ABUNDANCE, HABITAT USE, AND GENETIC STRUCTURE OF COYOTES (*CANIS LATRANS*) IN SOUTH CAROLINA, USA

by

JORDAN L. YOUNGMANN

(Under the Direction of Gino J. D'Angelo)

ABSTRACT

Coyotes (*Canis latrans*) colonized the eastern USA during the last century, impacting local ecosystems and restructuring trophic dynamics. In the southeastern USA, coyotes exert competitive pressure on mesopredator guilds and consumptive impacts on prey populations, including important game species such as white-tailed deer (*Odocoileus virginianus*) and wild turkeys (*Meleagris gallopavo*). However, population dynamics, including resident and transient life strategies, make managing coyote populations difficult. A growing body of research advocates for state agencies to reassess harvest recommendations for impacted game populations as opposed to relying on lethal coyote control. Further understanding of coyote population dynamics and behavior is necessary to effectively respond to this top predator on the southeastern USA landscape. From 2019 – 2021, I employed non-invasive genetic sampling, spatial capture-recapture analysis, DNA metabarcoding diet analysis, and population genetics to assess coyote density, resource selection, diet, and genetic structure in South Carolina, USA, a state that has experienced recent deer declines concurrent with coyote colonization. I found that coyote densities existed heterogeneously across the state in relation to the availability of open/early successional landcover types. Densities across all sites were 8.73 coyotes/100km^2 in

2019 and 8.20 coyotes/100km^2 in 2020 but site-specific densities ranged from 1.74 – 27.48 coyotes/100km^2. Coyote movements across Alabama, Georgia, and South Carolina, USA revealed foraging behavior associated with open/early successional landcover and an avoidance of primary and secondary roads. Coyotes also shifted foraging towards forest landcover types during April – June, potentially in response to the availability of fawns. Metabarcoding analysis revealed that coyotes consumed a variety of vertebrate prey species from May – June, including a large proportion of deer, but showed low levels of wild turkey consumption. Finally, I documented genetic panmixia among coyote populations across South Carolina, USA with low levels of relatedness at local geographic scales. Future management of coyotes should recognize the difficulties of controlling a far-reaching mosaic of coyote populations and seek to reassess management goals for game species, especially in regions that support high coyote densities.

INDEX WORDS: coyote, *Canis latrans*, DNA metabarcoding, foraging, non-invasive genetic sampling, population genetics, resource selection, spatially explicit capture-recapture

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by

JORDAN L. YOUNGMANN

B.S., Hobart College, 2010

M.S., Mississippi State University, 2018

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by

JORDAN L. YOUNGMANN

Major Professor:	Gino J. D'Angelo
Committee:	Michael J. Chamberlain
	Richard B. Chandler
	John C. Kilgo
	Stacey L. Lance
	Karl V. Miller

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
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DEDICATION

This dissertation is dedicated to my wife, Sarah, and to my two children, Linden and Hollis. If you are to pair yourself with another person, find someone who is smarter, wiser, and more lighthearted than yourself. I cannot express the luck I possess in having found this absolute gem of a wife. As for my children, thanks for the wonderful gift of perspective, nothing compares to your laughter and joy.

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CHAPTER 1

INTRODUCTION

Coyotes (*Canis latrans*) are a mid-sized canid that have lived in North America since the Pleistocene (Tedford et al. 2009, Wang and Tedford 2010). Although the historic range of coyotes has been typified by periodic expansion and contraction (Hody and Kays 2018), coyotes are recognized as endemic to the western United States of America (USA) prior to European colonization (Young and Jackson 1951, Hody and Kays 2018). However, over the last century, coyotes have emigrated out of the western USA and Mexico, and eastward across North America (Moore and Parker 1992, Hody and Kays 2018). Coyotes colonized eastward along two fronts: northward through the Upper Midwest, USA into Canada and the northeast USA, and southward into the southeastern USA and along the eastern seaboard (Hody and Kays 2018). Expansion of coyotes into the eastern USA was made possible partially through the extirpation of apex carnivores such as wolves (*Canis* spp.) and mountain lions (*Puma concolor*) east of the Mississippi River. Coyotes have now occupied the eastern USA temperate forests since at least the second half of the 20th century and are currently present in all states east of the Mississippi River (Moore and Parker 1992, Hody and Kays 2018).

Along the eastward expanding colonization routes, the leading front of immigrating coyotes hybridized with endemic wolf species. North of the Great Lakes, coyotes introgressed with gray and eastern wolves (*C. lupus, C. lyacon*, vonHoldt 2016*a*,*b*) and along the Gulf of Mexico and into southeastern USA, coyotes introgressed with remnant populations of red wolves (*C. rufus*, Hinton et al. 2019, Heppenheimer et al. 2020). Recent genetic analyses demonstrated

that these colonization fronts have met in Virginia or North Carolina (Bozarth et al. 2011, Heppenheimer et al. 2018). As a result of admixture with wolves and selective pressure due to rapid range expansion, eastern coyotes are morphologically larger than western coyotes (Hinton et al. 2019). In the absence of endemic top predators, coyotes have become one of the few large predators on the eastern landscape (Gompper 2002; Roemer et al. 2009). The ecological role coyotes now play in the eastern USA has become an important topic of research, with implications for wildlife management and conservation.

Traditionally recognized as generalist mesopredators, coyotes exhibit a broad and varied diet, including white-tailed deer (*Odocoileus virginianus*, hereafter deer), small mammals such as the cotton rat (*Sigmodon* spp.), lagomorphs, soft mast including *Rubus* spp. and persimmons (*Diospyros virginiana*), and insects (Schrecengost et al. 2008, Swingen et al. 2015, Cherry et al. 2016, Ward et al. 2018, Hinton et al. 2021, Jensen et al. 2022). Dietary overlap with sympatric species such as bobcats (*Lynx rufus*), red foxes (*Vulpes vulpes*), and gray foxes (*Urocyon cinereoargenteus*) has resulted in competition between coyotes and endemic mesopredators in the eastern USA (Egan et al. 2021, Webster et al. 2021, Dyck et al. 2022). In some cases, coyotes have outcompeted conspecific canids with broad scale declines in gray fox populations and localized extirpation of red foxes (Major and Sherburne 1987, Harrison et al. 1989, Levi and Wilmers 2012, Egan et al. 2021). Competitive exclusion of bobcats by coyotes has also been documented, especially when prey availability is limited (see Dyck et al. 2022 for a full review).

In the southeastern USA, it is well documented that coyotes predate heavily on deer, an important game species in the USA (Saalfield and Ditchkoff 2007, Kilgo et al. 2012, Chitwood et al. 2014). Although predation was first observed on fawns, it is now clear that adult deer are also susceptible to coyote predation, though the prevalence of such events is debated (Chitwood

et al. 2014, Cherry et al. 2016, Ward et al. 2018). Increased predation by coyotes acted concurrently with an increased emphasis on harvest of antlerless deer across southeastern states during the 1990s and 2000s (Adams et al. 2009, Kilgo et al. 2012). Predation and harvest have exerted additive downward pressure on deer populations in some areas and may partially explain the decline in some deer populations observed in portions of the southeastern USA over the last few decades (Kilgo et al. 2010, Chitwood et al. 2015, Nelson et al. 2015). Although there is some debate about the extent of impacts coyotes have had on eastern deer populations following coyote colonization (Bragina et al. 2019*a*, *b*, Kilgo et al. 2019), local evidence of high fawn mortality has raised concerns over how to manage a novel predator to best mitigate depredation on important game species.

Although VanGilder et al. (2009) documented that intensive coyote removal may increase fawn recruitment over a short timeframe at limited geographic scale, subsequent research in the southeastern USA indicated that coyote control may not be as effective at broader spatial scales (Kilgo et al. 2014, Gulsby et al. 2015, Kilgo et al. 2017). Kilgo et al. (2012, 2014) and Robinson et al. (2014) have suggested that reductions in antlerless harvest may be a more viable response to deer population declines than predator control. However, Chitwood et al. (2015) indicated reduction in antlerless harvest may not be sufficient in scenarios with low deer-density and high predation of neonates. Considering the relative ineffectiveness of coyote removal, future management decisions may be dependent on improved understanding of coyote abundance across each state, which will better inform whether deer harvest regulations are in need of reevaluation.

Coyote abundance may vary across the landscape due to habitat preferences (Kays et al. 2008, Schrecengost et al. 2009, Hinton et al. 2015, Cherry et al. 2017, Ward et al. 2018).

Although Kays et al. (2008) documented a preference by coyotes for forested landscapes in the Adirondacks, NY, coyote abundance was most associated with disturbed forests with open canopies. However, in the southeastern USA coyotes prefer open, often agricultural landscapes (Schrecengost et al. 2009, Hinton et al. 2015, Cherry et al. 2017). In a study encompassing 10,530 km^2 across southeastern Alabama and the Savannah River area of Georgia and South Carolina, Ward et al. (2018) found that vegetative density influenced prey use by coyotes across the landscape where predation of deer was associated with less dense vegetation. The association between prey availability and coyote abundance has been thoroughly documented in the literature, where density, reproductive rates, and juvenile dispersal have all been linked with resource availability (Bekoff and Gese 2003). If habitat can influence both abundance and prey use by coyotes, estimation of variation in coyote densities across broad geographic areas should incorporate differences in landscape-level characteristics.

Estimating abundances of predators can be difficult due to their cryptic behavior and relatively low densities on the landscape. Past research used scent stations (Goff 1979, Twiss 2006, VanGilder et al. 2009), howl elicitations (Sharp 1981, Okoniewski and Chambers 1984, Crawford et al. 1993, Cherry et al. 2017), and track and scat deposition surveys (Goff 1979, VanGilder et al. 2009, Kilgo et al. 2014) to estimate or index abundance and density of coyote populations. Non-invasive genetic sampling has also been used to estimate abundance of wild populations (Taberlet and Luikart 1999, Waits 2004). Non-invasive genetic techniques primarily use DNA collected from scat to identify individuals and build encounter histories for mark-recapture models (Bozarth et al. 2015, Garwood et al. 2015, Gulsby et al. 2015, Gulsby et al. 2016, Morin et al. 2016).

Since the colonization of the eastern USA, study of 2nd and 3rd-order resource selection by coyotes has been a primary area of research (Thornton et al. 2004, Crimmins et al. 2012, Hinton et al. 2015, Hickman et al. 2016, Stevenson et al. 2019). However, coyote foraging behavior is still poorly understood (Mastro 2011, Mastro et al. 2011). Whereas behavioral studies in the western USA are aided by direct observation of coyote movements in open landscapes, the eastern USA is hindered by heavily forested regions that do not allow for ground truthing remote movement data to ascertain individual behaviors such as searching, hunting, and traveling. Furthermore, how landscape characteristics influence foraging in heterogeneous environments such as the eastern USA is unknown (Ward et al. 2018). A fine-scale understanding of resource selection during foraging could help explain demographic processes of pack stability, dispersal, and density of coyote populations as well as provide a mechanism by which coyotes consume prey.

Finally, coyote demographics in the southeastern USA have been described as a dynamic population maintained by mobile, transient coyotes interspersed among localized, resident coyotes (Hinton et al. 2015, Kierepka et al. 2017, Morin and Kelly 2017). Mills and Allendorf (1996) proposed the "one migrant per generation" hypothesis which posits that anywhere from 1 to 10 immigrants are required to maintain gene flow between populations. Transient coyotes have been documented to disperse over 200 km (Hinton et al. 2012, Sasmal et al. 2019). Thus, coyotes in the southeastern USA demonstrate weak genetic structure due to high levels of gene flow across the region (Kierepka et al. 2017, Hinton et al. 2019). Although studies in California have identified population structure along habitat clines (Sacks et al. 2008), research on Southeastern coyotes has found little genetic structure (Heppenheimer et al. 2017, Kierepka et al. 2017, and Hinton et al. 2019). However, Heppenheimer et al. (2017) and Hinton et al. (2019)

assessed populations across the entire east coast and the entire southeastern USA, respectively, while Kierepka et al. (2017) only looked at populations within the Savannah River Site (SRS) in South Carolina, USA. Determining the extent to which coyote populations are maintained by transient individuals may require assessing genetic structure at an intermediate scale where single individuals may connect distant groups but may not exceed the threshold required to maintain gene flow at a level that surpasses genetic drift (Mills and Allendorf 1996). Similar to Sacks et al. (2008), an intermediate scale study of coyotes in the southeastern USA may provide geographic coverage that encompasses multiple subpopulations while also being at a fine enough scale to detect genetic structure.

I studied coyote populations at two scales: individual sample sites ranging from 300-1200 km^2 and across the state of South Carolina. South Carolina has experienced rapid coyote population expansion and several studies documenting the effects of coyotes on deer have been conducted within the state (Kilgo et al. 2010, 2012, 2014). In Chapter 2, I used non-invasive genetic sampling and spatially explicit capture-recapture to estimate coyote densities among three ecoregions. I assessed whether broad landscape-level characteristics were important to variation in coyote density. In Chapter 3, I used movement data from a previous tri-state study across Alabama, Georgia, and South Carolina to investigate foraging behavior of coyotes across four seasons. In Chapter 4, I used DNA metabarcoding to investigate coyote consumption of vertebrates in South Carolina during deer fawning and turkey nesting and poult-rearing. Finally, in Chapter 5, I assessed population genetic structure of coyotes in South Carolina to determine the spatial scale at which populations are maintained through transient individuals. These individual components of my study add to the existing literature on coyote population dynamics in a meaningful way, as well as provide state agencies with an effective methodology to assess

coyote abundance across a broad geographic scale. Both management of coyote populations and responsive management of game species relies on research that evaluates coyotes across a broad geographic extent.

South Carolina encompasses three major ecoregions: Piedmont, Southeastern Plains, and Middle Atlantic Coastal Plain (Omernik 1987). Ecoregions are based on abiotic and biotic characteristics and represent categorically different landscapes (Griffith et al. 2002). Coyotes have been present in South Carolina since the 1980s (Hill et al. 1987, Mayer et al. 2005) and have rapidly spread across the state (Hody and Kays 2018, Ruth and Cantrell 2021). Coyotes harvested during fall and winter deer season or reported taken by trapping efforts provide an index of abundance across the state and show a steady population increase until around 2014, followed by a plateau and slight decrease (Figure 1.1). Abundance estimates for coyotes were previously conducted at the USA Department of Energy Savannah River Site (SRS) where coyote densities were estimated using howl surveys to be 80-150 coyotes/100km^2 (Schrecengost 2007). However, a state-wide survey of coyote populations has not been conducted even though assessment of coyote populations across broader geographic regions has been suggested to better capture demographic characteristics of this mobile species (J. W. Hinton and M. J. Chamberlain, unpublished report). I endeavored to study coyotes across South Carolina to assess populations that have stabilized following colonization and rapid expansion.

LITERATURE CITED

Adams, K., J. Hamilton, and M. Ross. 2009. QDMA's Whitetail Report. Quality Deer Management Association, Bogart, GA, USA.

Bekoff, M., and E. M. Gese. 2003. Coyote (*Canis latrans*). Pages 467–481 *in* G. A. Feldhamer, B. C. Thompson, and J. A. Chapman, editors. Wild mammals of North America: biology, management, and conservation. Second edition. Johns Hopkins University Press, Baltimore, Maryland, USA.

Bozarth, C. A., F. Hailer, L. L. Rockwood, C. W. Edwards, and J. E. Maldonado. 2011. Coyote colonization of northern Virginia and admixture with Great Lakes wolves. Journal of Mammalogy 92:1070–1080.

Bozarth, C. A. B. Gardner, L. L. Rockwood, and J. E. Maldonado. 2015. Using fecal DNA and spatial capture-recapture to characterize a recent coyote colonization. Northeastern Naturalist 22:144–162.

Bragina, E. V., R. Kays, A. Hody, C. E. Moorman, C. S. DePerno and L. S. Mills. 2019*a*. Effects on white-tailed deer following eastern coyote colonization. Journal of Wildlife Management 83:916–924.

Bragina, E. V., R. Kays, A. Hody, C. E. Moorman, C. S. DePerno and L. S. Mills. 2019*b*. White-tailed deer and coyote colonization: a response to Kilgo et al. (2019). Journal of Wildlife Management 83:1641–1643.

Cherry, M. J., K. L. Turner, M. B. Howze, B. S. Cohen, L. M. Conner, and R. J. Warren. 2016. Coyote diets in a longleaf pine ecosystem. Wildlife Biology 22:64–70.

Cherry, M. J., P. E. Howell, C. D. Seagraves, R. J. Warren, and L. M. Conner. 2017. Effects of land cover on coyote abundance. Wildlife Research 43:662–670.

Chitwood, M. C., M. A. Lashley, C. E. Moorman, and C. S. DePerno. 2014. Confirmation of coyote predation on adult female white-tailed deer in the southeastern United States. Southeastern Naturalist 13:N30–N32.

Chitwood, M. C., M. A. Lashley, J. C. Kilgo, C. E. Moorman, and C. S. DePerno. 2015. White-tailed deer population dynamics and adult female survival in the presence of a novel predator. Journal of Wildlife Management 79:211–219.

Crawford, B. A., M. R. Pelton, and K. G. Johnson. 1993. Techniques to monitor relative abundance of coyotes in east Tennessee. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 47:62–70.

Crimmins, S. M., J. W. Edwards, and J. M. Houben. 2012. *Canis latrans* (Coyote) habitat use and feeding habits in central West Virginia. Northeastern Naturalist 19:411–420.

Dyck, M. A., E. Wyza, and V. D. Popescu. 2021. When carnivores collide: a review of studies exploring the competitive interactions between bobcats *Lynx rufus* and coyotes *Canis latrans*. Mammal Review mam.12260. doi:10.1111/mam.12260.

Egan, M. E., C. C. Day, T. E. Katzner, and P. A. Zollner. 2021. Relative abundance of coyotes (*Canis latrans*) influences gray fox (*Urocyon cinereoargenteus*) occupancy across the eastern United States. Canadian Journal of Zoology 99:63–72.

Garwood, T., R. Ziobro, K. Keene, A. Waller, and J. N. Pauli. 2015. Diet, habitat use, and size of an urban population of coyotes obtained noninvasively. Urban Naturalist 5:1–8.

Goff, G.R. 1979. Analysis and evaluation of three indices of eastern coyote abundance. Thesis, College of Environmental Science and Forestry, Syracuse, New York, USA.

Gompper, M. E. 2002. Top carnivores in the suburbs? Ecological and conservation issues raised by colonization of northeastern North America by coyotes. BioScience 52:185–190.

Griffith, G. E., J. M. Omernik, J. A. Comstock, M. P. Shafale, W. H. McNab, D. R. Lenat, J. B. Glover, and V. B. Shelburne. 2002. Ecoregions of North Carolina and South Carolina. US Geological Survey, Reston, VA, USA.

Gulsby, W. D., C. H. Killmaster, J. W. Bowers, J. D. Kelly, B. N. Sacks, M. J. Statham, and K. V. Miller. 2015. White-tailed deer fawn recruitment before and after experimental coyote removals in central Georgia. Wildlife Society Bulletin 39:248–255.

Gulsby, W. D., C. H. Killmaster, J. W. Bowers, J. S. Laufenberg, B. N. Sacks, M. J. Statham, and K. V. Miller. 2016. Efficacy and precision of fecal genotyping to estimate coyote abundance. Wildlife Society Bulletin 40:792–799.

Harrison, D. J., J. A. Bissonette, and J. A. Sherburne. 1989. Spatial relationships between coyotes and red foxes in eastern Maine. Journal of Wildlife Management 53:181–185.

Heppenheimer, E., K. E. Brzeski, J. W. Hinton, B. R. Patterson, L. Y. Rutledge, A. L. DeCandia, T. Wheeldon, S. R. Fain, P. A. Hohenlohe, R. Kays, B. N. White, M. J. Chamberlain, and B. M. vonHoldt. 2018a. High genomic diversity and candidate genes under selection associated with range expansion in eastern coyote (*Canis latrans*) populations. Ecology and Evolution 8:12641–12655.

Heppenheimer, E., D. S. Cosio, K. E. Brzeski, D. Caudill, K. Van Why, M. J. Chamberlain, J. W. Hinton, and B. vonHoldt. 2018b. Demographic history influences spatial patterns of genetic diversity in recently expanded coyote (*Canis latrans*) populations. Heredity 120:183–195.

Heppenheimer, E., K. E. Brzeski, J. W. Hinton, M. J. Chamberlain, J. Robinson, R. K. Wayne, and B. M. vonHoldt. 2020. A genome-wide perspective on the persistence of red wolf ancestry in southeastern canids. Journal of Heredity 111:277–286.

Hickman, J. E., W. D. Gulsby, C. H. Killmaster, J. W. Bowers, M. E. Byrne, M. J. Chamberlain, and K. V. Miller. 2016. Home range, habitat use, and movement patterns of female coyotes in Georgia: implications for fawn predation. Journal of the Southeastern Association of Fish and Wildlife Agencies 2:144–150.

Hill, E. P., P. W. Sumner, and J. B. Wooding. 1987. Human influences on range expansion of coyotes in the Southeast. Wildlife Society Bulletin 15:521–524.

Hinton, J. W., M. J. Chamberlain, and F. T. Van Manen. 2012. Long-distance movements of transient coyotes in eastern North Carolina. American Midland Naturalist 168:281–288.

Hinton, J. W., F. T. Van Manen, and M. J. Chamberlain. 2015. Space use and habitat selection by resident and transient coyotes (*Canis latrans*). PLoS ONE 10:e0132203.

Hinton, J. W., E. Heppenheimer, K. M. West, D. Caudill, M. L. Karlin, J. C. Kilgo, J. J. Mayer, K. V. Miller, M. Walch, B. vonHoldt, and M. J. Chamberlain. 2019. Geographic patterns in morphometric and genetic variation for coyote populations with emphasis on southeastern coyotes. Ecology and Evolution 9:3389–3404.

Hinton, J. W., K. Rountree, and M. J. Chamberlain. 2021. Diet of coyotes on the Tensas River National Wildlife Refuge during the white-tailed deer pre-fawning and fawning seasons. Southeastern Naturalist 20:245–258.

Hody, J. W., and R. Kays. 2018. Mapping the extension of coyotes (*Canis latrans*) across North and Central America. ZooKeys 759:81–97.

Jensen, A. J., C. J. Marneweck, J. C. Kilgo, and D. S. Jachowski. 2022. Coyote diet in North America: geographic and ecological patterns during range expansion. Mammal Review mam.12299.

Kays, R. W., M. E. Gompper, and J. C. Ray. 2008. Landscape ecology of eastern coyotes based

on large-scale estimates of abundance. Ecological Applications 18:1014–1027.

Kierepka, E. M., J. C. Kilgo, and O. E. Rhodes, Jr. 2017. Effect of compensatory immigration on the genetic structure of coyotes. Journal of Wildlife Management 81:1394–1407.

Kilgo, J. C., H. S. Ray, C. Ruth, and K. V. Miller. 2010. Can coyotes affect deer populations in southeastern North America? Journal of Wildlife Management 74:929–933.

Kilgo, J. C., H. S. Ray, M. Vukovich, M. J. Goode, and C. Ruth. 2012. Predation by coyotes on white-tailed deer neonates in South Carolina. Journal of Wildlife Management 76:1420–1430.

Kilgo, J. C., M. Vukovich, H. Scott Ray, C. E. Shaw, and C. Ruth. 2014. Coyote removal, understory cover, and survival of white-tailed deer neonates. Journal of Wildlife Management 78:1261–1271.

Kilgo, J. C., C. E. Shaw, M. Vukovich, M. J. Conroy, and C. Ruth. 2017. Reproductive characteristics of a coyote population before and during exploitation. Journal of Wildlife Management 81:1386–1393.

Kilgo, J. C., M. J. Cherry, S. S. Ditchkoff, W. D. Gulsby, and K. V. Miller. 2019. Coyotes and white-tailed deer populations in the east: A comment on Bragina et al. (2019). Journal of Wildlife Management 83:1636–1640.

Levi, T., and C. C. Wilmers. 2012. Wolves–coyotes–foxes: a cascade among carnivores. Ecology 93:921–929.

Major, J. T., and J. A. Sherburne. 1987. Interspecific relationships of coyotes, bobcats, and red foxes in western Maine. Journal of Wildlife Management 51:606–616.

Mastro, L. L. 2011. Life history and ecology of coyotes in the Mid-Atlantic States: a summary of the scientific literature. Southeastern Naturalist 10:721–730.

Mastro, L. L., E. M. Gese, J. K. Young, and J. A Shivik. 2012. Coyote (*Canis latrans*), 100 + years in the east: a literature review. Addendum to the Proceedings of the 14th Wildlife Damage Management Conference 14:129–131.

Mayer, J. J., L. D. Wike, and M. B. Caudell. 2005. Furbearers. Pages 366–373 *in* J. C. Kilgo and J. I. Blake, editors. Ecology and management of a forested landscape: fifty years on the Savannah River Site. Island Press, Washington, DC, USA.

Mills, S. K., and F. W. Allendorft. 1996. The one-migrant-per-generation rule in conservation and management. Conservation Biology 10:1509–1518.

Moore, G. C., and G. R. Parker. 1992. Colonization by the eastern coyote (*Canis latrans*). Pages 23–37 *in* A. H. Boer, editor. Ecology and management of the eastern coyote. Wildlife Resources Unit, University of New Brunswick, Fredericton, New Brunswick, Canada.

Morin, D. J., M. J. Kelly, and L. P. Waits. 2016. Monitoring coyote population dynamics with fecal DNA and spatial capture–recapture. Journal of Wildlife Management 80:824–836.

Morin, D. J., and M. J. Kelly. 2017. The dynamic nature of territoriality, transience and biding in an exploited coyote population. Wildlife Biology 2017:wlb.00335.

Nelson, M. A., M. J. Cherry, M. B. Howze, R. J. Warren, and M. L. Conner. 2015. Coyote and bobcat predation on white-tailed deer fawns in a longleaf pine ecosystem in southwestern Georgia. Journal of the Southeastern Association of Fish and Wildlife Agencies 2:208–213.

Okoniewski, J. C., and R. E. Chambers. 1984. Coyote vocal response to an electronic siren and human howling. Journal of Wildlife Management 48:217–222.

Omernik, J. M. 1987. Ecoregions of the conterminous United States. Annals of the Association of American Geographers 77:118–125.

Robinson, K. F., D. R. Diefenbach, A. K. Fuller, J. E. Hurst, and C. S. Rosenberry. 2014. Can managers compensate for coyote predation on white-tailed deer? Journal of Wildlife Management 78:571–579.

Roemer, G. W., M. E. Gompper, and B. Van Valkenburgh. 2009. The ecological role of the mammalian mesocarnivore. BioScience 59:165–173.

Ruth, C. R. and J. Cantrell. 2021. South Carolina deer harvest report. South Carolina Department of Natural Resources, Columbia, USA.

Saalfeld, S. T., and S. S. Ditchkoff. 2006. Survival of neonatal white-tailed deer in an exurban population. Journal of Wildlife Management 71:940–944.

Sacks, B. N., S. K. Brown, and H. B. Ernest. 2008. Population structure of California coyotes corresponds to habitat-specific breaks and illuminates species records. Molecular Ecology 13:1265–1275.

Sasmal, I., C. E. Moorman, M. B., Swingen, S. Datta, and C. S. DePerno. 2018. Seasonal space use of transient and resident coyotes (*Canis latrans*) in North Carolina, USA. Canadian Journal of Zoology 97:326–331.

Schrecengost, J. D. 2007. Home range and food habits of the coyote (*Canis latrans*) at the Savannah River Site, South Carolina. Thesis, University of Georgia, Athens, USA.

Schrecengost, J. D., J. C. Kilgo, D. Mallard, H. S. Ray, and K. V. Miller. 2008. Seasonal food habits of the coyote in the South Carolina Coastal Plain. Southeastern Naturalist 7:135–144.

Schrecengost, J. D., J. C. Kilgo, H. S. Ray, and K. V. Miller. 2009. Home range, habitat use and survival of coyotes in western South Carolina. American Midland Naturalist 162:346–355.

Sharp, D. W. 1981. Siren-elicited responses of coyotes in western Kentucky and Tennessee. Proceedings of the Annual Conference of Fish and Wildlife Agencies 35:273–280.

Stevenson, E. R., M. A. Lashley, M. C. Chitwood, J. E. Garabedian, M. B. Swingen, C. S. DePerno, and C. E. Moorman. 2019. Resource selection by coyotes (*Canis latrans*) in a longleaf pine (*Pinus palustris*) ecosystem: effects of anthropogenic fires and landscape features. Canadian Journal of Zoology 97:165–171.

Swingen, M. B., C. S. DePerno, and C. E. Moorman. 2015. Seasonal coyote diet composition at a low-productivity site. Southeastern Naturalist 14:397–404.

Taberlet, P., and G. Luikart. 1999. Non-invasive genetic sampling and individual identification. Biological Journal of the Linnean Society 68:41–55.

Tedford, R. H., X. Wang, and B. E. Taylor. 2009. Phylogenetic systematics of the North American fossil *Caninae* (Carnivora: Canidae). Bulletin of the American Museum of Natural History 325:1–218.

Thornton, D. H., M. E. Sunquist, and M. B. 2004. Ecological separation within newly sympatric populations of coyotes and bobcats in south-central Florida. Journal of Mammalogy 85: 973–982.

Twiss, R. R. 2006. The eastern coyote (*Canis latrans*) in Connecticut: relative abundance indices and public perception. Thesis, University of Connecticut, Storrs, USA.

VanGilder, C. L., R. G. Woods, and K. V. Miller. 2009. Effects of an intensive predator removal on white-tailed deer recruitment of northeastern Alabama. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 63:11–16.

vonHoldt, B. M., J. A. Cahill, Z. Fan, I. Gronau, J. Robinson, J. P. Pollinger, B. Shapiro, J. Wall,

and R. K. Wayne. 2016a. Whole-genome sequence analysis shows that two endemic species of North American wolf are admixtures of the coyote and gray wolf. Science Advances 2:e1501714.

vonHoldt, B. M., R. Kays, J. P. Pollinger, and R. K. Wayne. 2016b. Admixture mapping identifies introgressed genomic regions in North American canids. Molecular Ecology 25:2443–2453.

Waits, L.P. 2004. Using noninvasive genetic sampling to detect and estimate abundance of rare wildlife species. Pages 211–228 *in* W. L. Thompson, editor. Sampling rare or elusive species: concepts, designs, and techniques for estimating population parameters. Island Press, Washington, DC, USA.

Wang, X. and R. H. Tedford. 2010. Dogs: Their fossil relatives and evolutionary history. Columbia University Press, Chichester, UK.

Ward, J. N., J. W. Hinton, K. L. Johannsen, M. L. Karlin, K. V. Miller, and M. J. Chamberlain. 2018. Home range size, vegetation density, and season influences prey use by coyotes (*Canis latrans*). PLoS ONE 13:e0203703.

Webster, S. C., J. C. Beasley, J. W. Hinton, and M. J. Chamberlain. 2022. Resident and transient coyotes exhibit differential patterns of movement behavior across heterogeneous landscapes in the southeastern United States. Ecology and Evolution 12:e8725.

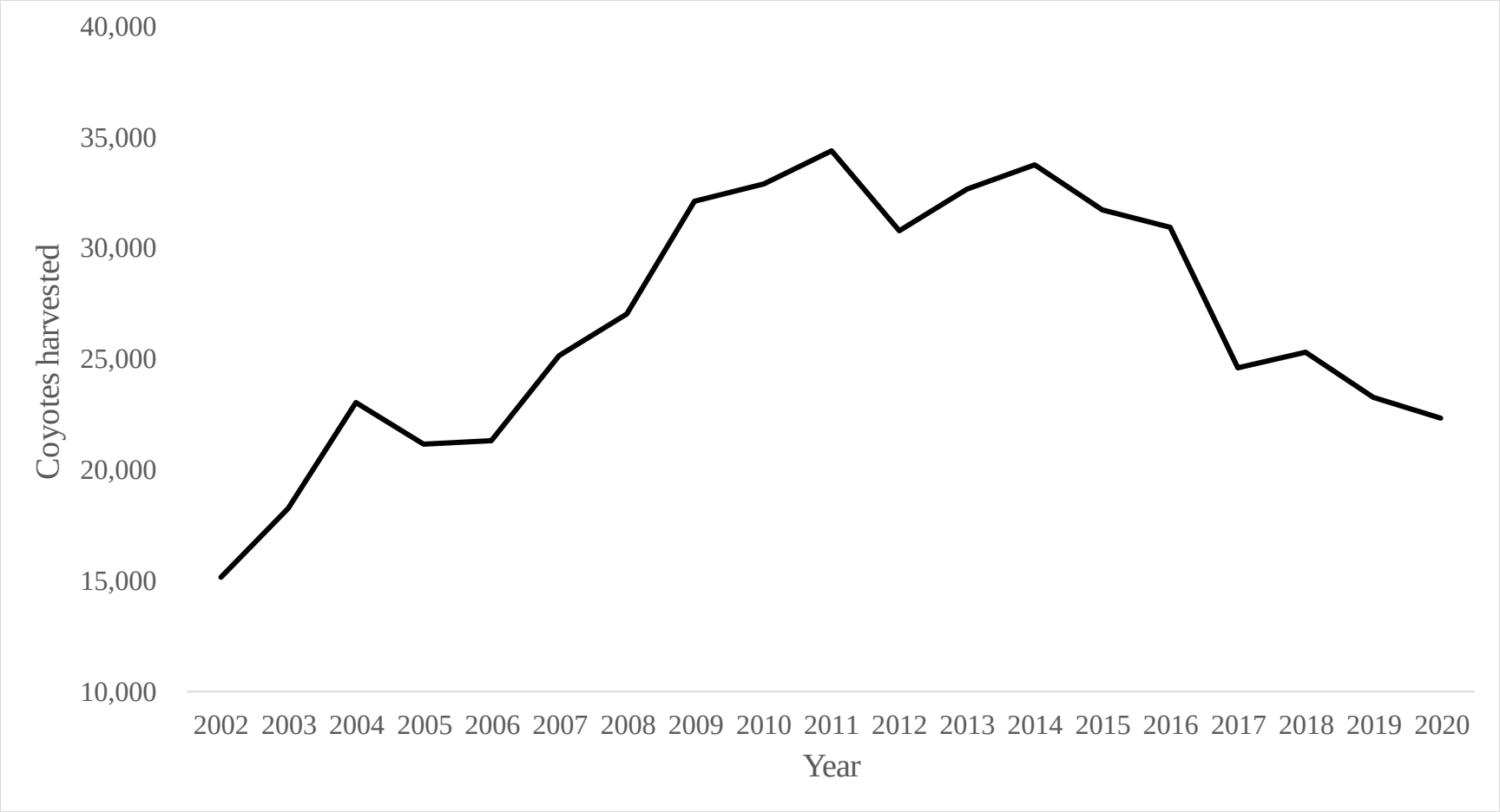

Figure 1.1. Combined coyote trapping and estimated harvest data from 2002 through 2020 in South Carolina. Data provided by C. Ruth, South Carolina Department of Natural Resources.

CHAPTER 2

DENSITY ESTIMATION OF COYOTES ACROSS SOUTH CAROLINA USING NON-INVASIVE GENETIC SAMPLING AND SPATIAL CAPTURE-RECAPTURE

Youngmann, J. L., J. C. Kilgo, S. L. Lance, R. B. Chandler, C. Ruth, J. Cantrell, and G. J. D'Angelo. 2023. To be submitted to the *Journal of Wildlife Management*.

ABSTRACT

Recent implementation of non-invasive genetic sampling and statistical tools such as spatially explicit capture-recapture have allowed for efficient estimation of population parameters, even among sparse distributions of cryptic species. Assessing population density is fundamentally important for understanding demographic processes and management of wildlife populations. As coyotes (*Canis latrans*) have colonized the eastern United States of America (USA) over the last century, accurate assessment of spatial variation in density has become vital for addressing ecosystem impacts that this novel predator may have on the eastern landscape. We employed non-invasive genetic sampling and spatially explicit capture-recapture to estimate coyote densities across 10 study sites in South Carolina, USA during the summers of 2019 and 2020. We collected a total of 272 coyote samples belonging to 171 unique individuals. We estimated coyote densities to be 8.73 coyotes/100km^2 in 2019 and 8.20 coyotes/100km^2 in 2020 across our study sites and observed that densities varied widely by site (1.74 – 27.48 coyotes/100km^2). Additionally, we determined that open/early successional land cover had a significant positive impact on coyote densities, likely a result of increased prey availability. Broadscale management of coyotes in the southeastern USA may be difficult due to the interplay between high- and low-density areas, resulting in rapid colonization by coyotes of exploited areas.

INTRODUCTION

Facilitated by the extirpation of large predators such as the eastern and red wolves (*Canis lyacon, C. rufus*) and cougar (*Puma concolor*), coyotes have expanded their range across most of North America over the last century (Hody and Kays 2018). Coyote expansion occurred along two major fronts; northward along the Great Lakes into New York and down the eastern seaboard, and southward along the Gulf Coast and up the eastern seaboard. Coyote colonization of the

eastern USA has resulted in limited hybridization with remnant populations of wolves as well as natural selection for larger individuals to facilitate rapid migration (Hinton et al. 2019). The eastern coyote is, therefore, relatively larger than endemic coyotes of western North America. Although coyotes are recognized as generalist carnivores, they are capable of predating large prey such as adult white-tailed deer (*Odocoileus virginanus,* hereafter referred to as deer; Chitwood et al. 2014). Since colonizing the eastern USA, coyotes have been implicated in population declines in prey species such as deer (Kilgo et al. 2010, 2012) and other predator populations such as red and gray foxes (Egan et al. 2021). Due to their size and impacts on endemic species, coyotes function as a top predator in the absence of wolves and cougars (Gompper 2002, Roemer et al. 2009).

Despite concerns that coyotes may be negatively impacting important game species in the southeastern USA, managers have struggled to mitigate the effects of increased coyote numbers through lethal control (Kilgo et al. 2014, Robinson et al. 2014, Gulsby et al. 2015). Coyotes exhibit resident and transient life history strategies where transient individuals maintain population dynamics across space, backfilling any loss of coyote numbers as space becomes available (Hinton et al. 2015, Kierepka et al. 2017, Morin and Kelly 2017). Although lethal control of coyotes has proven effective at small spatial scales (VanGilder et al. 2009), several studies have shown that the lethal removal of individuals results in rapid replacement of coyote numbers, likely via transient response (Kilgo et al. 2014, 2017, Gulsby et al. 2015). Additionally, coyotes have been shown to exhibit density dependent fecundity, resulting in more offspring in response to lowered population densities as a function of resource availability (Sterling et al. 1983, Conner et al. 1998). Therefore, biologists in the southeastern USA have begun to recognize the difficulty of broadscale lethal control to stable populations of coyotes. Instead,

management of games species, such as deer, should take into account coyote densities across a broad scale (Kilgo et al. 2010, 2012, 2014, Robinson et al. 2014). In regions of high coyote densities, changing deer harvest regulations may be an appropriate response to declines in deer herd numbers, including the reduction of antlerless harvest (Kilgo et al. 2010, 2012, Robinson et al. 2014).

Accurate estimation of population size and density is important for understanding population demographics and appropriately managing species (Williams et al. 2002, Royle et al. 2013, Morin et al. 2016). Advancements in statistical analysis such as spatial capture-recapture (SCR) and genetic technologies like non-invasive genetic sampling (NGS) have made it possible to quantify population size in species formerly considered too cryptic to effectively survey (Efford 2004, Waits and Paetkau 2005, Royle et al. 2013, Morin et al. 2016). Predator populations are particularly difficult to quantify because they often exist at low densities across the landscape and are challenging to trap using traditional capture-recapture methodologies (Balme et al. 2009). The use of unique identifiers, including individual distinctions in pelage such as spots or stripes, have allowed researchers to use camera traps to conduct population surveys of some cat species such as tigers, leopards, and jaguars (Royle et al. 2013). However, canids typically do not exhibit unique markings that allow for camera surveys to be used effectively. Instead, past researchers have assessed canid populations, including wolves, foxes, wild dogs, and coyotes, with NGS through scat collection or hair snares (Stenglein et al. 2010, Bozarth et al. 2015, Morin et al. 2016, Lonsinger et al. 2018, Roffler et al. 2019, Srivathsa et al. 2021). NGS studies have aided in the management of canid species across the globe.

Many studies have sought to understand resource selection of coyotes in the forested landscapes of the eastern USA. However, landcover influences on coyote population density are

still poorly understood. It is generally accepted that coyotes prefer open habitats, including early successional landcover and agricultural areas, while avoiding forest cover (Hinton et al. 2015, Ward et al. 2018, Youngmann et al. 2022). Cherry et al. (2016) used howl surveys to index coyote abundance in relation to landcover and posited that coyote numbers increased with open landcover, likely as a result of coyote preference for those landcover types. Kays et al. (2008) used NGS to estimate coyote abundance in the Adirondacks, New York, USA and found that abundance increased with openings in canopy cover within a heavily forested environment. Additional estimates of coyote densities in the eastern USA have used spatial capture-recapture and NGS but have not explicitly attempted to relate variation in coyote densities to landcover (Bozarth et al. 2015, Morin et al. 2016, Murphy et al. 2018, Kluever et al. 2022).

We conducted a SCR analysis using NGS during the summers of 2019 and 2020 across South Carolina, USA to estimate coyote densities in relation to key landscape-level covariates. By assessing coyote populations at a broad spatial scale, we hoped to provide a comprehensive understanding of coyote densities now that populations have stabilized following colonization of South Carolina (Ruth and Cantrell 2021). We hypothesized that coyote densities would increase with open/early successional habitats and agricultural land use (Hinton et al. 2015, Cherry et al. 2016*a*, Ward et al. 2018, Youngmann et al. 2022). Open/early successional and agricultural habitats may increase the likelihood of high coyote densities by providing key resources, including prey availability. We also predicted that coyote densities would be lower in areas of forest cover, likely as a result of lower prey densities and avoidance of these land cover types (Cherry et al. 2016*a*, Morin et al. 2016). We sought to capture variation in coyote densities across a broad spatial scale to better inform management of game species like deer. Our primary objective was to provide managers with a tool for predicting where coyote populations are most

likely to have outsized impacts on game species, so that harvest regulations can be adjusted accordingly.

STUDY AREA

We assessed coyote densities across eight study sites in 2019 and two additional areas in 2020 for a total of 10 sites. Our study sites were a mixture of private and public lands located in the Piedmont, Southeastern Plains, and Middle Atlantic Coastal Plains ecoregions across South Carolina (Fig. 2.1). The Piedmont ecoregion was predominately oak-hickory-pine (*Quercus-Carya-Pinus*) forests before being largely cultivated for cotton, corn, tobacco, and wheat farming (Griffith et al. 2002). More recently, many areas in the Piedmont have been converted to natural and planted pine stands. The Piedmont experiences mean annual temperatures around 15°C and average 1229 mm of rainfall each year (Wiken et al. 2011). Our study sites within the Piedmont included the Long Cane and Enoree Ranger Districts of the Sumter National Forest (NF), and the Davis Land and Timber property. In 2020 we also sampled the Liberty Hill Wildlife Management Area (WMA) and surrounding private lands.

Our study sites within the Southeastern Plains included the Savannah River Site, a U.S. Department of Energy National Environmental Research Park; Fort Jackson; and the Carolina Sandhills National Wildlife Refuge (NWR) and Sandhills State Forest (SF) complex. The Southeastern Plains is characterized by sandy soils and prior to cultivation was mostly longleaf pine (*Pinus palustris*) forest (Griffith et al. 2002). Currently the Southeastern Plains are covered in cultivated cropland and pasture/hay with large regions of production pine. Notably, the Savannah River Site differs from the surrounding ecoregion by being predominately cultivated pine plantations with river drainages surrounded by bottomland hardwoods (Kilgo and Blake

2005). The Southeastern Plains experience mean annual temperatures around 16°C and average 1358 mm of rainfall each year (Wiken et al. 2011).

The Middle Atlantic Coastal Plain is typified by lowland swamps, marshes, and estuaries, with pine plantations in areas of higher elevation. The Middle Atlantic Coastal Plain experiences mean annual temperatures around 15.5°C and average 1229 mm of rainfall each year (Wiken et al. 2011). Our study sites in the Middle Atlantic Coastal Plain included the complex of private and state-owned public lands around the Webb Center and WMA and the Marsh and Woodbury WMA complex. In 2020 we also sampled the public and private lands around the Ernest F. Hollings Ashepoo-Combahee-Edisto Basin NWR and Donnelly WMA (hereafter called ACE Basin).

METHODS

Sampling methodology

We sampled transects at each study site across two-week sessions during July and August of 2019 and 2020. Coyotes typically raise pups from May-August (Gese et al. 1988, Smith et al. 1981, Kilgo et al. 2017, Sasmal et al. 2018) and space use by coyotes in the southeastern USA during the summer months is localized with both resident and transient individuals exhibiting limited movement and small home range sizes (Sasmal et al. 2018). Additionally, coyote populations experience the lowest levels of mortality in the summer (M. J. Chamberlain, unpublished data). Due to these demographic trends, coyote populations during the summer may be assumed to be closed-an important assumption for capture-recapture studies (Royle et al. 2013). By June, pups should be weaned and precocious; sampling during this time captures coyote populations at their yearly peak population size (Harrison et al. 1991, Mastro et al. 2012).

Our sampling efforts were conducted across spatially and temporally independent sessions (sites and year) and sampling occasions, wherein we repeatedly sampled within each session. We conducted an initial sweep of each transect at the beginning of a sampling session to remove all accumulated scat present from before the start of sample collection. We then sampled transects within each site every three days for a total of four sampling occasions in 2019 and five sampling occasions in 2020. We drove transects at approximately 10 km/hr, using the edges of the road as the boundary of our transect sampling area. On several sampling occasions, unforeseen circumstances such as weather and vehicular malfunctions required that an additional day of sampling was necessary to complete that occasion. Accordingly, we grouped multiple sampling days as one occasion in our analysis. For cases where we were unable to complete sampling for a given transect, we censored those sections from our analysis for that sampling occasion.

We collected scat samples using either a wooden stick, which was discarded after each sampling, or forceps that were sanitized with alcohol wipes and a butane lighter. We collected 0.4 mL of the outer portion of scat and placed it into a 2-mL tube containing 1.6 mL of DETs (DMSO/EDTA/Tris/salt) buffer (Frantzen et al. 1998, Stenglein et al. 2010). The remainder of the scat was sealed in a Ziploc freezer bag and stored on ice in the truck before being transferred to a -20°C freezer for storage. Whole scat was collected as a backup in case of DNA extraction failure with the DETs-preserved samples. We recorded the GPS coordinates of each sample along with the general appearance of the scat and any pertinent information concerning the location and condition of the sample.

Laboratory methodology

We extracted each sample using the Qiagen Mini Stool Kit (Qiagen, Valencia, California, USA), following the manufacturer's protocols with the exception that we filtered the eluted product a second time through the final filtration step to maximize the concentration of nuclear DNA (nDNA). Samples were amplified using a 12-primer multiplex described in Stenglein et al. (2010). We followed the run specifications laid out in Stenglein et al. (2010) and Morin et al. (2016) with the exception that we reduced the number of PCR cycles to 30 repetitions. This was done because we found that our samples were amplifying too strongly to be accurately scored (unpublished data). The Stenglein et al. (2010) lab protocols were developed using scat samples exhibiting a range of degradation and, therefore, required a high number of cycles to produce satisfactory amplification. Our sampling methodologies ensured that samples were no more than 3-4 days old and, therefore, contained high levels of quality DNA.

We used the multi-tube approach to run four separate replicate PCRs on each sample. PCR products were analyzed on a 3130x machine at either the University of Georgia or Cornell University and we observed no difference in PCR replicates between the two machines. We scored each sample using Geneious 2022.2.2 (http://www.geneious.com/) and confirmed consensus genotypes using *ConGenR* in Program R (Lonsinger and Waits 2015). Heterozygote loci were required to be observed in two separate replicates, while homozygote loci were required to be observed in three separate replicates. Matching genotypes were determined using a threshold of seven loci matches to address the probability of identity based on likelihood of siblings. Finally, we used *ConGenR* to identify recaptures across all sites and years.

Additionally, we used a species-specific mitochondrial DNA (mtDNA) control-region multiplex to identify the species of each scat (De Barba et al. 2014). Several gray fox samples

amplified at seven loci or more, but we removed these samples. Finally, we used eight known coyotes trapped from the Francis Marion National Forest and 25 known domestic dogs to run an additional genetic assignment test on all canid samples using Structure 2.3.4 (Pritchard et al. 2000, Falush et al. 2003). We assumed two populations and ran 10 iterations with a burn-in of 50,000 repetitions followed by 150,000 repetitions. We then removed all individuals identified as dog through our STRUCTURE analysis.

SCR analysis

To convert sampling transects into individual trap locations to be used in *secr* (Efford 2022), we first discretized linear transects into individual points using the *Points on a Line* function in ArcGIS (Morin et al. 2016, Murphy et al. 2018). We placed points 500m apart along transects and uniquely identified each point as a trap location. We then used the *Near* function in ArcGIS to associate each sample to the closest trap. We then input data into *secr* as a capture file, which included samples with their corresponding individual ID, trap location, sample session, occasion, and sex ID, and a trap file, which included trap ID, x and y coordinates, and a usage code denoting which occasions had been sampled.

SCR models

For our statewide estimates of densities, we constructed capture histories using 'count' detectors and sample year as our primary sample session with four sampling occasions in 2019 and five sample occasions in 2020. We combined all sites into a single session per year in order to share parameters across sites and assess the influence of landcover covariates across our sampling region. We buffered our traps by four times the half-normal sigma estimate produced using the *secr* function rpsv and corroborated our buffer using the *secr* function suggest.buffer (Efford 2022). Both functions gave buffer estimates of approximately 2800 meters in 2019 and 5500

meters in 2020. We chose to use a buffer of 5500 meters for both sessions to conservatively ensure that there was low likelihood of detecting animals at the edge of their home range. Using our buffer, we then created "trapbuffer"-type habitat masks for each session using a grid spacing of 500 meters. Finally, we included sex ID as a categorical individual covariate using the hcov function in *secr*, which allows for a hybrid mixture model including unknown individuals.

Using all sites combined, we initially fit all combinations, including additive terms, of session and sex ID as covariates for the detection parameters g0 and sigma and ranked these models using Akaike's model selection (AIC). We held density as a constant for this initial determination of best fit for detection probability. We then used our top detection model to estimate densities with combinations of three landcover covariates: forest cover, open and early successional, and agriculture. Landcover covariates were derived from reclassified 2019 National Land Cover Database categories where forest cover was deciduous, evergreen, and mixed forest, open and early successional was shrub, scrub, and grassland, and agricultural was pasture and row crops (Dewitz and U.S. Geological Survey 2021). Forest cover, available open and early successional, and agricultural landscapes have been shown to be important in both coyote resource selection as well as variation in coyote abundance (Kays et al. 2008, Hinton et al. 2015, Cherry et al. 2016*a*, Ward et al. 2018, Youngmann et al. 2022). We extracted habitat covariates from our derived landcover raster layers to our session-specific habitat masks using the addCovariates function in *secr*.

Density was first estimated across all sites with session being defined by sample year. We ranked models using AIC and used model coefficients to assess the influence of landcover on coyote densities across South Carolina. We then used our top density model to estimate site and

year specific densities at all sample sites to compare among sites and produce abundance estimates using the *secr* function region.N.

RESULTS

Using a mask buffer of 5.5 km around our transects, we effectively sampled 642,025 hectares in 2019 and 761,575 hectares in 2020, comprising approximately 7.7 – 9.2% of South Carolina's landmass. Our sampling efforts covered four of the five major ecoregions of South Carolina and our sample sites ranged from 32,800 hectares (Davis Land and Timber) to 123,100 hectares (Enoree National Forest). We collected a total of 240 samples in 2019 across eight sites and four sampling occasions and 299 samples in 2020 across 10 sites and five sampling occasions. Using mtDNA, we identified 382 samples as coyote or domestic dog, 30 samples as bobcat, 40 samples as gray fox, two samples as red fox, two samples as multiple predators, and were unable to identify species for 83 samples (84.60% species identification success). The number of nDNA loci necessary for accurate delineation between siblings was seven loci ($P_{ID(sibs)} = 0.00066$) and we successfully obtained consensus genotypes for 313 coyote or domestic dog samples for an individual identification success of 81.9%. We observed an average allelic dropout rate of 4.10% and an average false allele rate of 0.93% indicating excellent genotype success with a probability of genotyping error equaling 10×6.40^{-6} across the four PCR replicates we used. We censored three samples that matched consensus genotypes across three separate sites (ACE Basin, Fort Jackson, and Carolina Sandhills) because we were unable to confirm if these represented a single individual found at all sites or laboratory error. Additionally, we removed 35 samples that had been found off of our pre-determined transects as well as two samples that were identified as domestic dog using our STRUCTURE genetic assignment. Our final capture history across 2019 and 2020 included 272 confirmed coyote samples with 184 unique individuals. We successfully

determined sex identification for 171 individuals (82 female, 89 male) with 13 individuals designated as unknown sex.

SCR analysis

Our top hybrid mixture model for detection parameters varied both g0 and sigma by sex with an AIC weight of 0.43 (Table 2.1). Additional competitive models included sigma varying by sex and session. We used our top detection model in subsequent estimations of densities across all sites and among individual sites.

The top density model included open/early successional, forest cover, and agriculture but not session followed by our open/early successional only model (Table 2.2). Open/early successional landcover was a significant positive influence on coyote densities across South Carolina (Table 2.3). Both forest cover and agriculture were not significant predictors, but forest cover was positively associated with density, whereas agriculture was negatively associated with density. Our top model produced a density estimate across South Carolina of 8.73 coyotes/100km^2 (95% CI: 6.64 – 11.47 coyotes/100km^2) in 2019 and 8.20 coyotes/100km^2 (95% CI: 6.23 – 10.79 coyotes/100km^2) in 2020 (Table 2.4).

Using our top model across all sites, we estimated individual densities for each sample site and year. We excluded Enoree National Forest in 2019, Marsh and Woodbury WMAs in 2019, and ACE Basin in 2020 due to sparse data. Density estimates ranged from 1.74 coyotes/100km^2 (Webb Complex in 2020) to 27.48 coyotes/100km^2 (Stephen Davis in 2019; Table 2.5).

DISCUSSION

Early density estimates of coyote populations across North America have varied widely, both temporally and geographically (Bekoff and Gese 2003, Mastro et al. 2012). Coyote densities in

the endemic range of the western USA range from 12 – 90 coyotes/100km^2 (Clark 1980, Knowlton et al. 1999, see Table 2.2 in Bekoff and Gese 2003), with an outlier estimate of 150 – 230 coyotes/100km^2 in the fall (Knowlton 1972). However, Knowlton et al. (1972) noted that coyotes likely exist at around 20 – 40 coyotes/100km^2 across their range. Lower estimates could be found at the northern edge of the coyote range (1 – 60 coyotes/100km^2) and along the eastern edge of the colonizing front of coyotes (0.3 – 56 coyotes/100km^2; see Table 2.2 in Bekoff and Gese 2003, see abstracts in Mastro et al. 2012, Hinton and Chamberlain 2022). More recently, high coyote densities have been reported in urban settings in the Midwest and eastern USA (40 – 350 coyotes/100km^2; Gehrt et al. 2011, Way 2011) and in South Carolina after complete colonization and rapid population growth (80 – 150 coyotes/100km^2; Schrecengost 2007). Conversely, Hinton and Chamberlain (2022) recently reported coyote densities in the Red Wolf Experimental Recovery Area of North Carolina, USA an order of magnitude lower than previous reports, likely due to intense lethal control by federal agencies to aid in the recovery of red wolves. Without using a spatially explicit methodology to estimate coyote densities, these previous studies employed a variety of analyses, including known or collared individual counts, howl surveys, scat deposition rates, number of trapped animals, and traditional capture-recapture methods. Therefore, these methods are limited by the inability to address fine-scale spatial variation in densities across a sampling region as well as post hoc estimation of boundary effects to density along study site perimeters (Royle et al. 2013). Understanding of coyote densities across time and space should be contextualized by region, historical relation to population flux such as colonization, size of study area, and methodology and data used. Therefore, we sought to employ SCR methods, in conjunction with habitat covariate predictors of density variation, to best address spatial heterogeneity of coyote densities across South Carolina.

Our density estimates across all sample sites in South Carolina were relatively low compared to some previous, non-spatially-explicit estimates, but comparable to more recent SCR estimates of coyotes in the eastern USA and endemic populations in the southwestern USA. Morin et al. (2016) and Bozarth et al. (2015) estimated coyote densities to be approximately 2 – 9 coyotes/100km^2 in Virginia, USA and Lonsinger et al. (2018) and Woodruff et al. (2021) estimated coyote densities to range from approximately 5 – 11 coyotes/100km^2 in Utah and Arizona, respectively. However, these authors noted that coyote densities may be limited by low productivity landscapes found in the Appalachian Mountains of Virginia or the deserts of southwestern USA. South Carolina should theoretically support higher densities of coyotes and the South Carolina Department of Natural Resources (SCDNR) estimated from hunter surveys that approximately 40 coyotes/100km^2 were harvested incidental to deer hunting during 2019 and 2020, the same years as our study. Murphy et al. (2016) estimated coyote densities from 5 – 9 coyotes/100km^2 in southwest Louisiana, which is a landscape relatively similar to those found at our study sites in South Carolina. Furthermore, in Oregon, Ruprecht et al. (2021) produced estimates similar to our higher density sites at approximately 25 coyotes/100km^2. However, Murphy et al. (2016) used both scat sampling as well as hair snares to collect NGS samples and attributed their higher estimates of density to including hair samples, which they hypothesized captured more transient coyotes than scat samples. Ruprecht et al. (2021) used hybrid models combining SCR and radio-collared individuals, but when they accounted for individual detection heterogeneity to model resident and transient status, their estimates were higher at almost 40 coyotes/100km^2 (Ruprecht et al. 2021). Importantly, Morin et al. (2016) noted that accounting for individual detection heterogeneity in coyotes may be crucial to accurately modeling the differences in space use and detection between resident and transient coyotes.

Failure to account for heterogeneity in detection may bias density estimates (Howe et al. 2013, Ruprecht et al. 2021). Considering that transient coyotes may comprise more than 30% of the population in the southeastern USA (Hinton et al. 2015, Morin and Kelly 2017), an inability to model detection heterogeneity could lead to underestimating coyote densities in the region. However, modeling detection heterogeneity is notoriously difficult due to the need for a large amount of data, which can be problematic for cryptic predators (Howe et al. 2013). We conducted a post-hoc analysis of detection parameters using the finite-mixture model class in *secr* (Borchers and Efford 2008), but consistently encountered a lack of fit for baseline detection models when individual study site was included and latent classes approaching a proportion of 99:1 when we combined data across all study sites. Our findings indicate limited support for multiple behavioral classes though our results may be due to a lack of sufficient data to appropriately model behavioral differences and, therefore, detection probabilities for resident and transient coyotes. These post-hoc analyses, in conjunction with our understanding of the literature, indicate that our density estimates may, consequently, be biased low due to our inability to incorporate detection heterogeneity. However, we note that our average density estimates compare to studies that incorporated assumed differences in detection between resident and transient individuals (Murphy et al. 2018) and estimates for our higher density sites approach estimates of studies modeling latent classes (Ruprecht et al. 2021).

An additional source of bias in our density estimates may result from timing our sampling efforts during July and August. We chose this sampling period because it should represent coyote populations at a stable yearly maximum following whelping and before juvenile dispersal and mortality due to incidental harvest during deer hunting season in the fall. We sampled later in the summer in hopes to better capture young-of-the-year pups as they became precocious and moved

beyond their den sites (Gese et al. 1988, Smith et al. 1981, Sasmal et al. 2018, Kilgo et al. 2017). However, it is possible that pups were not yet mobile enough to be readily captured using our sampling methodology of surveying dirt and gravel roads. Indeed, genetic estimates of pair-wise relatedness within our sample sites reveal low levels of relatedness (unpublished data), potentially due to insufficiently sampling individuals whelped during the spring. If we did, indeed, fail to effectively sample pups, and breeding pairs can produce 4-6 pups per year in our region (Kilgo et al. 2017), we may have underestimated resident coyote densities during an annual population maximum.

However, our understanding of coyote biology provides a useful reference point for interpreting coyote densities. Resident packs in the southeastern USA maintain relatively large territories ranging around 10 – 30 km^2 (Holzman et al. 1992, Schrecengost et al. 2009, Hinton et al. 2015, Ward et al. 2018, Webster et al. 2022). Therefore, 3 – 10 packs could feasibly occupy 100 km^2 if territories perfectly adjoined each other. If each pack contains a breeding pair, several juveniles, and 4 – 6 pups (Kilgo et al. 2017), it is conceivable that pack sizes can reach numbers of 8 – 10 coyotes. This would mean that upwards of 100 resident coyotes could reside inside 100 km^2, given the availability of usable landcover. If 30% of coyotes in the southeastern USA are transient, this would mean that approximately 150 coyotes/100km^2 could theoretically exist at such a spatial scale. However, these calculations rely on several assumptions that are rarely true. For instance, territory and pack size is related to resource availability, and home ranges in the southeastern USA tend to be large, while pack size can be much lower than 10 coyotes. Additionally, pack territories do not perfectly adjoin and interstitial space between territories provides room for transient individuals to exist (Ward et al. 2018, M. J. Chamberlain, unpublished data). It is, therefore, likely that coyotes exist at lower average densities relative to

available space and resources. Hinton and Chamberlain (2023) conducted a similar post hoc assessment of their data and concluded that, in the absence of federal control of coyote populations, the Red Wolf Experimental Recovery Area could feasibly support upwards of 22 coyotes/100km^2, although they speculated the number to be around 8 – 12 coyotes/100km^2. Our estimates in South Carolina, which ranged from 1.7 – 27.5 coyotes/100km^2, reflect variation in density correlated to heterogeneous landcover and, although they may be biased low, are biologically feasible based on coyote biology and previous literature.

Open/early successional land cover was a significant predictor of densities across our study sites. This finding supports a myriad of studies showing selection for open landscapes across the eastern USA by coyotes (Hickman et al. 2015, Hinton et al. 2015, Ward et al. 2018, Sasmal et al. 2019, Youngmann et al. 2022). Increased resource availability should result in higher population densities, and previous studies have shown that food abundance is important for regulating coyote population size (see Bekoff and Gese 2003 for further discussion). Consumption of lagomorphs and small mammals by eastern coyotes has been well documented (Schrecengost et al. 2008, Kelly et al. 2015, Cherry et al. 2016*b*, Ward et al. 2018, Jensen et al. 2022) and these prey species have been shown to select for open/early successional landscapes. Indeed, Ward et al. (2018) documented an increase in the consumption of lagomorphs in open landscapes by coyotes and Youngmann et al. (2022) observed persistent year-round use of open/early successional landcover by coyotes during foraging bouts. Areas of open landcover should confer increased resource availability for coyote populations across a large spatial extent and, therefore, result in higher carrying capacity for increased population densities.

We found that forested land cover did not have a statistically significant impact on density, although we did observe a positive relationship between coyote densities and forest

cover, similar to estimates of abundance by Kays et al. (2008) and Cherry et al. (2016*a*). Kays et al. (2008) rejected the hypothesis that forested landscapes were unable to sustain coyote populations but documented increased abundance in areas of reduced canopy cover within forested systems. Morin et al. (2016) found coyote densities in the low-productivity, heavily forested Appalachian region of Virginia comparable to several of our density estimates from similarly forested study sites, including the Enoree and Long Cane Ranger Districts and the Marsh/Woodbury WMAs. However, our two highest density sites, Davis Land and Timber and the SRS, are also heavily forested. This discrepancy may be explained by the relative intensity of land management among these sites, with Davis Land and Timber and the SRS receiving higher rates of timber harvest and thinning than other federal and state lands. Although our landcover covariates did not have the resolution capable of distinguishing variation in forest structure, it is likely that coyote densities increased with the availability of open forest landscapes within Davis Land and Timber and SRS. Additionally, interspersion of open/early successional landcover within available forest cover may positively impact coyote densities, although previous research has indicated habitat fragmentation is associated with lower coyote abundance (Crooks and Soule 1999, Cherry et al. 2016). Finally, there is little space available to coyotes without forest cover in the heavily forested eastern USA and yet coyote populations have expanded and thrived. Coyotes may use areas of forest systems with decreased canopy cover which may concentrate certain prey populations such as lagomorphs and small mammals (Kays et al. 2008). Additionally, Youngmann et al. (2022) documented a shift in resource selection among coyotes in Alabama, Georgia, and South Carolina where packs sought out forest cover for foraging or denning and pup-rearing during the spring. Forested landscapes clearly can support coyote

populations in the eastern USA, although forest structure may have important impacts on coyote density.

The insignificant effect we observed of agriculture on coyote densities may be a function of insufficiently sampling agricultural landscapes (Fig. 2.1). However, our findings are similar to Cherry et al. (2016*a*) who reported that coyote abundance was not influenced by row crops. A key difference between our methods is that Cherry et al. (2016*a*) included pasture with their open landcover covariate, which they found was a primary predictor of coyote abundance, whereas we included pasture in our agriculture covariate. This may explain the high standard error we observed around our agriculture covariate beta estimate. Coyote densities may be positively influenced by open pasture but negatively impacted by cultivated crops. Alternatively, pasture and row crops may not be selected for by coyotes for foraging because they do not provide sufficient resources (Youngmann et al. 2022). However, coyotes have been shown to select for agriculture (Hinton et al. 2015) and the effect on densities may be highly variable.

Coyotes have been documented to be heterogeneously arranged across the landscape (Kays et al. 2008, Gulsby et al. 2015, Cherry et al. 2016*a*) and our findings show variation in coyote densities across relatively small geographic distances. Heterogeneous densities at local scales, coupled with the dual life strategy of resident and transient individuals, allow coyote populations to quickly colonize available space (Kilgo et al. 2014, 2017, Gulsby et al. 2015, Hinton et al. 2015, Kierepka et al. 2017). A heterogeneous mosaic of population densities across local spatial scales has fundamental implications for management of coyotes in the eastern USA. Previous research on the efficacy of lethal control has found mixed success in coyote populations across the southeastern USA. Concerted local efforts to remove coyotes has been shown to result in decreased coyote numbers and increased fawn recruitment (VanGilder et al. 2009), but the

long-term effects of these efforts may be negligible due to rapid recolonization of treatment areas (Kilgo et al. 2014, Gulsby et al. 2015). For instance, Kilgo et al. (2014) conducted a coyote removal study at the SRS from 2010 – 2012 where they removed an average of 158 coyotes per year across three 32 km^2 treatment units. Although they observed initial reductions in relative abundance of coyotes within treatment areas (78%), subsequent genetic and demographic data revealed immediate backfill from transient individuals to pre-treatment levels (Kierepka et al. 2017, Kilgo et al. 2017). Our projected densities at the SRS during 2019 – 2020 were the highest of any of our study sites except for Davis Land and Timber and may, in fact, be lower than peak densities experienced by Kilgo et al. (2014) during post-colonization. Therefore, areas of high density within SRS may have also allowed rapid backfill of available space created by trapping efforts (Kierepka et al. 2017, Kilgo et al. 2017). Relative success of lethal control as a management tool for coyotes may rely on the heterogeneous array of densities in and around a treatment area. However, coyote population dynamics operate on a large scale due to the highly mobile life strategy of transiency, and it is unlikely that local reductions in coyote populations would be sustainable. With the ubiquitous presence of coyotes across South Carolina, heterogeneously arranged across the landscape, lethal control is simply not feasible at a regional or state-wide scale.

Finally, a notable takeaway from this study is the success of a modified lab methodology from the standard protocol described by Stenglein et al. (2010), which may enable natural resources departments with limited resources to more easily conduct density estimates. Unlike Stenglein et al. (2010), we ran all samples for only four PCR replicates to determine consensus genotypes. This was done to streamline workflow and increase lab efficiencies. We observed relatively high rates of genotype success compared to previous NGS studies of coyote

populations across North America using this methodology. Lonsinger et al. (2018) reported higher rates of individual identification success during the winter in Utah, USA (93.3%) and comparable rates during the summer (82.8%), while Woodruff et al. (2020) reported a success rate of (~78%) across the fall and summer in Arizona, USA. Both of these studies were conducted in southwestern North America, where arid climates may help to preserve nDNA. Morin et al. (2018) observed consensus genotype success rates at 57.7% in western Virginia, USA across multiple seasons and Bozarth et al. (2015) reported a 53% success rate in northern Virginia, USA across 2 years of sampling. These studies were conducted in southeastern North America, similar to our study area of South Carolina. Our genotype success can likely be attributed to our sampling design, where sampling occasions were conducted at 3-day intervals across a two-week session. This meant that samples were rarely more than three days old upon preservation in DET buffer. Previous NGS methodologies have used sampling intervals from 1 – 8 days (Woodruff et al. 2021), 6 – 10 days (Bozarth et al. 2015), 14 days (Lonsinger et al. 2018), and 1 month (Morin et al. 2016). By sampling every three days across a 2-week closed session, we were able to efficiently conduct NGS density surveys of coyote populations at 8-10 sites across South Carolina during the summers of 2019 – 2020. Our resulting genotype success and density estimates show that short-term, concerted sampling efforts can produce useful density estimates of an elusive carnivore and may be employed by natural resource agencies with limited resources.

MANAGEMENT IMPLICATIONS

We documented a heterogeneous landscape of coyote densities across South Carolina as a function of landcover type. Highly variable densities, coupled with coyotes' dual life strategy of residency and transiency allows them to quickly colonize available space, which explains why

population control has historically failed. However, open/early successional land cover – the very same land cover types that managers are encouraged to provide for deer, turkeys, quail, and rabbits – had a significant and positive impact on coyote density across our study sites. We recommend state agencies assess harvest recommendations while considering reductions in antlerless harvest in areas of high coyote densities. This research provides an improved understanding of the spatial distribution of coyote populations across the landscape, and aids managers in developing alternative ways to support populations of game species of interest. However, our density estimates may have been biased low due to unmodeled detection heterogeneity and the inability to capture young-of-the-year. Continued research on the impacts of resident and transient individuals on coyote density estimates is warranted to assess the efficacy of spatial capture-recapture methods in this study system.

LITERATURE CITED

Balme, G. A., L. T. B. Hunter, and R. Slotow. 2009. Evaluating methods for counting cryptic carnivores. Journal of Wildlife Management 73:433–441.

Bekoff, M., and E. M. Gese. 2003. Coyote (*Canis latrans*). Pages 467–481 *in* G. A. Feldhamer, B. C. Thompson, and J. A. Chapman, editors. Wild mammals of North America: biology, management, and conservation. Second edition. Johns Hopkins University Press, Baltimore, Maryland, USA.

Borchers, D. L., and M. G. Efford. 2008. Spatially explicit maximum likelihood methods for capture-recapture studies. Biometrics 64:377–385.

Bozarth, C. A., B. Gardner, L. L. Rockwood, and J. E. Maldonado. 2015. Using fecal DNA and spatial capture-recapture to characterize a recent coyote colonization. Northeastern Naturalist 22:144–162.

Cherry, M. J., P. E. Howell, C. D. Seagraves, R. J. Warren, and L. M. Conner. 2016*a*. Effects of land cover on coyote abundance. Wildlife Research 43:662–670.

Cherry, M. J., K. L. Turner, M. B. Howze, B. S. Cohen, L. M. Conner, and R. J. Warren. 2016*b*. Coyote diets in a longleaf pine ecosystem. Wildlife Biology 22:64–70.

Chitwood, M. C., M. A. Lashley, C. E. Moorman, and C. S. DePerno. 2014. Confirmation of coyote predation on adult female white-tailed deer in the southeastern United States. Southeastern Naturalist 13:N30–N32.

Clark, F. W. 1972. Influence of jackrabbit density on coyote population change. The Journal of Wildlife Management 36:343–356.

Conner, M. M., M. M. Jaeger, T. J. Weller, and D. R. McCullough. 1998. Effect of coyote removal on sheep depredation in northern California. Journal of Wildlife Management 62:690–699.

Crooks, K. R., and M. E. Soulé. 1999. Mesopredator release and avifaunal extinctions in a fragmented system. Nature 400:563–566.

De Barba, M., J. R. Adams, C. S. Goldberg, C. R. Stansbury, D. Arias, R. Cisneros, and L. P. Waits. 2014. Molecular species identification for multiple carnivores. Conservation Genetics Resources 6:821–824.

Dewitz, J., and U.S. Geological Survey. n.d. National Land Cover Database (NLCD) 2019 Products (ver. 2.0; June 2021): U.S. Geological Survey data release.

Efford, M. 2004. Density estimation in live-trapping studies. Oikos 106:598–610.

Egan, M. E., C. C. Day, T. E. Katzner, and P. A. Zollner. 2021. Relative abundance of coyotes (*Canis latrans*) influences gray fox (*Urocyon cinereoargenteus*) occupancy across the eastern United States. Canadian Journal of Zoology 99:63–72.

Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587.

Frantzen, M. a. J., J. B. Silk, J. W. H. Ferguson, R. K. Wayne, and M. H. Kohn. 1998. Empirical evaluation of preservation methods for faecal DNA. Molecular Ecology 7:1423–1428.

Gehrt, S. D., J. L. Brown, and C. Anchor. 2011. Is the urban coyote a misanthropic synanthrope? The case from Chicago. Cities and the Environment (CATE) 4(1):1–25.

Gompper, M. E. 2002. Top carnivores in the suburbs? Ecological and conservation issues raised by colonization of northeastern North America by coyotes. BioScience 52:185–190.

Griffith, G. E., J. M. Omernik, J. A. Comstock, M. P. Shafale, W. H. McNab, D. R. Lenat, J. B. Glover, and V. B. Shelburne. 2002. Ecoregions of North Carolina and South Carolina. US Geological Survey, Reston, VA, USA.

Gulsby, W. D., C. H. Killmaster, J. W. Bowers, J. D. Kelly, B. N. Sacks, M. J. Statham, and K. V. Miller. 2015. White-tailed deer fawn recruitment before and after experimental coyote removals in central Georgia. Wildlife Society Bulletin 39:248–255.

Hansen, S. J. K., J. L. Frair, H. B. Underwood, and J. P. Gibbs. 2015. Pairing call-response surveys and distance sampling for a mammalian carnivore. Journal of Wildlife Management 79:662–671.

Harrison, D. J., J. A. Harrison, and M. O'Donoghue. 1991. Predispersal movements of coyote (*Canis latrans*) pups in eastern Maine. Journal of Mammalogy 72:756–763.

Hickman, J. E., W. D. Gulsby, C. H. Killmaster, J. W. Bowers, M. E. Byrne, M. J. Chamberlain, and K. V. Miller. 2015. Home range, habitat use, and movement patterns of female coyotes in Georgia: implications for fawn predation. Journal of the Southeastern Association of Fish and Wildlife Agencies 2:144–150.

Hinton, J. W., and M. J. Chamberlain. 2022. Evidence of reduced abundance, density, and survival of coyotes under federal management for red wolf recovery. Ecosphere 13:ecs2.4216.

Hinton, J. W., E. Heppenheimer, K. M. West, D. Caudill, M. L. Karlin, J. C. Kilgo, J. J. Mayer, K. V. Miller, M. Walch, B. vonHoldt, and M. J. Chamberlain. 2019. Geographic patterns in morphometric and genetic variation for coyote populations with emphasis on southeastern coyotes. Ecology and Evolution 9:3389–3404.

Hinton, J. W., F. T. van Manen, and M. J. Chamberlain. 2015. Space use and habitat selection by resident and transient coyotes (*Canis latrans*). PLOS ONE 10:e0132203.

Hody, J. W., and R. Kays. 2018. Mapping the expansion of coyotes (*Canis latrans*) across North and Central America. ZooKeys 759:81–97.

Holzman, S., M. J. Conroy, and J. Pickering. 1992. Home range, movements, and habitat use of coyotes in southcentral Georgia. The Journal of Wildlife Management 56:139.

Howe, E. J., M. E. Obbard, and C. J. Kyle. 2013. Combining data from 43 standardized surveys to estimate densities of female American black bears by spatially explicit capture–recapture. Population Ecology 55:595–607.

Jensen, A. J., C. J. Marneweck, J. C. Kilgo, and D. S. Jachowski. 2022. Coyote diet in North America: geographic and ecological patterns during range expansion. Mammal Review 52:480–496.

Kays, R. W., M. E. Gompper, and J. C. Ray. 2008. Landscape ecology of eastern coyotes based on large-scale estimates of abundance. Ecological Applications 18:1014–1027.

Kelly, J. D., C. H. Killmaster, and J. W. Bowers. 2015. Seasonal and spatial variation in diets of coyotes in central Georgia. Journal of the Southeastern Association of Fish and Wildlife Agencies 2:296–302.

Kierepka, E. M., J. C. Kilgo, and O. E. Rhodes. 2017. Effect of compensatory immigration on the genetic structure of coyotes. Journal of Wildlife Management 81:1394–1407.

Kilgo, J. C., and J. I. Blake. 2005. Ecology and management of a forested landscape: fifty years on the Savannah River Site. Island Press, Washington, D.C..

Kilgo, J. C., H. S. Ray, C. Ruth, and K. V. Miller. 2010. Can coyotes affect deer populations in southeastern North America? Journal of Wildlife Management 74:929–933.

Kilgo, J. C., H. S. Ray, M. Vukovich, M. J. Goode, and C. Ruth. 2012. Predation by coyotes on white-tailed deer neonates in South Carolina. Journal of Wildlife Management 76:1420–1430.

Kilgo, J. C., M. Vukovich, H. Scott Ray, C. E. Shaw, and C. Ruth. 2014. Coyote removal, understory cover, and survival of white-tailed deer neonates. Journal of Wildlife Management 78:1261–1271.

Kilgo, J. C., C. E. Shaw, M. Vukovich, M. J. Conroy, and C. Ruth. 2017. Reproductive characteristics of a coyote population before and during exploitation. Journal of Wildlife Management 81:1386–1393.

Kluever, B. M., M. B. Main, S. W. Breck, R. C. Lonsinger, J. H. Humphrey, and J. W. Fischer. 2022. Using noninvasive genetics for estimating density and assessing diet of urban and rural coyotes in Florida, USA. Urban Naturalist 9:1–24.

Lonsinger, R. C., P. M. Lukacs, E. M. Gese, R. N. Knight, and L. P. Waits. 2018. Estimating densities for sympatric kit foxes (*Vulpes macrotis*) and coyotes (*Canis latrans*) using noninvasive genetic sampling. Canadian Journal of Zoology 96:1080–1089.

Lonsinger, R. C., and L. P. Waits. 2015. ConGenR: rapid determination of consensus genotypes and estimates of genotyping errors from replicated genetic samples. Conservation Genetics Resources 7:841–843.

Mastro, L. L., E. M. Gese, J. K. Young, and J. A Shivik. 2012. Coyote (*Canis latrans*), 100 + years in the east: a literature review. Addendum to the Proceedings of the 14th Wildlife Damage Management Conference 14:129–131.

Morin, D. J., and M. J. Kelly. 2017. The dynamic nature of territoriality, transience and biding in an exploited coyote population. Wildlife Biology 2017:wlb.00335.

Morin, D. J., M. J. Kelly, and L. P. Waits. 2016. Monitoring coyote population dynamics with fecal DNA and spatial capture-recapture: estimating coyote population parameters. Journal of Wildlife Management 80:824–836.

Murphy, S. M., B. C. Augustine, J. R. Adams, L. P. Waits, and J. J. Cox. 2018. Integrating multiple genetic detection methods to estimate population density of social and territorial carnivores. Ecosphere 9:e02479.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Robinson, K. F., D. R. Diefenbach, A. K. Fuller, J. E. Hurst, and C. S. Rosenberry. 2014. Can managers compensate for coyote predation of white-tailed deer?. Journal of Wildlife Management 78:571–579.

Roemer, G. W., M. E. Gompper, and B. Van Valkenburgh. 2009. The ecological role of the mammalian mesocarnivore. BioScience 59:165–173.

Roffler, G. H., J. N. Waite, K. L. Pilgrim, K. E. Zarn, and M. K. Schwartz. 2019. Estimating abundance of a cryptic social carnivore using spatially explicit capture–recapture. Wildlife Society Bulletin 43:31–41.

Royle, J. A., R. B. Chandler, R. Sollmann, and B. Gardner. 2013. Spatial Capture-Recapture. Academic Press, Waltham, Massachusetts, USA.

Ruprecht, J. S., C. E. Eriksson, T. D. Forrester, D. A. Clark, M. J. Wisdom, M. M. Rowland, B. K. Johnson, and T. Levi. 2021. Evaluating and integrating spatial capture–recapture models with data of variable individual identifiability. Ecological Applications 31:e02405.

Ruth, C. R. and J. Cantrell. 2021. South Carolina deer harvest report. South Carolina Department

of Natural Resources, Columbia, USA.

Sasmal, I., C. E. Moorman, M. B. Swingen, S. Datta, and C. S. DePerno. 2019. Seasonal space use of transient and resident coyotes (*Canis latrans*) in North Carolina, USA. Canadian Journal of Zoology 97:326–331.

Schrecengost, J. D. 2007. Home range and food habits of the coyote (*Canis latrans*) at the Savannah River Site, South Carolina. Thesis, University of Georgia, Athens, USA.

Schrecengost, J. D., J. C. Kilgo, D. Mallard, H. S. Ray, and K. V. Miller. 2008. Seasonal food habits of the coyote in the South Carolina Coastal Plain. Southeastern Naturalist 7:135–144.

Srivathsa, A., R. G. Rodrigues, K. B. Toh, A. Zachariah, R. W. Taylor, M. K. Oli, and U. Ramakrishnan. 2021. The truth about scats and dogs: Next-generation sequencing and spatial capture–recapture models offer opportunities for conservation monitoring of an endangered social canid. Biological Conservation 256:109028.

Stenglein, J. L., M. De Barba, D. E. Ausband, and L. P. Waits. 2010. Impacts of sampling location within a faeces on DNA quality in two carnivore species. Molecular Ecology Resources 10:109–114.

Stenglein, Jennifer L., L. P. Waits, D. E. Ausband, P. Zager, and C. M. Mack. 2010. Efficient, noninvasive genetic sampling for monitoring reintroduced wolves. Journal of Wildlife Management 74:1050–1058.

Sterling, B., W. Conley, and M. R. Conley. 1983. Simulations of demographic compensation in coyote populations. Journal of Wildlife Management 47:1177–1181.

VanGilder, C. L., G. R. Woods, S. Hwy, R. Spring, and K. V. Miller. 2009. Effects of an intensive predator removal on white-tailed deer recruitment in northeastern Alabama.

Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 63:11–16

Waits, L. P., and D. Paetkau. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. Journal of Wildlife Management 69:1419–1433.

Ward, J. N., J. W. Hinton, K. L. Johannsen, M. L. Karlin, K. V. Miller, and M. J. Chamberlain. 2018. Home range size, vegetation density, and season influences prey use by coyotes (*Canis latrans*). PLOS ONE 13:e0203703.

Way, J. G. 2011. Record pack-density of eastern coyotes/coywolves (*Canis latrans* x *lycaon*). American Midland Naturalist 165:201–203.

Webster, S. C., J. C. Beasley, J. W. Hinton, and M. J. Chamberlain. 2022. Resident and transient coyotes exhibit differential patterns of movement behavior across heterogeneous landscapes in the southeastern United States. Ecology and Evolution 12:e8725.

Wiken, E., F. Jimenez Nava, and G. Griffith. 2011. North American Terrestrial Ecoregions – Level III. Commission for Environmental Cooperation, Montreal, Canada.

Williams, B. K., J. D. Nichols, and M. J. Conroy. 2002. Analysis and management of animal populations. Academic Press, London, United Kingdom.

Woodruff, S. P., D. R. Eacker, and L. P. Waits. 2021. Estimating coyote densities with local, discrete Bayesian capture-recapture models. Journal of Wildlife Management 85:73–86.

Youngmann, J. L., J. W. Hinton, N. W. Bakner, M. J. Chamberlain, and G. J. D'Angelo. 2022. Recursive use of home ranges and seasonal shifts in foraging behavior by a generalist carnivore. Ecology and Evolution 12:e9540.

Table 2.1. Model ranking using Akaike's Information Criterion scores corrected for small sample size (AICc) for detection parameters g0 and sigma for spatial capture-recapture estimates of coyote (*Canis latrans*) populations in South Carolina, USA during summer 2019 and 2020.

Detection model	Detection function	*N*	logLik	AICc	ΔAICc	*w*
g0 ~ sex, sigma ~ sex	halfnormal	6	-1218.26	2448.96	0.00	0.43
g0 ~ 1, sigma ~ sex	halfnormal	5	-1219.56	2449.43	0.47	0.34
g0 ~ 1, sigma ~ sex + session	halfnormal	6	-1219.21	2450.87	1.91	0.17
g0 ~ sex + session, sigma ~ sex + session	halfnormal	8	-1217.98	2452.71	3.75	0.07
g0 ~ session, sigma ~ session	halfnormal	6	-1224.08	2460.59	11.63	0.00
g0 ~ 1, sigma ~ session	halfnormal	5	-1225.15	2460.61	11.65	0.00
Null	halfnormal	4	-1226.69	2461.59	12.63	0.00
g0 ~ sex, sigma ~ 1	halfnormal	5	-1226.06	2462.44	13.48	0.00
g0 ~ session, sigma ~ 1	halfnormal	5	-1226.19	2462.69	13.73	0.00
g0 ~ sex + session, sigma ~ 1	halfnormal	6	-1225.71	2463.86	14.90	0.00

Table 2.2. Model ranking using Akaike's Information Criterion scores corrected for small sample size (AICc) for spatial capture-recapture density estimates of coyote (*Canis latrans*) populations in South Carolina, USA during summer 2019 and 2020.

Density model	Detection function	*N*	logLik	AICc	ΔAICc	*w*
D ~ Agriculture + Forest + Open	halfnormal	9	-1177.20	2373.36	0.00	0.44
D ~ Open	halfnormal	7	-1179.60	2373.79	0.43	0.36
D ~ Agriculture + Forest + Open + session	halfnormal	10	-1177.17	2375.52	2.16	0.15
D ~ Open + session	halfnormal	8	-1180.44	2377.64	4.28	0.05
D ~ Forest	halfnormal	7	-1212.52	2439.62	66.26	0.00
D ~ Forest + session	halfnormal	8	-1212.30	2441.37	68.01	0.00
D ~ Agriculture	halfnormal	7	-1214.59	2443.78	70.42	0.00
D ~ Agriculture + session	halfnormal	8	-1214.34	2445.44	72.08	0.00
D ~ session	halfnormal	7	-1218.02	2450.63	77.27	0.00
Null	halfnormal	4	-1226.69	2461.59	88.23	0.00

Table 2.3. Coefficient estimates for the top density model of coyotes across South Carolina, USA during summer 2019 and 2020.

Model variable	β	SE	95% CI
D	-9.64	1.19	-11.97, -7.30
D.Agriculture	-6.08	11.50	-28.63, 16.47
D.Forest	1.65	1.39	-1.07, 4.37
D.Open	4.64	1.18	2.32, 6.96
g0	-4.06	0.20	-4.45, -3.68
g0 - Male	0.51	0.30	-0.08, 1.10
sigma	7.51	0.09	7.33, 7.69
sigma - Male	-0.67	0.14	-0.95, -0.40
pmix - Male	0.81	0.26	0.29, 1.32

Table 2.4. Parameter estimates of coyote densities (D), g0, sigma, and sex ratio (pmix) across South Carolina, USA during summer 2019 and 2020. Density estimates are derived by dividing projected abundance estimates for each year by the state space (642025 hectares in 2019 and 761575 hectares in 2020).

Parameter	Estimate	SE	95% CI
D - 2019[a]	8.73	1.22	6.64, 11.47
D - 2020[a]	8.20	1.15	6.23, 10.79
g0 - Female	0.017	0.003	0.012, 0.025
sigma – Female[b]	1825.91	166.95	1526.91, 2183.46
pmix - Female	0.31	0.06	0.21, 0.43
g0 - Male	0.03	0.006	0.018, 0.043
sigma – Male[b]	931.11	102.28	751.24, 1154.05
pmix - Male	0.69	0.06	0.57, 0.79

[a]coyotes/100km^2
[b]meters

Table 2.5. Site information and density estimates for sample sites across South Carolina, USA used to estimate coyote densities during summer 2019 and 2020.

Site	Year	Detections	Animals	Sample Space (km^2)	D (coyotes/100km^2)	SE	Lower CI	Upper CI	Expected N	SE	Lower CI	Upper CI
ACE Basin	2020[a]	2	2	671								
Carolina Sandhills	2019	4	4	920	6.68	2.51	3.28	13.60	61.46	23.05	30.18	125.14
	2020	25	17	920	10.10	2.79	5.94	17.19	92.96	25.67	54.65	158.13
Enoree National Forest	2019[a]	1	1	1231								
	2020	7	6	1231	4.00	1.75	1.77	9.08	49.30	21.52	21.74	111.78
Fort Jackson	2019	9	7	522	5.70	2.32	2.65	12.29	29.75	12.11	13.81	64.10
	2020	20	12	522	8.26	2.50	4.63	14.75	43.09	13.02	24.14	76.90
Liberty Hill	2020	14	9	524	9.33	3.27	4.79	18.16	48.89	17.12	25.10	95.23
Long Cane National Forest	2019	6	5	850	3.49	1.62	1.47	8.31	29.68	13.78	12.49	70.58
	2020	21	12	850	8.16	2.54	4.50	14.81	69.36	21.57	38.24	125.81
Marsh and Woodbury WMAs	2019[a]	1	1	639								
	2020	6	3	639	2.58	1.55	0.87	7.67	16.48	9.91	5.55	48.96
Savannah River Site	2019	43	34	1090	23.69	5.24	15.43	36.37	258.12	57.12	168.15	396.23
	2020	39	34	1090	17.72	3.78	11.73	26.79	193.11	41.14	127.78	291.83
Stephen Davis	2019	26	23	328	27.48	6.68	17.18	43.95	90.13	21.91	56.36	144.15
	2020	32	16	328	15.13	4.39	8.67	26.40	49.62	14.39	28.43	86.61
Webb Complex	2019	14	10	923	8.11	2.87	4.14	15.91	74.87	26.50	38.18	146.80
	2020	3	3	923	1.74	1.01	0.60	4.99	16.03	9.29	5.58	46.04

[a]Sparse data

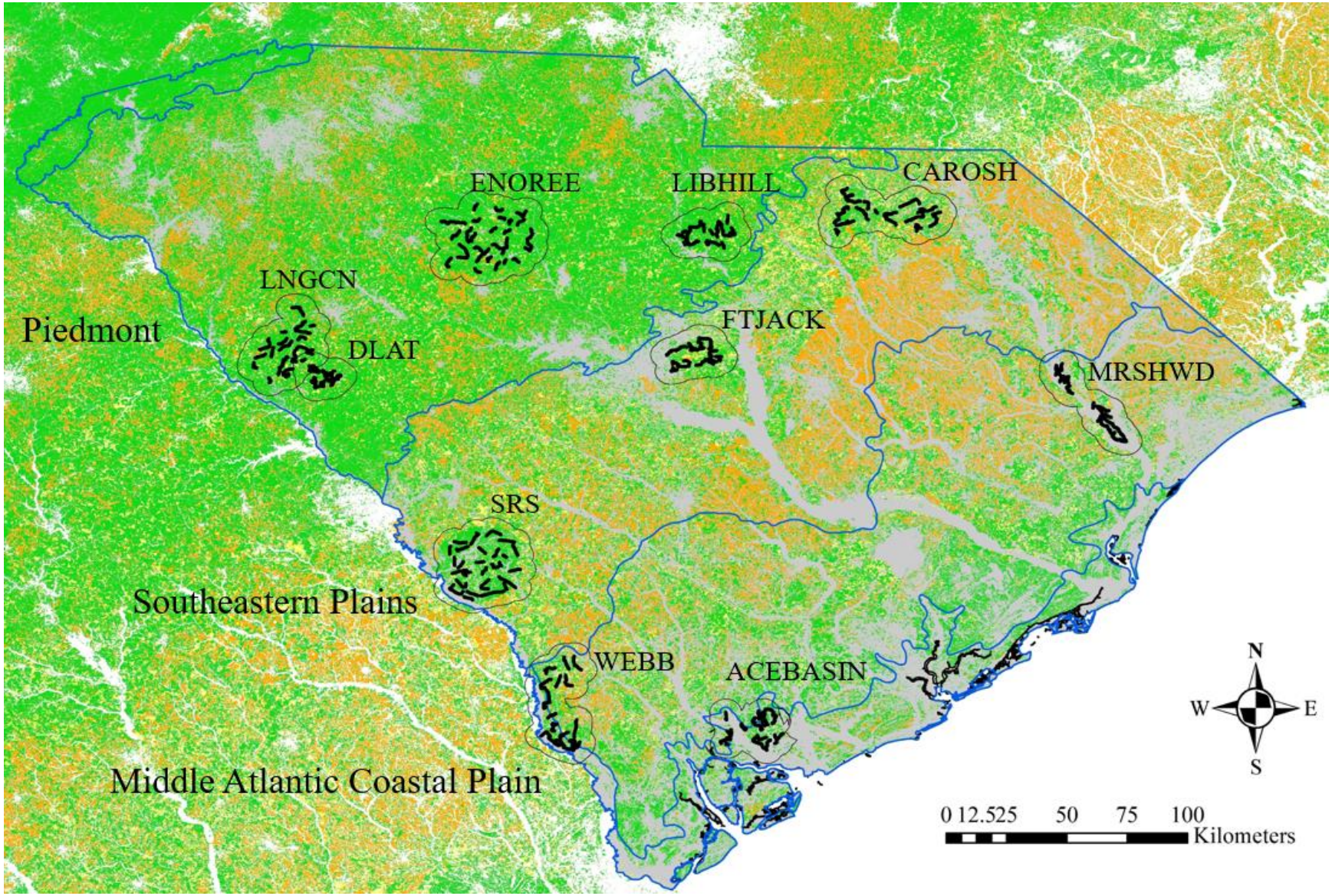
ENOREE
LIBHILL
CAROSH
LNGCN
Piedmont
DLAT
FTJACK
MRSHWD
SRS
Southeastern Plains
WEBB
ACEBASIN
Middle Atlantic Coastal Plain
N
W
E
S
0 12.525 50 75 100
Kilometers

Figure 2.1. Sample sites across South Carolina used for non-invasive genetic sampling and spatial capture-recapture estimates of coyote densities during 2019 and 2020. Landcover including forest (green), agriculture (orange), and open/early successional (yellow) were used as predictors of coyote density. LNGCN, Long Cane Ranger District Sumter National Forest; DLAT, Davis Land and Timber; ENOREE, Enoree Ranger District Sumter National Forest; LIBHILL, Liberty Hill Wildlife Management Area and private lands; SRS, Savannah River Site; FTJACK, Fort Jackson; CAROSH, Carolina Sandhills National Wildlife Refuge and State Forest; WEBB, Webb Complex; ACEBASIN, Ernest F. Hollings Ashepoo-Combahee-Edisto Basin NWR and Donnelly WMA; MRSHWD, Marsh and Woodbury Wildlife Management Areas.

CHAPTER 3

RECURSIVE USE OF HOME RANGES AND SEASONAL SHIFTS IN FORAGING BEHAVIOR BY A GENERALIST CARNIVORE

Youngmann, J. L., J. W. Hinton, N. W. Bakner, M. J. Chamberlain, and G. J. D'Angelo. 2022. Recursive use of home ranges and seasonal shifts in foraging behavior by a generalist carnivore. Ecology and Evolution 12:e9540. Reprinted here with permission of the publisher.

ABSTRACT

Coyotes (*Canis latrans*) colonized the southeastern United States over the last century as large predators, including the red wolf (*Canis rufus*) and eastern cougar (*puma concolor*), were extirpated from the region. As a generalist carnivore, the coyote preys on white-tailed deer (*Odocoileus virginianus*) and various smaller mammals, birds, and vegetation. While resource selection by coyotes has been well documented at the home-range scale, little is known about their foraging behavior, which is an important factor in thoroughly understanding influences of coyotes on prey and sympatric carnivores. We assessed 3rd-order resource selection of coyotes at sites across Alabama, Georgia, and South Carolina during 2015-2016. Using GPS collars, we tracked 41 resident coyotes across 4 calendar seasons and identified suspected foraging areas using recursive analysis where individuals repeatedly returned to known locations. We found that resident coyotes selected for open landcover types throughout the year, while avoiding primary and secondary roads. Additionally, resident coyotes avoided forested landcover types while selecting for forest edges except from April – June when they foraged within interior forest away from edges. Previous studies have documented substantive predation rates on white-tailed deer neonates by coyotes, and that fawn mortality may increase in forested landscapes away from forest edge. Our findings indicate that foraging coyotes may select forest cover types during spring where fawns are more vulnerable to predation. Additionally, there has been debate in the literature as to how coyotes obtain consistent levels of deer in their diets outside of fawning and fall hunting seasons. Our study indicates that use of road-kill carcasses by coyotes was an unlikely explanation for the presence of deer in coyote diets throughout the year, as coyotes in our study were not observed using roads during foraging excursions.

KEYWORDS

Canis latrans, coyote, foraging, recursive behavior, resource selection, scavenging, space use

INTRODUCTION

Animals maintain home ranges through repeated visitations to areas in a systematic manner (Van Moorter et al. 2009, 2016). By maintaining a home range, animals minimize risks associated with navigating unfamiliar areas, while improving survival and fitness by revisiting areas that contain critical resources. Revisiting areas promotes disproportionate space use in home ranges, which minimizes extensive, wide-ranging movements that would destabilize use of distinct core areas that shape the home range (Merkle et al. 2014, Morin and Kelly 2017, Van Moorter et al. 2016, McKeown et al. 2020). Depending on species, animals respond to spatio-temporal dynamics of resources using various movement strategies that can range from nomadism (Teitelbaum and Mueller 2019) to sedentatism (Sells and Mitchell 2020). Understanding how recursive behaviors shape these movement strategies is an emerging area of ecological study that examines the repeated use of areas for resource acquisition (Ohashi and Thomson 2005, Berger-Tal and Avgar 2012, Riotte-Lambert et al. 2013, Berger-Tal and Bar-David 2015).

Recursive behavior is routinely documented in a variety of species, occurring when animals remember where resources are located within a heterogenous landscape (Berger-Tal and Avgar 2012, Berger-Tal and Bar-David 2015). Path recursion results from nonrandom movements in which animals monitor areas of foraging and repeatedly return to resource rich areas while ignoring resource poor areas (Berger-Tal and Bar-David 2015). Although traditional resource selection analyses have made tremendous advancements in associating animal movements to available resources within their home ranges (Boyce and McDonald 1999, Boyce et al. 2002, Manly et al. 2002), recursive analysis narrows the focus to areas of repeated use by

animals. Therefore, recursive analysis can directly link a mechanism driving animal decision-making (i.e., foraging, den site selection, travel corridors) and resource availability within home ranges.

As noted by Berger-Tal and Bar-David (2015), recursive movements are nearly universal in animals, but the phenomenon is given limited appreciation by researchers because there is little cross-referencing among studies using different methodologies and nomenclature. Their synthesis reported parallels in studies on traplining behavior of pollinators, path recursion of migratory ungulates, and predator-prey interactions under the landscape of fear framework. Whether the study of recursive movements is conducted directly or through other parallel lines of research, it provides a powerful tool for us to understand behavioral and ecological processes. For example, McKeown et al. (2020) used recursive analysis to study movement behavior underlying the formation of red fox (*Vulpes vulpes*) home ranges in southeastern Sweden, whereas Buderman et al. (2018) incorporated recursive behaviors into resource selection functions (RSFs) to examine cougar (*Puma concolor*) ecology in the wildland-urban landscape of the Front Range of Colorado, USA.

In the United States, research on coyotes (*Canis latrans*) has increased significantly over the last several decades because of the species' recent range expansion (Hody and Kays 2018, Hinton et al. 2019), role as the top canid predator in most regions (Gompper 2002, Kilgo et al. 2010, Robinson et al. 2014), ability to live in urban areas (Gehrt et al. 2009, Lombardi et al. 2017, Breck et al. 2019), and hybridization with red wolves (*Canis rufus*; Nowak 2002, Bohling and Waits 2015, Hinton et al. 2018) and eastern wolves (*Canis lycaon*; Wilson et al. 2000, Benson et al. 2012, Rutledge et al. 2012). In the southeastern United States, average home range sizes reported for resident coyotes are relatively large (range = 5.2 – 85.0 km^2; Hinton et al.

2015, Hickman et al. 2016, Ward et al. 2018, Mastro et al. 2019, Stevenson et al. 2019, Chamberlain et al. 2021) and consist of a diversity of land cover ranging from open anthropogenic (i.e., urban and agriculture) to dense vegetation cover (Hinton et al. 2015, Hickman et al. 2016, Ward et al. 2018, Stevenson et al. 2019). Recent studies of coyotes in the southeastern United States reported that coyotes tend to select early successional vegetation communities and open landcover types (Hinton et al. 2015, Stevenson et al. 2019) and primarily consume mammalian prey and fruit (Schrecengost et al. 2008, Cherry et al. 2016, Hinton et al. 2017, 2021, Ward et al. 2018). Ward et al. (2018) suggested that coyote home ranges were stable year-round because of differential use of heterogenous resources (i.e., prey and land cover) and population dynamics of their preferred prey prevented coyotes from overexploiting limited resources within their home ranges. This observation aligns with some key prerequisites of recursive behavior in which foraging areas should show recovery after depletion, and that coyotes have spatio-temporal memory to track the rate of resource recovery in their home ranges (Berger-Tal and Bar-David 2015). Indeed, because coyotes actively defend their home ranges from conspecifics, presumably they have substantive knowledge of local conditions and spatio-temporal patterns of revisitation to areas by coyotes (i.e., recursion behavior) underlies how coyotes form and maintain home ranges.

Despite previous work in the region on 2nd and 3rd-order resource selection by coyotes (Thornton et al. 2004, Crimmins et al. 2012, Hinton et al. 2015, Hickman et al. 2016, Stevenson et al. 2019), how coyote foraging is influenced by landscape characteristics remains poorly understood (Ward et al. 2018). A review of the breadth of coyote research across the eastern United States identified foraging ecology as an important area of future research (Mastro 2011, Mastro et al. 2011). However, due to the densely forested landscapes of the eastern United

States, it is difficult to assess coyote behavior without the use of remote VHF and GPS technologies and novel approaches to analyzing movement data may best capture important ecological behaviors. When correlating prey used by coyote packs with land cover characteristics of home ranges and mean monthly temperatures (e.g., season), Ward et al. (2018) found that vegetation density and season influenced which prey coyotes consumed. For example, coyote consumption of deer and rabbits was negatively correlated with vegetation density, whereas consumption of small mammals and fruit was positively correlated with vegetation density. However, when accounting for coyote consumption of fawns, Ward et al. (2018) reported that season was the most important factor and land cover provided little to no information. Given that our movement data were collected from the same study animals used by Ward et al. (2018), we believe our recursive analysis provides further insights into their findings and coyote foraging behavior in general.

Our objective was to assess the relationship between foraging behaviors of coyotes and landcover using a recursive analysis combined with RSFs in which we assumed that coyotes repeatedly visited areas to acquire resources. Ward et al. (2018) correlated prey use by coyotes with size of coyote home ranges as well as land cover types in their home ranges. The intent of the study was to blend traditional diet analysis with resource selection to make stronger inferences about factors influencing coyote diets. We build off Ward et al. (2018) by using recursive analysis to identify potential foraging areas within the coyote home ranges reported in their study and correlating land cover characteristics with these foraging areas rather than frequency of occurrence of prey types in pack diets. To accomplish this, we used nocturnal and crepuscular locations from coyotes studied by Ward et al. (2018), which consisted of 41 resident coyotes from 15 pack territories across Alabama, Georgia, and South Carolina, USA. In this

region, coyotes were not typically active outside their loafing areas during the day (Hinton et al. 2015, Ward 2017) and previous research has shown coyotes to be predominately nocturnal while foraging (Andelt and Andelt 1981, Holzman et al. 1992, Grinder and Krausman 2001), so we assumed most foraging occurred between dusk and dawn.

Due to the exploratory nature of our analysis and its use of previously published data, we had no genuine *a priori* hypotheses and worked under a predictive modeling framework rather than a hypothetico-deductive one (Freedman 1983, Tredennick et al. 2021). Indeed, our intent was not to conduct analyses on a familiar dataset and then report our goals as *a priori* hypotheses. Nevertheless, we believe our assessment is an important step for improving study designs and hypotheses investigating coyote ecology and their interactions with prey. We built global models for each season using land cover covariates known to be important to 2nd and 3rd order coyote resource selection (Table 3.1). Coyotes in the southeastern United States have been shown to prefer open, early successional and agricultural landcover, while avoiding forested landcover (Chamberlain et al. 2000, Holzman et al. 1992, Schrecengost et al. 2009, Hinton et al. 2015, Hickman et al. 2016, Cherry et al. 2017, Ward et al. 2018, Stevenson et al. 2019). It is hypothesized that more open landscapes mirror the environments of coyotes in the western United States. We predicted that recursive movements associated with coyote foraging would be linked with these open landcover types, while coyotes would avoid forests for foraging. Several studies have shown that edge features are an important factor in home-range selection (Tigas et al. 2002, Hinton et al. 2015, Webster et al. 2022) and it is generally understood that coyotes use landscape edges for hunting and navigation. We predicted that forest edges would be important for recursive movements during foraging bouts. Additionally, Chamberlain et al. (2021) found that coyotes used high density vegetation for both foraging as well as denning in the spring and

we predicted that recursive behavior would be associated with increased vegetative density throughout the year. Finally, to assess if coyote consumption of deer occurred primarily through scavenging of roadkill, we used recursive analysis to correlate revisitation of areas proximate to roads during fall and winter when deer experience greater road mortality due to increased movements associated with their breeding season. Several diet studies have questioned if scavenging of roadkill is an important foraging strategy for coyotes and we predicted that we would see a negative correlation between recursive movements and proximity to roads.

MATERIALS AND METHODS

Study area

We conducted research across approximately 16,200 km^2 of public and private lands in Alabama, Georgia, and South Carolina, USA. Our study area was comprised of two distinct populations of coyotes: Alabama and the contiguous Georgia-South Carolina complex on both sides of the Savannah River (hereafter, SRA; Fig. 3.1). The Alabama population was in the Southeastern Plains ecoregion along the southern border of the Piedmont ecoregion, whereas the SRA population occurred along the boundary between the Piedmont and the Southeastern Plains (Omernik 1987).

The Southeastern Plains and Piedmont ecoregions were characterized by a mixture of upland hardwoods and pines (*Pinus* spp.), and bottomland hardwoods along drainage systems (Griffith 2010). However, the Southeastern Plains included more loblolly pine forests (*P. taeda*) with the addition of oak-hickory-pine woodlands. Land use across both ecoregions was largely loblolly and shortleaf pine (*P. echinate*) plantations and agriculture dominated by cotton, corn, tobacco, soybeans, and peanuts, with the Southeastern Plains generally containing more land in agriculture. Our study area experienced mild, mid-latitude humid subtropical climate with mean

annual temperatures around 17°C (Griffith 2010). Annual rainfall averaged 136 cm in the Southeastern Plains and 123 cm in the Piedmont (NOAA 2018).

Available food for coyotes included deer, eastern wild turkeys (*Meleagris gallopavo*), rabbits (*Sylvilagus* spp.), squirrels (*Sciurus* spp.), eastern woodrats (*Neotoma floridana*), hispid cotton rats (*Sigmodon hispidus*), mice (*Peromyscus* spp.), shrews (*Blarina* spp., *Sorex* spp.), voles (*Microtus* spp.), wild pig (*Sus scrofa*), armadillos (*Dasypus novemcinctus*), opossums (*Didelphis virginiana*), insects, persimmons (*Diospyros virginiana*), blackberry (*Rubus* spp.), wild plums (*Prunus* spp.), pokeweed (*Phytolacca americana*), wild grape (*Vitis* spp.), muscadine (*Vitis rotundifolia*), and black cherry (*Prunus serotina*). Ward et al. (2018) documented diets of resident coyotes across our study sites and reported that of 1226 scat samples, 40.7% contained deer, 25.1% rabbits, 24.5% other small mammals, and 27.5% fruits such as persimmons, wild grape, muscadine, blackberry, dewberry, and pokeweed. Insects, armadillos, livestock, opossum, raccoon, birds, reptiles, human trash, and wild pigs were minor components of coyote diets.

Sampling design

We trapped coyotes during January–February 2015–2016 using offset MB-550 foothold traps (Minnesota Trapline Products Inc., Pennock, Minnesota, USA) and used catchpole, muzzle, and hobbles to restrain them. We fitted coyotes with mortality-sensitive G2110E satellite collars (Iridium; Advanced Telemetry Systems, Isanti, Minnesota, USA) programmed to collect 4-hour interval fixes. Locations were transmitted every 3 days to an Advance Telemetry Systems website center through an Iridium Satellite system. Our research was conducted under approval of the University of Georgia Institutional Animal Care and Use Committee (A2014 08-025-R2) and we followed guidelines published by the American Society of Mammologists (Sikes et al. 2011) and best management practices for trapping furbearers in the United States (White et al.

2021). For further information concerning field protocols and diet assessment see Ward et al. (2018).

Data analyses

To determine landscape-level variables that influenced coyote foraging, we used a resource selection framework (Manly et al. 2002). First, we split our data across 4 seasons: spring (April-June), summer (July-September), fall (October-December), and winter (January-March). To account for seasonal changes in sunlight, we censored diurnal locations using the program 'suncalc' in Program R 4.1.0 (Thieurmel and Elmarhraoui 2019, R Core Team 2020) by calculating dusk and dawn timestamps for each day and only including nocturnal and crepuscular locations between those times. We conducted our analyses on dusk to dawn locations to identify foraging locations for each individual coyote and excluded diurnal locations, which are likely associated with denning and loafing behaviors (Holzman et al. 1992).

We conducted recursion analyses using the program 'recurse' in Program R 4.1.0 (Bracis et al. 2018, R Core Team 2020) using a 100-m buffer around each location to determine the number of times an individual returned to an area (hereafter, recursions) by season. We chose this buffer radius as a conservative estimate of the forage area size likely associated with foraging behaviors. Webster et al. (2022) recently calculated first-passage time (FPT) for a dataset that included the same individual coyotes as those in our study. They reported an average FPT radius around 1500-m using the same 4-hr interval GPS collars. However, Bracis et al. (2018) warns against using a recursive buffer that results in many overlapping circles and suggests that behaviors such as foraging may require smaller buffers to best capture recursive movements. As a test of our buffer size, we ran recursive analyses at 100-m, 250-m, 500-m, 750-m, and 1000-m radius intervals. We observed that buffers 250-m and 500-m created recursion

polygons that closely mirrored the 50% KUD pack home range, while buffers 750-m and 1000-m closely mirrored, or exceeded, the 95% pack home range. Chamberlain et al. (2021) used first-passage time analysis to quantify behavioral states of resident and transient coyotes. They noted that the largest mean variance for all movement paths in their study was 164.7 m and we felt comfortable using the aforementioned 100-m buffer size in our analyses. Additionally, fix accuracy for our collars was 20 meters, making our buffer size appropriate to account for GPS error. Finally, for subsequent RSF analysis, we combined areas of high recursion to create foraging patches that were typically larger than our 100-m buffer, making our choice of buffer size simply a tool for identifying areas of high return (Fig. 3.2).

To determine used and random areas for RSFs, we first combined locations from all individuals within a pack to calculate seasonal 95% fixed kernel density estimates from utilization distributions for coyote pack home ranges using the *kernelUD* function in package 'adehabitatHR' under the ad hoc smoothing parameter in Program R 4.1.0 (Calenge 2006, R Core Team 2020, Fig. 3.2). We then selected pack GPS locations by season with total associated revisits calculated by 'recurse' within the upper-quartile range of recursions to represent points of high foraging use across pack home ranges. We buffered these locations by 100 m in R using the 'raster' package with the dissolve function to create used areas and extracted all GPS locations from within these areas as used locations (Fig. 3.2). To obtain summary statistics for recursion areas, we summed the total number of recursions per buffered location and used the package 'recurse' in Program R 4.1.0 to estimate how much time (hours) each coyote spent within that buffered area, and the time since the last previous visit to that location. Time inside a buffered area is calculated in the package 'recurse' using linear interpolation between adjacent locations, which can allow for estimates smaller than the GPS fix intervals (Bracis et al. 2018).

Once foraging areas were identified, we used the *spsample* function to sample all cells across the 95% kernel utilization distribution (KUD) pack home range to represent resource availability (Pebesma and Bivand 2005, R Core Team 2020). Systematic sampling of availability has been found to most accurately assess resource availability and produced satisfactory model convergence (Benson 2013).

Our landcover covariates were chosen based on their known importance in 2nd and 3rd-order selection of cover types by coyotes in the southeastern United States (Table 3.1). To determine the influence of various landscape characteristics and landcover types on recursive movements, we obtained spatial data on vegetation density, distance to primary and secondary roads, and distance to landcover types. We identified and grouped primary and secondary roads using 2019 USGS Tiger/Line data (Topologically Integrated Geographic Encoding and Referencing). We used the normalized difference vegetation index (NDVI; Pettorelli et al. 2005) to estimate vegetation density and the 2016 National Land Cover Dataset (NLCD; Homer et al. 2015) to assess landcover types. We reclassified NLCD data to combine deciduous, evergreen, and mixed forests into a forest class; shrub/scrub and herbaceous into an open class; and hay/pasture and cultivated crops into an agriculture class. We also included distance to forest edge as a covariate because our study sites were predominately forested, and forest edge comprised most obvious ecotones within pack home ranges. We used the Euclidean Distance function in ArcGIS to calculate distance to roads and each landcover type for every 30-m x 30-m pixel across our study area. We extracted landscape-level covariates across used and random locations to develop RSFs.

To assess the explanatory power of landscape covariates on foraging behavior, we conducted generalized linear mixed effect models in which use was a binary (1 = use, 0 =

random) response variable and landscape covariates were explanatory variables using the package 'lme4' in Program R 4.1.0 (Douglas et al. 2015). We tested our covariates by season for collinearity using the Spearman's correlation test, but all combinations retained a value of $r < 0.6$. Additionally, we included individuals nested within packs as a random intercept to address variation among individuals and packs. Due to the exploratory nature of our study, we chose to not employ a model selection methodology but, instead, used a global model from each season using the covariates selected for their known importance in eastern coyote ecology. Finally, we employed a modified version of Boyce et al. (2002) k-fold cross-validation method described in Roberts et al. (2017) to assess model performance. We blocked our data by pack so that each fold contained spatially independent individuals and averaged Spearman's rank correlation tests for each fold to assess model performance.

RESULTS

In our recursive analysis, we used 41 resident coyotes monitored via GPS collars during 2015-2016, 23 in the Alabama region and 18 in the SRA region. Across all seasons, recursions in our used points ranged from 1 (never returning to the location) to 37 (8.1 ± 5.9, mean ± SE, Supplementary Table 3.1). Recursions were highest during spring (9.8 ± 6.5) and lowest during fall (7.3 ± 5.4). Time since return and time spent within a foraging area had positively skewed distributions, so we present median and interquartile ranges (IQR). Coyotes returned to foraging areas at a median interval of every 7.5 days (IQR = 4.1-16.0 days, Supplementary Table 3.1). By season, coyotes returned to foraging areas at the shortest interval during spring (median = 5.9, IQR = 3.2-13.2 days) and the longest interval during summer (median = 8.6, IQR = 5.0-17.5 days). Individuals stayed within a recursive area for a median length of 4.5 hours (IQR = 2.6-7.1 hours, Supplementary Table 3.1). Across seasons, coyotes remained within recursive areas least

during summer (median = 3.8, IQR = 2.3-5.4 hours) and most during spring (median = 6.3, IQR = 3.9-8.9 hours).

Resource selection by season

By season, we assessed 3rd-order resource selection of 41 individual coyotes across 15 packs within purported forage patches. Coyotes selected for agriculture during the fall and winter while neither selecting or avoiding it during the spring and summer (Table 3.2, Fig. 3.3). Coyotes avoided forest while selecting for forest edge in all seasons except spring, where they selected for forest while avoiding forest edge. Coyotes selected for open, early successional landcover while avoiding roads for every season. Finally, coyotes selected for less dense vegetation in the summer, higher vegetative density during the spring and winter, and showed no selection preference for vegetative density in the fall. Our models performed relatively well with average Spearman's rank correlation values ranging from 0.62 to 0.87 for each season (Table 3.2).

DISCUSSION

We used recursive analysis to identify areas where coyotes were presumed to be foraging and found that coyotes exhibited disproportional use of their home ranges because of their preferential use of land cover types. By censoring movement data to nighttime activity and using sites of recursive behavior as our selected foraging areas, we sought to identify foraging excursions among resident coyotes. A disadvantage of our methodology is that behaviors associated with recursive movements can be difficult to interpret. We recognize that our results may capture additional behaviors such as traveling, denning, and resting. However, our characterization of recursive movements during nocturnal and crepuscular hours as foraging behavior is aligned with studies that reported increased foraging activity of coyotes during these hours (Andelt and Andelt 1981, Holzman et al. 1992, Grinder and Krausman 2001). We found

that coyotes used recursive areas at intervals ranging from approximately 4 days to more than 2 weeks. Coyotes foraged within these areas for a median length of approximately 4 hours, the time interval that our GPS collars returned location fixes. Stated differently, our findings suggest that coyotes often moved and foraged continuously throughout their home range, rather than remaining within an area >4 hours. We believe this pattern may reflect a movement strategy by coyotes to minimize time spent in areas, so as to reduce their exposure to mortality risks (predominately anthropogenic across our study area) while allowing prey to sufficiently recover between foraging bouts under a landscape of fear framework (Brown et al. 1999, Laundre et al. 2001).

Our general findings are consistent with other studies of coyote habitat selection conducted throughout the southern United States, in which coyotes typically select for open, early successional land cover (Holzman et al. 1992, Hinton et al. 2015, Cherry et al. 2016, Stevenson et al. 2019). Coyotes revisited open land cover across all seasons while exhibiting seasonal variation in revisitation to agriculture and forests. We found that coyotes selected for agriculture during revisitations only during fall and winter. Hinton et al. (2015) reported that coyotes used agricultural fields during the spring and summer as daytime loafing areas when planted crops grew high enough to provide cover. However, our findings indicate that agricultural fields were not used for foraging during this time. Cherry et al. (2016) found increased consumption of crops in coyote diets during fall and when fruit was locally unavailable. Coyotes may forage in agricultural fields during fall and winter to supplement their diet with residues from crops found in harvested fields, which could further explain selection of agricultural cover by coyotes during winter, when plant consumption was at its lowest across packs presumably due to lack of native vegetation (Ward et al. 2018). However, it is also likely

that coyotes exploit agricultural-forest edges to hunt prey and consumption of crops is either acquired passively through consumption of prey or through scrounging in preferred foraging areas.

Coyotes selected areas of increased vegetation density during spring and winter revisitations, while avoiding areas of high vegetative density during the summer. Selection for areas with dense vegetation during spring was also observed in an independent study of coyote movements in north-central Georgia, approximately 125 km and 275 km from our SRA and Alabama study areas, respectively (Chamberlain et al. 2021). Using a behavioral state model, Chamberlain et al. (2021) reported that resident coyotes selected areas with dense vegetation density when resting or foraging during the pup-rearing season. Therefore, it was not surprising to us that coyotes exhibited selection for dense vegetation during spring in our study for two reasons. First, coyotes whelp and care for small pups during March through June, and likely relied on vegetation cover for concealing their pups (Andelt 1985, Harrison and Gilbert 1985, Way et al. 2001, Mastro 2011, Mastro et al. 2011, Chamberlain et al. 2021). Second, coyotes prey on deer and likely select cover types during spring that increase their ability to find fawns (Ward et al. 2018, Chamberlain et al. 2021). Deer are known to place their fawns in areas where fawns are concealed by vegetation (Gulsby et al. 2018, Shuman et al. 2018). As coyote pups and deer fawns increase in age and body size, their mobility improves, and they become more independent of their parents while relying less on vegetation concealment for security. Coyotes likely respond to this improved mobility by reorienting their movements to forest edges and adjacent cover types for the remainder of the year.

We documented selection for forested cover by coyotes and avoidance of forest edge during spring revisitations, suggesting that coyotes foraged under forest cover during this season.

Our findings suggest that coyotes shift their selection towards interior forest during spring, possibly as both pup-rearing and foraging strategies. Gulsby et al. (2017) reported a positive relationship between coyote depredation of fawns and mean patch size of forest, although they stated that reasons for the relationship between fawn survival and edge habitat, landscape heterogeneity, and forest patch size remain unclear. We believe selection by coyotes for interior areas of forest during their pup rearing season could be a mechanism by which fawns become more susceptible to predation in forested landscapes during peak fawning in the southeastern United States (April-June).

Finally, we documented avoidance of roads by resident coyotes throughout the year during revisitation bouts. Avoidance of roads by resident coyotes was also observed by Hinton et al. (2015) when they accounted for space use status (i.e., resident vs. transient) of study animals. Considering we only observed movements of resident coyotes, we believe these findings support Hinton et al.'s (2015) conclusions that transient coyotes (i.e., dispersing animals) rely more on road networks as movement corridors than do residents. Regardless, year-round avoidance of roads by resident coyotes provides some insight into how coyotes may acquire deer in their diets throughout the year. For example, scavenging roadkill carcasses has often been implied as a means for coyotes to acquire adult deer (Chamberlain et al. 1999, Schrecengost et al. 2008, Crimmins et al. 2012). Considering deer-vehicle collisions typically peak during the fall and winter (Steiner et al. 2014, Stickles et al. 2015), we expected coyote foraging to shift towards roads during fall and winter to capitalize on the availability of roadkill carcasses. However, road avoidance by coyotes was similar from spring to fall before increasing during winter, when roadkill carcasses would have been better preserved along roads. Furthermore, roads are a primary source of mortality for many wildlife species and there is no shortage of animal

carcasses for coyotes to scavenge along roadsides. If scavenging was an important foraging strategy for coyotes to acquire food, we would have expected to see recursive movements along roadways. Instead, we observed avoidance of roads by coyotes, which supports Hinton et al.'s (2017) suggestion that mortality risks for coyotes when traveling along roadways outweighed benefits of feeding on roadkill carcasses. Finally, Ward et al. (2018) found that consumption of deer by our study animals was associated with smaller home range sizes, indicating that coyote space use was negatively correlated with consumption of deer. It is unlikely that reduced space use would improve the ability of coyotes to locate carcasses spread intermittently along roadways. Nevertheless, deer were consumed year-round by coyotes and the negative correlation between space use and consumption of deer implies that , regardless of season, coyotes selected land cover types where deer were most vulnerable to direct predation. During the spring, this would be land cover types where fawns were present, and during the rest of the year, areas where juvenile or adult deer were more accessible.

An important limitation to our study is the inability to distinguish individual behaviors using our movement dataset. Although we censored our data to most accurately capture known periods of coyote foraging (Andelt and Andelt 1981, Holzman et al. 1992, Grinder and Krausman 2001), it is likely that our recursive analysis captures some additional behaviors such as denning, territory marking, use of cover, and traveling. Previous work on movement recursions has often relied on ground truthing areas of repeated use to corroborate den or kill sites, water holes, and other areas of interest (Berger-Tal and Bar-David 2015, Buderman et al. 2018, McKeown et al. 2020). Berger-Tal and Bar-David (2015) recognized the need for experimental validation of theoretical models because of the correlative association between recursion and behavior. Due to the plastic and mobile nature of our study species, future work is

necessary to further tease out behavioral states and associated recursive movements. This may include behavioral-state modeling with finer scale data to focus recursive analysis on empirically determined behaviors as well as 4th-order resource selection that correlates diet consumed with the landscape covariates associated with recursive foraging patches, similar to Ward et al. (2018). Additionally, little work has been done to ground truth denning sites of coyotes in the eastern United States (Mastro 2011, Mastro et al. 2011), which would help to better understand denning behavior of coyote packs during the spring and how they forage and use resources during that time. Here, our work attempts to provide a methodology using GPS data to explore recursive behavior in coyotes and seeks to inform hypotheses for future work on coyote ecology in the eastern United States.

CONCLUSIONS

Our research demonstrates that recursive analysis can be used to empirically explore foraging behavior of coyotes and can be used to identify movement corridors and suitable habitat for other species. Recursive movements and the rate of revisitations to foraging areas are necessary for coyotes to stabilize their movements and form home ranges. Furthermore, bounded space use and recursive movements to foraging areas demonstrate that resident coyotes exploit food resources within the spatial scale of their home ranges. Additionally, changes in selection across seasons may reveal a mechanism by which coyotes exhibit prey-switching in response to changing availability of food resources. Although assessing the cognitive abilities of coyotes was beyond the scope of this study, it is obvious that memory-based foraging and limits to vagility are responsible for how coyotes interact with landscape heterogeneity and resource dispersion as well as limiting the size of their home ranges.

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CONFLICT OF INTEREST

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

Jordan L. Youngmann: Conceptualization (equal); Data curation (equal); Formal analysis (lead); Investigation (equal); Methodology (equal); Visualization (lead); Writing – original draft (lead); Writing – review & editing (equal). **Joseph W. Hinton**: Conceptualization (equal); Data curation (equal); Formal analysis (supporting); Investigation (equal); Methodology (equal); Project administration (equal); Supervision (equal); Writing – original draft (supporting); Writing – review & editing (equal). **Nicholas W. Bakner**: Conceptualization (equal); Formal analysis (supporting); Investigation (equal); Methodology (equal); Writing – original draft (supporting); Writing – review & editing (equal). **Michael J. Chamberlain**: Conceptualization (equal); Funding acquisition (lead); Project administration (equal); Resources (lead); Supervision (equal); Writing – original draft (supporting); Writing – review & editing (equal). **Gino J.**

D'Angelo: Conceptualization (equal); Supervision (equal); Writing – original draft (supporting); Writing – review & editing (equal).

DATA ACCESSIBILITY STATEMENT

The data files for all movement analyses are available upon request or can be accessed on Dryad (https://doi.org/10.5061/dryad.z8w9ghxgh).

REFERENCES

Andelt, W.F., and Andelt, S. H. 1981. Habitat use by coyotes in southeastern Nebraska. – J. Wildl. Manage. 45:1001–100

Andelt, W. F. 1985. Behavioral ecology of coyotes in South Texas. – Wildlife Monogr. 94: 3–45.

Benson, J. F. et al. 2012. Spatial genetic and morphologic structure of wolves and coyotes in relation to environmental heterogeneity in a Canis hybrid zone. – Mol. Ecol. 21: 5934–5954.

Benson, J. F. 2013. Improving rigour and efficiency of use-availability habitat selection analyses with systematic estimation of availability. – Methods Ecol. Evol. 4: 244–251.

Berger-Tal, O. and Avgar, T. 2012. The glass is half-full: overestimating the quality of a novel environment is advantageous. – PLoS ONE 7: e34578.

Berger-Tal, O. and Bar-David, S. 2015. Recursive movement patterns: review and synthesis across species. – Ecosphere 6: 1–12.

Bohling, J. H. and Waits, L. P. 2015. Factors influencing red wolf–coyote hybridization in eastern North Carolina, USA. – Biol. Conserv.184: 108–116.

Boyce, M. S. and McDonald, L. L. 1999. Relating populations to habitats using resource selection functions. – Trends Ecol. Evol. 14: 268–272.

Boyce, M. S. et al. 2002. Evaluating resource selection functions. – Ecol. Model. 157: 281–300.

Bracis, C. et al. 2018. Revisitation analysis uncovers spatio-temporal patterns in animal movement data. – Ecography 41: 1801–1811.

Breck, S. W. et al. 2019. The intrepid urban coyote: a comparison of bold and exploratory behavior in coyotes from urban and rural environments. – Sci. Rep. 9: 1–11.

Brown, J. S. et al. 1999. The ecology of fear: optimal foraging, game theory, and trophic interactions. – J. Mammal. 80: 385–399.

Buderman, F. E. et al. 2018. Time-varying predatory behavior is primary predictor of fine-scale movement of wildland-urban cougars. – Mov. Ecol. 6: 1–16.

Calenge, C. 2006. The package adehabitat for the R software: a tool for the analysis of space and habitat use by animals. – Ecol. Model. 197: 516-519.

Chamberlain, M. J., and Leopold, B. D. 1999. Dietary patterns of sympatric bobcats and coyotes in central Mississippi. – Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 53: 204–219.

Chamberlain, M. J. et al. 2000. Spatial-use patterns, movements, and interactions among adult coyotes in central Mississippi. – Can. J. Zoology 78: 2087–2095.

Chamberlain, M. J. et al. 2021. Fine-scale movements and behaviors of coyotes (*Canis latrans*) during their reproductive period. – Ecol. Evol. 11: 9575–9588.

Cherry, M. J. et al. 2016. Can coyote predation risk induce reproduction suppression in white-tailed deer? – Ecosphere 7: e01481.

Cherry, M. J. et al. 2017. Effects of land cover on coyote abundance. – Wildl. Res. 43: 662–670.

Crimmins, S. M. et al. 2012. *Canis latrans* (coyote) habitat use and feeding habits in Central West Virginia. – Northeast. Nat. 19: 411–420.

Douglas, B. et al. 2015. Fitting linear mixed-effects models using lme4. – J. Stat. Softw. 67: 1–48.

Freedman, D. A. 1983. A note on screening regression equations. – Am. Stat. 37: 152–155.

Gehrt, S. D. et al. 2009. Home range and landscape use of coyotes in a metropolitan landscape: conflict or coexistence? – J. Mammal. 90: 1045–1057.

Gompper, M. E. 2002. Top carnivores in the suburbs? – Bioscience 52: 185–190.

Griffith, G.E. 2010. Level III North American terrestrial ecoregions: United States descriptions. Commission for Environmental Cooperation. Montreal, Quebec, Canada.

Grinder, M. I., and Krausman, P. R. 2001. Home range, habitat use, and nocturnal activity of coyotes in an urban environment. – J. Wildl. Manage. 65: 887–898.

Gulsby, W. D. et al. 2017. Landscape heterogeneity reduces coyote predation on white-tailed deer fawns. – J. Wildl. Manage. 81: 601–609.

Gulsby, W. D. et al. 2018. Behavioral response of white-tailed deer to coyote predation risk. – Ecosphere 9: e02141.

Harrison, D. J., and Gilbert, J. R. 1985. Denning ecology and movements of coyotes in Maine during pup rearing. – J. Mammal. 66: 712–719.

Hickman, J. E. et al. 2016. Home range, habitat use, and movement patterns of female coyotes in Georgia: implications for fawn predation. – J. Southeast. Assoc. Fish Wildl. Agencies 2: 144–150.

Hinton, J. W. et al. 2015. Space use and habitat selection by resident and transient coyotes (*Canis latrans*). – PLoS ONE 10(7): e0132203.

Hinton, J. W. et al. 2017. Using diets of Canis breeding pairs to assess resource partitioning between sympatric red wolves and coyotes. – J. Mammal. 98: 475–488.

Hinton, J. W. et al. 2018. Size-assortative choice and mate availability influences hybridization between red wolves (Canis rufus) and coyotes (*Canis latrans*). – Ecol. Evol. 8: 3927-3940.

Hinton, J. W. et al. 2019. Geographic patterns in morphometric and genetic variation for coyote populations with emphasis on southeastern coyotes. – Ecol. Evol. 9: 3389–3404.

Hinton, J. W. et al. 2021. Diet of coyotes on the Tensas River National Wildlife Refuge during the white-tailed deer pre-fawning and fawning seasons. – Southeast. Nat. 20: 245–258.

Hody, J. W., and Kays, R. 2018. Mapping the extension of coyotes (*Canis latrans*) across North and Central America. – ZooKeys 759: 81–97.

Holzman, S. et al. 1992. Home range, movements, and habitat use of coyotes in southcentral Georgia. – J. Wildl. Manage. 56: 139–146.

Homer, C. et al. 2015. Completion of the 2011 National Land Cover Database for the conterminous United States–representing a decade of land cover change information. – Photogramm. Eng. Remote Sens. 81: 345–354.

Kilgo, J. C. et al. 2010. Can coyotes affect deer populations in southeastern North America? – J. Wildl. Manage. 74: 929–933.

Lombardi, J. V. et al. 2017. Coyote, fox, and bobcat response to anthropogenic and natural landscape features in a small urban area. – Urban Ecosyst. 20: 1239–1248.

Laundré, J. W. et al. 2001. Wolves, elk, and bison: reestablishing the "landscape of fear" in Yellowstone National Park, U.S.A. – Can. J. Zool. 79: 1401–1409.

Manly, B. F. J. et al. 2002. Resource selection by animals: statistical analysis and design for field studies. Second Edition. Kluwer, Boston, Massachusetts, USA.

Mastro, L. L. 2011. Life history and ecology of coyotes in the Mid-Atlantic states: a summary of the scientific literature. – Southeast. Nat. 10: 721–730.

Mastro, L. L. et al. 2011. Coyote (*Canis latrans*), 100+ Years in the East: A literature review. Addendum to the Proceedings of the 14th Wildlife Damage Management Conference (2012).

Mastro, L. L. et al. 2019. Home range and habitat use of West Virginia *Canis latrans* (Coyote). –

Northeast. Nat. 26: 616–628.

McKeown, B. et al. 2019. Does recursive use of resource locations shape a home range? Exploring the red fox's cognitive map. – Wildlife Biol. 2020(1).

Merkle, J. A. et al. 2014. A memory-based foraging tactic reveals an adaptive mechanism for restricted space use. – Ecol. Lett. 17: 924–931.

Morin, D. J., and Kelly, M. J. 2017. The dynamic nature of territoriality, transience and biding in an exploited coyote population. – Wildlife Biol. doi: 10.2981/wlb.00335.

National Oceanic and Atmospheric Administration [NOAA]. 2018. Climate at a glance: national time series. <https://www.ncdc.noaa.gov/cag/>. Accessed 19 Aug 2018.

Nowak, R. M. 2002. The original status of wolves in eastern North America. – Southeast. Nat. 1: 95-130.

Ohashi, K., and Thomson, J. D. 2005. Efficient harvesting of renewing resources. – Behav. Ecol. 16: 592–605.

Omernik, J. M. 1987. Ecoregions of the conterminous United States. – Ann. Am. Assoc. Geogr. 77: 118–125.

Pebesma, E., and Bivand, R. S. 2005. S classes and methods for spatial data: the sp package. – R news. 5: 9 – 13.

Pettorelli, N. et al. 2005. Using the satellite-derived NDVI to assess ecological responses to environmental change. – Trends Ecol. Evol. 20: 503–510. https://doi.org/10.1016/j.tree.2005.05.011

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>. Accessed 23 Sep 2020.

Riotte-Lambert, L. et al. 2013. Periodicity analysis of movement recursions. – J. Theor. Biol. 317: 238–243.

Roberts, D. R. et al. 2017. Cross-validation strategies for data with temporal, spatial, hierarchical, or phylogenetic structure. Ecography. 40: 913–929.

Robinson, K. F. et al. 2014. Can managers compensate for coyote predation of white-tailed deer? – J. Wildl. Manage. 78: 571–579.

Rutledge, L. Y. et al. 2012. Intense harvesting of eastern wolves facilitated hybridization with coyotes. – Ecol. Evol. 2: 19–33.

Schrecengost, J. D. et al. 2008. Seasonal food habits of coyote in the South Carolina Coastal Plain. – Southeast. Nat. 7: 135–144.

Schrecengost, J. D. et al. 2009. Home range, habitat use and survival of coyotes in western South Carolina. – Am. Midl. Nat. 162: 346–355.

Sells, S. N. and Mitchell, M. S. 2020. The economics of territory selection. – Ecol. Model. 438: 109329.

Shuman, R. M. et al. 2018. Resource selection by parturient and post-parturient white-tailed deer and their fawns. – J. Southeast. Assoc. Fish Wildl. Agencies 5: 78-84.

Sikes, R. S. and Gannon, W. L. 2011. The Animal Care and Use Committee of the American Society of Mammalogists. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. – J. Mammal. 92: 809–823.

Steiner, W. et al. 2014. A review on the temporal pattern of deer–vehicle accidents: Impact of seasonal, diurnal and lunar effects in cervids. – Accid Anal Prev 66:168–181.

Stevenson, E. R. et al. 2019. Resource selection by coyotes (*Canis latrans*) in a longleaf pine (*Pinus palustris*) ecosystem: effects of anthropogenic fires and landscape features. – Can. J. Zool. 97: 165–171.

Stickles, J. H. et al. 2015. Using deer-vehicle collisions to map white-tailed deer breeding activity in Georgia. – J. Southeast. Assoc. Fish Wildl. Agencies 2: 202–207.

Teitelbaum, C. S. and Mueller, T. 2019. Beyond migration: causes and consequences of nomadic animal movements. – Trends Ecol. Evol. 34: 569–581.

Thieurmel, B. and A. Elmarhraoui. 2019. suncalc: compute sun position, sunlight phases, moon position and lunar phase. R package version 0.5.0. <https://CRAN.R-project.org/package=suncalc>. Accessed 2 Dec 2021.

Thornton, D. H. et al. 2004. Ecological separation within newly sympatric populations of coyotes and bobcats in south-central Florida. – J. Mammal. 85: 973–982.

Tigas, L. A. et al. 2002. Behavioral responses of bobcats and coyotes to habitat fragmentation and corridors in an urban environment. – Biol. Conserv. 108:299–306.

Tredennick, A. T. et al. 2021. A practical guide to selecting models for exploration, inference, and prediction in ecology. – Ecology 102:e03336.

Van Moorter, B. et al. 2009. Memory keeps you at home: a mechanistic model for home range emergence. – Oikos 118: 641–652.

Van Moorter, B. et al. 2016. Movement is the glue connecting home ranges and habitat selection. – J. Anim. Ecol. 85: 21–31.

von Holdt, B. M. et al. 2016. Whole-genome sequence analysis shows that two endemic species of North American wolf are admixtures of the coyote and gray wolf. – Sci. Adv. 2: e1501714.

Ward, J. N. Space use and resource selection by coyotes in the southeastern United States. University of Georgia. 2017.

Ward, J. N. et al. 2018. Home range size, vegetation density, and season influences prey use by coyotes (*Canis latrans*). – PLoS ONE 13: e0203703.

Way, J. G. et al. 2001. Eastern coyote denning behavior in an anthropogenic environment. – Northeast Wildlife 56: 18–30.

Webster, S. C., J. C. Beasley, J. W. Hinton, and M. J. Chamberlain. 2022. Resident and transient coyotes exhibit differential patterns of movement behavior across heterogeneous landscapes in the southeastern United States. – Ecol. Evol. 12:e8725.

White, H. B. et al. 2021. Best management practices for trapping furbearers in the United States. – Wildl. Monogr. 207: 3–59.

Wilson, P. J. et al. 2000. DNA profiles of the eastern Canadian wolf and the red wolf provide evidence for a common evolutionary history independent of the gray wolf. – Can. J. Zool. 78: 2156–2166.

Table 3.1. A selection of landcover covariates that potentially influence foraging behavior of coyotes in Alabama, Georgia, and South Carolina during 2015-2016.

Covariate	Biological Importance	References
Agriculture	Resident coyotes select for agriculture when choosing home ranges (2^{nd}-order selection) and use agriculture within their home ranges (3^{rd}-order selection).	Hinton et al. 2015, Ward et al. 2018
Open, early successional	Coyotes select for open, early successional habitat in their home ranges (3^{rd}-order selection)	Hinton et al. 2015, Cherry et al. 2017, Stevenson et al. 2019
Forest	Coyotes avoid forest cover in their home ranges (3^{rd}-order selection)	Chamberlain et al. 2000, Holzman et al. 1992, Schrecengost et al. 2009, Hinton et al. 2015, Hickman et al. 2016
Forest edge	Coyotes are known to forage along habitat edges and edge is an important factor in home-range selection (2^{nd}-order selection)	Tigas et al. 2002, Hinton et al. 2015
NDVI	Coyotes may select high density vegetation for foraging	Ward et al. 2018, Chamberlain et al. 2021
Roads	Resident coyotes avoid roads in their home range selection (3^{rd}-order selection) but may use them for scavenging roadkill	Chamberlain et al. 1999, Schrecengost et al. 2008, Crimmins et al. 2012, Cherry et al. 2016

Table 3.2. Parameter estimates from top generalized linear mixed models for 3rd-order resource selection functions for radio-collared coyotes in Alabama, Georgia, and South Carolina during 2015-2016. Shown are coefficient estimates (β), standard error (SE), 95% confidence intervals (CI), *z*-scores, and *P*-values. Also included is the average Spearman's rank correlation coefficient (ρ) for each seasonal global model.

Season	Model variables	β	SE	95% CI	*z*	*P*	ρ
Spring	Distance to Forest	-0.074	0.026	-0.125, -0.023	-2.840	0.005	0.62
	Distance to Agriculture	-0.031	0.027	-0.084, 0.022	-1.155	0.248	–
	Distance to Open/Early	-0.407	0.029	-0.463, -0.352	-14.291	<0.001	–
	Distance to Roads	0.201	0.031	0.141, 0.261	6.526	<0.001	–
	NDVI[1]	0.101	0.024	0.054, 0.147	4.267	<0.001	–
	Distance to Forest Edge	0.063	0.028	0.009, 0.117	2.275	0.023	–
Summer	Distance to Forest	0.452	0.028	0.398, 0.506	16.419	<0.001	0.65
	Distance to Agriculture	0.000	0.026	-0.051, 0.051	-0.013	0.990	–
	Distance to Open/Early	-0.139	0.024	-0.185, -0.092	-5.865	<0.001	–
	Distance to Roads	0.136	0.031	0.076, 0.196	4.457	<0.001	–
	NDVI	-0.116	0.017	-0.150, -0.082	-6.660	<0.001	–
	Distance to Forest Edge	-0.534	0.035	-0.603, -0.466	-15.264	<0.001	–
Fall	Distance to Forest	0.532	0.029	0.474, 0.590	18.080	<0.001	0.69
	Distance to Agriculture	-0.183	0.026	-0.234, -0.132	-7.062	<0.001	–
	Distance to Open/Early	-0.293	0.024	-0.340, -0.245	-12.097	<0.001	–
	Distance to Roads	0.087	0.026	0.035, 0.138	3.306	0.001	–
	NDVI	-0.008	0.016	-0.040, 0.025	-0.459	0.646	–
	Distance to Forest Edge	-0.415	0.037	-0.488, -0.342	-11.164	<0.001	–
Winter	Distance to Forest	0.529	0.034	0.463, 0.595	15.705	<0.001	0.87
	Distance to Agriculture	-0.117	0.030	-0.176, -0.057	-3.837	<0.001	–
	Distance to Open/Early	-0.216	0.025	-0.266, -0.167	-8.561	<0.001	–
	Distance to Roads	0.307	0.028	0.252, 0.362	10.921	<0.001	–
	NDVI	0.219	0.025	0.170, 0.268	8.750	<0.001	–
	Distance to Forest Edge	-0.709	0.041	-0.790, -0.629	-17.198	<0.001	–

[1]Normalized Difference Vegetation Index

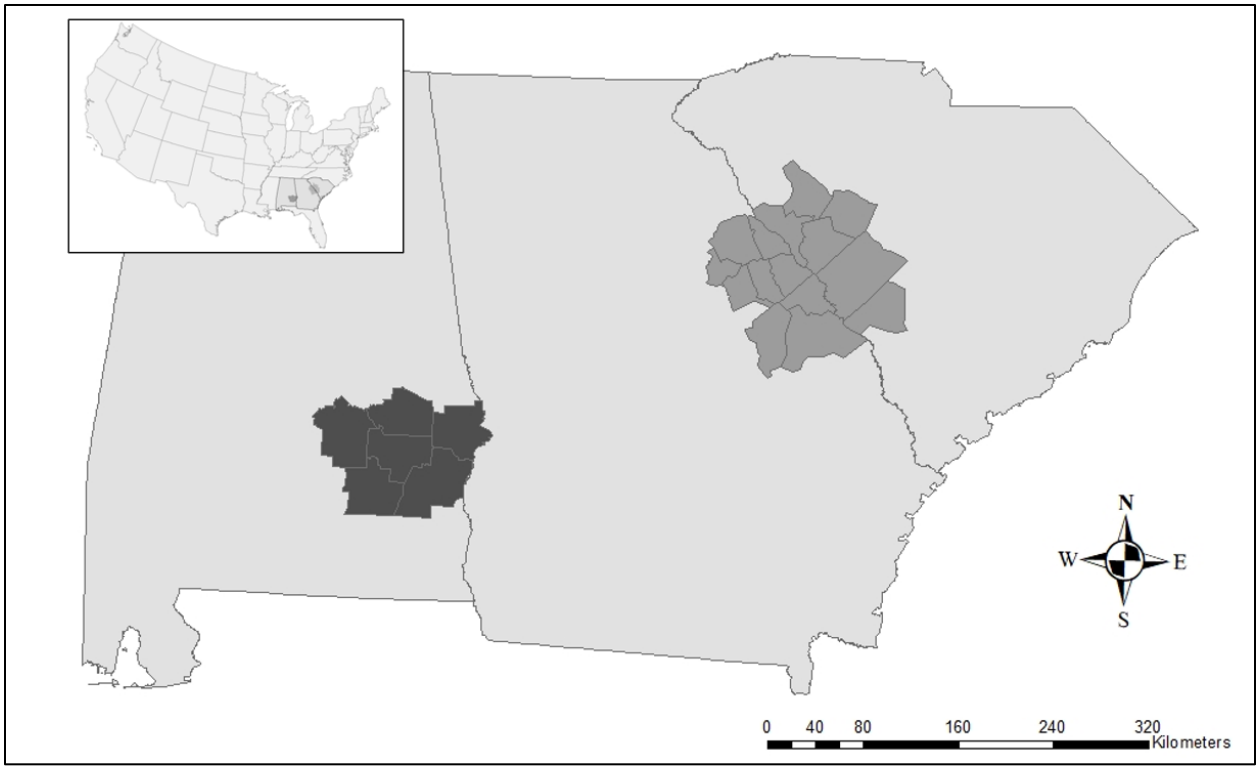

Figure 3.1. Map showing study sites in Alabama, Georgia, and South Carolina, USA used to assess recursive movements of coyotes in 2015-2016. The Alabama site is in dark grey on the Alabama-Georgia line and the Savannah River Area (SRA) site is in light grey on the Georgia-South Carolina line.

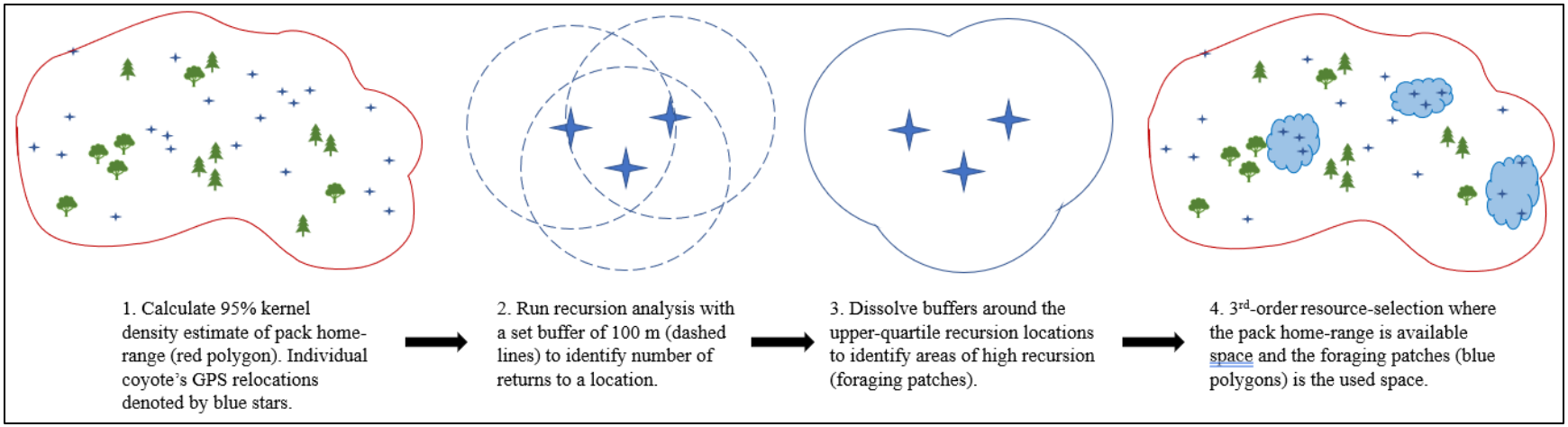

Figure 3.2. A workflow schematic detailing how 3rd-order resource selection is derived from individual coyote foraging movements using the program 'recurse' in Program R 4.1.0 (Bracis et al. 2018).

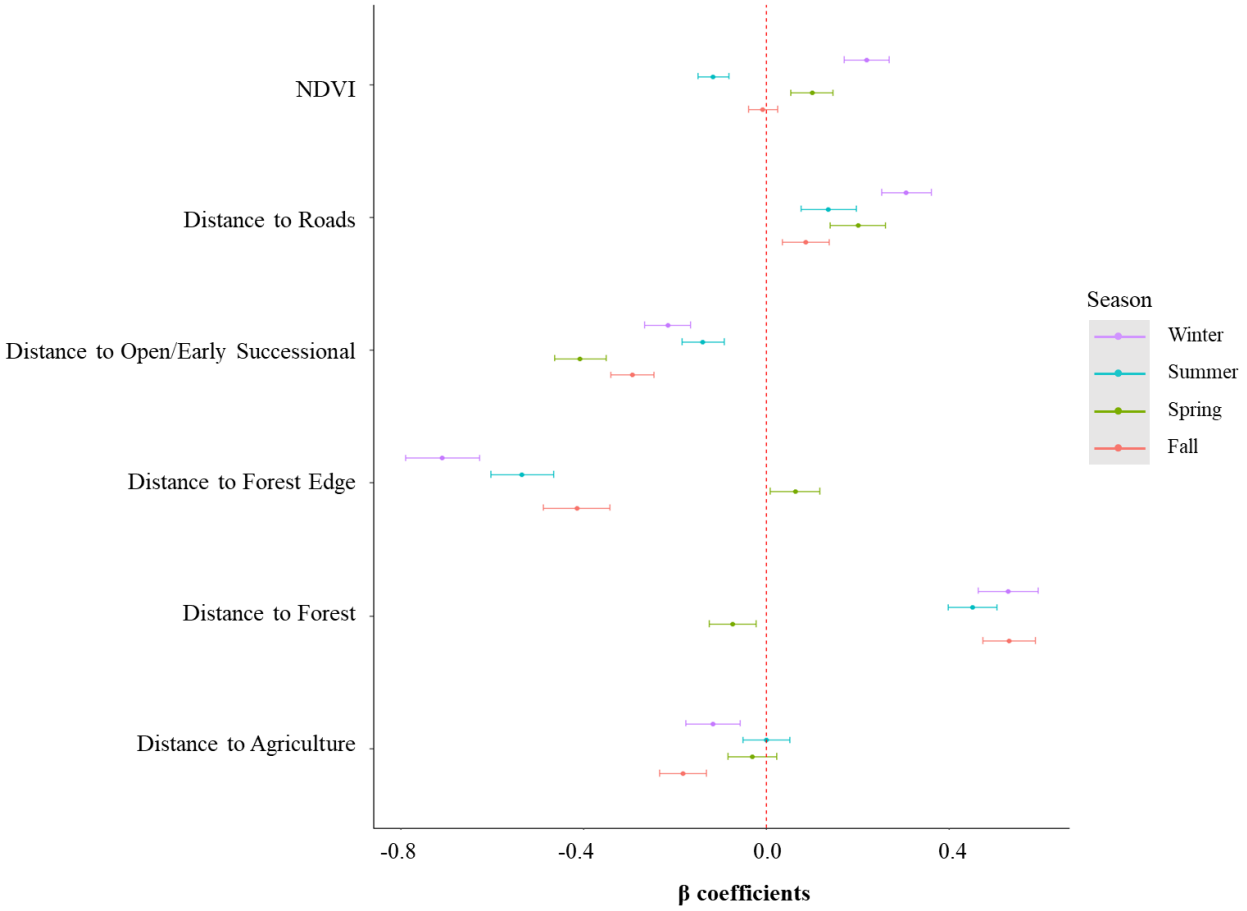

Figure 3.3. Parameter estimates with 95% confidence intervals for 3rd-order resource selection functions across 4 seasons for radio-collared coyotes in Alabama, Georgia, and South Carolina during 2015-2016. For all distance-based variables, negative values infer selection. For the Normalized Difference Vegetation Index (NDVI), positive values infer selection.

Supplementary Table 3.1. Summary statistics of recursions, time since last visit, time spent within recursion site, and frequency of occurrence of diet items found in pack scat radio-collared coyotes in Alabama, Georgia, and South Carolina during 2015-2016. Recursion Q3 cutoff is the third-quartile limit used to select high recursion GPS points to create forage patches. Summary statistics are otherwise derived from all GPS locations extracted from within the recursion patches.

		Recursion	Recursions		Time Since			Time Inside			Average frequency of occurrence of diet			
Season	Pack	Q3 Cutoff	Mean	SD	Q1	Median	Q3	Q1	Median	Q3	Deer[a]	SM[b]	Rabbit	Plants
Fall	Burks Mountain	3	5.6	3.5	6.4	8.7	16.7	1.4	2.2	4.7	NA	NA	NA	NA
Fall	Childs	4	12.2	7.5	2.5	5.3	23.8	4.7	6.5	8.9	0.0	0.0	0.7	1.0
Fall	Clayton Creek	3	4.9	2.6	12.9	30.5	89.1	1.4	2.4	3.9	0.5	0.1	0.2	0.5
Fall	Dunn	5	7.0	3.2	4.4	6.6	9.5	2.7	3.6	4.6	NA	NA	NA	NA
Fall	Gaston	1	2.7	1.2	2.9	3.8	13.4	1.0	1.6	2.8	NA	NA	NA	NA
Fall	Grant	3	4.2	2.3	4.2	7.7	13.8	2.2	3.4	5.3	NA	NA	NA	NA
Fall	Hwy 82	3	3.8	1.5	13.4	16.7	31.2	1.1	1.7	3.5	0.4	0.1	0.3	0.7
Fall	Joyes	1	4.3	3.9	3.0	4.7	8.9	1.6	3.0	5.3	NA	NA	NA	NA
Fall	Kitchens	6	6.3	1.8	4.1	8.0	12.1	2.9	3.7	4.5	0.3	0.1	0.5	0.4
Fall	Mt. Calvary	3	5.5	3.7	6.1	11.2	18.8	1.5	2.8	4.7	1.0	0.0	0.0	0.0
Fall	Parker Hill	4	6.6	3.4	3.2	5.3	8.6	3.5	4.5	5.6	NA	NA	NA	NA
Fall	Pines Grove	6	8.2	3.5	4.1	6.1	10.4	3.2	4.5	6.4	0.5	0.0	0.5	0.0
Fall	Saluda	4	6.6	3.3	6.2	9.5	13.3	1.6	2.2	3.7	0.4	0.1	0.3	0.3
Fall	Soap Creek	7	8.6	3.4	5.3	7.2	10.4	3.5	4.5	5.4	NA	NA	NA	NA
Fall	Zion Chapel	1	5.8	4.4	3.9	6.4	10.1	2.1	4.3	6.9	NA	NA	NA	NA
Spring	Burks Mountain	4	9.3	6.1	3.5	9.3	21.4	3.9	6.4	12.7	NA	NA	NA	NA
Spring	Childs	3	7.8	3.7	5.0	6.6	10.7	2.5	4.0	5.8	0.0	0.5	0.0	0.5
Spring	Clayton Creek	4	6.0	3.6	13.4	47.7	114.0	4.9	8.3	11.8	0.5	0.3	0.4	0.0
Spring	Dunn	8	11.0	5.0	3.2	4.2	7.0	3.6	6.0	8.8	0.2	0.4	0.2	0.4
Spring	Gaston	3	5.3	2.4	4.5	8.7	14.0	2.8	4.3	6.9	NA	NA	NA	NA
Spring	Grant	11	22.0	8.5	1.3	2.2	4.2	7.4	9.4	13.3	1.0	0.2	0.0	0.2
Spring	Hwy 82	4	4.2	1.4	6.1	13.5	20.2	1.2	1.9	2.5	0.3	0.4	0.1	0.5

Spring	Kitchens	2	4.1	2.2	4.0	6.4	8.2	3.4	3.9	5.2	0.2	0.2	0.2	0.3
Spring	Mt. Calvary	6	9.0	4.1	1.7	5.2	9.1	4.5	6.6	10.5	0.9	0.0	0.0	0.1
Spring	Parker Hill	11	13.7	5.2	4.8	5.8	8.1	4.7	8.4	9.1	NA	NA	NA	NA
Spring	Pines Grove	5	12.9	6.7	2.8	4.1	8.5	5.2	6.7	8.2	0.3	0.3	0.2	0.5
Spring	Saluda	7	10.4	4.1	3.2	4.8	6.7	5.1	7.0	10.2	0.5	0.1	0.5	0.2
Spring	Soap Creek	5	7.3	2.7	2.8	5.0	9.5	4.3	5.8	7.7	NA	NA	NA	NA
Spring	Zion Chapel	5	9.9	5.3	3.3	4.5	13.0	5.6	7.0	8.9	0.1	0.1	0.3	0.3
Summer	Burks Mountain	3	7.9	3.4	5.7	7.1	10.5	2.4	3.6	5.5	NA	NA	NA	NA
Summer	Childs	4	11.9	5.3	4.1	5.4	7.8	3.6	4.6	5.4	0.5	0.0	0.0	1.0
Summer	Clayton Creek	5	6.3	2.4	23.4	60.0	89.4	2.8	4.1	7.4	0.0	0.5	0.5	0.1
Summer	Gaston	3	4.1	1.8	5.1	11.1	18.4	1.5	2.2	3.8	NA	NA	NA	NA
Summer	Grant	4	5.1	2.1	4.1	8.1	13.1	3.8	4.6	6.2	0.5	0.2	0.0	0.5
Summer	Hwy 82	3	4.0	1.9	5.9	13.1	20.1	2.2	3.2	4.5	0.4	0.3	0.1	0.5
Summer	Kitchens	5	6.0	2.1	7.6	10.8	16.1	2.1	2.8	4.2	0.3	0.3	0.0	0.9
Summer	Mt. Calvary	5	6.0	2.1	7.0	9.4	14.2	2.3	4.2	6.8	1.0	0.0	0.0	0.0
Summer	Parker Hill	5	4.9	0.9	2.9	3.8	5.2	3.8	5.2	18.5	NA	NA	NA	NA
Summer	Pines Grove	3	12.7	6.0	2.8	4.8	6.9	3.3	4.4	5.7	0.5	0.1	0.3	0.9
Summer	Saluda	6	7.3	3.0	4.9	8.1	13.0	2.6	4.0	5.6	0.3	0.8	0.1	0.0
Summer	Soap Creek	3	5.0	2.6	4.7	8.5	14.4	1.6	2.2	3.0	NA	NA	NA	NA
Summer	Zion Chapel	2	6.2	4.6	10.9	28.5	60.4	1.5	2.7	4.5	0.1	0.3	0.3	0.4
Winter	Burks Mountain	3	5.1	2.7	4.9	8.3	15.9	1.3	2.6	5.1	NA	NA	NA	NA
Winter	Childs	3	9.5	8.7	3.9	9.0	16.3	3.0	5.6	8.6	0.0	1.0	0.0	0.0
Winter	Clayton Creek	6	8.2	3.5	6.8	12.6	53.6	2.6	4.4	8.7	0.5	0.5	0.2	0.0
Winter	Dunn	4	8.0	3.6	3.0	5.2	8.6	2.2	4.2	5.4	0.4	0.6	0.1	0.0
Winter	Gaston	1	1.4	0.5	0.3	2.7	5.6	1.8	7.0	13.5	NA	NA	NA	NA
Winter	Grant	4	5.5	1.9	3.3	6.1	10.7	4.2	5.8	9.3	0.6	0.1	0.2	0.0
Winter	Hwy 82	2	3.6	2.8	3.8	6.9	15.0	1.1	2.3	4.8	0.5	0.5	0.0	0.0
Winter	Joyes	2	4.1	2.8	3.7	7.0	13.6	2.8	4.1	4.8	NA	NA	NA	NA
Winter	Mt. Calvary	6	9.9	5.1	9.8	21.0	46.5	3.4	4.8	6.7	0.7	0.2	0.0	0.0
Winter	Parker Hill	8	14.3	5.8	3.9	4.3	5.6	8.7	10.3	11.4	0.5	0.4	0.1	0.0
Winter	Pines Grove	3	7.6	4.7	5.3	9.8	19.0	2.4	4.5	8.2	0.6	0.3	0.3	0.0
Winter	Saluda	12	18.6	7.8	2.6	3.5	7.2	5.3	6.5	7.1	NA	NA	NA	NA

Winter	Soap Creek	2	3.7	1.8	5.6	10.4	24.3	1.8	2.6	3.6	0.4	0.3	0.5	0.1
Winter	Zion Chapel	1	6.7	3.1	2.8	5.5	13.7	3.4	6.3	9.4	0.2	0.2	0.5	0.0

[a]White-tailed deer
[b]Small mammals

CHAPTER 4

ASSESSING SPRINGTIME VERTEBRATE PREY OF SYMPATRIC MESOPREDATORS IN THE SOUTHEASTERN UNITED STATES USING METABARCODING ANALYSIS

Youngmann, J. L., S. L. Lance, J. C. Kilgo, C. Ruth, J. Cantrell, and G. J. D'Angelo. 2023. Submitted to *Plos ONE* December 2022. Revisions submitted May 2023.

ABSTRACT

Coyotes (*Canis latrans*) colonized the eastern United States over the last century and formed a 3-species predator guild with bobcats (*Lynx rufus*) and gray foxes (*Urocyon cinereoargenteus*) across much of the southeastern United States. Diets among the three species vary along with respective impacts on game species such as white-tailed deer (*Odocoileus virginianus*) and wild turkeys (*Meleagris gallopavo*). To determine predation impacts on vertebrate prey and dietary overlap in consumption of prey items, we assessed diets of coyote, bobcat, and gray fox during spring, coinciding with white-tailed deer fawning and wild turkey nesting and brood rearing. We sampled across three sites along the Savannah River in South Carolina from mid-May through mid-June of 2020-2021. We collected 180 scat samples along 295.9 kilometers (71.1 – 122.4 km/site) of unpaved secondary roads and used DNA metabarcoding to determine vertebrate diet items. We identified predator species of scat using DNA metabarcoding and species-specific mtDNA fragment analysis (153 were coyote, 20 bobcat, and seven gray fox). Overall, we found evidence that two species, coyote and bobcat, consumed deer while all three consumed turkeys. Frequency of deer in the diet varied across sites for coyotes from 62 – 86% and wild turkey was present with a frequency of occurrence of 9% for coyotes, 5% for bobcats, and 14% for gray fox. Vertebrate diet specialization was evident across predator species with high frequency of deer in coyote diets, rabbits and small mammals in bobcat diets, and herpetofauna in gray fox diets. During deer fawning and wild turkey nesting and brood rearing, dietary overlap appears to be mediated by disparate selection of prey items, which reduced competition among coyotes, bobcats, and gray foxes. Use of DNA metabarcoding may augment our understanding of dietary preferences within this predator guild by providing increased resolution of diet composition among important game species.

INTRODUCTION

Coyotes (*Canis latrans*), bobcats (*Lynx rufus*), and gray foxes (*Urocyon cinereoargenteus*) are mesopredators comprising a three-predator guild that has been well-documented across their sympatric range [1–4]. Range overlap among these species has increased over the last century as coyotes have colonized the eastern United States [5,6], in part due to the extirpation of top predators such as the red wolf (*Canis rufus*) and eastern cougar (*Puma concolor*). In areas lacking larger predators such as black bears (*Ursus americanus*), coyotes and bobcats are apex predators within the trophic hierarchy of the eastern United States, due to their size and consumption of ungulates such as white-tailed deer (*Odocoileus virginianus*) [3,7,8]. The success of coyotes in the eastern United States has implications for other species, including other mesopredators. For example, recent research has documented marked declines in gray fox populations in the region, potentially as a result of exploitative and interference competition with colonizing coyote populations including overlap in diet and spatial exclusion [4,9,10].

Assessing diets is an important step towards understanding how coyotes, bobcats, and gray foxes compete, and how this predator guild influences the food web in the eastern United States. While many studies have reported considerable overlap in space use and diet among coyotes, bobcats, and gray foxes [2,4,11–16], differences in hunting strategies and differing trophic functions may potentially alleviate intraguild competition. For example, bobcats are obligate carnivores that hunt via ambush tactics, whereas coyotes and gray foxes are generalist omnivores that hunt cursorily and supplement their diet with non-animal foods [1,17–22]. Bobcat diets in the eastern United States are largely comprised of small mammals, including squirrels (*Sciurus* spp., *Glaucomys* spp., *Sigmodon hispidus*), lagomorphs, and white-tailed deer [13,22,23]. Coyotes in the eastern United States tend to exhibit greater diversity in food choice

than bobcats, consuming white-tailed deer, small mammals such as the cotton rat (*Sigmodon* spp.), lagomorphs, soft mast including *Rubus* spp. and persimmons (*Diospyros virginiana*), and insects [13,17,19–21,24,25]. There has been little research conducted on gray fox diets in the eastern United States, especially since coyote colonization, but studies in the western United States have shown them to be generalist omnivores with a diet that is similar to coyotes [26–28]. Recent stable isotope analysis has shown overlap in the diet of coyotes and gray foxes in the eastern United States [4,29]. Through assessment of the vertebrate species found in coyote, bobcat and gray fox diets, we hope to better understand how this predator guild interacts and its role in shaping predator-prey dynamics in the eastern United States.

In the southeastern United States, the coyote-bobcat-gray fox predator guild is thought to be negatively impacting two important game species: white-tailed deer and wild turkey (*Meleagris gallopavo*). Thus, assessing the diet of all three predator species will provide data on intra-guild competition as well as inform management of game species. Population trajectories of white-tailed deer have stabilized or declined in some southeastern states, coincident with rises in coyote populations [30]. Past studies have reported high rates of white-tailed deer fawn mortality due to predation, especially in the southeastern United States [31–34]. Additionally, coyote, bobcat, and gray fox depredation of turkey hens and poults during nesting and brood rearing periods [35–37] C. Ruth, South Carolina Department of Natural Resources, personal communication] may be partially responsible for declining wild turkey populations over the last decade [38–41]. Although these predators may predate hens, particularly during the spring [35–37,42–44], there is scant evidence across diet studies that they rely on wild turkeys as a food source [12,15,45]. Better understanding of predator impacts on populations of wild turkeys and

white-tailed deer is vital for agencies to effectively conserve and manage predator and prey species alike.

Diet analyses have traditionally used morphometric identification of remnant prey items in either scat or stomach contents [46]. However, analysis of both host and prey species through visual identification of scat is often inaccurate [13,47]. Morin et al. [13] documented high rates of misclassification of coyote and bobcat scat, which led to incorrectly attributing diet to the wrong predator. Although some recent studies have used genetic methodologies [11,14,29,48,49], Monterroso et al. [47] identified only 8% of 400 diet studies that used genetics to identify predator species. Morphometric identification of diet items within scat also can be biased because of differences in digestion efficiency and the inability to classify trace amounts of remnant prey items. For instance, traditional diet analyses have typically failed to show substantial predation on avian species by coyotes, bobcats, or gray foxes, even during periods when ground nesting birds are on their nests [23,50]. However, coyotes, bobcats, and gray foxes do appear to have consistent, but low, presence of avian species in their diets [26,27,51,52]. The general lack of avian species in scat studies may be due to low predation rates or the inability of morphometric diet analyses to identify avian remains. Several methodological studies have shown disparities in identification of avian remains between analysis of stomach contents and morphometric scat analysis, possibly due to differential rates of digestion [53,54]. To address past shortcomings in sampling methods, we used DNA metabarcoding, which uses genetic sequencing to identify both the host and varied prey species contained within each scat sample. This method may provide further resolution in determining the dietary composition of each species [47,55,56] and has recently been used successfully to study coyote diets [57,58]. DNA

metabarcoding may allow better understanding of how coyotes, bobcats, and gray foxes function as competitors within their guild and as apex predators on the landscape.

To assess intraguild competition among coyotes, bobcats, and gray foxes and to better understand predation levels on game species, we sampled scat during the fawning period of white-tailed deer and nesting of wild turkeys. Our objective was to compare vertebrate diet items using metabarcoding analysis among these sympatric predators to assess the potential for competition among intraguild interactions in the southeastern United States. We hypothesized that coyotes and gray foxes would exhibit higher diversity in their diets than bobcats due to their generalist diet preferences. Additionally, we hypothesized that we would observe higher levels of dietary overlap between coyotes and gray foxes due to their similar diets and hunting strategies. We also predicted that because genetic methods can be more precise than visual identification of prey items [13,47,55,56], we would find higher frequency of avian prey within predator diets than previously reported.

Furthermore, because coyote scat analysis can be used to assess the influence of landcover covariates on coyote diet [20,21], we sought to replicate Hinton et al. [21] to address three additional hypotheses. Firstly, forest cover would positively affect the consumption of deer and wild turkeys. Forest cover is a predominant landcover type within our study region and previous research has documented increased predation of fawns in relation to forest patch size and availability, although causality has been difficult to quantify [59,60]. Limited understory vegetation in closed canopy forests should increase the ability of coyotes to find fawns and turkeys. Secondly, Julian date would positively influence consumption of fawns as previously reported in the southeastern United States [32,61,62]. Finally, consumption of other prey items would be negatively correlated with the consumption of deer and wild turkeys. Coyotes exhibit

prey switching behavior to optimize foraging by capitalizing on high quality resources and fawns experience heavy predation rates in the springtime, likely due to their availability and vulnerability [20,21].

STUDY AREA

We assessed diets of coyote, bobcat, and gray fox populations in three Level III ecoregions in South Carolina, USA: the Davis Land and Timber property in the Piedmont, the U.S. Department of Energy's Savannah River Site in the Southeastern Plains, and the South Carolina Department of Natural Resources' Webb Complex in the Middle Atlantic Coastal Plain (Fig 1) [63]. The Davis Land and Timber property is entirely privately owned, Savannah River Site is a National Environmental Research Park, and the Webb Complex is a mixture of state-owned wildlife management areas and privately owned lands. The Piedmont ecoregion lies between the Blue Ridge Mountains and the Southeastern Plains. Originally dominated by oak-hickory-pine (*Quercus-Carya-Pinus*) forests, the Piedmont has experienced extensive cotton, corn, tobacco, and wheat farming [64]. However, much of the region is now covered by both natural and planted pine stands. Mean annual temperature in the Piedmont is approximately 15°C with a mean annual precipitation of 1229 mm [65]. The Southeastern Plains is typified by sandy soils and was comprised historically of mostly longleaf pine (*Pinus palustris*) forest although it now contains extensive amounts of cultivated cropland and pasture/hay with large areas of pine plantations [64]. However, the Savannah River Site is almost entirely forested in planted pine, with bottomland hardwoods scattered throughout [66]. Mean annual temperature in the Southeastern Plains is ~16°C with a mean annual precipitation of 1358 mm [65]. The Middle Atlantic Coastal Plain contains lowland plains filled with swamps, marshes, and estuaries. Also originally covered in longleaf pine, many areas have been converted to pine plantations [64].

Mean annual temperature in the Middle Atlantic Coastal Plain is ~15.5°C with a mean annual precipitation of 1229 mm [65].

METHODS

Ethics statement

Our research only involved the non-invasive collection of scat with no live capture, handling, or killing of animals. We were, therefore, exempt from the University of Georgia Institutional Animal Care and Use Committee protocols.

Sampling methods

We sampled each site from mid-May through mid-June during 2020 and 2021, coincident with fawning in white-tailed deer and nesting in wild turkeys within this region [32,67]. We established 71.1 km of transects at Davis Land and Timber, 122.4 km at Savannah River Site, and 102.4 km at the Webb Complex. We conducted our sampling along secondary dirt and gravel roads that were evenly spaced and limited in vehicle traffic. We collected scat on the first day and then every three days for 10 total sampling days over a 31-day period. This ensured that each scat collected was $\leq$ 3 days old except for the first day of collection. We conducted each sampling pass by driving a vehicle along each transect at ~8 km/hr looking for scat.

We used a new wooden sampling stick or sterilized forceps to place an approximately 0.4-mL cross-section of scat into a 2 mL tube containing 1.6 mL of DETs (DMSO/EDTA/Tris/salt) buffer [68]. We recorded the sample ID, GPS coordinates, and appearance (i.e., consistency, color, contents, etc.). We placed the remaining scat into a sealed plastic bag marked with the date, GPS coordinates, and sample ID. We stored buffered samples at ambient temperature. We extracted DNA from each sample using Qiagen's QIAamp DNA Stool Mini Kit (Qiagen, Valencia, California, USA) and methodologies were conducted in two

Qiagen QIAcubes. We followed Qiagen's extraction protocols except for the final step where we eluted with 50 µl Buffer ATE and then pipetted each sample by hand back onto the filter and spun the elution product back through the filter tube to ensure that all DNA was captured from the extraction process. We then sent extracted samples to Jonah Ventures (Boulder, CO) for metabarcoding sequencing and analysis.

Metabarcoding analysis

Although coyotes and gray foxes are recognized as generalist omnivores, we only assessed mesopredator diets using a vertebrate metabarcoding primer. Kluever et al. [57] recently reported that metabarcoding analysis of coyotes in Florida resulted in inconclusive plant diet due to the inability to ascertain how plant DNA may have ended up in scat. They noted that the predominate plant species detected was *Pinus* spp., potentially due to pollen/seeds/spores deposition. As our sampling period was during the growing season in the Southeastern United States and samples were likely subjected to significant pollen deposition and our primary research objective was to document predation by mesopredators on vertebrate prey species, we decided to only assess vertebrate prey items. However, we recognize this minimizes any inference we might make concerning dietary overlap among bobcats, coyotes, and gray foxes.

We sent extracted DNA to Jonah Ventures for library preparation, sequencing, and bioinformatic analysis. Jonah Ventures used a segment of the Actinopterygii rRNA 12S gene (Ac12S) for PCR amplification with forward and reverse primers containing a 5' adaptor sequence for indexing and Illumina sequencing [69]. The 25 µL PCR reactions followed Promega PCR Master Mix specifications (Promega catalog # M5133, Madison, WI), including 12.5ul of Master Mix, 0.5 µl of each primer, 1.0 µl of gDNA, and 10.5 µl of DNase/RNase-free H_2O. The PCR cycling conditions started with denaturation at 94°C for 3 minutes, followed by

45 cycles of 30 seconds at 94°C, 30 seconds at 52°C, and 1 minute at 72°C, and a final elongation at 72°C for 10 minutes. Amplicons were cleaned through incubation with Exo1/SAP for 30 minutes at 37°C followed by inactivation at 95°C for 5 minutes and stored at -20°C. To incorporate Illumina adaptors and individual sample barcodes they performed a second round of PCR using Promega Master mix, 0.5 µM of each primer and 2 µl of cleaned DNA from the first PCR reaction. The second PCR cycling conditions consisted of an initial denaturation of 95°C for 3 minutes followed by 8 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds. To standardize sample concentrations before sequencing the indexed amplicons were cleaned and normalized using SequalPrep Normalization Plates (cat#A10510-01; Life Technologies, Carlsbad, CA) and following manufacturer's protocols. The final pool consisted of 5µl of each normalized sample. Jonah Ventures conducted three independent replicates of PCR to increase the likelihood of identifying prey sequences.

Sample library pools were sequenced on an Illumina MiSeq (San Diego, CA) in the Colorado University Boulder BioFrontiers Sequencing Center using the v2 500-cycle kit (cat# MS-102-2003). All bioinformatic processing was done by Jonah Ventures and followed the methods detailed in Craine [70]. In general, sequences were demultiplexed, primers removed, and low-quality reads were discarded. Then taxonomy was assigned to each ESV by mapping them against GenBank reference data [71] and Jonah Ventures voucher sequences records. The consensus taxonomy was generated by first considering 100% matches, and then going down in 1% steps until matches were present for each ESV [70].

Data preparation

To prepare data for analysis, we first removed sequences that contained base pair matches with reference databases at less than 90% and any samples that contained 0 reads. We then identified

the likely host species as sequences that contained the greatest reads within a sample. In cases where a clear predator species had fewer reads than a concurrent prey species, we identified the host species as the predator. We then matched host predator species identified through the metabarcoding sequence with an independent species identification methodology using a mitochondrial DNA (mtDNA) control-region multiplex described in De Barba et al. [72]. We included this step due to the occurrence of rare cases of introgression between coyotes and domestic dogs during colonization of the Eastern United States and some admixture remains in the region [73,74]. We used the independent, mitochondrial species identification to verify coyote, bobcat, and gray fox samples and to rectify discrepancies between samples identified as coyote and those identified as domestic dog. Specifically, in cases where metabarcoding sequences identified as domestic dog, but mtDNA identified as coyote, we assigned that sample as coyote. Similarly, in cases where metabarcoding sequences identified a sample as coyote, but mtDNA identified as domesticated dog, we assigned that sample as coyote. Cases of admixture between coyotes and domestic dogs should be most prevalent in coyote individuals, not free-roaming dogs. We assumed that evidence of admixture in our samples (i.e., confusion between metabarcoding and mtDNA species identification) is more likely found in coyote samples, not domestic dog samples. In a few cases, multiple predators were identified within a sample, with no clear indication of which was the host species based on the number of metabarcoding reads present or mismatches with mtDNA identification. Therefore, we removed those samples as cases of unknown predators. Finally, we removed or rectified spurious sequence identifications that either did not match a known endemic species or could be clearly assigned to a known endemic species.

Statistical analysis

Frequency of occurrence

Frequency of occurrence (FO) is a readily used metric for analyzing diets by averaging the occurrence of an individual prey species across all samples [27,46,75,76]. To calculate FO, we first categorized prey species into eight major groups: deer, wild turkey, lagomorphs, squirrels, small mammals (*Microtus* spp., *Sigmodon hispidus*, etc.), birds, herpetofauna, and other. For each predator we divided the total occurrence of each group by the number of samples collected and multiplied by 100 to present FO as a percentage. For comparison among sample sites, we conducted chi-squared contingency table analysis using absolute FO as described in Wright [77] to avoid pseudoreplication.

Prey diversity and dietary overlap

We used a suite of ecological indices to compare diet diversity and overlap among the predator guild in our study. Due to low sample sizes for both bobcat and gray fox, we were only able to compare diet diversity and overlap among sample sites for coyotes. We used uniquely identified prey species to calculate a paired differences index (PDI), specialized diet (*d*), and species specificity index in the package 'bipartite' in Program R [78,79] to assess the breadth and specificity of predator diets. Diet specialization of a species can be estimated using PDI and ranges from 0 (generalist) to 1 (specialist) [80,81]. Estimates of *d* assess the degree a predator relies on a single prey item as opposed to a random selection of available prey wherein 0 denotes no specialization and 1 denotes complete specialization [82]. Using the eight FO categories of prey items, we estimated Shannon's Diversity Index and Pianka's index of niche overlap (*O*) in the package 'spaa' to assess dietary partitioning and explore the possibility of exploitative competition among sympatric predators [83].

Landcover models

Ward et al. [20] used GPS-collared individuals to identify core areas of coyote pack home-ranges. They then systematically sampled core areas for scat to relate diet composition to pack-level landcover covariates. Resident coyote packs showed limited home-range overlap and by pooling scat across pack core areas, Ward et al. [20] avoided pseudoreplication through the non-independence of scat from the same individual or social group. Hinton et al. [21] was unable to identify pack home-ranges, but instead used a home-range estimator to identify clustering across their scat locations. Hinton et al. [21] used these clusters as a heuristic for individual pack ranges and modeled the influence of canopy cover on coyote diet with scat clusters used as a random variable to account for pseudoreplication.

In order to identify areas of clustered scat locations similar to the methodology described in Hinton et al. [21], we calculated 50% kernel density estimates (KDEs) with the h-plugin smoothing parameter using the 'adehabitatHR' package for R (Version 3.6.3) [84]. We limited our models to coyote scat due to low sample sizes in both bobcat and gray fox samples. We censored scat outside of our 50% KDEs and modeled turkey and deer presence in scat as a binomial response variable of 1 or 0. Our explanatory variables included mean forest cover across each KDE cluster calculated from the 2019 National Land Cover Database (NLCD) [85] by grouping the deciduous forest, mixed forest, and coniferous forest landcover groups together. We also included the Julian date of scat collection as a continuous variable along with FO of prey including deer, wild turkeys, rabbits, small mammals, and birds within each KDE cluster. We removed squirrels and herpetofauna from our analysis due to the low presence found within our scat samples. Finally, we included both the individual KDE cluster and the sample site as random variables to account for pseudoreplication. We constructed three groups of generalized

linear mixed models (GLMMs) to address the influence of forest cover, Julian date, and other diet items on turkey and deer consumption. We conducted our analysis in the package lme4 in Program R [86] and used Akaike's information criterion adjusted for small sample sizes (AICc) to select the best approximating models [87].

RESULTS

We collected 222 scat samples during the spring of 2020 and 192 scat samples during the spring of 2021 for a total of 414 samples. After removal of samples containing no purported prey species, no reads, or reads below our 90% identity threshold, we assessed 208 samples collected across our three sample sites (50.2% of total samples collected). Using both metabarcoding reads and mitochondrial species assignment, we found consensus identification of predator for 20 bobcats, 153 coyotes, and seven gray foxes (Table 1). One bobcat sample and 10 coyote samples were missing peaks for mtDNA identification and were only identified from metabarcoding sequences, and nine coyote samples were identified as dog through metabarcoding but as coyote through mtDNA. All gray fox samples were identified through both metabarcoding and mtDNA methods. In addition, we identified 10 domestic dog samples through both metabarcoding and mtDNA methods and two raccoon samples using metabarcoding sequences. We did not identify any red fox (*Vulpes vulpes*) samples across our three sites for either 2020 or 2021. Finally, we were unable to distinguish among purported predator species for 16 samples due to a combination of mixed metabarcoding reads and mitochondrial identification and therefore removed these samples from further analysis. Due to low sample sizes for bobcats and gray foxes, we pooled all sites and years across species to conduct comparisons among predators while using only coyote samples to compare among sites.

Diets across predator species

Frequency of occurrence

We found that coyote samples exhibited the highest FO for deer consumption across sample sites and years (68.0%), followed by bobcats (25.0%), while gray foxes showed no consumption of deer (Table 2, Fig 2). All predators consumed wild turkeys, with gray foxes having the highest FO at 14.3%, followed by coyotes (9.2%) and bobcats (5.0%). Bobcats had the highest FO of lagomorphs (35.0%), followed by coyotes (13.7%), while gray foxes showed no consumption of lagomorphs. Similarly, only bobcats (FO = 30.0%) and coyotes (FO = 2.0%) consumed squirrels. All predators consumed small mammals, mostly hispid cotton rats (*Sigmodon hispidus*) with highest FO for bobcats (35.0%), followed by gray fox (28.6%), and coyotes (12.4%). Beyond consumption of wild turkeys, all predators ate additional avian species with highest FO for gray foxes at 28.6%, followed by bobcats (15.0%) and coyotes (6.5%). Herpetofauna made up the majority of species found in gray fox samples with a FO of 57.1% while coyote samples only had a FO of 0.7% and bobcats consumed no herpetofauna. There were several species that we grouped into a category of "other". We identified armadillos (*Dasypus novemcinctus*) in 13 coyote scats, cattle (*Bos taurus*) appeared in six coyote scats and invasive wild pigs (*Sus scrofa*) appeared in four coyote scats, comprising a FO of 8.5%, 3.9%, and 2.6%, respectively. Finally, we did find potential evidence of intraguild predation or scavenging; bobcats and coyotes had one sample each containing gray fox and one coyote sample contained bobcat.

Prey diversity and dietary overlap

All three predators behaved as specialists across sites with PDI ranging from 0.86 (bobcats) to 0.96 (coyotes). Gray foxes relied the most on a single vertebrate prey group in relation to a random selection of other diet items based on specialized diet (d = 0.71), followed by coyotes

(0.35), and bobcats (0.34). However, coyotes had the highest species specificity index (bobcats: 0.36, coyotes: 0.52, gray foxes: 0.45), meaning that coyotes relied more heavily on a single resource than the other two predators. Bobcats exhibited the highest diversity in vertebrate diet items with Shannon diversity equaling 2.03, followed by coyotes (1.80), then gray foxes (1.52). Finally, estimates of Pianka's niche overlap revealed low levels of vertebrate dietary overlap between gray foxes and coyotes (0.14) and gray foxes and bobcats (0.32), with moderate overlap between coyotes and bobcats (0.61).

Coyotes among sites

Frequency of occurrence

We detected no differences in vertebrate diet for coyotes among sample sites or across years except for consumption of lagomorphs among sites ($\chi^2 = 7.61$, $P < 0.05$). Coyote scat at the Webb Complex contained more deer with a FO of 85.7%, followed by Davis Land and Timber (67.6%), and Savannah River Site (62.1%, Fig 3). Coyotes at all three sites showed consumption of wild turkeys: scat at the Webb Complex and Davis Land and Timber contained an FO of 9.5%, followed by Savannah River Site at 8.6%. Davis Land and Timber coyote scats had greater levels of FO of lagomorphs (21.6%), followed by Savannah River Site (6.9%) and the Webb Complex (4.7%). Consumption of squirrels was only documented at the Savannah River Site (1.7%) and Davis Land and Timber (2.7%). Consumption of small mammals, including hispid cotton rats, ranged from 4.8% FO (Webb Complex) to 17.6% (Davis Land and Timber). Consumption of avian species other than wild turkey was low at all three sites (FO 4.7 – 6.9%). Consumption of herpetofauna only occurred in one scat sample each at Savannah River Site and the Webb Complex. All six occurrences of cattle were found at Davis Land and Timber, while

invasive wild pigs were consumed at Savannah River Site and the Webb Complex. Bobcat and gray fox appeared only once each in scat collected at Davis Land and Timber.

Prey diversity and dietary overlap

Coyotes at all three sites during the spring behaved as specialists with paired difference indices ranging from 0.94 (Davis Land and Timber) to 0.97 (Webb Complex). Shannon diversity of vertebrate diet ranged from 1.32 at the Webb Complex to 1.74 at Davis Land and Timber. However, Davis Land and Timber exhibited the most specialization in coyotes with *d* equaling 0.13, followed by the Webb Complex (0.10) then Savannah River Site (0.09). Species-specificity indices of vertebrate diet ranged from 0.48 (Davis Land and Timber) to 0.65 (Webb Complex).

Generalized linear mixed models

We identified eight individual clusters of scat across our three study sites: three at Davis Land and Timber, two at Savannah River Site, and three at the Webb Complex. We censored any scat outside of our 50% KDE clusters and ran GLMM models using 110 remaining samples. Use of deer and turkeys by coyotes were negatively correlated with each other (Table 4) as our top model for turkey in coyote diet included only deer FO as an explanatory variable, and conversely, our top model for deer in coyote diet included only turkey FO as an explanatory variable (Table 3). While other variables appeared in additional competitive models (ΔAICc < 2.0) for turkey and deer in coyote diet, beta estimates were not significant and tended to have high variance, potentially due to low sample size (Table 3).

DISCUSSION

We collected scat during the springtime in South Carolina, USA to assess diet of coyotes, bobcats, and gray foxes through DNA metabarcoding. Using genetic methodologies to accurately identify the host predator of each collected sample [13], we observed a dramatic discrepancy in

sample sizes between the three mesopredators, with coyote samples far exceeding either bobcat or gray fox samples. Across South Carolina, annual harvest records reveal a decline in gray fox harvest concurrent with an increase in coyote harvest since the early 1990s and consistently low bobcat harvest across years (J. Butfiloski, Furbearer and Alligator Program Coordinator, SCDNR, unpublished data). Annual scent-station furbearer surveys on the Savannah River Site from 1991-2014 also show a decline in gray fox visitations as coyote visitations increased while bobcat visitations were uniformly lower over the same timespan (M. Caudell, SCDNR, unpublished data). Additionally, although previous literature in the southeastern USA has documented limited spatial segregation between these mesopredators [2], competition with coyotes, a larger, generalist predator, may lead to competitive exclusion of bobcats and gray foxes [3,9]. Coyote abundance, therefore, may have been higher than bobcats or gray foxes across our study sites, although we observed low levels of dietary overlap across our mesopredator guild. Finally, we sampled secondary dirt and gravel roads for scat, which may have biased our collection towards coyotes, which are known to use road systems for travel and territory marking [88]. Our comparison of diet among coyotes, bobcats, and gray foxes should be qualified by the uneven sampling success we observed among mesopredators and future research addressing dietary overlap in this predator guild should seek to maximize detection of bobcat and gray fox scat.

Contrary to our predictions, we observed low levels of dietary overlap between coyotes and gray foxes from mid-May to mid-June. The low levels of overlap were largely driven by higher levels of deer consumption by coyotes and herpetofauna consumption by gray foxes, which may have been specific to the time of year our sampling occurred and the fact that we only assessed vertebrate prey items. Because we were specifically interested in deer and turkey

consumption, we focused sampling during fawning for deer [32] and nesting for turkey [67]. Previous studies have indicated that coyotes and gray foxes increase their reliance on fruit in June and July [17,19,20,25,26,28], which coincides with the reduction of fawn availability in the region as fawn survival increases after approximately 8-10 weeks of life [32,34]. Seasonal selection of differing prey may result in dietary partitioning among these generalist omnivores during the spring. Although we did not assess plant consumption, we predict that dietary overlap between coyotes and gray foxes may increase as coyotes begin consuming a broader range of small mammals and fruits during the rest of the year [26]. Such overlap has been documented across the sympatric range of coyotes and gray foxes and may lead to competitive exclusion in some cases [4,9,10,26,51,89]. Further study in the southeastern United States should examine dietary overlap between coyotes and gray foxes throughout the year, including consumption of plants, to see whether they continue to occupy different dietary niches or whether overlap increases as fawns become less susceptible to predation during the late summer [32,34].

We documented moderate levels of dietary overlap between bobcats and coyotes across our study sites due to a shared pool of prey species. Davis Land and Timber was the one site where coyotes consumed more rabbits than at the other two sites and was privately owned containing higher percentages of managed open landcover types, which provide more suitable habitat for rabbits. Higher densities of rabbits may have increased coyote consumption of lagomorphs and could lead to increased intraguild competition with bobcats. In general, however, our findings were similar to previous diet studies, which documented higher frequencies of deer occurrence by coyotes than by bobcats and higher frequencies of rabbits, squirrels, and small mammals by bobcats than by coyotes [11,26,90]. Differences in diet between bobcats and coyotes likely reflect alternative hunting strategies (ambush vs. cursorial) and speak

to each species occupying differing functional roles (specialist vs. generalist). Changes in relative abundance of shared prey species may increase direct competition between coyotes and bobcats as they are forced to focus to a greater extent on similar available food sources [3]. However, our study did not address coyote consumption of plant items, which would reduce dietary overlap with bobcats.

Our findings also provide insight on the impacts of mesopredator predation on game species such as white-tailed deer and wild turkey. Coyote prey-switching behavior presumably optimizes diet based on resource availability and we observed a negative relationship between FO of deer and turkeys in clusters of coyote scat. However, low sample size may have contributed to this relationship with turkey consumption by coyotes only moderately correlated with deer consumption. The fawning window of deer in this region has been documented to be up to 90 days [62] J. Kilgo, United States Forest Service, unpublished data] and newly born fawns would have been present on the landscape throughout our study period. Although increased consumption of fawns during the spring may result in lower predation on other species such as turkeys during the same time period, our data suggest that all three species are eating turkeys, specifically, and avian species, generally, more often than previously reported [12,26,27,51,52]. As we predicted, our use of DNA metabarcoding may have improved our ability to detect turkey and other avian prey in scat. However, we were unable to determine whether consumption of turkeys occurred through predation of eggs, poults, or adult turkeys. Coyotes, bobcats, and gray foxes are all known to predate adult hens, especially during the spring when nesting and brooding hens are vulnerable [35,37,42–44] and our sampling window may have missed predation of vulnerable hens during the first part of the reproductive period of wild turkeys in South Carolina. However, low presence of turkey in predator scat and

specialization in other prey species indicate that turkeys are likely not an important component of diet in this three-predator guild.

Our data provide additional support that coyotes of the southeastern United States are specializing in deer during the spring. In fact, our finding of an FO of 68% for deer is higher than previous studies finding that coyote scat contains approximately 20 – 60% FO of deer [17,19,20,24,25,52,90]. We cannot say whether coyotes were preying on fawns, preying on adults, or scavenging. However, previous studies were able to differentiate between fawns and adult deer through comparison of guard hair sizes and reported that spring consumption of deer by coyotes was overwhelmingly focused on fawns [17,19,20,24,25,90]. Regardless, it is clear that coyotes are preferentially consuming deer during these months. Furthermore, we did not find evidence that either Julian date or forest cover increased FO of deer in scat clusters. Instead, our data suggests that deer consumption by coyotes was ubiquitous throughout our sampling period and high across our study sites. Region-wide impacts on deer populations due to coyote predation have been thoroughly discussed over the last two decades [30,32,59,91–93] with some arguing that decreases in antlerless harvest may be necessary to mitigate the effects of coyote consumption of fawns in the southeastern United States [30,32].

Our findings document a temporal window wherein coyote vertebrate diets consist largely of white-tailed deer, resulting in a reduction of dietary overlap with other sympatric predators, most notably, gray foxes. Coyotes, bobcats, and gray foxes occupy shared space within the forested landscape of the eastern United States [1,2]. Resource partitioning is necessary to reduce competition among coyotes, bobcats, and foxes if they are to avoid competitive exclusion, which is a growing concern for dwindling endemic populations such as the gray fox [4,9,10]. We recognize that our study is limited in its inference due to small sample

sizes for both bobcats and gray foxes and the lack of plant and non-invertebrate diet in our analyses, but we believe that important conclusions can be drawn from our data regarding the implications of having three mesopredators sharing the southeastern United States landscape. Our findings indicate that these three species share certain similar vertebrate dietary traits during the spring (i.e., consumption of small mammal and some avian prey). However, their dietary overlap is reduced through variable consumption of specific prey items including deer for coyotes, small mammals for bobcats, and herpetofauna for gray foxes. Additionally, while coyotes prey heavily on fawns across the southeastern United States, specialization on fawns by coyotes may lessen intraguild dietary competition with the other sympatric predators.

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LITERATURE CITED

1. Chamberlain MJ. Ecological relationships among bobcats, coyotes, gray fox, and raccoons and their interactions with wild turkey hens. Dissertation, Mississippi State University. 1999.

2. Chamberlain MJ, Leopold BD. Overlap in space use among bobcats (*Lynx rufus*), coyotes (*Canis latrans*) and gray foxes (*Urocyon cinereoargenteus*). Am Midl Nat. 2005;153: 171–179. doi:10.1674/0003-0031(2005)153[0171:OISUAB]2.0.CO;2

3. Dyck MA, Wyza E, Popescu VD. When carnivores collide: a review of studies exploring the competitive interactions between bobcats *Lynx rufus* and coyotes *Canis latrans*. Mammal Rev. 2021; mam.12260. doi:10.1111/mam.12260

4. Webster SC, Chamberlain MJ, Hinton JW, Beasley JC. Isotope analysis reveals dietary overlap among sympatric canids. J Mammal. 2021;102: 1222–1234. doi:10.1093/jmammal/gyab077

5. Nowak RM. The original status of wolves in eastern America. Southeast Nat. 2002;1: 95–130. doi:10.1656/1528-7092(2002)001[0095:TOSOWI]2.0.CO;2

6. Hody JW, Kays R. Mapping the expansion of coyotes (*Canis latrans*) across North and Central America. ZooKeys. 2018;759: 81–97. doi:10.3897/zookeys.759.15149

7. Gompper ME. Top carnivores in the suburbs? Ecological and conservation issues raised by colonization of northeastern North America by coyotes. BioScience. 2002;52: 185–190. doi:10.1641/0006-3568(2002)052[0185:TCITSE]2.0.CO;2

8. Roemer GW, Gompper ME, Van Valkenburgh B. The ecological role of the mammalian mesocarnivore. BioScience. 2009;59: 165–173. doi:10.1525/bio.2009.59.2.9

9. Egan ME, Day CC, Katzner TE, Zollner PA. Relative abundance of coyotes (*Canis latrans*) influences gray fox (*Urocyon cinereoargenteus*) occupancy across the eastern United States. Can J Zool. 2021;99: 63–72. doi:10.1139/cjz-2019-0246

10. Bauder JM, Allen ML, Ahlers AA, Benson TJ, Miller CA, Stodola KW. Long-term data reveal equivocal evidence for intraguild suppression among sympatric canids. Biodivers Conserv. 2022. doi:10.1007/s10531-022-02465-y

11. Thornton DH, Sunquist ME, Main MB. Ecological separation within newly sympatric populations of coyotes and bobcats in south-central Florida. J Mammal. 2004;85: 973–982. doi:10.1644/BEH-020

12. Melville HIAS, Conway WC, Morrison ML, Comer CE, Hardin JB. Prey selection by three mesopredators that are thought to prey on eastern wild turkeys (*Meleagris gallopavo sylvestris*) in the Pineywoods of east Texas. Southeast Nat. 2015;14: 447–472. doi:10.1656/058.014.0306

13. Morin DJ, Higdon SD, Holub JL, Montague DM, Fies ML, Waits LP, et al. Bias in carnivore diet analysis resulting from misclassification of predator scats based on field identification: bias and uncertainty in carnivore scat identification. Wildl Soc Bull. 2016;40: 669–677. doi:10.1002/wsb.723

14. Smith JA, Thomas AC, Levi T, Wang Y, Wilmers CC. Human activity reduces niche partitioning among three widespread mesocarnivores. Oikos. 2018;127: 890–901. doi:10.1111/oik.04592

15. Chamberlain MJ, Leopold BD. Dietary patterns of sympatric bobcats and coyotes in central Mississippi. Proc Annu Conf Southeast Assoc Fish Wildl Agencies. 1999;53: 204–219.

16. Melville HIAS, Conway WC, Morrison ML, Comer CE, Hardin JB. Home-range interactions of three sympatric mesopredators in east Texas. Can J Zool. 2015;93: 547–557. doi:10.1139/cjz-2014-0322

17. Schrecengost JD, Kilgo JC, Mallard D, Ray HS, Miller KV. Seasonal food habits of the coyote in the South Carolina Coastal Plain. Southeast Nat. 2008;7: 135–144. doi:10.1656/1528-7092(2008)7[135:SFHOTC]2.0.CO;2

18. Whitaker J, Ruckdeschel C, Chamberlain A, Bakken L. Food of bobcats and coyotes from Cumberland Island, Camden County, Georgia. Ga J Sci. 2015;73. Available: https://digitalcommons.gaacademy.org/gjs/vol73/iss2/2

19. Cherry MJ, Turner KL, Howze MB, Cohen BS, Conner LM, Warren RJ. Coyote diets in a longleaf pine ecosystem. Wildl Biol. 2016;22: 64–70. doi:10.2981/wlb.00144

20. Ward JN, Hinton JW, Johannsen KL, Karlin ML, Miller KV, Chamberlain MJ. Home range size, vegetation density, and season influences prey use by coyotes (*Canis latrans*). Yue B-S, editor. PLOS ONE. 2018;13: e0203703. doi:10.1371/journal.pone.0203703

21. Hinton JW, Rountree K, Chamberlain MJ. Diet of coyotes on the Tensas River National Wildlife Refuge during the white-tailed deer pre-fawning and fawning seasons. Southeast Nat. 2021;20: 245–258. doi:10.1656/058.020.0201

22. Landry SM, Roof JE, Rogers RE, Welsh AB, Ryan CW, Anderson JT. Dietary patterns suggest West Virginia bobcats are generalist carnivores. J Fish Wildl Manag. 2022. doi:10.3996/JFWM-22-001

23. Godbois I, Jones J, Conner L, Warren R. Bobcat diet on an area managed for northern bobwhite. Proc Southeast Assoc Fish Wildl Agencies. 2003;57: 222–227.

24. Swingen MB, DePerno CS, Moorman CE. Seasonal coyote diet composition at a low-productivity site. Southeast Nat. 2015;14: 397–404. doi:10.1656/058.014.0219

25. Kelly JD, Killmaster CH, Bowers JW. Seasonal and spatial variation in diets of coyotes in central Georgia. J Southeast Assoc Fish Wildl Agencies. 2015;2: 296–302.

26. Neale JCC, Sacks BN. Food habits and space use of gray foxes in relation to sympatric coyotes and bobcats. 2001;79: 1794–1800.

27. Larson RN, Morin DJ, Wierzbowska IA, Crooks KR. Food habits of coyotes, gray foxes, and bobcats in a coastal southern California urban landscape. West North Am Nat. 2015;75: 339–347. doi:10.3398/064.075.0311

28. Masters HM, Maher CR. Diet reveals potential for competition and coexistence among coyotes (*Canis latrans*), red foxes (*Vulpes vulpes*), and gray foxes (*Urocyon cinereoargenteus*). Can J Zool. 2022;100: 90–97. doi:10.1139/cjz-2021-0103

29. Warsen SA, Frair JL, Teece MA. Isotopic investigation of niche partitioning among native carnivores and the non-native coyote (*Canis latrans*). Isotopes Environ Health Stud. 2014;50: 414–424. doi:10.1080/10256016.2014.897946

30. Kilgo JC, Ray HS, Ruth C, Miller KV. Can coyotes affect deer populations in southeastern North America? J Wildl Manag. 2010;74: 929–933. doi:10.2193/2009-263

31. Saalfeld ST, Ditchkoff SS. Survival of neonatal white-tailed deer in an exurban population. J Wildl Manag. 2007;71: 940–944. doi:10.2193/2006-116

32. Kilgo JC, Ray HS, Vukovich M, Goode MJ, Ruth C. Predation by coyotes on white-tailed deer neonates in South Carolina: coyote predation on fawns. J Wildl Manag. 2012;76: 1420–1430. doi:10.1002/jwmg.393

33. Jackson AM, Ditchkoff SS. Survival estimates of white-tailed deer fawns at Fort Rucker, Alabama. Am Midl Nat. 2013;170: 184–190. doi:10.1674/0003-0031-170.1.184

34. Chitwood MC, Lashley MA, Kilgo JC, Moorman CE, Deperno CS. White-tailed deer population dynamics and adult female survival in the presence of a novel predator: Deer Population Dynamics. J Wildl Manag. 2015;79: 211–219. doi:10.1002/jwmg.835

35. Byrne ME, Chamberlain MJ. Survival and cause-specific mortality of adult female eastern wild turkeys in a bottomland hardwood forest. Southeast Nat. 2018;17: 345–356. doi:10.1656/058.017.0216

36. Ulrey EE, Cedotal CA, Chamberlain MJ, Collier BA. Spatial distribution of potential wild turkey nest predators in west-central Louisiana. Wildl Soc Bull. 2022;46: e1285. doi:10.1002/wsb.1285

37. Chamberlain MJ, Miller DA, Leopold BD, Hurst GA. Predation rates on wild turkey hens in a hardwood bottomland forest and a mixed forest in Mississippi. Proc Southeast Assoc Fish Wildl Agencies. 1996;50: 428–435.

38. Porter WF, Healy WM, Backs SE, Wakeling BF, Steffen DE. Managing wild turkeys in the face of uncertainty. Proc Natl Wild Turk Symp. 2011;10: 1–9.

39. Casalena MJ, Schiavone MV, Bowling AC, Gregg ID, Brown J. Understanding the new normal: wild turkeys in a changing northeastern landscape. Proc Natl Wild Turk Symp. 2015;11: 45–57.

40. Byrne ME, Chamberlain MJ, Collier BA. Potential density dependence in wild turkey productivity in the southeastern United States. Proc Natl Wild Turk Symp. 2015;11: 329–351.

41. Eriksen RE, Brown TA, Scott KB, Hughes TW, Akridge MD, Penner CS. Status and distribution of wild turkeys in the United States: 2014 status. Proc Natl Wild Turk Symp. 2015;11: 7–18.

42. Palmer WE, Hurst GA, Stys JE, Smith DR, Burk JD. Survival rates of wild turkey hens in loblolly pine plantations in Mississippi. J Wildl Manag. 1993;57: 783. doi:10.2307/3809080

43. Miller DA, Burger LW, Leopold BD, Hurst GA. Survival and cause-specific mortality of wild turkey hens in central Mississippi. J Wildl Manag. 1998;62: 306. doi:10.2307/3802293

44. Little AR, Benson JF, Chamberlain MJ, Conner LM, Warren RJ. Survival and cause-specific mortality of female eastern wild turkeys in two frequently-burned longleaf pine savannas. Wildl Biol. 2016;22: wlb.00855. doi:10.2981/wlb.00264

45. Wagner GD, Hill EP. Evaluation of southeastern coyote diets during the wild turkey reproductive season. Proc Annu Conf Southeast Assoc Fish Wildl Agencies. 1994;48: 173–181.

46. Klare U, Kamler JF, Macdonald DW. A comparison and critique of different scat-analysis methods for determining carnivore diet: Comparison of scat-analysis methods. Mammal Rev. 2011;41: 294–312. doi:10.1111/j.1365-2907.2011.00183.x

47. Monterroso P, Godinho R, Oliveira T, Ferreras P, Kelly MJ, Morin DJ, et al. Feeding ecological knowledge: the underutilised power of faecal DNA approaches for carnivore diet analysis. Mammal Rev. 2019;49: 97–112. doi:10.1111/mam.12144

48. Reed SE. Non-invasive methods to assess co-occurrence of mammalian carnivores. Southwest Nat. 2011;56: 231–240. doi:10.1894/F13-JKF-14.1

49. Witczuk J, Pagacz S, Gliwicz J, Mills LS. Niche overlap between sympatric coyotes and bobcats in highland zones of Olympic Mountains, Washington. J Zool. 2015;297: 176–183. doi:10.1111/jzo.12270

50. Wagner GD. Coyote diet in areas of wild turkey abundance during the wild turkey reproductive season. Thesis, Mississippi State University. 1993.

51. Neale JCC, Sacks BN. Resource utilization and interspecific relations of sympatric bobcats and coyotes. Oikos. 2001;94: 236–249. doi:10.1034/j.1600-0706.2001.940204.x

52. Jensen AJ, Marneweck CJ, Kilgo JC, Jachowski DS. Coyote diet in North America: geographic and ecological patterns during range expansion. Mammal Rev. 2022; mam.12299. doi:10.1111/mam.12299

53. Cavallini P, Volpi T. Biases in the analysis of the diet of the red fox (*Vulpes vulpes*). Wildl Biol. 1995;1: 243–248. doi:10.2981/wlb.1995.0030

54. Balestrieri A, Remonti L, Prigioni C. Assessing carnivore diet by faecal samples and stomach contents: a case study with Alpine red foxes. Open Life Sci. 2011;6: 283–292. doi:10.2478/s11535-010-0106-1

55. Gosselin EN, Lonsinger RC, Waits LP. Comparing morphological and molecular diet analyses and fecal DNA sampling protocols for a terrestrial carnivore: noninvasive diet analysis methodologies. Wildl Soc Bull. 2017;41: 362–369. doi:10.1002/wsb.749

56. Shi Y, Hoareau Y, Reese EM, Wasser SK. Prey partitioning between sympatric wild carnivores revealed by DNA metabarcoding: a case study on wolf (*Canis lupus*) and coyote (*Canis latrans*) in northeastern Washington. Conserv Genet. 2021;22: 293–305. doi:10.1007/s10592-021-01337-2

57. Kluever BM, Main MB, Breck SW, Lonsinger RC, Humphrey JH, Fischer JW. Using noninvasive genetics for estimating density and assessing diet of urban and rural coyotes in Florida, USA. Urban Nat. 2022;9: 1–24.

58. Henger CS, Hargous E, Nagy CM, Weckel M, Wultsch C, Krampis K, et al. DNA metabarcoding reveals that coyotes in New York City consume wide variety of native prey species and human food. PeerJ. 2022;10: e13788. doi:10.7717/peerj.13788

59. Gingery TM, Diefenbach DR, Wallingford BD, Rosenberry CS. Landscape-level patterns in fawn survival across North America: Landscape-Level Patterns in Fawn Survival. J Wildl Manag. 2018;82: 1003–1013. doi:10.1002/jwmg.21456

60. Gulsby WD, Kilgo JC, Vukovich M, Martin JA. Landscape heterogeneity reduces coyote predation on white-tailed deer fawns: Landscape Characteristics Affect Fawn Predation. J Wildl Manag. 2017;81: 601–609. doi:10.1002/jwmg.21240

61. Kilgo JC, Vukovich M, Scott Ray H, Shaw CE, Ruth C. Coyote removal, understory cover, and survival of white-tailed deer neonates: Coyote Control and Fawn Survival. J Wildl Manag. 2014;78: 1261–1271. doi:10.1002/jwmg.764

62. Michel ES, Strickland BK, Demarais S, Belant JL, Kautz TM, Duquette JF, et al. Relative reproductive phenology and synchrony affect neonate survival in a nonprecocial ungulate. Crocker D, editor. Funct Ecol. 2020;34: 2536–2547. doi:10.1111/1365-2435.13680

63. Omernik JM. Ecoregions of the conterminous United States. Ann Assoc Am Geogr. 1987;77: 118–125. doi:10.1111/j.1467-8306.1987.tb00149.x

64. Griffith GE, Omernik JM, Comstock JA, Shafale MP, McNab WH, Lenat DR, et al. Ecoregions of North Carolina and South Carolina. (2 sided color poster with map, descriptive text, summary tables, and photographs). US Geol Surv Rest VA. 2002.

65. Griffith GE. Level III North American terrrestrial ecoregions : United States descriptions. Prep North Am Comm Environ Coop. 2010; 1–64.

66. Kilgo JC, Blake JI. Ecology and management of a forested landscape: fifty years on the Savannah River Site. Washington: Island Press; 2005.

67. Chamberlain MJ, Wightman PH, Cohen BS, Collier BA. Gobbling activity of eastern wild turkeys relative to male movements and female nesting phenology in South Carolina. Wildl Soc Bull. 2018;42: 632–642. doi:10.1002/wsb.932

68. Frantzen M a. J, Silk JB, Ferguson JWH, Wayne RK, Kohn MH. Empirical evaluation of preservation methods for faecal DNA. Mol Ecol. 1998;7: 1423–1428. doi:10.1046/j.1365-294x.1998.00449.x

69. Evans NT, Olds BP, Renshaw MA, Turner CR, Li Y, Jerde CL, et al. Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. Mol Ecol Resour. 2016;16: 29–41. doi:10.1111/1755-0998.12433

70. Craine JM. Seasonal patterns of bison diet across climate gradients in North America. Sci Rep. 2021;11: 6829. doi:10.1038/s41598-021-86260-9

71. Benson DA. GenBank. Nucleic Acids Res. 2004;33: D34–D38. doi:10.1093/nar/gki063

72. De Barba M, Adams JR, Goldberg CS, Stansbury CR, Arias D, Cisneros R, et al. Molecular species identification for multiple carnivores. Conserv Genet Resour. 2014;6: 821–824. doi:10.1007/s12686-014-0257-x

73. Adams JR, Leonard JA, Waits LP. Widespread occurrence of a domestic dog mitochondrial DNA haplotype in southeastern US coyotes. Mol Ecol. 2003;12: 541–546. doi:10.1046/j.1365-294X.2003.01708.x

74. Hinton JW, Heppenheimer E, West KM, Caudill D, Karlin ML, Kilgo JC, et al. Geographic patterns in morphometric and genetic variation for coyote populations with emphasis on southeastern coyotes. Ecol Evol. 2019;9: 3389–3404. doi:10.1002/ece3.4966

75. Zabala-Albizua J, Zuberogoitia I. Badger, Meles meles (*Mustelidae, Carnivora*), diet assessed through scat-analysis: a comparison and critique of different methods. Folia Zool - Praha-. 2003;52: 23–30.

76. Hinton JW, Ashley AK, Dellinger JA, Gittleman JL, van Manen FT, Chamberlain MJ. Using diets of Canis breeding pairs to assess resource partitioning between sympatric red wolves and coyotes. J Mammal. 2017;98: 475–488. doi:10.1093/jmammal/gyw233

77. Wright B. Use of chi-square tests to analyze scat-derived diet composition data. Mar Mammal Sci. 2009;26: 395–401. doi:10.1111/j.1748-7692.2009.00308.x

78. Dormann CF, Gruber B, Fründ J. Introducing the bipartite Package: Analysing Ecological Networks. 2008;8.

79. Dormann CF. How to be a specialist? Quantifying specialisation in pollination networks. Netw Biol. 2011;1: 1–20.

80. Poisot T, Lepennetier G, Martinez E, Ramsayer J, Hochberg ME. Resource availability affects the structure of a natural bacteria–bacteriophage community. Biol Lett. 2011;7: 201–204. doi:10.1098/rsbl.2010.0774

81. Poisot T, Bever JD, Nemri A, Thrall PH, Hochberg ME. A conceptual framework for the evolution of ecological specialisation: Evolution of ecological specialisation. Ecol Lett. 2011;14: 841–851. doi:10.1111/j.1461-0248.2011.01645.x

82. Blüthgen N, Menzel F, Blüthgen N. Measuring specialization in species interaction networks. BMC Ecol. 2006;6: 9. doi:10.1186/1472-6785-6-9

83. Pianka ER. Niche overlap and diffuse competition. Proc Natl Acad Sci. 1974;71: 2141–2145. doi:10.1073/pnas.71.5.2141

84. Calenge C. The package “adehabitat” for the R software: A tool for the analysis of space and habitat use by animals. Ecol Model. 2006;197: 516–519. doi:10.1016/j.ecolmodel.2006.03.017

85. Dewitz J, U.S. Geological Survey. National Land Cover Database (NLCD) 2019 Products (ver. 2.0; June 2021): U.S. Geological Survey data release.

86. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J Stat Softw. 2015;67: 1–48. doi:10.18637/jss.v067.i01

87. Burnham KP, Anderson DR. Model selection and multimodel inference: a practical information-theoretic approach. 2nd ed. Springer; 2002.

88. Fedriani JM, Fuller TK, Sauvajot RM, York EC. Competition and intraguild predation among three sympatric carnivores. Oecologia. 2000;125: 258–270. doi:10.1007/s004420000448

89. Nelson MA, Cherry MJ, Howze MB, Warren RJ, Conner LM. Coyote and bobcat predation on white-tailed deer fawns in a longleaf pine ecosystem in southwestern Georgia. 2015; 6.

90. Bragina EV, Kays R, Hody A, Moorman CE, Deperno CS, Mills LS. Effects on white-tailed deer following eastern coyote colonization. J Wildl Manag. 2019;83: 916–924. doi:10.1002/jwmg.21651

91. Bragina EV, Kays R, Hody A, Moorman CE, DePerno CS, Mills LS. White-tailed deer and coyote colonization: a response to Kilgo et al. (2019). J Wildl Manag. 2019;83: 1641–1643. doi:10.1002/jwmg.21766

92. Kilgo JC, Cherry MJ, Ditchkoff SS, Gulsby WD, Miller KV. Coyotes and white-tailed deer populations in the east: A comment on Bragina et al. (2019). J Wildl Manag. 2019;83: 1636–1640. doi:10.1002/jwmg.21735

Table 4.1. Sample sizes of scat used for dietary analysis at sites along the Savannah River in South Carolina, USA for three mesopredators: coyotes (*Canis latrans*), bobcats (*Lynx rufus*), and gray foxes (*Urocyon cinereoargenteus*) during May-June 2020-2021.

Predator	**Savannah River Site**	**Davis Land and Timber**	**Webb Complex**	**Total**
Bobcat				
2020	1	5	3	9
2021	0	9	2	11
Coyote				
2020	21	43	5	69
2021	37	31	16	84
Gray Fox				
2020	0	5	2	7
2021	0	0	0	0

Table 4.2. Number of prey species identified in predator scat using DNA metabarcoding at three sites along the Savannah River, South Carolina, USA. Scat was collected during May-June 2020-2021.

		Predator		
Diet category	Scientific name	Bobcat	Coyote	Gray fox
White-tailed deer	*Odocoileus virginianus*	5	104	0
Wild turkey	*Meleagris gallopavo*	1	14	1
Rabbit	*Sylvilagus floridanus*	7	21	0
Squirrel	Sciurus spp.	6	3	0
Small mammal	Microtus spp.	1	0	0
	Scalopus aquaticus	0	3	0
	Sigmodon hispidus	7	17	2
Avian	*Antrostomus vociferus*	0	5	0
	Cardinalis cardinalis	0	0	2
	Colinus virginianus	1	0	0
	Passiformidae	2	0	0
	P. erythrophthalmus	0	4	0
	Vireo	1	1	0
Herpetofauna	*Anolis carolinensis*	0	0	1
	Hylidae dryophytes	0	0	3
	Plestiodon laticeps	0	1	0
	Alligator mississippiensis	0	1	0
Other	*Bos taurus*	0	6	0
	Dasypus novemcinctus	0	13	0
	Lynx rufus	0	1	0
	Procyon lotor	0	1	0
	Sus scrofa	0	4	0
	Urocyon cinereoargenteus	1	1	0

Table 4.3. Top five models for turkey (*Meleagris gallopavo*; top) and white-tailed deer (*Odocoileus virginianus*; bottom) consumption by coyotes (*Canis latrans*) at three sites along the Savannah River, South Carolina, U.S from May-June 2020-2021. Includes number of variables (*k*), log-likelihood (LL), change in Akaike's information criterion adjusted for small sample sizes (ΔAICc), and AICc weights (ω_i). Deer = white-tailed deer frequency of occurrence (FO); Turkey = turkey FO, Rabbit = rabbit FO; Small Mammals = small mammal FO; Bird = avian FO; Forest = mean forest cover; Julian = Julian date.

Turkey	Model	*k*	LL	ΔAICc	ω_i
	Deer	4	-36.25	0.00	0.19
	Null	3	-37.91	1.16	0.10
	Rabbit	4	-36.86	1.22	0.10
	Forest + Deer	5	-35.98	1.66	0.08
	Small Mammals	4	-37.15	1.79	0.08

Deer	Model	*k*	LL	ΔAICc	ω_i
	Turkey	4	-54.16	0.00	0.50
	Julian + Turkey	5	-53.78	1.44	0.24
	Forest + Turkey	5	-53.99	1.87	0.20
	Global	7	-53.94	6.27	0.02
	Julian + Turkey + Rabbit + Small Mammals + Bird	8	-53.41	7.55	0.01

Table 4.4. Parameter estimates for the top model for turkey (*Meleagris gallopavo*; top) and white-tailed deer (*Odocoileus virginianus*; bottom) consumption by coyotes (*Canis latrans*) at three sites along the Savannah River, South Carolina, U.S from May-June 2020-2021. Includes regression coefficients (β), standard error (SE), 95% confidence intervals (CI), *z*-scores, and *P*-values.

Turkey	Model variable	β	SE	95% CI	*z*	*P*
	Intercept	0.44	1.47	-2.45, 3.33	0.30	0.77
	Deer FO	-3.68	2.20	-8.00, 0.63	-1.67	0.09

Deer	Model variable	β	SE	95 % CI	*z*	*P*
	Intercept	5.07	1.50	2.13, 8.01	3.38	<0.01
	Turkey FO	-32.13	10.78	-53.25, -11.01	-2.98	<0.01

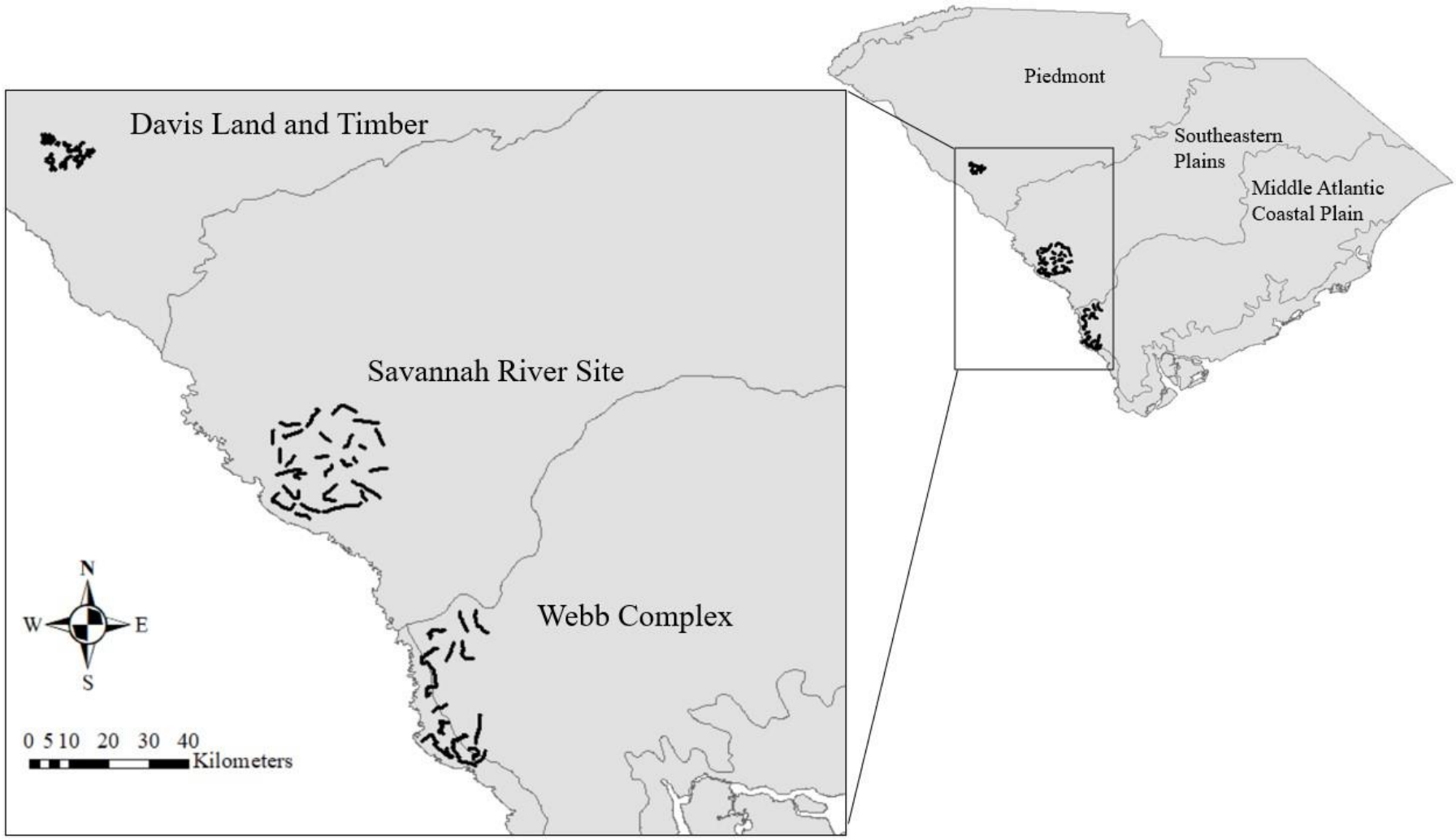

Figure 4.1. Study sites along the Savannah River, South Carolina, USA where scat was collected to assess diet of coyotes (*Canis latrans*), bobcats (*Lynx rufus*), and gray foxes (*Urocyon cinereoargenteus*) during May-June 2020-2021. Inset shows sample transects used to collect scat for diet analysis.

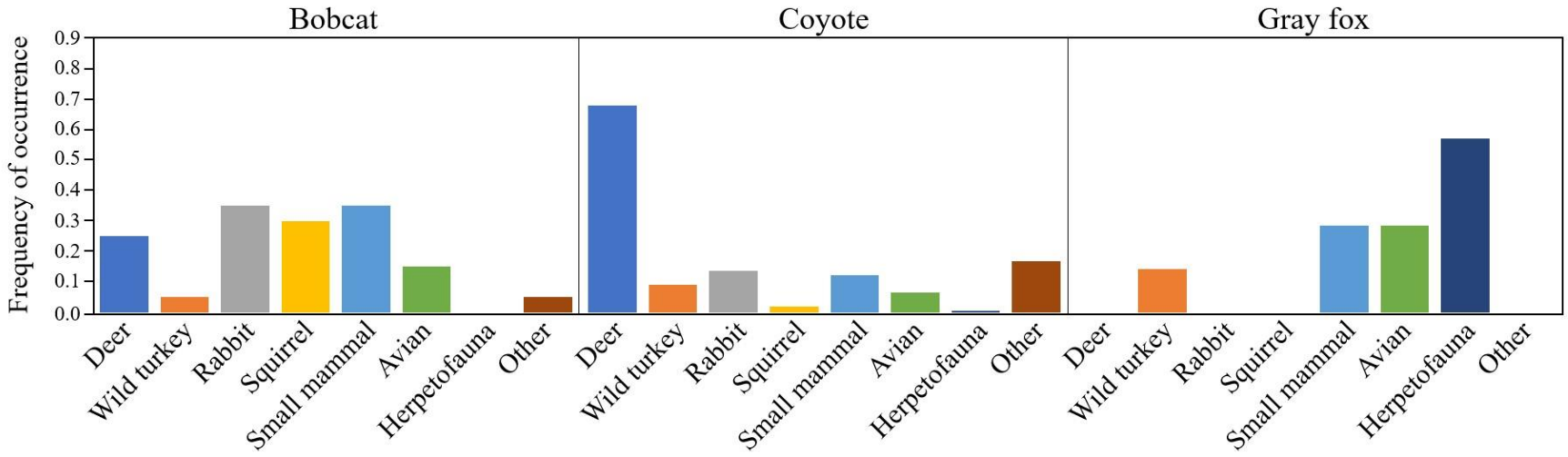

Figure 4.2. Frequency of occurrence for prey items of coyotes (*Canis latrans*), bobcats (*Lynx rufus*), and gray foxes (*Urocyon cinereoargenteus*) along the Savannah River, SC during May-June 2020-2021.

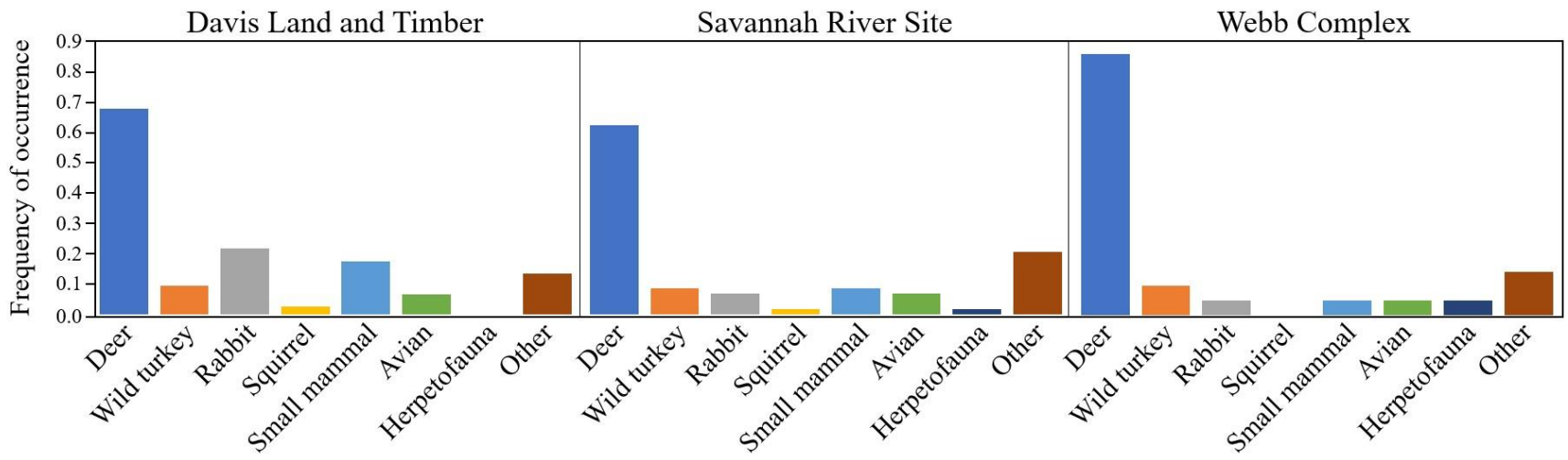

Figure 4.3. Frequency of occurrence for prey items of coyotes (*Canis latrans*) across the Davis Land and Timber, Savannah River Site, and Webb Complex study sites along the Savannah River, SC during May-June 2020-2021.

CHAPTER 5

GENETIC PANMIXIA IN RECENTLY COLONIZED COYOTE POPULATIONS ACROSS SOUTH CAROLINA, USA

Youngmann, J. L., S. L. Lance, J. C. Kilgo, C. Ruth, J. Cantrell, and G. J. D'Angelo. 2023. To be submitted to *Southeastern Naturalist*.

ABSTRACT

Coyotes (*Canis latrans*) are a highly mobile, generalist species that occupy a wide variety of habitats and ecoregions across North America. Assessments of population genetic diversity and structure among the recently colonized populations of coyotes in the eastern United States of America (USA) have revealed broad genetic structure concordant with immigration routes, high genetic diversity, and panmixia across local spatial scales. However, few studies have assessed eastern coyote genetics at an intermediate scale that would provide information on population dynamics of this mobile animal. We sampled 10 populations across South Carolina, USA during summer 2019-2020 using noninvasive genetic sampling and 10 microsatellite loci to identify individuals. We assessed genetic diversity and pairwise relatedness as well as genetic structure across our populations to determine whether there were landscape-level differences in genetic differentiation and the scale of coyote population structure across the state. We found high levels of observed heterozygosity (0.599 – 0.872) and allelic richness (3.99 – 5.12) across our sites but low levels of relatedness. We observed low genetic differentiation (pairwise F_{ST}: 0.010 – 0.066) and insignificant isolation by distance with no evidence of genetic structure, indicating genetic panmixia. Coyote populations seem to be operating at a spatial extent greater than South Carolina and gene flow is likely maintained by unrelated, transient individuals. Future research and management of coyotes in the heterogeneous landscapes of the southeastern USA should account for the geographic scale that populations encompass.

INTRODUCTION

Population-level genetic structure can be maintained by extrinsic barriers to movement such as geographic delineations and habitat clines, or intrinsic processes such as morphological or behavioral limits to home range size and dispersal (Wright 1943, Mills and Allendorf 1996).

Generally, at least one immigrant per generation is required to maintain genetic similarity between populations and frequent genetic exchange can result in panmixia (Mills and Allendorf 1996). For instance, low genetic structure and panmixia as a result of high dispersal has been documented in the Pacific walrus (*Odobenus rosmarus divergens*; Beatty et al. 2020), big brown bats (*Eptesicus fuscus*; Richardson et al. 2021), and coyotes (*Canis latrans*; Kierepka et al. 2017, Heppenheimer et al. 2018*b*). Understanding the extent and scale of gene flow among populations is necessary for defining some aspects of wildlife management and understanding demographic processes such as population dynamics and dispersal.

Coyotes offer an opportunity to investigate population connectivity through genetics due to their behavioral plasticity, rapidly expanding range, and ability to travel long distances (Hinton et al. 2012, Hody and Kays 2018). Past research in the endemic range of the western United States of America (USA) has shown natal-biased dispersal by coyotes that encourages genetic structure along habitat-specific interfaces (Sacks et al. 2004, 2005). Recent studies have documented genetic structure within densely populated urban settings, likely due to anthropogenic barriers to movement, genetic bottlenecking, and genetic drift (Rashleigh et al. 2008, Damm et al. 2015, DeCandia et al. 2019, Henger et al. 2020). However, coyotes have dramatically increased their historic range by colonizing the eastern USA over the last century (Hody and Kays 2018) and studies have documented dispersal movements >300 km (Hinton et al. 2012). As such, recent investigations of genetic diversity in colonized populations of coyotes have shown weak signals of substructure in the eastern USA. Genetic panmixia is evident across localized spatial scales (Damm et al. 2015, Bohling et al. 2017, Kierepka et al. 2017, Heppenheimer et al. 2018*b*) and population structure generally adheres to historic colonization

routes at large spatial scales (i.e., east of the Mississippi River; (Heppenheimer et al. 2018*a*, *b*, Hinton et al. 2019).

Furthermore, coyotes exhibit a dual life-history strategy of residency and transiency, which may also impact local population genetic structure. Resident individuals hold relatively stable territories while transient individuals disperse in search of available space and resources (Hinton et al. 2015, Morin and Kelly 2017), thus potentially increasing geneflow. Efforts to lethally control local populations of coyotes have resulted in mixed success, with evidence of rapid back-fill of space by transient individuals from outside the area of management (Kilgo et al. 2014, Gulsby et al. 2015). Both Williams et al. (2003) and Kierepka et al. (2017) observed little change in genetic structure after coyote removal efforts, likely due to compensatory reproduction and immigration from genetically similar populations. High rates of gene flow, therefore, should play an important role in maintaining genetic diversity while minimizing structure in coyote populations of the eastern USA. However, most investigation of population genetics in eastern coyotes has occurred at either large spatial scales (vonHoldt et al. 2016*a*, *b*, Heppenheimer et al. 2018*a*, *b*, Hinton et al. 2019) or was locally constrained (Damm et al. 2015, Kierepka et al. 2017). Two studies, Bohling et al. (2017) in West Virgina, USA and portions of Virginia, USA and Berkman et al. (2019) in New York, USA studied coyotes at a state-wide scale and found evidence of panmixia and high gene flow due to dispersal. Assessing genetic structure at an intermediate scale, such as across a state, may provide insight into the extent that coyote population dynamics operate across the heterogeneous landscape of the eastern USA.

Our objective was to assess coyote population genetics across the state of South Carolina, USA. We predicted that genetic diversity would be high due to gene flow and rapid population expansion (Heppenheimer et al. 2018*a*). We also assessed relatedness within coyote populations

across South Carolina to determine kinship dynamics within each of our study sites. We predicted that kinship would be highly variable due to the presence of resident and transient individuals. Finally, we sampled populations throughout three major ecoregions to determine if there were landscape-level differences in genetic structure similar to those found in other portions of the coyote range (Sacks et al. 2004, 2005). We predicted that population structure would be low (Heppenheimer et al. 2018*b*) as a result of coyotes' generalist behavior and high dispersal capabilities. Our research bridges the gap between previous population structure studies of eastern coyotes by assessing genetic characteristics across an intermediate spatial scale and informs our understanding of coyote populations.

STUDY AREA

We assessed coyote population genetic structure across 10 study sites in South Carolina (Fig. 1). Study sites were a mixture of private and public lands located in the Piedmont, Southeastern Plains, and Middle Atlantic Coastal Plains ecoregions across South Carolina. The Piedmont ecoregion lies between the Blue Ridge Mountains and the Southeastern Plains. Originally dominated by oak-hickory-pine (*Quercus-Carya-Pinus*) forests, the Piedmont has experienced extensive cotton, corn, tobacco, and wheat farming (Griffith et al. 2002). However, much of the region now contains both natural and planted pine stands. Mean annual temperature in the Piedmont is approximately 15°C with a mean annual precipitation of 1229 mm (Wiken et al. 2011). Our study sites within the Piedmont included the Long Cane and Enoree Ranger Districts of the Sumter National Forest (NF), and the Davis Land and Timber property. In 2020 we also sampled the Liberty Hill Wildlife Management Area (WMA) and surrounding private lands.

Our study sites within the Southeastern Plains included the Savannah River Site, a U.S. Department of Energy National Environmental Research Park, Fort Jackson, and Carolina

Sandhills National Wildlife Refuge (NWR) and Sandhills State Forest (SF) complex. The Southeastern Plains is typified by sandy soils and was comprised historically of mostly longleaf pine (*Pinus palustris*) forest although it now contains extensive amounts of cultivated cropland and pasture/hay with large areas of pine plantations (Griffith et al. 2002). However, the Savannah River Site is almost entirely forested in planted pine, with bottomland hardwoods scattered throughout (Kilgo and Blake 2005). Mean annual temperature in the Southeastern Plains is ~16°C with a mean annual precipitation of 1358 mm (Wiken et al. 2011).

The Middle Atlantic Coastal Plain contains lowland plains filled with swamps, marshes, and estuaries. Also originally covered in longleaf pine, many areas have been converted to pine plantations (Griffith et al. 2002). Mean annual temperature in the Middle Atlantic Coastal Plain is ~15.5°C with a mean annual precipitation of 1229 mm (Wiken et al. 2011). Our study sites in the Middle Atlantic Coastal Plain included the complex of private and state-owned public lands around the Webb Center and WMA, the Marsh and Woodbury WMA complex, and the Bonneau Ferry WMA.

METHODS

Sampling methodology

We sampled transects at each site across a 2-week period during July and August of 2019 and 2020 except for at Bonneau Ferry WMA where tissue samples were taken from trapped and euthanized coyotes. We conducted an initial sweep of each transect at the beginning of the sampling period to remove all accumulated scat present from before the start of the sampling session. We then sampled transects every 3 days for a total of 4 sampling occasions in 2019 and 5 sampling occasions in 2020. We drove transects at approximately 10 km/hr, using the edges of the road as the boundary of our transect sampling area. We collected scat samples using either a

wooden popsicle stick, which was discarded after each sampling, or forceps that were sanitized with alcohol wipes and a butane lighter. We collected 0.4 mL of the outer portion of scat and placed it into a 2 mL tube containing 1.6 mL of DETs (DMSO/EDTA/Tris/salt) buffer (Frantzen et al. 1998, Stenglein et al. 2010a). The remainder of the scat was sealed in a Ziploc freezer bag and stored on ice in the truck before being transferred to a -20°C freezer for storage. Whole scat was collected as a backup in case of DNA extraction failure with the DETs-preserved samples. We recorded the GPS coordinates of each sample along with the general appearance of the scat and any pertinent information concerning the location and condition of the sample.

Laboratory methodology

We extracted each sample using the Qiagen Mini Stool Kit (Qiagen, Valencia, California, USA), following the manufacturer's protocols with the exception that we filtered the eluted product a second time through the final filtration step to maximize the concentration of nDNA. Samples were amplified using a 10-primer microsatellite multiplex described in Stenglein et al. (2010b) with the addition of two sex primers (Seddon 2005). We followed the run specifications laid out in Stenglein et al. (2010b) and Morin et al. (2016) with the exception that we reduced the number of PCR cycles to 30 repetitions. This was done because we found that our samples were amplifying too strongly to easily scored (unpublished data). The Stenglein et al. (2010b) lab protocols were developed using scat samples exhibiting a range of degradation and, therefore, required a high number of cycles to produce satisfactory amplification. Our sampling methodologies ensured that samples were no more than 3-4 days old and, therefore, contained high levels of quality DNA. We included a negative control on each 96-well PCR plate to detect cross-contamination.

We used the multi-tube approach to run four separate replicate PCRs on each sample (Taberlet et al. 1996). PCR products were prepared with formamide and LIZ500 size standard and analyzed with 3730xl capillary machines (Applied Biosystems Inc., Foster City, CA) at either the University of Georgia Genomics and Bioinformatics Core or the Cornell University Institute of Biotechnology Genomics Core. We observed no difference in PCR replicates between the two laboratories. We scored each sample using Geneious 2022.2.2 (http://www.geneious.com/) and confirmed consensus genotypes using *ConGenR* in Program R (Lonsinger and Waits 2015). Heterozygote loci were required to be observed in two separate replicates, while homozygote loci were required to be observed in three separate replicates (Taberlet et al. 1996). Matching genotypes were determined using a threshold of seven loci matches to address the probability of identity based on likelihood of siblings. Finally, we used *ConGenR* to identify recaptures across all sites and years.

All samples were additionally screened with a 6-primer, species-specific mitochondrial DNA (mtDNA) control-region multiplex to identify the species of each scat (De Barba et al. 2014). We used the PCR specifications outlined in De Barba et al. (2014) and visualized results using Geneious 2022.2.2 (http://www.geneious.com/). Several gray fox samples amplified at seven loci or more, but we removed these samples. Finally, we used eight known coyotes trapped from the Bonneau Ferry WMA and 25 known domestic dogs to run an additional genetic assignment test on all canid samples using STRUCTURE 2.3.4 (Pritchard et al. 2000, Falush et al. 2003). We assumed two populations and ran 10 iterations with a burn-in of 50,000 repetitions followed by 150,000 repetitions. We then removed all individuals identified as dog through STRUCTURE.

Genetic analysis

Genetic diversity

We assessed allelic richness (A_R), which standardizes estimates of allelic diversity by sample size, using the package HIEFERSTAT (Goudet et al. 2022) implemented in program R. We estimated observed (H_O) and expected (H_E) heterozygosity as well as linkage disequilibrium for each study site in ARLEQUIN v.3.5.2.2 (version 3.5.2.2; Excoffier and Lischer 2010) using 10,000 permutations to measure linkage disequilibrium and tested significance using Bonferroni's correction for multiple comparisons ($p \leq 0.001$). We used GENEPOP (version 4.7.5; Rousset 2008) to estimate inbreeding coefficients (F_{IS}) for each study site. Finally, to estimate relatedness within study sites, we used EIMIDB9 (version 1.0.0.0; Wang 2022). EIMIDB9 uses a joint-likelihood estimator to reduce biases associated with small sample sizes and high numbers of closely related individuals (Wang 2022).

Genetic structure

We calculated Reynold's et al. (1983) linearized pairwise F_{ST} among study sites using ARLEQUIN (version 3.5.2.2; Excoffier and Lischer 2010) and estimated significance over 10,000 permutations using Bonferroni's correction for multiple comparisons ($p \leq 0.001$). We also used ARLEQUIN to assess genetic differentiation among and within our three major ecoregions using an analysis of molecular variance (AMOVA) and 10,000 permutations. We used GENEPOP (version 4.7.5; Rousset 2008) to perform a Mantel test estimating isolation by distance (IBD) between F_{ST} and geographic distance using 10,000 permutations to test for significance. Before conducting tests of IBD we used the *near* function in ArcGIS to produce a pairwise geographic distance matrix.

We used STRUCTURE (version 2.3.4; Pritchard et al. 2000, Falush et al. 2003) to assess genetic structure through a Bayesian assignment methodology. We modified the default settings to account for uneven sampling among our study sites by employing an initial admixture coefficient of 0.1 (1/10 populations) and uncorrelated allele frequencies (Wang 2017). We then ran 10 iterations with a burn-in of 50,000 followed by 150,000 repetitions. We assessed the most likely number of groups (K) using Evanno's ΔK (Evanno et al. 2005) implemented in STRUCTURE HARVESTER (Earl and vonHoldt 2012). However, in cases of negligible population structure, ΔK is unable to find a best fit for K = 1, or panmixia, because it relies on the rate of change in the likelihood distribution. Therefore, we also used the mean estimate of likelihood for each K value to assess a best fit at K = 1. We visualized STRUCTURE results in CLUMPAK (Kopelman et al. 2015).

Finally, we conducted an analysis of principal components using the Discriminant Analysis of Principal Components (DAPC) implemented in ADEGENET (Jombart 2008). While STRUCTURE and estimates of genetic differentiation use assumptions about ancestry, admixture, and population genetic theory such as Hardy-Weinberg equilibrium and linkage disequilibrium, DAPC operates outside of population genetic principles, serving as a complement to traditional analyses of genetic structure. We used a Bayesian Information Criterion (BIC) to determine the best fit for number of groups, typically estimated as nearest to the elbow of the BIC curve produced by the *find.cluster* function.

RESULTS

We successfully genotyped 204 coyote individuals across our 10 study sites with sample sizes ranging from 4 to 69 coyotes per site (Table 5.1). Coyotes across our sample sites exhibited high genetic diversity with allelic richness ranging from 3.99 (Bonneau Ferry WMA) to 5.12

(Carolina Sandhills) and observed heterozygosity ranging from 0.599 (Bonneau Ferry WMA) to 0.872 (Liberty Hill; Table 5.1). We observed F_{IS} from -0.065 (Liberty Hill) to 0.252 (Enoree NF) and significant linkage disequilibrium at pairwise loci from 0 to 8 (Davis Land and Timber).

Estimates of kinship showed right-tailed relatedness distributions for all of our sites (Fig. 5.2). Pairwise relationships were predominantly skewed towards 0, meaning that coyotes within sites were largely unrelated to each other. Bonneau Ferry WMA was the only site that showed signs of a bimodal relatedness distribution with a slight peak around r = 0.3, indicating multiple full sibling or parent-offspring relationships.

Genetic structure

We observed negligible population structure among ecoregions and among our study sites. F_{ST} ranged from 0.010 (Liberty Hill and the Carolina Sandhills) to 0.066 (Bonneau Ferry WMA to Long Cane NF; Table 5.2). Estimates of molecular variance revealed that 97.30% of the genetic variation was found within individual study sites whereas differences between study sites within ecoregion accounted for only 3.08% of the variation (Table 5.3). Variation between ecoregions was -0.38%, meaning that there was more similarity between ecoregions than differences between study sites or individuals. Similarly, IBD revealed positive but insignificant genetic differentiation as a function of geographic distance ($p = 0.167$) with an R^2-value of 0.053 and slope of 4×10^{-5} (Fig. 5.3).

Bayesian clustering analysis revealed a best fit of K=8 using Evanno's ΔK followed by K=2 (Fig. 5.4). However, estimates of mean likelihood probability distributions for each value of K showed support for a highest likelihood at K=1. Although K=2 revealed heterogeneous ancestry within each coyote study site, K=8 showed no population structure (Fig. 5.5). Best fit for number of clusters using principal components was 3 clusters (Fig. 5.6), which clearly split

individuals into distinct groups (Fig. 5.7). However, most study sites were evenly divided among groups with little intrapopulation structure evident (Table 5.4).

DISCUSSION

Across sample sites, we observed comparable estimates of allelic richness and heterozygosity to previous studies of eastern coyotes (Bohling et al. 2017, Heppenheimer et al. 2018*b*). Coyotes began colonizing South Carolina by the late 1970s and quickly became established across the state by the 1990s (Ruth and Cantrell 2021). Harvest reports of coyotes killed during the fall and winter deer season in South Carolina showed a steady increase during the 2000s until a peak in harvest around 2014 (C. Ruth, unpublished data). Coyote numbers have, therefore, experienced rapid growth across a largely contiguous spatial scale and may have recently stabilized. Population genetic theory stipulates that rapid population growth should mitigate the effects of genetic bottlenecking (Nei et al. 1975), which has been shown in other species including white-tailed deer (DeYoung et al. 2003). In the case of eastern coyotes, previous studies have confirmed low levels of introgression by wolf species (*Canis* spp.) along the colonizing front of coyotes moving eastward (Kays et al. 2010, vonHoldt et al. 2016*a*, *b*, Way and Lynn 2016, Heppenheimer et al. 2018*a*, Hinton et al. 2019). Admixture with other canid species likely has contributed to high genetic diversity found in eastern coyotes. Finally, the mobile nature of coyotes has undoubtedly contributed to our observations of high heterozygosity and allelic richness, likely aiding in high rates of gene flow across the state. Therefore, a confluence of historical and demographic processes is responsible for the high genetic diversity of coyotes throughout South Carolina.

Estimates of kinship between individuals showed a majority of coyotes within our study sites were unrelated to each other, with only a handful of related individuals, usually first sibling

and parent-offspring pairs. Way et al. (2001) observed kinship among packs to consist of unrelated breeding individuals and their offspring in northeastern USA following colonization. Williams et al. (2003) found low levels of relatedness in exploited coyote populations with a high turnover rate. It should be noted that we sampled during July and August, when pups are becoming precocious and detectable using our sampling methodologies and population-wide mortality is relatively low (Harrison and Gilbert 1985, Harrison et al. 1991, Sasmal et al. 2019). Pups will begin to disperse during the fall (Harrison et al. 1991), concurrent with the onset of hunting season where a majority of coyote mortality occurs (M. J. Chamberlain, personal communication). The resulting transiency and mortality should provide an influx of new individuals into exploited populations (Williams et al. 2003, Kierepka et al. 2017, Kilgo et al. 2017), meaning that our estimates of relatedness during the summer likely represent local kinship at its highest point of the year. As such, predominately unrelated pairwise relationships between coyotes at each of our study sites speak to high rates of immigration and emigration and the influence of transient individuals on maintaining gene flow.

Our analyses of genetic structure revealed little evidence of differentiation, even between geographically disparate study sites. Unlike past studies in California, USA, we did not observe any structure between ecoregions (Sacks et al. 2004, 2005). Notably, ecoregions within South Carolina represent no functional impediment to coyote dispersal and may not present distinctive alternatives during natal-biased dispersal. Instead, coyotes across our study sites exhibited classic signs of genetic panmixia, including low genetic differentiation and ancestry grouping approaching K = 1. Similarly, previous estimates of population structure in the southeastern, USA has found genetic panmixia across local, intermediate, and broad scales (Damm et al. 2015, Bohling et al. 2017, Kierepka et al. 2017, Hinton et al. 2019). Even at localized spatial scales, we

determined that many individuals are unrelated by descent. Previous attempts to lethally control colonized populations of coyotes in the eastern USA has shown that coyotes quickly respond to local-scale removal through immigration (Kilgo et al. 2014, Gulsby et al. 2015, Kierepka et al. 2017). Furthermore, coyotes in the Southeast have been documented to travel >300km (Hinton et al. 2012) and 30% of coyotes can be expected to be transient at any given time (Hinton et al. 2015, Ward et al. 2018). Therefore, coyote population dynamics likely are strong contributing factors in mitigating genetic structure, even across hundreds of kilometers, and the one immigrant per generation rule indicates that coyote dispersal maintains a shifting mosaic of gene flow across a broad geographic area (Mills and Allendorf 1996). Our findings indicate that coyote populations may be panmictic (i.e., random mating) at a scale larger than geographic boundaries of the state of South Carolina.

Our study was limited by several factors that make further assessment of coyote population genetics across our study sites difficult. First, we did not collect samples from outgroups from either the coyote endemic range of the western USA or other states in eastern USA. A lack of genetic outgroups can impact the ability of genetic structure analyses such as STRUCTURE to delineate between ancestry groups and may have led to our observation of a best fit of 1 for K. However, lack of outgroups typically leads to inflated signals of genetic structure, perhaps as seen in our marginal support for K = 2, and the preponderance of evidence in our data instead indicates panmixia. We also observed low sample sizes across several of our study sites, likely as a result of sparse coyote densities. Low sample sizes may have reduced our ability to accurately describe genetic diversity and could also impact estimates of genetic differentiation and structure. Finally, although we sampled later in the summer to maximize our chances of collecting scat from coyote pups while avoiding fall mortality during hunting season

and juvenile dispersal, we may not have sufficiently obtained samples from young-of-the-year individuals. By sampling only adult coyotes, we may have underestimated pairwise kinship and missed important genetic structure among coyote packs. Future research would benefit from sampling during multiple seasons to obtain a representative sample size for each site, as well as collecting samples from other regions of the coyote range to contextualize genetic structure among populations.

Highly mobile animals are capable of maintaining genetic connectivity over a broad spatial scale. Rapid range expansion also facilitates gene flow, but colonizing populations typically exhibit evidence of genetic bottlenecking and drift due to sparse distribution of individuals along the colonization front (Welles and Dlugosch 2018). Having recently expanded their range throughout the eastern USA, coyotes have established robust populations across eastern North America. Although past assessment of eastern coyote genetics has revealed population structure along immigration routes (Heppenheimer et al. 2018*b*, Hinton et al. 2019), evidence of genetic bottlenecking has largely been relegated to fragmented populations inside urban areas (Rashleigh et al. 2008, DeCandia et al. 2019, Adducci et al. 2020, Henger et al. 2020). Instead, rural populations of coyotes have been shown to exhibit high allelic richness and heterozygosity (Bohling et al. 2017, Kierepka et al. 2017, Heppenheimer et al. 2018*a*). Rapid population expansions can result in increased genetic diversity, and it is likely that high rates of gene flow across broad geographic space have resulted in a genetically diverse and generally homogenous population structure within eastern coyotes. Our findings corroborate these previous reports while further emphasizing the scale at which coyote populations may be interacting with each other in the southeastern USA.

LITERATURE CITED

Adducci, A., J. Jasperse, S. Riley, J. Brown, R. Honeycutt, and J. Monzón. 2020. Urban coyotes are genetically distinct from coyotes in natural habitats. Journal of Urban Ecology 6:1–11.

Beatty, W. S., P. R. Lemons, S. A. Sethi, J. P. Everett, C. J. Lewis, R. J. Lynn, G. M. Cook, J. L. Garlich-Miller, and J. K. Wenburg. 2020. Panmixia in a sea ice-associated marine mammal: evaluating genetic structure of the Pacific walrus (*Odobenus rosmarus divergens*) at multiple spatial scales. A. Baird, editor. Journal of Mammalogy 101:755–765.

Berkman, L. K., J. L. Frair, P. E. Marquardt, D. M. Donner, J. C. Kilgo, and C. M. Whipps. 2019. Spatial genetic analysis of coyotes in New York State. Wildlife Society Bulletin 43:21–30.

Bohling, J. H., L. L. Mastro, J. R. Adams, E. M. Gese, S. F. Owen, and L. P. Waits. 2017. Panmixia and limited interspecific introgression in coyotes (*Canis latrans*) from West Virginia and Virginia, USA. Journal of Heredity 108:608–617.

Damm, D. L., J. B. Armstrong, W. M. Arjo, and A. J. Piaggio. 2015. Assessment of population structure of coyotes in east-central Alabama using microsatellite DNA. Southeastern Naturalist 14:106–122.

De Barba, M., J. R. Adams, C. S. Goldberg, C. R. Stansbury, D. Arias, R. Cisneros, and L. P. Waits. 2014. Molecular species identification for multiple carnivores. Conservation Genetics Resources 6:821–824.

DeCandia, A. L., C. S. Henger, A. Krause, L. J. Gormezano, M. Weckel, C. Nagy, J. Munshi-South, and B. M. vonHoldt. 2019. Genetics of urban colonization: neutral and adaptive

variation in coyotes (*Canis latrans*) inhabiting the New York metropolitan area. Journal of Urban Ecology 5:1–12.

DeYoung, R. W., S. Demarais, R. L. Honeycutt, A. P. Rooney, R. A. Gonzales, and K. L. Gee. 2003. Genetic consequences of white-tailed deer (*Odocoileus virginianus*) restoration in Mississippi. Molecular Ecology 12:3237–3252.

Earl, D. A., and B. M. vonHoldt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4:359–361.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology 14:2611–2620.

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564–567.

Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587.

Frantzen, M. a. J., J. B. Silk, J. W. H. Ferguson, R. K. Wayne, and M. H. Kohn. 1998. Empirical evaluation of preservation methods for faecal DNA. Molecular Ecology 7:1423–1428.

Futuyma, D. J., and G. Moreno. 1988. The evolution of ecological specialization. Annual Review of Ecology and Systematics 19:207–233.

Griffith, G. E., J. M. Omernik, J. A. Comstock, M. P. Shafale, W. H. McNab, D. R. Lenat, J. B. Glover, and V. B. Shelburne. 2002. Ecoregions of North Carolina and South Carolina. US Geological Survey, Reston, VA, USA.

Gulsby, W. D., C. H. Killmaster, J. W. Bowers, J. D. Kelly, B. N. Sacks, M. J. Statham, and K. V. Miller. 2015. White-tailed deer fawn recruitment before and after experimental coyote removals in central Georgia. Wildlife Society Bulletin 39:248–255.

Harrison, D. J., and J. R. Gilbert. 1985. Denning ecology and movements of coyotes in Maine during pup rearing. Journal of Mammalogy 66:712–719.

Harrison, D. J., J. A. Harrison, and M. O'Donoghue. 1991. Predispersal movements of coyote (*Canis latrans*) pups in eastern Maine. Journal of Mammalogy 72:756–763.

Henger, C. S., G. A. Herrera, C. M. Nagy, M. E. Weckel, L. J. Gormezano, C. Wultsch, and J. Munshi-South. 2020. Genetic diversity and relatedness of a recently established population of eastern coyotes (*Canis latrans*) in New York City. Urban Ecosystems 23:319–330.

Heppenheimer, E., K. E. Brzeski, J. W. Hinton, B. R. Patterson, L. Y. Rutledge, A. L. DeCandia, T. Wheeldon, S. R. Fain, P. A. Hohenlohe, R. Kays, B. N. White, M. J. Chamberlain, and B. M. vonHoldt. 2018*a*. High genomic diversity and candidate genes under selection associated with range expansion in eastern coyote (*Canis latrans*) populations. Ecology and Evolution 8:12641–12655.

Heppenheimer, E., D. S. Cosio, K. E. Brzeski, D. Caudill, K. Van Why, M. J. Chamberlain, J. W. Hinton, and B. vonHoldt. 2018*b*. Demographic history influences spatial patterns of genetic diversity in recently expanded coyote (*Canis latrans*) populations. Heredity 120:183–195.

Hinton, J. W., M. J. Chamberlain, and F. T. van Manen. 2012. Long-distance movements of transient coyotes in eastern North Carolina. American Midland Naturalist 168:281–288.

Hinton, J. W., E. Heppenheimer, K. M. West, D. Caudill, M. L. Karlin, J. C. Kilgo, J. J. Mayer, K. V. Miller, M. Walch, B. vonHoldt, and M. J. Chamberlain. 2019. Geographic patterns in morphometric and genetic variation for coyote populations with emphasis on southeastern coyotes. Ecology and Evolution 9:3389–3404.

Hinton, J. W., F. T. van Manen, and M. J. Chamberlain. 2015. Space use and habitat selection by resident and Ttransient Coyotes (*Canis latrans*). PLOS ONE 10:e0132203.

Hody, J. W., and R. Kays. 2018. Mapping the expansion of coyotes (*Canis latrans*) across North and Central America. ZooKeys 759:81–97.

Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405.

Kays, R., A. Curtis, and J. J. Kirchman. 2010. Rapid adaptive evolution of northeastern coyotes via hybridization with wolves. Biology Letters 6:89–93.

Kierepka, E. M., J. C. Kilgo, and O. E. Rhodes. 2017. Effect of compensatory immigration on the genetic structure of coyotes. Journal of Wildlife Management 81:1394–1407.

Kilgo, J. C., and J. I. Blake. 2005. Ecology and management of a forested landscape: fifty years on the Savannah River Site. Island Press, Washington, DC.

Kilgo, J. C., M. Vukovich, H. Scott Ray, C. E. Shaw, and C. Ruth. 2014. Coyote removal, understory cover, and survival of white-tailed deer neonates. Journal of Wildlife Management 78:1261–1271.

Kilgo, J. C., C. E. Shaw, M. Vukovich, M. J. Conroy, and C. Ruth. 2017. Reproductive characteristics of a coyote population before and during exploitation. Journal of Wildlife Management 81:1386–1393.

Kopelman, N. M., J. Mayzel, M. Jakobsson, N. A. Rosenberg, and I. Mayrose. 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Molecular Ecology Resources 15:1179–1191.

Lonsinger, R. C., and L. P. Waits. 2015. ConGenR: rapid determination of consensus genotypes and estimates of genotyping errors from replicated genetic samples. Conservation Genetics Resources 7:841–843.

Mills, L. S., and F. W. Allendorf. 1996. The one-migrant-per-generation rule in conservation and management. Conservation Biology 10:1509–1518.

Morin, D. J., and M. J. Kelly. 2017. The dynamic nature of territoriality, transience and biding in an exploited coyote population. Wildlife Biology 2017:wlb.00335.

Morin, D. J., M. J. Kelly, and L. P. Waits. 2016. Monitoring coyote population dynamics with fecal DNA and spatial capture-recapture. Journal of Wildlife Management 80:824–836.

Nei, M., T. Maruyama, and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1–10.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Rashleigh, R. M., R. A. Krebs, and H. V. Keulen. 2008. Population structure of coyote (*Canis latrans*) in the urban landscape of the Cleveland, Ohio area. 108:54–59.

Reynolds, J., B. S. Weir, and C. C. Cockerham. 1983. Estimation of the coancestry coefficient: Basis for a short-term genetic distance. Genetics 105:767–779.

Richardson, J. L., S. Michaelides, M. Combs, M. Djan, L. Bisch, K. Barrett, G. Silveira, J. Butler, T. T. Aye, J. Munshi-South, M. DiMatteo, C. Brown, and T. J. McGreevy Jr.

2021. Dispersal ability predicts spatial genetic structure in native mammals persisting across an urbanization gradient. Evolutionary Applications 14:163–177.

Rousset, F. 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources 8:103–106.

Sacks, B. N., S. K. Brown, and H. B. Ernest. 2004. Population structure of California coyotes corresponds to habitat-specific breaks and illuminates species history. Molecular Ecology 13:1265–1275.

Sacks, B. N., B. R. Mitchell, C. L. Williams, and H. B. Ernest. 2005. Coyote movements and social structure along a cryptic population genetic subdivision. Molecular Ecology 14:1241–1249.

Sasmal, I., C. E. Moorman, M. B. Swingen, S. Datta, and C. S. DePerno. 2019. Seasonal space use of transient and resident coyotes (*Canis latrans*) in North Carolina, USA. Canadian Journal of Zoology 97:326–331.

Stenglein, J. L., M. De Barba, D. E. Ausband, and L. P. Waits. 2010a. Impacts of sampling location within a faeces on DNA quality in two carnivore species. Molecular Ecology Resources 10:109–114.

Stenglein, Jennifer L., L. P. Waits, D. E. Ausband, P. Zager, and C. M. Mack. 2010b. Efficient, noninvasive genetic sampling for monitoring reintroduced wolves. Journal of Wildlife Management 74:1050–1058.

Seddon, J. M. 2005. Canid-specific primers for molecular sexing using tissue or non-invasive samples. Conservation Genetics 6:147–149.

Taberlet, P., S. Griffin, B. Goossens, S. Questiau, V. Manceau, N. Escaravage, L. P. Waits, and

J. Bouvet 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Research 24:3189–3194.

vonHoldt, B. M., J. A. Cahill, Z. Fan, I. Gronau, J. Robinson, J. P. Pollinger, B. Shapiro, J. Wall, and R. K. Wayne. 2016*a*. Whole-genome sequence analysis shows that two endemic species of North American wolf are admixtures of the coyote and gray wolf. Science Advances 2:e1501714.

vonHoldt, B. M., R. Kays, J. P. Pollinger, and R. K. Wayne. 2016*b*. Admixture mapping identifies introgressed genomic regions in North American canids. Molecular Ecology 25:2443–2453.

Wang, J. 2017. The computer program structure for assigning individuals to populations: easy to use but easier to misuse. Molecular Ecology Resources 17:981–990.

Wang, J. 2022. A joint likelihood estimator of relatedness and allele frequencies from a small sample of individuals. Methods in Ecology and Evolution 13:2443–2462.

Ward, J. N., J. W. Hinton, K. L. Johannsen, M. L. Karlin, K. V. Miller, and M. J. Chamberlain. 2018. Home range size, vegetation density, and season influences prey use by coyotes (*Canis latrans*). PLOS ONE 13:e0203703.

Way, J. G., P. J. Auger, I. M. Ortega, and E. G. Strauss. 2001. Eastern coyote denning behavior in an anthropogenic environment. Northeast Wildlife 56:18–30.

Way, J. G., and W. S. Lynn. 2016. Northeastern coyote/coywolf taxonomy and admixture: A meta-analysis. Canid Biology & Conservation 19:1–7.

Welles, S. R., and K. M. Dlugosch. 2018. Population genomics of colonization and invasion. Pages 655–683 *in* O. P. Rajora, editor. Population Genomics. Population Genomics,

Springer International Publishing, Cham. <https://link.springer.com/10.1007/13836_2018_22>. Accessed 6 Feb 2023.

Wiken, E., F. Jimenez Nava, and G. Griffith. 2011. North American Terrestrial Ecoregions – Level III. Commission for Environmental Cooperation, Montreal, Canada.

Williams, C. L., K. Blejwas, J. J. Johnston, and M. M. Jaeger. 2003. Temporal genetic variation in a coyote (*Canis latrans*) population experiencing high turnover. Journal of Mammalogy 84:177–184.

Wright, S. 1943. Isolation by distance. Genetics 28:114–138.

Table 5.1. Summary statistics for genetic diversity of coyotes (*Canis latrans*) across 10 study sites in South Carolina, USA from July-August 2019-2020.

Study Site	n	A_R (8 alleles)	H_O	H_E	LD*	F_{IS}
Carolina Sandhills	25	5.12	0.789	0.856	0	0.079
Enoree NF	7	5.06	0.634	0.845	0	0.252
Fort Jackson	18	4.90	0.831	0.836	1	0.001
Liberty Hill	9	4.95	0.872	0.825	0	-0.065
Long Cane NF	18	4.85	0.765	0.834	4	0.082
Marsh Woodbury	4	5.00	0.850	0.836	0	-0.020
Savannah River Site	69	4.93	0.724	0.824	7	0.116
Stephen Davis	32	5.03	0.764	0.843	8	0.093
Webb Groton Westervelt	14	4.81	0.707	0.812	1	0.127
Bonneau Ferry WMA	8	3.99	0.599	0.743	3	0.194

*Significant with a Bonferroni correction of $p \leq 0.001$ for multiple comparisons

Table 5.2. Pairwise estimates of genetic differentiation (F_{ST}) among coyotes (*Canis latrans*) sampled across 10 study sites in South Carolina, USA from July-August 2019-2020.

	Carolina Sandhills	Fort Jackson	Savannah River Site	Enoree NF	Liberty Hill	Long Cane NF	Davis Land and Timber	Marsh/ Woodbury WMAs	Webb Groton Westervelt	Bonneau Ferry WMA
Carolina Sandhills	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------
Fort Jackson	0.024*	----------	----------	----------	----------	----------	----------	----------	----------	----------
Savannah River Site	0.029*	0.025*	----------	----------	----------	----------	----------	----------	----------	----------
Enoree NF	0.012	0.028	0.020	----------	----------	----------	----------	----------	----------	----------
Liberty Hill	0.010	0.020	0.028	0.018	----------	----------	----------	----------	----------	----------
Long Cane NF	0.026*	0.027	0.026*	0.040	0.021	----------	----------	----------	----------	----------
Davis Land and Timber	0.017	0.036*	0.029*	0.032	0.035	0.040*	----------	----------	----------	----------
Marsh/Woodbury WMAs	0.015	0.023	0.025	0.029	0.015	0.031	0.014	----------	----------	----------
Webb Complex	0.038*	0.028	0.016	0.022	0.019	0.033*	0.042*	0.019	----------	----------
Bonneau Ferry WMA	0.025	0.041	0.039*	0.030	0.040	0.066*	0.050*	0.038	0.054	----------

*Significant with a Bonferroni correction of $p \leq 0.001$ for multiple comparisons

Table 5.3. Analysis of molecular variance (AMOVA) comparing genetic variation of coyotes (*Canis latrans*) from 10 study sites across three ecoregions in South Carolina, USA from July-August 2019-2020.

Source of variation	d.f.	Sum of squares	Variance of components	Percentage of variation
Among ecoregions	2	16.07	-0.02	-0.38
Among study sites within ecoregions	7	57.57	0.12	3.08
Within study sites	398	1568.41	3.94	97.30
Total	407	1642.05	4.05	

Table 5.4. Three clusters of coyote (*Canis latrans*) individuals across 10 study sites in South Carolina, USA from July-August 2019-2020, assessed using Discriminate Analysis of Principle Components (DAPC).

	DAPC Cluster		
Site	1	2	3
Carolina Sandhills	9	11	5
Enoree NF	2	2	3
Fort Jackson	6	4	8
Liberty Hill	4	2	3
Long Cane NF	5	5	8
Marsh/Woodbury WMAs	0	3	1
Savannah River Site	27	14	28
Davis Land and Timber	5	22	5
Webb Complex	1	3	10
Bonneau Ferry WMA	6	0	2

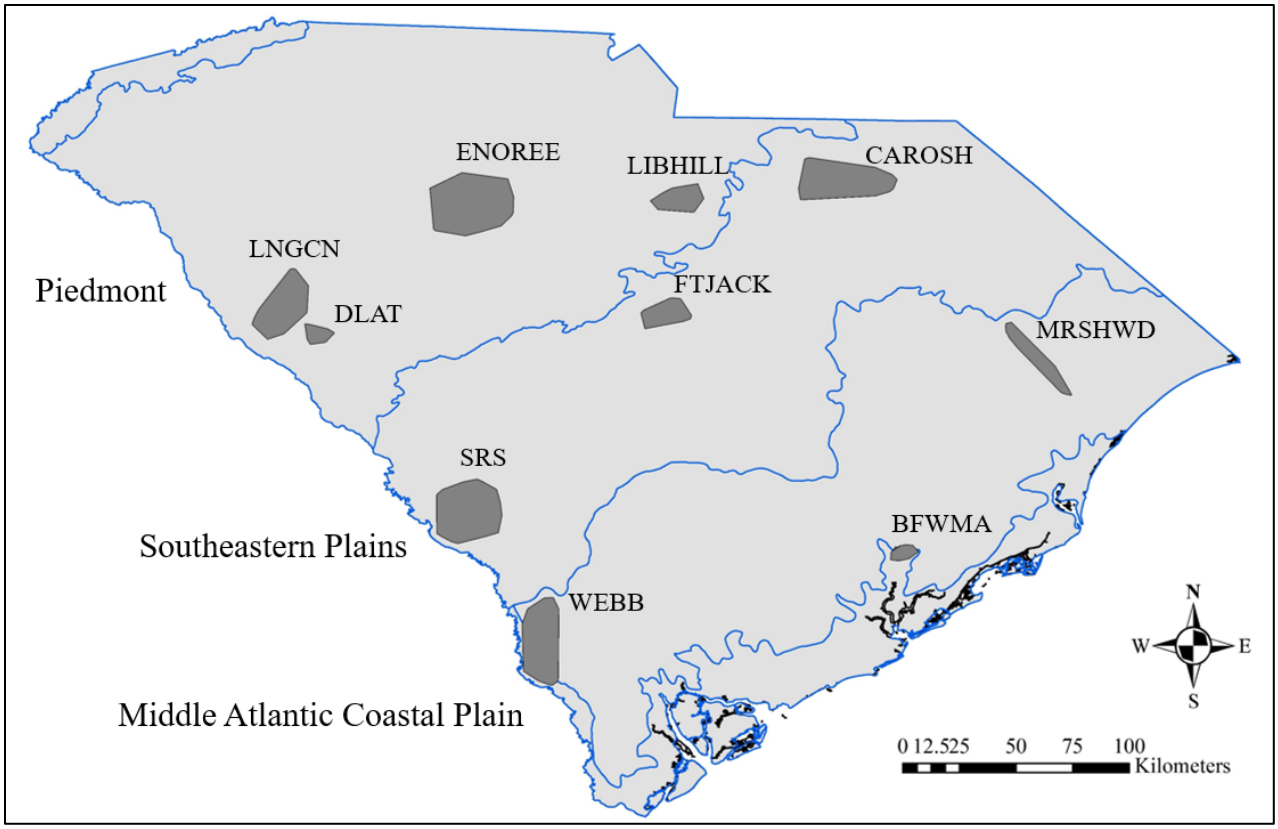

Figure 5.1. Study sites across South Carolina, USA used to assess coyote (*Canis latrans*) population genetics across three major ecoregions during July-August 2019-2020. LNGCN, Long Cane Ranger District Sumter National Forest; DLAT, Davis Land and Timber; ENOREE, Enoree Ranger District Sumter National Forest; LIBHILL, Liberty Hill Wildlife Management Area and private lands; SRS, Savannah River Site; FTJACK, Fort Jackson; CAROSH, Carolina Sandhills National Wildlife Refuge and State Forest; WEBB, Webb Complex; BFWMA, Bonneau Ferry Wildlife Management Area; MRSHWD, Marsh and Woodbury Wildlife Management Areas.

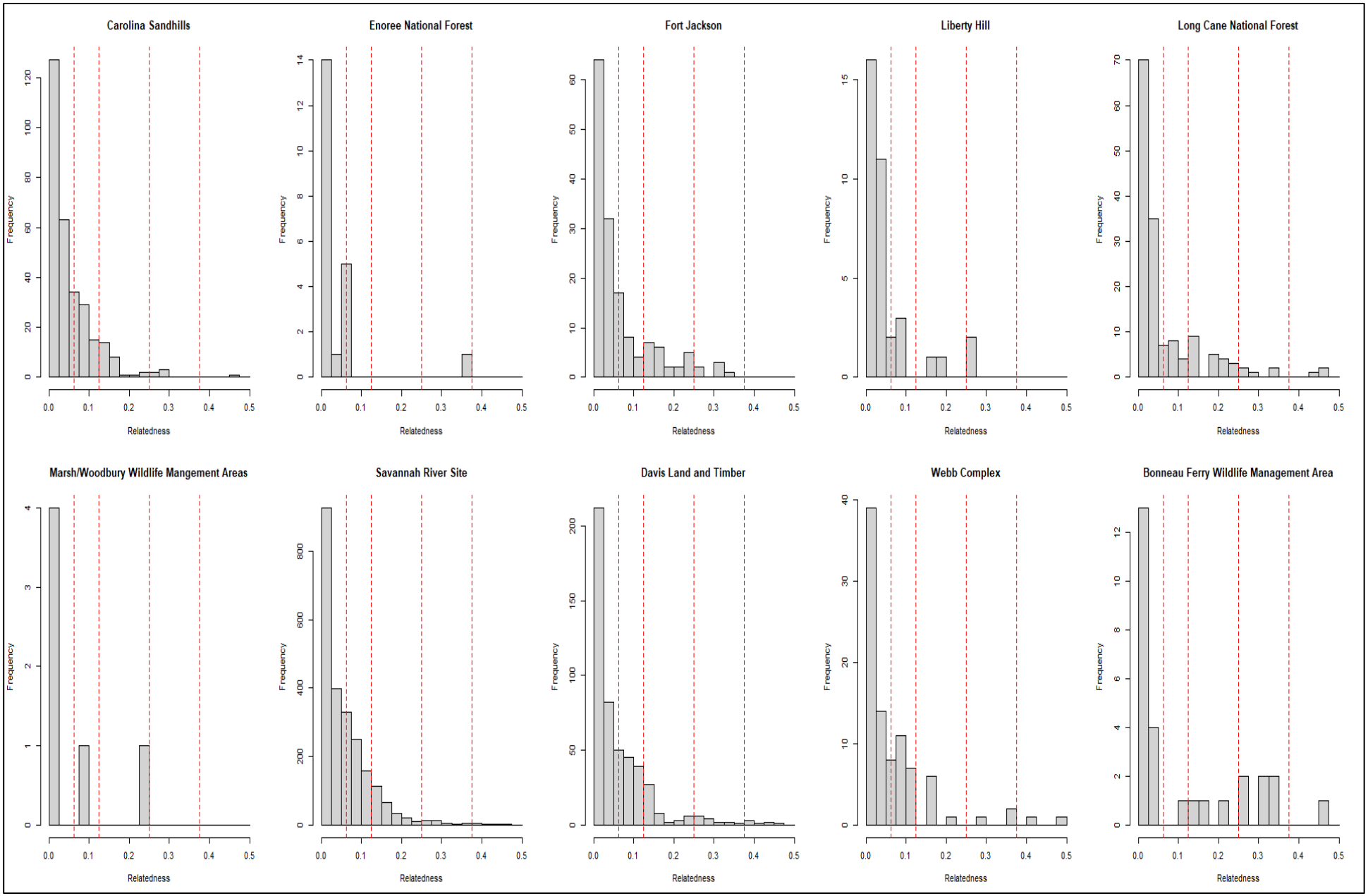
Carolina Sandhills
Enoree National Forest
Fort Jackson
Liberty Hill
Long Cane National Forest
Marsh/Woodbury Wildlife Mangement Areas
Savannah River Site
Davis Land and Timber
Webb Complex
Bonneau Ferry Wildlife Management Area
Frequency
Relatedness

Figure 5.2. Pairwise estimates of relatedness among coyotes (*Canis latrans*) at 10 study sites across South Carolina, USA from July-August 2019-2020. Vertical dotted lines indicate threshold values for unrelated individuals ($r = 0$), full cousins ($r = 0.0625$), half siblings ($r = 0.125$), full siblings or parent/offspring ($r = 0.25$), and full siblings whose parents are also full siblings ($r = 0.375$).

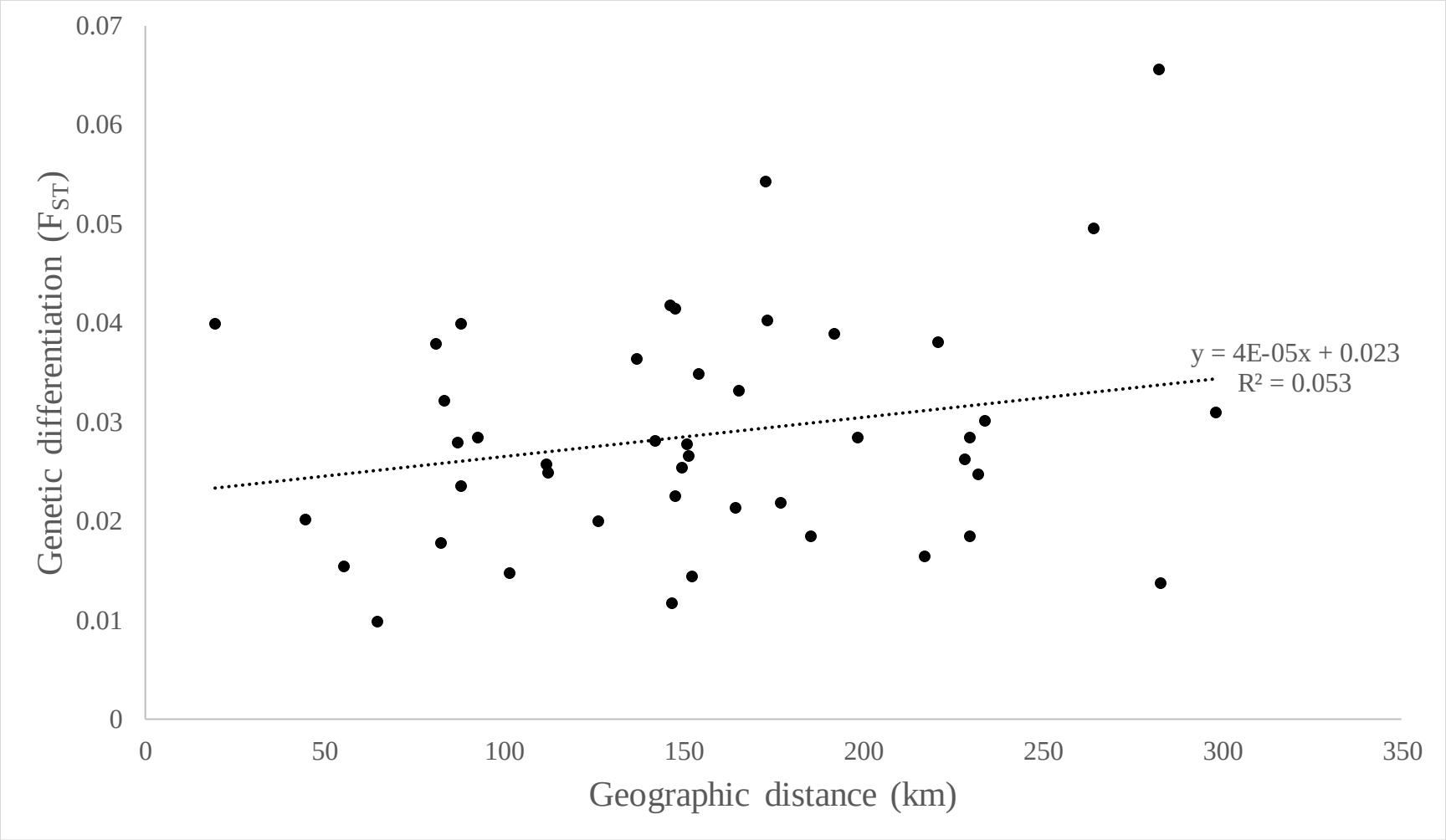

Figure 5.3. Genetic isolation by distance showing the correlation between geographic distance and genetic differentiation among coyotes sampled at 10 study sites in 2019-2020 across South Carolina, USA.

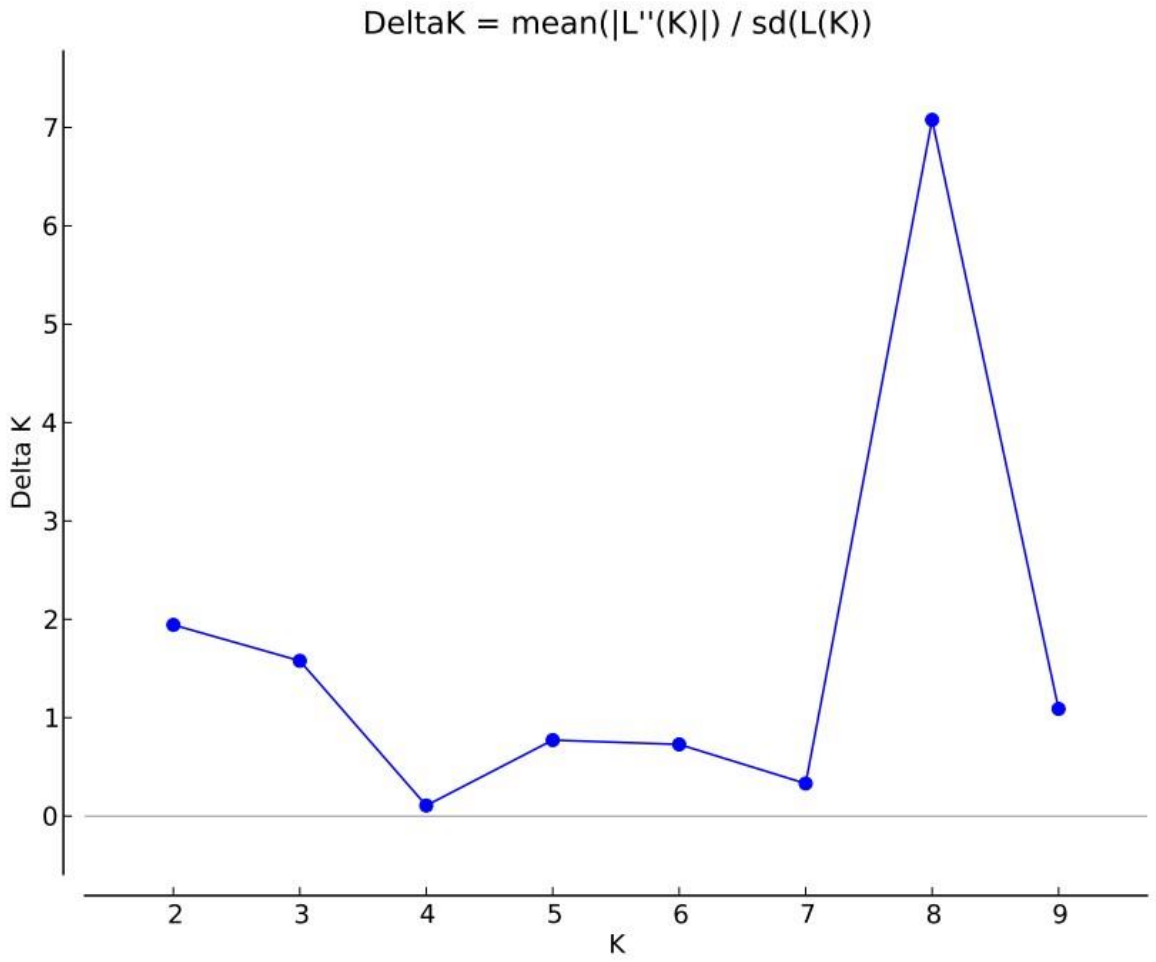

Figure 5.4. Delta K estimates for best fit of purported clusters in STRUCTURE's Bayesian clustering among coyotes (*Canis latrans*) sampled from 10 study sites in South Carolina, USA.

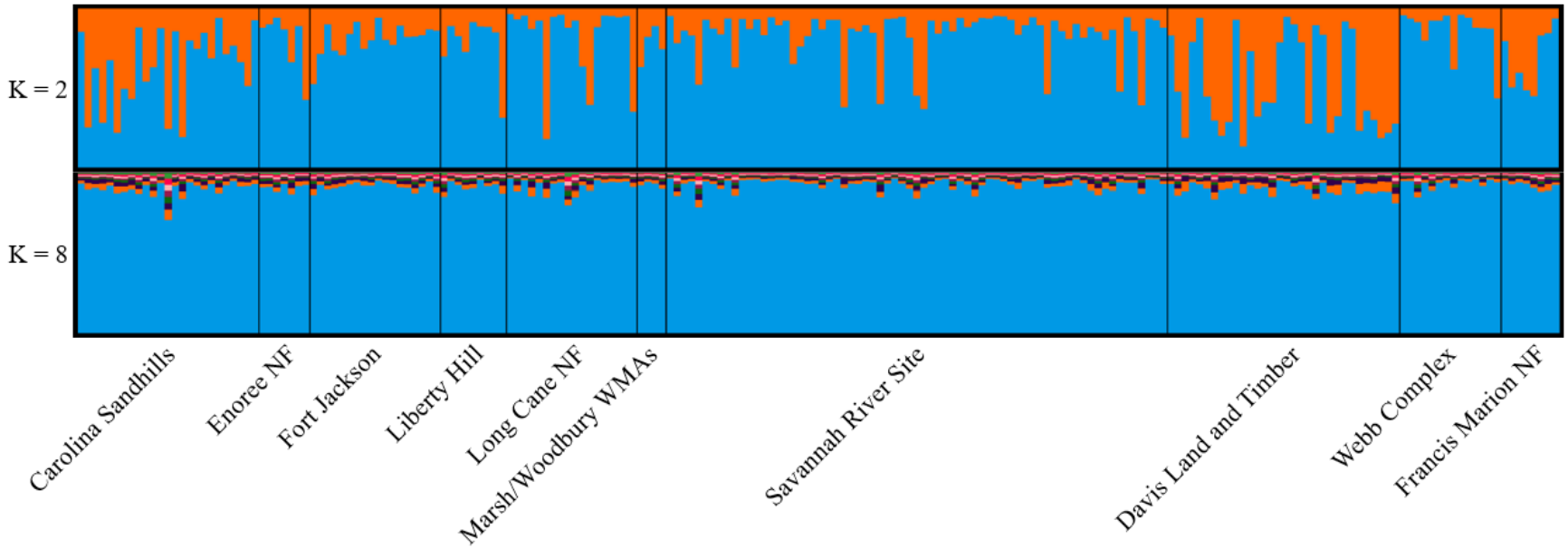

Figure 5.5. Estimates of ancestry coefficients among coyote (*Canis latrans*) individuals from 10 study sites sampled across South Carolina, USA from July-August 2019-2020 using the program STRUCTURE. Estimates are broken into 2 purported groups (K = 2, top) and 8 purported groups (K = 8, bottom) as indicated by a best fit of Evanno's delta K. Vertical bars represent individual coyotes, while colors indicate purported ancestry groups.

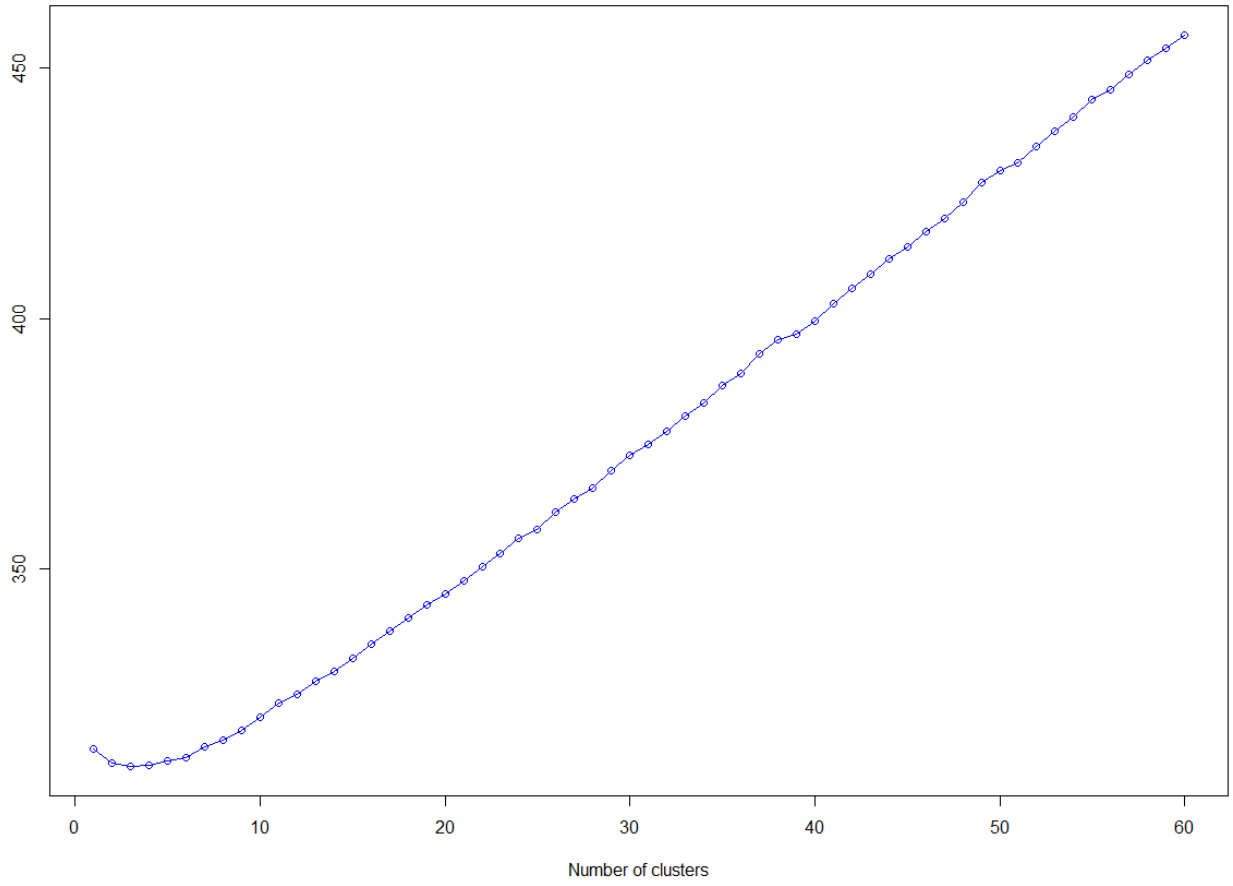

Figure 5.6. Bayesian Information Criterion (BIC) used to determine the best fit for number of groups of coyotes (*Canis latrans*) sampled across 10 study sites in South Carolina, USA from July-August 2019-2020. Best fit is estimated as nearest to the elbow of the BIC curve.

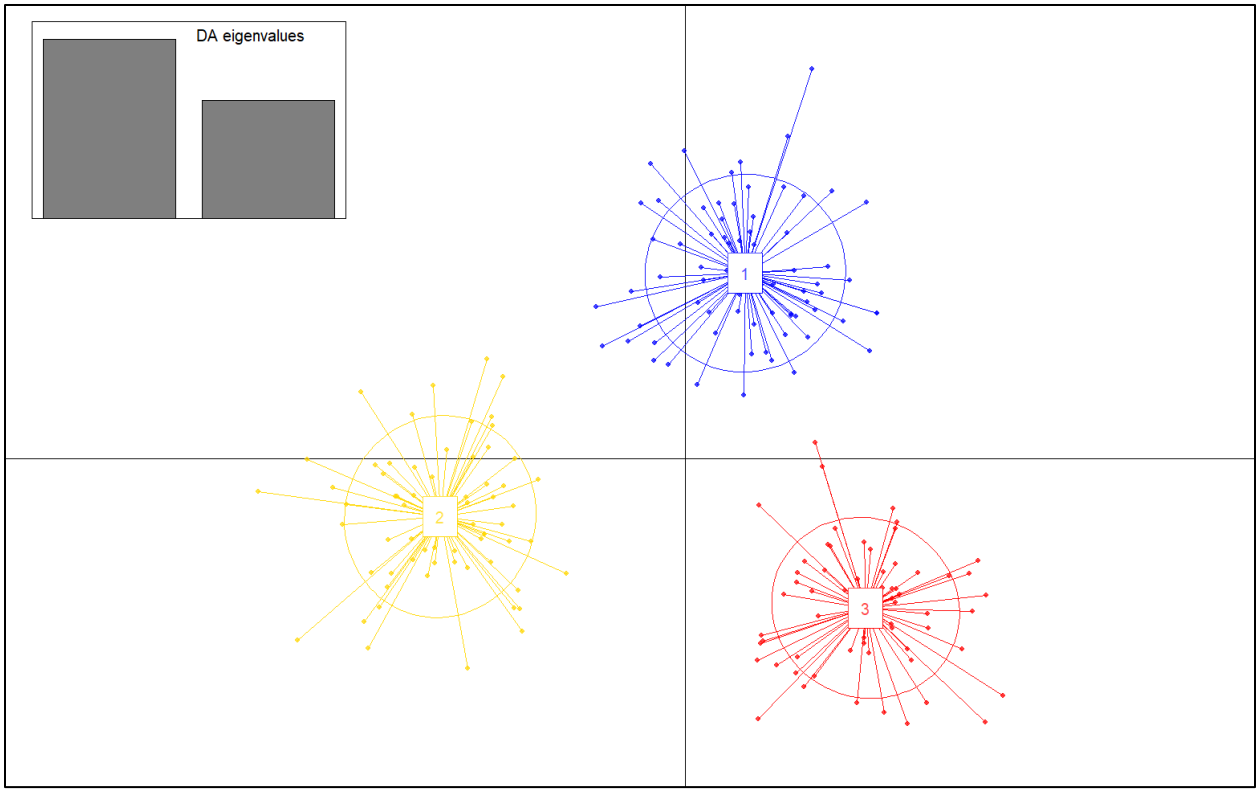

Figure 5.7. Three clusters of coyote (*Canis latrans*) individuals across 10 study sites in South Carolina, USA from July-August 2019-2020, assessed using Discriminate Analysis of Principle Components (DAPC).

CHAPTER 6

CONCLUSIONS AND MANAGEMENT IMPLICATIONS

Coyotes have become ubiquitous across the landscape of the southeastern USA following rapid immigration over the last half century. Previous research has attempted to contextualize how coyotes impact native ecosystems and the potential detrimental effects on sympatric predators and game species. However, studies have mostly evaluated coyote populations either at a broad scale (ie., genetic studies across the eastern seaboard) or at a local scale (<1000km^2 and/or <3 sites). Although resident coyotes maintain stable territories, approximately 30% of individuals in the southeastern USA exhibit transient behavior, often traveling long distances in search of available resources to establish a home range. Furthermore, heterogeneous landcover should impact coyote density through resource availability, space, and cover. Therefore, coyote populations may operate at a spatial scale intermediate to previous studies. I assessed coyotes across South Carolina, USA at 10 study sites ranging from 300 – 1200 km^2 to evaluate coyote density, foraging behavior, diet, and genetic structure.

In Chapter 2, I found that coyote populations exhibited a wide range of densities across South Carolina even between geographically proximate study sites. Density was positively associated with open/early successional land cover, likely due to the increased availability of prey in those habitat types. However, forest and agricultural landcover types did not significantly explain variation in coyote density. Due to the relationship between density-dependence and dispersal in coyote populations, forest and agricultural landscapes may serve as pseudo-sinks and open/early successional landcover as sources. Therefore, future research should assess whether

coyote source-sink dynamics may contribute to dispersal across a heterogeneous landscape. Management of coyotes in the eastern USA may be difficult due to the interplay between high- and low-density areas, resulting in rapid colonization by coyotes of exploited areas. I recommend further research on demographic rates such as survival and mortality, immigration and emigration, and fecundity between purported source and sink patches.

In Chapter 3, I investigated foraging behavior of coyotes at sites across Alabama, Georgia, and South Carolina using GPS collars. Using recursive analysis, I identified purported foraging areas within pack home ranges where individuals repeatedly returned to known locations during nighttime movements. Similar to previous literature, I found that resident coyotes selected for open landcover and avoided primary and secondary roads. Additionally, resident coyotes avoided forest cover except during the spring when they shifted selection into interior forest. I posit that coyotes may be shifting their selection into forest during the fawning period of white-tailed deer in order to capitalize on a significant resource pulse. Additionally, previous literature has hypothesized that coyotes scavenge deer carcasses to obtain the persistent levels of deer found in a myriad of diet studies. However, I show that resident coyotes are not selecting for primary and secondary roads during foraging bouts, making scavenging an implausible explanation for deer consumption. Continued research is required to understand whether coyotes in the southeastern USA are actively predating on juvenile and adult deer year-round.

In Chapter 4, I assessed vertebrate diets of coyotes, bobcats, and gray foxes during white-tailed deer fawning and wild turkey nesting and brood rearing using the novel approach of DNA metabarcoding. I found that coyotes and bobcats consumed deer, with deer occurring at high frequencies in coyote scat, and all three mesopredators consumed turkeys, albeit at low

frequencies. Specialization in vertebrate diet resulted in mesopredators selecting different prey, perhaps minimizing dietary overlap. Although it is clear that coyotes heavily predate on deer during peak fawning, there is minimal evidence that coyotes, bobcats, or gray fox rely on turkeys during the same time period. My findings support previous literature showing that it is unlikely that coyote predation is a significant factor in turkey mortality during nesting and brood rearing.

Finally, in Chapter 5, I assessed population genetics across coyote populations in South Carolina to determine whether there were ecoregion-level clines in genetic structure and to assess the scale at which dispersal may influence geneflow among coyotes across the state. I found high levels genetic diversity across sites but low levels of relatedness, similar to previous studies. I observed negligible genetic differentiation within and among ecoregions and insignificant isolation by distance. My findings indicate the coyote population genetics in South Carolina are panmictic, likely maintained through dispersal by transient individuals at a large geographic scale.

My research corroborates previous literature showing that coyotes in the southeastern USA exhibit plastic behavior, including prey-switching in response to seasonal changes in resource availability, dispersal that facilitates large-scale geneflow, and a mosaic of population densities in relation to landcover. Future research and management of coyotes in the heterogeneous landscapes of the southeastern USA should account for the geographic scale that populations encompass. It is unlikely that localized management will have a substantive effect on mitigating the impacts of coyote populations. Instead, where possible, I recommend using management techniques designed to increase fecundity, neonate survival, and recruitment of game species of interest.