

Parental assessment in captive breeding groups of *Mauremys leprosa* and *Emys orbicularis*.

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Captive breeding risks

Endangered species conservation programs use captive breeding to increase offspring recruitment released back in to the wild environment. In the Iberian turtles *Mauremys leprosa* and *Emys orbicularis* (Fig. 1 and Fig. 2) reproduction groups usually include around ten adults kept in the same enclosure. In such situations there is a significant risk of inbreeding, because only a subset of these animals might be actually reproducing. Thus, the correct identification of the relative contribution of each adult to the next generation may be a key point to ensure the long term conservation success.



Figure 1. *Mauremys leprosa*

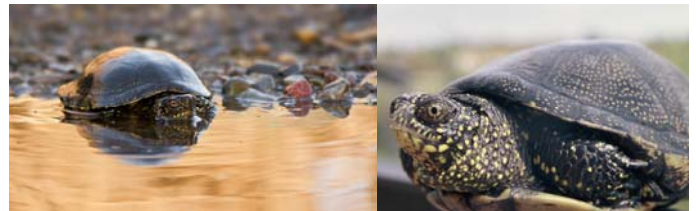


Figure 2. *Emys orbicularis*

Genetic markers for parental assessment

Microsatellites or short tandem repeats (STR) are polymorphic DNA loci containing repeated motifs from 2 to 7 nucleotides. The number of these repeats for a specific locus may differ, producing alleles of varying length. This polymorphism can be analyzed with a high resolution electrophoresis. STR are widely used, and the same set of 13 STR is currently used to perform human forensic identification and parental tests in most countries of the world (El-Alfy&Abd El-Hafez, 2012).

In this work we selected a set of five STR with cross amplification in Mediterranean turtles (King&Julian 2004, Tab. 1, Fig. 3) for parental assessment.

Table 1. STR set parameters

Species	STR	Detected alleles	5' tag	Expected Sizes	Annealing T
<i>Mauremys leprosa</i>	B08	4	VIC	205-215	65→55°C
	D16	7	NED	165-220	65→55°C
	D87	2	6-FAM	215-220	65→55°C
	D88	7	PET	125-155	56,5 °C
	D114	6	VIC	90-115	59,2 °C
<i>Emys orbicularis</i>	B08	6	VIC	185-205	65→55°C
	D16	11	NED	160-225	65→55°C
	D87	11	6-FAM	185-260	65→55°C
	D88	10	PET	135-185	56,5 °C
	D114	5	VIC	110-165	59,2 °C

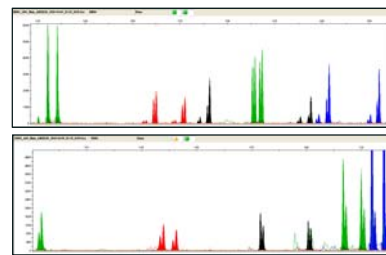


Fig. 3. (A) *E. orbicularis* and (B) *M. leprosa* STR set

STR results

Informative power of each marker and the combined resolution of the set within each reproductive group may be inferred by heterozygosity levels and by the combined non exclusion probabilities (Table 2). These values indicate that this set of 5 markers does not have enough resolution to be used in open populations. However, it can provide with valuable information in closed groups, specially if one of the parental is known.

Table 2. STR resolution values, including Observed Heterozygosity (Ho), Expected Heterozygosity (He), the Polymorphic information content (PIC) and the combined non exclusion probabilities for the first parental (NE-1P) and the second (NE-2P).

Species	Group	STR	N alleles	Ho	He	PIC	NE-1P	NE-2P
<i>Mauremys leprosa</i>	Zoo de Barcelona	B08	3	0,5	0,53	0,424	0,159	0,044
		D16	7	0,833	0,909	0,812		
		D87	2	0,5	0,53	0,368		
		D88	8	0,833	0,924	0,83		
		D11	4	0,667	0,561	0,476		
	C.R.A.R.C.	B08	4	0,556	0,575	0,48	0,172	0,047
		D16	8	0,667	0,856	0,786		
		D87	2	0,333	0,503	0,362		
		D88	7	0,714	0,813	0,73		
		D11	5	0,375	0,775	0,682		

Species	Group	STR	N alleles	Ho	He	PIC	NE-1P	NE-2P
<i>Emys orbicularis</i>	CRT Riudarenes	B08	3	1	0,642	0,516	0,104	0,021
		D16	8	0,75	0,892	0,816		
		D87	5	0,571	0,802	0,704		
		D88	7	0,75	0,85	0,77		
		D11	6	0,75	0,767	0,68		
	CRT Baix Ter	B08	3	0,4	0,611	0,492	0,165	0,04
		D16	8	0,7	0,816	0,751		
		D87	5	0,5	0,721	0,635		
		D88	7	0,8	0,8	0,73		
		D11	6	0,8	0,684	0,62		

Parental assessment

In *E. orbicularis*, only one couple (in a group of 3 males and 7 females) might have produced more than 60% of 12 offspring. Preliminary results in *M. leprosa* showed that none of the sampled adults was a compatible parental. The analysis of 8 offspring with known mothers showed inconsistencies in marker STR D16, suggesting the presence of null alleles. Thus, in both species, a second set of markers is currently being tested to increase the parental assessment signification. If the low contribution per adult is confirmed, some management changes would be required to improve captive breeding in Iberian aquatic turtles.

LITERATURE

El-Alfy & Abd El-Hafez (2012) Journal of Genetic Engineering and Biotechnology 10, 101–112; King&Julian (2004) Conservation Genetics 5, 719–725

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