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### Cover Page Footnote

The authors thank Arkansas Game and Fish Commission Wildlife Management Division for data collection. They also thank Dr. Angela Holland for advice and review of earlier versions of this manuscript.

# Factors Affecting the Sex Ratio of White-tailed Deer (*Odocoileus virginianus*) Fetuses in Arkansas

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Running Title: White-tailed deer fetal sex-ratios

## Abstract

Health of the mother is known to affect the sex-ratio of the offspring in sexually dimorphic mammals. Both density-dependent and environmental factors (conditions at present, or seasonal conditions the year before) could impact the health of the mother. There are however two opposing hypotheses for whether a mother in good health should invest more in a strong son (that can father many offspring) or in a daughter (that can stay in the same area and use good resources). Fetuses from 1,208 female white-tailed deer (*Odocoileus virginianus*) were collected over a 10-year period (2002-2012), across 75 counties in Arkansas. For each adult female deer, we estimated age, recorded live and dressed mass, calculated the kidney fat index (KFI), back-calculated the date of conception, and recorded the number and sex of fetuses. Our objective was to investigate whether female Arkansas deer that are in good health tend to produce more male or female fetuses, in addition to investigating which factors affect conception date in those same females. We developed a generalized linear model, with a binomial response (male versus female fetuses), to examine the effects of conception date, age and mass of the mother, number of fetuses, and KFI on fetal sex ratios. We found that mass of the mother had the greatest impact on fetal sex-ratios, with heavier females producing more male fetuses ( $\beta = 0.217 \pm 0.008$ ,  $P = 0.001$ ). Female body condition (KFI) did not impact fetal sex-ratios. In addition, we developed a generalized model with mass of the doe, age of the doe, and an interaction between the two variables, to see how these physical properties influence conception date. We found a significant relationship between mass and conception date ( $\beta = -0.699 \pm 0.190$ ,  $P < 0.001$ ), and age and conception date ( $\beta = -7.524 \pm 2.411$ ,  $P = 0.001$ ), as well as the interaction term ( $\beta = 0.149 \pm 0.050$ ,  $P = 0.003$ ). Older females tended to conceive closer to the average conception date of the area, whereas younger, light mass females, tended to conceive later than average. We hope that the results of our study will form

a baseline against which deer condition in Arkansas can be measured in the future.

## Introduction

Maternal investment plays an important role in the success of offspring (Lee and Moss 1986), and it has been shown that natural selection favors parents who invest equally in their sons or daughters (Fisher 1999). Although it is assumed that sex-ratios at birth are 50:50, sex ratios in many mammals frequently deviate from 50:50 and often the health of the mother plays a key role in dictating the sex ratio at conception in sexually dimorphic mammals (Burke and Birch 1995, Garroway and Broders 2007). Much evidence has been put forward to show that the health of the mother affects the sex-ratio of offspring at birth (Trivers and Willard 1973). Some evidence suggest that offspring produced by females in good condition will have an advantage that is carried into adulthood. This advantage should be more pronounced and valuable in males than females (Trivers and Willard 1973), which then should have natural selection favoring higher male: female offspring ratios when mothers are in good condition. However, in contrast to this hypothesis is the idea that females in poor health (that are resource-stricken) should invest in sons, who are more likely to disperse and hence not be a competitor for resources (local resource competition hypothesis) (Clark 1978). Hence, if the Trivers-Willard hypothesis holds true then mothers in good health should invest more in male offspring that can father many offspring, but if the local resource competition hypothesis holds true then females will produce more males (that could disperse) when the mothers are in poor condition and resources are limited (Caley and Nudds 1987). Recently, others have shown that it is less about condition of the female or conception date, but rather the actual birth date of the offspring that influences the offspring sex-ratios (Saalfeld et al. 2007). One would expect birth date to be somewhat related to conception date in most mammals, and specifically one would

expect white-tailed deer (*Odocoileus virginianus*; hereafter deer) that conceive earlier to be more likely to give birth earlier, and hence are more likely to produce male offspring (Saalfeld et al. 2007).

Timing of conception is important not only because it could potentially influence offspring sex-ratios, but also because of direct impact on parturition dates and consequent fitness of offspring (Saalfeld et al. 2007). In ungulates, many factors influence conception dates, including age and physical condition of the female in white-tailed deer (Verme 1983, Jacobson 1994, Karns et al. 2014). In roe deer (*Capreolus* newborn deer or pregnant mothers could influence fitness and potentially sex-ratios of offspring (Tosa et al. 2018). Poor resource quality often leads to lower mass of juveniles going into winter, and decreased survival for those lighter mass individuals (Gaillard et al. 1997). Hence, forage quality could influence mass of the doe (i.e. female deer), but also conception timing, and all of these factors could be influenced in turn by the age of the mother (Rhodes et al. 1991, Verme 1983).

Age and the hierarchical status of the female in the herd (Pusey et al. 1997), together with the presence of mature males (Komers et al. 1999) are known to also affect the date at which female deer conceive. In general, younger females are expected to have later conception dates than older females (>1.5 year-old) who tend to conceive earlier (Rhodes et al. 1991). In elk (*Cervus elaphus*) the main factor influencing conception date is body condition of the mother, and better condition females will breed earlier, irrespective of age (Cook et al. 2004). Karns et al. (2014) showed that the relationship between mass and conception date varied depending on the age of the female.

There is great support for the balancing of age structure and sex ratios within deer populations (Miller and Marchinton 1995), and consequently there are several theories regarding which factors influence sex ratios of offspring. White-tailed deer harvest-management is a challenge for many state agencies, and knowing which dates to set for hunting seasons, how to distribute limits, and which harvest restrictions by sex will yield the most desirable results, all aid in managing a valuable resource (McShea 2012). Moreover, knowing the impacts of female harvest, and conception rates, on sex ratios of offspring may further aid in management efforts.

Our main objective was two-fold, first we wanted to investigate whether fetal sex-ratios in Arkansas white-tailed deer vary based on the health of the female or whether other factors, such as conception date, play a greater role in dictating the sex ratios of offspring, and

*capreolus*) productivity of the habitat and population density (Pettorelli et al. 2002), as well as age structure of male deer (Newbolt et al. 2017) greatly influenced conception date. Because of the influence of environmental factors on female white-tailed deer body condition, and consequent fitness, timing of conception is often dictated by environmental conditions (McGinnes and Downing 1977, Kie and White 1985, Simmard et al. 2014). Quality and quantity of forage is mostly determined by the harshness of the preceding season (either dry and hot summers, or cold, deep-snow winters), and the availability of good resources to second, we wanted to understand which biological factors most influence conception date in female white-tailed deer in Arkansas. We expected older females to conceive earlier than younger females, and because variation in conception timing could be partially influenced by the condition of the mother, we also expected that timing of conception will impact fetal sex ratios.

## Methods

### Data collection

Within the months of February and March from 2002 to 2012, Arkansas Game and Fish Commission (AGFC) staff collected a total of 1,839 white-tailed deer for determining statewide herd health conditions and breeding chronology (N= 1,512 female deer). This was done in conjunction with the agency's statewide chronic wasting disease (CWD) monitoring efforts. Deer were harvested from both public lands (i.e. state-owned wildlife management areas, national wildlife refuges, and state and federal parks) and from private lands in cooperation with participating private landowners [primarily those participating in the Deer Management Assistance Program (DMAP)].

Data recorded from collected female deer included the date of collection, the location of collection (i.e. GPS coordinate, hunting zone, and deer management unit), age estimated by the tooth replacement-wear method (Severinghaus 1949), un-eviscerated mass (to the nearest kilogram) using hanging scales, and both paired kidney-fat weights and paired kidney weights (g) for kidney fat index (KFI) calculations (calculated as the weight of the paired kidney-fat divided by the weight of the paired kidneys, recorded as a percentage) (Monson et al. 1974). Lastly, the number and sex of all fetuses present were recorded, and the crown-rump length of all fetuses extracted were measured for determining conception and parturition dates (Hamilton et al. 1985). We used live mass of the does, since not all dressed-

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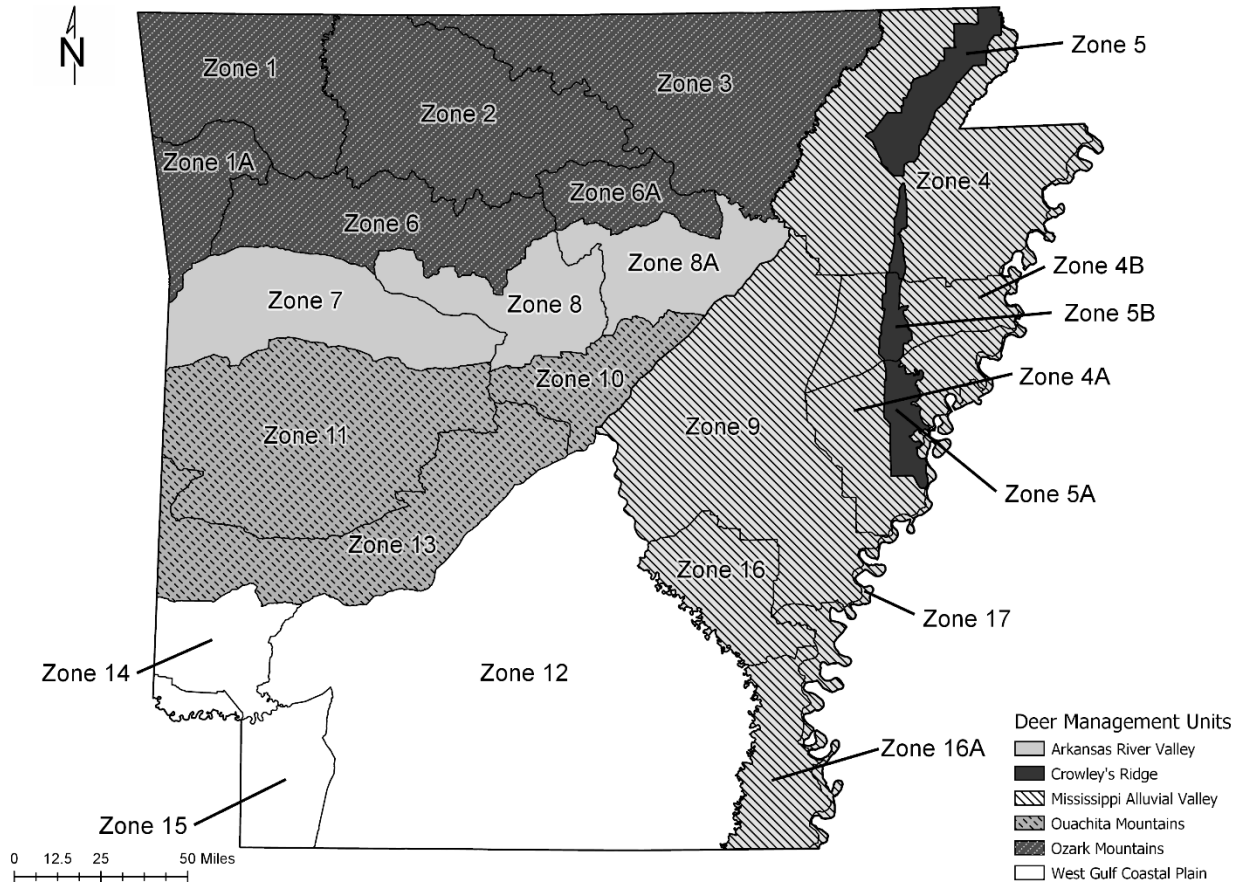


Figure 1. Arkansas deer hunting zones and deer management units

mass measurements were recorded. To ensure there was no bias in using live weight of females with different numbers and age fetuses we did a correlation analysis between live and dressed mass ( $r = 0.95$ ) and concluded there is very little discrepancy and that using live mass should not influence our analyses. Two types of spatial scales were used during the analysis; for the first spatial scale females were sorted into twenty-five study areas, which represented the twenty-five distinct hunting zones as defined by AGFC (Figure 1). The second spatial scale utilized six, geographically distinct deer management units (DMU). Deer management units are larger than the hunting zones, but are delineated by AGFC to encompass similar habitats and deer densities (Fig. 1). Deer age categories started at half a year old, and increased by 1 year up to 5.5 years old. All Deer estimated to be older than 5.5 years were lumped into the 5.5+ years old category. We calculated the number of days from average conception date, by calculating the mean date of conception for all deer within a hunting zone (by year) and then used the absolute number of days from that mean date to assign a “number of days

from average conception date” value to each deer (Kearns et al. 2014). For example, if the average conception date for a zone was November 15<sup>th</sup> then a female with a conception date of November 20<sup>th</sup> will get a value of +5 (5 days later than the average conception date), whereas a female that conceived on November 12<sup>th</sup> will get a value of -3 (3 days earlier than the average conception date).

#### Data Analysis

To investigate which factors affected fetal sex-ratios we developed a generalized linear model with a binomial distribution and included age and mass of the doe, number of fetuses per doe, KFI, and conception date. In addition, we developed a generalized linear model to test the effect of age and mass of the doe, and the interactive effect of age and mass, on conception date. Location of deer (deer management units) was used included in all models to account for regional differences. All data analysis was done in Program R version 4.0.3 (R Core Team 2020).

## Results

After accounting for missing values, we used data from 1,208 does from the 25 deer zones in our analyses. Average deer mass was 46.84 kg (SD = 8.28 kg) and the average age was 3.37 years old (SD = 1.48 years). The majority of does fell within the 2.5- and 3.5-year-old age classes (n = 394 and n = 388 respectively), with only 62 females being older than 5.5 years. Of the does used in the analyses, the majority had twin fetuses (n = 799), followed by single fetuses (n = 370), 37 had triplets and 1 doe had four fetuses. There was no correlation between KFI and mass ( $r = 0.32$ ), but in general KFI was lowest for lighter mass females (<35 kg).

### Fetal sex ratio

Overall there were more male fetuses (1,237) than females (1,069), resulting in a 1: 0.86 sex ratio. The only variable that significantly impacted fetal sex ratios was mass of the doe ( $\beta = 0.021 \pm 0.008$  SE,  $P = 0.00$ ). Heavier females were more likely to produce male offspring (Fig.2). Interestingly, the number of fetuses per female showed a slight negative (albeit non-significant) relationship with the number of male fetuses that are produced ( $\beta = -0.174 \pm 0.099$  SE,  $P = 0.079$ ). When the number of fetuses exceeded 2, the fetal sex ratio was in favor of females. Contrary to our expectations, neither conception date ( $\beta = 0.001 \pm 0.003$  SE,  $P = 0.64$ ) nor KFI ( $\beta = -0.001 \pm 0.001$  SE,  $P = 0.92$ ) impacted fetal sex ratios. None of the other variables impacted fetal sex-ratios.

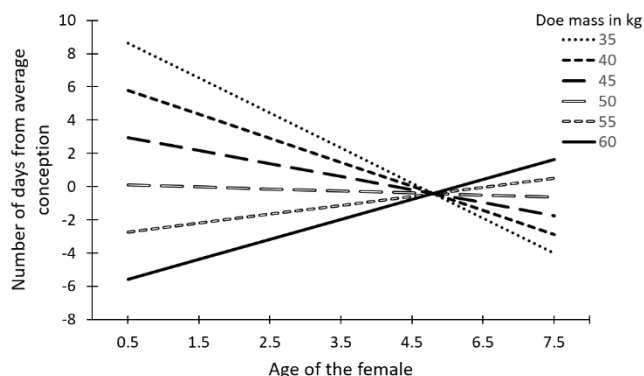


Figure 2. Predicted distribution of conception date of female white-tailed deer in Arkansas, displayed as the number of days earlier (negative values) or later (positive values) than average that a female conceived, as it relates to age of the doe and mass of the doe (N=1,208), from data collected 2002-2012.

### Conception date

Early to mid-November was the most common conception-date range for does across Arkansas (November 6<sup>th</sup> – November 16<sup>th</sup>), but the exact peak varied among regions. The greatest predictor of conception date was mass of the doe ( $\beta = -0.699 \pm 0.190$  SE,  $P < 0.001$ ), age of the doe ( $\beta = -7.525 \pm 2.411$  SE,  $P = 0.001$ ), and the interaction of these two terms ( $\beta = 0.148 \pm 0.050$  SE,  $P = 0.003$ ). Younger females (less than 2.5 years old) of lower mass (under 45 kg) tended to conceive later than average, and older females tended to conceive earlier than the average conception date for their location (Figure 2). Female deer between 3.5 and 5.5 years old were predicted to have conception dates that fell around the average for the area that they occur. Females around 50 kg were predicted to conceive right at the average of the area, regardless of what their age is.

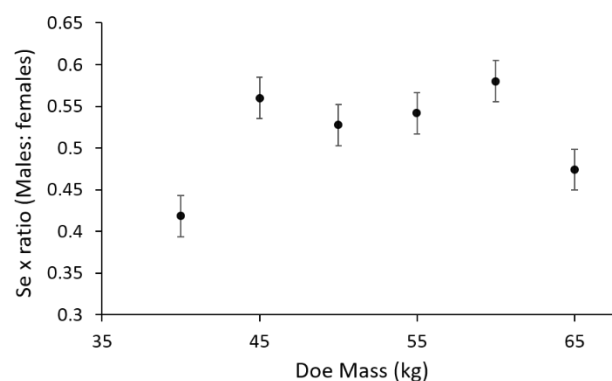


Figure 3. Average fetal sex ratio  $\pm$  SE (males: females) as it relates to mass of the doe, (N=1,208), from data collected 2002-2012.

## Discussion

We expected body condition (KFI) to influence fetal sex ratios, but instead found mass of the female to be the strongest predictor of fetal sex ratios in white-tailed deer. Heavier female mule deer (*Odocoileus hemionus*) tend to produce more male offspring; however, those females also had high KFI values (Kucera 1991). Although mass could be an indicator of better body condition, the non-significance of KFI in our model suggests otherwise. Cameron (2004) showed that although there is great variation in offspring sex-ratio studies, body condition at time of conception seems to be the major predictor of offspring sex, with females in better overall condition at time of conception tending to produce more males. Our results do not support those findings. We did not include factors, such as the social

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structure of the adults in our models, and it is known that demographics, and specifically adult sex ratios, could influence offspring sex ratios of large ungulate species (Cassinello and Gomendio 1996; Hamel et al. 2016). As adult sex-ratios became more male biased, female bighorn sheep (that were in good body condition, heavier, and older) had fewer male offspring (Hamel et al. 2016). In addition, our focus on fetal sex-ratios rather than live offspring sex-ratios, may result in the discrepancies with previous works.

The most pronounced pattern regarding conception date was seen in lower mass females. Young females with lower mass were predicted to conceive later than average. This makes sense since female white-tailed deer fawns typically do not reach sexual maturity until around 32 kg, and are not likely to reach this mass before the average day of conception during their first breeding season. The majority of the females in our study were above 35 kg, but even at 40 kg females younger than 3.5 years conceived much later than the other does in the same the area. These data are in line with Green et al. (2017) who showed that female fawns conceived later than adult females, and Rhodes et al. (1991) also showed that younger females had much later conception dates than adults. Based on these results it would make sense that mass of the female impacts conception date, which was true for our study. Heavier and older females are known to have earlier parturition dates and greater reproductive success (Plard et al. 2014), and because body mass of the mother is known to influence survival of juveniles (Gaillard et al. 1997; Michel et al. 2015), and also the condition of the juveniles into adulthood (Douhard et al. 2013), it seems likely that the advantage lies in conceiving in an optimum window of time (Clements et al. 2011). Our results mirror those in Alabama, where heavier females conceived closer to the average conception date, and younger, lighter females were farther away from average (Karns et al. 2014).

### Conclusion

Our study showed that conception dates of female white-tailed deer were influenced by age and body mass of the doe. Heavier females, especially those bred latest in the season, were most likely to produce male offspring. Kidney fat index did not show up as a significant predictor of fetal sex-ratios, and does not directly point to body condition of the female influencing sex ratios. Hence, we cannot conclude that our findings are in line with the Trivers-Willard hypothesis, but Sheldon and West (2004) showed that physiological and morphological data collected from

female post-conception (as in our case), rarely conform to Trivers-Willard hypothesis. Instead they suggest to focus on pre-conception behavioral data to make inferences on offspring sex-ratios.

From a management perspective, specifically in Arkansas, the goals of white-tailed deer management are focused on sustainability and balance (i.e. between both sex and age-class, with available habitat, and with sociological carrying capacity) of the species. If we assume that higher number of male fetuses vs females fetuses translates into more males being born and reaching the harvestable population (assuming that fawn survival is equal between sexes), then it is theoretically possible that more male deer could be harvested and a balanced adult sex-ratio could still be maintained. More importantly however, is a better understanding of biological factors for female deer which may in turn influence breeding chronology and reproductive output in regards to the number and sex of fetuses produced. This information may provide critical information for wildlife managers who are trying to manage for balanced deer herds through harvest management recommendations and season frameworks.

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