2021-06-04

Population Dynamics of Mule Deer (Odocoileus hemionus): Maternal Effects and De Novo Genome

Sydney Lamb
Brigham Young University

Population Dynamics of Mule Deer (*Odocoileus hemionus*):

Maternal Effects and De Novo Genome

Sydney Lamb

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

Brock R. McMillan, Chair
Randy T. Larsen
Paul B. Frandsen

Department of Plant and Wildlife Sciences

Brigham Young University

ABSTRACT

Population Dynamics of Mule Deer (*Odocoileus hemionus*):
Maternal Effects and De Novo Genome

Sydney Lamb
Department of Plant and Wildlife Sciences, BYU
Master of Science

Population dynamics of large ungulates are complex and vary with fluctuations in factors such as predation, resource availability, human disturbance, and weather (Gaillard et al. 1998, Forrester and Wittmer 2013). These regulating factors exhibit similar effects on ungulate populations by changing vital rates such as birthrate, death rate, emigration or immigration (Gaillard et al. 2000). To better understand the mechanisms influencing population change, it is useful to involve tools from multiple disciplines (Krausman et al. 2013). Here we explore population dynamics of mule deer (*Odocoileus hemionus*) through the lenses of two distinct fields: population ecology and genomics. In the first chapter we examine the influence of maternal effects on offspring fitness. In the second chapter we present a high-quality, chromosome-level reference genome for mule deer. We expect results from each of these studies to provide valuable resources for continued research and conservation of mule deer.

Keywords: mule deer, maternal effects, ungulate genomics, whole genome

ACKNOWLEDGEMENTS

Thanks to Dr. Brock McMillan and Dr. Randy Larsen for providing me the opportunity to work on this amazing project. Thank you for your confidence in me, for your support, for giving me ample opportunities to grow and for helping me reach my goal of becoming a wildlife biologist. I will always cherish your mentorship and friendship. Thanks also to Dr. Paul Frandsen for guiding me through the world of conservation genomics. You helped me step out of my comfort zone and succeed.

Jackson and Tansy Chandler, Daniel Sallee, and Tabitha Hughes – thank you for hundreds of hours spent looking for fawns, teaching me to take my work boots off occasionally, and all the good times at the swimming hole. There never was a better field crew. Thanks to Adam Taylor for his super computer expertise and passion for wildlife. Your work was crucial to publishing the mule deer genome.

I am grateful to the members of Sportsman for Fish and Wildlife and the Mule Deer Foundation. Your friendship, funding, hours spent in the field, and passion for wildlife made this project possible.

Thanks to the personnel of the Utah Division of Wildlife Resources for funding this project and creating footprints worth following. I feel grateful to be among your ranks as a wildlife biologist.

Thanks to my family for supporting me in the pursuit of my dreams. Mom and Dad – I am grateful for the hunting heritage you passed to me and your constant belief in my ability to do hard things. Uncle Jim – thanks for the many rides in your truck and always answering the phone when I was in a bind.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

Maternal Effects on Birth Weight, Growth, and Survival of Mule Deer Fawns

Sydney Lamb, Randy T. Larsen, Paul B. Frandsen, Brock R. McMillan
Department of Plant and Wildlife Sciences, Brigham Young University, Provo, UT
Master of Science

ABSTRACT

Maternal effects are the influence of maternal phenotype and the maternally-provided environment on the phenotype of her offspring. Frequently, maternal effects are manifest both pre- and post-parturition. Pre-parturition effects are primarily direct allocation of energy to the offspring that is in utero. Post-parturition effects can include direct (e.g., nursing and defending offspring) and indirect (e.g., selection of habitat that is relatively safe or has high nutritional value) influences. While both direct and indirect effects are often discussed, there is a paucity of information on the relative importance of each type due to the difficulty in monitoring mothers prior to parturition and mother-offspring relationships after parturition in free-ranging animals. Our objective was to determine the importance of direct maternal effects on birth weight, growth rates, and survival of mule deer (*Odocoileus hemionus*) fawns through the first 18 months of life. We determined the effect of maternal condition on birth weight (pre-parturition direct effect). We also examined the post-parturition direct effect of maternal condition on growth rates and survival of fawns. Direct maternal effects were evident both pre- and post-parturition; dams in better condition produce offspring with greater mass at birth, higher rate of growth, and better survival. Our findings demonstrate that maternal condition influences fawn health from gestation through recruitment. These links highlight the importance of considering direct maternal effects when examining population dynamics and reproductive success in long-lived mammals

INTRODUCTION

Maternal effects are the influence of maternal phenotype and "the maternally provided environment" on the phenotype of her offspring (Bernardo 1996, Wolf and Wade 2009). Life history theory states that females must balance their probability of survival between maintenance functions, and current and future reproductive events (Festa-Bianchet et al. 1998, Marshall and Uller 2007). When resources are abundant, females may produce larger or more offspring by allocating more energy toward current reproduction (Haywood and Perrins 1992, Bardsen et al. 2008). However, when resources are scarce, females often allocate less energy to current reproduction (e.g., produce smaller and fewer offspring) to maximize lifetime fitness (Smith and Fretwell 1974, Einum and Fleming 2000). Producing smaller offspring potentially decreases current maternal reproductive success in exchange for enhancing a future reproductive bout. Therefore, the influence of maternal effects may have a positive or negative effect on the fitness potential of the offspring (Kirkpatrick and Lande 1989, Marshall and Uller 2007, Freeman et al. 2013).

Identifying the life-history traits of offspring that are influenced by maternal effects can be difficult, as there are numerous mechanisms driving both life-history traits and maternal effects (Kirkpatrick and Lande 1989, Benton et al. 2001). Maternal condition and subsequent energy allocation have been shown to influence offspring traits in multiple species, including size at birth (Feiner et al. 2016), growth rate (Haywood and Perrins 1992), survival (Duquette et al. 2014), age at first reproduction and fecundity. Nonetheless, maternal effects may disappear during ontogeny (Gendreau et al. 2005) or be masked by the influence of environmental conditions in long-lived species (Hewison and Gaillard 1999). Therefore, determining the duration of maternal effects is complex.

Ungulates may provide a model system to study the duration of maternal effects because they are iteroparous and long-lived (Freeman et al. 2013). Short-term maternal effects, from birth to weaning, exist in some ungulate species (Kojola 1993, Wauters et al. 1995, Festa-Bianchet and Jorgenson 1998). Long-lasting maternal effects may also be present. An intergenerational maternal effect exists on growth and size at maturity in white-tailed deer (*Odocoileus virginianus*; Monteith et al. 2009). Additionally, maternal effects last into adulthood for antler growth in elk (*Cervus canadensis*) and mule deer (*Odocoileus hemionus*), a sexually selected trait that influences fitness potential (Freeman et al. 2013). Despite evidence for both short- and long-term maternal effects in ungulate species, maternal effects experienced by offspring from early life through recruitment are not well understood.

Maternal effects in early life can manifest both pre- and post-parturition (Bernardo 1996). However, there has been little differentiation between effects in utero or during offspring rearing. Pre-parturition effects are primarily direct allocation of energy to the offspring that is in utero (Robbins and Robbins 1979). Conversely, both direct and indirect maternal effects can influence offspring post-parturition. For example, direct effects include energy allocation such as milk to nursing young and indirect effects include protection of young (Wolf and Wade 2009), habitat selection, and maternal care other than nursing (Bernardo 1996). While both direct and indirect effects are often discussed, there is a paucity of information on the relative importance of each type due to difficulty in monitoring mothers prior to parturition and mother-offspring relationships after parturition in free-ranging animals.

Here we focus on direct maternal effects in mule deer fawns. Our objective was to determine the importance of direct maternal effects on birth weight, growth rates, and survival of fawns through the 18 months of life. More specifically, we examined potential effects of

maternal body condition. We hypothesized that maternal condition would be directly translated to offspring both while in utero and throughout the lactation period. We predicted that greater maternal condition would correlate to increased birth weight, growth, and survival of fawns. Additionally, we hypothesized that the duration of direct maternal effects would last through six months of age. We predicted that greater maternal condition would lead to larger body sizes of six month old fawns and that larger fawns would have a greater likelihood of survival to recruitment.

MATERIALS AND METHODS

Study Area

We conducted this study on the Cache Management Area in northern Utah, USA (Figure 1). This area comprised a portion of the Bear River Range and consisted of Wasatch-Cache National Forest, some state and private lands. The topography includes steep mountains, deep canyons and high mountain valleys with elevation that ranges from 1,300 to 3,000 meters. The area is comprised of high elevation coniferous forest and lower elevation shrub steppe habitats. High elevation forests are predominantly comprised of lodgepole pine (*Pinus contorta*), Engelmann spruce (*Picea englemannii*), and white fir (*Abies concolor*). Low elevation shrub steppe is dominated by sagebrush (*Artemisia tridentate*), bitterbrush (*Purshia tridentate*), snowberry (*Symphoricarpos albus*), chokecherry (*Prunus virginiana*), and aspen (*Populus tremuloides*). Potential competitors on the landscape include elk (*Cervus canadensis*), pronghorn (*Antilocapra americana*), moose (*Alces alces*), domestic cattle (*Bos taurus*), and domestic sheep (*Ovis aries*). Major predators include coyote (*Canis latrans*) and mountain lion (*Puma concolor*). Average annual temperature ranged from 7-27° C during the summer and -10-2° C during the

winter, with average annual precipitation of 80 cm per year, the majority of which occurred during winter (PRISM Climate Group, Oregon State University).

Adult Female Capture

During March of 2018-2020, we (by way of a private capture crew) captured adult female deer via helicopter net-gunning (Barrett et al. 1982, Krausman et al. 1985, White and Bartmann 1994). Individuals were hobbled, blindfolded and transported to one of four processing stations spread around the study area. We assessed weight, body size (hind foot length, chest and neck girth), body condition (ingesta-free body fat; Cook et al. 2004, Cook et al. 2010), and age (estimation based on tooth wear; Severinghaus 1949, Robinette et al. 1957) of each captured female prior to release. We determined pregnancy via transabdominal ultrasonography (E.I. Medical Imaging portable ultrasound; Smith and Lindzey 1982). Pregnant females were fitted with GPS collars equipped with Neolink technology and a vaginal implant transmitter (VIT; Model M3930U, Advanced Telemetry Systems Inc., Isanti, MN, USA) with a temperature and light sensitive switch. Neolink radio-paring technology in the GPS collar monitors the status of the VIT and, subsequently, the neonate collar. This technology allows remote monitoring of both VITs and neonate collars.

Neonate Capture and Monitoring

When VITs were expelled from deer during parturition and the VIT detected light or a temperature below 32°C, the VIT broadcast a birth message to the GPS collar. The birth message sent to the GPS collar triggered an email alerting us of the birthing event. The very high frequency (VHF) beep pattern of the VIT also changed from a 30 ppm pattern to a 60 ppm

pattern. When an email alert was received, we waited at least 6 hours to allow time for bonding between adult and offspring before beginning our search for neonates (Heffelfinger et al. 2018). We used a combination of the GPS location, from the adult collar at the time the VIT was expelled, and radio-telemetry to locate the VIT and parturition site. Once the VIT was located we performed a systematic search to locate the neonatal fawn(s).

Upon discovery of neonates, we fitted them with a Neolink series VHF, mortality-sensing, drop-off radio collar (Model M4230BU, Advanced Telemetry Systems, Isanti, MN, USA). We recorded several measurements including weight, chest girth, hind foot length, and new hoof growth (to estimate the age of the neonate(s); Haugen and Speake 1958, Robinette et al. 1973, Sams et al. 1996, Lomas and Bender 2007). We handled neonates with nitrile gloves, kept handling time to a minimum, and replaced them at site of capture in order to reduce the transfer of human scent and the likelihood of maternal abandonment.

When the adult female collar and the neonate collar were more than 150 meters apart for 12 hours an absence warning was sent via email. If the neonate collar remained motionless for 8 hours, a mortality warning was also sent and the VHF beep-pattern increased from 30 to 60 ppm. We attempted to locate neonates within 24 hours of a mortality notification in order to determine cause of mortality. After locating a collar in mortality, we searched for the fawn carcass and examined evidence found at the mortality site. We used field or lab necropsies to determine cause of death and classified mortalities into the following causes using criteria from the literature: bobcat predation, cougar predation, coyote predation, unknown predation, malnutrition/disease, accident, capture related, and unknown (White 1973, Gese and Grothe 1995, Stonehouse et al. 2016). All handling of animals was approved by the Institutional Animal

Care and Use Committee at Brigham Young University, and was in accordance with guidelines from the American Society of Mammalogists (Sikes and Gannon 2016).

Juvenile Recapture

For clarity, we use the term 'neonate' to describe offspring from zero to six months, and the term 'fawn' or 'juvenile' for all ages there after. A subset of neonates were recaptured, weighed and fitted with GPS collars in December of each year (via the same helicopter net-gunning procedure described above) at six months of age. This recapture enabled us to examine growth rates, attach a permanent GPS collar, monitor juveniles beyond six months of age, and obtain complete life-history data of selected individuals. Because the number of neonates available for recapture was limited, we sampled additional animals with unknown parentage to increase sample size.

Statistical Analysis

To determine the influence of maternal effects on birth weight and growth rate of neonates, and body size at six months, we used generalized linear models in program R (version 4.0.2). Variables included maternal age and condition, neonate birth weight, sex, presence of twin, hoof growth, capture date, year of birth, and fawn growth. We formulated a list of *a priori* models for each of the response variables including birth weight, growth rate, and body size at six months of age. Prior to construction of models, we evaluated multicollinearity among all variables and did not include variables that were correlated ($r > |0.50|$) in the same model. We evaluated and ranked *a priori* models using Akaike's Information Criteria and AICc weights (Akaike 1973, Burnham and Anderson 2002). We considered strongly competing models to be

those with ΔAICc <2. In the event of competing models, we averaged models that carried >5% AICc weight.

We evaluated survival from birth to six months of age, and from six months of age to 18 months of age using Cox Proportional Hazard (CPH) regression models. CPH models allow for estimates of survival for each individual, based on sampled variables and varying time components (Cox 1972, Fox 2002). Time components for survival to six months included monthly survival from zero to six months of age, and a comparison of survival between the first month of life and months two through six following parturition. Sampled variables included maternal condition, neonate sex, birth weight, presence of twin, and days from peak parturition. We modeled survival to 18 months of age daily. Because some of the additional juveniles sampled had unknown parentage, variables included in this model were limited to sex, year, and capture weight. We determined the most influential factors associated with neonate survival to six months and juvenile survival to 18 months. Similar to the generalized linear models, we first formulated *a priori* models and then ranked them based on minimization of Akaike's Information Criteria and AICc weights (Akaike 1973, Burnham and Anderson 2002).

RESULTS

Between March of 2018 and March of 2020 we captured and collared 89 female mule deer. Of these adults, 22 individuals were captured in two consecutive years and one individual was captured all three years for a total of 112 capture events. Average ingesta-free body fat (IFBF) for adult females was 6.06% in 2018, 4.34% in 2019, and 6.26% in 2020. Ultrasonography revealed that 106 of 112 (95%) individuals captured were pregnant. We captured neonates from 61 collared adult females. In three fawning seasons we located 104 neonates and captured and

collared 98 individuals (6 were stillborn). Over the course of the study we observed 39 sets of twins, and 20 singletons. Of neonates captured, 49 were male and 49 were female. Parturition dates ranged from 28 May to 29 June. Mean date of parturition was June 8th, 14th, and 9th during 2018-2020, respectively. Between December of 2018 and December of 2020 we captured and collared 59 juvenile fawns. Of juveniles captured, 27 were individuals originally captured as neonates. Average weight was 30.5 kg in 2018 (SE = 1.2), 31.0 kg in 2019 (SE = 0.8), and 33.6 kg in 2020 (SE = 1.0).

We examined the influence of maternal effects on birth weight for 98 neonates. Out of 23 candidate models, the top model examining neonate birth weight accounted for 78.4% of the AICc weight compared to 15.7% for the second most supported model (no competing models, ΔAICc<2; Table 1-1). The most supported model included influence of twin, sex, hoof growth, maternal condition (i.e., IFBF) and maternal age (Table 1-2). As predicted, birth weight of neonates was positively influenced by maternal condition. On average for females with the lowest IFBF, neonates weighed 3.0 kg at birth (SE = 0.1). For females with higher IFBF, neonates averaged 3.5 kg at birth (SE = 0.1; Figure 1-2). Presence of a twin was correlated with lower birth weight. On average, neonates associated with a twin weighed 0.2 kg less than those that were singletons. Age was in the top model but it did not account for much of the model weight. Hoof growth was also associated with greater birth weight. Measurement of hoof growth is used to determine age of neonate at capture, and older neonates would be heavier than those captured closer to parturition. Males were also associated with greater birth weight (Table 1-2).

We examined growth rates of 27 individuals recaptured at six months of age. We had four competing models (ΔAICc<2) and averaged all models that carried >5% AICc weight. The averaged models had a cumulative weight of 65.3% (Table 1-3). Variables in the averaged

models included maternal condition, birth weight, sex, and twin. Maternal condition was positively related to growth at six months. On average neonates from females in better condition (higher IFBF) grew at a rate of 5.2 kg per month (SE = 0.2), while those from females in poorer condition grew at a rate of 4.4 kg per month (SE = 0.2; Figure 1-3).

Our top CPH model for neonate survival to six months accounted for 50% of the AICc weight (no competing models, ΔAICc<2.0) and included the influence of maternal condition, a variable time component of month one versus month 2-6, and whether the neonate was born before or after the peak of parturition (Table 1-5). Neonate survival to six months was positively influenced by maternal condition. Neonates from females in better condition had a higher likelihood of survival than neonates from females in poor condition (Figure 1-4). There was a lower likelihood of survival in month one compared to months two through six, especially for fawns born after peak parturition (Table 1-6).

Further, we examined the influence of maternal effects on body size at six months for 59 individuals. The top model, with 43.7% of the AICc weight included maternal age and birth weight (Table 1-7). This is not surprising as birth weight is a life-history trait that can have long-lasting effects (Monteith et al. 2009), and reproductive success and maternal experience increase with age (Ozoga and Verme 1986, Festa-Bianchet 1988). After accounting for the effect of maternal age and birth weight, the next ranked model, with 26% of the AICc weight, included maternal condition (Table 1-8). While the effect was not as strong as at birth, juveniles from females in better condition weighed 4.1 kg more than juveniles from females in poor condition (SE = 1.3; Figure 1-5).

Our top CPH model for survival from six to 18 months accounted for 68% AICc weight. Our most supported survival model included the influence of weight and year (Table 1-9). As

predicted, larger fawns had a greater likelihood of survival (Table 1-10). Fawns weighing 35 kg at six months had at least a 50% probability of survival (Figure 1-6). Year also influenced survival. Environmental conditions were poor in 2018, northern Utah experienced a hot, dry summer followed by a large snowpack during winter. The influence of poor environmental conditions could not be overcome by weight.

DISCUSSION

The study of maternal effects to determine phenotypic quality of offspring has become foundational for understanding life-history characteristics (Kirkpatrick and Lande 1989, Bernardo 1996). Until recently, technological restraints have limited the ability to monitor free-ranging ungulates and their offspring post-weaning. Here, for the first time, we connect the influence of maternal condtion to indicators of health in mule deer fawns from birth to recruitment. Direct maternal effects on neonatal mule deer were evident during both pre- and post-parturition. Consistent with our prediction, better maternal condition correlated to increased birth weight, growth and survival of fawns to six months. Additionally, the effects of condition lasted through early life and influenced juvenile body weight and survival through the first 18 months of life.

Mule deer experienced a pre-parturition direct effect of allocation of energy to offspring in utero, manifest as birth weight. Consistent with findings from previous studies of mule deer, the condition of the female during mid-pregnancy is correlated to the size and nutritional condition of the neonate at birth (Hudson and Browman 1959, Short 1970, Heffelfinger et al. 2018). Birth weight is one of the most influential factors on growth, survival and reproductive success (Monteith et al. 2014). Our findings indicate that the condition of a female during

gestation has a significant impact on offspring birthweight. Fawns born from females in good condition are likely to be approximately 40% heavier at birth, which could lead to greater potential fitness.

Post-parturition direct effects were also present through the lactation period, as demonstrated by the influence of maternal condition on growth. Here we see that there is likely a correlation between quality or quantity of milk and maternal condition during gestation. The amount and quality of milk produced by the mother is important for growth and development of young (Cook et al. 2004, Tollefson et al. 2010, Tollefson et al. 2011). Adult females in better condition likely produce more or better milk, which in turn provides greater energy returns to nursing fawns. While there are many environmental factors that may also influence growth to six months, few studies have been able to recapture free-ranging juveniles to monitor growth at this scale.

We were able to clearly link post-parturition, direct maternal effects to survival of juvenile mule deer. There is evidence in other ungulate species that heavier females give birth earlier (Cameron et al. 1993, Keech et al. 2000). Our research creates a direct link between female condition, birth timing, and survival to six months. Fawns have a higher likelihood of survival if their mother is in good condition and if they are born at or before peak parturition. Specifically, fawns from females in the best condition, born before peak parturition have a roughly 50% greater chance of survival than fawns from females in poor condition, born after peak parturition (Figure 1-4). Further research could investigate direct influences of female condition on gestation length.

Our results do not support the assertion that maternal effects weaken after maternal care ends (Gendreau et al. 2005). Maternal care lessens after weaning, which occurs during late

summer and early fall for mule deer (Bowyer 1991, Tollefson et al. 2011). Here we see that maternal condition still influences offspring post-weaning, as fawns from mothers in better condition were heavier when recaptured in early winter (Figure 1-5). Further, the influence of maternal condition persists through the first 18 months of life. Small sample size of recaptured individuals with known parentage inhibited us from directly examining the influence of maternal condition on survival to 18 months. Nonetheless, our data demonstrate that weight at six months of age influences survival to 18 months. Therefore, we infer a connection between maternal condition, size at six months of age, and survival to 18 months of age.

Mule deer are a model species to study the importance and persistence of maternal effects because they are long-lived and have numerous offspring over their lifespan. However, it has been difficult to monitor free-ranging adult females and their offspring to examine effects over time. Our ability to capture and recapture both adults and juveniles provided a clear linkage between direct maternal effects and recruitment of mule deer. We demonstrated that maternal effects were present both pre- and post-parturition. We found that maternal nutritional condition directly influences fawn health during gestation, lactation, and early life. Herd health and reproductive potential cannot rely solely on population size or sex ratio but must also include metrics of maternal health.

LITERATURE CITED

Akaike, H. 1973. Information theory as an extension of the maximum likelihood principle. Akademiai Kiado, Budapest, Hungary.

Bardsen, B. J., P. Fauchald, T. Tveraa, K. Langeland, N. G. Yoccoz, and R. A. Ims. 2008. Experimental evidence of a risk-sensitive reproductive allocation in a long-lived mammal. Ecology **89**:829-837.

Benton, T. G., E. Ranta, V. Kaitala, and A. P. Beckerman. 2001. Maternal effects and the stability of population dynamics in noisy environments. Journal of Animal Ecology **70**:590-599.

Bernardo, J. 1996. Maternal effects in animal ecology. American Zoologist **36**:83-105.

Bowyer, R. T. 1991. Timing of parturition and lactation in southern mule deer. Journal of Mammalogy **72**:138-145.

Burnham, K. P., and D. R. Anderson. 2002. A practical information-theoretic approach. Model selection and multimodel inference **2**.

Cameron, R. D., W. T. Smith, S. G. Fancy, K. L. Gerhart, and R. G. White. 1993. Calving success of female caribou in relation to body weight. Canadian Journal of Zoology **71**:480-486.

Cook, J. G., B. K. Johnson, R. C. Cook, R. A. Riggs, T. Delcurto, L. D. Bryant, and L. L. Irwin. 2004. Effects of summer-autumn nutrition and parturition date on reproduction and survival of elk. Wildlife Monographs:1-61.

Cox, D. R. 1972. Regression models and life-tables. Journal of the Royal Statistical Society: Series B (Methodological) **34**:187-202.

Duquette, J. F., J. L. Belant, N. J. Svoboda, D. E. Beyer Jr, and P. E. Lederle. 2014. Effects of maternal nutrition, resource use and multi-predator risk on neonatal white-tailed deer survival. Plos One **9**:e100841.

Einum, S., and I. A. Fleming. 2000. Highly fecund mothers sacrifice offspring survival to maximize fitness. Nature **405**:565-567.

Feiner, Z. S., H. Y. Wang, D. W. Einhouse, J. R. Jackson, E. S. Rutherford, C. Schelb, C. S. Vandergoot, T. G. Zorn, and T. O. Höök. 2016. Thermal environment and maternal effects shape egg size in a freshwater fish. Ecosphere **7**:e01304.

Festa-Bianchet, M. 1988. Age-specific reproduction of bighorn ewes in Alberta, Canada. Journal of Mammalogy **69**:157-160.

Festa-Bianchet, M., J.-M. Gaillard, and J. T. Jorgenson. 1998. Mass-and density-dependent reproductive success and reproductive costs in a capital breeder. The American Naturalist **152**:367-379.

Festa-Bianchet, M., and J. T. Jorgenson. 1998. Selfish mothers: Reproductive expenditure and resource availability in bighorn ewes. Behavioral Ecology **9**:144-150.

Fox, J. 2002. Cox proportional-hazards regression for survival data. An R and S-PLUS companion to applied regression **2002**.

Freeman, E. D., R. T. Larsen, K. Clegg, and B. R. McMillan. 2013. Long-Lasting Effects of Maternal Condition in Free-Ranging Cervids. Plos One **8**:10.

Gendreau, Y., S. D. Côté, and M. Festa-Bianchet. 2005. Maternal effects on post-weaning physical and social development in juvenile mountain goats (Oreamnos americanus). Behavioral Ecology and Sociobiology **58**:237-246.

Gese, E. M., and S. Grothe. 1995. Analysis of coyote predation on deer and elk during winter in Yellowstone National Park, Wyoming. American Midland Naturalist **133**:36-43.

Haugen, A. O., and D. W. Speake. 1958. Determining age of young fawn white-tailed deer. The Journal of Wildlife Management **22**:319-321.

Haywood, S., and C. M. Perrins. 1992. Is clutch size in birds affected by environmental conditions during growth? Proceedings of the Royal Society of London. Series B: Biological Sciences **249**:195-197.

Heffelfinger, L. J., K. M. Stewart, A. P. Bush, J. S. Sedinger, N. W. Darby, and V. C. Bleich. 2018. Timing of precipitation in an arid environment: Effects on population performance of a large herbivore. Ecology and Evolution **8**:3354-3366.

Hewison, A. M., and J.-M. Gaillard. 1999. Successful sons or advantaged daughters? The Trivers–Willard model and sex-biased maternal investment in ungulates. Trends in Ecology & Evolution **14**:229-234.

Hudson, P., and L. G. Browman. 1959. Embryonic and fetal development of the mule deer. The Journal of Wildlife Management **23**:295-304.

Keech, M. A., R. T. Bowyer, M. Jay, V. Hoef, R. D. Boertje, B. W. Dale, and T. R. Stephenson. 2000. Life-history consequences of maternal condition in Alaskan moose. The Journal of Wildlife Management:450-462.

Kirkpatrick, M., and R. Lande. 1989. The evolution of maternal characters. Evolution **43**:485-503.

Kojola, I. 1993. Early maternal investment and growth in reindeer. Canadian Journal of Zoology-Revue Canadienne De Zoologie **71**:753-758.

Lomas, L. A., and L. C. Bender. 2007. Survival and cause-specific mortality of neonatal mule deer fawns, north-central New Mexico. Journal of Wildlife Management **71**:884-894.

Marshall, D. J., and T. Uller. 2007. When is a maternal effect adaptive? Oikos **116**:1957-1963.

Monteith, K. L., V. C. Bleich, T. R. Stephenson, B. M. Pierce, M. M. Conner, J. G. Kie, and R. T. Bowyer. 2014. Life-history characteristics of mule deer: Effects of nutrition in a variable environment. Wildlife Monographs **186**:1-62.

Monteith, K. L., L. E. Schmitz, J. A. Jenks, J. A. Delger, and R. T. Bowyer. 2009. Growth of male white-tailed deer: consequences of maternal effects. Journal of Mammalogy **90**:651-660.

Ozoga, J. J., and L. J. Verme. 1986. Relation of maternal age to fawn-rearing success in white-tailed deer. Journal of Wildlife Management **50**:480-486.

Robbins, C. T., and B. L. Robbins. 1979. Fetal and neonatal growth patterns and maternal reproductive effort in ungulates and subungulates. The American Naturalist **114**:101-116.

Robinette, W. L., C. H. Baer, R. E. Pillmore, and C. E. Knittle. 1973. Effects of nutritional change on captive mule deer. Journal of Wildlife Management **37**:312-326.

Sams, M. G., R. L. Lochmiller, E. C. Hellgren, W. D. Warde, and L. W. Varner. 1996. Morphometric predictors of neonatal age for white-tailed deer. Wildlife Society Bulletin:53-57.

Short, C. 1970. Morphological development and aging of mule and white-tailed deer fetuses. The Journal of Wildlife Management:383-388.

Sikes, R., and W. Gannon. 2016. the Animal Care and Use Committee of the American Society of Mammalogists. 2016. Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy **97**:663-688.

Smith, C. C., and S. D. Fretwell. 1974. The optimal balance between size and number of offspring. The American Naturalist **108**:499-506.

Stonehouse, K. F., C. R. Anderson, M. E. Peterson, and D. R. Collins. 2016. Approaches to Field Investigations of Cause-specific Mortality in Mule Deer. Colorado Parks and Wildlife.

Tollefson, T. N., L. A. Shipley, W. L. Myers, and N. Dasgupta. 2011. Forage quality's influence on mule deer fawns. The Journal of Wildlife Management **75**:919-928.

Tollefson, T. N., L. A. Shipley, W. L. Myers, D. H. Keisler, and N. Dasgupta. 2010. Influence of Summer and Autumn Nutrition on Body Condition and Reproduction in Lactating Mule Deer. Journal of Wildlife Management **74**:974-986.

Wauters, L. A., S. A. de Crombrugghe, N. Nour, and E. Matthysen. 1995. Do female roe deer in good condition produce more sons than daughters. Behavioral Ecology and Sociobiology **37**:189-193.

White, M. 1973. Description of remains of deer fawns killed by coyotes. Journal of Mammalogy **54**:291-293.

Wolf, J. B., and M. J. Wade. 2009. What are maternal effects (and what are they not)? Philosophical Transactions of the Royal Society B-Biological Sciences **364**:1107-1115.

FIGURES

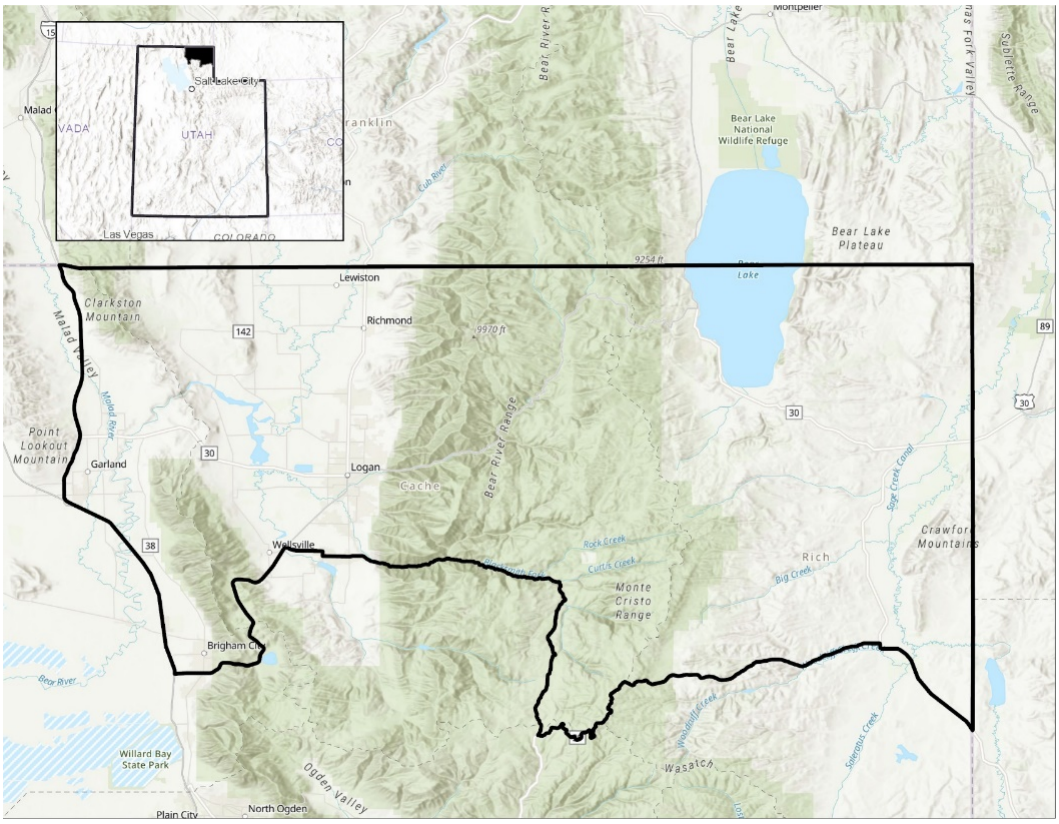

Figure 1-1. Cache management unit located in northern Utah, USA where we assessed the influence of maternal condition on birth weight, growth rate, and survival of mule deer fawns (*Odocoileus hemionus*) during 2018-2020.

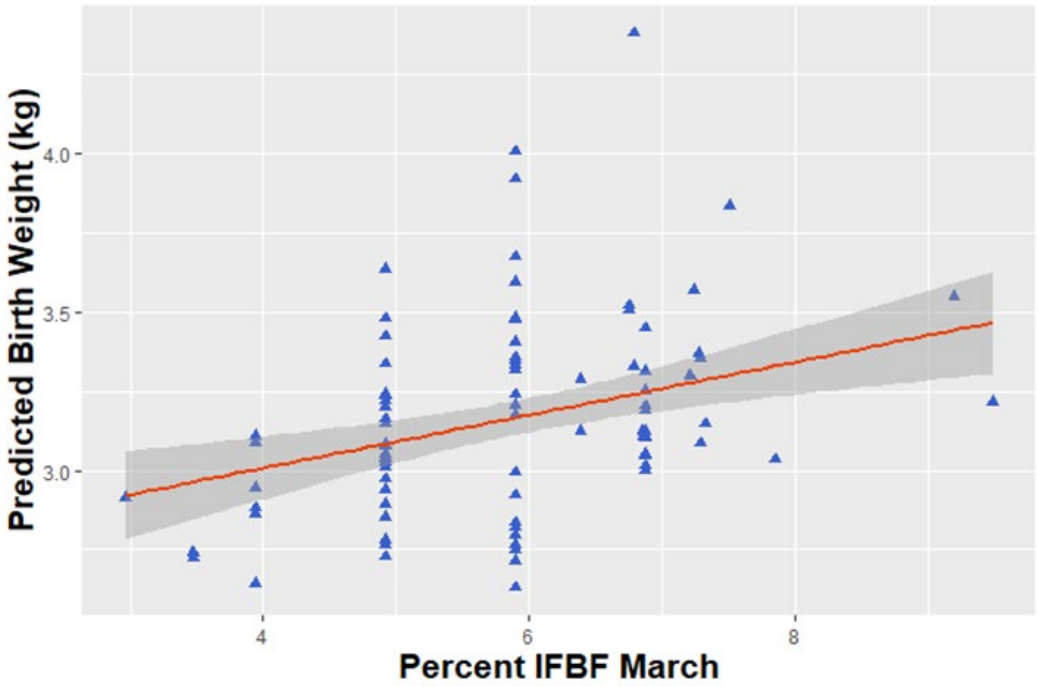

Figure 1-2. Predictive model for birth weight of neonatal mule deer based on maternal condition in March, according to the top model from AICc selection. Top model included twin, sex, IFBF, hoof growth, and adult age; northern Utah, USA, 2018-2020.

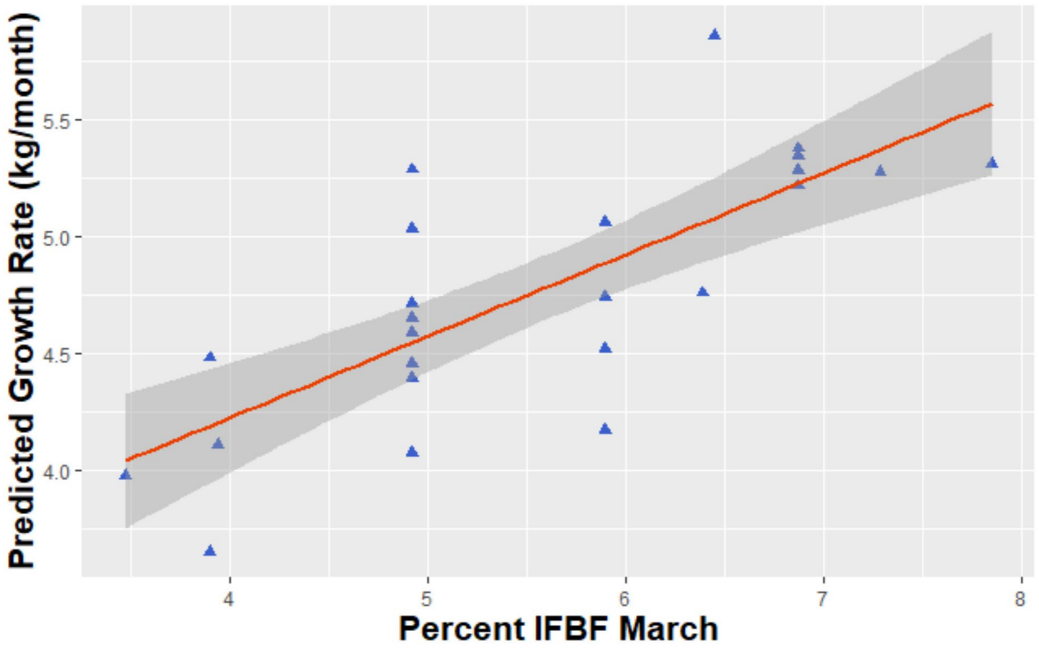

Figure 1-3. Predictive model for growth of neonatal mule deer to six months based on maternal condition in March, according to model average of all models carrying >5% AICc weight. Variables in the averaged models included IFBF, birth weight, sex, and twin; northern Utah, USA, 2018-2020.

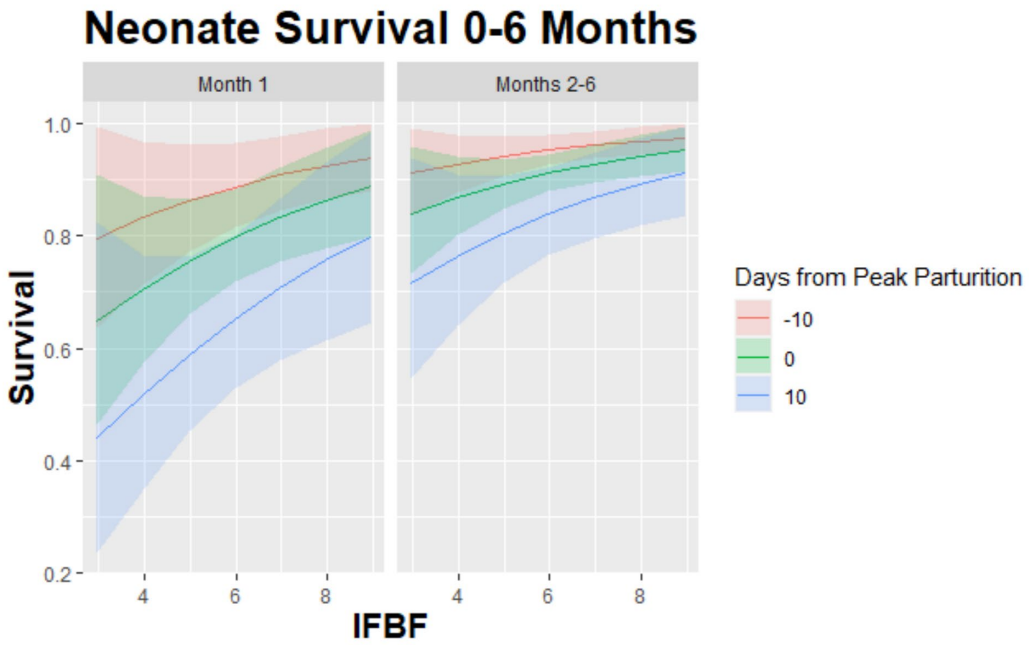

Figure 1-4. Predictive model for survival of neonatal mule deer to six months based on maternal condition, month, and birth timing; northern Utah, USA, 2018-2020.

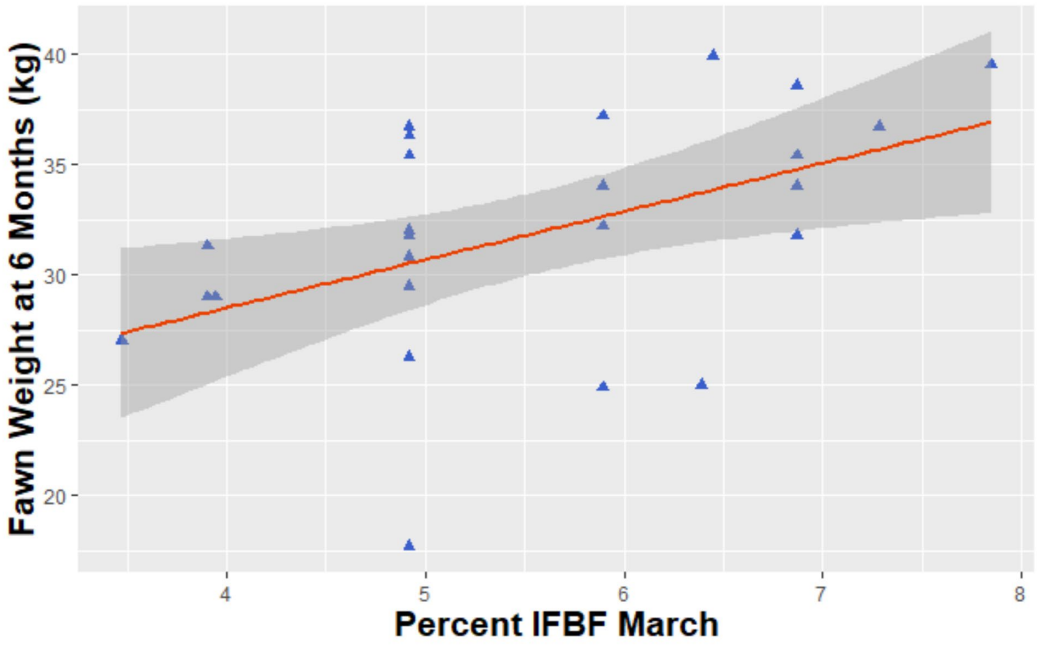

Figure 1-5. Predictive model for weight of mule deer fawns at six months based on maternal condition, age, and neonate birth weight; northern Utah, USA, 2018-2020.

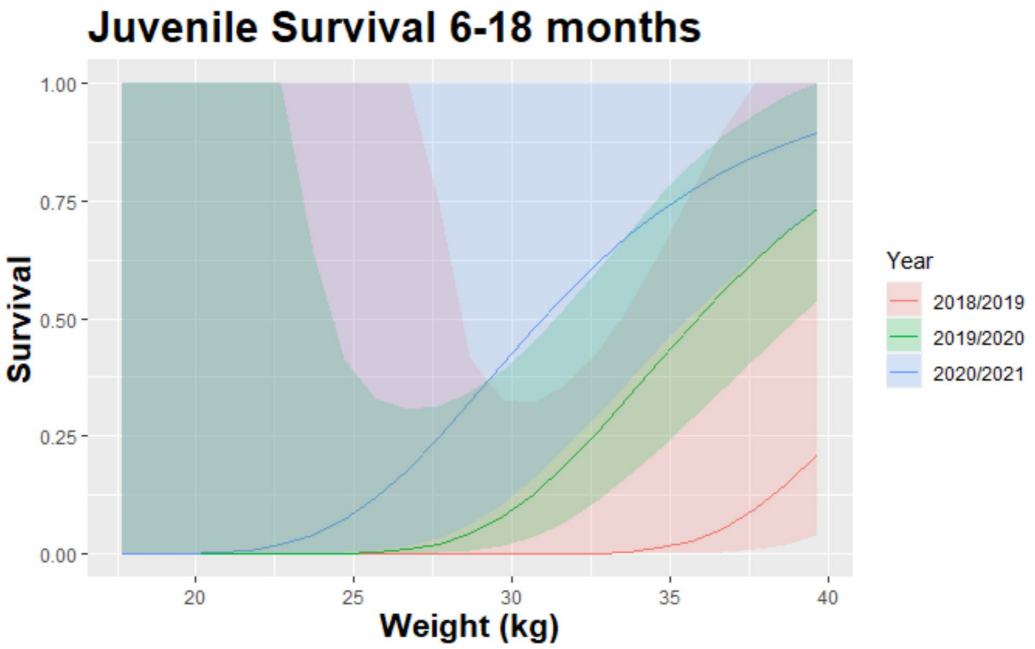

Figure 1-6. Predictive model for survival of mule deer fawns from six months to 18 months based on juvenile weight and year, according to the top model from AICc selection; northern Utah, USA, 2018-2020.

TABLES

Table 1-1. AICc model selection results of 23 candidate models for birth weight of neonatal mule deer in northern Utah, USA, 2018-2020.

	d.f.	AICc	ΔAICc	Weight
Twin+Sex+IFBF+Hoof+Adult Age	7	215.9	0.00	0.784
Year+Twin+Sex+IFBF+Hoof+Adult Age+Capture Date	10	219.2	3.25	0.154
Year+Twin	5	224.4	8.47	0.011
Year+IFBF	5	225.2	9.27	0.008
Twin+IFBF	4	225.2	9.31	0.007

Table 1-2. Top model (based on AICc) for birth weight of neonatal mule deer in northern Utah, USA, 2018-2020.

	Estimate	Std. Error	t-value
Intercept	2.3621	0.4178	< 0.001
Twin	-0.3006	0.1601	0.0633
Sex	0.0156	0.1288	0.9033
IFBF	0.1346	0.0528	0.0124
Hoof	0.1502	0.0384	< 0.001
Adult Age	-0.0394	0.0335	0.2425

Table 1-3. AICc model selection results for 27 candidate models for growth of neonatal mule deer to six months in northern Utah, USA, 2018-2020. Top model included IFBF and birth weight.

	d.f.	AICc	ΔAICc	Weight
IFBF+BW	4	60.0	0.00	0.399
IFBF+Sex+BW	5	62.6	2.56	0.111
IFBF+Twin+BW	5	63.1	3.04	0.087
BW	3	64.0	3.93	0.056
IFBF	3	64.3	4.24	0.048

Table 1-4. Output from averaged models (based on AICc) for growth of neonatal mule deer in northern Utah, USA, 2018-2020.

	Estimate	Std. Error	z-value
Intercept	1.1755	0.9784	0.2513
IFBF	0.2659	0.1352	0.0572
BW	0.6377	0.2489	0.0149
Sex	0.0291	0.1281	0.8273
Twin	0.0021	0.1139	0.9861

Table 1-5. AICc model selection results for survival of neonatal mule deer to six months in northern Utah, USA, 2018-2020. Top model included month one vs months 2-6, birth timing around peak parturition, and IFBF.

	d.f.	AICc	ΔAICc	Weight
Mo1+EvL+IFBF	3	570.87	0.00	0.50
Month+EvL+IFBF+Month^2	4	573.73	2.87	0.12
Month+EvL+IFBF	3	574.15	3.29	0.10
Halves+EvL+IFBF	3	574.33	3.46	0.09
Halves+EVL	2	575.40	4.53	0.05

Table 1-6. Top model (based on AICc) for survival of neonatal mule deer to six months in northern Utah, USA, 2018-2020.

	Estimate	Std. Error	z-value
Mo1	-0.8939	0.2866	0.0028
EvL	0.0638	0.0239	0.0017
IFBF	-0.2148	0.1225	0.0721

Table 1-7. AICc model selection results for weight of juvenile mule deer at six months in northern Utah, USA, 2018-2020. Top model included adult age and neonate birth weight.

	d.f.	AICc	ΔAICc	Weight
Adult age +BW	4	60.0	0.00	0.437
Adult age+BW+IFBF	5	62.6	2.56	0.244
Adult age+BW+mean	5	63.1	3.04	0.119
IFBF+BW	3	64.0	3.93	0.070

Table 1-8. Output from top two models (based on AICc) for weight of juvenile mule deer at six months in northern Utah, USA, 2018-2020.

	Estimate	Std. Error	t-value
Intercept	19.8057	4.7419	0.0003
Adult age	-1.2318	0.3673	0.0026
BW	5.6940	1.3103	0.0002
Intercept	14.6083	6.1820	0.0270
Adult age	-0.9605	0.4193	0.0315
BW	5.2764	1.3330	0.0006
IFBF	0.9182	0.7139	0.2112

Table 1-9. AICc model selection for survival of juvenile mule deer from 6-18 months in northern Utah, USA, 2018-2020. Top model included year and weight.

	d.f.	AICc	ΔAICc	Weight
Year+Weight	3	208.44	0.00	0.68
Sex+Year+Weight	4	209.97	1.53	0.32
Year	2	224.73	16.28	0.00

Table 1-10. Top model (based on AICc) for survival of juvenile mule deer in northern Utah, USA, 2018-2020.

	Estimate	Std. Error	z-value
Year 2019/2020	-1.6127	0.4441	6.06E-05
Year 2020/2021	-2.6421	0.6591	0.0003
Weight	-0.2112	0.0507	1.44E-05

CHAPTER 2

De novo Chromosome Level Assembly of the Mule Deer Genome

Sydney Lamb, Brock R. McMillan, Randy T. Larsen, Paul B. Frandsen
Department of Plant and Wildlife Sciences, Brigham Young University, Provo, UT
Master of Science

ABSTRACT

The mule deer (*Odocoileus hemionus*) is an ungulate species that ranges from western Canada to central Mexico. Mule deer are an essential source of food for many predators, are relatively abundant, and commonly make broad migration movements. A clearer understanding of the mule deer genome can help facilitate knowledge of its population genetics, movements, and demographic history, aiding in conservation efforts. While mule deer are excellent candidates for population genomic studies because of their large population size, continuous distribution, and diversity of habitat, few genomic resources are currently available for this species. Here, we sequence and assemble the mule deer genome into a chromosome-level assembly using long-read sequencing and Hi-C for use in future research. We also provide a genome annotation and compare demographic histories of the mule deer and whitetail deer using PSMC. We expect this assembly to be a valuable resource in the continued study and conservation of mule deer.

INTRODUCTION

The mule deer (*Odocoileus hemionus*) is a mid-sized ruminant (50-90 kg; Renecker and Samuel 1991, Mysterud 2000), ranging from the Yukon Territory in Canada to Central Mexico. Mule deer can be found in boreal forests, high and low elevation desert shrublands, subalpine forests, woodlands, prairies, and a variety of other habitats with subspecies and types frequently inhabiting different habitats (Wallmo 1981). They belong to the Cervidae family, one of the most speciose families in the suborder Ruminantia (Anderson and Wallmo 1984). Eleven subspecies of mule deer have been recognized but are grouped into two morphologically distinct types, mule deer (*O. h. hemionus, fulginatus, californicus, inyoensis, eremicus, crooki, peninsulae, sheldoni,* and *cerrosensis*) and black-tailed deer (*O.h. columbianus*, and *sitkensis*; Latch et al. 2009). While the two types are well-supported by morphological and DNA evidence, little divergence has been observed among the subspecies within each type (Cronin 1991, Carr and Hughes 1993). This is likely due to large population sizes and the frequency of long-distance dispersal by individual deer (Brown 1992, Alerstam et al. 2003).

Characteristics such as large population size, diversity of habitat and capacity for long distance dispersal make mule deer a good candidate species for genomic study (Luikart et al. 2003, Hohenlohe et al. 2010, Schwartz et al. 2010). However, limited genomic resources are available. Currently, genetic resources available for *Odocoileus spp*. are limited to a variety of microsatellite loci (Bishop et al. 1994, DeWoody et al. 1995, Jones et al. 2000) and molecular resources gleaned from the bovine genome (Haynes and Latch 2012, Brauning et al. 2015, Powell et al. 2016). Recently, Russell et al. published the first draft whole genome sequence assembly and a species diagnostic SNP panel specifically for mule deer (Russell et al. 2019).

However, this assembly was based on low-coverage short-read sequencing (Illumina) and was assembled using a reference based approach, limiting identification of large structural variants.

Here, we report a high-quality, chromosome-level draft reference genome for mule deer assembled from a combination of long-read (PacBio) and short-read (Illumina) sequence data and scaffolded using Hi-C. Our goal was to develop whole genome resources that will aid in better understanding population dynamics. These resources would help address questions related to mating systems, parentage assignment, relatedness, estimation of demographic parameters, population genetic analysis, and assessment of population viability (Miller et al. 2019). We also provide an annotation and estimate demographic histories of both the white-tail and mule deer using the Pairwise Sequentially Markovian Coalescent (PSMC) model. We discuss how this new genome assembly can be applied to conservation and management of mule deer.

MATERIALS AND METHODS

Sample collection and DNA preparation

A tongue biopsy was collected within 2 hours post mortem from a single female mule deer that was removed for depredation purposes from Woodland Hills, Utah (40°00’ N 111° 38’ W). The biopsy was immediately stored on ice and frozen at -80° Celsius within 12 hours of collection. The sample remained frozen at -80° Celsius until DNA extraction and sequencing were performed. Genomic DNA was extracted from the tongue tissue using proteinase K and a Qiagen Genomic Tip Kit for High Molecular Weight DNA following Qiagen’s extraction protocol (Qiagen, Valencia, CA, USA). After extraction, the DNA was visualized with pulsed-field gel electrophoresis to evaluate the DNA strands for sufficient length required by SMRT (single-molecule real-time) sequencing using PacBio Sequel II (Rhoads and Au 2015).

Sequencing and Assembly

The DNA extractions were successful on the first attempt and the pulsed-field gel showed sufficient DNA length, with a band around 50 kbp. The extracted DNA was sheared to 65 kbp and then size-selected for fragments greater than 32 kbp using a Sage Science BluePippin. The size-selected DNA was prepared into a PacBio library using the SMRTbell® Express Template Preparation Kit 2.0 (PacBio, USA). The library was sequenced across two PacBio Sequel II 8M SMRT cells (PN: 101-389-001). Each run was performed at the Brigham Young University DNA Sequencing Center (Provo, Utah).

Extracted DNA was prepared into a paired-end Illumina library with a fragment size of 500 bp. The library was prepared using the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina, and the manufacturer's instructions were followed as outlined in the kit manual (New England BioLabs, Inc., USA). The library was sequenced across two Illumina HiSeq 2500 lanes with 2x150 bp paired-end sequencing at the Brigham Young University DNA Sequencing Center (Provo, Utah).

We converted the PacBio subreads BAM file to FASTQ using Samtools v.1.9 (Li et al. 2009). We used WTDBG2 v.2.5-1 to assemble the reads into contigs with the command parameters "-x sq -g 2.3G -t 80 -L5000" due to the assembler's speed and accuracy comparable to other long read assemblers (Ruan and Li 2020). Reads shorter than 5000 bp were removed and not used in the assembly using the "–L5000" parameter in WTDBG2. The approximate genome size was estimated using a previous reference guided mule deer genome, and the parameter was set to 2.3 Gbp. The consensus sequence was derived using the command "wtpoa-cns -t 80 -i"

(Kundu et al. 2019). The genome assembly was performed on the Fulton Supercomputer (Brigham Young University, Provo, Utah, USA).

Genome Polishing

We performed an initial error correction step by remapping the PacBio long reads back to the WTDBG2 contig assembly sequence using Minimap2 v.2.17-r941 " -ax map-pb -t 40" and sorting, indexing, and converting the alignment file with the command "sort -o -T reads.tmp" and "index reads.sorted.bam" in Samtools v.1.9 into BAM format. We performed Racon error correction twice using "-u -t 80" parameters with the PacBio reads, with a separate alignment file created for each run.

We conducted genome polishing with high fidelity short-read data by first mapping Illumina reads to the Racon corrected consensus assembly. We first trimmed adapters from the Illumina sequences using Trim Galore v.0.6.4. We then mapped Illumina reads to the Racon corrected assembly using BWA v.0.7.17-r1188 and sorted and indexed the alignment file with Samtools v.1.9. We used Pilon v.1.23 to correct indel erros using "--vcf --tracks --fix indels -- diploid" parameters. We then ran a second round of indel correction by repeating the steps above on the output from the first round of Pilon.

We generated assembly statistics using the assembly_stats script (Trizna 2020). We used BUSCO v. 3.0.2 used to evaluate the recovery of universal single copy orthologs using the mammalia_odb9 ortholog set (Simão et al. 2015)

Chromosome-level Scaffolding

High-throughput chromosome conformation capture (Hi-C) was performed to provide chromosome level scaffolding for the consensus genome (Figure 2-3). In brief, in situ Hi-C data was aligned to a draft genome assembly using the Juicer pipeline (Durand et al. 2016). The 3D-DNA pipeline (Dudchenko et al. 2017) was used to error-correct, anchor, order and orient the pieces in the draft assembly. The 3D-DNA visualization module, in conjunction with Juicer Tools, was used to create contact maps for the draft and the final genome assemblies. As a rule, 3D-DNA was run without parameter tuning. Instead, a manual review step using Juicebox Assembly Tools aka JBAT (Dudchenko et al. 2018, Robinson et al. 2018) was employed to polish the genome assembly output by 3D-DNA. The Hi-C scaffolding was performed by DNA Zoo Consortium (Houston, TX, USA).

Genome annotation

RepeatMasker software (http://www.repeatmasker.org/) was used with the NCBI engine to estimate the overall repeat content of the genome (Tarailo-Graovac and Chen 2009). Repeat databases were built using RepeatModeler with parameters "BuildDatabase -name -engine ncbi && RepeatModeler -engine ncbi -pa 8 -database". RepeatMasker v.4.1.1 was used to identify repeats using the parameters "-pa 16 -gff -nolow -lib".

We performed homology-based gene prediction using Gene Model Mapper (GeMoMa) v.1.6.4 with the existing *Odocoileus virginianus* (Keilwagen et al. 2019) genome annotation used as a reference; the following command was used "GeMoMa -Xmx50G GeMoMaPipeline threads=40 outdir=annotation_out GeMoMa.Score=ReAlign AnnotationFinalizer.r=NO o=true

t=mule_deer.fa i=white_tail a=GCF_002102435.1_Ovir.te_1.0_genomic.gff g=GCF_002102435.1_Ovir.te_1.0_genomic.fna".

Historical demography

We used the Pairwise Sequentially Markovian Coalescent (PSMC) v.0.6.5-r67 to estimate the demographic history of the mule deer (Li and Durbin 2011) . We re-aligned Illumina reads to the final assembly with BWA and sorted and indexed the alignment file in Samtools. We used mpileup and bcftools to call heterozygous sites using the command "samtools mpileup -C50 -uf" and "bcftools call -c" respectively. Additionally, Bcftools v.1.11 was used with the vcfutils.pl utility and the following parameters "vcf2fq -d 10 -D 90". We then used PSMC v.0.6.5-r67 to generate the demography history with the following command "fq2psmcfa -q20 | psmc -N25 -t15 -r5 -p "4+25*2+4+6", which we visualized with psmc_plot.pl with a generation time of 5 years and a mutation rate of $3.22x10^{-8}$ using "psmc_plot.pl -p -u 3.22e-08 -g 5". To compare demographic histories with the other most common North American deer species, the white-tailed deer (*Odocoileus virginianus*), we followed the same process described above. We downloaded the *O. virginianus* assembly from NCBI (accession: NC_015247). We downloaded the raw Illumina reads from the sequence read archive (SRA) using fastq-dump, a utility within SRAtoolkit v.2.10.9, with the following parameters "fastq-dump --gzip --skip-technical --readids --read-filter pass --dumpbase --split-e –clip". Because fastq-dump alters read names, individual read names were corrected to match in both the forward and reverse fastq files by removing ".1" from the end of the forward reverse identifier and ".2" from the end of the reverse sequence identifier.

RESULTS

Sequencing results

We recovered 239 giga-bases of PacBio subread data (~90x coverage) from the two PacBio Sequel II SMRT cells. The first SMRT cell generated 114.19 Gbp of subread data with a mean polymerase read length of 23,861 bp and a read n50 of 31,007 bp. The second SMRT cell generated 125.82 Gbp of subread data with a mean polymerase read length of 29,002 bp and a read n50 of 46,596 bp (Hebert et al. 2018). The Illumina sequencing run yielded ~690 million reads equaling 87.2 Gbp of raw sequence data. Using the homology-based gene prediction technique, we successfully identified 21,983 full-length genes.

Assembly

We successfully assembled a de novo genome of a female mule deer (Figure 2-1). The assembled genome has a total length of 2.61 Gbp with a gc content of 42.8% and a contig N50 of 28.6 Mbp (Table 2-1). The longest contig was roughly 96.5 Mbs. After Hi-C scaffolding, we successfully placed 93.45% of the total base pairs into chromosomes. We successfully identified 92.7% of BUSCO genes in the assembly, with 90.5% single copy and 2.2% of duplicated, comparable to other recently published cervid genomes (Table 2-2; Ba et al. 2020).

Demographic History

We used a PSMC analysis to compare historic population trends of *O. hemionus* and *O. virginianus*. In comparing the PSMC analysis, we observe that *O. hemionus* and *O. virginianus* have divergent demographic histories. As effective population size for *O. hemionus* increases, the effective population size of *O. virginianus* appears to decrease, and vice versa. The effective

population size of *O. hemionus* has been in a constant decline since the most recent glacial period roughly 500,000 years ago. Two possible explanations for this decline may include overall population decline or population fragmentation. This appears to be in contrast to *O. virginianus* which has shown increases in effective population size since the same time period. While both deer species inhabit the same continent, and even possess some overlapping habitat, it appears that the species react differently to environmental changes (Figure 2-2).

DISCUSSION

Re-use potential

Our high-quality draft genome of the mule deer represents an advance in available genomic data for the *Odocoileus* genera. With a total length of 2.6 Gb and a contig N-50 of 28.6Mbs, this de novo draft assembly can serve as a base for future conservation and research. Due to the importance of deer on both the ecosystem level and to local economies, a continued effort to conserve these populations is vital (Hobbs 1996). Our hope is that this genome can further our understanding of *O. hemionus*, and subsequently lead to more effective management of the species. Use of this genome may provide insight into the impact of anthropogenic barriers on gene flow, the possibility of species divergence in isolated populations, and the presence of multiple paternity (Carling et al. 2003, LaCava et al. 2020).

LITERATURE CITED

Alerstam, T., A. Hedenström, and S. Åkesson. 2003. Long-distance migration: evolution and determinants. Oikos **103**:247-260.

Anderson, A. E., and O. C. Wallmo. 1984. Odocoileus hemionus. Mammalian species:1-9.

Ba, H., Z. Cai, H. Gao, T. Qin, W. Liu, L. Xie, Y. Zhang, B. Jing, D. Wang, and C. Li. 2020. Chromosome-level genome assembly of Tarim red deer, Cervus elaphus yarkandensis. Scientific data **7**:1-8.

Bishop, M. D., S. M. Kappes, J. W. Keele, R. T. Stone, S. Sunden, G. A. Hawkins, S. S. Toldo, R. Fries, M. D. Grosz, and J. Yoo. 1994. A genetic linkage map for cattle. Genetics **136**:619-639.

Brauning, R., P. J. Fisher, A. F. McCulloch, R. J. Smithies, J. F. Ward, M. J. Bixley, C. T. Lawley, S. J. Rowe, and J. C. McEwan. 2015. Utilization of high throughput genome sequencing technology for large scale single nucleotide polymorphism discovery in red deer and Canadian elk. bioRxiv:027318.

Brown, C. G. 1992. Movement and migration patterns of mule deer in southeastern Idaho. The Journal of Wildlife Management:246-253.

Carling, M. D., P. A. Wiseman, and J. A. Byers. 2003. Microsatellite analysis reveals multiple paternity in a population of wild pronghorn antelopes (Antilocapra americana). Journal of Mammalogy **84**:1237-1243.

Carr, S. M., and G. A. Hughes. 1993. Direction of introgressive hybridization between species of North American deer (Odocoileus) as inferred from mitochondrial-cytochrome-b sequences. Journal of Mammalogy **74**:331-342.

Cronin, M. A. 1991. Mitochondrial and nuclear genetic relationships of deer (Odocoileus spp.) in western North America. Canadian Journal of Zoology **69**:1270-1279.

DeWoody, J., R. Honeycutt, and L. Skow. 1995. Microsatellite markers in white-tailed deer. The Journal of heredity **86**:317-319.

Dudchenko, O., S. S. Batra, A. D. Omer, S. K. Nyquist, M. Hoeger, N. C. Durand, M. S. Shamim, I. Machol, E. S. Lander, and A. P. Aiden. 2017. De novo assembly of the Aedes aegypti genome using Hi-C yields chromosome-length scaffolds. Science **356**:92-95.

Dudchenko, O., M. S. Shamim, S. S. Batra, N. C. Durand, N. T. Musial, R. Mostofa, M. Pham, B. G. St Hilaire, W. Yao, and E. Stamenova. 2018. The Juicebox Assembly Tools module facilitates de novo assembly of mammalian genomes with chromosome-length scaffolds for under $1000. bioRxiv:254797.

Durand, N. C., M. S. Shamim, I. Machol, S. S. Rao, M. H. Huntley, E. S. Lander, and E. L. Aiden. 2016. Juicer provides a one-click system for analyzing loop-resolution Hi-C experiments. Cell systems **3**:95-98.

Haynes, G. D., and E. K. Latch. 2012. Identification of novel single nucleotide polymorphisms (SNPs) in deer (Odocoileus spp.) using the BovineSNP50 BeadChip. Plos One **7**:e36536.

Hebert, P. D., T. W. Braukmann, S. W. Prosser, S. Ratnasingham, J. R. DeWaard, N. V. Ivanova, D. H. Janzen, W. Hallwachs, S. Naik, and J. E. Sones. 2018. A Sequel to Sanger: amplicon sequencing that scales. BMC genomics **19**:1-14.

Hobbs, N. T. 1996. Modification of ecosystems by ungulates. The Journal of Wildlife Management:695-713.

Hohenlohe, P. A., P. C. Phillips, and W. A. Cresko. 2010. USING POPULATION GENOMICS TO DETECT SELECTION IN NATURAL POPULATIONS: KEY CONCEPTS AND

METHODOLOGICAL CONSIDERATIONS. International Journal of Plant Sciences **171**:1059-1071.

Jones, K., K. Levine, and J. Banks. 2000. DNA-based genetic markers in black-tailed and mule deer for forensic applications. California Fish and Game **86**:115-126.

Kundu, R., J. Casey, and W.-K. Sung. 2019. Hypo: super fast & accurate polisher for long read genome assemblies. bioRxiv.

LaCava, M. E., R. B. Gagne, S. M. L. Stowell, K. D. Gustafson, C. A. Buerkle, L. Knox, and H. B. Ernest. 2020. Pronghorn population genomics show connectivity in the core of their range. Journal of Mammalogy **101**:1061-1071.

Latch, E. K., J. R. Heffelfinger, J. A. Fike, and O. E. Rhodes Jr. 2009. Species-wide phylogeography of North American mule deer (Odocoileus hemionus): cryptic glacial refugia and postglacial recolonization. Molecular Ecology **18**:1730-1745.

Li, H., and R. Durbin. 2011. Inference of human population history from individual whole-genome sequences. Nature **475**:493-496.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin. 2009. The sequence alignment/map format and SAMtools. Bioinformatics **25**:2078-2079.

Luikart, G., P. R. England, D. Tallmon, S. Jordan, and P. Taberlet. 2003. The power and promise of population genomics: From genotyping to genome typing. Nature Reviews Genetics **4**:981-994.

Miller, W. L., J. Edson, P. Pietrandrea, C. Miller-Butterworth, and W. D. Walter. 2019. Identification and evaluation of a core microsatellite panel for use in white-tailed deer (Odocoileus virginianus). BMC genetics **20**:49.

Mysterud, A. 2000. The relationship between ecological segregation and sexual body size dimorphism in large herbivores. Oecologia **124**:40-54.

Powell, J. H., S. J. Amish, G. D. Haynes, G. Luikart, and E. K. Latch. 2016. Candidate adaptive genes associated with lineage divergence: identifying SNPs via next-generation targeted resequencing in mule deer (Odocoileus hemionus). Molecular Ecology Resources **16**:1165-1172.

Renecker, L. A., and W. Samuel. 1991. Growth and seasonal weight changes as they relate to spring and autumn set points in mule deer. Canadian Journal of Zoology **69**:744-747.

Rhoads, A., and K. F. Au. 2015. PacBio sequencing and its applications. Genomics, proteomics & bioinformatics **13**:278-289.

Robinson, J. T., D. Turner, N. C. Durand, H. Thorvaldsdóttir, J. P. Mesirov, and E. L. Aiden. 2018. Juicebox. js provides a cloud-based visualization system for Hi-C data. Cell systems **6**:256-258. e251.

Ruan, J., and H. Li. 2020. Fast and accurate long-read assembly with wtdbg2. Nature methods **17**:155-158.

Russell, T., C. Cullingham, A. Kommadath, P. Stothard, A. Herbst, and D. Coltman. 2019. Development of a Novel Mule Deer Genomic Assembly and Species-Diagnostic SNP Panel for Assessing Introgression in Mule Deer, White-Tailed Deer, and Their Interspecific Hybrids. G3: Genes|Genomes|Genetics **9**:911-919.

Schwartz, M. K., K. S. McKelvey, S. A. Cushman, and G. Luikart. 2010. Landscape genomics: a brief perspective. Pages 165-174 Spatial complexity, informatics, and wildlife conservation. Springer.

Simão, F. A., R. M. Waterhouse, P. Ioannidis, E. V. Kriventseva, and E. M. Zdobnov. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics **31**:3210-3212.

Tarailo-Graovac, M., and N. Chen. 2009. Using RepeatMasker to identify repetitive elements in genomic sequences. Current protocols in bioinformatics **25**:4.10. 11-14.10. 14.

Trizna, M. 2020. assembly_stats 0.1. 4. Zenodo. doi **10**.

Wallmo, O. C. 1981. Mule and black-tailed deer of North America. University of Nebraska Press, Lincoln, Nebraska, USA.

FIGURES

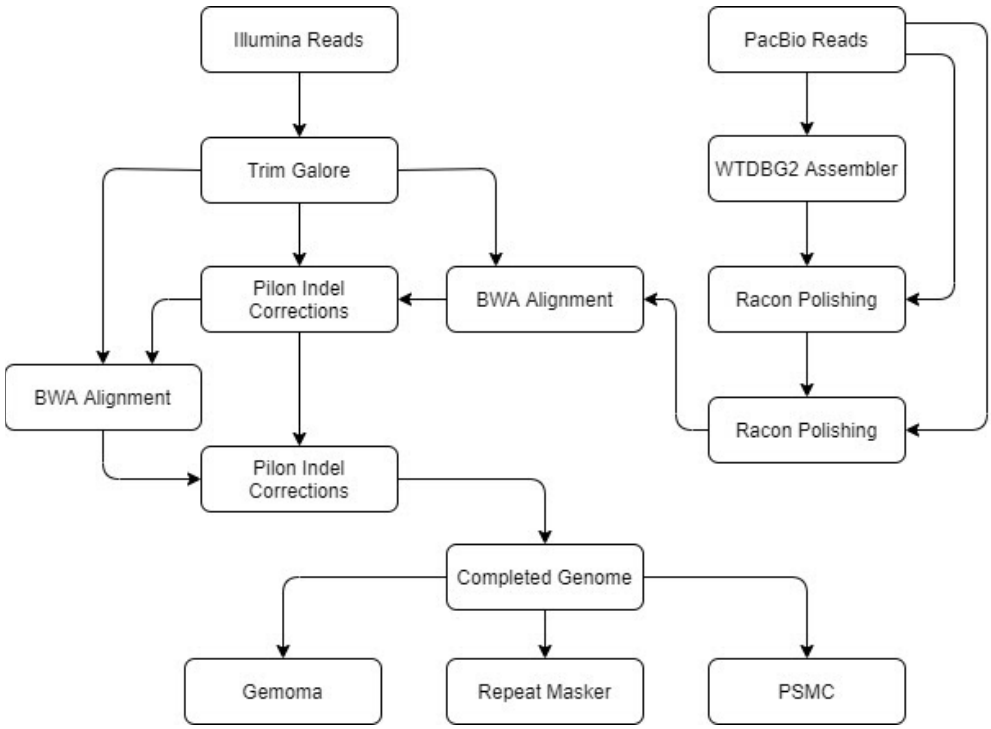

Figure 2-1. Summary chart of mule deer (*Odocoileus hemionus*) genome assembly. The genome was assembled using WTDBG2.

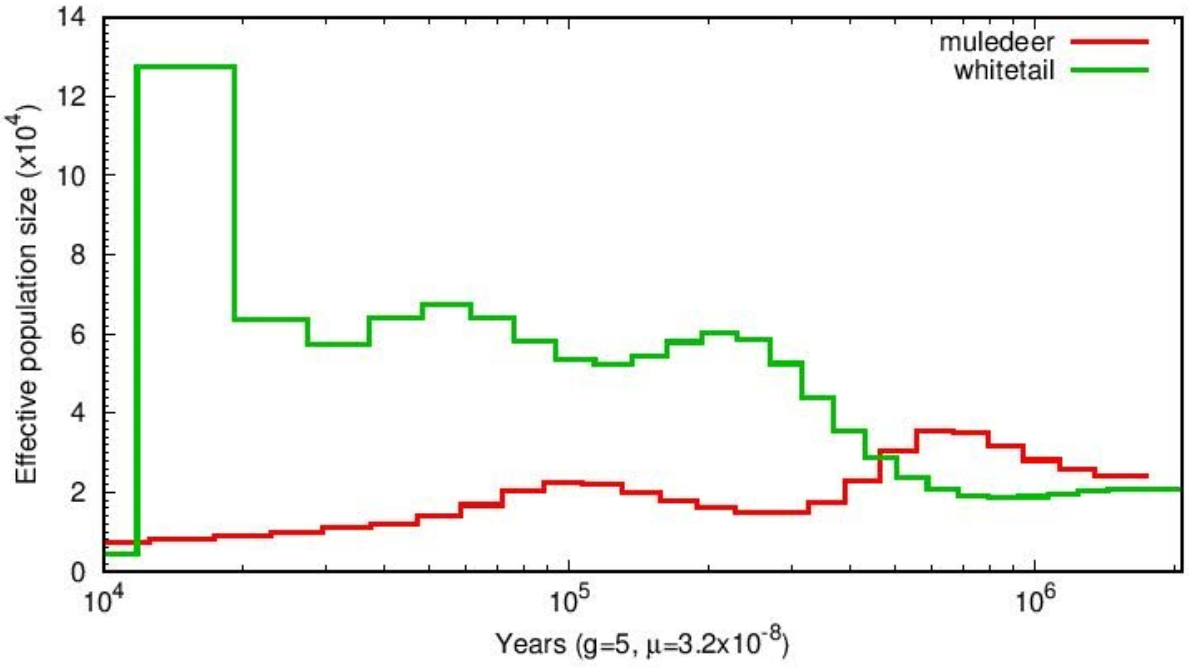

Figure 2-2. Effective population size reconstructions estimated with PSMC for *Odocoileus virginianus* and *O. hemionus.*

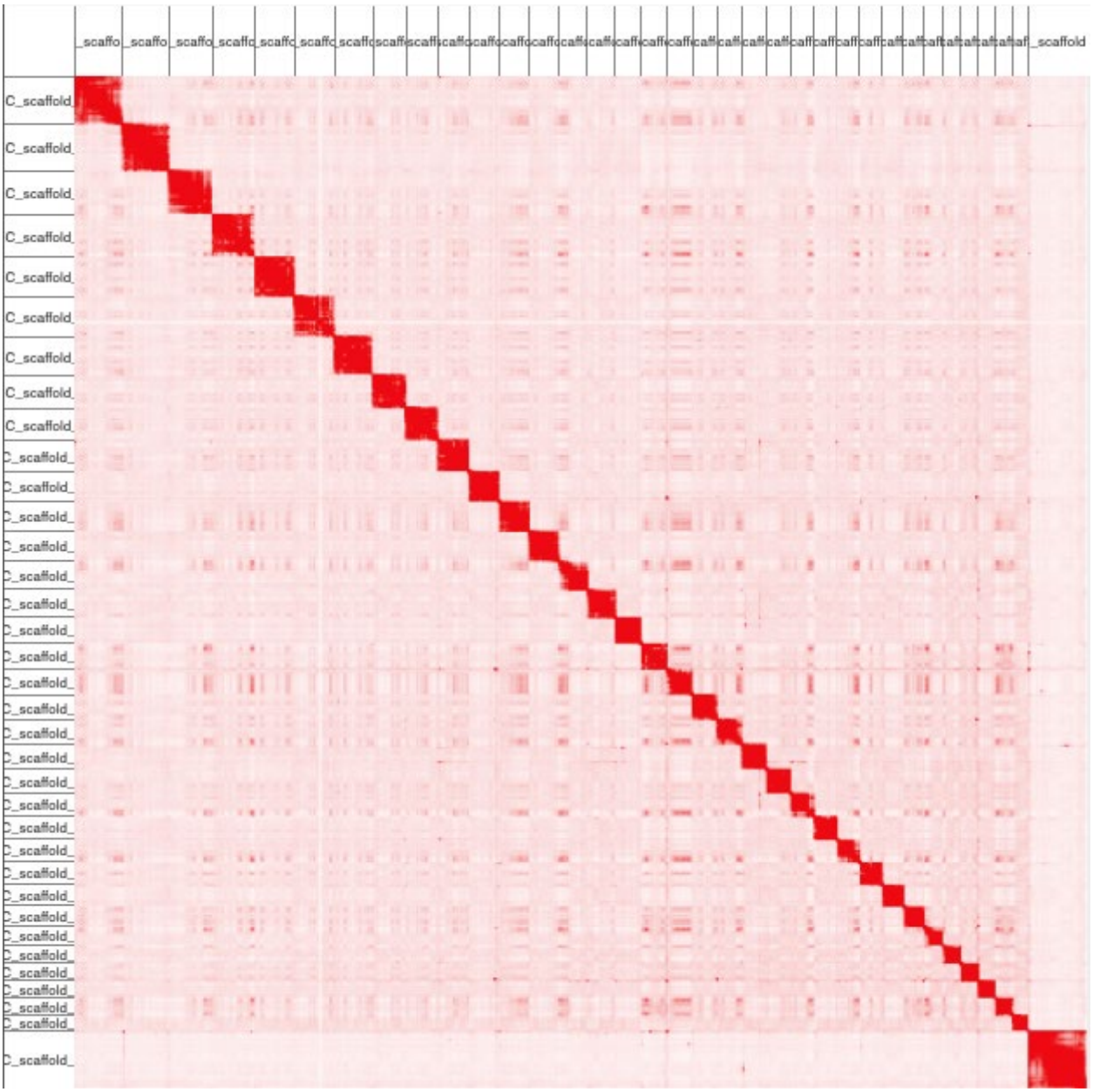

Figure 2-3. Hi-C contact map of the 35 chromosome-length scaffolds for *O.hemionus* genome assembly. 93.45% of genome is held in these chromosome length scaffolds.

TABLES

Table 2-1. Metrics of *O. hemionus* genome assembly.

Statistic	Contig Statistics	Scaffold Statistics
L10	3	2
L20	7	4
L30	11	7
L40	17	10
L50	25	13
N10	70,176,608	112,788,759
N20	61,054,283	102,607,815
N30	53,515,511	91,764,684
N40	41,741,510	76,003,873
N50	28,571,243	72,141,738
Longest	96,500,000	139,401,535
Mean	434,345	473,570
Median	23,108	20,939
Sequence Count	6,007	5,510
Shortest	1,531	1,000
GC Content	41.8	41.8
Total Base Pairs	2,609,110,763	2,609,372,263

Table 2-2. BUSCO statistics for *O. hemionus* genome assembly.

BUSCO Statistic	Number Identified	Percent Identified
Complete	3806	92.70%
Complete and Single-Copy	3714	90.50%
Complete and Duplicate	92	2.20%
Fragmented	114	2.80%
Missing	184	4.50%
Total	4104	100%