UNIVERSITY OF CENTRAL OKLAHOMA
Edmond, Oklahoma
Jackson College of Graduate Studies

Observations on the Genetic Diversity of Bobcat (*Lynx rufus*) Populations in Oklahoma

A THESIS
SUBMITTED TO THE GRADUATE FACULTY
In partial fulfillment of the requirements
for the degree
MASTERS OF SCIENCE IN BIOLOGY

By
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Edmond, Oklahoma
2022

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Acknowledgments

Funding for this research was provided by the University of Central Oklahoma (UCO) Office of Research and Sponsored Programs RCSA grant; Oklahoma Department of Wildlife (ODWC), Wildlife Restoration Grant; and the UCO College of Mathematics and Science Dean's Molecular Fund. I would like to thank J. Davis (ODWC) for obtaining the tongue samples, Dr. W. S. Fairbanks (Oklahoma State University) for providing tissue subsamples, and C. Smith, T. Gray, C. Jennings, K. Patatanian, and R. Frank for assistance in the lab. I would like to thank Dr. Michelle Haynie for serving as my academic advisor during this project and for providing advice and support during my time at UCO. I would also like to thank Dr. Victoria Jackson, Dr. Allyson Fenwick, and Dr. Christopher Butler for serving on my committee and providing advice during my academic career. I would also like to thank my mom and my brother, Michael, for supporting me during my time at UCO, as well as Dr. Daniel Brooks, for inspiring me to pursue a Master's degree.

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Abstract

Bobcats (*Lynx rufus*) have been recorded in a majority of counties within the state of Oklahoma, and are regularly harvested for their pelts statewide. There have been a limited number of studies on bobcats in Oklahoma due to annual hunting seasons, human alterations to the environment, and regular long-distance dispersals of male bobcats, questions have been raised regarding the genetic diversity and structure of the population in the state. To better understand the current genetic diversity of Oklahoma's bobcats, tongue samples from harvested individuals were collected by the Oklahoma Department of Wildlife Conservation and deposited in laboratories at the University of Central Oklahoma and Oklahoma State University. I extracted DNA from tissue samples collected across 25 counties and performed fragment analyses using 10 microsatellite loci, plus a sex-determining locus. This allowed me to assess the current genetic structure of bobcats within the state, as well as levels of genetic diversity. My data shows that there is currently a high level of heterozygosity across the state, representing a sustainable level of genetic diversity. Results of genetic structure analyses indicate that there is a single population distributed across the state, allowing for the maintenance of genetic diversity through high levels of gene flow.

Chapter 1

Background: Natural History & Conservation of Bobcats

Bobcats (*Lynx rufus*) are mesocarnivores, averaging 869 mm in total body length for males, and 786 mm in females (Jackson, 1961; Sikes and Kennedy, 1992). This sexual dimorphism is also seen in the tail (148 mm for males, 137 mm for females), hind foot (170 mm in males, 155 mm in females), and ear lengths (66 mm in males, 63 in females), with the body weight ranging from 6.8 kg in females to 9.6 kg in males. Their fur is short, dense, and soft (McCord and Cardoza, 1982). Coloration is generally yellowish to reddish brown, with black spots and black tipped guard hairs across the body. The venter is cream colored with black spots, the forelegs are tawny with black bars, and the face contains a black nose pad, white vibrissae, and black striped ruff and forehead. The dorsal surface of the ear is black with a white spot in its center and tufts of hair on their tips, and two tuffs of vibrissae are found on each side of the head (Banfield, 1987). Coloration is variable, depending on habitat, with lighter fur within desert or arid environments, and darker fur in more forested areas (Cahalane, 2005). Bobcats are related to other medium-sized cats within the Americas (Canadian lynx, *L. canadensis*), as well as in Europe and Asia (Iberian lynx, *L. pardinus*; Eurasian lynx, *L. lynx*), but are genetically and morphologically distinct from lynxes (Johnson et al., 2006). Bobcats can live up to 15.5 years in the wild on average, although significantly longer lifespans have been recorded in captivity (Feldhamer et al., 2004). The number of recognized subspecies has changed over time, with 11 subspecies being described in the 20th century. In 2017, bobcat taxonomy was revised following genetic research, and currently only two subspecies are recognized, *Lynx rufus rufus* (found east of the Great Plains) and *Lynx rufus fasciatus* (found west of the Great Plains) (Kitchener et al., 2017).

Bobcats are fairly widespread within the United States, having known populations in nearly all of the contiguous 48 states with the exception of Delaware (Gooliaff et al., 2018). They have not migrated into Alaska due to natural barriers, nor have they been introduced to Hawaii or other American territories (Applegate and Bahrt, 1993). There are populations in southern Canada and northern Mexico, although various natural factors have limited their expansion north or south (Larivière and Walton, 1997). Historically, bobcats have been widespread and abundant throughout their range, although they were extirpated from Midwestern and north Atlantic regions during the previous century because they were considered a threat to agricultural activities (Woolf and Hubert, 1998). Over recent decades, bobcats have recovered their range in this portion of the country (Popescu et al., 2021). Historic populations along the east coast of the US were also reduced due to human development, and while bobcats have returned to this region, they have not been widely recorded within sections of the Eastern Seaboard (Rose et al., 2020).

Bobcats have been recorded in a wide variety of habitats across their full continental range, including forests, grasslands, shrublands, and deserts, but are not limited to these environments (Conner and Leopold, 1996). This generalist behavior and ability to inhabit different ecosystems has aided the species by allowing a greater geographic distribution than habitat specialists such as ocelots (*Leopardus pardalis*; Anderson and Lovallo, 2003). In spite of this adaptability, bobcats prefer woodlands, including deciduous, coniferous, and mixed forests (Fuller et al., 1985a). However, deserts, grasslands, shrublands, and swamps provide enough resources and space to support bobcat populations. The habitat must be large enough to allow female bobcats to establish a home range, marked by scent, to aid in the care of offspring through availability of cover for den maintenance and enough prey for both the female and juveniles to sustain themselves (Preuss and Gehrig, 2008). Males and females prefer different habitats during different seasons, with adult bobcats moving to higher elevations during warmer summer months and returning to lower elevations during colder winter months in regions with hilly or mountainous terrain (Koehler and Hornocker, 1989). They also are more selective of habitat type in winter. Bobcats are similar to other large and medium sized cat species in that they are generally solitary animals, with the exception of females caring for a litter and during the reproductive season. An average sex ratio of adults is 1:1, although variation in sex ratio is known to occur in different habitats due to climate and resource availability (Foote, 1945).

Bobcats often are a keystone species in their environments, and serve as a significant predator for several mammal and bird species (Tewes et al., 2002; Rose and Prange, 2015). Bobcats have a varied carnivorous diet, being able to hunt and consume small and medium prey, such as rodents, rabbits, and birds (Labisky and Boulay, 1998; McKinney and Smith, 2007). They also consume the occasional large prey in the form of deer and pronghorn, either as carrion or by taking down juveniles, though there have been records of adult deer be successfully killed. Commonly recorded prey items are lagomorphs, followed by large rodents such as muskrats (*Ondatra zibethicus*) and woodchucks (*Marmota monax*), with smaller rodents and ground dwelling mammals being consumed on an opportunistic basis (Jones and Smith, 1979; Biggins and Biggins, 2006). The consumption of larger mammals such as ungulates depends on the size of the bobcat and the presence of smaller prey that may be easier to capture, as well as the season. A majority of records of bobcats actively hunting deer occur during winter, possibly due to the limited number of smaller prey items (Delibes and Hiraldo, 1987). The most common birds consumed by bobcats are Galliformes, while other bird groups such as Passeriformes and Gruiformes are consumed less regularly (Fritts and Sealander, 1978a). There have been records of bobcats consuming other carnivores such as red foxes (*Vulpes vulpes*) and striped skunks (*Mephitis mephitis*), although far more infrequently than the consumption of herbivores (Hamilton and Hunter, 1939; Witmer and DeCalesta, 1986). Bobcats will also scavenge on carrion and bird eggs as well as the occasional fish, amphibian, or reptile depending on the availability of other food (Beasom and Moore, 1977). Adult bobcats are solitary hunters, with almost no record of them hunting in groups even when attempting to take down larger prey (Kautz et al., 2019).

The diverse diet has also assisted in the bobcat's ability to inhabit a wide variety of habitats and avoid competing with other mesocarnivores and larger carnivores within North America, which can include areas that have been heavily altered by humans (Lewis et al., 2015). However, bobcats will compete with other carnivores, such as coyotes (*Canis latrans*) and red foxes, for food and territory (Peers et al., 2013). These species as well as larger carnivores, such as grey wolves (*C. lupus*) and mountain lions (*Puma concolor*), might kill bobcats either as prey or to remove them from a territory (Hamilton and Hunter, 1939; Gipson and Kamler, 2002). Females and juveniles are particularly vulnerable to being killed (Gashwiler et al., 1961). Despite having a fairly wide diet, it is more specialized towards smaller prey due to the bobcat's size, allowing them to avoid most direct competition with larger predators, and their fur coloration and smaller size make it easier for the bobcats to avoid larger animals through camouflage (López-Vidal et al., 2014). Adults might defend their territory or dens from predators, although this is less common than attempting to avoid other predators (Allen et al., 2016). They have also been recorded climbing trees and entering bodies of water to avoid predators, which in turn limits the risk of injury (Wilson et al., 2010). Bobcats are mainly nocturnal, with peak activity at 1800 to 2400 hours, and 0400 to 1000 hours (Kirby et al., 2010). They are also less active during midday, typically spending this time in their dens. Bobcats can serve as a host to a variety of endoparasites and ectoparasites, such as nematodes, trematodes, and helminths, as well as viruses and bacteria such as rabies and pneumonia (Progulske, 1952).

Females' home ranges tend to be smaller than those of males, but tend to be more permanent and exclusive to a female and her offspring, at least until the offspring reach sexual maturity. Male home ranges tend to be larger in size and overlap with those of other males and females (Pollack, 1950). Bobcats are able to move through most environments, thus increasing access to potential territories (Millions and Swanson, 2007). Mountainous environments serve as a formidable barrier, because while bobcats can cross mountain ranges, it takes more time and can be more hazardous due to steepness and temperature, increasing chances of mortality (Crowe, 1975). The presence of mountain ranges can result in the long-term separation of populations, and thus formation of genetically unique groups (Koscinski et al., 2009). Dense forests and jungles, as well as tundra, also are barriers to bobcats (McCord, 1974a). Dense forested habitats, although home to a variety of prey species, limit the movement of bobcats due to the volume of plant material. Within the United States, this can result in the development of genetically unique populations, while in Central America it serves to limit the overall range of the species, as bobcats have not been reported outside of Mexico (Sánchez-Cordero et al., 2008).

Bobcats reach sexual maturity around their second year, although some females have been reported to reproduce during their first year (Johnson and Holloran, 1985). Adult males are considered to be sexually mature based on their body mass rather than their age, with larger males able to reproduce sooner than smaller ones (Fritts and Sealander, 1978b). Males also show variation in sperm production depending on the season, with more sperm being formed in September or October through early summer, and late summer and early fall having lower sperm counts (Gañán et al., 2009). Larger and older males are less affected by this than younger males due to their greater overall body size and the greater testicular volume (Winegarner and Winegarner, 1982). Bobcats are sexually active from maturity until their death (Pollack, 1950).

The reproductive season for bobcats generally begins around February or March and ends around July, although variation is present in different latitudes and longitudes due to differences in climate (Duke, 1954). Males will enter a female's range during the reproductive season and will perform various behaviors with a female, such as bumping or chasing. Males also perform vocalizations, such as hissing and screaming, to evaluate the female's interest in mating (McCord, 1974b). Males will generally enter a female's range if the female has presented signals that she is in estrous through scent markings, which are essential because the estrous cycle only lasts a few days (Mehrer, 1975). If the female is receptive, the male will grasp her neck in his mouth and begin copulation, and the two may copulate multiple times during the day (Mellen, 1991). Once the male has completed mating, he will move into another territory. Because both males and females mate with multiple individuals, pairings are not maintained after sexual intercourse (Gashwiler et al., 1961). Having multiple copulation partners helps to ensure that males produce multiple offspring and pass on their genetic material, and females receive the most optimal DNA allowing for the development of healthy offspring (Mehrer, 1975). Females typically choose which males they copulate with and there are limited reports of forced copulations (McNitt et al., 2020).

After a successful impregnation, the female will enter a natal den to give birth following a gestation period of approximately 63 days (Hemmer, 1976). These dens are typically dry, hidden, and difficult for other predators to access to improve chances of the offspring's survival, and are commonly found in rocky areas and caves, although bobcats have occasionally been recorded utilizing abandoned beaver dens (Zezulak and Schwab, 1979). There are records of bobcats making use of abandoned human structures for natal dens, such as storage sheds and buildings (Bailey, 1979). A natal den will be used several times in the bobcat's life, especially if it is of good quality, and the bobcat will also maintain a series of auxiliary dens while rearing litters (Bailey, 1979). Auxiliary dens do not typically offer the same protection as a natal den, but do provide sanctuary during times when the natal den may not be accessible, such as during severe weather that force the litter from the natal den (Rollings, 1945). All bobcats maintain resting sites within their territory which serve as temporary shelters while moving across their range (Bailey, 1974).

Female bobcats will typically give birth to a litter of 1 to 6 individuals, averaging about 2 offspring per litter (Johnson and Holloran, 1985). Newborn bobcats weigh between 280 and 340 grams, and are born blind and will not gain sight until after a period of 3 to 11 days (Young, 1978). Offspring will nurse for at least 2 months after birth, and may begin to eat solid foods, along with nursing, 4 months after birth (Young, 1978). Deciduous dentition will begin to emerge after 11 to 14 days, and teeth will have fully emerged after 9 weeks, with permanent dentition emerging at 16 to 19 weeks of life and being fully developed at 34 weeks (Winegarner and Winegarner, 1982). Young bobcats will begin to follow their mother around her range at about 3 months, and will begin to travel alone at 6 months, although they will typically remain close to the den site (Kitchings and Story, 1984). Juvenile bobcats typically hunt their own prey in autumn of their first year of life, and will begin to disperse from their mother's range before the next litter is born (Kamler et al., 2000).

Subadult bobcats will begin to move from their mother's range at about 1 year of age and establish a home range of their own (Kitchings and Story, 1984). They follow natural and human-made trails, such as roads, cliffs, and ditches to travel across territories (McCord, 1974a). Home ranges must contain enough space to move, enough prey and, if it is a female, locations for dens and resting sites, including brush and rock piles (Nielson and Woolf, 2002). This range must also be free of other bobcats (Plowman et al., 2006). Once a home range has been located, a bobcat will identify it as their territory by using a variety of scent markers, including urine, feces, scrapes on plants, and anal gland secretions (Bailey, 1974). A female's range is about 9.7 km in size, with males inhabiting 97.6 km, with the difference in size relating to the female maintaining a permanent range while the male is more transient (Miller and Speake, 1979). Adult bobcats will move on average between 1 to 7 km per day to locate food, travel between rest sites, look for potential mates in the case of males, and disperse to find new home ranges (Knowles, 1985). Home range size depends on sufficient availability of prey, and may grow or shrink due to fluctuations in prey populations, as well as during reproductive seasons (Litvaitis et al., 1986). If changes in the environment prevent movement of males, the pressure on bobcat populations to maintain gene flow and population recruitment will increase, and make the continued presence of the species in the region more difficult. Bobcats might migrate out of habitats that cannot support them with either food or cover, and a small enough population with either too few males or females may die off. This could have cascading effects within a habitat, as the removal of a major predator can alter the presence of plants and animals within an environment (Kauhala et al., 1999).

Bobcats are sought after by fur trappers and sport hunters for their high value pelts, making this species a significant furbearer in states in which it is found (Tumlison and McDaniel, 1986). Historically, bobcats have been hunted by humans for their fur rather than as a source of food, because they do not provide as much sustenance compared to larger mammals, while the fur has been used for the creation of clothing due to its ability to retain heat (McGee, 1987). Various indigenous nations used bobcat pelts for day-to-day clothes, as well as decorative and practical ornaments. For example, tribes in the lower Columbian River Region of North America use bobcats to create robes and quivers (McGee, 1987). European settlers and fur trappers initially focused on other mammals due to their size and quality of fur, such as otters (*Lontra canadensis*), minks (*Neogale vison*), fishers (*Pekania pennanti*), and beavers (*Castor canadensis*; Ray, 1987). Over time, as populations of other furbearers began to decline, and due to the fur trade being one of the major industries in the American colonies, fur trappers began to expand both the range of trapping and the number of species that were harvested (Ray, 1987). An estimated 2,655,000 bobcats were harvested for their fur in the 19th century, and although the number of bobcats hunted annually was reduced in the 20th century, bobcat pelts are still sought after, with legal harvests being allowed in 38 states (Obbard et al., 1987). Harvest levels fluctuate over time, (e.g., 46,000 pelts in the 1960s, and 114,000 pelts in the 1980s), as do the price of the pelts, going from $203 in 2003, to $150 in 2021 (Obbard et al., 1987). Trade in pelts is still a source of income for fur trappers, although the fur market has shrunk in recent years due to a reduction in the number of trappers (Lavoie et al., 2009). It is unknown how annual harvests affect the genetic and population health of bobcats, as records and research on these are limited.

One of the greater threats to bobcats is removal of individuals for carnivore control to limit effects of predation on domestic livestock, such as sheep, goats, and poultry. Bobcats may hunt domestic animals due to the concentration of prey items on farm and ranch land. Agriculture provides an easier source of food especially when natural food availability is low, with lower risks to the health of the bobcat (Young, 1978). To limit the possible consumption of domestic animals, a variety of methods have been used to support the removal of predators, including a bounty system, although this has largely been phased out (Young, 1978). Current practices include trapping with the use of snares or gripping traps such as legholds and body grippers, cages, shooting, and laying out poison traps for bobcats to consume (Hansen, 2007). Non-lethal methods to prevent the loss of livestock to bobcat predation have also been shown to be effective, including the installation of newer fencing and sheds, as well as practicing night penning, maintaining larger herds, and the greater use of guard dogs to ward off predators (Hansen, 2007). Programs to compensate farmers and ranchers for lost livestock have been considered and partially implemented (e.g., Livestock Assistance Program, Livestock Compensation Program, and Livestock Indemnity Program, which cover for animals lost due to natural disasters and predation), with the goal of reducing removal of carnivores by providing financial assistance for animals killed, although currently they are limited in scope and funding (US Department of Agriculture (USDA), 2014).

Bobcats are also under pressure from human development across their continental range. Property that has been altered to support cropland inhibits the bobcat's ability to both move across and inhabit the area due to a reduction in available prey and cover (Lesmeister et al., 2015; Lewis et al., 2015). Bobcats have been sighted in suburban and urban environments, although usually in areas of mixed development or locations with easy access to cover, such as large parks or undeveloped lots (Harrison, 1998). More heavily developed areas, such as large city centers, tend to have low or no bobcat populations resulting from rarity of prey and shelter, as well as the greater presence of other predators, such as domestic dogs (Ordeñana et al., 2010). The greater use of roadways within urban areas, as well as highways across North America, are a significant cause of mortality for bobcats as they are moving across their range or attempting to establish new ranges (Lovallo and Anderson, 1996). Vehicular impacts can kill bobcats almost instantly, making it harder for a habitat to support a stable bobcat population, and could prevent bobcats from inhabiting more developed areas (Foster and Humphrey, 1995). There have been efforts to reduce wildlife deaths in developed regions, including the use of vegetation barriers around cropland and the installation of wildlife crossings over or under major roadways, which allow bobcats to safely move across human-made barriers and reduce lethal interactions between humans and bobcats (McCollister and Van Manen, 2010).

Currently, bobcats are considered to be a species of Least Concern by the International Union of Conservation of Nature (IUCN), although obtaining accurate counts has been difficult due to the bobcat's solitary nature and their preference for habitats with dense cover (Kelly et al., 2016). Additionally, there are factors that could affect the overall health of bobcat populations, ranging from habitat loss to the creation of artificial barriers, which increase the possibility of inbreeding due to a loss of individuals and restricted breeding populations over time (Smith et al., 1991). Harvest of bobcats is typically handled by state wildlife agencies, as well as the US Fish and Wildlife Service (USFWS), which also manages federal wildlife management policies. Predator control is managed by the Wildlife Services arm of the USDA, which also manages compensation programs. The use and export of bobcat pelts is regulated by the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) and the Lacy Act, which puts limits on the sale and import of game material, and forbids the sale of animal products from threatened and illegally harvested animals, although enforcement can be difficult. Although bobcats are considered to be of Least Concern, as stated earlier, there are concerns that certain groups or subspecies may be threatened or even endangered, such as populations reintroduced to Cumberland Island, Georgia (Diefenbach et al., 2006). In those cases, the subspecies would be protected by the Endangered Species Act (ESA), although more genetic and population research is required to determine both the taxonomic status of a population and the number of individuals within it. Monitoring the activity and genetic health of populations can aid in improving land management and sporting practices and policies, mitigating possible negative effects of human activity and allowing the continued presence of bobcats within their range (Bradley and Fagre, 1988).

Chapter 2

Introduction

Bobcats (*Lynx rufus*) have a wide distribution across North America, being found in Canada, Mexico, and the United States (Lawhead, 1984; Hansen, 2007). Their presence within the United States is well documented, and currently populations are found within all of the contiguous 48 states with the exception of Delaware (Woolf and Hubert, 1998). Bobcats are harvested on an annual basis throughout most of their range for their fur, which may have an effect on the genetic health of the species in portions of their range (Kapfer and Potts, 2012). Studies on other species have shown that overharvesting of a population, for example consuming coconut crabs as a food source, can lead to changes in a population's genetics and physiology (Yorisue et al., 2020). Similar situations have been recorded for mammals, such as the overharvest of sea otters (*Enhydra lutris*) for their pelts, which resulted in severe population declines that the species is still recovering from (Doroff et al., 2003). Similar studies on bobcats are needed to determine what, if any, effect annual harvests may be having on the species.

Bobcat Population Structure

Bobcats are solitary and are only found together when mating or rearing young (Cochrane et al., 2006). Adult male bobcats move away from their natal home ranges to establish territories that they will defend (Sculley et al., 2018), and these may overlap with multiple female territories (Plowman et al., 2006). Females are philopatric and each tends to remain in a single territory, rarely changing her home range during her lifetime. Movement and establishment of territories in new areas can have an impact on the surrounding community because bobcats can assist in reducing and maintaining low population numbers of rodents and other mammals (Koehler and Hornocker, 1989).

Movement of individuals also can lead to the formation of new populations within the species' geographic range and can impact the genetic structure of the population through the combination of unique genetic pairings (Fritts and Sealander, 1978b). Mating between dispersing bobcats allows for the maintenance of genetic variation and can limit the effects of inbreeding (Schwartz et al., 2003). However, due to the 1:1 sex ratio seen in most populations and male-biased dispersal, there is concern that the removal of male bobcats, through roadside fatalities, poisoning, and harvesting, might have a negative effect on the genetic diversity of some populations (Johnson et al., 2006).

Bobcat Harvesting

Bobcat pelts carry a high monetary value, and are sought after by hunters and trappers (Lavoie et al., 2009). Removal of a large number of individuals from a population has been known to severely alter the genetic variation of a species (Newsome et al., 1989). Species' genetic diversity can be impacted by an increase in inbreeding within a population due to a decreasing number of individuals, which in turn leads to decreasing fitness within a group (Millions and Swanson, 2007). The loss of predators in a community can have cascading effects that might be detrimental to the health of an ecosystem (Preuss and Gehring, 2008), such as an increase in populations of rodents, rabbits, and deer, which in turn can limit the growth of plant life within a habitat (Petraborg and Gunvalson, 1962), as well as the expansion of other carnivores into a region which can alter bird and herbivore populations (Kauhala et al., 1999).

Assessing Genetic Diversity

Genetic sampling is one method of determining population diversity and structure within a habitat (Coen and Schrieir, 2017). This can be accomplished through the collection of waste samples (urine and feces) that are used to mark an individual's territory, as well as blood, tissue, and fur samples which can provide more viable DNA samples (Bischof and Swenson, 2012). Genetic samples can provide information on the origin of a population by tracing migration patterns, and on its current genetic structure (Wultsch et al., 2015). It also can allow for tracing of unique genetic features and even identifying possible diseases within the population. Long term studies have previously shown that older adult males are favored by hunters and trappers due to the size and quality of the pelts, which could alter the social structure of local populations (Allen et al., 2018). Observing genetic traits can assist in evaluating a population's degrees of hybridization, inbreeding, and variation within and between sub-populations (Ruell et al., 2009). Large, multi-state studies have been performed utilizing genetic methods, providing evidence that inbreeding levels were low and that migration between populations was occurring within a region where the bobcat's numbers were in a state of long-term recovery (Anderson et al., 2018). However, similar work has been limited within Oklahoma.

Oklahoma Bobcats

Bobcats are found in every county within Oklahoma, and they are considered to be a keystone predator in their communities (Rolley and Warde, 1985). The state has a regulated trapping season for bobcats, and due to the value of the pelt, a large number of the cats are hunted. Hunting removes their alleles from the gene pool and potentially decreases the genetic diversity found in the state, a circumstance that is possibly impacting other furbearers (Hiller et al., 2011). The fur trapping season starts on 1 December and ends on 28 or 29 February, with a limit of 20 bobcats per season per trapper. Various hunting methods are allowed, including several types of traps (smooth-jawed spring traps and enclosed trigger traps), firearms, and archery equipment. The season can lead to the removal of 9-10 thousand individuals from the state's total population (Chrisman et al., 2019). For example, 10,506 bobcats were trapped in the 2018-2019 season. Trapping, combined with losses from other natural and human-caused factors, can have a detrimental effect on bobcat populations (Crowe, 1975).

Limited work has been done to study the possible effect this harvest has had on Oklahoma's bobcat population. Data generated in this study could be utilized to observe the current genetic diversity of the population and inform future wildlife management practices (Elizalde-Arellano et al., 2012). For example, studies from 1977 to 1981 within the Ouachita National Forest showed lower density of bobcats compared to other regions of the United States due to low prey abundance, yearling pregnancy, and juvenile survival rate (Rolley, 1985). Regular harvest was the only non-natural form of mortality recorded in this study, although the research was limited in range and time period (Rolley, 1985). Despite limited studies, the overall population in Oklahoma appears to be stable based on the frequency of sightings. Having knowledge of the genetic diversity and structure is needed to determine what impact current hunting policies or other factors are having on the population (Homyack et al., 2008). Methods utilized in previous studies included roadside kill surveys and the counting of track lines to determine the presence of bobcats, but this does not provide a full account of the state's population numbers (Davis, 2016; Howery et al., 2018). If removal of large numbers of bobcats is having a negative impact on the population, then it might be necessary to alter the state's game policy to limit further damage to the population (Diefenbach et al., 2015).

Objectives

Obtaining and analyzing tissue samples from bobcats collected by trappers will allow for a better understanding of the genetic diversity of Oklahoma's bobcats (Nielsen and Woolf, 2002). The data generated by this project will be utilized to determine the current genetic diversity and population structure of bobcats in Oklahoma, and will be used in future research to assess changes in diversity between trapping seasons and inform possible wildlife management practices. Comparing samples from different parts of the state could establish a broader data set on the overall variation of bobcats and provide a base-line for genetic structure from various locations in Oklahoma that may be affected by the annual harvest. The objectives of this research were to:

- Identify the current levels of genetic variation in bobcats in Oklahoma
- Determine the baseline genetic diversity of bobcats in the state
- Determine if there is genetic structure present in the population

Materials and Methods

Sample Collection and DNA Extraction

A total of 324 tongue samples from 41 counties (Appendix A, Fig. 1) were collected from legally harvested bobcats during the 2018-2019 trapping season by the Oklahoma Department of Wildlife Conservation (ODWC) and were sent to Oklahoma State University (OSU) for parasite testing. Subsamples of tongue tissues were obtained and sent to UCO. Of these samples, 220 individuals from 25 counties (Appendix B, Fig. 1) were selected for this project based on the county of origin, number of samples from each county, and region of Oklahoma. I divided the state into 3 regions: eastern, ranging from the Ouachita Mountains to the Osage hills; central, ranging from the Sandstone Hills to the Red Bed Plains; and western, ranging from the Gypsum Hills to the High Plains. Samples were selected to obtain an even representation of each region. Some counties were excluded due to a lack of available specimens. DNA was extracted from the tongue samples using Qiagen DNeasy Tissue Extraction kits following manufacturer protocols.

PCR Amplification and Genotyping

Each sample was amplified via polymerase chain reaction (PCR) for 10 microsatellite loci (Appendix A, Table 1; Menotti-Raymond et al., 1999; Carmichael et al., 2000; Faircloth et al., 2005), and one Y chromosome microsatellite for sex identification (sex-determining region of the Y, SRY; Hanson et al., 2007). Loci were selected following a pilot study using 19 loci which had been shown to work in previous bobcat and lynx studies (Menotti-Raymond and O'Brien, 1995; Menotti-Raymond et al., 1997, 1999, 2005; Carmichael et al., 2000; Faircloth et al., 2005), and the 10 selected loci were determined to be the most effective at identifying variation in this population. The PCR master mix for all markers, including the Y-chromosome marker, consisted of 13.6 microliters (ul) of water, 2.5 ul of GoTaq 5x Flexi Buffer combined with dye (Promega), 2.5 ul of 8mM dNTPs, 2 ul of 25 mM $MgCl_2$, 1 ul each of 10 mM forward and reverse primers, and 0.2 ul of GoTaq Flexi DNA Polymerase (100 ul) with a concentration of 50 ul. Each sample was plated using 9.5 ul formamide (Applied Biosystems), 1 ul ROX500 size standard (Applied Biosystems), and 1 ul PCR product, and genotyped on an ABI 3500 Genetic Analyzer. Genotypes were scored using GeneMapper 5.4 (Applied Biosystems). After an initial round of genotyping, samples that presented issues, such as dye blowout or excess stutter, were amplified and genotyped again to confirm allele calls.

DNA Analyses

Allele frequencies, observed and expected heterozygosity, polymorphic information content (PIC) for each locus, and the total population, were estimated using CERVUS 3.0.7 (Marshall et al., 1998; Slate et al., 2000; Kalinowski et al., 2007, 2010). Parameters for CERVUS were: 10,000 tests performed, with proportion of mistyped loci at 0.01, truncated at zero equaling 1, relaxed confidence 80, strict confidence 90, corrected likelihood of 1, and likelihood error rate of 0.01. FSTAT 2.9.4 (Goudet, 1995, 2001) was used to estimate Hardy-Weinberg Equilibrium (HWE), linkage disequilibrium (LD), and allelic richness. Parameters for FSTAT were: nominal level of 5/100 over 1000 iterations for HWE and LD, allelic richness based on a minimal sample size of 154, P-value for F_{IS} at 200 randomizations, and P-value for genetic disequilibrium at 900 permutations. The number of unique genetic clusters represented by the samples was estimated using STRUCTURE 2.3.4 (Pritchard et al., 2000; Falush et al., 2003, 2007; Hubisz et al., 2009). Parameters for STRUCTURE were: a burn-in period of 100,000; MCMC reps of 1,000,000 after burnin; K = 1 to 25; and 5 iterations of each test of K. STRUCTURE results were uploaded to Structure Harvester (Earl and vonHoldt, 2012) to determine the most likely value of K that best fit the data based on the Evanno et al. (2005) method. STRUCTURE results also were uploaded to CLUMPAK (Kopelman et al., 2015) to average the five independent runs for each value of K and produce a single bar graph for each K value. Additionally, population structure was assessed by assigning each sample to a "population" based on the collection county, with the number of populations assumed as 25.

Results

For the 220 individuals that were used in this project, a total of 2,200 amplification products were genotyped (not including re-amplified markers or the SRY marker). In total, 271 reactions failed to generate genetic information and were deleted from the data set. The remaining 1,929 reactions, representing complete or partial genetic profiles for 213 individuals (Appendix B, Table 2), were analyzed as described above. Results of the SRY analyses identified 134 males and 86 females within the data set (Appendix B, Table 2), and of the total tissues there were 211 males, 130 females, and 6 that could not be determined.

Genetic Diversity

The number of alleles per locus ranged from 14-25 (Appendix B, Table 3). Mean observed heterozygosity was 0.8775, whereas mean expected heterozygosity was 0.9029. Mean polymorphic information content (PIC) was 0.8629. Allelic richness ranged from 13 (for locus BC1AT) to just under 25 (for locus LC110) and was similar to the number of observed alleles for each locus. All loci were in HWE, although seven loci (FCA90, FCA77, FCA391, FCA96, FCA126, FCA132, and FCA742) were approaching significance. Additionally, no loci were in LD, although one pair (LC110xFCA77) approached significance (P value for 5% nominal value = 0.01111). Gene diversity of each locus, estimated using FSTAT, was greater than 0.800 (LC110 – 0.903; BC1AT – 0.821; FCA90 – 0.907; BCE5T – 0.848; FCA77 – 0.907; FCA391- 0.833; FCA96 – 0.923; FCA126 – 0.898; FCA132 – 0.931; FCA742 – 0.801).

Genetic Structure

Results from the Structure with no preassigned collection data indicated two distinct clusters, as well as an admixture group, within the state (Appendix A, Figs. 2, 3). Admixture was determined with the 80:20 rule, with individuals with a value of 0.8 or higher being assigned to either cluster 1 or cluster 2, as appropriate, and lower than 0.8 being assigned to the admixture group. In total, there were 120 individuals in Cluster 1, 41 in Cluster 2, and 59 in the Admixture group, with the three groups being located across the state with no association to geographic regions (Appendix A, Fig. 2). Additionally, when county assignments were used, two distinct groups, as well as an admixture group as priors, were detected (Appendix A, Fig. 4). In total, 18 individuals belonged to group 1, 134 belonged to group 2 and 68 belonged to the admixture group. There were no noticeable barriers between clusters and individuals from each cluster, as well as admixture individuals, were found in eastern, central, and western counties, and in some counties all groups were present (Appendix A, Fig. 2).

Discussion

Genetic Diversity

The examination of 220 samples from eastern, western, and central portions of the state allowed me to assess genetic diversity and structure across the state. A high level of heterozygosity was detected, and while there were a sizeable number of homozygous individuals, it did not appear to impact the overall genetic diversity of bobcats in the state. The high degree of heterozygosity for each locus and across all loci is indicative of a high degree of genetic diversity. Additionally, allelic richness was high and similar to the number of observed alleles, indicating both past and future genetic diversity is good for this population. These data provide support for high genetic diversity within the bobcat population across the whole state, and suggest that the population is genetically stable.

High levels of homozygosity can be caused by a number of factors, such as genetic drift and inbreeding, both of which are small population effects. Small populations could form as a result of overharvesting, such with mountain lion populations in Colorado (Logan and Runge, 2021). Inbreeding and the assorted detrimental traits such as physical deformities (malformed limbs, limitations to sensory capabilities, limited effectiveness of the immune system) and mental deficiencies could affect a bobcat's ability to survive, which has been reported in other cat species, including the cheetah (*Acinonyx jubatus*; Merola, 1994; Lacy, 1997). The level of homozygosity observed within the Oklahoma population is small enough to not cause concern and is offset by the current variation in the population.

The current genetic makeup of bobcats in the state could indicate that mortality events such as fur harvests have a limited effect on the genetic diversity of this species in Oklahoma. My data supports the notion that this species is able to sustain a moderate to high level of diversity, even with the regular loss of individuals from natural (age, predation, illness) and anthropogenic (hunting, vehicular impact) deaths. This could be due to the wide distribution and presence of bobcats across the state, allowing groups to sustain themselves through the influx of individuals from other populations. The behavior and physical traits of bobcats (pelage, smaller size, use of dens, nocturnal hunting and movement) might also limit the impact of loss of individuals through reduced predation and competition with other carnivores (Fuller et al., 1985b). Maintenance of a den or shelter as well as care of fur also increases the chances of surviving severe weather events and seasonal changes, further reducing the likelihood of mortality and improving the chances of reproduction (Kamler and Gipson, 2004). While it is difficult to know for certain if these are having a direct effect on the population, they could aid in maintaining the high levels of diversity within Oklahoma.

Bobcats establish a home range upon reaching adulthood to maintain access to resources and reduce chances of inbreeding with siblings or parents. The current population seems to be sizeable enough to allow for adult bobcats to locate prospective mates without breeding with related individuals, and the number of males moving through territories might contain a high level of diversity, allowing for the maintenance of high levels of heterozygosity. During the 2019 season, more males than females were harvested, and genetic variation was still present within the population. There might be enough space within Oklahoma to allow for a large number of bobcats to locate and maintain territories. The ability to support many individuals improves the sustainability of populations by supporting enough adults to limit familial interbreeding, which has been recorded in other vertebrates, including trout (Neville et al., 2009). This could be a significant factor in the current genetic makeup in the state's bobcats.

Genetic Structure

STRUCTURE results indicated there were two distinct genetic clusters in Oklahoma, but after a full review of the data, this appears to be the result of a bias in the program (Janes et al., 2017). The reliability of the program does not appear to be a significant issue, and the initial results could be due to the use of a default ancestry or unbalanced sampling, though more observations are needed to confirm this (Wang, 2016). Therefore, a single panmictic population most likely occurs in Oklahoma, with gene flow within and between regions. The presence of different clusters and admixture individuals in different counties might signify the lack of genetic barriers for bobcats in Oklahoma. Physical barriers, such as roads and fences, have been recorded blocking the distribution of local mammal populations, reducing the number of adults within a population, and altering genetic diversity in a habitat, and this may have an effect on bobcats (Jaeger and Fahrig, 2004). Due to the high levels of heterozygosity and lack of structure, these possible barriers do not seem to be reducing gene flow in Oklahoma. There is also the possibility that bobcats are migrating into Oklahoma from neighboring states, serving as a regular source of genetic diversity. Bobcats emigrate from their natal range upon reaching maturity, and although physical barriers do exist (e.g., the Red River along the Texas/Oklahoma border), the size of state borders would enable movement between different states. Immigrants bring new genetic material to local populations, increasing heterozygosity and improving genetic health, which can help a species sustain itself (Hasselgren et al., 2018). The ability of bobcats to avoid barriers and move freely is likely a significant factor in the current genetic makeup in the state.

Conclusions

The current dataset allowed me to determine the contemporary genetic diversity and structure of bobcats from 2019. Obtaining and analyzing samples from subsequent harvest seasons would allow researchers to monitor possible fluctuations in genetic variation and structure over multiple years. Collecting this information would allow researchers to observe changes in heterozygosity and allelic richness, and quantify the effects of factors such as the removal of many individuals from the population. Being able to monitor possible alterations to the population's genetic makeup would allow state and federal agencies to manage continued harvesting of bobcats without inbreeding or other small population effects.

Being able to monitor and identify immigration of bobcats from neighboring states would increase available data on both bobcat movements and interbreeding among populations. This information would clarify possible causes of genetic diversity within bobcat populations, as well as increasing data on the formation and sizes of home ranges, including territories that cross state lines through the use of other tools such as radio collars and game cameras. Collecting this data could assist state wildlife agencies in managing local populations by classifying genetic groups that are present in different states, which could aid in preserving unique populations or contribute to continued genetic diversity.

Future Directions

While the number of tissues used for this study provides a data set for a large percentage of the state, there are 100 samples from the 2018-2019 collection effort that need to be analyzed. Analyzing the remaining 2019 samples would expand the amount of baseline genetic data for the state and may provide further evidence of genetic diversity. Analyzing the remaining samples would also increase the number of counties with genetic data records, as well as provide additional information on the distribution of genetic clusters within Oklahoma. This would assist in showing connections between groups, as well as help clarify the effects or lack of barriers that may impede gene flow. However, it is unlikely that this data would alter the conclusion that a single population exists within Oklahoma.

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Appendix A

List of Tables

Table 1. -- List of loci used in this study. The primer name, citation source, published allele range, and optimal annealing temperature are provided

Primer Name	Source	Allele Size Range	Annealing Temp. (°C)
BC1AT	Faircloth et al. 2005	318	50-60
BCE5T	Faircloth et al. 2005	261	50
FCA77	Menotti-Raymond et al. 1999	143-155	40-62
FCA90	Menotti-Raymond et al. 1999	93-120	58-60
FCA96	Menotti-Raymond et al. 1999	184-224	53
FCA132	Menotti-Raymond et al. 1999	137-153	54-60
FCA126	Menotti-Raymond et al. 1999	139-145	58-60
FCA742	Menotti-Raymond et al. 1999	123-175	50-60
FCA391	Menotti-Raymond et al. 1999	237-273	56-58
LC110	Carmichael et al. 2000	91-103	50-56

Table 2. -- Allele calls for all loci and all individuals used in this study. For each specimen, allele calls for all 10 loci, collection county, and sex identification, based on SRY genotyping, are provided. Asterisks (*) represent missing data.

Specimen	LC110	BC1AT	FCA90	BCE5T	FCA77	FCA391	FCA96	FCA126	FCA132	FCA742	County	Sex
1	79/84	314/314	106/108	265/272	138/140	200/204	181/189	126/142	161/163	108/112	McCurtain	F
2	78/78	314/322	104/109	268/268	136/143	200/204	192/192	128/131	161/169	108/116	McCurtain	F
3	78/78	314//318	99/105	265/272	139/143	204/208	181/182	130/133	165/172	108/108	McCurtain	F
4	78/78	310/322	101/106	258/258	139/143	200/200	181/192	120/123	163/171	108/108	McCurtain	F
5	78/84	314/318	105/105	272/272	141/143	208/208	192/192	*/*	163/171	108/116	McCurtain	M
7	78/78	310/318	101/110	257/265	144/146	204/208	189/189	126/130	165/169	112/120	McCurtain	F
8	78/78	310/314	100/103	268/268	145/147	199/212	174/192	131/134	165/173	108/116	McCurtain	M
9	78/89	314/322	107/110	265/276	140/143	208/212	176/176	127/129	170/172	108/112	McCurtain	M
10	78/89	310/310	107/109	258/258	142/144	200/200	181/190	126/128	*/*	108/116	McCurtain	F
11	78/85	306/310	102/107	268/276	141/143	208/208	176/179	129/142	170/174	104/108	McCurtain	F
12	75/94	310/310	103/111	265/268	140/144	203/207	176/183	123/142	*/*	104/108	McCurtain	M
13	78/93	296/296	100/102	265/268	140/144	200/209	180/180	127/131	*/*	112/112	McCurtain	F
14	78/93	310/310	109/111	268/272	140/140	208/208	181/181	125/125	*/*	108/108	McCurtain	M
15	82/93	302/318	105/108	257/260	135/140	203/211	192/192	129/129	*/*	104/108	McCurtain	M
16	75/78	310/310	102/102	268/276	140/142	203/203	175/181	129/131	*/*	104/108	McCurtain	M
17	78/82	304/314	103/106	257/26	144/146	194/209	181/19	125/125	*/*	108/112	McCurtain	F

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18	78/82	300/304	103/105	258/258	142/142	200/209	188/192	*/*	*/*	108/116	McCurtain	F
19	*/*	306/318	103/105	258/258	136/136	201/205	180/194	123/123	*/*	108/108	McCurtain	F
20	75/78	300/304	108/108	268/272	135/144	199/212	188/193	131/133	*/*	104/108	McCurtain	M
21	73/75	296/304	100/100	258/276	142/146	199/208	180/180	133/135	*/*	108/108	McCurtain	F
22	72/79	306/310	103/107	257/272	143/145	201/209	*/*	124/124	*/*	*/*	McCurtain	M
23	73/81	306/306	101/109	265/265	141/143	201/213	*/*	128/131	*/*	*/*	McCurtain	F
24	73/73	314/322	106/108	257/276	141/146	204/208	*/*	123/126	*/*	*/*	McCurtain	M
25	77/80	306/318	119/121	265/268	139/142	203/207	*/*	131/131	*/*	*/*	McCurtain	F
27	73/81	310/318	112/120	258/268	141/146	203/208	*/*	125/127	*/*	*/*	McCurtain	F
28	76/81	310/314	116/127	265/276	142/146	204/208	*/*	125/129	*/*	108/108	McCurtain	F
29	76/81	310/314	100/112	268/276	133/141	207/208	*/*	131/133	*/*	*/*	McCurtain	M
30	76/76	310/318	104/110	265/276	133/141	202/204	*/*	133/135	*/*	*/*	McCurtain	F
31	76/76	306/306	103/109	268/272	139/146	202/207	180/189	125/130	*/*	108/108	McCurtain	M
32	73/81	302/310	101/103	268/272	140/146	208/212	190/190	131/133	*/*	*//	McCurtain	F
33	74/82	310/314	106/110	268/268	142/142	201/205	183/192	131/131	*/*	108/121	McCurtain	M
34	74/82	314/318	110/110	261/272	140/140	207/208	176/176	133/137	*/*	112/116	McCurtain	F
35	82/94	*/*	110/110	261/268	142/146	199/203	180/183	142/144	160/160	112/112	McCurtain	M
36	74/82	314/318	102/102	265/268	132/134	208/212	176/189	137/140	*/*	119/119	McCurtain	F
37	78/86	310/310	110/110	272/276	140/144	200/204	181/181	127/132	160/160	108/112	McCurtain	M

38	74/78	310/326	103/109	268/276	133/133	207/212	183/189	132/138	*/*	114/119	McCurtain	M
39	74/78	302/306	107/107	268/268	140/148	201/209	194/194	129/132	*/*	114/119	McCurtain	F
40	78/86	310/314	107/110	258/272	134/138	203/208	185/193	121/137	*/*	114/114	McCurtain	M
41	74/78	310/322	107/107	265/268	142/146	208/213	190/194	130/132	160/160	107/107	McCurtain	F
42	74/82	310/314	102/110	268/272	144/144	200/208	180/192	121/121	173/177	119/119	McCurtain	M
43	72/78	310/314	104/110	272/276	142/146	209/209	*/*	*/*	166/170	110/112	McCurtain	M
44	75/82	302/318	107/110	268/272	135/140	204/208	187/187	*/*	170/172	114/114	McCurtain	M
45	78/82	306/306	102/108	261/265	140/140	203/212	180/194	120/133	158/160	110/114	McCurtain	M
46	75/94	302/306	100/100	257/261	134/138	200/209	*/*	113/142	169/169	111/116	McCurtain	M
47	94/94	314/318	103/103	258/265	144/148	203/211	181/192	121/138	168/172	104/116	McCurtain	F
48	75/82	310/318	101/109	258/268	140/140	199/212	187/187	121/142	166/166	*/*	McCurtain	F
49	75/79	302/314	104/110	265/268	144/146	203/208	181/192	131/133	172/172	114/114	McCurtain	F
50	76/82	310/318	103/105	268/272	140/144	200/204	185/185	121/142	168/170	114/116	McCurtain	F
51	78/82	310/310	101/103	261/276	131/145	203/208	182/189	121/144	171/178	114/123	Atoka	F
52	75/82	310/314	106/106	272/276	137/142	208/208	188/188	*/*	170/170	119/119	Atoka	F
53	*/*	306/314	110/110	265/265	139/146	203/208	*/*	131/131	168/172	112/116	Atoka	F
54	77/82	310/314	101/102	265/268	141/141	208/212	*/*	*/*	162/164	112/116	Atoka	M
55	77/79	318/322	103/104	261/268	139/146	203/203	*/*	135/142	170/172	108/108	Atoka	F
56	77/82	306/306	109/109	261/272	141/146	203/203	*/*	135/142	170/170	*/*	Atoka	M
57	74/82	310/314	102/102	265/27	141/141	211/215	184/19	133/137	162/164	112/116	Atoka	F

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58	77/94	310/314	109/109	268/273	139/141	207/207	188/188	135/142	170/172	108/116	Atoka	M
59	75/77	310/310	102/107	268/272	139/146	207/207	186/191	*/*	172/179	108/108	Atoka	F
60	76/79	306/314	102/108	268/272	139/139	198/203	*/*	138/142	162/168	116/116	Atoka	F
61	79/82	314/314	102/104	268/268	139/139	210/214	185/185	128/128	164/166	108/112	Atoka	M
62	78/82	310/314	102/104	272/276	141/141	212/216	*/*	127/129	160/172	108/108	Atoka	F
63	75/78	310/310	102/106	270/273	144/146	204/204	192/192	127/135	161/173	108/108	Atoka	F
64	74/78	314/322	107/110	266/273	141/141	204/216	*/*	127/131	165/167	116/116	Atoka	M
65	75/83	306/318	90/111	258/270	141/141	203/208	*/*	129/133	165/165	108/108	Atoka	F
66	74/78	314/318	108/110	257/272	137/141	208/208	*/*	128/133	163/172	108/108	Atoka	M
67	74/77	314/330	102/104	272/276	139/149	208/212	*/*	129/133	163/173	104/108	Atoka	F
68	82/94	310/310	102/102	268/268	142/146	200/205	*/*	127/127	165/172	112/116	Atoka	M
69	82/87	314/322	109/112	250/263	139/146	208/208	*/*	127/127	167/167	104/112	Atoka	M
70	77/87	314/318	109/109	258/273	137/142	204/212	192/192	130/130	169/174	112/116	Atoka	M
71	87/94	306/310	100/104	273/277	137/142	208/212	176/176	127/131	167/172	108/108	Beaver	F
72	87/94	310/318	102/104	268/272	139/144	199/212	174/174	125/127	173/173	104/104	Beaver	F
73	74/78	310/326	108/110	265/268	142/146	207/211	176/192	131/133	163/172	108/108	Beaver	M
74	74/78	298//306	106/110	268/276	137/144	199/208	178/178	135/135	163/174	108/112	Beckham	M
75	74/74	310/322	108/110	257/265	137/139	208/208	192/192	129/133	167/170	*/*	Beckham	M
76	75/78	314/322	104/104	272/276	144/147	199/208	176/180	123/135	172/172	*/*	Blaine	M

77	74/82	318/318	104/108	268/272	144/146	199/208	176/178	129/131	170/170	*/*	Blaine	M
78	74/77	314/318	102/110	276/276	141/144	212/212	185/192	129/129	160/160	*/*	Blaine	F
79	75/77	298/318	102/108	268/276	146/149	208/208	176/180	127/127	161/168	108/116	Blaine	M
80	77/77	310/334	106/106	268/276	144/146	208/212	190/190	129/131	163/165	108/108	Blaine	M
81	77/77	306/314	103/110	268/276	137/139	208/212	178/192	129/131	172/174	108/108	Blaine	F
82	77/78	310/318	108/110	272/276	134/147	212/212	179/192	129/129	165/165	*/*	Blaine	M
83	70/74	310/314	105/105	272/276	138/148	189/189	187/191	135/137	177/178	114/119	Blaine	M
84	75/79	298/314	100/105	258/265	141/143	203/212	187/193	127/136	170/170	107/107	Blaine	M
85	75/79	306/318	104/104	268/272	139/149	208/212	180/181	129/129	173/178	114/119	Blaine	M
86	78/82	306/318	100/103	265/268	151/158	208/208	180/194	135/140	170/172	119/124	Blaine	M
87	*/*	310/318	107/109	265/268	132/141	203/212	185/195	135/142	162/164	107/116	Blaine	M
88	*/*	314/318	104/109	268/272	129/138	194/208	*/*	129/133	162/162	112/116	Blaine	M
89	*/*/	298/306	110/110	261/265	146/148	191/191	176/191	127/129	174/178	115/119	Blaine	M
90	**	306/310	107/109	272/272	132/141	190/190	179/182	123/133	162/162	103/107	Blaine	M
91	*/*	306/318	108/108	257/268	141/151	208/216	*/*	137/140	162/172	107/107	Blaine	M
92	*/*	306/310	108/108	268/272	143/146	207/211	174/178	128/138	162/162	110/119	Blaine	M
93	74/78	*/*	106/111	270/273	138/140	*/*	*/*	127/133	167/167	108/108	Blaine	M
94	76/82	*/*	109/109	238/250	131/148	189/189	*/*	133/133	161/171	108/112	Blaine	M
95	74/82	*/*	101/101	266/270	138/140	188/188	*/*	126/131	162/162	104/108	Blaine	M
96	77/82	318/318	103/103	272/27	138/143	188/188	*/*	124/130	167/167	108/112	Blaine	M

				2								
97	74/82	*/*	110/110	268/268	136/140	208/217	*/*	*/*	163/173	108/112	Blaine	M
98	79/84	*/*	100/103	265/268	136/140	192/192	175/175	139/139	163/163	107/115	Blaine	F
99	83/87	*/*/	100/102	268/273	136/140	199/212	*/*	139/139	163/173	108/116	Blaine	M
100	79/83	**	103/105	268/272	138/144	194/194	*/*	138/138	163/163	108/108	Blaine	M
101	78/81	*/*	101/109	268/268	136/140	188/188	175/175	*/*	163/163	108/112	Blaine	F
102	77/91	*/*	101/106	261/272	140/140	189/189	*/*	139/139	163/163	108/108	Blaine	M
103	83/89	*/*	105/107	257/276	136/140	208/208	176/176	125/128	162/165	108/116	Blaine	M
104	75/78	*/*	107/111	273/273	141/146	208/208	175/179	127/135	162/162	120/120	Blaine	M
105	75/78	*/*	109/109	276/276	139/141	199/208	181/181	125/125	162/162	*/*	Blaine	M
106	75/79	*/*	104/107	261/272	142/142	204/208	178/180	126/126	162/162	107/111	Blaine	M
107	78/89	*/*	104/106	272/272	140/142	208/208	175/192	131/135	162/162	107/111	Caddo	M
108	75/96	*/*	102/104	257/276	139/139	208/212	178/180	125/131	161/161	107/107	Canadian	M
109	89/96	*/*	104/104	268/272	141/144	200/205	174/182	*/*	162/167	103/107	Canadian	M
110	83/96	*/*	102/109	261/265	139/141	208/208	176/179	133/133	163/172	107/107	Carter	M
111	89/96	*/*	100/109	265/272	149/151	208/208	176/194	129/133	162/167	116/116	Carter	M
112	78/96	*/*	100/109	265/265	142/144	194/217	181/192	128/135	162/162	*/*	Carter	M
113	76/83	306/318	102/108	265/268	*/*	204/208	174/176	131/131	172/174	103/111	Carter	M
114	78/78	310/314	100/111	265/272	139/141	*/*	180/182	127/130	162/164	108/112	Carter	M
115	82/96	306/306	103/104	265/272	*/*	208/208	*/*	125/131	168/172	107/115	Carter	M

116	79/79	306/314	100/103	268/272	139/141	199/212	*/*	127/127	210/217	103/111	Carter	M
117	79/79	306/322	84/84	*/*	141/141	200/204	*/*	125/125	164/164	107/115	Carter	F
118	75/78	306/314	104/109	268/272	*/*	200/204	*/*	124/129	163/163	107/115	Carter	F
119	75/78	298/314	102/108	268/268	*/*	208/208	*/*	125/130	162/162	107/111	Carter	F
120	78/83	306/318	100/104	265/272	144/147	208/212	174/176	129/131	163/163	107/115	Carter	M
121	78/78	310/314	102/104	258/265	*/*	199/212	*/*	*/*	167/169	107/111	Carter	M
122	75/79	318/322	108/109	265/268	144/146	194/208	193/193	129/129	164/170	103/115	Carter	M
123	81/83	314/318	*/*	261/276	141149	208/208	176/192	131/131	163/167	104/116	Carter	M
124	73/81	304/318	107/111	272/272	141/141	208/212	188/190	127/131	163/173	112/116	Carter	M
125	75/83	306/318	104/107	261/272	139/141	208/212	176/180	125/125	173/173	112/116	Carter	M
126	77/78	314/314	*/*	265/268	139/141	208/208	192/192	127/129	163/173	104/104	Carter	F
127	75/78	318/330	107/107	272/272	137/141	204/208	183/183	129/129	167/173	104/108	Carter	M
128	75/75	310/330	*/*	268/276	139/141	*/*	*/*	121/129	163/169	108/108	Carter	F
129	75/83	310/318	106/109	268/272	139/141	208/208	175/180	129/131	171/175	104/116	Cherokee	F
130	75/83	310/318	*/*	268/272	139/141	208/208	179/188	125/129	161/167	104/116	Cherokee	F
131	*/*	310/318	*/*	265/272	139/141	208/212	188/188	125/129	173/175	116/116	Cherokee	F
132	73/75	304/318	102/107	272/276	139/141	208/208	*/*	129/131	167/175	116/116	Cherokee	F
133	83/83	298/314	100/109	265/268	139/141	*/*	190/192	129/133	163.167	108/112	Choctaw	M
134	75/78	310/318	102/102	265/272	139/141	204/208	176/176	125/127	165/165	112/116	Choctaw	M
135	78/83	314/314	102/111	265/268	139/146	204/212	*/*	131/131	169/172	112/112	Choctaw	M

136	77/79	306//318	102/102	265/272	*/*	208/208	178/178	127/129	165/172	108/108	Choctaw	M
137	75/95	310/318	107/111	265/265	146/146	200/200	179179	125/125	165/165	112/112	Choctaw	M
138	78/83	310/314	111/111	265/276	139/141	*/*	*/*	129/131	169/169	108/112	Choctaw	F
139	75/79	310/314	*/*	250/267	132/141	*/*	*/*	134/136	165/171	108/108	Choctaw	F
140	75/83	310/314	109/109	258/269	141/146	*/*	181/183	127/131	165/176	108/120	Choctaw	M
141	75/77	318/322	*/*	268/272	139/146	207/208	176/189	131/131	161/169	108/112	Choctaw	M
142	78/78	314/318	109/109	265/268	139/139	204/208	192/192	125/127	163/169	108/112	Choctaw	M
143	75/78	306/314	104/111	268/268	139/146	*/*	176/181	129/135	161/169	108/112	Choctaw	M
144	78/78	314/314	102/110	258/272	*/*	208/208	192/192	125/125	163/169	108/116	Choctaw	M
145	75/78	310/314	104/104	265/272	139/144	207/207	181/183	125/129	165/172	104/116	Choctaw	M
146	75/78	314/318	102/102	278/278	146/149	208/208	181/194	125/135	161/167	108/112	Coal	M
147	75/78	310/314	104108	258/265	142/142	198/208	179/179	135/135	167/167	108/116	Coal	F
148	74/78	306/314	*/*	*/*	141/141	204/208	*/*	129/135	161/161	*/*	Coal	M
149	78/79	310/314	*/*	268/272	139/139	208/212	179/179	125/133	167/172	108/108	Creek	M
150	75/83	310/318	*/*	261/268	141/146	208/208	*/*	129/129	167/174	108/112	Creek	M
151	78/78	310/318	108/108	250/267	139/141	208/208	*/*	125/131	161/173	108/112	Creek	F
152	75/75	322/322	102/109	258/272	141/141	208/208	*/*	125/129	167/169	108/116	Creek	M
153	78/79	310/314	102/109	265/265	128/137	208/208	178/187	133/135	164/168	*/*	Creek	M
154	78/83	314/314	102/102	240/250	128/142	208/212	178/178	125/135	168/168	*/*	Creek	M
155	78/95	310/322	100/104	268/276	146/149	208/208	176/176	129/133	166/172	*/*	Custer	F

156	76/78	298/310	100/100	273/273	139/141	208/212	174/174	131/131	166/166	*/*	Custer	M
157	75/78	310/318	108/108	*/*	137/139	*/*	181/181	129/133	161/170	*/*	Custer	M
158	75/84	310/314	108/108	268/268	137/137	200/212	176/176	127/129	161/161	*/*	Custer	M
159	75/95	306/318	102/102	273/273	137/139	204/204	178/178	127/131	161/161	*/*	Custer	F
160	78/78	314/314	104/104	261/261	139/139	208/208	182/182	127/131	161/161	*/*	Custer	F
161	*/*	298/322	107/107	268/276	139/149	200/200	*/*	121/127	161/161	*/*	Custer	M
162	*/*	310/314	109/109	268/268	139/141	207/208	188/188	129/133	160/160	*/*	Custer	F
163	78/78	310/318	102/110	273/273	139/141	208/208	178/178	127/131	165/171	108/108	Custer	M
164	78/84	306/310	102/108	261/268	141/141	200/204	181/192	125/129	171/172	108/116	Custer	F
165	75/78	306/322	100/100	276/276	137/139	208/208	176/183	125/131	163/172	112/116	Dewey	M
166	78/79	306/314	102/109	268/272	137/139	204/212	178/178	129/129	167/172	108/108	Dewey	F
167	78/78	310/318	108/111	273/276	139/141	200/200	185/190	127/127	161/163	*/*	Dewey	M
168	76/78	310/322	104/109	273/273	139/141	199/204	181/181	131/133	171/173	104/104	Dewey	F
169	75/78	306/306	102/102	272/276	139/141	208/208	176/183	131/131	163/171	108/112	Dewey	F
170	75/84	306/322	102/102	276/276	137/141	208/208	176/176	127/131	171/173	108/116	Dewey	F
171	78/84	318/318	104/107	268/276	137/141	204/208	180/180	129/131	163/167	108/116	Dewey	M
172	76/79	306/318	104/110	265/268	141/141	208/208	179/179	125/129	165/172	112/120	Dewey	F
174	86/94	*/*	107/109	261/268	159/159	199/208	180/180	*/*	170/173	*/*	Ellis	F
175	73/79	306/318	100/109	265/270	159/159	208/208	192/192	*/*	165/170	*/*	Ellis	F
176	71/76	318/318	102/111	265/27	159/159	208/208	174/17	*/*	160/172	108/108	Ellis	F

				6			4					
177	72/78	306/310	102/102	268/276	141/159	199/208	*/*	*/*	160/172	108/112	Ellis	F
178	77/79	306/318	88/109	268/276	137/159	199/204	182/182	129/129	165/168	104/108	Ellis	F
188	75/82	*/*	105/107	272/272	159/159	204/204	*/*	*/*	160/169	*/*	Garfield	M
189	75/79	*/*	100/102	273/273	159/159	204/204	*/*	*/*	160/172	*/*	Garfield	M
190	75/79	314/314	104/104	268/272	159/159	200/208	*/*	*/*	167/175	*/*	Garfield	F
191	73/75	*/*	105/107	265/268	159/159	212/212	*/*	*/*	173/173	*/*	Garfield	M
192	75/79	298/298	102/102	261/268	159/159	208/208	*/*	*/*	170/173	*/*	Garfield	M
196	76/82	310/318	109/111	265/272	141/141	204/212	*/*	127/135	164/164	108/116	Greer	M
202	85/93	*/*	100/105	*/*	159/159	203/208	*/*	*/*	*/*	108/116	Haskell	F
203	85/93	*/*	102/104	*/*	159/159	212/213	*/*	*/*	*/*	109/116	Haskell	F
204	85/93	*/*	102/110	*/*	159/159	204/208	*/*	*/*	*/*	109/109	Haskell	M
205	85/93	*/*	100/102	*/*	159/159	208/212	*/*	*/*	*/*	108/116	Haskell	F
206	85/93	*/*	102/102	*/*	158/158	204/212	*/*	*/*	*/*	108/112	Haskell	M
207	85/93	*/*	108/108	*/*	158/158	204/208	*/*	*/*	*/*	112/112	Hughes	F
208	85/93	*/*	102/109	*/*	159/159	200/214	*/*	*/*	*/*	108/116	Hughes	F
209	85/93	*/*	104/109	*/*	159/159	204/208	*/*	129/135	*/*	104/108	Hughes	M
210	85/93	*/*	102/104	*/*	159/159	203/208	*/*	*/*	*/*	112/116	Hughes	M
211	85/93	*/*	104/109	*/*	159/159	200/206	*/*	125/133	*/*	108/117	Hughes	M
212	*/*	310/318	106/108	268/276	143/146	207/211	178/185	123/131	164/164	108/112	Jackson	M
213	*/*	318/326	99/102	256/267	146/148	189/203	169/169	125/135	164/164	108/112	Jackson	M
214	75/78	306/314	104/107	268/272	140/146	208/211	180/186	127/127	165/168	112/116	Jackson	M
215	73/78	318/338	102/110	265/268	158/158	199/202	176/176	125/133	*/*	104/108	Jackson	M
216	76/83	306/334	104/107	265/272	134/141	207/211	183/183	129/129	164/164	108/112	Jackson	M

238	78/96	306/306	108/110	272/276	137/142	203/212	174/176	125/129	164/164	108/108	Kiowa	F
239	76/83	306/314	106/108	272/272	142/144	208/216	180/192	129/129	164/164	108/116	Kiowa	F
240	76/82	314/318	96/100	257/276	141/141	203/208	176/180	129/129	163/163	104/116	Kiowa	F
241	*/*	318/322	109/109	265/268	139/144	196/204	176/183	133/133	164/164	108/116	Kiowa	M
242	82/96	298/318	102/110	265/265	141/141	203/219	181/190	127/127	163/163	108/116	Latimer	M
277	70/79	302/318	100/100	261/268	141/146	208/212	181/195	133/133	164/164	108/116	Okfusske	F
278	70/75	302/306	100/102	268/272	132/149	208/212	176/179	127/133	163/163	108/108	Okfusske	M
279	74/79	318/322	104/111	261/265	146/146	204/208	178/180	*/*	164/164	112/120	Okfusske	M
280	74/83	314/318	100/106	261/273	139/144	203/208	181/192	127/127	164/164	112/116	Okfusske	M
281	77/92	314/322	101/110	260/268	145/147	199/207	178/178	*/*	166/172	116/116	Okmulgee	M
282	79/92	310/314	101/102	268/272	138/141	208/212	176/176	*/*	163/165	108/117	Okmulgee	F
283	75/78	314/314	103/104	265/273	142/145	211/215	174/176	*/*	169/169	*/*	Okmulgee	M
284	77/79	314/314	86/106	261/269	146/149	203/208	174/183	*/*	165/170	108/108	Okmulgee	M
285	77/82	310/318	103/109	269/272	144/146	203/204	176/176	*/*	169/169	112/121	Okmulgee	M
294	73/79	306/310	104/110	268/272	134/142	208/216	181/184	129/133	165/168	108/116	Pittsburg	M
295	*/*	306/306	100/102	272/276	132/141	203/208	175/178	129/133	163/165	108/108	Pittsburg	F
296	73/76	314/318	102/107	269/269	141/143	208/212	*/*	131/131	165/169	104/108	Pittsburg	F
297	73/79	306/314	102/109	265/272	144/146	203/207	173/173	125/131	165/169	104/108	Pittsburg	M
298	*/*	314/322	100/104	261/268	142/144	203/208	176/184	123/131	206/210	108/112	Pittsburg	M
318	81/92	306/310	86/107	268/27	143/143	200/212	178/17	*/*	163/170	108/116	Washita	M

				6			8					
319	77/79	306/314	107/111	265/276	139/142	200/208	176/176	*/*	168/172	104/112	Washita	M
320	78/92	306/310	104/107	276/276	139/141	199/202	174/174	*/*	163/167	108/112	Washita	F
321	79/84	314/314	102/107	265/268	144/146	204/208	176/176	*/*	165/172	108/108	Washita	F
322	77/82	310/322	106/111	269/276	137/143	200/217	180/180	*/*	161/171	108/108	Washita	M

Table 3. -- Cervus results for the total population. The locus name, alleles per locus (k), number of individuals genotyped (N), observed heterozygosity (Hobs), expected heterozygosity (HExp), polymorphic information content (PIC), and null allele frequency [F(Null)] are provided for each locus

Locus	k	N	HObs	HExp	PIC	F(Null)
BC1AT	14	186	0.812	0.821	0.794	0.0027
BCE5T	20	207	0.787	0.848	0.829	0.0316
FCA126	23	181	0.707	0.898	0.886	0.1179
FCA132	24	182	0.643	0.93	0.922	0.181
FCA391	28	211	0.682	0.832	0.818	0.099
FCA742	17	183	0.672	0.8	0.777	0.0848
FCA77	24	213	0.742	0.907	0.898	0.0982
FCA90	24	210	0.714	0.907	0.897	0.1184
FCA96	23	154	0.545	0.922	0.914	0.2553
LC110	25	204	0.877	0.903	0.894	0.0135

Appendix B

List of Figures

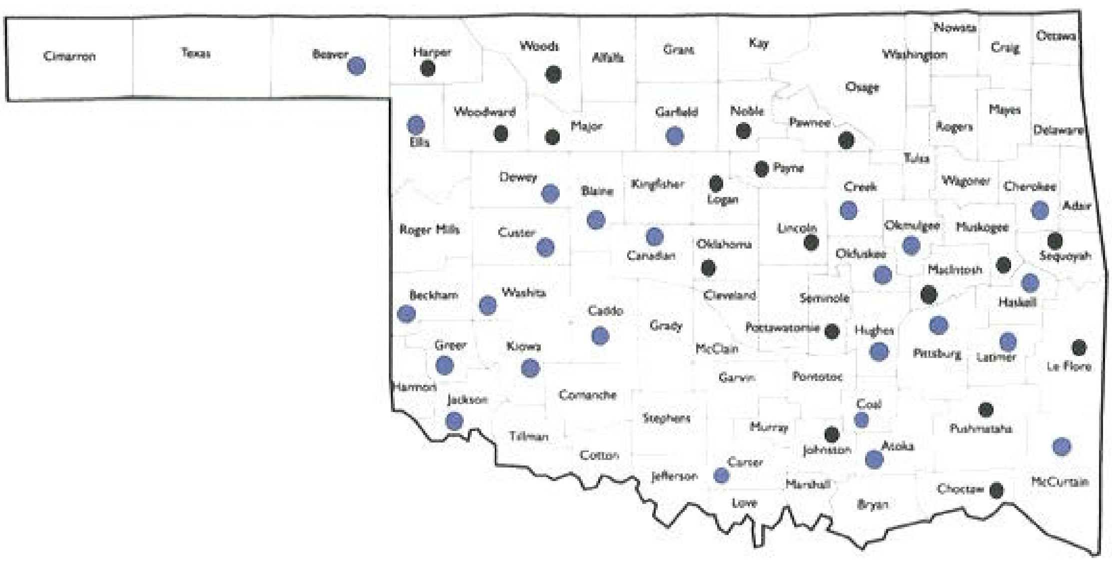

Figure 1. -- Map of Oklahoma showing counties where tissue samples were collected. Blue dots indicate counties included in this study.

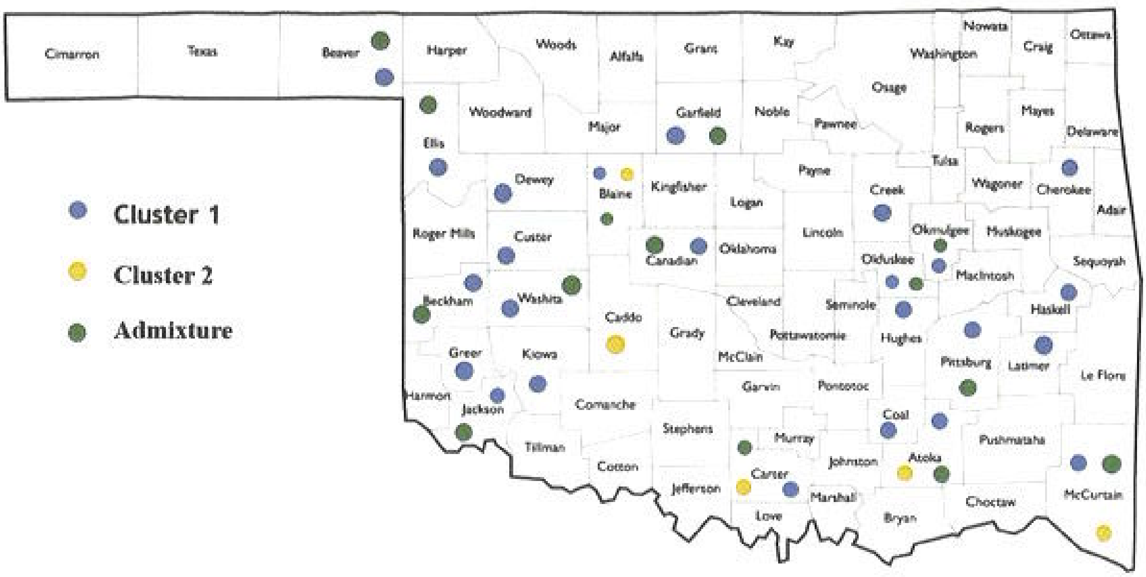

Figure 2. -- Map showing the location of genetic clusters identified using Structure. Blue dots are cluster 1, yellow dots are cluster 2, and green dots represent localities where admixture individuals were identified.

K=2

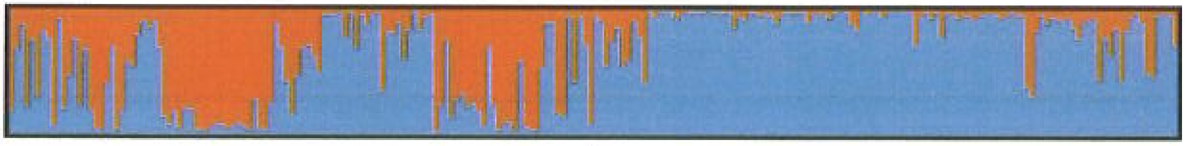

Figure 3 -- Graph of K = 2 STRUCTURE results generated using CLUMPAK, with K representing the possible subpopulations present in the overall bobcat population.

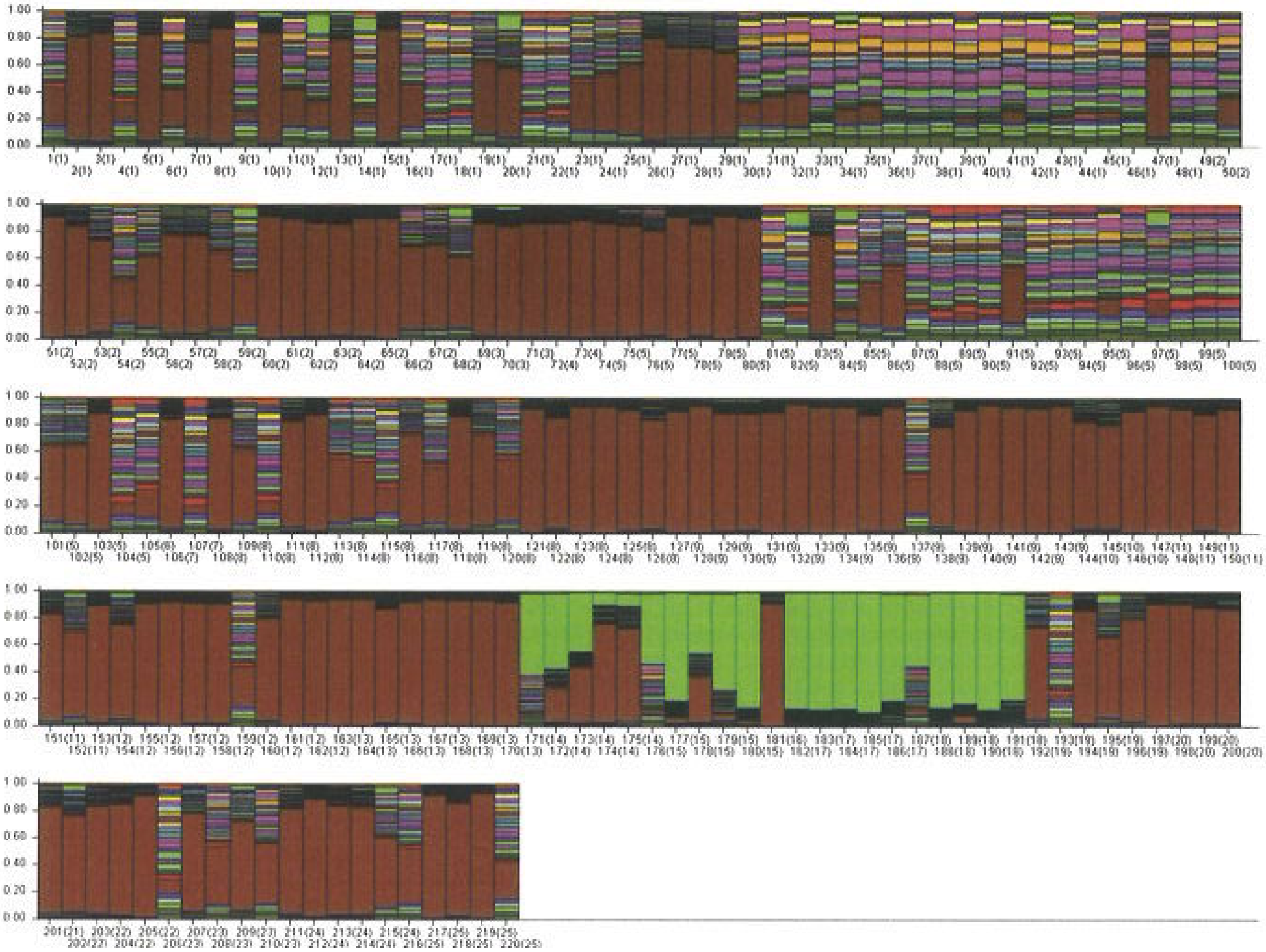

Figure 4. -- Graph of the Structure results assigning samples to collection counties. Three groups are evident (brown, green, and admixed – multiple colors). Each color is associated with a different county of collection.