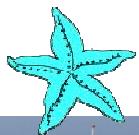




School of Marine Sciences

Conservation of Green Sea Turtles through Genetics and Genomics

Dr. Yaron Tikochinski



June - 24 - 2014



Mikhmoret marina

Green Sea Turtles in Israel: On the verge of extinction

About 10 nesting females along the Israeli shore
(about 200 Km)



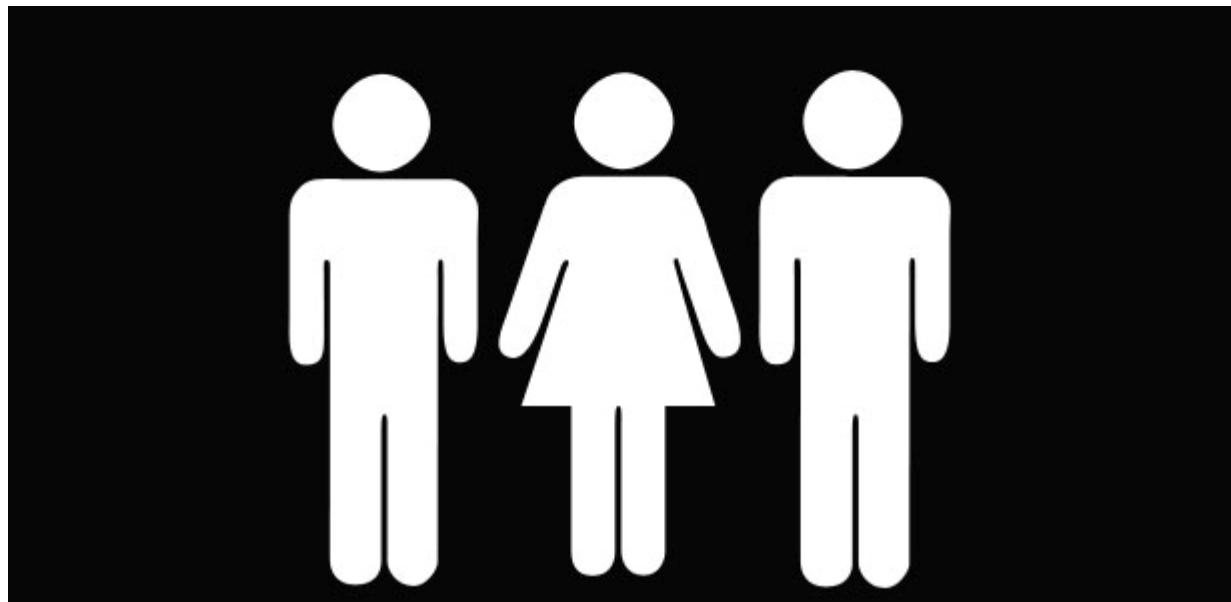
About Sea Turtles:

- Philopatric



About Sea Turtles:

- polyandry



The Sea Turtle Rescue Center



- Locate
- Heal
- Release

Sea Turtles Rescue Center: Save and Heal



Sea Turtles Rescue Center: Feed and Bread



Let's Increase the Numbers

“I can make my own people”

Jerry Seinfeld

Breeding Stock



The Sea Turtle Rescue Center



A Large Variable Population



“Population with no Variation will
not survive Evolution”

C. T. Urtle



A Stable, Strong Population

“My boys can swim!”

George Costanza
(marine biologist)

Genetic Variability of Green Turtles

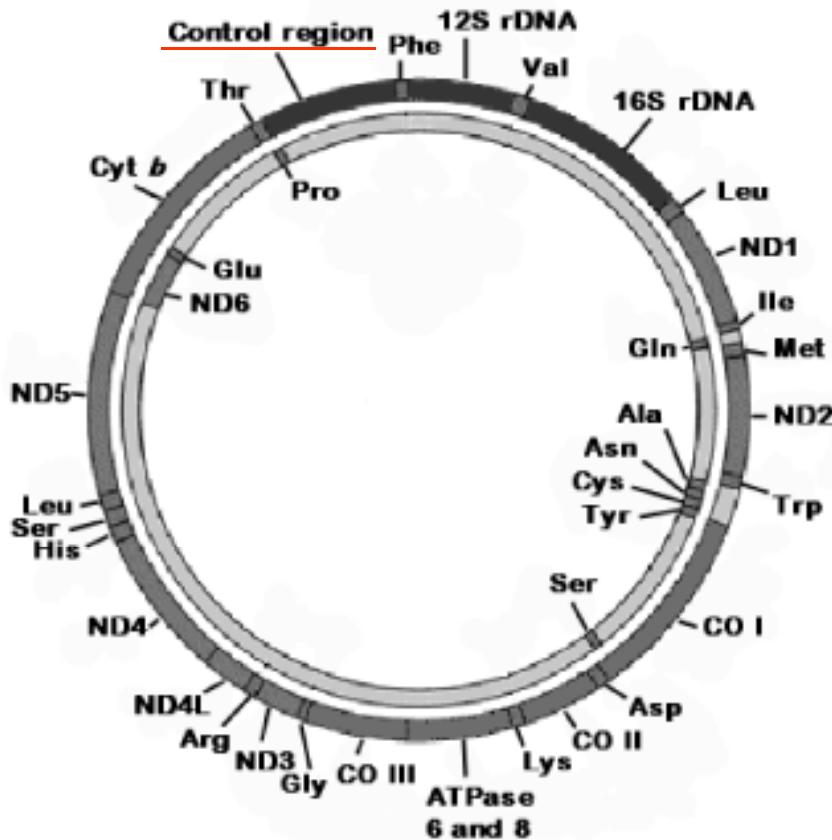


Figure 1 - Schematic representation of the circular molecule of the “conserved” vertebrate mitochondrial genome. Genes outside and inside the circle are transcribed in the H and L strands, respectively. Protein-coding genes are represented as follows: Cyt b - cytochrome b; CO I, CO II and CO III - subunits I, II and III of the cytochrome oxidase; ND1-6 - subunits 1 to 6 of the NADH reductase. tRNA are represented by their three-letter amino acid abbreviations.



- Mitochondrial DNA D-Loop
- 600bp at the 5'
- 70 haplotypes worldwide
- All Mediterranean (but 2)
CM-A13
- Genomic STR's show variability

Genomic STR's

- Genomic STR's show variability
- Difficult to analyze – can't tell a mother's genotype by her offspring



- Back to mtDNA?
- Longer Fragments?

The Mitochondrial D-loop



ACACAGGAATAAAAGTGTCCACACAAACTAACCTAAATTCTCTGCCGTGCCAACAGAACAAATACCC
GCAATACCTATCTATGTATTATTGTACATCTACTTACCAATAGCATATGACCAGTAATGTTAACAG
TTGATTGGCCCTAACACATAAAAAATCATTGAATTACATAAAATATTTAACACATGAATATTAAGCAG
AGGATTAAAAGTGAAATGACATAGGACATAAAACTATTAACTCAACCATGAATATCGTCACAGT
AATTGGTTATTCCTAAATAGCTATTCACGAGAAATAAGCAACCCCTGTTAGTAAGATAAACATTACCA
GTTTCAAGCCCATTCACTGTGGCGTACATAATTGATCTATTCTGGCCTCTGGTAGTTTCAGGCA
CATACAAGTAACGACGTTCAATTGTTCCCCTTAAAAGGCCTTGGTGAATGAGTTCTACATTAAAT
TTATAACCTGGCATACGGTAGTTACTTGATATAGTAGTTTTCTCTGTGTTCTCAGGCCAC
ATAACTGATACTGCCGATTCACTGAAACTGGACTTACGTTAAATATGATTGGCGTGCAAACGTGATT
ATGGTATTATTAAGTTAATGCTTAAAGACATAGAATTTCACAATTAAACCTAAACAATGATCTACACC
TAACTCATTATTAACTGTACTTTAGCTAAACCCCCCTACCCCCGTTAAAGTCAACACCAGCCGCTAT
AGCCATTACTCTGCCAAACCCCTAAATCCGAGACTGACCAAACGTGACATAATATCAACTGCATAAGC
ATCACACAAATCAATAGGATACTTACACTAATATTAAAAAGTACTATACAATTCAAAACACCTCTACCA
CACCTCAACCAATATATATATATTACATTATATATATATATTATATATTATATATAAT

AT

DNA Repeats – a Source for Polymorphism



- Mutations' hot spots
- Evolutionary shortcuts

Polymorphism Emerging

The mitochondrial D-loop:
PCR with fluorescent primers.



The 3' end has length polymorphism:
115, 117, 119, 121, 123, 125, 127 bp

AT Repeats - Aligned



New Haplotyping

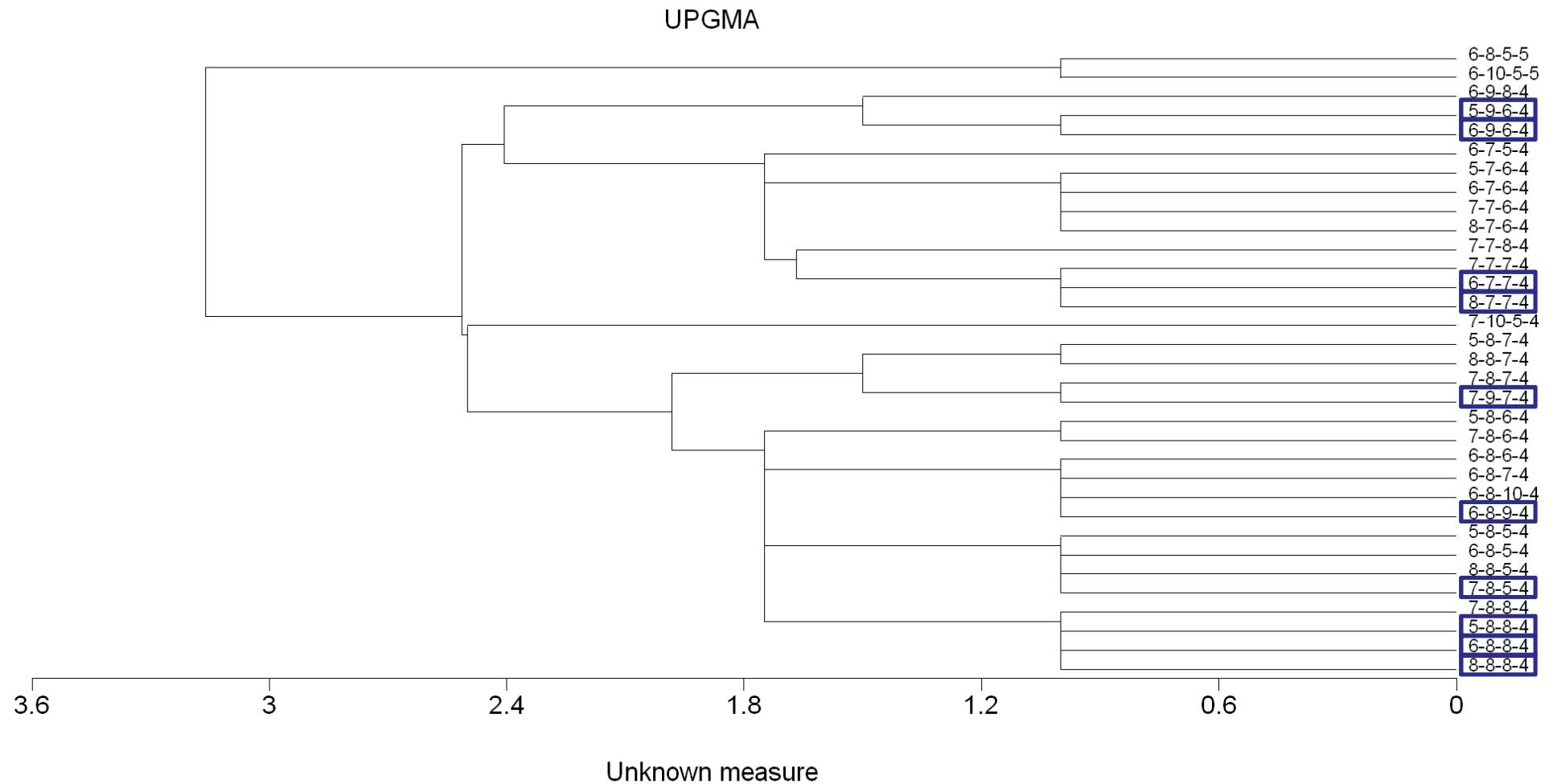
- 34 Haplotypes (+1)
(mostly non-Israeli)

- Can we use them?
 - Polymorphic
 - Reliable
 - Reproducible
 - Kinship

STR1	STR 2	STR 3	STR 4	Stranded	Local*
8	8	8	4	0	1
8	7	7	4	16	47
7	9	7	4	0	1
7	8	5	4	3	1
6	9	6	4	4	16
6	8	9	4	6	12
6	8	8	4	37	11
6	7	7	4	0	1
5	9	6	4	0	2
5	8	8	4	3	3
8	8	7	4	2	
8	8	5	4	1	
8	7	6	4	2	
7	10	5	4	2	
7	8	8	4	5	
7	8	7	4	2	
7	8	6	4	4	
7	7	8	4	4	
7	7	7	4	2	
7	7	6	4	4	
6	10	5	5	1	
6	9	8	4	1	
6	8	10	4	2	
6	8	7	4	8	
6	8	6	4	14	
6	8	5	5	2	
6	8	5	4	54	
6	7	6	4	3	
6	7	5	4	2	
5	8	7	4	1	
5	8	6	4	4	
5	8	5	4	4	
5	7	6	4	1	
				194	95

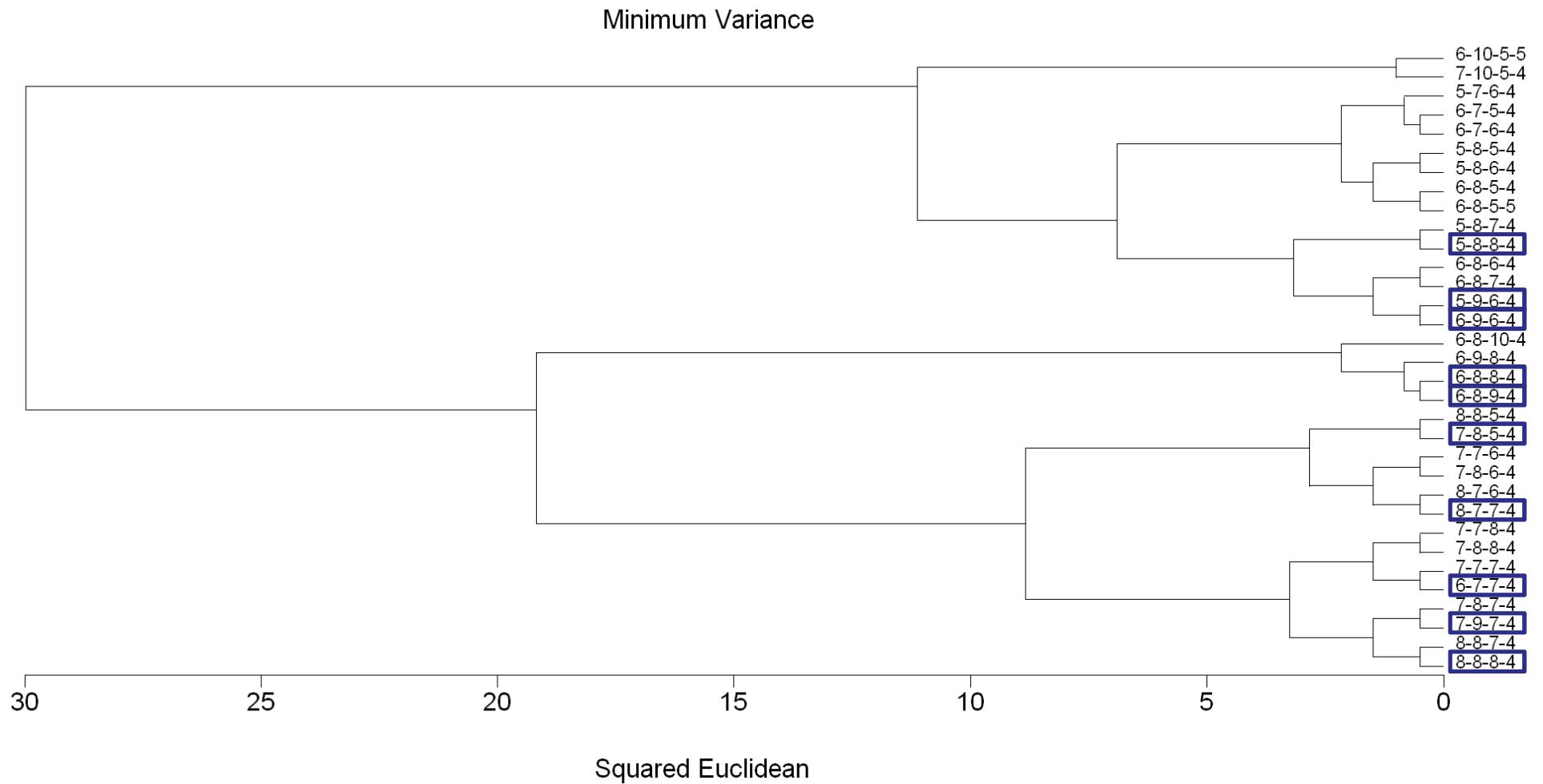
Mediterranean/Israeli Green Turtles

Tree



Mediterranean/Israeli Green Turtles

Tree



Does it Tell a True Story?

- DNA never lies
- Scientists should always doubt

Analysis

Israeli

Haplotype	Close Linkage*	Stranded turtles
8 8 8 4	5	0
8 7 7 4	4	16
7 9 7 4	2	0
7 8 5 4	7	3
6 9 6 4	4	4
6 8 9 4	5	6
6 8 8 4	9	37
6 7 7 4	5	0
5 9 6 4	3	0
5 8 8 4	6	3
69		

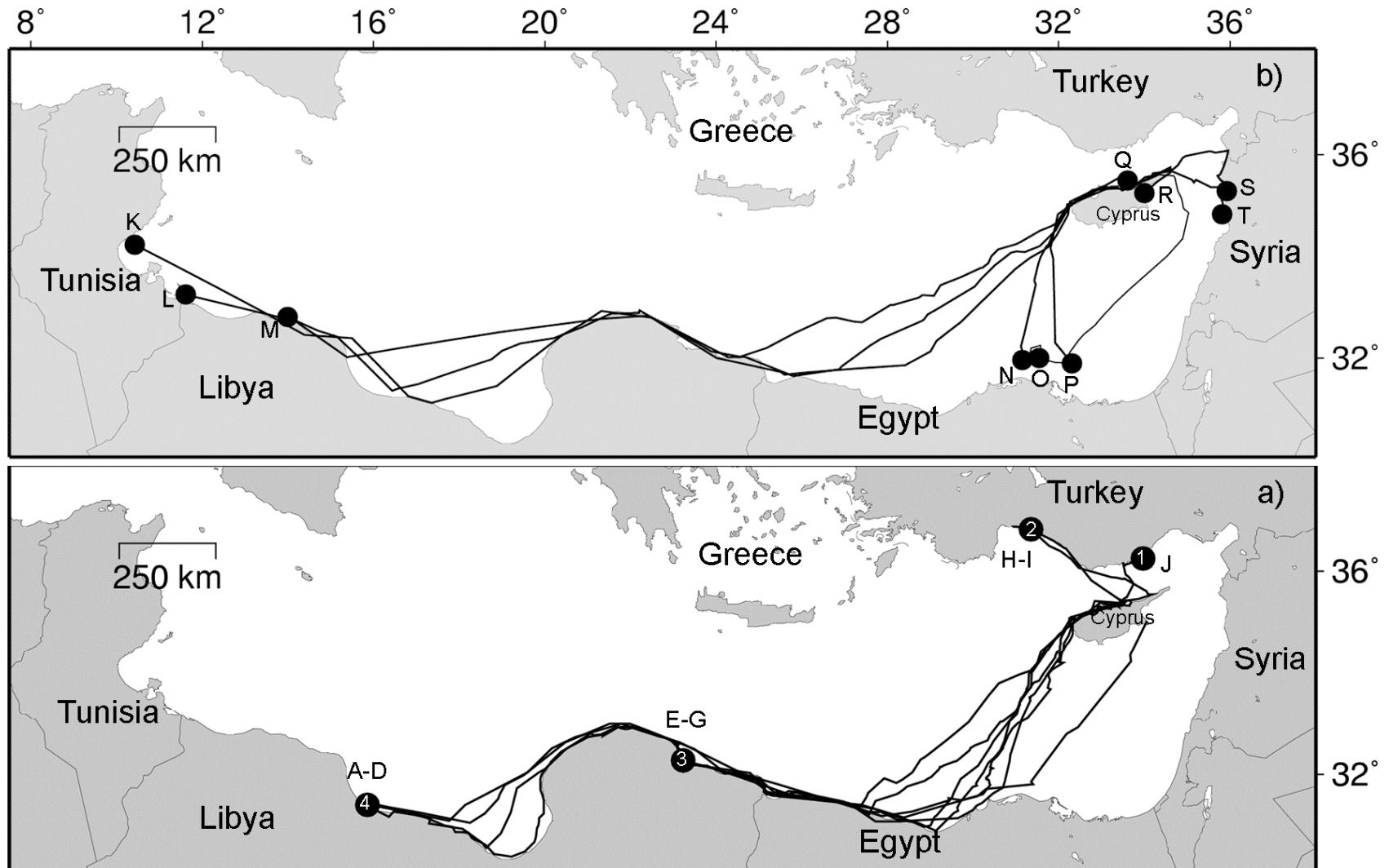
Other

Haplotype	Close Linkage*	Stranded turtles
8 8 7 4	6	2
8 8 5 4	5	1
8 7 6 4	4	2
7 10 5 4	1	2
7 8 8 4	7	5
7 8 7 4	8	2
7 8 6 4	6	4
7 7 8 4	3	4
7 7 7 4	6	2
7 7 6 4	6	4
6 10 5 5	1	1
6 9 8 4	2	1
6 8 10 4	5	2
6 8 7 4	9	8
6 8 6 4	9	14
6 8 5 5	2	2
6 8 5 4	10	54
6 7 6 4	7	3
6 7 5 4	3	2
5 8 7 4	6	1
5 8 6 4	7	4
5 8 5 4	6	4
5 7 6 4	5	1
125		

Haplotype	Israel	Stranded	Turkey	N. Cyprus	S. Cyprus
6 8 8 4	1	37	17	12	20
8 7 7 4	1	16	0	0	0
6 8 7 4	1	8	8	4	0
6 8 9 4	1	6	0	0	0
6 9 6 4	1	4	1	5	1
7 8 5 4	1	3	3	0	0
5 8 8 4	1	3	0	0	0
8 8 8 4	1	0	0	0	0
7 9 7 4	1	0	0	0	0
6 7 7 4	1	0	0	0	0
5 9 6 4	1	0	0	0	0
6 8 5 4	0	54	27	0	0
6 8 6 4	0	14	11	16	0
7 8 8 4	0	5	1	1	1
7 8 6 4	0	4	1	1	0
7 7 8 4	0	4	0	0	0
7 7 6 4	0	4	2	0	0
5 8 6 4	0	4	0	0	0
5 8 5 4	0	4	0	0	0
6 7 6 4	0	3	1	1	0
8 8 7 4	0	2	0	0	0
8 7 6 4	0	2	2	0	0
7 10 5 4	0	2	0	0	0
7 8 7 4	0	2	0	0	0
7 7 7 4	0	2	3	0	0
6 8 10 4	0	2	0	0	0
6 8 5 5	0	2	0	0	0
6 7 5 4	0	2	2	0	0
8 8 5 4	0	1	0	0	0
6 10 5 5	0	1	0	0	0
6 9 8 4	0	1	2	0	1
5 8 7 4	0	1	0	0	0
5 7 6 4	0	1	0	0	0
7 10 6 4	0	0	0	1	0
	11	194	87	41	23

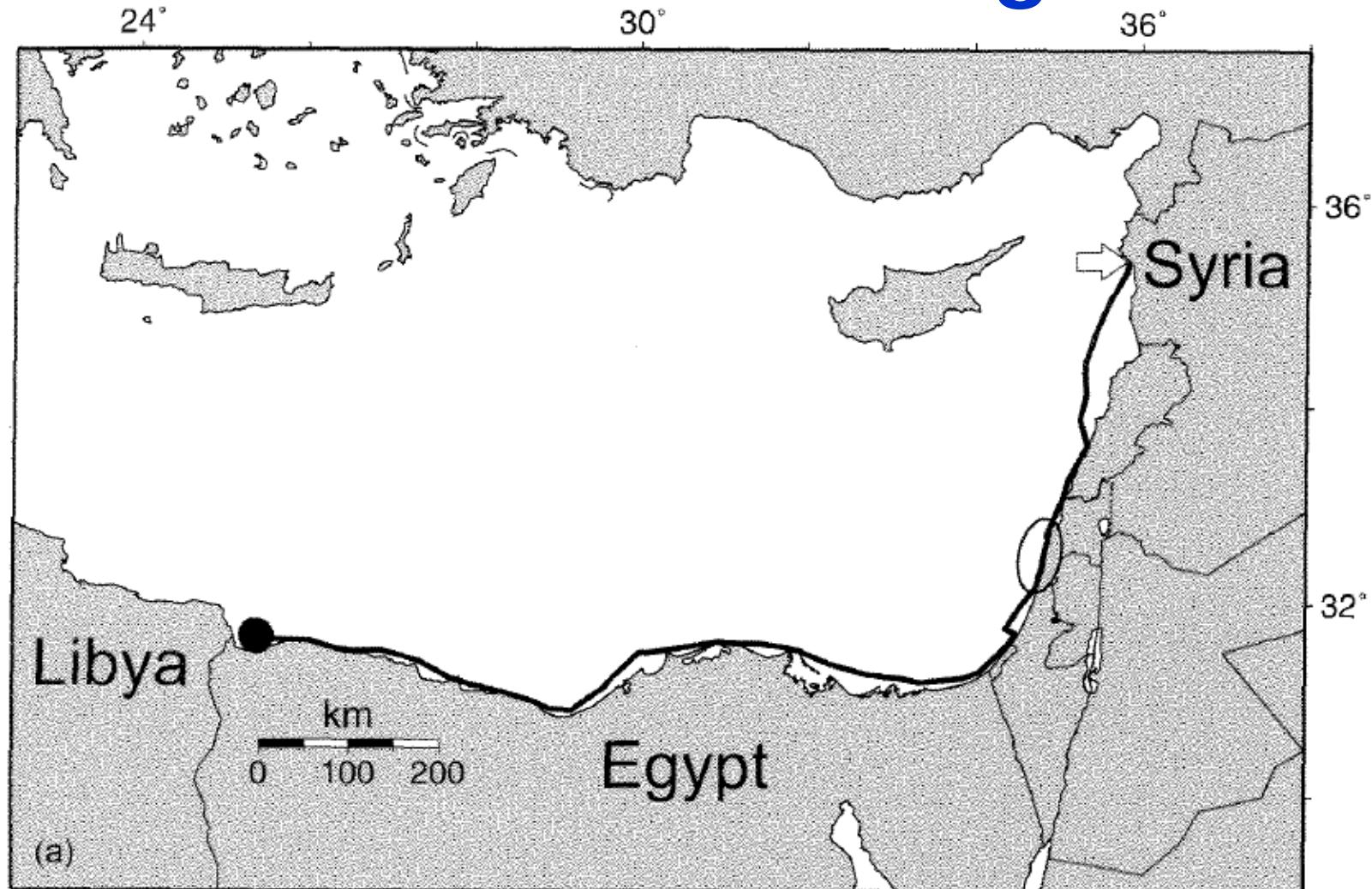
Mediterranean Green Turtles New Haplotyping

Mediterranean Green Turtles Satellite Tracking



Broderick et al., 2007

Mediterranean Green Turtles Satellite Tracking



Rees et al., 2008

Can We Use This Storyteller Outside the Mediterranean?

Atlantic – same pattern

Pacific - ?

Genetic Variability of Indo-Pacific Green Turtles

	STR 1		STR 2		STR 3		STR 4		STR 5		STR 6		
CACCCA	A T A T A T	A A T T	A T A T A T A T A T A T A T A T		T	A T A T A T A T A T A T A T A T	T	A T A T A T A T A T A T A T	T	A T A T A T A T A T A T	T	A T A T A T A T A T A T A T A T	A A T A - G
CACCCA	A T A T A A	A A T T	A T A T A T A T A T A T A T A T		T	A T A T A T A T A T A T	T	A T A T A T A T A T	T	A T A T A T A T A T	T	A A T A - G	
CAACCA	A T A T A T	A A T T	A T A T A T A T A T A T A T A T A T		T	A T A T A T A T A T A T A T A T	T	A T A T A T A T A T A T A T	T	A T A T A T A T A T	T	A A T A T G	
CAACCA	A T A T A T	A A T T	A T A T A T A T A T A T A T A T A T A T		T	A T A T A T A T A T	T	A T A T A T A T A T	T	A T A T A T A T	T	A A T A T G	
CAACCA	A T A T A T	A A T T	A T A T A T A T A T A T A T A T A T A T		T	A T A T A T A T A T	T	A T A T A T A T A T	T	A T A T A T A T	T	A A T A T G	
CAACCA	A T A T A T	A A T T	A T A T A T A T A T A T A T A T A T A T		T	A T A T A T A T A T	T	A T A T A T A T A T	T	A T A T A T A T	T	A A T A T G	
CAACCA	A T A T A T	A A T T	A T A T A T A T A T A T A T A T A T A T		T	A T A T A T A T A T	T	A T A T A T A T A T	T	A T A T A T A T	T	A A T A T G	
CAACCA	A T A T A T	A A T T	A T A T A T A T A T A T A T A T A T A T		T	A T A T A T A T A T	T	A T A T A T A T A T	T	A T A T A T A T	T	A A T A T G	
CAACCA	A T A T A T	A A T T	A T A T A T A T A T A T A T A T A T A T		T	A T A T A T A T A T	T	A T A T A T A T A T	T	A T A T A T A T	T	A A T A T G	

Repeats in Other Sea Turtles

Loggerhead: ATATT

Conventional:	3 Haplotypes
Repeat Haplotyping:	48-108 repeats
	30 Haplotypes (250 turtles)

Repeats in Other Sea Turtles

Hawksbill:
CATATATAT

Conventional:	? Haplotypes
Repeat Haplotyping:	10,23,30 repeats 3 Haplotypes (8 turtles)

Repeats in Other Sea Turtles

Olive Ridley:

ATATT and ATATTATT

Variation in repeat length and heteroplasmy of the mitochondrial DNA control region along a core–edge gradient in the eastern spadefoot toad (*Pelobates syriacus*)

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*School of Marine Sciences, Ruppin Academic Center, Michmoret 40297, Israel, †Department of Zoology, Tel Aviv University, Tel Aviv 69978, Israel, ‡Department of Genetics, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Abstract

Peripheral populations are those situated at the distribution margins of a species and are often subjected to more extreme abiotic and biotic conditions than those at the core. Here, we hypothesized that shorter repeat length and fewer heteroplasmic mitochondrial DNA (mtDNA) copies, which are associated with more efficient mitochondrial function, may be related to improved survival under extreme environmental conditions. We sampled

Defining Aims

Why do we look at the DNA?

Conservation of Biodiversity

Defining Aims

Sea turtles need our help in order to
survive as species

A stable population needs genetic
variation

Defining Aims

We look at DNA in order to evaluate
genetic polymorphism

500bp +300bp +STR's (genomic and
mitochondrial)

This is just a glimpse!

Our Initiative – Sea Turtle Genome

www.seaturtlegenome.com

The screenshot shows the homepage of the Sea Turtle Genome website. At the top, there is a horizontal menu bar with links to "About The Project", "Data", "Collaborators", and "Contact Us". Below the menu is a large banner featuring a photograph of a sea turtle on a sandy beach with waves crashing behind it. In the upper right corner of the banner, there is a logo for the Ruppin Academic Center, which includes a circular emblem and the text "המרכז האקדמי רופין" and "Ruppin Academic Center". To the right of the banner, there is a small link to "english |" and icons for email and printing. The main title "Sea Turtle Genome" is prominently displayed in large white letters across the center of the banner. Below the banner, the page content begins with a section titled "About The Project". The text describes the collaboration between the Ruppin Academic Center in Israel and the University of New Mexico in the USA for sequencing the genome of the green sea turtle, Chelonia mydas. It also mentions the seven marine turtle species in existence today and their status as threatened or endangered. The text continues to discuss the protection of green sea turtles under international conventions like IUCN and CITES, the challenges they face from human activities, and their unique reproductive behaviors. A note at the bottom states that the sequencing and comparative analysis is not funded by any external organization apart from the collaborators listed.

About The Project Data Collaborators Contact Us

Sea Turtle Genome

english |

About The Project

The Marine Sciences School of the Ruppin Academic Center in Israel has started collaboration with the Dept. of Molecular Genetics and Microbiology of the University of New Mexico, USA, for sequencing the genome of the green sea turtle, *Chelonia mydas*. It is one of seven marine turtle species in existence today, abundant in all tropical and subtropical oceans. Six from the family Cheloniidae (*Chelonia mydas* or green sea turtle, *Eretmochelys imbricata* or hawksbill sea turtle, *Natator depressus* or flatback sea turtle, *Caretta caretta* or Loggerhead sea turtle, *Lepidochelys kempii* or Kemp's ridley sea turtle, *Lepidochelys olivacea* or olive ridley sea turtle) and one from the family Dermochelyidae (*Dermochelys coriacea* or leatherback sea turtle). All species of sea turtles are listed as threatened or endangered. Green sea turtles have been listed as endangered worldwide since 1982 by the World Conservation Union (IUCN) and are protected under the Bern Convention and CITES. Though it is illegal to collect, harm or kill them, green turtles are still in danger because of several human actions. They get caught in fishing nets, they suffer from pollution, their habitats are decreasing due to real estate development and they are still hunted as adults, juvenile or eggs in some countries. The green turtles are known to exhibit high levels of philopatry and nesting females are beach-specific in their return to their natal hatching origin. Sea turtles travel long distances, they have polyandric reproduction and sperm conservation. They have temperature-dependent sex determination. Blood sample was taken from one of the males (Buddha) from the breeding stock at the Israeli Sea Turtle Rescue Center. DNA and RNA were extracted from it. The DNA has just been sequenced to 2x coverage. The sequencing and comparative analysis is not funded yet by any external organization apart from the collaborators listed.

Sea Turtle Genome - Status

We have completed 2X coverage

BGI (China) Published the genome

We are starting a transcriptome

We look for collaborators

What Can We Do With the Sea Turtle Genome

A lot:

Easily find polymorphic sites (STR's)

Genes ↔ Traits

Genes ↔ Diseases

Gene expression

Population Studies

Variability

Variability

Variability

Mitochondrial D-loop

Short Tandem Repeats

Library construction for STRs

Extract

Digest

Clone

Find STRs

Screen Population

Can we do it better?

The Alternative

Rational:

One individual will show population
polymorphism

The Alternative

2x Genome of 1 specimen

Isolate all STR

Locate site specific pairs

Isolate heterozygote sites

The Algorithm

164

276 >gnl|ti|1228074423_171967670 GTGC

112 >gnl|ti|1286273056 177629148 GTGC

11

145

35 >gnl|ti|1236036399 172623525 AACTA

AACACCAGAGAACTAAACaactaaactaaactaaactaaactaaactaaactaACAAACTGAAAAGCA
ACAGA

180 >gnl|ti|1241113976 173210823 AACTA

Primer design

60

8_>gnl|ti|1262525131_175488122 AT

TCTTCAAGTAACTTACTGGTGCCTTAAGTCCTGTTAGGCCAGTCTCatatatatGTATATAAAATT
TCTATTTCATGAAAGAGTGTGCTGTGCATGCCAACT

68->gnl|ti|1203695794_169077269 AT

Forward Primer

TTAAGTCCTGTTAGGCCAGTCT

Reverse Primer

GCATGCACAGCAGCACTTTCA

11

58

88->gnl|ti|1249504736_174204011 AC

AGCTGATTTGTACTTTGATAGTATCATCACATTCTAACAGTTacacacacacacacacacac
act
TCCATCCTGCAAATTGAAGTGAAAGCACCCAGACTTCTCTACCC

30 >gnl|ti|1203651562 168979272 AC

AGCTGATTTGTACTTTGATAGTATCATCACATTCTAACAAAGTTacacacacacacacacac
acacacacactTCCATCCTGCAAATTGAAGTGAAGCACCCAGACTTCTTCTACCC

Forward Primer

AGTATCATCACATTCTAACAAAGT

Reverse Primer

GAAAGTCTGGGTGCTTCACCTC

11



Thank you for your attention



Looking beyond the horizon

Thanks:

Yaniv Levy

Adi Barash

Raphael Bendelac

Alon Daya

Adam Friedmann

Uzi Motro

Marina Friling

Renanel Pickholtz



Yakup Kaska

Lucy Wright

Prof. Brendan Godley

Annette Broderick

Andreas Demetropoulos

Genome Project:

Jeremy Edwards

