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A parthenogenetic *Varanus*

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Abstract. We report on a case of parthenogenesis in the varanid lizard *Varanus panoptes*. Parthenogenesis was observed in a female kept alone for three years. A clutch was deposited from which a single egg could be secured and incubated. Incubation was successful and a male specimen hatched. Obviously the newborn was produced without contribution of a father. After the unisexual reproduction, the mother was kept with males and bisexual reproduction was observed, too. We performed DNA Fingerprinting and showed that the parthenogen and its mother exhibit almost identical DNA patterns. The bisexually produced offspring has only a subset of bands in common with the mother and another subset in common with the father. Thus DNA Fingerprinting is in accordance with our observations and confims parthenogenesis.

We compare our results with existing cytological models of parthenogenesis and point out the following: 1. The mode of parthenogenesis described here is facultative, as the mother was able to reproduce in the bisexual mode as well. 2. The parthenogen is male and hence not a clone of the mother. 3. Almost complete heredity of maternal Fingerprint markers. All these points considered our case seem to fit to no known model of parthenogenesis exactly. But an additional recombination could result homogamety (would explain the sex of the parthenogen) while expressing almost all maternal bands.

Introduction

Parthenogenesis is a rare phenomenon in higher organisms (Cuellar, 1977). While absent in mammals (Surani, 1995), parthenogenesis is reported from birds (Astaurov and Demin, 1972), reptiles, amphibians and fishes (Vrijenhoek et al., 1989). This author listed cases of parthenogenesis in the families Gekkonidae, Teiidae, Uromastycidae, Chamaeleontidae, Xanthusiidae, and the snake *Ramphotyphlops*. Böhme (1975) and Frye and Madden (1995), found indications for parthenogenesis in the families Corytophanidae and Iguanidae, respectively. Since then parthenogenesis has been reported in the snake families Acrochordidae (Magnusson, 1979; Dubach et al., 1997), Crotalidae, Colubridae (Scalka and Vozenilek, 1986; Schuett et al., 1997) and Boidae (Groot et al., 2003; Kuhn and Schmidt, 2004).

In bisexual reproduction (fig. 1) a meiosis occurs, and a quadruplet of gametes (the oocyte and 3 polar bodies) is formed. Two gametes contain the information of half of the maternal genome and two the other half. Diploidy is restored by fusion of the oocyte with a paternal spermatozoon. Cell division is initialized by the entrance of the spermatozoon.

In parthenogenesis (fig. 1) either a fusion of two maternal gametes or clones of the mother yield the diploid parthenogen. In Automixis the parthenogen inherits either the complete genome of the mother (central fusion), or two copies of one half (terminal fusion). The subsequent cell divisions are initialized by maternal substances (Peacock, 1952). Soumalianen et al. (1987) describe several mechanisms how somatic ploidy level can be restored in parthenogenesis and yield some biological examples.

The most frequent case in reptiles is endomitosis (Smith, 1992) or premeiotic doubling (Suomalianen et al., 1987): during endomitosis a tetraploid cell develops after premeiotic doubling. It follows the production of four clones of the mother. This mode can be found in Gekkonidae, Teiidae and Lacertidae. In these

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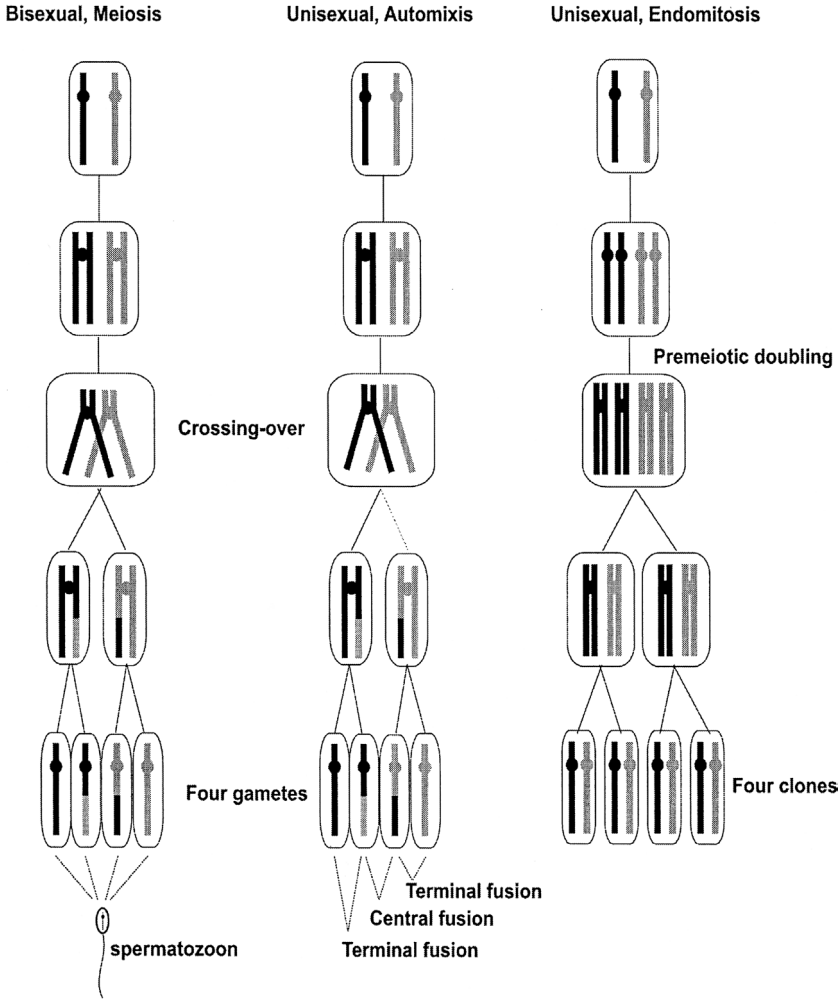


Figure 1. The fate of chromosomes in bisexual (left) and unisexual (middle, right) reproduction.

all-female populations males are possible, but are extremely rare (Darevsky et al., 1978). The biology of species following this mode is particularly well studied as reviewed in Darevsky et al. (1985). All these cases appear to be associated with hybridisation, polyploidy, and female offspring (Darevsky et al., 1985). We stress that these all-female populations are comparatively noticeable in the field.

In other cases like in *Acrochordus*, *Nerodia*, *Thamnophis*, *Crotalus*, and *Python* (Magnusson, 1979; Scalka and Vozenilek, 1986; Dubach et al., 1997; Schuett et al., 1997; Groot et al., 2003; Kuhn and Schmidt, 2004), parthenogenesis is a rarely observed phenomenon and only single cases are reported. This is due to the spontaneous occurrence of this type (Cuellar, 1974): females that usually reproduce bisexually reproduce unisexually when there are isolated. As parthenogens produced in this manner are unliklely to be recognized in nature, all observations of this phenomenon (including this report) stem from captive specimens. Generally herpetologists note facultative parthenogenesis when a clutch is deposited after the mother had been without a male for a long time.

The genetic and cytological background of this type remains poorly known and findings so

far point at different cylolocigal mechanisms. Facultative parthenogenesis is a rare phenomen, and thorough investigations on numerous individuals are lacking. This type was discovered in birds (domestic turkeys, Olsen, 1975), and described as automixis (automixis with terminal fusion, Suomalian et al., 1987). From Olsen's studies, it is known that two meiotic divisions occur as usual, and produce a quadruplet of gametes (the ovum and three polarbodies). The last meiotic division yields an ovum and its sister cell – the second polar body. The latter functions in a manner analogous to a spermatozoon, by entering the ovum and fertilizing it (Olsen, 1976). As consequence diploidy is restored and the zygote is homogametic (ZZ or WW). WW is not viable as reported from other species (Kiblisky and Reig, 1966; Olsen, 1976). The combination ZZ produces a male offspring. Schuett et al. (1997) described four cases of facultative parthenogenesis in *Thamnophis* and *Crotalus* and associated it with automictic parthenogenesis. They summarized the features of their report: 1) a high incidence of abortive events and developmental abnormalities; 2) the production of only diploid males; 3) parthenogens possess only a subset of the genes of the dam and no paternal genes; 4) high levels of homozygosity and limited levels of heterozygosity; 5) tendency for parthenogens to be less vigorous, and to have abnormal sex organs.

Important features of parthenogenesis depend on the chromosomal systems. Heterogamy is the male determining chromosomal state in the XX-XY system and the female determining state in the ZZ-ZW system. All *Varanus* species studied so far exhibit the ZZ-ZW system of chromosomes. However heterochromosomes are absent in the subgenus *Varanus* (King and King, 1975), the subgenus to which *Varanus panoptes* belongs. Nevertheless, it is likely that the sex is determined in this subgenus in a similar way as in other *Varanus* species, despite of the absence of discernible heterochromosomes.

Material and methods

Observations. On Dec. 10, 1994 one of the authors received two young specimens of *Varanus panoptes* with unknown locality from the German Customs authority. Their total lengths measured 25 and 28 cm respectively, indicating a very young age of the two specimens (the maximum length of adults is 160 cm). For the first three weeks both specimens were housed in one cage. As the bigger specimen exhibited an aggressive behaviour towards the smaller one, both were separated in different cages. This was the last contact with another specimen. The further development of both proceeded normally.

Five years later, on 12th June 1998, one specimen died. A probable cause of death was egg binding, as a thorough inspection of the specimen showed eggs in its oviduct. Moreover, by inspecting the cage two impaired eggs were found. An attempt to rescue the eggs failed. This specimen had never been with a male and was kept alone since the separation from its sister. One month later on July, 1998 a clutch of four eggs was discovered in the cage of the sister (M). This time one egg was rescued while the other three eggs were damaged and discarded. The egg was transferred into an incubator. After an incubation period of 270 days (at temperatures between 26-28°C), a young *Varanus panoptes* hatched (April 18, 1999). The putative parthenogen measured 13 cm (head-body) and 17 cm (tail). Its body mass was 33.7 g. The hatchling was healthy, fed on arthropods and developed normally. It never showed any indications of weakness or being less vigorous as compared to other subadults.

In the meantime its mother (M) was introduced to several males. Matings were observed and M produced bisexual offspring from which the siblings B1 and B2 could be included in this analysis. Their putative father (F) and two randomly chosen males (U1, U2) were also included.

DNA-Fingerprinting in reptiles. To substantiate our observation, we employed DNA-Fingerprinting. DNA-Fingerprinting is a suitable and frequently used method to trace relationships between parents and their offspring and is based on the detection of simple repeated multilocus motifs spread over the entire genome. A series of bands is produced in one individual pattern. Normally the bands are inherited in roughly equal parts from both parents. Exceptions from that scheme are due to crossing-overs or spontaneous mutations influencing restriction sites, multilocus motifs, or adjacent regions. However, these modifications are expected to play a minor role in DNA-Fingerprint patterns and generally refer to single bands only. Perfect matches between patterns identify individuals, twins or clones.

A special feature of sex bias of multilocus motifs is known from some reptiles and should be considered in minisatellite Fingerprinting. Numerous reptile species follow the ZZ-ZW type of chromosomal heterogamy. In contrast to the XX-XY system, the female sex is heterogametic. At least in some colubroid snakes (Jones and Singh, 1981; Epplen et al., 1983; Levinson et al., 1985), multilocus motifs are enriched in the pericentric region of the female W-chromosome. These repeated DNA-sequences came out as Bkm (banded krait minor) satellite and may cause a bias between the number of female and male bands in DNA-Fingerprints (Lenk and Joger, 1994).

Laboratory methods. DNA was extracted from blood samples collected by caudal vein puncture (Joger and Lenk, 1997), and stored in 99% ethanol. DNA isolation, digestion by restriction enzymes (Hinf I and Hind III), agarose electrophoresis, capillary transfer to a nylon membrane (Biodyne B) followed standard protocols established in our laboratory (Swatschek et al., 1993, 1994). Nylon membranes were prehybridized in hybridisation mixture (5× SSPE, 0.1% SDS, 1% powdered milk, 5× Denhardt's solution; Sambrook et al., 1989), for 2 hours at 39°C. Then the hybridisation mixture was decanted and fresh hybridisation mixture containing 10 pmol/mL of the digoxigenated oligonucleotide probe $(GGAT)_4$ (Fresenius) was added. Hybridisation was carried out at 39°C overnight. Membranes were washed three times with 6× SSC for 30 minutes each.

Further detection employed immunological methods: DNA/DNA-hybrids were detected by an antibody which was raised against digoxigenin (Boehringer). This antibody was coupled to a phosphatase which in turn produced a coloured precipitate at the sites of hybridisation (Boehringer). After colour reactions were completed, nylon membranes were documented and processed by the Bioprofil system (Fröbel, Lindau).

Sexing. The phenotypic sex of the parthenogen was determined by anaesthetising the specimen with Isofluran. Sexual organs were everted manually and their length was taken as an indicator of sex.

Results

The DNA-Fingerprint profile of the five specimens is shown in fig. 2. The mother M and its unisexual offspring P show highly congruent patterns. In the HinfI treated samples no difference between M and P is visible. In the HinfI/HindIII treated samples an additional band, absent in the mother is visible on all three patterns of the offspring (P, Bl, B2, fig. 2b). We traee it back either to minor genetic alterations, as mentioned above, or to other method-related artefacts. Apart from that, there is a perfect match between both patterns.

B1 and B2, the bisexually produced offspring, show multiple differences in patterns relative to M. As expected, they share some of the bands with their mother and some bands with their putative father F. Hence F is obviously the real father of B1 and B2. The unrelated males (U1 and U2) exhibit different patterns. The band sharing (table 1) between M and P is high 94-100%. Band sharing between B1/B2 and their parents were mediate (mother: 44-74%, father: 56-73%). The band sharing between these specimens and the two unrelated males is low (10-32%).

Sexing the parthenogen P indicates it as a male. The everted hemipenes are shown in fig. 3.

Table 1. Band sharing frequency of Minisatellite patterns. Probe is $(GGAT)_4$. Upper right cut with HinfI, lower left cut with HinfI plus HindIII. Only predominant bandings were used.

Individuals							
–	**M**	**P**	**B1**	**B2**	**F**	**U1**	**U2**
M	–	1.00	0.56	0.50	0.10	0.29	0.12
P	0.94	–	0.56	0.50	0.10	0.29	0.12
B1	0.74	0.70	–	0.30	0.60	0.29	0.24
B2	0.44	0.42	0.48	–	0.73	0.26	0.21
F	0.27	0.25	0.56	0.71	–	0.35	0.32
U1	0.22	0.2 1	0.29	0.30	0.35	–	0.20
U2	0.11	0.11	0.10	0.10	0.12	0.40	–

Discussion

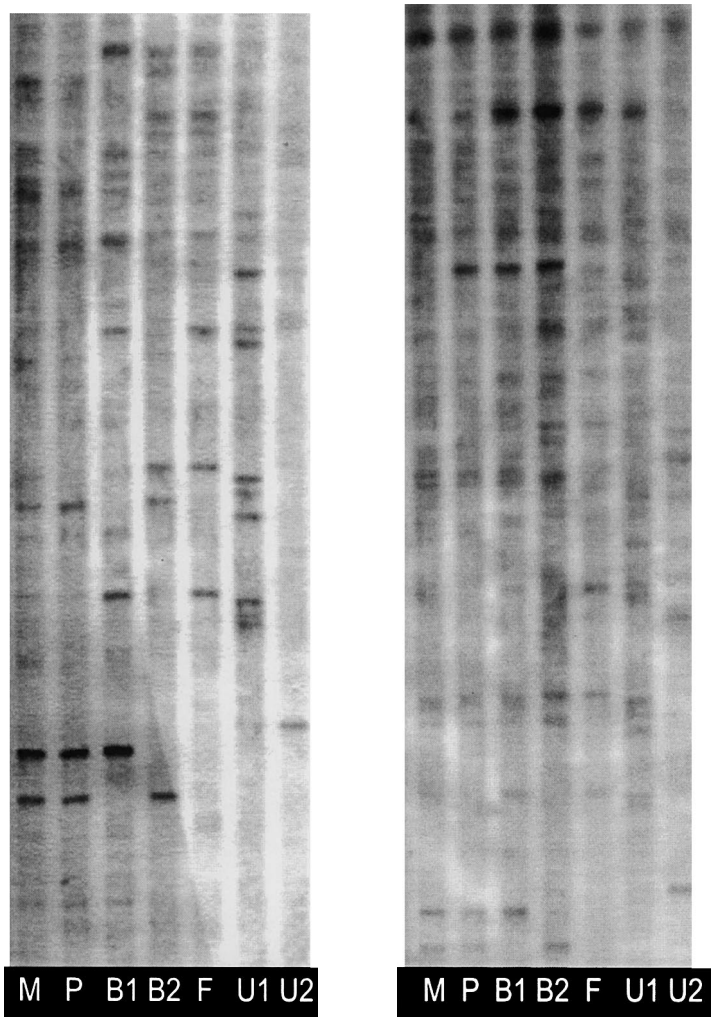
Our designation "parthenogenesis" is based on following:

i) Observation of reproduction of a female that never had been together with a male.
ii) Fingerprint patterns of the parthenogen are at least 94% identical with the mother.

We conducted no cytological experiments to analyze the mechanism of parthenogenesis, but discuss our results in comparison with other parthenogenetic models.

Facultative parthenogenesis

We designate our case as facultative parthenogenesis, since reproduction in *Varanus panoptes* is usually known to be bisexual. Additionally the mother was able to switch from unisexual to bisexual mode of reproduction, which confirms the facultative parthenogenesis.



(a) (b)

Figure 2. Fingerprint Blots: with the probe $(GGAT)_4$. Cut with endonucleases (a) HinfI and (b) HinfI/HindIII. From left to right: M (the mother), P (the parthenogen), B1 (bisexual offspring of M), B2 (bisexual offspring of M), F (father of B1 and B2), U1 (an unrelated male), U2 (an unrelated male).

Maleness of the parthenogen and chromosomal system

The change of sex from mother to offspring is only possible in the ZZ-ZW system where the heterogametic mother could pass on Z or W chromosomes or both. In the XX-XY system the mother (XX) can pass on X-chromosomes only, and thus can produce only daughters in parthenogenesis. In the ZZ-ZW system the mother can produce both, daughters and sons. As the sex changed from M to P *Varanus panoptes* express the ZZ-ZW system.

Quasi-clonal heredity of DNA-Fingerprint patterns

The parthenogen exhibits a pattern almost identical with the maternal one. M transmitted almost all her Fingerprint markers to P, suggesting that both chromosome sets are transmitted from mother to her son – a feature typical for a clonal heredity. This feature is similar to the case in

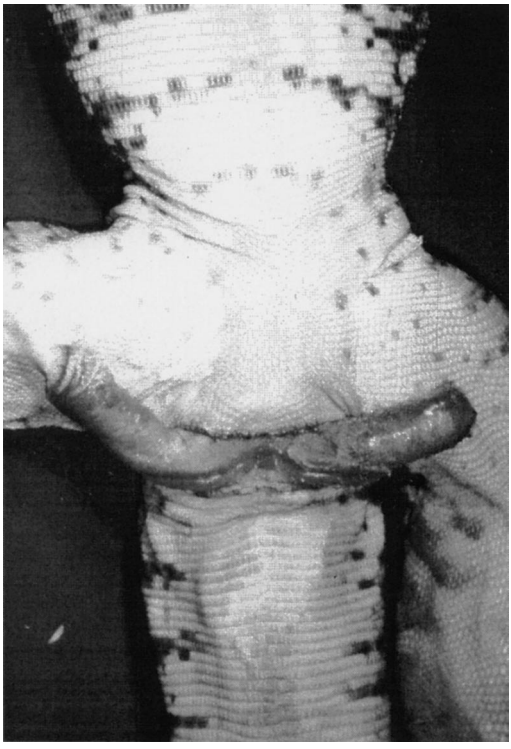


Figure 3. The two everted hemipenes of P: evidence for male sex.

Acrochordus (Dubach et al., 1997) and *Python* (Groot et al., 2003), where a high proportion of mother's markers are passed on to the unisexual offspring.

Heterozygosity of the mother

If both parents are related and homozygous for the loci encoding the Fingerprint patterns, the bisexual as well as the unisexual offspring would have the same bandings as its mother. The bisexual offspring shows a Figerprint-pattern consisting of roughly equal parts of maternal and paternal bandings and does not present the complete maternal pattern. As a consequence both parents must be heterozygous.

The observed mode of parthenogenesis

The close Fingerprint identity between the mother-son pair as well as heterozygosity of the mother confirm the observations of parthenogenesis in *Varanus panoptes*. But no known model would explain our case sufficiently. The maleness and the inheritance of almost all maternal Fingerprint bands by the parthenogen suggest that a recombination (intrachromosomal or interchromosomal) has happened where the sex-determining genes were involved. As a consequence a transmission of two Z-chromosomes and the remaining maternal genome could have been happened. This would fit to our observations. We do not know whether the indiscriminate sex chromosomes of *Varanus panoptes* play a role in this mechanism (would facilitate crossing over), but we think that it is important to mention it in this context. Alternatives to the genetically determined mode of sex is temperature dependent and hormonal sex determination. But these models are not known from *Varanus* species.

Is facultative parthegenesis an artefact or plays it a role in nature?

Facultative parthenogenesis has never been observed in the field, as parthenogens are hardly to discover. All known cases of facultative parthenogenesis were discovered by reproductions in captivity. However, the complexity of this mode makes it likely that it happens not by chance. Also it occurs in a wide varity of reptile species and thus may have a natural origin.

Absence of males appears to be a central point in discovering the mechanism, but on the other hand it may also be the trigger for it. Dubach et al. (1997) assume that female File snakes reproduce unisexually in small ponds when males are lacking. Generally speaken facultative parthenogenesis would be especially meaningful in situations when males are temporarily missing. Thinkable are situations dealing with maintnance, increase and range expansion of populations. The fact that the mother of the parthenogen switches back to the normal mode of reproduction would be concordant with these assumptions. Additionally, we like to stress that the sister of M produced fatherless eggs, too. It could be a hint that this

parthenogenesis was not an exceptional case in this species.

In the future, we are going to observe both the mother and her son to give answers to the main questions focussed on this hypothesis, such as: Is the parthenogen fertile? Does it mate with its mother? What is the result of this mating? We hope to be able to answer some of these questions in future.

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