Follow Maus' Paws: Defining the Common genes in mitochondrial DNA control region of Modern Native Egyptian Mau

(2012) Intel International Science and Engineering Fair - Intel ISEF

A B S T R A C T

The purpose of this project is to compare the DNA of present day spotted cats in Egypt with Egyptian Mau, standard breds overseas, and ultimately with that from a mummified cat, to see if there are any markers in either or both of the modern day cats to the original Mau cats. The Egyptian Mau - ***Felis silvestris ornate***- is the only spotted domestic cat that developed naturally. Egyptian Mau Cats are the oldest known descendents of domesticated cats in existence since Pharaonic time; it is probably recognized from 3500 B.C. This study was addressed the Egyptian Mau as a heritage and culture identity of Egypt. The experiment was designed to find an answer the question whether, the Egyptian Mau originally recognized at Egypt. The experiment was planned on three steps. Firstly, samples collected (hair shafts) that have been taken from cats in Egypt, United States of America and ancient cat mummy from the Egyptian Museum. Then the second step was through extracting the DNA from samples and amplifying certain gene by PCR technique. Finally, the third was done by determining specific PCR product DNA sequence by using DNA sequencer. The results showed that DNA that isolated from native Egyptian Mau was compact band of 1600 bp. These purified DNA sequence were subjected to PCR reaction by specific primers to CR mtDNA region and yield an amplified 492 bp sequence which conducted to DNA sequencer. Our Data also showed that we successfully isolated a pure DNA and CR mtDNA amplified sequence.

1- INTRODUCTION:

Cats are wonderful animals! It happens to be remarkable how well these animals have adapted to domesticity. Three thousand years ago, the ancient peoples of Egypt developed an extraordinary respect for the African wild cats that inhabited their Valley, These cats called Maus, Mau is the Egyptian word means cat.

The Purpose:

The purpose of this study is; compare the DNA of present day spotted cats in Egypt to see if there are any markers or common genes of the modern day cats. It thought that this study could possible importance in upgrading the image of feral cats in Egypt.

The Problem:

Many scientist have been doubting that the native modern Egyptian-Mau have no markers of the ancient cats. The breed – Egyptian Mau – has nothing to do with Egyptian cats nowadays. And the only interests of scientist is where cats first domesticated in Egypt, Cyprus or was it someplace else and the Egyptians were the main breeders of cat and also for mummies.

Maus Nowadays are currently unrecognized, and often suffer from endemic feline diseases. In fact they are not protected animals or valued like they were in ancient times. Outside of Egypt, many Maus are also endangered due to interbreeding and lack of new bloodlines.

And it is endangered not only because the gene pool of the breed outside Egypt weakening

Made by: MOATAZ A. ABDELGHAFAR

due to excessive in-breeding (little new blood), but in Egypt the increase of life threatening feline health Diseases threaten the continuing existence of this breed.

So this study is established hoping to back the ancient Egyptians proper care of these wonderful cats. Scientists and around the world neglected to work on the cats in Cairo and Alexandria feral cats, however these cats from what I saw many of them are really distinct and the hybrid of them have a great genetic diversity. And also from what I saw that these new genes pool are not exist anywhere else. I also do not recommend that the whole world considering the Egyptian Mau as breed is the only mummified cats, this study to give the world's attention to the wonderful feral cats in Egypt which in need support, however they were pure bred or even hybrid one. As it is the origin to the whole world Egyptian Maus.

Background Information:

The Egyptian Mau:

It is believed that these cats are descendents of the sacred cats of Egypt, It is the only naturally occurring spotted cat, this cat has a consistent spotted pattern. It is possible that a spotted gene (Sp). This gene (Sp) that breaks up the pattern into smaller patches that manifest as spots. A cat that has only one copy of the dominant spotting modifier.

The native Egyptian Mau is recognized for its slightly worried expression, which I think you can see in the above photograph particularly. Figure no.1, the native Egyptian Mau appears to demonstrate its ancient origins in some of the uniquely different anatomical features that it possesses. Apparently this cat is very sensitive to the air temperature and understandably it prefers very warm temperatures.

Egyptian Mau breed traits:

- Natural (not human-bred) spot pattern
- Speed (an Egyptian Mau has been clocked running 30 mph.
- Gooseberry green eyes (some yellow is common; orange exists but is considered a flaw in terms of showing).
- Bands" on the legs and tail.
- ."Belly flap" (2 bags of skin in front of the knee)5-
- "Somewhat feral" personality; they can be difficult to socialize, but are extremely loyal when socialized, and tend to be "one-person" cats.

It's importance in science :

- There is no doubt cats are very important to the Egyptian culture, past and present. feral cats are all over Egypt. I think they are very important to the ecosystem with keeping vermin under control. And according to (Egyptian Mau Pattern and Color Genetic – 2007 – by Constance A. Carroll PhD. Egyptian Maus has a vital role in the Nile agriculture ecosystem.

- The interest to science is where cats first domesticated in Egypt or was it someplace else and the Egyptians were the main breeders of cat – for mummies.

- From what I saw Maus is a Egyptian Heritage that could possibly be the window to ancient Egypt. It's an ancient living moving treasure.

2- METHOD AND MATERIALS:

2.1 Sample Collection:

This study extends 26 hair shafts samples from the pure bred of Egyptian Mau. With an additional 20 samples of hair shafts representing hybrid bred of feral cats, samples were collected from cats at EMRO (Egyptian Mau Rescue organization) samples were from 20-25 hair roots samples.

2.1.1 Steps of having samples:

The cats were selected based on the criteria of EMBAC – the Egyptian Mau Breed Advisory Committee.

Following the standard of points how to remark the Egyptian Mau? (council meeting - 29th October 2008).

- Coat, glove, and mask must be worn before dealing with cats.
- Tables must be cleaned with alcohol spray before and after each participant (Cat).
- Glove must be withdrawn thoroughly between each collection to prevent any cross- contamination of DNA samples.
- You must deal with cats gently and smoothly, and also be patient with them until they came to you.

2.2. DNA Extraction:

Using Alliance BIO pure™ Genomic DNA Tissue Extraction Kit the DNA was isolated. Following the Tissue protocol of the kit (page2).

2.2.1 Tissue Dissociation:

20-25 hair roots about 0.5 cm was cut up then grinned by a Micropestle. 200 µl of GT Buffer was added then the tissue homogenized again.

2.2.2 Lysis:

To the previous tube, 20 µl of Proteinase K was added and mixed by vortex. The samples were incubated at 60 ˚C for 30 min. 200 µl GBT Buffer was added and mixed by vortex for 5 sec each sample, then the tube incubated again at 70 ˚C for 20 min. the samples were centrifuged for 2 min at full speed and the supernatant was transferred to a new eppendorf tube.

2.2.3 DNA binding:

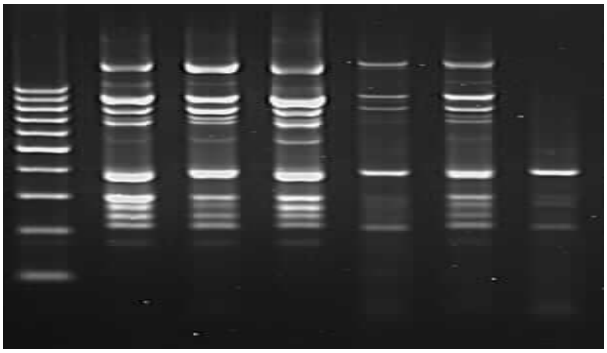
- 200 µl of absolute ethanol was added to the supernatant and vortexed for 10 sec. GD column was placed in a 2ml collection tube. Then the mixture transferred to the GD column, centrifuged at full speed for 2 min. the flow-through was discard and the GD column transferred to a new collection tube.

2.2.4 Wash:

- 400 µl of W1 Buffer was added to GD column, the tubes centrifuged at full speed for 30 sec. to the column, 600 µl of Wash Buffer was added and centrifuged at full speed for 30 sec, then the flow through was discarded. Finally the tube centrifuged for 3 min to dry the column.

1. DNA elution.

The dired column was transferred to a clean 1.5 micro-centrifuge tube (eppendorf). 50 µl of preheated Elution Buffer (at 70 ˚C for 20 min) was added to the center of the column and allowed to stand for 5 min until the elution buffer was absorbed by the matrix. Finally the tube was centrifuged for the last time at full speed for 30 sec to elute purified DNA. After extracting the DNA, The samples were divided into two groups.



The first group has been sent to UC (university of California) to be amplified and sequencing.

The second group was subjected to RAPD assay.

2.3. The first group:

Sequencing:
The 100 µl of DNA was amplified using PCR primer STRs; JHmtF3- gatagtgcttaatcgtgc and JHmtR3 - gtcctgtggaacaatagg -. In order to define the mtDNA control region as this region is required for sequencing.

2.4. The second group:

A 50 µl of the extracted DNA was amplified using PCR primer MP77 to restrict the DNA to separate segments of bands to be run on agar ose gel for RAPD profiling.

3- Results :

The data showed that RAPD profile with at least one lost or gained band. All random cats gained a band of 600, 300 and 200 while standard lost a band of 500 b.p

Samples ID	1	2	3	1/	2/	3/
Samples (77MP)	R 1	R2	R 3	S1	S2	S3
Molecular Weight						
bp 1000	1	1	1	1	1	0
bp 900	1	1	1	0	1	0
bp 800	1	0	0	0	1	0
bp 700	1	0	1	0	1	0
600bp	1	1	1	0	0	0
bp 500	0	0	0	1	1	1
bp 400	0	1	0	1	1	1
bp 300	1	1	0	0	0	0
bp 200	1	1	1	0	0	0
bp 100	0	0	0	0	0	0

And also that data confirmed that the similarity between random and standard cats that may be 30% only when we used this primer.

4- Discussion:

This Research was firstly established to answer a question whether the native Egyptian Mau is really Egyptian Mau breed or as Dr. Lyons mentioned that's just a stolen name and we need to find another new name and head to upper Egypt for cats.

It's believe that when you are Egyptian you see directly your heritage belongs to you. The Egyptian Mau were in Egypt 4000 years ago, and presently it said that the cats in modern Egypt has no relation with the ancient mummified cats, and these cats has nothing

genetically in common with ancient breed.

It was hoped to find large percentage of similarities between the native Egyptian Mau, but unfortunate the previous results showed that the similarities among cats in modern Egypt only 30 % This data required further investigation such as extraction of PCR product and DNA sequence to determine the gained sequence that may be reflect on morphology and behavior.

As this comparative study established on four stages :

- Native Egyptian mau.
- Overseas maus.
- Compare the Native Egyptian mau against the overseas maus.
- The ancient mummified cats.

And this year work was only on the native Egyptian Mau – the first stage - , this year it was accepted to define the common genes among the native Egyptian Mau in order to access to these genes in the overseas Maus.

Then the second stage was to define the common genes in the overseas Maus in order to access to these genes in the native Egyptian Mau. The both first stage and second stage will determine the similarities and differences between the native Egyptian Mau and the overseas Maus.

The new will turn to the final stage and the results of the third step will compared with the mummified cats based on the results of Dr. Lyons study on mummified cats.

While extermination it found that data was vary between repeated events specifically in the DNA extraction. The DNA extraction was repeated 11 times using four protocols.

The first protocol:

- Cut 10-15 hair roots about 0.5 cm into 1.5 eppendorf tube.
- Add 50 µl of 200 mM NaOH solution.
- Then boil the tubes in water bath

  94 ˚C .

- Cool at room temperature and add 50 µl of solution containing 200 mM HCL + 100 mM Tris-HCL .

The observation was that the DNA didn't extracted after repeating the protocol three times each time experimented on four samples.

It found that the DNA didn't extracted as the homogenizing step was missed. And probably concentration of the solution wasn't accuracy correct.

The second protocol:

- Cut 10-15 hair roots about 0.5 cm into 1.5 eppendorf tube.
- Use 50 µl of following to lysis buffer, 10mM Tris, 50 mM KCL, 0.5 Tween.
- Then add 10 µ of 20 µg/ml solution of proteinase K in mM Tris-HCL
- Vortex for 30 sec.
- Centrifuge at 13000 for one sec.
- Incubate overnight in a 56 ˚C.
- Incubate again after cooling for 10 min at 94 ˚C.
- Cool down at room temperature.
- Centrifuge at 13000 for one sec.

Again this protocol does not given the required. As the DNA couldn't isolated even after repeating this protocol four times each time contained three samples. It thought that we could not managed in isolate the DNA as we didn't homogenizing the samples.

The third protocol was the kit's protocol where it found the homogenizing step. However we couldn't isolate the DNA except after five times repeating the protocol each time on one sample. And probably that is due to the homogenizing step and depend on how long you were homogenizing as the longer you homogenizing the better results of DNA extracted.

5. conclusion :

It was expected that the study will determine the common genes among native Egyptian Mau estimating the similarities through RAPD analysis however out results was not expected as to be this little percentage of similarities which matched Dr. Lyons words about being the Egyptian Mau at present following genetically the Mediterranean seas like from Israel and Turkey. Sadly the Results showed the Egyptian Mau is facing an extinction in Egypt. It is believe that this is the suite time to take action and starting save this cat.

It is believe that Egyptian Mau is playing a vital role in the Biodiversity of the Egyptian environments. It also believe that the ancient Egyptians worshiped Maus for nothing.

Made by: MOATAZ A. ABDELGHAFAR

Follow Maus' Paws: Defining the Common genes in mitochondrial DNA control region of Modern Native Egyptian Mau
(2012) Intel International Science and Engineering Fair - Intel ISEF

This study hoping to be continued within monitoring the specific role of Egyptian Mau in the biodiversity of Egypt. Also this study was hoped to continue in additional three stages. But after the gotten results the further studies on how to rescue the Egyptian Maus and Egyptian Cats from extinction. Through sequencing the 30 % the common genes among the Native Egyptian Mau. Then compare this the mentioned percentage with the published papers of dr. Lyons on the genome bank of the mummified cats, by this method the rest of unknown 70% of the genes.

However this results will support the mission of EMRO (Egyptian Mau Rescue Organization) that they have a scientific paper mentioned the similarities among the native Egyptian Mau only 30%. This will support the awareness public and the Egyptian citizen with the situation.

Our environment is a cycle this cycle perhaps In the grand time scale of our life of the Egyptian, these effects may be currently seen as the equivalent of storm clouds gathering on the horizon. But one day the storm is coming anyway. That's why it's time to take a real Action. Unless we start loving and caring about heritage and environment.

The biodiversity is also Biological diversity is the resource upon which families, communities, nations and future generations depend. It is the link between all organisms on earth, binding each into an interdependent ecosystem, in which all species have their role. It is the cycle of life.

The Egypt's natural assets are made up of plants, animals, land, water, the atmosphere AND peopel! Together we all form part of the Egyptian's ecosystems, which means if there is a biodiversity crisis, it is reflected on our health and livelihoods are at risk too.

However my study results is not positive, it is believe that it came in the right time to prove that extinction of the Egyptian. And also to pay attention to many expressions which missed among Egyptians i.e. the biodiversity and rescuing the endangered animals.

It is predicted that this results will change the most Egyptians thinking about the Egyptian Maus, and the role of bastet in the ancient Egypt, and how these affected the life style in past. This is the time to act and knew that Egypt has been endowed with a unique variety of ecosystems and a corresponding variety of wildlife. This ecosystem need to our attention and car.

Saving the Egyptian Maus seems to be big, however it is a crisis situation, it does not mean that we are in hopeless situation. It only required to move, there are lots of things you can

do to help ease the pressure on this decline and loss of biodiversity.

6. acknowledgment:

Alexandria University,

University of California,

American University at Cairo,

New Castle University

Special Thanks to the following organizations:

EMRO (Egyptian Mau Rescue Organization)

Bibliotheca Alexandrina

ACA (Alley Cat Allies)

GCCF (The Governing Council of the Cat Fancy)

EMBAC (Egyptian Mau Breed Advisory Committee)

Egyptian Mau Club at UK

Also Special thanks to those people who assisted and supported this study.

Dr. Doaa A. Ghareeb

Dr. Lisle A. Lyons

Dr. Sailma Ikram

Dr. mahmoud Ibrahem

Dr. Ahmed Sultan

Dr. Ahmed Zaki

And thanks to everyone support that project:

M. Sarah Elshater

Ms. Rosalind Smith (EMRO)

M. Heba Elgamal (PSC)

Follow Maus' Paws: Defining the Common genes in mitochondrial DNA control region of Modern Native Egyptian Mau

(2012) Intel International Science and Engineering Fair - Intel ISEF

Ms. Amanda (ACA)

Mr. Michael Broad (pictures-of-cats.org)

Mr. mark Globary (EMBAC)

Dr. Boshra Salim

Dr. Mina

7. References:

1. R.A. Grahn, J.D. Kurushima, N.C. Billings, J.C. Grahn, J.L. Halverson, E. Hammer, C.K. Ho, T.J. Kun, J.K. Levy, M.J. Lipinski, J.M. Mwenda, H. Ozpinar, R.K. Schuster, S.J. Shoorijeh, C.R. Tarditi, N.E. Waly, E.J. Wictum, L.A. Lyons - (2010)- FELINE NON REPETITIVE MITOCHONDRIAL DNA CONTROL REGION DATABASE FOR FORENSIC EVIDENCE, 2010 Elsevier Ireland Ltd
2. Bateson Patrick, and C.Turner Dennis, (2000), _THE DOMESTIC CATS_ Cambridge Univesity press.
3. M.Butler John (2005), _FORENSIC DNA TYPING_ ElSevier Acadmic press. Accessed in Mon, 17th Jan 2012.
4. A.Micker David, A.Freyer Greg, and A.Crotty David (2003,) _DNA science_ CSH Laboratory press. Accessed in Tue, 10th Jan 2012.
5. Laboratory Animal, Institution of laboratory animal research (ILAR), Commission on life science, National research: http://dels.nas.edu/ilar/
6. Robert Plomin, John C.Defies, E.MacClearn Gerald, and Rutter Michael, (1997) – BEHAVIORALGENETICS – W.H. Free and Company.
7. J. Malek, The Cats in Ancient Egypt, British Museum, London, 1993
8. M.J. Lipinski, L. Froenicke, K.C. Baysac, N.C. Billings, C.M. Leutenegger, A.M. levy, M. Longeri, T. Niini, H.Ozpinar, M.R. Slater, N.C. Pedersen, L.A. Lyons (2007), THE ASCENT OF CAT BREEDS: GENETIC EVALUATIONS OF BREEDS AND WORLDWIDE RANDOM-BRED POPULATIONS - Elsevier Ireland Ltd & ScienceDirect.
9. Melissa Bateson, (2003), The pronze Egyptian Mau - past, present and future - Egyptian Mau culb.com –
10. Anne-Charotteronn, Analysis of nucleotide Variation in Non-human Primates (2007), ACTA UNIVERSITATIS UPSALIENSIS UPPSALA.
11. Mohammadreza Nassiri, Shahrokh Ghovvati, Arash Alipour, Hamid Mohebati, POPULATION GENETIC DATA ANALYSIS AND ALLELE FREQUENCIES FOR STR LOCI IN IRANIAN TURK AND SISTANI ETHNICS. University of Mashhad Press Iran.
12. M. Culver, (2001) M. A. Menotti-Raymond and S. J. Brien – PATTERN OF SIZE HOMOPLASY AT 10 MICROSATELLITE LOCI IN PUMAS, Society for Molecular Biology and Evolution.
13. E. Randi, M. Pierpaoli, M. Beaumont, B. Ragni, (2001) Genetic Identification Of Wild And Domestic Cats (*Felis Silvestris)* And Their Hybrid Using Bayesian Clustering Methods, Society for Molecular Biology and Evolution.
14. J. C. Redfearn, C. J. Woolverton, Kent State University Center for Public Health Preparedness, Activity appropriate for: Laboratory.
15. K. Muller, C. Burgger, R. Klein, E. Miltner, F. Reuther, P. Wiegand, (2008), STR typing of hairs from domestic cats, Forensic Science. Int. Genet.
16. C. R. Tarditi, R. A. Grahn, J. J. Evans, J. D. Kurushima, L. A. Lyons, Mitochondrial DNA sequencing of cat hair: an informative forensic tool, Forensic Science press.
17. D. R. Smith, W. M. Brown, (1991),
A MITOCHONDRIAL DNA RESTRICTION ENZYM ECLEAVAGE MAP FOR THE SCORPION *HADR UR US ARIZONENSIS (IURIDAE)*, The Journal of Arachnology.
18. P. Pineau, M. Henry, R. Suspene, A. Marchio, A. Dettai, R. Debruyne, T. Petit, k. Alexis,

A. Dejean, S. W. Hobson, and J. P. Vartanian, (2004), A Universal Primer Set for PCR Amplification of Nuclear Histone H4 Genes from All Animal Species _ Society for Molecular Biology and Evolution.

19. Stephen J. O'Brien, Eric S. Lander, Mark E. Haskins, Urs Giger, Niels C. Pederson, David E. Wildt5, William J. Murphy, Naoya Yuhki and Marilyn Menotti-Raymond, (2002), Sequencing the Genome of the Domestic Cat –from p.6 to p. 9, NHGRI White Paper.
20. S. A. Miller, J. P. Harly, (2007), Zoology, McGrwa Hill press.
21. Clark S. (1999) thinking with demons, Oxford: Clarendon Press.
22. Salima Ikram, Divine Creatures: Animal Mummies in Ancient Egypt.
23. Salima Ikram (2004), The Mummified Cats.
24. C. A. Carroll, Egyptian Mau pattern and Color Genetics.
25. M. Broad, (last update 2010) Cat history, pictures-of-cats.org.
26. Michael Broad, Egyptian cat Worship, pictures-of-cats.org
27. Anne Gregory, (2008) The Egyptian Mau Breed Advisory Committee.