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Received 2 Apr 2013 | Accepted 22 Aug 2013 | Published 18 Sep 2013

DOI: 10.1038/ncomms3488

Single-male paternity in coelacanths

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Latimeria chalumnae, a 'living fossil,' is of great scientific interest, as it is closely related to the aquatic ancestors of land-living tetrapods. *Latimeria* show internal fertilization and bear live young, but their reproductive behaviour is poorly known. Here we present for the first time a paternity analysis of the only available material from gravid females and their offspring. We genotype two *L. chalumnae* females and their unborn brood for 14 microsatellite loci. We find that the embryos are closely related to each other and never show more than three different alleles per locus, providing evidence for a single father siring all of the offspring. We reconstruct the father's genotype but cannot identify it in the population. These data suggest that coelacanths have a monogamous mating system and that individual relatedness is not important for mate choice.

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Coelacanths, an ancient lineage of fish, were thought to be extinct for more than 60 mya until Marjorie Courtenay-Latimer discovered an individual on a fishing trawler in 1938 later to be scientifically described as *Latimeria chalumnae*¹. As this very first report from South Africa, more than a hundred individuals have been found off the East African Coast, most of them on the Comoros^{2,3}. Researchers refer to coelacanths as 'living fossils' because of their nearly unchanged morphology since the late Devonian, approximately 400 mya ago⁴. They are of major scientific interest as they represent a very basal group of the gnathostomes. Consequently, a lot of studies investigated the phylogenetic relationship between coelacanths, lungfish and tetrapods^{5–8}. Also, the divergence of the two coelacanth species *L. chalumnae* in Africa and *Latimeria menadoensis* in Indonesia has attracted quite a lot of scientific attention^{9–12}.

The nearly unchanged morphology of coelacanths for the last 400 mya^{4,13} has led to the conclusion that their behaviour, ecology and genetics are very likely unchanged since the Devonian, and several molecular studies have confirmed their slow rate of evolution at the genetic level^{14–17}. However, recent population genetic studies revealed genetic divergence between individuals among and within the field sites and thus a potential for adaptation in *L. chalumnae*^{18,19}. Still they present an opportunity to get fascinating insights into the ecology and behaviour of a very old animal group.

Because they have such a high scientific value, coelacanth ecology has been studied quite extensively despite their relatively secluded habitat. Several studies on coelacanth biology have revealed many details about their life histories and ecology. We know that *Latimeria* occurs in water depths below 100 m (refs 20–22). They prefer caves to rest during the day^{23,24}. They are active at night and ambush predators but have a very low metabolic rate probably allowing for long periods without food²⁵. Coelacanths have rather large home ranges, but low dispersal rates and population sizes seem small^{20,23,26}. In fact, despite their ability to adapt to different habitats, there is no doubt that *Latimeria* has to be considered a rare and an endangered species^{20,27}.

As presented, *L. chalumnae* is quite an extensively studied species; however, a lot of questions remain, so far, unresolved. For example, free swimming juveniles are rarely seen and do not live together with the adults, leading to the assumption that they withdraw to other habitats possibly to escape cannibalistic adults^{28,29}. Also, the reproductive behaviour of coelacanths is largely unknown. The capture of gravid females with full-term embryos (Table 1) has proven that *L. chalumnae* are ovoviviparous and give birth to live young^{30,31}. Embryos have large yolk sacs that are retained almost until birth. Morphological structures to directly nurture the embryo (exclusively or in addition to the maternally provisioned yolk) have so far not been discovered^{28,31–33}. The period of embryogenesis is estimated to last approximately 3 years²⁰ meaning a very large investment for the female carrying the young. The internal fertilization, necessary for intrauterine development of the eggs, however, functions without externally visible copulatory organs. Male *L. chalumnae* might possess a cloaca³⁴ that could even be used as an eversible copulatory organ³¹; however, matings have so far never been observed and even the existence of the cloaca has been debated³⁵.

The absence of external copulatory organs means that females cannot easily be forced into copulations³⁶. As females invest a lot of time and energy into each clutch, it seems likely that they should optimize the offsprings' fitness by being selective about their mating partners. Females might even choose to mate with several mates to ensure fertilization of the full clutch, to enhance the genetic variability of the offspring or to make sure that the fittest male sires her clutch.

In this paper, we present the first molecular analyses of gravid females and their offspring. We use microsatellites to investigate the genetic diversity of the two only available coelacanth broods. We find a low genotypic variability and high relatedness of the offspring of each female. We show that each clutch was sired by a single male, with no evidence for multiple paternities. From reconstruction of the paternal genotypes, we show that females do not use relatedness criteria (preference or avoidance of closely related individuals for mating) for mate choice.

Results

Genetic diversity. Embryos in both females were well developed and seemed close to being born soon. Both females had roughly the same number of embryos: 26 (CCC 162–Mozambique) and 23 (CCC 253–Zanzibar). In both clutches, a maximum of three alleles per locus are found. Mean homozygosity per locus (HL) is very similar in all groups: full population HL = 0.47 ± 0.20 s.d., Mozambique HL = 0.46 ± 0.15 s.d., Zanzibar HL = 0.42 ± 0.17 s.d.). Consequently, no significant difference is detected between the groups (ANOVA: SQ = 0.059, FG = 2, MQ = 0.029, $F = 0.895$, $P = 0.411$).

In the 26 Mozambique embryos, 22 different genotypes were identified all of which were different from the maternal genotype. Four genotypes were represented by two individuals each (Table 2). This was most likely due to the very close relatedness of the siblings and relatively low marker resolution. Similarly, in the 23 Zanzibar embryos, 19 different genotypes could be identified, also all of them being different from the maternal genotype. Again, four genotypes were present, each in two different individuals (Table 3).

The levels of relatedness within the clutches are very high (Mozambique $R = 0.60 \pm 0.23$; Zanzibar $R = 0.68 \pm 0.15$ mean \pm s.d.). Not surprisingly, they are significantly higher than the level of relatedness over the entire *Latimeria* population ($R = -0.121 \pm 0.45$ mean \pm s.d.) (ANOVA results: SQ = 310.7, FG = 2, MQ = 155.3, $F = 902.4$, $P < 0.001$; a Scheffé *post hoc* test reveals a significant difference between the overall population and the clutches) (Fig. 1).

Paternity analysis. The low number of alleles per locus and the high level of individual relatedness within the broods indicate a single father for each clutch. This hypothesis is confirmed by the programme GERUD that calculated that a single male could have sired all offspring within each clutch. The reconstructed male genotypes were compared with all genotypes from our whole-population data set using the programme COLONY but could not be identified within the sample. For both clutches, the most closely related genotypes to the fathers' are found to be from the Comoros. The reconstructed male from the Mozambique clutch share 11 alleles at 7 loci with an individual found at the Comoros ($R = 0.211$). The reconstructed male of the Zanzibar clutch is closely related ($R = 0.45$) to a different animal from the Comoros. Their genotypes share 14 alleles at 9 loci. The parents of both clutches are not more closely or less related to each other than the rest of the population (Fig. 1).

Discussion

Phylogenetically, the occurrence of viviparity in coelacanths is very interesting. Although it is generally assumed that viviparity has evolved several times independently within the fishes³⁷, recent findings of viviparity being widespread in placoderms³⁸ might suggest that internal fertilization and the bearing of live young rather was the ancestral state for all gnathostomes³⁹ and was lost rather than gained during evolution. At least the strategy of producing young with large yolk provisions rather than very

Table 1 | List of all documented cases of gravid *Latimeria chalumnae.**

Individual number	Size (cm)	Weight (kg)	Capture date	Location	Eggs/ juveniles number	Size/weight range	Developmental stage	Current location	References
CCC 8	142	41	Nov-54	Anjouan/ Comoros	Eggs			MNHN Paris	3
CCC 10	166	78.5	Mar-55	Anjouan/ Comoros	Eggs	1-cm diameter		MNHN Paris	3
CCC 13	154	60	May-56	Grande Comore/ Comoros	Eggs	2-cm diameter		MNHN Paris	3
CCC 20	180	95	Jan-60	Grande Comore/ Comoros	Eggs	7-cm diameter		MNHN Paris	3
CCC 29	160	65	Jan-62	Anjouan/ Comoros	5 juveniles	30.1–32.7 cm	Yolk-sac	AMNH New York	3,31
CCC 79	163	78	Jan-72	Anjouan/ Comoros	19 eggs	8.5–9-cm diameter; 300–334 g		MNHN Paris	51
CCC 146	161	82	Jun-87	Anjouan/ Comoros	ca. 30 eggs			Discarded	
CCC 154	168	?	Jun-89	Grande Comore/ Comoros	59 eggs	Chicken-sized	(Vitellogenic)	Canadian Museum of Nature, Ottawa	
CCC 159	164	80	Apr-91	Grande Comore/ Comoros	67 eggs	Small		SAIAB, Grahamstown South Africa	52
CCC 162	179	98	Aug-91	Quelimane/ Mozambique	26 juveniles	30.8–35.8 cm, 410–502 g	Late term, some without yolk-sac	Natural Museum of Maputo / Mozambique	53
CCC 166 / 167	178	78	Nov-91	Anjouan/ Comoros	19 juveniles	ca. 28 cm		Fishing School Anjouan, 18 juveniles discarded	52
CCC 171	165	69	Mar-95	Grande Comore/ Comoros	Eggs			Galawa Beach Hotel/Comoros	54
CCC 178	170	77	Apr-01	Malindi/Kenya	17 eggs	7.5–8.7 cm, 115–274 g	Unfertilized	National Museums of Kenya, Nairobi	55
CCC 179	160	73	Jul-01	Tsandamba/ Madagascar	2 juveniles			IHSM Tulear, Madagascar	52
CCC 199	161	85	Nov-04	Tanzania	23 eggs?			TITECH Japan	52
CCC 202	184	105	Jan-05	Kigombe/ Tanzania	36 juveniles	5 cm	Early state	TAFIRI Dar es Salaam, Most juveniles rotten + discarded	52,56
CCC 206	162	69	Dec-08	Bagamoyo/ Tanzania	30–40 eggs	5–7 cm (visible in CT-scan)		TITECH Japan	52
CCC 215	131	51	May-07	Sulawesi/ Indonesia	25 eggs	3-cm diameter		Sam Ratulangi Univ., Manado, Indonesia	52
CCC 224	175	80	Dec-07	Mwambani/ Tanzania	23 juveniles		Yolk-sac	10 juveniles TITECH Japan, all other juveniles rotten	52
CCC 252	155	60	Jul-05	Fiherenamasay/ Madagascar	2 eggs			Musée de la Mer Tulear, IHSM Tulear	52
CCC 253	176	86.5	Jul-09	Ras Nungwi, Zanzibar	23 juveniles	35.7–37.1 cm, 500 g	Late term with yolk-sac	All discarded	52
CCC 255	168	70	Dec-08	Kilwa/Tanzania	30–40 eggs	5–7-cm diameter		TITECH Japan	52
CCC 259 (MW15)	159	79	Jan-10	Nyuli (Mwambani)/ Tanzania	23 (25) eggs		Fertilized	Rotten + discarded	52
CCC 283	184	79	Sep-10	Karange/ Tanzania	19 juveniles	29.0–31.0 cm	Yolk-sac	Sea Products Ltd, all juveniles rotten	52
CCC 294	170	85	Jul-11	Soalara/ Madagascar	2 juveniles	12 cm, 258 g		Copefrito Society Tulear	52

The number, size and weight and developmental stages of the eggs or juveniles found in the oviduct are provided as well as the current location of the animals and the origin of the information. Females and offspring analysed for this study are marked in bold typeface. CCC = Coelacanth Conservation Council inventory of known specimens of the living coelacanth caught since 1938 (ref. 52). *For each female the CCC identity number, the size and weight, capturing date and location are given.

Table 2 | Genotypes in family 1 (Mozambique)*.

Gen	#	Locus																							
		MS1	MS2	MS3	MS4	MS5	MS6	MS7	MS8	MS10	MS11	MS14b_2	MS16	MS18	GTH06										
Mozambique																									
a	1	1	3	1	2	1	2	1	1	1	2	1	2	1	1	1	2	1	1	1	1	1	1		
b	1	1	3	1	2	1	2	1	1	1	2	1	2	1	1	1	2	1	1	1	1	2	1	1	
c	1	1	3	1	2	1	2	1	1	1	2	1	2	1	1	1	2	2	2	1	1	1	1	1	
d	1	1	3	1	2	1	2	1	1	1	2	2	2	1	1	1	2	1	1	1	1	2	2	1	1
e	1	1	3	1	2	1	2	1	1	1	2	2	2	1	1	2	1	1	1	1	1	1	2	0	0
f	1	1	3	1	2	1	2	1	2	1	2	2	2	1	1	1	2	1	1	1	2	2	1	1	1
g	1	1	3	1	2	2	2	1	1	1	2	1	2	1	1	2	1	1	1	2	2	2	1	2	1
h	1	2	3	2	3	2	2	1	2	1	2	1	2	1	1	1	1	1	1	2	1	2	1	1	1
i	1	2	3	2	3	2	2	1	2	1	2	1	2	1	1	1	1	1	1	1	2	2	1	1	1
j	1	2	3	2	3	2	2	1	2	1	2	1	2	1	1	1	1	1	1	1	2	2	1	2	1
k	1	2	3	2	3	1	2	1	2	1	2	1	2	1	1	1	1	1	1	1	1	2	1	2	1
l	1	3	3	2	3	2	2	1	2	1	2	2	2	1	1	1	1	1	1	1	1	2	1	1	1
m	1	3	3	2	3	2	2	1	2	1	2	2	2	1	1	1	1	1	1	1	1	2	2	1	1
n	1	3	3	2	3	2	2	1	2	1	2	1	2	1	1	1	1	1	1	1	1	2	2	1	2
o	1	3	3	2	3	2	2	1	2	1	2	1	2	1	1	1	1	1	1	1	2	2	1	2	1
p	1	3	3	2	3	2	2	1	2	1	2	1	2	1	1	1	1	1	1	1	2	2	2	1	1
q	1	3	3	2	3	1	2	1	2	1	2	1	2	1	1	1	1	1	1	1	1	2	2	1	2
r	1	3	3	2	3	2	2	1	2	1	2	2	2	1	1	1	1	1	1	2	1	2	1	2	1
s	2	1	3	1	2	1	2	1	1	1	2	2	2	1	2	1	1	2	1	1	1	2	2	1	1
t	2	1	3	1	2	1	2	1	1	1	2	2	2	1	2	1	1	2	1	1	1	2	2	1	2
u	2	2	3	2	3	2	2	1	2	1	2	1	2	1	1	1	1	1	1	1	2	1	2	1	2
v	2	3	3	2	3	2	2	1	2	1	2	1	2	1	1	1	1	1	1	1	2	1	2	1	1
CCC 162	2	3	2	2	2	2	1	1	2	2	1	2	1	1	1	1	1	1	1	2	2	2	1	2	1
Father†	1	3	1	3	1	2	1	2	1	1	2	1	2	1	1	1	1	1	1	1	2	1	1	1	1

*The genotypes (Gen) found in the embryos, the number of times the genotype was observed (#) and the alleles (coded as numbers) present at each locus are given.
†Reconstructed genotype based on the offspring's allelic combinations.

Table 3 | Genotypes in family 2 (Zanzibar)*.

Gen	#	Locus																					
		MS2	MS3	MS4	MS5	MS6	MS8	MS11	MS14	MS16	MS18	GTH06											
Zanzibar																							
a	1	1	1	1	2	1	2	1	2	1	1	1	1	1	1	2	3	1	2	1	1	1	2
b	1	1	1	1	2	1	2	1	1	1	1	1	1	1	1	1	2	1	2	1	2	1	2
c	1	1	1	1	2	1	2	1	2	1	1	1	1	1	1	2	3	1	2	1	1	1	3
d	1	1	1	1	2	1	2	1	2	1	1	1	1	1	1	2	2	1	2	1	2	1	3
e	1	1	1	1	2	1	2	1	1	1	1	1	1	1	1	3	1	2	1	1	1	1	2
f	1	1	1	1	3	1	2	1	2	1	1	1	1	1	1	2	2	1	2	0	0	1	2
g	1	1	1	2	2	1	2	1	1	1	1	1	1	1	1	3	1	2	1	1	1	1	3
h	1	1	1	2	2	1	2	1	2	1	1	1	1	1	1	3	1	2	1	1	1	1	3
i	1	1	1	2	3	1	2	1	1	1	1	1	1	1	1	3	1	2	1	2	1	2	2
j	1	1	1	2	3	1	2	1	2	1	1	1	1	1	1	2	1	2	1	1	1	1	2
k	1	1	2	1	2	1	2	1	2	1	1	1	1	1	1	0	0	1	2	1	2	1	2
l	1	1	2	1	3	1	2	1	2	1	1	1	1	1	1	2	1	2	1	2	1	2	1
m	1	1	2	1	3	1	2	1	2	1	1	1	1	1	1	3	1	2	1	1	1	1	2
n	1	1	2	2	3	1	2	1	2	1	1	1	1	1	1	2	2	1	1	1	2	1	3
o	1	1	2	2	3	1	2	1	2	1	1	1	1	1	1	2	1	2	1	2	1	2	3
p	2	1	1	1	2	1	2	1	1	1	1	1	1	1	1	2	1	2	1	2	1	1	3
q	2	1	1	2	2	1	2	1	1	1	1	1	1	1	1	3	1	2	1	2	1	2	2
r	2	1	2	1	3	1	2	1	2	1	1	1	1	1	1	2	1	2	1	2	1	2	2
s	2	1	2	1	3	1	2	1	2	1	1	1	1	1	1	3	1	2	1	1	1	1	3
CCC 253	Mother	1	1	1	2	2	1	2	1	1	1	1	1	1	1	2	3	1	2	1	2	1	2
Father†		1	2	2	3	1	1	1	1	1	1	1	1	1	1	2	1	2	1	1	1	1	3

*The genotypes (Gen) found in the embryos, the number of times the genotype was observed (#) and the alleles (coded as numbers) present at each locus are given.
†Reconstructed genotype based on the offspring's allelic combinations.

many small eggs seems to be at least as old as 300 million years, as it has been reported also from the Carboniferous coelacanth *Rhabdoderma*²⁹.

Like every other ecological trait, the internal fertilization and development of juveniles has costs as well as benefits. The main

advantage of internal development, of course, is the enhancement of offspring survival. The main disadvantage of internal embryonic development is the increased costs of reproduction per individual offspring for the female. Females suffer reduced fecundity and might even face higher predation pressure due to

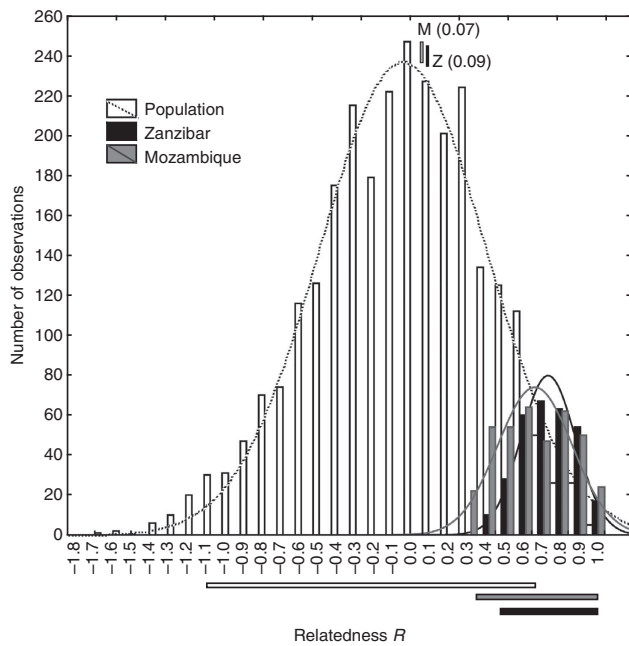


Figure 1 | Distribution of individual relatedness. The distribution of *R* values and 95% confidence intervals (bars below the graph) are given for the total population (light bars; *n* = 70) of *Latimeria chalumnae* and within the clutches (Zanzibar—black bar (*n* = 23); Mozambique—grey bar (*n* = 26)). The relatedness values of the parents of both clutches are given (values in brackets) and their position within the relatedness values of the total population is shown (black bar—Z = Zanzibar parents; grey bar—M = Mozambique parents).

lower mobility during pregnancy^{40,41}. In coelacanths, the benefits of live-bearing seem to compensate the large disadvantages to the female. Even though an estimated 3-year developmental phase²⁸ means a great investment into each embryo from the mother, this might be advantageous as the offspring might escape cannibalistic attacks from adults²⁹.

Another interesting aspect of the bearing of live young is the internal fertilization mode in coelacanths. Internal fertilization without copulatory organs is rare among the viviparous fishes but can be found, for example, in the Goodeidae⁴² (even though they do have a differentiated anal fin⁴³). Coelacanths completely lack external copulatory organs or even modified structures, although they clearly have internal fertilization. For a mating to be successful even without male copulatory organs, matching body sizes of the mating partners seem to be the key factor that influences the reproductive output to a great amount⁴².

All these considerations (high maternal costs per offspring, fertilization rate correlated to morphology) lead to the conclusion that females should choose their mating partners very carefully to optimize the offspring number and survival. Multiple mating seemed as a way to ensure the highest potential level of successful fertilization, the highest level of genetic variability in the offspring as well as ensuring the inheritance of the best genes in the offspring⁴⁴. However, contrary to our expectations, both investigated clutches were clearly sired by a single male. Even though sample size is low, this finding might indicate that coelacanths have a monogamous mating system. Monogamous mating systems are most commonly found in species where the father also provides parental care or where there is no opportunity for polygamy⁴⁵. As in coelacanths, we have no information about any parental care after birth. It seems more likely that females do not mate multiply, because they do not have

Table 4 | Primers used for microsatellite genotyping.

Microsatellite name	Primers sequence	Annealing temp (°C)	Reference
MS1	F: 5'-ATTATTCAATAAAAACGGCTATCTT-3' R: 5'-GGAAACAGTGCAAAGTACGAC-3'	54	17
MS2	F: 5'-CCTACTACTAGGCTCCTTCT-3' R: 5'-AACGAAGTACAGCTTATGAGG-3'	50	17
MS3	F: 5'-ATGTCTATCGTTATCTAGGGC-3' R: 5'-GATTTATATTGGAGCTGCTTTC-3'	56	17
MS4	F: 5'-CAATAATTGCACATAGGCAATC-3' R: 5'-GTCCTTACACGTCAAATTGTAC-3'	52	17
MS5	F: 5'-CACACCGATACATCTGTCAAT-3' R: 5'-TGTAACCTGCATAGCGTGC-3'	56	17
MS6	F: 5'-GCCTATAGTTGGGCCTTCT-3' R: 5'-TTTACGCTTTTGTGTTGTGC-3'	60	17
MS7	F: 5'-TTTTGGGTTCTTTGCATCA-3' R: 5'-CGTGATATGCAAAGCAGATG-3'	54	17
MS8	F: 5'-CGAATATATTAGAATTACGGGGTGT-3' R: 5'-CTTGCATGTAGCCAGTTTGC-3'	60	17
MS10	F: 5'-TCTGTTGCTGGATTTTGTAAA-3' R: 5'-TTCTGAGTAGCCATTCGTCAG-3'	54	17
MS11	F: 5'-GACCCCATCTGTGTCTGGTT-3' R: 5'-CACATCCAAGACATGGCAAC-3'	60	18
MS14b_2	F: 5'-TCAGAGCTAGGCTAAACTACCATG-3' R: 5'-GAGATTGGGAATAAATTTGCGTG-3'	62	18
MS16	F: 5'-ACTGGAGGGATTCAGGTGTG-3' R: 5'-AAAATTGGTGTGGTGCACAG-3'	62	18
MS18	F: 5'-CCTCCCCGAAAACAAAT-3' R: 5'-ATGTCAAGGTGGCAGTCCTT-3'	60	18
GTH06	F: 5'-CCTGCAGCTCAAATTCAGTTTGCT-3' R: 5'-M13-GCTTTCCAGCTGGCCCTTCTCC-3'	56	18

To amplify locus GTH06 a M13 (5'-CACGAGCTGTAAACGAC-3') tail was added to the 5' end of the reverse primer.

the opportunity or because multiple matings do not provide enough benefits to outweigh the potential costs. Potential costs to multiple mating could be the enhanced energy expense needed to search for mates and the potentially enhanced predation and infection risks during mating and mate search.

Another very interesting finding of our study was the relatedness pattern found between parents: mother and father of a clutch were not closer or had less relatedness than the majority of random pairs of the *Latimeria* population. This might mean that females avoid mating with close relatives; however, it could also mean that mating occurs randomly with respect to the overall genotypic relatedness. Either the probability to meet a close relative for mating is very low⁴⁶ or other male traits might be more important than relatedness, for example, morphology (see above) or parasite resistance. The absence of female choice based on relatedness could mean that in coelacanth, no mechanism for kin recognition is implemented. This in turn would facilitate the occurrence of cannibalism (see above), which seems to be a realistic threat to juvenile *Latimeria* and might be the reason for the evolution of viviparity as well as behavioural adaptations in the group.

In conclusion, we found that *L. chalumnae* juveniles are very closely related to each other and their genotypes could be explained by assuming that they were sired by a single male. Coelacanth therefore appear to present a monogamous mating system. The relatedness pattern of the parents seemed random and did not convey evidence for genetically based female choice. Due to their mating system, morphological traits could be more important for the reproductive success. This study provided new insights into coelacanth reproductive behaviour and therefore proved once more how extraordinary these animals are.

Methods

Samples. We used material from a female *L. chalumnae*, which was captured in 1991 off the coast of Mozambique. This female was found to be pregnant with 26 fully developed embryos. We also secured material from a second pregnant female that was captured in 2009 in Zanzibar bearing 23 late-term embryos (Table 1).

Molecular analyses. We extracted DNA from all embryos and both mothers using a 20% Chelex solution protocol⁴⁷ and genotyped the samples for 14 nuclear microsatellite markers^{17,18}. We performed PCR reactions in a total volume of 10 µl, with 1 × buffer, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.2 µM of each primer (Table 4) and 0.05 U Taq polymerase (EuroTaq – BioCat, Heidelberg, Germany). PCR conditions were set to 5 min of denaturing at 95 °C, 40 cycles of denaturing at 95 °C for 30 s, 30-s primer specific annealing temperature (50 °C–MS2; 52 °C–MS4; 54 °C–MS1, MS7, MS10; 56 °C–MS3, MS5, GTH06; 60 °C MS6, MS8, MS11, MS18; 62 °C–MS14, MS16) and 72 °C for 30-s extension, followed by a final extension of 5 min at 72 °C. For locus GTH06, we adapted a M13 protocol. We added the M13-tail (5'-CACGACGTTGAAAACGAC-3') to the 5' end of the reverse primer. Primer concentrations were GTH06F – 10 pmol, M13 primer with 800 nm fluorescent label 10 pmol and GTH06_Rtailed 2.5 pmol µl⁻¹. PCR products were analysed on a Licor 4300 DNA Analyser (Licor Biosciences, Lincoln, USA). Genotyping was done using the programme Saga2 (Licor Biosciences, Lincoln, USA). In addition, we visually inspected allele sizes and corrected them manually if necessary.

We analysed the Mozambique clutch using the full set of 14 microsatellites (Table 2). We genotyped 11 of the 14 loci for individuals from Zanzibar (no data could be obtained from MS1, MS7 and MS10) (Table 3). For the comparison to our dataset of adult individuals (*N* = 70) representing the entire known *Latimeria chalumnae* population 10 MS loci could be considered.

Statistical analyses. We counted the number of alleles and genotypes in the clutches. We reconstructed paternal genotypes using the programme GERUD⁴⁸ and searched for the genotypes in the reference population using the programme COLONY⁴⁹. We calculated individual relatedness and homozygosity levels per locus using the programme STORM⁵⁰. We compared *Latimeria* clutches to an already available data set of adult *L. chalumnae* individuals from the east coast of Africa¹⁸. We used STATISTICA (StatSoft Inc., Tulsa, USA) to compare the groups (ANOVA) and to produce graphs (histograms).

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Acknowledgements

This work was funded by the University of Bochum (Professor Tollrian), by the University of Würzburg and by the Deutsche Forschungsgemeinschaft (DFG Fr369/22-1). Our work was supported by the Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit Bonn-Bad Godesberg, by the CNDRS Moroni and the ACEP South Africa. We are grateful to A. Koehlin (Basel), Dr H. Vischer (Basel), S. Riedmiller (Tanga), S. Gessert and S. Fricke. We acknowledge the help of Dr A. Capral from the Natural History Museum Maputo for obtaining the female coelacanth from Mozambique. Logistic support was provided by C. Hack (Deputy Head of Mission of German Embassy Daressalam), Dr G. Nanyaro (Ministry for Livestock Development, Daressalam) and K. Fruehinsfeld (German Embassy, Tananarive).

Author contributions

K.P.L. did the molecular and statistical analyses and wrote the manuscript. K.B. did part of the molecular analyses. K.H. helped gathering the tissue samples, provided the information on captured gravid coelacanth females and drafted the manuscript. J.S. helped gathering the tissue samples. P.S., Z.e.K. and B.P.N. provided access to the animals and logistic support. H.F. gathered the tissue samples and designed the study. M.S. designed the study and drafted the manuscript.

Additional information

Competing financial interests: The authors declare no competing financial interests.

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How to cite this article: Lampert, K. P. *et al.* Single-male paternity in coelacanth. *Nat. Commun.* 4:2488 doi: 10.1038/ncomms3488 (2013).