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Facultative parthenogenesis discovered in wild vertebrates

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Facultative parthenogenesis (FP)—asexual reproduction by bisexual species—has been documented in a variety of multi-cellular organisms but only recently in snakes, varanid lizards, birds and sharks. Unlike the approximately 80 taxa of unisexual reptiles, amphibians and fishes that exist in nature, FP has yet to be documented in the wild. Based on captive documentation, it appears that FP is widespread in squamate reptiles (snakes, lizards and amphisbaenians), and its occurrence in nature seems inevitable, yet the task of detecting FP in wild individuals has been deemed formidable. Here we show, using microsatellite DNA genotyping and litter characteristics, the first cases of FP in wild-collected pregnant females and their offspring of two closely related species of North American pitviper snakes—the copperhead (*Agkistrodon contortrix*) and cottonmouth (*Agkistrodon piscivorus*). Our findings support the view that non-hybrid origins of parthenogenesis, such as FP, are more common in squamates than previously thought. With this confirmation, FP can no longer be viewed as a rare curiosity outside the mainstream of vertebrate evolution. Future research on FP in squamate reptiles related to proximate control of induction, reproductive competence of parthenogens and population genetics modelling is warranted.

Keywords: terminal fusion automictic parthenogenesis; Squamata; Serpentes; microsatellite DNA genotyping

1. INTRODUCTION

The discovery of facultative parthenogenesis (FP) in vertebrates can be traced to the late 1800s with reports

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of parthenogenetic development in domestic fowl (*Gallus domesticus*) [1]. However, it was not until reproductive studies began on commercial strains of turkey (*Meleagris gallopavo*) that a robust research programme was developed to understand the genetic and physiological processes governing this unorthodox form of reproduction (reviewed in Olsen [2]). One major finding was poultlets that hatched from unfertilized eggs were always male. The resulting outcome was attributed to the sex-chromosomal system (ZW) shared with many non-avian reptiles but differing from that of most mammals, fishes and other vertebrates (XY), where males are homogametic (ZZ) and females are heterogametic (ZW) [2]. In this avian system, ZZ and ZW are the only viable conceptus outcomes [2], with the combination of WW cells postulated to result in developmental failure. However, recent analyses that FP in boid snakes has demonstrated that WW cells produce viable progeny [3,4]. With the discovery that all the parthenogenetic offspring produced by females were male, early investigators of FP surmised that the second polar body, a meiotic product that typically degenerates, essentially behaves like a spermatozoon to activate and fertilize the ovum and restore diploidy, a process known as terminal fusion automixis [5]. The resulting parthenogen is therefore not a clone of the mother, but a half clone [5]. This genome wide homozygosity may explain the variety of developmental abnormalities, including stillbirths and deformities, which have been reported in certain parthenogenetic litters [4,6–8] (see the electronic supplementary material, table S2). Despite reports of developmental abnormalities, a parthenogenetic strain of the domestic turkey was developed in which most males appeared normal and reproduced successfully [2]. These results indicate that the ability to reproduce by way of FP is heritable and, as hypothesized by Olsen *et al.* [9], potentially linked to a single autosomal recessive gene.

Understandably, FP held little interest to most evolutionary biologists since it was known only in domesticated birds. This view began to shift with reports of FP in snakes [3,4,6,7,10–12], lizards, including the endangered Komodo dragon [8] and sharks [13,14]. In all instances, females had long been removed from the wild or were captive bred; therefore, ovulation and gestation were restricted to captivity (see the electronic supplementary material, table S2), resulting in FP being considered as a captive syndrome. With this string of events, the study of FP is gaining momentum as a research programme rather than just being viewed as rare cases of incidental reproductive peculiarities [5,10,15,16]. Nonetheless, despite these recent advances, whether FP exists in wild vertebrates remains unknown [10,15], primarily because of the formidable effort required to address this question. According to Avise [17], many progeny for which a mother is known must be genotyped, progeny possessing only alleles that could be inherited from the mother must be identified, and the possibility that the mother mated a male who shared her alleles at the loci surveyed must be eliminated in the analysis.

Among vertebrates, squamate reptiles represent lineages seemingly predisposed to FP [17], as evidenced by the growing number of reports in captivity [3,4,6,7,10–12]. From these reports, a specific set of

Table 1. Genotypes of the mothers and offspring at seven (*A. contortrix*) and eight (*A. piscivorus*) microsatellite loci.

species	ID	Ac4335	Ac6790	Ac7679	Ac3113	Ac5683	Ac6673	Ac6402	Ac7363
<i>A. contortrix</i>	mother	169/178	—	231/240	213/213	232/244	140/170	133/149	206/242
	offspring	178/178	—	240/240	213/213	232/232	140/140	149/149	206/206
<i>A. piscivorus</i>	mother	164/164	186/195	197/209	200/206	248/248	129/129	149/153	221/221
	offspring	164/164	195/195	197/197	200/200	248/248	129/129	153/153	221/221

characteristics commonly observed within litters have been identified that appear indicative of FP. These features, however, appear to differ in relation to the phylogenetic position of the lineage in question (reviewed in Booth & Schuett [10]). In caenophidian snakes, a derived clade containing most of the extant species [18], FP results in the production of unusually small litters that are composed entirely of males with low offspring viability and large numbers of developmental failures [6,7,10]. Here, we detected these litter characteristics in two litters from wild-captured North American pitviper snakes belonging to the clade Caenophidia—the copperhead (*Agkistrodon contortrix*) and cottonmouth (*Agkistrodon piscivorus*). Microsatellite DNA analysis was then used to test the hypothesis that these resulted from FP. While long-term sperm storage has been documented in females of both species [19,20], our present analysis excludes this as a competing hypothesis. Adding support to our findings, FP recently has been described in a captive-born copperhead [10] and has been suspected in captive cottonmouths [19], a suspicion we confirm here in a zoo-born individual (see the electronic supplementary material).

2. METHODS SUMMARY

From a total of 22 litters from wild-collected pregnant *A. contortrix*, and 37 litters from wild-collected pregnant *A. piscivorus*, two litters were selected for molecular analysis following the identification of litter characteristics indicative of FP [10] (a single male offspring, multiple yolked ova). DNA was extracted from ethanol-preserved blood and screened at seven (*A. contortrix*) and eight (*A. piscivorus*) microsatellite loci, following protocols outlined by Booth & Schuett [10]. In order to calculate the probability that a male sired the progeny and contributed identical alleles as the mother, population allele frequencies were derived from adult *A. contortrix* ($n = 63$) and *A. piscivorus* ($n = 45$) collected within the source populations. Population inbreeding coefficient (F_{IS}) was calculated using FSTAT [21].

3. RESULTS AND DISCUSSION

Unambiguous microsatellite genotypes were obtained at seven loci in *A. contortrix* and eight loci in *A. piscivorus*. Despite maternal heterozygosity at six and four loci, respectively (table 1), and collectively high population allelic diversity and heterozygosity (see the electronic supplementary material, table S3), homozygosity for one of the maternal alleles was observed uniformly across all loci in the progeny of both species. In the absence of unique paternal alleles in the progeny, the probability that a male contributed identical alleles as the mother and thus the progeny of these litters resulted from sexual reproduction was infinitesimally small (*A. contortrix* $p = 7.989 \times 10^{-16}$; *A. piscivorus* $p = 2.416 \times 10^{-8}$). This is supported by the rarity of multiple homozygous genotypes, as found in the progeny (table 1), within the populations from which

the specimens were derived (see the electronic supplementary material, table S4). No evidence was found supporting significant inbreeding within the population of *A. contortrix* ($F_{IS} = -0.011$), whereas F_{IS} was positive within the population of *A. piscivorus* (0.120), potentially reflecting female philopatric behaviour. Despite this elevated F_{IS} within *A. piscivorus*, no individuals other than the respective mother possessed genotypes compatible with the progeny, nor did any individual captured possess a composite genotype exhibiting homozygosity across all loci. Combined with the litter characteristics (male-only progeny and high numbers of developmental failures), we are confident that our findings represent the first cases of FP in wild vertebrates.

The homozygous nature of the offspring in contrast to the respective maternal heterozygosity indicates an automictic form of parthenogenesis, and probably involves terminal fusion automixis as recently inferred for long-term captive *A. contortrix* [10] and *A. piscivorus* (see the electronic supplementary material). This contrasts with obligate parthenogenetic vertebrates species (one blind snake, various lizards) recorded in nature, the majority of which use an apomictic form in which offspring develop directly from mitotic divisions of unreduced ova (reviewed in Avise [17]).

The evolutionary and ecological significance of FP in wild-living squamates and other vertebrates hinges on whether parthenogens show reproductive competency [6,10]. This is an assumption we will test with the copperhead and cottonmouth (Zoo Atlanta) parthenogens in this study if they attain adulthood. Hedrick [22] hypothesized that the production of viable parthenogens would be extremely rare in outbred individuals because of the likelihood that individuals resulting from such a genome-wide reduction in heterozygosity would suffer an elevated frequency of homozygosity at deleterious genes. Kearney *et al.* [23] reviewed potential examples of this, which include the reduction of immune function associated gene diversity and the altered ability to contend with increased parasite load. While we cannot comment as to the current health of the wild-produced cottonmouth parthenogen (released shortly after birth with no apparent health issues), the copperhead parthenogen has remained healthy (normal size, growth and feeding; figure 1). Likewise, the captive cottonmouth parthenogen at Zoo Atlanta (see the electronic supplementary material) remains healthy.

The conditions under which we report these natural instances of FP questions the role of FP in nature and the conditions under which it may be considered adaptive. A long-standing hypothesis—a reproductive advantage under conditions of isolation from potential mates [5,15]—is not supported in these instances given the population demography and significant



Figure 1. Female *Agkistrodon contortrix* and parthenogenetic male offspring described in this study.

male-biased sex ratios often observed in these species [24]. It may, however, be envisioned that under circumstances where a female becomes isolated from a suitable mate, FP may permit the establishment of a population prior to the introduction of genetically diverse conspecifics. In the light of the growing evidence of widespread FP in captive squamates, confirmation under natural conditions removes the prevailing dogma that FP is strictly a captive syndrome, thus warranting future research into the proximate control of induction, reproductive competence and population genetics modelling.

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