The kin selection hypothesis in a lekking mole cricket: assessing nested patterns of relatedness

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Loosely defined, a lek is a male mating aggregation visited by females primarily for the purpose of fertilization. No consensus has been forged that successfully explains how and why leks have evolved across the full breadth of lekking taxa. The two major conceptual explanations (cooperation and competition) are both intricately intertwined in any given system and exhibit varying levels of plasticity based on an organism's environment and life history. The kin selection hypothesis suggests that if females prefer larger leks (as is often the case), unattractive males may aggregate with their attractive relatives in order to boost the latter's reproductive success, while effectively sacrificing any opportunities of their own. Here we develop microsatellite markers to genetically evaluate the kin selection hypothesis by measuring relatedness and precise spatial locations of males in a population of the lekking prairie mole cricket (*Gryllotalpa major* Saussure). Results indicate that neighbouring males are often highly related, suggesting that kin selection may play some role in this system. However, because leks are comprised of multiple kin groups, if kin selection is operating it is likely doing so at a smaller scale than predicted by the kin selection hypothesis of lek formation. The high levels of subgroup relatedness within this species likely occurs as a passive process due to male viscosity, but the functional implications of this interesting genetic organization remain unknown. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **00**, 000–000.

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INTRODUCTION

Lekking is a behaviour traditionally thought to be shaped by competition (Brown, 1964; Bradbury, 1981; Avery, 1984), as indeed may be true in some of the classical lek systems. However, with the discovery of new and unique variations of the lek, which do not always follow the traditional patterns, we have been forced to reassess previous assumptions. For example, in some species of manakin (Passeriformes: Pipridae), males exhibit obligate cooperative courtship and are unable to even perform courtship rituals without a male partner (Foster, 1977). Perhaps even more intriguing is the suggestion that cooperation may play a substantial role in lek formation even within classical lekking species. Kokko & Lindström (1996) established the mathematical

framework for kin selection as a potential driver of lek mating, and shortly thereafter Petrie, Krupa & Burke (1999) presented empirical evidence that peacocks, *Pavo cristatus*, preferentially lek in close proximity to relatives that are randomly separated at birth.

Both parties may accrue indirect inclusive fitness benefits when less attractive males forego their own reproduction in order to increase the number and success of attractive close relatives' reproductive opportunities (Hamilton, 1964). According to the lek-kin selection hypothesis, unattractive males join leks to boost the desirability of their attractive relatives – because larger leks are often more attractive to females (Kokko & Lindström, 1996). In support of this idea are the observations that most lekking species display extreme variance in male mating success (known in the literature as 'mating skew' – see Höglund & Alatalo, 1995) and that female visitation

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is more or less positively correlated with lek size, although not always on a per-capita basis (Isvaran & Ponkshe, 2013). When mating skew is high, unattractive males may have a very bleak reproductive outlook and thus have very little to lose by forgoing sex altogether – a strategy that may not be worth the cost if mating opportunities are evenly distributed. Because lekking males have the opportunity to advertise alone but often choose not to do so, it has been hypothesized that kin selection may play a role in selecting for male lekking behaviour (Höglund, 2003).

Due to the difficulties of gauging the tradeoffs unattractive males potentially undertake in the kin selection model *in situ*, almost all empirical tests of the kin selection hypothesis only ask whether males in a given lek are more highly related to each other than by chance. The resulting prediction is the existence of significantly higher levels of relatedness within leks than between them (Höglund, 2003). However, increased levels of competition between related neighbours may cancel out the potential for kin selection, even if individuals do not disperse (Queller, 1994; Griffin & West, 2002). The 'philopatry' hypothesis, developed to explain manakin behaviour, predicts that even though some genetic structure may be present, average relatedness within a lek should be equal to that of the entire population (comprised of multiple leks, see Francisco, Gibbs & Galetti, 2009). Importantly, this hypothesis suggests that kin groups will sometimes occur by chance, but not often enough to create a pattern at the level of the entire population. However, these predictions only apply in 'semi-viscous' populations where organisms freely disperse within a population, but rarely do so across a larger metapopulation, a phenomenon that occurs in several tropical understory-dwelling birds (Bates, 2000). In leks with truly viscous dispersal, we predict that males will tend to remain in the lek closest to where they hatch, creating a pattern of elevated relatedness within leks, due to the presence of brothers. In larger leks, there would be a potential for several kin groups to exist. An idea that has been little explored suggests that nested patterns of relatedness may result from kin selection at a smaller scale within the lek (multiple clusters of related individuals, see Shorey *et al.*, 2000); although, the mechanisms that enable females to discriminate between groups within a lek have not been considered. Additionally, the presence of multiple kin groups within a single lek would imply that kin selection does not drive lek evolution, but may rather be a response to lekking behaviour.

Only one study has explored lek relatedness outside of avians (in moor frogs, but they may not even truly lek, see Knopp *et al.*, 2008) and there are no clear patterns across taxa (sometimes support for related neighbours is mixed even within a species, such as in black grouse, see Lebigre *et al.*, 2014). Partial support for the kin selection hypothesis has been found in some species such as peafowl (Petrie *et al.*, 1999) and facultatively lekking wild turkey (Krakauer, 2005), but in others no clear pattern exists (such as in manakins, see Lebigre *et al.*, 2014). This finding implies that kin selection is more likely to be a result than a cause of lekking behaviour if it truly does exist in some of these systems. Several studies have discovered genetic substructure nested within a lek (Shorey *et al.*, 2000; Segelbacher *et al.*, 2007; Francisco *et al.*, 2009), suggesting the existence (and importance) of kin clusters at a smaller scale than originally expected under the kin selection hypothesis. Interpretation of these results is confounded by significant differences in many of the lekking systems studied; although, this point is often overlooked (Höglund & Alatalo, 1995).

Over the past 20 years the prairie mole cricket, *Gryllotalpa major* Saussure (Orthoptera: Gryllotalpidae), has been used as a contrasting model to study lek behaviour (Hill, 1998, 1999; Hill & Shadley, 2001; Hoffart, Jones & Hill, 2002; Hill, Wells & Shadley, 2006; Howard & Hill, 2006, 2007, 2009; Howard, Mason & Hill, 2008; Hill *et al.*, 2009; Howard *et al.*, 2011). Although similar to many lekking birds, one important difference is *G. major's* short lifespan (which includes one mating season of 1–2 months), enabling researchers to effectively rule out delayed direct benefits such as dominance inheritance. Similarly, we note that male burrows are never reused and do not represent a valuable resource; they erode rapidly once unoccupied (~weeks) and it is possible that horn shapes must be 'tuned' to frequency of the caller (Bennet-Clark, 1987). However, Sæther (2002) argued that direct benefits can be ruled out in almost no studies in which related individuals prefer to lek together. The only exception stands as Krakauer's (2005) work on wild turkeys, *Meleagris gallopavo*, in which support was found for all three requirements necessary to prove the existence of kin selection: (1) brothers display together, (2) subordinate/unattractive males help dominant/attractive relatives gain more matings, and (3) the indirect benefit to the helper surpasses the cost of forgoing his own reproduction. Direct benefits (in this case, future reproductive opportunities) were precluded by the complete (100%) mating skew found within kin groups and the lack of dominance inheritance (Krakauer, 2005).

Predation is unlikely to explain *G. major's* lekking behaviour due to the fact that they advertise from underground burrows. This leaves two potential

explanations for joining a lek: kin selection and immediate increased mating opportunity. We focus on the former because data on female behaviour and male mating success is not available. Furthermore, due to the construction and maintenance of a single calling burrow at a time, relative positions of *G. major* males within the lek are extremely stable over the course of the intense, brief calling season (Hill, 1998, 1999). Females, conversely, may experience high mortality through predation during their flight in search of calling mates. Choice for male call components such as amplitude, frequency, or chirp rate likely operates in *G. major* as in other crickets (Forrest & Green, 1991; Wagner & Reiser, 2000); male attractiveness is extremely repeatable and skew in female attractions is high (Howard *et al.*, 2011). *G. major* males also retain normal flight wings (a feature lost in some other mole cricket species), so we assume they are capable of flight, although it has never been observed. This potential difference in predation pressure linked to the travelling sex is one possible explanation for the highly male-biased operational sex ratio observed.

Here, following the development and application of 15 species-specific microsatellite DNA markers, we undertake the first genetic study evaluating the kin selection hypothesis in a lekking insect. Using the prairie mole cricket as a model, we expect to observe higher relatedness within than between leks if close relatives are lekking together. If male dispersal is limited, these patterns may result from the passive process of natal philopatry (through limited dispersal) rather than active choice of a lek from which to advertise; although, kin selection may operate in either situation. Conversely, if we find no patterns of relatedness, we can assume that the spatial organization of individuals is random and can thus rule out the possibility for kin selection explaining male aggregation in this species.

MATERIAL AND METHODS

SAMPLE COLLECTION

Preliminary tissue samples were collected from two individuals in 2012 from White Oak, OK (36.622825, −95.274093) and Paintbrush Prairie Conservation Area, MO (38.548495, −93.259620) for microsatellite primer design (see Supporting Information, Data S1). Precise location data were not gathered for 2012 specimens. Tissue samples for relatedness analysis were collected during the 2013 and 2014 seasons from White Oak, OK, USA. For these males, each active burrow (i.e. those containing a calling male) was marked with a surveyor's flag, numbered, the date of first activity recorded, and precise location logged using a Trimble Pro-XRT differential GPS receiver with a Nomad handheld unit (Trimble Navigation Ltd. Sunnyvale, CA, USA). All males were collected within prairie habitat during the calling season (April–May). In general, tissue was removed from males' wing-tips in a non-destructive fashion.
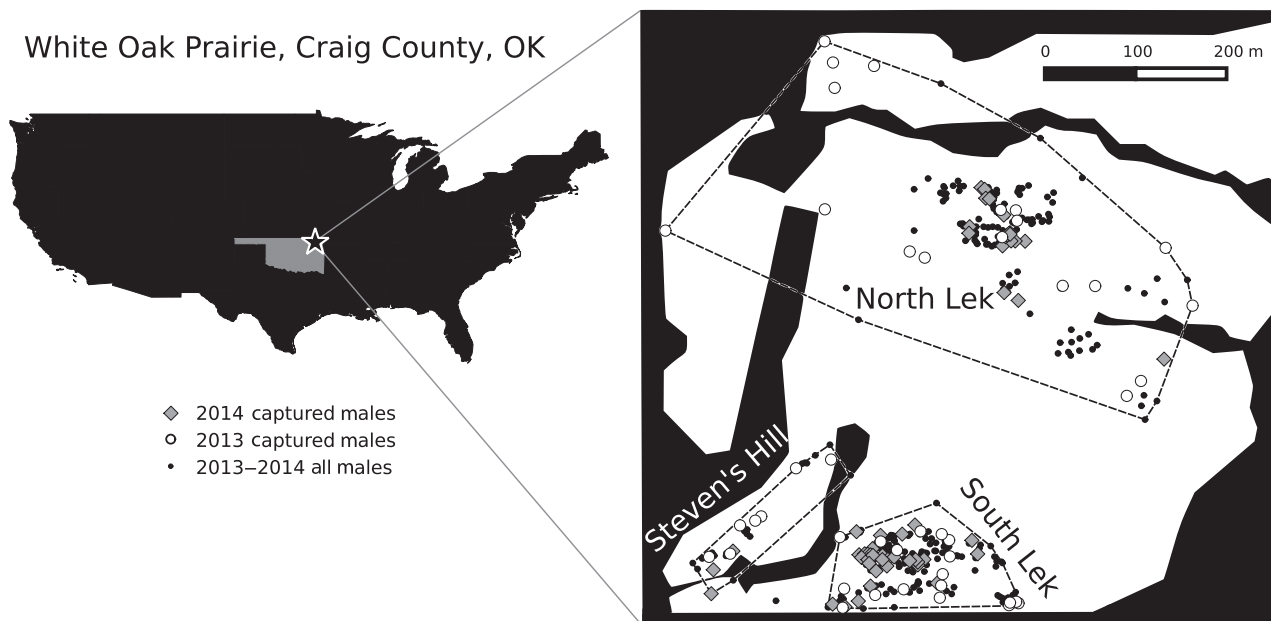

Figure 1. Location and layout of White Oak Prairie, Craig County, OK subdivided into general lek areas based on behavioral observations. Solid black area denotes unsuitable habitat (rocks, shrubs, forest, ponds). Sampled male burrows identified in white (2013) or gray (2014). Unsampled but active burrows are denoted by black dots.

Lek areas were delineated based on behavioural observations of individual clustering over several years (Hill, 1999) and assigned names: North, South, and Steven's Hill (Fig. 1).

MICROSATELLITE CHARACTERIZATION AND ANALYSIS

Microsatellite markers were designed via a high-throughput shotgun sequencing method, similar to Castoe *et al.*, 2010 (see Supporting Information, Data S1). Markers were subsequently optimized for use with low-yield wing-tip DNA (see Supporting Information, Table S1) and MICRO-CHECKER v. 2.2.3 software (Van Oosterhout *et al.*, 2004) was used to check for potential scoring errors and to estimate null allele probabilities (Supporting Information, Table S2). Tests for departures from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium

were performed using Genepop on the Web v. 3.4 (Raymond & Rousset, 1995). Genotypes were obtained for a total of 98 male crickets (2013: N = 45, 2014: N = 53, out of an estimated ~150 adult males in the population each year) and markers not meeting appropriate statistical criteria were left out of the analysis (Supporting Information, Table S2). Genetic data analysis (GDA; Lewis & Zaykin, 2001) was used to generate summary population statistics across each putative lek [mean number of alleles (N_A), expected (H_E) and observed (H_O) heterozygosity; see Table 1].

GENETIC STRUCTURE AND RELATEDNESS

Genetic differentiation between sampled years was assessed through F_{ST} (θ analogue – see Weir & Cockerham, 1984) using FSTAT version 2.9.3.2 (Goudet, 2001). STRUCTURE v2.3.4 (Pritchard, Stephens & Donnelly, 2000) and spatial principal component analysis (sPCA from the *adegenet* package in R, Jombart, 2008) were used to identify population subdivision within the combined 2 year dataset. While the former simply looks for genetic clusters, the latter is able to uncover spatial gradients in the distribution of genotypes (Jombart *et al.*, 2008). COANCESTRY software (Wang, 2011) was employed to calculate Wang's triadic relatedness values and test for significant group differences (see r, Table 1). COLONY (Jones & Wang, 2010) was used to classify sibling relationships, and distances between individuals were measured in QGIS (QGIS Development Team, 2012). Relatedness-by-distance relationships were tested for significance using Pearson correlation tests (Table 2, Pearson, 1895). An unequal-variance *t*-test (one tailed, Welch, 1947) was used to test for variation in mean dispersal of related vs. nonrelated individuals. See Supporting Information (Data S1) for more detailed molecular methods including software parameters.

Table 1. Population summary statistics by lek (North, South, Steven's hill)

Statistic	Population	2013	2014	Combined
N	All	44.6	52.8	97.4
	North Lek	18	18	35.7
	South Lek	19.7	32	51.8
	Steven's Hill Lek	7	2.7	9.9
A	All	8.7	8.7	9.8
	North Lek	5.3	5.3	8.3
	South Lek	5	6.3	8
	Steven's Hill Lek	4	2	5.3
H_E	All	0.75	0.74	0.75
	North Lek	0.65	0.54	0.74
	South Lek	0.64	0.66	0.74
	Steven's Hill Lek	0.67	0.39	0.73
H_O	All	0.74	0.67	0.7
	North Lek	0.67	0.52	0.71
	South Lek	0.64	0.68	0.69
	Steven's Hill lek	0.67	0.44	0.75
r	All	0.07	0.08	0.07
	North Lek	0.07	0.10***	0.08*
	South Lek	0.11***	0.09***	0.08***
	Steven's Hill Lek	0.13**	0.04	0.12***

N = average number of individuals genotyped per locus; A = mean number of alleles per locus; H_E, expected heterozygosity; H_O, observed heterozygosity; r = Wang's triadic relatedness estimator (Wang 2007), differences between lek and overall population means of r tested via 1,000,000 bootstraps.
* is mean difference > CI-95%; **CI-97.5%; ***CI-99% (null = no difference).

RESULTS

SUMMARY POPULATION STATISTICS

All loci proved polymorphic, with an average of 9.3 alleles per locus (range = 5–22, calculated using 15 loci). MICRO-CHECKER indicated that null alleles were likely at loci GM-2, GM-6, and GM-26 across leks and years (Supporting Information, Table S2). We removed these three loci from any subsequent analyses, but retained GM-30 for analysis (including for values in Table 1) because the possibility of null alleles for this locus was driven by only one of the leks, and thus likely to result from excess homozygosity and high relatedness. Overall, both years were found to display a highly significantly (P < 0.000) deviation from HWE at all loci, resulting from a heterozygote

Table 2. Pearson's product-moment correlations between relatedness and distance for each lek (North, South, Steven's Hill)

	2013 North Lek	2014 North Lek	2013 South Lek	2014 South Lek	2013 Steven's Hill	2013 Overall	2014 Overall
Correlation	−0.051	−0.168	−0.473	−0.222	−0.425	−0.161	−0.096
P-value	0.530	0.038*	6.75e-11***	1.3e-06***	0.055	6.58e-07***	4.88e-04***

Significant values in bold.
*P = < 0.05, ** is P ≤ 0.025, and *** is P ≤ 0.01.
Leks correspond to those in Figures 1 & 2.

deficit ($P < 0.000$, see Table 1 for expected and observed heterozygosity). The high variability exhibited at locus GM-29 (see N_A, Supporting Information, Table S1) may generate a type I error due to the sample size required to observe all allelic combinations, resulting in significant deviations from HWE. Thus we retained it for our analyses despite its being flagged for possible null alleles in two of the leks (Supporting Information, Table S2). No evidence of linkage disequilibrium was detected.

POPULATION GENETIC STRUCTURE

No significant genetic differentiation was found between the 2013 and 2014 datasets, with an overall F_{ST} of 0.005 (95% CI: 0.00–0.01). This was supported by STRUCTURE analysis on the combined dataset, which failed to separate samples by year. STRUCTURE results indicate that while there is no highly divergent population structure, subtle kin patterns exist across the study site. Peaks in delta k were found at 2, 5, and 8 using the Evanno method (Evanno, Regnaut & Goudet, 2005). Secondary peaks are often ignored in STRUCTURE analyses because, instead of reflecting divergent population structure, they often represent kin groups. We tested this assumption by mapping the STRUCTURE assignments for the above k values, which show a clear pattern of spatial autocorrelation/variance [global multiple regression over Moran's Eigenvector Maps (MEMs), $P = 0.01$ for $k = 2$
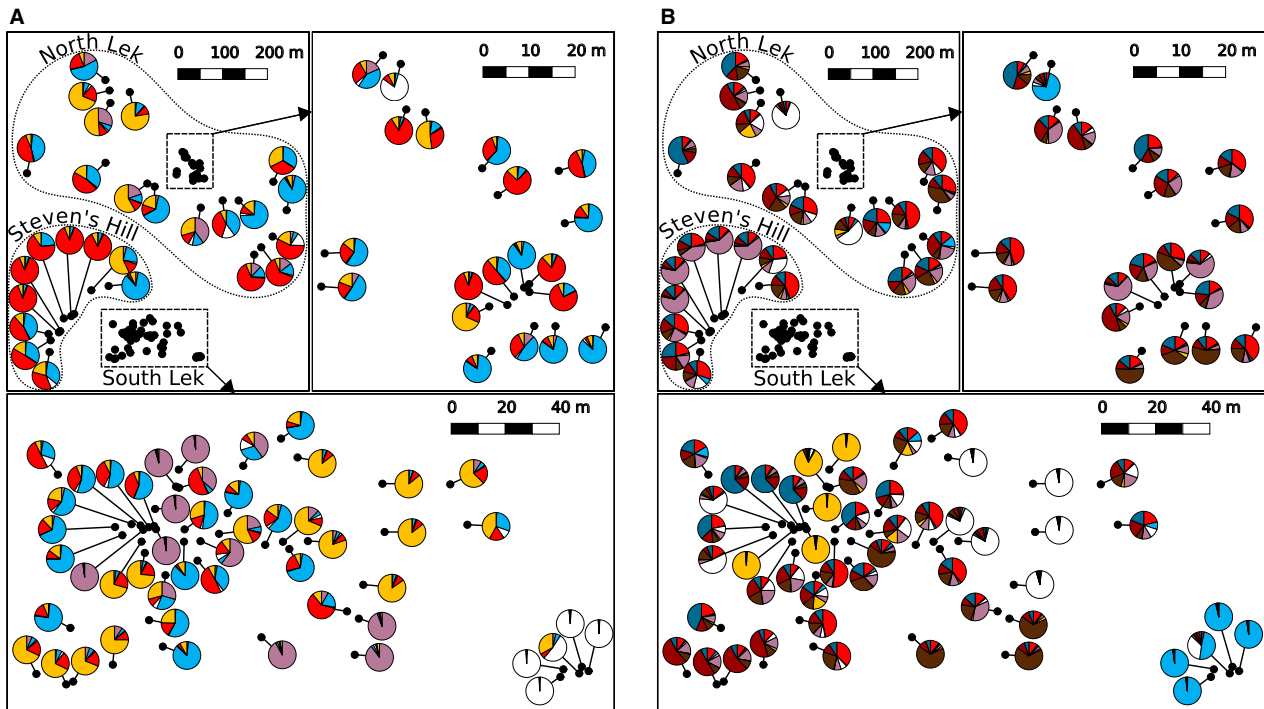

Figure 2. Structure assignment maps for A) k = 5, and B) k = 8. Pies represent STRUCTURE cluster assignment probabilities, with attached dots indicating precise male sampling site. Upper left tile of each shows the entire population with the upper right and bottom tiles representing zoomed insets of the central portion of the north lek and the entire south lek respectively.

(see Supporting Information, Fig. S1), $P = 0.007$ for $k = 5$ (Fig. 2A), and $P = 0.0004$ for $k = 8$ (Fig. 2B)]. sPCA reveals similar global structure across White Oak, and also shows clear genetic gradients across the population [global MEMs were again significant ($P = 4e-05$); see Supporting Information, Fig. S2]. A plot of the autocorrelation against the variance shows a drop off after the first seven spatial principal components (see Supporting Information, Fig. S3), demonstrating that these seven represent organized genetic structure as opposed to noise.

RELATEDNESS

We typically observe at least three separate lekking areas at White Oak every year (defined in Fig. 1), based solely upon individual male spatial location at the site (insufficient males were collected from Steven's Hill in 2014 for relatedness analyses of this group). To address the predictions set forth by the kin selection and philopatry hypotheses, we compared mean relatedness values within leks to the population as a whole. Using 1 000 000 bootstrap replicates, we tested for differences between years and found no significant difference. Comparing relatedness within leks to that of the population as a whole yields several significant differences, the general trend showing increased relatedness within subgroups (Fig. 3, Table 1). In exploring relatedness vs. Euclidean distance (Fig. 4), the Pearson correlation tests revealed significant negative relationships for each year overall and separately in a majority of the leks (Table 2). The negative relatedness-by-distance relationship is highly significant for the 2013 Steven's Hill lek if a single outlier (and likely part of a separate lek) is removed from the dataset ($P = 0.0002$). The plots of relatedness-by-distance (Fig. 4) display a very similar trend across years and
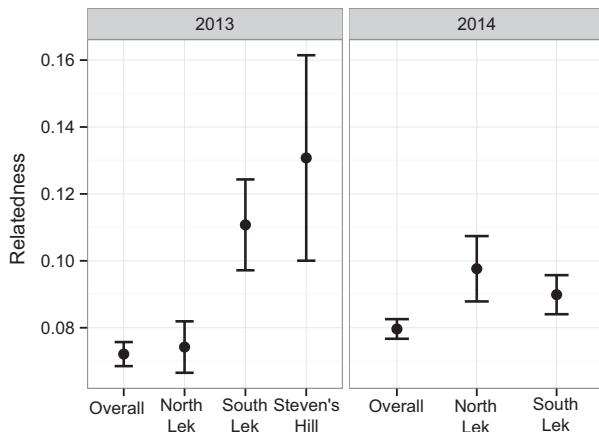
it is clear that the linear correlation detected by the Pearson tests is primarily the result of spikes in the relatedness of close neighbours (at below ~50 m, a sign that immediate male kin remain spatially clustered). Thus the true relationship would be better described by an exponential decay function, a pattern clearly observed in a majority of the lek plots (Fig. 4, Table 2). We attribute the secondary increase in relatedness over longer distances to low sample size (in Steven's Hill 2013 this pattern is driven by a single data point). Additionally, because relatedness values are pairwise, dispersal of one male in a family group may add several points, as seen in the secondary peak in the south lek 2013 data that is driven by only 2–3 males (Fig. 4).

KIN DISPERSAL

Male full siblings on average occupied burrows much closer to each other than half-siblings (one-tailed *t*-test, unequal variances, $P < 0.0001$) or less related males ($P < 0.0001$). Average distance between individuals classified as full-sibs ($N = 29$ pairs) was 22.73 ± 19.82 m, while that between individuals classified as half-sibs ($N = 45$ pairs) was 223.95 ± 248.53 m. Average distance between all pairs of captured males from the population not classified as full- or half-sibs (each year analysed separately) was 376.85 ± 262.40 m ($N = 2201$ pairs).

DISCUSSION

Results suggest that at least three different levels of spatially autocorrelated genetic structuring occur in prairie mole crickets (at $K = 2$, 5, and 8 clusters, with potentially more), including lineages that are mainly confined to specific leks in the population and also highly related male kin clusters that appear temporally stable across years. Nested spatial organization was confirmed in previous work (Hill, 1999). Under a scenario of female-biased dispersal and male multiple mating, the average distance between full-sibs, who originate from the same maternal brood 100% of the time, should be less than that between half-sibs, who originate from the same brood only if they share a mother. We speculate that the kin clustering indicated here is likely the result of extreme male viscosity, as opposed to the re-association of sib-groups during lek formation.

Although we cannot rule out dispersal and subsequent re-association of male kin (possibly via chemical recognition cues, which have been shown to operate in other crickets – see Simmons, 1990), we find this unlikely for three reasons. First, only one recaptured male was found more than a few metres
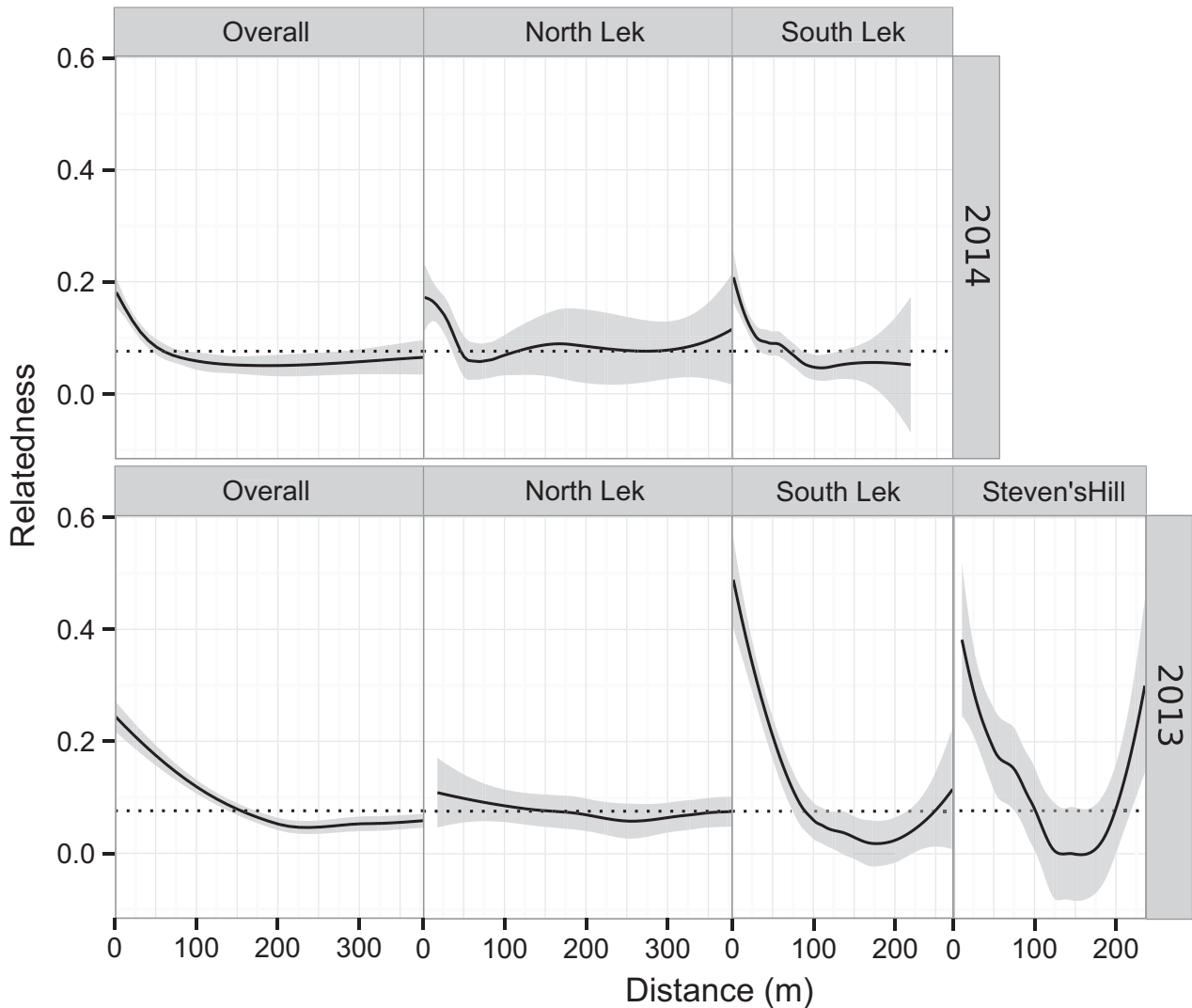
Figure 3. Population and lek relatedness means by year with standard error bars.

Figure 4. Loess regressions of pairwise relatedness-by distance with gray standard error area, implemented in R (R Development Core Team, 2011). Relatedness values estimated using Wang's triadic method (Wang 2007). The first plot in each row shows values for the entire population, with subsequent plots displaying values for each lek area for the year denoted at right.

from his original burrow (but still adjacent to his old one, $N = 4$ recaptured males). Second, no records exist of *G. major* adult males flying to lights, conspecific calls, or sound traps although males of other mole cricket species are readily found in this manner (Walker & Forrest, 1989). Thirdly, while males may somehow be able to evaluate a few immediate neighbours for relatedness, siblings are more likely to be adjacent than to be nearest neighbours, and full-sibs on average occur closer together than half-sibs, who in turn are closer than nonsiblings (all suggesting the pattern is a result of 'passive' processes). Therefore, we conclude that the genetic structure observed is likely due to passive processes rather than active choice to display near relatives.

DEFINING THE LEK

Our study site likely consists of multiple functional leks of between five and ~50 interacting individuals, given an estimate of an 80–100 m effective signal range (Hill, 1998; Howard *et al.*, 2008), which is comparable to previously published lek sizes (Hill, 1999). Using this range to delimit leks in our population would identify more than ten separate leks, many including only a handful of individuals (especially in the northern half of the site). However we are unable to sample every male in the population, leaving us unable to effectively test genetic differentiation at that scale. Instead, we chose to focus on a somewhat larger scale within the population, where

we have consistently observed three isolated lekking arenas, annually, over a period of more than 20 years. These three areas have been designated as follows: (1) the North Lek, with burrows widely dispersed in smaller aggregations (clearly consisting of several leks) across an area of ~16 ha, and separated from the other areas by a marsh; (2) the south lek, covering an area of ~ 2.5 ha, likely comprising two leks; and (3) a smaller lek we refer to as Steven's Hill, covering an area of ~1.1 ha and located to the northeast of the south lek, which probably represents two functional leks (separated from the south lek by a woody swathe of shrubs, see Fig. 1). While environmental factors probably prevent males from advertising in these woody/marshy boundary areas or traversing them in underground tunnels, they do not appear to represent a significant barrier to gene flow (probably primarily through female mating flight), given the results of STRUCTURE analysis.

STRUCTURE analyses discerned two widespread groups distributed across the entire population, along with secondary groups of five and eight clusters that likely represent cohesive kin groups that exist over at least two non-overlapping generations (2013–2014). sPCA, sibship analysis, and relatedness-by-distance correlations all support the existence of kin clusters within leks, even though it is clear gene flow is occurring between leks. Several studies have noted that STRUCTURE analyses resulting in an unexpectedly high value for K or secondary peaks in delta k are caused by the program picking up the signature for kin groups (von Holdt *et al.*, 2008, 2010). Because families and populations are patchy in occurrence, we should expect nested (or step-wise) genetic patterns to be common in nature. The results of sPCA show significant patterns of genotype distribution across the spatial matrix, consisting of extensive fine-scale population structure as expected under a kin group scenario. This research suggests that isolated kin groups occur and remain somewhat structured (over at least two generations) due to limited male dispersal within an otherwise admixing *G. major* population.

PHILOPATRY HYPOTHESIS

While our data do not fit the philopatry hypothesis of Francisco *et al.* (2009), we clearly see a pattern indicative of male philopatry, likely due to highly viscous dispersal of males. Francisco *et al.* (2009) find that related manakins lek together by chance as a result of limited dispersal, yet the pattern is random and within-lek relatedness is not significantly elevated. In contrast, we find that *G. major* relatedness within leks is significantly elevated. The short

distances between full siblings, coupled with subtle spatio-genetic clustering, suggest highly limited dispersal in *G. major* males, and we argue that the predictions set forth by Francisco *et al.* (2009) only apply to a subset of organisms that are highly dispersive across a limited environment (e.g. tropical forest understory-dwelling birds). We interpret the non-significance in the difference of mean relatedness levels across the north lek vs. the population as a whole as a by-product of our sampling scheme (more widespread during 2013) and that the area we defined as the north lek consists of more than one functional lek, as indicated by STRUCTURE results (Fig. 2). Indeed, it is the largest and least aggregated of our three subgroups. This sampling scheme resulted in fewer sampled nearest neighbours and thus fewer full siblings, depressing mean subgroup relatedness. A more closer look at the data shows that these within-group relatedness levels are driven more by the close proximity of highly related kin than by a linear relatedness-by-distance effect, which is a common feature throughout the population. Likewise, an almost identical relationship can be discerned separately in each of the three leks (Fig. 3) and confirms an earlier report of spatial clustering at closest nearest neighbour distances (Hill, 1999).

KIN SELECTION HYPOTHESIS

Three general components are necessary to prove the existence of kin selection in leks: (1) males must preferentially lek with relatives, (2) brothers must help each other gain more mating opportunities, and (3) indirect benefits must outweigh the costs of some males forgoing reproduction. Our results are similar to those found in white-bearded manakins, where elevated relatedness was found within subgroups (Shorey *et al.*, 2000). However, the subgroups delineated by Shorey *et al.* (2000) do not coincide with the actual lek. If kin selection is present at the scale of an entire lek, we would expect to see a somewhat linear correlation between relatedness and Euclidean distance (and we would expect a drop off wherever lek boundaries are found). While initial statistical tests on *G. major* do reveal a linear relatedness-by-distance correlation, visualization of the data shows that there is a clear reduction in relatedness after ~50 m with a subsequent plateau, indicating that a negative exponential function better fits the data. This shows that while there is elevated relatedness within leks, perhaps we are not defining lek boundaries correctly with respect to functional scale in the quest to uncover kin selected benefits. Shorey *et al.* (2000) also reported significant genetic structuring within leks. We suggest the possibility that smaller kin clusters are operating within leks and that if kin

selected benefits are present, they must be occurring at a different scale than generally assumed in the lekking-kin selection hypothesis (Kokko & Lindström, 1996). Males at White Oak Prairie are aggregated to a statistically significant degree at three levels of scale (the lek, smaller groups within the lek, and the very nearest neighbours within this smaller grouping; Hill, 1999). We would not expect to see multiple stratified kin groups within a lek if kin selection was a causative factor in the evolution of leks; although, we should note that our study focused on a large, dense natural population of G. major.

Kin selection may continue to operate if increased per-capita matings occur within segregated kin clusters – perhaps those that are denser (but see Howard et al., 2011). Patterns of female preference/attraction have not been studied for this system, so it is unknown whether males in larger leks accrue proportionally more mating opportunities. In a setting with smaller, more isolated, leks (as is common across G. major's range) flying females may effectively discriminate between kin groups through their choice of lek while in flight, based on characteristics of the males' sexual advertisement, or through more passive means such as dispersal distance. Like in males, however, we suggest the likelihood that any potential discrimination of relatedness by females in G. major occurs through a passive process. Unless females disperse significantly farther than males underground or undergo multiple risky mating flights (during the short calling season), it is unlikely for them to evaluate many partners via chemical recognition cues, which generally require physical contact. Furthermore, females of many lekking species are thought to have few mates during a season (Höglund & Alatalo, 1995). To our knowledge, no precedent for acoustic kin recognition exists for insects; although, acoustic environment can influence choosiness (Swanger & Zuk, 2015). In theory, inclusive fitness benefits may drive active female preference for related individuals (Kokko & Ots, 2006), but this has never consistently been demonstrated over multiple contexts (Reynolds et al., 2014). In most cases examined, organisms prefer unrelated mates, such as in field crickets (Simmons, 1989). Like in many other systems, it is likely that female preferences in G. major are highly dependent on the environment, where inbreeding, mate density, and predation risk may each significantly influence mate-choice behaviour (Jennions & Petrie, 1997).

In working with the prairie mole cricket it is sometimes difficult for researchers to define the boundaries of a lek, suggesting G. major females may have the same problem. G. major males cannot see each other, and likely have neurological constraints on how many other male callers they can discriminate at any one time (Pollack, 1988), especially if substrate vibrations are the main channel through which this occurs (Hill & Shadley, 1997, 2001). Groups of two to three G. major males are often observed alternating their calls, and we doubt that males have direct contact beyond these immediate neighbours. Yet, their airborne calling songs likely propagate at levels above the hearing threshold of G. major to distances up to 80–100 m (Hill, 1998; Howard et al., 2008). We note that it is highly unlikely that males of this species have any interactions with those on the opposite side of a lek. G. major females also probably filter out males that are quieter or farther away (Pollack, 1988); although, attractiveness of certain males is highly consistent – suggesting the possibility that females may regularly fly over closer, unattractive males to reach sexy ones (Howard et al., 2011). The 'hotspot' hypothesis of lek evolution suggests that males may choose a lek location simply in response to chances of encountering females (Bradbury, Gibson & Tsai, 1986). We have been unable to evaluate this hypothesis, as spatial patterns of female emergence remain unknown. It is probable that female mole crickets limit their mate sampling to a subset of males within a lek, potentially resulting in a lack of selection on preference for larger leks. An alternative, the 'hotshot' hypothesis, attributes lek formation to male preference to advertise near a 'hotshot' male. The elevated relatedness observed between G. major male neighbours (suggesting that they do not travel very far), makes the 'hotshot' scenario an unlikely fit for this system. The most parsimonious explanation is that spatial patterns of male kin clustering and female attraction both result from a passive process in a heterogeneous environment – one from which we cannot yet rule out kin selection.

Overall, our results suggest that G. major males tend to remain in close kin clusters despite occasional male dispersal occurring throughout populations. Significant relatedness-by-distance correlations, elevated relatedness levels within subgroups, and spatial autocorrelation of genotypes all point to a system where male neighbours tend to be highly related to each other. While males may have the ability to disperse (via flight – they have normal flight wings), they appear to be preferentially philopatric. Kin-selected benefits, if they occur in this system, likely are a passive by-product of population viscosity rather than active choice to display with relatives. Furthermore, it is possible that G. major lekking behaviour is simply the result of limited male dispersal, although it is clear that dynamic processes are operating within leks (ex. different tiers of genetic and spatial organization, see Hill, 1999).

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AUTHOR CONTRIBUTIONS

KTK established the experimental design, and collected all data. KTK and WB provided molecular reagents and performed analyses. The study was conducted on the field site used by PSMH to gather data for most of the extant literature on this species. All three authors contributed to the writing and editing of the paper.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Structure assignment map for $k = 2$.
Figure S2. Spatial distribution maps of the first four spatial principal components from sPCA with Delaunay neighbour networks connections (A–G represent spatial principal components 1–7 respectively).
Figure S3. Screeplot of the spatial (Moran's I) and variance components of sPCA eigenvalues which can assist in determining how many spatial principal components (sPCs) contain valuable information.
Table S1. Characteristics of 15 microsatellite DNA loci developed for the prairie mole cricket and screened for a total of 98 specimens collected at White Oak Prairie, Craig County, Oklahoma, USA.
Table S2. Chakraborty null allele estimates by lek calculated via MICRO-CHECKER software.
Data S1. Molecular methods.

SHARED DATA

Raw Illumina reads of this article are available on GenBank website (accession nos. SRR2068349-SRR2068365) and in the Dryad digital repository (Keane *et al.*, 2015).