SPATIAL GENETIC STRUCTURE OF FOUR BOBCAT POPULATIONS IN THE SOUTHEASTERN U.S.

by

ALLISON ELAINE REID

(Under the Direction of Robert J. Warren and C. Joseph Nairn III)

ABSTRACT

The bobcat (*Lynx rufus*) is a species of conservation and economic importance. However, little is known regarding certain aspects of the bobcat's life history, such as dispersal characteristics and social organization, both of which will influence the spatial genetic structure of populations. Predictions based on the bobcat's mating system suggest that the bobcat may exhibit sex-biased dispersal and philopatry. This thesis examined the spatial genetic structure within a well-studied population of bobcats in southwestern Georgia as well as the spatial genetic structure between four populations in Georgia and Florida. We found little evidence of sex-biased dispersal and philopatry, and our data indicate that the four populations were one panmictic unit. Our results suggest that dispersal patterns cannot be predicted from a species' mating system alone. Moreover, because bobcat populations lack differentiation across large spatial scales, a landscape-level approach to bobcat management may be appropriate.

INDEX WORDS: bobcat, *Lynx rufus*, microsatellites, relatedness, kinship, spatial genetic structure, isolation by distance, dispersal, philopatry

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ALLISON ELAINE REID

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ALLISON ELAINE REID

Co-Major Professors:	Robert J. Warren C. Joseph Nairn III
Committee:	L. Michael Conner John P. Carroll

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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The ordinary citizen today assumes that science knows what makes the community clock tick; the scientist is equally sure that he does not. He knows that the biotic mechanism is so complex that its workings may never be fully understood.

- Aldo Leopold, *A Sand County Almanac*

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CHAPTER 1

INTRODUCTION: DISPERSAL, PHILOPATRY, AND SPATIAL GENETIC STRUCTURE

Introduction

In polygynous mammalian species, dispersal is expected to be male-biased, whereas females are thought to be the sedentary, or philopatric, sex (Greenwood 1980, Waser and Jones 1983). Philopatry occurs when an individual remains near or within its natal home range past the age of independence (Waser and Jones 1983, Ratnayeke *et al*. 2002). As such, philopatry has the potential to cluster closely related individuals and likely contributes to the evolution of cooperative traits among the philopatric sex via kin selection (Hamilton 1964, Greenwood 1980, Waser and Jones 1983).

Philopatry has been well-documented for social, mammalian species, largely in the interest of studying altruism, cooperation, and group-living (Waser and Jones 1983). Philopatry is a behavioral attachment to a site, not an individual or a group of conspecifics, and, as such, gregariousness, or group-living, is not a requisite (Waser and Jones 1983). Few studies, however, have examined this behavior for solitary mammals (Ratnayeke *et al*. 2002, Cutrera *et al*. 2005, Janečka *et al*. 2006) and most evidence of philopatry for solitary species appears anecdotal (Waser and Jones 1983).

The bobcat (*Lynx rufus*) is a solitary, polygynous mammal, and, based on knowledge of the bobcat's mating system, *a priori* predictions of the resultant dispersal and philopatric patterns can be made. Although there is some evidence to suggest that philopatry occurs in this species, either the sample size of the study was small (Janečka *et al*. 2006) or the reports were anecdotal (Chamberlain and Leopold 2001, Anderson and Lovallo 2003). By examining spatial genetic structure, this thesis investigated whether the bobcat conforms to predictions that sex-biased dispersal and philopatry occurs for this species.

Literature Review

Philopatry and Dispersal

Dispersal is the transference of an individual from its natal area to the area where it might reproduce (Howard 1960), and dispersing individuals are thought to move into the first unchallenged site near their natal home range (Murray 1967). This movement of individuals is both a behavioral process and a life history trait that affects populations and, ultimately, species, both ecologically and evolutionarily (Dieckmann *et al*. 1999). Dispersal, or lack thereof, can impact a population's dynamics (Dieckmann *et al*. 1999), spatial distribution (Howard 1960), social organization (Greenwood 1980, Waser and Jones 1983), and genetic structure (Chesser 1991a,b), as well as local adaptation and speciation (Slatkin 1987).

Dispersing animals commonly exhibit a sex bias in which one sex disperses, while the other is sedentary or philopatric (Greenwood 1980, Dobson 1982, Waser and Jones 1983). Various definitions of philopatry are present in the scientific literature. Philopatry can be defined as either the nondispersal of young from a natal home range (Waser and Jones 1983), or the short-range dispersal into adjacent areas (Greenwood 1980). Depending on the accepted definition, philopatry can be confounded by instances where offspring disperse into vacant, adjacent home ranges (Waser and Jones 1983). Some have even specified that, in order for a species to be considered philopatric, both sexes must display limited dispersal (Shields 1987). Moreover, individuals may be classified as philopatric, though not all will remain faithful to a site for their entire lifetime (Greenwood 1980, Waser and Jones 1983). These discrepancies are important to note, as they will affect the interpretation of results; however, it is not the purpose of this study to reconcile conflicting definitions or elucidate how one may distinguish between philopatry and "cheap rent" – that is, short-distance, less-risky dispersal into a familiar area.

Philopatry, as used here, occurs when an individual remains near or within its natal home range beyond the age of independence (Waser and Jones 1983, Ratnayeke *et al*. 2002).

Generally, sex-biased dispersal is presumed to relate to the mating system of the organism as well as the associated differential costs and benefits of dispersal and philopatry between the sexes (Greenwood 1980, Dobson 1982, Lieberg and von Schantz 1985, Shields 1987). Within mammals, the predominant mating system is polygyny, and typically males disperse, whereas females are philopatric (Greenwood 1980). Additionally, differences in dispersal distances occur between the sexes: males are expected to disperse further than females, and the differences in distance dispersed are attributed to polygyny as well (Greenwood 1980).

With polygyny, a male's reproductive success is more variable than that of a female's, and his reproductive fitness is limited by access to females and the number of matings he can acquire (Greenwood 1980, Sandell 1989). The greater variation in male reproductive output causes intrasexual competition to be more intense for this sex, resulting in a large portion of males denied access to females and greater male dispersal as they search for potential mates (Greenwood 1980, Dobson 1982). Females, on the other hand, are responsible for investing in the offspring and gain the most from being sedentary (Greenwood 1980). Additional investigations are necessary before it is known whether mating systems prove to be the best predictor of sex-biased dispersal (Greenwood 1980).

Aside from greater fitness through reproductive success afforded to dispersing males, several other potential benefits of dispersal exist. By dispersing, males provide their progeny with outbreeding enhancement (Shields 1987) and avoid any inbreeding depression (Shields 1987, Pusey and Wolf 1996). Males may also escape locally crowded situations that would otherwise have a direct impact on their individual fitness (Shields 1987). In addition, although sex-biased dispersal may not reduce competition, it does reduce competition with kin in terms of resources and breeding opportunities (Shields 1987), and therefore, has strong inclusive fitness consequences (Griffin and West 2002). However, dispersal is not free of costs. These costs include outbreeding depression (Howard 1960, Shields 1987), decreased fitness resulting from the risks and energy expenditure associated with dispersal (Waser and Jones 1983, Shields 1987), and decreased fitness of the relatives of dispersers, as they do not benefit from dispersing individuals' cooperation (Shields 1987).

While dispersal affords males potentially greater fitness through reproductive success, philopatry benefits females in several ways. As the sedentary sex, females acquire a familiarity with the site and its resources (Greenwood 1980, Shields 1987). Also, an individual's natal area is likely to have suitable habitat and available mates as opposed to the uncertainty of surrounding areas; by remaining within that natal area, an individual maximizes its reproductive success (Murray 1967). Because of the decreased risk and energy use associated with a sedentary lifestyle, females experience increased survival and fecundity (Shields 1987). Additionally, as sedentary individuals, females may experience some optimal level of inbreeding that maintains locally adapted gene complexes (Shields 1987). However, as with dispersal, there are potential costs associated with philopatry. Costs include inbreeding depression (Shields 1987) as well as crowding and competition, particularly with relatives (Shields 1987, Griffin and West 2002)

There are two corollaries associated with sex-biased philopatry: kin clustering and increased tolerance (Waser and Jones 1983). The clustering of related females in a polygynous species produces conditions in which cooperation and altruistic traits between relatives may evolve, resulting in increased direct and indirect fitness (Hamilton 1964, Greenwood 1980). Thus, increased tolerance between neighboring relatives may result and is most commonly noted as increased home range overlap between individuals of the sedentary sex (Waser and Jones 1983). However, this idea warrants further discussion in that home range overlap is known to occur between nonrelatives of the sedentary sex (Waser and Jones 1983). It is possible that home range occupants may not be able to detect and exclude all trespassers or that the individuals, although they occupy overlapping ranges, may not share the space temporally (Waser and Jones 1983). Alternatively, philopatry and limited dispersal will lead to groups comprised of close relatives, regardless of kin selection, and individuals do not necessarily derive benefits from being surrounded by kin (Greenwood 1980, Griffin and West 2002).

In the end, both sexes would derive benefits from philopatry and familiarity with an area (Greenwood 1980). The sex that disperses is the result of a conflict between the sexes, wherein the relative costs and benefits of dispersal and philopatry to the sexes determine the outcome (Greenwood 1980), and natural selection will favor philopatry when dispersal costs are high (Perrin and Mazalov 2000). Not surprisingly, departures from the general prediction of male sex-biased dispersal and female-biased philopatry can result. Philopatric tendencies can be temporarily male-biased, and in some cases, no sex bias exists (Greenwood 1980, Waser and Jones 1983). Females may prefer to disperse in polygynous species, such as when a male monopolizes a group of females (Greenwood 1980, Lieberg and von Schantz 1985). In this case, the average length of the male's tenure is long enough that his daughters will become sexually mature while he is still dominant (Greenwood 1980, Lieberg and von Schantz 1985).

Proximate mechanisms also affect natal dispersal and the decision to move, including population density and associated resource competition (Christian 1970, Crowe 1975, Aars and Ims 2000, Perrin and Mazalov 2000) as well as parental aggression toward juveniles and social subordination (Christian 1970). The influence of proximate mechanisms may negate any sex-biased dispersal tendencies. For example, male-biased dispersal is not anticipated when female reproduction is limited by resource competition (Aars and Ims 2000, Perrin and Mazalov 2000). Thus, dispersal decisions are not made for universal reasons and it is unlikely that dispersal evolved for any single reason (Shields 1987). Ultimate and proximate causes of dispersal and philopatry likely differ between species, populations, and individuals; rather than there being one reason for dispersal, there are often several interacting forces (Waser and Jones 1983, Waser 1985, Dobson and Jones 1985, Shields 1987).

Spatial genetic structure

Populations are frequently structured into groups of varying genetic similarity (Wright 1969, Sugg *et al.* 1996, Balloux and Lugon-Moulin 2002). Across space, this genetic structuring, or non-random distribution of alleles, can result from limited dispersal, selection, genetic drift, and/or population history and is referred to as spatial genetic structure (Vekemans and Hardy 2004, Alberto *et al.* 2005). Limited dispersal, or in this case, philopatry, can cause genetic structuring both within and between populations (Chesser 1991a,b).

Within a continuously distributed population, sex-biased dispersal and philopatry cause kin clustering, and as a result, genetic relatedness among males and among females is expected to differ. Specifically, relatedness among the philopatric sex is predicted to be higher overall than among the dispersing sex (Surridge *et al.* 1999), and the philopatric sex will have related individuals in close proximity (e.g., Ishibashi *et al.* 1997, Knight 1999, Surridge *et al.* 1999, Cutrera *et al.* 2005, Fredsted *et al.* 2005). Between-population genetic analysis can also reveal information regarding the patterns of sex-biased dispersal and spatial genetic structure, as the dispersing sex should be more genetically similar across populations than the philopatric sex (Goudet *et al.* 2002). Additionally, between-population analyses may reveal another form of genetic structure termed isolation by distance, in which limited gene dispersal causes neighboring populations to be more genetically similar than distant populations (Hardy and Vekemans 1999, Balloux and Lugon-Moulin 2002, Vekemans and Hardy 2004).

Ultimately, understanding the distribution of gene flow through dispersal and its influence on genetic variation both within and between populations is important in terms of conservation. Gene flow will homogenize the genetic variation between populations (Balloux and Lugon-Moulin 2002). Gene flow also has the ability to aid in restoring genetic variation to small, isolated populations (e.g., Westemeier *et al*. 1998, Ingvarsson 2001, Madsen *et al*. 2004, Pimm *et al*. 2006). However, with isolation or the presence of barriers, a lack of gene flow may accelerate local adaptation and speciation (Slatkin 1987), or it may contribute to the risk of extinction for small populations by means of genetic drift and inbreeding depression (e.g., Hendrick and Kalinowski 2000, Higgins and Lynch 2001, Frankham *et al*. 2004). Also, the distribution of gene flow and genetic variation among populations may allow for the recognition of movement corridors and, potentially, their subsequent preservation (Ernest *et al*. 2003). Within populations, an understanding of the complimentary nature of social behavior and genetics is necessary. If a species exhibiting sex-biased philopatry was examined genetically without regard to its social behavior, conservation plans may call for the mixing of individuals from many populations in order to boost this sex's genetic variation (Sugg *et al*. 1996). However, in doing so, the resulting evolutionary benefits from cooperative behavior would be reduced, potentially creating dire demographic consequences for the population (Sugg *et al*. 1996). Thus, supplementing genetic data with demographic data and vice versa provides for a stronger analysis of population structure (Fredsted *et al*. 2005).

Bobcat Ecology and Life History

Bobcats are the most widely distributed felid in North America (Anderson 1987). They occur throughout the United States (McCord and Cardoza 1982) and are only absent from one state - Delaware (Woolf and Hubert 1998). The occupancy of such a large area is evidence that this felid has adapted to a wide range of environmental conditions and habitats, including deserts, subtropical swamps, mountain ranges, and seasonal snow (McCord and Cardoza 1982, Anderson 1987, Anderson and Lovallo 2003). The bobcat's flexibility also extends to its diet, which includes lagomorphs, small rodents, deer, birds, carrion, and occasional insects and vegetation (McCord and Cardoza 1982, Anderson 1987, Anderson and Lovallo 2003).

Bobcats are solitary carnivores (Anderson 1987, Anderson and Lovallo 2003). Generally, individuals of solitary species are found alone and do not cooperate, except during mating and kitten rearing (Waser and Jones 1983, Sandell 1989). Although solitary animals, including bobcats, may interact infrequently with conspecifics, they reside in a complex social network, recognizing neighbors and adjusting their spatial distribution and habitat use accordingly (Waser and Jones 1983, Anderson and Lovallo 2003). For bobcats, social and spatial organization are patterned around home ranges (McCord and Cardoza 1982, Anderson 1987, Anderson and Lovallo 2003), which, as used here, are areas where an animal conducts its basic activities of mating, caring for young, and food gathering (Burt 1943).

Similar to other solitary felids, home range occupancy derives from a territorial system termed "land tenure" that is based on prior rights and mutual avoidance (Bailey 1974, 1981; Anderson and Lovallo 2003). The maintenance of the adult bobcat's territory and social structure is aided by scent marking with urine, feces, scrapes, and anal gland excretions (Bailey 1974, McCord and Cardoza 1982). Scent marking allows residents to advertise land occupancy, thereby keeping intruders out and maintaining boundaries (Bailey 1974). In this case, vacancy in land occupancy would be easily recognized by the disappearance of scent markings (Bailey 1974).

The possession of a home range is a necessity to bobcats. Home ranges promote familiarity with an area and its resources, allowing land-use partitioning, increased hunting success, and the preservation of energy (Conner *et al*. 1999, Nielson and Woolf 2001). Land tenure reduces intraspecific competition for resources, such as breeding areas for males and denning sites for females (Nielson and Woolf 2001). Occupancy of a home range also provides for greater reproductive success, particularly for females. In one study, only females that possessed a home range produced kittens (Knick 1990).

Bobcat home ranges vary in size depending on numerous factors, though prey availability, prey abundance, and habitat quality may be the most important determinants (Bailey 1974, Anderson 1987, Anderson and Lovallo 2003). Bobcat home range size was found to increase after a habitat transformation that left the area less productive for bobcat prey (Marshall and Jenkins 1966, Buie *et al*. 1979). Similarly, the habitat suitability index model for the bobcat notes the importance of particular vegetation cover in bobcat habitat and relates the cover's significance to its ability to support prey populations (Boyle and Fendley 1987).

Seasonal variation, in relation to climate, prey abundance and availability, and differing social pressures, can impact home range size (Anderson 1987, Anderson and Lovallo 2003). Males increase home range size during the winter breeding season to maximize reproductive opportunities, whereas females restrict their movements during denning in the winter and spring (Anderson 1987, Lovallo and Anderson 1996). Female home ranges may be especially small in the summer season as females use their home ranges more intensively to provide for their young (Bailey 1979, 1981; Conner *et al*. 1992).

Home ranges of male bobcats are larger than those of females (Anderson 1987, Anderson and Lovallo 2003). The differences in home range size between the sexes are attributed to the bobcat's polygynous mating system (Anderson 1987, Anderson and Lovallo 2003). The most important resource for a solitary female carnivore is nutriment, whereas for males, it is mating opportunities (Sandell 1989). As a result, female bobcat home range size is strongly influenced by prey availability (Anderson 1987, Anderson and Lovallo 2003). For males, the prevalence of mating opportunities, or the number of females within his range, influences home range size (Anderson 1987, Anderson and Lovallo 2003).

Inter- and intra-sexual overlap occurs between bobcat home ranges (McCord and Cardoza 1982, Anderson 1987). Male home ranges regularly overlap one or more females as well as ranges of other males (Bailey 1974; McCord and Cardoza 1982; Anderson 1987, 1988; Nielson and Woolf 2001). The polygynous mating system of bobcats and the importance of procurement of matings for males and resources for females is likely the reason for inter-sexual home range overlap (Sandell 1989).

Although females are generally expected to occupy exclusive home ranges, several studies have reported female intra-sexual home range overlap (e.g., Rucker *et al*. 1989, Conner *et al*. 1992, Chamberlain and Leopold 2001, Nielson and Woolf 2001). The overlap between female home ranges has been considered a breakdown in land tenure resulting from the distribution of resources (Bailey 1974, Fendley and Buie 1986, Sandell 1989), density of conspecifics (Fendley and Buie 1986), and/or climate (Bailey 1981). It has been suggested that female intra-sexual overlap is tolerated within the outlying portions of the home range

(Chamberlain and Leopold 2001, Neilson and Woolf 2001). Chamberlain and Leopold (2001) suggest that kinship may affect the degree of female home range overlap by allowing greater overlap when adjacent females are relatives. Home range overlap between related females may be a consequence of philopatry (see previous section).

In the absence of environmental disturbance or instability (Knick 1990), juveniles are the predominantly dispersing age group (Anderson and Lovallo 2003). Although knowledge of juvenile bobcat dispersal and movement is limited, a few general ideas can be gleaned from the literature. Juveniles tend to disperse from their natal ranges in the winter or spring in the year following their birth (Griffith *et al.* 1980, Kitchings and Story 1984, Griffith and Fendley 1986, Knick 1990), though the exact timing of dispersal is variable (Anderson and Lovallo 2003). Juvenile bobcat dispersal apparently relates to adult intolerance during the breeding season (Griffith *et al.* 1980, Kitchings and Story 1984, Griffith and Fendley 1986, Anderson 1987). As would be predicted from their polygynous mating system (Greenwood 1980), young males are known to disperse earlier (Bailey 1981) and farther than young females (Robinson and Grand 1958, Kitchings and Story 1984), and bobcats may exhibit a male-biased natal dispersal (Janečka *et al.* 2006). The juvenile population may fill vacant home ranges left open by death or resident removal (Crowe 1975, Griffith and Fendley 1986, Knick 1990), acquire part of their mother's home range (Anderson and Lovallo 2003), or disperse long distances (Knick and Bailey 1986).

Justification

Multiple justifications hold for this thesis. Broadly, bobcats possess both conservation and management importance. These felids may serve as apex predators in parts of their range, particularly in the forested ecosystems of the Southeast (Conner *et al.* 2000). Apex predators may regulate populations of medium-sized omnivores and increase the survival of shared prey items (Rogers and Caro 1998, Courchamp *et al*. 1999, Crooks and Soulé 1999). As such, bobcats may benefit economically important prey species, such as bobwhite quail (*Colinus virginianus*), though additional studies are needed (Doughty 2004). Equally significant, bobcats were listed under Appendix II of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) in 1975 (Anderson 1987, Woolf and Hubert 1998, Anderson and Lovallo 2003). Bobcats are maintained under CITES protection because of their similar appearance to other endangered felids and their potential to be overexploited without the regulation of trade (Anderson and Lovallo 2003). Thus, proper understanding of the bobcats' social and genetic structure has the potential to aid in management and conservation decisions for bobcats as well as other species that may benefit from the presence of this apex predator.

Specific to this study, the examination of the relationship between spatial distribution and relatedness will provide more information regarding the life history characteristics of bobcats. For instance, it can be determined whether sex-biased dispersal and philopatry is occurring in a species, or more accurately, a particular population. Data regarding dispersal and philopatry are difficult to obtain in the field both logistically and financially. By using genetic techniques as indirect methodology, researchers can begin to approach these questions more feasibly. Moreover, determining whether sex-biased dispersal occurs within bobcat populations will test Greenwood's (1980) hypothesis of sex-biased dispersal and philopatry based on predictions made from the species mating system. Lastly, other studies examining dispersal and philopatry characteristics in solitary felids generally involve larger-sized cats, unstable prey base, and/or metapopulations (e.g., Beier 1995, Poole 1997, Sweanor *et al*. 2000, Ferreras *et al*. 2004).

This study presented the opportunity to investigate an unexploited population of medium-sized, solitary felids that survive on a stable prey base and were not considered part of a metapopulation.

Objectives

1. Examine within-population spatial genetic structure and determine whether sex-biased dispersal and philopatry are taking place by testing if:
    a. adult females are more related to one another than males;
    b. adult female relatedness declines through space;
    c. adult female first-degree relatives settle closer to one another than unrelated adult females;
    d. adult female home range overlap is associated with individual relatedness.
2. Examine between-population genetic structure and determine if:
    a. populations are genetically different;
    b. populations fit the expected isolation by distance model;
    c. sex-biased dispersal is occurring and adult males are more genetically similar across populations than adult females.

Thesis Format

This thesis was written in manuscript format. Chapter 1 is an introductory chapter that summarizes the current scientific knowledge regarding sex-biased dispersal, philopatry, spatial genetic structure, and bobcat ecology. Chapters 2 and 3 are manuscripts that will be submitted for publication. Chapter 2 investigates within-population spatial genetic structure, sex-biased dispersal, and philopatry for a population of bobcats at the Joseph W. Jones Ecological Research Center in southwestern Georgia. Chapter 3 examines between-population spatial genetic structure, sex-biased dispersal, and philopatry among four bobcat populations from southern Georgia and northern Florida. Chapter 4 provides a succinct review of all findings and conclusions.

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CHAPTER 2

FINE-SCALE SPATIAL GENETIC STRUCTURE OF A BOBCAT POPULATION IN SOUTHWESTERN GEORGIA[1]

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[1] Reid, A.E., B.C. Faircloth, R.J. Warren, and L.M. Conner. To be submitted to *Molecular Ecology*

Abstract

Polygynous mammalian species typically exhibit male-biased dispersal and female-biased philopatry. Hypothesized dispersal patterns can be tested by examining the genetic similarity between individuals within the context of their spatial distribution. Sex-biased dispersal and philopatry may cause kin clustering of the sedentary sex, thereby affecting the spatial organization of genotypes and causing the philopatric sex to be more closely related across small spatial scales than the dispersing sex. The bobcat (*Lynx rufus*) is a solitary, polygynous species and is thought to evince male-biased dispersal and female-biased philopatry. We tested this hypothesis by determining genetic relatedness, obtained from six microsatellite loci, between individual bobcats (n = 62 individuals) and compared these relatedness values to individual spatial distribution gathered from capture locations or radio-telemetry. We found that females were not more related to one another than males, and females were no closer to relatives than would be expected by chance. Our results demonstrate that dispersal patterns cannot be predicted from a species' mating system alone, rather, proximate factors, such as habitat quality and individual density, should be considered as well as a landscape-level perspective for wider-roaming species.

Key words: bobcat, *Lynx rufus*, microsatellites, relatedness, kinship, spatial genetic structure

Introduction

For many species, dispersal commonly exhibits a sex bias, in which one sex disperses, while the other is sedentary or philopatric (Greenwood 1980, Dobson 1982, Waser and Jones 1983). Various definitions of philopatry are present in the scientific literature. Philopatry can be described as either nondispersal of young from a natal home range (Waser and Jones 1983), or their short-range dispersal into adjacent areas (Greenwood 1980). Depending on the accepted definition, philopatry may be confounded by instances where offspring disperse into vacant, adjacent home ranges. Moreover, individuals may be classified as philopatric, but not all will remain faithful to a site for an entire lifetime (Greenwood 1980, Waser and Jones 1983). For instance, philopatry may occur in individuals past weaning, although not beyond sexual maturity (Waser and Jones 1983). Philopatry, as used here, occurs when an individual remains near or within its natal home range past the age of independence (Waser and Jones 1983, Ratnayeke *et al*. 2002).

Ultimately, sex-biased dispersal relates to the mating system as well as the associated competition for mates and the differential costs and benefits of dispersal and philopatry between the sexes (Greenwood 1980, Dobson 1982, Lieberg and von Schantz 1985, Shields 1987). Mammals are predominantly polygynous, and with this mating system, males generally disperse, whereas females are philopatric (Greenwood 1980). In polygyny, a male's reproductive success is more variable than that of a female's, and his reproductive fitness is limited by his access to females and the number of matings he can acquire (Greenwood 1980, Sandell 1989). Variation in male reproductive output causes intra-sexual competition to be more intense for this sex, resulting in more males being denied access to females and greater dispersal as they search for potential mates (Greenwood 1980, Dobson 1982). Females, however, are typically responsible for investing in the offspring and derive the greatest benefits from being sedentary (Greenwood 1980).

By remaining near or within their natal home ranges, philopatric individuals are settling in proximity to related individuals, and this clustering of relatives may produce conditions in which cooperation and altruistic traits between relatives evolves (Hamilton 1964), including tolerance (Waser and Jones 1983). Tolerance between individuals of the sedentary sex is commonly noted as increased home range overlap (Waser and Jones 1983). However, home range overlap is known to occur between nonrelatives of the sedentary sex as well (Waser and Jones 1983). Alternatively, philopatry and limited dispersal may lead to groups comprised of close relatives, and these individuals may not necessarily derive fitness benefits by being surrounded by kin (Greenwood 1980, Griffin and West 2002).

Hypothesized dispersal patterns can be tested by examining the genetic similarity between individuals within the context of their spatial distribution (Peakall *et al*. 2003). Within a continuously distributed population, sex-biased dispersal and philopatry cause kin clustering of the sedentary sex, resulting in a nonrandom spatial distribution of genotypes (i.e., spatial genetic structure) (e.g., Ishibashi *et al*. 1997, Knight *et al.* 1999, Surridge *et al*. 1999, Cutrera *et al*. 2005). As a result of restricted dispersal and philopatry, the spatial genetic structure of the philopatric sex and the dispersing sex are expected to differ (Peakall *et al*. 2003, Fredsted *et al*. 2005). Additionally, the overall genetic relatedness among males and among females should differ as a result of sex-biased dispersal: relatedness within the philopatric sex is predicted to be higher than relatedness within the dispersing sex (Surridge *et al*. 1999).

In examining the relationship between spatial distance and genetic relatedness in mammalian systems, species with well-defined social systems have received the most attention, likely in the interest of studying altruism, cooperation, and group-living (Waser and Jones 1983). Philopatry is attachment to a site, not an individual or a group of conspecifics, and, as such, gregariousness, or group-living, is not a requisite (Waser and Jones 1983). Recent studies have examined the spatial genetic structure of species with less-defined social systems, for example, Soay sheep (*Ovis aries*) (Coltman *et al.* 2003), talar tuco-tucos (*Ctenomys talarum*) (Cutrera *et al.* 2005), and raccoons (*Procyon lotor*) (Ratnayeke *et al.* 2002). Additionally, at the time of this writing, we were aware of only one study examining this relationship in a population of Texas bobcats (Janečka *et al.* 2006), a species thought to exhibit philopatry and sex-biased dispersal.

The bobcat, a solitary, polygynous species, is the most widely dispersed native felid in North America (Anderson 1987, Anderson and Lovallo 2003). For bobcats, social and spatial organization are thought to be patterned around home ranges (Anderson and Lovallo 2003). We define home ranges as areas in which an animal conducts basic activities of mating, caring for young, and food gathering (Burt 1943). Similar to other solitary felids, home range occupancy derives from a territorial system termed "land tenure" that is based upon prior rights and mutual avoidance (Bailey 1974, 1981; Anderson and Lovallo 2003). Home range size varies for numerous reasons, including prey availability, seasonal variations, and sex of the individual. Males tend to occupy larger home ranges than females (Anderson 1987, Anderson and Lovallo 2003). Bobcat home ranges are smaller in areas of high prey availability and abundance (Fendley and Buie 1986, Knick 1990). Bobcats are also known to vary the size of their home ranges seasonally, in relation to climate, prey abundance, and social pressures (Anderson 1987, Lovallo and Anderson 1996, Anderson and Lovallo 2003).

Inter- and intra-sexual overlap occurs between bobcat home ranges (McCord and Cardoza 1982, Anderson 1987). Male home ranges regularly overlap the ranges of one or more females as well as the ranges of other males (Bailey 1974; McCord and Cardoza 1982; Anderson 1987, 1988; Nielson and Woolf 2001). Although females are generally expected to occupy exclusive home ranges, several studies have reported female intra-sexual home range overlap (e.g., Rucker *et al.* 1989, Conner *et al.* 1992, Chamberlain and Leopold 2001, Nielson and Woolf 2001). The overlap between female home ranges has been considered a breakdown in land tenure resulting from the distribution of resources (Bailey 1974, Fendley and Buie 1986, Sandell 1989), density of conspecifics (Fendley and Buie 1986), and/or climate (Bailey 1981). Chamberlain and Leopold (2001) suggest that kinship may affect female overlap by allowing greater overlap when adjacent females are relatives, and this would be expected if females were exhibiting philopatric behavior (Waser and Jones 1983).

In the absence of environmental disturbance or instability (Knick 1990), juveniles are the predominantly dispersing age group (Anderson and Lovallo 2003). Although knowledge of juvenile bobcat dispersal and movement is limited, some general information is available in the literature. Though the exact timing of dispersal is variable (Anderson and Lovallo 2003), juveniles tend to disperse from their natal ranges in the winter or spring of the year following their birth (Griffith *et al.* 1980, Kitchings and Story 1984, Griffith and Fendley 1986, Knick 1990). Juvenile bobcat dispersal is thought to occur as a result of adult intolerance during the breeding season (Griffith *et al.* 1980, Kitchings and Story 1984, Griffith and Fendley 1986, Anderson 1987). The juvenile population may fill empty home ranges left vacant by death or the removal of a resident (Crowe 1975a, Griffith and Fendley 1986, Knick 1990), acquire part of their mother's home range (Anderson and Lovallo 2003), or disperse long distances (Knick and Bailey 1986). As would be predicted from their polygynous mating system (Greenwood 1980), young males are known to disperse earlier (Bailey 1981) and farther than young females

(Robinson and Grand 1958, Kitchings and Story 1984), and bobcats may exhibit male-biased dispersal and female-biased philopatry (Chamberlain and Leopold 2001, Anderson and Lovallo 2003, Janečka *et al* 2006).

Based on knowledge of the bobcat's mating system and past research, *a priori* predictions of their dispersal and philopatric patterns can be made. Although some studies suggest that philopatry may occur in this species, either the sample size was small (Janečka *et al.* 2006) or reports appear anecdotal (Chamberlain and Leopold 2001, Anderson and Lovallo 2003). Determining pedigrees or dispersal patterns using existing field-based techniques presents difficulties when studying wide-ranging animals in natural populations (Hughes 1998, Mossman and Waser 1999, Van De Casteele *et al*. 2001). Resultantly, we chose to use a genetics-based approach to analyze dispersal, philopatry, and relatedness in a population of bobcats.

By examining fine-scale, spatial genetic structure using six, polymorphic microsatellite loci and radio-telemetry data, we investigated whether bobcats conform to predictions of philopatry and sex-biased dispersal based on their breeding system. If philopatry was occurring, we predicted that overall, adult females would be more related to each other than adult males were to one another. In addition, we expected that individual females living in close proximity would be more related than those living farther apart, and thereby, result in a negative association between relatedness and spatial distance. Our predictions applied only to the adult population because the source population of the juveniles examined was unknown. When conducting sex-biased dispersal tests, juveniles need to be excluded because pre-dispersal juveniles will not exhibit genetic differences between the sexes (Goudet *et al*. 2002). However, juveniles were included in this study's analyses for comparative purposes only.

Methods

Study Area

Ichauway, formerly a hunting plantation, serves as a privately owned, 11,735 ha outdoor research facility for the Joseph W. Jones Ecological Research Center (collectively, the Jones Center). The Jones Center is located in Baker County, Georgia, 16 km south of Newton, Georgia. This site is located in the Dougherty Plain physiographic province of the southeastern Gulf Coastal Plain. Elevations of 27 to 61 m are characteristic of the area (Boring 2001). The Flint River travels along 22 km of Ichauway's eastern boundary, and approximately 24 km of the Ichawaynochaway Creek flows through the study area (Boring 2001). The climate of the area varies from hot, humid summers to short, mild, wet winters. Average daily temperatures span 11.1°C in the winter to 27.2°C in the summer, and average annual precipitation is 132 cm (Boring 2001).

The Jones Center includes an assemblage of habitats with a majority of acreage in longleaf pine woodlands and limesink wetlands. Oldfield grasses (e.g., *Andropogon* spp.) and wiregrass dominate the understory (Goebel *et al*. 1997). Overall, the plant taxa are highly diverse; more than 1,000 vascular plant species have been cataloged on site (Drew *et al*. 1998).

Prescribed fire is the primary habitat management tool used on the Jones Center, and burning occurs on 4,000 to 6,000 ha throughout the entire site on a two-year rotation, usually during winter and early spring (Godbois *et al*. 2004). Among its many functions, prescribed fires open the canopy and promote an herbaceous understory (Boring 2001). Additional management practices are utilized on site, including supplemental feeding of bobwhite quail (*Colinus virginianus*), the disking of fields to improve food availability, and limited predator removal following quail hunting season (March-May). Removed predators include: raccoons (*Proyon* *lotor*), opossums (*Didelphis virginianus*), striped skunk (*Mephitis mephitis*), coyote (*Canis latrans*), red fox (*Vulpes vulpes*), and gray fox (*Urocyon cinereoargenteus*). Bobcats were harvested prior to 1999, after which harvesting ceased. Limited bobcat harvest resumed in January 2005 as part of a concomitant bobcat study (Lynch 2005).

Bobcat Capture and Telemetry

From December 2000 through March 2005, we trapped bobcats using baited #3 Victor Soft Catch traps (Woodstream Corp., Lititz, PA) and baited #1.75 Oneida Victor coil-spring traps (Victor Inc., Ltd., Cleveland, OH.). Once netted and secured, captured bobcats received an intramuscular injection of ketamine hydrochloride (10 mg/kg body weight). We recorded sex, weight, total body length, hind foot length, ear length, and tail length. Individual bobcats were classified as either adult or juvenile based on secondary sex characteristics, length, and weight (Crowe 1975b). All captured adults received a 180 g VHF radio-collar (Advanced Telemetry Systems, Isanti, MN), and adults and juveniles obtained a uniquely numbered ear tattoo. Starting in November 2003, 3 mm ear punches were taken from both ears of each captured bobcat using a sterile, disposable, dermal biopsy punch (Anthony Products, Inc., Indianapolis, IN). Although trapping occurred throughout the site, areas with radio-collared bobcats captured prior to November 2003 were targeted to obtain a tissue sample from previously captured and radio-collared individuals. Bobcats were held for approximately 24 hours before being released to the capture site, ensuring a full recovery from the anesthetic. All trapping and handling procedures were approved by the University of Georgia Institutional Animal Care and Use Committee (IACUC #A990159).

With radio-telemetry, we monitored radio-collared bobcats beginning two to seven days following their release. We obtained telemetry locations via triangulation, taking ≥ 2 azimuthal locations from GPS-referenced locations using a 3-element Yagi antenna (Sirtrack, New Zealand) and a hand-held receiver (Wildlife Materials Inc., Carbondale, IL.). In an effort to minimize error, no more than 15 minutes were allowed between gathering consecutive bearings (White and Garrott 1990).

Telemetry data collection was structured around calendar-based seasons, all four of which were characterized by differing environmental and social conditions (e.g., Gehrt and Fritzell 1998, Ferreras *et al*. 2004), though the exact timing of social events varies with latitude (Anderson and Lovallo 2003). Generally, winter (December - March) is characterized by matings and pregnancy of females, spring (March - June) with birth and lactation, summer (June - September) with dependence of kittens, and fall (September - December) with the beginning of kitten independence (Bailey 1979).

Analyses of Spatial Distribution

We used the FORTRAN program EPOLY (L.M. Conner, personal communication) to convert radio-telemetry locations into Universal Transverse Mercator (UTM) coordinates. From these coordinates, bivariate median centers were calculated for all tissue-sampled bobcats both by season and by year. Bivariate median center calculations were calculated in Fidelity (L.M. Conner, personal communication). For seasonal calculations, all telemetry points were used. If the bobcat was a juvenile and did not receive a collar, we used the coordinates of the nearest known, GPS-referenced telemetry station as the coordinates of the capture location. Yearly centers were only calculated for individuals with $\geq$ 30 telemetry points per season (a complete seasonal home range), for at least two seasons that year, using only the seasons that met this specification. Years were defined from winter of one year through fall of the following year, based on calendar seasons.

Because of a general lack of travel impediments imposed by topography or environmental characteristics, Euclidean distances were used to calculate distances between bivarate median centers or capture locations. Euclidean distances were calculated using the R-package (Legendre and Vaudor 1991) and SPAGeDi 1.2 (Hardy and Vekemans 2002).

Ninety-five percent minimum convex polygon home ranges were calculated in CALHOME (Kie *et al*. 1996) and used in the analysis of home range overlap. Minimum convex polygon (MCP) home ranges are established using the boundary of telemetry points, and, as a result, the distribution of locations is ignored and the home range may encompass areas not actually utilized by the animal (Harris *et al*. 1990). Because of the disadvantages associated with MCP home ranges, we calculated the index of overlap, which accounts for the occurrence of each bobcat in the overlap area. The index of overlap was calculated as follows: product of the % area overlap and the % point overlap for all pairs overlapping bobcats which had been sampled for tissue (Cochrane 2003). Indices of overlap were only calculated for adult bobcats with complete seasonal home ranges.

DNA Analysis and Population Genetics

For genetic analysis, we collected 3 mm tissue punches from both ears of all captured bobcats beginning in November 2003 (n = 64). Prior to punching, we shaved bobcat ears to reduce the amount of hair on the sample, cleansed the ears with alcohol pads, and later treated the ears with a topical antibiotic after tissue acquisition. Following collection, all tissue samples were stored at -20°C. We extracted DNA from the tissue samples using Qiagen DNEasy DNA isolation kits (Qiagen, Inc. Valencia, CA). To aid in tissue digestion of the remaining hair, we added DTT (1,4-Dithio-DL-threitol) (100 mg/mL) along with Proteinase K, according to manufacturer instructions, to each sample and digested the tissue overnight. After extraction, we quantified DNA concentration using 10 DNA samples and PicoGreen® dsDNA Quantitation Reagent (Molecular Probes, Inc., Eugene, Oregon).

We tested eight polymorphic microsatellite loci for the bobcat, identified and designed by Faircloth *et al*. (2005). Locus bcb12d was omitted during the study because it produced inconsistent genotypes. Another, bch6t, was dropped from analysis because of high error rates (see below). Polymerase chain reactions (PCRs) for all loci were executed using 10 μl volumes. Final concentrations for all reagents were as follows: 0.25 U Jumpstart Taq DNA polymerase, 1X Reaction Buffer, 1.5 mM MgCl2, 0.5 mM dntps, 1 mM 'pigtailed' primer (Brownstein *et al*. 1996), 0.1 mM CAG or M13 reverse tagged primer, 0.9 mM dye labeled tag, and 56 ng DNA. We used three touchdown thermal cycling programs (Don *et al*. 1991) with annealing temperatures spanning 7.5°C (50-42.5°C, 55-47.5°C, 60-52.5°C) (Faircloth *et al*. 2005). Cycling conditions were as follows: 94°C for 1 min, 15 cycles at 95°C for 20 s, highest annealing temperature for 20 s followed by a 0.5°C decrease in temperature for each consecutive cycle, 72 °C extension for 1 min, 15 cycles at 95°C for 20 s, 42.5°C, 47.5°C, 52.5°C, respectively, annealing for 20 s, 72°C extension for 1 min, finishing with a 10 min extension at 72°C. We loaded PCR product on a 5.6% polyacrylamide gel and scored using an ABI 377-96 sequencer with GENESCAN Rox500 florescent size standard (Applied Biosystems, Inc., Foster City, CA) that was run with every sample. We ran positive and negative controls during amplification and scoring.

We analyzed our results using GENESCAN and GENEMAPPER software (Applied Biosystems, Inc., Foster City, CA). Calculations of allele frequencies were done in Cervus version 2.0 (Marshall *et al*. 1998), and analysis of linkage disequilibrium and Hardy-Weinberg equilibrium were done using Genepop 3.4 (Raymond and Rousset 1995). The Hardy-Weinberg exact test was calculated using either the Markov chain method with default values (dememorization = 10,000, batches = 100, iterations = 5,000) or complete enumeration, where possible. A global test, using Fisher's method, for population-wide Hardy-Weinberg equilibrium was also conducted. We preformed *a posteriori* sequential Bonferroni corrections (Rice 1989) for locus-specific linkage disequilibrium and Hardy-Weinberg equilibrium estimates.

The Wildlife and Fisheries Research and Education Facility at Mississippi State University provided us with known-cross bobcat tissue samples to verify Mendelian inheritance of our microsatellite loci.

Relatedness and Kinship Estimates

We calculated relatedness estimates using Relatedness 5.0 (Queller and Goodnight 1989). The program's calculation uses a regression-based approach to estimate relatedness, r_{xy} (Queller and Goodnight 1989). That is, r_{xy} is the expected fraction of alleles that are identical by descent and shared between two individuals (Blouin 2003). Using this equation, relatedness values can range from -1.0 to +1.0 (Ralls *et al*. 2001). A positive r_{xy} value indicates that the pair, or group, of individuals share more alleles identical by descent than would be expected by chance, and a negative value indicates that the pair of individuals share fewer alleles than would be expected (Surridge *et al*. 1999, Ralls *et al*. 2001). First-degree relatives are expected to have a pairwise relatedness value of 0.5, however, the estimated values will be highly variable (Queller and Goodnight 1989, Ralls *et al*. 2001).

With six microsatellite loci, we estimated pairwise relatedness for all bobcat pairs and group relatedness for all females (including juveniles), all males (including juveniles), adult females, and adult males. As used in the analyses of this paper, an adult bobcat has a complete seasonal home range of $\geq$ 30 telemetry points (a complete home range), and the 'all' category includes juveniles, adults with incomplete home ranges, and adults with complete home ranges. The adult bobcats used for the group relatedness analysis had at least one complete seasonal home range during the course of the study.

Because bobcats were sampled throughout the study site, sampling across multiple groups of potential relatives, a bias correction was not done for pairwise or group estimates, and individuals were weighted equally (Queller and Goodnight 1989). To estimate confidence intervals around group relatedness estimates, we used the jackknife procedure across loci (Queller and Goodnight 1989).

We used Kinship 1.3.1 (Goodnight *et al*. 1994, Goodnight and Queller 1999) to determine the likelihood that a pair of individual genotypes fit either a hypothesized primary relationship or null relationship. The simulation procedure within the program was used to calculate the likelihood ratios necessary to reject the null hypothesis as well as calculate the Type II error rate (Goodnight *et al*. 1994, Goodnight and Queller 1999). Using 20,000 simulations, we tested our data for first-degree relationships (parent-offspring, full sibling). Other relatedness categories, such as second-degree relatedness (half sibling, avuncular, etc.) were tested, but not included in the analysis because the Type II error rate for these relationships was high.

Spatial Genetic Structure

We calculated the average distance between individuals based on their relationship category, as calculated in Kinship, of either a first-degree relationship or not related (i.e., not a first-degree relationship). We did not do this analysis on a seasonal basis because the sample size of first-degree relatives by season was small; instead, we choose to do an overall distance analysis that would reveal general patterns. For pairs of individuals that occurred together in more than one season, we used the average spatial distance between the two individuals across seasons. If the pair, however, was temporally together only once, then that one distance was used. To account for the discrepancy in the number of seasons a pair may have appeared together, we calculated a weighted average, rather than the arithmetic average. We determined the weighted average distance between first-degree relatives as well as the weighted average distance between unrelated individuals and compared the distances using confidence intervals. Various groupings were analyzed: all bobcats (including juveniles), all females, all males, all adults, adult females, and adult males. The adult category included only individuals with at least one complete seasonal home range and only used data from seasons with complete home range data.

Under restricted dispersal, genetic and spatial data are auto-correlated (Sokal and Wartenberg 1983, Barbujani 1987, Hardy and Vekemans 1999, Smouse and Peakall 1999, Diniz-Filho and Telles 2002, Peakall *et al.* 2003, Vekemans and Hardy 2004), and as such, we used two spatial autocorrelation statistics to examine the relationship between spatial and genetic data, the Mantel test (Mantel 1967) and Moran's I statistic and its associated correlogram. We used the program 'zt' (Bonnet and Van de Peer 2002) to perform the Mantel tests. This program calculates the Pearson correlation coefficient (Bonnet and Van de Peer 2002), here referred to as r_m. The significance of each correlation was obtained through 10,000 permutations. Yearly analyses were done for all adults, adult females, and adult males. Seasonal tests were conducted for all bobcats, all females, all males, all adults, adult females, and adult males. Because we predicted that adult females would be more related at short distances with relatedness declining through space (a negative correlation), tests were one-tailed when the relationship between spatial distance and relatedness was examined for adult females. However, tests for other groupings were two-tailed because no predictions were made.

Using SPAGeDi, Moran's *I* statistic for multilocus genotypes was calculated for distance classes using Euclidean distances based on bivariate median centers. With a typical range of -1 to +1, a significant positive value for Moran's *I* indicates genetic similarity within a particular distance class, while a negative number represents genetic dissimilarity (Barbujani 1987, Diniz-Filho and Telles 2002). These values are then examined across distance classes, resulting in a correlogram (Hardy and Vekemans 1999, Diniz-Filho and Telles 2002). Because equalizing the sample size between distance intervals resulted in distance intervals that were too large for our analysis (data not presented), we used 11 distance classes in our analysis, 10 distance intervals 1,000 m each, from 0 to 10,000 m and an additional distance interval for all distances greater than 10,000 m.

Confidence intervals of 95% were calculated for Moran's *I* statistic in each distance interval. Values outside the 95% confidence interval indicate significant spatial genetic structure, while values within the confidence interval do not differ from a random distribution. Yearly analyses were done for all adults, adult females, and adult males. Seasonal tests were conducted for all bobcats, all females, all males, all adults, adult females, and adult males. If females were exhibiting philopatry, we expected adult females to have a positive spatial autocorrelation in short distance classes with spatial autocorrelation declining across space, as described by Sokal and Waretenberg (1983), Smouse and Peakall (1999), Diniz-Filho and Telles (2002), Peakall *et al.* (2003), Berthier et al. (2005), and Fredsted *et al.* (2005). No predictions for other groupings were made.

Lastly, we examined the relationship between pairwise home range overlap indices and pairwise relatedness with the Spearman rank correlation coefficient, r_s (Ratenayeke *et al.* 2002, Comer *et al.* 2005). We calculated Spearman correlation coefficients by season for all adults, adult females, and adult males. Spearman coefficients were obtained only for seasons with ≥ 5 instances of pairwise overlap for the particular category under consideration because smaller sample sizes would be greatly affected by extreme values. In addition, we calculated a Spearman coefficient for average overlap indices and pairwise relatedness values across all seasons for all adults, adult males, and adult females. If a pair occurred together more than one season, we used an average of the overlap indices between that pair. If overlap indices increased with increasing pairwise relatedness values, a positive Spearman correlation coefficient would result. The significance of Spearman correlation coefficients was determined by using a one-tailed test for analyses with only adult females or two-tailed tests for other groupings. Juveniles were not considered in any of the Spearman coefficient analyses because they did not have associated telemetry data.

Error Estimation

We calculated our genotyping error rate twice, the first time as an attempt to determine where problems may be occurring, and the second time as a final estimate after correcting any problems noted with the first estimate. We used Python 2.3 (www.python.org/2.3.1) to randomly choose 15% of our error samples for both estimates out of all possible samples. Samples used for our error estimates included bobcat samples from the Jones Center and samples from other locations in the Southeast (see Chapter 3).

We ran error samples blindly through the PCR and analysis process. Aside from being blind samples, error samples were treated as if they were regular, known samples in an attempt to be consistent. As such, if error samples produced low peak heights, split peaks, or were nonamplifying, these samples were run one additional time, and this "reliable" genotype was used in our error estimates. These first attempts were not errors *per se*, as they were not mismatching genotypes, just low-quality genotypes, and the error caused by nonamplification or split peaks is not likely to be represented in our data as these issues were monitored and corrected. The errors we were interested in testing were the errors that may not be noticed or controlled as easily without a blind run, such as false alleles, false homozygotes/heterozygotes, or binning problems.

Results

Population Genetics

Based on the known cross samples, Mendelian inheritance at all loci was confirmed. Two samples from the Jones Center (R14 and R24) were eliminated from the all analyses because they either did not amplify during PCR or gave inconsistent genotypes. All other samples (n = 62) amplified at all loci. All six loci were polymorphic and the number of alleles per locus ranged from three to eight, with a mean expected heterozygosity of 0.694 (Table 2.1). No significant linkage disequilibrium was found between loci following a sequential Bonferroni correction. Only one locus, bcg8t, significantly differed from the Hardy-Weinberg equilibrium ($P < 0.01$) following a sequential Bonferroni correction, possibly as a result of null alleles or allelic dropout. The global test in Genepop revealed that this population did not differ significantly from Hardy-Weinberg equilibrium ($P = 0.0528$). A global test was run a second time without bcg8t to determine whether this locus was causing the small p-value, and the resulting value from this second test was $P = 0.856$.

Relatedness and Kinship Estimates

Pairwise relatedness values (n = 1,891) ranged from -0.620 to 0.844. Because averaging pairwise relatedness values for a particular grouping is not entirely accurate as it averages across ratios, rather than taking the average of a ratio as Relatedness 5.0 does, group relatedness values were determined separately (Fig. 2.1). Adult females were not more related as a group than the adult males. The relatedness of adult females did not differ from zero, whereas adult males did differ from zero. When all females were compared to all males, the two groups did not differ from one another, nor do they differ from zero.

Using Kinship, we assigned bobcats one of two categories: first-degree relationship (parent-offspring, full sibling) category or unrelated category. We only tested for a first-degree relationship because our Type II error rate was high (Table 2.2). Out of 1,891 pairs of bobcats, 148 were designated as first-degree relatives.

Spatial Genetic Structure

The weighted average distances separating pairs of first-degree relatives and unrelated pairs were compared within various groups (Table 2.3). A total of 99 out of 148 pairs of first-degree relatives occurred together in the same season, and these 99 pairs were utilized in our weighted-average distance calculations. The distance between unrelated bobcats compared to first-degree relatives did not differ, and the absence of distance differences was true for the groups analyzed: all bobcats, all females, all males, all adults, adult females, and adult males.

Seasonal and yearly Mantel tests were conducted for various age and sex groups, totaling 83 different Mantel tests (Table 2.4). The correlation for all bobcats was significantly negative for one out of 15 seasons. The ‘all’ females category exhibited a significant negative correlation for two out of 10 seasons. The correlation for adult females was significantly negative for only three out of 15 seasons, and adult females exhibited a negative correlation for a total of eight out of 15 seasons. In three out of the four years examined, adult females displayed a negative spatial-genetic relationship, though none of these relationships was significant. All of the female groupings examined had negative median correlations. The ‘all’ males category had a negative median correlation and no Mantel tests were significant. Adult males exhibited a positive spatial-genetic relationship for eight out of 11 seasons and for all three years; however, none of these relationships was significant.

Moran's *I* statistic and correlogram results for all bobcats, all males, all females, all adults, adult males, and adult females combined were similar to those obtained from the Mantel tests. Eighty-six correlograms were examined, but only six had values within the first distance class that fell outside of the 95% confidence interval (Fig 2.2). Although these six correlograms displayed significant Moran's *I* statistics in short distance intervals, the correlograms varied as to what seasons and groupings displayed this pattern as well as which direction the Moran's *I* statistic was significant. Interestingly, five out of the six correlograms were categories containing juveniles. All other correlograms lacked the positive spatial autocorrelation expected under restricted dispersal described by Sokal and Waretenberg (1983), Smouse and Peakall (1999), Diniz-Filho and Telles (2002), Peakall *et al*. (2003), Berthier et al. (2005), and Fredsted *et al*. (2005) and, instead, were similar to those described by Diniz-Filho and Telles (2002) exhibiting no spatial genetic structure.

Home Range Overlap

We calculated a total of 18 Spearman coefficients (Table 2.5), and only one produced a significant result – adult females in spring 2004. In general, the direction of the relationship between overlap and relatedness varied for adult bobcats, adult females, and adult males. However, when overlap indices were averaged across seasons and analyzed using Spearman correlations, adult females fit their expected patterns, whereas males exhibited a negative correlation, though neither of these correlations was significant.

Error estimates

For the first error estimate**,** seven loci were checked for error (Table 2.6). An allele never noticed at the bch6t locus appeared in seven out of 16 error samples. We felt this allele was likely a false allele, since one sample contained this allele in addition to its heterozygote genotype. To be sure, a second run was done with these same samples. Only one sample of those seven still produced this allele during the second run, and as such, we felt confident that it was a false allele. The samples from the second run of bch6t were used for the first error calculation.

A total of 127 genotypes was checked for errors, 18 of which produced genotype mismatches. Only one of these errors appeared to result directly from running the incorrect sample, and the cause of the one instance of error at bc1at remains unclear. The remainder of the errors all resulted from false homozygotes (n = 8 genotypes) (Fig. 2.3), false alleles (n = 1 genotype), and binning problems that resulted in incorrect allele sizing (n = 7). Three loci (bch6t, bcd1t, and bcg3d) had relatively high error rates as compared to other loci, and all errors, aside from the incorrect sample being run, were represented with these loci. Bins were adjusted for bcd1t and bcg3d; however, bch6t was subsequently disregarded. Not only did bch6t have the complication of a false allele, the distribution of bch6t's alleles was nearly continuous, and as such, it was difficult to adjust bins accordingly and to accurately size alleles.

For the second error estimate, only bcd1t and bcg3d were run, because only these two loci had bins that were adjusted and corrected in GENEMAPPER. The error rate was lowered, with only one genotype mismatch at bcg3d out of the 36 genotypes total checked for error (Table 2.6). This one error resulted from the error sample being incorrectly scored as a homozygote.

Thus for the final error rate, the uncorrected loci from the first estimate and the corrected loci from the second estimate were combined, and four out of 109 genotypes did not match.

Discussion

Philopatry, Dispersal, and Spatial Genetic Structure

Overall, we found little evidence of sex-biased dispersal occurring within this population. As a group, adult females were not more related to one another than the adult male group was to one another. Additionally, adult, first-degree relatives were not significantly closer than adult, unrelated individuals. Using Mantel tests, adult females had a negative correlation between pairwise spatial distance and genetic relatedness, though this pattern did not have consistent statistical significance across seasonal and yearly data. Adult males tended to have a more positive correlative relationship, though the results were not significant.

Results obtained from correlograms were similar to those from Mantel tests. Adult female correlograms lacked the predicted positive spatial autocorrrelation described by Sokal and Waretenberg (1983), Smouse and Peakall (1999), Diniz-Filho and Telles (2002), Peakall *et al*. (2003), Berthier et al. (2005), and Fredsted *et al*. (2005), rather the correlograms resembled those without spatial genetic structure (Diniz-Filho and Telles 2002). Six correlograms exhibited spatial autocorrelation in short-distance classes, five of which had positive spatial autocorrelation. Interestingly, all positive, short-distance autocorrelation resulted in correlograms that included juveniles. These juveniles were likely the offspring of the sampled adults and had yet to disperse. The winter 2004 and winter 2005 seasons contained the greatest proportions of juveniles, so it is not surprising that these seasons had significant correlograms, as well as two significant Mantel tests examining the all female category.

Finally, when overlap indices were compared to pairwise relatedness values, we expected females to have a positive relationship. Conversely, statistically significant patterns were not observed other than one instance for females in spring 2004. Though there was a lack of statistical significance, this relationship between home range overlap and relatedness exhibited a positive trend, particularly for all adults and adult females, whereas adult males exhibited a negative relationship. Home range overlap may be indicative of tolerance between individuals (Waser and Jones 1983), and as such, as relatedness increases, overlap is expected to increase (Chamberlain and Leopold 2001). However, sample sizes were small for these analyses, and one or two pairs of females with high relatedness values may have skewed the data. Furthermore, instances of overlap occurred between individuals that had lower/negative relatedness values. Thus, for our study population, home range overlap may not have resulted from increased tolerance; rather individuals may not have been able to exclude all intruders from their home range (Waser and Jones 1983). Also, overlap between individuals can be affected by resource distribution, whether that resource is limiting, fluctuating, (Bailey 1974) or in excess (Pierce *et al*. 2000). Further analyses with larger sample sizes should be completed to better consider the relationship between relatedness, overlap, and habitat quality.

The lack of spatial genetic patterning can eventuate for numerous reasons; in particular, our methods may not have been powerful enough to detect instances of philopatry. The occurrence of sex-specific philopatry and sex-biased dispersal may have been too limited to generate female spatial genetic structure. In particular, one pair of females possessing a first-degree relationship was, based on bivariate median centers, within 250 m of one another. Both females were adults; however, only one of the two bobcats had a complete home range. This one incident provides evidence that philopatry may take place on site, but likely in a limited capacity.

By supplementing this study with demographic data and following individuals from kittenhood into and through adulthood, the extent of philopatry can be better defined. Additionally, we did not acquire tissue samples for all bobcats on site, and so it is possible that other instances of philopatry were missed for this study.

Though Greenwood (1980) was uncertain whether his mating system hypothesis would serve as good predictor of dispersal patterns, estimations of biased dispersal patterns are usually defined in the context of the prevalent mating system (Greenwood 1980, Dobson 1982, Waser and Jones 1983, Lieberg and von Schantz 1985, Shields 1987). However, Greenwood's (1980) hypothesis is an extension of the parental investment hypothesis, originated by Bateman (1948) and refined by Trivers (1972), which predicts the sex roles of males and females based on reproductive investment. Some argue that the parental investment hypothesis is antiquated and ignores contrary evidence from the field (e.g., Gowaty 2003), and as such, predicting dispersal patterns based on the mating system may not be appropriate. Additionally, other hypotheses regarding the ultimate causes of natal dispersal, such as resource competition with kin (Shields 1987) and inbreeding avoidance (Pusey and Wolf 1996) have been proposed, along with numerous proximate mechanisms, including social subordination (Christian 1970) as well as population density and associated competition (Christian 1970, Crowe 1975a, Aars and Ims 2000, Perrin and Mazalov 2000). Accordingly, individual dispersal decisions are not made for universal reasons; causes of dispersal and philopatry likely result from an interaction of various factors, thereby differing between species, populations, and individuals (Waser and Jones 1983, Dobson and Jones 1985, Waser 1985, Shields 1987). In essence, it is important that every dispersal and philopatry study examine the study population within its own context.

On the Jones Center, suitable bobcat habitats were believed to be saturated during this study (L.M. Conner, personal communication), providing little space for home range establishment by dispersing juveniles within the study area. Furthermore, bobcats on site were not harvested between 1999 and January 2005. According to Crowe (1975a), removing part of a population would make territories available for dispersing juveniles, thus reducing juvenile dispersal and competition. In general, short-distance dispersal is likely to be sex-biased, whereas long distance dispersal usually occurs to colonize vacant sites and reduce competition, and does not exhibit a sex bias (Goudet *et al*. 2002). It is therefore possible that bobcats at the Jones Center were not displaying sex-biased, short-distance dispersal because of resource competition, in which case both sexes of juveniles would be expected to disperse (Dobson 1982). During winter 2005, bobcat removal recommenced for a concurrent bobcat ecology study (Lynch 2005). This removal study may open previously occupied territories, allowing juveniles to settle on site and reducing competition between bobcats, and thereby future analyses may find different dispersal patterns. Further analyses are required to determine how bobcat removal may affect spatial genetic structure as opposed to those populations not experiencing removal.

The methodologies of this study can be improved upon for future bobcat population genetics studies. A landscape-level approach, that is, a larger-scale approach that considers the habitat occupied by the population and surrounding landscapes that influence dispersal and gene flow (Sork *et al*. 1999), would enhance any subsequent genetic studies on this site. Not only would a landscape-level approach better define the breeding population, it would also aid in determining how adjacent areas and their management may be influencing bobcats on the Jones Center. Lastly, forthcoming bobcat genetics studies would benefit by utilizing more loci in the analysis as well as a larger sample size of individuals, and in doing so, gain statistical power for tests examining sex-biased dispersal and sex-biased philopatry.

Error

The true genotype of an individual is not perceivable and has to be estimated indirectly with molecular analysis (Bonin *et al*. 2004). Thereby, genotyping errors, defined as a mismatch between two or more genotypes of the same sample analyzed independently, arise when the estimated genotype is not consistent with the true genotype (Bonin *et al*. 2004). Error rates are generally not mentioned in molecular analysis studies, though error is thought to be common and pernicious, possibly leading to incorrect conclusions (Hoffman and Amos 2005), as happened during a study of chimpanzee reproductive behavior (Gagneux *et al*. 1997, Constable *et al*. 2001). Thus, following recommendations similar to those proposed by Bonin *et al*. (2004), we minimized our error and estimated our error rate.

We attempted to minimize error via numerous means: we extracted DNA in a room separate from that used for amplifications; we ran positive and negative controls during amplification and scoring; we replicated questionable samples (nonamplifying samples, odd profiles/additional alleles, low peak heights, split peaks) and discarded the sample at that locus after three runs if it produced inconsistent or nonamplifying genotypes; we eliminated suspicious markers; and we used an automated process while manually checking samples. Despite our efforts, we still experienced genotyping error.

Unfortunately, genotyping error is the bane of molecular analysis. Error is not limited to the use of noninvasive genetic samples (i.e., hair, feather tips, or scat), and has the potential to affect studies utilizing higher quality DNA sources (Jeffery *et al*. 2001, Bonin *et al*. 2004, Hoffman and Amos 2005). Genotyping errors can originate during multiple stages of the genotyping process as well as from various factors, and because of their multiple sources, errors cannot be eliminated entirely (Bonin *et al*. 2004).

Not surprisingly then, instances of error were found at all except two loci, following error correction. Although the possibility of laboratory or human error cannot be neglected in any case of genotype mismatch (Fernado *et al*. 2003), only one out of 163 genotypes is, with certainty, attributed to human error. Other errors included a false allele noticed in bch6t, binning problems, and false homozygotes.

False alleles result from a polymerase-slippage error or electrophoresis artifact and are not part of the true genotype (Taberlet *et al*. 1996, Fernando *et al*. 2003). False alleles are noticed because they will, at times, produce samples with greater than 2 alleles (Miller *et al*. 2002). This form of error can be mitigated by reamplifying samples with a suspicious allele to determine their true genotype.

Binning-related problems were also a source of error. In order to size alleles in GENEMAPPER, bins are constructed to contain samples within a certain allele size range, and the bins then represent the alleles. However, if bins are not constructed adequately and are too close together, a problematic sample may move from one bin (or allele) to the next, causing a mismatched genotype for a sample. This problem can be monitored with repeat amplification of samples (Fernando *et al*. 2003) as well as estimating error, and usually, this situation can be ameliorated by subsequently adjusting bins. Our binning-related problems suggest that GENEMAPPER may have difficulty with similar-sized alleles, particularly if one allele occupies a wide bin.

Another source of error, false homozygotes, was noted two different ways within our data set: 1) we may have called a sample a homozygote, but the first error estimate called it a heterozygote or 2) we may have labeled a sample as heterozygote, though the first error estimate called it a homozygote (Fig. 2.3). Both of these instances would result in the sample being mistakenly called a homozygote at some point, hence our use of the term ‘false homozygote’. This type of error results from the preferential amplification of only one of the two alleles, referred to as allelic dropout, and it is thought to result from the stochastic sampling of template DNA for the PCR mixture (Jeffery *et al*. 2001, Miller *et al*. 2002). Additionally, false homozygotes may result from binning-related problems within GENEMAPPER, particularly if a bin is too wide or if the program does not note one of two alleles. False homozygotes were noticed for some samples prior to conducting our error estimates, as they usually had a suspicious peak on one side (Fig. 2.4). In this case, samples were rerun and verified to be heterozygotes. The excess of homozygotes caused by allelic dropout can also be estimated by departures from Hardy-Weinberg equilibrium at a locus (Xu *et al*. 2002). However, after questionable samples were rerun and corrections made following the first error estimate, the two loci rerun for error, bcd1t and bcg3d, do not differ from the Hardy-Weinberg equilibrium, and therefore, did not have an excess of false homozygotes.

We were confident that our miscalled, false homozygote samples were not the result of stutter. Stutter tends to have a typical pattern of several, shorter repeat length peaks trailing up to the true peak, whereas the true alleles of a heterozygote are of comparable amplification. Loci prone to stutter will produce samples with greater than two alleles (Fernando *et al*. 2003), and aside from the false alleles of bch6t, we did not have samples with greater than two alleles. As such, our false homozygote samples did not appear to result from stutter.

The problems associated both with binning, false homozygotes, and stutter might be avoided by choosing loci of greater repeat length. As a result, alleles will not be as closely spaced, making the definition of an allele and its bin easier. Some have suggested that dinucleotides are more prone to stutter than tri- or tetra nucleotides (Schwengel *et al*. 1994). By preferentially avoiding dinucleotide loci, a study may be less conflicted by instances of stutter as well as genotyping error.

Regrettably, few programs were available at the time of this writing that incorporate error in relatedness estimates. One program, Relpair 2.0 (Boehnke and Cox 1997, Epstein *et al*. 2000), will incorporate error into likelihood estimates for a hypothesized dyad relationship. However, the program uses an allele-specific error rate and applies the rate equally across all of the data (Boehnke and Cox 1997, Epstein *et al*. 2000), and this supposition did not fit our results, as it appeared that certain alleles and loci were more error prone than others.

Ultimately, the effect of errors on relatedness and relationship estimates depends on whether the estimate excludes incompatible genotypes during the analysis (Wang 2004). Therefore, estimators of pairwise relatedness and kinship estimators using a likelihood approach are little affected, whereas parentage determination using likelihood approaches is susceptible to error and incorrect conclusions (Wang 2004). As such, although error poses a potential problem, we felt that our data and results are somewhat robust. The only estimation in our study significantly affected by error was the exclusion of genotypes during estimation of parental relationships in Kinship. However, we estimated a hypothesized first-degree dyad relationship with full-sibling pairs as well as parent-offspring pairs, so pairs fitting a first-degree relationship not included in parentage analysis may have been picked up during full-kinship analysis.

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Table 2.1. Summary characteristics for the six microsatellite loci used in all analyses. Table includes starting annealing temperature, number of alleles (A), observed heterozygosity (H_O), expected heterozygosity (H_E), *P*-value for Hardy-Weinberg equilibrium (P_{HW}), and polymorphic information content (P_{IC}).

Locus	**Annealing start temp. (°C)**	**A**	**H_O**	**H_E**	**P_{HW}**	**P_{IC}**
bc1at	50	8	0.758	0.782	0.370	0.742
bcd1t	50	3	0.661	0.576	0.515	0.495
bcd8t	60	6	0.500	0.509	0.905	0.481
bce5t	50	8	0.839	0.800	0.964	0.765
bcg3d	60	6	0.742	0.678	0.398	0.611
bcg8t	50	8	0.611	0.821	<0.01*	0.791

* Indicates locus out of Hardy-Weinberg equilibrium following a sequential Bonferroni correction.

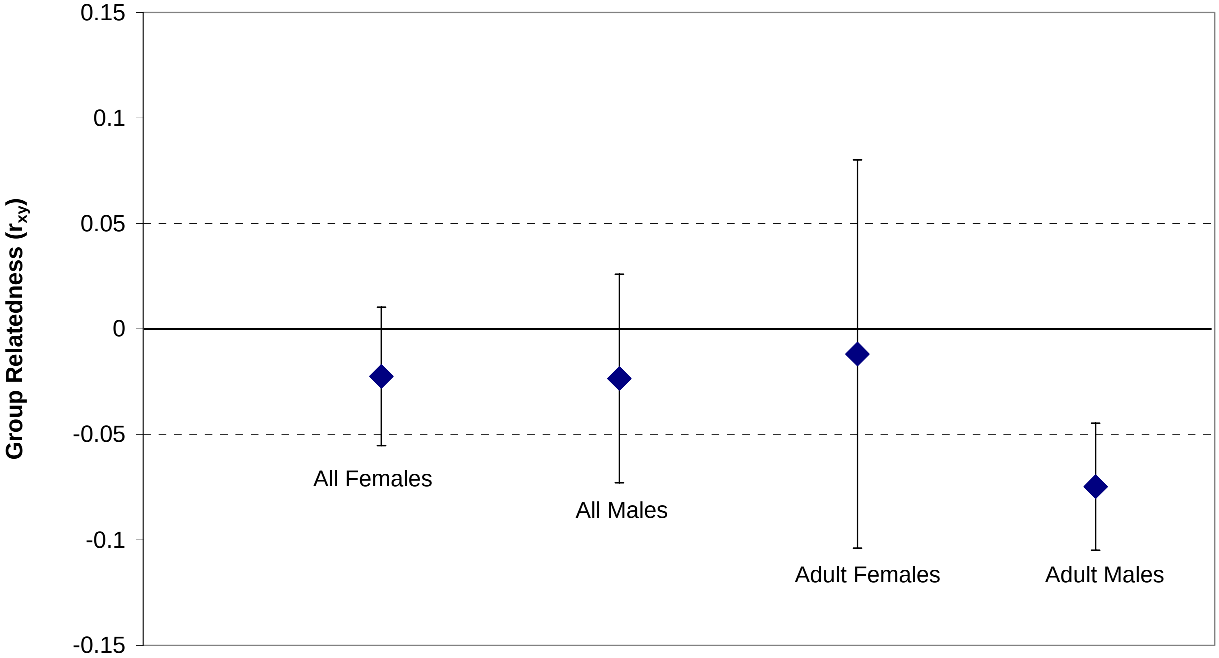


Figure 2.1. Within-group relatedness values for various age and sex groupings of bobcats. The 'All' category includes juvenile and adult bobcats, while the 'Adult' category includes only adult bobcats with at least one complete seasonal home range.

Table 2.2. Summary statistics for the two relationship categories. Table includes sample size (n), Type I error rate, Type II error rate, and the average pairwise relatedness (r_{xy}) of each category.

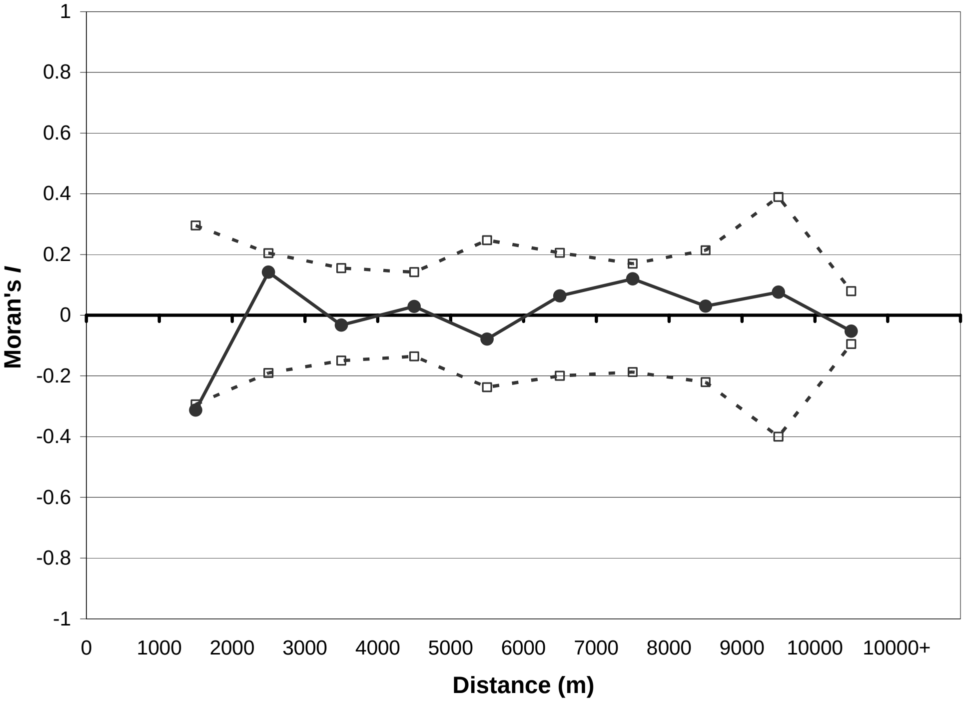
Relationship	**n**	**Type I**	**Type II**	**r_{xy}**
First-degree	148	0.05	0.159	0.411
Unrelated	1743			0.004

Table 2.3. Weighted average distances separating various age and sex groupings of bobcats. The ‘All’ category includes juvenile and adult bobcats, while the ‘Adult’ category includes only adult bobcats with at least one complete seasonal home range. Table includes sample size (n_{pairs}), weighted average distance between individuals (m), 95% confidence interval, and the range of weighted pairwise distance values.

Group	n_{pairs}	Range	Distance (m)	95% CI (±m)
All First-degree	99	245-16786	6533	788
All Unrelated	1153	0-11529	6718	107
All Female First-degree	27	246-12914	6149	1388
All Female Unrelated	325	506-19184	7166	391
All Male First-degree	21	523-11248	5141	3229
All Male Unrelated	228	760-16102	6213	445
All Adult First-degree	8	2258-11575	5467	2525
All Adult Unrelated	146	2684-16101	6637	585
Adult Female First-degree	7	2258-11576	5301	3026
Adult Female Unrelated	107	479-15588	6911	687
Adult Male First-degree	1		6023	
Adult Male Unrelated	39	2280-16102	5539	1198

Table 2.4. Mantel test results for various age and sex groupings of bobcats. Table includes the time scale of the mantel test conducted (seasonal or yearly data), the number of mantel tests analyzed for a particular time (n_{mantel}), the number of individual cats per mantel test (n_{cats}), the mantel (Pearson) correlation coefficient (r_m), the season or year that obtained a significant correlation coefficient (significant time), the number of individual bobcats for a significant test, and the *P*-value of a significant correlation. Significance was determined at $\alpha \leq 0.05$.

Grouping	**Time**	n_{mantel}	n_{cats}	r_m	**Significant Time**	n_{cats}	r_m	***P*-value**
All cats	seasonal	13	6 to 39	-0.0961 to 0.0386	Winter 2005	39	-0.0669	0.0130
				median = -0.0265				
All Females	seasonal	10	6 to 22	-0.270 to -0.00234	Winter 2004	13	-0.270	0.0136
				median = -0.0909	Winter 2005	22	-0.165	0.0130
All Males	seasonal	8	5 to 16	-0.203 to 0.138	None			
				median = -0.0195				
All Adults	seasonal	15	5 to 24	-0.0947 to 0.211	None			
				median = -0.0145				
	yearly	4	9 to 20	-0.0240 to 0.0131	None			
				median = -0.0173				
Adult Females	seasonal	15	4 to 16	-0.258 to 0.214	Spring 2004	14	-0.172	0.0403
				median = -0.0614	Winter 2004	9	-0.258	0.0441
					Winter 2005	16	-0.189	0.0145
	yearly	4	5 to 16	-0.229 to 0.00676	None			
				median = -0.131				
Adult Males	seasonal	11	4 to 7	-0.0318 to 0.480	None			
				median = 0.234				
	yearly	3	4 to 5	0.220 to 0.350	None			
				median = 0.320				



Moran's *I*
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7000
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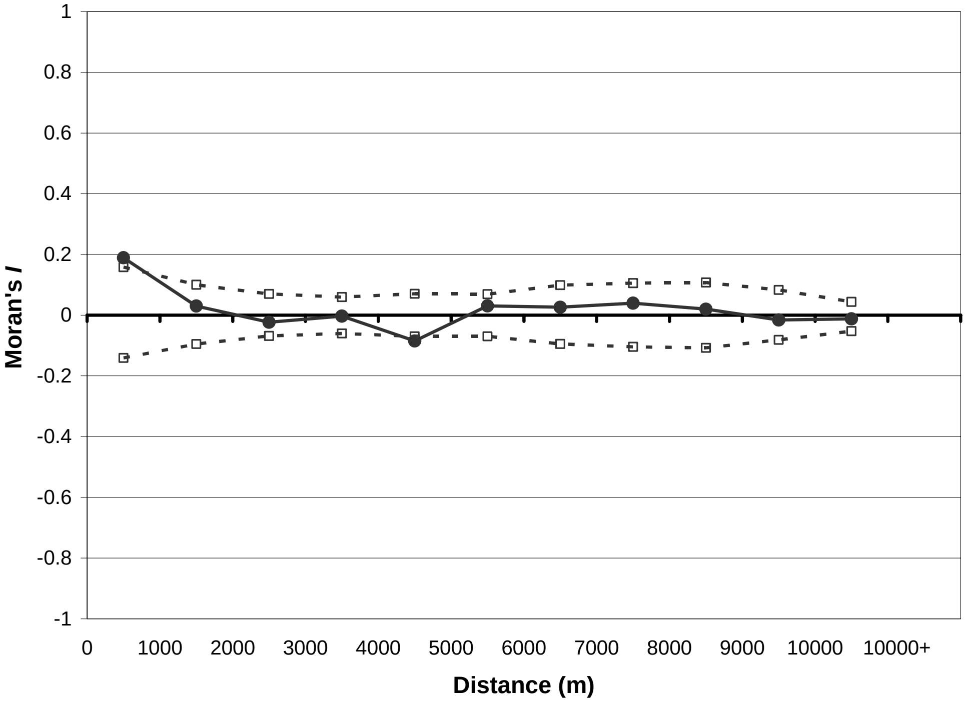
10000
10000+
Distance (m)

a.



Moran's *I*
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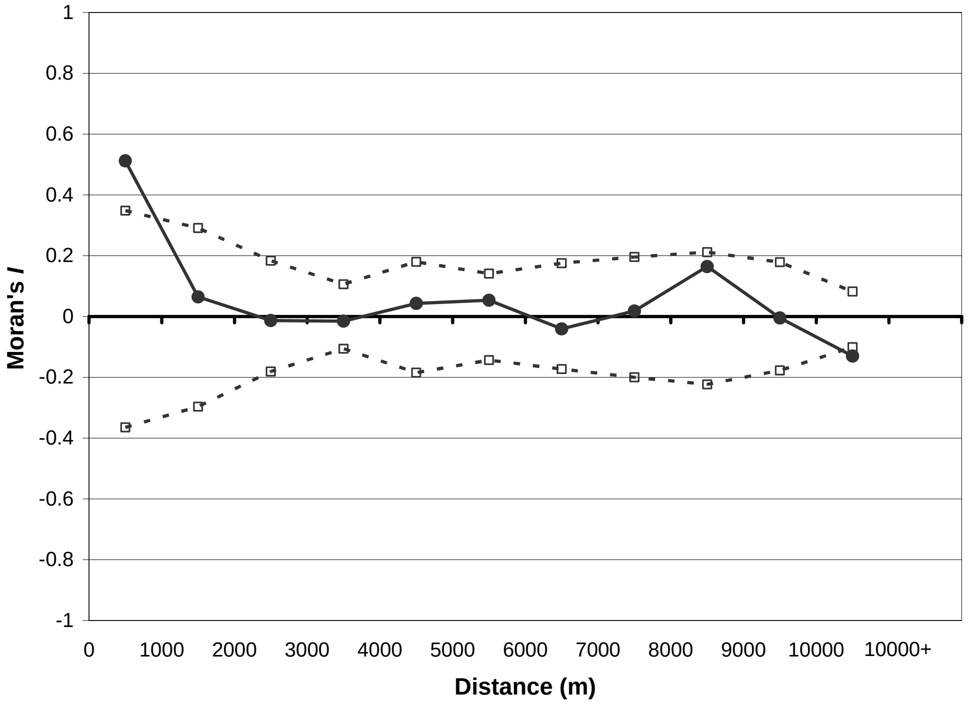
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Distance (m)

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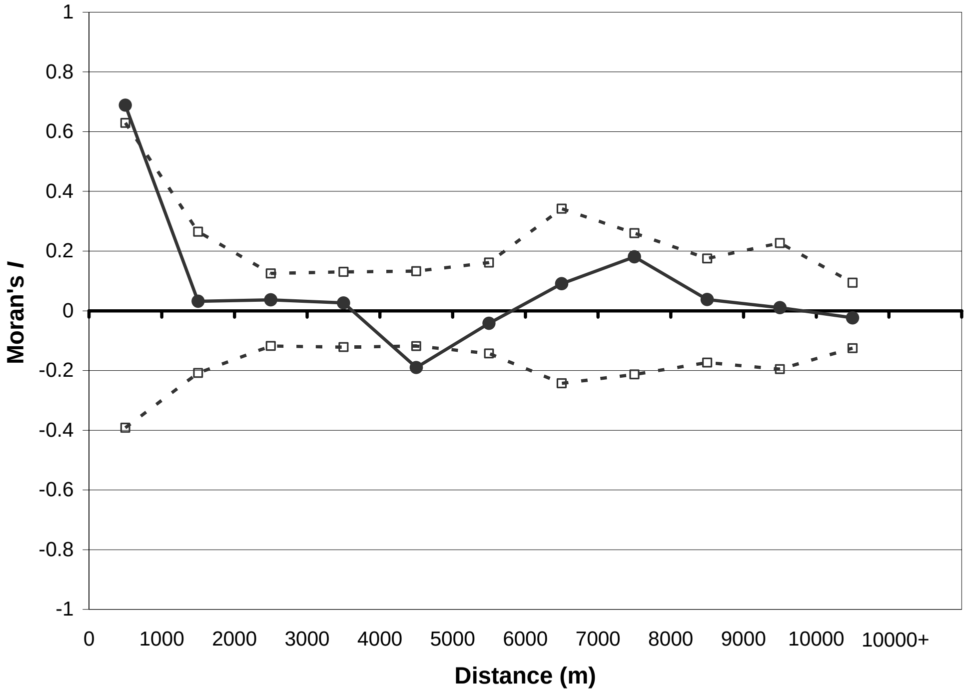
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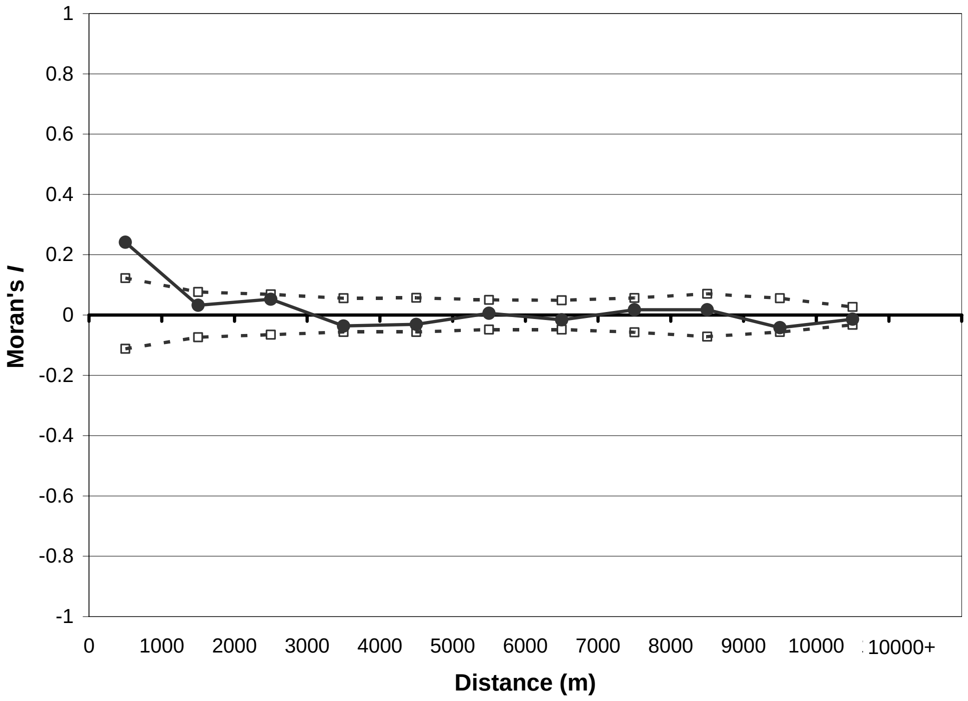
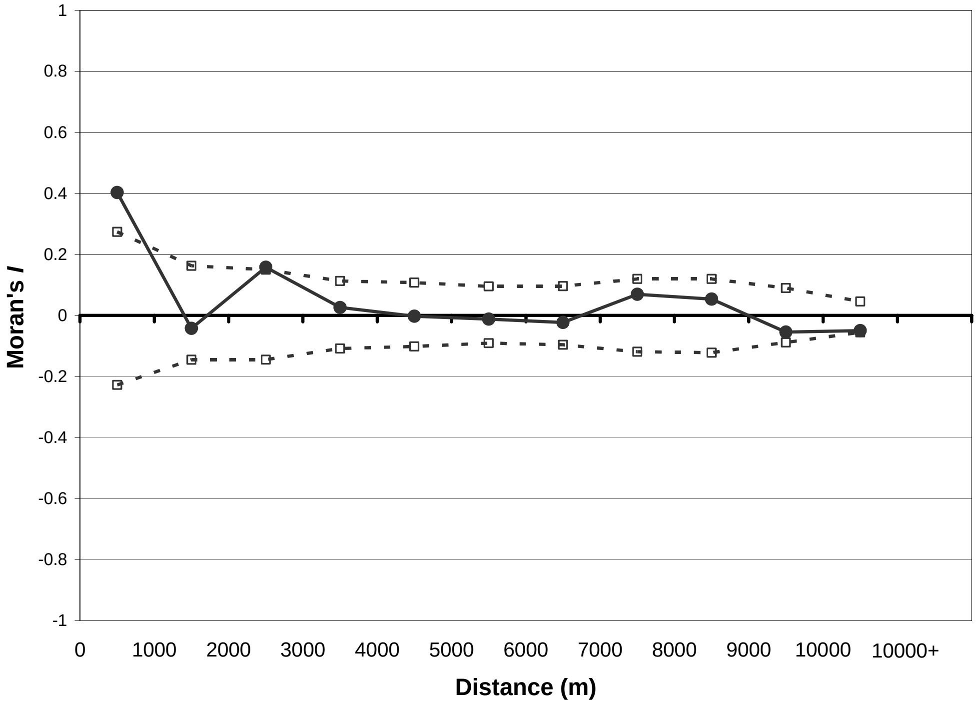
Distance (m)

d.



e.



f.

Figure 2.2. Six correlograms with significant positive spatial genetic autocorrelation in short distance classes. a. Spring 2004, adult males. b. Winter 2004, all bobcats. c. Winter 2004, all females. d. Winter 2004, all males. e. Winter 2005, all bobcats. f. Winter 2005, all females.

Table 2.5. Association between overlap indices and relatedness. Table includes the time scale of the data analyzed, the number of correlations ($n_{correlations}$), the number of bobcat pairs used in each correlation analysis (n_{pairs}), the Spearman correlation coefficients, and season or average that obtained statistical significance. Significance was determined at $\alpha \leq 0.05$.

Grouping	**Time**	**$n_{correlations}$**	**n_{pairs}**	**r_s**	**Significant Time**	**n_{pairs}**	**r_s**	***P*-value**
All Adults	seasonal	9	5 to 32	-0.600 to -0.320 median = 0.112	None			
	average	1	79	0.0565	None			
Adult Females	seasonal	5	5 to 14	-0.631 to 0.714 median = 0.196	Spring 2004	8	0.714	0.02330
	average	1	22	0.285	None			
Adult Males	seasonal	1	5	0.100	None			
	average	1	14	-0.187	None			

Table 2.6. Error estimates for seven bobcat microsatellite loci. Table includes error rates as the percent of mismatched genotypes. Probable causes of error are listed. Error estimates were conducted twice for 2 loci.

Locus	**Repeat type**	**1st run error rate (% genotype mismatch)**	**Cause(s) of error**	**2nd run error rate (% genotype mismatch)**	**Causes of error**
bc1at	tetranucleotide	6	unknown - human?	NA	NA
bcd1t	trinucleotide	11	false homozygote	5	false homozygote
bcd8t	tetranucleotide	5	incorrect sample	NA	NA
bce5t	tetranucleotide	0	NA	NA	NA
bcg3d	dinucleotide	22	false homozygote, binning	0	NA
bcg8t	dinucleotide	5	false homozygote	NA	NA
bch6t	dinucleotide	50	false homozygote, binning, false allele	NA	NA

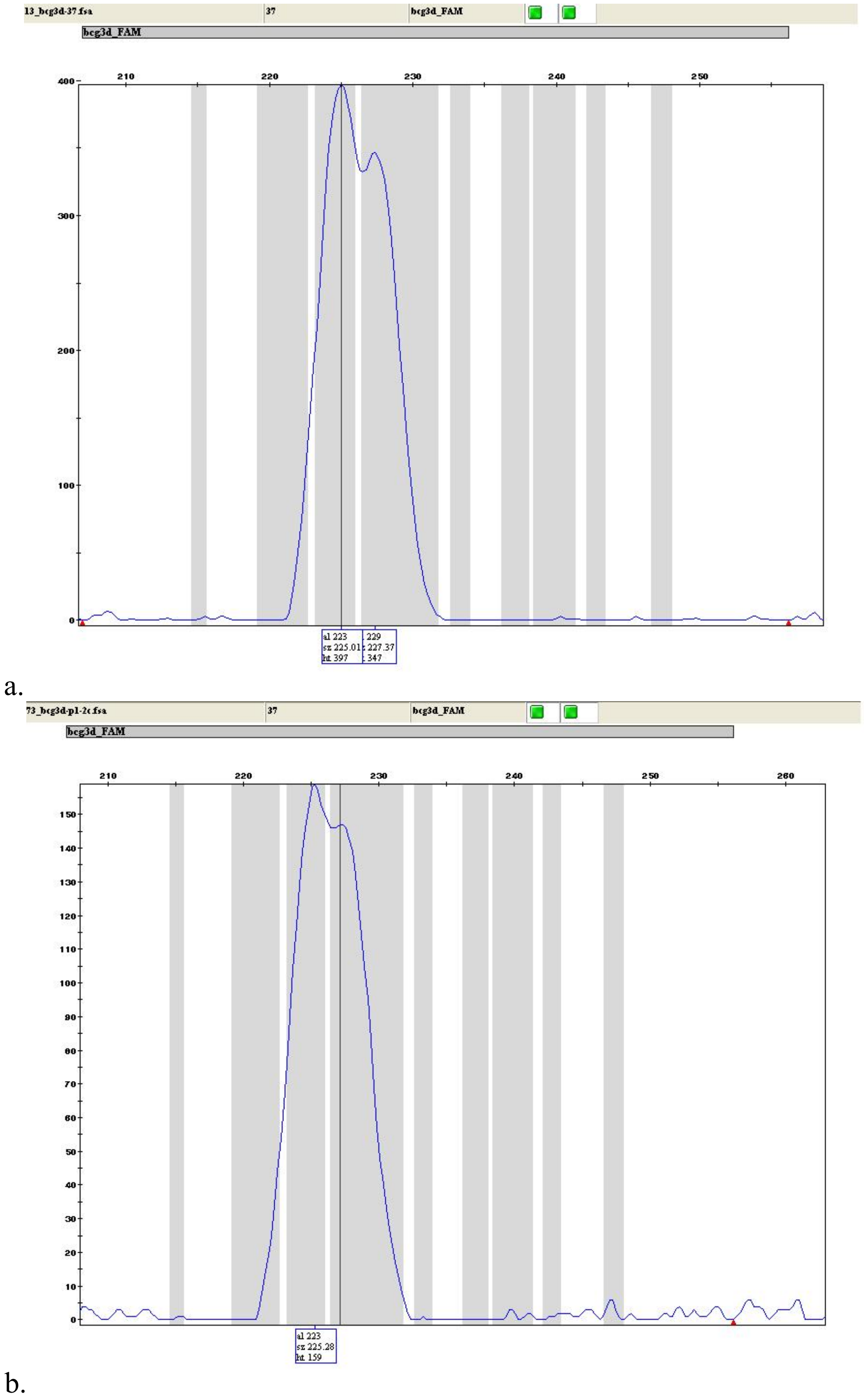


Figure 2.3. False homozygote in GENEMAPPER. Sample 37, locus bcg3d. a. Sample run scored correctly as a heterozygote. b. First error run, scored incorrectly as a homozygote. Grey areas represent the bins used to define alleles.

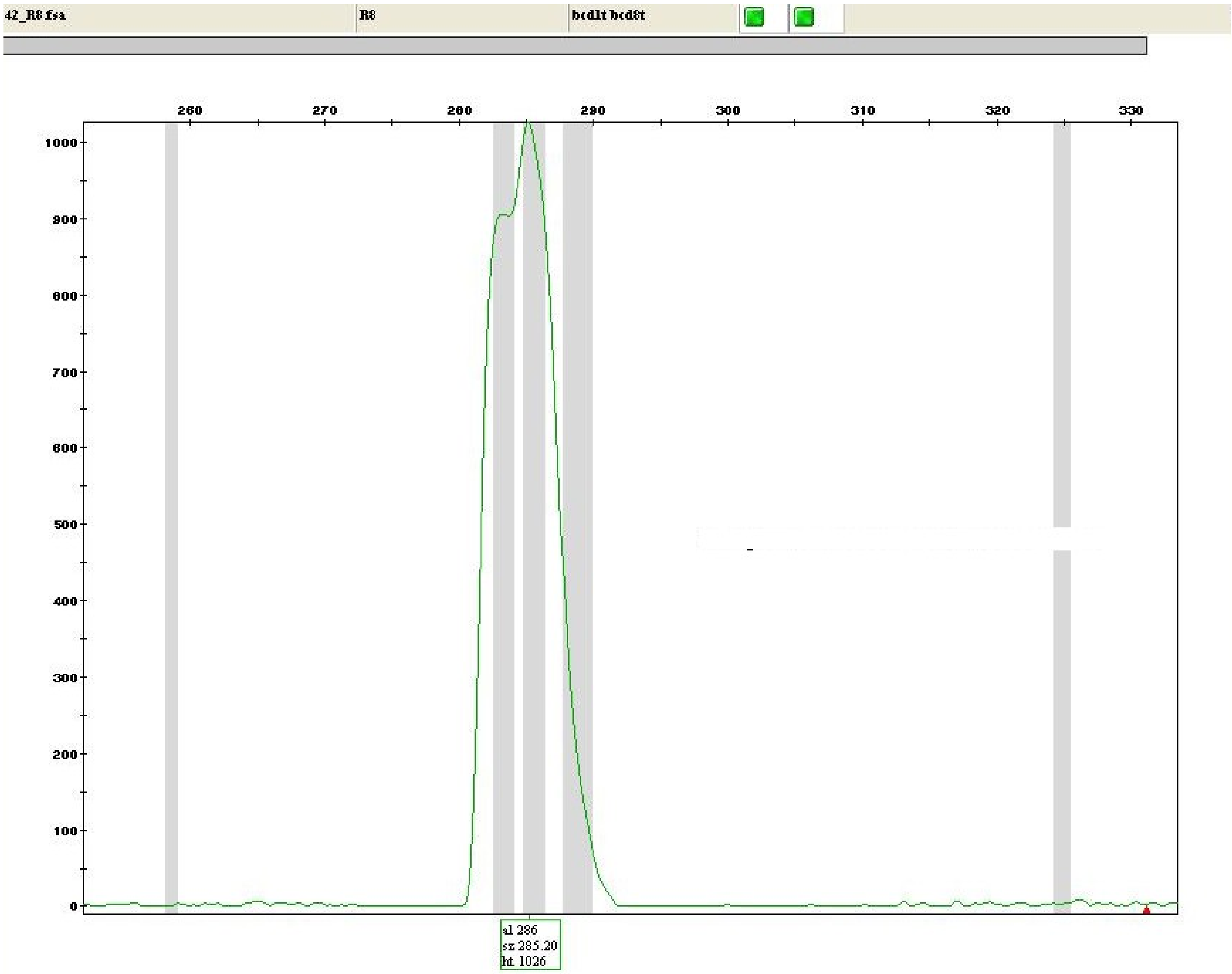


Figure 2.4. Picture of sample with a suspicious peak. Sample R8 at locus bcd1t, and only one out of two peaks was called. Suspicious peaks such as these suggested that the sample may have been a heterozygote, and, as a result, a sample with such a profile was rerun. Grey areas represent the bins used to define alleles.

CHAPTER 3

BETWEEN-POPULATION SPATIAL GENETIC STRUCTURE OF FOUR SOUTHEASTERN BOBCAT POPULATIONS[2]

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[2] Reid, A.E., B.C. Faircloth, R.J. Warren, and L.M. Conner. To be submitted to *Journal of Mammalogy*.

Abstract

Populations demonstrate genetic structure, resulting from various factors, particularly limited dispersal. Between-population genetic structuring often fits the isolation by distance model, which predicts that populations in close proximity to one another will be more similar genetically than populations that are farther apart. Additionally, existent genetic structure between populations can be examined to deduce whether sex-biased dispersal is taking place. We explored population genetic structure between four sampled bobcat (*Lynx rufus*) populations throughout southern Georgia and northern Florida using F_{ST}, R_{ST}, isolation by distance analysis, and STRUCTURE. We used various sex-biased dispersal estimators, including sex-specific F_{ST}, mean corrected assignment indices, and variance of corrected assignment indices to deduce whether sex-biased dispersal was occurring. Two pairs of populations differed significantly using F_{ST} calculations, though all other estimators suggested that the four sampled bobcat populations were one panmictic unit. Furthermore, we found no evidence supporting the occurrence of sex-biased dispersal between our sampled populations. Our results suggest that bobcats are widely dispersing, and management for this species should occur at larger spatial scales.

Keywords: bobcat, *Lynx rufus*, microsatellites, population structure, dispersal, philopatry

Introduction

Dispersal is the transference of an individual from its natal area to the area where it might reproduce (Howard 1960), and a dispersing individual is thought to move into the first unchallenged site near its natal home range (Murray 1967). Dispersal commonly exhibits a sex bias in which one sex disperses, while the other is sedentary or philopatric (Greenwood 1980, Dobson 1982, Waser and Jones 1983). Philopatry, as used here, occurs when an individual remains near or within its natal home range beyond the age of independence (Waser and Jones 1983, Ratnayeke *et al*. 2002). Within mammals, males are commonly the dispersing sex, while females are philopatric, and this patterning is thought to be associated with the predominant mammalian mating system of polygyny (Greenwood 1980). Though sex-biased dispersal and philopatry predictions are generally made from the prevalent mating system, other ultimate and proximate factors influence the dispersal patterns as well, including resource competition with kin (Shields 1987), inbreeding avoidance (Pusey and Wolf 1996), social subordination (Christian 1970), and population density and associated competition (Christian 1970, Crowe 1975, Aars and Ims 2000, Perrin and Mazalov 2000).

Dispersal is both a behavioral process and a life history trait producing ecological and evolutionary consequences for populations and, ultimately, species (Dieckmann *et al*. 1999). Dispersal, or lack thereof, can impact a population's dynamics (Dieckmann *et al*. 1999), spatial distribution (Howard 1960), and social organization (Greenwood 1980, Waser and Jones 1983). Dispersal, when successful and resulting in reproduction, is the primary conduit of gene flow (Slatkin 1987). Limited gene flow through limited dispersal can induce genetic structuring both within and between populations (Slatkin 1987, Chesser 1991a,b), as well as contribute to local adaptation and speciation (Slatkin 1987).

Populations are often genetically structured with varying degrees of genetic similarity through space (Wright 1969, Sugg *et al*. 1996, Balloux and Lugon-Moulin 2002). While numerous mechanisms contribute to genetic structure, such as selection or population history, it is gene flow through dispersal and breeding systems that are the predominant influencing factors (Chesser 1991b), particularly at local spatial scales (Vekemans and Hardy 2004). One common form of genetic structuring between populations is isolation by distance (Balloux and Lugon-Moulin 2002). In the isolation by distance model, limitations on dispersal distances prevent individuals from mating with more spatially distant members of their species, and instead, individuals mate within a confined area, or neighborhood (Sokal and Wartenberg 1983). As a result, spatially close populations are expected to exhibit greater genetic similarity than those that are farther apart (Wright 1969, Sokal and Wartenberg 1983, Balloux and Lugon-Moulin 2002, Vekemans and Hardy 2004). Additionally, examination of spatial genetic structure between populations can reveal patterns of sex-biased dispersal, as the dispersing sex should be more genetically similar across populations than the philopatric sex (Goudet *et al*. 2002).

Gene flow, and thereby dispersal, is an important evolutionary mechanism. This exchange of genetic material is generally perceived as a ‘constraining’ force, impeding local adaptation and speciation by homogenizing between-population genetic differences (Slatkin 1987, Balloux and Lugon-Moulin 2002), though it may at times serve as a creative force, generating new polymorphisms and gene combinations for natural selection to act upon (Slatkin 1987, Balloux and Lugon-Moulin 2002). Gene flow can counteract the process of genetic drift, which affects small, isolated populations by reducing genetic diversity and increasing levels of inbreeding, reducing evolutionary potential, and fixing deleterious mutations (e.g., Westemeier *et al*. 1998, Hendrick and Kalinowski 2000, Higgins and Lynch 2001, Ingvarsson 2001, Frankham *et al*. 2004, Madsen *et al*. 2004, Pimm *et al*. 2006). Genetic structuring between populations, generated in part by gene flow or lack thereof, also has conservation implication. For instance, measures of genetic diversity among subpopulations or population groupings have aided management decisions by informing managers where conservation efforts should focus to preserve the most genetic diversity or genetic distinctiveness (e.g., Moritz 1994, Crandall *et al*. 2000).

While studies examining between-population genetic structure and isolation by distance exist for myriad taxa, including various plant species (e.g., Hamilton 1997, Galloway and Fenster 2000), invertebrate species (e.g., Magalon *et al*. 2005, Trouve *et al*. 2005), and various vertebrate species (e.g., Rassmann *et al*. 1997, Baer 1998, MacDougall-Shackleton and MacDougall-Shackleton 2001, Rueness *et al*. 2003), we were unaware of any published work examining between-population genetic structure for bobcats (*Lynx rufus*), a species of conservation concern and management importance in the United States. Additionally, studies examining sex-biased dispersal and philopatry in mammalian taxa are limited to species with well-defined social systems (Waser and Jones 1983). Recent studies have begun to examine the spatial genetic structure of species with less-defined social systems, for example, Soay sheep (*Ovis aries*) (Coltman *et al*. 2003), talar tuco-tucos (*Ctenomys talarum*) (Cutrera *et al*. 2005), and raccoons (*Procyon lotor*) (Ratnayeke *et al*. 2002). At the time of this writing, we were aware of only one study examining this relationship in a population of Texas bobcats (Janečka *et al*. 2006), a species thought to exhibit philopatry and sex-biased dispersal.

The bobcat, a solitary, polygynous species, is the most widely dispersed native felid in North America (Anderson 1987, Anderson and Lovallo 2003). They occur throughout the United States (McCord and Cardoza 1982) and are only absent from one state – Delaware (Woolf and

Hubert 1998). Despite their seemingly ubiquitousness, bobcats are a species of conservation concern. Bobcats were listed under Appendix II of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) in 1975 because of harvest pressure exerted on this species (Anderson 1987, Woolf and Hubert 1998, Anderson and Lovallo 2003). As a result, member countries, including the United States had to demonstrate that international trade would not be a detriment to bobcat survival (Woolf and Hubert 1998). Bobcats are maintained under CITES protection because of their similar appearance to other endangered felids and their potential to be overexploited without the regulation of trade (Anderson and Lovallo 2003). Currently, bobcats are listed as endangered in Indiana, Iowa, New Jersey, and Ohio (Woolf and Hubert 1998, Anderson and Lovallo 2003). With the extirpation of large carnivores throughout the United States, these medium-sized felids may now serve as apex predators in parts of their range (Conner *et al*. 2000), and as such, they may regulate populations of medium-sized omnivores (Rogers and Caro 1998, Courchamp *et al*. 1999, Crooks and Soulé 1999) and benefit economically important prey species (Doughty 2004).

Although dispersal is the primary means for gene flow and contributes to population genetic structure, knowledge of juvenile bobcat dispersal and movement is limited. A few general ideas can be gleaned from the literature, however. The initiation of juvenile dispersal relates to adult intolerance during the breeding season (Griffith *et al*. 1980, Kitchings and Story 1984, Griffith and Fendley 1986, Anderson 1987). The juvenile population may fill vacant home ranges left open by death or resident removal (Crowe 1975, Griffith and Fendley 1986, Knick 1990), acquire part of their mother's home range (Anderson and Lovallo 2003), or disperse long distances, as evidenced by a young male's dispersal of 182 km (Knick and Bailey 1986). As would be predicted from their polygynous mating system (Greenwood 1980), young males are known to disperse earlier (Bailey 1981) and farther than young females (Robinson and Grand 1958, Kitchings and Story 1984), and bobcats may exhibit a male-biased natal dispersal and female-biased philopatry (Chamberlain and Leopold 2001, Anderson and Lovallo 2003, Janečka *et al* 2006, Chapter 2).

Because of the depauperate level of knowledge available regarding bobcat dispersal and population genetic structure and the potential of this information to aid in management and conservation decisions, we investigated the genetic structure and sex-biased dispersal between four bobcat populations in the southeastern United States using six microsatellite loci. While we made no *a priori* predictions regarding the level of population differentiation, we believed that our sampled populations would fit the isolation by distance model. That is, populations in spatial proximity would be more genetically similar than populations farther apart. Moreover, based on the bobcat's polygynous mating system and limited evidence for sex-biased dispersal and philopatry (Chapter 2), we predicted that bobcats would exhibit a male-biased dispersal and female-biased philopatry, resulting in genetic similarity between males across populations and genetic dissimilarity between females across populations.

Methods

Study Area

Bobcat samples were collected from four locations in southern Georgia and northern Florida: Joseph W. Jones Ecological Research Center at Ichauway (Jones Center) (Chapter 2), Tall Timbers Research Station, Pebble Hill Plantation, and Pinebloom Plantation.

Tall Timbers Research Station (Tall Timbers) is a 1,568 ha research facility located in Leon County, Florida. The site is in the Red Hills physiographic region of the Gulf Coastal Plain of northern Florida. Upland pine forests composed of longleaf, shortleaf (*P. echinata*), and loblolly (*P. taeda*) dominate the landscape. Bottomland hardwood drains and fallow fields comprise the remainder of the site. Common bottomland species include oaks (*Quercus* spp.), sweet gum (*Liquidambar styraciflua*), black gum (*Nyssa sylavatica*), and hickories (*Carya* spp.). Old-field vegetation is the prevalent ground cover.

Prescribed fires occur on a yearly, rotational basis throughout the Tall Timbers site to reduce hardwood cover and promote an herbaceous ground cover. Mechanical practices are utilized to limit the encroachment of hardwoods in pine forests. Supplemental feeding for bobwhite quail and predator removal are also common management practices.

Pebble Hill Plantation (Pebble Hill) is a privately-owned 1,246 ha quail plantation in Thomas and Grady counties, Georgia. Pebble Hill is located in the Red Hills physiographic region in the Gulf Coastal Plain of southern Georgia. The landscape is mainly composed of upland pine forests. Longleaf pine and loblolly pine dominate the overstory component of the forests, and wiregrass and old-field vegetation comprise the understory of the upland forests. Hardwoods, such as oak, sweet gum, and black gum, are found throughout the site, primarily in riparian areas.

Prescribed burns, commercial thinning, disking, and mechanical removal of hardwoods are the prevalent management practices. For game species, such as bobwhite quail and white-tailed deer, food plots are planted and supplemental feeding is conducted. Predator removal occurs on site.

Pinebloom Plantation (Pinebloom) is an approximately 8000 ha, privately-owned plantation located in Baker County, Georgia. This site resides in the Dougherty Plain physiographic province of the southeastern Gulf Coastal Plain. Slash pine (*P. elliotti*) dominates the overstory and old-field vegetation dominates the understory. Fallow fields comprised of ragweed (*Ambrosia* spp.) are also found throughout the site.

Management regimes on site are similar to those conducted by Pebble Hill. Prescribed burns, commercial thinning, disking, and mechanical removal of hardwoods are the prevalent management practices. For game species such as bobwhite quail and white-tailed deer, food plots are planted and supplemental feeding is conducted. Predator removal occurs on site.

Site staff at each location provided UTM center coordinates for each site. With these coordinates, we calculated Euclidean distances between sites using SPAGeDi 1.2 (Hardy and Vekemans 2002) (Table 3.1).

Bobcat Capture, Tissue Collection, and DNA Analysis

Bobcat capture and tissue acquisition on the Jones Center followed methodologies described by Chapter 2. Bobcat collection on Tall Timbers, Pebble Hill, and Pinebloom took place as part of a coordinated predator removal study occurring on these three sites. Bobcats were lethally removed using baited foothold traps, cage traps, or spotlighting with firearms. Samples obtained for this study were captured during March to September 2003 and March to September 2004. Pebble Hill and Pinebloom were sampled in 2003, while Pinebloom and Tall Timbers were sampled in 2004. Sex of the bobcat, date of removal, and method of removal were recorded. No age data were provided.

We obtained neck muscle tissue from each carcass for subsequent genetic analysis. Only one individual was processed at a time to avoid sample contamination and confusion. Instruments were sterilized between sampled individuals using 10% bleach solution, distilled water, and alcohol. A fine layer of muscular tissue was removed from each neck prior to sample collection in the location where the sample was to be taken, which prevented bacteria or other substances present on the outer layers of muscle from contaminating our samples. We collected two tissue samples of approximately 25 mg from each individual for genetic analysis. DNA extraction and amplification procedures, population genetics calculations, and determination of genotyping error rates were reported in Chapter 2.

Population Structure

We quantified the genetic differentiation between populations by calculating pairwise F_{ST} using Weir and Cockerham's estimator (1984), as implemented in FSTAT 2.9.3 (Goudet 2001). Statistical significance of these pairwise values was tested using randomized permutations. Levels of significance were adjusted with the sequential Bonferroni method (Rice 1989) as implemented in FSTAT. Using RST CALC (Goodman 1997), we calculated pairwise R_{ST} values, which are based on a different mutational model than F_{ST} (Balloux and Lugon-Moulin 2002).

To examine whether our populations conformed to the isolation by distance model, we regressed pairwise estimates of $F_{ST}/(1-F_{ST})$ against the natural log of distance (Rousset 1997) using ISOLDE in Genepop 3.4 (Raymond and Rousset 1995). Significance of the correlation was determined using the Mantel permutation procedure in ISOLDE with 10,000 permutations. If spatial distance and genetic differentiation between populations conform to the isolation by distance model, a significant positive correlation is expected because genetic differentiation between populations increases with spatial distance (Wright 1969, Sokal and Wartenberg 1983, Balloux and Lugon-Moulin 2002, Vekemans and Hardy 2004).

In order to test genetic population structure with the aforementioned methods, *a priori* groups need to be defined, and these group definitions may involve some subjectivity based on pre-existing conditions such as geographic separation or phenotypic differences (Pritchard *et al.*

2000, Evanno *et al*. 2005). However, groupings based on particular characteristics may not reflect actual genetic differentiation (Pritchard *et al*. 2000, Evanno *et al*. 2005). As such, an alternative Bayesian clustering approach was used to test population structure as implemented in the program STRUCTURE (Pritchard *et al*. 2000). This program attempts to cluster multilocus genotypes into groupings of individuals in such a way that the groupings are not in Hardy-Weinberg or linkage disequilibrium (Pritchard *et al*. 2000, Evanno *et al*. 2005). Using Markov Chain Monte Carlo (MCMC) methods, STRUCTURE tests user-defined values for the number of populations (K), in which individuals are assigned probabilistically to populations and the log probability of the data for a value of K, log (Pr(X|K)), is calculated, allowing the user to determine the most likely population clustering (Pritchard *et al*. 2000, Evanno *et al*. 2005).

We tested for the presence of genetic clusters using 120 genotypes without the use of geographic data in STRUCTURE 2.1 (Pritchard *et al*. 2000). We tested values of K from one to eight populations and ran seven independent runs for each value of K. We assumed admixture between subpopulations and correlated allele frequencies (Falush *et al*. 2003). For each run, we used a 75,000 burn-in period and 1,000,000 MCMC repetitions.

Sex-biased Dispersal

In our analyses, we used a combination of methodologies to examine sex-biased dispersal: sex-specific F_{ST}, mean corrected assignment index (*mAIc*), and variance of AIc (*vAIc*) (e.g., Goudet 2001, Goudet *et al*. 2002, Comer 2005, Cutrera *et al*. 2005). All calculations were done in FSTAT, which uses a randomization approach to test for a sex-biased dispersal (Goudet 2001). We used 10,000 iterations and a one-sided test to evaluate our hypothesis of male-biased dispersal in bobcats. With these methods, the dispersing sex is expected to have a lower F_{ST}

value as a result of gene flow, a lower assignment index (i.e., is less likely to originate from that population) and a higher assignment index variance than the philopatric sex (Goudet *et al*. 2002).

Notably, tests for sex-biased dispersal require the exclusion of all predispersing individuals. Because dispersal occurs in juvenile bobcats (Anderson and Lovallo 2003), this test required the removal of juveniles from the analyses. However, age data were available for the Jones Center bobcats only, and as such, juveniles from the Jones Center were the only pre-dispersal individuals removed from the sex-biased calculations.

Results

Population Genetics

We collected tissue from a total of 120 individuals (n = 64 from the Jones Center, n = 34 from Pinebloom, n = 12 from Tall Timbers, and n = 5 from Pebble Hill). Three samples were dropped from the entire study (n = 2 from the Jones Center and n = 1 from Pinebloom) because they produced inconsistent genotypes or did not amplify during PCR. All except four individuals were genotyped at all loci, and each of those four did not produce a genotype at one locus (n = 1 sample from Pebble Hill at bcg8t, n = 3 from Pinebloom at bcg3d, bcd1t, and bcg8t).

Based on the known cross samples, Mendelian inheritance at all loci was confirmed. We found polymorphism across all loci and all populations (Table 3.2). There was no significant linkage disequilibrium between loci following a sequential Bonferroni correction. Only one locus in one population, bcg8t in the Jones Center bobcats, differed significantly from the Hardy-Weinberg equilibrium ($P < 0.01$) following a sequential Bonferroni correction, possibly as a result of null alleles, allelic dropout, or small sample size. The global test in Genepop revealed that this population did not differ significantly from Hardy-Weinberg equilibrium ($P = 0.0530$). We ran the global test a second time without bcg8t to determine whether this locus was causing the small P-value, and the resulting value from this second test was $P = 0.856$. Based on global tests, all other populations were within Hardy-Weinberg equilibrium. Genotyping error results were described in Chapter 2.

Population Structure

We calculated pairwise F_{ST} and pairwise R_{ST} to determine population differentiation. Pairwise F_{ST} ranged from -0.0135 to 0.0547 for our four bobcat populations, while pairwise R_{ST} values ranged from -0.00230 to 0.0328 (Table 3.3). The Jones Center and Tall Timbers bobcat populations differed significantly from one another based on their pairwise F_{ST} estimate, as did the Tall Timbers and Pinebloom bobcat populations. However, there were no significant differences between populations using R_{ST}.

We tested our populations' conformation to the isolation by distance model by regressing $F_{ST}/(1-F_{ST})$ against the natural log of distance. We found no evidence to suggest that our four bobcat populations correspond to this model ($P = 0.290$).

We used a Bayesian clustering technique as implemented in STRUCTURE to test population differentiation and structure as well. The highest mean log probability occurred for $K = 2$ with $\log \Pr(X|K) = -2031$, and the second highest mean log probability was for $K = 1$ with $\log \Pr(X|K) = -2034$. The values of the mean log probability for $K \geq 3$ were $\log \Pr(X|K) < -2883$. The data suggest that our four populations were one panmictic unit: the log likelihoods for $K = 1$ and $K = 2$ differ by very little, and in all tests, from $K = 1$ to $K = 8$, individuals were assigned symmetrically across populations.

Sex-biased Dispersal

For our sex-biased dispersal tests, we dropped all known juveniles from the Jones Center sample (Table 3.4). We tested for the presence of sex-biased dispersal using F_{ST}, mean corrected assignment indices (*mAIc*), and variance of the corrected assignment indices (*vAIc*). Using these three estimators, we found no evidence of a sex-biased dispersal (Table 3.5). The results of *mAIc*, *vAIc*, and F_{ST} did not differ significantly between males and females. The only test to fit the expected patterning was F_{ST}, in which females were more differentiated between populations than males.

Discussion

Population Structure

Our genetic data were analyzed both with and without the context of the populations' geographic locations. When we conducted our genetic analyses of populations grouped by geographic locations with *F*-statistics and *R*-statistics, we obtained mixed results. While no pairwise R_{ST} values were statistically significant, two pairwise F_{ST} values were: F_{ST} between Tall Timbers and the Jones Center (0.0547) and the F_{ST} between Tall Timbers and Pinebloom Plantation (0.0474). The fact that the F_{ST} values and R_{ST} values differed was not atypical, as the estimators will not complement one another depending on the mutation method and rate, level of gene flow, and sample size (Balloux and Goudet 2002). Though neither serves as a best estimator for estimates of population differentiation as determined by microsatellites (Balloux and Goudet 2002, Balloux and Lugon-Moulin 2002), F_{ST} was likely a more appropriate estimator for our data in that we had small sample size and a limited number of loci (Gaggiotti *et al.* 1999) and, as other results suggest, our populations were weakly structured (Balloux and Goudet 2002).

In general, F_{ST} values that fall between 0 and 0.05 suggest weak genetic differentiation and values between 0.05 and 0.15 allude to a moderate differentiation (Wright 1978, Hartl and Clark 1997). The pairwise F_{ST} between Tall Timbers and the Jones Center fell just slightly above the demarcation for moderate differentiation and the pairwise F_{ST} between Tall Timbers and Pinebloom indicated weak differentiation. The resultant differentiation may have developed through numerous means. Both significant pairwise F_{ST} values were for population pairs that were separated by 84 km and 66 km (Table 3.1), the longest and third longest distances out of all pairwise distances. These larger distances may have contributed to restricted gene exchange between populations. We could not exclude juveniles from these population-wide calculations, and higher F_{ST} values (greater differentiation estimates) may result when sampling the population before rather than after juvenile dispersal (Basset *et al*. 2001). Lastly, Tall Timbers had a small sample size (n = 12), though it was by no means our smallest sample size (Pebble Hill n = 5). The small sample may have been biased, and thereby did not effectively capture the genetic makeup of Tall Timbers. Thus, significant differentiation between Tall Timbers and two other populations may be an artifact of small sample size.

When examining our populations for isolation by distance patterning, our results were not significant, suggesting genetic differentiation between populations was not correlated with geographic distance for our four bobcat populations. The lack of isolation by distance patterning may have emerged for several reasons. We tested the isolation by distance hypothesis using Euclidian distances, however, several studies have utilized distances more appropriate for their study organism in addition to Euclidean distances, such as riparian distances or distances that incorporate landscape features (i.e., landscape distances) (e.g., Goossens *et al*. 2005, Vignieri 2005). At the time of this study we were unaware of any landscape characteristics that would emanate in travel impediments, restrictions, or corridors between populations. However, it is possible that a more appropriate measure of bobcat travel exists and would serve as a better fit for the isolation by distance model. Moreover, long distance dispersal is recognized as slowing or preventing population differentiation (Wright 1943), thus if bobcats were regularly dispersing distances equal to or greater than those separating our sampled populations, a typical isolation by distance pattern would not be generated. Additionally, population differentiation measures are influenced by sample size and the number of loci used (Gaggiotti *et al*. 1999, Balloux and Goudet 2002), thus an augmentation of our within-population sample size as well as an increase in the number of loci used may produce results that better fit the isolation by distance model. Lastly, a lack of isolation by distance patterning may indicate that the populations have not reached the assumed gene flow/genetic drift equilibrium (Slatkin 1993).

When the genetic data were analyzed without the context of its geographic information using the Bayesian clustering technique implemented in STRUCTURE, we found that the mean log likelihood was highest for K = 2, though K = 1 was similar, and individuals were assigned symmetrically to all populations across all tests (K = 1 to K = 8). Pritchard *et al*. (2000) advise researchers to choose the smallest value of K that captures the major genetic structure and to be wary of inferred structure based on small differences in log probabilities of K. Moreover, while K = 2 had a slightly higher log probability, individuals were assigned symmetrically to all populations, and there was no obvious biological interpretation of that result (Pritchard *et al*. 2000). Thus, our data obtained using a clustering approach implied that our four populations were one panmictic unit.

Without *a priori* information, our results suggested one population, whereas results based on population assumptions, as implemented using F_{ST} calculations, suggest that two pairs of populations differ significantly from one another. Measures of genetic differentiation such as F_{ST} and R_{ST}, reflect long-term evolutionary averages over hundreds or thousands of years and may not reflect contemporary gene flow (Koenig *et al*. 1996, Bossart and Prowell 1998, Sork *et al*. 1999), particularly in human-altered and fragmented landscapes (Peakall *et al*. 2003). As compared to our F_{ST} results, it is likely that STRUCTURE was more effectual in capturing the true genetic structure between populations. The Bayesian clustering approach as implemented in STRUCTURE does not require *a priori* assumptions of populations (Pritchard *et al*. 2000). Assuming that sampled populations are effectively populations may not be accurate, as perceived geographic clusters of individuals may not be representative of actual genetic differences (Evanno *et al*. 2005).

Sex-biased dispersal

We tested for sex-biased dispersal using sex-specific F_{ST}, sex-specific mean corrected assignment indices, and sex-specific assignment index variances. Under the hypothesis of sex-biased dispersal, males and females fit their expected patterns for the sex-specific F_{ST} test only, and none of the three tests produced significant results. Thus, our tests provided no evidence that sex-biased dispersal was occurring between our populations.

Sex-biased dispersal tests necessitate the exclusion of juveniles from all analyses (Goudet *et al*. 2002). However, because age data were only available for one population, we were unable to exclude juveniles from all populations. Failure to exclude juveniles would reduce the power of these tests, since inclusion of juveniles in their source populations would cause males and females to be more similar to one another within a population and exhibit greater genetic differences between populations. Moreover, these tests require strong sex-biased dispersal, with a ratio between the sexes of dispersing individuals greater than 80:20, otherwise, a sex-biased dispersal would likely go unnoticed (Goudet *et al*. 2002). Thus, it is possible that our populations exhibit sex-biased dispersal, but not at extreme enough rates to pick up using the statistical tests currently available. Lastly, sex-biased tests are dependant upon the sampling scheme. Sufficient immigrants must be sampled to detect a dispersal bias (Goudet *et al.* 2002), and our sample sizes may not have been large enough to capture immigrant individuals.

Predictions of sex-biased dispersal and philopatry are usually defined in the context of the prevalent mating system as well as the associated mate competition and the differential costs and benefits of dispersal and philopatry between the sexes (Greenwood 1980, Dobson 1982, Waser and Jones 1983, Lieberg and von Schantz 1985, Shields 1987). However, Greenwood's (1980) hypothesis is an extension of the parental investment hypothesis, originated by Bateman (1948) and refined by Trivers (1972), which predicts the sex roles of males and females based on reproductive investment. Some argue that the parental investment hypothesis is antiquated and ignores contrary evidence from the field (e.g., Gowaty 2003), and as such, predicting dispersal patterns based on the mating system may not be appropriate. Additionally, other hypotheses regarding the ultimate causes of natal dispersal, such as resource competition with kin (Shields 1987) and inbreeding avoidance (Pusey and Wolf 1996) have been proposed, along with numerous proximate mechanisms, including social subordination (Christian 1970) as well as population density and associated competition (Christian 1970, Crowe 1975, Aars and Ims 2000, Perrin and Mazalov 2000). Accordingly, individual dispersal decisions are not made for universal reasons; causes of dispersal and philopatry likely result from an interaction of various factors and, thereby, differ between species, populations, and individuals (Waser and Jones 1983, Dobson and Jones 1985, Waser 1985, Shields 1987). In essence, deviations from the predicted sex-biased dispersal and philopatry in mammals would not be unusual, and patterns of dispersal should be considered within the context of the characteristics of the populations under examination.

Overall, we found little evidence for between-population structure and no indication that sex-biased dispersal was taking place. Future analyses examining between-population structure and sex-biased dispersal in bobcats would benefit from the inclusion of additional loci and larger sample sizes (Gaggiotti *et al*. 1999, Balloux and Goudet 2002, Goudet *et al*. 2002). Because the inclusion of the predator removal samples from Tall Timbers, Pinebloom, and Pebble Hill were not originally considered in our study design, we were unable to effectively collect large samples sizes as well as age data for these populations. However, a larger sample size as well as a greater number of loci may capture a more representative depiction of genetic differentiation between populations as well as between dispersing and philopatric individuals. Inclusion of more populations may also aid in determining whether bobcat populations at a particular spatial scale (local or regional) fit the isolation by distance model, as doing so may effectively capture the dispersal patterns of this species. Further, an examination of the influence of different management regimes, such as bobcat harvest and removal (Crowe 1975), on bobcat movement, dispersal patterns, and genetic differences between populations may prove interesting. Finally, the lack of between-population genetic differences across a broad spatial scale suggests that bobcats within our four populations were widely dispersing, and as such, bobcat management should consider a landscape-level approach. This larger-scale approach considers the habitat occupied by the population and surrounding landscapes that influence dispersal and gene flow (Sork *et al*. 1999), and in doing so, incorporates surrounding lands and management regimes.

Error

Ultimately, the effects of error on our population differentiation and sex-biased dispersal estimates were likely minor. Samples used to calculate the error rate were drawn randomly from all available samples, and as such, the error rate was representative of our entire sample and no population was free of error. After false allele, false homozygote, and binning-related problems were corrected, our final error estimates were relatively small. Thus, because our error estimates were relatively small and error was not limited to one population, our population differentiation and sex-biased dispersal tests were robust to error.

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Table 3.1. Pairwise distances (km) between the four bobcat study sites.

Population	Jones Center	Pebble Hill	Tall Timbers	Pinebloom
Jones Center	-	61.8	66.7	23.0
Pebble Hill	61.8	-	17.7	75.8
Tall Timbers	66.7	17.7	-	84.5
Pinebloom	23.0	75.8	84.5	-

Table 3.2. Summary characteristics for the six microsatellite loci used in all analyses. Table includes starting annealing temperature (which were consistent across populations), number of alleles (A), observed heterozygosity (H_O), expected heterozygosity (H_E), and *P*-value for Hardy-Weinberg equilibrium (P_{HW}).

Population	**Locus**	**Annealing start temp. (°C)**	**A**	**H_O**	**H_E**	**P_{HW}**
Jones Center	bc1at	50	8	0.758	0.782	0.370
	bcd1t	50	3	0.661	0.576	0.515
	bcd8t	60	6	0.500	0.509	0.905
	bce5t	50	8	0.839	0.800	0.964
	bcg3d	60	6	0.742	0.678	0.398
	bcg8t	50	8	0.611	0.821	<0.01*
	Global					0.053
Pebble Hill	bc1at	-	6	1.000	0.911	1.000
	bcd1t	-	2	0.200	0.467	0.333
	bcd8t	-	4	0.600	0.733	0.365
	bce5t	-	4	0.800	0.711	1.000
	bcg3d	-	5	0.800	0.822	0.343
	bcg8t	-	5	0.750	0.893	0.124
	Global					0.570
Tall Timbers	bc1at	-	6	0.833	0.732	0.632
	bcd1t	-	2	0.500	0.507	1.000
	bcd8t	-	4	0.583	0.471	1.000
	bce5t	-	5	0.583	0.667	0.843
	bcg3d	-	6	0.750	0.670	0.877
	bcg8t	-	8	0.750	0.764	0.623
	Global					0.998
Pinebloom	bc1at	-	8	0.848	0.785	0.736
	bcd1t	-	3	0.563	0.650	0.526
	bcd8t	-	5	0.545	0.556	0.974
	bce5t	-	9	0.848	0.815	0.275
	bcg3d	-	6	0.719	0.747	0.804
	bcg8t	-	9	0.719	0.874	0.028
	Global					0.435

* Indicates locus out of Hardy-Weinberg equilibrium following a sequential Bonferroni correction.

Table 3.3. F_{ST} (below diagonal) and R_{ST} (above diagonal) estimates for six microsatellite loci examined from bobcats in Georgia and Florida. Significance was determined at $\alpha \leq 0.05$.

Population	Jones Center	Pebble Hill	Tall Timbers	Pinebloom
Jones Center	-	0.0328	-0.0023	0.0105
Pebble Hill	0.0201	-	0.0013	-0.0115
Tall Timbers	0.0547*	0.0179	-	0.0226
Pinebloom	0.0045	-0.0135	0.0474*	-

* Indicates statistical significance

Table 3.4. Population data used for sex-biased dispersal test. The Jones Center includes adult bobcats only.

Population	n	M	F
Jones Center	38	13	25
Pebble Hill	5	2	3
Tall Timbers	12	5	7
Pinebloom	33	21	12

Table 3.5. Sex-biased dispersal test results for four bobcat populations. Table includes the number of individuals of each sex (n), sex-specific F_{ST}, sex-specific mean corrected assignment indices (*mAIc*), sex-specific variance of corrected assignment indices (*vAIc*), and associated *P*-values.

Group	n	F_{ST}	*mAIc*	*vAIc*
Females	47	0.0457	0.0239	6.275
Males	41	0.0129	0.0274	5.268
P-value		0.0839	0.547	0.683

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS: DISPERSAL, PHILOPATRY, AND SPATIAL GENETIC STURCTURE IN SOUTHEASTERN BOBCATS

Bobcats (*Lynx rufus*) are a species of conservation and economic importance. These felids are apex predators in parts of their range, particularly the forested ecosystems of the Southeast (Conner *et al*. 2000), and therefore, may benefit prey species of economic importance, such as the bobwhite quail (*Colinus virginianus*) (Doughty 2004). Additionally, bobcats are protected under the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES), Appendix II, because of their similar appearance to endangered felids and their potential to be overexploited without the regulation of trade (Anderson 1987, Woolf and Hubert 1998, Anderson and Lovallo 2003). Surprisingly little is known regarding certain aspects of the bobcat's life history, such as its dispersal characteristics and social organization, both of which will influence the spatial genetic structure in populations (Chesser 1991a,b). Predictions based on the bobcat's mating system would suggest that the bobcat may exhibit sex-biased dispersal and philopatry (Greenwood 1980, Waser and Jones 1983), and one study (Janečka *et al*. 2006) as well as anecdotal evidence (Chamberlain and Leopold 2001, Anderson and Lovallo 2003) lend credence to this prediction. By examining the spatial genetic structure within a well-studied population of bobcats at the Jones Center and the spatial genetic structure between bobcat populations in southern Georgia and northern Florida, this thesis presented the opportunity to investigate some of the little known aspects of the bobcat's life history.

One of our objectives was to examine within-population spatial genetic structure, which would demonstrate whether bobcats fit the expected mammalian pattern of male-biased dispersal and female-biased philopatry. Within a continuously distributed population, sex-biased dispersal and philopatry cause kin clustering of the sedentary sex, resulting in a nonrandom spatial distribution of genotypes (i.e., spatial genetic structure) (e.g., Ishibashi *et al*. 1997, Knight *et al.* 1999, Surridge *et al*. 1999, Cutrera *et al*. 2005). Although no predictions were made regarding the male segment of the population, we expected that overall, adult females would be more related to one another within the population than males, first-degree adult female relatives would be closer spatially than unrelated adult females, and adult females would demonstrate a negative association between relatedness and spatial distance.

Our other objective was to examine the between-population genetic structure of our four bobcat populations, which would elucidate whether populations across a relatively large spatial scale were genetically different, whether these populations fit the isolation by distance model, and whether sex-biased dispersal was occurring between populations. Differences in dispersal rates between populations are expected to lead to the isolation by distance patterning, in which spatially close populations are more genetically similar than those that are further apart (Wright 1969, Sokal and Wartenberg 1983, Balloux and Lugon-Moulin 2002, Vekemans and Hardy 2004). The presence of male sex-biased dispersal would cause males between populations to be more genetically similar than females between populations (Goudet *et al*. 2002). Although we did not make predictions regarding the level of differentiation between populations, we supposed that our four sampled populations would fit the isolation by distance model. Additionally, we expected these populations would exhibit sex-biased dispersal and philopatry, thereby resulting in genetic similarity among males across space.

The results of our within-population spatial genetic structure analyses did not provide evidence of sex-biased dispersal and philopatry. Adult females were not more related as a group than adult males, though the male group relatedness did differ from zero; adult female first-degree relatives did not settle closer to one another than unrelated pairs of adult females; and adult females had a negative relationship between spatial distance and relatedness, while adult males had a positive relationship, though these associations were not significant.

Our between-population genetic analyses suggested that our four populations were actually one panmictic unit, demonstrating that our sampled bobcats were widely dispersing animals. Genetic similarity over a broad spatial area implies that populations throughout that area may be interacting. We found that our sampled populations did not fit the isolation by distance model, meaning that genetic similarity between populations was not related to spatial proximity. Furthermore, there was no significant evidence supporting the occurrence of sex-biased dispersal, rather males and females did not differ from one another across space. However, the sexes fit their expected F_{ST} patterning – females had a larger F_{ST} value across populations than males.

While a past study and published literature indicate that bobcats evince male-biased dispersal and female-biased philopatry, reports were anecdotal (Chamberlain and Leopold 2001, Anderson and Lovallo 2003) or the sample size was small (Janečka *et al*. 2006). Additionally, dispersal is influenced by many ultimate and proximate factors, and as such, dispersal patterns are likely to differ between individuals, populations, and species (Waser and Jones 1983, Dobson and Jones 1985, Waser 1985, Shields 1987). Although predictions made based on the bobcat's mating systems suggest that sex-biased dispersal may eventuate, and there is evidence for sex-biased dispersal, it was not unexpected that our populations might exhibit a different patterning. Our results demonstrate that dispersal patterns cannot be predicted from a species' mating system alone, rather other ultimate and proximate factors, such as inbreeding avoidance (Pusey and Wolf 1996) and population density and associated competition (Christian 1970, Crowe 1975, Aars and Ims 2000, Perrin and Mazalov 2000), should be considered.

Aside from differing ultimate and proximate factors influencing dispersal, there are innumerable other reasons why our populations did not fit expectations, for both our within- and between-population analyses. Most obvious was our sampling of genetic variability via microsatellite loci and individuals. Increasing the number of individuals sampled in a population would more effectively capture the genetic variability of that population as well as potentially sample any immigrants or philopatric individuals. Thus, by sampling a larger number of individuals, estimates of population differentiation and sex-biased dispersal are generally improved (Gaggiotti *et al*. 1999, Goudet *et al*. 2002). Moreover, when sampling individuals, age data needs to be collected and made available in order to exclude juveniles and accurately test for sex-biased dispersal (Goudet *et al*. 2002). Age data were only available for one of the four populations used in this study. By utilizing a larger number of loci, variance around relatedness estimates would be reduced (Queller and Goodnight 1989, Blouin *et al*. 1996) and genetic variability of populations would be better sampled, thereby lessening the variance and improving the power of population differentiation and sex-biased dispersal tests (Gaggiotti *et al*. 1999, Balloux and Goudet 2002, Goudet *et al*. 2002). However, an increase in the number of loci used may increase the genotyping error rate of the study (Waits and Leberg 2000, McKelvey and Schwartz 2004).

Lastly, our sampled bobcat populations may not fit the expected population differentiation and sex-biased dispersal patterning because of the bobcat's ability to disperse long distances (e.g., Knick and Bailey 1986). Widely dispersing animals may not generate localized spatial genetic structure (Peakall *et al*. 2003) because dispersal across great distances prevents differentiation between populations (Wright 1943). Additionally, our methods may not have been powerful enough to detect sex-biased dispersal when there is a low bias in dispersal (Goudet *et al*. 2002). In winter 2005 on the Jones Center, two adult females possessing a first-degree relationship were within 250 m of one another based on a trapping location and a home range center, and only one of the two females was radio-collared and had a delineated home range. This one incident suggests that philopatry may be occurring, though in a limited capacity. By supplementing this study with demographic data and following individuals from kittenhood into and through adulthood, the extent of philopatry can be better defined. It remains to be seen whether bobcats regularly follow sex-biased dispersal and philopatric patterning or if this one incident resulted from nothing more than chance.

Unfortunately, we were unable to exclude all error from our study, though we did reduce the error rate considerably. It is possible that the error rate affected our relatedness estimates, population differentiation estimates, and sex-biased dispersal tests. However, we believe that even with the known error, our data and results are robust. The effects of error on pairwise relatedness estimates are expected to be minimal because these estimates do not involve any exclusion of pairs (Wang 2004). Our population differentiation estimates were only minimally influenced by error as well because our error rate was relatively small and error occurred across all populations.

In summary, although there were some instances where our sampled bobcats fit their expected patterns, overall, we found no significant evidence that sex-biased dispersal was taking place. Moreover, our results suggest that bobcat populations sampled within our spatial scale were not genetically differentiated, implying that bobcat management at local spatial scales should consider managing bobcats across a larger spatial scale and taking a landscape-level perspective. Additionally, certain management practices, such as bobcat removal, may influence bobcat movement (Lynch 2005) by creating home range openings near a juvenile's natal area, thus negating the need to disperse off site and allowing juveniles to settle near their natal area (Crowe 1975). Further analyses are needed to determine how management practices influence bobcat social and spatial organization. Studies should also examine how habitat quality, as reflected by dominant cover types and prey abundance, influences female home range overlap. While home range overlap may be indicative of tolerance between philopatric relatives (Waser and Jones 1983), we found little evidence of philopatry. Thus, female home range overlap may be resulting from other causes, such as the distribution of resources (Bailey 1974, Fendley and Buie 1986, Sandell 1989), density of conspecifics (Fendley and Buie 1986), and/or climate (Bailey 1981). We plan to investigate female home range overlap as it relates to habitat quality and genetic relatedness in a forthcoming study. Finally, studies would benefit from the inclusion of more loci, individuals, and populations, and though these additions should clarify the bobcat's dispersal patterning, future studies might also consider how habitat characteristics or corridors influence the populations' conformance to the isolation by distance model.

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