Natural variation, laboratory selection, and genomics of desiccation tolerance in *Drosophila*

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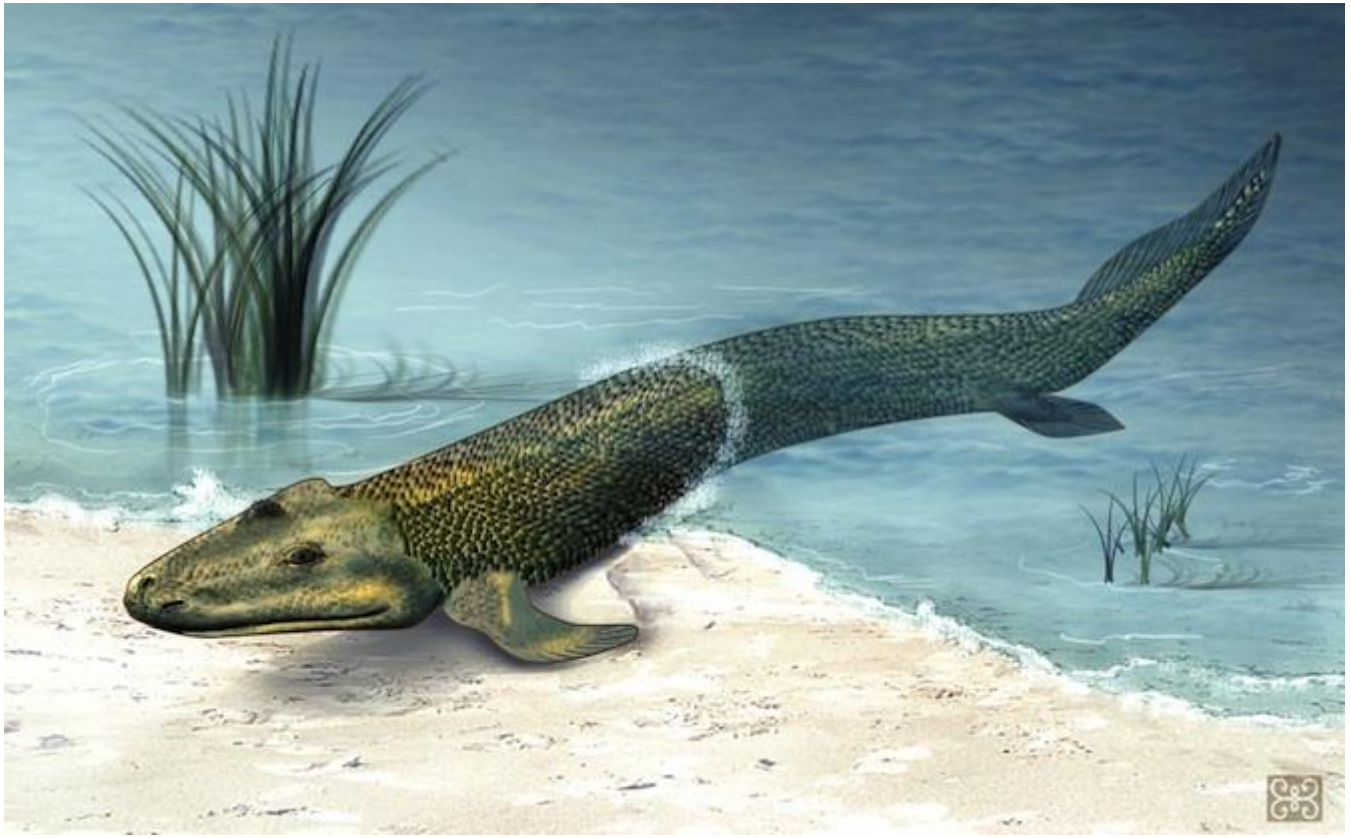
Water to terrestrial life

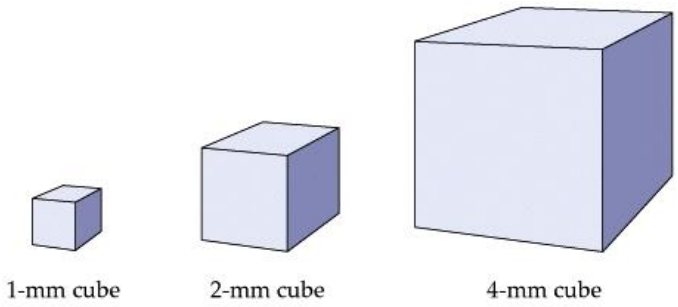


Challenge is…

How to conserve it???

30 percent; arid

Size and Water loss relations



Surface area	6 sides × 1^2 = 6 mm^2	6 sides × 2^2 = 24 mm^2	6 sides × 4^2 = 96 mm^2
Volume	1^3 = 1 mm^3	2^3 = 8 mm^3	4^3 = 64 mm^3
Surface area-to-volume ratio	6/1	3/1	1.5/1

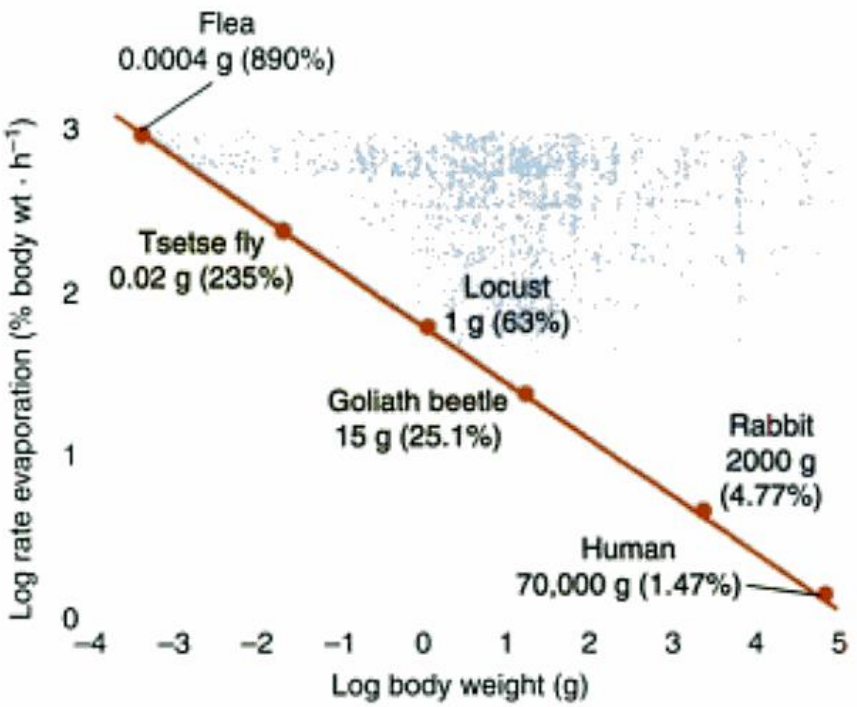


Figure 14-2 Small animals dehydrate more rapidly than large animals because of their high surface-to-mass (and thus surface-to-volume) ratios. This log-log plot shows the amount of water, as a percentage of body weight, that is lost per hour under hot desert conditions versus body weight. [Adapted from Edney and Nagy, 1976.]

Chemical Nature of Water

Resistance to desiccation can involve one or more adaptive mechanisms

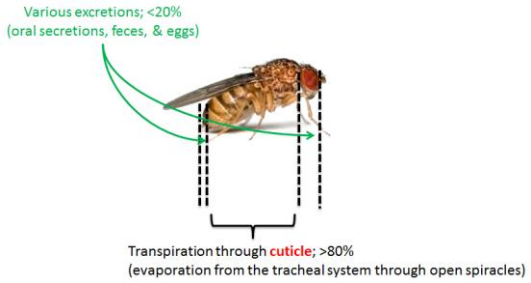
This resistance could be accomplished in three ways:

by increasing the amount of water in the body,

by tolerating the loss of a greater percentage of body water (dehydration tolerance)

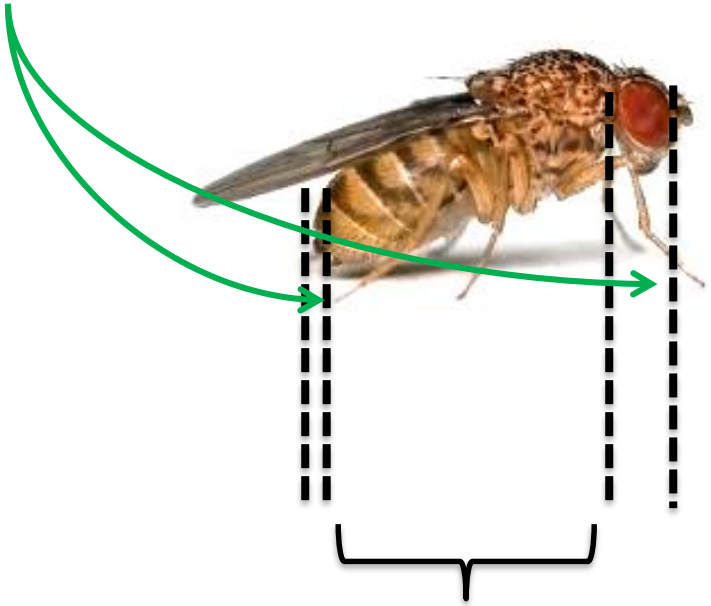
or

By reducing rates of water loss.



Insects can lose water through various routes…

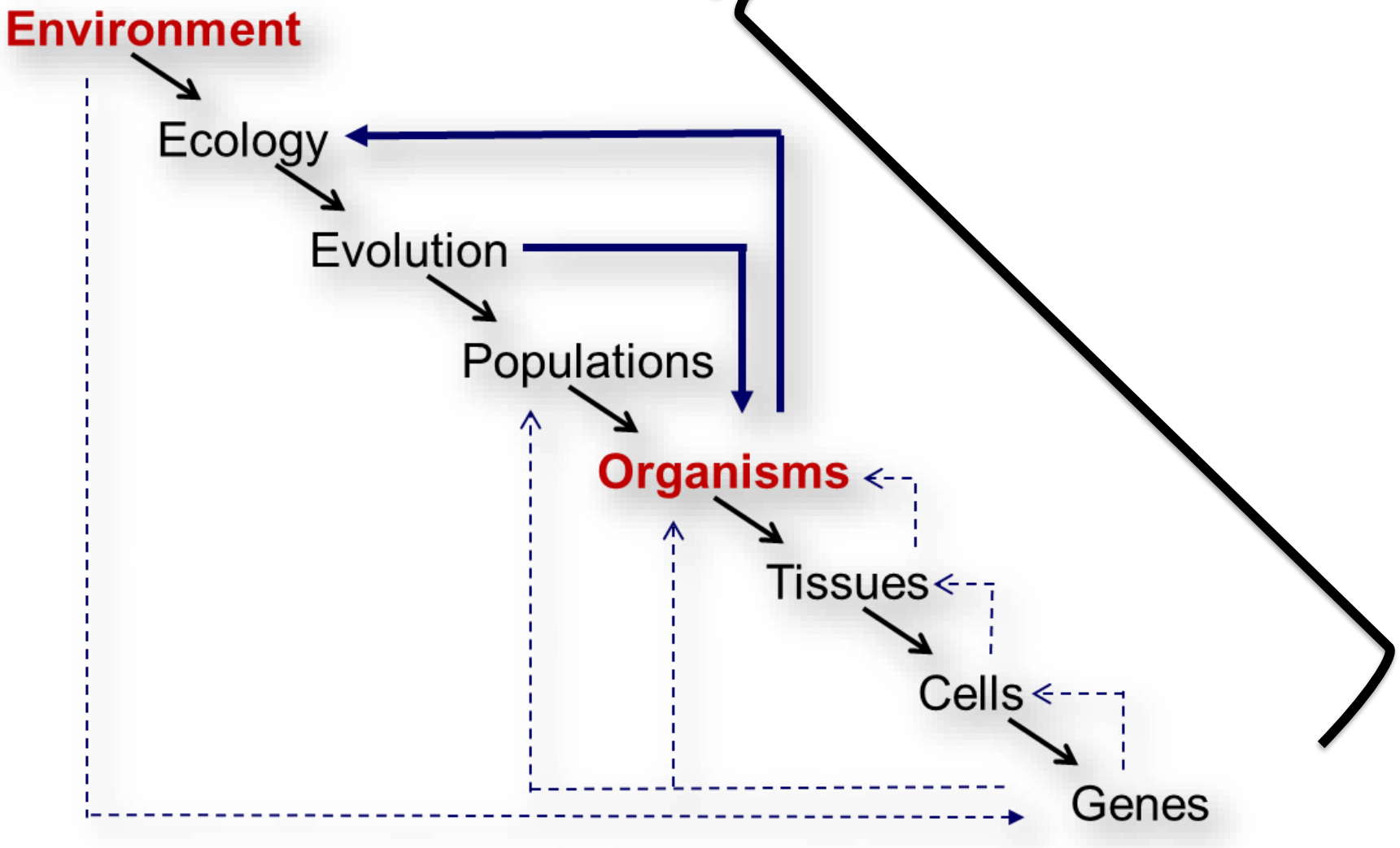
Various excretions; <20%
(oral secretions, feces, & eggs)



Transpiration through **cuticle**; >80%
(evaporation from the tracheal system through open spiracles)

Gibbs et al., *2003. J Exp Biol*

Water Balance



Desiccation tolerance in *Drosophila*…

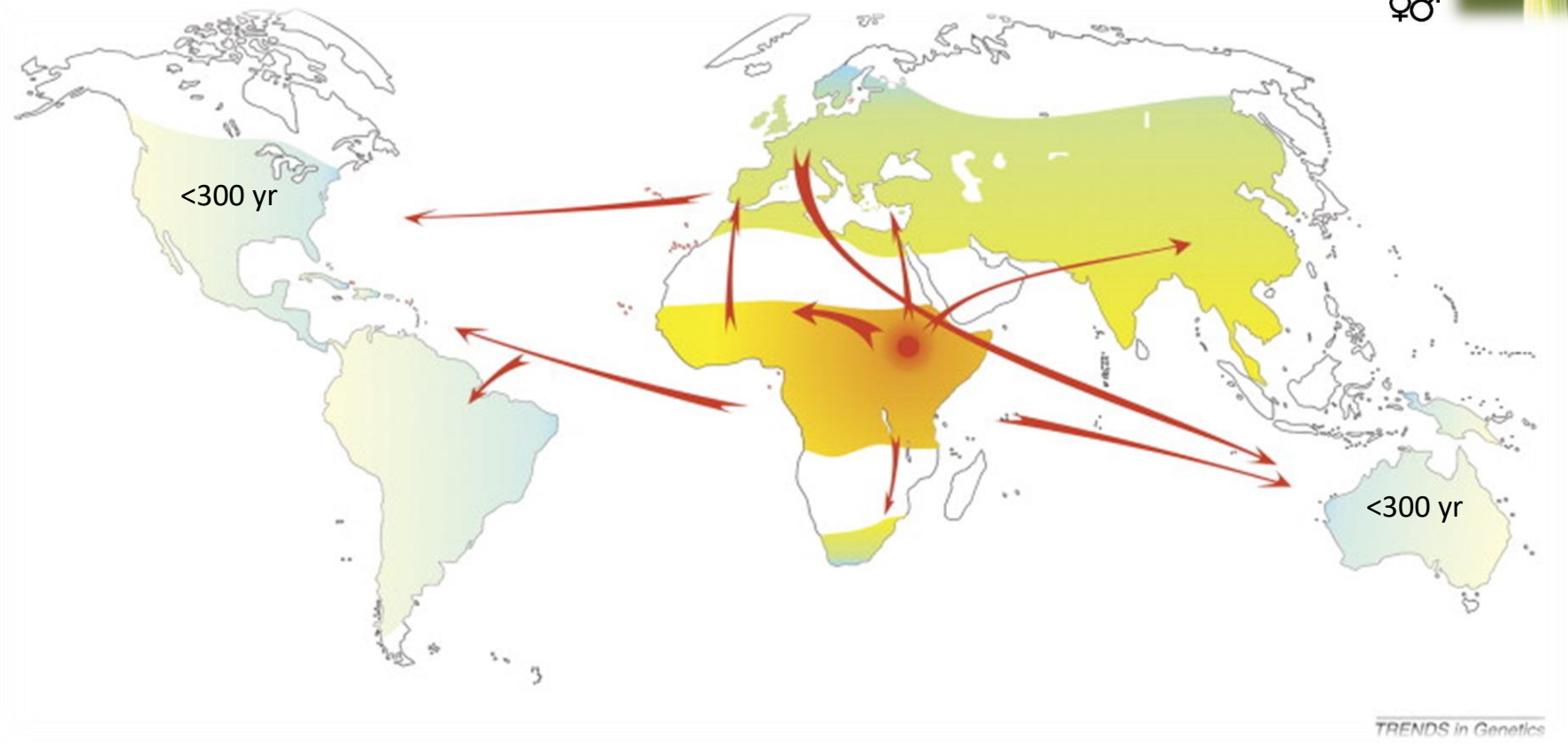
I. **Natural variation**
II. **Artificial selection**
II. **Genomics**

ecology » organism » genes

Drosophila melanogaster as a model organism

‘colonization’

♀♂



I. Natural Variation

Desiccation tolerance

Six geographical locations at four different thermal conditions

- developmental temperatures of

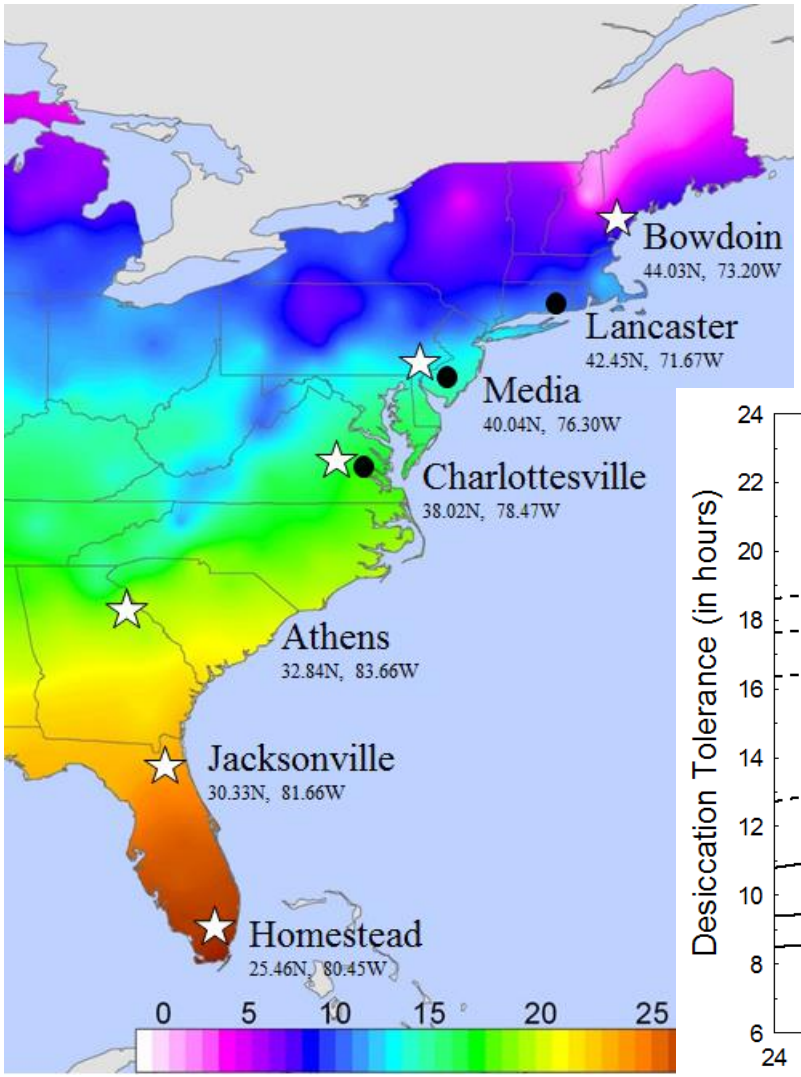
18 ° C

25 ° C

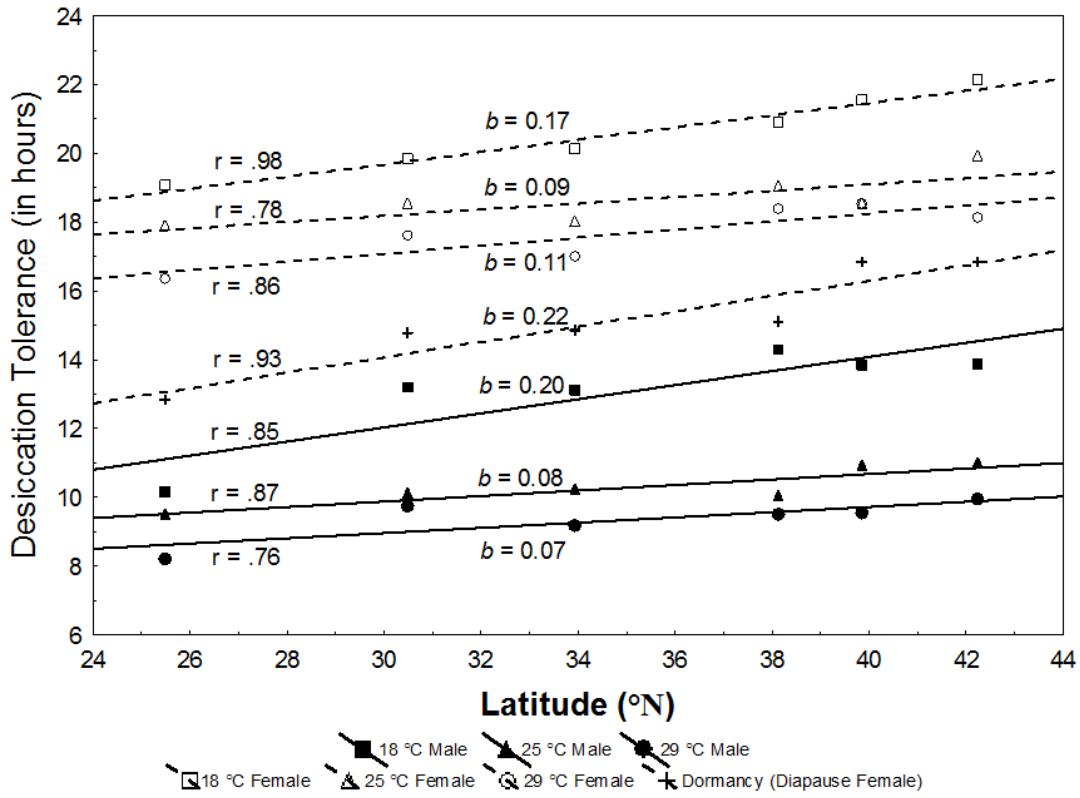
29 ° C

&

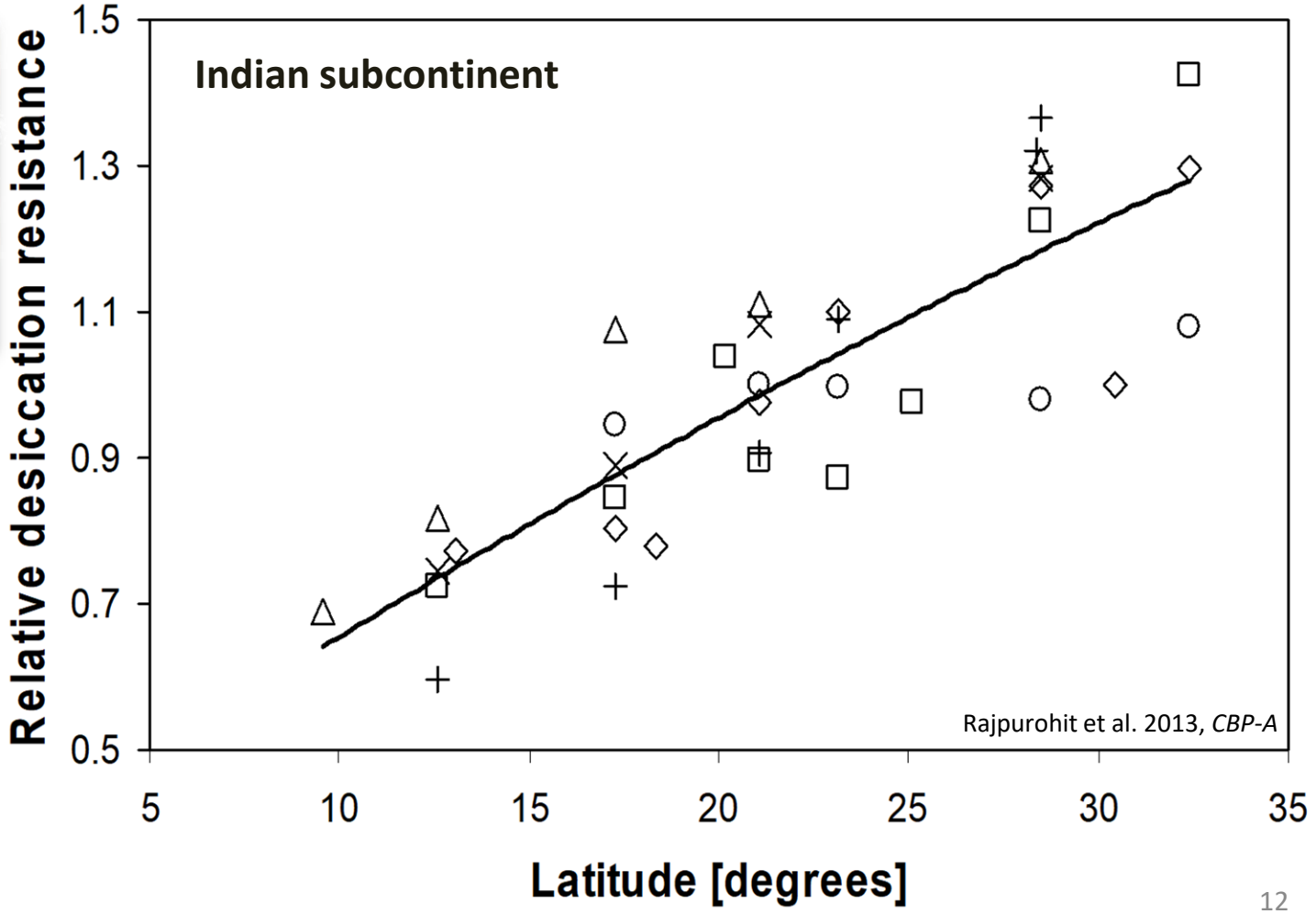
- adult female exposure to dormancy-inducing conditions of 11˚C, 9L:15D)



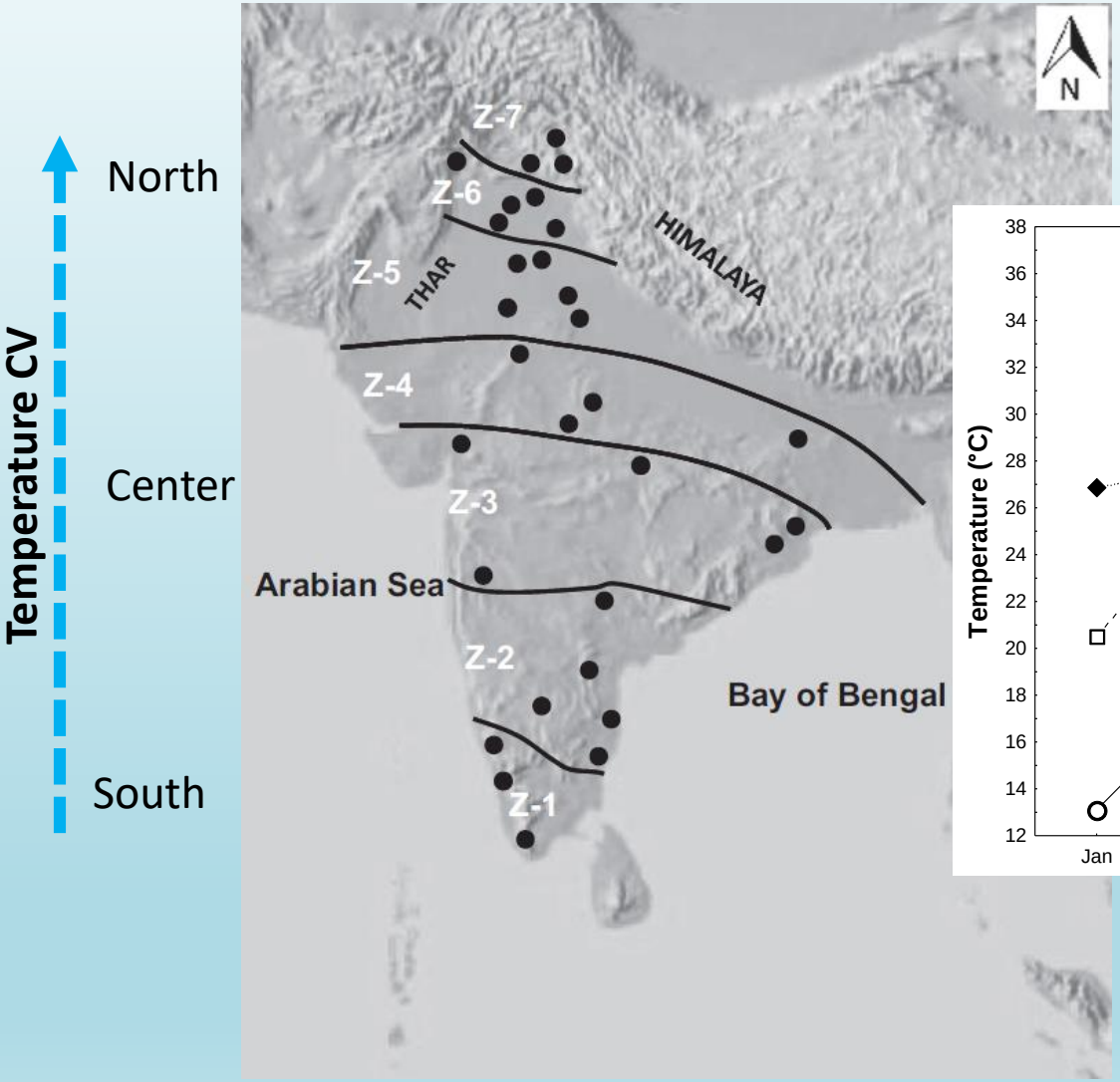
Molecular Ecology 2018, under review



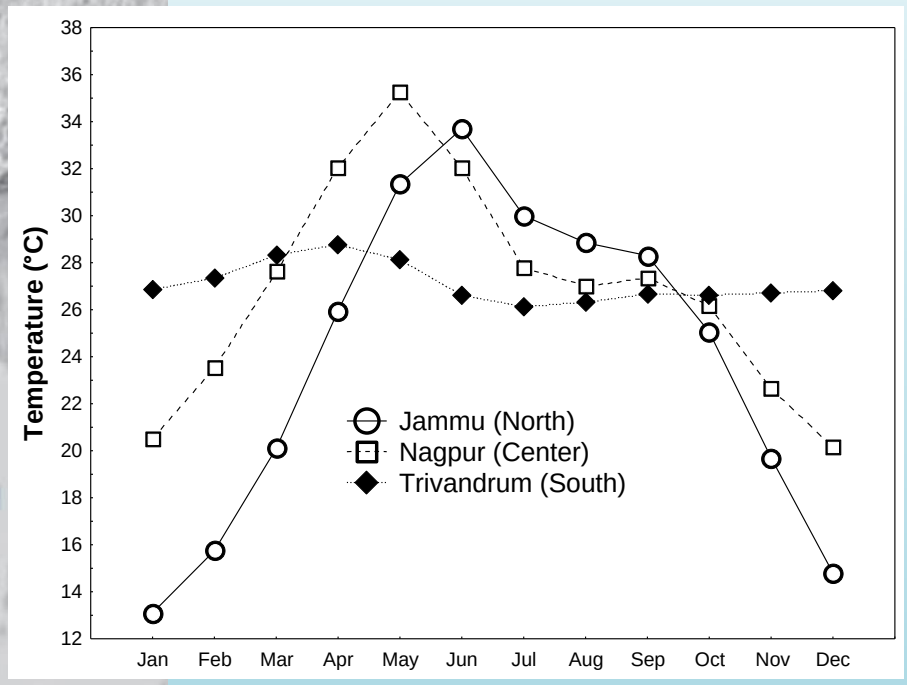
Geographic cline of relative desiccation tolerance of six drosophilid species from the Indian subcontinent. Values are relative to the mean survival time for all populations assayed within each species.



Seasonal variations; Indian subcontinent

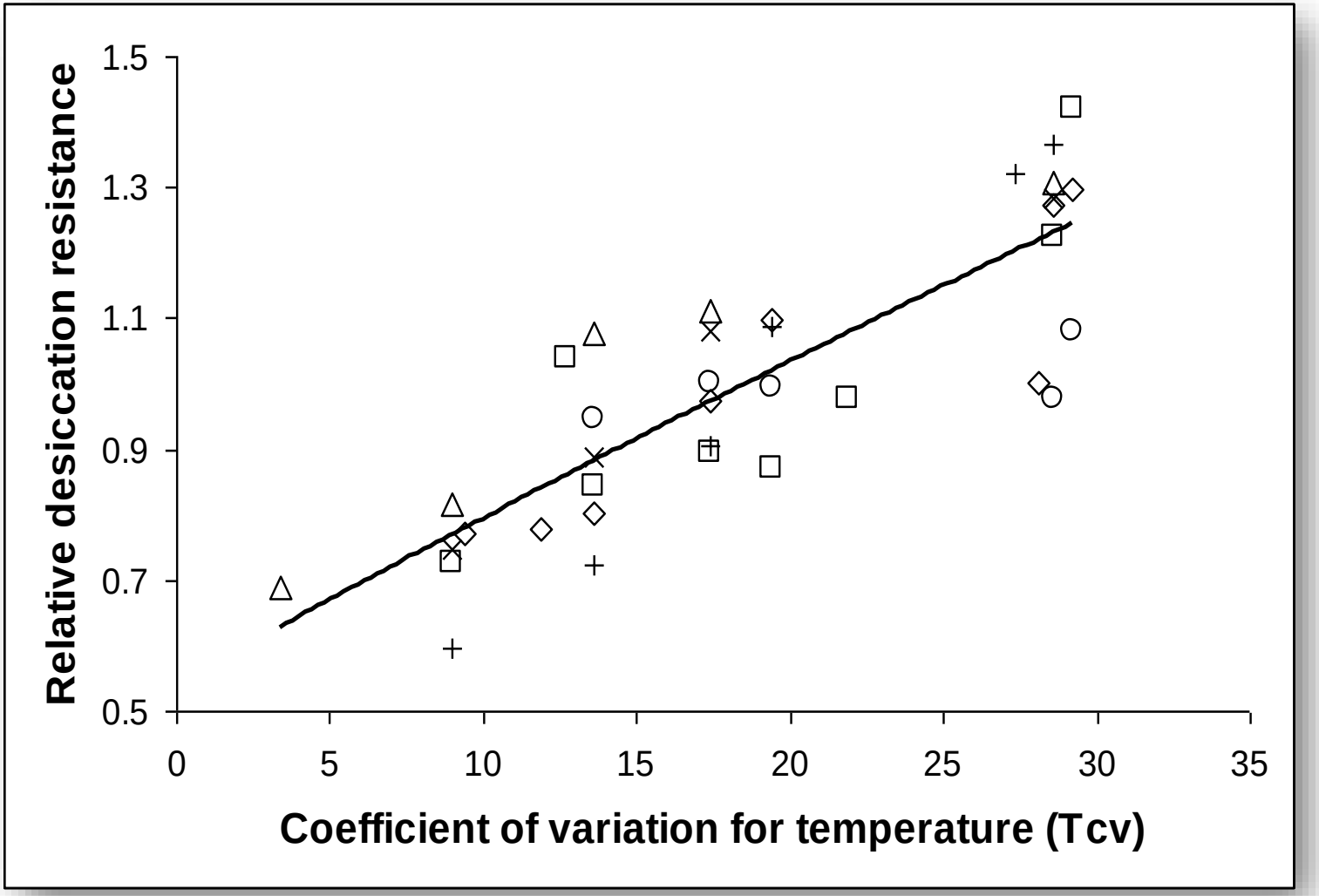


Rajpurohit and Nedved 2013, *JTB*



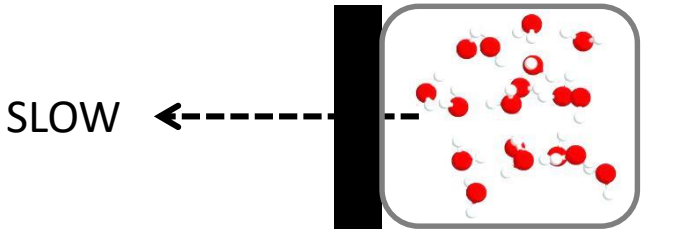
Rajpurohit et al. 2013, *CBP-A*

Climatic cline of relative desiccation resistance of six drosophilid flies. The values are relative to the mean survival time separately in each species.

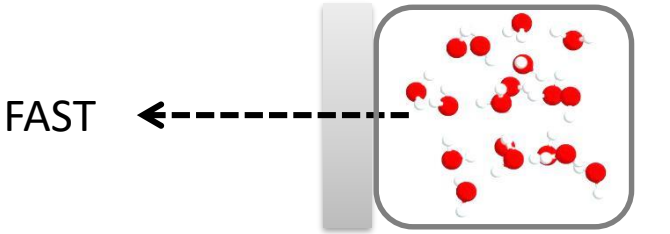


Rajpurohit et al., 2013; *CBP*

Reducing rate of water loss (Do molecules matter?)



Case ‘A’ membrane



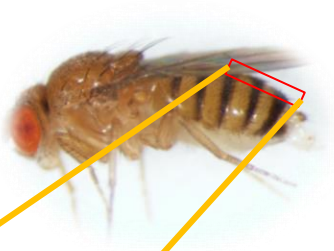
Case ‘B’ membrane

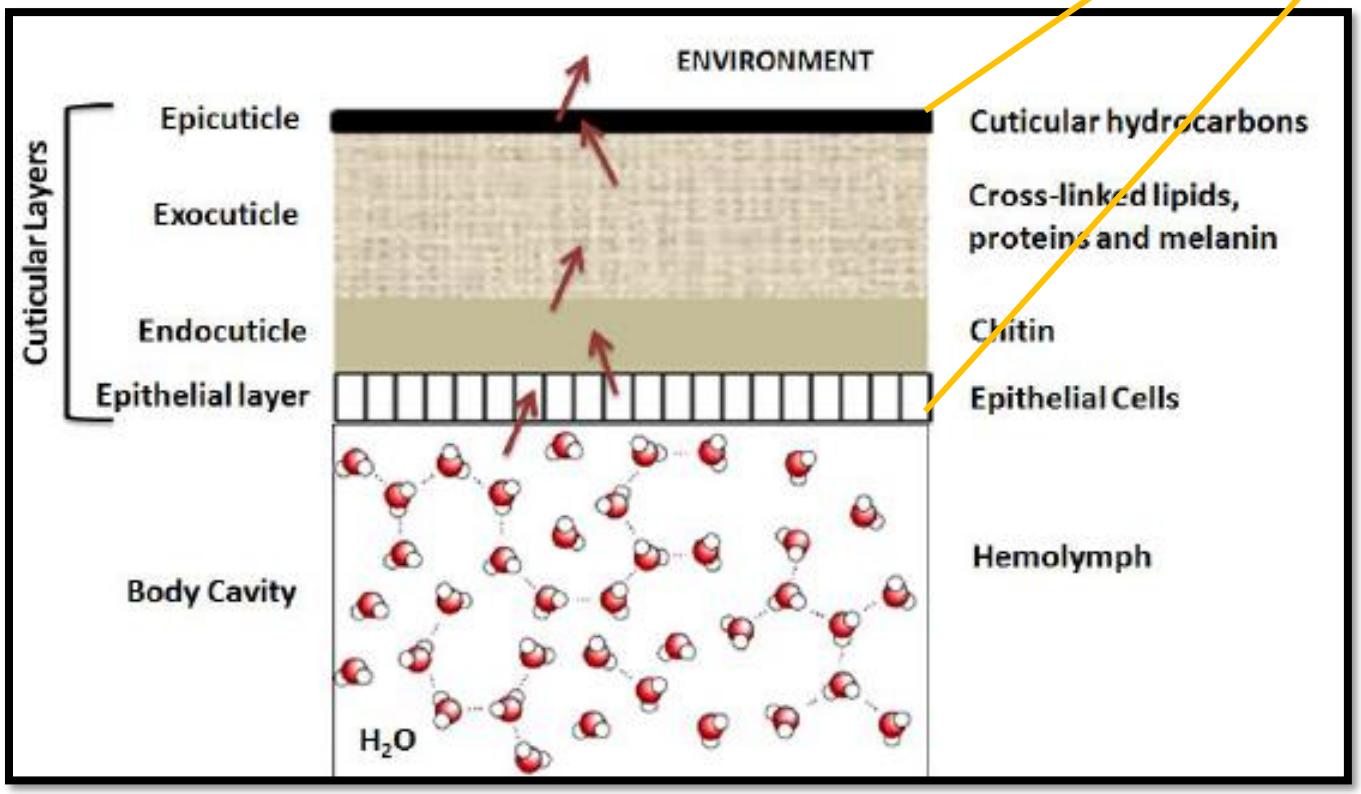
Where are these compounds located?

Melanin

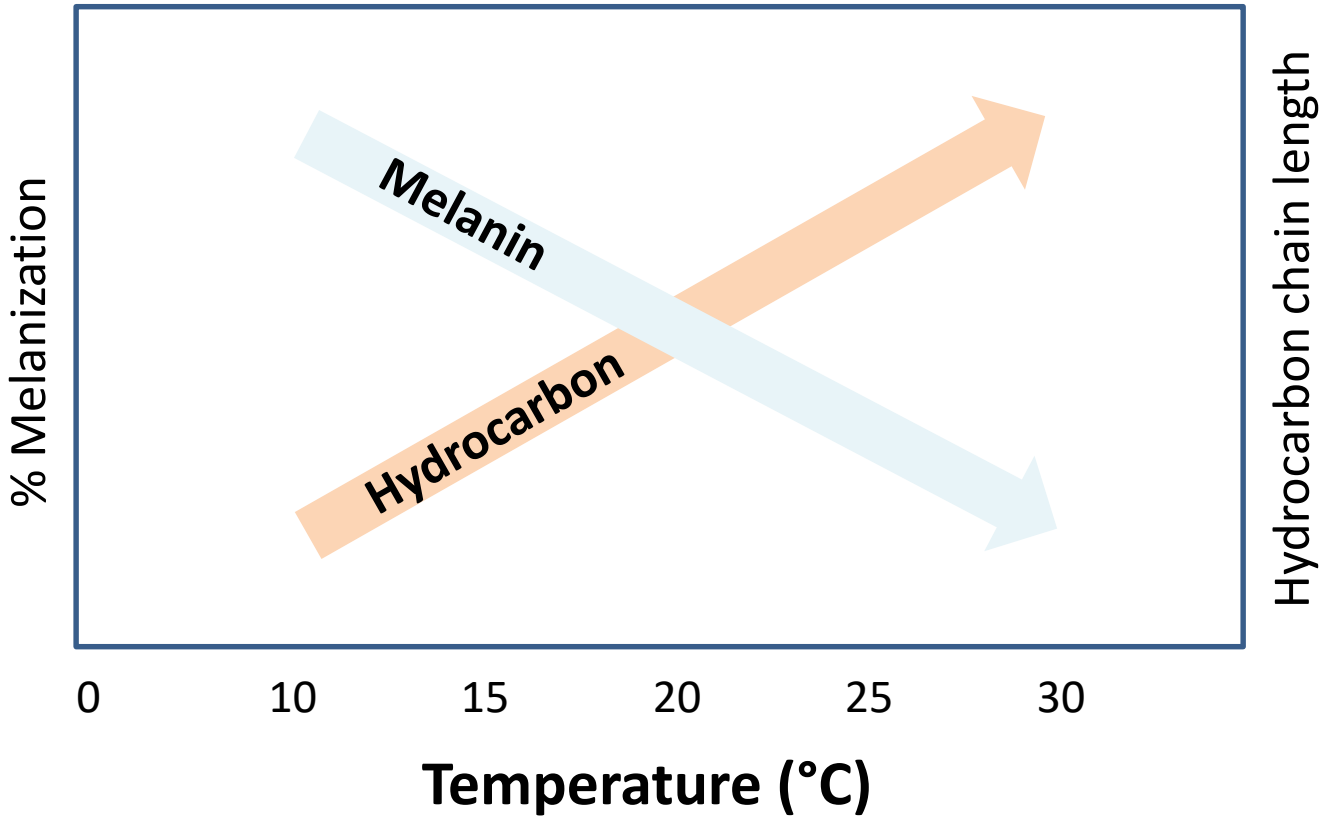
&

Hydrocarbons





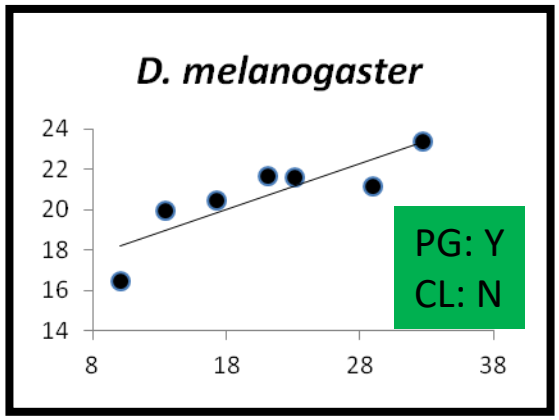
Temperature relations of melanin & hydrocarbons

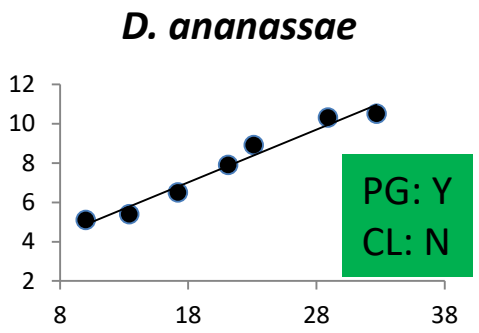


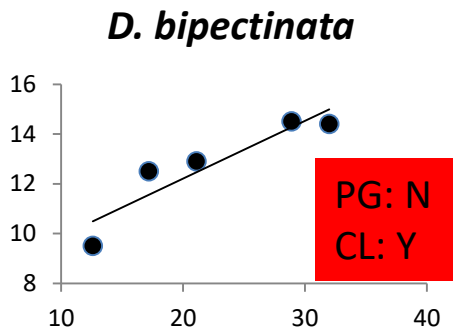
Status of **desiccation tolerance** in natural populations; tropical; Indian subcontinent

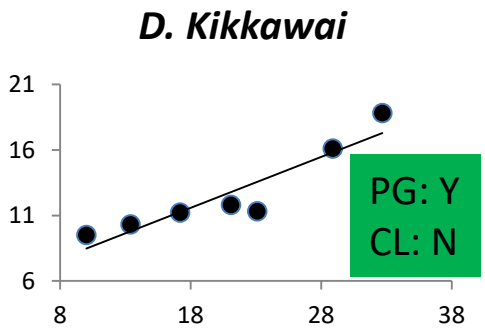
(PG: Pigmentation; CL: cuticular lipids)

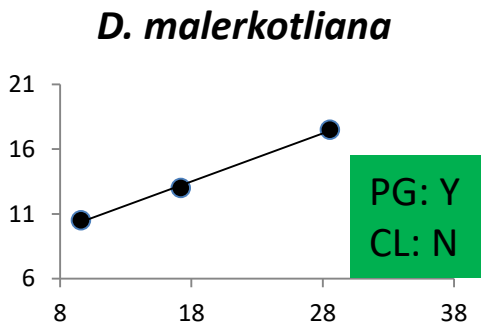
Desiccation tolerance (hours)

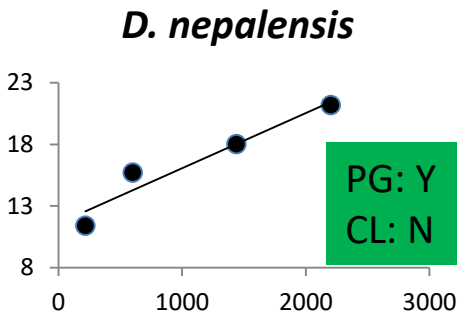


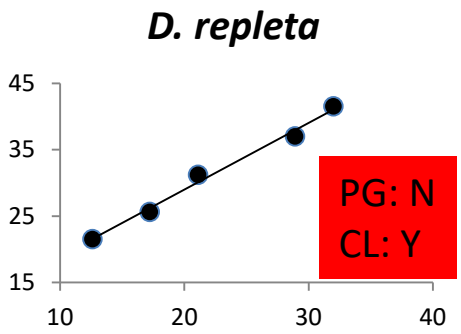


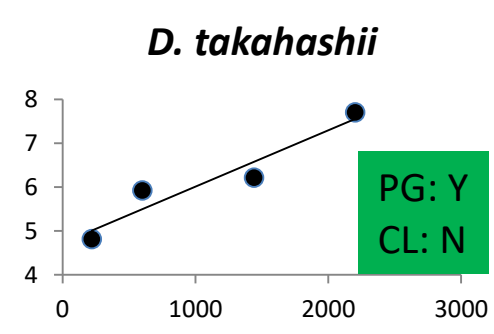


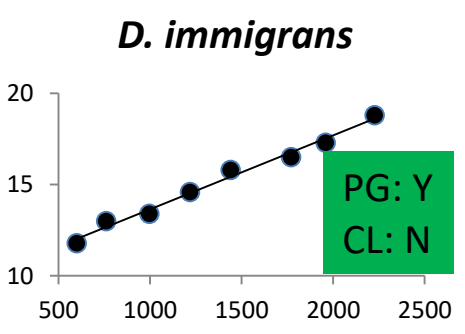












Latitude (°N)/altitude (meters a.s.l.)

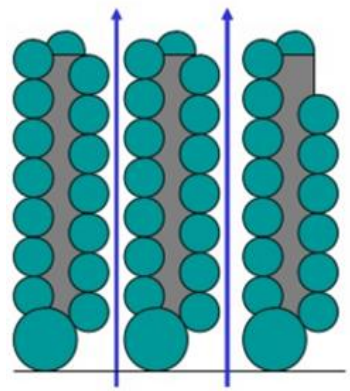
reviewed in *JTB 2013 & Scientific Data 2017*

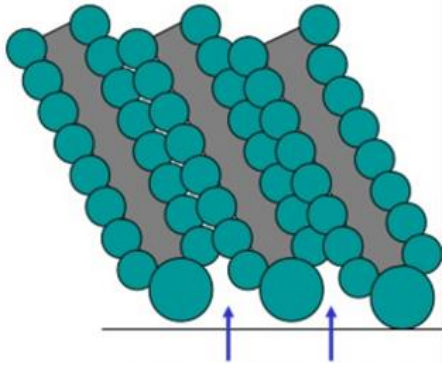
Control of Water Movement

Melanin

?

Hydrocarbons





Water Water

Water is retained

-hydrophobic nature of these compounds

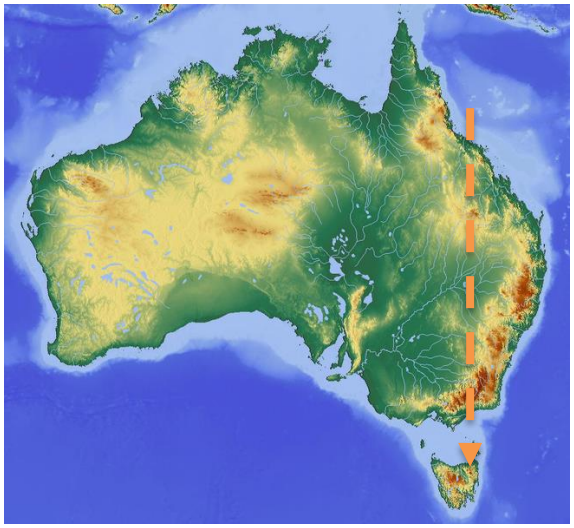
II. Artificial Selection

(testing ecological hypotheses)

Using Experimental Evolution to Test an Ecological Hypothesis

India

Australia



USA

Summary…

- Drier & variable climate in northern/southern (higher latitude)
- Northern/Southern (higher Lat) populations of *Sophophora* are more desiccation-resistant
- Northern/Southern (higher Lat) populations are **darker** (*i.e.* more **melanin**)

Functional Hypotheses for Variation in Melanization

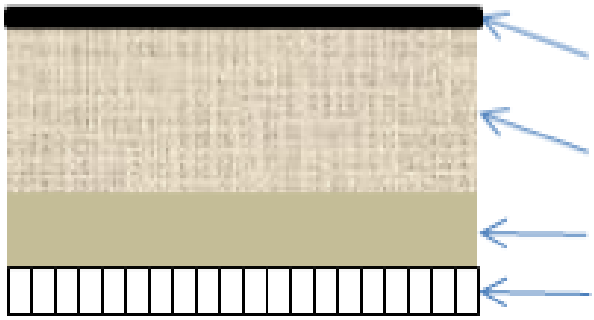
Temperature regulation

Pathogen resistance

Crypsis

Reproduction

Cuticular waterproofing



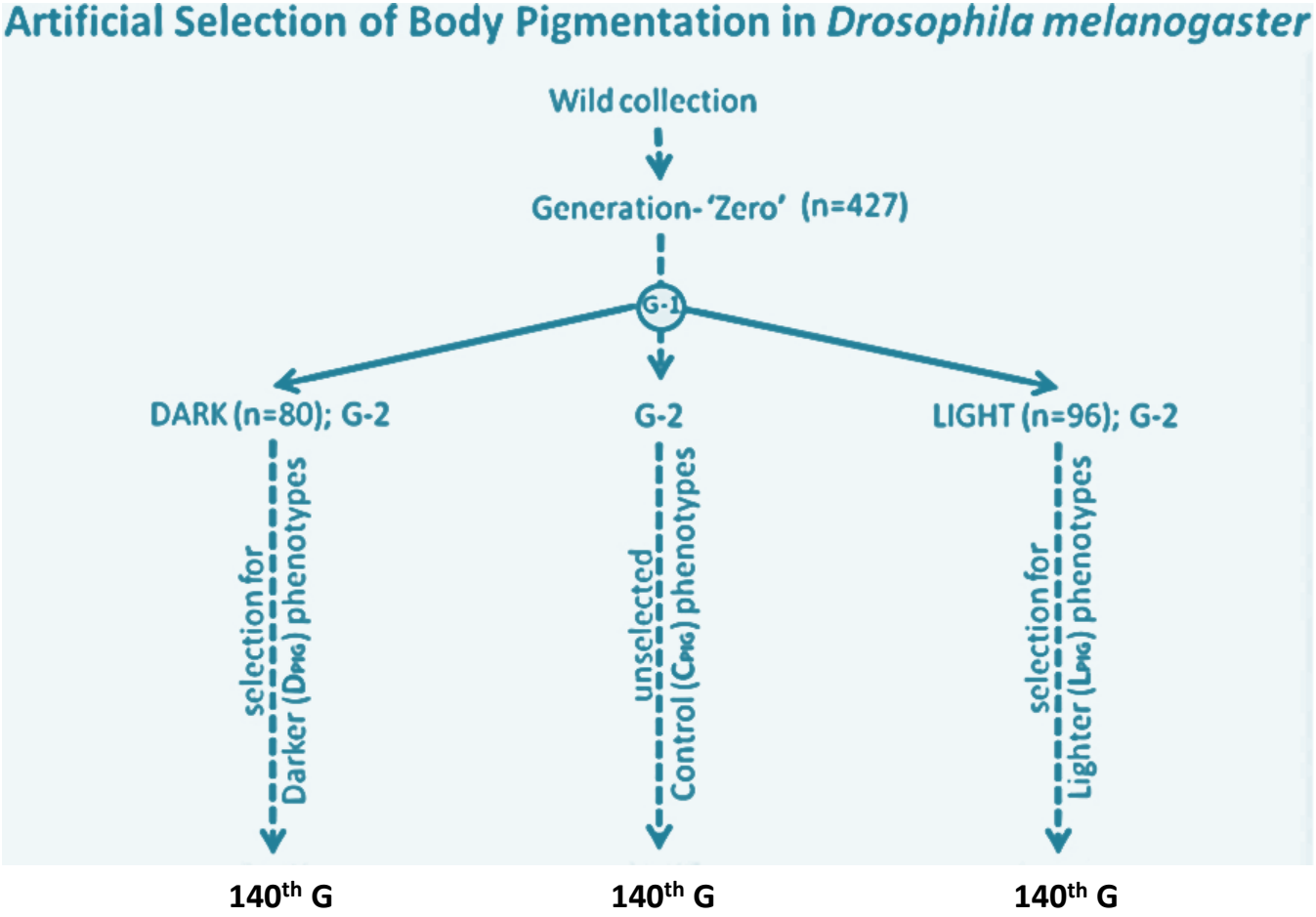
Epicuticle: Complex layer. The outermost layer and probably the most important layer. Involved in water proofing and general impermeability of cuticle.

Exocuticle: Region in which cross-linking of proteins occurs to give cuticle hardness. Melanin and other pigments found in this area. Sclerotization mainly occurs here.

Endocuticle: Contains most of the chitin. Little crosslinking of proteins.

Epithelium

Artificial Selection for LIGHT or DARK Pigmentation in *Sophophora melanogaster*



-at room temperature
-on regular fly media
-generation time 21 days

3-fold replication

BJLS 2012

Laboratory *Natural* Selection for Desiccation Resistance in *Sophophora melanogaster*

Desiccation Selection:

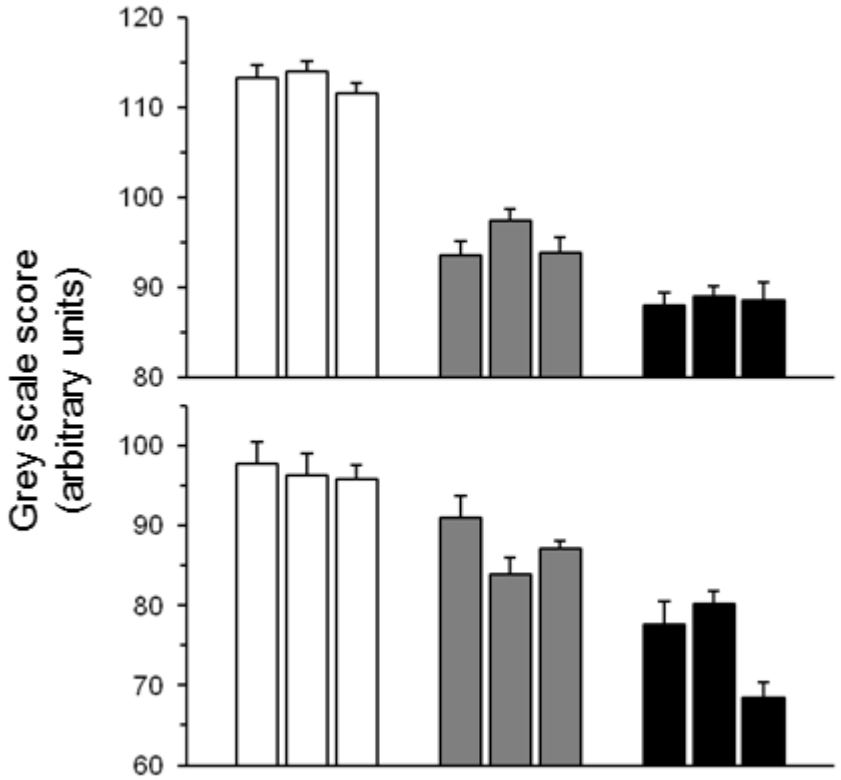
- ~9,000 four-day old *S. melanogaster*
- Replace food with desiccant
- Return food after ~85% mortality
- Use survivors to found next generation
- Unstressed control populations; 3-fold replication

If Pigmentation and Desiccation Resistance are Functionally Linked, Then:

- Dark-selected flies will be more desiccation resistant; light-selected will be less so

- Desiccation-selected populations will be darker than non-selected control populations

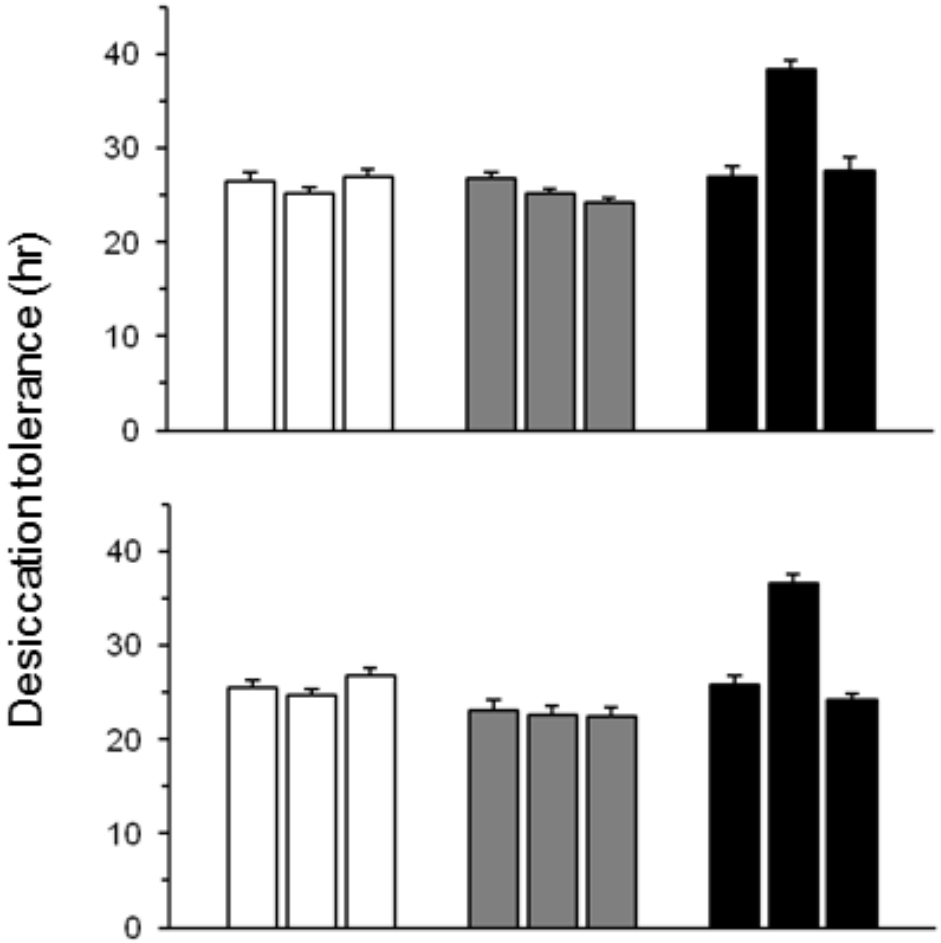
40 (140+) Generations of Selection for Light and Dark Pigmentation



BJLS 2012

PLoS One 2016

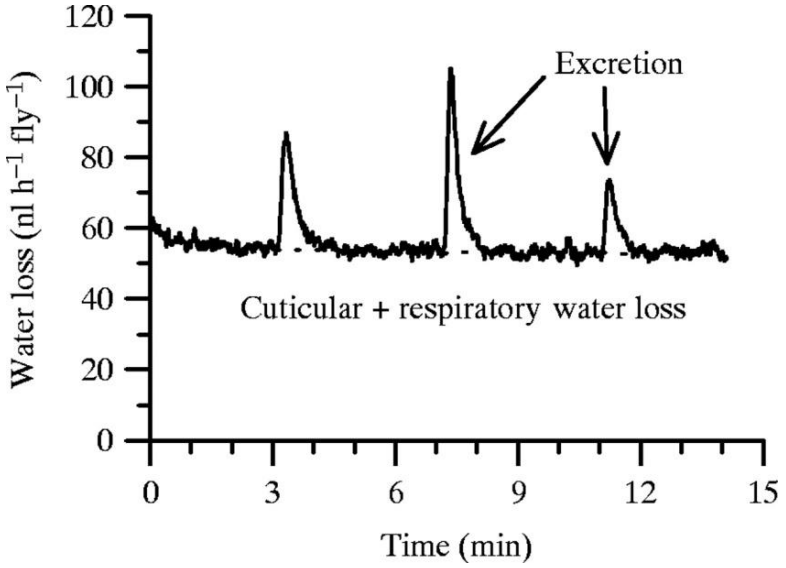
Pigmentation Selection Does Not Affect Desiccation Resistance



N.S.

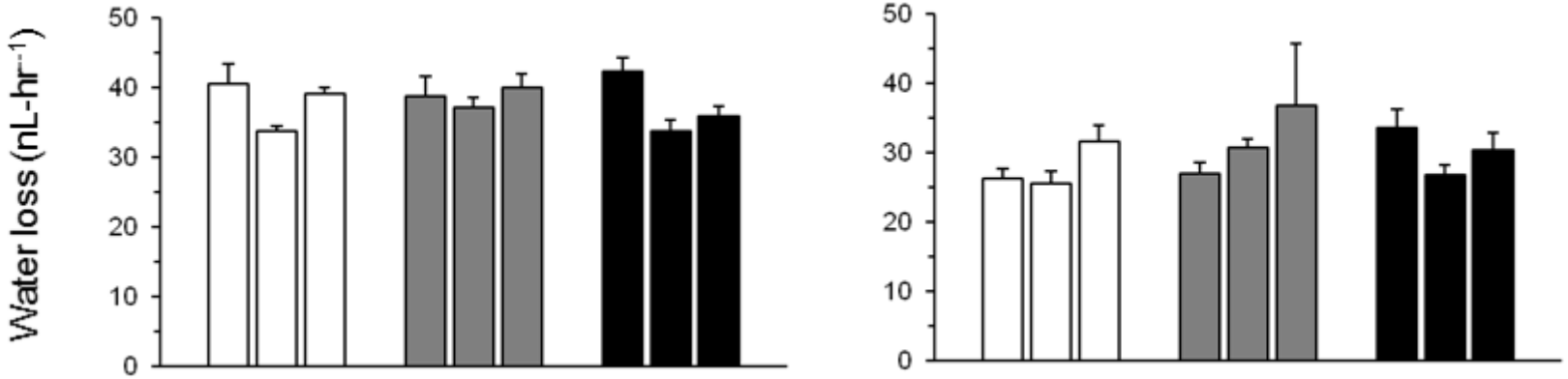
PLoS One 2016

Respirometry: water loss rate measurement



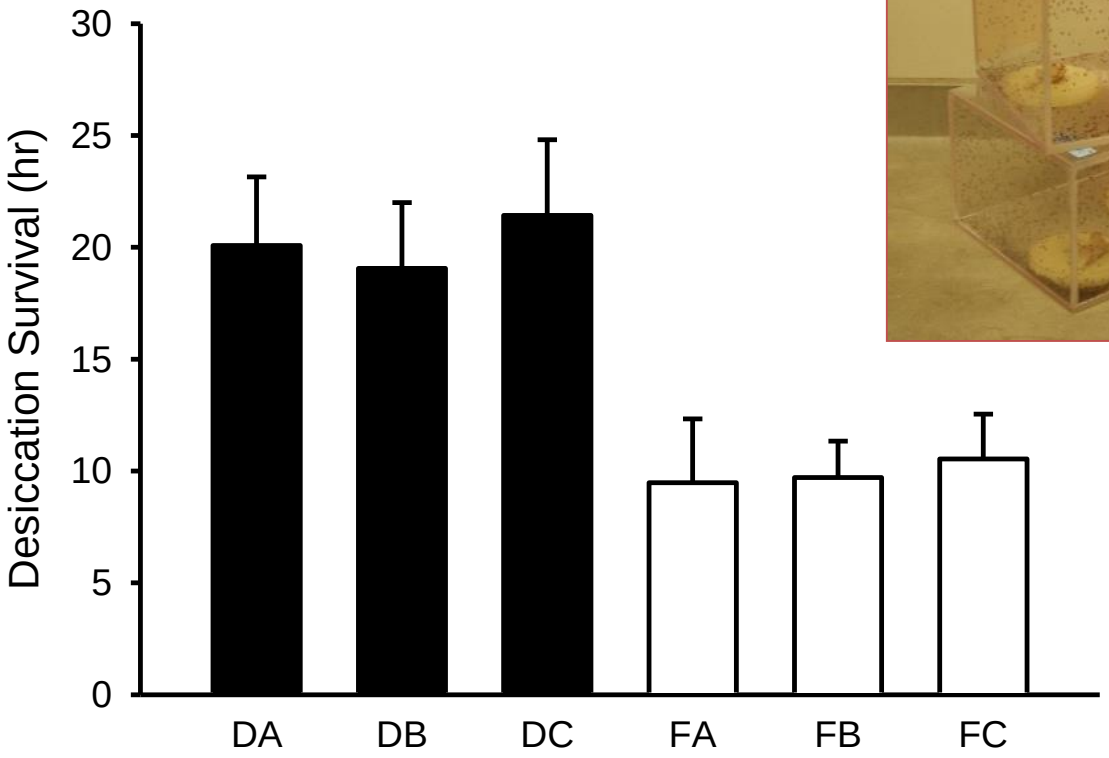
Male

Female



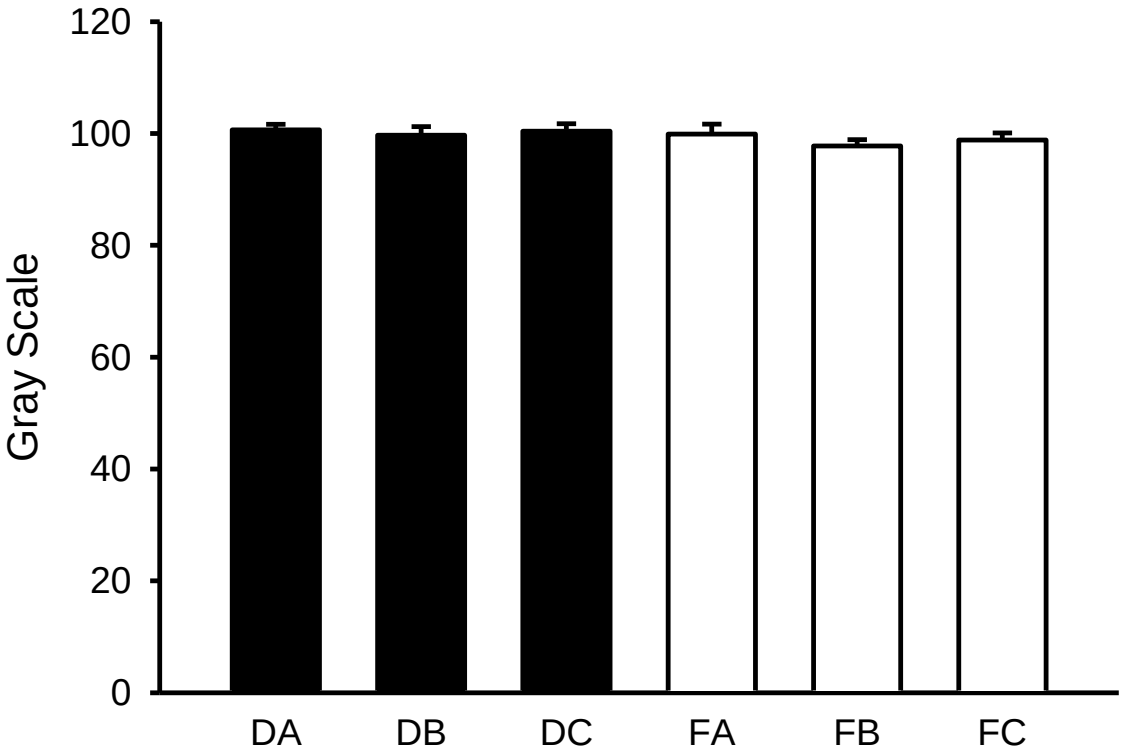
PLoS One 2016

Desiccation-Selected Flies Survive Dry Conditions Longer than Non-selected Controls



PLoS One 2016

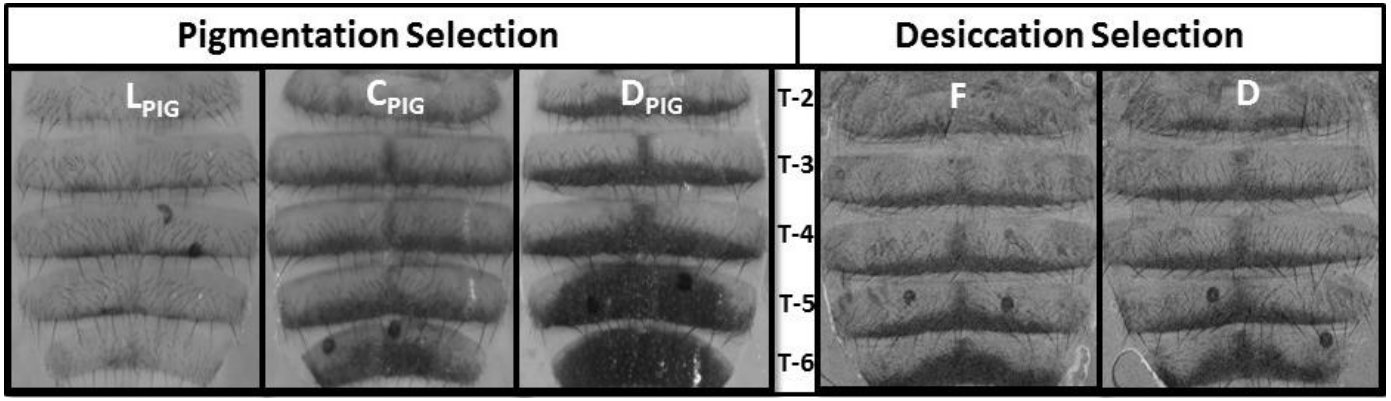
Desiccation-Selected Flies are Not Darker than Controls



PLoS One 2016

Predictions

G-40 G-140



• Dark-selected flies will be more desiccation resistant; light-selected will be less so

NO

• Desiccation-selected populations will be darker than non-selected control populations

NO

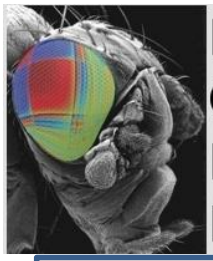
but…..

Pigmentation-selected lines exhibited unexpected correlated evolution of:

- body size

- glycogen content

III. GWAS



Drosophila Genetics Reference Panel 2

192 Lines

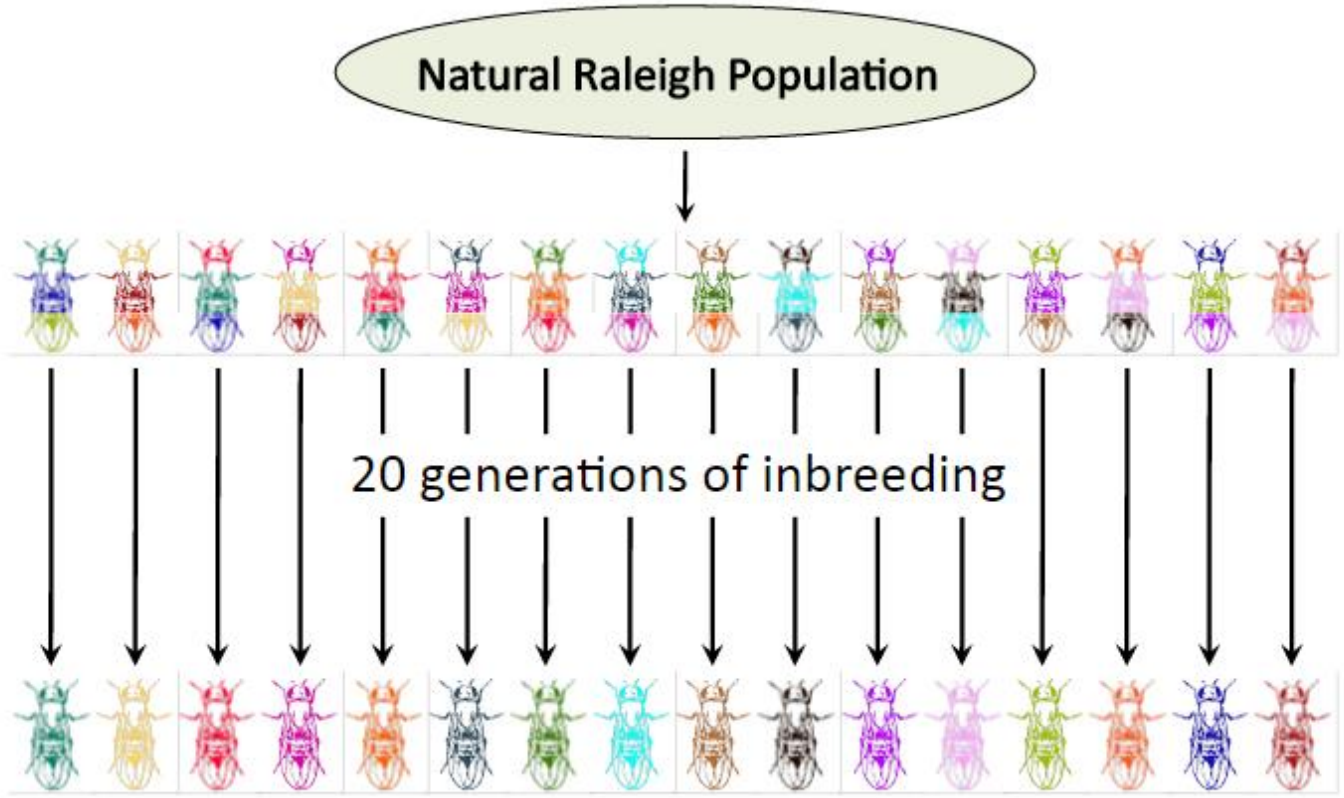
↓

which have been inbred to near Homozygosity

↓

fully sequenced

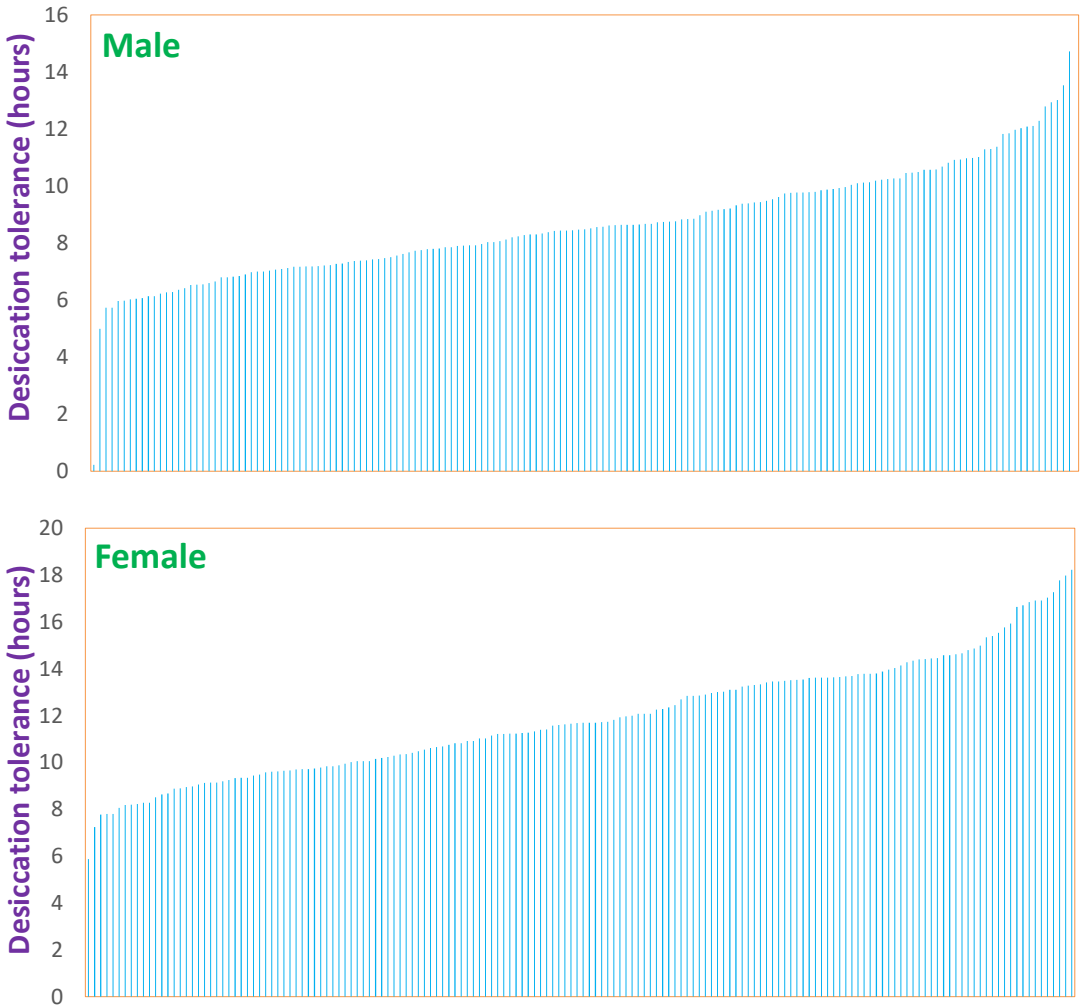
Drosophila Genetic Reference Panel (DGRP)



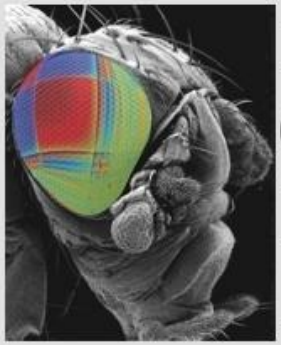
Mackay *et al.*, *Nature*, 2012; Huang *et al.*, *Genome Research*, 2014

Status of Desiccation tolerance in DGRP Lines

~162 Lines (Location: Raleigh; Lat:35.77° N ;Long:78.63° W)



Molecular Ecology 2018, under review



Drosophila Genetics Reference Panel 2

Project Description

The *Drosophila* Genetic Reference Panel (DGRP) is a population consisting of more than 200 inbred lines derived from the Raleigh, USA population. The DGRP is a living library of common polymorphisms affecting complex traits, and a community resource for whole genome association mapping of quantitative trait loci.

This webtool allows users to perform genome wide association studies (GWAS) by providing phenotypes.

Submit Phenotype Data for GWAS

Please complete steps I-III

I. Email address for results:

II. Upload phenotype file (Be sure to place male phenotype before female phenotype if providing a two-column file):
Choose File No file chosen
Click to view example phenotype files and format.

III. Execute Genome Wide Association Study: Execute GWAS

DGRP data

All DGRP data are open and can be found here

FAQ

If you are experiencing difficulties or have general questions about this site, please visit the FAQ

Browser track

UCSC Genome Browser track of DGRP2 data

Citation:

If you use these data and/or this website for analyses, please cite the following manuscript:

Mackay *et al.*, 2012

Huang, Massouras, *et al.*, 2014

Genome Wide Association

SNPs identified from the genome-wide association study for desiccation tolerance in *Drosophila melanogaster*, with annotation information.

 # These SNPs were annotated as being in or nearby 20 genes and one non-coding region

ID	AvgMixedPval	FBgn	Gene Name
3L_22526049_SNP	1.26E-06		
3R_667649_SNP	1.49E-06		
3R_442763_SNP	1.50E-06	FBgn0087012	5-HT2
X_9339800_INS	2.74E-06	FBgn0261260	mgl
3L_5473554_SNP	3.08E-06	FBgn0035607	CG4835
2L_11391909_SNP	3.21E-06	FBgn0263764	CG43681
3R_23199754_SNP	3.60E-06		
3L_5473561_INS	3.74E-06	FBgn0035607	CG4835
3R_5598721_SNP	5.21E-06	FBgn0037750	Whamy
3R_24407226_SNP	5.86E-06	FBgn0000659	fkh
2L_4358249_SNP	7.91E-06		
3L_12383501_SNP	9.82E-06	FBgn0036279	Ncc69
2L_16519498_SNP	1.18E-05	FBgn0032587	CG5953
3R_21728606_SNP	1.45E-05	FBgn0051089	CG31089
3L_9396890_SNP	1.49E-05		
2L_551066_SNP	1.53E-05	FBgn0005660	Ets21C
3L_523916_SNP	1.77E-05	FBgn0001316	klar
3R_13186623_SNP	1.91E-05	FBgn0038498	beat-IIa
3R_674796_SNP	2.20E-05		
3R_632820_SNP	2.95E-05	FBgn0260794	ctrip
3L_7482778_SNP	3.20E-05		
3R_23720187_SNP	3.26E-05	FBgn0085382	CG34353
3L_14512611_SNP	3.40E-05	FBgn0087007	bbg
2L_16076165_SNP	3.86E-05	FBgn0013433	beat-Ia
2L_10534268_SNP	4.01E-05	FBgn0032266	CG18302
2L_10534270_SNP	4.22E-05	FBgn0032266	CG18302
3R_443008_SNP	4.26E-05	FBgn0087012	5-HT2
2L_534795_SNP	4.71E-05	FBgn0003963	ush
2L_7973561_SNP	6.76E-05	FBgn0085450	Snoo
2L_7974079_SNP	8.55E-05	FBgn0085450	Snoo
2R_11948370_SNP	8.79E-05		
3R_5595870_SNP	9.11E-05	FBgn0037747	CG8481
2L_10049930_SNP	9.77E-05	FBgn0053301	CG33301

Molecular Ecology 2018, under review

Genome Wide Association

Genes containing SNPs associated with desiccation tolerance in DGRP lines. GO Biological Process information was obtained using GOrilla (Eden et al. 2009).

ID	Gene Name
FBgn0087012	5-HT2
FBgn0087007	bbg
FBgn0013433	beat-Ia
FBgn0038498	beat-IIa
FBgn0032266	CG18302
FBgn0051089	CG31089
FBgn0053301	CG33301
FBgn0085382	CG34353
FBgn0263764	CR43681
FBgn0035607	CG4835
FBgn0032587	CG5953
FBgn0037747	CG8481
FBgn0260794	ctrip
FBgn0005660	Ets21C
FBgn0000659	fkh
FBgn0001316	klar
FBgn0261260	mgl
FBgn0036279	Ncc69
FBgn0085450	Snoo
FBgn0003963	ush
FBgn0037750	Whamy

Gene ontology (GO) enrichment analysis played these 20 genes in various GO biological categories that broadly included *metabolism*, *signaling*, *cuticle development* and *transport*.

Molecular Ecology 2018, under review

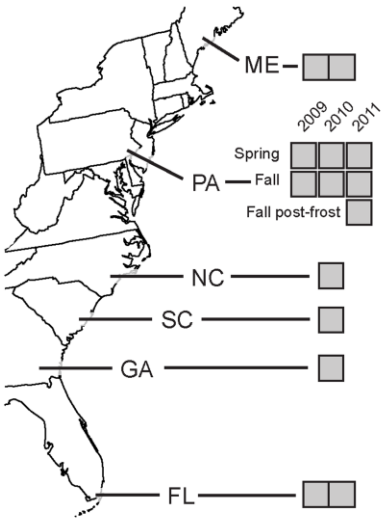
Some of the interesting candidate genes…

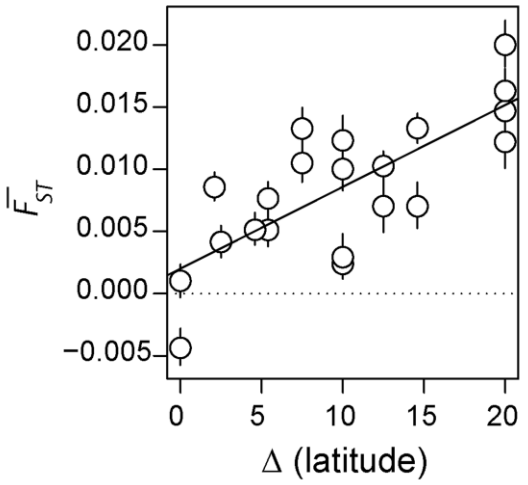
One such gene, *5-HT2*, is a 5-hydroxytyramine (5-HT, serotonin) receptor. Serotonin is an important diuretic hormone in insects (Martini *et al.* 2004), indicating that DGRP lines may differ in urine production.

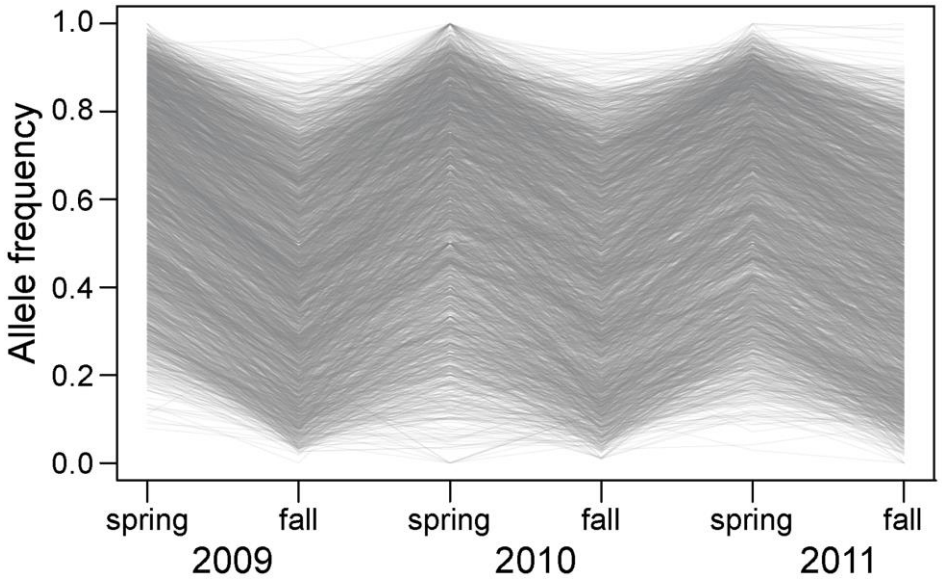
Two additional candidates (*mgl*, *CG4835*) are functionally linked to chitin metabolism. Serotonin also mediates cuticular plasticization (Reynolds 1975); together these findings suggest that cuticular permeability may be an important factor affecting desiccation tolerance.

Metabolic differences also appear to be important, as SNPs mapped to or close to three genes implicated in lipid metabolism (*klar*, *CG18302*, *CG31089*)

Genomic Evidence of Rapid and Stable Adaptive Oscillations over Seasonal Time Scales in *Drosophila*







Bergland et al. 2014, *PLoS Genetics*

Are genes identified (among North American populations) being associated with desiccation tolerance in DGRP show increased signatures of spatially varying selection relative to the rest of the genome?

If YES

…we can plan some finer scale experiments

Acknowledgement

Labs

Allen Gibbs
University of Nevada, USA

Paul Schmidt
University of Pennsylvania, USA

Collaborators…

Dmitri Petrov
Stanford University, USA

Eran Gefen
Haifa University, Israel

Alan Bergland
University of Virginia, USA

Queensland Art Gallery

Thanks