



## FEMALES CHOOSE MULTIPLE MATES IN THE LEKKING GREATER PRAIRIE-CHICKEN (*TYMPANUCHUS CUPIDO*)

BRIAN D. HESS, PETER O. DUNN,<sup>1</sup> AND LINDA A. WHITTINGHAM

Department of Biological Sciences, University of Wisconsin-Milwaukee, Lapham Hall S181,  
3209 N. Maryland Avenue, Milwaukee, Wisconsin 53201, USA

**ABSTRACT.**—In lek mating systems, females visit aggregations of displaying males and appear to have unrestricted opportunity to choose and mate with any male. Behavioral observations of lekking birds indicate that females generally mate once and that only a small percentage of males gain a majority of copulations. However, genetic analyses of paternity have revealed that multiple males sometimes sire young within one brood. We analyzed paternity of 25 broods of Greater Prairie-Chickens (*Tympanuchus cupido pinnatus*) breeding in an isolated population in central Wisconsin. We found multiple paternity in 44% of broods (1–4 sires brood<sup>-1</sup>) and a positive relationship between the number of sires and female home-range size prior to nesting. Multiple paternity was apparently not influenced by local breeding experience: native Wisconsin and translocated females from Minnesota did not differ in the likelihood of multiple mating. The high incidence of multiple paternity may reduce the variance in male reproductive success and increase the effective population size, which may be important for the maintenance of genetic diversity in small, isolated populations. Received 27 April 2011, accepted 23 September 2011.

Key words: Greater Prairie-Chicken, grouse, home range, lek, multiple paternity, sexual selection, *Tympanuchus cupido*.

### Les femelles de *Tympanuchus Cupido* choisissent des partenaires multiples dans les arènes

**RÉSUMÉ.**—Dans les systèmes de parade en arène, les femelles visitent des agrégations de mâles en parade et semblent avoir la possibilité de choisir librement et de s'accoupler avec n'importe quel mâle. Des observations comportementales d'oiseaux en parade dans une arène ont indiqué que les femelles ne s'accouplent généralement qu'une fois et que seul un petit pourcentage de mâles obtient la majorité des copulations. Cependant, des analyses génétiques de la paternité ont révélé que parfois plus d'un mâle engendrent des jeunes dans une même couvée. Nous avons analysé la paternité de 25 couvées de *Tympanuchus cupido pinnatus* se reproduisant dans une population isolée du centre du Wisconsin. Nous avons trouvé une paternité multiple dans 44% des couvées (1–4 géniteurs couvée<sup>-1</sup>) et une relation positive entre le nombre de géniteurs et la taille du domaine vital des femelles avant la nidification. La paternité multiple n'était apparemment pas influencée par l'expérience reproductive locale : les femelles natives du Wisconsin et celles transférées du Minnesota n'ont pas différé quant à la probabilité d'accouplement multiple. L'incidence élevée de la paternité multiple peut réduire la variance du succès reproducteur des mâles et augmenter la taille effective de la population, laquelle peut être importante pour maintenir la diversité génétique dans les petites populations isolées.

LEKKING SPECIES ARE KNOWN for extraordinary sexual dimorphism in size, plumage color, and ornamentation, which presumably reflects strong sexual selection. In classical lekking species, males cluster on display grounds, females visit these aggregations solely to assess males and mate with them, and males do not provide any resources toward reproduction other than their genes (Bradbury and Gibson 1983). Sexual selection is expected to be strong because only a small percentage of males gain most of the copulations, which produces high variance in male reproductive success (e.g., Lebigre et al. 2007, Nooker and Sandercock 2008). Increasing variance in male reproductive success is often thought to reduce effective population size and increase the loss of genetic

variation through drift (Hill 1972). However, this prediction is dependent on the distribution of paternity within and among broods (i.e., the level of multiple mating; Lotterhos 2011). Females are thought to have unrestricted opportunity to mate with any male on the lek and, thus, unrestricted ability to choose the best male (Bradbury and Gibson 1983). Consistent with this idea, behavioral observations at leks indicate that each female generally mates with only one male (Alatalo et al. 1996, Semple et al. 2001), and genetic analyses show that usually only a single male sires a female's entire brood (Table 1). Indeed, females of lekking birds have been generally characterized as monogamous with low copulation rates, and multiple mating is expected to be rare (Birkhead and

<sup>1</sup>Address correspondence to this author. E-mail: pdunn@uwm.edu

TABLE 1. Multiple paternity in lekking species of birds ( $n$  = number of clutches with multiple paternity/clutches sampled).

Species	Clutches with multiple paternity (%)	$n$	Source
Ruff ( <i>Philomachus pugnax</i> )	50.0	17/34	Lank et al. 2002
Wild Turkey ( <i>Meleagris gallopavo</i> )	35.1	34/97	Thuman and Griffith 2005
Greater Prairie-Chicken ( <i>Tympanuchus cupido</i> )	45.2	14/31	Krakauer 2008
Greater Prairie-Chicken ( <i>Tympanuchus cupido</i> )	44.0	11/25	Present study
Buff-breasted Sandpiper ( <i>Tryngites subruficollis</i> )	40.4	19/47	Lanctot et al. 1997
Wire-tailed Manakin ( <i>Pipra filicauda</i> )	18.4	7/38 <sup>a</sup>	Ryder et al. 2009
Greater Sage-Grouse ( <i>Centrocercus urophasianus</i> )	20.0	2/10	Semple et al. 2001
	7.3	14/191	Bush 2009
Blue-crowned Manakin ( <i>Lepidothrix coronata</i> )	5.0	1/20	Durães et al. 2009
Black Grouse ( <i>Tetrao tetrix</i> )	4.2	2/48	Lebigre et al. 2007
	0.0	0/11	Alatalo et al. 1996

<sup>a</sup> Mixed paternity was based on the number of females (38) with multiply sampled broods, because some broods contained only one egg.

Møller 1992). However, genetic analysis of paternity in two species of lekking shorebirds revealed that females may mate multiply because broods were often sired by more than one male (Lanctot et al. 1997, Lank et al. 2002, Thuman and Griffith 2005; Table 1). Although these results call into question the generality of female monogamy in lekking species, it remains unknown whether multiple mating is frequent in other lekking species.

Multiple mating is common among avian species that form pair bonds, presumably because female mate choice is restricted by the availability of unpaired males and extrapair mating allows females to produce offspring sired by a higher-quality or a more genetically compatible male than her social mate (reviewed in Griffith et al. 2002, Kempenaers 2007). However, in lek mating systems it is less clear how females would benefit from mating with multiple males. Although female choice may be unconstrained, the ability of females to choose the best male assumes that they can accurately assess male quality. If female assessment criteria are subject to error (Yasui 1998), then multiple mating could reduce the cost of mating solely with poor-quality or incompatible males. Females mating multiply could rely on sperm competition or postcopulatory cryptic female choice (e.g., a “genetically loaded raffle”) to select a suitable male (Griffith and Immler 2009, Pryke et al. 2010). Multiple mating could also result as a consequence of females making multiple visits to a lek or visiting multiple leks and identifying more than one high-quality male (Lank et al. 2002). Other potential benefits in lekking (and other) species include fertilization insurance (Jennions and Petrie 2000) or genetic diversification of offspring (Lank et al. 2002).

We used genetic analyses to determine the extent of multiple mating by females in a lekking grouse, the Greater Prairie-Chicken (*Tympanuchus cupido pinnatus*; hereafter “prairie-chickens”; see Table 1 for scientific names of other species compared in our study). In Wisconsin, prairie-chickens are found in two small populations (~1,500 individuals total) that went through a population bottleneck in the late 1950s, which led to a loss of genetic variation (Bellinger et al. 2003). To increase genetic diversity, adult female prairie-chickens were translocated to Wisconsin from Minnesota, where genetic variation is greater (Johnson et al. 2004). This translocation provided an opportunity to experimentally test whether previous local breeding experience influences female mating

behavior and the incidence of multiple mating. Furthermore, radiotracking allowed us to test the hypothesis that multiple mating is more likely to occur when females have larger home ranges that encompass more leks.

## METHODS

We studied female prairie-chickens breeding at the Buena Vista Grassland (44°20'15", 89°38'49") in Portage County, Wisconsin, during the 2007–2009 breeding seasons (March–May). Prairie-chickens are nonmigratory, and the population in central Wisconsin is isolated by >500 km from the nearest population in Minnesota. We focused on two groups of females: (1) adult females translocated to Wisconsin that had previous breeding experience in Minnesota and (2) adult resident (locally born) females. Hereafter, these groups are referred to as “translocated” or “resident” females, respectively. Translocated females were captured in Minnesota in late summer after known breeding attempts and released at the Buena Vista study site during August and September (Hull et al. 2011) 5 to 6 months prior to their first breeding season in Wisconsin. Resident females were captured in April at leks with walk-in funnel traps or in June and July at night by spotlighting (Toepfer et al. 1987). The three resident females caught in April could have been breeding for the first time, but the seven other resident females were caught as adults during the previous summer and, thus, were in at least their second breeding season. A small (50  $\mu$ L) blood sample was taken from the brachial vein of each female and stored in lysis buffer for paternity analysis.

We used 12-g necklace-type radiotransmitters (Telemetry Solutions, Concord, California; Amstrup 1980) to track the movements of 24 translocated females during their first breeding season in Wisconsin and 15 resident females. Nests were located by monitoring radiotagged females, and most females (93%) began laying by 7 May. When unknown, nest initiation date was estimated by candling eggs early in incubation (Westerskov 1950) or by backdating from the date of hatch (Johnson et al. 2011). When chicks reached 5 weeks of age, brooding females were captured along with their chicks by spotlighting at night. A small blood sample (50  $\mu$ L) was collected from all chicks. In addition, we collected unhatched eggs and eggshell fragments from nests after

they hatched or were abandoned or depredated. This enabled us to determine hatching success and acquire DNA from eggshell membranes for young that could not be sampled as chicks because of nest loss. Although 39 females were radiotracked, we were only able to recover DNA and genotype at least 2 eggs or chicks in 25 broods (mean ± SE = 8.5 ± 0.68 individuals brood<sup>-1</sup>; range: 2–15).

**Parentage analysis.**—DNA was extracted from blood using a saturated salt solution (Miller et al. 1988) and from eggshells using a standard phenol chloroform procedure or a DNeasy Blood and Tissue kit (Qiagen, Madison, Wisconsin). A total of six microsatellite loci designed from other species were optimized for prairie-chickens and used to determine parentage. These included LLS4 (Red Grouse [*Lagopus lagopus scoticus*]; Piertney and Dallas 1997), ADL44 and ADL230 (Domestic Chicken [*Gallus gallus*]; Bouzat et al. 1998), and SGCA6, SGCA9, and SGCA11 (Greater Sage-Grouse; Taylor et al. 2003). These polymorphic loci had a combined exclusion probability ( $P_{et}$ ) of 0.9996 (Table 2). All polymerase chain reaction (PCR) amplifications contained 20–75 ng of genomic DNA, 0.25 μM (LLSD4) or 0.50 μM (ADL44, ADL230, SGCA6, SGCA9, SGCA11) fluorescently labeled forward primer and unlabeled reverse primer, 50 mM KCl, 10 mM Tris, 3.75 mM MgCl<sub>2</sub> (1.5 mM MgCl<sub>2</sub> for LLS4 and ADL230; 3.0 mM MgCl<sub>2</sub> for ADL44), 0.2 mM dNTPs, and 1 unit of *Taq* polymerase for a total reaction volume of 10 μL. Thermal cycling profiles for SGCA6, SGCA9, and SGCA11 consisted of 36 cycles of 95°C for 1 min, 54°C for 1 min (62°C for SGCA6), and 72°C for 1 min (Taylor et al. 2003). Thermal cycling profiles for LLS4 and ADL44 (ADL230) consisted of one cycle at 94°C for 3 min followed by 32–40 cycles at 94°C for 30 s, 48°C for 30 s (46°C for ADL230) and 72°C for 30 s followed by 5 min at 72°C (Bellinger et al. 2003). The PCR products were run on an ABI 377 automated sequencer with a fluorescently labeled ladder in each lane. Two individuals for which allele sizes were known were run on each gel. Allele sizes were determined using GENOTYPER software (PE Biosystems, Foster City, California).

**Number of sires.**—DNA samples were not available for males in the population, so we estimated the number of sires in each brood using two methods. First, using the single-locus minimum method, the maternal alleles were subtracted from the genotypes of her offspring and the number of remaining paternal alleles was divided by two and rounded up to provide a minimum estimate of the number of sires (Fiumera et al. 2001). One brood contained a single genotype not matching the maternal genotype at three loci. This individual was assumed to be the result of egg dumping and was not included in paternity analyses. All other offspring matched the putative mother at all loci.

Counting the number of paternal alleles often underestimates the number of sires (Sefc and Koblmüller 2009), and therefore we also estimated the number of sires using the computer program COLONY, version 2.0 (Jones and Wang 2009). COLONY uses a maximum-likelihood method to assign all individuals within a sample into distinct genetic groups (full-sibs, half-sibs, unrelated) of varying sizes. For this analysis, both males and females were considered polygamous and each brood was entered as a separate group of maternal siblings (i.e., no egg dumping). Allele frequencies of the six microsatellite loci were estimated from the genotypes of 96 unrelated adult females (40 from Minnesota and 56 from Wisconsin, which were input together). The

TABLE 2. Number ( $n$ ) of alleles, allele size range, expected ( $H_e$ ) and observed ( $H_o$ ) heterozygosity, and probability of exclusion ( $P_e$ ) for six microsatellite loci calculated from unrelated adult female Greater Prairie-Chickens ( $n = 96$  females; 40 translocated, 56 resident).

Locus	Alleles ( $n$ )	Sizes (base pairs)	$H_e$	$H_o$	$P_e$
LLSD4	21	182–230	0.914	0.917	0.817
ADL44	8	178–185	0.793	0.884	0.589
ADL230	10	102–122	0.833	0.833	0.635
SGCA6	23	299–355	0.926	0.916	0.840
SGCA9	23	324–368	0.911	0.874	0.813
SGCA11	10	356–276	0.702	0.604	0.488
Mean	15.8		0.847	0.838	$P_{et}$ : 0.999

allelic dropout rate was set at zero, and the rate of mutations was set at 0.01. COLONY can overestimate sire numbers when used with markers of low genetic diversity; however, our microsatellite loci were highly polymorphic (Table 2), minimizing the possibility of overestimating sire number (Sefc and Koblmüller 2008). COLONY and the single-locus minimum methods are biased in opposite directions, so congruency provides strong support for the estimated number of sires (Sefc and Koblmüller 2008). A similar analysis in COLONY was used to identify paternal siblings across broods for nests from 2007 ( $n = 23$ ), which had the largest sample of any year. Thus, we could estimate the number of different males siring young in these broods and the total number of young each male sired.

**Female home-range size.**—We also used radiotelemetry to track and estimate female home-range size. The study area (195 km<sup>2</sup>) was divided into north and south halves, and females in each half of the study area were located on successive days. Each day, the direction traveled was reversed. Using a global-positioning-system receiver, locations of individuals were determined by triangulations of at least three bearings (all recorded within 1 km) that were used to calculate the maximum-likelihood estimator (all bearings given equal weight) using LOCATE III software (Nams 2006). We also compared triangulated estimates with known locations for 31 transmitters (hidden decoys or dead birds). This showed that 90% of triangulated estimates were within 350 m of actual locations (average [± SE] difference = 170 ± 18.4 m). Note that this is much less than the average minimum distance between neighboring leks at our study site (1,329 ± 119.7 m,  $n = 35$  in 2007; 1,552 ± 114.7 m,  $n = 27$  in 2008).

We estimated home-range size for all resident and translocated females located at least five times (11.2 ± 0.63 locations bird<sup>-1</sup>) between 0530 and 0900 hours CDT from 1 April to the onset of egg laying ( $n = 33$ ). Home-range size was estimated as the 95% contour of a fixed-kernel-density estimator using the HRT extension for ARCGIS (Rodgers et al. 2007). The kernel method uses a distribution of location data to construct a three-dimensional home range, with the third dimension representing time spent in a given part of that range (Seaman et al. 1999). Home-range estimates used reference validation smoothing because it yields conservative estimates and low levels of bias across small sample sizes (Seaman et al. 1999). There was no correlation between estimated home-range size and

number of locations in our sample ( $r^2 = 0.08$ ,  $F = 2.8$ ,  $P = 0.10$ ,  $n = 33$  females tracked). We included the number of locations for each radiotracked female as a covariate in analyses of home-range size. Locations of females and all leks on the study area were recorded in ARCGIS, version 9 (ESRI, Redlands, California). All means are presented  $\pm$  SE, and all tests were two-tailed.

## RESULTS

**Multiple paternity.**—Multiple paternity was common: 44% of broods (11 of 25) were sired by multiple males (Table 3). Multiple paternity varied from two sires in 28% of broods (7 of 25) to three or four sires in 16% of broods (4 of 25). Females were equally likely to have multiply sired broods regardless of their local breeding experience. We detected multiple paternity in 47% of broods (7 of 15) of translocated females and 40% of broods (4 of 10) of resident females (Fisher's exact test,  $P > 0.99$ ; Table 3). We genotyped 80% of the recovered eggs (227 of 284;  $9.1 \pm 0.63$  eggs clutch<sup>-1</sup>) and 71% of eggs laid (227 of 319). Although it was not possible to extract DNA from all eggs laid, the detection of multiple paternity (coded yes or no) was not related to the percentage of eggs genotyped in each clutch (logistic regression,  $\chi^2 = 1.0$ ,  $df = 1$ ,  $P = 0.32$ ,  $n = 25$  nests).

The number of sires per brood was similar for translocated and resident females (Table 3). We estimated one to four sires ( $1.7 \pm 0.23$ ) per brood for the 15 translocated females, and one to three sires ( $1.6 \pm 0.28$ ) per brood for the 10 resident females ( $t = 0.19$ ,  $df = 23$ ,  $P = 0.85$ ; Table 3). The single-locus minimum method and COLONY estimated the same number of sires for most broods (92%, 23 of 25). For the remaining two broods, COLONY estimated one more sire than the single-locus minimum method; however, both methods considered these to be multiply sired broods.

For our analysis of paternal siblings, COLONY estimated that 30 different males explained the genotypes of 201 eggs from 23 broods in 2007 (the year with more extensive sampling). These 30 males sired 1 to 19 offspring ( $6.7 \pm 0.78$ ) and obtained paternity in one to four broods ( $1.6 \pm 0.14$ ). Almost half of the males (47%, 14 of 30) sired young in at least two broods, but just three males (10%) sired young in three or four broods.

**Hatching success.**—As an index of fertilization success we examined hatching success for broods in relation to multiple paternity. Among nests that were not abandoned or depredated ( $n = 15$ ), 96% of eggs (182 of 189) hatched. Seven unhatched eggs occurred in five different clutches. Thus, hatching failure was infrequent and, not surprisingly, unrelated to the presence of multiple paternity in the brood. Multiple paternity occurred in 1 of 5 broods with at least

one unhatched egg, and in 6 of 10 broods in which all eggs hatched (Fisher's exact test,  $P = 0.28$ ). The power of this test was low because of the limited number of nests that successfully hatched young and the low hatching failure. Assuming that these proportions remain the same (20% vs. 60%), a sample size of 30 broods would be sufficient to detect a significant positive relationship between hatching success and the occurrence of multiple paternity.

**Female home-range size.**—Females with larger home ranges had more leks within their respective home ranges (logistic regression,  $\chi^2 = 8.9$ ,  $P < 0.01$ ,  $n = 33$ ), but only marginally so after controlling for the number of telemetry locations ( $\chi^2 = 3.5$ ,  $P = 0.06$ ). The number of sires was also positively related to the number of leks within the home range in logistic regressions using both the single-locus minimum ( $\chi^2 = 7.8$ ,  $P = 0.02$ ,  $n = 22$ ) and COLONY ( $\chi^2 = 6.3$ ,  $P = 0.03$ ,  $n = 22$ ) estimates of the number of sires (number of telemetry locations was not significant:  $P > 0.81$ ). Female home-range size did not differ significantly between translocated (least squares mean [LSM] =  $7.9 \pm 1.6$  km<sup>2</sup>,  $n = 23$ ) and resident (LSM =  $3.5 \pm 2.5$  km<sup>2</sup>;  $F = 2.03$ ,  $P = 0.16$ ,  $n = 10$ ) females in a model that controlled for the number of telemetry locations. Similarly, there was no difference between translocated (LSM =  $2.2 \pm 0.38$ ,  $n = 23$ ) and resident (LSM =  $1.5 \pm 0.60$ ,  $n = 10$ ) females in the number of leks within their home ranges ( $F = 0.94$ ,  $P = 0.34$ ).

## DISCUSSION

Multiple mating was common in Greater Prairie-Chickens: 44% of females produced broods sired by more than one male. The likelihood of multiple paternity in a brood was not influenced by the female's local breeding experience, but females with larger home ranges had more sires in their broods. The frequent occurrence of multiple paternity in our population may reduce the skew in male reproductive success and increase the effective population size, which could reduce the strength of sexual selection and help maintain genetic diversity in this small population.

**Multiple mating.**—It is generally expected that multiple paternity will be rare when female mate choice is unconstrained as it is in lek mating systems and females can reliably assess male compatibility or quality (Birkhead and Møller 1992). The occurrence of multiply sired broods in our population of prairie-chickens (44%) was higher than in other species of grouse (Alatalo et al. 1996, Semple et al. 2001, Lebigre et al. 2007, Bush 2009), but similar to that in Wild Turkeys (Krakauer 2008) and lekking species of waders (Lanctot et al. 1997, Lank et al. 2002, Thuman and Griffith 2005; Table 1). There are few data on the number of sires per brood from other lekking species, but like Ruffs (1.6 sires; Lank

TABLE 3. Multiple paternity in broods of translocated and resident female Greater Prairie-Chickens ( $t$  and  $P$  values are for a  $t$ -test comparing females translocated from Minnesota with resident females from Wisconsin; \*Fisher's exact test).

Females	Nests	Eggs	Offspring genotyped	Genotyped samples nest <sup>-1</sup>	Sires nest <sup>-1</sup>	Nests with >1 sire
Total	25	284	227	$9.1 \pm 0.6$	$1.64 \pm 0.17$	11 (44%)
Translocated	15	171	135	$9.0 \pm 0.8$	$1.67 \pm 0.23$	7 (47%)
Resident	10	113	92	$9.2 \pm 1.0$	$1.60 \pm 0.28$	4 (40%)
					$t = 0.19$	
					$P = 0.85$	$P > 0.99^*$

et al. 2002), female prairie-chickens appeared to have an unusually high number of sires per clutch (average = 1.6, maximum = 4). By contrast, multiple paternity occurred in 7% of Greater Sage-Grouse broods (14 of 191) and there was a maximum of two sires per brood (Bush 2009).

Variance in male reproductive success is thought to be large in lekking species because most females mate with only a few males. For example, in an observational study of prairie-chickens just 19% of males obtained 87% of copulations (Nooker and Sandercocock 2008). However, the actual pattern of paternity may differ from what is observed, and multiple mating could reduce this extreme skew in male reproductive success in lek mating systems. We were unable to sample males during our study and, thus, we cannot directly measure the influence of female multiple mating on the variance in male reproductive success. However, the relatively large number of males siring young in our sample of broods (30 males sired 201 young; 6.7 young sire<sup>-1</sup> in an average clutch of 13.3 eggs) suggests that multiple mating by female prairie-chickens may reduce the skew in male reproductive success. By contrast, male reproductive success appears to be more skewed in the Greater Sage-Grouse (Bush 2009) because 36 males sired 443 young, for an average of 12.3 young sire<sup>-1</sup> from an average clutch of 7.9 eggs (Schroeder et al. 1999). These results suggest that even in some mating systems that appear highly polygynous, a variety of mating tactics (e.g., sneaker males in fish; Jones et al. 2001) or sperm competition can result in a lower-than-expected variance in male mating success, potentially reducing the strength of sexual selection.

*Factors influencing multiple mating.*—If female mate choice is unconstrained in lekking species, it is not clear why they would choose to mate with multiple males. One possibility is that females increase their probability of encountering a high-quality male when they visit multiple leks or visit the same lek multiple times (Lank et al. 2002). For example, multiple paternity in Ruffs is associated with females mating during multiple visits to the same lek (Lank et al. 2002). A study of Greater Prairie-Chickens in Colorado showed that 85% of females (67 of 79) visited two or more different leks and 71% (79 of 111) visited the same lek at least twice (Schroeder 1991). Consistent with these results, we found that females with larger home ranges had more leks within their home range and more sires in their brood. However, if females cannot identify genetically compatible or high-quality males prior to copulation, multiple mating may be a mechanism to produce postcopulatory sperm competition that results in fertilization by more compatible males (Griffith and Immler 2009). For example, Gouldian Finches (*Erythrura gouldiae*) mated with males of both compatible and incompatible color morphs, but only the sperm from the compatible male fertilized the eggs (Pryke et al. 2010).

Multiple mating may also benefit females by ensuring fertilization of their eggs. For example, some female tits (*Parus major* and *Cyanistes caeruleus*) have entire clutches sired only by extrapair males, which suggests that multiple mating ensures fertilization (Krokene et al. 1998). Multiple mating may also help to ensure fertilization when preferred males copulate frequently, leaving them temporarily depleted of sperm (Preston et al. 2001). By contrast, most female Black Grouse mate only once with a single male and, thus, rely on a single insemination to fertilize their

entire clutch (Lebigre et al. 2007). Even for fertilized eggs, multiple mating might also increase hatching success (Whittingham and Dunn 2010) by reducing the proportion of offspring with deleterious recessive alleles, which could be especially important in populations with low genetic diversity, such as prairie-chickens in Wisconsin (Johnson et al. 2004). Finally, multiple mating may occur as a consequence of disrupted breeding, such as interrupted copulations on the lek that lead to remating (Alatalo et al. 1996) or stored sperm fertilizing eggs in subsequent clutches after loss of an initial clutch (Oring et al. 1992).

*Conservation implications.*—The effective population size (the number of breeding individuals,  $N_e$ ) is usually much lower than the actual population size ( $N$ ), especially in polygynous species (Nunney 1993). As a consequence, the loss of genetic variation through drift might be relatively large in small isolated populations of lekking species, such as prairie-chickens in Wisconsin (see also Stiver et al. 2008). However, multiple mating could slow the loss of genetic variation by increasing  $N_e$  if females mating multiple times choose males that would otherwise be excluded from breeding (Pearse and Anderson 2009). For example, in small populations of lekking European Treefrogs (*Hyla arborea*), low variance in male fertilization success (75% of males mated) led to a relatively higher  $N_e$  and lower-than-expected genetic drift (Broquet et al. 2009).  $N_e$  generally increases with the average number of mates per female and a more equitable distribution of paternity within broods (Lotterhos 2011). However,  $N_e$  can either increase or decrease in relation to a monogamous system at low levels of multiple mating, such as two mates per female (similar to the 1.6 mates female<sup>-1</sup> in prairie-chickens), depending on demographic variables such as the variance in female reproductive success (Lotterhos 2011). Thus, the ultimate effects of lek mating systems on  $N_e$  will require a more detailed synthesis of both demographic (e.g., Stiver et al. 2008) and genetic data.

#### ACKNOWLEDGMENTS

This project was part of a translocation program initiated by the Society of *Tympanuchus cupido pinnatus* (STCP) and the Wisconsin Department of Natural Resources with the assistance of the University of Minnesota-Crookston (UMC), University of Wisconsin-Madison, and University of Wisconsin-Milwaukee. We especially thank J. Toepfer (STCP) and N. Emery (UMC) for catching and radiotagging the females and collecting blood samples, and T. J. Hauge, B. Sadler, and A. Steinke for assistance with radiotracking and trapping chicks. G. Dahl, D. Drake, S. Hull, L. Kardash, C. Nemeč, K. Rosenthal, and D. Sample provided technical support and assisted in the field, and M. Ambaradar, J. Bollmer, J. Eimes, G. Hoebel, E. Tuttle, and two anonymous reviewers provided helpful comments on the manuscript or assistance in the lab. Financial support was provided by the Animal Behavior Society, the Ruth Walker Fund of the University of Wisconsin-Milwaukee, the Wisconsin Society for Ornithology, and the Wisconsin Department of Natural Resources (DNR). This work was approved by IACUC at UW-Madison (A3368-01) and UW-Milwaukee (09-10 no. 15), and permits were issued by the Wisconsin DNR (Wisconsin Endangered and Threatened Species Permit no. 626).

## LITERATURE CITED

- ALATALO, R. V., T. BURKE, J. DANN, O. HANOTTE, J. HÖGLUND, A. LUNDBERG, R. MOSS, AND P. T. RINTAMÄKI. 1996. Paternity, copulation disturbance and female choice in lekking Black Grouse. *Animal Behaviour* 52:861–873.
- AMSTRUP, S. C. 1980. A radio-collar for game birds. *Journal of Wildlife Management* 44:214–217.
- BELLINGER, M. R., J. A. JOHNSON, J. TOEPFER, AND P. DUNN. 2003. Loss of genetic variation in Greater Prairie Chickens following a population bottleneck in Wisconsin, U.S.A. *Conservation Biology* 17:717–724.
- BIRKHEAD, T. R., AND A. P. MØLLER. 1992. *Sperm Competition in Birds*. Academic Press, London.
- BOUZAT, J. L., H. H. CHENG, H. A. LEWIN, R. L. WESTEMEIER, J. D. BRAWN, AND K. N. PAIGE. 1998. Genetic evaluation of a demographic bottleneck in the Greater Prairie Chicken. *Conservation Biology* 12:836–843.
- BRADBURY, J. W., AND R. M. GIBSON. 1983. Leks and mate choice. Pages 109–138 in *Mate Choice* (P. Bateson, Ed.). Cambridge University Press, Cambridge, United Kingdom.
- BROQUET, T., J. JAQUIÉRY, AND N. PERRIN. 2009. Opportunity for sexual selection and effective population size in the lek-breeding European treefrog (*Hyla arborea*). *Evolution* 63:674–683.
- BUSH, K. 2009. Genetic diversity and paternity analysis of endangered Canadian Greater Sage-Grouse (*Centrocercus urophasianus*). Ph.D. dissertation, University of Alberta, Edmonton.
- DURÃES, R., B. A. LOISELLE, P. G. PARKER, AND J. G. BLAKE. 2009. Female mate choice across spatial scales: Influence of lek and male attributes on mating success of Blue-Crowned Manakins. *Proceedings of the Royal Society of London, Series B* 276:1875–1881.
- FIUMERA, A. C., Y. D. DEWOODY, J. A. DEWOODY, M. A. ASMUSSEN, AND J. C. AVISE. 2001. Accuracy and precision of methods to estimate the number of parents contributing to a half-sib progeny array. *Journal of Heredity* 92:120–126.
- GRIFFITH, S. C., AND S. IMMLER. 2009. Female infidelity and genetic compatibility in birds: The role of the genetically loaded raffle in understanding the function of extrapair paternity. *Journal of Avian Biology* 40:97–101.
- GRIFFITH, S. C., I. P. F. OWENS, AND K. A. THUMAN. 2002. Extrapair paternity in birds: A review of interspecific variation and adaptive function. *Molecular Ecology* 11:2195–2212.
- HILL, W. G. 1972. Effective size of populations with overlapping generations. *Theoretical Population Biology* 3:278–289.
- HULL, S., D. SAMPLE, D. DRAKE, S. FANDEL, L. KARDASH, O. LEDÉE, AND S. SCHWAB. 2011. The Wisconsin Greater Prairie-Chicken program: Integrating research, management, and community outreach in the 21st century. *Passenger Pigeon* 73:89–99.
- JENNIONS, M., AND M. PETRIE. 2000. Why do females mate multiply? A review of the genetic benefits. *Biological Reviews* 75:21–64.
- JOHNSON, J. A., M. R. BELLINGER, J. TOEPFER, AND P. O. DUNN. 2004. Temporal changes in allele frequencies and low effective population size in Greater Prairie-Chickens. *Molecular Ecology* 13:2617–2630.
- JOHNSON, J. A., M. A. SCHROEDER, AND L. A. ROBB. 2011. Greater Prairie-Chicken (*Tympanuchus cupido*). In *The Birds of North America Online* (A. Poole, Ed.). Cornell Lab of Ornithology, Ithaca, New York.
- JONES, A. G., D. WALKER, C. KVARNEMO, K. LINDSTRÖM, AND J. C. AVISE. 2001. How cuckoldry can decrease the opportunity for sexual selection: Data and theory from a genetic parentage analysis of the sand goby, *Pomatoschistus minutus*. *Proceedings of the National Academy of Sciences USA* 98:9151–9156.
- JONES, O., AND J. WANG. 2009. COLONY: A program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources* 10:551–555.
- KEMPENAERS, B. 2007. Mate choice and genetic quality: A review of the heterozygosity theory. *Advances in the Study of Behavior* 37:189–278.
- KRAKAUER, A. H. 2008. Sexual selection and the genetic mating system of Wild Turkeys. *Condor* 110:1–12.
- KROKENE, C., K. RIGSTAD, M. DALE, AND J. T. LIFJELD. 1998. The function of extrapair paternity in Blue Tits and Great Tits: Good genes or fertility insurance? *Behavioral Ecology* 9:649–656.
- LANCOT, R. B., K. T. SCRIBNER, B. KEMPENAERS, AND P. J. WEATHERHEAD. 1997. Lekking without a paradox in the Buff-breasted Sandpiper. *American Naturalist* 149:1051–1070.
- LANK, D. B., C. M. SMITH, O. HANOTTE, A. OHTONEN, S. BAILEY, AND T. BURKE. 2002. High frequency of polyandry in a lek mating system. *Behavioral Ecology* 13:209–215.
- LEBIGRE, C., R. V. ALATALO, H. SIITARI, AND S. PARRI. 2007. Restrictive mating by females on Black Grouse leks. *Molecular Ecology* 16:4380–4389.
- LOTTERHOS, K. E. 2011. The context-dependent effect of multiple paternity on effective population size. *Evolution* 65:1693–1706.
- MILLER, S. A., D. D. DYKES, AND H. F. POLESKY. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* 16:1215.
- NAMS, V. O. 2006. *Locate III User's Guide*. Pacer Computer Software, Tatamgouche, Nova Scotia.
- NOOKER, J. K., AND B. K. SANDERCOCK. 2008. Phenotypic correlates and survival consequences of male mating success in lek-mating Greater Prairie-Chickens (*Tympanuchus cupido*). *Behavioral Ecology and Sociobiology* 62:1377–1388.
- NUNNEY, L. 1993. The influence of mating system and overlapping generations on effective population size. *Evolution* 47:1329–1341.
- ORING, L. W., R. C. FLEISCHER, J. M. REED, AND K. E. MARSDEN. 1992. Cuckoldry through stored sperm in the sequentially polyandrous Spotted Sandpiper. *Nature* 359:631–633.
- PEARSE, D. E., AND E. C. ANDERSON. 2009. Multiple paternity increases effective population size. *Molecular Ecology* 18:3124–3127.
- PIERTNEY, S. B., AND J. F. DALLAS. 1997. Isolation and characterization of hypervariable microsatellites in Red Grouse *Lagopus lagopus scoticus*. *Molecular Ecology* 6:93–95.
- PRESTON, B. T., I. R. STEVENSON, J. M. PEMBERTON, AND K. WILSON. 2001. Dominant rams lose out by sperm depletion. *Nature* 409:681–682.
- PRYKE, S. R., L. A. ROLLINS, AND S. C. GRIFFITH. 2010. Females use multiple mating and genetically loaded sperm competition to target compatible genes. *Science* 329:964–967.
- RODGERS, A. R., A. P. CARR, H. L. BEYER, L. SMITH, AND J. G. KIE. 2007. HRT: Home range tools for ArcGIS, version 1.1. Ontario Ministry of Natural Resources, Centre for Northern Forest Ecosystem Research, Thunder Bay, Ontario.

- RYDER, T. B., P. G. PARKER, J. G. BLAKE, AND B. A. LOISELLE. 2009. It takes two to tango: Reproductive skew and social correlates of male mating success in a lek-breeding bird. *Proceedings of the Royal Society of London, Series B* 276:2377–2384.
- SCHROEDER, M. A. 1991. Movement and lek visitation by female Greater Prairie-Chickens in relation to predictions of Bradbury's female preference hypothesis of lek evolution. *Auk* 108:896–903.
- SCHROEDER, M. A., J. R. YOUNG, AND C. E. BRAUN. 1999. Sage grouse (*Centrocercus urophasianus*). In *The Birds of North America*, no. 425 (A. Poole, P. Stettenheim, and F. Gill, Eds.). Birds of North America, Philadelphia.
- SEAMAN, D. E., J. J. MILLSPAUGH, B. J. KERNOHAN, G. C. BRUNDIGE, K. J. RAEDEKE, AND R. A. GITZEN. 1999. Effects of sample size on kernel home range estimates. *Journal of Wildlife Management* 63:739–747.
- SEFC, K. M., AND S. KOBLMÜLLER. 2009. Assessing parent numbers from offspring genotypes: The importance of marker polymorphism. *Journal of Heredity* 100:197–205.
- SEMPLE, K., R. K. WAYNE, AND R. M. GIBSON. 2001. Microsatellite analysis of female mating behaviour in lek-breeding sage grouse. *Molecular Ecology* 10:2043–2048.
- STIVER, J. R., A. D. APA, T. E. REMINGTON, AND R. M. GIBSON. 2008. Polygyny and female breeding failure reduce effective population size in the lekking Gunnison Sage-Grouse. *Biological Conservation* 141:472–481.
- TAYLOR, S. E., S. J. OYLER-McCANCE, AND T. W. QUINN. 2003. Isolation and characterization of microsatellite loci in Greater Sage-Grouse (*Centrocercus urophasianus*). *Molecular Ecology Notes* 3:262–264.
- THUMAN, K. A., AND S. C. GRIFFITH. 2005. Genetic similarity and the nonrandom distribution of paternity in a genetically highly polyandrous shorebird. *Animal Behaviour* 69:765–770.
- TOEPFER, J. E., J. A. NEWELL, AND J. MONARCH. 1987. A method for trapping prairie grouse hens on display grounds. Contribution no. 2144, Montana Agricultural Experimental Station, Missoula.
- WESTERSKOV, K. 1950. Methods for determining the age of game bird eggs. *Journal of Wildlife Management* 14:56–67.
- WHITTINGHAM, L. A., AND P. O. DUNN. 2010. Fitness benefits of polyandry for experienced females. *Molecular Ecology* 19:2328–2335.
- YASUL, Y. 1998. The 'genetic benefits' of female multiple mating reconsidered. *Trends in Ecology & Evolution* 13:246–250.

*Associate Editor: E. M. Tuttle*