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# MULTIPLE PATERNITY IN THREE WILD POPULATIONS OF EASTERN MASSASAUGA (*SISTRURUS CATENATUS*)

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*Abstract*.**—Multiple paternity is widespread among animals. Within snakes, multiple paternity has been well-documented with the exception of the family Elapidae. However, variation in the frequency of multiple paternity among populations is poorly documented and warrants further investigation. Here, we provide evidence for multiple paternity in three wild populations of the Eastern Massasauga (*Sistrurus catenatus*). We documented multiple paternity in six of 12 Pennsylvania litters, five of 12 Michigan litters, and two of two Illinois litters. Female body size did not influence the likelihood of multiple paternity. However, an increase in female size correlated with increased litter size. Including this study, multiple paternity is now documented in 21 snake species belonging to 15 genera and four families. These results have implications for the captive management and conservation of this endangered rattlesnake. Specifically, captive breeding programs, such as the Eastern Massasauga Species Survival Plan (SSP®), might consider providing opportunities for multiple paternity.**

*Key Words*.—Eastern Massasauga SSP®; microsatellite DNA; phylogenetically widespread; snakes

## INTRODUCTION

Multiple paternity occurs when more than one male sires the offspring of a single litter (Jellen and Aldridge 2011). It is a phenomenon that occurs across numerous animal taxa (Taylor et al. 2014) including, among vertebrates: fish (Avise et al. 2002), amphibians (Roberts and Byrne 2011), reptiles (Uller and Olsson 2008), birds (Griffith et al. 2002), and mammals (Clutton-Brock 1989). Among snakes, multiple paternity has been documented in four families: Pythonidae (one species), Homalopsidae (two species), Colubridae (14 species), and Viperidae (four species, Wusterbarth et al. 2010; reviewed in Jellen and Aldridge 2011; Simonov and Wink 2011; Meister et al. 2012; and Clark et al. 2014). One notable exception to the widespread occurrence of multiple paternity in snakes is its ostensible absence in six marine Elapids (Fig. 1), perhaps due to some aspect of the unique ecology of these exclusively marine species (Lukoschek and Avise 2011). Within a number of species for which it has been documented, instances of multiple paternity appear common. Typically, more than half of litters are sired by two or more males (Table 16.1 in Jellen and Aldridge 2011). However, variation in the frequency of multiple paternity among populations is poorly documented with analysis of multiple populations having occurred in only three species (Jellen and Aldridge 2011).

Many snake species have promiscuous mating systems and there is little debate as to the adaptive advantages this breeding strategy confers to males (Rivas and Burghardt 2005). However, the adaptive advantages to females are less apparent. While direct benefits, including nuptial gifts and parental care, are not thought to apply to snake mating systems (Uller and Olsson 2008), indirect genetic benefits may ultimately increase female fitness. For example, multiple mating may allow females to Trade-Up by mating with higher quality males (Pitcher et al. 2003) and promote sperm competition among rival males, which becomes especially likely if sperm quality is heritable or linked to other beneficial genes (Klemme et al. 2014). Sperm competition may permit a Bet-Hedging strategy in which multiple sires produce a genetically diverse litter better suited to respond to environmental stochasticity (Calsbeek et al. 2007). Additionally, cryptic female choice may reduce genetic incompatibility (Tregenza and Wedell 2000). Alternatively, females may simply mate with multiple males as a way to reduce injury to themselves (Shine et al. 2005), reduce male harassment (Lee and Hays 2004), or as a genetic correlation to selection favoring multiple mating in males (Halliday and Arnold 1987).

Examining the relationship between variables such as female size, litter size, and the relative frequency of multiple paternity may help elucidate general patterns of

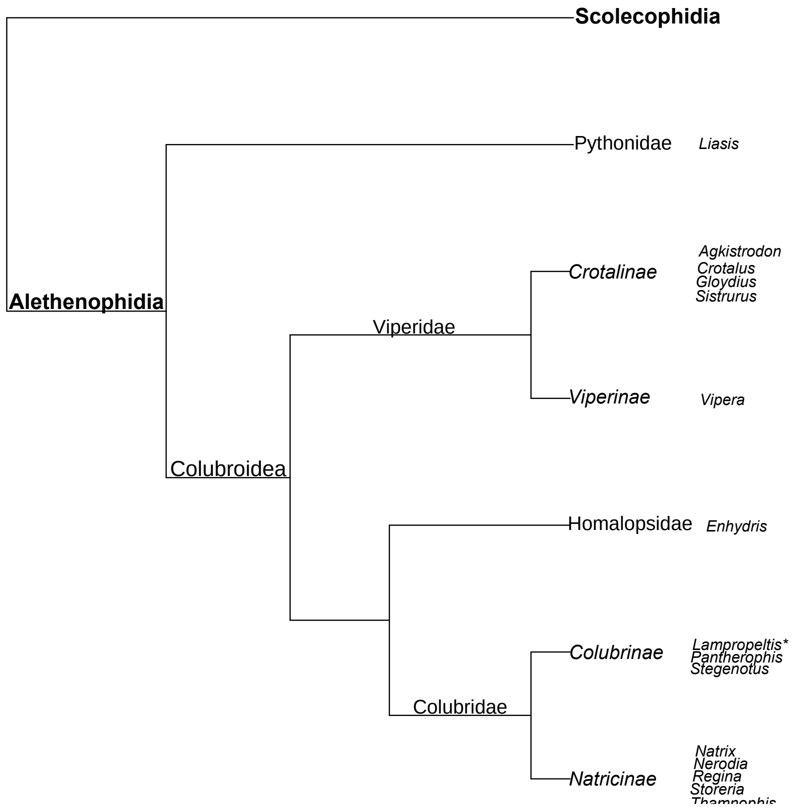

**FIGURE 1.** Cladogram (modified from Pyron et al. 2010) of snakes showing genera in which multiple paternity has been confirmed (Wusterbarth et al. 2010; Jellen and Aldridge 2011; Lukoschek and Avise 2011; Simonov and Wink 2011; Meister et al. 2012; and Clark et al. 2014). In *Lampropeltis* (asterisk), multiple paternity has only been documented in captivity.

reproductive behavior and physiology. Due to their size, larger females may produce larger litters (Luiselli et al. 1995; Gignac and Gregory 2005), which would present a fitness advantage to those females and any males who successfully mate with them. In such instances, males would benefit from preferentially seeking larger females. Given a large sample size and variable suite of microsatellite loci, such a pattern may be detectable as an increase in the frequency of multiple paternity with increasing female size. However, this would need to be weighed against the increased probability of detecting multiple paternity in larger litters. Within litters, there may be a tradeoff between litter size and offspring size with larger litters producing smaller offspring due to maternal size constraints (Rohr 2001; Gignac and Gregory 2005). Alternatively, we may find that the frequency of multiple paternity, rather than female size, better explains variance in litter size (Bryja et al. 2008). This second scenario would be most plausible in those species lacking long-term sperm storage wherein all ova may not be fertilized from a single mating event.

Like many other viperids, Eastern Massasaugas (*Sistrurus catenatus*; Fig. 2) mate during the late summer and fall, and females give birth to live young the following summer (Szymanski 1998). Consequently, spermatozoa must remain viable within the oviduct for approximately eight months before fertilization (Aldridge et al. 2008), a scenario that makes sperm competition particularly likely within this species. Although the details of sperm storage in the Eastern Massasauga are not known, branched tubular sperm storage structures are present in other viperids, including *Agkistrodon*, *Cerastes*, *Crotalus*, and *Vipera* (Sever and Hamlett 2002; Siegel et al. 2011) and sperm storage has been documented in *Crotalus* (Almeida-Santos and Da Graça Salomão 1997).

Historically, the Eastern Massasauga was found in wetlands and surrounding upland habitat in Illinois, Indiana, Iowa, Michigan, Minnesota, New York, Ohio, Pennsylvania, Wisconsin in the USA, and in Ontario, Canada. However, habitat destruction and modification have resulted in substantial declines in extant populations and extirpations of others. As a result, the Eastern Massasauga has been proposed for listing as threatened under the U.S. Endangered Species Act and is classified as endangered or threatened in every state and province where it occurs except Michigan, where it is a species of special concern (Szymanski 1998; U.S. Fish

**FIGURE 2.** A litter of Eastern Massasaugas (*Sistrurus catenatus*) photographed upon their release at our Cass County, Michigan study site. (Photographed by Eric T. Hileman).

and Wildlife Service 2015). To address this decline, a zoo-based captive-breeding program, the Eastern Massasauga Species Survival Plan (SSP®), is underway that may one day allow reintroduction into the wild (http://www.emrssp.org/ [Accessed 5 April 2016]). For captive-breeding to succeed, a better understanding of the reproductive biology of wild populations is needed. In this paper, we achieve three objectives: (1) we provide evidence for the occurrence of multiple paternity in a previously untested species, the Eastern Massasauga, (2) we compare the frequency of multiple paternity among Eastern Massasauga populations, and (3) we suggest how this information might be incorporated into the Eastern Massasauga SSP®.

## MATERIALS AND METHODS

We collected gravid females from the wild at three study sites: Butler and Venango Counties, Pennsylvania; Cass County, Michigan; and Piatt County, Illinois, USA. From Pennsylvania, we collected 26 gravid females (2003–2006), 19 from Michigan (2011–2012), and two from Illinois (2002 and 2004). We housed females individually in glass aquaria, provided a thermal gradient, gave access to water *ad libitum*, and offered food (one thawed mouse from a commercial supplier) weekly.

Following parturition, we sampled blood from caudal vessels of each female, and blood, shed skins, or tissues (in the case of still-borns) from each offspring. We froze tissue samples immediately or preserved them in ethanol and stored them at –4° C until DNA extraction. Following tissue sampling, we released females and live-born offspring at their original capture locations. Next, we selected a subset of litters consisting of at least five offspring for paternity analysis to increase our power to detect multiple paternity. This resulted in sample sizes of 12 for Pennsylvania, 12 for Michigan, and two for Illinois.

We extracted genomic DNA from blood samples using Qiagen DNeasy® Blood & Tissue Kits (Qiagen, Inc., Valencia, California, USA) and from other tissues using Qiagen Puregene® Core Kit A. We genotyped snakes from Illinois at six microsatellite loci (*Scu*-01, *Scu*-05, *Scu*-07, *Scu*-26, *Scu*-106, *Scu*-125; Gibbs et al. 1998; H. Lisle Gibbs, pers. comm.) as part of a separate study (Jaeger 2014). For the Pennsylvania and Michigan populations, we genotyped snakes at a subset of loci (*Scu*-01 and *Scu*-106) based on relatively high measures of allelic richness and observed heterozygosity (Table S1, Supplemental Data). We amplified the DNA in 20-µL volumes containing: 1x GoTaq Flexi Buffer (Promega Corp., Madison, Wisconsin, USA), 2 U of GoTaq DNA Polymerase, 250 µM of each dNTP, 1.5 mM $MgCl_2$, 1 µM of each primer, 0.1 mg/mL bovine serum albumin, and 50–100 ng of genomic DNA. The PCR program consisted of an initial incubation at 94° C for 2 min; four cycles of denaturation at 94° C for 20 s, annealing at locus-specific temperature for 20 s, and extension at 70° C for 5 s; 40 cycles of denaturation at

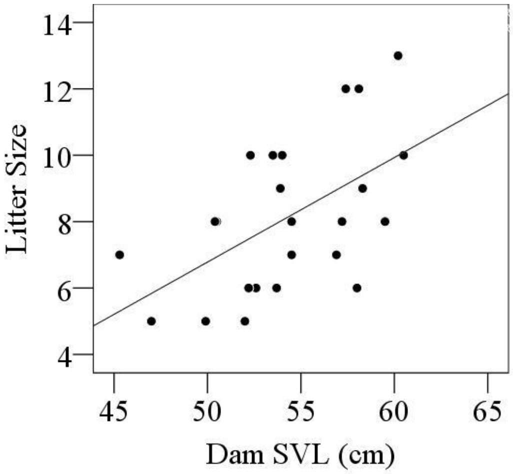

**FIGURE 3**. Relationship of litter size to dam snout-vent length of Eastern Massasaugas (*Sistrurus catenatus*) from Pennsylvania and Michigan, USA.

94° C for 15 s, annealing at locus-specific temperature for 20 s, and extension at 70° C for 5 s; and a final extension at 70° C for 1 min. Locus-specific annealing temperatures were 51, 54, 59, 59, 58, and 56° C for *Scu*-01, *Scu*-05, *Scu*-07, *Scu*-26, *Scu*-106, and *Scu*-125, respectively.

We visualized PCR products on 1% agarose gels to ensure successful amplification. We analyzed successful reactions using an ABI 310 Prism Genetic Analyzer (Applied Biosystems®, Foster City, California, USA). To determine microsatellite genotypes, we used GeneMapper v4 (Applied Biosystems®) and verified them by eye. As a check for genotyping errors, we re-amplified and genotyped 24% of the Pennsylvania samples, 16% of the Michigan samples, and 42% of the Illinois samples.

To determine the number of sires per litter, we used Gerud version 2.0. This program estimated the number of sires and the probable genotypes of those sires based on population allele frequency data from a population in Clinton Co., Illinois, USA. In cases where the dam had a probable null allele, we were not able to use Gerud and instead subtracted the maternal alleles from those of each of her offspring to obtain the possible sire alleles. In those instances, if the number of remaining unique paternal alleles across the litter exceeded two, we determined that multiple paternity had occurred. In cases where the mother is heterozygous, the burden of proof was even greater, as we would need to detect five unique parental alleles before declaring multiple paternity (two unique alleles from the dam and at least three unique alleles from the sires). Furthermore, if two homozygous sires contributed to a litter, our methods would count them as a single sire. Thus, assuming mutations have a negligible effect (Estoup et al. 2002), the frequencies reported here are likely underestimates of the prevalence of multiple paternity in this species (Tables 2–4, Supplemental Data). Next, we compared the frequency of multiple paternity between the Pennsylvania and Michigan litters using a chi-square test of independence. Small sample size prevented statistical analysis of the Illinois population. We also assessed the correlation between dam snout-vent length (SVL) and litter size. Finally, we compared SVL between dams of singly and multiply sired litters using a two-sample *t*-test, and checked normality assumptions using a Shapiro-Wilk goodness-of-fit test. For all statistical tests, we set the significance *a priori* at $\alpha = 0.05$.

## RESULTS

Mean litter size was 8.3 ± 2.1 (SD) in the Pennsylvania group and 7.8 ± 2.2 (SD) in the Michigan group. The two Illinois litters numbered eight and nine offspring. For each litter, we genotyped dams and 5–13 offspring. Based on analysis in Gerud v2.0 or the presence of three or more paternal alleles, we found evidence of multiple paternity in each of the three examined populations. The frequency of multiple paternity was 50% (six of 12 litters) in the Pennsylvania population and 42% (five of 12 litters) in the Michigan population. In the Illinois population, multiple paternity was found in both (two of two) litters (Table S2, Supplemental Data). The frequency of multiple paternity did not differ significantly between Pennsylvania and Michigan populations ($n = 24$, $\chi^2 = 0.33$, df = 1, $P = 0.56$). We found a significant association between dam SVL and litter size (litter size = -8.958 + 0.315*dam SVL, $r^2 = 0.30$, $t = 3.102$, df = 22, $P = 0.005$; Fig. 3). In the pooled Michigan and Pennsylvania samples, we found SVL to be normally distributed ($W = 0.978$, $P = 0.870$) and found there was no significant difference in dam SVL between singly and multiply sired litters ($n = 24$, $t = 0.797$, df = 22, $P = 0.434$).

## DISCUSSION

Our results document the occurrence of multiple paternity in the Eastern Massasauga and increases the number of viperid species for which multiple paternity is known to occur in the wild. Among snakes, multiple paternity is now known to occur in 21 species representing 12 genera and four families. Our results indicate that the occurrence of multiple paternity within the Eastern Massasauga is geographically widespread, occurring in populations in Illinois, Michigan, and Pennsylvania. In this way, the Eastern Massasauga is similar to the Adder (*Vipera berus*; Ursenbacher et al. 2008), Northern Watersnake (*Nerodia sipedon*; Prosser et al. 2002), and Common Gartersnake (*Thamnophis sirtalis*; Garner et al. 2002), the other snake species in which multiple paternity was assessed in multiple populations. Long-term sperm storage has been suggested as a probable reproductive mechanism for many species of New World pitvipers, including the Eastern Massasauga (Schuett 1992), and may facilitate the occurrence of multiple paternity by extending the period over which successful mating may occur.

We did not detect differences in the frequency of multiple paternity between sites. However, larger sample sizes would be necessary unless such differences were dramatic. Furthermore, the frequencies we reported for the Michigan and Pennsylvania populations are likely an underestimate because we only analyzed two variable microsatellite loci for those populations. The frequency of multiple paternity has been found to vary among sites in Common Gartersnakes (*Thamnophis sirtalis*; Garner et al. 2002) and Blanding's Turtles (*Emydoidea blandingii*; Anthonysamy et al. 2014) and among years in Brown Smoothhound Sharks (*Mustelus henlei*; Chabot and Haggin 2014), but whether this variation is due to differences in ecology or population density is unknown.

We found no evidence that larger females were more likely to have multiply sired litters than smaller females. Such a pattern might be expected if larger females are more attractive to males (e.g., as in Common Gartersnakes; Shine et al. 2001). However, given that larger females tend to produce larger litters, there could be a fitness advantage for males to preferentially mate with larger females. Definitively resolving questions of mate choice will require direct observations of mating behavior, as genetic patterns cannot account for all mating attempts (Prosser et al. 2002) and female post-copulatory mate choice may result in single paternity even in litters of females that mate multiple times (Friesen et al. 2014). Furthermore, to better understand the significance of multiple paternity in snake mating systems, future work should examine additional elapid snakes, to determine the extent of single paternity in this family (Lukoschek and Avise 2011). We suggest analyses of basal alethenophidian and scolecophidian snakes, and of closely related lizards, to determine whether the capacity for multiple paternity is the ancestral condition in snakes (Rivas and Burghardt 2005; Uller and Olsson 2008).

The frequent occurrence of multiple paternity that we observed has implications for captive breeding programs, such as the Eastern Massasauga SSP®. Providing females with opportunities to mate with multiple males might increase the proportion of viable offspring by reducing the chances of genetic incompatibility between males and females (Madsen et al. 1996). Furthermore, opportunities for multiple paternity could help maintain representation of a larger proportion of male founders over time and increase the effective size of the breeding population, thereby lessening the effects of inbreeding and maintaining adaptive potential within litters (Calsbeek et al. 2007; Moore et al. 2007). How opportunities for multiple paternity are provided may require careful consideration. In another viperid, the Copperhead (*Agkistrodon contortrix*), agonistic encounters among males resulted in elevated corticosterone levels in losers, possibly reducing their success in courtship and mating (Schuett et al. 1996). Thus, housing adult males singly and providing females with males in succession (rather than simultaneously) might better serve the goals of captive breeding. When initiated, the Eastern Massasauga SSP included small numbers of potential breeders distributed among numerous participating institutions (Joanne Earnhardt, pers. comm.). As the captive population grows, we recommend that the breeding program be modified to allow for multiple paternity and genetic monitoring be implemented to identify successful sires, perhaps through a zoo-academic collaboration (Fernandez and Timberlake 2008).

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**CHRISTOPHER A. PHILLIPS** is Research Project Leader and Curator of Amphibians and Reptiles at the Illinois Natural History Survey and an affiliate Professor in the Department of Animal Biology and the Department of Natural Resources and Environmental Sciences at the University of Illinois, Champaign, Illinois, USA. His research program centers on how populations of amphibians and reptiles arrived at their current spatial distributions and how they maintain (or fail to maintain) these distributions using molecular markers, field observations, field manipulations, and laboratory experiments. (Photographed by Mike Dreslik).

**BRADLEY J. SWANSON** is Professor of Biology, Director of Environmental Studies, and Director of the ATCG Wildlife Forensics Laboratory at Central Michigan University, Mt. Pleasant, Michigan, USA. His research focuses on the use of genetics to answer ecological and conservation related questions. (Photographed by Carm Crisci).

**RICHARD B. KING** is a Presidential Research Professor at Northern Illinois University, DeKalb, Illinois, USA. His interests center on ecological and evolutionary processes at local and regional scales and the conservation biology of Midwestern amphibians and reptiles. (Photographed by Kristin Stanford).

# SUPPLEMENTAL DATA

**TABLE S1**. Allelic richness and observed heterozygosity for loci used in this study (Gibbs et al. 1998; Jaeger 2014).

| Loci | Allelic Richness | Observed Heterozygosity |
|---|---|---|
| Scu-01 | 11 | 0.49 |
| Scu-05 | 9 | 0.44 |
| Scu-07 | 9 | 0.46 |
| Scu-26 | 7 | 0.63 |
| Scu-106 | 6 | 0.54 |
| Scu-125 | 7 | 0.48 |

**TABLE S2.** Microsatellite DNA genotypes of dams, offspring, and inferred sires among Eastern Massasaugas (*Sistrurus catenatus*) from study sites in Pennsylvania, Michigan, and Illinois. In cases where sire genotypes could not be inferred (see text), paternal alleles are listed. For Michigan Dam 2, both dam and sire genotypes were inferred using Gerud. Missing genotypes are indicated by dashes. Multiple paternity is indicated by asterisks adjacent to sire number or sire alleles.

| | Microsatellite DNA Locus | | | | | |
|---|---|---|---|---|---|---|
| | *Scu*-01 | *Scu*-106 | *Scu*-05 | *Scu*-07 | *Scu*-26 | *Scu*-125 |
| Pennsylvania Dam 1 | 147/150 | 117/117 | | | | |
| Offspring 1 | 147/147 | 117/123 | | | | |
| Offspring 2 | 150/150 | 117/123 | | | | |
| Offspring 3 | 150/150 | 117/123 | | | | |
| Offspring 4 | 147/150 | 117/117 | | | | |
| Offspring 5 | 147/147 | 117/121 | | | | |
| Offspring 6 | 147/150 | 117/117 | | | | |
| Offspring 7 | 147/150 | 117/117 | | | | |
| Offspring 8 | 147/150 | 117/121 | | | | |
| Offspring 9 | 147/150 | 117/117 | | | | |
| Sire 1* | 147/147 | 121/121 | | | | |
| Sire 2* | 147/150 | 117/123 | | | | |
| Pennsylvania Dam 2 | 154/null | 117/123 | | | | |
| Offspring 1 | 147/154 | 123/129 | | | | |
| Offspring 2 | 156/156 | 123/129 | | | | |
| Offspring 3 | 147/147 | 117/123 | | | | |
| Offspring 4 | 147/147 | 117/123 | | | | |
| Offspring 5 | 147/154 | 117/129 | | | | |
| Offspring 6 | 147/147 | 123/129 | | | | |
| Offspring 7 | 147/147 | 117/123 | | | | |
| Offspring 8 | 147/154 | 117/129 | | | | |
| Offspring 9 | 154/154 | 123/129 | | | | |
| Offspring 10 | 156/156 | 123/129 | | | | |
| Offspring 11 | 154/154 | 117/117 | | | | |
| Offspring 12 | 152/154 | 123/129 | | | | |
| Sire 1* | 147/152 | - | | | | |
| Sire 2* | 154/156 | 117/129 | | | | |
| Pennsylvania Dam 3 | 150/152 | 117/117 | | | | |

| | | |
|---|---|---|
| Offspring 1 | 147/152 | 117/125 |
| Offspring 2 | 150/150 | 117/125 |
| Offspring 3 | 150/152 | 117/125 |
| Offspring 4 | 150/150 | 117/117 |
| Offspring 5 | 150/150 | 117/125 |
| Offspring 6 | 147/150 | 117/125 |
| Offspring 7 | 147/152 | 117/117 |
| Sire | 147/150 | 117/125 |
| Pennsylvania Dam 4 | 147/147 | 117/117 |
| Offspring 1 | 147/147 | 117/117 |
| Offspring 2 | 147/147 | 117/117 |
| Offspring 3 | - | 117/123 |
| Offspring 4 | 145/147 | 117/117 |
| Offspring 5 | 147/147 | 117/117 |
| Offspring 6 | 147/147 | 117/117 |
| Offspring 7 | 147/147 | 117/117 |
| Offspring 8 | 147/150 | 117/117 |
| Offspring 9 | 147/147 | 117/117 |
| Offspring 10 | 147/147 | 117/117 |
| Sire 1* | 147/147 | 117/117 |
| Sire 2* | 145/150 | 117/123 |
| Pennsylvania Dam 5 | 147/147 | 117/125 |
| Offspring 1 | - | 117/125 |
| Offspring 2 | 145/147 | 117/117 |
| Offspring 3 | 145/147 | 117/117 |
| Offspring 4 | 147/147 | 117/125 |
| Offspring 5 | 145/147 | 117/117 |
| Offspring 6 | 147/147 | 117/125 |
| Offspring 7 | - | 117/123 |
| Offspring 8 | 147/147 | 125/125 |
| Sire 1* | 145/145 | 117/117 |
| Sire 2* | 147/147 | 123/125 |
| Pennsylvania Dam 6 | 150/null | 125/null |
| Offspring 1 | 150/150 | 117/125 |
| Offspring 2 | 150/150 | 117/125 |
| Offspring 3 | 150/150 | 117/117 |
| Offspring 4 | 145/145 | 117/125 |
| Offspring 5 | 147/150 | 117/125 |
| Offspring 6 | 150/150 | 117/125 |
| Offspring 7 | 147/150 | 117/125 |
| Offspring 8 | 150/150 | 117/125 |
| Sire 1* | 150/150 | 117/117 |
| Sire 2* | 145/147 | 125/125 |
| Pennsylvania Dam 7 | 147/147 | 123/null |
| Offspring 1 | 147/147 | 117/123 |
| Offspring 2 | 145/147 | 125/125 |

| | | |
|---|---|---|
| Offspring 3 | 147/147 | 117/123 |
| Offspring 4 | 147/147 | 117/123 |
| Offspring 5 | 147/147 | 117/123 |
| Offspring 6 | 147/147 | 117/123 |
| Offspring 7 | 147/152 | 117/123 |
| Sire Alleles* | 145, 147, 152 | 117, 125 |
| Pennsylvania Dam 8 | 156/156 | 123/123 |
| Offspring 1 | 156/156 | 117/123 |
| Offspring 2 | 156/156 | 117/123 |
| Offspring 3 | 156/156 | 117/123 |
| Offspring 4 | 156/156 | 117/123 |
| Offspring 5 | 154/156 | 117/123 |
| Offspring 6 | 154/156 | 117/123 |
| Offspring 7 | - | 117/123 |
| Offspring 8 | 154/156 | 117/123 |
| Offspring 9 | 154/156 | 117/123 |
| Sire | 154/156 | 117/117 |
| Pennsylvania Dam 9 | 147/147 | 117/123 |
| Offspring 1 | 147/147 | 117/117 |
| Offspring 2 | 147/147 | 117/117 |
| Offspring 3 | 147/147 | 117/117 |
| Offspring 4 | 145/147 | 117/123 |
| Offspring 5 | 147/147 | 123/129 |
| Offspring 6 | 145/147 | 123/129 |
| Offspring 7 | 147/147 | 117/123 |
| Offspring 8 | 145/147 | 117/129 |
| Offspring 9 | 145/147 | 123/129 |
| Offspring 10 | 145/147 | 123/129 |
| Sire | 145/147 | 117/129 |
| Pennsylvania Dam 10 | 147/150 | 117/125 |
| Offspring 1 | 147/147 | 117/117 |
| Offspring 2 | 145/147 | 125/125 |
| Offspring 3 | 145/147 | 117/125 |
| Offspring 4 | 145/147 | 125/125 |
| Offspring 5 | 145/147 | 125/125 |
| Sire | 145/147 | 117/125 |
| Pennsylvania Dam 11 | - | 117/117 |
| Offspring 1 | 147/147 | 117/117 |
| Offspring 2 | 145/147 | 117/117 |
| Offspring 3 | 147/147 | 117/117 |
| Offspring 4 | 145/147 | 117/117 |
| Offspring 5 | 145/147 | 117/117 |
| Sire | 147/147 | 117/117 |
| Pennsylvania Dam 12 | 150/null | 125/125 |
| Offspring 1 | 150/150 | - |
| Offspring 2 | 150/150 | 117/125 |

| | | |
|---|---|---|
| Offspring 3 | 150/150 | 117/125 |
| Offspring 4 | 147/150 | 117/125 |
| Offspring 5 | 147/150 | 117/125 |
| Offspring 6 | 147/147 | 117/125 |
| Offspring 7 | - | 117/125 |
| Offspring 8 | 147/147 | 117/125 |
| Offspring 9 | 147/147 | 117/125 |
| Offspring 10 | 147/147 | - |
| Sire Alleles | 147, 150 | 117 |
| Michigan Dam 1 | 145/147 | 121/125 |
| Offspring 1 | 145/147 | 123/125 |
| Offspring 2 | 145/147 | 123/125 |
| Offspring 3 | 145/147 | 121/123 |
| Offspring 4 | 145/147 | 123/125 |
| Offspring 5 | 147/147 | 123/125 |
| Offspring 6 | 147/147 | 121/123 |
| Offspring 7 | 147/147 | 121/123 |
| Offspring 8 | 145/147 | 123/125 |
| Offspring 9 | 147/147 | 123/125 |
| Offspring 10 | 147/147 | 121/123 |
| Sire | 147/147 | 123/123 |
| Michigan Dam 2 | 147/149 | 123/125 |
| Offspring 1 | 149/149 | 121/123 |
| Offspring 2 | 147/147 | 125/125 |
| Offspring 3 | 147/149 | 123/123 |
| Offspring 4 | 147/147 | 121/123 |
| Offspring 5 | 147/149 | 123/123 |
| Offspring 6 | 147/149 | 121/123 |
| Offspring 7 | 147/149 | 123/123 |
| Offspring 8 | 147/149 | 123/123 |
| Offspring 9 | 147/147 | 121/123 |
| Offspring 10 | 147/147 | 121/123 |
| Offspring 11 | 147/149 | 123/123 |
| Offspring 12 | 145/147 | 123/125 |
| Offspring 13 | 147/149 | 121/123 |
| Sire 1* | 147/147 | 121/121 |
| Sire 2* | 145/147 | 123/125 |
| Michigan Dam 3 | 145/151 | 123/125 |
| Offspring 1 | 145/145 | 123/125 |
| Offspring 2 | 145/147 | 123/125 |
| Offspring 3 | 151/151 | 123/123 |
| Offspring 4 | 151/151 | 123/123 |
| Offspring 5 | 145/147 | 123/123 |
| Offspring 6 | 145/145 | 121/123 |
| Offspring 7 | 147/151 | 123/125 |
| Offspring 8 | 145/145 | 121/125 |

| | | |
|---|---|---|
| Offspring 9 | 145/145 | 123/125 |
| Sire 1* | 145/147 | 121/123 |
| Sire 2* | 151/151 | 123/123 |
| Michigan Dam 4 | 145/null | 125/125 |
| Offspring 1 | 145/147 | 125/127 |
| Offspring 2 | 151/151 | 125/125 |
| Offspring 3 | 145/147 | 125/125 |
| Offspring 4 | 143/145 | 125/127 |
| Offspring 5 | 143/145 | 125/125 |
| Offspring 6 | 151/151 | 125/125 |
| Offspring 7 | 151/151 | 125/125 |
| Sire Alleles* | 143, 147, 151 | 125, 127 |
| Michigan Dam 5 | 147/147 | 123/125 |
| Offspring 1 | 147/147 | 125/125 |
| Offspring 2 | 147/147 | 125/125 |
| Offspring 3 | 147/147 | 123/125 |
| Offspring 4 | 147/147 | 125/125 |
| Offspring 5 | 147/147 | 125/125 |
| Sire | 147/147 | 125/125 |
| Michigan Dam 6 | 145/147 | 123/125 |
| Offspring 1 | 145/147 | 123/125 |
| Offspring 2 | 147/147 | 123/125 |
| Offspring 3 | 147/147 | 125/125 |
| Offspring 4 | 145/147 | 123/123 |
| Offspring 5 | 145/147 | 123/125 |
| Offspring 6 | 145/147 | 123/125 |
| Offspring 7 | 147/147 | 125/125 |
| Offspring 8 | 145/147 | 123/125 |
| Sire | 147/147 | 123/125 |
| Michigan Dam 7 | 147/151 | 123/125 |
| Offspring 1 | 147/147 | 123/123 |
| Offspring 2 | 147/147 | 123/125 |
| Offspring 3 | 147/147 | 121/125 |
| Offspring 4 | 147/151 | 123/123 |
| Offspring 5 | 151/151 | 121/125 |
| Offspring 6 | 147/147 | 123/123 |
| Offspring 7 | 147/147 | 121/123 |
| Offspring 8 | 151/151 | 121/125 |
| Sire | 147/151 | 121/123 |
| Michigan Dam 8 | 147/147 | 123/125 |
| Offspring 1 | 147/151 | 123/125 |
| Offspring 2 | 147/151 | 123/123 |
| Offspring 3 | 147/147 | 125/127 |
| Offspring 4 | 147/147 | 125/125 |
| Offspring 5 | 147/147 | 125/127 |
| Offspring 6 | 147/147 | 125/127 |
| Sire 1* | 147/151 | 123/123 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Sire 2* | 151/151 | 125/127 | | | | |
| Michigan Dam 9 | 145/145 | 121/125 | | | | |
| Offspring 1 | 145/145 | 123/125 | | | | |
| Offspring 2 | 145/145 | 123/125 | | | | |
| Offspring 3 | 145/145 | 121/125 | | | | |
| Offspring 4 | - | 121/123 | | | | |
| Offspring 5 | 145/145 | 125/125 | | | | |
| Offspring 6 | 145/147 | 121/123 | | | | |
| Offspring 7 | 145/145 | 125/127 | | | | |
| Offspring 8 | - | 121/123 | | | | |
| Sire 1* | 145/147 | 123/125 | | | | |
| Sire 2* | 145/47 | 123/127 | | | | |
| Michigan Dam 10 | 145/147 | 121/125 | | | | |
| Offspring 1 | - | - | | | | |
| Offspring 2 | 145/147 | 125/125 | | | | |
| Offspring 3 | 145/147 | 121/121 | | | | |
| Offspring 4 | - | 125/125 | | | | |
| Offspring 5 | 147/149 | 121/121 | | | | |
| Offspring 6 | - | - | | | | |
| Offspring 7 | - | - | | | | |
| Offspring 8 | 145/147 | 121/125 | | | | |
| Sire | 145/149 | 121/125 | | | | |
| Michigan Dam 11 | 147/null | 124/124 | | | | |
| Offspring 1 | 145/145 | 124/126 | | | | |
| Offspring 2 | 145/145 | 124/124 | | | | |
| Offspring 3 | 147/147 | 124/124 | | | | |
| Offspring 4 | 145/145 | 124/124 | | | | |
| Offspring 5 | 147/147 | 124/124 | | | | |
| Offspring 6 | 145/145 | 124/126 | | | | |
| Sire | 145/147 | 124/126 | | | | |
| Michigan Dam 12 | 149/null | 125/125 | | | | |
| Offspring 1 | 145/145 | 125/125 | | | | |
| Offspring 2 | 145/149 | 125/125 | | | | |
| Offspring 3 | 145/145 | 125/125 | | | | |
| Offspring 4 | 145/149 | 125/125 | | | | |
| Offspring 5 | 145/145 | 125/125 | | | | |
| Offspring 6 | 145/149 | 125/125 | | | | |
| Sire Alleles | 145 | 125 | | | | |
| Illinois Dam 1 | 150/null | 125/125 | 188/216 | 168/172 | 173/177 | 189/189 |
| Offspring 1 | 162/162 | 125/125 | 188/188 | 168/172 | 173/177 | 189/203 |
| Offspring 2 | 150/150 | 123/125 | 188/188 | 168/172 | - | 189/189 |
| Offspring 3 | 150/150 | 125/125 | 188/188 | 168/172 | 173/177 | 189/203 |
| Offspring 4 | 152/152 | 123/125 | 188/188 | 168/168 | 173/177 | 189/203 |
| Offspring 5 | 152/152 | 125/125 | 188/216 | 168/172 | 173/177 | 189/203 |
| Offspring 6 | 150/150 | 117/125 | 188/188 | 168/172 | 171/177 | 189/203 |
| Offspring 7 | 150/152 | 125/125 | 188/188 | 168/172 | 171/177 | 189/189 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Offspring 8 | 152/152 | 125/125 | 188/188 | 168/170 | 173/177 | 189/189 |
| Sire Alleles* | 150, 152, 162 | 117, 123, 125 | 188 | 168, 170 | 171 | 189, 203 |
| Illinois Dam 2 | 143/145 | 123/125 | 188/188 | 166/null | 171/171 | 195/195 |
| Offspring 1 | 141/143 | 119/123 | - | 166/166 | 171/173 | 195/195 |
| Offspring 2 | 143/143 | 117/125 | 188/188 | 166/166 | 171/173 | 168/195 |
| Offspring 3 | 143/145 | 119/123 | 188/188 | 166/166 | 171/177 | 195/195 |
| Offspring 4 | 141/143 | 119/123 | 188/195 | 168/168 | 171/177 | 195/195 |
| Offspring 5 | 143/145 | 125/125 | 188/188 | 166/166 | 171/177 | 168/195 |
| Offspring 6 | 141/143 | 119/123 | 188/195 | 166/166 | 171/173 | 195/195 |
| Offspring 7 | 141/143 | 125/125 | - | 166/166 | 171/177 | 195/195 |
| Offspring 8 | 141/143 | 125/125 | 188/188 | 168/168 | 171/171 | 195/195 |
| Offspring 9 | - | 123/125 | 188/188 | 168/168 | 171/177 | 195/195 |
| Sire Alleles* | 141, 143 | 117, 119, 125 | 188, 195 | 166, 168 | 171, 173, 177 | 168, 195 |