Quantifying movement behavior, the first step of sexual selection, in the lizard *Anolis sagrei*.

Ambika Kamath[1,2,3] and Jonathan B. Losos[1]

1. Department of Organismic and Evolutionary Biology and Museum of Comparative Zoology, Harvard University, 26 Oxford Street, Cambridge MA 02138
2. *Present Address*: Department of Ecology, Evolution and Marine Biology, University of California Santa Barbara, Santa Barbara, CA 93106
3. Author for correspondence. Email: ambikamath@gmail.com

Abstract

How individuals move through their environment dictates which other individuals they encounter, determining their social and reproductive interactions and the extent to which they experience sexual selection. Specifically, females rarely have the option of mating with all males in a population—they can only choose among the males they encounter. Further, quantifying phenotypic differences between the males that females encounter and those that sire females' offspring lends insight into how social and reproductive interactions shape male phenotypes. We used an explicitly spatiotemporal Markov chain model to estimate the number of potential mates of *Anolis sagrei* lizards from their movement behavior, and used genetic paternity assignments to quantify sexual selection on males. Females frequently encountered and mated with multiple males, offering ample opportunity for female mate choice. Sexual selection favored males that were bigger and moved over larger areas, though the effect of body size cannot be disentangled from last-male precedence. Our approach corroborates some patterns of sexual selection previously hypothesized in anoles based on describing them as territorial, whereas other results, including female multiple mating itself, are at odds with territorial polygyny, offering insight into discrepancies in other taxa between behavioral and genetic descriptions of mating systems.

Keywords: mating system, territory, territoriality, polygyny, anole, encounter rates

Introduction

Sexual selection is a layered process, with animals sequentially having to overcome intrasexual competition, intersexual mating preferences, and, for males, post-copulatory competition and choice before achieving reproductive success [1, 2]. Decades of research have spawned a vast literature on each of these aspects of sexual selection. However, a crucial but little-appreciated component of measuring sexual selection in natural populations involves quantifying the very first step of the mating sequence, namely encountering potential mates. How individuals move across space through time directly influences the number and phenotypic distribution of potential mates they encounter [2–4]. Moreover, by bringing about encounters between potential mates as well as between potential competitors, individuals' movement patterns set the stage for subsequent sexual selection through male-male competition and female choice [5, 6]. Documenting animals' movement patterns, to understand how often and which members of the opposite sex are encountered by individuals, is thus fundamental to discovering the extent to which sexual selection can act in the wild. Concurrently, quantifying phenotypic differences between potential mates (individuals encountered) and actual mates (individuals whose offspring are borne), yields insight into the nature of selection imposed by social and reproductive interactions.

In particular, individuals' movement patterns determine the potential for female mate choice to drive sexual selection [7, 8]. Female mate choice has been studied extensively, yielding vigorous debate surrounding the precise mechanisms by which it arises, acts, and is maintained across a range of taxa (reviewed in [9–11]). Common to all models of female choice, however, is the idea that females can choose among males. But whether and to what extent individual females in fact have such a choice, and therefore the extent to which female choice can drive sexual selection, depends in large part on how many males they encounter [3, 12, 13].

In studies of sexual selection, examinations of movement behavior are often restricted to considering how females sample among males in taxa where female mate choice is already acknowledged to be important. For example, searching behavior is often thought to be pertinent to sexual selection in species where females must visit and sample from among males in leks or at fixed display sites (e.g. [8, 14]). However, similar measurements of movement behavior, and of encounters between potential mates, are equally relevant to understanding the opportunity for sexual selection

in other animal species as well, including those where female choice is not usually considered a major selective pressure [13, 15].

Unexpected opportunities for female choice are often uncovered in species in which earlier behavioral descriptions of mating systems, based on movement patterns as well as social interactions, are found to be inconsistent with more recent genetic descriptions of mating patterns [16]. For example, most birds were widely regarded as monogamous prior to the advent of genetic tools that revealed frequent extrapair copulation [17]. Occasionally, these inconsistencies have prompted researchers to reexamine animals' movement patterns to reconcile behavioral and genetic descriptions of mating patterns (e.g. [18, 19]). For example, tracking the movement behavior of red deer revealed that females move long distances between harems unexpectedly often, demonstrating the possibility of female choice in a system where sexual selection was thought to be dominated by male-male competition [20]. In general, though, discrepancies between behavioral and genetic descriptions of mating systems remain common—consider how often species are described as "socially monogamous," for example, but "genetically promiscuous." These discrepancies imply that we remain without complete or correct descriptions of movement and social behaviors that account for polygynandry in many species, and are thus unable to fully understand how sexual selection has shaped and is shaped by these behaviors.

In this paper, we develop an explicitly spatiotemporal approach to estimate encounters between potential mates from observations of the movement behavior of male and female *Anolis sagrei* lizards. Our first goal is to investigate if females encounter multiple males, which could offer females the possibility of mate choice. This possibility has previously been considered unlikely in most anoles, which have widely been described as territorial and polygynous despite genetic data revealing widespread multiple mating (reviewed in [21]). Our second goal is to characterize sexual selection in this population by examining the predictors of male reproductive success at two levels. First we ask if the number of potential mates encountered by males is associated with their phenotype (the spatial extent of their movement and body size). Second, we test three hypotheses to understand the phenotypic differences between potential mates (males encountered by females) and actual mates (males that sire females' offspring). We first examine if females bear offspring sired by the males they encounter more often [22]. Then, we ask if males encountered later in the breeding season are more likely to sire offspring than males encountered earlier ("last-male precedence"; [23]). Finally, given widespread sexual selection in animals for larger males [24] as well as pronounced

male-biased sexual size dimorphism in *A. sagrei*, we ask if females disproportionately bear offspring sired by larger males. We find evidence that females both encounter and mate with multiple males, and that sexual selection acts on movement behavior, male-female interactions, and body size in male *A. sagrei*, suggesting the possibility for complex polygynandrous reproductive dynamics including both pre- and post-copulatory female mate choice. We discuss how some of our results are consistent with previous descriptions of *A. sagrei* as territorial while others are unlikely to have been considered from within the framework of territoriality. Finally, we consider how paying attention to movement behavior can allow for the reconciliation of diverse types of behavioral and genetic studies of animal mating systems in many taxa.

Methods

Field sampling and egg collection

Anolis sagrei is a low-perching arboreal lizard native to Cuba and the Bahamas that has been established in Florida for nearly a century [25, 26]. It co-occurs with the native, higher-perching *Anolis carolinensis* throughout most of its range in the US, including in our sampled population; however, few interactions between the two species were observed.

Anolis sagrei lizards were captured, marked, and monitored to estimate their movement patterns in the University Gardens on the University of Florida campus in Gainesville, FL, from March 4, 2015 to May 25, 2015 between 0900 and 1800 hours. The start date of sampling coincided approximately with the increased activity of these lizards after the winter and the beginning of the breeding season; we concluded sampling at about the time at which female *A. sagrei* began laying eggs (based on our observations in 2014 of hatchlings appearing at the end of June, after an approximately month long incubation period [27]). We caught almost every lizard within a 7140 m^2 area and marked each captured individual with a unique bead tag [28], which allowed us to subsequently observe and identify individuals from a distance without disturbing them (in total, 4% of observations were of unmarked individuals). When captured, we measured each individual's snout-vent length (SVL) as a measure of body size, and removed ~2-3 cm of tail tissue for genetic analysis. At each subsequent observation of a lizard, we noted its identity as well as the time and location of the observation. We avoided observing the same individual more frequently than once

per hour, allowing ample time for lizards to resume normal behavior if disturbed by us. Approximately once a month, we recaptured and re-measured males to estimate the average growth rate of males in this population.

We measured distances between the locations at which lizards were observed to triangulate their relative positions. In areas of continuous vegetation, locations more than 1m apart were considered distinct. We also mapped the location of all trees within the site at which lizards were not observed, to include all trees to which a lizard could potentially have moved in our estimations of their movement patterns (Figure S1).

At the end of the observation period (which, based on 2014 observations, was approximately when females began laying eggs), we captured 36 marked females and brought them into captivity. Females were housed singly under established anole husbandry conditions [29] until mid-November. Each cage contained a pot of soil in which the resident female laid eggs fertilized by sperm stored from her copulations in the field. Eggs were incubated for two to ten days, after which embryos were dissected from them for genetic analysis.

Movement pattern analysis

Analyses were carried out in R v. 3.3.2 [30]. We used a discrete-time Markov chain to model lizards' movements between the mapped locations in the site. We divided daytime hours (0800 to 2000 hours; anoles are diurnal, so we assumed that the lizards did not move at night) over the sampling period (83 days) into 996 hour-long blocks. Observations were assigned to the bin closest to the time of the observation. Transition probabilities (P_{ij}) between locations i and j were modelled as exponentially declining with the distance between the locations (d_{ij}), with rows of the transition matrix then normalized to sum to one (N is the total number of locations):

$$P_{ij} = \frac{e^{\lambda dij}}{\sum_{j=1}^{N} e^{\lambda dij}} \qquad \text{(Equation 1)}$$

In other words, lizards were modelled as less likely to move to distant locations than to close locations, with a total probability of 1 of moving from each location to *some* location within the site, including staying at the same location. The value of the exponential decline parameter λ was estimated by maximizing the likelihood of the observed data (including only pairs of consecutive observations of lizards, i.e. no assumptions were made while fitting the model regarding mortality or

emigration after the last observation of a lizard) using the *bbmle* package [31]; separate models were fit for males and females.

The probability that a lizard was at a particular location at a particular hour was inferred using the Markov chain model described above. Methodological details can be found in the Supplementary Information, but briefly, this probability depended not only on where that lizard was seen previously but also where it was seen next. We thus calculated, for each lizard, a matrix of probabilities that it occupied a particular location at a particular hour, for all locations and hours. Then, we performed element-wise multiplication of pairs of these matrices to calculate the probability of co-occurrence at each one hour-long time bin, for every possible pair of lizards. Encounters were categorized for each pair at each hour ("yes/no") from these probabilities by setting cutoffs based on the co-occurrence probabilities calculated for pairs of lizards observed in the same location within an hour of one another. For each hour, we inferred an encounter between a pair of lizards if their co-occurrence probability was larger than the cutoff.

To quantify potential mating opportunities for each individual, we calculated the number of females encountered by each male and the number of males encountered by each female, as estimated by our model. The proportion of females that encounter multiple males and the mean number of males encountered by females reveal the extent to which multiple mating by females is possible in this population.

We quantified the spatial extent of an individual's movement by calculating the mean of the distances from each observation of the individual to the centroid of all observations of the individual (mean distance from centroid). Lower mean distance from the centroid indicates smaller spatial extent. We jittered points randomly within a 0.5 m radius along both the X and Y axis of our site before calculating mean distance from the centroid, to account for the 1 m resolution at which locations were mapped (Figure S1).

We estimated a growth curve for males by fitting a logistic equation to males' SVL measured initially and at recaptures, using nonlinear least squares regression [32] (see Supplementary Information). We used this logistic growth curve to estimate the SVL of each male on the day of each of his inferred encounters, based on his SVL at the nearest capture, to test for sexual selection on male body size and for male avoidance of size-matched males (see below).

163 *Parentage analysis*

164 DNA was extracted from the 36 females housed in captivity, all 161 sampled males, and 383
165 offspring using an AutogenPrep 965. Six microsatellite regions were amplified for these individuals
166 (see Table S1 for primer and amplification protocol details; [33, 34]), with fragment analysis
167 performed at the DNA Analysis Facility at Yale University. Alleles were scored manually after
168 examining chromatogram peaks in Geneious v10.0.9 [35].

169 Parentage analyses were performed in CERVUS v3.0.7 [36]. High proportions of null alleles
170 were estimated at three loci (Table S1); following [37, 38], we retained these loci in the analysis but
171 typed apparent homozygotes at only one allele, with the other allele coded as missing. All offspring
172 had known mothers, and we provided as candidate sires for each offspring the list of males that its
173 mother was estimated to have encountered, as described above. In the simulation to determine
174 LOD score cutoffs for assignment, we provided a genotyping error rate of 0.01 (based on mother-
175 offspring mismatch across all loci); the proportion of loci typed was 0.81. Simulations were run with
176 23 candidate sires and the proportion of sires typed set to 0.75, based on the maximum number of
177 males encountered by any female in the population (17 males). These parameters were chosen after
178 running preliminary analyses with simulations in which the proportion of sires typed was set to 0.25,
179 0.5, or 0.75 and the number of candidate sires set correspondingly to 68, 34, or 23; we chose the
180 parameter combination with the closest match between the proportion of sires typed and the
181 observed assignment rate [39]. We performed further analyses to test the effects of restricting the
182 number of candidate sires relative to the whole population, and of providing all sampled males as
183 candidate sires for all offspring; we found that simply restricting the number of sires did not inflate
184 paternity assignments and that results downstream analyses were unaffected by excluding the 18
185 offspring whose paternity assignments differed in the analysis where all males were provided as
186 candidate fathers (see Supplementary Information).

187 *Hypothesis Testing*

188 The number of potential mates encountered and spatial extent (mean distance to the
189 centroid) had right-skewed distributions, and were therefore log-transformed before parametric
190 analyses; SVL was analyzed untransformed. We compared spatial extent between males and females
191 using a t-test weighted by the number of observations per individual, and investigated if variation in

192 males' spatial extent was related to body size, using a linear regression of SVL at first capture vs.
193 mean distance to the centroid, weighted by the number of observations per individual.

194 Next, we examined if the number of females encountered by males varied with the spatial
195 extent of males' movement (mean distance from the centroid) and with mean male body size at their
196 encounters with females, using a multiple linear regression weighted by the number of observations
197 per individual.

198 To assess if males avoided size-matched males, we examined the differences in estimated
199 SVL between pairs of males estimated to encounter one another, and compared these differences to
200 the differences in estimated SVL between randomly chosen pairs of males. For males that were
201 estimated to encounter one another, we used the logistic growth curve to estimate their SVL on the
202 day of the encounter. We initially sampled five random pairs per pair of males estimated to
203 encounter one another, estimated their SVLs on the same day as the corresponding encounter, and
204 eliminated random pairs in which either individual had an estimated SVL less than the minimum
205 observed SVL. We then repeated this random sampling a total of 3000 times to assess if the number
206 of male-male encounters among size-matched males (estimated SVL difference of 0-2 mm) was
207 significantly lower than expected by chance.

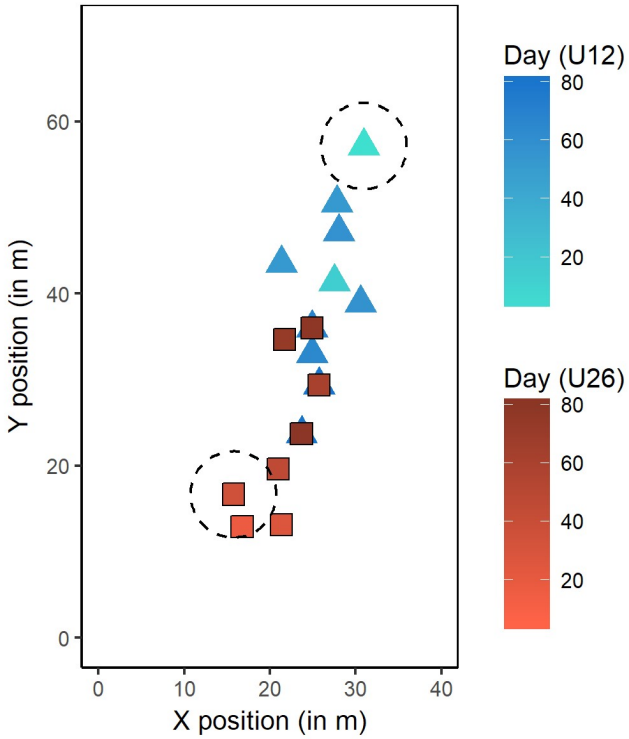
208 We used a resampling approach to examine (1) if males who sired individual females'
209 offspring encountered the mother significantly more often than males who encountered the same
210 females but did not sire offspring (encounter rate hypothesis), (2) if males who sired individual
211 females' offspring encountered those females later than males who encountered the same females
212 but did not sire offspring (last-male precedence hypothesis) and (3) males who sired individual
213 females' offspring were bigger than males who encountered the same females but did not sire
214 offspring (body size hypothesis).

215 We first calculated the difference between means of the number of encounters between
216 male-female pairs for sires and non-sires across all offspring. We also calculated, for each male-
217 female pair, the last hour at which the pair encountered one another and maximum SVL estimated
218 for the male across all encounters between the pair as an estimate of male body size, and then
219 calculated the difference between mean hour of last encounter and mean body size between sires
220 and non-sires. We then recalculated these differences between means after randomly assigning each
221 offspring a sire from the set of males that encountered the mother of that offspring. Random sire

assignments were performed in two ways. To address hypothesis (1), we sampled uniformly from the list of males encountered by each mother. Then, to address hypotheses (2) and (3), we sampled in proportion to the rate at which each male encountered each mother. The former allowed us to test if sires encountered mothers more often than did non-sires, and the latter allowed us to test if later-encountered males and bigger males sired offspring more often than earlier-encountered males and smaller males, after accounting for variation across males in encounter rates. Each resampling was repeated 10000 times.

Results

A total of 253 individuals (161 males, 92 females) were caught and marked during the sampling period, and were observed a total of 5629 times. The number of observations per individual ranged from one to 128; the median number of observations per individual for males and females was 11 and 15 respectively. An example of two individuals' locations through time is shown in Figure 1.



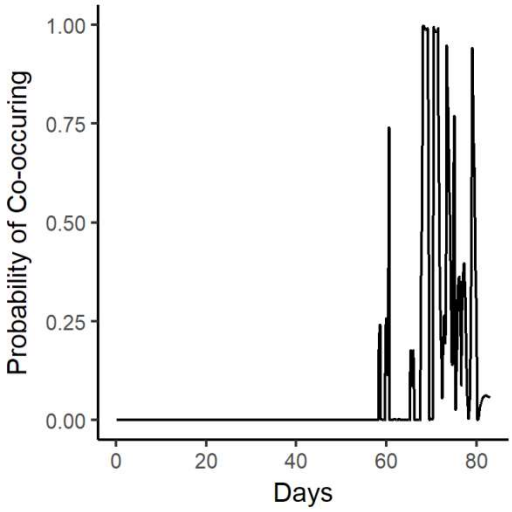


Figure 1. Examples of the locations through time for two individuals, U12 and U26 (left), and the probability of their co-occurrence as estimated by the Markov chain model (above). The dashed circles have a diameter of 10 m, which matches previous estimates of the territory size of male *Anolis sagrei*.

We used a Markov chain to model lizards' movements between locations in the site, where transition probabilities were modelled as exponentially declining with the distance between locations. We estimated λ values of -0.78 and -1.27 for males and females respectively (see Equation 1), indicating that males were more likely than females to move longer distances. Using this Markov chain model to estimate individuals' movement patterns and thereafter the probabilities of their co-occurrence (see Figure 1 for an example), we calculated that females encountered 5.1 ± 3.7 males (mean ± S.D.) and males encountered 2.9 ± 3.0 females; 78% of females and 60% of males encountered multiple individuals of the opposite sex (Figure 2). Males encountered an average of 4.5 ± 3.6 other males.

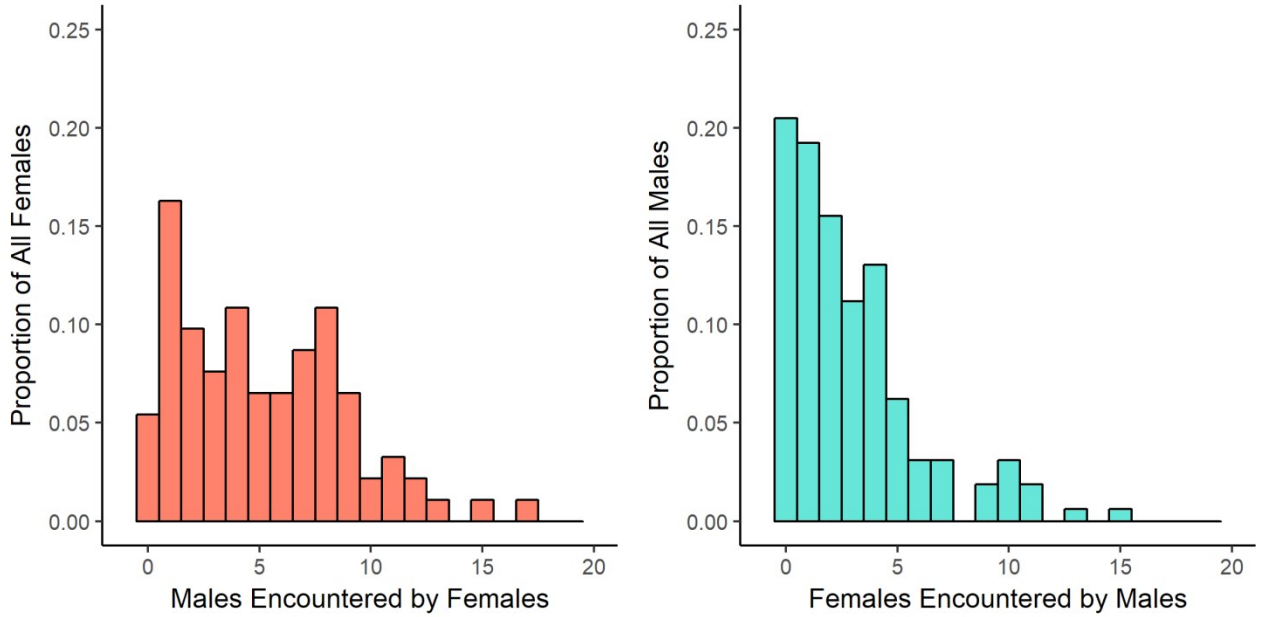


Figure 2. Histograms of the number of males encountered by females (left) and females encountered by males (right).

The mean distance from the centroid of all of an individual's locations ranged from 0.2 m to 41.3 m for males and from 0.2 m to 20.8 m for females (mean ± standard deviation for males vs. females: 6.8 ± 7.0 m vs. 2.7 ± 3.3 m; $t = 8.1$, $df = 208.7$, $P < 0.001$). This measure of spatial extent was weakly associated with SVL at initial capture for males ($r^2 = 0.04$, $F_{1, 135} = 4.91$, $P = 0.03$).

To estimate male body size at their encounters with females using a logistic growth curve, we recaptured 68 males and re-measured their SVLs a total of 94 times, with 32 ± 15 (mean ± SD) days elapsed between measurements. The mean difference in estimated SVL between pairs of males estimated to encounter one another (7.1 ± 4.5 mm) was comparable to the mean difference between randomly chosen pairs of males (7.4 ± 5.7 mm). However, observed size differences were underrepresented in the smallest (0-2 mm) category compared to random pairwise size differences (0.11 vs. 0.18 ± 0.002; $P <$ 0.0003; Figure 3).

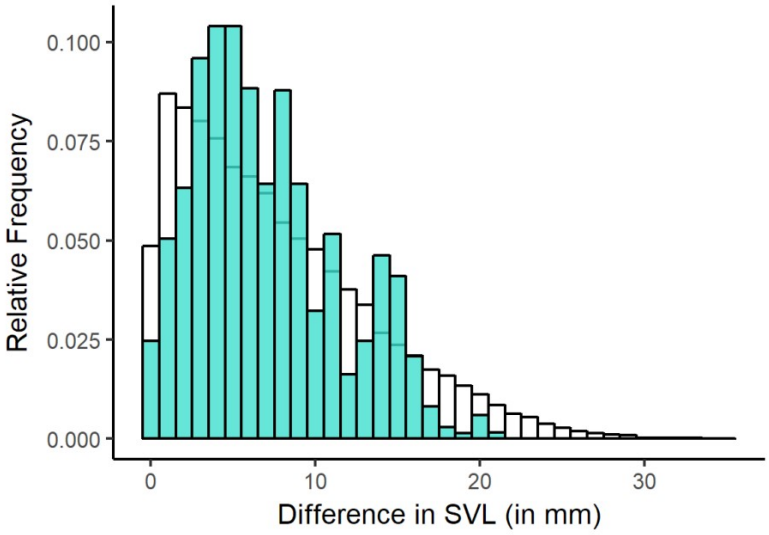


Figure 3. Estimated SVL differences between male pairs estimated to co-occur (blue bars), compared with pairwise SVL differences between randomly chosen pairs of males, with random males' sizes estimated on the same days at the observed co-occurrences (white bars).

Males that encountered more females had a greater spatial extent ($r^2 = 0.10$, $F_{1, 113} = 13.0$, P <0.001; Figure 4) and were larger in size on average at their encounters with females ($r^2 = 0.09$, $F_{1, 113}$ = 13.0, P <0.001; Figure 4); the interaction between spatial extent and SVL was not significant (r^2 = 0.0, $F_{1, 113} = 0.03$, $P = 0.87$)

Under the parentage analysis in which we set the potential sires for each offspring to be the males estimated to have encountered their mother, paternity was assigned to 84% of all offspring (323 individuals) at an 80% confidence level. We found that 64% of mothers bore offspring sired by more than one male; including offspring with unassigned sires, this proportion rose to 81%. Using a resampling approach to calculate p-values, we found that sires of offspring encountered mothers significantly more often than did non-sires (mean number of encounters between mothers and sires: 102 ± 140; non-sires: 40 ± 65; $P < 0.0001$). Accounting for variation across males in how often they encounter mothers, we found that sires encountered females significantly later than non-sires (mean ± SD of the last hour of encounter for sires: 892 ± 110; non-sires: 605 ± 258; $P < 0.0001$) and were

280 significantly bigger than non-sires (mean ± SD of the maximum male SVL across encounters for
281 male-female pairs, for sires: 57.8 ± 3.0 mm; non-sires: 53.2 ± 5.6 mm; $P < 0.0001$; Figure 5).

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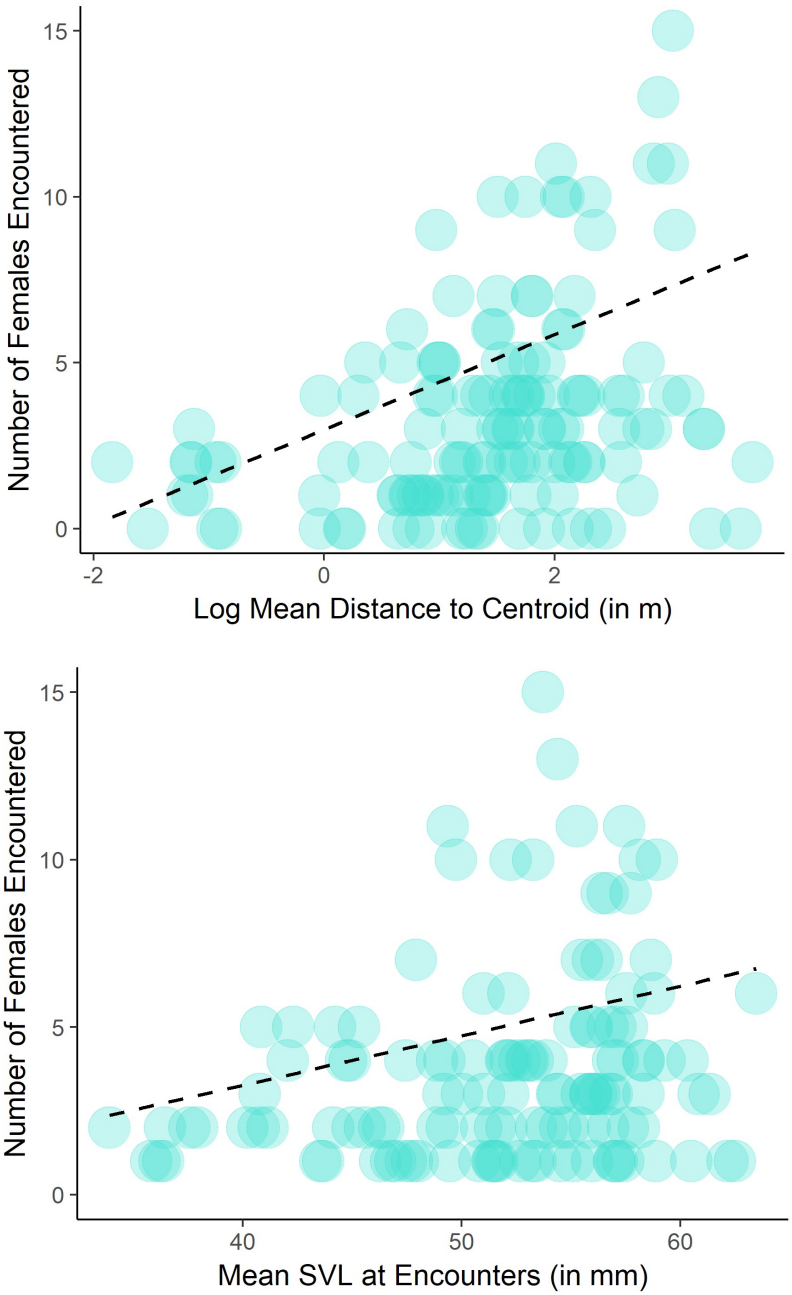

Figure 4. Relationship between the number of females encountered by males and the males' spatial extent (top; measured as the mean distance to the centroid) and males' mean estimated SVL across encounters (bottom).

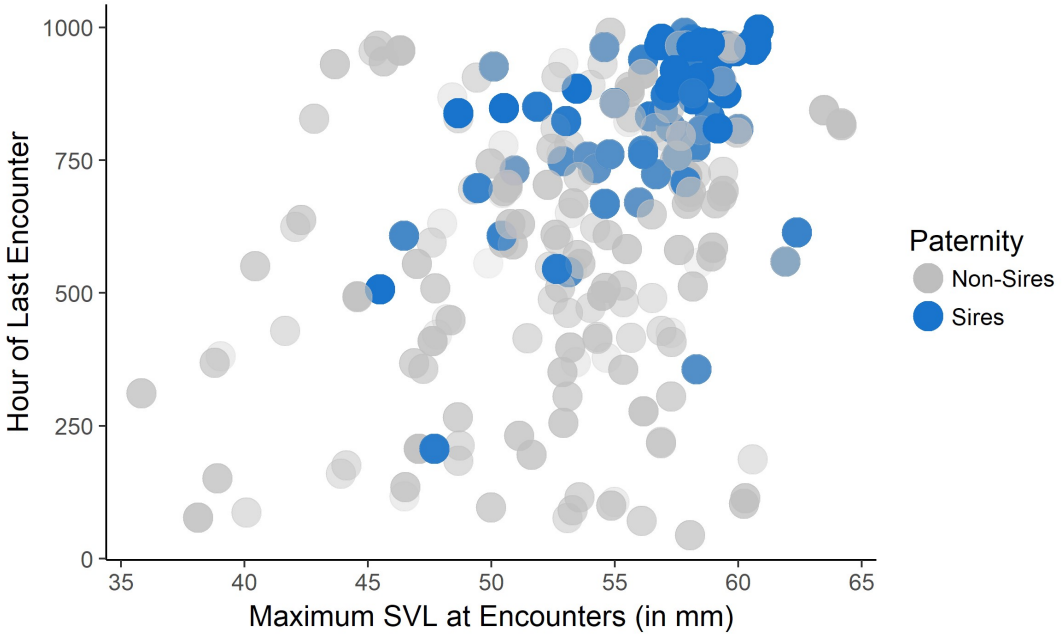

Figure 5. Maximum male snout-vent length (SVL) and the hour of last encounter for male-female pairs, colored by whether or not the male sired any of the female's offspring.

Discussion

How animals move through space determines how many and which other individuals they encounter, setting the stage for all of their subsequent social and reproductive interactions and ultimately determining their reproductive success. Understanding animals' movement patterns and the encounters they bring about is thus a key step in characterizing the mating system of a population or species, and is essential for determining how behavior both facilitates and is subject to sexual selection. In particular, female mate choice can contribute to sexual selection in natural populations only if individuals' movement patterns give females the opportunity to choose among mates. Developing a spatiotemporal characterization of the movement patterns of a population of *A. sagrei* lizards, we found not only that a majority of males (60%) encountered multiple females but also that a majority of females (78%) encountered multiple males over the first three months of the breeding season, indicating the potential in *A. sagrei* for complex polygynandrous mating patterns with ample scope for female choice.

Consistent with previous genetic descriptions of anole mating systems [40–42], we found that a majority of females (64%–81%) bore offspring sired by more than one male. However, our

results are at odds with most previous behavioral descriptions of movement patterns and mating systems in *Anolis* lizards, which leaned heavily on, and were constrained by, the framework of territoriality. These behavioral descriptions were often coupled with an implicit expectation that anoles mate strictly polygynously, i.e., males mate with multiple females, but females mate with just the one male in whose territory they reside (reviewed in [21]). Consequently, field studies have often implied that the opportunity for female choice in anoles is limited because it is precluded by territoriality (e.g. [43–45], but see [46]). Concurrently, despite varying evidence as to whether female choice is even possible in natural populations, laboratory studies have offered females the choice between males to assess precopulatory mate preference [47–49] and have mated females with multiple males to assess postcopulatory sexual selection [50, 51]. Our demonstration that individual *A. sagrei* lizards in a natural population move such that females frequently encounter and mate with multiple males indicates that female anoles definitely have the opportunity to exercise post-copulatory mate choice and might also exercise pre-copulatory mate choice. This calls into question the utility of territorial polygyny as a description of these lizards' mating system. More generally, discrepancies between behavioral and genetic descriptions of mating systems are widespread across animals (e.g. [16, 17]), and our results suggest that the re-examination of animals' movement behavior may help to reconcile these discrepancies.

In territorial species, such re-examinations could further prompt us to revisit explanations of aspects of animals' behavior that have long been interpreted as characteristic of territorial polygyny, to discern if they might also be consistent with female choice. For example, we found that males encounter size-matched males less often than expected at random, which is consistent with male body size in anoles determining the outcome of male-male fights over territory ownership and access to mates [52, 53] and larger males subsequently excluding other large males from their territories [54, 55]. In this context of territoriality, smaller males are hypothesized to evade detection by larger territorial males, residing in their territories and attempting to 'sneak' copulations with resident females. But in taxa where females choose mates based on male body size (as may be the case in this species; see below), larger males that retain smaller neighbors can accrue a mating advantage compared to males with neighbors of equal size (e.g. [56, 57]. Thus, males may engage in agonistic interactions to exclude size-matched but not smaller males from their vicinity, arranging themselves spatially relative to other males in a manner conducive to their success in the arena of female choice [58].

330 That said, territoriality is a multi-faceted concept, pertinent not only to reproductive
331 interactions but also to habitat selection and space use behavior [59]. Our results support long-
332 standing views in some of these facets. We found that males are more likely to move greater
333 distances and had a greater spatial extent than did females, which is consistent with previous
334 estimates of sex differences in territory size (e.g. [60, 61]), and with evidence for male-biased
335 dispersal in anoles [62, 63]. But the spatial extent of individuals in this population is substantially
336 higher than previous estimates of territory size in this and ecologically similar species (e.g. ~3 m^2 for
337 females and ~10 m^2 to ~14 m^2 for males [60, 64, 65], compared with minimum convex polygons
338 with mean areas of 36 m^2 for females and 225 m^2 for males in this study). Although it is always
339 possible that lizards in our site behaved unusually or, more generally, that movement behavior varies
340 across populations and species, the discrepancy may also be due in part to limited spatial and
341 temporal sampling in previous studies leading to underestimates of anole space use. Limited
342 sampling may also partially underlie why female multiple mating has rarely been considered
343 important in past research on anole territoriality—studies with limited spatial or temporal sampling
344 extents will be constrained in what behaviors they can detect (reviewed in [21]). Indeed, subsampling
345 from our dataset shows that if we had limited our spatial or temporal sampling extent to match the
346 median sampling of previous studies, we would have detected a greatly reduced number of male-
347 female pairs whose home ranges overlap with one another (see Supplementary Information). In
348 sum, we posit that while certain tenets of territoriality are well-supported in anoles, previous studies
349 have likely underestimated the complexity of *Anolis* lizards' movement patterns and social
350 interactions by being constrained by a territorial framework. Future studies of movement patterns
351 that explicitly do not depend on a territorial framework, conducted in a diversity of populations and
352 species across a range of habitats and densities, will be necessary to determine if we are correct.
353 Moreover, it remains unknown if similar problems afflict other species that have long been
354 described as territorial.

355 Larger males not only encountered more females but were also more likely to sire offspring
356 than smaller males, suggesting strong sexual selection for larger body size in male *A. sagrei*. These
357 results are consistent with conventional wisdom in anoles [66], and, more generally, with evidence
358 from across animals that body size in males predicts reproductive success [24]. However, most
359 previous evidence in favor of this hypothesis in anoles is based on the estimation of mating patterns
360 from home range area and overlap within the framework of territoriality (e.g. [60, 67]. More recent

genetic evidence includes both marginal [51, 68] and strong [41] trends towards larger males siring more offspring. All results from our study, which integrates behavioral and genetic data, point to sexual selection favoring larger body size in males, helping to explain the marked male-biased sexual size dimorphism seen in this species. As a species with largely indeterminate growth, body size in these lizards can be an indicator of age [43, 69], or the ability to survive and thrive, suggesting an adaptive reason for females to choose to mate with, or bear offspring sired by, larger males [70].

Selection on body size is difficult to disentangle from last-male precedence—because males are smaller at earlier times in the breeding season, it is possible that large males sire more offspring simply because they have encountered and mated with females more recently. Last-male precedence is found in many animals [23], including reptiles (e.g. [71]), but previous evidence from laboratory experiments in *A. sagrei* suggests instead that the first male to mate with a female is more likely to sire offspring than a later male [72]. More targeted laboratory experiments could readily separate size effects from mating order effects, lending insight into the mechanisms by which post-copulatory mate choice operates in these animals.

Additionally, sexual selection may act on movement behavior—males with larger spatial extents encountered more females than males with smaller spatial extents. Because male body size and spatial extent were only very weakly correlated, the results do not indicate strong ontogenetic shifts in movement behavior. Thus, it appears that there are multiple ways for males to achieve reproductive success—they can grow large, they can traverse large areas, or both [73].

However, understanding how the effects of body size, spatial extent, and last-male precedence integrate with the processes of males first encountering females and then siring their offspring is not necessarily straightforward. In particular, it remains unknown, but is unlikely, that a single movement or mating strategy can be adaptive in the face of such social complexity. Instead, animals may make decisions about movement depending on their social and environmental context, as opposed to adopting fixed patterns of space-use [5, 7, 8, 74]. Such context-dependent decision making is often referred to as the maintenance of "alternative mating strategies" (reviewed in [75]) though this variation need not be strictly discrete (e.g. [73, 76]). Individual-based models that incorporate the various sequential, compounding influences on reproductive success could reveal if males can make adaptive, context-dependent decisions to move or stay at particular locations based on their phenotype and the social and environmental situations they find themselves in.

391 Discerning if the decision rules used by individuals in a species are consistent across variable
392 habitats and populations likely represents a promising way of understanding animal mating systems
393 [77, 78]. The Markov chain approach to modelling movement patterns and estimating interactions
394 presented here could be modified in future studies of anoles as well as other taxa to incorporate
395 both social and environmental variation influencing these decision rules—transition probabilities
396 between locations could be scaled according to habitat preferences or the occupancy of a location by
397 specific individuals, for example. Finer spatial and temporal scales of location sampling, made
398 possible with the advancement of automated methods of tracking animals in the wild [79], could also
399 allow for individual-level parameterization of any movement model, potentially improving the
400 accuracy with which encounters are estimated. For example, 26 individual offspring (7% of total)
401 were assigned sires not among the males estimated to encounter their mothers (see Supplementary
402 Information)—it is possible that a greater temporal resolution of location observations would have
403 detected the movements bringing about these encounters and subsequent matings. At present,
404 however, the size and cost of tracking or receiving devices and the spatial resolution of GPS data
405 limit the range of taxa for which such methods can be employed.

406 Ultimately, even finer scale location sampling will be insufficient for determining which
407 encounters in fact lead to matings—discovering this crucial aspect of animals' behavior will depend
408 on focal animal observations of encounters in natural or naturalistic conditions. For a male, the gap
409 between encountering a female and siring her offspring can include the sequential gauntlets of male-
410 male competition, female mating preferences, and post-copulatory competition and choice [1, 2].
411 Disentangling effects on mating success at these various levels will be essential to fully
412 understanding how sexual selection shapes behavior, and will require close observation of animal
413 interactions prior to and including mating [80–83].

414 However, movement behavior is a precursor to bringing about any of these interactions—it
415 comprises the first step of sexual selection and its quantification is therefore necessary for
416 understanding the shape that sexual selection can take. In this paper, we develop a spatiotemporal
417 framework to quantify movement behavior to estimate encounters between potential mates. This
418 framework reveals an infrequently-recognized opportunity for female mate choice in *Anolis sagrei*,
419 demonstrates that larger males are favored across multiple levels of sexual selection, and shows
420 evidence for sexual selection on movement behavior and the timing of male-female encounters. We

hope that similar frameworks centered on movement behavior can help to organize disparate studies that approach sexual selection at different levels in a variety of animal taxa.

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Statement of Authorship:

AK and JBL designed the study, AK collected data and performed analyses, AK and JBL wrote the manuscript.

Data Accessibility Statement:

Data and code are currently available on GitHub (https://github.com/ambikamath/anolismovt).

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Supplementary Information

Modelling lizard locations.

As described in the main text, we modelled lizards' movements between locations using a Markov chain model, where transition probabilities were fitted as exponentially declining with the distance between locations (Equation 1 in main text). If **M** is the transition matrix of the Markov chain model, and an individual lizard was observed at location A_m within hour T_m and then later at location A_n within hour T_n, then we calculated the probability that that individual was found at a particular location (L) within the hour $T \in (T_m, \ldots, T_{n-1})$ as follows:

$$\mathrm{P}(\mathrm{L}, \mathrm{T} | \mathrm{A_m}, \mathrm{T_m}, \mathrm{A_n}, \mathrm{T_n}) = \frac{\mathbf{M}^{\mathrm{T}-(\mathrm{Tm})+1}[\mathrm{A_m}, \mathrm{L}] \times \mathbf{M}^{(\mathrm{Tn})-\mathrm{T}+1}[\mathrm{L}, \mathrm{A_n}]}{\sum_{\mathrm{i}=1}^{N} \mathbf{M}^{\mathrm{T}-(\mathrm{Tm})+1}[\mathrm{A_m}, \mathrm{A_i}] \times \mathbf{M}^{(\mathrm{Tn})-\mathrm{T}+1}[\mathrm{A_i}, \mathrm{A_n}]}$$

N is the total number of locations mapped; in our site, we mapped a total of 318 locations, which included not only locations at which lizards were observed but also trees within the site on which lizards were not observed but to which they might have moved (Figure S1). For the hours preceding the first observation and following the last observation for each individual, lizards were assumed to be equally likely to be anywhere in the site (i.e. the appropriate row or column of the transition matrix in equation 1 was replaced by a unit vector). In sum, after this procedure, we obtained, for each lizard, a vector of probabilities of the lizard being in each of the 318 locations within each hour during the sampling period.

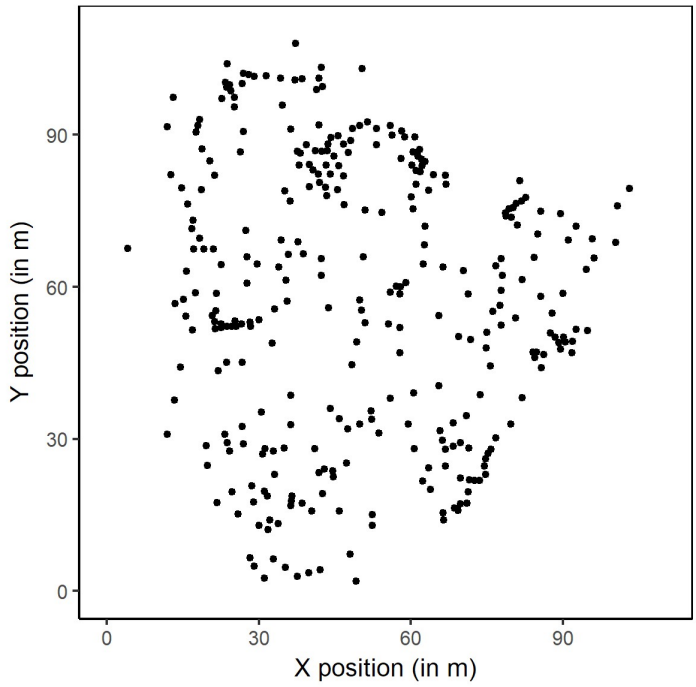

Figure S1. Map of all locations at which lizards were observed. In areas of continuous vegetation, locations more than 1m apart were considered distinct. We also mapped the location of all trees within the site at which lizards were not observed, to include all trees to which a lizard could potentially have moved in our estimations of their movement patterns, amounting to a total of 318 locations.

Estimating Encounters

To estimate whether or not a pair of lizards encountered one another, we first performed an element-wise multiplication of the two matrices of the individuals' probabilities of being at each location within each hour, and then summed probabilities across all locations for each hour in the resultant matrix for the pair. Probabilities were summed across locations so that two individuals that may be moderately likely to co-occur at multiple locations could still be estimated as encountering one another.

The calculated probabilities of observed co-occurrences, defined as a pair of lizards observed in the same location within one hour of each other, were used to set the probability cutoff by which we defined whether or not other pairs of lizards encountered one another. These cutoffs depended on location. The 318 locations in this site were not uniformly distributed, with some locations clustered more closely together than others (Figure S1). When the probability of movement declines with distance, as modeled here, it is easier to leave a focal location that is close to other locations than it is to leave a relatively isolated location. Consequently, the estimated probability of remaining at the former location will be lower compared to the probability of leaving the latter location. We therefore set probability cutoffs to vary by the locations at which lizards in the pair were seen, on the basis of the focal locations' proximity to nearby locations First, for the location of each observed

co-occurrence, we calculated the mean distance to the ten closest locations to it, and saw that, as expected, the calculated probability of an encounter at that location between two lizards observed to co-occur increased with increasing distance to the ten closest points (Figure S2). We therefore binned locations on the basis of these distances, into 1 m increments. The minimum probability calculated for observed co-occurrences in each bin, denoted by the black horizontal lines in Figure S2, was set as a cutoff for estimating whether or not an encounter took place, for all pairs of lizards for all hours in the sampling period. Of course, using such a distance-based probability cutoff to estimate encounters meant that we needed to assign a distance to all pairs of lizards for all hours. To do so, at each hour we considered the previous and next locations at which the lizards in the pair were observed, and found the minimum of the means of the ten closest distances for these locations. For defining encounters (as a binary "yes/no" variable) for each pair of lizards at each hour, we assessed if the probability of their encounter at that hour was higher than the appropriate probability cutoff based on the assigned distance.

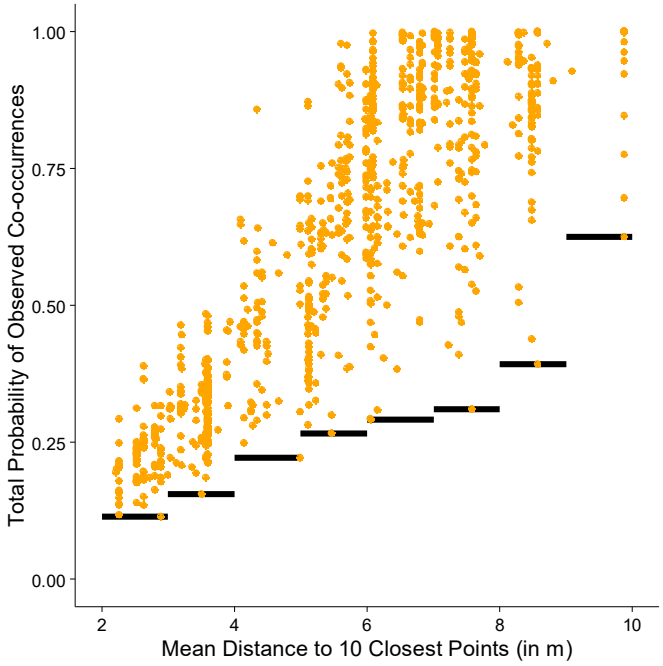

Figure S2 Cutoffs (black horizontal lines) for defining encounters, based on the mean distance from each location to the ten closest locations. Points indicate the sum of probabilities of co-occurrences across all locations, for pairs of individuals at hours where the two were observed at the same location within an hour of one another.

To assess the sensitivity of the number of male-female encounters to the probability cutoff described above, we repeated analyses using the 25% quartile instead of the minimum probability in each bin; a higher cutoff is more stringent, and will recover fewer encounters, but we were curious whether females would still be estimated as encountering multiple males across the sampling period. Not surprisingly, conservatively increasing the probability threshold for defining encounters from the minimum probability of observed co-occurrences to the 25% quartile reduced estimates of the number of potential mates encountered by both females (3.4 ± 3.1) and males (2.0 ± 2.1), but the potential for females to mate with multiple males remained.

Because boundary effects of a closed site might artificially increase the number of encounters, we also assessed if the number of male-female encounters was sensitive to treating the site as closed, by repeating the analyses described above with a simulated buffer of 50 locations, placed randomly within 20 m around the perimeter of the site. Allowing individuals to move to these locations did not alter estimates of the average number of males encountered by females (5.1 ± 3.7) or females encountered by males (2.9 ± 3.0).

Growth Curve for Males

We estimated the growth curve of males in this population by fitting the following logistic equation (with parameters *a* and *r*) to the observed data, using nonlinear least squares regression (Schoener and Schoener 1978):

$$\mathrm{L2} = \frac{\mathrm{aL_1}}{\mathrm{L_1} + (\mathrm{a} - \mathrm{L_1})e^{\mathrm{rD}}}$$

where L_1 and L_2 are the SVL of an individual measured at two successive captures, and D is the number of days between the two captures. We used this logistic growth curve model to estimate the SVL of each male on the day of each of his encounters, based on his SVL at the nearest capture. Parameters for the logistic growth curve for male SVL (Equation 3) were estimated to be a = 63.7 and r = -0.016. Snout-vent lengths predicted by the model were highly correlated with measured SVLs ($r^2 = 0.92$).

Paternity analysis

Tests for sexual selection were performed on the paternity assignments in which candidate sires for offspring were the list of males their mother was estimated to have encountered (described in main text). However, we ran two further analyses. First, to assess if high paternity assignment rates in the main analysis were a consequence of restricting the number of candidate sires relative to the whole population, we also ran ten replicates of the analysis in which we sampled potential sires randomly from all 161 males, preserving the number of potential sires for each offspring individual and supplying the same group of potential sires for all offspring of a single female. Randomizing the identity but not the number of males encountered by each mother yielded 18% paternity assignment on average (13% to 24%, at an 80% confidence level), suggesting that the four-fold difference in

assignment rates was due to identifying potential sires with greater accuracy based on their spatiotemporal movement patterns (assignment rate of 84% in the main analysis).

Second, we ran an analysis in which all sampled males were provided as candidate sires for all offspring. Simulation parameters specified for this analysis were 161 candidate sires and 0.96 proportion of males typed, based on the proportion of observations in the field that were of marked males. Paternity assignments were concordant for 80% of the individuals that were assigned paternity by both analyses. Of the remaining 39 individuals, the LOD scores for 21 mother-sire pairs differed between analyses by less than 1; these were considered false positives from the second analysis, and the assignments of the first paternity analysis were retained for downstream analysis. For the remaining 18 offspring, with mother-sire pairs where the LOD scores between the two analyses differed by more than 1, we found that results were not altered if we conducted downstream hypothesis testing both including and excluding their paternity assignments from the first analysis.

Twenty-six individuals that were assigned paternity only when all males were provided as candidate sires. For these individuals, we calculated the minimum distance between any locations at which the mother and putative sire were observed, to assess the minimum distances that these individuals would have had to move to mate with one another. The minimum distance between locations at which mothers and sires of these 26 offspring were observed (regardless of *when* these lizards were found at those locations) ranged from 0 m to 56 m, with a median of 8.7 m.

Subsampling in Time and Space

To understand the effect of limiting sampling in space or time on male-female home range overlap (i.e. how mating patterns have often been assessed in previous studies of anoles), we asked if a purely spatial predictor— the overlap in minimum convex polygons (MCPs) between pairs of individuals—is affected by subsampling from our dataset to match either the median area (400 m^2) or the median duration of sampling (4 weeks) or both of previous studies (see Table S2 for studies and sample areas and durations included in the calculation of the medians).

On average, in the whole dataset, female MCPs overlap with those of 12.8 ± 8.7 males, whereas male MCPs overlap with those of 8.1 ± 6.7 females. In subsamples of a randomly selected area of 400 m^2 (repeated 404 times), we calculated that females overlapped a mean of 5.5 ± 3.1 males

765 and males overlapped a mean of 2.3 ± 1.2 females. In subsamples of a duration of four weeks (for
766 each of a possible 55 start dates), we calculated that females overlapped a mean of 4.5 ± 1.0 males
767 and males overlapped a mean of 3.4 ± 0.8 females. In subsamples with a randomly selected area of
768 400 m^2 and duration of four weeks, we calculated that females overlapped a mean of 2.4 ± 1.9 males
769 and males overlapped a mean of 1.5 ± 1.0 females. Thus, limited sampling in space or time decreases
770 the number of mates inferred from spatial overlap of MCP estimates of home range. That said, at all
771 scales of subsampling, we recovered that females overlap with multiple males, possibly suggesting
772 that our study population may be dissimilar to many of those studied previously, and also hinting
773 that limited sampling in space and time is not the full explanation for why previous studies of *Anolis*
774 territoriality have largely ignored the potential for females to encounter and mate with multiple
775 males (but see Tokarz 1998).

Table S1

Locus	Reference	Primers (5' – 3')	Amplification Conditions*	Null allele frequency (for bolded loci, apparent homozygotes typed at only one allele)	Number of alleles
S70	Bardeleben et al. 2004	GCAAGAGGTAGCCTCAGC GTTATCAGTACGAGGCACTG	TD1	-0.002	18
S77	Bardeleben et al. 2004	GAGTAAAGGTCTGGGTCAGG GCAGTACAAATACCACAGAGC	TD1	-0.006	16
AC8	Wordley et al. 2011	CCCAATAGAGGAAAGGGACC AGAATCACGCCTTCTGCTTT	57.7°C	**0.053**	10
AC19	Wordley et al. 2011	GAAAAGTAGTGGGGCATTGG AGTTTCCCAAGAAAACCCGT	TD1	0.007	16
AC45	Wordley et al. 2011	GCATCACCAGTCCCTTTG GCTTTGGTTGAGTTGTATTGT TTG	57.7°C	**0.11**	6
G11	J. Kolbe (pers. comm.)	GGTGCCACTGGGTATTCTGT CTCAATCCTGCTGTCACCTG	TD2	**0.16**	19

*Amplification conditions:

1. 57.7°C: 95°C for 2', 35 cycles of 94°C for 45s, 57.7°C for 45s, 72°C for 1', 72°C for 10'.
2. TD1: 95°C for 5', 10 touchdown cycles of 95°C for 30s, 55°C – 50°C for 30s, 72°C for 45s, 25 cycles of 95°C for 30s, 50°C for 30s, 72°C for 45s, 72°C for 5'.
3. TD2: 95°C for 5', 10 touchdown cycles of 95°C for 30s, 64°C – 55°C for 30s, 72°C for 45s, 25 cycles of 95°C for 30s, 55°C for 30s, 72°C for 45s, 72°C for 5'.

Table S2. Sampling area and duration of previous studies on *Anolis* home ranges

Note that though the median duration is 3.5 weeks, a more conservative value of 4 weeks was used in the subsampling analysis above.

Study	**Sampling Area (in m²)**	**Sampling Duration (in weeks)**
Evans 1938	150000	3
Greenberg and Noble 1944	25	47
Gordon 1956	400	52
Sexton et al. 1963	200	4
Rand 1967	425	5
Jenssen 1970	930	8
Jackson 1973	14000	1
Philibosian 1975	420	0.2
Stamps 1977	9.5	5
	34	13
	47	13
	37	15
Hicks and Trivers 1983	12000	9
Ruby 1984	441	28
Fleishman 1988	169	10
Jenssen and Nunez 1998	36	1
Tokarz 1998	137	5
Pereira et al. 2002	12	1
	70	1
Paterson 2002	400	1
McMann and Paterson 2003	400	1
Calsbeek 2009	1500	2
Johnson et al. 2010	500	3
Nicholson and Richards 2011	14000	52
Bush et al. 2016	875	3
Schoener and Schoener 1982	100	–
Fitch and Henderson 1976	–	1
Fitch and Henderson 1987	–	2
Fobes et al. 1992.	–	1.5
Median	**400**	**3.5**

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