Monitoring genetic differences in *Drosophila melanogaster* populations from the "Evolution canyon" (Israel) and their responses to global warming.

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Introduction and objectives

Introduction:

Current global warming is a concerning matter of study. This phenomenon effects are expected to cause extreme conditions that will lead to ecological and evolutionary changes. A poleward shift (6.1km per decade) of many species distribution is already occurring (Parmesan and Yohe. 2003) but, the genetic basis of this shift is yet to be known. Therefore, a solution to study genetic variation responses to global warming is focusing in *D.melanogaster* populations from the remarkably contrasted slopes (North facing slope, NFS, and South Facing Slope, SFS) of the Evolution Canyon (EC).

Objectives:

❑Finding genetic differences of *D.melanogaster* EC populations and, whether or not, these are due to the contrasting slope differences.

❑Determine possible responses to Global warming of *D.melanogaster* EC's and world's populations.

Materials and Methods:

An extensive research in online article databases, such as the "Web of Science" or "Google Scholar", provided the necessary information for this project. Key words taped in order to find information were: Global warming, *Drosophila melanogaster,* Evolution canyon, differentiation and candidate genes.

Evolution Canyon

Parameter	Interslope divergence
Vegetation	NFS: Savanoid formations→*Cerationia siliqua, Pistacia lentiscus* and savannah grasses. SFS: Mediterranean brushwood forest →*Quercus calliprinos* and *Pistacea palestina.*
Temperature	June: 0.7ºC interslope variation. October: 3ºC interslope variation.
Relative humidity	May-August: ± 1.4% variation. November-January: 4.4% to 7.3% variation.
Radiation	SFS receives 200% to 800% more radiation than the NFS.

Table 1. Data on Evolution Canyon characteristics. (Pavlícek *et al.*2002).

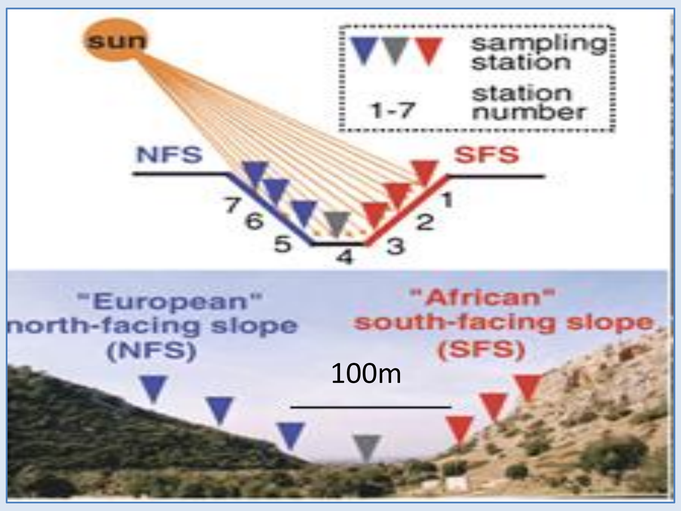

Figure 1. Schematic diagram of the lower Nahal Oren Canyon (EC 1). Higher radiation on SFS incidence is represented. It is also shown the differences in vegetation within the two slopes, SFS and NFS. (Nevo. 2012).

Selection
OVERLAPS
Migration!

Results and Discussion

Hsp70Ba

Mutation

P- Element Insertion to promoter → Mutant Hsp70Ba^{p}

(Michalak *et al.* 2001).

SFS →1.2% flies ≠ NFS → 39% flies

(Michalak *et al.* 2001).

- Interslope difference → Distribution.
- Flies travelling for 15km in 15h across desert (Singh and Rhomberg. 1987) plus poleward shift → Migration(EC).
- Expression of hsp genes related to transposons presence in the promoter (Hoffman. 2008). → transposon with higher heat tolerance maintained through global warming.

Period

Mutation

23a ACGGGC ACTGGT(ACAGGT ACAGGT).....(ACTGGA ACTGGA ACTGGA)[ACCGGG ACAGGA ACTGGA ACCGGA ACAGGA ACTGGA ACCGGA ACAGGA ACTGGA]ACGGGC
ACAGGT.....(ACAGGC ACAGGC ACAGGC)ACTGGA.....ACAGGC

23b ACGGGC ACTGGT(ACAGGT ACAGGT ACAGGT)(ACTGGA ACTGGA ACTGGA)[ACCGGG ACAGGA ACTGGA].....ACGGGA
ACAGGT ACAGGC ACTGGA ACGGGA ACAGGC ACTGGA(ACAGGC ACAGGC ACAGGC).....ACTGGC.....ACAGGC

22a ACGGGC ACTGGT(ACAGGT ACAGGT ACAGGT)(ACTGGA ACTGGA ACTGGA)[ACCGGG ACAGGA ACTGGA].....ACGGGA
ACAGGT ACAGGC ACTGGA ACGGGA ACAGGC ACTGGA(ACAGGC).....ACGGGC ACAGGC ACAGGC

22b ACGGGC ACTGGT(ACAGGT ACAGGT ACAGGT)(ACTGGA ACTGGA ACTGGA)[ACCGGG ACAGGA ACTGGA].....ACGGGA
ACAGGT ACAGGC ACTGGA ACGGGA ACAGGC ACTGGA(ACAGGC ACAGGC).....ACTGGA .....ACAGGC

21 ACGGGC ACTGGT(ACAGGT ACAGGT ACAGGT)(ACTGGA ACTGGA ACTGGA)[ACCGGG ACAGGA ACTGGA ACCGGA ACAGGA ACTGGA] .....ACGGGA
ACAGGT.....(ACAGGC ACAGGC ACAGGC)ACTGGA.....ACAGGC

20 ACGGGC ACTGGT(ACAGGT ACAGGT).....(ACTGGA ACTGGA ACTGGA)[ACCGGG ACAGGA ACTGGA ACCGGA ACAGGA ACTGGA].....ACGGGA
ACAGGT.....(ACAGGC ACAGGC ACAGGC)ACTGGA.....ACAGGC

17 ACGGGC ACTGGT(ACAGGT ACAGGT).....(ACTGGA ACTGGA ACTGGA)[ACCGGG ACAGGA ACTGGA].....ACGGGA
ACAGGT.....(ACAGGC ACAGGC ACAGGC)ACTGGA.....ACAGGC

14 ACGGGC ACTGGT(ACAGGT ACAGGT ACAGGT)(ACTGGA).....ACGGGA

Figure 2. Sequences of the thr-gly repeats of the fifth exon of per gene of *D.melanogaster* from the EC. 23a is the flybase sequence, the others are variants found in the same EC. There are also given the n=20 (20) sequence deletions, including the one in the 18-bp motif (square brackets). An underlined silent mutation is also documented. (Zamorzaeva *et al.* 2005).

SFS→14% n=20 flies ≠ NFS → 39% n=20 flies

(Zamorzaeva *et al.* 2005)

- Interslope difference → Distribution.
- Poleward shift → Migration (EC).
- Depending on the number of thr-gly repeats, the circadian cycle of the fly will be optimal for a certain temperature (Sawyer *et al.* 2006).

Desaturase 2

Mutation

A aattgtaatttatggttgcttctggttgttaaaggcgaaccaaagtcaagttgctattacgtgagcttatatgtttaagcttcagtgattccaatggcagtaaaaatagagaaaatcgtgagattcggtcgggccaagaaccaagattgcatcgtgcaaggatatcaatcaaatta
B aattgtaatttatggttgcttctggttgttaaaggcgaaccaaagtcaagttgctattacgtgagcttatatgtttaagcttcagtgattccaatggcagtaaaaatagagaaaatcgtgagatgc.....aagattgcatcgtgcaaggatatcaatcaaatta
C aattgtaatttatggttgcttctggttgttaaaggcgaaccaaagtcaagttgctattacgtgagcttatatgtttaagcttcagtgattccaatggcagtaaaaatagagaaaatcgtgagatgc.....aagattgcatcgtgcaaggatatcaatcaaatta
D aattgtaatttatggttgcttctggttgttaaaggcgaaccaaagtcaagttgctattacgtgagcttatatgtttaagcttcagtgattccaatggcagtaaaaatagagaaaatcgtgagatgc.....aagattgcatcgtgcaaggatatcaatcaaatta

A ttcgagaaaggataaggaccgttccctgaacattaaaattgataaggactgttaacttaaatacgataaggataatagtgctaagtgattgagtgcagtgaaagaaagtgacgatatggcgccctactcaaggatataccatcaggataagtcctccagagaaacgggtg
B ttcgagaaaggataaggaccgttccctgaacattaaaactgataaggactgttaacttaaatacgataaggataatagtgctaagtgattgagtgcagtgaaagaaagtgacgatatggcgccctactcaaggatataccatcaggataagtcctccagagaaacgggtg
C ttcgagaaaggataaggaccgttccctgaacattaaaactgataaggactgttaacttaaatacgataaggataatagtgctaagtgattgagtgcagtgaaagaaagtgacgatatggcgccctactcaaggatataccatcaggataagtcctccagagaaacgggtg
D ttcgagaaaggataaggaccgttccctgaacattaaaactgataaggactgttaacttaaatacgataaggataatagtgctaagtgattgagtgcagtgaaagaaagtgacgatatggcgccctactcaaggatataccatcaggataagtcctccagagaaacgggtg

A tccttttcgaggacgatgcgcaaacagtcgacggtgacctgaccaccgacagattccagttgaaacgagctgagaagcgtcggctaccctagtctggcggaatataatcctgttcgcattggtccacctggctgctctatatggcctgcactccatattcacaaggg
B tccttttcgaggacgatgcgcaaacagtcgacggtgacctgaccaccgacagattccagttgaaacgagctgagaagcgtcggctaccctagtctggcggaatataatcctgttcgcattggtccacctggctgctctatatggcctgcactccatattcacaaggg
C tccttttcgaggacgatgcgcaaacagtc.....tga.....gagctgagaagcgtcggctaccctagtctggcggaatataatcctgttcgcattggtccacctggctgctctatatggcctgcactccatattcacaaggg
D tccttttcgaggacgatgcgcaaacagtc.....tga.....gagctgagaagcgtcggctaccctagtctggcggaatataatcctgttcgcattggtccacctggctgctctatatggcctgcactccatattcacaaggg

Figure 3. Promoter and exon 1 regions of the desaturase2 gene in *D.melanogaster.* (A) Flybase data. (B) N allele. Sequence with one deletion: 16-bp deletion in promoter. (C) D allele. Sequence with two deletions: 16-bp deletion in promoter; deletion in exon 1 consists of 5 bp – Stop codon TGA – 29 bp. (D) D. allele. Sequence with two deletions: 16-bp deletion in promoter; deletion in exon 1 consists of 10 bp – Stop codon TGA – 24 bp. Promoters are underlined. (Zamorzaeva *et al.* 2009).

D allele: higher mating speed. Heterozygous (ND) females have ↑ mating success and ↑ sexual attractiveness than homozygous.

SFS→ Monomorphic ≠ NFS→ Polimorphic

(Zamorzaeva *et al.* 2009)

- Higher mating success and sexual attractiveness of the heterozygous females (ND) plus the higher survival of the heterozygous forms in stressful (cooler) conditions might be the cause for the abundance of the D allele in the NFS. (Weisman *et al*. 2007).
- Poleward shift → Migration (EC).
- Heat stress tolerance (Weisman *et al*. 2007). → Further studies required.

Conclusions

1) The EC is a suitable model to monitor adaptive evolving responses in the largest possible number of species.
2) Candidate genes are one of the best tools to determine genetic variations in the EC.
3) Further studies on several interslope differentiations are needed so as to discover the genetic basis.
4) Needed evidences of heat-stress tolerant *D.melanogaster* loci and their response to global warming.

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