of brood locations. From these MCPs, we computed the mean size of each brood home range, and we derived the radius of a circle covering an area of identical size.

Using data for broods with verified amalgamation status, we classified all broods as amalgamated (1) or non-amalgamated (0), independent of the period(s) during which they

were captured. We then created a matrix of pairwise amalgamation status between all broods captured. Possible values were (A) if both broods compared were amalgamated, (B) if either of the pair was amalgamated, and (C) if neither brood being compared was amalgamated. We created similar pairwise matrices of euclidean distance (*Distance*) between the central point of each brood's distribution of spatial locations and difference in Julian hatch dates ($\Delta_{HatchDate}$) of all captured broods.

We divided every value in the *Distance* matrix by twice the mean home range radius (estimated above) and rounded these to integer values. Similarly, we divided the time difference matrix ($\Delta_{HatchDate}$) by twice the length of time during which we captured broods (2 $x$ 14 days), rounding again to integer values. In both cases we multiplied the denominator by 2 to account for adjacency in time and distance periods.

Once transformed to categorical measures, we unfolded each pairwise matrix, and we examined the effect of year, $\Delta_{HatchDate}$, *Distance*, and the interaction between *Distance* and $\Delta_{HatchDate}$ on the occurrence of post-hatch brood amalgamation using the LOGISTIC procedure in SAS 9.1 with the GLOGIT link function (SAS Institute 2004). We ranked and selected models using $AIC_c$, and we averaged across all parameters in the candidate model set ($w_i > 0.0$) to incorporate the effects of model selection uncertainty on parameter estimates (Akaike 1974; Burnham and Anderson 2002).

### 4.3.8 Non-genetic Indicators of Brood Amalgamation

During 2001-2003, we observed that variance in the weight of captured broods appeared to indicate their amalgamation status. In order to examine this apparent trend, we used the classification of non-amalgamated and amalgamated broods to separate all captured chicks into 3 classes: non-amalgamated broods, broods of mixed relationships, and amalgamated broods.

The non-amalagamated class comprised those chicks in verified, non-amalgamated broods where both parentage analysis and relationship inference indicated all individuals were either

full- or half-siblings. The mixed class comprised broods that were verified as amalgamations but contained individuals unrelated or related at a low level (first-cousins) to a majority of the brood. The amalgamated class comprised chicks from broods verified as amalgamations. For each of these classes, we computed the mean (±95% CI) of the standard deviation in brood weight at the first and second capture periods.

### 4.3.9 Relatedness Computation

We computed the background level of relatedness ($\bar{x} \pm 95\%$ CI) during each year of the study by taking the mean of all pairwise relatedness calculations across our entire sample (adults and offspring) during each breeding season. To investigate the relatedness within and between brood mates and amalgamated chicks and between amalgamated chicks and the inferred parents of a brood, we used a maximum likelihood estimator of relatedness adjusted for the presence of null alleles (Appendix A; Milligan 2003; Thompson 1975; Wagner et al. 2006; Weir et al. 2006) similar to the relationship estimation procedure used above. We examined the relatedness between brood mates and amalgamated chicks and among brood mates and amalgamated chicks using the mean relatedness (±95% CI) across all pairwise, within brood comparisons. Similarly, to analyze the relatedness between inferred parents of a brood and amalgamated chicks, we computed the mean relatedness (±95% CI) between all pairwise comparisons when we successfully assigned either or both parents to a particular brood.

## 4.4 Results

During 2001-2003, we captured, radio-tagged, and sampled 621 adult/sub-adult and 841 bobwhite chicks. We genotyped 96% and 93% of those birds, respectively (Table 5.1). Our overall rate of genotyping error was 0.008 (± 0.003; $n_{loci} = 16$; Appendix B).

Following Bonferroni correction (Rice 1989), CV-PA3E deviated from Hardy-Weinberg equilibrium in 2002. Linkage analysis indicated that 2 loci were linked (CV-PA1F and CV-PA12A) and 1 locus (CV-P1F2) exhibited problems with Mendelian inheritance, likely due to the presence of null alleles. We dropped CV-PA12A and CV-P1F2 from the data set. We then re-ran parentage assignment and conducted all subsequent analyses with the truncated data ($n_{loci} = 14$).

Analysis of genetic identity indicated that 8, 1, and 1 wing-banded chick(s), captured during 2001-2003 and initially recorded as amalgamated individuals, were actually recaptures of previously marked birds. In all cases, these identities were attributable to tag loss, corresponding to a tag-loss rates of 0.04, 0.004, and 0.02 during the 10-13 day inter-capture period for each year, respectively. We removed these individuals from the data set prior to subsequent analyses. We identified no captures of 1-2 potentially amalgamated chicks sharing full- or half-sib relationships with a majority of the brood with which they were captured.

We captured 80 broods at 1-4 days of age and 50 broods at 10-14 days of age during 2001-2003. Of these broods, we confirmed the occurrence of amalgamation in 6 and 16, respectively (Table 4.2). These numbers differ slightly from Faircloth et al. (2005) as a result of our exclusion of identical samples and the inclusion of broods for which the amalgamation status could not be verified in the original study. We provide the mean posterior probability of brood amalgamation during each year of the study in Figure 4.1 and the posterior probability of male and female brooding in Figure 4.3.

We classified 39, 27, and 6 broods into the unmixed, unknown, and amalgamated categories, respectively, at first capture periods and 21, 10, and 13 broods into the same categories at second capture periods during 2001-2003. Non-amalgamated broods possessed a lower variance in chick weight than amalgamated broods at both first and second captures (Figure 4.2). The variance in weight of chicks in the mixed class did not differ from that of non-amalgamated broods suggesting that these individuals were not amalgamated chicks.

The average number of locations per brood was 27 (± 4), 16 (± 2), and 18 (± 2). After excluding broods with insufficient numbers of locations ($< 95\% CI_{lower}$), we estimated brood home range using location data from 14, 26, and 10 broods during 2001-2003, respectively. Brood home range did not differ during the study (Figure 4.4), and we used the mean, overall radius of all calculated home ranges (69 m) for spatiotemporal analyses.

Year, difference in hatch date, and distance best explained variation in the status of broods being compared (Table 4.3). After model averaging, only year ($\beta_{Year_{2001_A}} = -2.4 \pm 1.4$; $\beta_{Year_{2001_B}} = -1.1 \pm 0.3$; $\beta_{Year_{2002_A}} = 1.5 \pm 0.7$; $\beta_{Year_{2002_B}} = 0.6 \pm 0.3$), difference in hatch date ($\beta_{\Delta_{HatchDate_A}} = -1 \pm 0.4$; $\beta_{\Delta_{HatchDate_B}} = -0.4 \pm 0.2$;), and the distance between broods when 1 of the pair were mixed ($\beta_{Distance_A} = -0.6 \pm 0.6$) affected amalgamation status relative to other broods.

Background levels of relatedness in the population differed slightly during the study period ($\hat{r}_{2001} = 0.06 \pm 0.0007$; $\hat{r}_{2002} = 0.06 \pm 0.0002$; $\hat{r}_{2003} = 0.05 \pm 0.0008$). Amalgamated chicks were not related to the broods with which they were captured at levels above the background relatedness in the population (Figure 4.7; $\hat{r}_{overall} = 0.06 \pm 0.007$), and the relatedness between amalgamated individuals was not different from that between non-amalgamated brood mates (Figure 4.6).

Using our parentage assignment procedures, we assigned both parents to 47 broods and single parents (male or female) to 26 broods ($\geq$ 1 parent assigned to 91% of broods). Using these assignments, we examined the relatedness between parents and amalgamated chicks in 11 and 3 broods captured during 2002 and 2003, respectively. No broods in 2001 were assigned a parent. Amalgamated chicks did not share a strong relationship with either inferred parent of the broods with which they were captured (Figure 4.8; $\hat{r}_{female_{overall}} = 0.04 \pm 0.02$; $\hat{r}_{male_{overall}} = 0.07 \pm 0.02$). The degree of relatedness between amalgamated chicks and the assigned male parent was above background levels of relatedness in the population during 2002.

## 4.5 Discussion

Our results demonstrate that post-hatch brood amalgamation occurs at a rate within the range we previously estimated for bobwhites (Faircloth et al. 2005), at both first and second capture occasions. The posterior probability of brood amalgamation (Figure 4.1) represents a more accurate estimate than presented in our prior work. Analysis of genetic identity between samples reduced the number of false positives in our original data set and allowed us to estimate a rate of tag loss from neonatal bobwhites, which may be useful in future bobwhite population models.

Our examination of variance in brood weight suggests that this technique allows determination of amalgamation status without the use of genetic data. Our results also show that broods in the "mixed" class (those composed of siblings and unrelated individuals) are not the result of amalgamation. The presence of unrelated individuals is best explained by intraspecific nest parasitism (Baskett 1947), reported (Rosene 1969; Stoddard 1931) but not verified for bobwhites using reliable techniques (MacWhirter 1989).

In light of hypotheses explaining the donation of offspring to other individuals, our results have several implications:

### 4.5.1 Amalgamated chicks are not related to adopting broods or parents

We previously suggested that brood amalgamation could potentially be explained by kin selection theory (Hypothesis 1a; Hamilton 1964*a*,*b*), which suggests that seemingly altruistic adoption events can be explained in the context of inclusive fitness benefits received by the adopting parent. A requirement of this theory is that the degree of relatedness between foster parents and amalgamated chicks be greater than the background level of relatedness in the population (West-Eberhard 1975). Our results indicate that the level of relatedness between foster parents and amalgamated chicks was $> 0.0$ but not greater than the background level of relatedness in the population, except for the comparison with adult males during 2002 - a difference we cannot explain. Similarly, the degree of relatedness between amalgamated

chicks and their brood mates was not different from the background level of relatedness in the population. These results suggest that kin selection is not responsible for this behavior, at least for bobwhites captured on our study site during the period examined.

In order to compare the relatedness of foster parents and amalgamated chicks, we used the genotypes of amalgamated chicks and those of the parent(s) assigned to the remainder of the brood. We believe this accurately represents the degree of relatedness between the parent(s) of each brood and amalgamated chicks, because we required at least one of the parents be located with the captured broods prior to assigning parentage to that individual. However, this comparison ignores the degree of relatedness between other adults roosting with the brood (Faircloth et al. 2005) and the amalgamated chicks. We were often unable to determine the identity (using radio-location) of these individuals prior to their flight from the capture area.

Additionally, our estimates of relatedness between brood mates, amalgamated chicks, and parents are subject to variance introduced as a function of estimator choice. Relatedness estimation in the absence of pedigree information is challenging (Blouin 2003; Milligan 2003; Wang 2007). Throughout this manuscript, we use a maximum likelihood estimator of relatedness (Milligan 2003; Thompson 1975; Wagner et al. 2006; Weir et al. 2006). We chose this algorithm because it exhibits lower root-mean square error and bias for most estimates of relatedness (Milligan 2003), particularly those of higher degree which we believed, *a priori*, may exist between members of amalgamated broods. The estimator exhibits a positive bias at the left-tail of the distribution of relatedness values (Milligan 2003), which likely inflates both our estimates of background relatedness and the degree of relatedness between assigned parents and amalgamated chicks or chicks within each brood (Figure 4.8, 4.7). Even in the presence of positive bias, we did not identify a strong degree of relatedness between foster parents and amalgamated chicks nor between amalgamated chicks and their brood mates.

### 4.5.2 Males are not more likely to be associated with amalgamated broods

Hypothesis 5a suggests that females may donate their offspring to a male mate, allowing the female to re-nest thus increasing the fitness of the mated pair. This situation is the brood rearing corollary of a hypothesized bobwhite mating system: rapid multi-clutch polygamy (Curtis et al. 1993; Emlen and Oring 1977). This hypothesis predicts that broods are attended by males to a greater degree than females. Males did not attend more broods than females nor did we find evidence of males attending more amalgamated broods than females (Figure 4.3). This result is again independent of other, adult birds that occasionally flushed from the capture area.

It is interesting to note that the trend of the data in 2001 and 2003 indicates that males attend more broods than females, and the correlation of these trends with low survival and reproductive output during these years (data not shown) is worthy of attention. Rates of male incubation were also high during these years (Chapter 5), suggesting that the difference may be due to nesting behavior rather than brooding behavior and the amalgamation of chicks.

### 4.5.3 Amalgamation of broods is affected by space and time

Hypothesis 1a and 2a (Eadie et al. 1988; Munro and Bedard 1977) state that brood amalgamation may be a function of accidental mixing between broods. Although we cannot address each specifically, our spatio-temporal analyses suggest that this is a plausible explanation. Year, hatch date, and inter-brood distance best explain the likelihood that either 1 or both of a pair of broods are amalgamated, relative to non-amalgamated broods. Furthermore, the likelihood of amalgamation is affected by year, which we believe is best explained by brood density across the landscape, an index of which is the number of broods captured (Table 4.2). Interestingly, inter-brood distance only affects the amalgamation probability of one brood of the pair being analyzed. This may indicate that the movement of chicks between broods is unidirectional: broods do not shuffle membership.

Throughout this manuscript, we have focused on explanations for the "donation" of offspring to other adult bobwhites. We have not addressed hypotheses explaining the receipt of additional chicks by unrelated adults, largely because our data do not support these analyses. Several of the hypotheses are best examined in a laboratory setting where the ability of adults to discriminate between young, hormone levels, and adoption tendencies can be objectively evaluated (*see* Riedman 1982). Our research and that of others (R. Cass, *personal communication*) suggest that hypotheses 3b and 4b (Eadie et al. 1988; Hamilton 1971; Nudds 1980) may best be tested using experimental manipulation of brood sizes and subsequent radio-monitoring of chicks to estimate size effects on individual survival. Studies of this nature have important implications for both predation theory (Foster and Treherne 1981; Hamilton 1971; Milinski 1984; Pulliam 1973; Wrona and Dixon 1991) and management of this species, which is declining (Brennan 1991; Butcher et al. 2007; IUCN 2007). Bobwhite broods provide an interesting system in which to investigate both the impact of behavior on predation avoidance and social foraging (Giraldeau and Caraco 2000) because immature individuals possess incomplete knowledge with respect to the avoidance of predation and the process of foraging.

## 4.6 Summary

Post-hatch brood amalgamation occurs frequently among bobwhite and may be a persistent feature of their biology. As such, this behavior deserves explanation, and several hypotheses attempt to explain this behavior in both bobwhite and similar, precocial species. Contrary to 2 of these hypotheses, we found: (1) amalgamated chicks are unrelated to their surrogate parents or brood-mates and (2) amalgamated broods are not more likely to be associated with male birds. Our results additionally indicate that spatio-temporal factors affect the probability of amalgamation: broods closer in space and time tend to amalgamate. This observation suggests that some brood mixing may be accidental - a proximate explanation. This observation does not, however, explain the movement of entire broods between attending

adults nor does it explain why adult individuals choose to accept young that are not their own. We think it likely that the ultimate factor(s) explaining amalgamation relate largely to variability in the environment, influenced by rates of predation and food availability. Brood amalgamation may be a means of mitigating the impact of some or all of these factors, potentially similar to the grouping instinct of adult birds during the non-breeding season.

## 4.7 Acknowledgments

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Table 4.1: Northern Bobwhites captured, radio-collared, and sampled for genetic analysis; number of individuals genotyped; and genotyping success during 2000-2003. We defined a successful genotype as amplification at $\geq 50\%$ of loci ($n_{loci} = 16$).

| Year | Captured | | Genotyped (%) | | Successfully Genotyped (%) | |
|---|---|---|---|---|---|---|
| | Adults | Chicks | Adults | Chicks | Adults | Chicks |
| 2001 | 179 | 230 | 162 (91%) | 215 (93%) | 160 (99%) | 214 (100%) |
| 2002 | 175 | 468 | 167 (95%) | 441 (94%) | 163 (98%) | 432 (98%) |
| 2003 | 267 | 143 | 266 (100%) | 129 (90%) | 263 (99%) | 129 (100%) |
| Overall | 621 | 841 | 595 (96%) | 785 (93%) | 586 (98%) | 775 (99%) |

Table 4.2: Number of brood captures, number of broods with verified amalgamation status, verification success rate, number of amalgamated broods, and the mean number of amalgamated chicks captured with a brood for all captures on Tall Timbers Research Station during 2001-2003.

| Year | Captures | | Verified (%Success) | | Amalgamated | | Amalgamated Chicks | |
|---|---|---|---|---|---|---|---|---|
| | 1-4 d | 10-12 d | 1-4 d | 10-12 d | 1-4 d | 10-12 d | Mean | ± 95% CI |
| 2001 | 20 | 9 | 18 (90%) | 8 (89%) | 1 | 1 | 2.0 | N/A |
| 2002 | 45 | 32 | 43 (95%) | 30 (94%) | 5 | 12 | 5.0 | 1.9 |
| 2003 | 15 | 9 | 14 (93%) | 5 (55%) | 0 | 3 | 4.6 | 1.7 |
| Overall | 80 | 50 | 75 (95%) | 43 (86%) | 6 | 16 | 4.6 | 1.4 |

Table 4.3: Model structure, $AIC_c$, $\Delta AIC_c$, model weight ($w_i$), relative likelihoods, and number of parameters for models of post-hatch brood amalgamation on Tall Timbers Research Station during 2001-2003.

| Model | $AIC_c$ | $\Delta AIC_c$ | $w_i$ | Relative $\ell$ | Parameters |
|---|---|---|---|---|---|
| PHBA(.) + Year + Distance + Hatch Date | 1939.99 | 0.00 | 0.51 | 1.00 | 10 |
| PHBA(.) + Year + Hatch Date | 1940.56 | 0.57 | 0.39 | 0.75 | 8 |
| PHBA(.) + Year + Distance + Hatch Date + Distance*Time | 1943.18 | 3.19 | 0.10 | 0.20 | 12 |
| PHBA(.) + Year + Distance | 1978.84 | 38.85 | 0.00 | 0.00 | 8 |
| PHBA(.) + Distance + Time + Distance*Hatch Date | 2030.49 | 90.50 | 0.00 | 0.00 | 8 |
| PHBA(.) + Hatch Date | 2034.77 | 94.78 | 0.00 | 0.00 | 4 |
| PHBA(.) + Distance + Hatch Date | 2040.29 | 100.30 | 0.00 | 0.00 | 6 |
| PHBA(.) + Distance | 2051.48 | 111.49 | 0.00 | 0.00 | 4 |

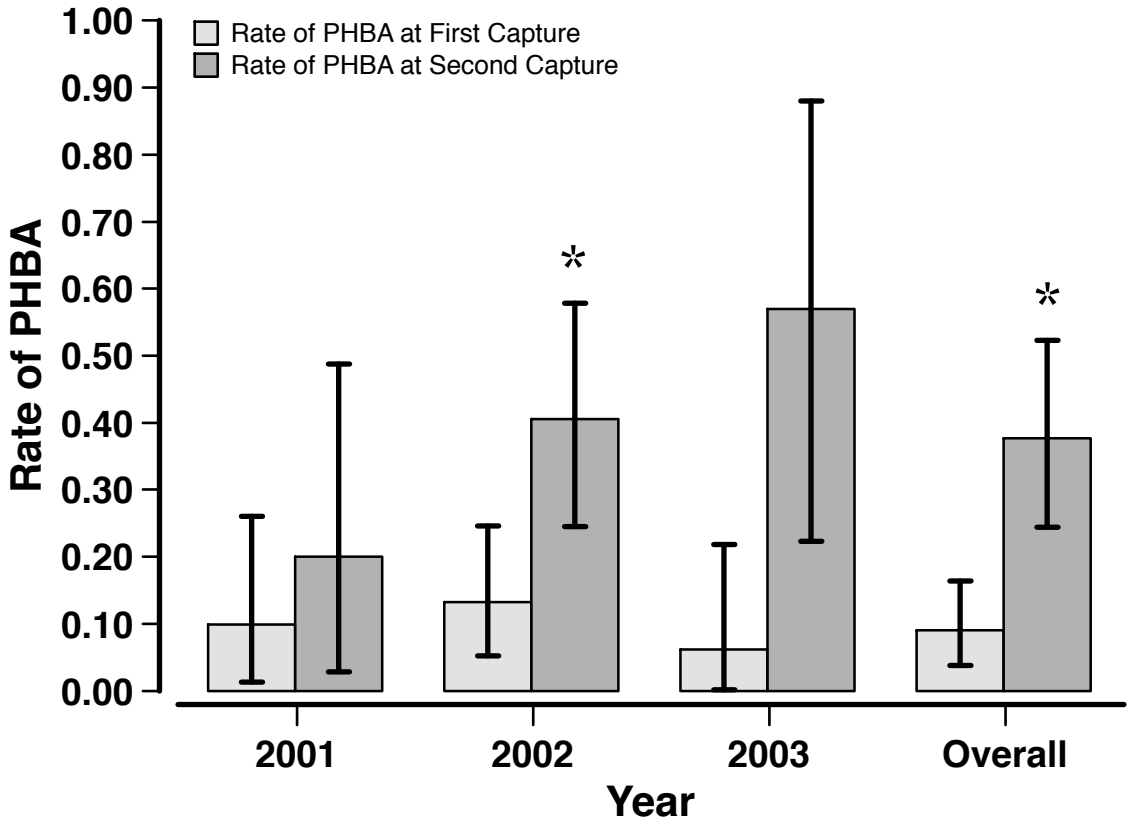


Figure 4.1: Posterior probability (± 95% CI) of post-hatch brood amalgamation (PHBA) for bobwhite broods captured during 2001-2003 on Tall Timbers Research Station. Asterisks indicate a significant difference between capture periods.

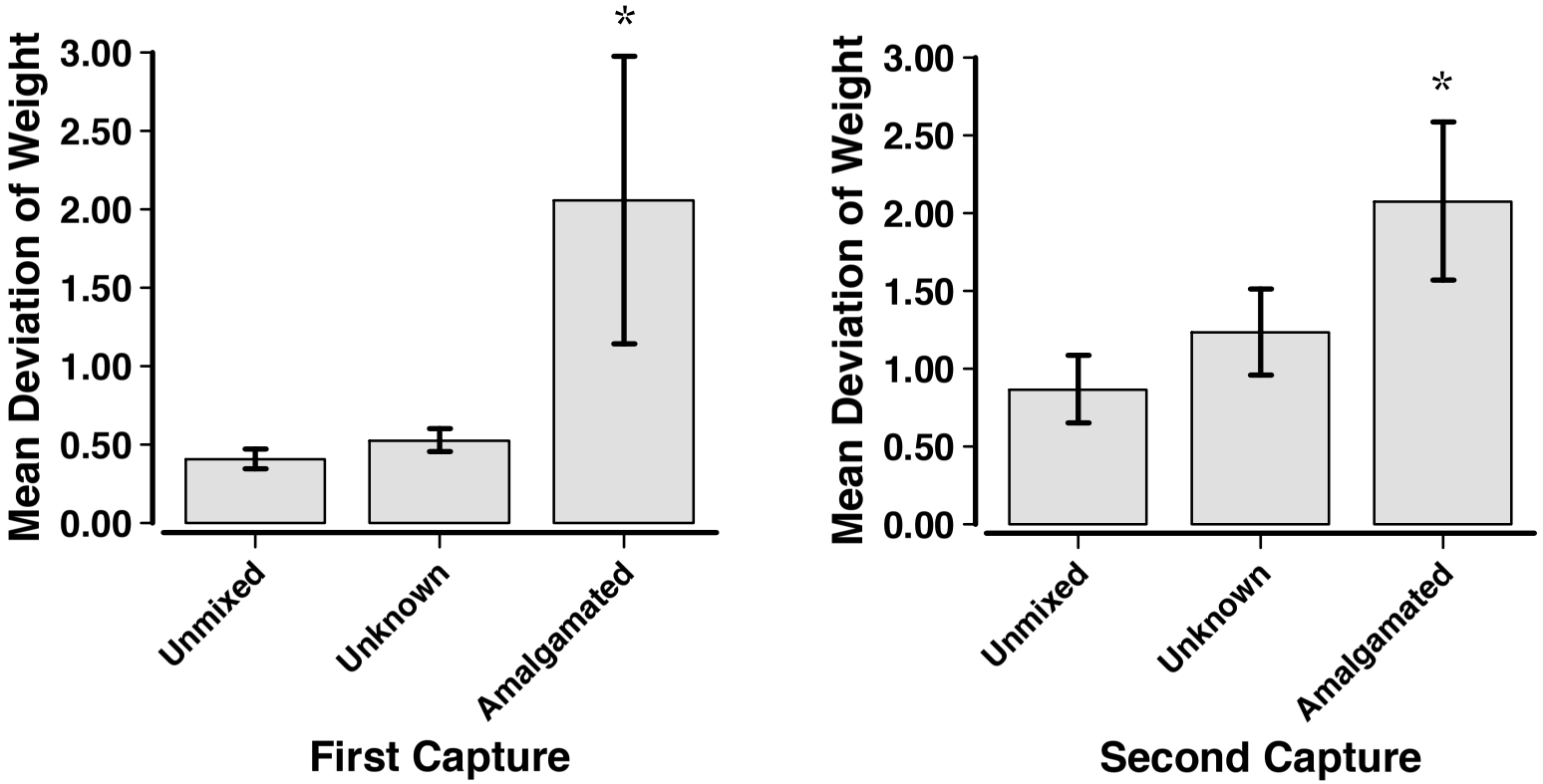


Figure 4.2: Mean standard deviation (± 95% CI) in brood weight for unmixed broods (full- and half-sibs only), broods with chicks of unknown origin, and amalgamated broods. Asterisks indicate a significant difference.

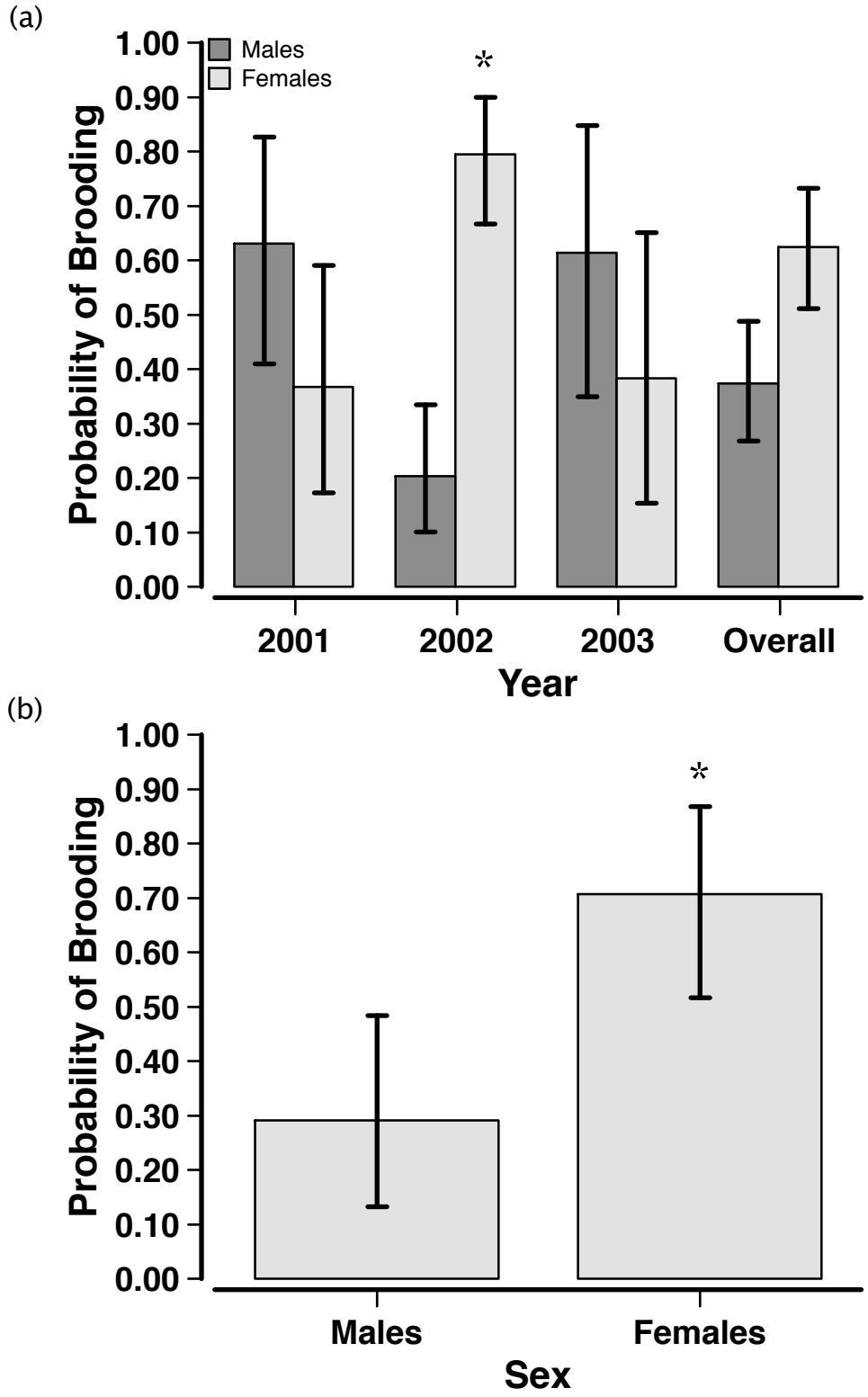


Figure 4.3: Posterior probability (± 95% CI) of: (a) all broods being captured with an adult male or female in each year of the study and (b) amalgamated broods being captured with an adult male or female during 2001-2003 on Tall Timbers Research Station.

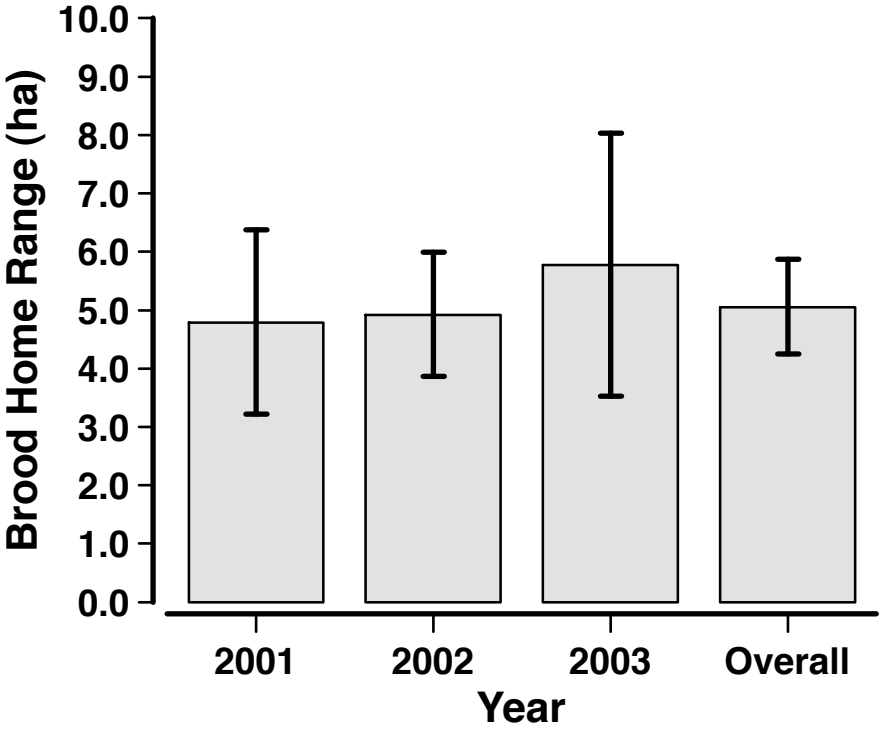


Figure 4.4: Mean (± 95% CI) brood home range for broods with > 15 locations collected during the 22-day post-hatch period 2001-2003 on Tall Timbers Research Station.

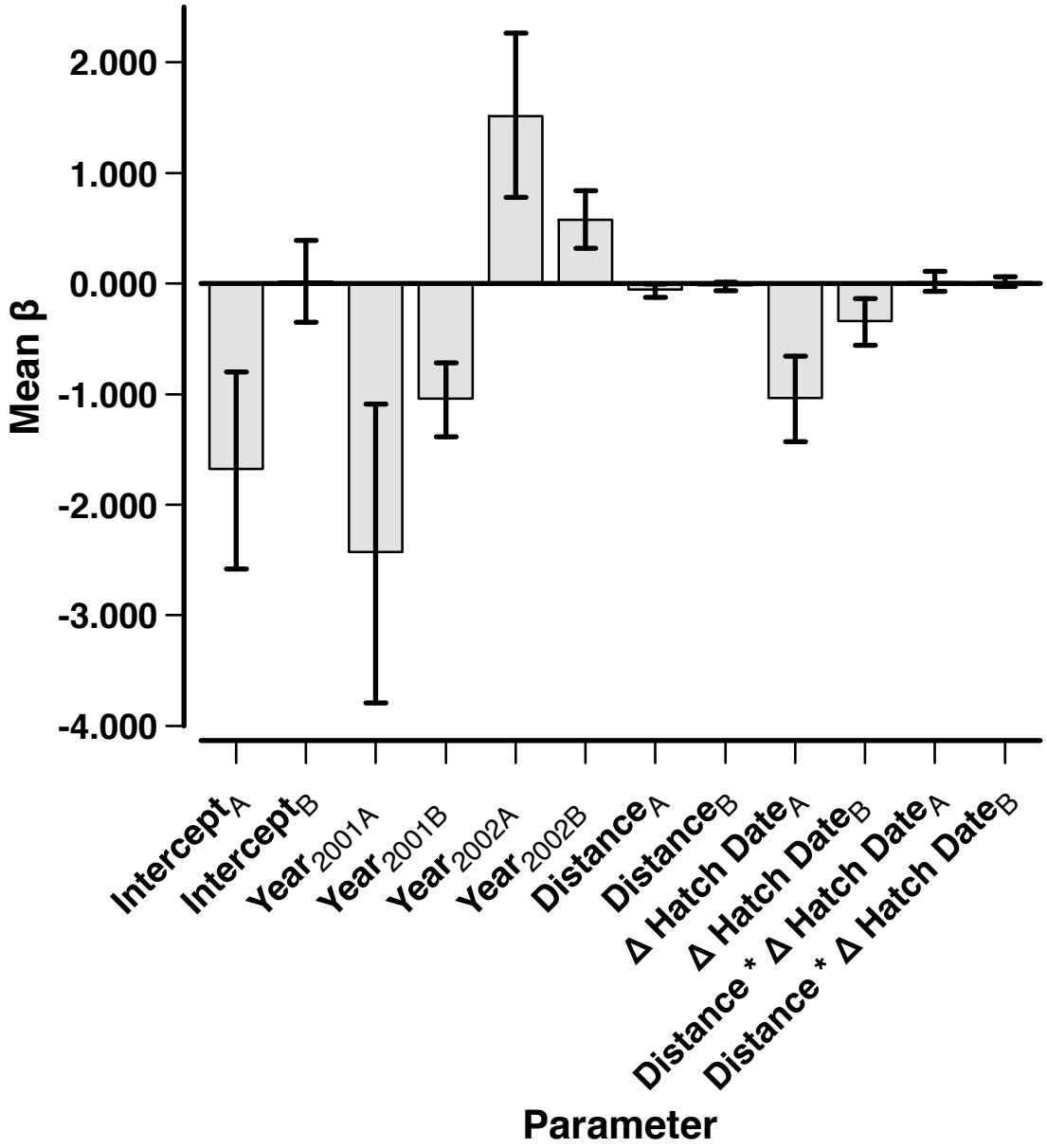


Figure 4.5: Model-averaged parameter estimates (± 95% CI) for models of pairwise probability of brood amalgamation on Tall Timbers Research Station during 2001-2003.

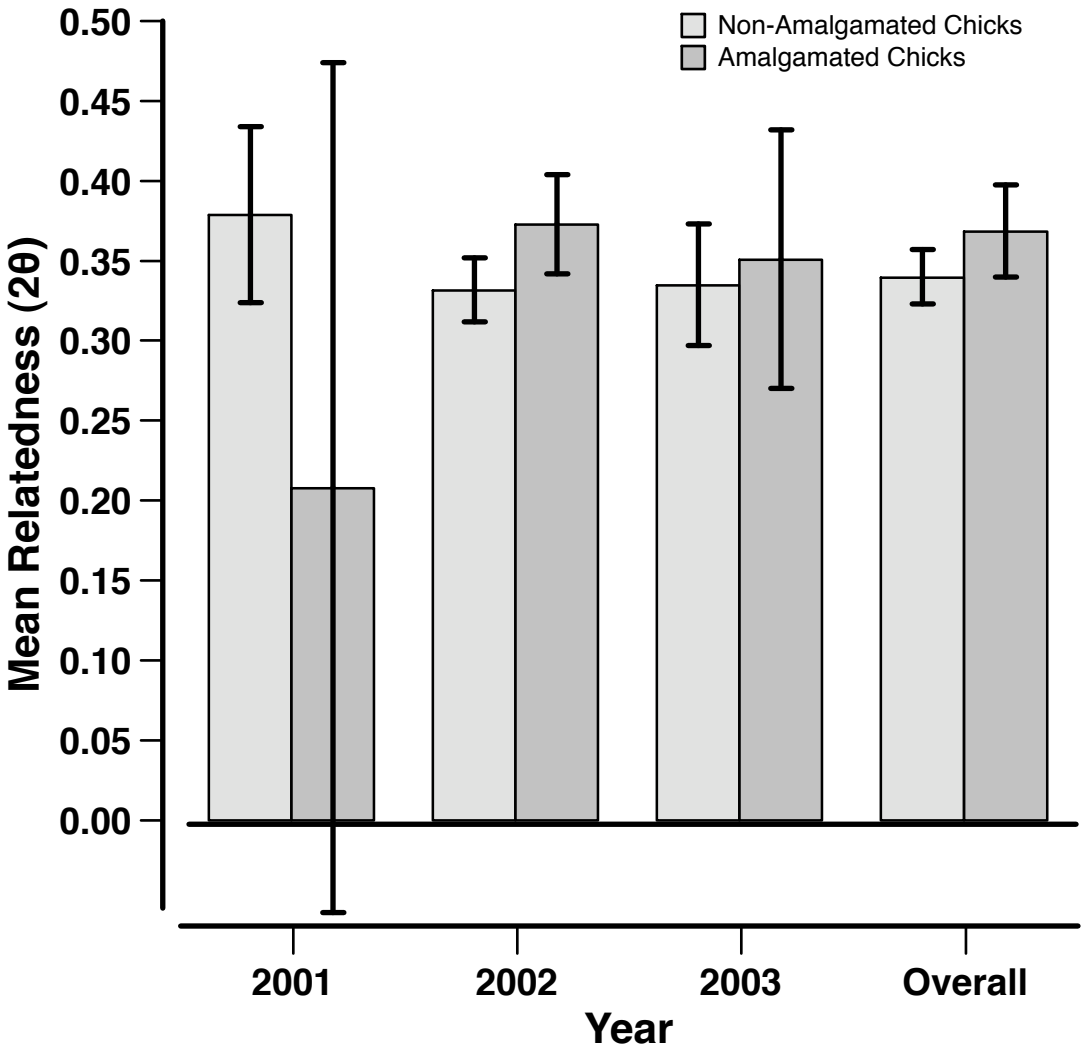


Figure 4.6: Mean within brood relatedness (± 95% CI) between (1) non-amalgamated chicks and (2) amalgamated chicks captured during 2001-2003 on Tall Timbers Research Station.

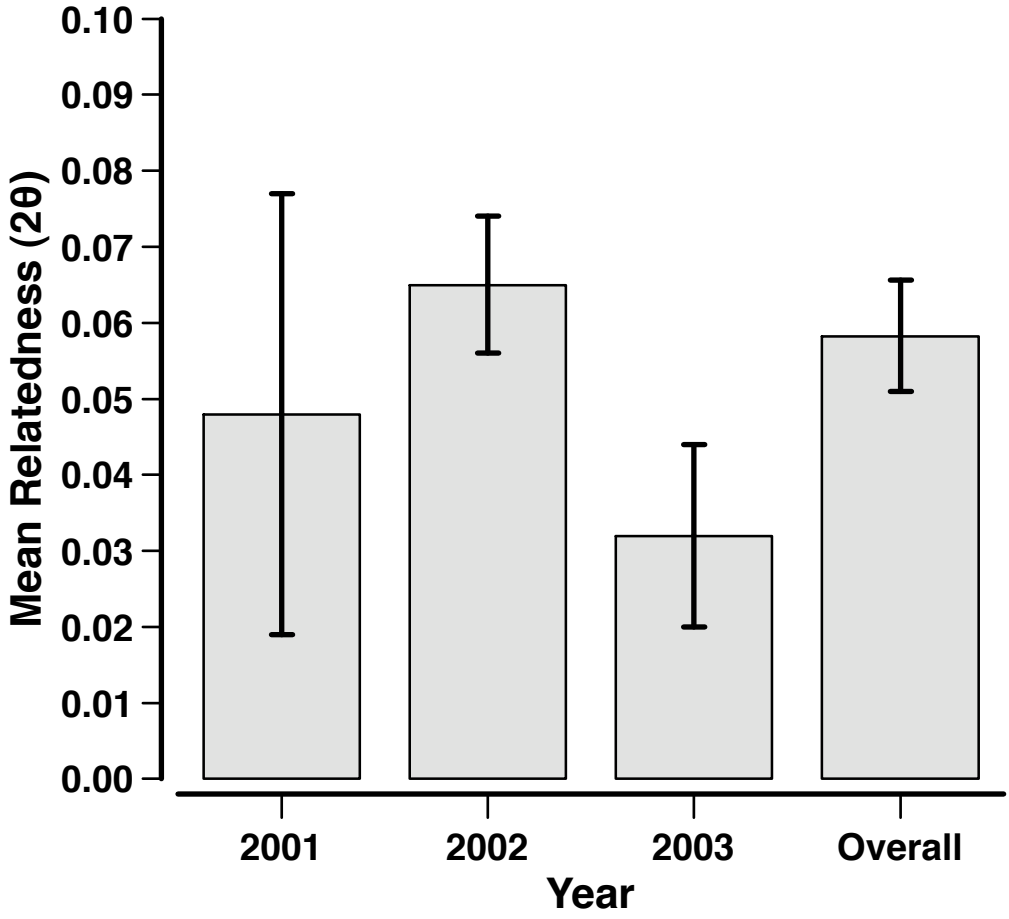


Figure 4.7: Mean within brood relatedness (± 95% CI) among non-amalgamated chicks and amalgamated chicks captured during 2001-2003 on Tall Timbers Research Station.

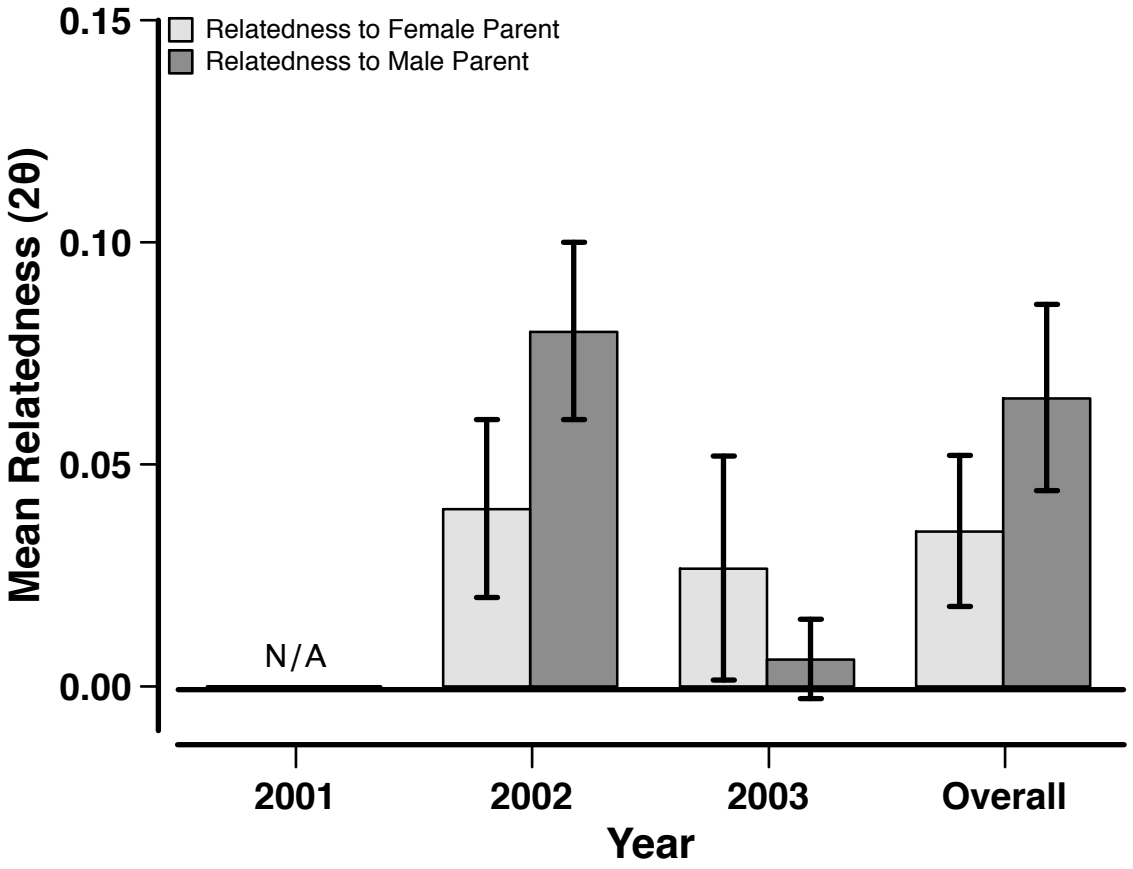


Figure 4.8: Mean (± 95% CI) relatedness between inferred parents, identified following our selection criteria, and amalgamated chicks captured during 2001-2003 on Tall Timbers Research Station.

# Chapter 5

## Reproductive Behavior of Northern Bobwhite (*Colinus virginianus*)[1]

[1]Faircloth, B. C., W. E. Palmer, T. M. Terhune, and J. P. Carroll. To be submitted to *The American Naturalist*

## 5.1 Abstract

Breeding behavior in Northern Bobwhites (*Colinus virginianus*) is classified as consisting of traits consistent with monogamy, promiscuity, and both rapid multiclutch and ambisexual polygamy. However, individual behavior has previously been quantified using only bobwhite social interactions. In this study, we combined field and molecular techniques to better assess social and genetic breeding behavior of individual bobwhites. Our results indicate that bobwhite are social, non-territorial, exhibit moderate rates of intra-specific nest parasitism, and often mate outside of the assumed pair-bond. Our results also show that behavior is not static: individual bobwhite females shift reproductive strategies within and among breeding seasons. Our results have important implications for the study of mating behavior, and we discuss their place within the study of mating behavior and recent work addressing adaptively flexible sex-roles.

## 5.2 Introduction

Mating systems and mating behavior are historically viewed as static entities. While mating systems are abstractions of reality (Gowaty 1985), species exhibit behaviors associated with a particular mating system and are thus assigned to that system. This is best emphasized by attempts to classify both mating systems and species into discrete, behavioral categories: monogamy, polygamy, multiple clutch polygamy, polyandry, and variants thereof (Avery and Ridley 1988; Emlen and Oring 1977; Parker and Burley 1997; Persson and Ohstrom 1989). It is thought that these systems are shaped by distinct, selective, environmental pressures resulting in an optimal strategy, manifested as the set of behaviors observed within the population.

As such, discrete combinations of environmental factors have historically been used to identify the mating system of a population. Similarly, specific mating systems should be predicted in particular situations (Emlen and Oring 1977). Selection for an optimal strategy

additionally suggests: (1) individuals do not exhibit multiple, competing strategies, and (2) individuals do not exhibit flexibility in their behavior (Darwin 1859). Extensions to theory explain the existence of multiple strategies within a population (Dobzhansky 1970; Maynard Smith and Price 1973) but not multiple strategies at the individual level.

Alternative explanations exist (Gowaty and Hubbell 2005; Hubbell and Johnson 1987; Sutherland 1985). Several (Hubbell and Johnson 1987; Sutherland 1985) explain variance in reproductive behavior as resulting from chance effects including encounter rate, survival, and post-mating latency (incorporating incubation, brooding, or other non-mating periods). Others propose that individual behavior may be the result of plasticity, selected to address variation in the environment (West-Eberhard 2003); individual assessment of breeding conditions (including survival, density of potential mates, and quality of mates); or a combination of chance effects, plasticity, and individual assessment of conditions (Gowaty and Hubbell 2005).

Northern Bobwhites (*Colinus virginianus*) are an interesting species in which to investigate reproductive behavior. Previous research suggests that mating behavior may be flexible at the population level, an observation supported by the independent assignment of several mating systems to bobwhite behavior: monogamy, promiscuity, and both rapid multiclutch and ambisexual polygamy (Baldini et al. 1952; Burger et al. 1995*b*; Curtis et al. 1993; Stettner et al. 1966; Stoddard 1931). However, observation of bobwhite social interactions suggests that a mixed reproductive strategy may exist within populations (Burger et al. 1995*b*, B. Faircloth, *unpublished data*). Furthermore, bobwhites differ from species commonly used to investigate mating behavior: they are not known to be territorial (Brennan 1999), intraspecific nest parasitism has been reported (Rosene 1969; Stoddard 1931) but not verified (MacWhirter 1989), males incubate nests (Burger et al. 1995*b*), the relationship between social and genetic mates is unknown, and the degree of extra-pair paternity has not been estimated (Brennan 1999).

In this manuscript, we use a combination of field and genetic methods to investigate the breeding biology of Northern Bobwhites. We examine the degree of territoriality among bobwhite males, females, and males and females; we estimate the rate of extra-pair paternity and intra-specific nest parasitism in the population; and we examine the influence of individual covariates on the rate of extra-pair paternity and intra-specific nest parasitism to identify parameters affecting these behaviors.

## 5.3 Methods

### 5.3.1 Capture, Sampling, and Radio-location of Adults and Sub-adults

We trapped, captured, and radio-collared adult and subadult bobwhite during October-June 2000-2003 on a 250 ha area of Tall Timbers Research Station, Tallahassee, FL according to methods described in Faircloth et al. (2005). We recorded the trap location, weight, sex, and age (adult or juvenile) of captured individuals; we collected 10 body feathers from the flank of each bird for subsequent genetic analysis; and we input all capture data to a relational database.

During the breeding season (April 15 - October 15), we located all birds by radio-location using the homing method (Kenward 2001; White and Garrott 1990) at least once per day, 7 days per week. At each location, we recorded the radio-frequency and band number of birds within 5 - 10 m of the bird being located. If we suspected a radio-tagged bird was present with an un-radioed individual, we attempted to flush the bird being located along with any associates. We identified nests when individuals were found in the same location >1 day. We flagged these areas >3 m from each nest, and we searched each area for the presence of a nest and to determine clutch-size when radio-location indicated the incubating bird was away from the nest.

We recorded daily locations of radio-collared adults, associated individuals of either sex, brood locations, and all nest locations on a GIS map of the study area. We converted each

location to UTM coordinates which were then input with all associated data (date, time, associated individuals) to a relational database.

Using unique radio-frequencies, associated band numbers, individual sex, and presence on the study area, we computed apparent rates of nesting, nesting success, renesting, double-clutching, and double clutch success following Burger et al. (1995*b*).

### 5.3.2 Capture of Chicks

We captured bobwhite chicks using capture procedures outlined in Smith et al. (2003) and Faircloth et al. (2005). During the breeding seasons of 2001-2003, we conducted 2 captures of broods at 1-4 and 10-13 days after hatching. At the initial capture in 2001, chicks were marked with patagial wing-bands (Carver et al. 1999). We weighed chicks to the nearest 0.1 g using a 20 g spring-scale (Pesola ®, Baar, Switzerland), and we collected 1.5 *m*m patagial microbiopsies from each chick for genetic analysis (Miltex Corp., York, PA). We treated the biopsy area with NeoPredef powder (Pfizer Animal Health, New York, NY), stored each sample in individual 1.5 *m*L microtubes containing a Tris-EDTA buffer (100*m*M Tris pH 8.0, 100*m*M EDTA pH 8.0, 10 *m*M NaCl, 1% SDS), and marked 10-13 day-old chicks with a patagial wing-band (Carver et al. 1999). We released chicks $\leq$ 5 m from the attending adult, as determined from radio-location.

Our brood capture procedures typically resulted in complete captures of a brood, released chicks rapidly returned to the attending parent(s), these procedures did not introduce observer bias by inducing subsequent amalgamation of broods, and the collection of patagial biopsies at 1-4 days of age does not affect neonatal survival (Evans et al. 2008; Faircloth et al. 2005).

### 5.3.3 Sampling of Unhatched Nests

When a radio-tagged bird was killed off-nest, we artificially incubated the contents of each nest for the remainder of the incubation period. We sampled chicks hatching from incubated

nests between 1 and 3 days post-hatch, following procedures outlined above. We included these individuals in our parentage assignment procedures and subsequent estimation of EPP and INP rates. Except where noted, we present these data separate from that derived from naturally incubated nests.

### 5.3.4 Microsatellite Genotyping

We extracted DNA from feathers or patagial biopsies using DNeasy kits (Qiagen Inc., Valencia, CA) with a modification to the digestion step, adding 25 $\mu$L of 100 $m$g/$m$L DTT (Dithiothreitol) along with proteinase K. We eluted DNA with either: 2 washes of 60 $\mu$L Buffer AE or 1 wash 120 $\mu$L Buffer AE. Prior to amplification, we treated all samples 1:3 (volume:volume) with 10% Chelex resin (BioRad Laboratories, Hercules, CA) to remove PCR inhibitors. We performed 96-well PCR amplifications of 16 microsatellite loci (Table 1.4; Faircloth et al. 2008; Schable et al. 2004) in 10 $\mu$L volumes using CAG- or M13R-tagged primers (Glenn and Schable 2005). Reaction concentrations were 0.5 U AmpliTaq Gold (Applied Biosystems, Foster City, CA), 1X Gold Buffer, 1X BSA (New England Biolabs, Ipswich, MA), 1.5 $m$M MgCl, 1.25 $m$M dNTPs, 0.5 $\mu$M untagged primer; 0.05 $\mu$M CAG or M13-reverse tagged primer, 0.45 $\mu$M dye-labelled tag (HEX, FAM, NED + CAG or M13-reverse), 3.3 $\mu$L ddH2O, and 2 $\mu$L DNA template (5-10 $n$g). We included multiple negative controls in each plate of PCR reactions.

We used one of two locus-dependent touchdown thermal cycling profiles (Don et al. 1991), each encompassing a 10 ℃ span of annealing temperatures (ranges: 60 - 50 ℃; 65 - 55 ℃). Cycling parameters included a Taq activation step at 95 ℃ for 5 min followed by 20 cycles at 95 ℃ for 20 sec; 60 or 65 ℃ for 30 sec minus 0.5 ℃ per annealing cycle; and 72 ℃ for 90 sec followed by 25 cycles at 95 ℃ for 20 sec; 50 or 55 ℃, respectively, for 30 sec; 72 ℃ for 90 sec. We used a final extension period of 10 min at 72 ℃.

We scored fragments using an ABI 3730xl sequencer (Applied Biosystems) with ROX500 fluorescent size standard. We sized fragments using GENEMAPPER version 4.0 software

(Applied Biosystems) and the Global Southern method (Applied Biosystems 2005). Initial tests indicated that the Global Southern method resulted in reduced intra-run variation in microsatellite fragment length relative to other sizing methods. We binned all fragments using the same binset to ensure consistency across years, and we discarded ambiguous genotypes from the data set and re-genotyped plates with failing negative controls.

To asses the rate of genotyping error, we randomly selected a 12% sample from the entire data set, assigned each individual a random identification string, and blindly genotyped, scored, and binned these samples (Hoffman and Amos 2005). We exported samples from the GENEMAPPER database, converted records to a useful format using GMCONVERT (Faircloth 2006), and imported data to a separate relational database. We re-assigned error samples their true identification and compared each to the corresponding non-error sample to compute the genotyping error rate on a per-locus and overall basis ($\bar{x} \pm 95\%$ CI).

### 5.3.5 Parentage Assignment and Linkage

Using CERVUS (Kalinowski et al. 2007; Marshall et al. 1998), we analyzed microsatellite data by year to test for Hardy-Weinberg equilibrium and infer parentage of all offspring. Along with genotypes for chicks captured during a particular year, we included genotypes of parents known to be alive at the start of breeding season. We used the delta approach (Marshall et al. 1998) to determine significance of parentage assignments at the 99% confidence level. We ran delta simulations for 100,000 iterations, using yearly estimates of the percent population captured (Table 1.3), twice the average number of opposite sex associates for incubating birds determined by radio telemetry ($n = 10$), and our overall estimate of genotyping error.

Following parentage inference, we conducted an additional parentage exclusion step using the parents inferred for a majority of each brood. This ensured that putative parents were not inappropriately excluded for certain parent-offspring combinations as a result of the contribution of the weight of the data. We assigned parents to members of a brood when: (1) one of the parents matched the adult with which each brood was captured, (2) the

inference/exclusion occurred at the 99% level, and (3) there were $\leq 2$ mismatches between parents and offspring across loci.

Using assignments, we identified 6 large families where parentage of all offspring was attributed to only two individuals ($\bar{x}_{offspring} = 8.5$). We used genotypes from these 6 family groups to test for linkage using twopoint analysis in CRIMAP (Lander and Green 1987) for all pairwise combinations of loci, and we assumed loci were linked when the log-odds for the pairwise-comparison was $> 3.0$.

### 5.3.6 Determination of Extra-pair Paternity (EPP) and Intra-specific Nest Parasitism (INP)

Prior to determining the rate of extra-pair paternity and number of extra-pair chicks within bobwhite broods, we excluded chicks within each brood arising as a result of amalgamation (Chapter 4), and we conducted our analyses using only chicks captured during the first period.

We determined that a brood contained extra-pair chicks when all of the following criteria were met:

1. the mother and father were assigned to $\geq 50\%$ brood members ($\geq 99\%$ trio confidence; $\leq 2$ trio mismatches), excluding brood members unrelated to either parent (trio LOD $< 0.00$; trio mismatch $> 2$) which we assumed represented eggs laid by other females.

2. the majority mother **was** the parent of individual offspring (candidate mother $\geq 99\%$ confidence; candidate mother mismatch $\leq 2$)

3. the majority father **was not** the parent of individual offspring (candidate father LOD $< 0.00$; candidate father mismatch $> 2$)

### Intra-specific Nest Parasitism

To determine rates of intra-specific nest parasitism, we examined the composition of broods relative to the parents assigned and the relatedness of individuals within the brood (see below). We did not genotype eggs taken directly from nests, with the exception of artificially incubated clutches.

We realize that amalgamated chicks can mimic the genetic profile of individuals assumed to be parasitic, violating the assumption that brood composition accurately reflects clutch composition. In order to address this potential bias, we excluded all known, amalgamated broods from our estimates of INP. We additionally ensured that the variance in the weight of broods examined did not indicate they were amalgamated (Chapter 4).

We determined that a brood contained chicks arising from nest-parasitism when all of the following criteria were met:

1. the mother was assigned to $> 50\%$ of brood members (candidate mother $\geq 99\%$ confidence; candidate mother mismatch $\leq 2$)

2. the majority mother was not the the parent of specific offspring (candidate mother LOD $< 0.00$; candidate mother mismatch $> 2$)

3. the parasitic chick(s) was unrelated to a majority ($> 50\%$) of brood-mates indicated using a maximum likelihood, relationship estimator (Appendix A; Milligan 2003; Thompson 1975; Wagner et al. 2006; Weir et al. 2006)

### EPP, Extra-pair fertilization (EPF), and INP Rates

After excluding broods not meeting the criteria outlined and assigning an indicator of status to remaining broods (1 = EPP or INP; 0 otherwise), and we used the status of each to estimate the posterior probability of EPP or INP using a bernoulli distribution and a non-informative, uniform prior (0,1) in WinBUGS (2 chains; 50,000 burn-in iterations; 50,000 simulations Lunn et al. 2000). We also computed the posterior probability of extra-pair

fertilization by modeling the number of extra-pair chicks per brood using a binomial distribution and a non-informative, beta prior (1,1). From these estimates, we computed the median (±95% CI) of the posterior probability of EPP, EPF, and INP. We additionally computed the mean ($\pm 95\% CI$) number of EPP or INP chicks per brood.

#### Relatedness of INP Offspring

To investigate the relationship between parasitic chicks and parents assigned to the brood with which they were captured, we used a maximum likelihood estimator of relatedness adjusted for the presence of null alleles (Appendix A; Milligan 2003; Thompson 1975; Wagner et al. 2006; Weir et al. 2006). We computed the background level of relatedness ($\bar{x} \pm 95\%$ CI) during each year of the study by taking the mean of all pairwise relatedness calculations across the entirety of our sample (adults and offspring) during each breeding season. We computed the relatedness between parasitic chicks as the mean relatedness (±95% CI) across all pairwise, within parasitic chick comparisons and the relatedness between parasitic chicks and parents as the mean pairwise relatedness (±95% CI) for each parent-chick combination.

### 5.3.7 Shifts in Mating Behavior

To investigate seasonal and yearly changes in individual mating behavior, we used parentage assignments to identify females that laid multiple, successful clutches, each genotyped and assigned a mate. As a result of small sample size, we combined artificially incubated and wild nests, and we combined yearly data.

We then assigned females to 1 of 4 behavioral categories: quasi-monogamy, serial multiple mating, multiple mating, and hybrid multiple mating. We defined: (1) quasi-monogamy as 2 pairs mating repeatedly *with* evidence of EPP at either attempt; (2) serial multiple mating as a female mating with different males at each attempt but without evidence of EPP at either (monogamy within attempts); (3) multiple mating as evidence of EPP at both attempts; and (4) hybrid multiple mating as females alternating between (1), (2) or (3).

We further classified hybrid multiple mating by investigating whether behavioral changes occurred within or among years.

### 5.3.8 Home-Range Overlap

To analyze the degree of male or female territoriality, we computed the degree of overlap between 95% kernel home ranges derived from the locations of radio-collared individuals during the breeding season. We removed nest coordinates repeated in the data set and individuals with $< 10$ locations (Kenward 2001). Using a Python (Oliphant 2007; van Rossum 2006) script and the ADEHABITAT package for R (Calenge 2006; R Development Core Team 2006), we estimated the smoothing parameter for each year of the study ($h_{year}$). We computed $h_{year}$ as the mean, LSCV-derived $h$ over all individuals where the algorithm converged (bivariate normal kernel; Kenward 2001).

To create a spatial representation of each home range, we generated 95% kernel volume contours for each individual using $h_{year}$ from above. We exported these contours to ArcGIS and used a Python script to compute the degree of overlap among all home ranges within each year by: selecting a focus individual from the data, identifying individuals and home ranges overlapping that of the focus individual, and computing the size of the intersecting polygons ($m^2$) on a per-individual basis. We imported these data to a relational database, assigning the sex to each individual in the data set. For male-male, female-female, and male-female comparisons, we computed mean range overlap ($\pm$ 95% CI) and the mean number of overlapping individuals ($\pm$ 95% CI) during 2001-2003, respectively.

### 5.3.9 Social Mating System

We used radio-location data to investigate the social mating system of bobwhites and the ability of opposite sex social interactions to predict the mating system. Using female parents assigned to a brood as the reference point, we tallied the number of unique, opposite sex associates with which this female was located during: (1) the entire breeding season and (2)

a period of 21 days prior to incubation. We used a period of 21 days to account for the laying duration of a typical bird [0.8 egg/day (Rosene 1969); mean clutch size during 2001-2003 = 12 eggs ($n = 154$)] and the maximum duration of sperm storage by bobwhites (5 days; Oswald 2004). Using the identity of these individuals, we computed the mean number ($\pm$ 95% CI) of opposite sex associates for female parents of EPP and non-epp broods. We additionally computed the percentage of nests for which the number of opposite sex associates ($> 1$) predicted the EPP status and the degree to which the most frequent opposite sex associate was assigned as the majority father of each brood.

### 5.3.10 Correlates of EPP and INP

Ecological correlates, including female condition (weight), habitat quality (home range size), operational sex ratio, and survival, are often used to explain the breeding behavior of species. We used logistic regression to examine the impact of these factors, in addition to sex of the incubating bird and male condition (weight), on the occurrence of both EPP and INP in bobwhites.

We created an index of the weekly operational sex ratio (OSR) in the population using a Python (Oliphant 2007; van Rossum 2006) program, in combination with data from radio-collared males and females. The program identified radio-collared males and females on the study area and excluded from the breeding population those individuals of either sex actively incubating a nest and those dying during the week of observation. We assumed males and females associated with broods belonged to the weekly breeding population provided that >50% of their locations were no longer on the nest, and we returned individuals with failing nests to the breeding population when the failure occurred prior to the midpoint of the week.

We estimated weekly, female survival during each month of the breeding season during 2001-2003 using the staggered entry, Kaplan-Meier model in program MARK (Kaplan and Meier 1958; White and Burnham 1999), and we computed the 95% and 50% kernel home ranges for each female, using methods identical to those detailed in Section 5.3.8.

We combined data collected from wild and artificially incubated nests, and we examined the effect of year; monthly survival; female age; female condition; female home-range (50% and 95% kernel); male incubation; male condition; and operational sex ratio 0, 1, 2, and 3 weeks prior to the onset of incubation on the occurrence of EPP. Similarly, we modeled the effect of year, monthly survival, female age, female condition, home range, male incubation, and operational sex ratio on the rate of INP. We built models containing only biologically relevant parameter combinations (*e.g.* a model could not contain both 50% and 95% home ranges) and analyzed all model combinations using a SAS (SAS Institute 2004) macro, PROC GENMOD, the binomial error distribution, and the logit link. We ranked and selected models using AIC (Akaike 1974; Burnham and Anderson 2002).

## 5.4 Results

During 2001-2003 we captured, radio-collared, and sampled 621 adult/sub-adult and 841 immature bobwhites. Our genotyping success was 98% and 99% for adult and immature birds, respectively (Table 5.1), and our overall rate of genotyping error was 0.008 ($\pm$ 0.003; $n_{loci} = 16$; Appendix B). Following Bonferroni correction (Rice 1989), CV-PA3E deviated from Hardy-Weinberg equilibrium in 2002, and we excluded 2 loci (CV-PA12A and CV-P1F2) from the study because of linkage and errors in Mendelian inheritance, respectively. After removing these loci, we re-ran our parentage assignment process with the truncated data ($n_{loci} = 14$).

During the breeding season we radio-located 178 and 180 male and female birds, respectively. The apparent nesting rate was 51% for females and 24% for males. Apparent nesting success for each sex was 64% for females and 67% for males (Table 5.2). We analyzed offspring from 85 wild broods and 20 artificially incubated broods. Using our parentage assignment criteria, we assigned both parents to 40 and 11 and a single parent to 34 and 5 wild and artificially incubated broods, respectively (Table 5.3).

Males and females shared genetic parentage with an average of 1.2 (± 0.1) mates during 2001-2003, and we found evidence of EPP in 23 wild broods and 7 artificially incubated broods (Figure 5.1). Extra-pair fertilizations resulted in 1.8 (±0.4) and 1.4 (±0.6) chicks per wild and artificially incubated broods (Figure 5.3a) corresponding to an EPF rate of 10-20% per brood during 2001-2003 (Figure 5.1).

Fourteen wild broods and 6 artificially incubated broods (Figure 5.2) contained 2.5 (±0.5) and 1.5 (±0.4) chicks arising from INP (Figure 5.3b), respectively. Background levels of relatedness in the population differed slightly during the study period ($\hat{r}_{2001} = 0.06 \pm 0.0007$; $\hat{r}_{2002} = 0.06 \pm 0.0002$; $\hat{r}_{2003} = 0.05 \pm 0.0008$). The degree of relatedness between chicks hatched from parasitic eggs and assigned parents did not differ from the background level of relatedness, and parasitic chicks tended to be full- or half-siblings of each other (Figure 5.4).

Mean proportions of male-male (0.22±0.02), female-female (0.22±0.02), and male-female (0.23±0.02) home range overlap were similar during 2001-2003 (Figure 5.5). The mean number of overlapping individuals per bird did not differ among male-male (22±2), female-female (22±2) and male-female (23±2) comparisons although there were yearly differences within each category (Figure 5.5).

Females laying nests containing EPP chicks did not differ from those laying non-EPP nests in the number of social mates 21 days prior to nesting or overall (Figure 5.6; artificially incubated birds not presented). Number of opposite sex associates poorly predicted the status (EPP or non-EPP) of wild or artificially incubated broods, but the most frequent, opposite sex associate predicted the majority, genetic father of broods with moderate success (Table 5.4).

We identified 13 females laying multiple nests during 2001-2003, and we assigned parents to 9 of these broods (69%). Two (22%) females exhibited quasi-monogamy, 2 females exhibited serial multiple mating (22%), 4 females exhibited multiple mating (44%), and 3 females exhibited a hybrid strategy (33%). The hybrid strategy, consisted largely of within-year shifts

in behavior ($n = 2$; 66%), although we identified a single individual shifting mating strategy among years.

Mean operational sex ratio during 2001-2003 (Female:Male) was 0.7 ($\pm$0.08), 0.9 ($\pm$0.04), and 1 ($\pm$0.07), respectively (Figure 5.8). We were unable to compute OSR during weeks 35 and 36 in 2002 due to missing data, but this did not affect subsequent analyses.

Survival probability did not differ between males and females, nor were there differences in weekly survival during each month of the study (Figure 5.7). However, models of weekly survival during each month of the study better described variation in the data (not shown), and we selected these estimates for use in subsequent analyses.

We examined 959 models of extra-pair paternity. No models of EPP were sufficiently supported by the data relative to one another ($w_i \leq 0.01$, $\beta_{AllParameters}$ include 0.0, Table 5.5) indicating that the parameters investigated do not explain variation in breeding behavior. As a result of poor model support, we did not model-average parameter estimates (Burnham and Anderson 2002).

Similarly, 479 models of intra-specific nest parasitism produced only a single model adequately explaining variation in the data relative to the other models tested (Table 5.6). This model indicated that female condition, indexed by body weight, and operational sex ratio 2 weeks prior to incubation affected the rate of intra-specific nest parasitsm ($\beta_{female_{weight}} = -15.1 \pm 12.9$; $\beta_{osr_2} = 7.2 \pm 5.9$).

## 5.5 Discussion

Our results indicate that breeding behavior in bobwhites is complex, incorporating a moderate degree of both male incubation and intra-specific nest parasitism and consisting of breeding between single, mated pairs; females and multiple males; males and multiple females; and females shifting reproductive strategies within and among breeding seasons.

In comparison to prior studies, our results show the assignment of bobwhites to contrasting mating systems (Baldini et al. 1952; Burger et al. 1995*b*; Curtis et al. 1993; Stettner

et al. 1966; Stoddard 1931) was largely correct. However, because these assignments were based on observations of *social* behavior, most were correct for the wrong reasons. The use of radio-location during the breeding season indicates that bobwhites have multiple, social mates. However, opposite-sex, social interactions poorly predict the occurrence of extra-pair mating in the population and underestimate the likelihood of a brood arising from a monogamous pairing. As a result, bobwhite social interactions may predict population-level trends but are an inaccurate predictor of individual reproductive outcomes.

Typically, territoriality and resource defense (Emlen and Oring 1977), the operational sex ratio (Clutton-Brock and Parker 1992; Emlen and Oring 1977), and correlates of environmental conditions (Orians 1969) are used to explain the breeding behavior or mating system of individuals within populations. Our results suggest that bobwhites are not territorial. Furthermore, our measures of operational sex ratio, survival, female condition, female habitat quality, sex of the incubating bird, and mate condition do not sufficiently explain variation in bobwhite reproduction. Similarly, no combination of parameters overwhelmingly explain variation in the rate of intra-specific nest parasitism with only a single model falling in the traditionally defined candidate set ($w_i > 0.10$; Royall 1997). This model suggests operational sex ratio 2 weeks prior to breeding and female condition (body weight) moderately explain variation in rate of intra-specific nest parasitism.

Interestingly, classical theory fails to incorporate aspects of intra-specific nest parasitism (Gowaty 1985). Although overlooked, this behavior provides direct fitness benefits to practicing females and their mates, by ensuring the success of at least some offspring or avoiding the costs associated with incubation. Additionally, it entails potential costs for females mistakenly incubating unrelated offspring. However, to view the acceptance of unrelated eggs in the nest as only a potential cost is shortsighted - overlooking the benefits, albeit minor, conferred by the presence of unrelated eggs on their surrogate broods. This is particularly true for a precocial species, where the cost of raising additional chicks is low. The mistaken

or purposeful acceptance of unrelated eggs in the nest may serve to reduce the predation risk of the surrogate clutch or resulting chicks (Foster and Treherne 1981).

Classical theory also fails to incorporate *individual* flexibility in behavior (Emlen and Oring 1977). Our data indicate 2 primary strategies explaining a majority of bobwhite mating behavior: reproduction between single pairs or mating with multiple individuals. The occurrence of 2, seemingly competing strategies within the population may be explained in the context of an evolutionary stable strategy or balancing selection (Dobzhansky 1970; Maynard Smith and Price 1973). However, our data also suggest the possibility of at least a third strategy: individual alteration of behavior within and among breeding seasons. This shifting strategy may best be explained in the context of behavioral plasticity (West-Eberhard 2003).

Interestingly, our results largely match prediction made by models of reproductive variability drawn from Hubbell and Johnson (1987); Sutherland (1985); West-Eberhard (2003) and put forth by Gowaty and Hubbell (2005). These models suggest that reproductive decisions result from the interaction between post-mating latency, individual survival, encounter rate, individual assessment of environmental conditions, and the ability of a species to flexibly express behavior. Although, the Gowaty and Hubbell (2005) model does not explicitly incorporate male incubation or intra-specific nest parasitism and subsequent effects on reproductive success, the structure of the model could be extended to address these aspects of bobwhite behavior.

The Gowaty and Hubbell (2005) model of behavior may largely explain proximate factors affecting reproductive decisions, but the ultimate explanation for the evolution of behavioral flexibility among bobwhites remains unexamined. It is logical to assume that flexibility benefits $r$-selected species in variable environments by allowing them to optimize their behavioral strategy in the presence of variable ecological factors including: survival (Burger et al. 1995*a*; Palmer and Wellendorf 2007; Terhune et al. 2007), food availability (Bookhout 1958; Stoddard 1931), nest predation/success (Burger et al. 1995*b*; Roseberry and Klimstra 1984), and population density (Errington 1934).

Given that behavior is flexible and potentially results from environmental variation, it is also logical to assume that techniques minimizing this variation, including supplemental feeding and predator management, may be used to test the hypothesis of adaptively flexible reproductive behavior. Until such time as: (1) simulation-based theory is extended to address behaviors including male incubation and intraspecific nest parasitism, and (2) tested by manipulative experimentation, the applicability of adapatively flexible behavior to systems will remain an interesting but untested pursuit.

## 5.6 Acknowledgments

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Table 5.1: Northern Bobwhites captured, radio-collared, and sampled for genetic analysis; number of individuals genotyped; and genotyping success during 2000-2003. We defined a successful genotype as amplification at $\geq 50\%$ of loci ($n_{loci} = 16$).

| Year | Captured | | Genotyped (%) | | Successfully Genotyped (%) | |
|---|---|---|---|---|---|---|
| | Adults | Chicks | Adults | Chicks | Adults | Chicks |
| 2001 | 179 | 230 | 162 (91%) | 215 (93%) | 160 (99%) | 214 (100%) |
| 2002 | 175 | 468 | 167 (95%) | 441 (94%) | 163 (98%) | 432 (98%) |
| 2003 | 267 | 143 | 266 (100%) | 129 (90%) | 263 (99%) | 129 (100%) |
| Overall | 621 | 841 | 595 (96%) | 785 (93%) | 586 (98%) | 775 (99%) |

Table 5.2: Apparent reproductive effort and success for bobwhite monitored during the breeding season of 2001-2003 on Tall Timbers Research Station. Table design and content following Burger et al. (1995*b*).

| | | | Nesting Rate | | Success | | Renest Rate[1] | | Double-clutch Rate[2] | | Double-clutch Success[3] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | Sex | $n$ | $n$ | % | $n$ | % | $n$ | % | $n$ | % | $n$ | % |
| 2001 | F | 43 | 17 | 39.5 | 9 | 52.9 | 3 | | 1 | | 1 | |
| | M | 50 | 17 | 34.0 | 9 | 52.9 | 1 | | 0 | | 0 | |
| 2002 | F | 67 | 45 | 67.2 | 32 | 71.1 | 11 | | 2 | | 2 | |
| | M | 64 | 12 | 18.8 | 10 | 83.3 | 0 | | 0 | | 0 | |
| 2003 | F | 52 | 20 | 38.5 | 12 | 60.0 | 5 | | 2 | | 2 | |
| | M | 41 | 8 | 19.5 | 6 | 75.0 | 1 | | 0 | | 0 | |
| Pooled | F | 162 | 82 | 50.6 | 53 | 64.6 | 19 | 65.5 | 5 | 9.4 | 5 | 9.4 |
| | M | 155 | 37 | 23.9 | 25 | 67.6 | 2 | 16.7 | 0 | 0.0 | 0 | 0.0 |

[1] Number and percent of birds failing on their initial nesting attempt and subsequently renesting.
[2] Number and percent of birds successfully hatching their first clutch and renesting.
[2] Number and percent of birds successfully hatching their first and second clutches.

Table 5.3: Parentage assignment success for bobwhite broods captured during 2001-2003 on Tall Timbers Research Station. "Successful" and "Incubated" denote whether chicks used in parentage assignment resulted from successful (wild) nests or nests hatched in an incubator, repectively.

| Year | Broods Analyzed | | 2 Parents Assigned (%) | | 1 Parent Assigned (%) | |
|---|---|---|---|---|---|---|
| | Successful | Incubated | Successful | Incubated | Successful | Incubated |
| 2001 | 20 | 12 | 8 (40%) | 7 (58%) | 8 (40%) | 3 (25%) |
| 2002 | 45 | 6 | 24 (53%) | 3 (50%) | 20 (44%) | 2 (33%) |
| 2003 | 15 | 2 | 8 (53%) | 1 (50%) | 6 (40%) | 1 (50%) |
| Overall | 85 | 20 | 40 (47%) | 11 (55%) | 34 (40%) | 5 (25%) |

Table 5.4: Frequency of correct paternal assignment and EPP status using the most frequent associate of female birds for broods captured on Tall Timbers Research Station and assigned genetic parents during 2001-2003.

| Nests | 21 Days Prior | | | Overall | | |
|---|---|---|---|---|---|---|
| | Father | EPP | Non-EPP | Father | EPP | Non-EPP |
| Wild | 69% | 56% | 38% | 61% | 100% | 13% |
| Artificially Incubated | 66% | 75% | 0% | 71% | 71% | 0% |

Table 5.5: Model structure, $AIC$, $\Delta AIC_c$, model weight ($w_i$), and number of parameters for models of extra-pair paternity (EPP) on Tall Timbers Research Station during 2001-2003.

| Model | $AIC$ | $\Delta AIC$ | $w_i$ | Parameters |
|---|---|---|---|---|
| $EPP(.) + female_{condition}$ | 65.95 | 0.00 | 0.01 | 2.00 |
| $EPP(.) + female_{age}$ | 66.21 | 0.26 | 0.01 | 2.00 |
| $EPP(.) + osr_2$ | 66.45 | 0.50 | 0.01 | 2.00 |
| $EPP(.) + mate_{condition} + osr_2$ | 66.73 | 0.78 | 0.01 | 3.00 |
| $EPP(.) + incubated_{male}$ | 66.74 | 0.79 | 0.01 | 2.00 |
| $EPP(.) + female_{age} + incubated_{male}$ | 66.75 | 0.80 | 0.01 | 3.00 |
| $EPP(.) + incubated_{male} + condition_{male}$ | 66.81 | 0.85 | 0.01 | 3.00 |
| $EPP(.) + osr_1$ | 66.83 | 0.88 | 0.01 | 2.00 |
| $EPP(.) + female_{age} + male_{condition}$ | 67.03 | 1.07 | 0.01 | 3.00 |
| $EPP(.) + kernel_{50\%}$ | 67.12 | 1.17 | 0.01 | 2.00 |
| $EPP(.) + female_{age} + incubated_{male} + male_{condition}$ | 67.24 | 1.28 | 0.01 | 4.00 |
| $EPP(.) + female_{condition}$ | 67.39 | 1.44 | 0.01 | 2.00 |
| $EPP(.) + male_{condition} + osr_1$ | 67.41 | 1.46 | 0.01 | 3.00 |
| $EPP(.) + incubated_{male} + male_{condition} + osr_2$ | 67.41 | 1.46 | 0.01 | 4.00 |
| $EPP(.) + kernel_{95\%}$ | 67.46 | 1.51 | 0.01 | 2.00 |
| $EPP(.) + survival_{month}$ | 67.46 | 1.51 | 0.01 | 2.00 |
| $EPP(.) + osr_3$ | 67.46 | 1.51 | 0.01 | 2.00 |
| $EPP(.) + osr$ | 67.51 | 1.55 | 0.01 | 2.00 |
| $EPP(.) + incubated_{male} + osr_2$ | 67.62 | 1.66 | 0.01 | 3.00 |
| $EPP(.) + survival_{monthly} + male_{condition}$ | 67.71 | 1.76 | 0.01 | 2.00 |
| $EPP(.) + female_{age} + osr_2$ | 67.75 | 1.80 | 0.01 | 3.00 |
| $EPP(.) + kernel_{95\%} + male_{condition}$ | 67.76 | 1.81 | 0.01 | 3.00 |
| $EPP(.) + \ldots$ | $\leq 67.76$ | $\geq 1.81$ | $\leq 0.01$ | |

Table 5.6: Model structure, $AIC$, $\Delta AIC_c$, model weight ($w_i$), and number of parameters for models of intra-specific nest parasitism (INP) on Tall Timbers Research Station during 2001-2003.

| Model | $AIC$ | $\Delta AIC$ | $w_i$ | Parameters |
|---|---|---|---|---|
| $INP(.) + female_{condition} + osr_2$ | 68.83 | 0.00 | 0.11 | 3 |
| $INP(.) + female_{condition} + incubated_{male} + osr_2$ | 70.55 | 1.71 | 0.05 | 4 |
| $INP(.) + age + female_{condition} + osr_2$ | 70.65 | 1.82 | 0.04 | 4 |
| $INP(.) + survival + female_{condition} + osr_2$ | 70.70 | 1.87 | 0.04 | 4 |
| $INP(.) + female_{condition} + kernel_{50\%} + osr_2$ | 70.83 | 2.00 | 0.04 | 4 |
| $INP(.) + female_{condition} + kernel_{95\%} + osr_2$ | 70.83 | 2.00 | 0.04 | 4 |
| $INP(.) + age + female_{condition} + incubated_{male} + osr_2$ | 72.40 | 3.57 | 0.02 | 5 |
| $INP(.) + survival + female_{condition} + incubated_{male} + osr_2$ | 72.50 | 3.67 | 0.02 | 5 |
| $INP(.) + female_{condition} + kernel_{95\%} + incubated_{male} + osr_2$ | 72.54 | 3.70 | 0.02 | 5 |
| $INP(.) + female_{condition} + kernel_{50\%} + incubated_{male} + osr_2$ | 72.54 | 3.71 | 0.02 | 5 |
| $INP(.) + survival + age + female_{cond} + osr_2$ | 72.57 | 3.73 | 0.02 | 5 |
| $INP(.) + age + female_{condition} + kernel_{50\%} + osr_2$ | 72.65 | 3.81 | 0.02 | 5 |
| $INP(.) + age + female_{condition} + kernel_{95\%} + osr_2$ | 72.65 | 3.82 | 0.02 | 5 |
| $INP(.) + survival + female_{condition} + kernel_{50\%} + osr_2$ | 72.70 | 3.87 | 0.02 | 5 |
| $INP(.) + survival + female_{condition} + kernel_{95\%} + osr_2$ | 72.70 | 3.87 | 0.02 | 5 |
| $INP(.) + age + female_{condition} + osr$ | 72.71 | 3.88 | 0.02 | 4 |
| $INP(.) + \ldots$ | $\leq 72.71$ | $\geq 3.88$ | $\leq 0.02$ | |

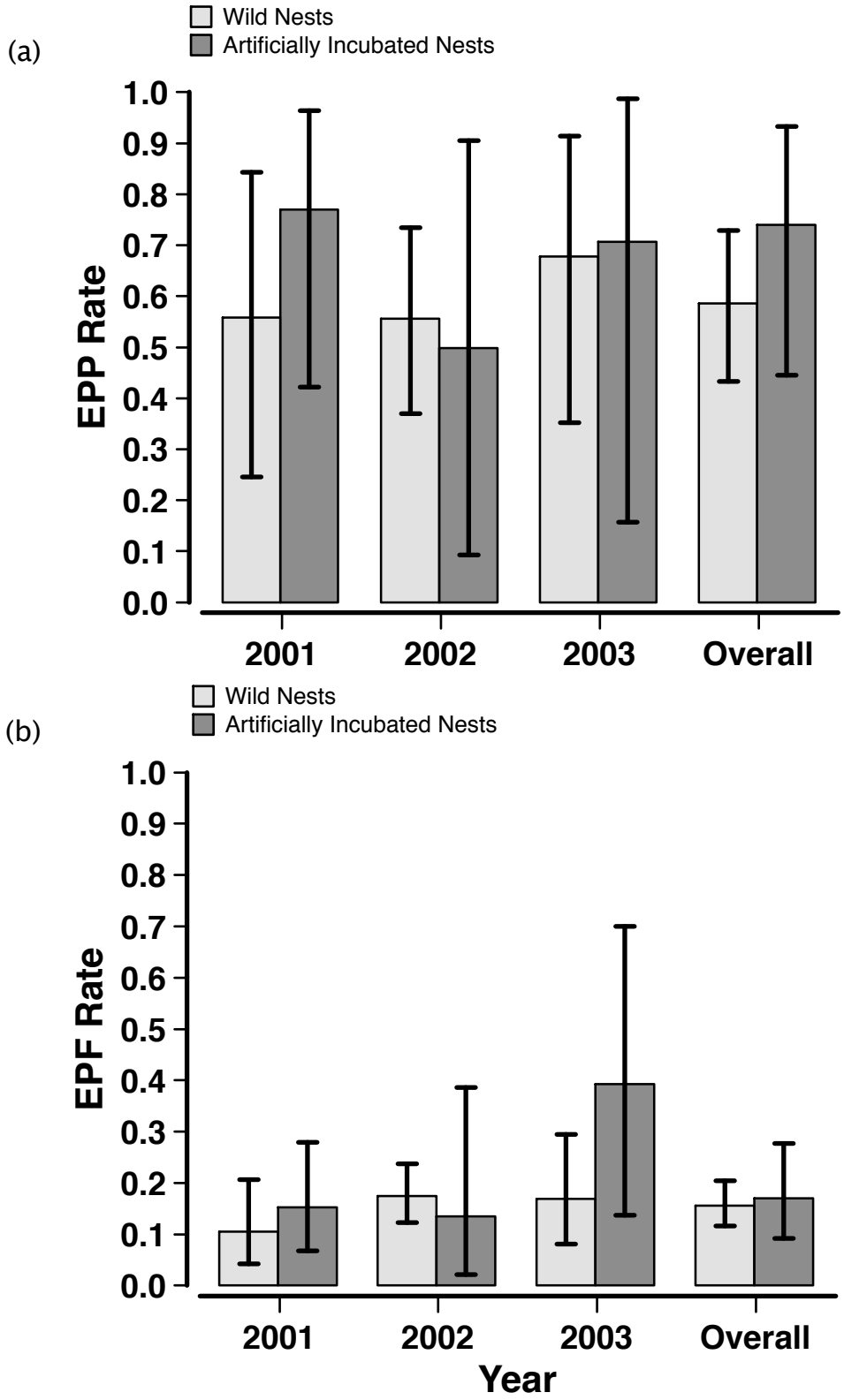


Figure 5.1: Median posterior probability (± 95% CI) of (a) extra-pair paternity (EPP) and (b) extra-pair fertilization (EPF) on Tall Timbers Research Station during 2001-2003.

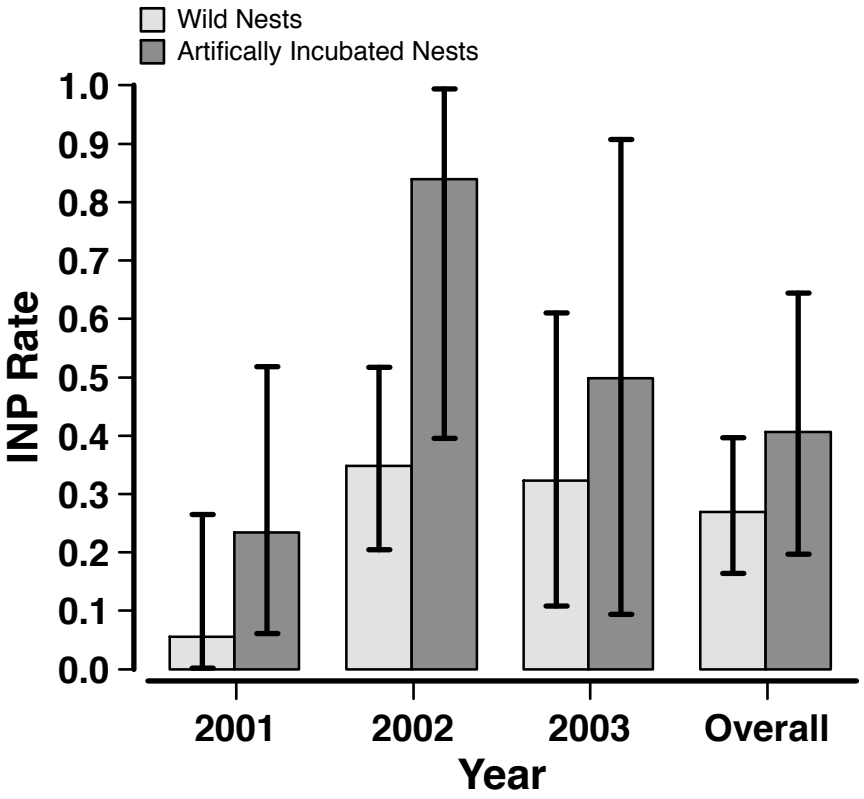


Figure 5.2: Median posterior probability (± 95% CI) of intra-specific nest parasitism (INP) on Tall Timbers Research Station during 2001-2003.

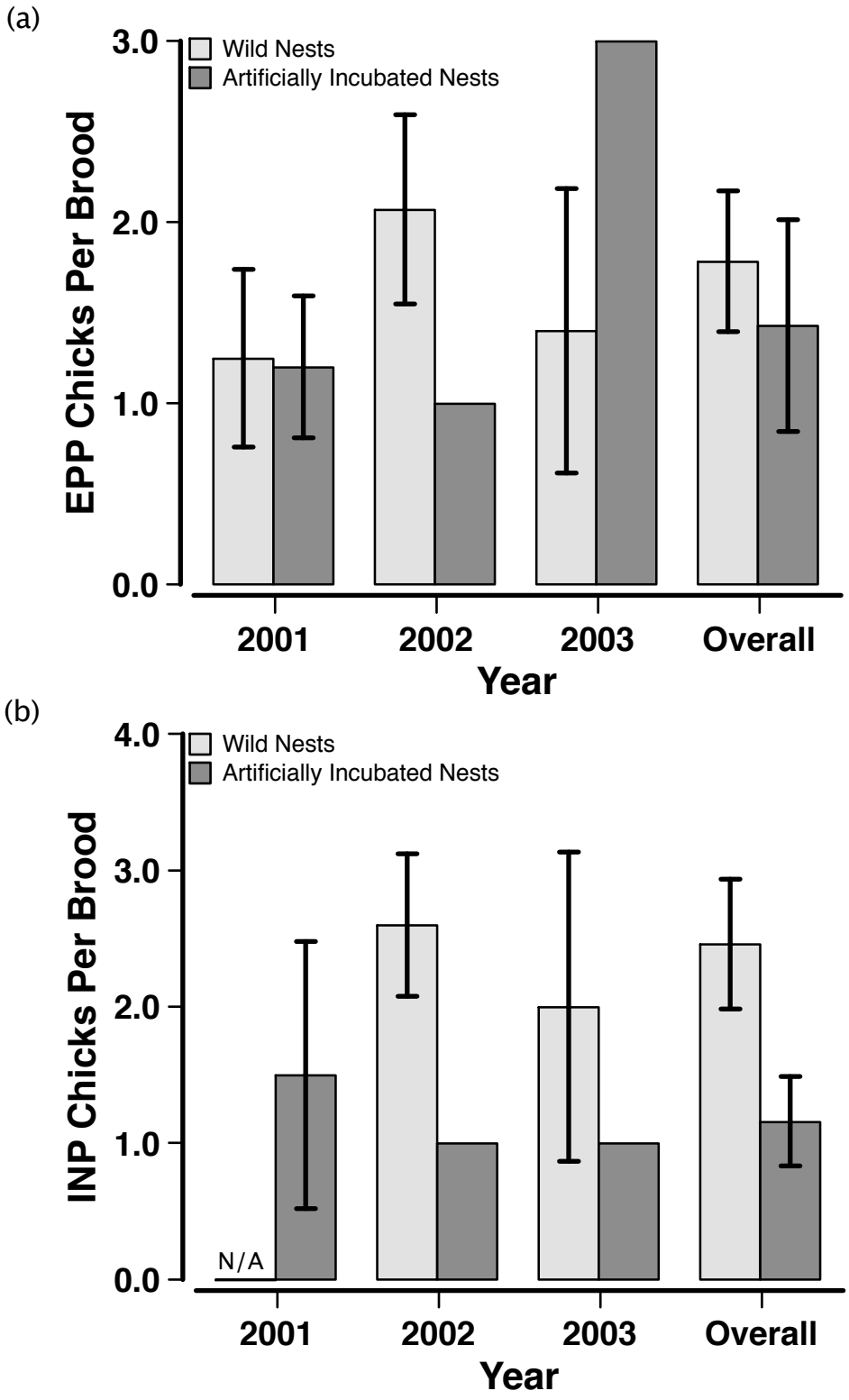


Figure 5.3: Mean number (± 95% CI) of chicks per brood arising from (a) extra-pair (EPP) matings and (b) intra-specific nest parasitism (INP) during 2001-2003 on Tall Timbers Research Station. A lack of confidence intervals indicates only a single brood contained chicks of either class.

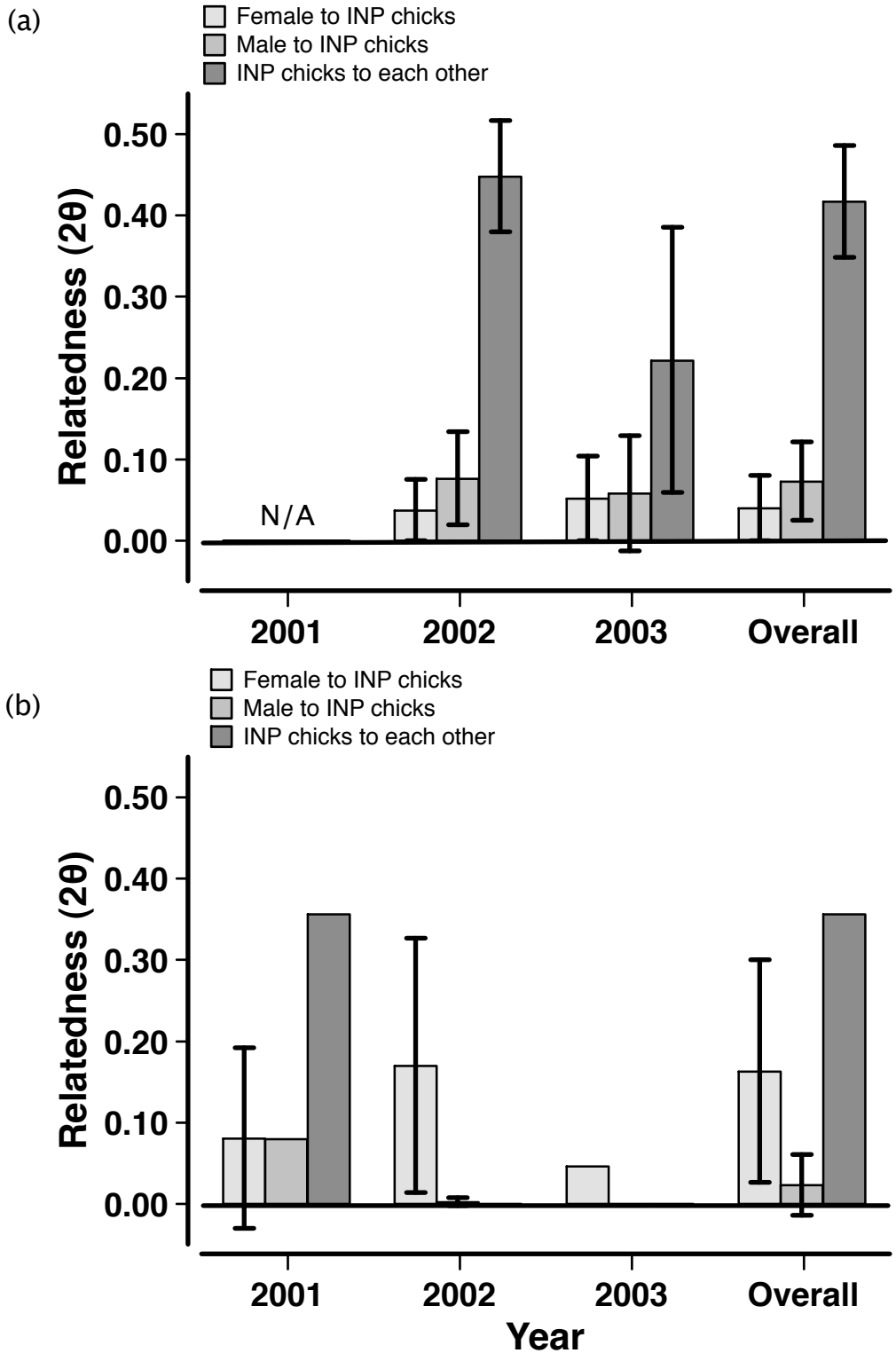


Figure 5.4: Mean relatedness (± 95% CI) between inferred parents and parasitic chicks in (a) wild and (b) artificially incubated broods collected during 2001-2003 on Tall Timbers Research Station. A lack of confidence intervals indicates single observations within categories.

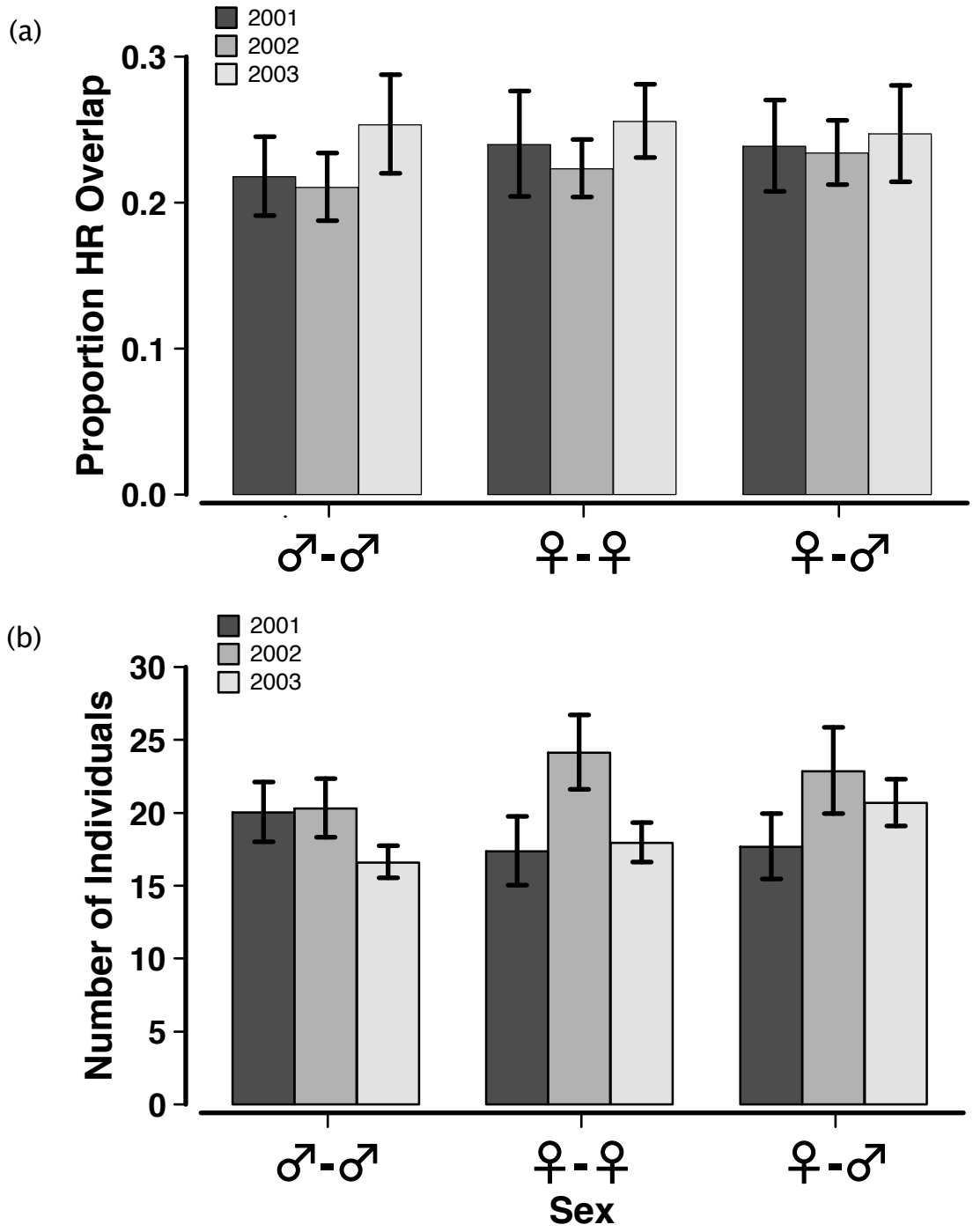


Figure 5.5: Mean proportion (± 95% CI) home range (HR) overlap (a) and (b) mean number (± 95% CI) of overlapping individuals for same sex and opposite sex pairings during 2001-2003 on Tall Timbers Research Station.

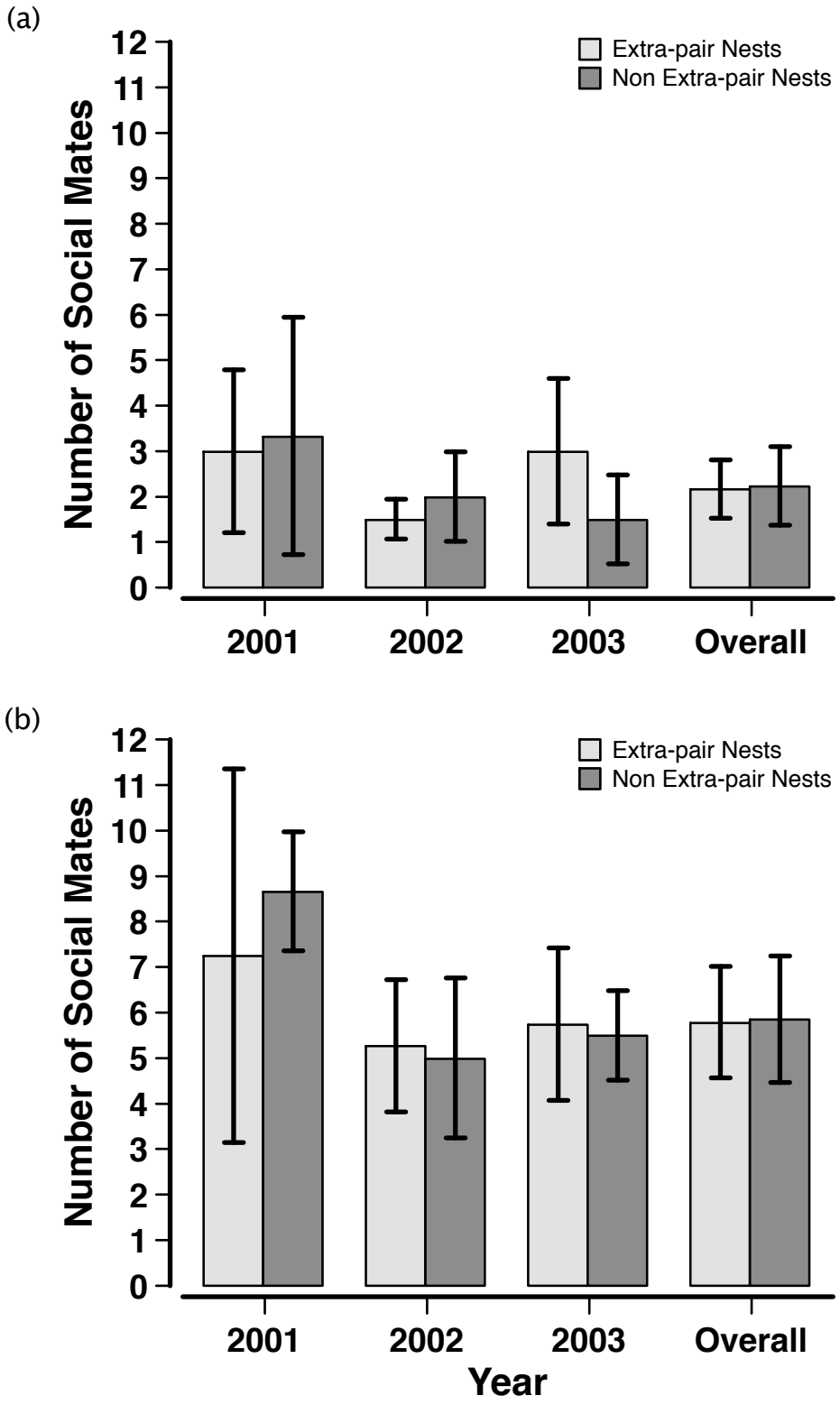


Figure 5.6: Mean number of social mates during (a) the period 3 weeks prior to incubation and (b) the duration of the breeding season for female parents of wild broods on Tall Timbers Research Station.

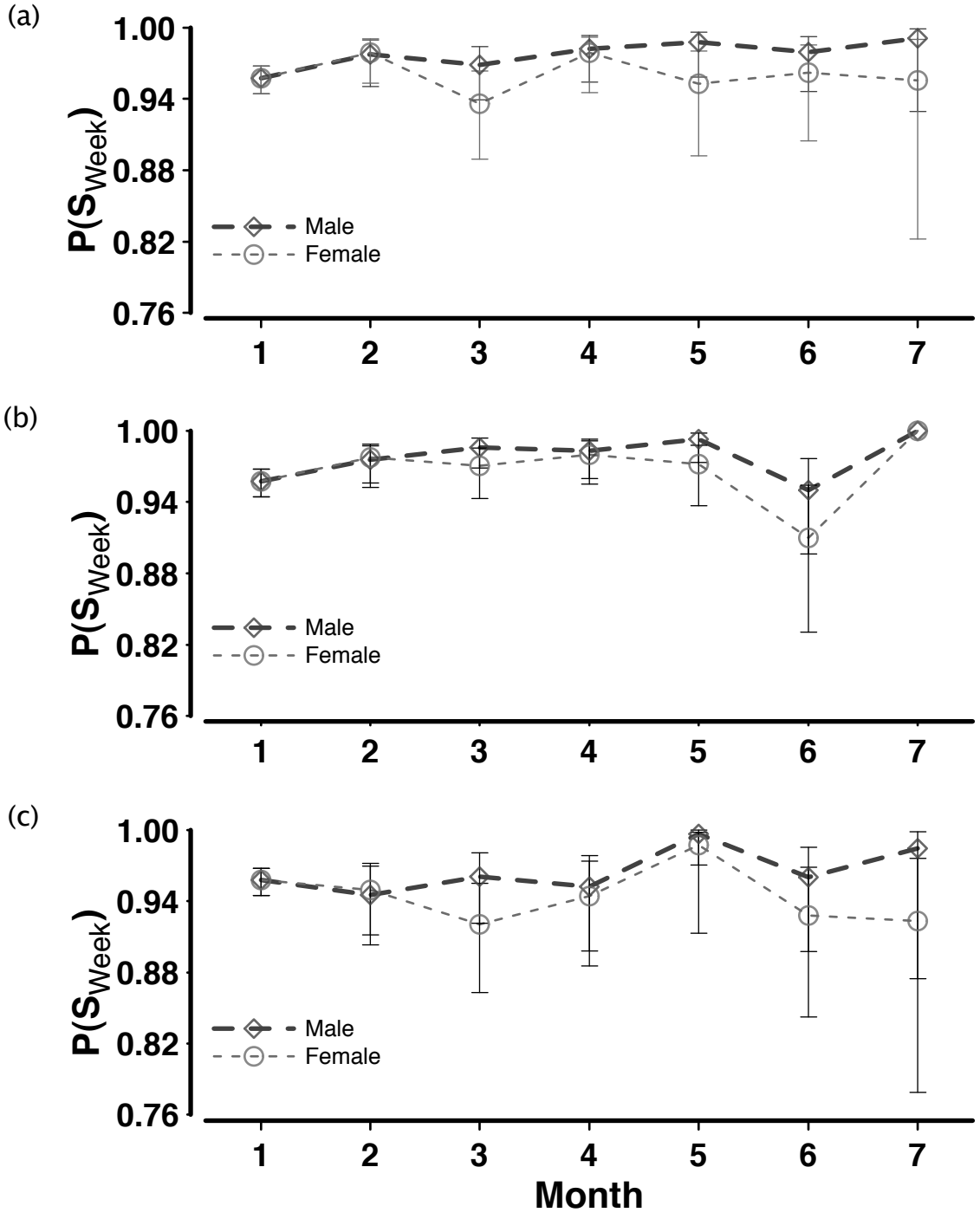


Figure 5.7: Estimated, weekly breeding season survival for each month of observation during 2001-2003 on Tall Timbers Research Station.

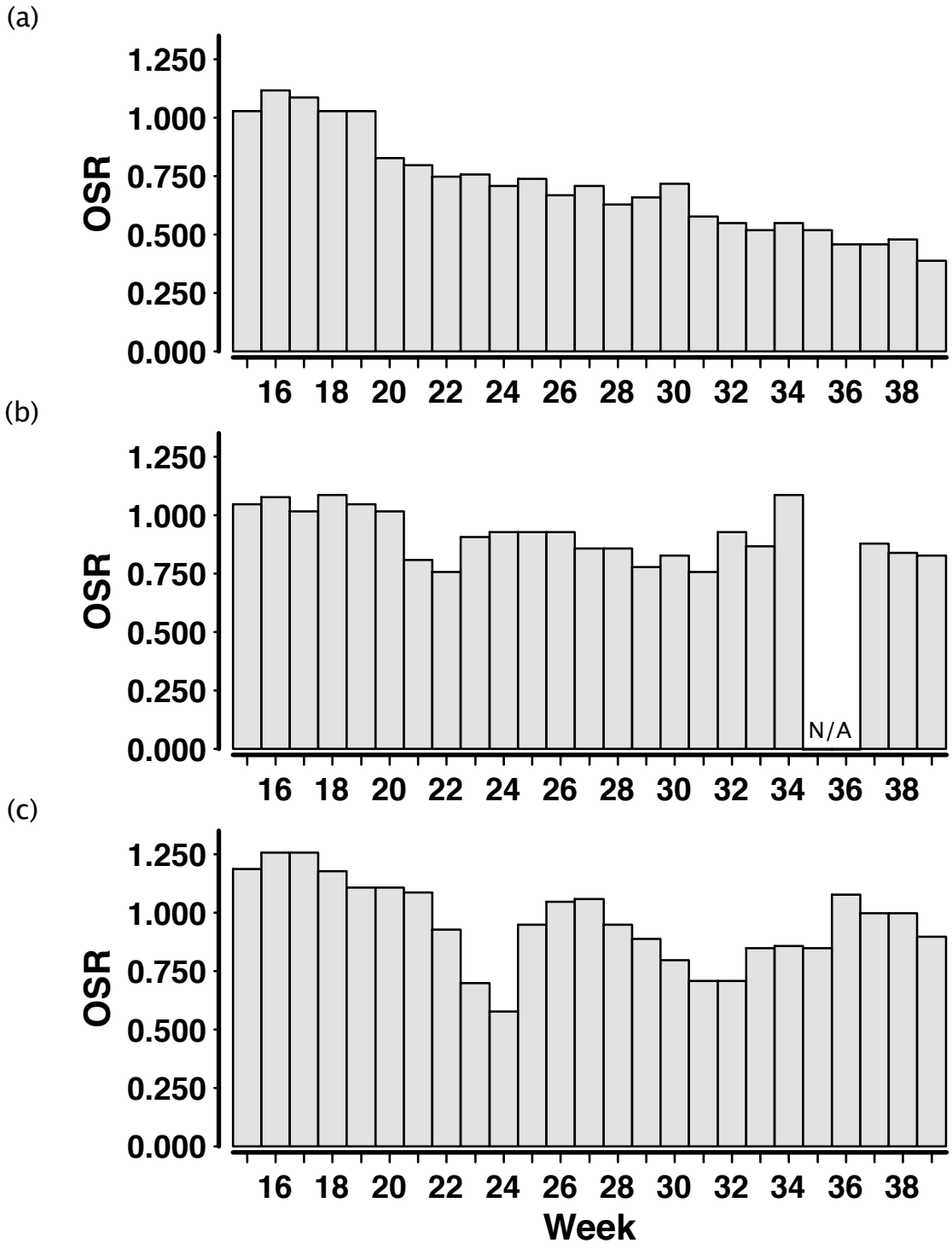


Figure 5.8: Operational sex ratio during 2001-2003 on Tall Timbers Research Station.

# Chapter 6

# Conclusions

Northern Bobwhite inhabit highly variable environments. Variability affecting individual fitness is best described by bobwhite survival (Palmer and Wellendorf 2007; Terhune et al. 2007, Figure 3.3) and reproduction rates (Burger et al. 1995, Table 5.2) and often manifested as predation, food availability, and disease. As in all species, variability in the environment drives selection for adaptations that confer increased fitness benefits (Darwin 1859). Behavior is but one of these adaptations.

To view behavior as a single adaptation is, of course, shortsighted. Behavior, as a general term, encompasses the suite of choices made and actions taken on the part of an individual. With respect to bobwhites, this includes decisions to live in a group, how and when to reproduce, and how to raise offspring. Each of these decisions can, of course, be dissembled into smaller, component parts.

Generally, we attempt to explain behavior by investigating the fitness gains derived from its expression. A subset of behavior and related fitness gains may result from inclusive effects - in essence taking an action that will increase the fitness of one's genes through the survival of a related individual.

It is from this standpoint that I investigated both bobwhite social groups and post-hatching brood amalgamation, in Chapters 2 and 4. Although perhaps appearing disconnected, the two behaviors are quite similar: individuals cooperate with others to accomplish a task, be it rearing of offspring, foraging, or avoiding predators.

Inclusive fitness and kin selection theory suggest these individuals should be relatives, sharing genetic heritage and cooperating for the greater good of their shared *genes*, potentially at a cost to themselves.

In both cases, my research suggests that this is not be true. Bobwhite social groups, while sometimes composed of relatives, do not derive a measurable increase in survival from the degree of their relatedness, suggesting that this is not the cause of their formation. Amalgamated broods are largely the combination of unrelated offspring by unrelated individuals, removing the potential for inclusive fitness gains.

With respect to bobwhite reproduction, I used similar techniques to accomplish a slightly different task: the description of bobwhite breeding behaviors. Previous research suggested that these behaviors were likely to be varied, perhaps as the result of variation at local or regional scales, but what I identified was an array of behavior within the same population and occasionally at the individual level: reproduction with single mates, reproduction with multiple mates, reproduction by individually shifting strategies, the use of conspecific nests, and an unexplained, but high degree of male incubation. Additionally, I could not identify a relationship between these behaviors and commonly-used explanatory factors, including territoriality and the operational sex ratio.

Setting aside the potential bias introduced by variance and error in measured parameters, the whole of these results brings one back to the theme introduced in the first sentence: behavior arose due to variation in the environment. Given that the environment is variable, we would expect behavior to be affected by changes in the environment, and we might expect that environmental variability selects for similar flexibility in behavior. Because we can take direct action to reduce variation in the environment through management practices, including supplemental feeding or predator control, we should use our knowledge of these techniques to investigate their effect on behavior. Assuming that flexibility in behavior may be the norm rather than the exception, we should expect these manipulations to result in potentially measurable effects.

For example, given a lack of inclusive effects of social group membership on fitness, the next step for social group research should revisit the work of Pulliam (1973), Hamilton (1971), and others. Using knowledge of the effects of supplemental feeding and predator control on variance in bobwhite survival, we should examine the effects of these practices on group formation, individual composition, group persistence, and individual survival. We should additionally conduct comparative studies throughout the range of bobwhites to investigate the impact of local and regional environmental factors on the expression of bobwhite behavior. And, we should create models of behavior, similar to those of Gowaty and Hubbell (2005), to generate predictions given variation in the environment that can be tested empirically.

Although results of this dissertation likely raise more questions than they answer with respect to bobwhite reproduction, the application of similar efforts to its study may provide rapid answers to common questions affecting many species: Does food availability reduce or increase multiple mating? Can predation affect the rate of intra-specific nest parasitism? Does the breeding system of bobwhite change at high densities? Can we directly manipulate natural flexibility in behavior for both the conservation and management of a species? And, if we can, is it a viable strategy among those impacting the conservation of a species?

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# Appendix A

## Estimation of Relatedness Using Maximum Likelihood

Throughout this manuscript, we used a maximum likelihood estimator of relationships and relatedness, adjusted for the presence of null alleles (Chapuis and Estoup 2007; Milligan 2003; Thompson 1975; Wagner et al. 2006; Weir et al. 2006). In all cases, we have assumed the population was non-inbred and utilized the 3-parameter likelihood estimate (Thompson 1975). The likelihood algorithm used for relatedness and relationship estimation is:

$$P(\{H_1^{(j)}, H_2^{(j)}\} \mid k) = \prod_{j=1}^{s} (k_0 P_0^{(j)} + k_1 P_1^{(j)} + k_2 P_2^{(j)}) \tag{A.1}$$

where $(H_1^{(j)}, H_2^{(j)})$ represent the genotypic states of the individuals being compared, $k = relationship \mid relatedness$, $s = n_{loci}$, and $(P_{0,1,2}^{(j)})$ represent the joint genotypic probabilities for each genotypic state. We computed joint genotypic probabilities (Table A.1) using (1) allele frequencies computed using microsatellite genotypes and adjusted for the presence of null alleles and (2) an estimate of null allele frequency ($\varepsilon_{null}$) derived using the EM algorithm (Chapuis and Estoup 2007; Dempster et al. 1977)

### A.0.1 Relationship estimation

To estimate relationships, we substituted identity by descent probabilities ($k_0, k_1, k_2$; Table A.2) for common relationship classes into the likelihood (brute-force), accepting those that minimized the negative log-likelihood given the joint genotypic probabilities for the states being compared (Table A.1).

### A.0.2 Relatedness estimation

To estimate relatedness, we used the downhill simplex algorithm (Nelder and Mead 1965), a numerical optimizaton technique implemented in Scipy (Ascher et al. 2001; Oliphant 2007; SciPy 2008; van Rossum 2006), to minimize the negative log-likelihood by solving for $k_{0,1,2}$ given the data (Table A.1). The MLE of $k_{0,1,2}$ for 2 individuals being compared were those values of $(k_0, k_1, k_2)$, subject to:

$$k_0 + k_1 + k_2 = 1 \tag{A.2}$$

and

$$0 \leq k_{0,1,2} \leq 1 \tag{A.3}$$

which minimized the negative log-likelihood (Equation A.1) given the data (Table A.1).

We transformed estimates of the probabilities of identity ($k_{0,1,2}$) to pairwise relatedness ($r$) using the following formulae:

$$\theta = \frac{k_1}{4} + \frac{k_2}{2} \tag{A.4}$$

$$r = 2\theta \tag{A.5}$$

and we used these estimates of $r$ in our analyses.

Table A.1: The joint genotypic probabilities at each genotypic state, corrected for the presence of null alleles. $\varepsilon_{null}$ refers to the locus-specific probability of a null allele, estimated in this manuscript using the EM Algorithm (Chapuis and Estoup 2007).

| Genotypic State | M | $H^1 : H^2$ | Joint Genotypic Probabilities | | |
|---|---|---|---|---|---|
| | | | $P_0$ | $P_1$ | $P_2$ |
| Hom/Hom | 2 | $H^1_{pp} : H^2_{pp}$ | $p^2 + (2p * \varepsilon_{null})$ | $p^3 + (2p^2 * \varepsilon_{null} + (p * \varepsilon_{null} * (p + \varepsilon_{null}))$ | $p^4 + (4p^3 * \varepsilon_{null} + 4p^2 * \varepsilon^2_{null})$ |
| Hom/Hom | 0 | $H^1_{pp} : H^2_{jj}$ | 0 | $0 + (pj * \varepsilon_{null})$ | $p^2j^2 + \varepsilon_{null}(2p^2j + 2pj^2) + 4pj * \varepsilon^2_{null})$ |
| Hom/Het | 1 | $H^1_{pp} : H^2_{pj}$ | 0 | $p^2j + (pj * \varepsilon_{null})$ | $2p^3j + (4p^2j * \varepsilon_{null})$ |
| Hom/Het | 0 | $H^1_{pp} : H^2_{jk}$ | 0 | 0 | $2p^2jk + (4pjk * \varepsilon_{null}$ |
| Het/Het | 2 | $H^1_{pj} : H^2_{pj}$ | $2pj$ | $pj * (p + j)$ | $4p^2j^2$ |
| Het/Het | 1 | $H^1_{pj} : H^2_{pk}$ | 0 | $pjk$ | $4p^2jk$ |
| Het/Het | 0 | $H^1_{pj} : H^2_{kl}$ | 0 | 0 | $4pjkl$ |

Table A.2: Identity by descent probabilities for common relationships used in relationship estimation. Taken from Weir et al. (2006).

| Relationship | $k_0$ | $k_1$ | $k_2$ |
|---|---|---|---|
| Twins | 1 | 0 | 0 |
| Full-siblings | $\frac{1}{4}$ | $\frac{1}{2}$ | $\frac{1}{4}$ |
| Parent-Offspring | 0 | 1 | 0 |
| Double First Cousins | $\frac{1}{16}$ | $\frac{4}{8}$ | $\frac{9}{16}$ |
| Half-siblings | 0 | $\frac{1}{2}$ | $\frac{1}{2}$ |
| First-cousins | 0 | $\frac{1}{4}$ | $\frac{3}{4}$ |
| Unrelated | 0 | 0 | 1 |

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# Appendix B

## Estimates of the genotype error rate

Table B.1: Per-locus estimates of the genotype error rate during 2001-2003 determined using a randomly selected and blindly genotyped subset of the bobwhite genetic samples collected during 2001-2003 on Tall Timbers Research Station. Asterisks indicate samples removed from the set of candidate markers due to linkage.

| Locus | | Error Rate |
|---|---|---|
| CV-P1A7 | | 0.000 |
| CV-P1F2 | | 0.011 |
| CV-P1F3 | | 0.005 |
| CV-P1H12 | | 0.000 |
| CV-P2D7 | | 0.003 |
| CV-PA12A | ** | 0.008 |
| CV-PA12G | | 0.011 |
| CV-PA1C | | 0.011 |
| CV-PA1F | ** | 0.013 |
| CV-P13E | | 0.013 |
| CV-PA3F | | 0.008 |
| CV-PA3G | | 0.016 |
| CV-PA5F | | 0.011 |
| CV-PBA4 | | 0.011 |
| CV-PBH5 | | 0.000 |
| CV-PCF5 | | 0.011 |