

Supplementary Methods

Reproductive histories of females.

Flora, one of the Chester females, was bred in captivity in Miami in October 1998 from the same clutch as Nessie and Raja. Flora has been reared in Chester since December 1999 (*i.e.* since she was 1 year old) without access to any male Komodo dragon. Flora was therefore approximately 7.5yrs old at reported parthenogenetic reproduction. Sungai, the London female, was captive bred in Yogyakarta Zoo, Indonesia in May 1995. She was reared in a group of siblings until Aug 1998, when at 3 yrs of age and still sexually immature according to considered opinion, she was moved, with her brother Kimaan, to Thoiry in France. Courtship was observed between Sungai and Kimaan and, though there was no confirmed intromission, it is possible that mating occurred and that sperm could have been stored by Sungai. Only infertile eggs were laid at Thoiry. Sungai was transferred to London in May 2005 having last cohabited with Kimaan in Jan 2003, which was 2.5 years prior to laying the clutch that resulting in parthenogens (when she was approximately 11 years old). Sungai was 11.5 years old when she laid the clutch that resulted in sexually produced offspring after mating with Raja.

Genetic profiling methods.

DNA was extracted from blood clots or tissue of adult and juvenile Komodo dragons using a standard proteinase-K protocol¹. Individuals were genotyped at 7 microsatellite loci (Table S1) using published thermal cycling conditions²; approximately 50 ng of DNA was used for a 10 µl PCR containing 75 mM Tris-HCl pH 8.9, 20 mM (NH₄)₂SO₄, 0.01% v/v Tween-20, 0.2 mM each dNTP, 3.0 mM MgCl₂, 20 pmol forward primer, 30 pmol reverse primer and 0.25 U *Taq* polymerase (ABgene). PCR products were pooled into one of two genotyping pools along with a GENESCAN-500 LIZ size standard (Applied Biosystems), separated by capillary electrophoresis on an ABI3100 automated sequencer (Applied Biosystems) and sized using the cubic model of

analysis in GENEMAPPER software (Applied Biosystems). All PCRs and electrophoretic separation was repeated twice and provided clear, unambiguous genotypes for every individual that were identical over both independent genotyping runs. Potential technical problems with scoring microsatellite loci such as null alleles are not present with these loci, since all alleles present in the maternal genotypes were recovered from the overall clutch genotypes and there were no PCR failures.

Parentage analysis.

(1) Exclusion of males as potential sires of the clutches occurs where there are unambiguous genetic differences among these. Thus, clutch 1 differs from Kimaan at K09 & K10, Regis at K07 and Raja K06, while clutch 2 differs from Regis at K06 & K09 and Raja at K09. Moreover, considering that the combined genotype of each clutch reconstructs that of their mother exactly, any male would have to be genetically identical to the female to produce the observed genotypes. None of the Komodo dragons possess identical genotypes, including the brother and sister Kimaan and Sungai that differ at locus K06 (see Table S1).

(2) Expected probabilities of homozygosity under sexual reproduction. If we just consider loci that are heterozygous in the females (homozygous loci are uninformative for this analysis), then because the offspring within each clutch are homozygous for different alleles that reconstruct the maternal genotype exactly, then the genotype of a potential father for a clutch must be heterozygous also (and identical to the mother). Flora has 5 heterozygous loci (K03, K05, K07, K08, K09), Sungai has 3 heterozygous loci (K03, K06, K07) (Table S1). The probability of a zygote receiving identical alleles (*i.e.* being homozygous) from heterozygous parents is 0.25 (from Hardy-Weinberg law, given that the frequency of each allele in a heterozygous, diploid parent is 0.5). Therefore the probability of obtaining a completely homozygous individual at independent loci in Flora is $P=0.00098$ (*i.e.* $0.25 \times 0.25 \times 0.25 \times 0.25 \times 0.25$ for 5 loci) and for Sungai is $P=0.0156$ (*i.e.* $0.25 \times 0.25 \times 0.25$ for 3 loci). Moreover, the probabilities of independently obtaining a clutch of

completely homozygous offspring under sexual reproduction are $P=9.93 \times 10^{-10}$ ($0.00098 \times 0.00098 \times 0.00098$ for Flora's 3 offspring) and $P=5.9 \times 10^{-8}$ ($0.0156 \times 0.0156 \times 0.0156 \times 0.00156$ for Sungai's 4 offspring). Sexual reproduction cannot ($P \ll 0.0001$) have produced these combinations of clutch genotypes. Sungai may have mated with Kimaan (see main text for details); since offspring from clutch 2 are homozygous, Kimaan is excluded as a parent of two offspring (4598, 4600 have a 137/137 genotype at locus K06) and can only be the father of the other two (4599, 4601) if the same alleles from each parent segregated together to produce completely homozygous genotypes, the probability of which at (3 heterozygous loci) is non-significant ($P=0.000244$) and thus the observed offspring must be parthenogenetic.

References

1. Sambrook, J. & Russell, D. *Molecular Cloning: A Laboratory Manual* (3rd edn.). Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY, USA (2001).
2. Ciofi, C. & Bruford, M.W. *Molecular Ecology* **7**, 134-136 (1998).