

**EFFECTS OF WIND POWER ON THE DEMOGRAPHY AND POPULATION
GENETICS OF THE GREATER PRAIRIE-CHICKEN**

QUARTERLY REPORT

Submitted by:

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Presented to:

**National Wind Coordinating Council
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NREL Subcontract Administrator:

Quarterly Report
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EXECUTIVE SUMMARY

Field.— Radio-transmitters were fitted to 48 new females in the spring of 2009 and 20 females with active radio-transmitters from previous years were monitored. In our first season of post-development monitoring, 69 nests were located; 39 at the impact site and 30 at the reference site. Apparent nest success was 20.9 and 41.3% at the impact and reference sites respectively. Apparent brood success was 42.9 and 30.0% at the impact and reference sites. Within successful broods, 49.9% of chicks survived from hatching until fledging. Monte Carlo simulations revealed that female prairie-chickens did not avoid wind turbines, high-capacity transmission lines, substations, access roads, improved roads, or unimproved roads when selecting nest sites during the first breeding season post-construction. However, given negative impacts of development on attendance of leks in close proximity to wind turbines (see previous report), delayed avoidance of the wind power facility by nesting hens are possible. We will assess the possibility of a lag effect. We documented 19 confirmed mortality events at Unit 3 during March – September; most mortalities (90%) were the result of predation. We collected >1600 locations of radio-marked birds during the quarter.

Laboratory.— Efforts in the lab this quarter focused on completing DNA sample extraction from the 2009 adult bird samples, and PCR amplification of extracted samples at all loci, including 13 new microsatellite markers. In addition, we began DNA extraction and amplification of the 2009 chick samples. Trapping efforts in 2009 were nearly entirely focused on Unit 3, and in 2009 we added 119 adult samples to our overall samples size of 632 adult birds collected over the three years of this study. Tracking and nest monitoring continued throughout the summer and resulted in the addition of 128 chicks from 24 broods to our paternity and dispersal analysis, which now includes data on 273 chicks from 53 broods sampled across all three research units over the three years

that data have been collected at all units. Overall genetic diversity across all three populations, using the 11 microsatellites for which we have data at all 3 research units, remains relatively high ($H_O = 0.71$, $H_E = 0.78$, $AR = 7.9$). In addition there continues to be some indication of genetic isolation of the three research units not consistent with equilibrium assumptions of isolation by distance (Table 3). We also completed molecular sexing of all chicks from 2007-2008 trapping seasons. Over the first two years of this study we sampled 146 chicks, 76 (52%) of which were male, which gives a juvenile male/female sex ratio of 1.1 males per 1 female. Finally, over the last quarter we have been working to increase the number of markers being used for our analysis at Unit 3 (Table 4). More microsatellites will increase the sensitivity of our analysis to better quantify any effects wind power development may have on dispersal of prairie-chickens across the landscape as well as increase our ability to unambiguously assign paternity and provide molecular identity of individuals. We have currently screened a total of 28 markers for use on Unit 3 samples, 24 of which have been found to be polymorphic; currently about 20% of the Unit 3 samples have been amplified at these additional markers.

Administration and Reporting.— As of 30 September, we have finished spending funds administered by NWCC. We have been awarded a \$149,546 State Wildlife Grant by US Fish and Wildlife Services and Kansas Dept. of Wildlife and Parks. Although the grant was to begin October 1, the contract is still under negotiation with our pre-awards administrators, but final approval appears imminent. We have also been awarded \$299,998 from the Department of Energy with a start date of 1 October. Although we have received assurances from the Washington DC office that we will receive funds, it is unclear when these funds will be available. The DOE contract office is mandated to process \$5B of Federal Recovery Act contracts before they can start processing other grant contracts. It is unclear if we will have a contract in place before the end of the calendar year. I have asked the KSU Division of Biology for gap funding until either of

these contracts is in place so that we do not have to lay off employees. Their decision is pending.

FIELD BASED HYPOTHESES

Hypothesis I) Lek Attendance: *Lek attendance on impact sites is not affected by wind-power development.*

Accomplishments Since Last Quarter

The lekking season occurs from March – May. No data were collected during this quarter to address this hypothesis.

Goals For Next Quarter

The lekking season occurs from March – May. No effort will be made to address this hypothesis in the upcoming quarter.

Hypothesis II) Avoidance of anthropogenic structures: *Prairie-chickens do not avoid wind-towers and/or other anthropogenic features on impact sites.*

Accomplishments Since Last Quarter

Radio-marked females were monitored ≥ 3 times per week from project trucks during the nesting and brood-rearing period (Apr–Aug) and ≥ 1 time per week during the rest of the year (Sep–Mar). Once a female localized in an area for three successive days, we used a portable radio receiver and handheld Yagi antenna to locate the incubating female. We flushed the female once in early incubation to count the eggs, to determine the stage of incubation, and to record the nest location with a GPS. Females with nests were monitored daily at a distance by triangulation of the radio signal. Once it was determined that the female was no longer tending the nest, we classified nest fate as successful (≥ 1 chick produced) or failed.

We used a GPS to record the locations of wind turbines, substations, high-capacity transmission lines, newly constructed wind park access roads, and non-wind related vertical structures (i.e., cell and weather towers) at the reference and impact sites of the Meridian Way study area. These locations were uploaded to a geographic information system (ArcMap 9.3, Environmental Systems Research Institute, Redlands,

CA) and overlaid onto a land-cover map of the study area obtained from the Kansas Applied Remote Sensing Program at Kansas University (<http://www.kars.ku.edu/products/ksid/index.shtml>). A roadway dataset that combined the 2006 Kansas State and Non-State Road System datasets (Kansas Department of Transportation: Bureau of Transportation Planning) was uploaded to the GIS. We created two additional layers containing nest locations from the study period prior to wind power development ($n = 63$) and after wind power development ($n = 69$) and calculated distances (m) from nests to each of the nearest wind turbine, substation, high-capacity transmission line, newly constructed wind park access road, state and local roads, and non-wind related vertical structures. For nests from the pre-construction period, distances were measured to where these features would eventually be constructed.

Distances from nests to anthropogenic features did not fit parametric distributions; therefore a Kruskal-Wallis test was used to test the null hypothesis that the distributions of nest-feature distances were similar for the pre- and post-construction nests. In addition, we used Monte Carlo simulations to determine the degree to which nest locations were impacted by the 7 anthropogenic features. A minimum convex polygon was drawn around the nests to define a biologically meaningful study area and a number of random points equal to the number of nests ($n = 69$) was selected inside this polygon using ArcMap 9.3. We created 10,000 simulated data sets of 69 random points within the study area. The distance to each of the nearest anthropogenic feature was measured for each random point. From each of the 10,000 random data sets, the shortest measured distance was selected and these distances were used to create distributions of distances from each type of feature to the nearest random point. We then compared the nearest distance from a nest location to a feature to the appropriate Monte Carlo distribution. A *P*-value was calculated as the proportion of nearest random points that were at least as far away from the feature as the observed nest. We repeated this process for the second closest nests, third closest, and so on up to the point when avoidance did not occur ($P > 0.05$). This approach is preferred to parametric comparisons such as one-way analysis of variance between nest proximities and random point proximities because (1) it lacks assumptions of parametric tests that may bias inferences of avoidance, (2) it is directly comparable to previous work assessing nesting avoidance of energy developments by

Lesser Prairie-Chickens (Pitman et al. 2005), and (3) it allows assessment of potential avoidance thresholds by systematically comparing the first, second...etc. closest nests to an associated random point distribution.

The 201 MW Meridian Way wind power facility includes 67 3-MW wind turbines, 26 km of 360 -kV transmission lines, 37 km of newly developed access roads, and 2 new substations (Fig. 1). We collected GPS locations for 132 nests; 63 nests were located during the 2-year pre-construction period (2007-08) and 69 nests were located during the first year of post-construction monitoring (2009). Median proximities between nests and wind turbines were 5.8 and 5.6 km for pre- and post-construction nests, respectively. The distribution of nest-nearest turbine distances did not differ between the pre-construction and post-construction samples ($H = 1.3$, $P = 0.2$, $df = 1$: Fig. 2). Results of Monte Carlo simulations revealed that nest placement during the first season of post-construction nesting was not influenced by any of the anthropogenic features evaluated. Indeed the closest nests were not any farther from the nearest feature than would be expected at random (Table 1); precluding further analysis of lower order nests. Notwithstanding, given the negative effects of the development on the attendance of leks in close proximity to turbines (see previous quarterly report), assessment of delayed impacts on nest placement will be necessary before inferences can be made regarding avoidance.

Goals For Next Quarter

The nesting and brood rearing period occurs during May – July. Therefore no data will be collected to address this hypothesis in the next quarter. However, extension of this analysis to additional nesting seasons will be necessary to assess delayed impacts of the wind power facility on nest site selection. ***Caution should be used when interpreting these results until lag effects can be evaluated.***

Hypothesis III) Impacts on Fecundity Rates: *Wind development will not reduce nest success or chick survival.*

Accomplishments Since Last Quarter

Radio-transmitters were fitted to 48 females in the spring of 2009 (see previous quarterly report). In addition, 20 females captured in previous years were still alive with active radio-transmitters and were monitored in 2009. Radio-marked hens were located by triangulation or homing ≥ 3 times/week from project trucks, an ATV, or on foot. When females localized in an area and their estimated location did not change for 3 successive days, we used portable radio receivers and handheld Yagi antennas to locate and flush the female so that the eggs could be counted and nest location recorded with a GPS unit. If the nest was first found during egg-laying, nest sites were visited again in < 2 weeks to determine final clutch size and nest status. During the first visit during incubation, eggs were removed and carried > 200 m from the nest and floated in a small container of luke-warm water to assess stage of incubation, estimate hatch date, and estimate the date of clutch initiation by backdating. Nest sites were not visited again until it was determined that the female had departed the nest and was located away from the nest for ≥ 2 consecutive days). Once the female departed the nest, we returned to the nest site and classified nest fate as successful (≥ 1 chick hatched and left the nest), failed (e.g., hail damage, trampled by livestock), depredated, or abandoned. For successful nests, hatchability was calculated as the percentage of eggs that hatched and produced chicks.

Within 3 days of hatching, chicks were captured off of the nests, counted, measured and fitted with uniquely marked patagial tags. After processing, chicks were immediately released at the capture site. Pre-fledging brood survival was estimated by conducting flush counts within 2 days of the 14th day post hatch (the average day of fledge) at dawn or dusk to determine the number of surviving chicks in the brood. For counts of 0 chicks, the brood hen was flushed again the following day to be certain no chicks remained in the brood.

In 2008, 69 nests were located at Unit 3 (39 impact, 30 reference). Mean clutch sizes were 13.3 (0.3) and 10.5 (0.6) for first nests and renests, respectively. Of 52 first nests, 14 nests successfully hatched ≥ 1 chick. Six of 14 renesting attempts successfully hatched. Apparent nest success for all nests combined was 29.4%. Apparent nest success

was 20.9% and 41.3% for the impact and reference sites, respectively. Median nest initiation dates for first nests was 23 April. Hatch rate of eggs in successful nests was $84.5 \pm 3.1\%$ chicks per egg and was similar between impact and reference areas (Table 1). Mean (\pm SE) clutch sizes for first and renests that were known to be complete were 12.9 ± 0.2 and 10.2 ± 0.7 , respectively.

Six of 17 broods successfully produced fledglings for an apparent brood survival rate to fledge of 35%. Apparent brood success differed between the impact and reference areas (Table 1). However, sample sizes were small. Within successful broods, 49.9% of chicks survived from hatching until fledging with greater apparent chick survival to fledge at the reference area (Table 1).

Goals For Next Quarter

We will use the nest survival model in Program MARK to generate maximum likelihood estimates of daily nest survival. Multiple model selection and inference will be used to evaluate the importance of multiple sources of variation on daily nest survival prior to wind power development. Explanatory variables will include: nest age, nest attempt (first or reneest), hen age, VOR (dm), COVER, and distances to anthropogenic features. We will calculate the expected nest survival probability by raising the daily nest survival estimate to an exponent equal to the mean incubation interval for prairie-chickens on the study site. The duration of laying and incubation periods will be determined from direct observations of successful nests discovered during laying, or from published values in the literature if necessary. Confidence intervals will be estimated with the delta method.

Systematic flush counts were used to estimate survival during the pre-fledging (0-14 days) and post-fledging periods (14-60 days). Because broods were not observed daily, we will use the nest survival model type in Program MARK to evaluate daily brood survival probabilities. Covariates will include hen age, a forb:grass cover index, VOR, and average home range distance to closest anthropogenic feature. Daily brood survival probabilities will be calculated using maximum likelihood estimates.

Hypothesis IV) Impacts on Breeding Habitat: *Placement of wind-towers and related structures does not impact the habitat use of breeding greater prairie-chickens.*

Accomplishments Since Last Quarter

We conducted an extensive analysis to evaluate the relationship between leks and habitat characteristics at multiple spatial scales in areas without wind power development. The following manuscript was accepted, revised and re-submitted to *Studies in Avian Biology*:

Gregory, A. J., L. B. McNew, T. J. Prebyl, B. K. Sandercock, and S. M. Wisely. *In press*. A multi-scale hierarchical modeling approach to mapping lek habitats of Greater Prairie-chickens in eastern Kansas. *Studies in Avian Biology*.

Goals For Next Quarter

The lekking season occurs during March-June. Therefore no activity is scheduled for this task.

Hypothesis V) Impacts on Survival: *Wind-power development does not increase mortality rates of greater prairie-chickens.*

Accomplishments Since Last Quarter

Radio-transmitters equipped with mortality switches were fitted to 48 females in the spring of 2009 (see previous quarterly report). In addition, 20 females captured in previous years were still alive with active radio-transmitters and were monitored in 2009. Radio-marked hens were located by triangulation or homing ≥ 3 times/week from project trucks, an ATV, or on foot. When a mortality signal was heard, the transmitter was located and retrieved. Probable causes of death were determined by field investigation and necropsies when carcasses are retrievable. However, scavenging often confounds the determination of mortality causes. Mortality events were classified as (1) predation, (2) hunter, (3) collision, or (4) unknown. Unknown causes included mortalities with conflicting or confounding signs of mortality (e.g., raptor flushed from carcass with mammalian chew marks).

The transmitters of 33 females were recovered and 19 mortalities were confirmed during 3 March – 30 September 2009. We could not determine whether 14 recovered

transmitters were the result of death or loss; therefore March – September survival was 51.5 – 72.1% at unit 3. The majority of mortality events (90%) were the result of predation (7 mammalian, 7 avian, 3 unknown). One bird died due to suspected collision with a fence, and one hen died as the result of capture. We have not documented any cases of hunting mortality in radio-marked females. We continue to track 25 birds at the time of this report. If appropriate, surviving birds that are recaptured in spring 2010 will be equipped with new transmitters and their survival status updated to alive from censored.

Goals For Next Quarter

We will continue to monitor prairie-chickens weekly for survival. A complete survival analysis will be conducted to estimate monthly and annual survival of females at impact and reference areas. We will begin trapping prairie-chickens in March 2010. Our goal will be to capture and equip ≥ 50 females at unit 3 (25 impact, 25 reference) with radio-transmitters and band $>80\%$ of the males on each lek trapped. Recaptures of marked birds will be recorded to estimate annual apparent survival via mark-recapture statistics.

Table 1. Reproductive statistics of female greater prairie-chickens at impact and reference areas of the Meridian Way Wind Power Facility, 2009

Statistic	Impact	Reference
No. of nests	30	39
Clutch Size		
First nests	13.0 (0.3)	14.1 (0.6)
Renests	10.1 (0.6)	11.2 (0.7)
Apparent nest success (%)	20.9	41.3
Hatch rate (successful nests; %)	83.4	85.4
Apparent brood success (%)	42.9	30.0
Fledglings per chick hatched	0.27	0.68

Table 2. Monte Carlo simulation results of distances (m) of greater prairie-chicken nests to anthropogenic features at the Meridian Way wind power facility in Cloud County, Kansas, 2009.

Feature	Median nest- feature distance	Near nest proximity to feature	P^a
Wind turbine	5,809	74	0.79
Access road	5,805	74	0.78
Transmission line	6,107	84	0.78
Substation	8,301	316	0.38
Improved road	3,919	107	0.47
Unimproved road	366	22	0.35

^a P -value = the proportion of 10,000 data sets in which the nearest random point was at least as far away as the nearest nest.

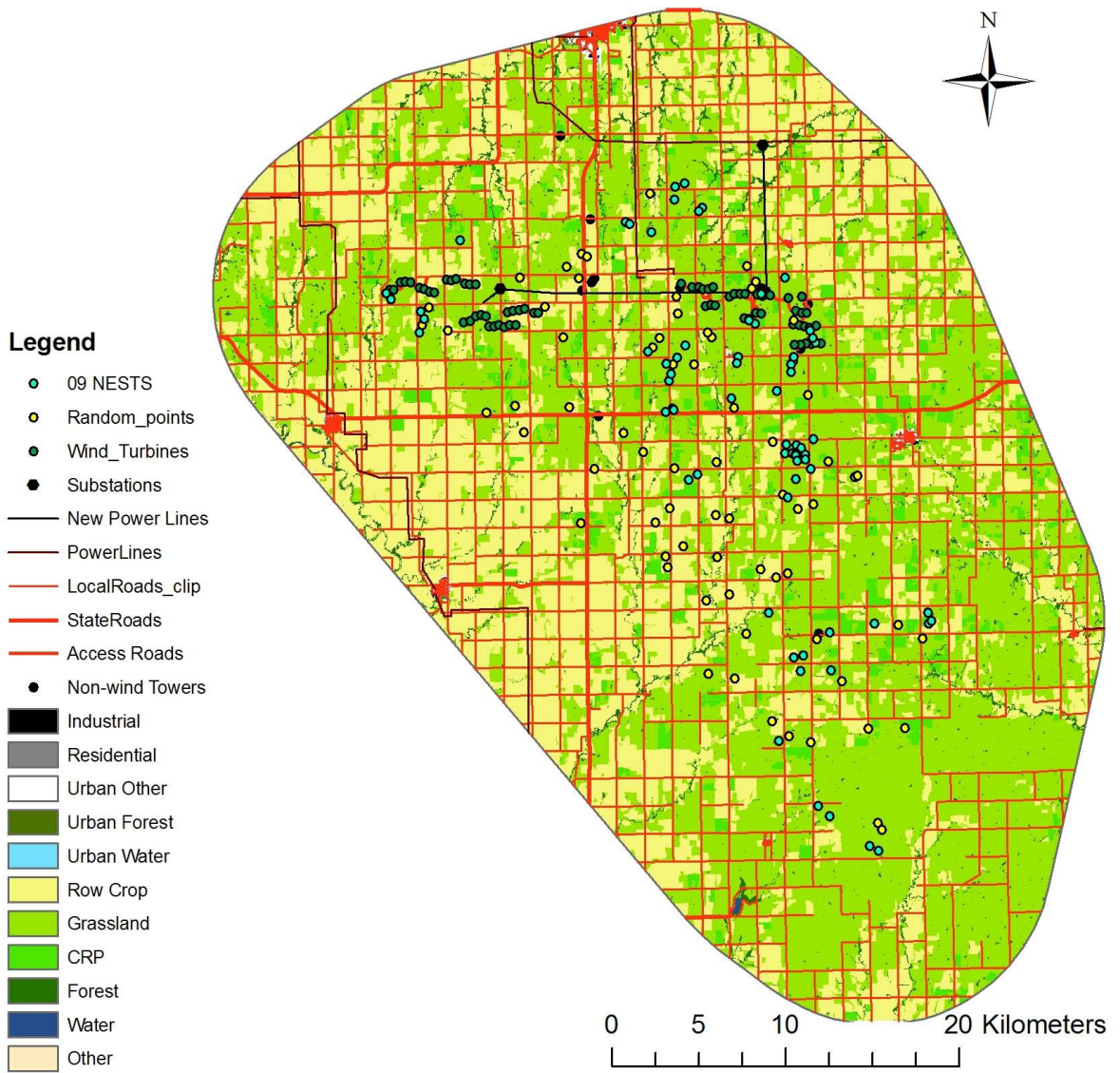


Figure 1. Locations of wind turbines and associated infrastructure, greater prairie-chicken nests, and random points at the Meridian Way wind power facility, Cloud County, Kansas.

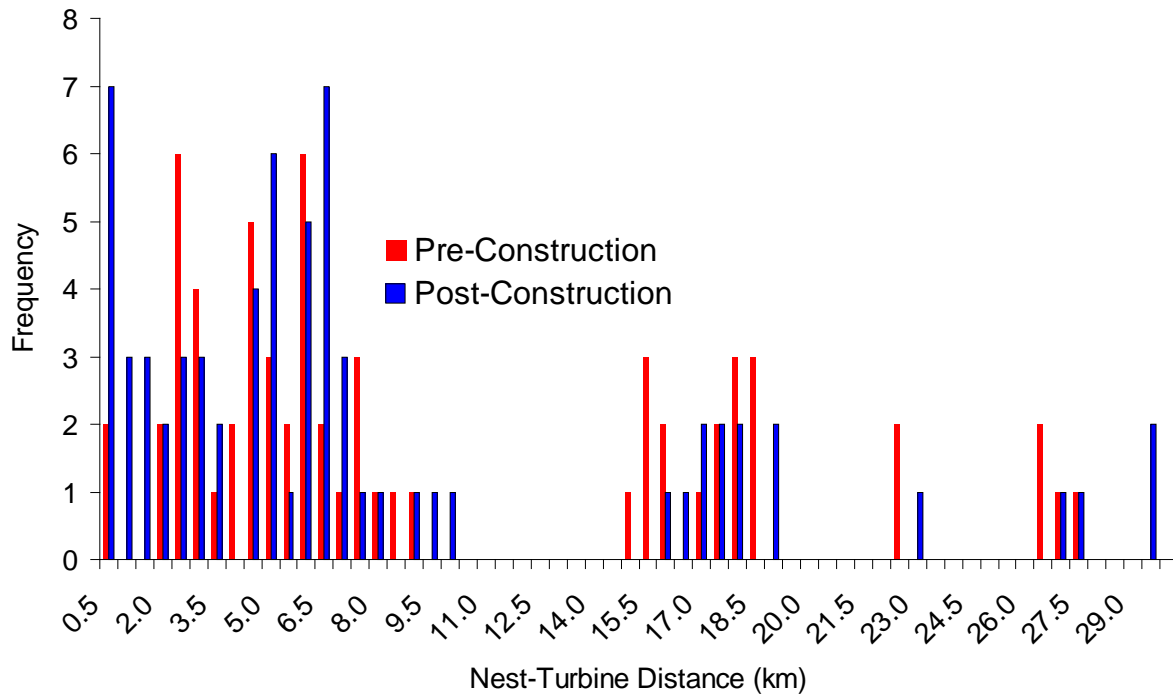


Figure 2. Distributions of distances from greater prairie-chicken nests to wind turbines for nesting periods before and after development of the Meridian Way wind power facility, Cloud County, Kansas.

LABORATORY BASED HYPOTHESES

Hypothesis I) Affects on breeding behavior: *There will be no change detected in the N_e/N .*

Accomplishments Since Last Quarter

We have incorporated data from the 2009 adult samples into our estimates of genetic diversity and effective population size both within and across all three research units (Table 3). To begin paternity and maternity analyses, we have also extracted DNA from all chick samples collected in 2009 ($n = 128$, from 24 broods all located in Unit 3), and have begun PCR amplification of the samples at all 24 loci currently in use for all samples (Table 4).

Goals for Next Quarter

We will continue molecular analysis of chick samples and begin parentage analysis.

Hypothesis II) Influence on Natal Dispersal: *There will be no differences detected in the dispersal patterns of prairie chickens pre and post wind-power development.*

Accomplishments Since Last Quarter

We completed mapping all anthropogenic structures associated with wind development across Unit 3, and continue to map other fine scale anthropogenic structures using a combination of field and remote sensing based methods. No other work on this objective was conducted during this time interval.

Goals for Next Quarter

Once molecular analysis of all samples at all loci is completed we will begin to assign putative genetic identity of all chicks caught and sampled in previous years to adults captured on lek sites during subsequent trapping seasons in an attempt to determine juvenile recruitment and dispersal patterns. In addition we will look for patterns of male prairie-chicken relatedness both within and between leks to aid in the determination of how males recruit to leks.

Literature Cited:

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- Cheng HH, Levin I, Vallejo RL, Khatib H, Dodgson JB, Crittenden LB, Hillel J. 1995. Development of a genetic map of the chicken with markers of high utility. *Poultry Science* 74:1855-1874.
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Table 3. Population genetic parameters for adult Greater Prairie-chickens in Kansas based on analysis with 11 microsatellite markers.

Deme	N	H_O	H_E	AR	N_e ± 95% CI	F_{IS}	Pairwise F_{ST}	Pairwise D	Distance Km
Unit 1	107	0.70	0.78	7.9	225 ± 55	0.10			
Unit 2	216	0.70	0.79	8.1	534 ± 142	0.11			
Unit 3	308	0.72	0.79	7.9	525 ± 71	0.09			
Unit 1 – Unit 2							0.013	0.23	320
Unit 2 – Unit 3							0.03	0.25	93
Unit 1 – Unit 3							0.012	0.44	413
Flint Hills Population	632				914 ± 119	0.10			

Partial Mantel test for isolation by distance using pairwise genetic distance and Euclidean distance between research units $R^2 = 0.32$, $P = 0.79$.

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Table 4. Microsatellite markers selected for use in population genetic analysis of Greater Prairie-chicken populations across Kansas.

Marker	NA	AR	HO	HWE	Species originally described from	Citation for microsatellite primer
ADL-146	7	5	0.57	0.051	A	Cheng et al. 1995
ADL-230	9	4	0.56	0.932	A	
ADL-123**					A	
ADL-142*	4	NA	0.13	NA	A	
ADL-144**					A	
BG-12	8	3	0.41	0.80	B	Peirtney and Hoglund 2001
BG-16	12	5	0.55	0.673	B	
BG-18	23	17	0.90	0.789	B	
BG-10*	8	NA	0.21	NA	B	
BG-14*	12	NA	0.96	NA	B	
BG-15*	6	NA	0.75	NA	B	
BG-20*	3	NA	0.32	NA	B	
LLSD-3	11	9	0.92	0.049	C	Piernney and Dallas 1997
LLSD-4	29	19	0.89	0.123	C	
LLSD-7	33	25	0.72	0.104	C	
LLST-1	8	5	0.73	0.229	C	
LLSD-2**					C	
SGCA-6	12	7	0.85	0.061	D	Taylor et al. 2003
SGCA-9	26	18	0.93	0.834	D	
SGTAT*	6	NA	0.76	NA	D	
SGCA-11*	6	NA	0.42	NA	D	
TTD-1*	7	NA	0.71	NA	B	Caizeguess et al. 2001
TTD-2*	9	NA	0.88	NA	B	
TTD-3*	5	NA	0.42	NA	B	
TTD-4**					B	

TTD-5**					B
TTD-6**					B
TTT-1*	7	NA	0.80	NA	
TTT-2*	6	NA	0.74	NA	

*indicates new primers recently added to the analysis. ** indicates primers recently added to the analysis for which no data currently exists. NA indicates that while data has been collected on the primer, too few samples have been analyzed to allow for a meaningful calculation of the parameter. Species letter codes are as follows: A = Domestic Chicken (*Gallus gallus*), B = Black Grouse (*Tetrao tetrix*), C = Red Grouse (*Lagopus lagopus scoticus*), D = Sage Grouse (*Centrocercus urophasianus*).