

FACTORS AFFECTING THE DEMOGRAPHY
OF A LEK-MATING BIRD: THE GREATER PRAIRIE-CHICKEN

by

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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Division of Biology
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2007

Abstract

Sexual selection via female choice and male-male aggression leads to elaboration of male traits. If male traits correlated with reproductive success are honest signals of male quality, survival costs may be associated with the expression of those traits. Testosterone (hereafter 'T') may enhance male breeding success, but T can also reduce immunocompetence and survival. Socially monogamous male birds with higher circulating T experience reproductive advantages, but the role of T in lek mating systems is largely unknown. To address these issues, I individually marked and conducted focal behavioral observations of greater prairie-chickens (*Tympanuchus cupido*) at five lek sites over a 5-year period. Females were fitted with radio-telemetry to monitor nesting success and survival. I examined the relationship between male traits and mating success using multinomial discrete choice models, a statistical method not previously applied to studies of sexual selection. Male mating success was highly skewed at greater prairie-chicken leks with 18.5% of males obtaining 87.2% of all successful copulations ($n = 108$ males; 85 copulations). Mating success was influenced most by male behavior, followed by several morphological attributes. The role of T was quantified using blood samples and by experimentally implanting a subset of males with T. T did not consistently affect mating success. Non-territorial males had lower T levels than territorial males. Among territory holders, T was unexpectedly negatively correlated with mating success. However, the odds of receiving a copulation were 4.3 times (0.42 to 45.3) greater for T-implanted males than males with sham implants. Future work should explore the interactions among the immune system, parasite load, and mating success of prairie-chickens. Annual survival of male prairie-chickens was not related to mating success, behavior, age or T level, suggesting there is no cost of increased male mating success. Like males, reproductive success of females was also highly skewed because $< 10\%$ of nests successfully hatched young. Comparisons of seasonal and annual survival rates indicate that females experience increased mortality during the breeding season relative to the nonbreeding season. Synthesis of field estimates of demographic parameters indicates prairie-chicken populations will decline without changes in rangeland management to reduce predator numbers or provide more nesting cover.

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Acknowledgments

I thank Brett K. Sandercock for his patience, support, and encouragement in the field and personally. I thank my committee, David Margolies, Sam Wisely, Kimberly With, and outside chair, Frank Blecha, for their encouragement and interest in my project. Expert statistical assistance was provided by Thomas Loughin. Robert Gibson and Timothy Parker gave helpful comments on previous drafts of Chapter 2. I thank the following organizations and people for allowing access to their prairie-chicken leks: Konza Prairie Biological Station, a property of The Nature Conservancy managed by the Division of Biology at Kansas State University; Rannells Flint Hills Prairie Preserve managed by Dr. Clenton Owensby in the Department of Agronomy at Kansas State University; and private lands owned by Grant Poole and James Hess. Tom VanSlyke and Kenny Berg provided logistical support. I thank Tracey Adamson, Jeremy Baumgardt, Amanda Behnke, Jarrod Bowers, Tara Conkling, Seth Cutright, DeVaughn Fraser, Chris Frey, Kyle Gerstner, Chod Hedinger, 'Hoogy' Hoogheem, Nichole Lambrecht, and Kara Oberle for field assistance. Funding for field work included: a NSF Kansas EPSCOR Grant, a research grant from the American Ornithologists' Union, and an NSF Doctoral Dissertation Improvement Grant (DEB-0608477). I was supported by the Konza Prairie NSF Long-Term Ecological Research Grant (DEB-0218210) and by the Division of Biology at Kansas State University. The research performed in the present study complied with the current laws of the countries in which they were performed and were conducted under the following research permits: Scientific, Education or Exhibition Wildlife Permit, Kansas Department of Wildlife and Parks (SC-118-2003, SC-068-2004, SC-078-2005, SC-072-2006), and Institutional Animal Care and Use Committee (Protocols 2079, 2351).

Many people have supported me personally during my graduate work. In particular, I have had overwhelming love and support from my parents, Ralph and Kay Nooker. They are truly the best parents and friends I could have hoped for. My church family at the Manhattan Seventh Day Adventist Church, especially Lauren Choi and Vernon and Ramona Zeller, helped me through a challenging time. I would also like to remember the many foster dogs in my life, and my late pointer, Jed, who remind me daily that nothing is better than a walk through the prairie with friends.

Dedication

This work is dedicated to my grandma, Eva Hoffman. I look forward to seeing her again in heaven.

CHAPTER 1 – Introduction

Behavioral ecology focuses on how behavior affects reproduction and survival, and includes the subfields of predator-prey interactions, resource competition, sociality and sexual selection. One aspect of sexual selection research involves the study of the different selective pressures exerted on males and females during reproduction (Emlen and Oring 1977). In socially monogamous songbirds, males defend a territory, attract a mate, guard their mate during their mate's fertile period, seek extra-pair copulations, and provide parental care. Socially monogamous females choose a social mate, seek extra-pair copulations, lay and incubate the eggs, and provide parental care. One drawback for studies of socially monogamous systems is that biparental care makes it difficult to disentangle the contributions of each sex to reproduction. Uniparental care, found in many polygamous and promiscuous mating systems, allows for the study of the proximate factors reproductive success of each sex independent from the contributions of the other sex. A complication of socially monogamous and polygamous systems is that resource defense (i.e., territoriality) affects the food and habitat resources available for reproduction. Most promiscuous mating systems lack resource defense by the sex that provides parental care. Sexual selection may be particularly intense in lek-mating systems, a unique form of promiscuity. In lek-mating systems, males only provide gametes to the females. Males are responsible for attracting a mate by displaying and defending small territories at lek sites; whereas, females are the sole caretakers of the nest and offspring. In lek mating grouse, the benefits of mate choice to females are primarily indirect (good genes for her offspring) because males are not defending food, nesting sites or any other resources required by females (Höglund and Alatalo 1995). Unlike monogamous systems where access to a particular male may be limited once he pairs with a female, lek-mating females are able to choose any male on the lek, regardless of the decisions of previous females. Another characteristic of lek mating systems is a high skew in male reproductive success. Because of this reproductive skew, sexual selection of male traits is intense in lek-mating systems, so links between male traits and male mating success should be relatively easy to discover.

Proximate factors affecting reproductive success vary by sex. In birds, males with brighter plumage, more elaborate ornaments, certain song characteristics, more aggressive behavior, or more intense displays are more successful than males without these qualities

(Brodsky 1988, Gibson et al. 1991, Fiske et al 1998, Duckworth 2006). Body condition and weather can influence reproduction in both sexes (Shorey 2002, Nooker et al. 2005). The hormonal factors mediating variation in male traits and mating success are relatively unknown. Testosterone (hereafter ‘T’) may play a key role in enhancing male breeding effort and success. T-dependent traits should be honest signals of male quality, because T can have immunosuppressive effects (Folstad and Karter 1992, Ditchkoff et al. 2001), leading to a negative correlation between circulating T and annual survival (Ketterson and Nolan 1999). Advantages during female choice and male-male competition are often associated with T-dependent traits, such as large fleshy head combs (in red jungle fowl *Gallus gallus*, Zuk et al 1995a,b), high-quality territories (Moss et al. 1994), and more vigorous courtship displays (Mateos and Carranza 1999). Experimental manipulations of T are needed to confirm causal links between T, male traits and mating success, especially in lek-mating species.

Only two studies of lek-mating species have experimentally implanted males with testosterone. T-implanted manakins increased rates of several display behaviors compared to sham-implanted controls (Day et al. 2006). Aggressive, display and territory attributes of T-implanted males of the lek-mating sharp-tailed grouse (*Tympanuchus phasianellus*) remained unchanged relative to unmanipulated control males, but lek attendance was higher for T-implanted males (Trobec and Oring 1972). Neither of these two studies examined mating success of T-implanted males relative to controls. No copulations were observed in the sharp-tailed grouse study (Trobec and Oring 1972), and the manakin study was conducted during the non-breeding season (Day et al. 2006).

Skew in male mating success has been widely documented in lek-mating systems; however, variation in female reproductive success is relatively unknown. Females are generally harder to capture and observe at lek sites. Their cryptic plumage and behavior makes them inconspicuous away from the lek. Telemetry offers the best opportunity for obtaining information during this portion of the life cycle. Knowledge of reproductive success is essential to determine the strength of sexual selection and to guide management of the population.

Survival estimates are another crucial piece of information for determining the consequences of sexual selection and to guide management decisions (Sandercock 2006). If sexually selected traits are honest signals of male quality, there may be survival costs to

possessing such traits. Annual survival of females is an important metric of any population-level monitoring or modeling scheme. Since the daily movements of males and females are largely independent of each other in lek-mating systems, it is important to calculate survival estimates separately for each sex.

Modern statistical techniques robustly test environmental and behavioral covariates when estimating survival and the degree of reproductive skew. *Cormack-Jolly-Seber* (CJS) models based on live encounter data improve upon return rates by separating apparent survival (ϕ) from the probability of encounter (p ; Sandercock 2006). The *nest survival* model in Program Mark improves upon Mayfield estimates (Mayfield 1975) because it relaxes the assumption of constant daily survival, is less sensitive to variation in nest monitoring efforts, and allows for daily survival rate to be modeled as a function of environmental covariates (Dinsmore et al. 2002). *Multinomial discrete choice* models tests how an individual chooses among a defined set of choices (Buskirk and Millspaugh 2006; Cooper and Millspaugh 1999; Moore and Swihart 2006). In the first application of multinomial discrete choice models to mate choice, I modeled how a female chooses a single male from all the available males on a lek. Male mating skew was quantified using *lambda values* to describe the intensity of sexual selection and allow for comparisons across leks regardless of lek size (Kokko and Lindström 1997). These four models have rarely been applied to lek-mating species.

The greater prairie-chicken (*T. cupido*) is a lek-mating grouse with economic importance as a gamebird. Male greater prairie-chickens perform ritualized courtship and territorial behaviors on small territories at lek sites (Schroeder and Robb 1993). Courtship displays consist of rapid foot-stomping while displaying feather and fleshy ornaments and emitting a low 3-note ‘boom’ vocalization. Territorial boundary disputes are frequent and often escalate to physical combat. The historic range of greater prairie-chickens extended from the prairie provinces of Canada, south to central Texas and east to western Ohio (Schroeder and Robb 1993, Ross et al. 2006). Loss and fragmentation of the tallgrass prairie have restricted sizable populations of prairie-chickens to four states (Silvy et al. 2004, Ross et al. 2006). Kansas is one of these four states with >5000 breeding birds, but prairie-chickens have declined 70% in Kansas during the past 20 years (Applegate and Horak 1999, Svedarsky et al. 2000). Recent declines in Kansas call

for contemporary estimates of prairie-chicken demography in order to guide rangeland management.

The lek-mating greater prairie-chicken is an ideal study species to examine reproduction and survival in the context of sexual selection for five reasons. Because males do not provide parental care and benefits to females are indirect, I can examine the contribution of each sex to reproduction without many confounding effects typical of socially monogamous systems. Second, plumage dichromatism of grouse is estrogen-dependent, not T-dependent (Kimball and Ligon 1999). The cryptic plumage of females is a derived condition within three basal orders of birds: Struthioniformes (ostriches), Anseriformes (ducks), and Galliformes (grouse). Derived female plumage allows study of T-dependent traits without the confounding effects of T on most plumage ornaments. Third, male mating success is highly skewed (18.5% of males obtained 87.2% of copulations; Chapter 2) and male-male aggression is intense (Schroeder and Robb 1993), making this species a good model to study variation in male reproductive success. Fourth, behavior as it relates to mating success has not been quantified for greater prairie-chickens. Lastly, most demographic estimates of greater prairie-chickens occurred 30 years ago (Horak 1985; Robel 1966, 1970a,b; Robel and Ballard 1974), before the increased use of annual burning for rangeland management (Robbins et al. 2002). There is need for modern estimates to guide prairie-chicken conservation.

The goals of this dissertation were to integrate behavioral ecology, population biology, experimental manipulations, and modern statistical techniques to understand how reproduction and survival vary between the sexes. During a 5-year field study, my research objectives have been: 1) to correlate male traits with mating success, 2) to determine the proximate role of T in mediating variation in male traits and mating success, 3) to examine the demography of prairie-chickens including which factors affect female reproduction, and 4) to analyze the survival consequences of reproduction in both sexes. My research results contribute to evolutionary ecology theory and the conservation biology of prairie-chickens, a declining grouse species.

This dissertation is organized into three core chapters. In Chapter 2, I used detailed behavioral observations and multinomial discrete choice models to quantify the skew in male mating success, to analyze the variation in male traits, to correlate male traits with mating success and to determine if survival costs were associated with male traits or mating success. I

found that behavioral traits exhibited more variation and were better predictors of male mating success than morphological traits or territory attributes. In Chapter 3, I quantified the natural variation in male T, examined links between T and male traits, mating success and survival, and tested for causal links between T and male attributes experimentally by comparing T-implanted males with sham-implanted controls. T was a better predictor of male mating success than behavioral traits. In Chapter 4, I estimated demographic parameters for radio-marked female prairie-chickens using nest survival and Cormack-Jolly-Seber mark-recapture models. Predation of nests and females during the breeding season appears to be the primary factor limiting population sizes of prairie-chickens. Chapter 5 is a summary of the major findings of these studies, a discussion of their theoretical and conservation importance, and suggestions for future research.

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CHAPTER 2 –
Correlates and Consequences of Male Mating Success in Lek-mating
Greater Prairie-Chickens (*Tympanuchus cupido*)

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Abstract

Female choice and male-male aggression are two modes of sexual selection that can lead to elaboration of male morphological and behavioral traits. In lek-mating species, male mating success is often strongly skewed, and it is puzzling why variation in male traits is still observed (lek paradox). If male traits correlated with reproductive success are honest signals of male quality, there may be survival costs associated with the expression of those traits. In this study, we determined which morphological, behavioral and territorial traits are correlated with male mating success and survival in the lek-mating greater prairie-chicken (*Tympanuchus cupido*). We introduce a novel application of multinomial discrete choice (MDC) models for analysis of female mate choice behavior. We found that behavioral and territorial attributes showed 6.5 times more variability among males than morphological traits. Both display and aggressive behaviors were strong predictors of male mating success, suggesting that both female choice and male-male aggression were important in determining mating success among male greater prairie-chickens. Moreover, annual survival of male prairie-chickens was independent of mating success and male traits. Since most morphological traits showed little variation (mean CV < 5%), the lek paradox applies only to behavioral traits of male greater prairie-chickens. Behavioral traits were the most important factor determining mating success of male prairie-chickens, but the proximate mechanism underlying this relationship is unknown. In the future, experimental manipulations of male hormones and parasite loads could bridge the proximate mechanisms and ultimate consequences of factors mediating male mating success in lek-mating birds.

Introduction

Sexual selection is an important driver of evolutionary change and leads to elaboration of male traits including morphology, behavior, and territory attributes. The intensity of sexual selection may be particularly high in lek-mating species because male mating success is often strongly skewed, where a subset of males receive the majority of copulations (Bradbury and Gibson 1983; Wiley 1991). Lek-mating is a rare mating system, but is found in a variety of taxa, including insects, birds, and mammals (Bradbury 1981; Höglund and Alatalo 1995). In lek-mating species, aggregated males perform displays at a communal lek site (Wiley 1991). Females visit lek sites to copulations, and may obtain indirect benefits such as good genes for offspring or possibly direct benefits such as reductions in copulation disturbance, transmittal of parasites, predation risk or the cost of searching for a mate (Reynolds and Gross 1990; Gratson et al. 1991; Höglund et al. 1992; but see Sæther et al. 1999). In most cases, females do not obtain food or nesting sites, so they must select mates based on male traits alone.

Three important factors affecting male mating success in lek-mating birds include morphology, behavior, and territorial position (Balmford 1991; Fiske et al. 1998). In lek-mating greater sage-grouse (*Centrocercus urophasianus*), females choose males based on their display characteristics, but aggressive behaviors, territory position and physical size have little or variable effects on female choice (Gibson and Bradbury 1985; Gibson et al. 1991; Gibson 1996; Spurrier et al. 1994). In black grouse (*Tetrao tetrix*), univariate analyses indicate that successful males are heavier, have larger combs (fleshy area above the eye), invest more in display and aggressive behaviors, and hold territories closer to the center of the lek than unsuccessful males (Alatalo et al. 1991; Alatalo et al. 1996b; Höglund et al. 1997; Rintamäki et al. 2000). However, male traits may be correlated, and some male traits may be non-significant predictors of mating success in multifactor analyses (Höglund and Alatalo 1995; Alatalo et al. 1996b).

If male traits are honest signals of male quality, they should be costly to produce or maintain (Wiley 1991; Jennions et al. 2001). Steroid hormones, such as testosterone, may be the proximate mechanism that mediates variation in male traits. If high testosterone levels enhance male traits at the cost of suppressing the immune system (Mougeot et al. 2004; Redpath et al. 2006), elaborate male traits should be correlated with reduced survival (natural vs sexual selection trade-off; Jennions et al. 2001). On the other hand, males may vary in their expression

of sexually selected traits relative to their ability to bear the costs of producing the trait (Jennions et al. 2001). In this case, there should be positive correlations among condition, male traits and survival. However, the links among male traits, mating success and survival are usually not examined in concert.

In this field study, we examined how male mating success correlates with male traits and survival in greater prairie-chickens (*Tympanuchus cupido*), a lek-mating species of prairie grouse. We improve on past work in three ways: 1) We describe the first quantitative field study of female mate choice in greater prairie-chickens; 2) We examine the survival consequences of sexually-selected traits; and 3) We present a new application of multinomial discrete choice (MDC) models to studies of mate choice. Past studies of the reproductive behavior of greater prairie-chickens have been qualitative only (Ballard and Robel 1974; Hamerstrom and Hamerstrom 1973; Robel 1966, 1970; Robel and Ballard 1974). In these studies, aggressive males in the center of the lek tended to receive more copulations. The roles of morphology and age on male mating success have not been addressed in previous studies of prairie grouse. We also determined if the consequence of increased mating success was reduced survival. Links among male traits, mating success and survival have been examined in one other lek-mating species but was limited to males greater than two years old (Alatalo et al. 1991). MDC models have not been used previously in studies of mate choice. This statistical method improves upon logistic regression and other procedures because mating success is not assumed to be normally distributed among males and it controls for pseudoreplication among males. The objectives of our study were threefold: 1) to quantify mating skew among male greater prairie-chickens, 2) to investigate morphological, behavioral and territorial correlates of male mating success simultaneously using MDC models, and 3) to examine the effects of male mating success on annual survival.

Methods

Overview

We observed greater prairie-chickens at lek sites between mid-March and mid-May, 2003-2006. All leks were located on cattle-grazed pastures in Riley and Geary Counties in northeast Kansas, USA (39° 05'N, 96° 34'W). Three leks were observed in 2003, and we

expanded our sampling effort to four leks in 2004-2006. In this study, we restricted our mate choice analyses to observations from three leks in 2003, four leks in 2004 and two leks in 2005 due to manipulations at the other leks for a separate study. Leks were visited in 2006 to determine annual return rates only.

Trapping and morphometrics

Birds were trapped at lek sites using walk-in funnel traps (Hamerstrom and Hamerstrom 1973, Toepfer et al. 1987). Males were given a unique combination of colored leg bands and tail markings using non-toxic permanent markers to aid in individual identification. Seven morphometric measurements were recorded during handling: body mass (± 1 g), four linear measurements of body size (lengths of tarsus plus the longest toe, keel, wing, tail; ± 1 mm for all), and two sexually dimorphic ornaments (comb area, measured as length x height, ± 1 mm²; pinnae length, feathers behind head erected during display, ± 1 mm). To minimize measurement variation between observers, one observer (JKN) measured >90% of males. Principal components analysis was not performed on morphometric data because these variables were weakly correlated ($|r| < 0.35$). We determined age-class as second-year (SY) or after-second-year (ASY) from the shape, coloration and wear of the outermost two primaries (numbers 9 and 10; Schroeder and Robb 1993). In SY birds, the outer primaries are retained from juvenile plumage, appear tapered with light brown dots on the leading edge extending to the tip, and contrast in coloration and wear with primaries 1 through 8. The outer two primaries of ASY birds have truncate tips, light brown dots on the leading edge that terminate > 2 cm from the tip, and do not contrast with the inner primaries.

Behavioral observations

Male greater prairie-chickens perform ritualized courtship and territorial behaviors on small territories at lek sites (Schroeder and Robb 1993). Courtship displays consist of rapid foot-stomping with tail fanning and raising of the pinnae feathers above the head. Displays culminate with the inflation of air sacs, a low 3-note 'boom' vocalization, and flicking of the tail and wing feathers. Flutter-jumps are a courtship display that consists of short flights (~ 3 m) and a 'clucking' vocalization, and are used to attract females when they are near the lek or on another

male's territory. Display behaviors are performed by unaccompanied males or can be directed at females. Territorial boundary disputes consist of two males facing each other at the territory boundary, frequently exchanging dry 'neh' calls. Fights often escalate to physical combat with males striking or pulling feathers from each other with their wings, beaks and feet. Aggressive behavior is easily distinguished from display behavior, because aggressive behavior is directed at another male and causes a reaction in the adjacent male. Males rarely boom during aggressive behavior.

Blinds were placed ~6 m from the edge of each lek. Grid stakes were placed at 6-m intervals and did not interfere with the movement of males or females. To control for the possible effects of observer, time of day and weather, observers were rotated among leks, the order that males were observed was varied systematically, and all observations were conducted during the peak of activity (30 minutes before sunrise to 3 hours after sunrise) on days without rainfall. Males were observed every other day on average to quantify display and aggressive behaviors. The behavior of individual males was recorded with continuous 10-min focal observations. Time spent in each of three main behavior categories (display, fighting, other) was calculated. Other behaviors included preening, foraging and roosting. Tallies were taken of the number of fights, males approached, boom vocalizations, and flutter-jump displays. Female presence or absence on the lek during the observation period was recorded for every focal observation.

Position of males relative to grid stakes and copulations were recorded during focal observations and periodic scans of the lek. Territorial positions of males were only recorded if associated with a boom vocalization or a territorial boundary dispute. When a pair attempts to copulate, adjacent males may attempt to disrupt the copulation. We determined that a copulation attempt was successful if females vigorously shook their wings and body feathers, preened their vent and departed the lek shortly after the copulation (Schroeder and Robb 1993). After unsuccessful copulations, females did not shake, remained on the lek, and continued to solicit males.

Statistical analyses

Statistical analyses were conducted in JMP IN (Ver. 4.0.4, SAS Institute, 2001), except where otherwise noted. Sample sizes varied among analyses because it was not possible to measure every attribute for all males. Descriptive statistics are presented as $\bar{x} \pm 1$ SD unless otherwise indicated. Some males survived between years and multiple records were included in the descriptive statistics of morphology, behavior and territory attributes. Our conclusions did not change if we selected one record per male at random (results not shown).

Lambda values of male mating skew allows the intensity of sexual selection to be quantified and compared across leks regardless of lek size (Kokko and Lindström 1997). This index varies from 0 to 1 where $\lambda = 0$ indicates random mating and $\lambda = 1$ indicates one male obtaining all the matings. Lambda values for male mating skew were calculated using Skew Calculator 2003 PC (Nonacs 2003). Mean skew per lek was calculated with one exception: lambda could not be calculated at Poole lek in 2004 because only one copulation was observed. The only value presented for the Poole lek is from 2005.

The six behavioral variables (% time displaying, % time fighting, and number of boom vocalizations, flutter-jump displays, fights, and males approached) were correlated. Eleven of 15 pairwise correlations were statistically significant ($|r| > 0.44$; $P < 0.01$). Thus, we used principal components analysis (PCA) to obtain two principal components of behavior that were statistically independent of each other. The Kaiser criterion (eigenvalues ≥ 1) was used to select the number of principal components to retain for use in our analysis (Kaiser 1960). Female presence has a large effect on male behavior in other species of lek-mating grouse (Wiley 1991; Höglund et al. 1997). To minimize variation among focal observations and to control for female presence, principal component scores were averaged separately for each male for observations when females were present or absent. Changes in behavior with respect to female presence were analyzed using a matched pairs analysis.

Territorial positions of males were plotted in ArcView (Ver. 3.3; Environmental Systems Research Institute, Inc., St. Charles, MO). Using the Animal Movement extension (Hooge et al. 1999), we obtained 95% kernel estimates of territory size from positions of each male and lek size from the pooled positions of all males (± 0.1 m²). Centroids of the 95% kernel estimates were determined using the XTools extension (Delaune 2003). Distance to lek center was defined

as the distance between the centroid of each males' range to the centroid of the entire lek (± 0.1 m).

Annual survival was estimated from return rates of male prairie-chickens to lek sites. Return rates are the product of multiple probabilities including: true survival (S), site-fidelity (F), and encounter rate (p) (Sandercock 2006). Our data indicated that F and p were close to unity (see Results), and we interpret return rates as estimates of true survival for male greater prairie-chickens. The SE of survival was calculated using the formula:

$$SE(\hat{S}) = \sqrt{\frac{\hat{S}(1 - \hat{S})}{N}}$$

where \hat{S} = annual survival and N = sample size of males. Logistic regression was used to analyze annual survival rates. Predictor variables included number of successful copulations received, mass, comb size, aggressive and display behavior when females were present and distance to the lek center.

The discrete choice model

Studies correlating mating success with male traits are complicated in lek-mating species because male mating success is highly skewed and males may be observed in multiple years. Previous studies have often used Pearson correlations or multiple regression to examine the effects of male traits on mating success (Höglund and Lundberg 1987; Hill 1991; Shorey 2002). Data on mating success among males in a lek frequently violate both the assumptions of normality and independence that underlie correlation and regression analyses. These problems can be avoided by using logistic regression to compare unsuccessful (0 copulations) and successful males (≥ 1 copulation; Gibson and Bradbury 1985; Höglund et al. 1997). However, use of a binomial response variable reduces the power to detect differences because males that received one or multiple copulations are pooled, and it still does not address the lack of independence in responses among males in a lek. An additional complication is that some males may be observed in more than one year, leading to possible pseudoreplication (Hurlbert 1984). Discrete choice models provide a solution to these issues by testing how an individual chooses among a defined set of choices (Buskirk and Millspaugh 2006; Cooper and Millspaugh 1999;

Moore and Swihart 2006). Discrete choice models use multinomial distributions to describe how females choose a mate from among a group of males in a lek. Each copulating female increases the copulation count of exactly one male, so that features such as skewed mating success among males and dependence among males' counts arise naturally from the model.

Three assumptions of the discrete choice model include: choices are equally accessible to the individual, the individual gains a 'benefit' from making the choice, and the 'benefit' is a function of the attributes of the resource (*sensu* 'satisfaction' in Cooper and Millsbaugh 1999). These three assumptions are likely to be met with respect to lek-mating prairie-chickens. First, forced copulations do not occur, and females have the ability to choose any male on a lek regardless of previous matings by that male. Moreover, there is no evidence that male prairie-chickens will refuse to mate with a soliciting female although this may be an issue for other lek-mating birds (Sæther et al. 2001). Second, females may gain indirect benefits of good genes for their offspring and possibly direct benefits such as avoiding harassment or reduced search costs (Reynolds and Gross 1990). Last, female benefits are presumably a function of male traits such as morphology, behavior or territory position.

Correlates of female choice were analyzed using a conditional logit multinomial discrete choice model (Proc MDC, SAS Ver. 9.1, SAS Institute 2003). The utility of a choice (U) in benefitting the individual is modeled as:

$$U_{ij} = x_{1,ij}\beta_1 + x_{2,ij}\beta_2 + \dots + x_{n,ij}\beta_n + \epsilon_j$$

where female i chooses among males j using characteristics of the choice j . β_n is the slope coefficient for explanatory variables, and ϵ is the error term. In this study, each lek represents a different set of choices (males) from which the females choose, which are represented in the model by different sets of values for the characteristics, x_1, \dots, x_n .

Each successful copulation was considered an independent choice of a female among the males at a lek. Studies of other lek-mating grouse indicate that females may copy choices of prior-mating females (Gibson et al. 1991; Gibson 1996; but see Spurrier 1994). To test for a possible role of female mate choice copying in greater prairie-chickens, we modified the best fit MDC model ($\Delta AIC = 0$) to examine correlates of female choice with respect to female mating order (a = first-mated or only females to copulate on a given day; b = females that copulated subsequently). We partitioned the model as follows:

$$\hat{U}_{ij} = x_{1_a}\beta_{1_a} + x_{2_a}\beta_{2_a} + \dots + x_{n_a}\beta_{n_a} + x_{1_b}\beta_{1_b} + x_{2_b}\beta_{2_b} + \dots + x_{n_b}\beta_{n_b} + \epsilon_j$$

where x_{1_a} and x_{1_b} are the contributions of x_{1_i} of first- and second-mated females, respectively. The resulting model AIC was compared to the best fit MDC model to determine model support. The paired estimates (x_{1_a} and x_{1_b} , for example) were also compared using a Z-statistic to determine if first- and second-mated females used different male traits to choose a mate.

In general, female prairie-chickens visited lek sites for short periods, were rarely available for capture, and a majority of the copulations were with unbanded females. However, we expected pseudoreplication among females to be low for three reasons. First, behavioral and genetic data indicate that in lek-mating species of grouse, females typically copulate only once to fertilize the entire clutch and do not copulate again unless to fertilize a re-nesting attempt (Alatalo et al. 1996a; Semple et al. 2001). Second, we included only successful copulations in our analyses. Last, annual survival of females is low (51-59%, Hamerstrom and Hamerstrom 1973; 33%, Chapter 4) and turnover of females was expected to be high.

In the global discrete choice model, male characteristics hypothesized to be linked to female choice included four behavioral indices (PC scores of display and aggression with and without females), five measurements of body size (mass, tarsus, keel, wing, and tail), two ornaments used in display (comb and pinnae), age class (SY or ASY, coded as 0 and 1), and two territory measurements (distance to lek center and size). Morphometric and territory measurements were standardized before analysis ($\bar{x} = 0$, $SD = 1$) so slope coefficients would be directly comparable with our PC-scores for components of behavior. Backward elimination was used to simplify the model, and the resulting models were evaluated using an information-theoretic approach (Burnham and Anderson 2002). Additional models where variables were added and subtracted from the minimum-AIC model were also considered. In all, 51 models were analyzed. Akaike weights (w_i), model-averaged estimates ($\hat{\theta}_a$), and weighted unconditional standard error [$se(\hat{\theta}_a)$] were calculated using formulae in Burnham and Anderson (2002; eqns. 4.1 and 4.9).

Results

Copulations observed

Over three years and 263 mornings of observations, 108 males were observed at four lek sites of greater prairie-chickens (9 lek-years). On average, 2.3 ± 2.9 females visited a lek each morning (range 0-18, $n = 263$ mornings). A majority of copulation attempts were unsuccessful (55.5% of 191 copulation attempts). Therefore, successful copulations were relatively infrequent events occurring at an average rate of 9.6 ± 7.5 successful copulations/lek/season. Lek size varied slightly (10-14 territorial males per lek), but male attendance was consistently high during the observation period with $92.4 \pm 14.6\%$ of all territorial males attending per day ($n = 263$ mornings). Male mating success was strongly skewed at each lek site (Figure 2.1). Lambda values for male mating skew ranged from 0.38-0.90 with a mean of 0.62 ± 0.17 (Figure 2.1). Overall, 18.5% of males obtained 87.2% of all successful copulations ($n = 108$ males; 85 copulations).

Of 85 successful copulations, 84.7% involved unbanded females. Of 10 banded females observed to copulate, eight females copulated once and two females mated multiple times. One female copulated with two different males with a one-month interval between copulations. The timing of the second copulation was consistent with a re-nesting attempt. The second female copulated successfully with the same male three times in the span of four days. We included only the first copulations in the MDC analyses.

Morphology

Of the 108 males observed over three years, 67 were captured and banded in the year of the observation, 13 were recaptured in years after initial capture, 14 were resighted only after initial capture, and 14 were unbanded. Thus, 80 handling records from 67 birds were used to describe morphometrics. Most morphological characters showed little variability ($CV < 9\%$; Table 2.1). Excluding comb size, the mean coefficient of variation in morphological characters was $4.8 \pm 2.3\%$. Comb size exhibited the most variability, with a coefficient of variation over 3 times greater than other morphological characters (Table 2.1).

Behavioral attributes

In total, 912 10-minute focal observations were conducted with 8.4 ± 3.3 observations per male each year ($n = 108$ males). Behavior showed greater variability among males ($CV > 30\%$) than morphological traits ($CV < 10\%$; Table 2.1). The first and second principal components of behaviors (PC1 and PC2) were extracted from a principal components analysis of six behavioral traits. Together, PC1 and PC2 explained 74.8% of the variance in male behavior (Table 2.2). Loadings on PC1 were high and positive for number of booms and time in display but negative for time in fights, so we interpreted PC1 as an index of ‘display’ behavior. Loadings on PC2 were high and positive for number of fights and number of males engaged, so we considered PC2 an index of ‘aggressive’ behavior. In a matched pairs analysis, both male display and aggression were greater if females were present on the lek ($t_{98} < -9.1$, $P < 0.001$).

Territory attributes

For nine lek-years, a total of 911 ± 211 male positions per year per lek were obtained. The 95% kernel estimates of lek size ranged from 0.10 to 0.27 ha with a mean of 0.15 ± 0.06 ha ($n = 9$ lek-years). The four leks were located on average 4.0 ± 2.0 km from each other (range 1.0 - 7.3 km). For 108 males, 74.6 ± 34.8 positions per male per year were obtained. Males showed strong fidelity to lek territories within a breeding season, and territory size averaged 385.3 ± 251.9 m² (range = 107.9 - 2078.1 m²). The two largest territories (1250.2 and 2078.1 m²) were due to peripheral males shifting territorial positions around the edge of the lek during the mating season. Male territories were located on average 13.1 ± 6.1 m from the center of the lek. Territory attributes had coefficients of variation similar to behavioral traits (46-65%, Table 2.1).

Results of MDC model

To be included in the multinomial discrete choice (MDC) analysis, males had to have a complete set of explanatory variables (behavioral, morphological and territorial measurements). This analysis was limited to 62 successful copulations among 20 successful and 43 unsuccessful males. Of the 51 models considered, 17 models had ΔAIC -values ≤ 2 (Table 2.3). Male mating success was consistently linked to behavior and body mass as indicated by the inclusion of these variables in all the minimum-AIC models and by their large slope coefficients ($\beta > 0.55$,

Figure 2.2). Male behavior when females were present was the strongest predictor of mating success, but behavior when females were absent was also important. Male mating success increased if males were more aggressive ($\beta = 0.80$) but displayed less ($\beta = -0.80$) when females were absent. Of the seven morphological traits, body mass ($\beta = 0.55$) and comb size ($\beta = 0.24$) made the largest contributions toward mating success, but the magnitude of slope coefficients were less than half of behavior (Figure 2.2). Of the two territorial metrics examined, neither distance from the lek center nor territory size correlated with mating success (Figure 2.2).

Female mate choice copying

Opportunities for mate-choice copying among female prairie-chickens were limited. Of the 57 morning observation periods when successful copulations occurred, only one female copulated on 40 mornings (70.2%), but multiple females were observed copulating on 17 mornings (29.8%). Of the subset of mornings where multiple females copulated, two or more females copulated with the same male on nine mornings (52.9%) and with different males on eight mornings (47.1%). At most, only 15 of the 85 successful copulations observed (17.6%) could be the result of mate choice copying because they involved a female mating with a male that had mated previously that same morning.

When the best fit MDC model ($\Delta AIC = 0$) was compared to a model that partitioned female choice between first- and subsequently-mating females, the partitioned model had a $\Delta AIC = 8.6$ and a $w_i < 0.01$. Thus, there is little evidence that females are copying the mating choices of previously mated females. Moreover, a comparison of the parameter estimates indicated that first- and subsequently-mated females were using the same cues to select a mate (estimates considered: mass, tail length, comb size and display and aggression with and without females; $Z < 1.63$; $P > 0.05$).

Annual survival

Return rates were determined for 64 newly-banded males and 31 returning males for a total of 95 bird-years. Return rates are the product of true survival (S), site-fidelity (F), and encounter rate (p). The encounter rate of male prairie-chickens was at unity ($p = 1$) because males were readily observed at leks and no males were overlooked for an intervening year

between breeding seasons (i.e., bird banded at time t , not seen in $t + 1$, but seen again at or after $t + 2$). In our study area, only 1 of 108 males (0.9%) was detected at more than one lek. This bird did not establish a territory on the second lek he visited. From these observations, we conclude that once males established a territory on a lek, they were unlikely to emigrate to a new lek, and that F was also close to 1. Therefore, return rates are effectively an estimate of true survival for male greater prairie-chickens in our study population.

Annual survival of males was $55.8 \pm 5.1\%$ (\pm SE; $n = 95$ bird-years) and was not affected by number of successful copulations received ($\chi^2 = 0.27$, $P = 0.60$, $n = 95$ bird-years), mass at capture ($\chi^2 = 0.98$, $P = 0.37$, $n = 80$), comb size ($\chi^2 = 0.47$, $P = 0.49$, $n = 75$), display behavior when females were present ($\chi^2 = 0.00$, $P = 1.00$, $n = 88$), aggressive behavior when females were present ($\chi^2 = 0.07$, $P = 0.79$, $n = 88$), or territory distance to lek center ($\chi^2 = 0.10$, $P = 0.75$, $n = 94$). Moreover, annual survival of males did not differ between age-classes (SY = $57.8 \pm 7.4\%$, $n = 45$; ASY = $55.1 \pm 7.1\%$, $n = 49$; Fisher's Exact $P = 0.84$).

Discussion

In this three-year study of greater prairie-chickens, male mating success was strongly skewed, similar to other lek-mating grouse (Wiley 1991; Höglund et al. 1997). This is the first study to calculate λ -values of male mating skew for greater prairie-chickens. Behavioral traits were better predictors of male mating success than morphological and territorial attributes, and showed the most variability among males. Remarkably, male mating success did not correlate with survival, suggesting that male traits preferred by females (i.e., behavior) may not be costly.

The importance of behavioral traits in determining male mating success may be a general pattern that is common to lek-mating species of birds. Our PC scores (PC1=Display, PC2=Aggression) were similar to a previous study of the lek-mating white-bearded manakin (*Manacus manacus*, Shorey 2002). In greater prairie-chickens, both display and aggressive behavior were important predictors of male mating success, contrary to previous studies of lek-mating birds where aggressive behavior did not correlate with mating success (Gibson and Bradbury 1985; Höglund and Lundberg 1987; Gibson et al. 1991; Alatalo et al. 1996b; Shorey 2002). Aggression may be particularly important in male-male competition if males establish dominance hierarchies at lek sites (Alatalo et al. 1991; Höglund et al. 1997), but the importance

of male-male competition might vary among species. In the absence of females, aggressive and display behavior of male prairie-chickens were positively and negatively correlated with mating success, respectively. We conclude that males that fight more but display less may be establishing dominance relationships in the absence of females, which females subsequently may use to select a mate (Bradbury and Gibson 1983).

Plumage characters had low predictive power for determining male mating success in prairie-chickens, with the exception of comb size (Gratson et al. 1991). Comb size was the only morphological trait with high variation ($CV = 26\%$) and it was included in 71% of the 17 models with $\Delta AIC \leq 2$. The low predictive power of most plumage characters in mate choice may be a general feature of galliform birds (Alatalo et al. 1991; Beani and Dessi-Fulgheri 1995; Rintamäki et al. 1998; Hagelin and Ligon 2001; Parker and Ligon 2003). We propose three possible explanations. First, male plumage ornamentation is a primitive character in Galliformes with the cryptic female plumage being a derived estrogen-dependent character (Kimball and Ligon 1999). Given that female plumage is a derived character, natural selection for cryptically colored females may be more intense than sexual selection for more elaborate male plumage. Second, natural selection may be relatively more important than sexual selection in determining the plumage of grouse (Bleiweiss 1997). Grouse living in similar habitats have more similar plumage than those with similar mating systems (Drovetski et al. 2006). Mating system correlates with sexual size dimorphism (Wiley 1974; Payne 1984), but not the degree of sexual plumage dichromatism in grouse (Drovetski et al. 2006). Lastly, low CV values may limit the statistical power to detect female choice.

Previous studies of lek-mating birds have frequently emphasized a link between territory position and male mating success (Höglund and Lundberg 1987; Gratson et al. 1991; Alatalo et al. 1996b; Shorey 2002). We did not find territory size or position to be an important predictor of male mating success, despite $CVs > 46\%$ and contrary to previous studies of prairie grouse (Robel 1966; Robel and Ballard 1974; Gratson et al. 1991). Our results support the hypothesis that direct female choice (via behavior) may be more important than indirect female choice (via territory; Sæther et al. 2005).

An unexpected result of our study was that male survival was not related to any of the traits examined (mating success, morphological, behavioral, or territorial traits), despite strong

evidence from previous studies showing that male characteristics correlate with survival due to condition-dependence or trade-offs between natural and sexual selection (Jennions et al. 2001). Yearling males had slightly higher survival than adult birds, but this difference was not significant. A trend for higher survival among yearlings than adults has been reported in previous studies of greater (Hamerstrom and Hamerstrom 1973) and lesser prairie-chickens (Hagen et al. 2005). Future work should determine if counteracting selective pressures may differentially affect males based on their mating success or behavior. For example, variation in predation among successful and unsuccessful males could arise from male position or dominance on and off the lek. If male traits are energetically costly (Vehrencamp et al. 1989), successful males may be better foragers than less successful males, and are able to off-set the costs of enhanced traits with greater food intake. Parasites have been shown to vary with mating success and male traits (Höglund et al. 1992), and may affect survival. In these examples, apparent survival would not vary with mating success due to multiple or compensatory selective pressures reducing the observed variation in survival.

Given the high skew in male mating success, the lek paradox asks the question “Why have male traits not been selected to fixation?” We have shown that the variability of morphological traits is much lower than the variation in either behavioral or territorial attributes. In the case of body size and male ornaments, such as the pinnae feathers, low CV (mean < 5%) may suggest that these traits have been under strong selection in the past. Territorial traits had high CV values, but were not used by females to select mates. Female greater prairie-chickens select males based on behavioral traits where adequate variation may still exist to base their decision.

Five hypotheses have been proposed as solutions to the lek paradox. One hypothesis is that the high skew in male mating success observed on a lek differs from the mating skew observed when examining the paternity in the offspring due to alternative mating tactics (Lanctot et al. 1997; Foster and Kokko 2006). This explanation may not apply to lek-mating grouse, because only low rates of multiple paternity have been reported (0-20% of broods) and copulations observed on the lek match the paternity of the brood (Alatalo et al. 1996a; Semple et al. 2001). Second, male mate choice or female-female aggression may maintain variability on leks if assortative mating occurs where high quality males mate with high quality females and

poor quality females mate with poor quality males (Rintamäki et al. 1998; Werner and Lotem 2006). This hypothesis is unlikely for greater prairie-chickens, because males never refused to copulate with a soliciting female, copulations were relatively rare events, and female-female aggression did not preclude multiple females from mating during the same morning. A third hypothesis states that if male-male competition was the sole determinant of skewed mating success, then the lack of female choice would negate the lek paradox (Beehler and Foster 1988; Höglund and Alatalo 1995). Seasonal mating success positively correlated with more aggressive males that displayed less frequently when females were not present, suggesting that male-male competition is important in greater prairie-chickens. However, female choice is also important because we found no evidence of forced copulations, and mating success is correlated with display behavior when females are present. Fourth, females may be obtaining direct benefits, such as reduced mate search costs (Reynolds and Gross 1990). Since most females in our population were unbanded, we are unable to evaluate the cost of mate searching for females. Future tests of this hypothesis will require better information on female movements and rates of lek visitation. A fifth hypothesis states that the lek paradox can be maintained with condition-dependence of sexually selected traits and high genetic variance in condition (Pomiankowski and Møller 1995; Turner 1995; Rowe and Houle 1996; Parker and Garant 2004). Our study shows that behavior is more variable and preferred by females more strongly than male body mass. If behavior proves to have high genetic variance, the genetic variance hypothesis may be the best explanation for the lek paradox in greater prairie-chickens.

In the future, research on the correlates of male mating success in lek-mating species will benefit from utilizing MDC models, quantifying the display and aggressive components of behavior, and determining the underlying physiological mechanisms mediating variation in male mating success and survival. MDC models are unique among statistical methods in that it directly models the process of female mate choice. In addition, MDC models control for pseudoreplication of males observed in multiple years and eliminates the problem of the non-normal distribution of male mating success. Past studies link male mating success of lek-mating species to components of display, but have not considered the role of aggressive behaviors. We showed the importance of aggression in determining male mating success, but were unable to examine the proximate causes. Variation in steroid hormones, such as testosterone, may underlie

the variation in male traits and survival. Testosterone has been linked to aggression, comb size and increased male mating success (Zuk et al 1995a,b; Alatalo et al. 1996b). In addition, male grouse with higher testosterone levels can have higher parasite loads, which may ultimately decrease survival (Redpath et al. 2006; Seivwright et al. 2005). A better understanding of the interactions among testosterone, parasite loads, mating success and survival in a lek-mating species would link the proximate mechanisms and ultimate consequences of factors mediating male mating success.

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Table 2.1 – Descriptive statistics for traits of male greater prairie-chickens observed at four leks near Manhattan, northeast Kansas during 2003-2005.

Category	Male Traits	Mean	SD	N	%CV
Morphology	Mass (g)	1034	60	80	5.8
	Tarsus (mm)	107	3	79	3.0
	Tail (mm)	96	5	78	5.3
	Keel (mm)	121	10	78	8.5
	Wing (mm)	230	4	80	1.9
	Pinnae (mm)	77	4	78	4.8
	Comb (mm ²)	229	59	75	25.7
Behavior ^a	Time displaying (%)	51	15	108	30.2
	Time fighting (%)	31	15	108	47.4
	Boom vocalizations (#)	30	10	108	38.8
	Flutter jump displays (#)	3	3	108	99.9
	Fights (#)	4	2	108	41.5
	Males engaged (#)	2	1	108	33.2
Territory	Territory Size (m ²)	385	252	108	65.4
	Distance to lek center (m)	13	6	108	46.4

^a Quantified during 10-min focal observations of males.

Table 2.2 – Eigenvectors of a principal component analysis of male behavior from 912 ten-minute focal observations of 108 male greater prairie-chickens on 2-4 leks per year. Observations were conducted during the breeding season (mid-March through mid-May) near Manhattan in northeast Kansas during 2003-2005. Principal component loadings > 0.4 are in bold.

Behavior	PC1 (Display)	PC2 (Aggression)
Percent time displaying	0.52	0.33
Percent time fighting	-0.52	0.08
Number of boom vocalizations	0.47	0.37
Number of flutter jump displays	0.34	0.06
Number of fights	-0.25	0.62
Number of males engaged	-0.26	0.60
Eigenvalue	2.75	1.73
Percent of variance explained	45.9	28.9

Table 2.3 – Comparison of multinomial discrete choice models that had $\Delta AIC \leq 2$ examining the effects of male morphology, behavior and territory attributes on mating success. Data included 62 copulations among 20 successful and 43 unsuccessful males on four greater prairie-chicken leks between 2003-2005 in northeast Kansas. Model abbreviations are as follows: morphological variables (m = mass at capture; c = comb area; t = tail; k = keel; r = tarsus; p = pinnae; w = wing; g = age class); behavioral variables (D = display with females; A = aggression with females; d = display without females; a = aggression without females); and territory variables (l = distance to center of lek, - = no effect).

Model	Morphology	Behavior	Territory	Deviance	K (# parameters)	AIC	ΔAIC	w_i
1	mct	DAda	-	132.7	7	146.72	0.00	0.09
2	mctk	DAda	-	130.7	8	146.73	0.01	0.09
3	mk	DAda	-	134.8	6	146.77	0.05	0.09
4	mc	DAda	-	134.8	6	146.85	0.13	0.08
5	mck	DAda	-	133.0	7	146.99	0.27	0.08
6	mtk	DAda	-	133.2	7	147.16	0.44	0.07
7	mctr	DAda	-	131.3	8	147.29	0.57	0.07
8	mcr	DAda	-	133.4	7	147.39	0.67	0.06
9	mt	DAda	-	135.4	6	147.43	0.71	0.06
10	mctk	DAda	l	129.6	9	147.63	0.91	0.06
11	mk	DAda	l	133.9	7	147.93	1.21	0.05
12	mct	DAd	-	136.1	6	148.14	1.42	0.04
13	mp	DAda	-	136.3	6	148.27	1.55	0.04
14	mct	DAda	l	132.6	8	148.63	1.91	0.03
15	mctp	DAda	-	132.7	8	148.69	1.97	0.03
16	mctw	DAda	-	132.7	8	148.72	2.00	0.03
17	mctg	DAda	-	132.7	8	148.72	2.00	0.03

Figure 2.1 - Mating success of male greater prairie-chickens observed at four leks from 2003-2005 near Manhattan in northeast Kansas. Bars and whiskers represent means and SD. Lambda values are means per lek. Males are ranked on the x-axis based on the number of copulations they received.

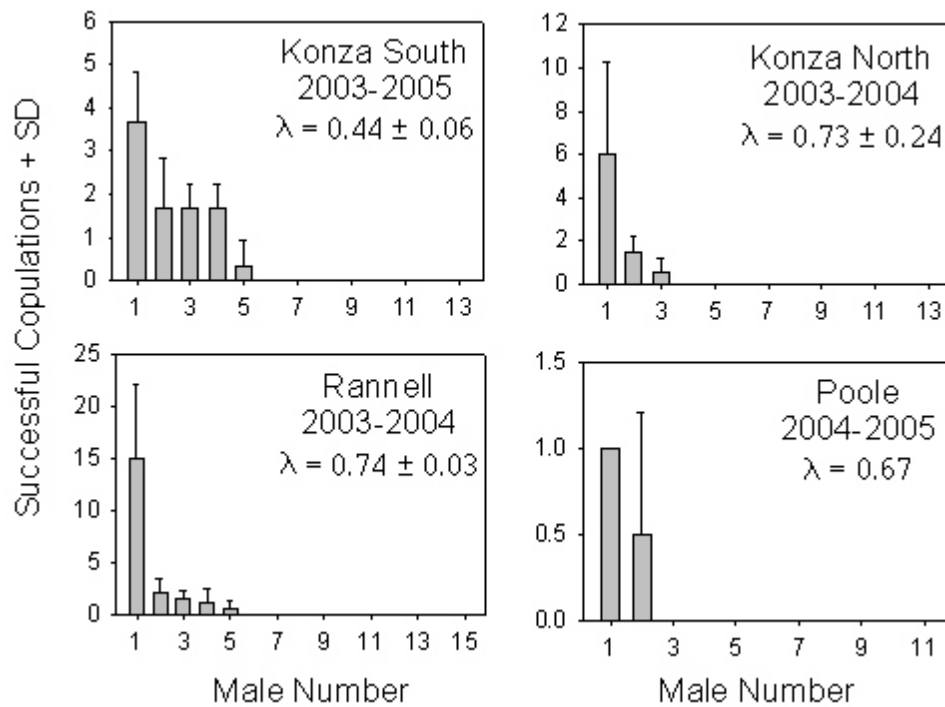
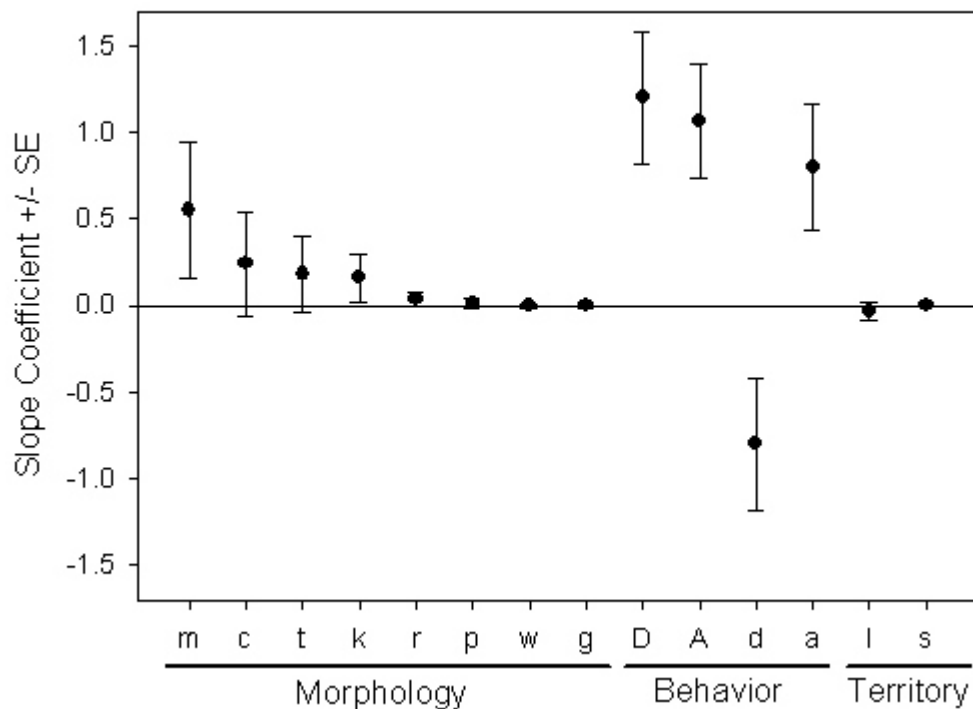


Figure 2.2 - Model-averaged estimates of the slope coefficients from a multinomial discrete choice model estimating the effect of male attributes on male mating success. Explanatory variables were standardized so slope estimates are directly comparable. Estimates of SE are unconditional and include uncertainty due to model selection. Data included 62 copulations among 20 successful and 43 unsuccessful male greater prairie-chickens from four leks between 2003-2005 near Manhattan in northeast Kansas. Abbreviations are as follows: morphological variables (m = mass at capture; c = comb area; t = tail; k = keel; r = tarsus; p = pinnae; w = wing; g = age class); behavioral variables (D = display with females; A = aggression with females; d = display without females; a = aggression without females); and territory variables (l = distance to center of lek, s = territory size).



CHAPTER 3 –

Testosterone: A Proximate Factor Mediating Mating Success in Male Greater Prairie-Chickens?

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Abstract

Testosterone (hereafter ‘T’) plays a key role in enhancing male breeding success, but T can also reduce immunocompetence and survival. Socially monogamous males with higher circulating T experience advantages during both female choice and male-male aggression. The role of T in lek-mating systems is largely unknown. Over three years, we quantified natural and experimental levels of T at five greater prairie-chicken (*Tympanuchus cupido*) leks with three objectives: 1) to quantify natural T variation in blood plasma and determine whether T levels are correlated with male traits and mating success; 2) to experimentally manipulate T to determine causal relationships between T and male traits and mating success; and 3) to analyze return rates to determine if increased T is costly. This project was one of the first manipulations of T in a lek-mating bird. Using a before-after control-impact (BACI) experimental design, changes in aggression, display behavior, territory attributes, mating success and survival were compared between 15 T-implanted males and 13 sham-implanted controls. Natural variation in T was a strong predictor of male mating success, but T was not related to any morphological, behavioral or territory traits considered. Comb area, tarsus length and distance to the center of the lek were also significant predictors of male mating success. Similar trends were found in the T-implant experiment: T-implanted males tended to gain more copulations than sham-implanted males, but the two groups did not vary with respect to their behavior or territory attributes. The odds of returning were 4.1 times higher in sham-implanted males than in T-implanted males, but return rates did not vary with respect to T. Future work should search for a link between T and male attributes because it is unlikely that females are assessing T levels directly. Possible sexually-

selected traits that may link T level to male mating success include parasite load, coloration of plumage and fleshy areas, and vocalizations.

Introduction

Life history trade-offs are ubiquitous in organismal biology but the proximate mechanisms driving variation in life history trade-offs are poorly understood (Ricklefs and Wikelski 2002). Steroid hormones may be one factor mediating life history trade-offs, such as the trade-off between mating and parental effort (Ketterson and Nolan 1999). Testosterone (hereafter ‘T’) plays a key role in enhancing male breeding effort and success. T-dependent traits should be honest signals of male quality, because T can have immunosuppressive effects (Folstad and Karter 1992, Ditchkoff et al. 2001) leading to a negative correlation between circulating T and annual survival (Ketterson and Nolan 1999). If T increases reproductive success but decreases survival, natural selection should confer an advantage to individuals who can balance these two fitness components.

Trade-offs between mating and parental effort create a continuum in avian mating systems including social monogamy, facultative polygyny, and promiscuity. In socially monogamous species of temperate songbirds, male T peaks during pair formation and drops during brood rearing (Ketterson et al. 1992, Ketterson and Nolan 1999). In facultatively polygynous species, males have high circulating T levels during pair formation and brood-rearing, leading to increased mating effort and reduced parental effort (Wingfield 1984). Lek-mating is a rare mating system found in a variety of taxa, including insects, birds, and mammals (Bradbury 1981). Lek mating is a type of mating system where males perform aggregated display at a lek site in which females visit to obtain copulations. Only females provide parental care, so T is predicted to remain high in lek-mating males during periods of territory establishment and female visitation (Wingfield et al. 1990). However, the available data from lek-mating grouse and manakins suggest that T is elevated only during the short period of peak female visitation to the lek (Alatalo et al. 1996b, Wikelski et al. 2003). In these two studies, sampling was limited to fewer than 30 individuals (Alatalo et al. 1996b, $n = 23$ males; Wikelski et al. 2003, $n \leq 27$ for each species). Larger sampling of T levels over the course of the breeding season are needed to confirm these predictions.

T may influence sexual selection through female choice and male-male competition. Because T peaks during periods of mating activity, T-mediated traits may be used by females to select a mate. Studies in galliformes have found T-dependent traits are often associated with female choice, such as large fleshy head combs (in red jungle fowl *Gallus gallus*, Zuk et al 1995a,b), high-quality territories (Moss et al. 1994), and more vigorous courtship displays (Mateos and Carranza 1999). Jungle fowl with larger combs were also more likely to attract multiple mates (Parker and Ligon 2003). T could also affect male fitness through male-male aggression. T-mediated male ornaments or behaviors often predict the winner of paired tests (Ligon et al. 1990, Hagelin 2002).

Several field studies in monogamous, territorial songbirds have used T-implants to determine experimentally how sex hormones influence male attributes, female choice, and male-male competition. In most studies, T-implanted males displayed more frequently and attracted more females than sham-implanted control males (Wingfield 1984, Ketterson et al. 1992, Hill et al. 1999). T manipulations have been conducted in one lek-mating passerine and three wild galliformes including one lek-mating grouse. T-implanted lek-mating golden-collared manakins (*Manacus vitellinus*) increase rates of several display behaviors compared to sham-implanted controls (Day et al. 2006). T-implanted male pheasants, with harem-defense polygyny, are more aggressive, have higher social rank, and increased frequency of male-male interactions (Briganti et al. 1999, Giudice and Ratti 2001). T-implanted males of the monogamous red grouse (*Lagopus l. scoticus*) had higher display rates and territory sizes compared to control males (Moss et al. 1994; Mougeot et al 2003a, b). Aggressive, display and territory attributes of T-implanted males of the lek-mating sharp-tailed grouse (*Tympanuchus phasianellus*) remained unchanged relative to unmanipulated control males, but lek attendance was higher for T-implanted males (Trobec and Oring 1972). These studies demonstrate that T may impact display rates, male-male aggression, and territory attendance of lek-mating grouse. Of the two manipulations of T in lek-mating birds, neither study examined mating success of T-implanted males relative to controls. No copulations were observed in the sharp-tailed grouse study (Trobec and Oring 1972), and the manakin study was conducted during the non-breeding season (Day et al. 2006).

Over three years, we examined how male traits, mating success and survival varies with natural and experimentally manipulated levels of T in male greater prairie-chickens (*T. cupido*), a

lek-mating bird. Our objectives were: 1) to quantify natural variation in T and determine whether T levels correlate with male traits and mating success; 2) to experimentally manipulate T to determine causal relationships between T and male traits and mating success; and 3) to analyze return rates to determine if increased T is costly. This project is one of the first experimental field studies of the interacting effects of T, male attributes, mating success and survival in a lek-mating bird. We predict that T will have a large impact on male aggression and mating success in lek-mating prairie-chickens, because peak T levels are significantly higher in lek-mating species as compared to monogamous species after controlling for phylogeny (Garamszegi et al. 2005).

Methods

We observed greater prairie-chickens at lek sites between mid-March and mid-May, 2004-2007. All leks were located on cattle-grazed pastures in Riley and Geary Counties in northeast Kansas, USA (39° 05'N, 96° 34'W). Four leks were observed in 2004-2005, and we expanded our sampling effort to five leks in 2006. In 2005-2006, a subset of males were given subcutaneous testosterone implants at two and four leks, respectively. Therefore, two leks were unmanipulated in 2005 and one in 2006. Leks were visited in 2007 to determine annual return rates only.

Trapping and Morphometrics

Birds were trapped at lek sites using walk-in funnel traps and drop nets (Hamerstrom and Hamerstrom 1973, Toepfer et al. 1987, Silvy et al. 1990, Schroeder and Braun 1991). Males were given a unique combination of colored leg bands and tail markings using non-toxic permanent markers to aid in individual identification. Seven morphometric measurements were recorded during handling: body mass (± 1 g), three linear measurements of body size (lengths of tarsus plus the longest toe, wing, tail; ± 1 mm for all), and two sexually dimorphic ornaments (comb area, measured as length x height, ± 1 mm²; pinnae length, feathers behind head erected during display, ± 1 mm). Principal components analysis was not performed on morphometric data because the seven variables were weakly correlated ($|r| < 0.35$). We determined age-class as second-year (SY) or after-second-year (ASY) from the shape, coloration and wear of the

outermost two primaries (numbers 9 and 10; Schroeder and Robb 1993). In SY birds, the outer primaries are retained from juvenile plumage, appear tapered with light brown dots on the leading edge extending to the tip, and contrast in coloration and wear with primaries 1 through 8. The outer two primaries of ASY birds have truncate tips, light brown dots on the leading edge that terminate > 2 cm from the tip, and do not contrast with the inner primaries.

Behavioral observations

Male greater prairie-chickens perform ritualized courtship and territorial behaviors on small territories at lek sites (Schroeder and Robb 1993). Courtship displays consist of rapid foot-stomping with tail fanning and raising of the pinnae feathers above the head. Displays culminate with the inflation of air sacs, a low 3-note 'boom' vocalization, and flicking of the tail and wing feathers. Flutter-jumps are a courtship display that consists of short flights (~3 m) and a 'clucking' vocalization, and are used to attract females when they are near the lek or on another male's territory. Display behaviors are performed by unaccompanied males or can be directed at females. Territorial boundary disputes consist of two males facing each other at the territory boundary, frequently exchanging dry 'neh' calls. Fights often escalate to physical combat with males striking or pulling feathers from each other with their wings, beaks and feet. Aggressive behavior is easily distinguished from display behavior, because aggressive behavior is directed at another male and causes a reaction in the adjacent male. Males rarely boom during aggressive behavior.

Blinds were placed ~6 m from the edge of each lek. Grid stakes were placed at 6-m intervals and did not interfere with the movement of males or females. To control for the possible effects of observer, time of day and weather, observers were rotated among leks, the order that males were observed was varied systematically, and all observations were conducted during the peak of activity (30 minutes before sunrise to 3 hours after sunrise) on days without rainfall. Males were observed every other day on average to quantify display and aggressive behaviors. The behavior of individual males was recorded with continuous 10-min focal observations. Time spent in each of three main behavior categories (display, fighting, other) was calculated. Other behaviors included preening, foraging and roosting. Tallies were taken of the number of fights, males approached, boom vocalizations, and flutter-jump displays. Female

presence or absence on the lek during the observation period was recorded for every focal observation.

Position of males relative to grid stakes and copulations were recorded during focal observations and periodic scans of the lek. Territorial positions of males were only recorded if associated with a boom vocalization or a territorial boundary dispute. When a pair attempts to copulate, adjacent males frequently attempt to disrupt the copulation. We determined that a copulation attempt was successful if females vigorously shook their wings and body feathers, preened their vent and departed the lek shortly after the copulation (Schroeder and Robb 1993). After unsuccessful copulations, females did not shake, remained on the lek, and continued to solicit males.

Testosterone Sampling

Since handling stress can affect circulating T, traps were monitored from a blind to determine how long each male was in a trap. We collected a blood sample immediately following the bird's removal from the trap or drop net and placed the sample on wet ice. Within 3 hours, the blood sample was centrifuged at 14,000 rpm for 5 min to separate the blood plasma from the red blood cells. The blood plasma was collected with a pipette, transferred to a new tube, and frozen at -20 C until it could be transferred to -70 C freezer.

We measured plasma testosterone concentrations in each blood sample using a commercially available kit for salivary testosterone enzyme immunoassay (EIA; Cat. #1-2402, Salimetrics LLC, State College, Pennsylvania). We followed the Salimetrics protocol for the EIA. All plasma samples were diluted 1:10 with assay dilutant (e.g., 15 μ L of plasma into 135 μ L of assay dilutant) for our assays.

We conducted a standard assay validation including assessment of parallelism, recovery of exogenous testosterone, intra- and interassay precision, and assay sensitivity (Jeffcoate 1981, Grotjjan and Keel 1996, O'Fegan 2000), to confirm that testosterone concentrations in prairie-chicken plasma were measured accurately and precisely. Validation assays were conducted on two pooled plasma samples (low and high) where each pooled sample consisted of plasma from three individuals. Parallelism ensures the assay maintains linearity under dilution, and recovery of exogenous testosterone verifies accurate measurement throughout the working range of the

assay (Jeffcoate 1981). We used the low and high controls from the kit and analyzed them in each of the assays. Inter-assay variation was calculated from these two controls. We calculated intra-assay variation by averaging the coefficient of variation (CV) of replicate tubes from 20 randomly chosen samples.

Methodological validation confirmed that variation in T levels represented variation among the males sampled. Serial dilutions (1:1, 1:2, 1:4, 1:8, and 1:16) of two pooled plasma samples yielded a displacement curve that was parallel (test of equal slopes, all $P > 0.6$) to the standard testosterone curve. Mean recovery of exogenous testosterone (range 38.4-240 pg/mL; levels chosen to correspond with expected plasma testosterone levels from actual samples) added to low and high pooled plasma samples were $109.9 \pm 1.1\%$ (\pm SE, $n=6$) for the low and $108.4 \pm 0.7\%$ (\pm SE, $n = 6$) for the high. Acceptable recovery of exogenous testosterone (within 90–110%) and demonstration of parallelism suggested no sample matrix effects (Jeffcoate 1981, Grotjhan and Keel 1996, O'Fegan 2000). Inter-assay variation for six assays was 9.14% and average intra-assay variation was 4.68%. The sensitivity of this assay is 1.0 pg/mL. Estimates of plasma T concentration were \log_{10} -transformed to normalize the data.

Testosterone Implant Experiment

To test for a link between T and male behavior, we used a Before-After Control-Impact Design (BACI; Conquest 2000). Behavior, territory attributes and mating success were compared before and after experimental application of implants filled with testosterone or left empty. T-implants were used following protocols developed for red grouse (Trobec and Oring 1972, Moss et al. 1994, Mougeot et al. 2003b, 2004). T-implants were constructed from 35-mm lengths of silastic tubing (Dow Corning, inner diameter = 1.47 mm, outer diameter = 1.95 mm, sealed with silastic glue; 30 mg testosterone propionate, Sigma-Aldrich, St. Louis, MO). The sham-treatment consisted of insertion of an empty silastic tube. The implant was inserted subcutaneously between the skin and breast muscles and the incision covered with “liquid bandage” to prevent infection (Band-Aide brand). Previous studies in red grouse have shown that T in similar implants last up to 5-6 weeks (Moss et al. 1994, F. Mougeot pers. comm.).

T was implanted in unsuccessful males to raise their level of circulating T to that of successful males. Banding and behavioral observations beginning in mid-March were used to

determine which males were successful and which were unsuccessful. Males were considered *successful* if they held territories that were completely surrounded by other males, fought often with many adjacent males, and obtained copulations previously. Males were considered *unsuccessful* if they held territories at the edge of the lek (at least 25% of territory not shared with another male), engaged in few fights, and obtained no copulations prior to implant. Unsuccessful males were alternately assigned to the T or the sham treatment. Using drop nets, sham and unsuccessful males were recaptured during the 1st and 2nd week of April, just before the peak of female visitation. The effect of the treatment was monitored by comparing blood serum analyses, behavioral observations, and territory attributes of T- and sham-implanted males before and after the treatment. In addition, a subset of T-implanted males were recaptured in early May, their implants were removed, dried in a desiccator at room temperature for 24 h, and weighed to determine the amount of T remaining.

In a previous analysis (Chapter 2), aggression scores derived from a principal components analysis were significantly different between successful and unsuccessful males (PC2 in Table 3.1, difference in aggression scores = 2.20, $F_{1,25} = 24.1$, $P < 0.001$). If implants raise T levels of unsuccessful males to be comparable to successful males, we predict a similar magnitude of difference between the aggression of T-implanted unsuccessful males and the aggression of sham-implanted successful males. Given this variation and at least 8 males in each of the two groups, we can expect to detect a similar difference in aggression scores over 95% of the time (power analysis in JMP, Version 4.0).

Statistical analyses

Statistical analyses were conducted in JMP IN (Ver. 4.0.4, SAS Institute, 2001), except where otherwise noted. Sample sizes varied among analyses because it was not possible to measure every attribute for all males. Descriptive statistics are presented as $\bar{x} \pm 1$ SD unless otherwise indicated.

The six behavioral variables (% time displaying, % time fighting, and number of boom vocalizations, flutter-jump displays, fights, and males approached) were correlated. Eleven of 15 pairwise correlations were statistically significant ($|r| > 0.44$; $P < 0.01$). Thus, we used principal components analysis (PCA) to obtain two principal components of behavior that were

statistically independent of each other. The Kaiser criterion (eigenvalues ≥ 1) was used to select the number of principal components to retain for use in our analysis (Kaiser 1960). Two principal components were retained (Table 3.1). Female presence has a large effect on male behavior in other species of lek-mating grouse (Wiley 1991, Höglund et al. 1997). To minimize variation among focal observations and to control for female presence, principal component scores were averaged separately for each male for observations when females were present or absent.

Territorial positions of males were plotted in ArcView (Ver. 3.3; Environmental Systems Research Institute, Inc., St. Charles, MO). Using the Animal Movement extension (Hooge et al. 1999), we obtained 95% kernel estimates of territory size from positions of each male and lek size from the pooled positions of all males ($\pm 0.1 \text{ m}^2$). Centroids of the 95% kernel estimates were determined using the XTools extension (Delaune 2003). Distance to lek center was defined as the distance between the centroid of a male's territory to the centroid of the entire lek ($\pm 0.1 \text{ m}$).

Annual survival was estimated from return rates of male prairie-chickens to lek sites. Return rates are the product of multiple probabilities including: true survival (S), site-fidelity (F), and encounter rate (p ; Sandercock 2006). F and p were close to unity in our study population (Chapter 2), and we interpret return rates as estimates of true survival for male greater prairie-chickens.

Changes in behavior, territory attributes, mating success and survival were compared among T-implanted and sham-implanted males before and after treatment, and among unmanipulated unsuccessful and unmanipulated successful males before and after the mean implant date. To analyze behavior, PC scores were standardized by female presence. Mean PC score, SD and number of observations were calculated for each male before and after treatment. Effect sizes were calculated in MetaWin Ver. 2.0 (Rosenberg et al. 2000). Differences among T-implanted, sham-implanted, unmanipulated unsuccessful, and unmanipulated successful males were analyzed using a categorical fixed effects model in MetaWin. Q_B statistics were used to test for variation in the mean effect size between different categories of males (Gurevitch and Hedges 1993). Changes in territory size and distance to lek center were analyzed using a matched pairs analysis in JMP IN. Mating success and return rates were analyzed using a Fisher's Exact test in

JMP IN. The odds ratio of mating success were calculated using Proc Freq in SAS (Ver. 9.1, SAS Institute 2003).

The Discrete Choice Model

Studies correlating mating success with male traits are complicated in lek-mating species because male mating success is highly skewed and males may be observed in multiple years. We used multinomial discrete choice (MDC) models to describe how females choose a mate from among a group of males in a lek (Chapter 2). Correlates of female choice were analyzed using a conditional logit multinomial discrete choice model (Proc MDC, SAS Ver. 9.1). The utility of a choice (U) in benefitting the individual is modeled as:

$$U_{ij} = x_{1,ij}\beta_1 + x_{2,ij}\beta_2 + \dots + x_{n,ij}\beta_n + \epsilon_j$$

where female i chooses among males j using characteristics of the choice j , β_n is the slope coefficient for explanatory variables, and ϵ is the error term. In this study, each lek represents a different set of choices (males) from which the females choose, which are represented in the model by different sets of values for the characteristics, x_1, \dots, x_n .

Each successful copulation was considered an independent choice of a female among the males at a lek. Studies of other lek-mating grouse indicate that females may copy choices of prior-mating females (Gibson et al. 1991; Gibson 1996; but see Spurrier 1994). In a previous study, we found no support for a model that quantified the effects of mate choice copying (Chapter 2). In addition, second-mated females used the same male traits to select a mate as first-mated females.

In general, female prairie-chickens visited lek sites for short periods, were rarely available for capture, and a majority of the copulations were with unbanded females. However, we expected pseudoreplication among females to be low for three reasons. First, behavioral and genetic data indicate that in lek-mating species of grouse, females typically copulate only once to fertilize the entire clutch and do not copulate again unless to fertilize a re-nesting attempt (Alatalo et al. 1996a; Semple et al. 2001). Second, we included only successful copulations in our analyses. Last, annual survival of females is low (51-59%, Hamerstrom and Hamerstrom 1973; 28-42%, Chapter 4) and turnover of females was expected to be high.

In the global discrete choice model, male characteristics hypothesized to be linked to female choice included four behavioral indices (PC scores of display and aggression with and without females), four measurements of body size (mass, tarsus, wing, and tail), two ornaments used in display (comb and pinnae), age class (SY or ASY, coded as 0 and 1), and two territory measurements (distance to lek center and size). Only copulations observed on unmanipulated leks were used in the MDC model. Morphometric and territory measurements were standardized before analysis ($\bar{x} = 0$, $SD = 1$) so slope coefficients would be directly comparable with our PC-scores for components of behavior. The \log_{10} of T values were calculated to obtain normally-distributed values. Backward elimination was used to simplify the model, and the resulting models were evaluated using an information-theoretic approach (Burnham and Anderson 2002). Additionally, we considered the minAIC model from Chapter 2 and the minAIC model including $\log_{10}T$. In all, 12 models were analyzed. Akaike weights (w_i), model-averaged estimates ($\hat{\theta}_a$), and weighted unconditional standard error [$se(\hat{\theta}_a)$] were calculated using formulae in Burnham and Anderson (2002; eqns. 4.1 and 4.9).

Results

Natural Variation in Testosterone

Over three years, 166 blood samples were collected from 100 individuals at five leks. T levels could not be quantified for 8 samples (4.8%). An average of 1.6 ± 0.9 samples were collected from 87 males and 10 females. $\log_{10}T$ varied by sex ($F = 23.38$, $P < 0.001$) and the amount of time the bird was in the trap ($F = 4.92$, $P = 0.03$), but was not affected by lek site ($F = 1.12$, $P = 0.11$), time of day with respect to sunrise ($F = 0.97$, $P = 0.34$) or either linear or quadratic seasonal trend ($F = 0.26, 0.28$, $P = 0.61, 0.60$, respectively; overall model $F_{10,137} = 4.01$, $P < 0.001$). T levels were 4.0 times higher in males (1.15 ± 0.91 ng/mL, $n = 82$) than in females (0.29 ± 0.18 ng/mL, $n = 10$). Time in trap was negatively correlated with $\log_{10}T$ levels, but the coefficient of determination was low ($r^2 = 0.03$). Since this correlation was unlikely to be biologically significant, this variation was not controlled in the analyses examining associations between testosterone, male traits, mating success, and survival.

Since there was no seasonal trend, average Log_{10}T levels of each male were compared with male traits and mating success. Some males were not observed on the lek following capture. These males had lower T levels than territory-holders (non-territorial males: 0.86 ± 0.85 ng/mL, $n = 14$; territorial males: 1.28 ± 0.96 ng/mL, $n = 81$; $F_{1,93} = 9.72$, $P = 0.002$).

No male behavior or morphological traits correlated with natural variation in T (Log_{10}T) among territorial males on unmanipulated leks. Neither male territory size or the territory's distance from lek center correlated with Log_{10}T (size: $F_{1,43} = 0.001$, $P = 0.97$; distance: $F_{1,43} = 0.001$, $P = 0.97$). Log_{10}T did not correlate with display or aggressive behavior when females were present (display: $F_{1,40} = 0.06$, $P = 0.81$; aggression: $F_{1,40} = 0.16$, $P = 0.69$) or when females were absent (display: $F_{1,44} = 1.22$, $P = 0.28$; aggression: $F_{1,44} = 0.12$, $P = 0.73$). Of the five morphological traits considered (mass, length of tarsus plus longest toe, keel, wing and tail), none were correlated with T levels ($F_{1,43} < 3.10$, $P > 0.09$). Two sexually dimorphic traits were not associated with Log_{10}T (pinnae: $F_{1,41} < 0.01$, $P = 0.99$; comb area: $F_{1,42} = 2.39$, $P = 0.13$).

The MDC model included 23 copulations among 12 successful and 26 unsuccessful males at five greater prairie-chicken leks over three years. Of the 15 models considered, two had ΔAIC values < 2 (Table 3.2). Mating success was correlated with natural variation in Log_{10}T , two morphological traits and one territorial trait as indicated by the minAIC model (Table 3.2). Log_{10}T had the largest slope coefficient (Figure 3.1), indicating that it was the strongest predictor of male mating success. Log_{10}T was positively correlated with mating success such that males with higher natural levels of T obtained more copulations (Figure 3.1; $t = 2.87$, $P = 0.004$). One sexually-selected trait and one measurement of body size were positively correlated with higher mating success (Figure 3.1; comb area: $t = 2.43$, $P = 0.02$; length of tarsus plus longest toe: $t = 3.24$, $P = 0.001$). Neither display nor aggressive behavior was correlated with mating success since it was not included in the minAIC model (Table 3.2; Figure 3.1). There was support for a model that included a negative, but nonsignificant, correlation between display behavior when females were absent and mating success during the breeding season ($t = -0.72$, $P = 0.47$; Table 3.2, Figure 3.1). Distance to lek center was negatively correlated with male mating success so that males closer to the center of the lek obtained more copulations (Figure 3.1; $t = -2.26$, $P = 0.02$).

Return rates did not vary with traits that affected male mating success. Survival did not vary with natural variation in Log_{10}T ($\chi^2 < 0.03$, $P = 0.86$, $n = 45$), comb area ($\chi^2 = 0.23$, $P = 0.63$, $n = 50$), tarsus length ($\chi^2 = 0.06$, $P = 0.80$, $n = 50$), or distance from the center of the lek ($\chi^2 = 0.01$, $P = 0.93$, $n = 66$). Males that displayed less when females were not present had higher return rates ($\chi^2 = 6.52$, $P = 0.01$, $n = 67$), even after Bonferroni correction ($P < 0.01$). The odds of returning were 2.1 times higher among males that did not receive a single copulation than among males that received one or more copulations (Odds Ratio 95% CI: 0.73 to 5.91; Fisher's exact test: $P = 0.19$).

Testosterone Implants

In 2005 and 2006, 15 T-implants and 13 sham-implants were conducted for a total of 28 males at five leks. There was no difference in Log_{10}T levels at the time of implant between T- and sham-implanted males (natural T levels of T-males: 1.33 ± 1.24 ng/mL, $n = 14$; sham-males: 1.84 ± 1.48 ng/mL, $n = 13$; $F_{1,25} = 0.94$, $P = 0.34$) or the date which the implant occurred (T-males: 10 April ± 4 , $n = 15$; sham-males: 12 April ± 5 , $n = 13$; $F_{1,26} = 1.67$, $P = 0.21$). After implant, the odds of T levels increasing were 2.0 times higher for of T-males than sham-implanted males (Odds Ratio 95% CI: 0.08 to 51.6). T-implanted males tended to have higher Log_{10}T levels (1.58 ± 0.93 ng/mL, $n = 6$) than sham-implanted males following implant (0.71 ± 0.33 ng/mL, $n = 2$; $F_{1,6} = 2.82$, $P = 0.14$). T-implants were removed from two experimental males 26 and 35 days after implant. The implants were completely empty. Therefore, behavioral observations >21 days post-implant were excluded from the behavioral analyses.

Before implant, males did not differ with respect to behavior, territory attributes, morphology or age. Male behavior before implanting did not differ between the two treatment groups (display with females: $F_{1,22} = 0.69$, $P = 0.41$; aggression with females: $F_{1,22} = 0.68$, $P = 0.42$; display without females: $F_{1,23} = 0.01$, $P = 0.94$; aggression without females: $F_{1,23} = 3.21$, $P = 0.09$). Neither territory size nor distance to lek center varied by treatment before the experiment (size: $F_{1,26} = 1.02$, $P = 0.17$; distance: $F_{1,22} = 0.20$, $P = 0.66$). Comb size, pinnae length, tarsus length, mass, wing length, and tail length did not differ between T- and sham-implanted males (all $F_{1,25} < 1.37$, $P > 0.25$). Males in the two treatments were the same age (Fisher's exact $P = 0.43$).

The testosterone treatment did not affect males' behavior or territory attributes. There was no difference between sham-implanted males and unmanipulated unsuccessful males with respect to behavior standardized for female presence (meta-analysis; display: $Q_B = 0.19$, $P = 0.66$; aggression: $Q_B = 2.36$, $P = 0.12$; $n = 23$ unsuccessful, 8 sham-implanted males) or territory attributes (matched pairs; size: $F = 0.20$, $P = 0.66$; distance to center: $F = 0.49$, $P = 0.49$; $n = 15$ unsuccessful, 11 sham), so they were pooled in subsequent analyses. Neither aggressive or display behavior differed between T-implanted males, unmanipulated unsuccessful males and unmanipulated successful males following treatment (display: $Q_B = 3.76$, $P = 0.15$; aggression: $Q_B = 5.44$, $P = 0.07$; Table 3.3). Using a matched pairs analysis comparing territory size and distance to lek center before and after treatment, no differences were detected among T-implanted, unsuccessful and successful males (matched pairs; size: $F = 1.39$, $P = 0.26$; distance to center: $F = 1.11$, $P = 0.34$; $n = 14$ T-implanted, 26 unsuccessful, 16 successful males). Comb size increased during the season in both T-implanted and unsuccessful males (T-males: $+25.4 \pm 69.0$ mm², $n = 5$; unmanipulated: $+21.5 \pm 17.7$ mm², $n = 4$; matched pairs $F = 0.01$, $P = 0.92$). No other morphometrics were expected to change during the course of this study because the birds do not molt during the breeding season.

T-implanted males tended to have higher mating success than sham-implanted males. Four of 15 T-implanted males (14.3%) received six copulations; whereas, only one of 13 sham-implanted males (3.6%) received a single copulation. The odds of receiving a copulation were thus 4.3 times (95% CI: 0.42 to 45.3) greater for T-implanted than sham-implanted males, but this difference was not significant (Fisher's exact $P = 0.33$). Two of 15 T-implanted males returned the subsequent year (13.3%); whereas, five of 13 sham-implanted males returned (38.5%). The odds of returning was 4.1 times (95% CI: 0.63 to 26.1) higher for sham-implanted than T-implanted males, but the probability of returning did not differ with respect to treatment (Fisher's exact $P = 0.20$). The return rate was 25% in both years of the study (2005-06: 3 of 12 males returned; 2006-07: 4 of 16 returned).

Discussion

Our research project is one of the first studies to demonstrate a direct link between T and mating success in a lek-mating bird. We improve on preceding studies of the natural variation of

T in a lek-mating species by analyzing T levels from >4 times the number of males examined previously ($n = 87$, this study; $n = 23$ males, Alatalo et al. 1996b; $n = 15-27$ per species, Wikelski et al. 2003). The T-implant experiment suggests that there may be a causal link between T and mating success, but low sample sizes and high variability limited the power to detect differences. Surprisingly, natural and experimental variation in T did not correspond with any male traits examined, including aggression. Since increased T was not linked to decreased survival, T may not be an honest signal of male quality in greater prairie-chickens.

T was related to male mating success, but not via any of the behavioral, morphological, or territorial traits considered. This lack of a relationship was surprising because it was unlikely that females were assessing T levels directly. We did not expect T to affect male plumage, because male plumage ornamentation is a primitive character in Galliformes with the cryptic female plumage being a derived estrogen-dependent character (Kimball and Ligon 1999). However, T may affect mating success via cumulative effects, unmeasured male traits, and territory establishment in fall. T may have small, statistically insignificant, effects on multiple male traits that, when taken together, influence male mating success (Ligon et al. 1998), but we were unable to test for such a cumulative effect. Secondly, T could also be related to male traits that were not measured, such as parasite load, UV reflectance, plumage color or vocalization attributes (Gibson et al. 1991; Mougeot et al. 2005b, 2006; Blas et al. 2006). Lastly, male prairie-chickens can be detected at lek sites throughout the year (Baines 1996, Salter and Robel 2000, pers. obs.). In the fall, juvenile males visit multiple leks and initiate territory establishment; whereas, adult males maintain or shift their territories closer to the lek center. T may enhance a male's fighting ability, suggesting that T may be a proximate mechanism mediating territory establishment in fall.

T increased male mating success, but did not decrease survival, suggesting that T, by itself, is not an honest signal of male quality. Since T was not related to any male traits examined, T levels are not likely to be under direct sexual selection. The average lifespan of a greater prairie-chicken is short (1.6 years, Robel and Ballard 1974) so cumulative detrimental effects may not have time to become apparent. In addition, high turnover of males at the lek (Chapter 2) indicates that there may be high rates of predation (Chapter 4), suggesting that

natural selection being a stronger determinant of male traits than sexual selection (Bleiweiss 1997; Drovetski et al. 2006).

Our T-implants did not have as large a magnitude of effect as we predicted. We predicted T-implanted males would be more aggressive than sham-implanted males. A study in domestic fowl detected the conversion of T to estradiol in the skin, producing female-like feathers on male chickens (George et al. 1981). We were not able to evaluate whether this occurred in our study, but estradiol also plays a role in aggression and territory defense (Soma et al. 2000). To separate the effects of T and estradiol, future work could use an enzyme inhibitor to prevent the conversion of T to estradiol (fadrozole, FAD blocks the aromatase enzyme; Soma et al. 2000, Mougeot et al. 2005a). Another hypothesis is that aggression did increase in our T-implanted males, but adjacent sham-implanted males increased their aggressive levels in response to repeated intrusions of T-implanted males (i.e. the challenge hypothesis, Wingfield et al. 1990). The overall effect would be no difference between the aggression levels of T- and sham-implanted males.

The inclusion of T in the MDC model reduced the effects of behavior on male mating success. Model 10 of this chapter (Table 3.2) includes the same variables as the minAIC model presented in Chapter 2 (aggressive and display behavior with and without females, mass, tail, comb). The slope coefficients of these two models are similar in magnitude and direction (data not presented), indicating that the results presented in this chapter are due to the inclusion of T in the model and not due to smaller sample sizes. T accounted for a large proportion of the variation in male mating success and was a better predictor of mating success than behavior. However, prairie-chicken territories were established and maintained through ritualized behavior (Schroeder and Robb 1993). The inclusion of distance to lek center in the analysis of male mating success suggested that certain specific aggressive behaviors, that were not quantified in our PCA of behavior, may be important in mediating male mating success. Structural size was an important component in mate choice decisions, but different aspects of size may be preferred by different females in different years. Females may be using different male traits to choose a mate on different leks, but we did not detect any differences among leks. We found a link between comb size and mating success similar to other studies (Rintamäki et al. 2000). However, we did not find a link between comb size and T as expected (Briganti et al. 1999,

Parker et al. 2002). Our research showed the importance of examining a suite of male traits since multiple traits were used by females to select a mate.

In conclusion, we found that T related to mating success, but we could not detect any male traits correlated with T that females might use to detect differences in T levels. To obtain a better understanding of the seasonal changes in T, multiple samples from individuals throughout the breeding season are needed. Fecal sampling would provide a non-invasive way to obtain such samples (Washburn et al. 2003, 2004; Wells et al. 2003). Future work should also examine parasites, UV plumage coloration and vocalizations as possible intermediary steps linking T to mating success.

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Table 3.1 - Eigenvectors of a principal component analysis of male behavior from 1332 ten-minute focal observations of 129 male greater prairie-chickens at 4-5 leks per year. Observations were conducted during the breeding season (mid-March through mid-May) near Manhattan in northeast Kansas during 2004-2006. Principal component loadings > 0.4 are in bold.

Behavior	PC1 (Display)	PC2 (Aggression)
Percent time displaying	0.53	0.26
Percent time fighting	-0.5	0.08
Number of boom vocalizations	0.49	0.31
Number of flutter jump displays	0.38	0.02
Number of fights	-0.21	0.65
Number of males engaged	-0.19	0.64
Eigenvalue	2.79	1.69
Percent of variance explained	46.4	28.2

Table 3.2 – Comparison of multinomial discrete choice models examining the effects of male testosterone (on a \log_{10} scale), morphology, behavior and territory attributes on mating success. Data included 23 copulations among 12 successful and 26 unsuccessful males on five greater prairie-chicken leks between 2004-2006 in northeast Kansas. Table abbreviations are as follows: S = parameters included in the survival model; Dev = deviance, K = number of parameters; AIC = Akaike’s Information Criterion; Δ AIC = difference of the AIC value in the given model compared to the minimum AIC model; and w_i = Akaike weights. Model abbreviations are as follows: testosterone variables (T = log-transformed testosterone levels), morphological variables (c = comb area; p = pinnae; r = tarsus; m = mass at capture; w = wing; t = tail; g = age class); behavioral variables (D = display with females; A = aggression with females; d = display without females; a = aggression without females); and territory variables (l = distance to center of lek, s = territory size), and - = none included.

Model	Testosterone	Morphology	Behavior	Territory	Dev	K	AIC	Δ AIC	w_i
1 - Step 10	T	rc	-	l	60.6	4	68.62	0.00	0.47
2 - Step 9	T	rc	d	l	60.1	5	70.07	1.45	0.23
3 - Step 8	T	rcp	d	l	59.2	6	71.17	2.56	0.13
4 - Step 7	T	rcp	d	ls	58.5	7	72.48	3.86	0.07
5 - Step 6	T	wrcp	d	ls	57.5	8	73.50	4.89	0.04
6 - Step 5	T	wrcpg	d	ls	56.4	9	74.43	5.81	0.03
7 - Step 4	T	twrcpg	d	ls	55.7	10	75.74	7.13	0.01
8 - min AIC ^a + T	T	mrc	DAda	-	59.9	8	75.89	7.28	0.01
9 - Step 3	T	twrcpg	Ad	ls	54.9	11	76.89	8.27	0.01
10 - min AIC ^a	-	mrc	DAda	-	62.9	7	76.92	8.30	0.01
11 - Step 2	T	twrcpmg	Ad	ls	54.4	12	78.37	9.75	0.00
12 - Step 1	T	twrcpmg	DAd	ls	54.1	13	80.13	11.51	0.00
13 - Global + T	T	twrcpmg	DAda	ls	54.0	14	82.00	13.38	0.00
14 - Global	-	twrcpmg	DAda	ls	59.7	13	85.71	17.09	0.00
15 - T only	T	-	-	-	92.3	1	94.33	25.71	0.00

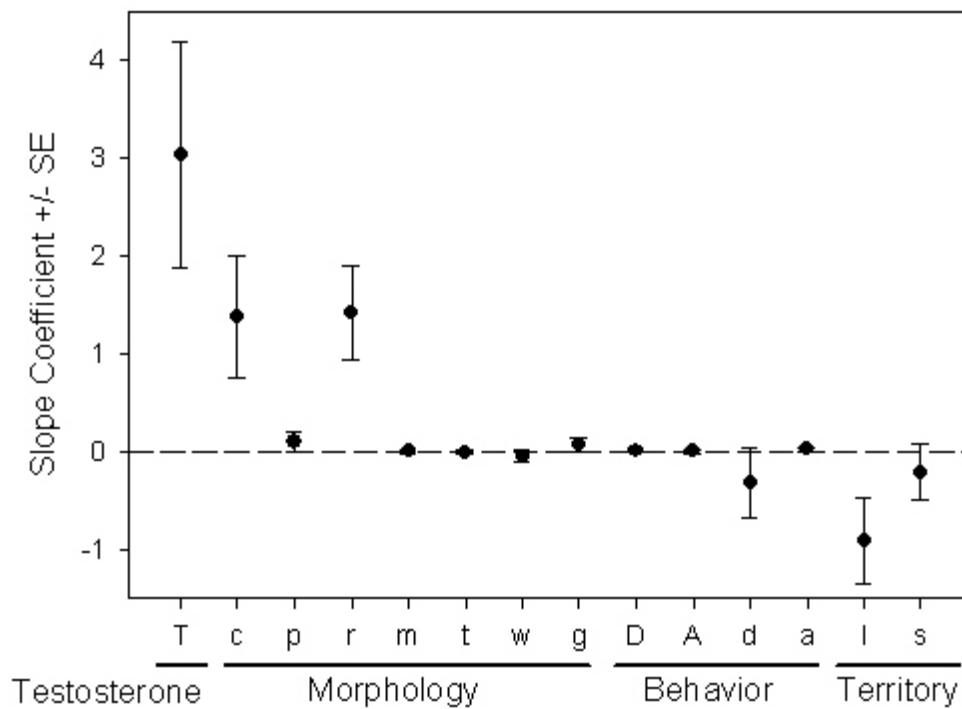
^a Model from Chapter 2

Table 3.3 - Comparison of effect sizes following a testosterone implant experiment of greater prairie-chickens. Data included 15 T-implanted males, 31 sham-implanted and unmanipulated unsuccessful males pooled, and 24 successful males on five leks between 2005-2006 in northeast Kansas. Display and aggressive behavior was quantified using a principal component analysis of behavior observed during 10-min focal observations and standardized for female presence (Table 3.1).

	Unsuccessful ^a			T-Implants			Successful		
	E+	95% CI	<i>n</i>	E+	95% CI	<i>n</i>	E+	95% CI	<i>n</i>
Display	0.22	-0.02 to 0.47	31	0.54	0.16 to 0.92	13	0.13	-0.11 to 0.38	24
Aggression	0.01	-0.24 to 0.25	31	-0.20	-0.57 to 0.16	13	0.26	0.01 to 0.51	24

^aSham-implanted males and unsuccessful unmanipulated males combined.

Figure 3.1 - Model-averaged estimates of the slope coefficients from a multinomial discrete choice model estimating the effect of male attributes on male mating success. Explanatory variables were standardized so slope estimates are directly comparable. Estimates of SE are unconditional and include uncertainty due to model selection. Data included 23 copulations among 12 successful and 26 unsuccessful males on five greater prairie-chicken leks between 2004-2006 in northeast Kansas. Abbreviations are as follows: testosterone variables (T = log-transformed testosterone levels), morphological variables (c = comb area; p = pinnae; r = tarsus; m = mass at capture; w = wing; t = tail; g = age class); behavioral variables (D = display with females; A = aggression with females; d = display without females; a = aggression without females); and territory variables (l = distance to center of lek, s = territory size).



CHAPTER 4 –
Breeding Ecology of Female Greater Prairie-Chickens
in Unfragmented Grasslands

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Abstract

Populations of greater prairie-chickens (*Tympanuchus cupido*) have been declining for at least 30 years due to the destruction and fragmentation of their tallgrass prairie habitat. Management of this species is hindered by a lack of contemporary demographic data analyzed with modern statistics. In this study, we examined nesting success and female movements to provide baseline demographic information for a population in natural, unfragmented prairie. Four leks were monitored for four years (2003-2006) in northeast Kansas. We fitted 43 females with radio-transmitters to monitor movements and to locate nests. On average, females nested 1.8 ± 1.3 km (\pm SD; $n = 19$ nests) and ranged up to 3.7 ± 1.9 km from the lek of capture ($n = 8$ females). Potential reproductive output was high because females laid large clutches (10.9 ± 1.7 eggs, $n = 24$), renested following clutch loss (22.2%, $n = 27$ females), and had high egg viability (88.6 \pm 13.3 % of eggs hatched; $n = 7$ nests). However, daily survival rate of nests was low (0.928, $n = 34$ nests) resulting in a predicted nest success rate of 7.4% for a 35-day exposure period. By applying known fate models to our telemetry data of 40 female prairie-chickens, we estimated a weekly survival rate of 0.970 resulting in a predicted survival rate of 45.7% for the 6-month breeding season. Using time-since-marking Cormack-Jolly-Seber models for live encounter data to control for transience, annual apparent female survival was 0.277 ± 0.081 (\pm SE) for 55 marked females after initial capture, and 0.424 ± 0.139 among females that returned at least once in a time-since-marking model. Comparison of seasonal and annual survival of females indicates that survival is 1.6 to 2.0 times higher during the nonbreeding season than the breeding season, presumably because females are susceptible to predation during incubation. Loss and fragmentation of native prairie would affect nesting up to 2 km and female movements up to 4 km from the disturbance. Conservation should protect large tracts of land and avoid disturbance

within 4 km of leks. Nest survival was unexpectedly low in greater prairie-chickens in natural habitats and may be the primary demographic factor limiting population viability. Rangeland practices that increase residual nesting cover or reduce predator impacts may be beneficial.

Introduction

Habitat loss and fragmentation have been the primary factors leading to population declines in grassland birds. Eastern tallgrass prairie is one of the most highly altered biomes in North America (Ryan et al. 1998). With declines between 82-99%, the destruction of tallgrass prairie exceeds those reported for any other major ecosystem in North America, including the bottomland hardwoods of south-central United States and the temperate rainforest of British Columbia (Samson and Knopf 1994). Greater prairie-chickens (*Tympanuchus cupido pinnatus*) are an indicator species for remaining North American tallgrass prairie. The historic range of greater prairie-chickens extended from the prairie provinces of Canada south to central Texas and east to western Ohio (Schroeder and Robb 1993, Ross et al. 2006). However, prairie-chickens have been extirpated or in danger of being extirpated from 15 states and provinces (Schroeder and Robb 1993). Kansas is one of only four states to hold >5,000 breeding birds (Silvy et al. 2004, Ross et al. 2006). However, prairie-chickens have declined 70% in Kansas during the past 20 years (Applegate and Horak 1999, Svedarsky et al. 2000). Recent declines in Kansas correspond with a shift in rangeland management of the tallgrass prairie from periodic burning and moderate cattle grazing to annual burning and early intensive cattle stocking (Robbins et al. 2002).

Movement and demographic information are critical to determining the size of management units for the conservation of greater prairie-chickens. Recent studies examining greater prairie-chicken movements at the landscape scale were located in a habitat that consisted of only 15% grasslands (Ryan et al. 1998). Studies in more extensive, contiguous habitat are needed to serve as a reference for field studies of relict populations of prairie-chickens in marginal habitats. The Flint Hills of Kansas are largely unplowed prairie due to the rocky substrate and offer one of the best localities for studying prairie-chicken ecology in contiguous habitat, but the most recent research was conducted over 20 years ago (Horak 1985; Robel 1966, 1970a,b; Robel and Ballard 1974).

One demographic factor that may have an important effect on the population dynamics of gamebirds is nest survival (Wisdom and Mills 1997). In greater prairie-chickens, nesting success ranges from 20-67% with considerable annual variation (Robel 1970a, Schroeder and Robb 1993). Most available estimates of nest survival for greater prairie-chickens have been reported as apparent nest survival (% of nests that hatch), uncorrected for the age of the nest when it was found. Nest survival modeling in Program Mark computes daily nest survival estimates while controlling for days of exposure and can yield estimates that are comparable between populations or management areas (Dinsmore et al. 2002, Rotella et al. 2004). The nest survival model improves upon Mayfield estimates (Mayfield 1975) because it relaxes the assumption of constant daily survival, is less sensitive to variation in nest monitoring efforts, and allows for daily survival rate to be modeled as a function of environmental covariates (Dinsmore et al. 2002).

Survival of juveniles and adults may also influence the rate of population change in grouse (Aldridge and Brigham 2001, Sandercock et al. 2005, Hannon and Martin 2006). Annual survival estimates of adults used in recent population models for greater prairie-chickens (Wisdom and Mills 1997, Peterson et al. 1998) are based upon return rates collected greater than 30 years ago from an isolated population in Wisconsin (Hamerstrom and Hamerstrom 1973). Modern estimates of adult annual survival are needed in light of the drastic population declines that have occurred in Kansas during the past 30 years (Applegate and Horak 1999). Cormack-Jolly-Seber (CJS) models based on live encounter data improve upon return rates by separating apparent survival (ϕ) from the probability of encounter (p). However, apparent survival (ϕ) is a minimum estimate of true survival (S) because it is the product of S and site fidelity (F ; Sandercock 2006). In addition to estimates of annual survival, seasonal estimates of breeding and nonbreeding survival are needed to determine the portion(s) of the annual cycle when birds are most vulnerable (Kirby and Cowardin 1986, Devries et al. 2003).

Conservation efforts would benefit from better information concerning the movement and demography of prairie-chickens in the core of their range (Applegate and Horak 1999, Peterson et al. 1998). In light of the greater prairie-chicken's most recent population declines in Kansas, our objectives were to: 1) estimate home range size using minimum convex polygons and kernel methods (Barg et al. 2005); 2) estimate nest survival using the nest survival procedure of Program Mark (Dinsmore et al. 2002); 3) estimate seasonal survival of radio-marked female

prairie-chickens during the breeding season using known fate models (Murray 2006, Mong and Sandercock 2007); and 4) estimate annual survival of female prairie-chickens using CJS models that control for the probability of encounter (Sandercock 2006). Our use of modern statistical techniques for analyzing nest and adult survival yields less biased estimates that can be compared among populations.

Methods

We monitored greater prairie-chickens at four lek sites between mid-March and mid-May, 2003-2006. All leks were located on cattle-grazed pastures in Riley and Geary Counties in northeast Kansas, USA (39° 05'N, 96° 34'W). We observed three leks in 2003, and expanded our sampling effort to four leks in 2004-2006. Prairie-chickens were captured and observed at lek sites between 2003-2005, and were resighted in 2006 only for estimation of apparent survival rates.

Trapping and morphometrics

Male and female prairie-chickens were trapped at lek sites using walk-in funnel traps and by spot-lighting at night (Hamerstrom and Hamerstrom 1973, Toepfer et al. 1987, Schroeder and Braun 1991). When we discovered nests of unbanded females, the female was captured by placing a mist net over the nest during incubation. All birds were given a unique combination of colored leg bands and tail markings to aid in individual identification. Seven morphometric measurements were recorded, including body mass (± 1 g) and two linear measurements of body size (lengths of tarsus plus the longest toe, keel; ± 1 mm). We determined age-class as second-year (SY) or after-second-year (ASY) from the shape, coloration and wear of the outermost two primaries (numbers 9 and 10; Schroeder and Robb 1993). In SY birds, the outer primaries are retained from juvenile plumage, appear tapered with light brown dots on the leading edge extending to the tip, and contrast in coloration and wear with primaries 1 through 8. The outer two primaries of ASY birds have truncate tips, light brown dots on the leading edge that terminate > 2 cm from the tip, and do not contrast with the inner primaries. A subset of males and females were fitted with necklace-style radio collars (Model RI-2BM, ca. 10.9 g, Holohil

Systems Ltd., Ontario, Canada; or Model TS - 25, ca. 15.6 g, Telemetry Solutions, Walnut Creek, CA; expected life was ~18 months and have 24 hour mortality sensors).

Estimation of home range size and distances

Prairie-chicken locations were estimated weekly using 2 methods. We triangulated bird positions using hand-held Yagi antennas by recording the position of the observer and a compass bearing to the bird. We also approached birds on foot to locate nests, broods and mortality events. Handheld global positioning system receivers (GPS, Model III Plus, Garmin International Inc., Olathe, KS 66062) were used to record locations of triangulation points, bird sightings and mortality events. Program LOCATE II was used to determine locations of radio-marked birds for each set of triangulation bearings ($n = 3$ or 4 bearings; Nams 2000, Millsbaugh and Marzluff 2001). All locations (in universal transverse mercator, UTM, coordinates) were imported into ArcView (Ver. 3.3; Environmental Systems Research Institute, Inc., St. Charles, MO 2002) for home range analyses.

We present both minimum convex polygon (MCP) and kernel estimates to provide measures of home range size that can be compared across studies. MCP estimates of home range size are often larger than kernel estimates even if using the same location data set (Girard et al. 2002, Barg et al. 2005). MCP methods require fewer locations but estimates may be affected by peripheral locations and include large areas that are not used by the organism (Barg et al. 2005). Kernel estimates are less affected by peripheral locations, and provide insight into areas of intense use, but require a greater number of locations per individual (> 15 , Girard et al. 2002). Both MCP and kernel home range estimates are influenced by the number of locations considered with biased estimates and large error associated with MCP estimates derived from < 10 positions/individual and kernel estimates derived from < 15 positions/individual (Girard et al. 2002). Bias can also occur in home range estimation using kernel estimates with species that exhibit a central-place behavior to a nest site within their home range (Mong 2005).

Home range for the entire breeding season was determined with two methods: MCP and 50% and 95% kernel estimates using the Animal Movement extension (Hooge et al. 1999). Calculation of MCP and maximum distance was restricted to birds with > 10 positions recorded. For birds with ≥ 15 positions, we also calculated 50% and 95% fixed kernel estimates. The nest

site location was only included once in our set of home range data points used to calculate kernel estimates. In kernel metrics, the smoothing parameter was determined using the Least Square Cross Validation (LSCV) method (Seaman and Powell 1996, Gitzen and Millspaugh 2003). We used the χ^2 approximation to the nonparametric Kruskal-Wallis test to examine sexual differences in home range size (JMP IN Ver. 4.0.4, SAS Institute, 2001). Three additional linear measurements were determined in ArcView using the Animal Movement extension: the distance between the nest site and the lek of banding, the distance between the nest site and the closest lek, and the maximum distance traveled from the lek of banding.

Nest monitoring

Nest contents were examined by flushing females once or twice during incubation. We restricted our nest visits to hot, dry afternoons to minimize scent trails. We recorded the position of the nest using handheld GPS receivers, marked the location using a flag placed 15-20 m from the nest in a random direction, and mapped nest locations relative to natural landmarks. We counted eggs to determine clutch size, and measured the maximum length (L) and width (W) of the eggs. Egg volume (V) was calculated as $V = kLW^2$ where constant k was set at 0.49 for spherical eggs of grouse (Hoyt 1979, Sandercock and Pedersen 1994). Eggs were also floated in a small cup of lukewarm water to estimate stage of incubation in a 24-day incubation period (Schroeder and Robb 1993). Buoyancy of eggs was related to stage of incubation as follows: horizontal on the bottom of the container = 0 days; oriented at 45° = 5 days; vertical orientation (90°) = 10 days; float to the surface = 13 days; ~18 mm diameter circle protrudes above the water surface = 21 days. On incubation days 22, 23 and 24, the initial stages of hatching can be detected as faint tapping, a star-shaped crack in the egg shell, and a small hole pip in the egg shell, respectively. Date of clutch initiation was estimated by back-dating from the stage of incubation and by assuming the laying period for a given clutch size was one egg per day. If buoyancy indicated that incubation had not yet been initiated (i.e., horizontal), we revisited the nest a week later to determine final clutch size and estimate start of incubation. If egg flotation indicated that incubation had been initiated (i.e., buoyancy $> 0^\circ$), we considered the clutch to be complete.

Nests were monitored every other day by triangulating the female's radio signal > 30 m from the nest site. If the female's signal was not heard from the nest location for two or three consecutive visits, the nest's contents were examined. Nests were visited daily at sunrise starting 1-2 days before the predicted day of hatching. Number of hatched and unhatched eggs were recorded. Nestlings were weighed (± 0.1 g) and measured (length of the tarsus, wing and total head from the back of the head to the tip of the beak). We marked hatchlings with individually numbered patagial wing tags to investigate local recruitment (Hannon et al. 1990).

The daily probability of nest survival (S) was estimated using the nest survival model in Program Mark (Ver. 4.1, White and Burnham 1999; Dinsmore et al. 2002, Rotella et al. 2004). Five assumptions of the nest survival model are (Dinsmore et al. 2002): stage of egg laying or incubation can be correctly determined when they are first found, nest fates are correctly determined, discovery and subsequent check do not influence nest survival, nest fates are independent, and homogeneity of daily nest survival rates. These assumptions were likely met in this study. Stage of incubation was determined by the buoyancy of eggs. Nest fates were relatively easy to determine. Successful nests contained eggs that were neatly pipped and chicks were found on subsequent checks of the female. Whereas, unsuccessful nests contained many fragmented egg shells usually with bloody remains and no chicks were accompanying the female. We minimized disturbance at the nest (approaching it only once or twice on hot, dry afternoons to minimize scent trails), but we cannot rule out possible observer effects completely. Nests were likely to be independent in this study because nests were not spatially clumped, and distances between first nests and renesting attempts of the same female were often > 1 km (see results). Daily nest survival rates were unlikely to be heterogeneous because all nests experienced the same climatic conditions and were probably exposed to similar predator communities.

Encounter histories for the nest survival analysis required five types of information: the date nest was located (k), the last date nest was checked and was active (l), the last date nest was checked (m), this was either the date the nest was discovered to be depredated or hatched, the fate of the nest (f), where 0 = hatched and 1 = failed, and the number of nests (n) with this encounter history. To model an effect of nest age on daily survival rate, we included a nest age covariate (age of nest when found). To transform our date information into an acceptable format for the

nest survival model, we determined the earliest date the first nest was found (22 April = $k = 1$) and calculated k , l and m relative to that date for all nests. Daily probability of survival was modeled as a linear function of day of season (lin), age of the nest (age), their interaction (*), main-effects (+), or as a constant (c).

Breeding survival of females

To measure survival within the breeding season, we monitored the subset of females that was given radio collars and modeled weekly probability of female survival (S) using the ‘nest survival’ procedure (Dinsmore et al. 2002) in Program Mark (White and Burnham 1999). Originally developed for nest data, this procedure can also be applied to “ragged” telemetry data for radio-marked birds (Hartke et al. 2006, Mong and Sandercock 2007). Birds that were never detected after first marking and presumably had failed radios were censored from analysis ($n = 7$ females). We assume that this censoring is random and noninformative, and had limited effect on our survival estimates (Hagen et al. 2006, Murray 2006). To test the assumption that censoring was random, we developed two models. The first model coded females that were not detected greater than 2 weeks into the study as ‘surviving’. A second model coded these females as ‘dead’. Weekly probability of survival was modeled as a function of mass at initial capture (mass), week since marking (t), a linear trend throughout the season (lin), a quadratic trend throughout the season (quad), constant (c), and additive (+) combinations of these factors.

Cause of mortality was grouped into four categories based on the following criteria (Hagen et al. 2007, Wolfe et al. 2007). Since it is difficult to make unambiguous statements about causes of mortality (Bumann and Stauffer 2002), we refer to “probable causes of mortality”. Cases where feathers, radios or leg bands were chewed, had obvious tooth marks, and were clumped (~1m) were considered to be the probable result of *mammalian* predators. Cases where feathers appeared cut and were spread over 2 m were considered probable *avian* predators. Cases where the carcass was intact, was located near the nest and had feathers that were pasted to the body as the result of trying to swallow the bird whole were considered the probable result of *snake* predation. Birds found dead on roadsides or near fences with evidence of trauma injuries were considered *collision* mortalities. We visited mortality locations as soon

as a mortality signal was detected, but we are unable to exclude the possibility of scavenging post-mortality.

Annual survival of females

We observed lek-mating behavior from a blind placed ~6 m from the edge of each lek. Between mid-March through early May, leks were observed every 1-2 days to record visits and the identity of color-banded and radio-marked females. We estimated apparent survival using time-since-marking Cormack-Jolly-Seber models for live encounter data in Program Mark (Ver. 4.1, White and Burnham 1999). Apparent annual survival (ϕ) was modeled as a function of time (t) or constant (c) for females overall, and separately for females after initial capture (ϕ^1) and for females that returned at least once (ϕ^{2+}) to determine if transience affected apparent survival. Resighting probability (p) was modeled as a function of time (t) or held constant (c). We were unable to test whether transmitters affected survival of female prairie-chickens, but no transmitter effects were recorded with the same field methods in lesser prairie-chickens (*Tympanuchus pallidicinctus*; Hagen et al. 2006). The global model for the mark-resight data was: ϕ^1, ϕ^{2+}, p_t . The variance inflation factor (\tilde{c}) was used to adjust for lack of fit and was estimated using bootstrap goodness-of-fit (GOF) tests using 1000 replicates on the global model (Lebreton et al. 1992, White et al. 2001).

Synthetic estimates

Prairie-chicken nests were discovered at different stages of the nesting cycle. To obtain an unbiased estimate of fecundity per nest (F_n) that controlled for variation in nest exposure and made full use of our demographic parameters, we calculated the mean number of female fledglings produced per female by:

$$F_n = \{[\text{BREED} * \text{TCL}_1 * \text{HATCH}_1] + [(1 - \text{HATCH}_1) * \text{RENEST} * \text{TCL}_2 * \text{HATCH}_2]\} * \text{ES} * \text{HS} * 0.5$$

where BREED = probability of breeding, TCL = total clutch laid, HATCH = probability of nests hatching at least one chick, RENESEST = probability of reneesting after clutch loss, ES = proportion of eggs that survived until hatching if the nest survived incubation, HS = proportion of eggs hatching if the nest survived incubation, subscripts 1 and 2 indicating estimates for first and second nests, and 0.5 is the assumed sex ratio of females per clutch. HATCH was calculated as

the product of estimates from the best fit nest survival model from the average laying date of first and second nests for 35 days or 24 days. Thirty-five days corresponds to the combined length of the egg laying (11 days, see Results) and incubation stages (24 days, Schroeder and Robb 1993); 24 days is the average length of incubation and would be the period of exposure if predation is negligible during egg laying.

We lacked direct estimates of juvenile survival. To estimate juvenile survival (S_{juv}) from hatching until the spring of the following year necessary for a stationary population, we used the equation:

$$S_{juv} = \frac{\lambda - S_{fem}}{F_n}$$

where λ is the rate of population change and was set to 1 for a stationary population, F_n was the seasonal estimate of fecundity per female, and S_{fem} was maximum annual apparent female survival as estimated from the Cormack-Jolly-Seber mark-resight models.

Statistical analyses

Components of fecundity were analyzed using procedures of Program JMP IN (Ver. 4.0.4, SAS Institute 2001). Correlates of laying date, clutch size, egg volume, and nestling size were analyzed using one-way ANOVAs. Egg volume and nestling measurements were averaged per female before analysis because eggs and nestlings within a clutch were not independent observations. Sample sizes varied among analyses because it was not possible to measure every parameter for all birds.

Models of survival in Program Mark were constructed with design matrices and the logit link function. The number of parameters were adjusted to match the model structure, which in most cases was the number of columns in the design matrix. Model selection was based on the information theoretic approach, and we considered the model with the lowest Akaike's Information Criterion value corrected for small sample sizes (AICc) to be the best supported by the data (Lebreton et al. 1992, Burnham and Anderson 2002). Models with $\Delta AICc \leq 2$ were considered equally parsimonious. When two or more models were equally parsimonious, we used the model averaging procedure in Program Mark to obtain overall parameter estimates

(Burnham and Anderson 2002, Cooch and White 2006). Akaike weights (w_i), model-averaged estimates ($\hat{\theta}_a$), and weighted unconditional standard error [$se(\hat{\theta}_a)$] were calculated using formulae in Burnham and Anderson (2002; eqns. 4.1 and 4.9). Descriptive statistics are presented as $\bar{x} \pm 1$ SD unless otherwise indicated.

Results

Between 2003-2005, we captured, banded and fitted 43 female and 4 male prairie-chickens with necklace radio collars. An additional 15 females were banded only. Radios were 1.2 to 1.8 % of female body mass at capture ($\bar{x} = 889 \pm 75$ g; $n = 32$) and 1.1 to 1.5 % of male body mass ($\bar{x} = 1007 \pm 62$ g; $n = 4$).

Home range size

Females moved a maximum distance of 3.7 ± 1.9 km from the lek they were banded ($n = 8$ females with ≥ 10 locations) and had minimum convex polygon (MCP) estimates of home ranges 395 ± 234 ha in size (range: 130 to 804 ha; $n = 9$ females). MCP estimates of male home ranges were 153 ± 151 ha (range: 51 to 327 ha; $n = 3$), and were similar in size to female MCP estimates ($Z = -1.47$, $P = 0.14$). For females, 95% kernel estimates of 575 ± 145 ha (range: 469 to 831 ha; $n = 5$) were 39.2% larger than the MCP estimates, with core use areas (50% kernel estimates; 95 ± 36 ha, range: 66 to 155 ha) that were 16.6% of the 95% kernel estimate ($n = 5$).

Nesting propensity

Of 24 females fitted with radio collars before egg laying and where 3 or more localities were obtained, nests were located for 19 females (79.2%). Two females presumably nested due to localization of movements and broody behavior, such as flying short distances when approached and emitting clucking calls (8.3%), but their nests were never located. The remaining three birds dropped their radios shortly after marking (12.5%). Thus, the probability of nesting (BREED) was 1.0 for female prairie-chickens.

During 2003-2005, 34 nests were found for radio-marked and unmarked females. One nest was depredated before the female could be captured and marked. Following nest failure,

22.2% of females laid a second nest (RENEST, $n = 27$ females). Females tended to be more likely to attempt second nests if first nests were destroyed during egg laying or early incubation ($\chi^2_{1,20} = 2.69, P = 0.10$; Figure 4.1), but timing of loss had no seasonal effect ($\chi^2_{1,20} = 0.12, P = 0.73$; overall logistic model $\chi^2_{1,20} = 3.42, P = 0.18$).

Nest locations

First nests were located 1.5 ± 1.0 km (range = 0.2 to 4.6 km) from the nearest lek ($n = 27$ nests) and 1.8 ± 1.3 km from the lek of capture (range = 0.2 to 4.7 km; $n = 19$ nests). Average distance between the first nests and renesting attempts within the same breeding season was 1.9 ± 1.6 km (range = 0.5 to 4.6 km; $n = 5$); whereas, the average distance between first nests in consecutive years was 1.7 ± 0.7 km (range = 1.2 to 2.5 km; $n = 3$).

Laying date

Average date of clutch initiation for first nests was 5 May \pm 13 days (range = 14 April to 3 June; $n = 27$). Laying date of first clutches did not correlate with female age, mass at capture, tarsus, or keel (all $P > 0.11$; model $F_{5,18} = 1.18, P = 0.36$). Average laying date of known re-nesting attempts was 24 May \pm 12 days (range = 4 May to 9 June; $n = 6$).

Clutch size

Average clutch size of first nests was 10.9 ± 1.7 eggs (TCL₁, range = 7 to 14 eggs; $n = 24$) and did not vary with respect to laying date, female age, mass, tarsus, or keel (all $P > 0.15$; model $F_{6,14} = 1.18, P = 0.12$). Average clutch size of renesting attempts was 10.8 ± 2.4 eggs (TCL₂, range 8 to 15; $n = 6$), and was not different from the clutch size of first nesting attempts ($F_{1,28} = 0.01, P = 0.92$). There was no evidence of interspecific nest parasitism by female ring-necked pheasants (*Phasianus colchicus*).

Egg measurements

Average egg length and width were 42.4 ± 1.1 mm and 31.7 ± 0.7 mm, respectively ($n = 32$ clutches). Average egg volume per female was 20.8 ± 1.2 cm³ ($n = 32$ clutches). Egg volume increased slightly with increasing female tarsus length ($P = 0.07$), but egg volume did not vary

with respect to laying date, clutch size, female mass, or female keel length or female age (all $F < 1.26$, $P > 0.31$; overall model $F_{7,18} = 0.77$, $P = 0.62$).

Nesting success and survival

Apparent nesting success was higher for re-nesting attempts (4 of 6 nests successful) than first nests (3 of 28 nests successful; Fisher's exact test $P = 0.01$, $n = 33$). Pooling first and second nesting attempts, apparent nesting success was low (20.6% ; $n = 34$). Of the 27 nests that failed to hatch, 85.2% of nests were depredated, 11.1% were destroyed by prescribed fires, and 3.7% were abandoned. Moreover, females were found depredated near the nest in 3 of 27 failed nesting attempts (11.1%). Probable cause of female mortality included snakes ($n = 1$) and mammals ($n = 2$). Of nests that survived until hatching, two clutches were partially depredated during incubation resulting in only 75.6 ± 35.0 % of eggs laid surviving until hatching (ES, $n = 7$ nests). Of nests surviving to hatching, egg hatchability was high and 89.3 ± 12.4 % of eggs produced chicks (HS, $n = 7$ nests).

Nest survival was modeled using data from 34 greater prairie-chicken nests. The model analyzed nest survival during an 84-day nesting period (22 April to 14 July). The best fit model included a linear effect of time on nest survival (Table 4.1). Model-averaged estimates revealed that nest survival increases throughout the nesting season from 0.868 ± 0.069 (\pm SE) during the first week (22 April) to 0.958 ± 0.023 (\pm SE) during the last week (15 July). Nest age had a positive, but nonsignificant effect on nest survival (Table 4.1, β estimate = 0.02 ± 0.01 \pm SE; 95% CI: -0.005 to 0.04). Using the constant model, nest survival throughout the entire season was 0.928 ± 0.013 (\pm SE; 95% CI: 0.897 to 0.951). On average, we found nests when they had been incubated four days, so our model predicted that our expected apparent survival should be 22.6% (0.928^{20}), which was close to our observed apparent survival of 20.6%. Assuming a 24-day incubation period, an average clutch size of 11 eggs, and constant risk of predation during laying and incubation, nest success was extrapolated to be 7.4% (0.928^{35}). Since we found most nests after the female initiated incubation, females may visit nests for only short periods during egg laying. The risk of nest predation during laying may be lower than during incubation if female attendance increases risk of detection by a predator. Under this scenario, predation exposure would be limited to the incubation period, resulting in a maximum estimate of nest

survival of 16.8% (0.928^{24}) for first nests and renesting attempts combined. Using daily survival rates from the best fit model that included a seasonal increase in daily survival rates, the proportion of nests surviving until hatching was 0.041 for first nests (HATCH₁) and 0.117 for renesting attempts (HATCH₂) for a 35-day exposure period. For a 24-day exposure period, the proportion of nests surviving until hatching was 0.088 for first nests (HATCH₁) and 0.196 for renesting attempts (HATCH₂).

Nestling measurements

Average total head length of hatchlings was 28.0 ± 0.6 mm ($n = 7$ broods) and was correlated with female mass ($\beta = 0.01 \pm 0.001$, $P < 0.01$) and the tarsus length ($\beta = -0.11 \pm 0.02$, $P = 0.02$), but not mean egg volume ($\beta = 0.18 \pm 0.19$, $P = 0.43$; model $F_{3,3} = 36.5$, $P = 0.01$). Average nestling tarsus, wing and mass were 18.6 ± 0.3 mm, 18.9 ± 1.4 mm, and 15.6 ± 1.4 g, respectively ($n = 7$ broods). None of these measurements varied with the female's mass ($P > 0.12$), the length of the female's tarsus ($P > 0.30$), or mean egg volume ($P > 0.11$; model $|F_{3,3}| < 1.74$, $P > 0.33$).

Recruitment

Fifty-one chicks were marked with patagial wing tags. Two chicks (3.9%), one male and one female, were recaptured the following spring at the lek closest to their nest. The male dispersed 1.5 km and the female dispersed 2.3 km.

Breeding survival of females

Of 43 females fitted with radio collars, seven females were never detected after capture and were censored from the analysis of breeding season survival. Four females were detected in years subsequent to the year of banding and were included twice in the data set of breeding season survival. Of the 40 females included in the survival analysis, seven were alive at the end of the breeding season (17.5%), six dropped their radio collars and were considered alive at last observation (15.0%), ten died (25.0%), and 17 were not detected (42.5%). Of the 10 females found dead, probable cause of mortality included four killed by mammals, two by raptors, two by collisions with a fence or vehicle, one by a snake, and one was of unknown cause.

The best fit model to the telemetry data for females was the constant model (S_c), regardless of whether females that were not detected greater than 2 weeks into the study were considered alive or dead (Table 4.2). If females that were not detected greater than 2 weeks into the study were considered alive, weekly apparent survival estimates were 0.970 ± 0.009 (\pm SE, 95% CI: 0.946 to 0.984). If this estimate is extrapolated, apparent survival would be 0.457 for the 6-month (26-week) breeding season (0.970^{26}). There was support for models that included an effect of mass and a linear trend (Δ AIC < 2.0; Table 4.2). Female mass at capture had a positive, but nonsignificant effect on female survival (β estimate = 0.27 ± 0.32 \pm SE; 95% CI: -0.36 to 0.90). There was a linear trend of female survival with survival estimates declining throughout the breeding season (week 1 = 0.978 ± 0.013 \pm SE; week 23 = 0.955 ± 0.032 \pm SE). If females that were not detected greater than 2 weeks into the study were considered dead, weekly survival estimates were 0.922 ± 0.014 (\pm SE, 95% CI: 0.889 to 0.946). If this estimate is extrapolated, survival would be 0.122 for the 24-week breeding season (0.922^{26}). There was support for models that included a quadratic time trend, an effect of mass and a linear time trend (Δ AIC \leq 2.01; Table 4.2), but the trends were not in the same direction as when undetected females were considered alive. The quadratic time trend showed a mid-season decrease in survival which fell to 0.893 ± 0.027 (\pm SE) with higher estimates at the beginning and end of the breeding season (0.980 ± 0.023 \pm SE). Female mass had a negative, but nonsignificant effect on female survival (β estimate = -0.10 ± 0.23 \pm SE; 95% CI: -0.55 to 0.35). There was a linear time trend of female survival with survival estimates increasing throughout the breeding season (week 1 = 0.920 ± 0.026 \pm SE; week 23 = 0.925 ± 0.037 \pm SE).

Annual survival of females

Fifty-five females were banded between 2003 and 2005, and 13 were resighted in subsequent years. One female was observed every year during the entire four-year study period. There was no evidence for overdispersion using bootstrap goodness-of-fit procedures (mean expected $\tilde{c} = 0.35$; $P = 0.74$). We set the variance inflation factor to 1 and used AICc for model selection. The best fit model included an interaction effect in apparent survival that included an effect of time separately for females after initial capture and for females that returned at least once, and a constant resighting probability (ϕ^1 , ϕ^{2+} , p_c , Table 4.3). Model-averaged estimates

showed considerable variation between years. Weighted average estimates of apparent survival of females after first capture was 0.277 ± 0.081 (\pm SE, range: 0.180 to 0.341) and in subsequent intervals was 0.424 ± 0.139 (\pm SE, range: 0.355 to 0.430). Overall estimates of apparent survival of females regardless of time since capture was 0.328 ± 0.083 (\pm SE, 95% CI: 0.189 - 0.506). Model-averaged estimates of resighting probability were constant throughout the study at 0.857 ± 0.174 (\pm SE; 95% CI: 0.270 - 0.990).

Nonbreeding survival of females

We calculated survival during the 6-month nonbreeding season (mid-September to mid-March) from our estimates of annual survival and 6-month breeding season survival (mid-March through mid-September). Annual survival was 0.424 for females that showed site-fidelity and breeding season survival was 0.456 for radio-marked females, so nonbreeding survival was $0.424/0.456 = 0.930$. Thus, survival during the nonbreeding season is 2.0 times higher than the breeding season ($0.930/0.456$). If we use our overall estimate of annual survival of females (0.328), survival during the nonbreeding season was $0.328/0.456 = 0.719$. This nonbreeding survival estimate is 1.6 times higher than survival during the breeding season ($0.719/0.456$).

Synthetic estimates

Our unbiased estimate of fecundity per nest (F_n) that controlled for variation in nest exposure was 0.24 and 0.47 female chicks per female using estimates of nest survival based on 35 and 24 day exposure periods, respectively. Assuming a constant rate of population growth ($\lambda = 1$) and annual female survival (S_{fem}) = 0.424, juvenile survival (S_{juv}) would have to be > 1.0 to maintain a stable population. A more realistic estimate for S_{juv} is 0.124 from a field study of lesser prairie-chickens (Pitman et al. 2006). Using this value for juvenile survival, the rate of population growth would be 0.45 or 0.48 using F_n values of 0.24 and 0.47, respectively. A λ value less than one predicts declines in population size. In order to maintain a stationary population for the field estimates of juvenile and adult survival, fecundity per nest would have to be increased to 4.64 female chicks per female. This is equivalent to nearly 100% nesting success since clutch size averages 11 eggs.

Discussion

Our field data show that greater prairie-chickens in natural grasslands experience high rates of predation on nests and incubating females. These results complement modeling studies that found that low nesting success may be the primary demographic factor limiting population viability in greater prairie-chickens (Wisdom and Mills 1997; Robel et al. 2003). We provide updated demographic rates in the core of the greater prairie-chicken range. Our parameter estimates are critical to conservation monitoring since previous estimates do not reflect land use changes or statistical advances of the past 20-30 years. Our estimates of nest survival control for varying exposure and produce biologically-meaningful estimates by including nest age as a covariate (Dinsmore et al. 2002). Our estimates of apparent survival account for the variation in the probability of encounter (Sandercock 2006). All females in our study attempted nesting, and hatchability of eggs was high suggesting that our study population is genetically healthy (Westemeier et al. 1998). Additional loss and fragmentation of native prairie could affect female greater prairie-chicken movements up to 3.7 km and nesting up to 1.5 km from the disturbance. Conservation efforts should focus on increasing nesting success and protecting contiguous blocks of habitat within 4 km of lek sites.

Prairie-chicken movements in our study area are larger than estimates in other locations. The average distance between first nests of a female in subsequent years was 1.7 km, a figure that two times greater than previously reported estimates (0.8 km; Schroeder and Braun 1993). Average distance between nests and the nearest lek (1.5 km) was also greater than previously reported distances (1.1 km, Robel 1970a; 0.5 km, Horak 1985). This could be due to the large amount of habitat available in Kansas. Our estimates of MCP home ranges of female prairie-chickens (~413 ha) were similar to previous estimates for birds in Colorado (213-624 ha using 75% probability contours; Schroeder 1991). Our estimates may be biased due to the relatively few number of positions and females used to obtain home range estimates. However, previous studies have shown that the number of locations is not as important as number of individuals (Girard et al. 2006), so future studies should focus on fitting transmitters on as many birds as possible. In addition, habitat selection studies should utilize kernel estimates to accurately define territory boundaries and size (Barg et al. 2005).

Nesting success is the primary variable limiting prairie-chicken population growth (Wisdom and Mills 1997), so it is important to have contemporary estimates to guide management decisions. Our estimates of the probability a female will initiate nesting (100%) was high and consistent with previous studies (90-100%; Wisdom and Mills 1997). However, our apparent nesting success (20.6%) was considerably lower than other reported rates (48%, Hamerstrom 1939; 26%, Robel 1970a; 30%, Horak 1985; 50%, Peterson and Silvy 1996) including those for the endangered subspecies of greater prairie-chicken (Attwater's prairie-chicken *T. c. attwateri*; 39%, Lockwood et al. 2005). Observed rates of nesting success are biased high because they do not consider the nests that were destroyed before discovered by an observer. Our estimate of apparent nest survival might have been more comparable to past estimates of nest survival if previous field studies also located nests during incubation but controlled for exposure before discovery (Green 1989). We report one of the first applications of nest survival models to greater prairie-chicken nesting success. The daily survival estimate of 0.928 derived from this model controls for varying exposure and allows for direct comparisons among studies. There was support for a model that included a linearly increasing trend of nest survival throughout the breeding season. This could be due to a combination of increased frequency of prescribed burning early in the breeding season and increased nesting cover later in the breeding season. If our estimate for daily nest survival is extrapolated to include the egg-laying and the entire incubation period, nesting success would be 7.4%. This value is considerably lower than apparent rates of nesting success, and indicates an urgent need for more proactive prairie-chicken conservation. Future management experiments could include predator removal (Schroeder and Baydack 2001), changes in rangeland management to increase nesting cover (Schroeder and Robb 1993), and limiting further habitat loss.

High nest failure may be why we observed higher rates of renesting (22.2%) than previous studies (0-17%; Wisdom and Mills 1997, Lockwood et al. 2005). Higher rates of renesting mitigated the effect of prescribed fires, but females moved long distances to locate unburned areas with suitable nesting cover for renesting attempts. In addition, we have shown that the probability of renesting varies with the timing of destruction of the initial nest: females are more likely to renest if their first nest was destroyed during laying or early incubation than if the nest was destroyed during late incubation similar to willow ptarmigan (*Lagopus lagopus*,

Robb et al. 1992). Prescribed fires should be set before mid-April to encourage re-nesting and allow adequate time for re-nesting.

We could not detect any relationships between female mass or size and egg size, clutch size, egg hatchability, or chick size, contrary to analyses for other grouse species (Moss et al. 1981, Robb et al. 1992, Martin and Horn 1993). Estimates of clutch size and egg hatchability were similar to previous studies (clutch size: 10.9 eggs, this study; 12.0, Hamerstrom 1939; 11.6, Robel 1970a; 10.7, Horak 1985; 11.3, Schroeder and Robb 1993; 12.1 Peterson and Silvy 1996; egg hatchability: 89%, this study; 98%, Hamerstrom 1939; 86-93%, Lutz et al. 1994; 89%, Peterson and Silvy 1996; 91%, Lockwood et al. 2005). We may have failed to detect a relationship between females and their eggs or offspring because weather has a greater impact on grouse survival and reproduction than individual variation (Dusek et al. 2002).

Seasonal estimates of survival indicate that female greater prairie-chickens are more vulnerable during the breeding season. A seldom-documented observation was high mortality among incubating hens (11.1%, this study; 9.2%, Hamerstrom 1939). Breeding season survival of females was 45.7% using known fate models. Breeding season survival of our population of greater prairie-chickens is somewhat higher than estimates for the endangered subspecies, Attwater's prairie-chicken (36%; Lutz et al. 1994). Our results suggest that predation is the proximate factor mediating female breeding season survival. This result is similar to a study on lesser prairie-chickens that found low female survival during the breeding season, especially among females attending broods (Hagen et al. 2007). However, other proximate factors, such as parasite loads, nutritional stress or increases in accidental deaths, could also be important (Hannon and Martin 2006). The annual estimates of apparent survival obtained from Cormack-Jolly-Seber models for live encounter data (42%) are comparable to return rates previously reported for prairie-chickens (41-56%, Hamerstrom and Hamerstrom 1973; 24-57%, Wisdom and Mills 1997), but improve upon previous estimates of return rates by controlling for probability of encounter (Sandercock 2006). Our estimates of apparent survival could be biased low if radio-transmitters negatively affected the survival of hens or if there is substantial emigration from our study site (Hagen et al. 2006, Sandercock 2006, Mong and Sandercock 2007). Radio-transmitters are not likely to have affected our results because similar transmitters did not reduce the survival of lesser prairie-chickens (Hagen et al. 2006) and the necklace

transmitters used in this study were lighter and less restrictive than the backpack transmitters used in early studies of greater prairie-chickens (Burger et al. 1991). We partially accounted for permanent emigration from our study site by using time-since-marking models that partition survival estimates between individuals that were only captured once with those that were captured or resighted multiple times (Sandercock 2006). The pattern of low breeding season survival, especially during incubation, as compared to higher nonbreeding survival may be a general feature of ground-nesting grouse and waterfowl (Kirby and Cowardin 1986, Devries et al. 2003, Hagen et al. 2007).

We estimated that juvenile survival (from hatching until the following spring) would have to be greater than 100% to maintain a stable population given our field estimates of other demographic parameters. Empirical estimates are more likely to be 12% to 38% ($38\% = 55\% \text{ brood survival} * 69\% \text{ post-brood survival}$, greater prairie-chickens, Wisdom and Mills 1997; 12.4%, lesser prairie-chickens, Pitman et al. 2006). Therefore, the greater prairie-chicken population examined in this study is unlikely to be maintained by current levels of fecundity and survival. Juvenile grouse mortality increases with resource extraction, agriculture, and grazing which create less area of high-quality forage (Hannon and Martin 2006). If few food resources are available, broods concentrate, increasing competition and predation. Every effort should be taken to create large areas with high quality brood forage in addition to increasing nesting success.

In the past 25 years, greater prairie-chicken populations have been extirpated or are in danger of being extirpated in 15 states and provinces and show declining trends in seven states (Schroeder and Robb 1993). Despite prairie-chicken's economic importance as a game bird and target for naturalists, current management decisions are based on demographic estimates that are 20 to 30 years old. We propose that increases in productivity, female survival during the breeding season, and juvenile survival from independence to spring are needed to maintain stable populations in the northern Flint Hills of Kansas. Our study was based on a single field site near Manhattan, Kansas, and further research is needed to determine if prairie-chicken demography varies in different regions of the Flint Hills. Land use in the Flint Hills of Kansas is changing due to encroachment of woody plants, increased development of wind power resources, and loss of conservation reserve program acreage in favor of ethanol-producing crops. Rangeland

practices that increase residual nesting cover or reduce predator impacts are needed, as well as experimental tests to confirm predation and nesting cover as the proximate mechanisms mediating variation in survival of nests and breeding females.

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Table 4.1 – Comparison of nest survival models examining the survival of greater prairie-chicken nests in northeast Kansas between 2003 to 2005 ($n = 34$). Table abbreviations are as follows: S = parameters included in the survival model; Dev = deviance, K = number of parameters; AICc = Akaike’s Information Criterion corrected for small sample sizes; Δ AICc = difference of the AICc value in the given model compared to the minimum AICc model; and w_i = Akaike weights. Model abbreviations are as follows: lin = linear time; age = nest age; c = constant; * = interaction; and + = main effects model.

Model	S	Dev	K	AICc	Δ AICc	w_i
1	lin	139.5	2	143.5	0.00	0.35
2	age	140.1	2	144.2	0.64	0.25
3	c	142.6	1	144.6	1.04	0.21
4	lin+age	139.4	3	145.5	1.95	0.13
5	lin*age	139.2	4	147.3	3.82	0.05

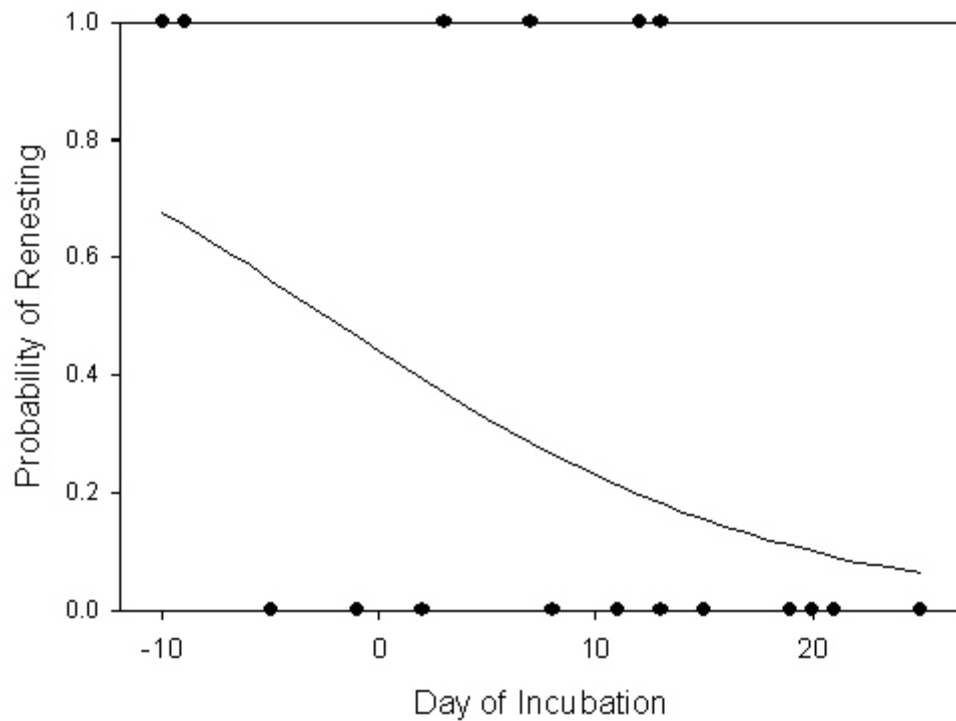
Table 4.2 – Comparison of nest survival models examining the survival of radio-marked females during the breeding season ($n = 40$ females). Females included in the analysis were marked at three greater prairie-chicken leks between 2003 to 2005 in northeast Kansas and were detected two weeks after banding. Seventeen birds were not detected during the period two weeks after banding and the end of the breeding season (1 September). Two data sets were analyzed: one assuming these birds were alive at the end of the study and the other assuming these birds were dead. Table abbreviations are as follows: S = parameters included in the survival model; Dev = deviance, K = number of parameters; AICc = Akaike’s Information Criterion corrected for small sample sizes; Δ AICc = difference of the AICc value in the given model compared to the minimum AICc model; and w_i = Akaike weights. Model abbreviations are as follows: t = time; mass = mass at initial capture; lin = linear seasonal trend; quad = quadratic seasonal trend; c = constant; and + = additive model.

Model	S	Dev	K	AICc	Δ AICc	w_i
Assuming birds that were not detected to have survived						
1	c	83.1	1	85.1	0.00	0.43
2	mass	82.4	2	86.5	1.35	0.22
3	lin	82.7	2	86.8	1.67	0.19
4	quad	81.7	3	87.8	2.72	0.11
5	quad+mass	81.4	4	89.5	4.39	0.05
6	t	69.5	23	119.1	34.01	0.00
Assuming birds that were not detected to have died						
1	c	182.9	1	184.9	0.00	0.40
2	quad	180.0	3	186.1	1.23	0.21
3	mass	182.7	2	186.7	1.83	0.16
4	lin	182.9	2	187.9	2.01	0.15
5	quad+mass	179.9	4	188.0	3.15	0.08
6	t	153.7	23	203.2	18.31	0.00

Table 4.3 – Comparison of Cormack-Jolly-Seber mark-resight models examining the apparent survival of individually marked females between breeding seasons ($n = 55$ females). Females were marked and observed at four greater prairie-chicken leks during a four-year period in northeast Kansas, 2003 to 2006. Table abbreviations are as follows: ϕ^1 = apparent survival for females after initial capture; ϕ^{2+} = apparent survival of females in subsequent intervals; p = resight probability; Dev = deviance, K = number of parameters; AICc = Akaike’s Information Criterion corrected for small sample sizes; Δ AICc = difference of the AICc value in the given model compared to the minimum AICc model; and w_i = Akaike weights. Model abbreviations are as follows: t = year and c = constant. Separate abbreviations for ϕ^1 and ϕ^{2+} indicates a time-since-marking model; whereas, an abbreviation between ϕ^1 and ϕ^{2+} indicates that the two classes were not modeled separately.

Model	ϕ^1	ϕ^{2+}	p	Dev	K	AICc	Δ AICc	w_i
1	t	t	c	1.6	6	90.2	0.00	0.28
2		c	c	11.0	2	90.4	0.19	0.25
3		t	c	10.1	3	91.7	1.53	0.13
4	t	t	t	0.7	7	91.8	1.57	0.13
5		c	t	8.9	4	92.8	2.59	0.08
6		t	t	6.8	5	93.0	2.83	0.07
7	c	c	c	10.0	4	93.9	3.71	0.04
8	c	c	t	8.0	5	94.3	4.06	0.04

Figure 4.1 – Probability of renesting as a function of the stage of development of the first nesting attempt when it was destroyed. First nests of 23 females were destroyed by natural causes from 2003 to 2005 near Manhattan in northeast Kansas. Points on the x-axis represent females that renested ($y = 1$) or did not attempt renesting ($y = 0$). Nests with negative values for day of incubation were destroyed during egg laying.



CHAPTER 5 – Conclusions

Sexual selection and natural selection result from differential reproductive success and survival. Lek mating systems provide a unique opportunity to study the various selective pressures of males and females without interactive effects found in monogamous mating systems. Greater prairie-chickens are a lek-mating species that is experiencing population declines in many parts of its range. Thus, a better understanding of the factors affecting mating and reproductive success is necessary for the successful management of this species. The objectives of this research were: 1) to determine which male traits correlate with mating success, 2) to identify the role of testosterone in determining the variation among males with respect to male traits and mating success, 3) to test whether male traits are honest signals of male quality by examining the correlation between male traits and annual survival, and 4) to examine prairie-chicken demography and the correlates of female reproductive success.

My field research analyzed the correlations between male traits and mating success, and provides the first detailed account describing how individual behavior affects male mating success of greater prairie-chickens (Chapter 2). I also presented the first application of multinomial discrete choice models for the analysis of mate choice data. Male prairie-chicken behavior showed high variability among males and had the greatest impact on mating success when compared to morphological or territorial traits. Both display and aggressive behaviors were strong predictors of male mating success, suggesting that both female choice and male-male aggression were important in determining mating success among male greater prairie-chickens. My research emphasizes the importance of examining multiple male traits in unison. In the future, comb color and vocalizations should be examined with respect to mating success and survival to determine if these traits may be honest signals of male quality.

I addressed links among testosterone, male traits and mating success in a lek-mating bird by examining natural variation and experimental increases in testosterone (hereafter ‘T’). I improve upon previous studies by analyzing a large number of plasma samples from unmanipulated males and using T-implants to determine causation. Sample sizes of the natural variation in T presented in Chapter 3 are 4 times those reported in previous studies, thereby increasing my statistical power to detect trends. My research is only the third study manipulating T levels in a lek-mating bird. Natural variation and experimental increases in T indicated that

testosterone was a strong predictor of mating success, but T was not related to any male morphological, behavioral or territorial traits considered. Future work should examine the link between T and male parasite load, coloration of plumage and vocalizations because it is unlikely that females are assessing T levels directly. To further elucidate seasonal trends in T levels, fecal analysis may provide a non-invasive method to obtain multiple samples from individual males.

I failed to detect correlations between annual survival and male traits or mating success. Annual survival of male prairie-chickens was independent of behavior, morphology, territory attributes, or T levels. Without survival costs associated with the expression of the male traits, these traits may not be honest signals of male quality. An alternative explanation is that high mortality due to predation may limit my ability to detect survival costs associated with the expression of sexually-selected traits. In other words, natural selection may be a stronger selective force than sexual selection in determining annual survival.

In Chapter 4, I presented the first demographic data in 30 years for greater prairie-chickens in Kansas, the core of their range. These metrics are needed because burning and grazing regimes have changed during the same time period. An interesting result of my study is that, like males, reproductive success of females was also highly skewed. Less than 10% of nests successfully hatched young. However, potential reproductive output was high since females laid large clutches, frequently re-nested following clutch loss, and had high egg viability. Comparisons of seasonal and annual survival rates indicate that survival is ~1.6 times higher during the nonbreeding season than the breeding season, presumably because of increased susceptibility to predation during incubation. Synthesis of field estimates of demographic parameters indicates prairie-chicken populations will decline without changes in rangeland management to reduce predator numbers and provide more nesting cover.

Taken together, this research and declining population trends provide a strong argument for prairie-chicken conservation in at least the northern Flint Hills of Kansas. High skew in both male and female reproductive success suggest that the effective population size of prairie-chickens is much less than the numbers observed on leks. Since only a small subset of adults are contributing offspring to successive generations, genetic diversity of prairie-chickens in the Flint Hills should be quantified and monitored. Previous studies in greater prairie-chickens have shown that low genetic diversity can have detrimental effects on reproduction. High rates of nest

and adult predation suggest that insufficient protective cover may be the primary cause of population declines. Habitat fragmentation may also increase the abundance of predator populations and create corridors for easy movement of predators across the landscape.

In summary, selection for intense male display and aggressive behavior may be a general trend in lek-mating birds. Testosterone appears to be a proximate mechanism mediating variation in male mating success, but how females utilize testosterone levels to choose a mate is still unknown. Variation in female reproductive success is probably a result of high predation pressure. Both nesting success and survival of juveniles and adults are depressed in our population located in the core of the range of the greater prairie-chicken. Rangeland management should be modified to reduce predator abundance, provide increased nesting cover, and improve survival of juveniles and adults.