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The Rise and Fall of Kin Structure in the Neotropical, Family-Living Buff-Breasted Wren (*Cantorchilus Leucotis*) in Gamboa, Panama

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THE RISE AND FALL OF KIN STRUCTURE IN THE NEOTROPICAL, FAMILY-LIVING BUFF-BREASTED WREN (*CANTORCHILUS LEUCOTIS*) IN GAMBOA, PANAMA

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Family-living species provide an exciting model to examine how natal dispersal and kin structure influence genetic structuring within local populations. Juvenile buff-breasted wrens (*Cantorchilus leucotis*) of both sexes delay dispersal and exhibit short-distance natal dispersal, which should lead to kin-structured populations in which relatives of both sexes occupy neighboring territories. Blood samples collected from juvenile and adult wrens in Gamboa, Panama were analyzed using microsatellite markers to determine whether related individuals are spatially clustered on neighboring territories, spatial clusters of relatives change over time, and if kin structure is sex-specific. Global and local spatial autocorrelation analyses detected genetic structuring among males over time, however this pattern was not prevalent among females. These spatial genetic patterns suggest that males may disperse shorter distances than females, which may lead to genetic structuring. When subject to genetic drift and isolation by distance, this kin structuring may increase the probability of population differentiation among behaviorally and geographically isolated populations.

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by

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INTRODUCTION

Dispersal is a fundamental life history trait that describes the movement of an individual from one location to another either temporarily or permanently (Greenwood 1980). The timing of movement of juveniles from the natal site, or natal dispersal, varies among species (Greenwood 1980). For many species, natal dispersal typically occurs when juveniles become nutritionally independent from their parents (Russell 2000, Russell et al. 2004). However, this contrasts with other species in which offspring delay natal dispersal past this developmental stage to remain with their parents on the natal territory (Ekman 2006). Why certain species delay natal dispersal has been widely debated; however, it appears that a combination of life history characteristics (e.g., long-lived and sedentary), ecological constraints (e.g., lack of breeding opportunities), and the benefits of philopatry (e.g., higher offspring survival) may favor these dispersal patterns (see Emlen 1982, 1995, Arnold and Owens 1998, Covas and Griesser 2007, Hatchwell and Komdeur 2000).

Social behavior in which relatives live in family groups may shape patterns of dispersal, as prolonged interactions among relatives may incur fitness advantages (Hamilton 1964, Koenig et al. 1992). Philopatry describes dispersal patterns in which individuals either remain on their natal territory or move short distances to nearby locations (Greenwood 1980). In social species, parents and their independent offspring may form families and continue to associate over extended periods of time (Koenig et al. 1992, Emlen 1994, 1995, Kokko and Ekman 2002, Covas and Griesser 2007).

Remaining on the natal territory with family members may have important costs, as individuals delay their own reproduction and often cooperate with their parents to raise siblings (Ekman 2006). However, individuals that delay dispersal to remain on their natal territory may benefit from a higher probability of survival and reproductive success (Walters et al. 1992, Ekman et al. 1999, Ekman et al. 2000, Griesser et al. 2006, Sparkman et al. 2010, Tarwater and Brawn 2010). When individuals that delay dispersal are more likely to obtain territories locally (see Kokko and Ekman 2002), the possibility exists that spatial clusters of relatives to form within a location over time. Once offspring disperse, a patchwork of spatial clusters of relatives may emerge if dispersal distances are short and these individuals recruit locally (Clobert et al. 2009, Sharp et al. 2011). Thus, it appears that dispersal and social behavior are intimately linked. They may influence the spatial distribution of individuals within a location and may ultimately lead to differences in spatial genetic patterns within a population over time (Chesser 1991, 1998, Clobert et al. 2009, Hatchwell 2010).

Philopatry and restricted spatial movements may lead to the formation of kin structure within a population (Greenwood 1980, Double et al. 2005, Ekman 2006). Patterns of kin clustering occur among several species of family-living birds exhibiting delayed natal dispersal including: apostlebirds, *Struthidea cinerea* (Woxvold 2006), white-breasted thrashers, *Ramphocinclus brachyurus* (Temple et al. 2006), Florida scrub jays, *Aphelocoma cœrulescens* (Coulon et al. 2008), white-winged choughs, *Corcorax melanorhamphos* (Beck et al. 2008), white-throated magpie-jays, *Calocitta formosa* (Berg et al. 2009), grey-crowned babblers, *Pomatostomus temporalis*

(Blackmore et al. 2011) and karoo scrub-robins, *Cercotrichas coryphaeus* (Ribeiro et al. 2012). Among these species, cooperative breeding is a predominant life history strategy (e.g., Berg et al. 2009, Blackmore et al. 2011). Families are primarily composed of a breeding pair and their retained independent offspring (e.g., Temple et al. 2006), although family groups may also be composed of extended relatives (i.e., Beck et al. 2008). The presence of kin clusters within these species suggests that this spatial genetic pattern may be common among social species in which individuals live in families and tend to remain philopatric to their natal territory. What remains unclear is whether variation in life history strategies, such as family-living and non-cooperatively breeding, also leads to patterns of kin clustering in species in which both sexes share similar dispersal patterns.

Sex bias in dispersal behavior occurs among most species and often leads to physical separation of male and female relatives when one sex disperses, which potentially limits inbreeding (Greenwood 1980, Double et al. 2005, Ribeiro et al. 2012). Differential costs of dispersal to males and females may drive sex-specific patterns which are predicted to occur when one sex benefits from defending resources or from familiarity with the natal site (Greenwood 1980). In mammals, males usually disperse while females remain philopatric to their natal territory and/or group (Greenwood 1980, e.g., Peakall et al. 2003, Archie et al. 2008). In birds, females typically disperse while males remain philopatric to their natal site (Greenwood 1980, e.g., Yaber and Rabenhold 2002), although exceptions do exist (e.g., McKinnon et al. 2006, Beck et al. 2008, Blackmore et al. 2011).

Spatial genetic structure forms predominantly within the philopatric sex and may lead to sex-specific spatial distribution of genetic variation (Double et al. 2005, Coulon et al. 2008). However, few studies investigate patterns of kin clustering in species in which both sexes are philopatric (e.g., Beck et al. 2008, Blackmore et al. 2011). For example, white-winged choughs do not display a sex bias in dispersal behavior and positive spatial genetic structure occurs among groups that composed of both male and female relatives (Beck et al. 2008). In contrast, grey-crowned babblers display genetic differentiation among social groups, but not between the sexes which suggests that males and females may benefit from delayed dispersal and philopatry (Blackmore et al. 2011). Both sexes display similar patterns of natal philopatry, however, differing spatial genetic patterns may ultimately reflect differences in the timing and distance of these behaviors (Blackmore et al. 2011). Thus, it remains unclear how a lack of sex-biased dispersal impacts spatial genetic structure and the extent to which philopatry of both sexes leads to inbreeding.

Little is known about the temporal stability of kin clustering in family-living species. Spatial genetic patterns may remain stable over time in family-living species, which typically display high adult survival and philopatry limiting turnover of existing breeding territories. If some lineages produce more philopatric offspring than others (Putland and Goldizen 2001, Double et al. 2005, Ekman 2006), then differences in reproductive success, recruitment and survival could lead to temporal fluctuations in spatial genetic structure. For example, Piertney et al. (2008) found cyclical patterns of kin structuring in the territorial red grouse, *Lagopus lagopus*, and the relatedness

among male territory holders varied dramatically over 10 years. Males on neighboring territories were first order relatives (e.g., siblings or father-son pairs) during nine years and certain lineages were found consistently over time while others were only present for a subset of years (Piertney et al. 2008). Double et al. (2005) also found that particular male superb-fairy wrens, *Malurus cyaneus*, contributed more offspring to the local reproductive population and that male relatives were spatially clustered on neighboring territories. Similar temporal patterns in spatial genetic structure have been found among rodent populations, although these studies primarily focus on genetic differentiation at broader geographic scales (Busch et al. 2009). Fluctuations in kin structure over time may be attributed to changes in population density, habitat characteristics, and social interactions among conspecifics (Piertney et al. 2008, Busch et al. 2009) however, the exact nature of these interactions remains unclear.

I studied spatial and temporal patterns of kin clustering in buff-breasted wrens, *Cantorchilus leucotis*, a family-living species in which both sexes delay natal dispersal, both sexes recruit into the local population when possible, and once territory holders, both sexes benefit from site familiarity (Gill and Stutchbury 2006, 2010). Buff-breasted wrens of both sexes are highly territorial, live in small nuclear families in which a male and female pair defend a territory year-round, and offspring of both sexes may disperse short distances from their natal territories (Gill and Stutchbury 2006, 2010), which should lead to kin structuring patterns among adults of both sexes. Juveniles remain on natal territories for an average of ten months post-fledging, before dispersing to breeding territories prior to the next breeding season (Gill and

Stutchbury 2010). Those offspring that recruit within the natal population often share at least one territory boundary with relatives (Gill and Stutchbury 2010), suggesting that kin structure could form within this population. Annual survival is high for both males and females (Gill and Haggerty 2012), which should lead to similar spatial genetic patterns over time. Previous studies contribute a detailed understanding of the dispersal, social and demography of buff-breasted wrens. By examining patterns of kin structure in buff-breasted wrens, we gain further insight into the genetic consequences of family-living, philopatry and short-distance natal dispersal.

I tested the hypothesis that family-living and short-distance natal dispersal of both sexes (Gill and Stutchbury 2006, 2010) leads to spatial genetic patterns in which neighboring territory holders are related and clusters of kin in space develop over time. If kin clusters are present, then the potential for inbreeding may exist and I will test whether territorial pairs are related. Behavioral and genetic approaches are used examine the following predictions: (1) kin structure is present, (2) sex-specific spatial genetic patterns exist, (3) these spatial clusters of relatives change over time, and (4) inbreeding occurs among territory holders. Investigation of spatial genetic structure among adult territory holders during six years over a 14 year time period will provide a better understanding of how these patterns may fluctuate temporally and will provide insight into the genetic consequences of social behavior and dispersal patterns. Current studies that investigate the consequences of delayed dispersal, philopatry and family living primarily focus on cooperatively breeding species. To my knowledge, this is the

first longitudinal study of the spatial genetic structure in a territorial and family-living species in which both males and females display similar dispersal behaviors.

METHODS

Study Area and Study Species

I studied a color-banded population of buff-breasted wrens around Gamboa, Panama (9º, 7' N, 79º, 42' W). This population has been the subject of intensive study since 1997 (e.g., Gill et al. 2005, Gill and Stutchbury 2006, 2010). Buff-breasted wrens are small (approximately 17 - 27 g), insectivorous passerines which inhabit second-growth forests ranging from Northern Panama to Northern Brazil (Ridgely and Gwynne 1989). Adults are long-lived (Gill and Haggerty 2012), form socially monogamous breeding pairs, and defend territories year-round (Gill and Stutchbury 2006). The study area consists of second-growth forest patches bordering the Panama Canal, the Chagres River, and the town of Gamboa (see Gill and Stutchbury 2005 for detailed description). Initially, the area of the study site was approximately 22 ha (15 - 24 pairs, 1997 - 1999) and was later expanded to approximately 76 ha (38 - 48 pairs, 2009 - 2011). Research was approved by Institutional Animal Care and Use Committee and conducted under research permits granted through Autoridad Nacional del Ambiente of Panama.

Wrens were caught in mist nets both passively and following song playback. Each bird received an individually numbered aluminum band and a unique combination of one to three color bands to permit individual identification during behavioral observations. Tarsus length (mm), unflattened wing chord (mm), and mass (g) were recorded for banded individuals. Adult wrens were sexed in the field by the presence of brood patches (females only), as well as size (males are larger than females within pairs) and sex-specific singing behavior (Gill and Vonhof 2006). Sex identification was also confirmed genetically (Griffiths et al. 1998, Jarvi and Farias 2006, see below). Hatch year birds, or fledglings, were distinguished by eye color (iris is gray in fledglings and brown in adults), presence of yellow gape, and vocalizations (Gill, unpublished data). Juveniles could be distinguished from adult pairs by behavior since offspring typically forage and sing alone (Gill et al. 2005).

DNA samples were collected from all banded individuals. To obtain blood samples, the brachial vein was punctured with a 26-gauge needle and approximately 20-200 µl of blood was collected in heparinized capillary tubes (Sheldon et al. 2008). From 1997-1999, DNA samples were placed into labeled microcentrifuge tubes with Queen's Lysis buffer and placed on ice until return from the field when they were frozen (protocol as described in Gill et al. 2005). Feather samples were also collected and stored in labeled envelopes for genetic analysis. During 2009 - 2010, DNA samples were placed into labeled microcentrifuge tubes (without buffer) and stored on ice until return from the field when approximately 20 µl was transferred to FTA cards (Whatman Ltd., see Smith and Burgoyne 2004); the remaining blood was processed

for other studies (Gill, unpubl.). In 2011, blood samples were processed in the field; approximately 20 - 100 µl of blood was transferred directly from heparinized capillary tubes to a FTA card.

A total of 192 banded and six unbanded individual territory holders was observed over six field seasons (some unbanded individuals encountered in more than one year). To provide snapshots of spatial genetic structure over time, banded territory holders present on the last survey day of each field season were included in analyses: 31 May 1997 (n=30), 24 June 1998 (n=47), 10 July 1999 (n=39), 24 March 2009 (n=58), 30 July 2010 (n=84) and 14 July 2011 (n=90). These dates differ between sampling periods due to differences in research effort and constraints on the time in which investigators were able to travel to the study site. During 1997, 1998, 1999, 2009, 2010, and 2011, 100 %, 100 %, 100 %, 98.36 %, 97.67 %, and 93.75 % of the territory holders observed within the study area were banded, respectively. Approximately 40.6 % (n=78) of banded territory holders were observed for one year only, whereas 38.0 % (n=73), 20.3 % (n=39), 0.5 % (n=1), and 0.5 % (n=1) were observed over two, three, four and six sampling periods, respectively. Given that most individuals were observed in at least two sampling periods, these data may not be independent across years.

Behavioral observations of banded birds were used to identify social relationships within groups. Social groups typically consisted of one breeding male and female, as well as their independent retained offspring (Gill and Stutchbury 2010). Two adult birds observed duetting and participating in territory defense were recorded as a

breeding pair. Adults interacting with fledglings were assumed to be the social parents and pairs or trios of fledglings were assumed to be siblings. In some social groups, offspring from the previous year remained on their natal territory, foraged in close proximity to the territorial pair and their offspring, and participated in territorial defense. Relationships observed in the field were compared to measures of genetic relatedness in order to confirm the accuracy of these data since certain aspects of behavior can be difficult to observe and may translate into differing genetic patterns among individuals (e.g., extra-pair paternity). Previous investigation of paternity within this population revealed extremely low extra-pair paternity of offspring (~3% of broods) (Gill et al. 2005).

Territory Mapping

During four sampling periods (18 April - 24 June 1998, 03 March - 10 July 1999, 18 May - 30 July 2010, and 13 May - 13 July 2011), behavioral observations of adult mated pairs were recorded to map the territory boundaries of all breeding pairs within the study area. Territories were not mapped during 2 February - 31 May 1997 and 2 February - 24 March 2009, however approximate territory locations were recorded. A total of 15, 24, 20, 38, 44 and 48 territories were observed in 1997, 1998, 1999, 2009, 2010 and 2011, respectively n (see Table 1). Territories were surveyed approximately every 5 - 14 days throughout field seasons. Once individuals were located, they were identified by color bands, and observed until they either disappeared from sight or

remained in the same place throughout the entire survey. Territories were visited more often if previous surveys yielded little to no observations due to difficulty in accessing the territory or in locating banded birds.

Table 1
Summary of the number of territories observed, mean territory size, and the mean distance to the nearest neighbor (NN) by year

Year	Number of Territories	Mean Territory Size (m^2)	Range Territory Size (m^2)	Mean NN Distance (m)	Range NN Distance
1997	15	3340.56 +/- 2750.87 SD	473 - 10345	94.39 +/- 29.55 SD	63.84 - 156.72
1998	24	5532.08 +/- 5075.67 SD	473 - 21125	88.86 +/- 33.01 SD	46.16 - 174.74
1999	20	7432.76 +/- 6367.54 SD	975 - 24885	82.93 +/- 20.00 SD	55.84 - 124.84
2009	32	1981.09 +/- 1362.67 SD	213 - 5683	128.25 +/- 88.54 SD	40.13 - 430.23
2010	44	2018.41 +/- 1545.73 SD	24 - 6628	97.94 +/- 58.08 SD	40.13 - 263.69
2011	47	1096.24 +/- 1036.88 SD	94 - 5073	98.68 +/- 71.11 SD	31.56 - 495.13

Territories were mapped in order to compare the spatial proximity and the genetic similarity between territory holders within the study area. Pairwise comparisons of territory centroids provided a measure of geographic distance which could then be compared to genetic distance between individuals using a spatial autocorrelation analysis. Movements by mated pairs were used to identify each pair's territory. Locations where disputes between neighboring pairs occurred were used to indicate

territory boundaries (Rabenold 1990). Responses of territory holders to intrusions include duetting and aggression (Gill et al. 2007); both behaviors were also observed during territorial disputes (Gill, pers. comm., Alessi, pers. obs.). Locations where adults were observed foraging, nesting, preening or duetting within the study site were recorded on paper maps during 1998 and 1999, and using GPS units (Garmin GPSmap 60CSx, Garmin Ltd., USA) during 2010 and 2011. Paper maps were scanned and the behavioral observations recorded were transcribed into ArcGIS 10.0. Latitude and longitude coordinates were calculated using the field calculator in ArcGIS 10.0.

Minimum convex polygons were created around territory boundaries and the centroid of each polygon was calculated in ArcGIS 10.0. Territories were not specifically mapped during 1997 and 2009; therefore observations from the subsequent field seasons were used to represent territory locations for these years. Centroids calculated during previous or following field seasons were used for territories with an insufficient number of observations either due to accessibility limitations or low accuracy of GPS coordinates (greater than +/-3.0 m). Each year, one to three territories were supplemented with centroid data from a different year to allow these territories to be included in analyses of spatial autocorrelation. Mean distance (+/- SD) between each territory and its nearest neighbor across all years was 98.05 m (+/- SD 15.73 m). To determine whether the position of each territory centroid differed between years, the distances between 1998 and 1999, as well as between 2010 and 2011 centroids were calculated for each territory. The centroid of the same territory remained relatively stable over time (1998 - 1999: mean=32.89 m +/- SD 23.06 m;

2010 - 2011: mean=25.74 m +/- SD 24.07 m). Mean territory size was 3,340.56 m^2 (+/- SD 2,750.87 m^2), 5,532.08 m^2 (+/- SD 5,075.67 m^2), 7,432.76 m^2 (+/- SD 6,367.54 m^2), 1,981.09 m^2 (+/- SD 1,362.67 m^2), 2,018.41 m^2 (+/- SD 1,545.73 m^2), and 1,096.24 m^2 (+/- SD 1,036.88 m^2) in 1997, 1998, 1999, 2009, 2010 and 2011, respectively (see Table 1).

DNA Extraction, PCR and Genotyping

For samples collected during 2009 - 2011, DNA was extracted primarily from frozen red blood cells and blood collected on FTA cards using a DNeasy Qiagen extraction kit following standard blood and tissue protocols. For samples collected during 1997 - 1999, DNA was previously extracted following the protocol described in Gill et al. (2005) or from feather samples using DNeasy Qiagen extraction kit following standard tissue protocol (e.g., Harvey et al. 2006).

I tested microsatellite markers originally developed for other bird species to obtain 11 polymorphic markers sufficiently variable to permit detection of genetic differences among individual buff-breasted wrens: TA-C3(B)-2, TA-A5-2, ThPl-27, TA-B4-2, SpuL4-30, TG04-004, ThPl-17, TG05-053, CpAAT51, TG04-012, and TG11-011 (Table 2). I confirmed the sex of all individuals using two independent markers, P8/P2 and ATP5 (Griffiths et al. 1998). In birds, females are the heterogametic sex and individuals with heterozygous genotypes were scored as female whereas individuals with homozygous genotypes were scored as male. The sex of an

individual observed in the field matched its genetic sex for 190 of the 192 banded territory holders (98.96 %). In the case of two of the 192 samples, both P8/P2 and ATP5 failed to amplify any alleles and the behavioral sex was used for subsequent analyses.

Table 2
Characteristics of microsatellite markers used for genotyping buff-breasted wrens

Locus	Repeat Motif	Na	Ta (°C)	Citation
ThPl-17	$(GT)_8$	6	54	Brar et al. 2007
ThPl-27	$(AC)_{15}$	10	54	Brar et al. 2007
TA-B4-2	$TGTC(TG)_8$	5	54	Cabe & Marshall 2001
TG11-011	$(AT)_9AA(AT)_6TA(AT)_3$	4	54	Dawson et al. 2010
TG05-053	$(T)_4GA(T)_6AA(T)_{16}AA(T)_4G(T)_6$	3	54	Dawson et al. 2010
CpAAT51	$(AAT)_{14}$	12	54	Hughes & Robinson 2001
TG04-012	$(GT)_4CT(GT)_5$	3	54	Dawson et al. 2010
TA-C3(B)-2	$(GT)_{19}N_{10}(TG)_2$	19	54	Cabe & Marshall 2001
TA-A5-2	$(AC)_7(AN)_3(AC)_3AT(AC)_2$	2	54	Cabe & Marshall 2001
TG04-004	$(AT)_{10}GT(AT)_7$	5	60	Dawson et al. 2010
SpuL4-30	$(GT)_{29}$	5	60	Haas et al. 2009

Polymerase chain reactions were performed in 25 µl reactions using Hot Start Ready-to-go PCR beads (GE Healthcare, Inc.), 0.8 - 2 µl of each primer, 2 µl of

template DNA and 19 - 23.2 µl of water. Annealing temperatures (T_a) of PCR reactions was either 54°C or 60°C depending on the microsatellite marker (Table 2). Individual markers were combined into multiplexes for PCR reactions and sequencing (MixA: TA-C3(B)-2, TA-A5-2; MixB: ThPl-27, TA-B4-2; MixC: SpuL4-30, TG04-004; MixD: ThPl-17, P8/P2, TG05-053; MixE: CpAAT51, ATP5; MixF: TG04-012, TG11-011). Forward primers were labeled with one of four fluorescent dyes including: 6-FAM, VIC, NED and PET. PCR profiles consisted of incubation at 95°C for 15 minutes, 3 cycles of amplification at 95°C for 30 s, 54°C or 60°C for 20 s, and 72°C for 5 s followed by 36 cycles of 95°C for 15 s, 54°C or 60°C for 20 s, and 72°C for 2 s, and a final extension step at 72°C for 30 minutes was added. PCR products from two multiplexes were loaded together and diluted with approximately 5 - 10 µl water (Mix A and B; Mix C and D; Mix E and F). Diluted PCR products (20 µl) were sent to Vanderbilt University DNA Sequencing Facility, TN for genotyping. GeneMarker 1.95 software was used to generate electropherograms and to create panels used to score alleles amplified by each microsatellite marker (SoftGenetics, State College, PA, USA). To ensure reliability of genetic data, genotyping error rate was estimated by replicating approximately 10% (n=24) of the total number of samples (Bonin et al. 2004). Genotyping error was estimated by comparing the number of mistyped alleles to the overall number of alleles replicated (Bonin et al. 2004)

Statistical Analyses

Significant differences between observed and expected allele frequencies within a population may reflect patterns of dispersal, genetic differentiation, non-random mating and natural selection (Excoffier and Heckel 2006). Allele frequencies, deviations from Hardy-Weinberg Equilibrium (HWE) and heterozygosity (observed and expected) were calculated separately by year for all banded territory holders present using Genalex 6 (Peakall and Smouse 2006). Tests for HWE were performed for each locus using 10,000 Markov chain steps. Linkage disequilibrium (LDE) was tested in order to determine whether non-random associations among loci occur (Excoffier and Lischer 2010). LDE tests were run using 10,000 permutations and a significance level of $p=0.05$ in Arlequin 3.5.1.3 (Excoffier and Lischer 2010). Heterozygote deficiencies were tested in MLRelate which uses a Monte Carlo randomization method ($n=999$ simulations performed) and U-statistic to determine significance (Guo and Thompson 1992, Rousset and Raymond 1995, Kalinowski et al. 2006). Significant p-values identify loci with heterozygote deficiencies that were attributed to null alleles and/or genotyping error (Kalinowski et al. 2006).

Spatial Genetic Structure and Spatial Clusters of Relatives

Spatial clusters of relatives describe a kin-structured pattern in which individuals within the focal population are non-randomly distributed near relatives. Since juvenile

buff-breasted wrens sometimes disperse short distances from natal territories to vacant breeding territories (Gill and Stutchbury 2010), a correlation is predicted to exist between genetic similarity and spatial proximity of individuals. Global spatial autocorrelation determines whether individuals within specified distance classes are more genetically similar than expected by chance (Smouse and Peakall 1999, Peakall and Smouse 2006). Local spatial autocorrelation analyses provide further resolution of spatial genetic structure by comparing a focal individual to its nearest neighbors in order to determine whether relatives are spatially clustered (Peakall and Smouse 2006). Global and local spatial autocorrelations were performed to test the hypothesis that spatial genetic structure exists across the study site. Data from 1997 - 1999 and 2009 - 2011 provided a series of snapshots in time of the spatial genetic structure over a 14-year time period. Banded territory holders present on the last survey day of each year were included in both global and local tests: 31 May 1997 (n=30), 24 June 1998 (n=48), 10 July 1999 (n=39), 24 March 2009 (n=60), 30 July 2010 (n=84) and 14 July 2011 (n=90). Each set of global analyses was performed by year and separately for (1) all territory holders, (2) male territory holders only, and (3) female territory holders only. Each set of local analyses was performed by year and separately for (1) male territory holders, and (2) female territory holders.

Global spatial autocorrelation analyses determine whether spatial genetic structure exists across the entire population or study site (Anselin 1995, Double et al. 2005). Specifically, this test measures the correlation between pairwise comparisons of genetic and geographic distances of individuals within each distance class. The genetic

similarity of individuals partitioned into predefined distance classes is compared to determine whether individuals within a given distance are more genetically similar than by chance (Double et al. 2005). Global autocorrelation coefficients (*r*) were calculated for each distance class using pairwise genetic and pairwise squared geographic distance matrices generated in Genalex 6 (Smouse and Peakall 1999, Peakall and Smouse 2006). Pairwise geographic matrices were calculated using territory centroids as the spatial coordinate for each focal individual. Distances between the focal territory and its nearest neighbor, or the territory centroid in closest proximity, were calculated in ArcGIS 10.0. Distance classes of 50 m were used since this estimate reflects a biologically meaningful distance class, since buff-breasted wren that obtained territories within the natal population often moved within one to two territories from their natal territory (Double et al. 2005, Gill and Stutchbury 2010). Global autocorrelation coefficients are bounded by -1 to +1 (Peakall et al. 2003). Tests for statistical significance were determined using 999 random permutation and 1,000 bootstrap estimates of autocorrelation coefficients to create a 95% confidence interval (Double et al. 2005, Peakall and Smouse 2006). Permutations were used to compare global (*r*) values to those permuted under the null hypothesis of no spatial genetic structure (Double et al. 2005, Peakall and Smouse 2006).

Upon obtaining a breeding territory, local recruits often shared a border with at least one relative (Gill and Stutchbury 2010), thus kin structure is expected to form among close neighbors. Local spatial autocorrelation of subsets of data may reveal the presence of spatially clustered, genetically similar individuals (Anselin 1995, Double et

al. 2005). To determine whether a correlation exists between genetically similar individuals and territory proximity, this test compares each focal individual to its nearest neighbors, or the closest individuals to the focal animal (Peakall and Smouse 2006). Two dimensional local spatial autocorrelation analyses (2D LSA) were performed in Genalex 6 (Peakall and Smouse 2006) for male and female territory holders present within the study site during each year sampled. Focal territories share a border with 1 - 4 territories within the study site. Four nearest neighbors was chosen in order to compare each focal individual to territories that were most likely to be adjacent, whereas 10 nearest neighbors was chosen in order to compare spatial genetic structure which might be present at farther distances from the focal territory. Genalex 6 calculates correlation coefficients (*lr*) and determines significance by using a one-tailed test (Peakall and Smouse 2006). Permutations were used to compare local (*lr*) values to those permuted under the null hypothesis of no spatial genetic structure (Double et al. 2005, Peakall and Smouse 2006).

Although local spatial autocorrelation analyses locate individuals that are genetically similar to their nearest neighbors, this analysis does not specify the actual relatedness or relationships among these individuals. Therefore, once identified, spatial clusters of relatives were further investigated to determine the exact nature of the relatedness between the focal individual and of its four nearest neighbors. Relatedness among each focal individual and its nearest neighbors was determined by calculating coefficients of relatedness (*r*) using MLRelate (Kalinowski et al. 2006). To determine the relatedness and relationships among spatial clusters, six years of data were

combined into two data sets (1997 - 1999 and 2009 - 2011) and analyses were performed separately in MLRelate. Heterozygote deficiencies were indicated by individual loci with significant p-values ($p<0.05$) which suggests the presence of null alleles and/or genotyping error (Kalinowski et al. 2006). MLRelate then uses the null alleles specified by the user to recalculate allele frequencies which can then be used to more accurately calculate measures of relatedness (*r*) and relationships. MLRelate uses k-coefficients (*k*) representing the genetic relationships between any two individuals to calculate coefficients of relatedness determine the probability that two individuals share alleles due to common ancestry and not simply by chance (see Kalinowski et al. 2006 for a detailed description). This method of calculating *r* assumes that the two individuals being compared are not inbred (Kalinowski et al. 2006).

I also used MLRelate to estimate the maximum log-likelihood of relationship (LnL(R)) of four relationship categories (Unrelated, Half-Siblings, Full-Siblings, Parent-Offspring) occurring between any two individuals within the population (Kalinowski et al. 2006). When the maximum likelihood of relationship was larger than that of the other possible relationships, the relationship with the highest likelihood may be accepted as the true relationship (Kalinowski et al. 2006). All maximum likelihood tests were performed using 999 permutations and 1,000 bootstraps.

Relatedness of Pairs of Territory Holders

Spatial clusters of relatives present within the study site may lead individuals to pair and breed with relatives, especially when philopatric individuals obtain local breeding territories and immigration rates are low. If spatial genetic patterns occur within the study population in both sexes, then it may be likely that territory holders are more genetically similar than expected by chance. The relatedness among breeding individuals within the study population was measured by (1) pooling all territory holders by year in order to calculate F_{IS}, and (2) by comparing pairwise relatedness coefficients to determine whether breeding pairs were significantly more related than expected by chance. Pairs of banded territory holders present on 31 May 1997 (n=15), 24 June 1998 (n=24), 10 July 1999 (n=19), 24 March 2009 (n=29), 30 July 2010 (n=40) and 14 July 2011 (n=42) were compared separately by year. Pairs of territory holders in which only one individual was banded were excluded from these analyses (1997: n=0; 1998: n=1; 1999: n=0; 2009: n=4; 2010: n=4; 2011: n=5). Pairs of territory holders were observed over multiple years making these data sets not entirely independent. However, analyzing F_{IS} and the proportion of pairs falling into each relationship category by year seems appropriate given that these individuals represent the reproductive potential of the study population during each time period.

Measures of the deviation of homozygotes from expected Hardy-Weinberg proportions, which assume mating is random, offer insight into patterns of inbreeding (Allendorf and Luikart 2008). When analyzing genotypic data within a population, F_{IS}

compares measures of the variance among genetically similar individuals to the total variance among all individuals within the population (Excoffier et al. 2005). Male and female territory holders were combined and F_{IS} values were calculated separately for each year in Arlequin 3.5.1.3 (Excoffier et al. 2005). An excess of homozygotes within the population is indicated by positive values of F_{IS}, whereas a deficit of homozygotes is reflected by negative values of F_{IS} (Allendorf and Luikart 2008). A high proportion of homozygotes could mean that individuals are reproducing with relatives leading to a decrease in genetic variation within a population.

Relatedness within each breeding pair was determined by calculating coefficients of relatedness (*r*) and by testing hypotheses of the specific relationships between territorial pairs using MLRelate (Kalinowski et al. 2006). For each territory, relatedness and relationships between male and female territory holders were compared using the same output and methods as described above. Values of (*r*) were calculated for all observed parent-offspring relationships to determine the overall accuracy of the estimates of relatedness (Nam et al. 2010).

Parentage Analysis

Parentage analyses were conducted in Cervus 3.0 (Kalinowski et al. 2007). Previously, Gill et al. (2005) assigned parentage using Cervus 3.0, thus this is an appropriate way to compare previous and current measures of parentage. Cervus 3.0 uses a maximum likelihood method to determine whether offspring are related to

candidate parents (Kalinowski et al. 2007). Offspring were compared to candidate parents present within the same sampling period to determine whether offspring-mother-father trios were significant at confidence levels of 80 % and 95 %. Bonferoni corrections were applied to Hardy-Weinberg to decrease the probability of Type I error when significant deviations from Hardy-Weinberg proportions occur by chance (Kalinowski et al. 2007). Cervus 3.0 treats null alleles as a type of genotyping error making it possible to incorporate these loci into parentage analyses (Kalinowski et al. 2007). Simulations of parentage (trio analysis) were performed using the following parameters: 90% of population sampled, 94.09% of loci typed and a 1.4% probability of mistyped alleles. Genotypes of mother-offspring pairs were compared to determine whether alleles mismatched and maternity could be assigned. Paternity was assigned to the male indicated in significant trios at 80% and 95% confidence intervals (high LOD). LOD scores are calculated by taking the natural log of the maximum likelihood value and positive values indicate that the candidate parents is more likely to be the true parents than by chance alone (Kalinowski et al. 2007).

RESULTS

Genetic Analyses

A mean of six alleles were observed per locus and mean observed heterozygosity was 0.568 (0.055 SE). Territory holders (n=192) were genotyped at eight to 11

microsatellite loci. Analysis of Hardy-Weinberg Equilibrium using Genalex 6 demonstrated that all loci tested were in HWE except ThPl-17 (1997), TA-C(3)-2 (during 1999 and 2010), ThPl-27 (2009 and 2010), and CpAAT51 (2010 and 2011). Tests for heterozygote deficiencies reveal an excess of homozygous genotypes at four loci during 2009 - 2011 (Thpl-27: p=0.00, TG11-11: p=0.002, Thpl-17: p=0.002 and TG05-053: p=0.003). These heterozygote deficiencies were interpreted as null alleles and were incorporated into allele frequency calculations and analyses of relatedness performed in MLRelate for the 2009 - 2011 data set only (Thpl-27, TG11-11, Thpl-17 and TG05-053). Independently replicating DNA extraction and PCR procedures for 24 samples at 11 loci revealed a genotyping error of approximately 1.4% (7 mismatching alleles/478 replicates).

Global Spatial Autocorrelation and Spatial Genetic Structure

Individual correlograms of each test of global spatial autocorrelation reflect the overall genetic structure within the study population over time. Under a null hypothesis, *r*-values that do not exceed the bounds of the 95% confidence interval created by 999 permutations indicate a lack of spatial genetic structure. Alternatively, values of *r* which exceed the upper bounds of the 95% confidence interval represent positive spatial genetic structure detected among all individuals compared within that particular distance class.

When all territory holders were compared, global spatial autocorrelation analyses revealed an absence of spatial genetic structure during 1997, 1998 and 1999 (Figure 2). In contrast, positive spatial genetic structure was detected among territory holders present during 2009, 2010 and 2011 (Figure 3). Distances at which spatial genetic structure was detected were similar across these years and ranged from 150 m - 200 m in 2009 and remained at 200 m in 2010 and 2011 (Figure 3). Since the average distance between neighboring territories was approximately 90 m, these results suggest that individuals within one to two territories are more related than expected by chance.

Significant spatial genetic autocorrelation was detected within distance classes of 150 m and 200 m during 2010 and 2011 among male territory holders (Figure 4) no spatial genetic structure was detected during 1997, 1998, 1999 or 2009 (Figures 4 and 5). Significant spatial genetic autocorrelation was detected at a distance class of 350 m for female territory holders present during 1997 (Figure 6) and at a distance class of 500 m (Figure 7). No significant patterns of spatial genetic autocorrelation were observed at any distance class during 1998, 1999, 2009 and 2011 (Figures 6 and 7). Although spatial autocorrelation results are known to vary depending on distance class selected for analyses, the influence of different distance classes on these data sets (e.g., 2011) was minimal (Figure 8).

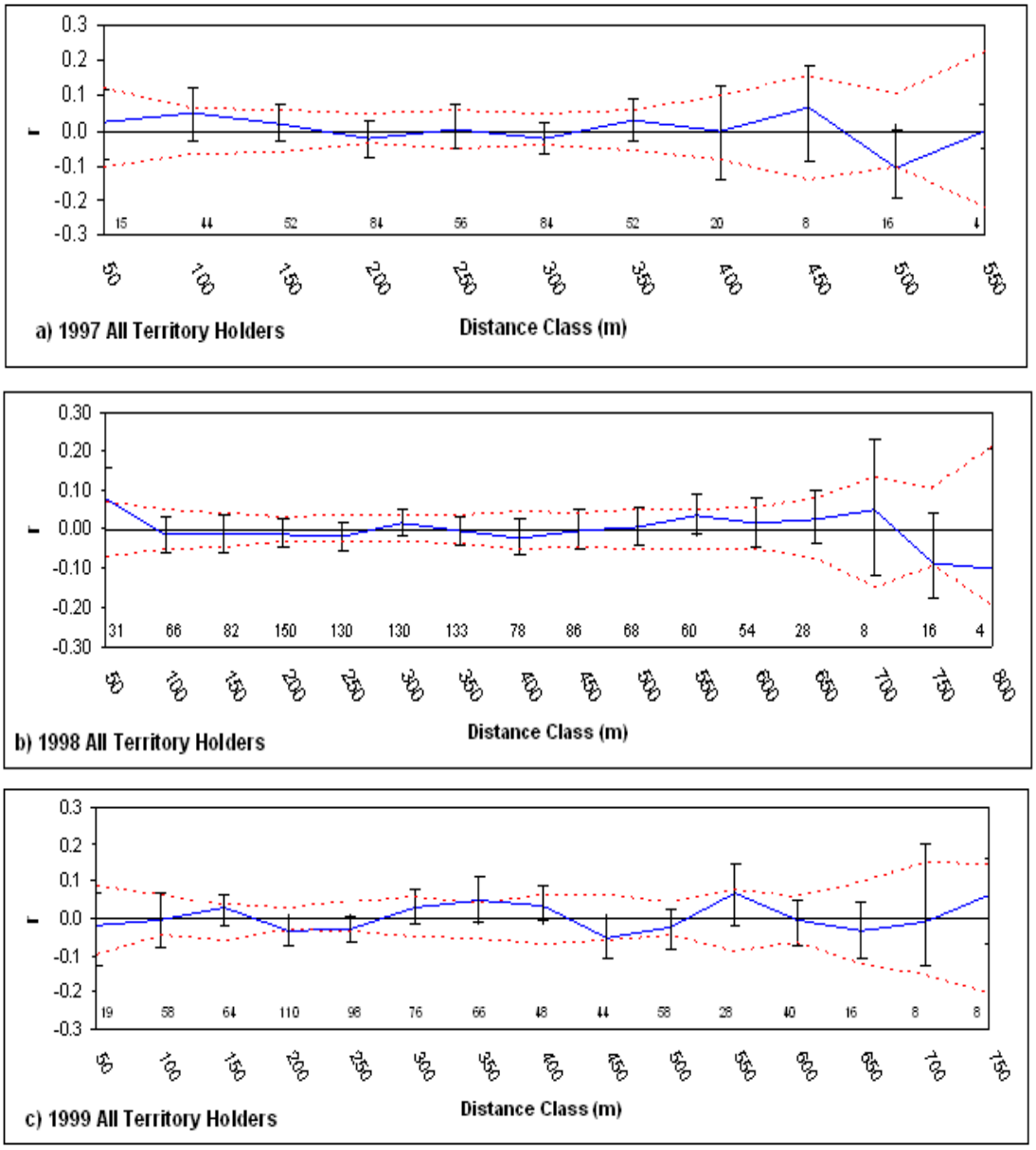

Figure 1. Global spatial autocorrelation analysis for all territory holders present during the first time period of the study: a) 31 May 1997, b) 24 June 1998 and c) 10 July 1999.

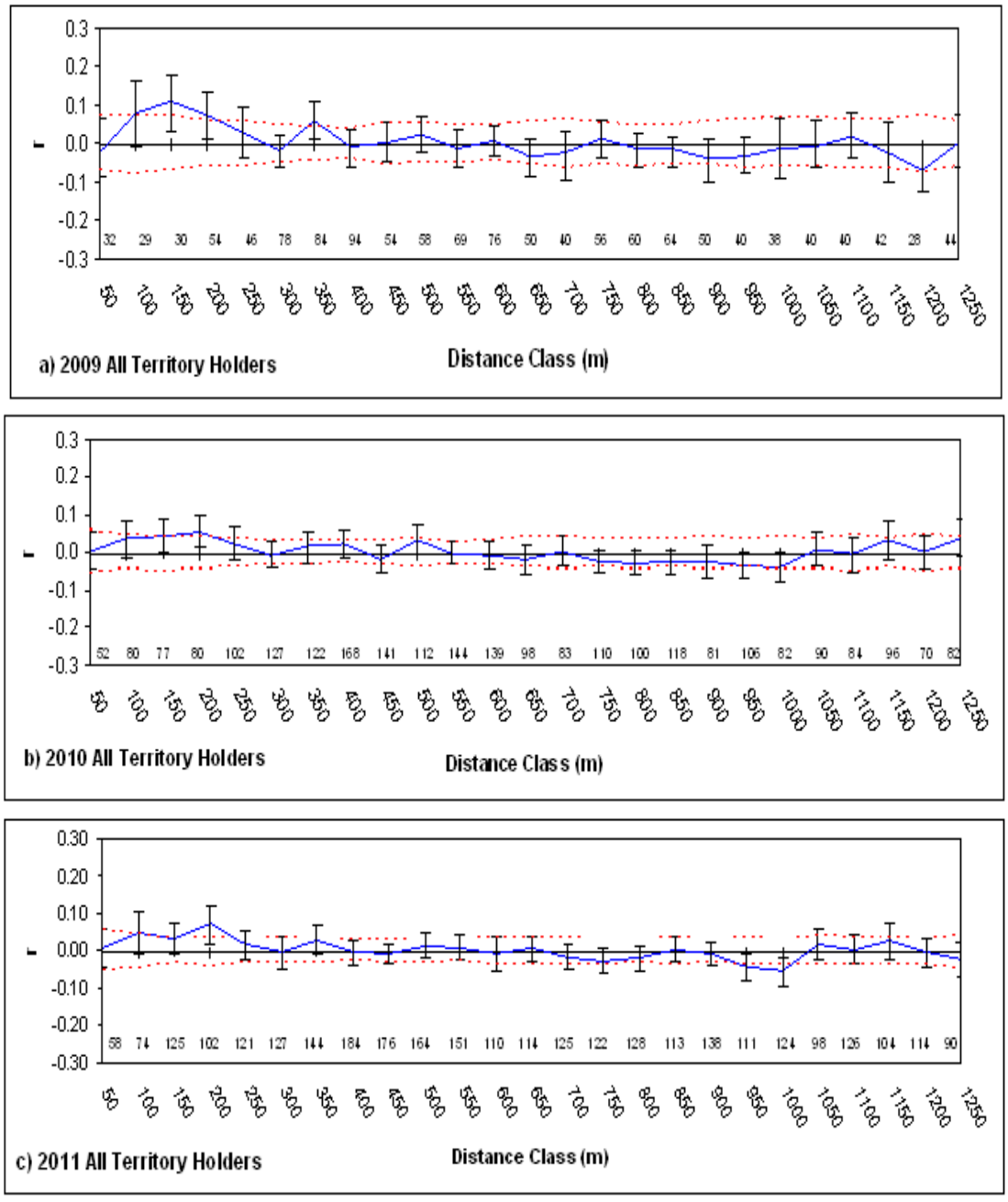

Figure 2. Global spatial autocorrelation analysis for all territory holders present during the second time period of the study: a) 24 March 2009, b) 30 July 2010 and c) 14 July 2011.

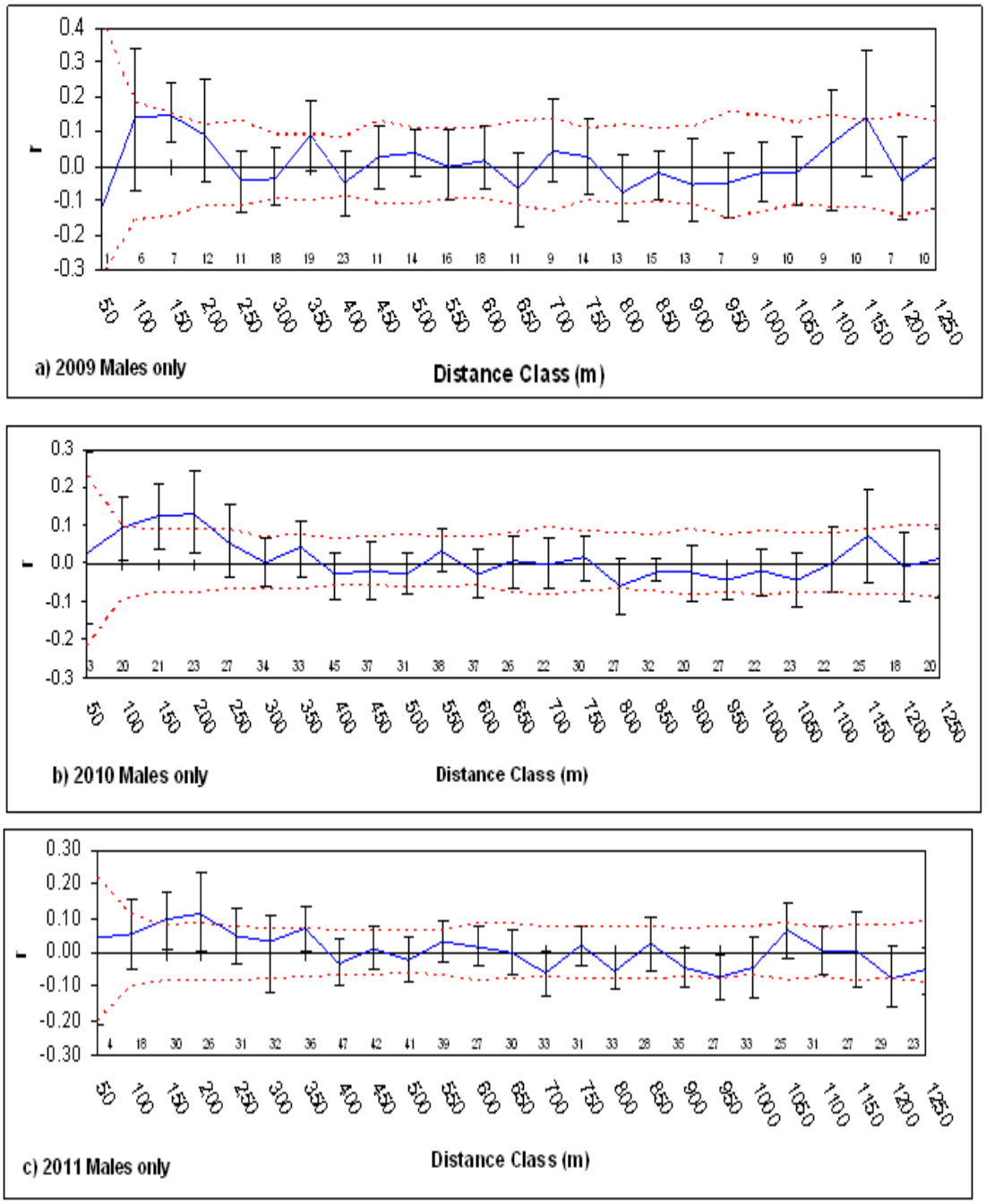

Figure 3. Global spatial autocorrelation analysis for all male territory holders present during the second time period of the study: a) 24 March 2009, b) 30 July 2010 and c) 14 July 2011.

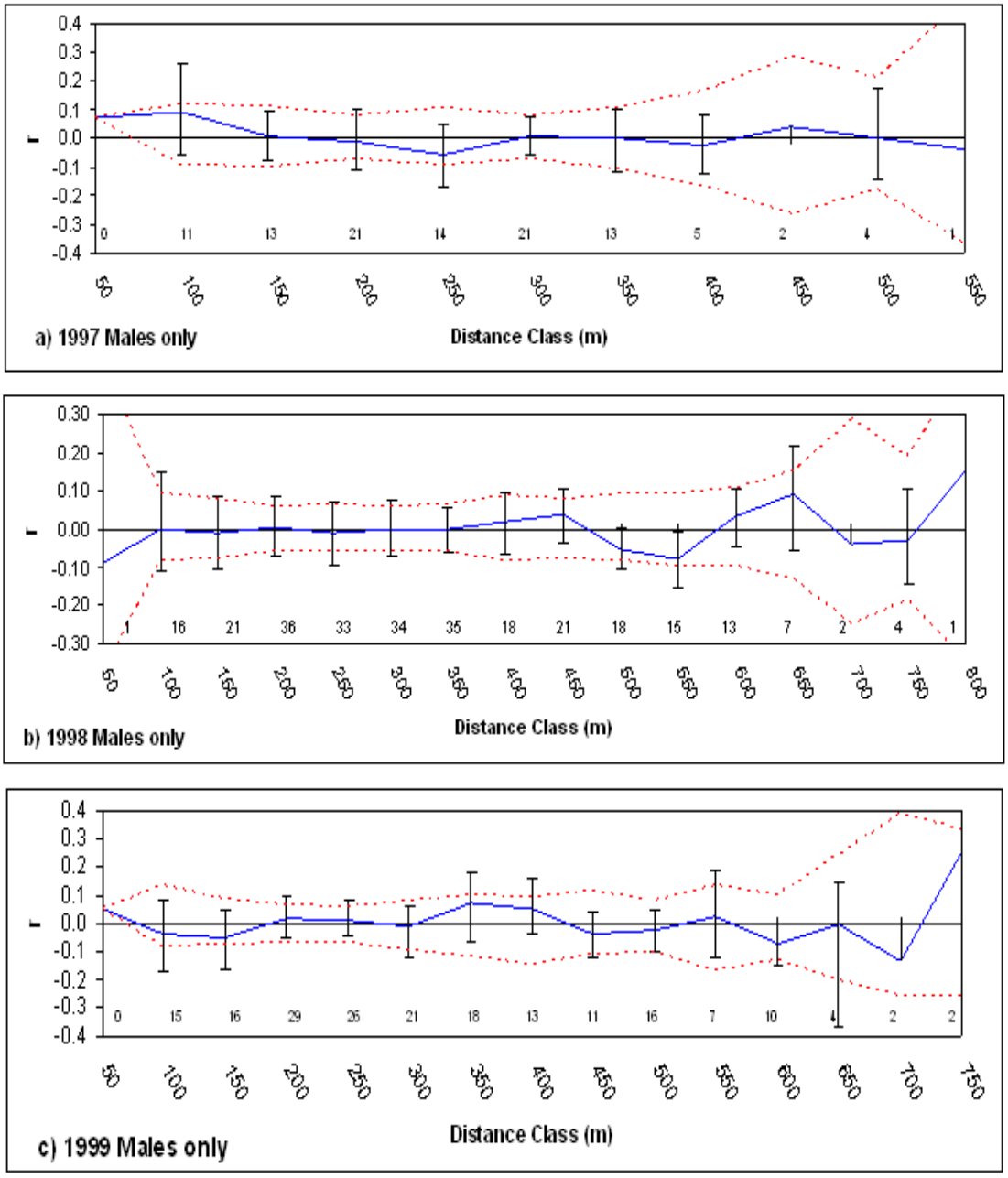

Figure 4. Global spatial autocorrelation analysis for all male territory holders present during the first time period of the study: a) 31 May 1997, b) 24 June 1998 and c) 10 July 1999.

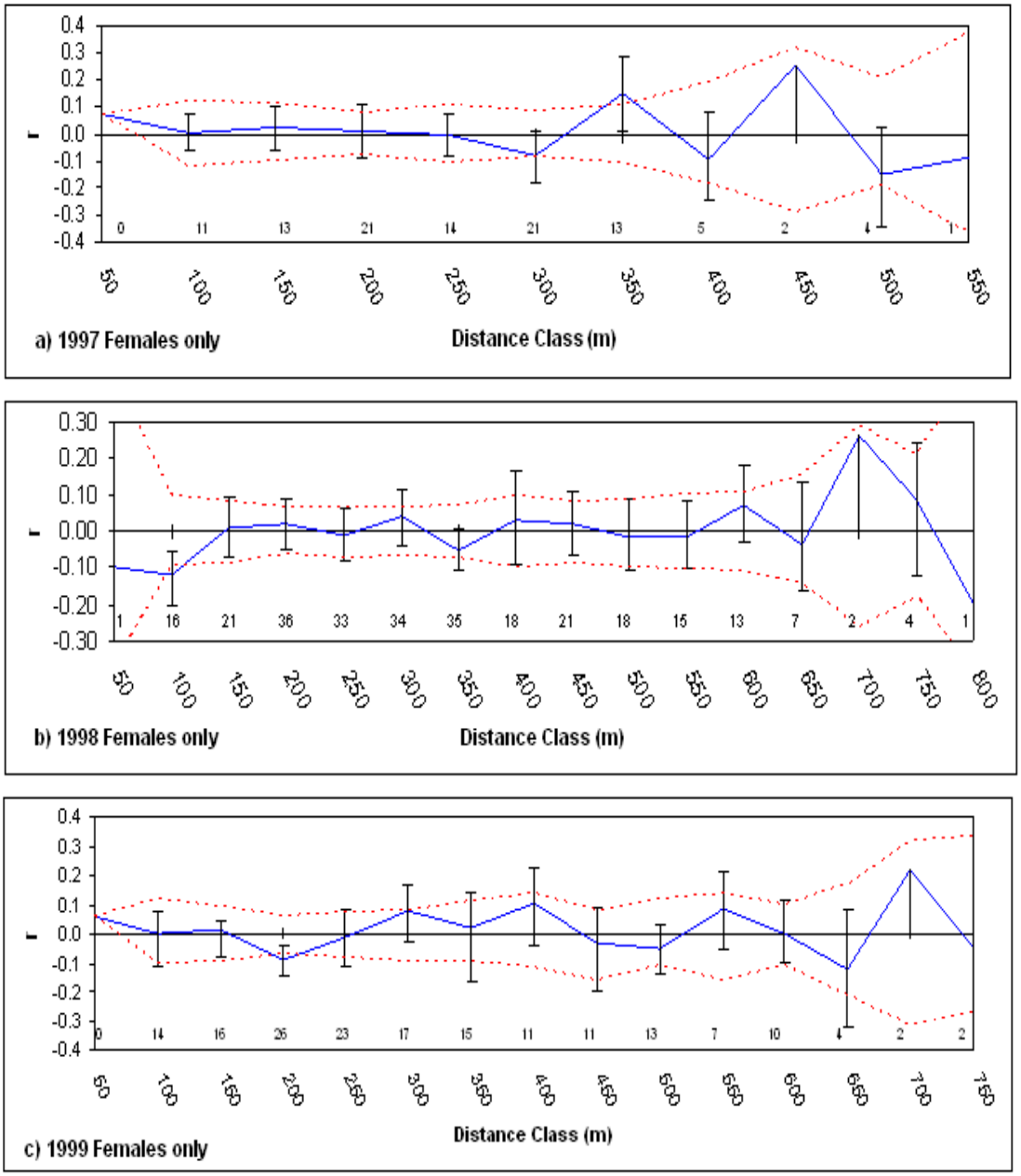

Figure 5. Global spatial autocorrelation analysis for all female territory holders present during the first time period of the study: a) 31 May 1997, b) 24 June 1998 and c) 10 July 1999.

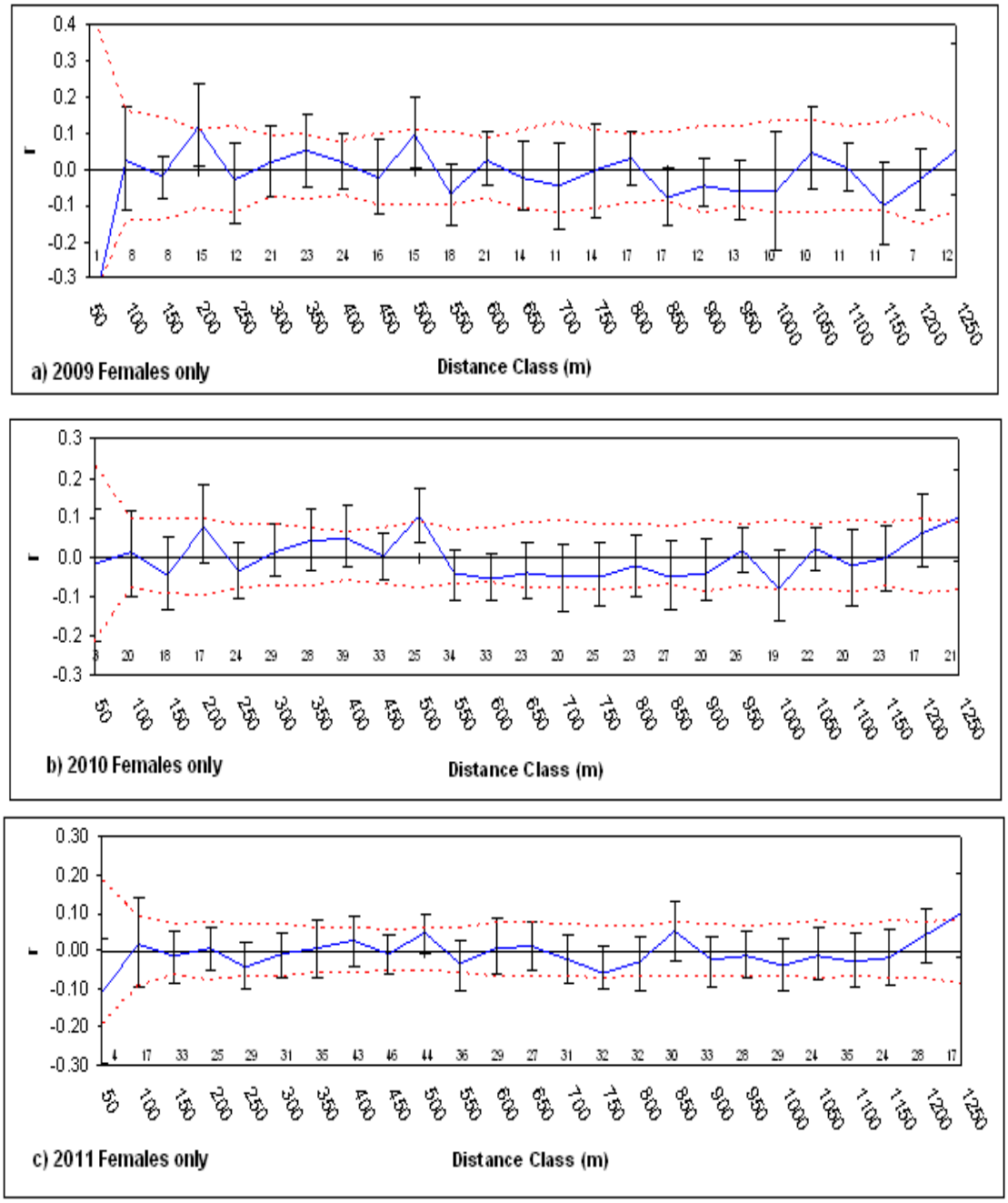

Figure 6. Global spatial autocorrelation analysis for all male territory holders present during the second time period of the study: a) 24 March 2009, b) 30 July 2010 and c) 14 July 2011.

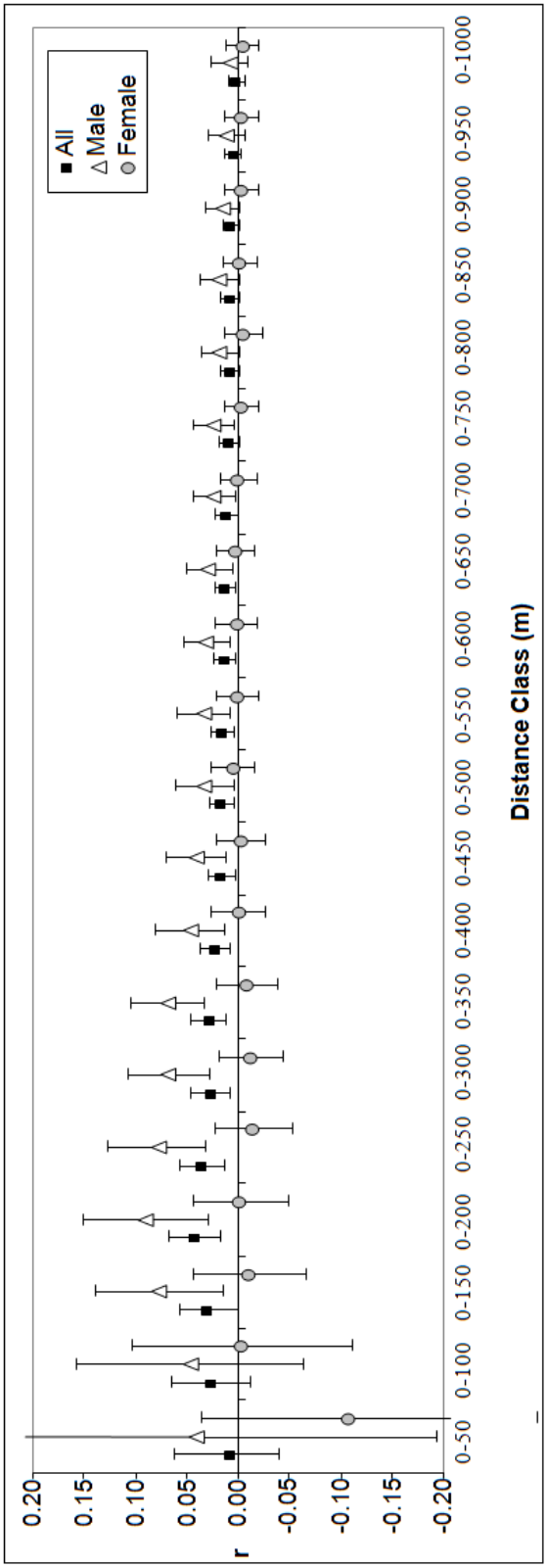

Figure 7. Influence of distance class size on genetic correlation (r) for territory holders present during 2011.

Local Spatial Autocorrelation and Spatial Clusters of Relatives

When male territory holders were each compared to their four nearest neighbors, local spatial autocorrelation analyses based on a one-tailed test revealed significant *lr* values for 13.3% of individuals present during 1997 (p=0.004 - 0.032), 8.3% in 1998 (p=0.025 - 0.032), 5.0% in 1999 (p=0.048), 17.2% in 2009 (p=0.002 - 0.025), 20.9% in 2010 (p=0.001 - 0.046) and 26.6% in 2011 (p=0.001 - 0.039) (see Table 3). Graphically, the x and y axes represent geographical distance in meters between individuals on each territory (e.g., Figure 9). Focal individuals which are unrelated (open circles) and related (solid triangles) are shown for each territory observed by year. One to two spatial clusters of relatives were detected among males during 1997 - 1999 (Figure 9). Five to 12 clusters were detected during 2009 - 2011 (Figure 10). Comparing focal males to their 10 nearest male neighbors produced similar number of spatial clusters to that of four nearest neighbors.

When female territory holders were each compared to their four nearest neighbors, two-dimensional local spatial autocorrelation analyses based on a one-tailed test revealed significant *lr* values for 9.6% of individuals in 2009 only (p=0.013 - 0.024, see Table 3). No spatial clusters of relatives were found among female territory holders during 1997 - 1999 (Figure 11) and zero to three clusters were detected during 2009 - 2011 (Figure 12). When focal females were compared to their 10 nearest neighbors, clusters of relatives were identified in three additional years. The number of clusters detected ranged from one in 1997 to five in 2010.

Table 3

Summary of the number of male and female territory holders, the number of spatial clusters detected, level of significance (p-value) and the range of lr values included in local spatial autocorrelation analyses of four nearest neighbors by year

Year	Sex	Number Territory Holders	*% lr* values Significant	p-values	Range *lr* values	Number of Clusters
1997	Male	15	13.3	0.004 - 0.032	-0.197 - 0.164	2
	Female	15	0	>0.200	-1.170 - 0.002	0
1998	Male	24	8.3	0.025 - 0.032	-0.273 - 0.154	2
	Female	24	0	>0.053	-0.170 - 0.129	0
1999	Male	20	5	0.048	-0.242 - 0.120	1
	Female	19	0	>0.111	-0.162 - 0.064	0
2009	Male	29	17.2	0.002 - 0.025	-0.102 - 0.295	5
	Female	31	9.6	0.013 - 0.024	-0.213 - 0.230	3
2010	Male	43	20.9	0.001 - 0.046	-0.150 - 0.421	9
	Female	41	0	>0.052	-0.214 - 0.159	0
2011	Male	45	26.6	0.001 - 0.039	-0.150 - 0.366	12
	Female	45	0	p>0.065	-0.239 - 0.125	0

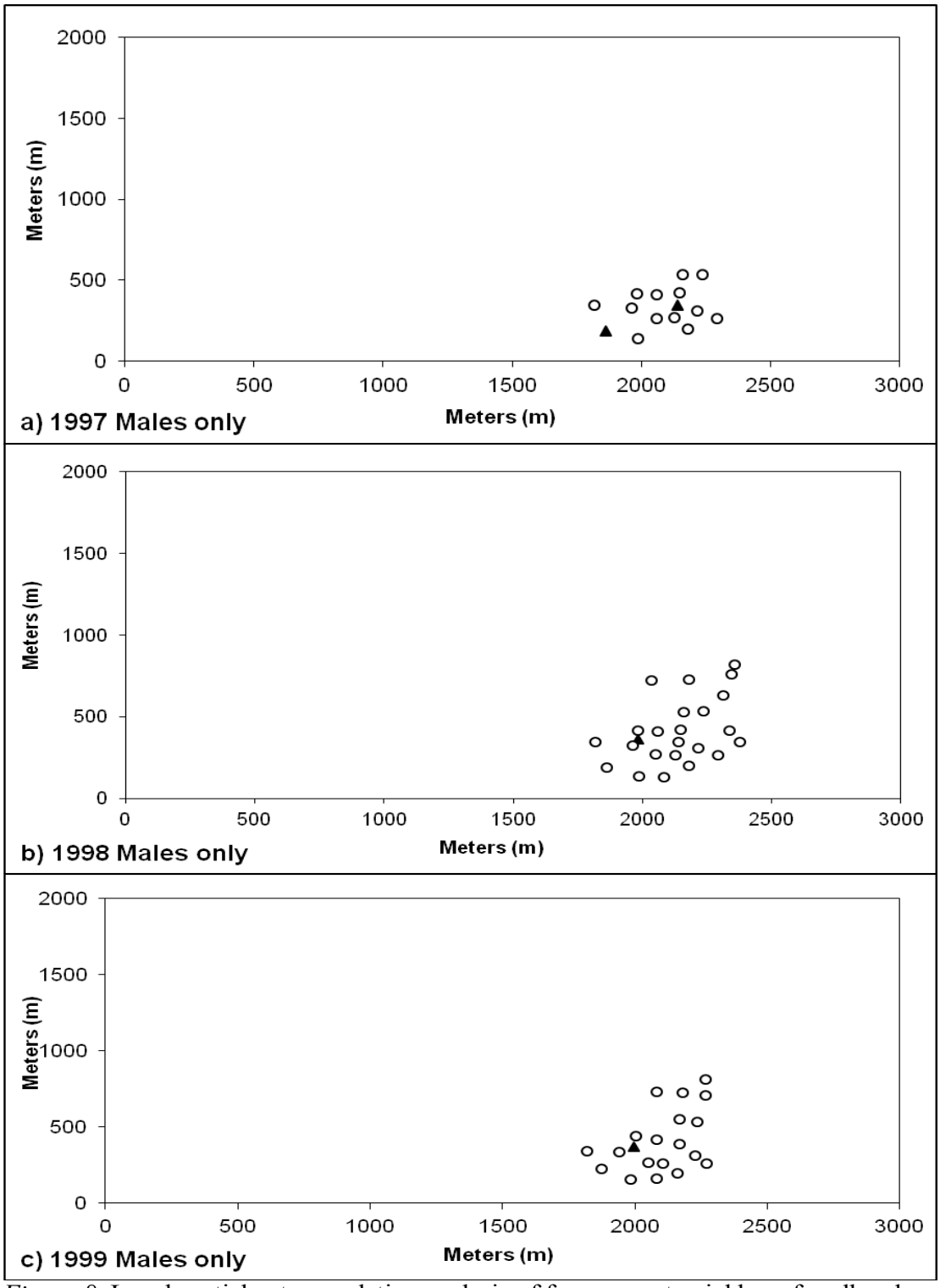

Figure 8. Local spatial autocorrelation analysis of four nearest neighbors for all male territory holders present during the first time period of the study: a) 31 May 1997, b) 24 June 1998 and c) 10 July 1999.

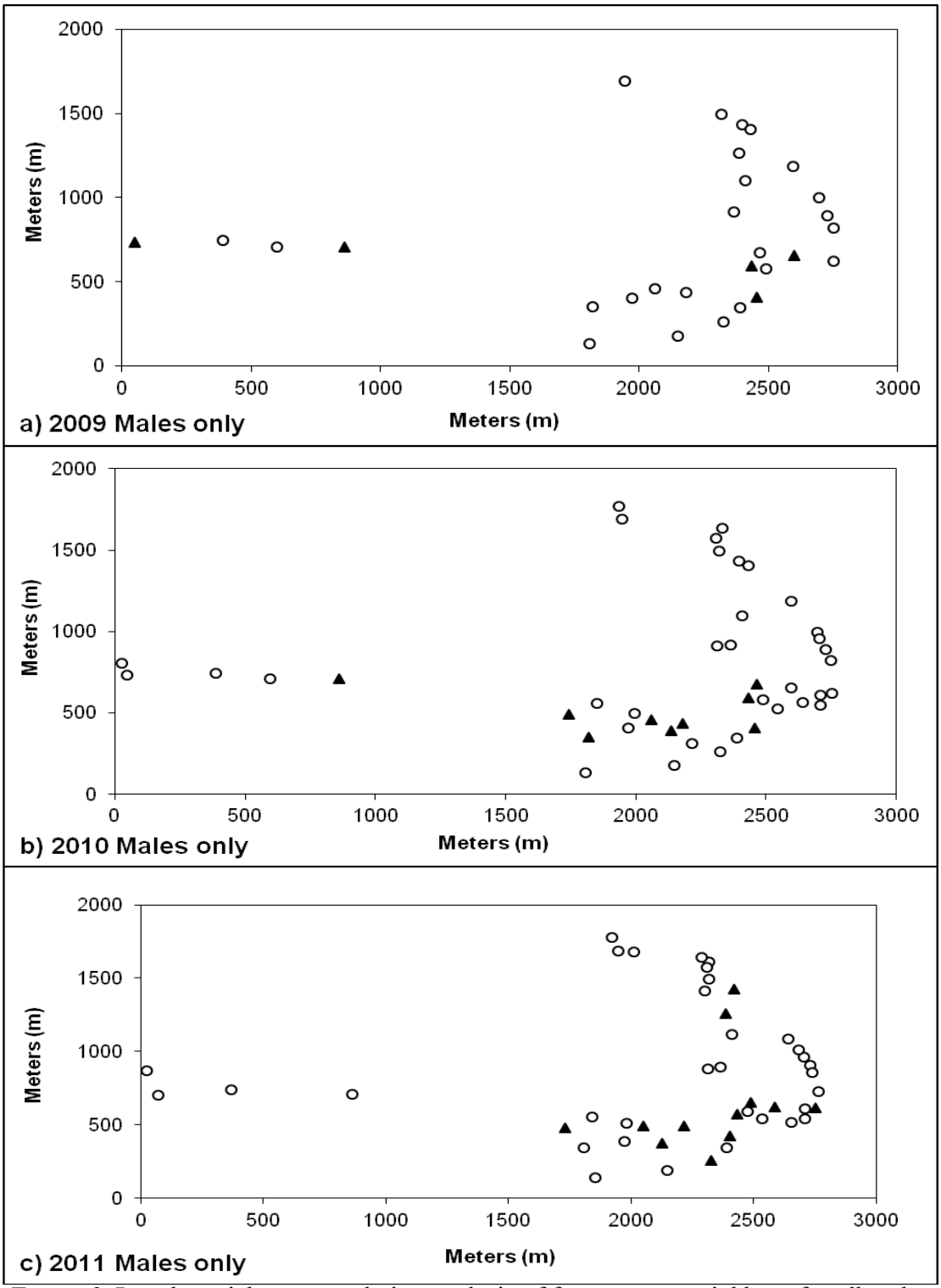

Figure 9. Local spatial autocorrelation analysis of four nearest neighbors for all male territory holders present during the second time period of the study: a) 24 March 2009, b) 30 July 2010 and c) 14 July 2011.

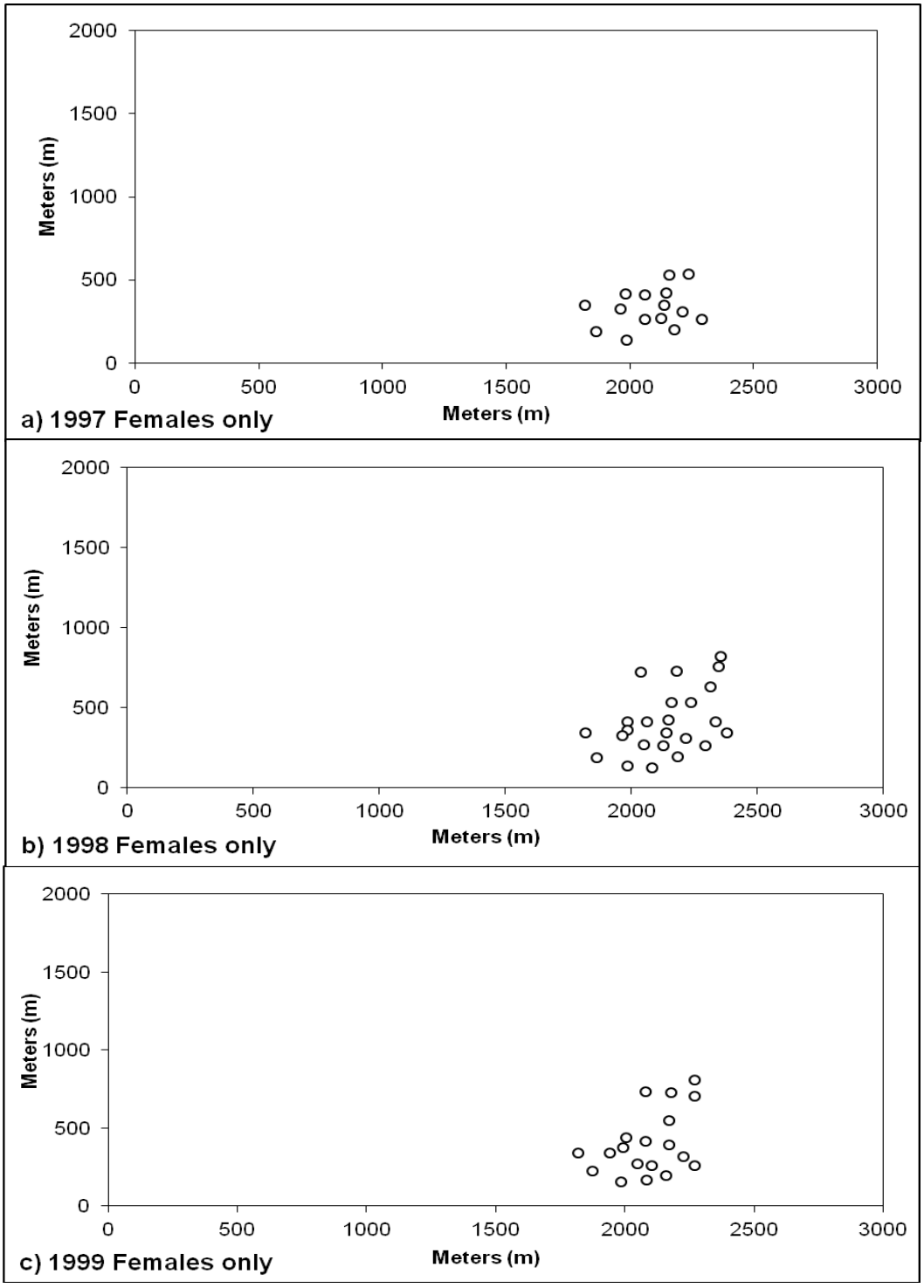

Figure 10. Local spatial autocorrelation analysis of four nearest neighbors for all female territory holders present during the first time period of the study: a) 31 May 1997, b) 24 June 1998 and c) 10 July 1999.

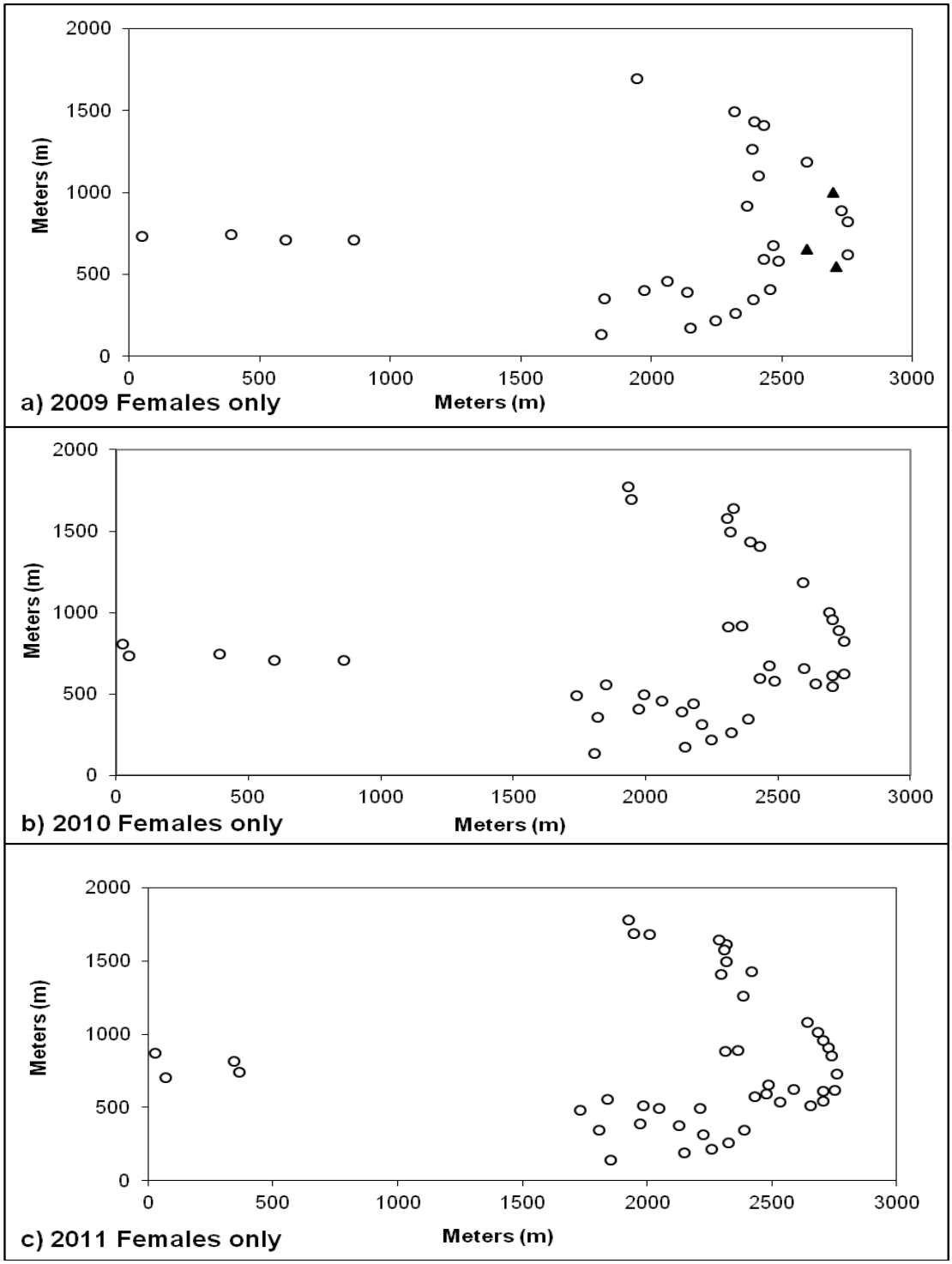

Figure 11. Local spatial autocorrelation analysis of four nearest neighbors for all male territory holders present during the second time period of the study: a) 24 March 2009, b) 30 July 2010 and c) 14 July 2011.

Relatedness of Territorial Pairs

Measures of F_{IS} were negative for all years (1997: -0.0062; 1998: -0.054; 1999: -0.034; 2010: -0.004; 2011: -0.005) except during 2009 (0.009) and values of F_{IS} did not significantly differ from zero in any year (1997: p=0.92; 1998: p=0.87; 1999: p=0.92; 2009: p=0.99; 2010: p=0.94; 2011 p=0.99). These results indicate that this population of territory holders is not inbred. MLRelate identified relationships between pairs of banded territory holders by using maximum likelihood estimates of relationship and by testing hypotheses of relationship between pairs of individuals. In all years, most territory holders were unrelated although the percentage of unrelated pairs varied considerably (66.6 - 89.5%). Of the pairs with some degree of relatedness, 10.5 - 26.7% of partnerships were comprised of half-siblings. A small proportion of partnerships were comprised of parent-offspring (4.8 - 6.7 %) and full-sibling relationships (0 - 2.5%), only varying slightly by year. Additionally, mean coefficient of relatedness (*r*) among all pairs present during each year was low (range=0.05 - 0.11).

Parentage Analysis

Parentage analyses performed in Cervus 3.0 were able to assign parent-offspring relationships with 80% and 95% confidence levels for 59 offspring-mother-father triads. Social mothers matched offspring at one or more alleles for all loci in cases

except one (n=58) and were thus concluded to be the genetic mother. Genetic analyses in Cervus 3.0 found the social father to be the most likely genetic father in all cases except one (n=58).

DISCUSSION

In this study, I examined spatial genetic patterns in buff-breasted wrens to assess the genetic consequences of short-distance natal dispersal of both sexes in a family-living, but non-cooperatively breeding species. This population of buff-breasted wrens showed significant spatial genetic structure that varied over time, which may reflect the outcome of patterns of dispersal. Males show stronger patterns of spatial genetic structure than females, which suggests that females disperse father distances than do males. Estimates of relatedness between territorial pairs revealed that a majority of pairs were unrelated, which suggests that inbreeding may be limited within this population. Together, these results suggest that kin structuring may be dynamic within this population and that factors other than short-distance natal dispersal and family-living may contribute to the development of spatial genetic patterns.

Spatial Genetic Patterns Among Territorial Adults

In buff-breasted wrens, previous behavioral observations indicate that both sexes are philopatric, thus positive spatial genetic structure was predicted to form among

both adult male and females. Global spatial autocorrelation analyses assess spatial genetic structure across the entire population while local spatial autocorrelation analyses determine whether spatial genetic clusters of relatives are present within the study site (Smouse and Peakall 1999, Peakall and Smouse 2006). In buff-breasted wrens, global spatial autocorrelation analyses detected positive spatial genetic structure among all territory holders at a distance of 150 - 200 m during 2010 - 2011; however these patterns were absent during 1997 - 1999 and in 2009. Both global and local spatial autocorrelation analyses reveal positive spatial genetic structure among both sexes. However, these patterns differ slightly between males and females. Among male territory holders, positive global spatial genetic structure was detected at distance classes of 150 - 200 m during 2010 and 2011, whereas among female territory holders, positive global genetic structure occurred at distance classes of 350 m and 500 m during 1997 and 2010, respectively. Local spatial autocorrelation analyses detected significant spatial clusters among territorial males and their four nearest neighbors in all years. In contrast, spatial clusters of relatives were only detected among female territory holders and their four nearest neighbors during one year. These results contrast previous behavioral observations of non-sex biased dispersal in buff-breasted wrens (Gill and Stutchbury 2010) and suggest that males may disperse shorter distances and settle locally more often than females.

In addition to detecting spatial clusters of relatives, local spatial autocorrelation analyses offer insight into the distribution of spatial clusters since relatives may be non-randomly distributed within a location. In buff-breasted wrens, spatial clusters of

relatives appear to be non-randomly distributed within the expanded study area during 2009 - 2011. Specifically, these clusters were found predominantly within one forest patch (i.e., original study area) and within a linear strip of forest along the Chagres River. Although spatial clusters of relatives were only detected among females during 2009, these clusters were also found within the same locations as male clusters. These "hot-spots" of spatial clusters of relatives have been described in superb fairy-wrens and white-winged choughs (Double et al. 2005, Beck et al. 2008), and it seems likely that further investigation using two dimensional spatial autocorrelation approaches may reveal similar patterns within other species. The non-random distribution of these spatial clusters of relatives, or "hot-spots," may reflect differential contribution of particular lineages to the overall spatial genetic structure of a population (Double et al. 2005). The presence of spatial clusters of relatives may have important implications for patterns of gene flow and genetic drift, as well as effective population size due to the non-random contribution of individuals to the genetic composition of the local population.

Unlike global tests, local spatial autocorrelation analysis specifically indicate which individuals are significantly related to their nearest neighbors, making it possible to further investigate the contribution of known individuals to the spatial genetic patterns observed within the study site. Since buff-breasted wrens are long-lived and remain on the same territory over multiple years (Gill and Haggerty 2012), the possibility exists that some families may contribute more than others to the population of reproductive recruits (Manel et al. 2003), as well as patterns of spatial genetic structure (Double et

al. 2005). For example, the only male cluster identified in 1998 and 1999 (focal individual: 30650, four nearest neighbors) was the father of a focal male identified in a cluster in 2010 (focal individual: 30698, four nearest neighbors). Another male banded in 1997 was found to be a relative of two male clusters identified during 2011 (focal individuals: 44148 and 44202, four nearest neighbors). Among females, spatial clusters of relatives were only identified during 2009 and all three clusters contained focal individuals which were banded after 1999. One of the "hot-spots" detected in superb-fairy wrens involved a male known to be highly productive, contributing 16 sons that survived to adulthood (Double et al. 2005). Thus, spatial clusters of relatives appear to result from long-lived and highly fecund individuals that produce offspring which survive and settle near their natal territories. Investigation of two dimensional spatial genetic patterns in other species may reveal further insight into the influences of specific individuals to kin clustering in family-living species.

These results in buff-breasted wrens differ from other studies that find global spatial genetic structure when both sexes are philopatric, in that we see sex-specific spatial genetic patterns despite previous behavioral observations of similar dispersal and settlement patterns among males and females. The spatial genetic structure found within buff-breasted wrens potentially differs from that found within white-winged choughs and white-breasted thrashers because analyses in these species included offspring present on focal territories (Temple et al. 2006, Beck et al. 2008), whereas this study includes only adults in analyses. In buff-breasted wrens, the average distance between territory centers was approximately 90 m, thus presence of global spatial

genetic structure among all territory holders suggests that individuals on adjacent territories are likely to be relatives. These results confirm previous behavioral observations that individuals within the study site often share at least one territory boundary with relatives (Gill and Stutchbury 2006). Few studies investigate the composition of genetic structure within the study site past these broad measures which compare individuals within distance classes rather than focal individuals specifically (Double et al. 2005, Beck et al. 2008).

Patterns of genetic structure that do not reflect demographic data potentially occur when there is a bias in detecting short distance dispersals or when males and females show slightly different dispersal patterns, which may be difficult to detect by observation (Woxvold et al. 2006). Both male and female buff-breasted wrens defend territories and display high site fidelity (Gill et al. 2005, 2007, 2008), thus both sexes should benefit from familiarity with their natal site (Greenwood 1980). In contrast to these predictions, global and local spatial genetic structure showed that these spatial genetic patterns differ among males and females, which suggests that dispersal distances may be underestimated by behavioral data. Differences in spatial genetic patterns indicate that dispersal is slightly sex-biased and farther dispersal distances by females may be favored, possibly in order to avoid mating with kin. Further investigation of genetic structure in species in which both sexes show similar patterns of dispersal may clarify the impact of these behaviors on the spatial distribution of same sex relatives and the possible mechanisms underlying these differences.

Genetic structure may reflect natal dispersal at different spatial scales (Ortego et al. 2011). Given that weak positive genetic structure was detected among females during some years, local spatial autocorrelation analyses were rerun to determine whether including more nearest neighbors would reflect kin structure if females dispersed slightly farther within the study area. Comparing focal males to their 10 nearest male neighbors produced similar number of spatial clusters to that of four nearest neighbors. Although the number of female clusters is smaller than that of male clusters, this pattern of relatedness at farther distances suggests that although females disperse within the study site, these spatial genetic patterns detect slight differences between male and female dispersal distances.

Changes in spatial genetic patterns over time may reflect cycling in population level processes, such as mortality and local settlement, as well as selection pressure on dispersal distance (Murrell et al. 2002, Matocq and Lacey 2004). This study shows changes in spatial genetic structure within this population within six sampling periods during 14 years. Since territory holders remain on the same territory over multiple years, spatial genetic structure was predicted to remain stable over time in buff-breasted wrens. In contrast with this prediction, male spatial genetic structure varied between the two time periods in that kin structure was absent during the early time period (1997 - 1999) and present during the later time period (2009 - 2011). Males were more often related to other adult males on neighboring territories during 2009-2011 than during 1997 - 1999. However, female spatial genetic structure was absent across sampling years. These results are consistent with other studies which present

data from multiple time periods in family-living and philopatric species (Double et al. 2005). Two snapshots in time of the local spatial genetic structure of superb-fairy wrens differ in the number of spatial clusters present (Double et al. 2005), which suggests that characteristics of spatial genetic structure vary over time. Fluctuations in reproductive success, survival and local settlement of individuals within the study site may contribute to changes in kin structure over time (Murrell et al. 2002, Matocq and Lacey 2004, Double et al. 2005), however further studies are required to determine whether these factors influence changes in spatial genetic structure in buff-breasted wrens.

If survival and reproductive success differs among lineages, then it seems likely that not all families contribute equally to the population of reproductive recruits (Manel et al. 2003). Mortality associated with particular a life-history stages may have important implications for the overall spatial genetic patterns if individuals differ in their contribution to future reproductive population (Beckerman et al. 2011). Within this population of wrens, individual survival is relatively high (approximately 70% during 1997-1999) and many offspring banded on their natal territory disperse to vacant territories within the study site (Gill and Stutchbury 2006). Adult survival is also similar among male and female territory holders (Gill and Haggerty, in press). If mortality promotes the formation of spatial genetic structure (Beckerman et al. 2011), then we might expect high female and male survival to lead to a lack of spatial genetic structure since this will limit vacancies via territory turnover and could mean that juveniles must disperse father distances to obtain a territory. A clearer understanding

of how ecological processes, such as life stage-specific mortality, influences patterns of kin structure over time is necessary to determine whether mortality leads to sex-specific spatial genetic patterns within this population.

The dispersal propensity, or ability, of a species may also influence patterns of spatial genetic structure. Ecological (e.g., habitat and foraging preferences) and morphological variables (e.g., wing size and shape) may directly influence the dispersal abilities of neotropical bird species, which may then lead to differences in spatial genetic structure among species. In an extensive survey of genetic differentiation of rain forest birds across South America, Burney and Brumfield (2009) found that species restricted to understory habitats showed higher levels of genetic differentiation than did canopy species. Wing characteristics may also influence dispersal propensity in that short, rounded wings, which allow individuals to maneuver through understory vegetation, may limit their ability to disperse longer distances (Moore et al. 2008, Burney and Brumfield 2009). Buff-breasted wrens have short, rounded wings and are unlikely to cross wide gaps between secondary-growth forest patches, which may mean that this species may show lower dispersal propensity. Boundaries of suitable habitat vary within the study site and might impose an upper limit on the ability of buff-breasted wrens to disperse, especially if individuals are unlikely to cross large patches of unsuitable habitat (e.g., a large regularly mowed field near the Chagres River). Variation in habitat characteristics which influence the dispersal movements and distances of individuals, could lead to differential patterns of spatial genetic structure. For example, spatial clusters of relative are found predominantly within a

rather large forest patch and are relatively absent within small linear patches of suitable habitat. Additional research is necessary to determine whether landscape characteristics influence patterns of dispersal and kin structure in buff-breasted wrens.

Relatedness of Territorial Pairs

The majority of territorial pairs consisted of unrelated individuals, thus the potential for inbreeding appears to be low. These results correspond with the weak global spatial genetic structure and sex-bias in spatial genetic structure among territory holders within this population. Additionally, the proportion of pairs which was unrelated fluctuated among years (66.6% - 89.5%). Changes in the relatedness of breeding pairs may arise when the proportion of related individuals which recruit into the reproductive population differs among years. In buff-breasted wrens, a lack of inbreeding among pairs of territory holders in combination with a lack of spatial genetic structure suggests that individuals may avoid mating with kin.

Benefits of Long Term Data Sets

Long-term behavioral and genetic data sets provide detailed insight into the nature of dispersal and genetic structure within a study population over time and potentially reveal changes in population structure that may not be detected by sampling over a shorter time period. However, these data sets are often difficult to obtain due to

logistical and economic constraints. In buff-breasted wrens, spatial genetic structure fluctuates over time which could lead to misrepresentations of these patterns if only subsets of these years were considered. For example, if we only sampled during the early time period it might appear that spatial genetic structure does not form within this species. In contrast, if only sampled during the later time period (2009 - 2011) were considered, than we might conclude that positive genetic structure is consistent and does not change over time. This is further illustrated by the yearly sampling of kin structuring male red grouse territory holders over 10 years (Piertney et al. 2008). Males on neighboring territories varied from unrelated during one year to highly related during other years (Piertney et al. 2008). Additionally, certain lineages were found consistently throughout the years while others were only present for a subset of years (Piertney et al. 2008). Furthermore, Piertney et al. (2008) demonstrate that that sampling continuous years for short durations of time may not provide a complete picture reflecting dynamic population structure. Thus, longitudinal data sets and the detailed knowledge of buff-breasted wren biology (e.g., Gill et al. 2005, Gill and Stutchbury 2006, 2010) offer further insight into the impact of fundamental life history behavior on patterns of spatial genetic structure within a population over time.

Conclusions

In conclusion, the spatial genetic structure present within this population reflects the complex interaction between sociality, dispersal and mating behavior in buff-

breasted wrens. In buff-breasted wrens, global and local spatial autocorrelation analyses reveal slightly different patterns of spatial genetic structure which suggests that both tests may be more appropriate to accurately measure kin structure, rather than global spatial autocorrelation alone. The long term study of the behavior and biology of buff-breasted wrens provides information which is critical to properly interpreting spatial genetic patterns and highlights the complexity of how patterns of sociality and dispersal translate into kin structure. Comparison of spatial genetic patterns across multiple years reveals fluctuations in kin structure which suggest that population dynamics may be cyclical in this species. Further research involving field experiments and computer modeling may provide insight into the extent to which density-dependent processes such as predation (i.e., mortality) and intraspecific interactions (e.g., cooperation versus competition) influence kin structuring patterns within this species.

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WESTERN MICHIGAN UNIVERSITY

Institutional Animal Care and Use Committee

Centennial
1903-2003 Celebration

Date: November 11, 2009

To: Sharon Gill, Principal Investigator
Maarten Vonhof, Co-Principal Investigator

From: Rob Eversole, IACUC Chair

Re: Changes to IACUC Protocol No. 07-11-01

This letter will serve as confirmation that the changes to your research project "Causes and Consequences of Delayed Dispersal in Neotropical Buff-breasted Wrens" requested in your memo dated November 4, 2009 (Student investigators Sarah Alessi, Ariel Cummings, Jennifer Davenport, Melanie Guigueno, Andrea Kryger, Rebecca Marshall and Jacob Williams added; five bird species (Carolina Wren, Northern Cardinal, Black-capped Chickadee, Yellow Warbler and Chipping Sparrow) added; egg collection and sampling added; hormone injection procedure added; hormone implant procedure added) have been approved by the Institutional Animal Care and Use Committee.

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