GENOMIC INVESTIGATION OF WILD PIG (*SUS SCROFA*) SOCIAL STRUCTURE AND MATING STRATEGIES

by

CHELSEA LETICIA TITUS

(Under the Direction of James C. Beasley)

ABSTRACT

Within the USA invaded range, wild pigs are destructive, invasive hybrids of European wild boar and domestic pigs. However, aspects of wild pig social structures and mating strategies have been largely understudied. For my research, I used genomic tools and samples collected over several years from disparate wild pig populations across southern USA to address these gaps. In Chapter 2, I found female sounders were the main social unit, but pseudo-solitary females and male-dominated bachelor groups occurred more often than previously described. While primarily relatives, some sounders included unrelated females. Bachelor groups were mainly young male kin. In Chapter 3, I found proportion of male European wild boar ancestry was negatively correlated with number of assigned litters, whereas male age was positively correlated. Collectively, my research suggests wild pig sociality and mating strategies are similar to those observed among European wild boar, but is complex, dynamic, and variable across invaded habitats.

INDEX WORDS: Genomics, Generalized Linear Mixed Effect Model, Reproductive Success, Social Organization, *Sus scrofa*, Wild Pigs

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DEDICATION

I dedicate this thesis first and foremost to my Mom, Dad, and Stepdad for fostering a love and respect for all creatures and flora under the sun. I also dedicate this to Dr. Lori S. Eggert for taking a risk on a shy weirdo with crazy hair and showing her that a love for nature and affinity for genetics need not be mutually exclusive. And most especially, I dedicate this thesis to William C. Moors for encouraging me to go back to school and pursuing my passion for wildlife, genetics, and doing good in the world. Without any of you, I would not be here.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Reliable life history data are needed to effectively develop appropriate conservation and management strategies for wildlife. For example, knowledge of a species' social structure and reproductive strategies are important areas of their life history and necessary for determining accurate life history tables to inform management decisions. Though life history information is widely available for many common species, these characteristics can change when species are moved to new areas or hybridize between source populations under different types of selection. Novel habitats can introduce different environmental pressures that may affect a species population dynamics or social organization, and variable source populations can introduce new traits that are better or less suited to the new environment. However, even for widespread species, the current literature investigating the ecology of invasive species lags behind the rate at which species are introduced to novel habits.

Species that live in groups with coordinated behavioral patterns (sociality) display benefits and costs to the individual group members (Silk 2007, Prox and Farine 2020). Sociality benefits include better resource access, "safety in numbers," or communal offspring care (Krause et al. 2002, Silk 2007). However, sociality may result in increased competition for mating opportunities, higher risk for disease transmission, or hinder their ability to hide from predators, thus affecting the fitness of individual group members (Moore et al. 1988, Krause and Godin

1995, Krause et al. 2002, Silk 2007, Cafazzo et al. 2016, Prox and Farine 2020). Therefore, when the benefits of social organization outweigh the costs, sociality will persist in the species (Krause et al. 2002, Silk 2007).

There is considerable variability in sociality throughout the animal kingdom, and social structures can vary both between and within species. Among mammals, three general social structures can be found. Subsocial mammals primarily live alone and are only found briefly with conspecifics for mating or offspring rearing purposes (e.g., jaguar – *Panthera onca*; Cavalcanti and Gese, 2009). Parasocial mammals are typically found living in a group cooperatively (e.g., African lion – *Panthera leo*; Dickinson and Koenig 2018). Eusocial mammals live in colonial cooperation, where almost all group members relinquish procreating in lieu of assisting a very small reproductive caste (naked mole-rats – *Heterocephalus glaber*; Nowak et al. 2010, Dickinson and Koenig 2018). Parasocial and eusocial structures can be further broken down into group organization and composition that drive interspecific interactions; ultimately driving management decisions. Nevertheless, group membership is associated with costs and benefits that can be spatially and temporally dynamic, promoting diverse social group characteristics across a species' range and history (Gehrt and Fox 2004). Bottlenose dolphins (*Tursiops aduncus*), for example, display spatially different social organization. Male bottlenose dolphin in Indian Ocean populations generally demonstrates temporary, individual based fission-fusion 'super-alliances', but this type of social group has not been recorded in any other populations (Connor et al. 2001). Comparably, red foxes (*Vulpes vulpes*) have displayed density-dependent social organization. While this species is typically monogamous, breeding pairs will recruit both related and unrelated individuals to care for their offspring when population densities are high

(Macdonald 1979). On the other hand, caribou (*Rangifer tarandus*) rates of fission and fusion among social groups differs prior to and amidst peak mating seasons (Body et al. 2015).

Aside from displaying a variety of social structures, mammals also display diverse mating strategies that are influenced by species-specific traits and external environmental pressures (Crook et al. 1976). Mating strategies range from life-long, monogamous pair bonding with equal and extensive paternal investment by both parents, to various forms of male and female promiscuity utilizing communal offspring care within social groups or barely any parental investment by either both or male parents (Crook et al. 1976, Clutton-Brock 1989, Silk 2007). Despite the strategy that is displayed, attributes that improve individual fecundity and offspring fitness arise from selective pressure on both males and females (Kodric-Brown and Brown 1984, Heisler et al. 1987, Clutton-Brock 1989). Nevertheless, study of female, rather than male, reproductive success makes up most of the literature. The ease in which scientists can observe maternal relationships compared to paternal ones may explain the dichotomy for many species, especially for promiscuous species where males provide little parental investment (Crook et al. 1976, Silk 2007). Paternal care notwithstanding, male genes heavily influence offspring fitness, and therefore females of promiscuous mating systems are most likely to choose mates with high survivability or attractiveness (Kodric-Brown and Brown 1984, Heisler et al. 1987, Jennions and Petrie 1997).

Among mammals, promiscuous males invest little effort in their own realized reproductive success compared to female conspecifics (Bronson 1985, Clutton-Brock 1989). Further, copulation does not equate to fitness. Therefore, males that will increase the survival of offspring (“good genes”) or provide male offspring with a reproductive advantage (“sexy sons”) are selected as mates (Weatherhead and Robertson 1979, Kodric-Brown and Brown 1984, Heisler et al. 1987, Jennions and Petrie 1997). Studies have found that “sexy” males or males with “good genes” display attributes that are correlated with the rate of reproductive success in some species, such as age or weight (Kodric-Brown and Brown 1984, Jennions and Petrie 1997, Frasier et al. 2007, Newbolt et al. 2017). Additionally, males that have advantageous fighting ability or weaponry, like dominance and large horns, or enhanced appealing traits, such as pelage, songs, and testes size, are often chosen by females as a mate (Kodric-Brown and Brown 1984, Heisler et al. 1987, Jennions and Petrie 1997). However, “sexy sons” or “good genes” selection is assumed under natural circumstances for relatively established taxa. Not much is understood about how sexual selection and mate choice changes for species in novel habitats or species experiencing introgression of genes artificially selected upon for domestication characteristics, such as increasing docility and productivity, by the intentional or accidental breeding of domestic stock with wild populations (e.g., Bactrian camels – *Camelus bactrianus*; plains bison - *Bison bison bison*).

Sus scrofa (domestic pigs, European wild boar, and invasive European wild boar-domestic pig hybrids [hereafter referred to as “wild pigs;” Keiter et al. 2016] are one of the most globally distributed species, with populations occurring on all continents but Antarctica (Barrios-Garcia and Ballari 2012, Lewis et al. 2019). As they are taxonomically the same, wild pigs among invaded ranges are believed to reflect many social and behavioral patterns observed in native European wild boar (*Sus scrofa spp.*). European wild boar social organization is primarily characterized as two social units: matriarchal sounders (female kin-based social groups) or males that are solitary with the exception of mating (Dardaillon 1988, Kaminski et al. 2005, Poteaux et al. 2009, Podgórski et al. 2014*a*, *b,* Battocchio et al. 2017, Beasley et al. 2018). Dardaillon (1988) reported another social unit comprised of young, likely dispersing, males growing toward solitary adulthood, which has since been occasionally observed in other populations. Sounders are typically described as several female kin with offspring; yet reports are based on conflicting observational studies (Dardaillon 1988, Poteaux et al. 2009, Podgórski et al. 2014*a*, *b*, Battocchio et al. 2017). Iacolina et al. (2009) reported wild boar social groups were mostly composed of unrelated females with offspring in Italy. Podgórski et al. (2014*a*, *b*) described social groups in Poland as a female and her offspring with a home range that overlaps those of neighboring female kin. Comparably, adult females were found to occasionally group together in populations of Japanese wild boar (*Sus scrofa leucomystax*), but are typically solitary, with the exception of mating or rearing their current offspring litter (Nakatani and Ono 1994). Further, the territories of male European wild boar will incorporate or overlap several sounder territories, and sounders often have overlapping, but temporally separated, home ranges (Kaminski et al. 2005, Podgórski et al. 2014*b*). Additionally, European wild boar in their native range reportedly display mate guarding during rutting season but quickly disassociate from mates when rut is over (Dardaillon 1988).

Wild pigs in their invaded North American range are a destructive invasive species descending from domestic pigs and European wild boar. Having been first introduced in 1539 as livestock, feral populations subsequently became widely established and have since hybridized with escaped European wild boar that were imported for hunting (Mayer and Beasley 2018, Mayer et al. 2020*a*). The resulting wild pig populations display significant admixture of domestic pig and European wild boar lineages with varying degrees of hybridization observed within and among them (Keiter et al. 2016; Smyser et al. 2020). Wild pigs are also highly promiscuous and gregarious, providing a fascinating opportunity to investigate the social organization and mating strategies in a taxa that has arisen from opposing selection pressures

(Wood and Barrett 1979, Mayer and Brisbin 2009, Beasley et al. 2018, Gray et al. 2020, Smyser et al. 2020).

Many aspects of wild pig social ecology and mating strategies remain understudied, despite the substantial environmental and economic impacts caused by this species. The extent to which wild pig social organization differs from that of native populations of European wild boar is difficult to predict and has not been described in the literature. Wild pigs are believed to reflect their European conspecifics, with sounders composed of related females with their offspring and males as solitary adults. It has been determined through observational studies that sounder size and composition is highly variable; from sounders of over 30 pigs to a solitary female with offspring, with 3-9 individuals typically reported (Mayer and Brisbin 2009, Poteaux et al. 2009, Beasley et al. 2018, Gaskamp et al. 2021). Although some studies have suggested access to water may affect group size, the inherent factors contributing to the diversity in wild pig sounder size are largely unexplored (Gabor et al. 1999; Gaskamp et al. 2021). It is of note that the studies typically do not share robust genetic unanimity and are restricted to behavioral observations (Gabor et al. 1999; Mayer and Brisbin 2009; Delgado-Acevedo et al. 2010). Similar to European wild boar, male social groups have been observed within wild pig populations, but rate of incidence, composition, and relationships among male groups are unknown and associations are assumed to be temporary (Gabor et al. 1999; Mayer and Brisbin 2009).

Furthermore, the available research on the reproductive biology and ecology for wild pigs primarily centers on females. Nevertheless, a holistic understanding of the reproductive ecology of this species requires exploration of male reproductive strategies. Similar to European wild boar, male territories will incorporate or overlap several sounder territories, and sounders often have overlapping, but temporally separated, home ranges (Gabor et al. 1999, Delgado et al. 2008, Sparklin et al. 2009, Gray et al. 2020, Clontz et al. 2021, Kilgo et al. 2021). Recently, research has supported male mate guarding and promiscuity among wild pigs, but common traits of successful males have yet to be described (Gabor et al. 1999, Delgado-Acevedo et al. 2010, VerCauteren et al. 2020). It is largely unknown how much wild pig reproductive traits are influenced by the introgression of domestic pig alleles (Chinn et al. Unpublished data). Unlike native European conspecifics, female wild pigs in the USA often reproduce at less than one year of age, bare larger litters, and produce multiple litters a year when conditions are ideal (Wood and Barrett 1979, Delgado-Acevedo et al. 2010, Chinn et al. 2021). Research has reported that *S. scrofa* can and do have litters that are sired by multiple males; however, the rate of multiple paternity is reportedly lower in European wild boar than wild pigs (Delgado et al. 2008, Delgado-Acevedo et al. 2010). In populations across the USA, wild pigs with higher European wild boar ancestry are proportionally shorter than their conspecifics with higher domestic ancestry, but the wild pigs with higher domestic ancestry have highly diverse physical characteristics (Mayer et al. 2020*b*). In particular, the length of the ear, head to body, and total length tends to be shorter in individuals with great ancestral associations to European wild boar, but these individuals tend to have longer snouts, longer hind feet, and higher shoulder height (Mayer et al. 2020*b*). Klimiene and Klimas (2010) reported that hybrids grew slower and differed in muscle composition from purely domestic pigs. Intriguingly, hybridized neonates and piglets with the striped naturalistic pelage of immature European wild boar have higher survival than conspecifics with non-striped pelage characteristic of immature domestic (Mayer et al. 2020*b*, Chinn et al. 2021). Within wild admixed populations in their native range, European wild boar only behaved aggressively toward group members that were domestic-boar hybrids and typically restricted the movement of morphologically different hybrid sounder members.

(Battocchio et al. 2017). Battocchio et al. (2017) suggested that European wild boar sounder members respond aggressively to hybrids to limit their prominence to predators (Battocchio et al. 2017).

A comprehensive overview of the organization and composition of wild pig social units and desirable reproductive traits is important for the holistic understanding of their life history, and ultimately impact how they are managed. There is limited knowledge on the influence of differences in resource availability on group size and membership. Additionally, little is known about the impacts of domestic versus wild ancestry on wild pig reproductive success. Further, within the current literature, there is little utilization of genetic tools to investigate prior suppositions that social organization and male reproductive success of wild pigs reflect that of European wild boar in their native range (Beasley et al. 2018, VerCauteren et al. 2020).

For my thesis, I used genetic samples collected over a several year period from wild pig populations in multiple discrete study areas across the southern USA to describe the composition of invasive wild pig social groups, as well as investigate the impact of age and genetic ancestry on individual male wild pig reproductive contribution. Currently, there has yet to be a study that utilizes robust genomic tools to understand invasive wild pig social group composition, fecundity of male invasive wild pigs, or the relatedness within male wild pig social groups. Because European wild boar evolved under natural selection in their native range and domestic pigs were bred under artificial selection, I predicted that the percentage male wild pig lineage associated with European wild boar ancestry would be positively correlated with the number of assigned litters. Likewise, many promiscuous mammals have found that older males sire more often than younger males. Hence, I predicted that male age would also be positively correlated with the number of assigned litters. This study will be the first to utilize single polymorphic loci (SNP) and robust genetic tools to describe social groups and examine the reproductive success of male wild pigs in the introduced USA range.

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CHAPTER 2

GENOMIC TOOLS REVEAL COMPLEX SOCIAL ORGANIZATION OF AN INVASIVE LARGE MAMMAL (SUS SCROFA)

Titus, C. L., Pierce, C. F., Smyser, T. J., Webb, S. L., and Beasley, J. C. 2021. To be submitted to *Biological Invasions*.

Abstract

A comprehensive understanding of sociality in wildlife is vital to optimizing conservation and management efforts. However, sociality is complicated, especially for widely distributed species that exhibit substantive behavioral plasticity. Often representing hybrids of European wild boar and domestic pigs, invasive wild pigs (*Sus scrofa*) are among the most adaptable and widely distributed large mammals. Wild pig social structure is believed to be similar to European wild boar, consisting of matriarchal groups (sounders) and solitary males. However, wild pig social structure is understudied with descriptions largely limited to observational studies. Using a hierarchical approach, we incorporate genomic tools to describe wild pig social group composition in two distinct ecoregions within their invaded range in North America, Savannah River Site, South Carolina (SRS) and Noble Research Institute in Oklahoma (NRI). Sounders were the most common social unit (SRS = 56.8%, NRI = 38.5%), but pseudo-solitary females (SRS = 31.1%, NRI = 23.1%) and male-dominated bachelor groups (SRS = 10.8%, NRI = 38.5%) occurred with greater frequency than previously reported. Though primarily composed of close female kin (SRS = 55.6%, NRI = 20.0%), some sounders included unrelated females - contrary to previous assumptions for this species (SRS = 4.4%, NRI = 0%). Bachelor groups were predominantly composed of young, dispersal-aged males and almost always included only close kin (SRS = 50.0%, NRI = 60.0%). Collectively, our study suggests social organization of wild pigs in their invaded range is similar to that described for wild boar but is complex, dynamic, and likely variable across invaded habitats.

Introduction

Sociality is the tendency for individuals to live within a group and exhibit coordinated behavioral patterns, conveying both benefits and costs to group members (Silk 2007; Prox and Farine 2020). Benefits of sociality may include “safety in numbers,” increased access to resources, or increased offspring survival (Krause et al. 2002; Silk 2007). On the other hand, group members may compete for mating opportunities (Cafazzo et al., 2016), be at greater risk of disease transmission (Moore et al., 1988), or be more visible to predators (Krause and Godin, 1995), all of which can influence their individual fitness (Krause et al. 2002; Silk 2007; Prox and Farine 2020). Thus, social structures persist in a species when the benefits of intraspecific interactions outweigh the costs (Krause et al. 2002; Silk 2007).

Social structures can be as varied as the animals that display them, with differences observed even within a taxon. Among mammals, social structures encompass subsocial species (i.e., primarily living alone, except briefly for mating or rearing offspring; jaguar – *Panthera onca*; Cavalcanti and Gese, 2009), parasocial species (i.e., primarily living in a group cooperatively; African lion – *Panthera leo*; Dickinson and Koenig 2018), and species with complex eusocial societies (i.e., hive or colonial cooperation, majority of colony members forgo reproduction to collectively care for and assist the minority reproductive caste; Damaraland mole-rats – Crytomys damarensis; Nowak et al. 2010, Dickinson and Koenig 2018). Among social species, group organization and composition are important life history traits that drive interspecific interactions, and ultimately conservation and management decisions. However, costs and benefits associated with group membership can be dynamic across both space and time, contributing to greater diversity in social organization across a species’ range (Gehrt and Fox 2004).

Describing social organization of a species becomes further complicated when the species is found globally in both domestic and free-living populations and in native and introduced ranges. Wild pigs (*Sus scrofa*) are one of the most widely distributed species globally, with populations of wild boar or wild pigs occurring on all continents except Antarctica (Barrios-Garcia and Ballari 2012; Lewis et al. 2019). Wild pigs include free-living domestic pigs (either released or escaped, feralized), introduced wild boar, but most frequently wild boar-domestic pig hybrids (hereafter referred to as "wild pigs;" Keiter et al. 2016). The social organization of European wild boar is complex, but social units are primarily believed to be characterized as matriarchal social groups (referred to as sounders) or solitary males that only temporarily associate with sounders to mate (Dardaillon 1988; Kaminski et al. 2005; Iacolina et al. 2009; Poteaux et al. 2009; Podgórski et al. 2014a, b; Battocchio et al. 2017; Beasley et al. 2018). Dardaillon (1988) documented a third social unit comprised of males believed to be young, dispersing individuals transitioning to solitary, breeding-aged adults, which has since been observed in other populations. Most studies have reported that sounders are typically composed of several closely related females and their offspring; however, such determinations have largely been made through observational studies and have yielded conflicting reports (Dardaillon 1988; Poteaux et al. 2009; Podgórski et al. 2014a, b; Battocchio et al. 2017). For example, Iacolina et al. (2009) found that wild boar social groups in Italy consisted mostly of unrelated females and their offspring. Podgórski et al. (2014*a*, *b*) found that social groups in Poland were comprised of individual females and their offspring, whose home ranges overlapped with those of neighboring relatives. Similarly, adult female Japanese wild boar (*S. s. leucomystax*) were found to occasionally group together but were typically either solitary or with their current litter of offspring (Nakatani and Ono 1994).

Within North America, wild pigs are a destructive invasive species descending from domestic pigs. Swine were first introduced as livestock in 1539 and subsequently established widespread feral populations, later hybridizing with escaped European wild boar that were introduced for sport hunting (Mayer and Beasley 2018; Mayer et al. 2020). As a result, contemporary wild pig populations have come to generally represent extensive admixture of wild boar and domestic pig lineages (Keiter et al. 2016; Smyser et al. 2020). Throughout the United States of America (USA), broad variability in the hybridization of domestic versus wild lineages has been observed both within and among wild pig populations (Smyser et al. 2020). With humans artificially selecting different phenotypic traits from specialized domestic lineages and natural selection of adaptive traits in hybridized wild populations, the extent to which wild pig social organization differs from that of European wild boar in their native range is difficult to predict and has not been described in the literature. Within the USA, wild pigs are presumed to reflect the social organization of European wild boar and sounders are assumed to be primarily composed of closely related females and their dependent offspring. Observational studies of wild pigs in the USA has demonstrated that sounders vary in size and demographic composition (Mayer and Brisbin 2009; Poteaux et al. 2009; Beasley et al. 2018; Gaskamp et al. 2021); ranging from a single female with offspring to sounders of over 30 pigs with 3-9 individuals typically reported. The underlying factors contributing to variance in sounder size are largely unexplored, although some studies have suggested water availability may influence group size in wild pigs (Gabor et al. 1999; Gaskamp et al. 2021). It is worth noting that these reports are often limited to behavioral observations and lack strong supported genetic agreement across studies (Gabor et al. 1999; Mayer and Brisbin 2009; Delgado-Acevedo et al. 2010). As with European wild boar, previous studies have observed male social groups within wild pig populations;

however, the frequency of occurrence, composition, and dynamics of these male coalitions is relatively unknown and thought to be temporary associations (Gabor et al. 1999; Mayer and Brisbin 2009).

A detailed description of the relatedness and demographic composition of wild pig social units (inclusive of solitary pigs and pig social groups) is important for understanding the broader population dynamics of this taxon. Little work has been done on wild pig social organization outside of the arid region in their USA range (Beasley et al. 2018), with limited understanding of how social group size and composition may differ with variations in resource distribution. Among the available studies, there is little utilization of genomic tools to investigate previously held assumptions of the relatedness displayed in social groups. However, the incorporation of genomics would help resolve the complexity of variation in group composition across different ecosystems. Using samples collected across two disparate ecoregions within the invaded range in the USA, our study aims to describe the composition of invasive wild pig social groups and the extent to which differences in habitat may influence the social organization of this species. Our study is the first to utilize robust genomic tools to understand invasive wild pig social group composition, as well as the first to investigate the relatedness composition of male wild pig social groups.

Methods and Materials

Study Areas

South Carolina

Wild pigs were captured on the Savannah River Site (SRS) near Aiken, South Carolina, USA from 2012-2019 as part of ongoing population control and research efforts. The SRS is an

802.67 km^2 restricted access site managed by the USA Department of Energy (DOE). Originally constructed for nuclear weapons manufacturing, the industrial footprint of SRS comprises a small portion of the landscape, with >90% of the site representing natural habitats including upland pine (*Pinus spp.*), cypress-tupelo (*Taxodium districhum-Nyssa aquaticus*) or oak-hickory (*Quercus spp.-Ulmus spp.*) bottomland hardwoods, shrub/herbaceous areas (*Myrica cerifera, Ilex spp.*, *Vaccinium corymbosum*), and upland/mixed forests (*Quercus spp.*, *Pinus spp.*; Imm and McLeod 2005). The climate is generally warm and humid, with average monthly daily high temperatures ranging from 15.4-33.4°C, average monthly relative humidity ranging from 63-80%, and average annual precipitation of approximately 1,200 mm (Imm and McLeod 2005). The wild pig population predates the establishment of SRS by the USA DOE in the 1950's. Individuals within the population are characterized as hybrids between Western heritage breeds and wild boar, however there is considerable variation in the extent of European wild boar ancestry among individuals (Mayer et al. 2020; Smyser et al. 2020). Since the establishment of the SRS, wild pigs have been intensively controlled to minimize damages through a combination of trapping, opportunistic shooting, and hunting with dogs (Mayer et al. 2020). Despite being managed since SRS's establishment, the wild pig population has expanded substantially over the past several decades (Mayer et al. 2020). Wild pigs are now abundant and widely distributed throughout SRS, with an estimated abundance of >5,000 individuals as of 2017 (Keiter et al. 2017).

Oklahoma

Wild pigs were captured from 2010-2017 in south-central Oklahoma (Carter and Love counties) as a component of ongoing research and control efforts at the Noble Research Institute's (NRI) Coffey Ranch (10.17 km^2), Oswalt Road Ranch (20.77 km^2), and Red River

Ranch (13.39 km^2), in addition to the Strate Ranch (3.90 km^2), Hoffman Ranch 99.35 km^2), and Ljungdahl Ranch (2.33 km^2). These ranches are found in the Cross Timbers and Prairies regions of Oklahoma and incorporates a mix of oak (*Quercus spp.*), elm (*Ulmus spp.*), and hickory (*Carya spp.*) forest and prairie habitat dominated by big and little bluestem (*Andropogon gerardii and Schizachyrium scoparium*, respectively), switchgrass (*Panicum vigatum*), and Indian grass (*Sorghastrum nutans*), as well as agricultural and rangelands. Wild pigs are widely distributed and abundant across the Cross Timbers and Prairies regions and have been intensively controlled since 2010 (using various methods; Stevens 2010; VerCauteren et al. 2020; Gaskamp et al. 2021). Recent studies estimate that there are approximately 1.6 million pigs in Oklahoma (Mayer 2014; VerCauteren et al. 2020b; Gaskamp et al. 2021).

Field Methods

We collected genetic samples from pigs that were captured as part of other research and control efforts, and detailed descriptions of capture and handling procedures have been described previously (e.g., Gaskamp et al. 2016, 2018, 2021, Keiter et al. 2017, Haydett et al. 2021, Yang et al. 2021). Briefly, we captured wild pigs from 2012 to 2019 at SRS with drop nets and/or corral traps, and from 2010 to 2017 at NRI using drop nets, corral traps, and suspended metal traps. We set and monitored corral traps at SRS primarily via cellular cameras to identify and target entire social groups or solitary males. For NRI, we used dual compartment corral traps with tripwires to trigger either a single spring or saloon style gate, suspended metal traps trigger using internet capable monitoring and offsite remote control, and we triggered drop net traps with a line-of-sight remote control (Gaskamp et al. 2021). Individuals were included in the study were trapped using live sampling and release, as part of other ongoing research projects, and samples collected from euthanized animals ancillary to other control and management activities. At time of capture, we recorded weight, sex, and age based on tooth rupture (0–0.5 year [piglet], 0.5–1 year [juvenile], 1–1.5 years [yearling], 1.5–3 years [subadult], and >3 years [adult], Matschke 1967; general weight estimates recorded in the field when tooth rupture was unavailable). Furthermore, hair (NRI) and/or tissue (i.e., pinnae, muscle, etc.; SRS, NRI) was also collected for genotyping. Tissue samples were stored in vials with 99.5% ethanol at -70°C or vials without ethanol at -18°C and hair samples were stored in paper coin envelopes at -18°C.

Social Unit Delineation

We assigned individuals captured at the same time/location to a social group if at least one breeding-age individual (e.g., yearling, subadult, or adult) was included in the trapping event. When available, we used telemetry or remote camera image data to validate group membership. We used the sex ratio to categorize social groups as either female dominant (including groups with equal sex ratio; sounders) or male majority (bachelor groups). We used other social unit descriptors (pseudo-solitary females and pseudo-solitary males) to classify breeding-aged individuals that were captured with extraneous group members (e.g., offspring, suitor males) and are essentially not a stable social group. We included all samples of individuals collected at each study site to determine population structure to adjust genomic pairwise relatedness; however, we only used samples of individuals captured as social groups (including pseudo-solitary individuals) to determine degrees of relatedness within groups. Because they were captured with other individuals (offspring and/or suitors), degrees of relatedness were also assigned to dyads of pseudo-solitary individuals.

Genetic Methods

Following extraction of DNA with MagMax DNA extraction kit (Thermo Fisher Scientific), we genotyped samples using GeneSeek's Genomic Profiler for Porcine 68,516 biallelic single nucleotide polymorphism (SNP) array (GeneSeek, a Neogen Company, Lincoln, Nebraska) developed with Illumina (San Diego, California) bead array chemistry (Ramos et al. 2009). We mapped biallelic single nucleotide polymorphisms to the Sscrofa11.1 reference genome assembly (Warr et al. 2019), removed unmapped or non-autosomal SNP, which provided 62,128 loci for analysis. We then implemented quality control measures using PLINK 1.9 (Purcell and Chang 2015) to remove loci out of Hardy-Weinberg equilibrium (p=1e-06), those with a call rate <0.99, and minor allele frequency <0.05 and pruned individuals missing >0.05 of loci. Furthermore, we removed loci in linkage disequilibrium (window size=50 loci, step size=5 loci, $r^2>0.5$). Following quality control measures, SRS retained 963 individuals genotyped at 17,474 SNP and NRI retained 401 individuals genotyped at 14,256 SNP.

Independently for each study area, we estimated pairwise genomic relatedness (r) for all sampled pigs using the methods described in Conomos et al. (2015, 2016). Specifically, through an iterative process executed in the R package Genesis (Gogarten et al. 2019; R Core Team 2020), we used PC-Air to conduct a principal component analysis (PCA) on a subset of unrelated individuals in the dataset. We then used PC-Relate to incorporate the principal components (PC) generated by PC-Air to adjust estimates of pairwise relatedness to account for any potential population structure in the study area with the number of PC to be retained determined by: 1) eigenvalues and associated scree plots and 2) self-kinship based on the expectation that values should be distributed around one (Simeone et al. 2011).

To quantify relatedness among wild pig social groups, we evaluated all dyads within each social group. For each dyad, we used the degrees of relatedness, as delineated in Ramstetter et al. (2017), to classify up to third-degree relatives. Based on these classifications, we considered first-degree relationships (r=0.177–0.354) to encompass parent-child and full, non-monozygotic siblings. Second-degree relationships (r=0.088–0.177) included grandparent-grandchild, avuncular, double-cousins, and half-siblings. Third-degree relationships (r=0.044–0.088) encompassed first-cousins, great-grandparent-great-grandchild, grand-avuncular, and half-avuncular (Ramstetter et al. 2017). More distant relationships (r<0.044) could not be confidently discerned as previous work that combined genetic estimates of relatedness with pedigree data demonstrated increasingly smaller expected ranges of relatedness values and decreasing overlap with observed relatedness values (Ramstetter et al. 2017). We assembled relatedness networks for each social group that was identified based on the degree of relatedness.

Hierarchal Analyses

For each study area, we then identified the following demographic composition for each group: group size, sex ratio, age composition, degree of relatedness composition, and mean group genomic relatedness. Given that wild pigs can reproduce year-round throughout much of their invaded range, breeding-aged boars (hereafter referred to as suitor males) are often captured with female-dominated social groups but are not actual group members. Unrelated suitor males were then identified as breeding-aged males that shared a pairwise relatedness of less than 0.0443 with all other group members they were captured with. Further, because sounders often are numerically dominated by juveniles due to the high reproductive capacity of this species, genomic relatedness values within groups can be highly dependent upon the occurrence and number of offspring present at the time of sampling. Therefore, to account for these factors and establish a comprehensive understanding of socio-genomic relationships within groups, we applied the following hierarchical approach in the analyses of our data: 1) all group membership included, 2) nonbreeding-aged individuals excluded (i.e., piglets and juveniles), and 3) nonbreeding-aged individuals and unrelated, breeding-aged males excluded (i.e., suitor males). We then compared how group demographic and relatedness composition differed between SRS and NRI datasets across each of these hierarchies.

Results

Savannah River Site

Adjusted pairwise genetic relatedness values were calculated for 963 individuals with 17,474 SNP and three PC (Supplementary Figure 2.1). We assigned 297 of the 963 genotyped individuals and an additional 4 individuals that were not genotyped to 77 social groups (e.g.: female dominated groups, male majority groups) for demographic analyses. The remaining individuals were excluded due to capture as solitary individuals, incomplete social groups (i.e., only piglets and/or juveniles were captured), or individuals that were missing age or other necessary capture information. For relatedness analyses, we excluded the four non-genotyped individuals. Overall, we found that female dominated groups were the most common group types across all analysis hierarchies; however, male majority groups remained present in all hierarchies (Supplementary Figure 2.4).

All Group Members Included

The first hierarchy of analysis considered all individuals captured together. This included all 301 individuals assigned to 77 social groups for demographic analysis and 296 individuals assigned to 76 groups for the genomic relatedness analysis. Based on demographic analysis, most social groups were classified as female dominated groups (83.1%; Supplementary Figure 2.4). The average group size (*n*) was 3.9 individuals (Table 2.1), and groups were typically composed of only breeding-aged (i.e., yearling or older; 51.9%; Figure 2.1), first-degree relatives (35.5%; Figure 2.2). However, 13.0% of all groups included at least one unrelated group member. Female dominated groups (sounders) had an average of 3.8 individuals and an average group r of 0.203 (Table 2.1). Sounders were most commonly composed of only breeding-aged (50.0%; Figure 2.1), first-degree relatives (33.3%; Figure 2.2). However, sounders that displayed a majority of first-degree dyads were the second most common relatedness composition (25.4%; Figure 2.2). Groups of only unrelated group members made up only 9.5% of all sounders. Interestingly, it was not uncommon for sounders to include at least one completely unrelated group member (17.2%), which were typically males. The remaining 16.9% of social groups were male majority groups (bachelor groups; Supplementary Figure 2.4). Bachelor groups had an average of 4.6 individuals and an average group r of 0.271 (Table 2.1). Bachelor groups were also typically composed of only breeding-aged (61.5%; Figure 2.1), first-degree relatives (46.2%; Figure 2.2). Unlike sounders, bachelor groups were unlikely to include an unrelated individual (7.7%; Figure 2.2). Only one bachelor group included a completely unrelated group member.

Nonbreeding-aged Group Members Excluded

The second hierarchy of analysis removed nonbreeding-aged group members (proposed offspring) from consideration. The remaining 144 group members assigned to 53 groups were considered for demographic analysis and 140 group members assigned to 52 groups were considered for relatedness analysis. The average group size decreased to 2.2 individuals and average group r decreased to 0.203 when compared to the results of the first hierarchy of analysis, however the range of r remained unchanged (Table 2.1). Furthermore, 28.4% of groups were composed of a single breeding-aged individual with nonbreeding-aged group members (i.e., pseudo-solitary individuals), and were excluded from the second hierarchy of analysis (Supplementary Figure 2.4). The average change in group r (Δr) was -0.004, but the largest Δr was -0.276. Groups of only first-degree (40.4%; Figure 2.2), female (60.8%; Supplementary Figure 2.4) relatives were the most common group composition. Under the second hierarchy of analysis, one bachelor group was reclassified as a sounder. The average sounder size was 2.2 individuals, and the average sounder relatedness was 0.198 (Table 2.1). Sounders of only first-degree relatives remained the most common group relatedness composition (38.6%; Figure 2.2). However, sounders of only unrelated group members were also common (15.9%; Figure 2.2), and 20.5% of sounders included at least one unrelated group member. Furthermore, bachelor groups accounted for 10.8% of the dataset analyzed under the second hierarchy (Supplementary Figure 2.4). Like sounders, the average group relatedness and average group size for bachelor groups also decreased (Table 2.1). Bachelor groups of only first-degree relatives also remained the most common group relatedness composition (50.0%; Figure 2.2). None of the bachelor groups included a completely unrelated group member.

Nonbreeding-aged And Unrelated Male Group Members Excluded

The third hierarchy of analysis removed both proposed offspring and suitor males from consideration, resulting in 133 group members assigned to 50 groups considered for demographic analysis and 129 group members assigned to 49 groups considered for relatedness analysis. Like the second hierarchy, 32.4% social groups were removed from consideration as pseudo-solitary individuals (Supplementary Figure 2.4). The average group size decreased to 2.1

individuals, but the average group r increased to 0.238 (Table 2.1). The largest Δr was -0.276 ($\Delta r_{average}$=-0.025). More than half of all groups were composed of only first-degree relatives (54.7%; Figure 2.4), and 3.8% of groups still included a completely unrelated group member. Without proposed offspring and suitor males, the average group size of sounders was 2.7 and average relatedness was 0.239 (Table 2.1). Sounders of only first-degree relatives were most common group demographic under the third hierarchy (55.6%; Figure 2.4). However, two of the remaining sounders (4.4%) still retained at least one unrelated group member. Another sounder consisted of an adult male and female that were first-degree relatives with an unrelated subadult female. The last group consisted of two adult females and one subadult female that were first-degree relatives associating with an unrelated adult female. Under the third hierarchy, 10.4% of the sampled groups were bachelor groups (Supplementary Figure 2.4). The average bachelor group size and average bachelor group relatedness did not change from the second hierarchy of analysis (Table 2.1). As with the second hierarchy, bachelor groups of only first-degree relatives remained the most common relatedness composition (50.0%; Figure 2.4), although one bachelor group was a pair of unrelated yearling males. Another bachelor group was composed of a subadult female with four subadult males that displayed variable degrees of relatedness within their group (Figure 2.3).

Noble Research Institute

We estimated adjusted pairwise genetic relatedness values for 401 individuals from southcentral Oklahoma using 14,256 SNP and 2 PC (Supplementary Figure 2.2). We assigned 386 of the 401 individuals to 40 social groups. The remaining 15 individuals were captured alone and excluded from analysis. Sounders were the most common group type under the first and second hierarchies of analysis but were evenly represented with bachelor groups under the third hierarchy of analysis (Supplementary Figure 2.4). Bachelor groups were more likely to have a higher group relatedness than sounders. Generally, southcentral Oklahoma social groups were typically either all first-degree relatives or displayed a majority of first-degree dyads and were not likely to include an unrelated group member; however, some groups were composed of only unrelated individuals (Figure 2.4). Furthermore, 31 social groups (334 individuals) were excluded due to incomplete social groups (i.e., only piglets and/or juveniles were captured) and/or individuals that were missing age or other necessary capture information. It is worth mentioning that among the excluded social groups, many were very large (Supplementary Table 2.1).

All Group Members Included

Under the first hierarchy, we considered all 13 social groups and their 67 group members for both demographic and relatedness analyses. Average group size was 5.2 individuals (Table 2.1). Sounders were slightly more common than bachelor groups (53.8% and 46.2%, respectively; Supplementary Figure 2.4). Southcentral Oklahoma groups were typically composed of only breeding-aged individuals (38.5%) or did not display an age majority (30.8%). Groups with a majority of nonbreeding-aged individuals were uncommon and those of a majority of breeding-aged individuals were rare (23.1% and 7.7%, respectively; Figure 2.1). The average group relatedness across all groups with all group members included was 0.197 (Table 2.1). The most common group relatedness composition were groups that displayed a majority of first-degree dyads (30.8%; Figure 2.4). However, groups of either only first-degree relatives or only unrelated individuals were not uncommon (23.1% and 23.1%, respectively; Figure 2.4). Only one group that included relatives had an unrelated group member (7.7%). Sounders represented

53.8% of all social groups, with the remaining consisting of bachelor groups (Supplementary Figure 2.3). The average sounder size was 4.1 individuals, and the average relatedness was 0.114 (Table 2.1). Most sounders were composed of only breeding-aged individuals (57.1%; Figure 2.1). Interestingly, nearly half of all sounders were typically composed of only unrelated group members (42.9%; Figure 2.4). However, sounders that included kin were unlikely to also include a completely unrelated group member. It must be considered that this may be an artifact of our low sample size. Bachelor groups made up 46.2% of all southcentral Oklahoma social groups (Supplementary Figure 2.4). The average bachelor group size was slightly larger than sounders (Table 2.1). Bachelor groups were typically composed of either a majority of nonbreeding-aged individuals or did not display an age majority (33.3% and 33.3%, respectively; Figure 2.1). As with average group size, the average bachelor group relatedness was also higher than sounders (Table 2.1). Bachelor groups of either a majority of second-degree or unrelated dyads were the most common group relatedness composition (16.7% and 16.7%, respectively; Figure 2.4). The only group with kin that included an unrelated group member was a bachelor group.

Nonbreeding-aged Group Members Excluded

After the second hierarchy of analysis, the average group size for southcentral Oklahoma greatly decreased (Table 2.1). Sounders remained more common than bachelor groups (46.2% and 38.5%, respectively; Supplementary Figure 2.4). However, 15.4% of social groups were found to be pseudo-solitary (Supplementary Figure 2.4). These females were likely caught with their dependent offspring; and were further removed from consideration. The average group r increased under the second hierarchy (Table 2.1). Groups generally experienced little change in group r ($\Delta r_{average}$=-0.028, Δr_{max}=-0.337). As with the first hierarchy of analysis, groups of only first-degree relatives were the most common group relatedness composition (45.5%; Figure 2.4).

Sounders had an average group size of 2.8 individuals and average group relatedness of 0.174 (Table 2.1). Sounders were most typically composed of a majority of first-degree dyads or only unrelated individuals (33.3% and 33.3%, respectively; Figure 2.4). However, sounders with kin did not also include an unrelated group member, they were only composed of close or distantly related individuals. Bachelor groups had an average group size of 3.2 individuals and average group relatedness of 0.349 (Table 2.1) under the second hierarchy of analysis. Unlike sounders, groups composed of only first-degree relatives were the most common among bachelor groups (60.0%; Figure 2.4), with a single bachelor group containing an unrelated member.

Nonbreeding-aged and Unrelated Male Group Members Excluded

For the third hierarchy of analysis, the average group size was 2.6 individuals and average group r was 0.260 (Table 2.1). With the removal of potential offspring and suitor males, 23.1% of social groups were removed from consideration (Supplementary Figure 2.4). Southcentral Oklahoma groups typically had a decrease in average group r from the first hierarchy of analysis ($\Delta r_{average}$=0.002, Δr_{max}=-0.327). Groups of only first-degree relatives were the most common (40.0%; Figure 2.4). The sounders that persisted under the third hierarchy accounted for 38.5% of the dataset (Supplementary Figure 2.4), had an average group size of 3 individuals, and average group relatedness of 0.172 (Table 2.1). Sounders of only first-degree relatives, majority of first-degree dyads, only unrelated group members, a majority of unrelated dyads, and no relatedness majority composition were all equally represented. Unlike SRS, no sounders that primarily were comprised of kin included an unrelated group member (Figure 2.4). Only one group under the third hierarchy of analysis included adult males (Figure 2.5). This group displayed variable relatedness among its members, and was composed of two adult males, two subadult females, and three yearling females. Bachelor groups comprised 38.5% of the dataset (Supplementary Figure 2.4). There was no change between the second and third hierarchy of analyses in the average or range of group size and relatedness (Table 2.1).

Discussion

Despite their widespread distribution and extensive ecological and economic impacts, the social organization of invasive wild pigs has been understudied and largely limited to observational behavioral data. Using robust genomic tools, our results revealed that previously held assumptions of invasive wild pig social organization were generally correct; however, these assumptions did not fully capture the diversity in group composition observed within populations. Across both geographic areas sampled for this study, wild pig social units consisted of female dominated social groups (sounders), solitary females (both with and without dependent offspring; pseudo-solitary females), male majority groups (bachelor groups), and solitary males. Aside from solitary males, female dominated groups were the most common social group observed across our two study sites. Although adult females in these groups were often close kin (first-degree relatives), many of these groups also included unrelated breeding-aged females – counter to previous assumptions. In southcentral Oklahoma, we captured groups comprised of only unrelated individuals, but groups of female kin did not include non-kin females. Even though there were many sounders of only unrelated females, kin-only sounders were still more common in southcentral Oklahoma. This tendency for females to form sounders with close kin has also been reported in European wild boar across their native range; however, few studies have found that these groups will recruit unrelated females (Kaminski et al. 2005; Iacolina et al. 2009; Podgórski et al. 2014b). We also found that many adult females were captured with only their offspring or unrelated breeding-aged males, suggesting the size and composition of groups is dynamic and variable within populations. Furthermore, males that were identified as unrelated capture members remained classified as unrelated under all three hierarchies of analysis, and thus could not have been sires of any piglets and/or juveniles present. Though they occurred less frequently, we also found that male majority groups were prevalent within populations and primarily comprised of related young (likely dispersing) individuals. Though our study can only describe social groups at the time of their capture, we have demonstrated invasive wild pigs display more complex and diverse social organization than previously believed.

Similar to wild boar (Iacolina et al. 2009; Podgórski et al. 2014a, b), matriarchal social groups (i.e., sounders) were the dominant social unit across both study sites. Sounders were highly variable in size depending on the number of offspring present, ranging from 2–45 individuals. The largest group captured at SRS had 16 individuals with an average group size of approximately 4 individuals when offspring and suitors were included. When these individuals were excluded, social groups at SRS were typically composed of two breeding-aged relatives. The largest group captured in southcentral Oklahoma was 45, however this group, as well as many other large social groups at this location, could not be included in our genomic dataset because age and/or sex was not recorded for many group members at time of collection. Therefore, we could not discern core group members from temporary associations (e.g., pre-dispersal aged offspring). In fact, >40% of groups captured in southcentral Oklahoma had over 10 individuals, and approximately 10% had over 20. Within their native range, average group size for European wild boar generally ranges from 4 to 9 individuals, depending on the location (Dardaillon 1988; Poteaux et al. 2009; Podgórski et al. 2014a, b; Battocchio et al. 2017), although studies do not always explicitly state whether offspring are included in these calculations. Thus, while groups at SRS were generally smaller than those reported for European wild boar, sounders in southcentral Oklahoma were more comparable in size to groups within the native range of this species. Studies of wild pigs in Texas and Oklahoma also have reported large social groups (>5 individuals; Gabor et al. 1999, Wehr et al. 2018, Gaskamp et al. 2021). The propensity for invasive wild pigs to form smaller groups in the Southeastern USA may be due to differences in resource distributions, management strategies, or genetic lineages compared to more arid regions of the USA. Productive bottomland swamps are abundant and widely distributed throughout much of the Southeastern USA and have a strong influence on wild pig movements and population dynamics (Keiter et al. 2017; Clontz et al. 2021). Furthermore, wild pigs on the SRS are intensively managed to reduce collisions with vehicles and minimize other damages (Beasley et al. 2014), and individuals within the SRS generally have lower wild boar ancestry than those individuals encountered in the NRI population (Smyser et al. 2020). More in-depth studies are needed to better understand how ancestry, management strategies, and landscape attributes, as well as other biotic and abiotic factors, may influence sounder sizes across the distribution of this species.

Interestingly, our data revealed wild pig group members are not always closely related to each other and may include unrelated individuals. Sounders at SRS were most commonly composed of only first-degree relatives; however, nearly half of all SRS sounders displayed variable relatedness. Many groups included group members that were first-degree relatives to some and distantly related or unrelated to others (Figure 2.3). Furthermore, 6.8% of SRS sounders included an unrelated breeding-aged female. Telemetry data collected for these groups as part of related studies (Beasley et al. 2021; Clontz et al. 2021) further confirmed these unrelated group members were not temporary associations, but established group members. Some populations of European wild boar also exhibit tolerance of unrelated females within sounders, but this tolerance is not shared across their range (Iacolina et al. 2009; Podgórski et al. 2014a, b). Conversely, southcentral Oklahoma sounders typically displayed dynamic relatedness, in which not all group members were closely related to all other group members (Figure 2.5).

Despite the dynamic relatedness exhibited within southcentral Oklahoma sounders, groups of kin were not likely to include a completely unrelated individual. Some studies on European wild boar have suggested frequent hunting or population control efforts may disrupt the social structure of sounders and increase the acceptance of an unrelated individual into social groups (Poteaux et al. 2009; Podgórski et al. 2014a). Though our two study sites both implement control strategies, the sites vary in the frequency and type of removal events. In particular, the majority of wild pigs removed from the SRS are captured in box traps or small corral traps, methods that often result in partial removal of sounders, whereas whole-sounder removal efforts are more common at NRI (Gaskamp et al. 2021). Disruptions to social units due to partial removal of sounders may facilitate the integration of unrelated members within social groups at the SRS and other areas that employ similar management strategies. Indeed, the majority of sows on the SRS translocated to novel areas as part of a study to assess the movement ecology of translocated wild pigs had integrated within a new (unrelated) sounder. These newcomers are adopted into their new sounders within 1-2 months following translocation (J. Smith, unpublished data). Interestingly, McIlraith (2021) found support for fission-fusion dynamics among members within sounders from four southern USA populations using GPS collars to estimate contact heterogeneity and cohesiveness of dyads (pairs of pigs) based on movement metrics. Wild pigs captured together, and subsequently collared, tended to be associated with one another more than pigs between sounders. However, even collared pigs from the same capture sounder exhibited irregular separation periods. Therefore, it may be possible that full descriptions of sounder relatedness reported herein may not have been available given the genetic ‘snapshot’ provided by our dataset, which consisted mostly of pigs captured and removed from the population. Future research is needed to link genetically related individuals with movement data to better understand fission-fusion dynamics and social structure. Landscape features also may influence the structure of sounders. Past studies have suggested habitat quality and access to resources could affect the size of sounders and the recruitment of an unrelated individual (Delgado et al. 2008; Gaskamp et al. 2021). Differences in landscape features between southcentral Oklahoma and SRS may account for the different relatedness compositions between the two sites. The SRS is generally wetter and more humid than southcentral Oklahoma and encompasses a mixture of upland and bottomland woodlands. Future studies should further investigate if differences in resource availability and/or removal pressure can account for the disparity in sounder relatedness between locations.

In addition to sounders, we observed individual adult females, which were captured with either dependent offspring, suitor males, or both in southcentral Oklahoma and SRS. This type of group demographic is thought to be composed of temporary associations as suitor males are not likely to remain with a female for more than a week or two and offspring often disperse as older juveniles or yearlings. Among these pseudo-solitary groups, some included offspring that were a few weeks old. These females may be temporarily separated from their sounder for parturition (Gabor et al. 1999; Mayer et al. 2020). This secretive behavior has been observed in studies of European wild boar (Dardaillon 1988; Kaminski et al. 2005; Poteaux et al. 2009). Indeed, we were able to confirm from camera data that some of these females had been in association with an uncaptured female prior to parturition (Clontz et al. 2021). However, other pseudo-solitary groups included offspring that were several months old, suggesting not all of these groups were temporary disassociations from larger groups during parturition. Gabor et al. (1999) demonstrated that female dispersal does occur, though rare. There remains a strong possibility that social organization for wild pig females may include both sounder and pseudo-solitary females, although the fission-fusion dynamics of wild pig social groups remains largely unknown. We are limited by our sampling strategy and can only describe the social organization of these individuals at the time of capture when no camera is available. Even so, through use of genomic data we can highlight the complexity of sounder composition and diversity in female social units with greater resolution than described in past studies.

Though not as common as female dominated groups, our study also revealed male majority groups (i.e., bachelor groups) are relatively common, and primarily composed of small, breeding-aged kin. Past studies have mentioned the observation of bachelor groups were rare (e.g., Gabor et al. 1999). However, these studies, as well as ours, largely targeted sounders or solitary adult males as part of ongoing research and management, and thus male-dominated groups undoubtedly occur more frequently than reported herein or in the prior literature. Although typically composed of only closely related males, a few bachelor groups in our study included a related female and one group included an unrelated female. Unlike sounders, bachelor groups were highly unlikely to include an unrelated group member, and none were composed of only unrelated individuals. Similar male coalitions have been observed in other species. Most notably in African lions, male crèche mates will take over and defend female prides (Packer and Pusey 1982). Male groups have also been observed in some raccoon (*Procyon lotor*) populations. Male raccoons of varying age and size have been observed traveling together and denning in the same tree (Gehrt and Fox 2004). Though uncommon in African elephants (*Loxodonta africana*), Asian elephants (*Elephas maximus*) frequently display bachelor herds that sometimes include females and are not dependent on relatedness of its members (Schneck 1991; Ahlering et al. 2011). Unlike raccoons and Asian elephants, wild pig bachelor groups in our study seem to be intolerant of unrelated individuals. However, the lack of capture groups composed of large, breeding-aged males suggests that bachelor groups are unlikely to be as cooperative as male African lion coalitions and are likely transitioning to become independent and solitary. Bachelor groups in European wild boar have been reported to be littermates that disperse from there natal groups for a short period before transitioning to become solitary (Dardaillon 1988). Wild boar females were also observed to disperse with their male litter mates on rare occasions (Kaminski et al. 2005). However, the behavior and frequency of bachelor coalitions in both wild pigs and European wild boar is an understudied phenomenon.

Collectively, our study suggests wild pig social organization is complex, dynamic, and likely variable across the species' introduced range. Wild pig social organization seems to mirror European wild boar in that they both are largely composed of matriarchal groups and tolerance of unrelated individuals varies by population. In addition to matriarchically dominated sounders and solitary males, pseudo-solitary females and bachelor coalitions were relatively common and should be included in future investigations of wild pig social organization. Interestingly, we found that sounders in southcentral Oklahoma, the drier and less humid study site, were less likely to include unrelated group members but were more likely to have groups composed of only unrelated individuals. It is unclear whether this observation was due to differences in resource distribution between sites or differences in culling strategies resulting in greater disruption of sounder composition at the SRS or an artifact of the dichotomous sample sizes between SRS and southcentral Oklahoma. Thus, future research is needed to individually elucidate the impacts of resource distribution and management strategies on wild pig and wild boar social dynamics, as these data could have direct implications to the development of more efficient management strategies for this destructive species. Further studies also are needed to better understand the dynamics and stability of associations of group members, as well as the frequency of pseudo-solitary females and male groups in wild pig populations.

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Table 2.1: Descriptive statistics under three hierarchies of analysis for wild pig (Sus scrofa) social groups (female dominated, male majority, pseudo-solitary female, and pseudo-solitary male) observed at the Savannah River Site (SRS), South Carolina, USA and Nobel Research Institution (NRI), Oklahoma, USA.

	Count[a]		**Min n**[b]		**Average n**[c]		**Max n**[d]		**Min r**[e]		**Average r**[f]		**Max r**[g]	
Group type	**SRS**	**NRI**	**SRS**	**NRI**	**SRS**	**NRI**	**SRS**	**NRI**	**SRS**	**NRI**	**SRS**	**NRI**	**SRS**	**NRI**
First Hierarchy: All Group Members														
Female Dominated	64	7	2	2	3.8	4.1	12	12	-0.056	-0.074	0.205	0.114	0.528	0.276
Male Majority	13	6	2	4	4.6	6.3	16	9	0.026	0.146	0.271	0.294	0.513	0.476
All	**77**	**13**	**2**	**2**	**3.9**	**5.1**	**16**	**12**	**-0.056**	**-0.074**	**0.216**	**0.197**	**0.528**	**0.476**
Second Hierarchy: Offspring Excluded														
Female Dominated	45	6	2	2	2.8	2.8	6	6	-0.056	-0.074	0.198	0.174	0.528	0.514
Male Majority	8	5	2	3	2.4	3.2	5	4	0.026	0.084	0.230	0.350	0.502	0.611
Pseudo-solitary Female	20	2												
Pseudo-solitary Male	1	0												
All	**74**	**13**	**1**	**1**	**2.2**	**2.7**	**6**	**6**	**-0.056**	**-0.074**	**0.203**	**0.253**	**0.528**	**0.611**
Third Hierarchy: Offspring, Suitors Excluded														
Female Dominated	42	5	2	2	2.7	3.0	5	6	-0.019	-0.009	0.239	0.172	0.528	0.276
Male Majority	8	5	2	3	2.4	3.2	5	4	0.026	0.084	0.230	0.349	0.502	0.611
Pseudo-solitary Female	23	3												
Pseudo-solitary Male	1	0												
All	**74**	**13**	**1**	**1**	**2.1**	**2.6**	**5**	**6**	**-0.019**	**-0.009**	**0.238**	**0.260**	**0.528**	**0.611**

[a] Total number of groups

[b] Minimum group size

[c] Average group size

[d] Maximum group size

[e] Minimum group relatedness

[f] Average group relatedness

[g] Maximum group relatedness

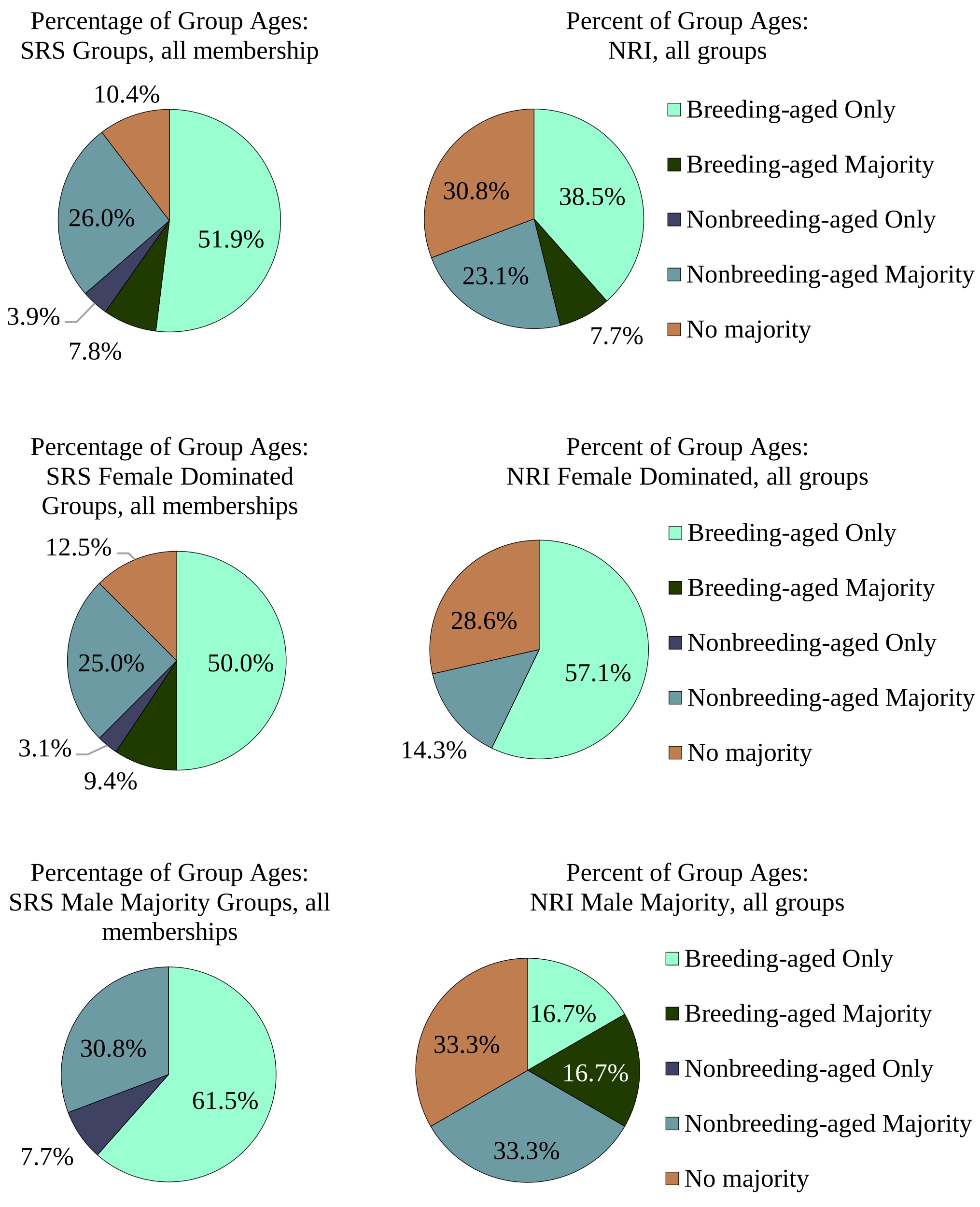


Figure 2.1: The percentage of majority age classes of wild pig (*Sus scrofa*) social groups at the Savannah River Site (left) and Noble Research Institute (right) under the first hierarchy (all group members) with all social group types (top), female dominated groups (middle), and male majority groups (bottom). The majority of age classes displayed in groups are shown as proportions of the total dataset for each study area.

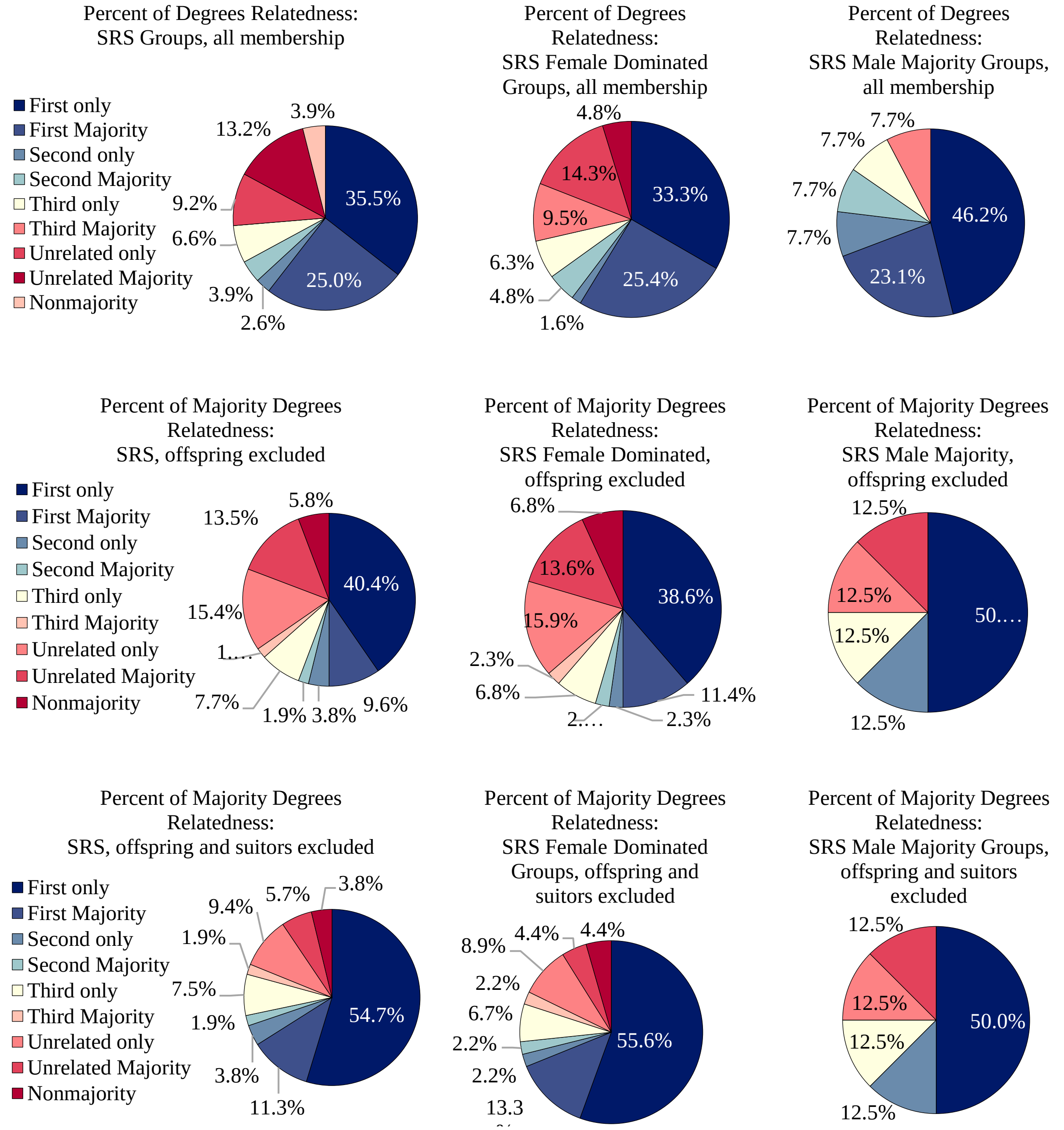


Figure 2.2: The percentage of the majority of degrees relatedness displayed in social groups at Savannah River Site with all group membership (top), proposed offspring excluded (middle), and proposed offspring and potential suitor males excluded (bottom) for all social groups (left), female dominated groups (middle), and male majority groups (right).

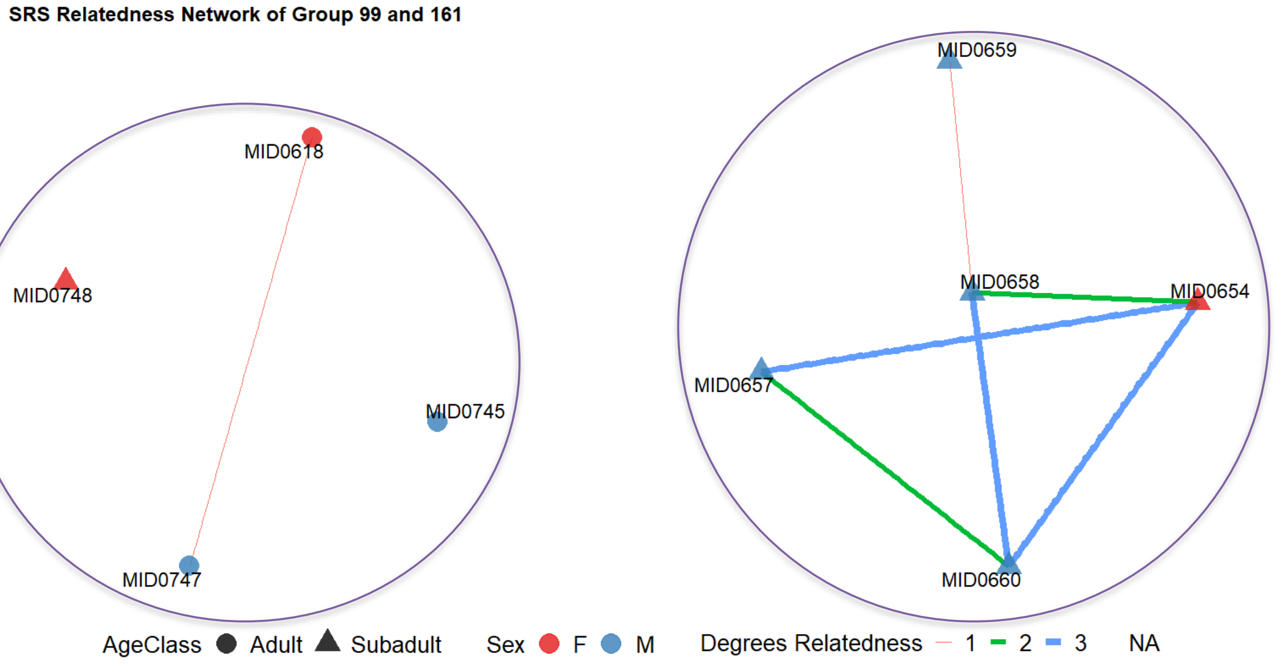
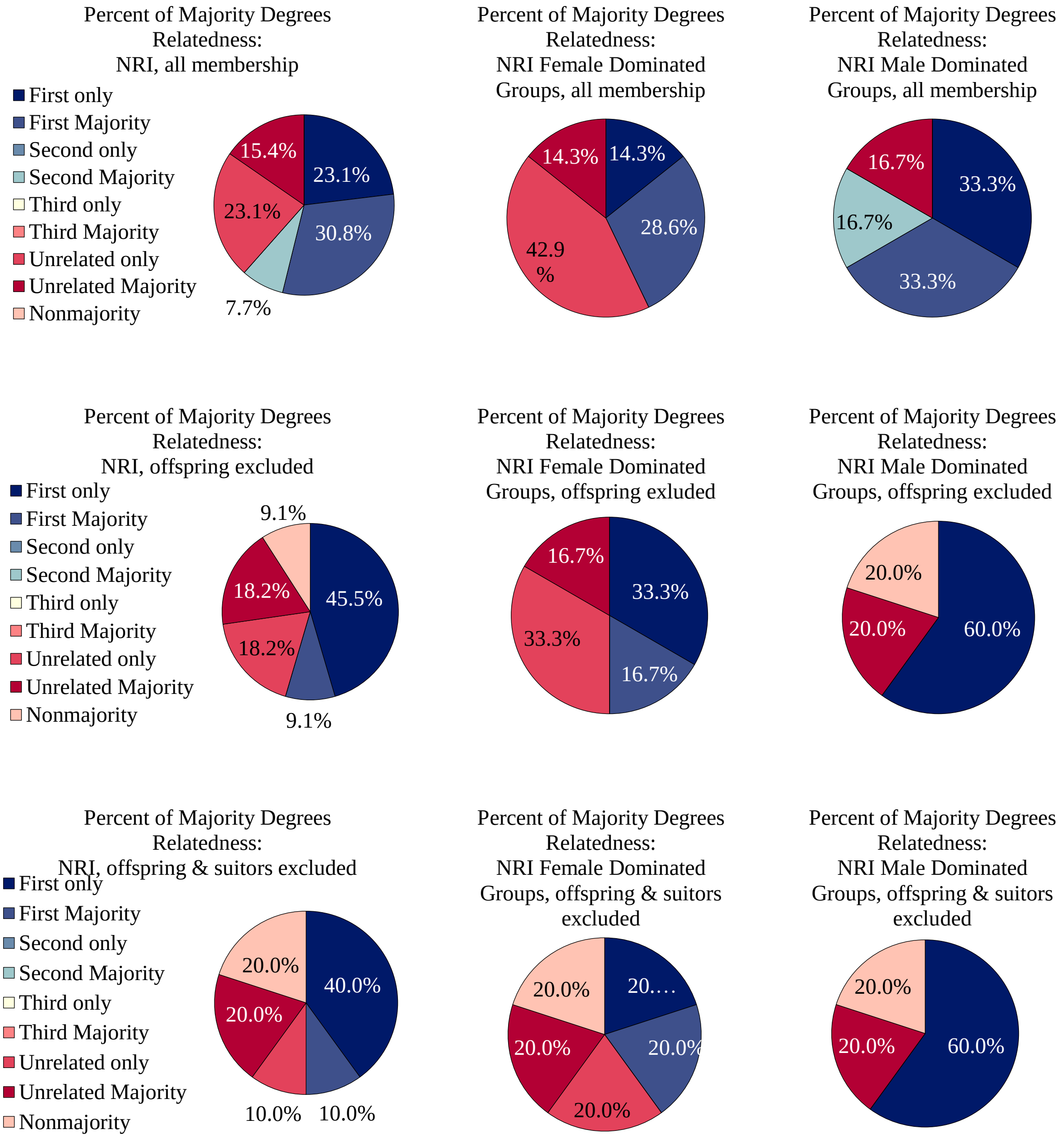


Figure 2.3: The plot of relatedness relationships observed in social groups 99 (left) and 161 (right) on the Savannah River Site. Each individual group member is represented by a point. The point shape indicates the group member's age class. The point color indicates the group member's sex. The line that connects two points indicate the degree of relatedness shared between the corresponding group members. Group members that are unrelated are classified as unrelated (NA) and are not connected by a line.



Percent of Majority Degrees Relatedness: NRI, all membership

Percent of Majority Degrees Relatedness: NRI Female Dominated Groups, all membership

Percent of Majority Degrees Relatedness: NRI Male Dominated Groups, all membership

Percent of Majority Degrees Relatedness: NRI, offspring excluded

Percent of Majority Degrees Relatedness: NRI Female Dominated Groups, offspring exluded

Percent of Majority Degrees Relatedness: NRI Male Dominated Groups, offspring excluded

Percent of Majority Degrees Relatedness: NRI, offspring & suitors excluded

Percent of Majority Degrees Relatedness: NRI Female Dominated Groups, offspring & suitors excluded

Percent of Majority Degrees Relatedness: NRI Male Dominated Groups, offspring & suitors excluded

Figure 2.4: The percentage of the majority of degrees relatedness displayed in all social groups(left), female dominated groups (middle), and male majority groups (right) at Noble Research Institute with all group membership (top), proposed offspring excluded (middle), and proposed offspring and potential suitor males excluded (bottom).

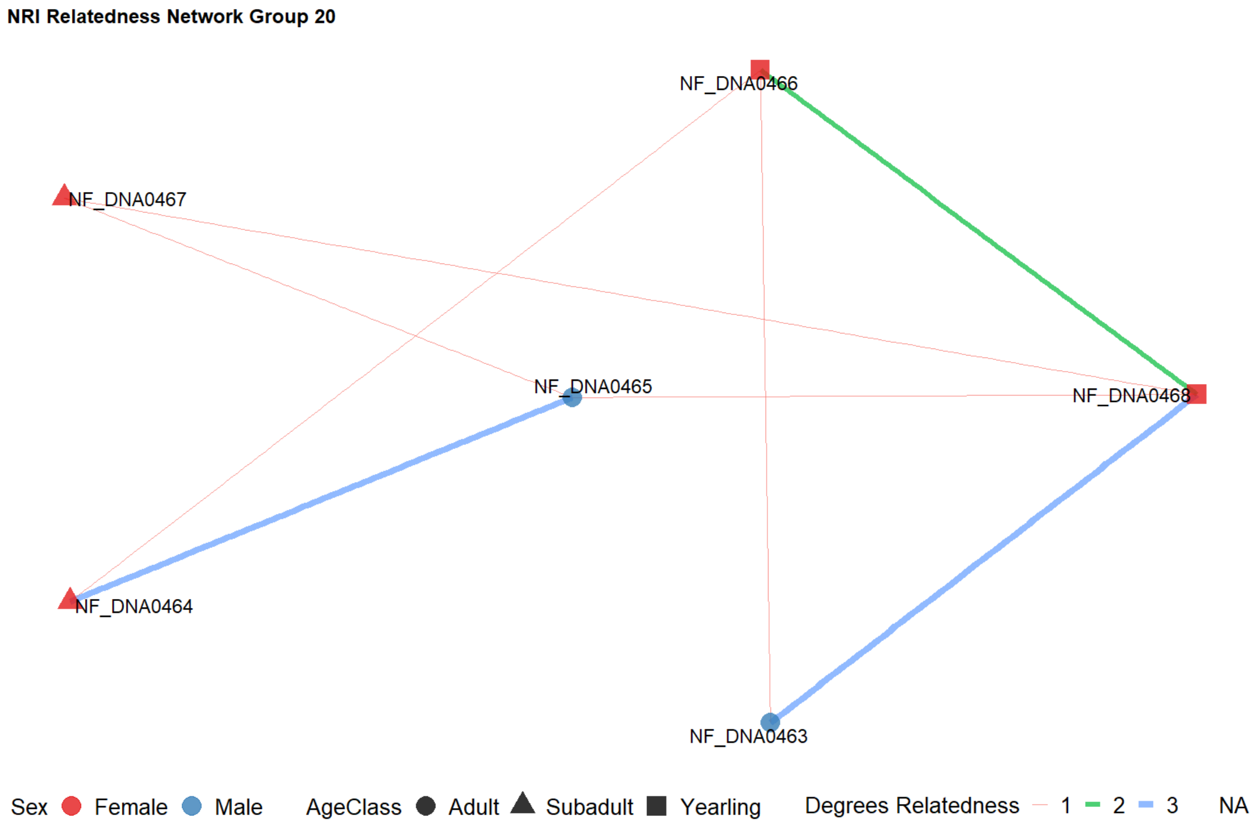


Figure 2.5: The plot of relatedness relationships observed in social group 20 at Noble Research Institute. Each individual group member is represented by a point. The point shape indicates the group member's age class. The point color indicates the group member's sex. The line that connects two points indicate the degree of relatedness shared between the corresponding group members. Group members that are unrelated are classified as unrelated (NA) and are not connected by a line.

CHAPTER 3

INFLUENCE OF AGE AND DOMESTIC PIG-WILD BOAR HYBRIDIZATION ON THE REPRODUCTIVE SUCCESS OF MALES IN A LARGE INVASIVE MAMMAL

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Abstract

A diverse array of mating strategies can be found among mammals. Mating strategies can range from life-long monogamy, with high maternal and paternal care, to male and female promiscuity and no paternal care of offspring. Promiscuous male alleles influence the fitness of their offspring without providing any paternal care, thus males are under selection pressure to increase their survivability or reproductive appeal. Wild pigs are a globally distributed large mammal with a highly polygynous mating system. Despite their extensive economic and environmental impacts, many aspects of their mating strategies and the extent to which introgression of domestic pig alleles influences reproductive traits in wild pigs is largely unknown. We used robust genomic tools on samples collected over a several year period from three discrete wild pig populations across the southern USA to investigate the effect of age and genetic ancestry on wild pig male reproductive output. We found the number of litters assigned to males to be negatively correlated with their proportion of European wild boar ancestry. Furthermore, we found male age to be positively correlated with the number of assigned litters, with more litters attributed to older males. Our study is the first to utilize single polymorphic loci to investigate male wild pig reproductive success in the invaded North American range.

Introduction

Mammals display a wide array of complex mating strategies that can be influenced by external environmental factors and species-specific attributes (Crook et al. 1976). These strategies range from life-long monogamy (pair bonding) and extensive investment in offspring by both parents, to various forms of male and female promiscuity with little to no offspring care (Crook et al. 1976; Clutton-Brock 1989; Silk 2007). Regardless of which strategies taxa use, there is often strong selective pressure on both males and females for attributes that promote individual fecundity and offspring fitness (Kodric-Brown and Brown 1984; Heisler et al. 1987; Clutton-Brock 1989). However, much of the literature focuses on understanding reproductive success of females, rather than males. This may be due in part to how much easier it is to observe maternal relationships than paternal relationships for many species, especially for promiscuous species where males provide little parental investment (Crook et al. 1976; Silk 2007). Despite the lack of paternal care in these mating systems, male genes can greatly influence the fitness of the next generation, and thus males that have high survivability or males that are more attractive are typically chosen by females as mates in a promiscuous system (Kodric-Brown and Brown 1984; Heisler et al. 1987; Jennions and Petrie 1997).

Males of many promiscuous mammals invest little effort in their own fecundity, especially when compared to females (Bronson 1985; Clutton-Brock 1989). However, successful copulation does not always guarantee fitness, and thus there is selection for males with “good genes” that will increase the survival of offspring or males that will produce “sexy sons” with a reproductive advantage (Weatherhead and Robertson 1979; Kodric-Brown and Brown 1984; Heisler et al. 1987; Jennions and Petrie 1997). Studies have found that in some species reproductively successful males that are either “sexy” (and thus produce “sexy sons”) or have “good genes” have specific traits, such as age, weight, or ornamental attributes, that are correlated with the rate of reproductive success (Kodric-Brown and Brown 1984; Jennions and Petrie 1997; Frasier et al. 2007; Newbolt et al. 2017). Further, females often mate with males that have advantageous fighting ability, like large, dominant males, or males with enhanced traits that show off to females, like pelage, courtship behaviors, or testes size (Kodric-Brown and Brown 1984; Heisler et al. 1987; Jennions and Petrie 1997). However, sexual selection for “sexy sons” or “good genes” is assumed in a natural setting where individuals have the free will to choose their mates. Little is known whether these trends in mate selection can be influenced by the introgression of genes artificially selected for increased reproductive output, tameness, or other traits often selected for during domestication, as in the case of native species that have been influenced by genetic introgression due to intentional or accidental breeding with domestic stock (e.g., wild pigs – *Sus scrofa*; wolves – *Canis lupus spp.*; European wildcat – *Felis silvestris spp.*; *Alectoris* partridges – *Alectoris spp.*; American Mink – *Neovison vison*; Bactrian camels – *Camelus bactrianus*).

Invasive wild pigs (i.e., feral domestic pigs, wild boar, and their hybrids; Keiter et al. 2016) are a globally distributed large mammal with a highly polygynous mating system. Wild pigs are an interesting case study in which to examine the effects of male attributes on reproductive success, as individuals display varying proportions of domestic pig ancestry, which have been anthropogenically selected for maximal productivity, and European wild boar ancestry, that follow natural selection for optimal fitness in the wild (Wood and Barrett 1979; Mayer and Brisbin 2009; Smyser et al. 2020). Despite the extensive economic and environmental impacts associated with this species, many aspects of their social ecology and mating strategies remain understudied (Beasley et al. 2018; Gray et al. 2020). Further, much of the available research focuses on the reproductive biology and ecology of females. However, research on male reproductive strategies is needed for a holistic understanding of the reproductive ecology of this species. Wild pig social organization is primarily characterized by matriarchal social groups and solitary males, although groups of dispersing young, predominantly males, also occur (Chapter 2, Dardaillon 1988, Gabor et al. 1999, Mayer and Brisbin 2009). Females and matriarchal groups have overlapping territories but often partition shared space temporally whereas male territories overlap the home ranges of several female or matriarchal groups (Gabor et al. 1999; Kaminski et al. 2005; Sparklin et al. 2009; Podgórski et al. 2014a, b; Gray et al. 2020; Kilgo et al. 2021; Clontz et al. 2021). Within their native range, European wild boar have been reported to display mate guarding behaviors during rut but quickly disassociate with females afterwards (Dardaillon 1988; Delgado et al. 2008; Gray et al. 2020). Recent studies have similarly found support for promiscuity and mate guarding among male wild pigs but have yet to identify common traits of reproductively successful males (Gabor et al. 1999; Delgado-Acevedo et al. 2010; VerCauteren et al. 2020).

The extent to which the introgression of domestic pig genes influences reproductive traits in wild pigs is largely unknown (Chinn et al. Unpublished data). However, contrary to wild boar in their native range, female wild pigs have larger litters, often can begin reproducing at < 1 year of age and can have multiple litters per year under favorable conditions (Wood and Barrett 1979; Delgado-Acevedo et al. 2010; Chinn et al. 2021). Additionally, studies have found that female wild pigs produce litters sired by multiple fathers, and the rate of litters with multiple paternity is higher in wild pigs than European wild boar (Delgado et al. 2008; Delgado-Acevedo et al. 2010). Within North America, wild pigs with domestic ancestry tend to display highly variable physical characteristics (e.g., pelage), but those of European wild boar ancestry are proportionally shorter than their conspecifics from feral origins (Mayer et al. 2020b). Specifically, descendants that arose from European wild boar ancestry have shorter total length, ear length, and head to body length, but longer snout length, hind foot length, and shoulder height (Mayer et al. 2020b). Klimiene and Klimas (2010) also found that the hybrids grew slower and had a different muscle composition (e.g., backfat, lean muscle, redness values, etc.) than their domestic conspecifics. Interestingly, hybrid neonates and piglets that display striped pelage (i.e., natural pelage coloration in wild boar) before transitioning into their mature coats have higher survival than their tri-colored spotted, black and white spotted, red/brown and spotted, and solid black conspecifics (Mayer et al. 2020b; Chinn et al. 2021). In their native range, European wild boar only displayed aggressive behaviors toward domestic-boar hybrid group members and morphologically different hybrids were typically restricted to the back of their group when observed in the wild. It has been suggested that these behaviors are in response to the hybrids being more visible to predators (Battocchio et al. 2017). Furthermore, recent studies have shown that despite the risks of increased predation associated with domestic pig traits among populations of European wild boar in the native range, some of these domestic traits may provide a heterozygotic advantage for females, such as increase litter size (Fulgione et al. 2016; Petrelli et al. 2021).

High resolution genetic tools are able to resolve the complex and variable mating strategies in a variety of mammals. However, there remains few studies available that utilize robust genetic tools to investigate previously held assumptions of the fecundity of male wild pigs (Beasley et al. 2018; VerCauteren et al. 2020). Nevertheless, the incorporation of genomics has the power to resolve observational cryptic relatedness that is often associated with paternally promiscuous species, as well as parse the influence of lineage on male success. For our study, we collected genetic samples over a several year period from wild pig populations in three discrete study areas across the southern USA to investigate the effect of age and genetic lineage (i.e., proportion of European wild boar) on the reproductive contribution of individual male wild pigs. Since European wild boar have evolved under natural selection for survival in the wild, we predicted the number of litters assigned to individual male wild pigs would be positively correlated with the proportion of lineage attributed to European wild boar ancestry. Furthermore, older males are found to sire more litters than younger males in many promiscuous mammal species. Thus, we predict the number of assigned litters would also be positively correlated with age of male wild pigs. This study will be the first to utilize single polymorphic loci (SNP) and robust genetic tools to investigate the reproductive success of male wild pigs in their invaded North American range.

Methods and Materials

Study Areas

South Carolina

Through ongoing population control and research efforts, wild pigs were sampled from 2012 through 2020 on the Savannah River Site (SRS) near Aiken, South Carolina, USA. The US Department of Energy (DOE) manages and restricts access to the 803 km^2 SRS area. The SRS was originally established for the manufacture of nuclear weapons, however the industrial footprint accounts for a small portion of the landscape. Approximately 90% of the land on the SRS is dominated by upland pine (*Pinus spp.*), cypress-tupelo (*Taxodium districhum-Nyssa aquaticus*) or oak-hickory (*Quercus spp.-Ulmus spp.*) bottomland hardwoods, herbaceous/shrub cover (*Vaccinium corymbosum, Ilex spp., Myrica cerifera*), and upland/mixed forests (*Pinus* *spp.*, *Quercus spp.*; Imm and McLeod 2005). The average monthly high temperatures range from 15.4-33.4°C, average monthly relative humidity ranges from 63-80%, and average annual precipitation is approximately 1,200 mm; providing a typically warm and humid climate (Imm and McLeod 2005). The DOE established the SRS in the 1950's, well after wild pig populations became established on the landscape. Currently, the SRS wild pig population is comprised of hybrids of Western heritage breeds of domestic pigs and wild boar, although there is considerable variability in the composition of wild boar and heritage breed ancestry among individuals (Mayer et al. 2020a; Smyser et al. 2020). Wild pigs have been intensively controlled since the establishment of the SRS to minimize damages through a combination of trapping and opportunistic shooting (Mayer et al. 2020a). Procedures, handling, and capture was conducted in compliance with University of Georgia's Animal Care and Use Committee under protocols A2012 08-004, A2015 05-004, A2015 12-017, and A2018 06-024.

Oklahoma

Wild pigs were captured at Noble Research Institute (NRI) from 2010 through 2017 at ranches in south-central Oklahoma, USA (Carter and Love counties) as part of continuing research and control efforts. The Coffey Ranch (10.17 km^2), Oswalt Road Ranch (20.77 km^2), and Red River Ranch (13.39 km^2), in addition to the Strate Ranch (3.90 km^2), Hoffman Ranch (9.35 km^2), and Ljungdahl Ranch (4.66 km^2) are found in the Cross Timbers and Prairies ecoregion, which is composed of a variegated habitat of oak-elm-hickory woods (*Quercus spp.-Ulmus spp.-Carya spp.*) and bluestem and grass prairie (*Andropogon gerardii and Schizachyrium scoparium*, respectively; switch grass – *Panicum vigatum* and Indian grass – *Sorghastrum nutans*; (Gaskamp et al. 2021). Similar to SRS, wild pigs in this region predate the acquisition of these ranches by NRI and are abundant and widely distributed (Mayer 2014; Gaskamp et al.

2021). The NRI has employed various methods to control wild pigs at these ranches since 2010 (Stevens 2010; Gaskamp et al. 2020, 2021).

Texas

The United States Department of Agriculture (USDA) captured wild pigs from 2016 through 2019 at Camp Bullis (CB), Joint Base San Antonio Bullis, San Antonio, Texas, USA as part of ongoing control and research efforts. Camp Bullis is a 112.9 km^2 military property situated in the Blackland Prairie and Edwards Plateau ecoregions in south-central Texas, USA. This area is composed of patches of rocky soil grasslands and cedar-oak woods (*Cedrus spp.*-*Quercus spp.*). The now abundant wild pig population at CB is believed to have been established sometime after 2006 from individuals that were released or immigrated nearby, well after the property's establishment (Snow et al. 2019). The military has since primarily implemented recreational hunting to control the population. However, recent control and research efforts have utilized corral and box traps, drop or cannon nets, and aerial shooting (Snow et al. 2019).

Field Collection

Detailed descriptions of capture and handling procedures for wild pigs in our dataset have been described elsewhere as part of past and current research and control efforts (e.g., CB – Snow et al. 2019; NRI - Gaskamp et al. 2021 and Haydett et al. 2021, SRS – Yang et al. 2021). From 2012 to 2020 at SRS, 2010 to 2017 at NRI, and 2016 to 2019 at CB, wild pigs were captured using a variety of strategies including corral traps, aerial shooting (helicopter), and/or drop-net traps that were baited with corn (*Zea mays*). Trapping often targeted female social groups, but males also were targeted for capture and were routinely captured opportunistically. At SRS, we set corral traps and used cellular cameras to identify and target entire social groups or solitary males. For NRI, we set tripwires in dual compartment corral traps to trigger either a single spring or saloon style gate and set and monitored drop net traps using line-of-sight remote control (Gaskamp et al. 2021). At CB, we used box and corral traps with either swing gates or drop gates, corral traps, and aerial shooting. Also at CB, one sample was collected as part of poacher confiscation. We used both lethal sampling as part of control and management events, as well as live sampling and release for ongoing research. When wild pigs were in hand, we recorded age (piglet – 0–0.5 year, juvenile – 0.5–1 year, yearling – 1–1.5 years, subadult – 1.5–3 years, and adult – >3 years) via tooth eruption (Matschke 1967), weight, and sex. For 18 individuals captured at NRI and 486 individuals captured at SRS we were unable to age via tooth eruption, and for these individuals age was determined by weight and expert opinion based on other morphological characteristics. We also collected hair (NRI) and tissue (i.e., pinnae, muscle, etc.; SRS, NRI, CB) for genotyping. Tissue samples were stored at -70°C in individual vials with 99.5% ethanol or at -18°C in individual vials without ethanol and hair samples were placed in individual paper coin envelopes stored at -18°C until DNA extraction.

Genetic Methods

We extracted DNA with MagMax DNA extraction kit (Thermo Fisher Scientific). Subsequently we genotyped animals with GeneSeek’s Genomic Profiler for Porcine (68,516 biallelic single nucleotide polymorphism (SNP) loci; GeneSeek, Lincoln, Nebraska) developed with Illumina (San Diego, California) bead array chemistry (Ramos et al. 2009). We mapped biallelic SNP loci to the Sscrofa11.1 reference genome assembly and pruned unmapped and non-autosomal loci from the dataset (Warr et al. 2019). Independently for each study site, we performed genetic quality control (QC) procedures using the program PLINK1.9 (Supplementary

Table 3.1; (Purcell and Chang 2015). Specifically, we filtered out loci that were out of Hardy-Weinberg equilibrium (p=1e-06), had minor allele frequencies <0.45, and a call rate of <0.99. We applied additional genetic QC filters to remove individuals missing >0.05 of all calls for the remaining loci. We also removed loci in linkage disequilibrium (window size = 50, step = 5, variance inflation factor >1.25) to ascertain the most informative loci for our analyses.

For each study site, we assigned individuals to cohorts based on their birth year. If individuals were not born the year they were sampled, birth year was approximated by subtracting their age at capture from the date they were captured. Since adults three years and older could not be delineated to finer age categories, we calculated approximate birth year as three years prior from their capture date. We used the program COLONY (Wang 2008; Jones and Wang 2010) to analyze each cohort and identify potential parents in each study area. We did not analyze the oldest cohort for each study area as there would be no candidate parents. For each cohort, we provided a list of candidate mothers (females assigned to cohorts ≥1 year older than the offspring list) and candidate fathers (males assigned to cohorts ≥1 year older than the offspring list). If no parent could be assigned from the list of candidates, the offspring was assigned a dummy parent(s). Wild pigs are capable of reproducing throughout the year across much of their range; however, mating typically peaks in fall and winter for most populations and often follows peak hard mast production (Chinn et al. Unpublished data). Thus, while delineation of cohorts at a finer temporal scale would be optimal for wild pigs, we cannot account for overlapping generations with COLONY. This is further complicated with datasets that are both large and long term, as well as supplied from different projects that have different aims and obstacles. We applied stringent parameters to analyze each cohort to minimize the potential for misassignment of candidate parents (Supplementary Table 3.2): females and males can both be polygamous, inbreeding may be present, clones are not present, and the species is diploid and dioecious. We analyzed each cohort using the program's full-likelihood method under COLONY's 'very high' likelihood precision, without sibship distribution priors, and 'very long' run length options. For run specifications, we allowed the allele frequency to be updated while finding the maximum likelihood (ML) configuration, sibship scaling based on the number of alleles and genotyping error rate per locus, and used defaults of 1 run and the default random number seed. We analyzed cohorts for each study area using the SNPs retained following QC (SRS = 540 loci, NRI = 609, and CB = 409), and loci type and error rates for each loci (dummy loci identification (mk@), codominant loci (0), allelic drop=0.0, and other genotyping errors rate=0.0001 calculated with samples that were run in duplicate). We estimated population allele frequencies for each cohort in each study area from their respective datasets during analysis with COLONY. For each cohort, we provided a list of offspring (cohort) and their respective genotypes, a list of candidate fathers (see above) with their respective genotypes and the probability that the true father was included (eq1), and a list of candidate mothers (see above) with their respective genotypes and the probability that the true mother was included (eq1).

$$\text{Eq1. } P = \frac{\mathrm{C}}{(\mathrm{D}\times\mathrm{A})\div 2}$$

Where P is the probability of the true parent (e.g. true father or true mother) was sampled and included in the dataset, C is the number of candidate parents (e.g. fathers or mothers), D is the population density of the study area (SRS=2.6 pigs/km^2, NRI=4.8 pigs/ km^2 (Supplemental Table 3.3), and CB=3.8 pigs/km^2 (Gaskamp et al. 2016, 2018, 2021; Keiter et al. 2017; Snow et al. 2019), S. Webb unpublished 2021), and A is the area of the study area in km^2 [SRS=780 km^2, NRI=10.37 km^2, and CB=112.9 km^2). Given that population density estimates can be influenced by the different methodologies used across our study areas, we tested the influence of variable density estimates on the output in COLONY (range= 0.9-10 pigs/km^2). We found that density estimates did not influence the assignments of candidate parents to offspring in our dataset Furthermore, we analyzed each cohort with 0 known paternal sibs, excluded paternities, paternal sibships, known maternal sibs, excluded maternities, and excluded maternal sibships (Supplementary Table 3.1). However, there remains a possibility that individuals born towards the end of their birth year are siblings to individuals born towards the beginning of the following year but were assigned a parent/offspring relationship. Conversely, pigs born at opposite ends of the same year may share a parent/offspring relationship but were assigned as siblings.

Data Analysis

We assessed paternal traits contributing to the number of sired litters for all males in our dataset using a generalized linear mixed model (GLMM) with a Poisson distribution and log transformation with the R package (R Core Team 2020) lme4 (Douglas Bates et al. 2015). The influence of high-capacity reproduction of domestic pig alleles on wild pig reproduction is not well understood and results are likely to be highly dependent on the occurrence and number of offspring present at the time of sampling. Furthermore, we may not have captured all offspring within a litter and/or some individuals may have perished prior to sampling. Thus, we used number of litters that each male sired as our response variable rather than the total number of offspring sired to achieve a more balanced experimental design. We used approximate age class at copulation for sires (age at capture for all other males with no identified litters) and percent of lineage attributed to European wild boar, as well as the interaction between age class and lineage, as our predictor variables (fixed effects). We also included study site and sample identification as random effects to account for variance across study sites and among sires that reproduced over multiple years, respectively, in our dataset. We evaluated models using AICc calculated with the R package MuMIn (Bartoń 2020) and considered all models within Δ2 AICc competitive.

Results

Genetic Analysis

Male wild pigs (n=483) accounted for 25% of our total dataset (n=1,918). Of the 483 males, we assigned 51 sires to 124 litters (221 offspring). On average, approximately a third of analyzed cohorts were individuals that had parents identified from the list of candidate parents (CB: 24.3%, NRI: 25.9%, SRS: 33.3%). An average of <15% of cohorts were assigned to sires from the list of candidate fathers (CB: 2.3%, NRI: 4.8%, SRS: 12.4%). Most sires only contributed to one litter (n=30); however, five sires were assigned to two litters, five to three litters, one to four litters, three to five litters, two to six litters, three to seven litters, one to eight litters, and one sire was assigned to nine litters. Collectively, male wild pigs had an average of 39.3% European wild boar ancestry (range=8.8-71.6%). Individually, SRS male wild pigs had an average of 23.5% (range=8.8%-41.9%), NRI male wild pigs had an average of 37.1% (range=26.6%-45.9%), and CB male wild pigs had an average of 53.6% (range=35.1%-71.6%) European wild boar ancestry. Individuals documented as sires within our dataset had a slightly lower proportion European wild boar lineage (29.6%; range=9.5-66.8%) than non-sires (40.4%; range=8.8-71.6%). This pattern was observed across study areas, as well (Figure 3.1). Of the litters where we could identify sires, litters were most commonly sired by adults (47%), although subadult and yearling males were documented siring litters as well, (23.5% and 29.4%, respectively). Interestingly, some males sired multiple litters with specific female partners over multiple years (SRS=3, CB=2). Some sires were estimated to have been born the year prior to at least one of their assigned litters (SRS=15, NRI=3). However, COLONY does not have the resolution to determine if theses assignments are truly sire-offspring or potentially full sibling relationships.

Data Analysis

Our GLMM analysis produced three supported models, including age class, the proportion of wild boar lineage, and the interaction between these variables (Table 3.1). Within these models, age class had a positive influence on the number of litters sired by males, whereas European wild boar lineage had a negative influence (Table 3.1). The top ranked model accounted for 56% of model weight, and included age class, lineage, and the interaction between these variables, which had a positive effect on number of litters sired (Table 3.1). Proportion of European wild boar lineage was not associated with the likelihood of a male siring a litter but did influence the number of litters they contributed if they did sire (Figure 3.1). Specifically of the males that did sire, the less European wild boar ancestry a sire possessed, the more litters they were likely to be assigned. Further, the proportion of sire lineage associated with European wild boar ancestry was negatively correlated with the number of assigned litters. When parsed out by age class, piglets and juveniles did not sire any litters (Figure 3.2). The remaining age classes all displayed an increasing number of assigned litters with a decreasing percentage of a male’s lineage that was attributed to European wild boar ancestry (Figure 3.2).

Discussion

Here we present the first study to incorporate robust genomic tools to investigate the reproductive success of male wild pigs in their invasive range with varying extents of introgression of wild boar and domestic lineage. As predicted, male wild pig reproductive success is more likely achieved in adulthood, as male age class had a positive effect on the number of litters sired within our dataset. The proportion of European wild boar ancestry in males also appears to play a role in their level of reproductive success, but surprisingly it was negatively correlated with the number of litters they contributed to. These data suggest both phenotypic and genotypic attributes may play important roles in the reproductive success of male wild pigs, which over time may interact to influence the genetic makeup of wild pigs throughout their invasive range.

Similar to other ungulates and mammals more broadly, adult wild pigs typically contributed to more litters than younger males. In white-tailed deer, older, more dominant bucks typically possess larger antlers until a certain age and display mate guarding behaviors during the rutting season, and as a result often outcompete younger males for mating opportunities (Newbolt et al. 2017). In naturalistic herds of North American bison, older and more dominant males outcompete younger, subdominant males in bellowing contests and guard females from satellite males during the rutting season (Wolff 1998). Similarly, mate guarding behaviors have been reported in both wild pigs and European wild boar (Gray et al. 2020). Male wild pigs are typically larger than females and display larger, continuously growing tusks (Mayer et al. 2020b). While adults contributed to the most litters in our study, some litters were attributed to males that were estimated to be subadults and yearlings at time of copulation. While we did not observe interactions between males and females prior to copulation, in promiscuous species, smaller, less dominant males often “sneak” past the guarding male to gain access to mating opportunities (Kodric-Brown and Brown 1984; Clutton-Brock et al. 1988; Clutton-Brock 1989; Pemberton et al. 1992).This alternative male reproductive strategy has been observed in other ungulates, especially in those that have displayed multiple paternity (Kodric-Brown and Brown 1984; Pemberton et al. 1992; Wolff 1998; Preston et al. 2003; Bro-Jørgensen 2011). Wild pigs and European wild boar have also reported to display multiple paternity (Delgado et al. 2008; Delgado-Acevedo et al. 2010). These males may be utilizing the "sneaky" alternative mating strategy. Future studies are needed to further characterize mating strategies employed by younger male wild boar and wild pigs to gain access to receptive females, as well as the extent to which successful mating opportunities in these individuals occur.

Offspring of wild boar with greater European wild boar lineage may have higher survival and be less likely to be bullied and their position restricted by group members (Battocchio et al. 2017; Chinn et al. 2021). However, hybrids with a greater proportion of domestic lineage may have accelerated growth compared to individuals that are dominated by wild boar alleles (Klimiene and Klimas 2010). There was considerable variation in the proportion of European wild boar lineage for both sires and non-sires. However, sires with less European wild boar lineage generally contributed to more litters than sires with more European wild boar lineage. Further, the interaction between age and lineage influenced the number of litters assigned to a male. The older the male and the less European wild boar lineage, the more litters the sire contributed to. A more in-depth study is needed to better parse the selection pressure occurring in this taxon.

While male mate choice was not the focus of our study, it is interesting to note that a few males sired multiple litters with a specific female over time, sometimes while also siring with other females during the same time frame. Like most wild pig management programs, management of wild pigs across our study areas was focused on removing female dominated sounders, with males removed more opportunistically in many cases. Thus, the frequency of repeated mating between male and female dyads may be underrepresented in our data. Adult male home ranges are typically larger than females and incorporate the home ranges of multiple sounders (Gabor et al. 1999; Kaminski et al. 2005; Podgórski et al. 2014a, b; VerCauteren et al. 2020; Clontz et al. 2021). Thus, such evidence of repeated breeding among male-female dyads suggests these females are likely residents within the corresponding male's home range. In the instances where the male sired multiple litters with the same female while also copulating with other females, the returning female mate is possibly a core female member of a sounder and the novel females are possible younger, subdominant or pre-dispersing sounder members (Chapter 2; Gabor et al. 1999, Poteaux et al. 2009). Indeed, investigation of movement data collected from a parallel study (Clontz et al. 2021) for a female and male that produced multiple litters in our study revealed extensive overlap in movements and home range (Figure 3.3). Conversely, Titus et al. (unpublished) found that suitors captured with wild pig sounders were unrelated to any of the other captured group members, including piglets and juveniles. Nevertheless, more extensive spatial and social data are needed across a larger geographic extent to better understand the underlying factors that influence whether males and females produce multiple litters.

This is the first study to investigate the reproductive success of male wild pigs in their invasive range using robust genomic tools. We found support that male reproductive success was positively correlated with male age. Conversely, male European wild boar lineage was negatively correlated with male reproductive success. We suggest future studies include spatial and social data to investigate mate choice and the potential for preferential mating in invasive wild pigs. Additionally, future studies should include other male ornamental or advertising attributes, such as tusks or weight, to further investigate predictors of male reproductive success.

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Table 3.1: Model selection results for the generalized linear mixed model analysis of male wild pig (*Sus scrofa*) age class, proportion of European wild boar ancestry, and the interaction between age class and ancestry on the number of litters assigned

	(Intercept)	**AgC[1]**	**EWB[2]**	**AgC:EWB[3]**	**df**	**logLik**	**AICc**	**delta**	**weight**
8	0.1925	+	-2.379	+	12	-153.827	332.3	0	0.562
4	0.3177	+	-3.472		8	-158.904	334.1	1.79	0.229
2	-1.054	+			7	-160.026	334.3	1.97	0.21
3	-1.092		-4.212		4	-212.305	432.7	100.38	0
1	-2.738				3	-213.47	433	100.67	0

[1] Age class

[2] Percentage of European wild boar ancestry

[3] Interaction between age class and percentage of European wild boar

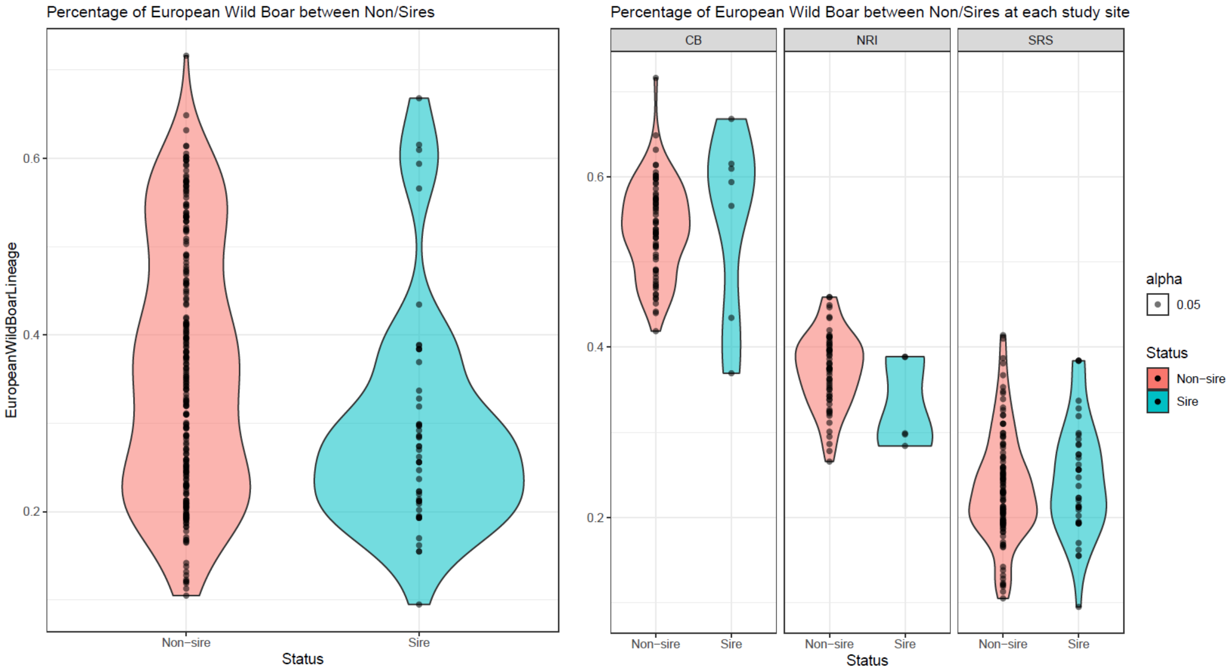

Figure 3.1: A violin plot of the distribution of European wild boar (*Sus scrofa spp.*) lineage among sire and non-sire wild pigs (*Sus scrofa*) for (left) the whole dataset and (right) each study site.

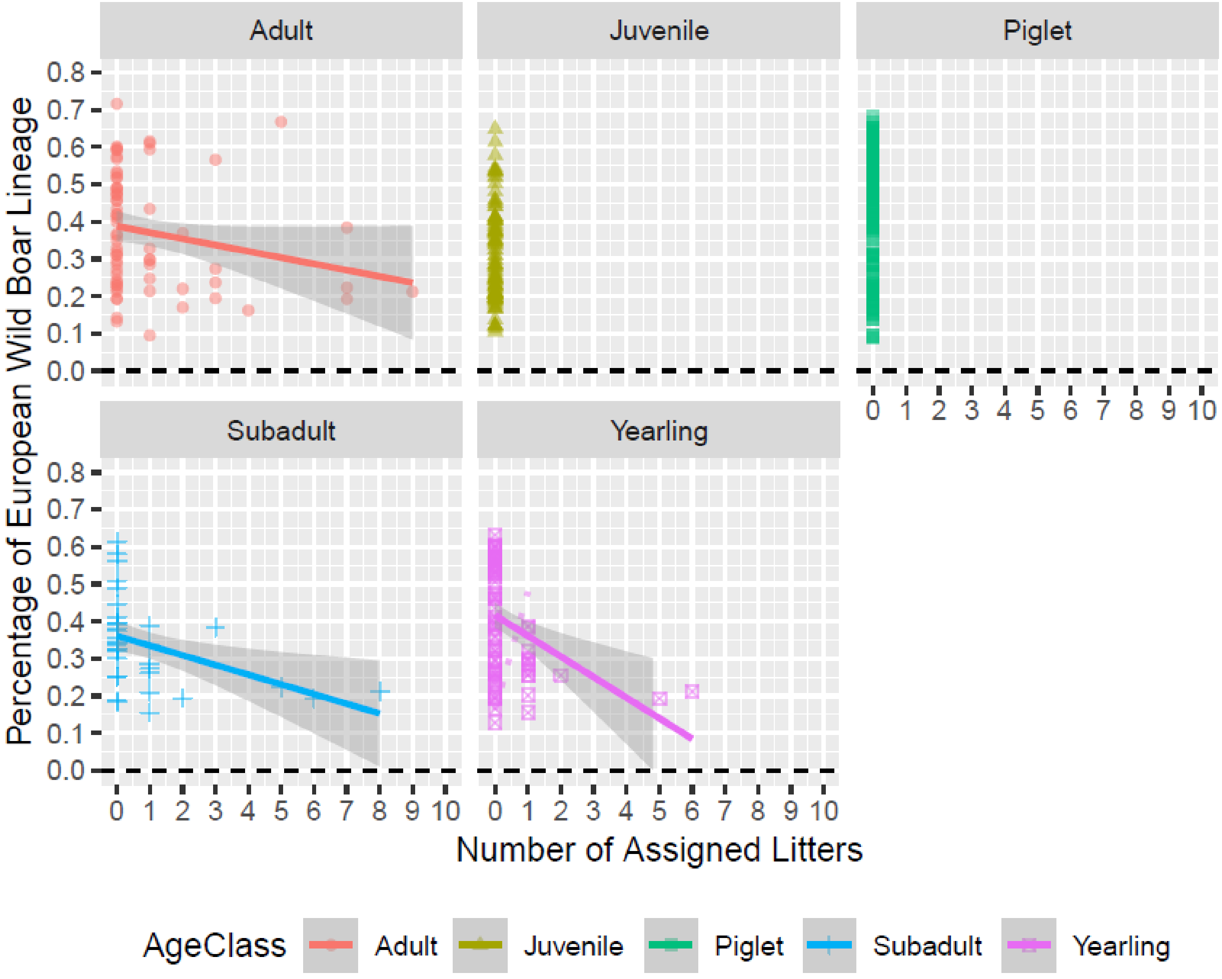

Figure 3.2: A plot of generalized linear mixed model analysis of the effects European wild boar (*Sus scrofa spp.*) lineage on the number of litters assigned to male wild pigs (*Sus scrofa*) for each age class.

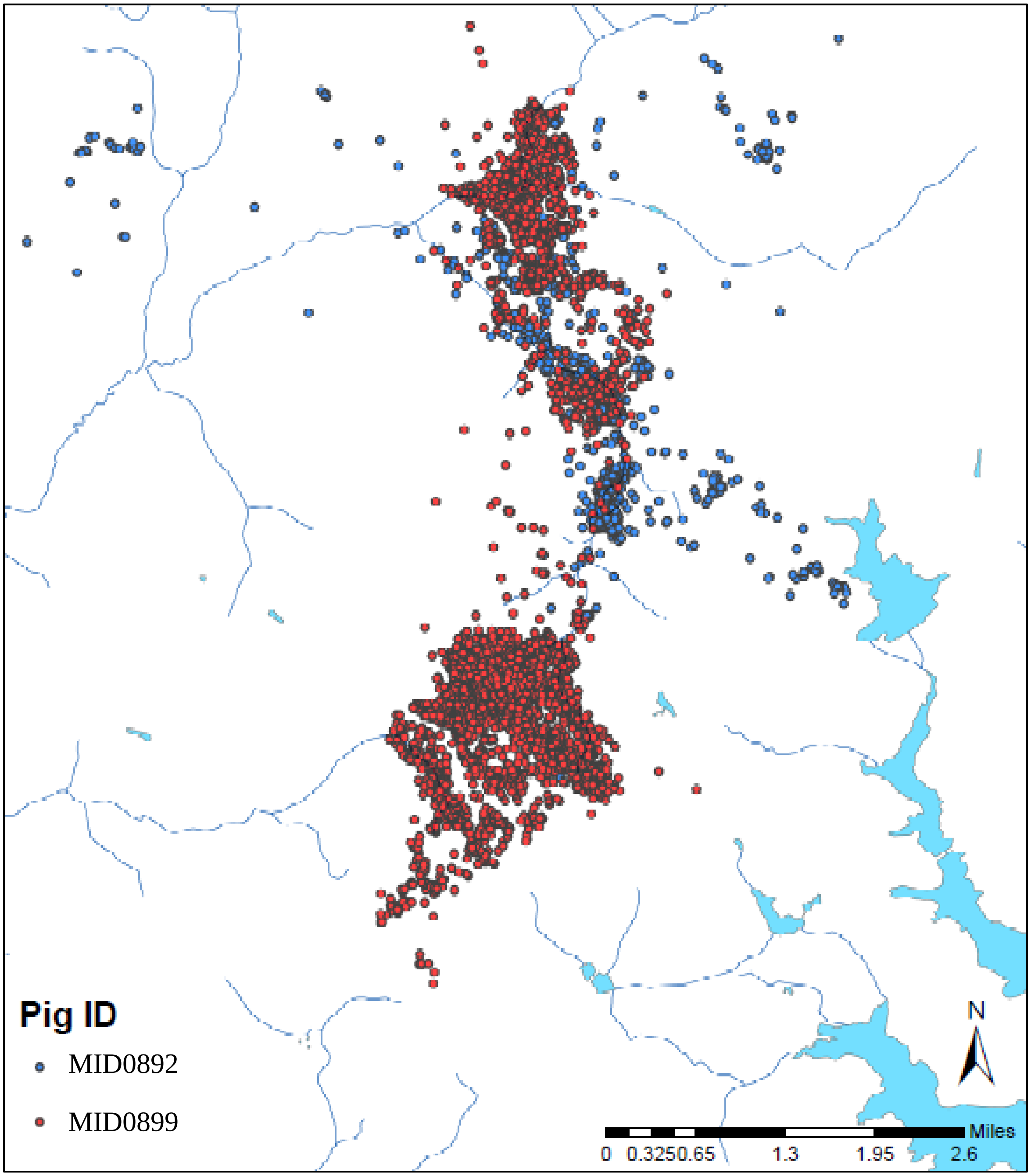

Figure 3.3: The spatial data of two wild pigs (*Sus scrofa*) that displayed recurrent procreation on the Savanna River Site. The spatial data of MID0892 (male) is displayed as blue points and spatial data of MID0899 (female) is displayed as red points.

CHAPTER 4

CONCLUSIONS

Despite their widespread distribution and extensive ecological and economic impacts, the social organization and mating strategies of invasive wild pigs has been understudied and largely limited to observational behavioral data. Using robust genomic tools, my research has shown that prior suppositions about invasive wild pig social organization were generally correct and male mating strategies are complex. However, assumptions about their social organization did not fully capture the nuances in group composition, nor the types of social units observed among populations. Although ancestry of male wild pigs contributed the individual level of reproductive success, proportion of their lineage associated with their European conspecifics was negatively correlated with the number of litters sired. Nonetheless, I have highlighted the need to continue research on this destructive taxon in their invaded range.

In Chapter 2, I found that wild pig social units consisted of sounders (female dominated social groups), solitary females (both with and without dependent offspring; pseudo-solitary females), male majority groups (bachelor groups), and solitary males across both geographic areas sampled for this study. Excluding solitary males, sounders were the most common social unit observed at Noble Research Institute (NRI) and Savannah River Site (SRS). Counter to previous assumptions, many sounders also included an unrelated breeding-aged female, though adult females in sounders were frequently close kin (first-degree relatives). I observed groups of only unrelated individuals within NRI, but groups comprised of female kin did not also include unrelated females. Kin-only sounders were still more common at NRI despite many sounders that were comprised of only unrelated females. This inclination for closely related females to form sounders has also been reported in populations of European wild boar in their native range, but few studies have reported these groups recruiting unrelated females (Kaminski et al. 2005, Iacolina et al. 2009, Podgórski et al. 2014*b*). I also observed many breeding-aged females captured with only offspring or unrelated breeding-aged males, suggesting sounder size and composition is diverse and dynamic within populations. Though occurring less often, I also observed many male majority groups within populations. These male majority groupings were principally related young (likely dispersing) individuals. Although my study can only delineate social groups at the time of capture, I have provided evidence that wild pigs exhibit more variable and complex social organization than previously considered.

Like European wild boar, sounders were the prevailing social unit at both study sites (Iacolina et al. 2009, Podgórski et al. 2014*a*, *b*). Sounders varied greatly in size ranging from 2–45 individuals, depending on the number of offspring present. The largest group size in the SRS dataset was 16 individuals and the average group size for SRS was approximately four individuals, before offspring and suitors were excluded. After offspring and suitors were excluded, social groups at SRS were typically two breeding-aged kin. The largest group size at NRI was 45 individuals, however this group, and many other big social groups at this location, could not be included in the dataset since age and/or sex was not recorded for the majority of their group members at time of collection. Consequently, I could not differentiate primary group members from ephemeral relationships (e.g., pre-dispersal aged offspring). In sum, more than 40% of NRI social groups were comprised of 10 or more individuals, and roughly 10% had over 20 individuals at time of capture. Depending on the location, European wild boar group size generally ranges from four to nine individuals in their native range (Dardaillon 1988, Poteaux et al. 2009, Podgórski et al. 2014*a*, *b*, Battocchio et al. 2017). However, studies do not always explicitly state whether offspring are excluded in these findings. Consequently, though SRS groups were primarily smaller than reports of European wild boar social groups, NRI sounders were more akin in group size to their conspecifics in their native range. Texas and Oklahoma studies of wild pigs also observed large social groups (five or more individuals; Gabor et al. 1999, Wehr et al. 2018, Gaskamp et al. 2021). Tendency for wild pigs to form smaller groups in SRS may be influenced by differences in management strategies, genetic lineages, or resource distributions not shared at NRI. Productive bottomland swamps are widespread across the Southeastern USA and have a large impact on the movement and population dynamics of wild pigs (Keiter et al. 2017, Clontz et al. 2021). Moreover, SRS wild pigs are extensively managed to reduce vehicle collisions and other damages, and individuals at this location generally have lower European wild boar ancestry than NRI individuals (Beasley et al. 2014, Smyser et al. 2020). Additional comprehensive research is required to better understand how management strategies, landscape attributes, and ancestry, as well as other factors, may affect the size of wild pig sounders across their invaded range.

Remarkably, my data uncovered that being a close relative is not required for wild pig social group membership, and social groups may include unrelated individuals. The SRS sounders were typically only groups of first-degree relatives; nevertheless, almost half of all SRS sounders demonstrated diverse relatedness among group membership. Numerous sounders consisted of group members that were first-degree relatives to some members and distantly related or unrelated to others (Figure 2.3). Moreover, 6.8% of SRS sounders had an unrelated breeding-aged female group member. Further confirmation that the unrelated females were established sounder members and not temporary associations were made using telemetry data collected for these sounders as part of related studies (Beasley et al. 2021, Clontz et al. 2021). Some native European wild boar populations also demonstrate tolerance of unrelated females within sounders, but this behavior is not displayed in all populations across their range (Iacolina et al. 2009, Podgórski et al. 2014*a*, *b*). On the contrary, NRI sounders generally exhibited diverse relatedness, where group members were not always closely related to all other group members (Figure 2.5).

Even though the diverse relatedness was observed within NRI sounders, social groups incorporating kin were unlikely to also have a group member that was completely unrelated to all other group members. Research on European wild boar suggests frequent hunting or population control strategies may disrupt sounder social structure and increase the tolerance of an unrelated sounder member (Poteaux et al. 2009, Podgórski et al. 2014*a*). Although my two study sites both employ control tactics, they vary in the type of removal and frequency of events. Specifically, SRS wild pigs are primarily removed using small corral traps or box traps. These methods often result in partial sounder removal, whereas whole-sounder removal tactics are more commonly employed at NRI (Gaskamp et al. 2021). This disturbance to social units due to incomplete sounder removal may facilitate the assimilation of unrelated members within social groups at the SRS, as well as other areas that use similar management tactics. Indeed, a recent translocation study on the SRS revealed many female wild pigs that were moved to new areas had assimilated into a new (unrelated) sounder after 1-2 months (J. Smith, unpublished data). McIlraith (2021) also provided GPS evidence of fission-fusion dynamics within sounders in four southern USA populations using contact heterogeneity estimates and movement metrics informed dyad (pairs of pigs) cohesiveness. When captured together, and subsequently collared, wild pigs typically associated with one another more than they did with pigs from other sounders. Nevertheless, collared pigs from the same sounder still exhibited irregular disassociations. Consequently, it is feasible that complete accounts of sounder relatedness described herein may not be attainable as a result of the genetic ‘snapshot’ supplied by my dataset, which consists primarily of pigs captured and subsequently removed from their respective populations. Future research is required to connect genetically related individuals and movement data in order to improve the understanding of fission-fusion dynamics and social organization. Sounders may also be impacted by landscape attributes. Previous research has suggested access to resources and habitat quality may influence sounder size and their tolerance unrelated individuals (Delgado et al. 2008, Gaskamp et al. 2021). Differences in relatedness compositions between NRI and SRS may be influenced by the different landscape features between the two sites. While generally wetter and more humid than NRI, the SRS also encompasses a mixture of upland and bottomland woodlands. Future research should investigate if differences in resource availability and/or removal pressure can explain the relatedness variation within sounders across different locations.

In addition to sounders, my dataset included pseudo-solitary, breeding-aged females that were captured with either suitor males, dependent offspring, or both at NRI and SRS. These females are believed to be temporary associations because suitor males typically do not remain with females for more than a couple weeks and offspring generally disperse as older juveniles or yearlings. Among the pseudo-solitary social units, there were females with offspring that were a few weeks old. These females may be temporarily disassociated from their sounder for parturition (Gabor et al. 1999, Mayer et al. 2020*a*). Secretive parturition has been documented in European wild boar in their native range (Dardaillon 1988, Kaminski et al. 2005, Poteaux et al. 2009). In fact, I was able to confirm that some pseudo-solitary females had associated with an uncaptured female before parturition using camera data from a related study (Clontz et al. 2021). Nevertheless, other pseudo-solitary females had offspring that were several months old, indicating this type social unit are not always temporary fissions from larger sounders during parturition. Gabor et al. (1999) determined that females will disperse on rare occasions. Hence, there remains a clear probability that female wild pig social organization includes both sounders and pseudo-solitary females, although the fission-fusion dynamics of wild pig social groups remains largely uncertain. My research into female wild pig social organization is limited by my sampling scheme, thus social organization can only be described for time of capture when no camera is available. All the same, I can highlight the complexity of sounder composition and diversity in female social units with greater resolution than described in past studies through the use of genomic data.

Although less prominent than sounders, my study also discovered that male majority groups (i.e., bachelor groups) are somewhat common, and primarily small, breeding-aged kin. Previous research mentions that the occurrence of bachelor groups was rare (e.g., Gabor et al. 1999). However, these studies, as well as ours, predominately focused on either sounders or solitary adult males. Hence male-dominated groups must occur more frequently than previously reported or herein. While typically consisting of only closely related male kin, some bachelor groups had female kin and one included an unrelated female. Contrary to sounders, bachelor groups seem highly intolerant of unrelated individuals, and none consisted of only unrelated individuals. Other species also display male coalitions; most notably, male African lions crèche mates work cooperatively to take over and defend female prides (Packer and Pusey 1982). Some raccoon (*Procyon lotor*) populations display male troupes with varying member age and group size have been reported traveling and denning together (Gehrt and Fox 2004). While rare among

African elephants (*Loxodonta africana*), bachelor herds that sometimes include females are common among Asian elephants (*Elephas maximus*), and these groups are not dependent on group relatedness (Schneck 1991, Ahlering et al. 2011). Contrary to the previous examples, wild pig bachelor groups in our study almost never included an unrelated individual. Nevertheless, the absence of groups inclusive of large, breeding-aged males in our dataset suggests that bachelor groups are unlikely to be long-term associations, but rather group members are likely dispersing and transitioning to solitary adulthood. Bachelor groups of European wild boar are reportedly littermates dispersing from natal groups briefly before transitioning into solitary adulthood (Dardaillon 1988). Female European wild boar littermates were also reported to occasionally disperse with these bachelor groups (Kaminski et al. 2005). Unfortunately, the frequency and behavior of bachelor groups is severely understudied in both wild pigs and European wild boar.

In Chapter 3, I present the first study to utilize genomic tools to examine the reproductive success of male wild pigs with diverse degrees of admixture of European wild boar and domestic pig ancestry in their invaded range. As I predicted, male reproductive success was positively correlated with age, as older males sired more litters within our dataset. Proportion of the male lineage attributed to European wild boar ancestry also appears to influence the individual level of reproductive success, but it was notably a negative correlation with the number of litters for which they contributed to. My study has highlighted the effect of age and lineage on the realized reproductive success of an invasive taxon descendent from domestic and natural sources.

Like many ungulates, adult wild pigs sire more litters than their younger counterparts. This pattern is seen in white-tailed deer (*Odocoileus virginianus*). As antler size increases every year with age, older bucks generally possess larger antlers; resulting in a competitive advantage over younger males during rut (Newbolt et al. 2017). Older males also outcompete younger males among naturalistic populations of American bison (*Bison bison*) in bellowing contests during the rutting season (Wolff 1998). Both white-tailed deer and American bison participate in mate guarding behaviors (Wolff 1998, Newbolt et al. 2017). Similarly, wild pigs have also been observed mate guarding (Gray et al. 2020). Male wild pigs are usually larger than females. Also, female tusks are relatively small and stop growing in adulthood, while males have continuously growing tusks that are ultimately larger (Mayer et al. 2020*b*). I found that adults sired the most litters in my study, but I found some litters were also attributed to subadult and yearling males. And though I could not observe pre-copulation behaviors between males and females in my study, smaller and less dominate males in promiscuous species will sometimes utilize an alternative “sneaky” mating strategy, where the usurping will bypass the guarding male to ascertain mating prospects (Kodric-Brown and Brown 1984, Clutton-Brock et al. 1988, Clutton-Brock 1989, Pemberton et al. 1992). Other ungulates have been reported to utilize this reproductive strategy, particularly species that display multiple paternity (Kodric-Brown and Brown 1984; Pemberton et al. 1992; Wolff 1998; Preston et al. 2003; Bro-Jørgensen 2011). Wild pigs and their European conspecifics are also documented to exhibit multiple paternity, although the rate at which this occurs differs between the two (Delgado et al. 2008; Delgado-Acevedo et al. 2010). In my dataset some sired litters at various age classes. These males may have employed the “sneaky” mating strategy when they sired at a younger age. Additional studies are necessary to further characterize mating strategies utilized among male wild pigs to further their reproductive output, and the rate of subdominant male reproductive success among wild pig populations.

As offspring, admixed wild pigs with a higher lineage proportion of European wild boar ancestry may have increased survivability and are less likely to be antagonized and restricted by their social group members (Battocchio et al. 2017, Chinn et al. 2021). Nonetheless, hybrid wild pigs that possess a larger proportion of domestic lineage typically grow faster than hybrids with more European wild boar ancestry (Klimiene and Klimas 2010). Although considerable variation in the degree of European wild boar ancestry was observed for both siring and non-siring males, sires with greater European wild boar ancestry generally assigned to fewer litters than sires with less European wild boar ancestry. Additionally, the interaction between age and lineage also played a role. The older the male and the less European wild boar ancestry they had, the more litters they sired. Nevertheless, to better elucidate the selection pressures on this taxon, a more in-depth study is needed.

Although mate choice was not an aim for my study, I did find that some of the males in my dataset sired more than one litter with a specific female across multiple years, occasionally producing litters with other females concurrently. Similar to other wild pig management programs, our study areas employ tactics that focus on the removal of sounders and males are typically removed opportunistically. And so, the rate of recurrent procreation between familiar mates is likely understated in my data. Furthermore, the home ranges of adult males are usually larger than females, encompassing complete or partial territories of more than one sounder or pseudo-solitary female (Gabor et al. 1999, Kaminski et al. 2005, Podgórski et al. 2014*a*, *b*, Gray et al. 2020, Clontz et al. 2021). Consequently, evidence of recurrent mating indicates these females are probably occupants of the territory of the corresponding sire. For cases in which a male sires multiple litters with one female while concurrently producing litters with other females, the familiar female could be a core member of a resident sounder and novel females are possibly subordinate or pre-dispersing sounder members (Gabor et al. 1999, Poteaux et al. 2009).

Nevertheless, further research that includes spatial and social data is needed to better explain the contributing factors that result in recurrent mating.

Altogether, my research provides evidence that wild pig social organization is dynamic, complex, and almost certainly variable across the introduced range, as well as indicates that age and ancestry influence male wild pig reproductive success. Wild pig social organization reflects that of their European conspecifics in that both are predominantly sounders and assimilation of unrelated group members differs across populations. In addition, pseudo-solitary females and bachelor groups seem common, highlighting the need to be included in future studies. Interestingly, I found that NRI sounders were unlikely to assimilate unrelated group members but formation of groups consisting of only unrelated individuals was common, and it is uncertain what factors influence the formation of groups or recruitment of group members. Furthermore, my research is the first to utilize robust genomic tools to investigate the social organization and male reproductive success of wild pigs in their invaded range, as well as the first to determine that age and ancestry is correlated with the level of reproductive output of male wild pigs. I found evidence that male reproductive success was positively correlated with male age and negatively associated with male European wild boar ancestry. Future studies are required to further investigate group dynamics and stability, as well as the rate of male groups and pseudo-solitary females in wild pig populations. Furthermore, I suggest that future studies include spatial and social data to investigate recurrent mating and the potential for preferential mating among wild pigs in their invaded. Additionally, future studies should include other male ornamental or advertising attributes, such as tusks or weight, to further investigate predictors of male reproductive success.

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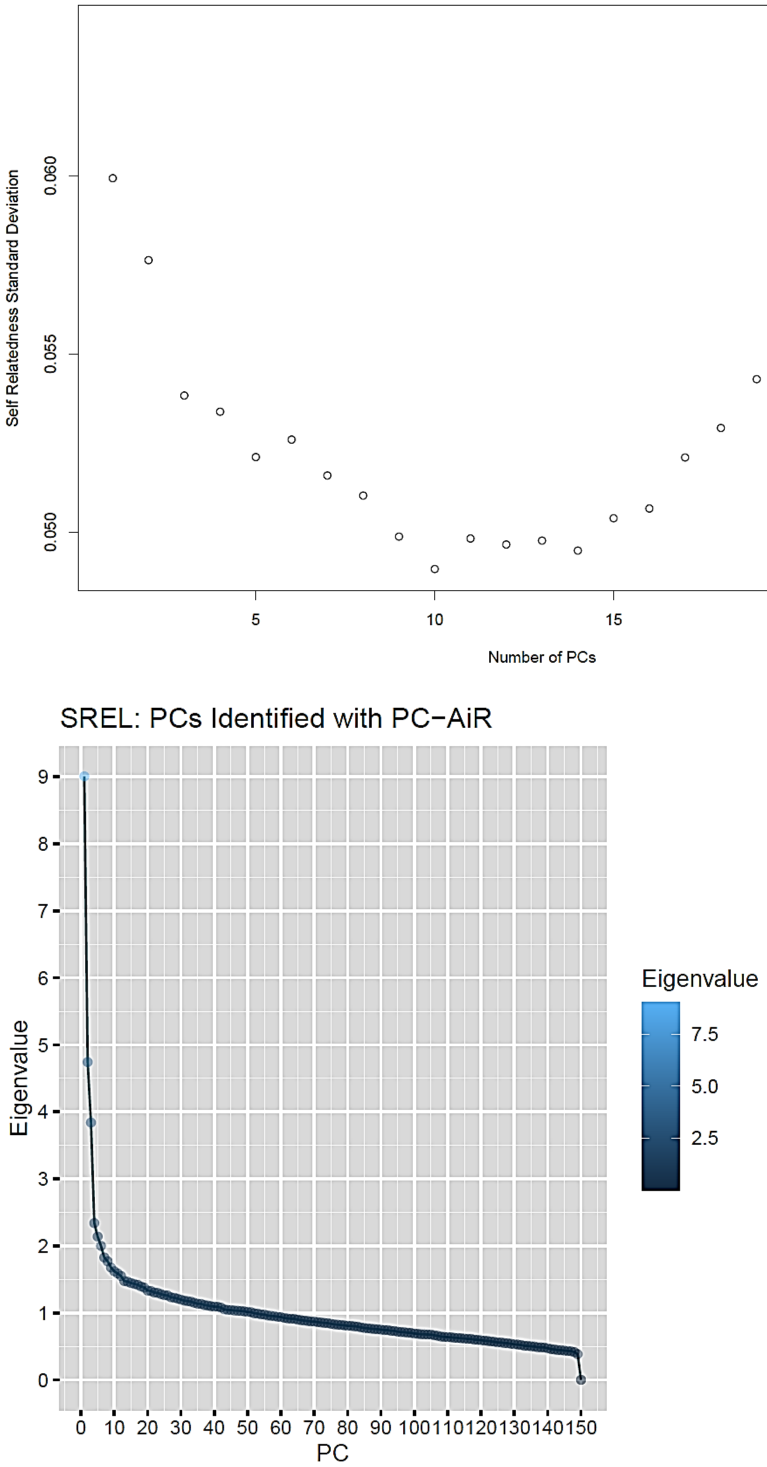
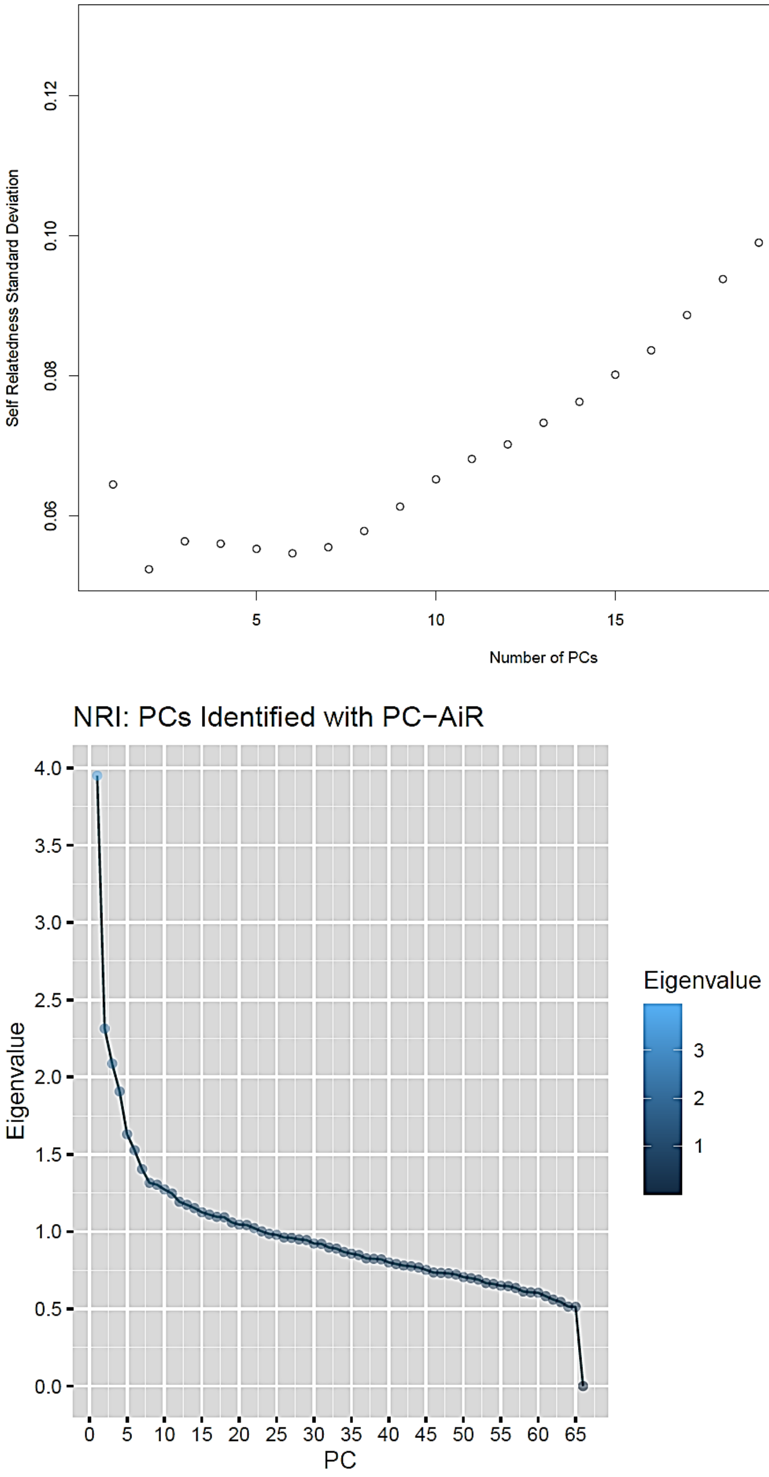
APPENDIX

Supplementary Table 2.1: Group size and count of each size of groups excluded from the southcentral Oklahoma (NRI) dataset.

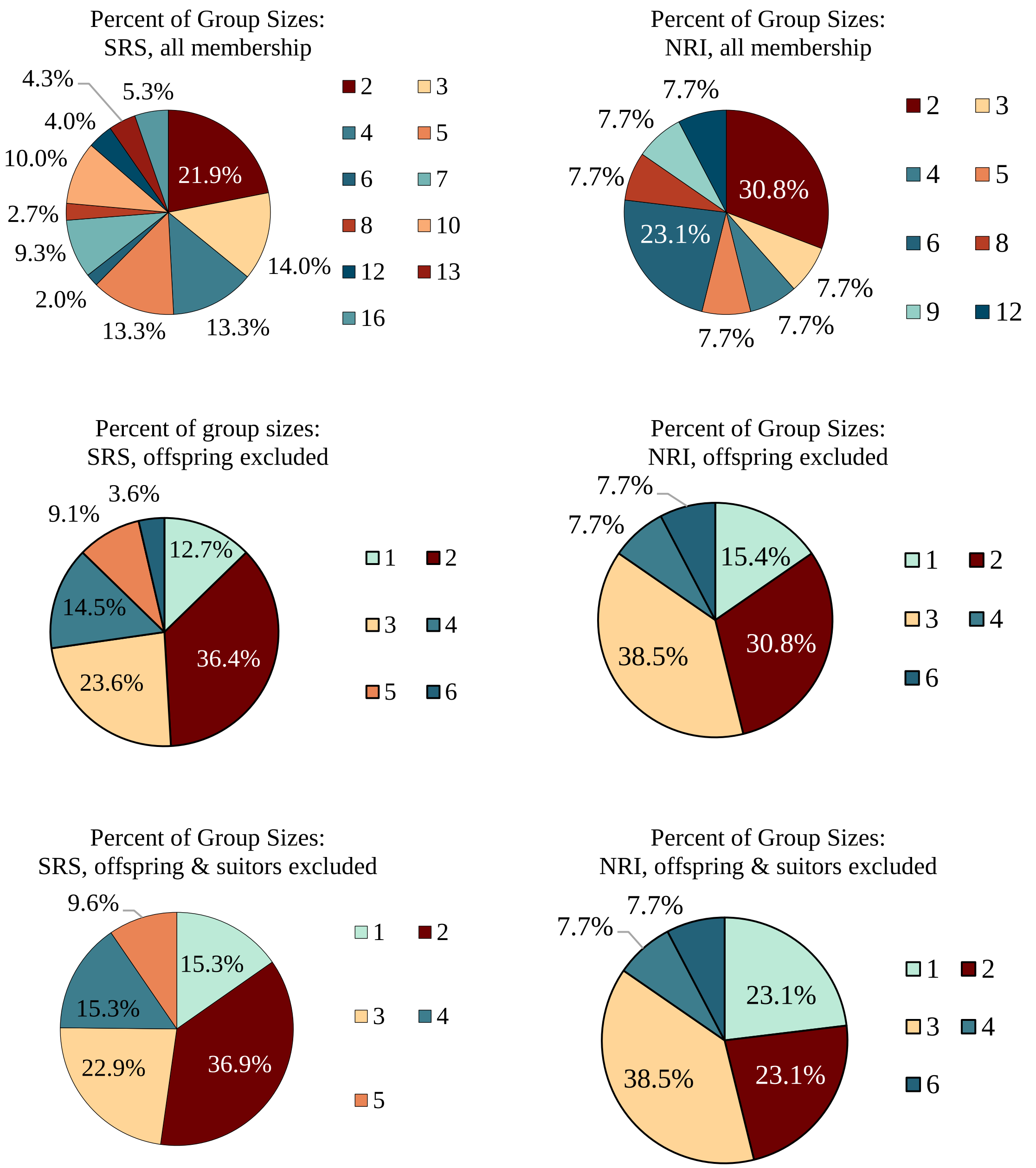
Group Size	**Count Group Size**
1	4
2	2
3	3
4	3
6	1
7	1
8	1
9	3
11	2
12	2
13	2
14	1
15	1
18	1
19	1
24	1
37	1
38	1
Total	**31**



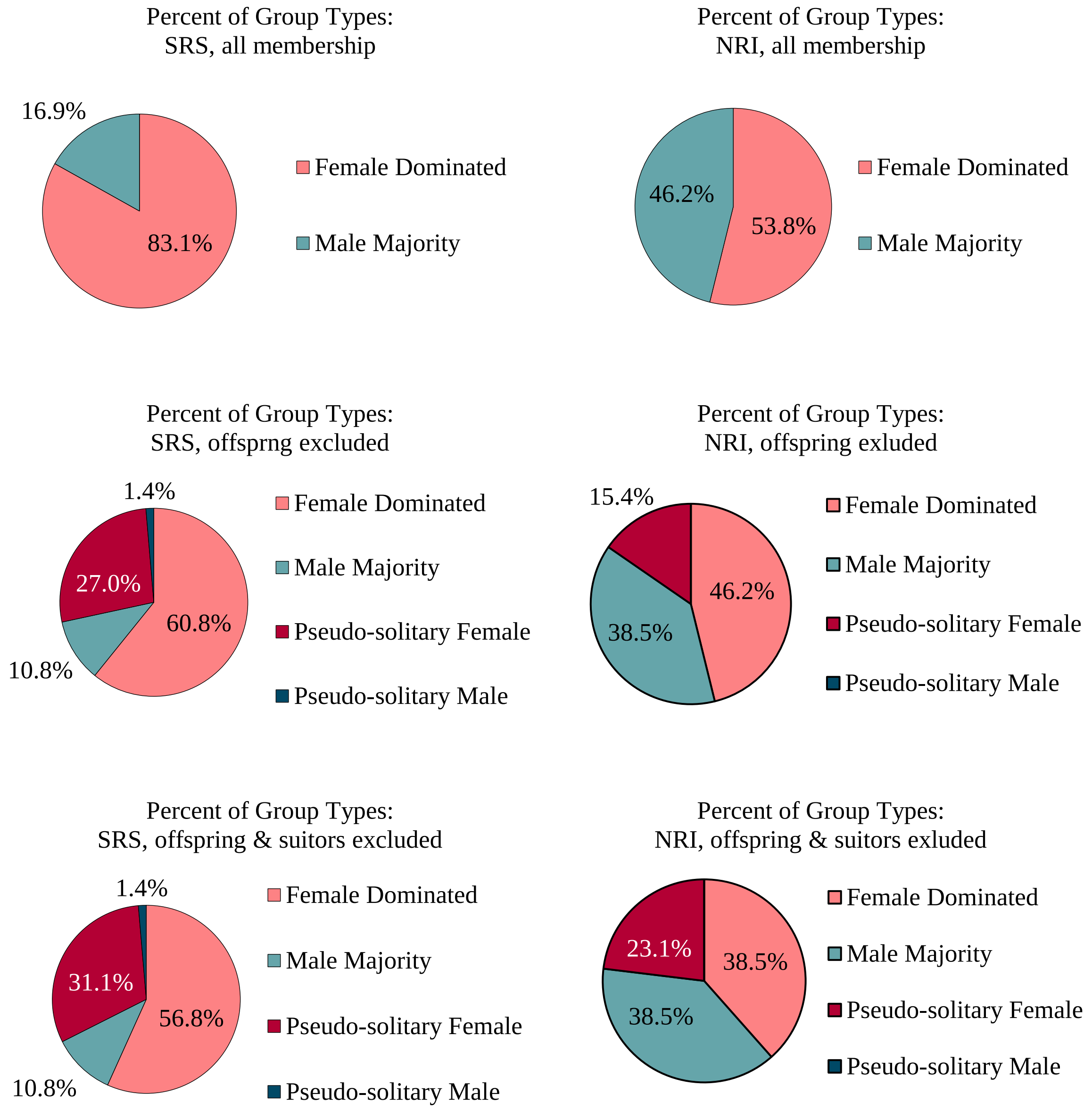
Supplementary Figure 2.1: Standard deviation (top) and scree plot of associated eigenvalues (bottom) of principal components identified for Savannah River Site. .



Supplementary Figure 2.2: Standard deviation (top) and scree plot of associated eigenvalues (bottom) of principal components identified for Noble Research Institute.



Supplementary Figure 2.3: Percentage of group sizes at SRS (left) and NRI (right) with all group membership (top), adult only group membership (middle), and group membership with proposed offspring and potential suitor males excluded (bottom). Group sizes are displayed as proportions of the total data set for each study area. Legends displaying group sizes and their corresponding colors are found to the right of each pie chart.



Supplemental Figure 2.4: Percentage of group types at SRS (left) and NRI (right) with all group membership (top), adult only group membership (middle), and group membership with proposed offspring and potential suitor males excluded (bottom). Group types are displayed as proportions of the total data set for each study area.

Supplementary Table 3.1: Genetic quality control (QC) parameters used in the program PLINK

Maximum missing variant call rate	0.01
Minimum minor allele frequency	0.45
Minimum Hardy-Weinberg equilibrium threshold	0.0000001
Maximum missing sample call rate	0.05
Variant pruning window size	50
Variant pruning window shift size	5
Maximum variant inflation factor	1.25

Supplementary Table 3.2: Parentage analysis parameters used in the program COLONY

Mating System - I	**Mating System - II**	**Species**	**Length of Run**
Female Polygamy	With Inbreeding	Dioecious	Very Long
Male Polygamy	Without Clone	Diploid	

Analysis Method	**Likelihood Precision**	**Run Specifications**		**Sibship Prior**
Full-Likelihood (FL)	Very High	Updated Allele Frequency:	Yes	No Prior
		Sibship Scaling:	Yes	
		Number of Run:	1	
		Random Number Seed:	1234	

Number of Loci	**Marker Types and Error Rates**	**Allele Frequency**
[# of SNP[a]]	mk@ 0@ 0.0@ 0.0001@	Unknown

Probability of father in candidates:

$$\frac{\text{[\# candidate males]}}{\text{([population density}^{b}\text{] * [area}^{b}\text{]) / 2}}$$

Probability of mother in candidates:

$$\frac{\text{[\# candidate females]}}{\text{([population density}^{\ddagger}\text{] * [area}^{\ddagger}\text{]) / 2}}$$

Number of Known Paternal Sibs:	0	**Number of Excluded Paternity:**	0	**Number of Excluded Paternal Sibships:**	0
Number of Known Maternal Sibs:	0	**Number of Excluded Maternity:**	0	**Number of Excluded Maternal Sibships:**	0

[a] Number of SNP used for analysis is determined for each study area during genetic quality control using PLINK (SRS: 540, NRI: 472, CB: 409)

[b] Population density and area of study are calculated from Snow et al., 2019 (Camp Bullis), Keiter et al. 2017 (Savannah River Site), and Gaskamp et al. 2016, 2018, 2021 (Nobel Research Institute)

Supplementary Table 3.3: Area and density values collected from various source to calculate average abundance across all ranches at Noble Research Institute, Oklahoma for use in COLONY.

Ranch	**Area[1] (Km^2)**	**Density[1] (pig/Km^2)**	**Area[2] (Km^2)**	**Density[2] (pig/Km^2)**	**Area[3] (Km^2)**	**Density[3] (pig/Km^2)**	**Area[4] (Km^2)**	**Density[4] (pig/Km^2)**	**Area[5] (Km^2)**	**Density[5] (pig/Km^2)**	**Area[6] (Km^2)**	**Density[6] (pig/Km^2)**
Coffey	10.11	18.7	10.11	1.8	10.24	7.9	10.24	13.3			10.17	10.4
Hoffman			9.45	1.2	9.30	0.9	9.30	2.0			9.35	1.4
Ljungdahl	4.66	4.3									4.66	2.1
Oswalt Road	20.93	4.9	20.28	1.9	20.93	2.9	20.93	6.2			20.77	4.0
Red River	13.16	14.4	13.85	3.4					13.16		13.39	8.9
Strate	3.90	1.8									3.90	1.8
										Average[7]	**10.37**	
										Average[7]		**4.8**
										COLONY[9]		**24.71**

[1] S. Webb, personal communication

[2] From Gaskamp et al. 2021 "Effectiveness and efficiency…" paper

[3] From Gaskamp et al. 2016 "Pseudorabies virus and …" paper

[4] From Gaskamp et al. 2018 "Damage caused to…" paper

[5] From Boyer et al. 2020 "Surficial soil damage …" paper

[6] Averaged all studies

[7] Average across ranches

[8] Variable used in COLONY (Area[8] x Density[8])