Organismal & Evolutionary Biology in Ocean Acidification Research

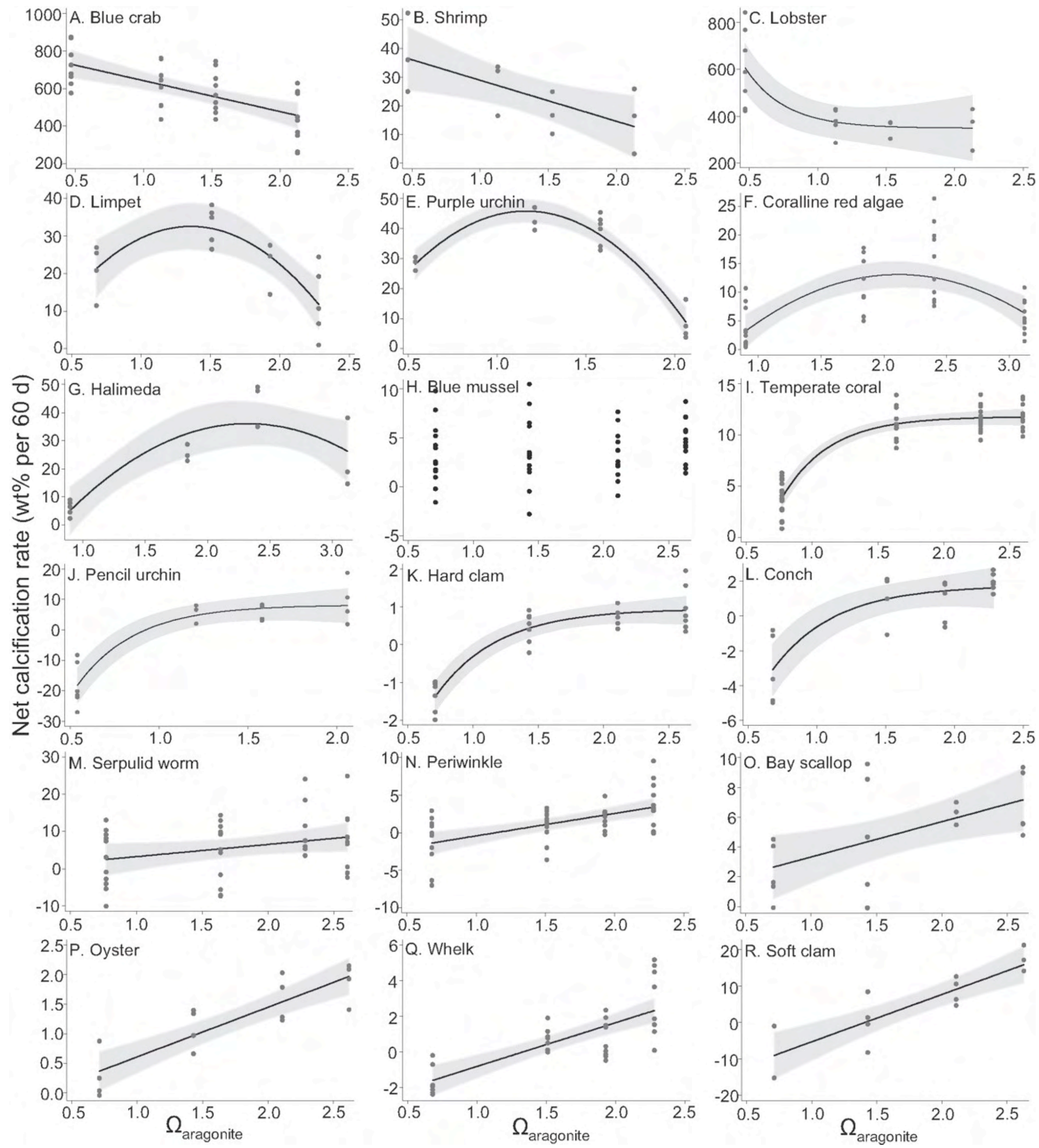
Ron Burton
Scripps Institution of Oceanography

Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification

Justin B. Ries, Anne L. Cohen and Daniel C. McCorkle

Geology 2009; 37:1131-1134

Calcification response patterns for 18 species of calcifying organisms subjected for 60 d to CO2-induced reductions in CaCO3 saturation state of seawater. Net rates of calcification(+)/dissolution(−) were estimated from buoyant weighing (verified with dry weight measured after harvesting) and are expressed as a percentage of the organisms' initial buoyant weight



Effects of high CO2 seawater on the copepod (Acartia tsuensis) through all life stages and subsequent generations

Published 27 June 2008 Science   Leave a Comment
Tags: biological response, crustaceans

We studied the effects of exposure to seawater equilibrated with CO2-enriched air (CO2 2380 ppm) from eggs to maturity and over two subsequent generations on the copepod Acartia tsuensis. Compared to the control (CO2 380 ppm), high CO2 exposure through all life stages of the 1st generation copepods did not significantly affect survival, body size or developmental speed. Egg production and hatching rates were also not significantly different between the initial generation of females exposed to high CO2 and the 1st and 2nd generation females developed from eggs to maturity in high CO2. Thus, the copepods appear more tolerant to increased CO2 than other marine organisms previously investigated for CO2 tolerance (i.e., sea urchins and bivalves). However, the crucial importance of copepods in marine ecosystems requires thorough evaluation of the overall impacts of marine environmental changes predicted to occur with increased CO2 concentrations, i.e., increased temperature, enhanced UV irradiation, and changes in the community structure and nutritional value of phytoplankton.

Kurihara H. & Ishimatsu A., 2008. Effects of high CO2 seawater on the copepod (Acartia tsuensis) through all life stages and subsequent generations. *Marine Pollution Bulletin* 56(6): 1086–1090.

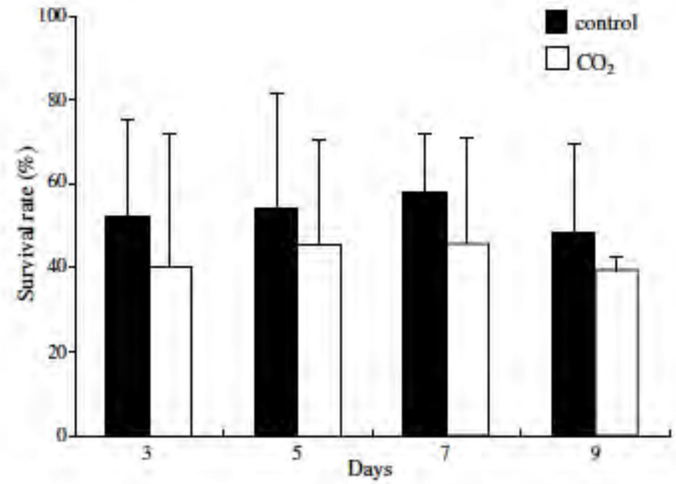


Fig. 1. Survival rate of the *Acartia tsuensis* eggs reared under control (CO_2 380 ppm) and high CO_2 (+2000 ppm) conditions until they developed into adults over a period of 9 days. There was no significant difference ($P > 0.05$) in the survival rate between the control and CO_2 group (two-way ANOVA, $P > 0.05$). Error bar = SD, $n = 4$.

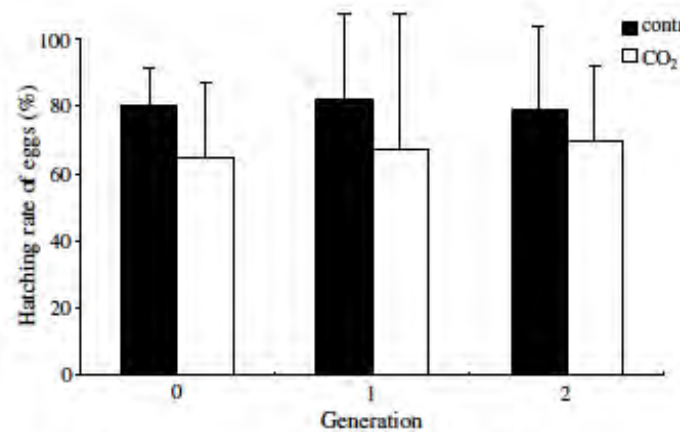


Fig. 5. Hatching rate of eggs produced by the female *Acartia tsuensis* of generations 0, 1 and 2. There was a significant difference in the hatching rate between control and CO_2 groups ($P < 0.05$, ANOVA), although there was no difference when compared separately for each generation (*t*-test). Error bar = SD.

The effect of ocean acidification and temperature on the fertilization and embryonic development of the Sydney rock oyster *Saccostrea glomerata* (Gould 1850)

LAURA M. PARKER[1], PAULINE M. ROSS[1],
WAYNE A. O'CONNOR[2]

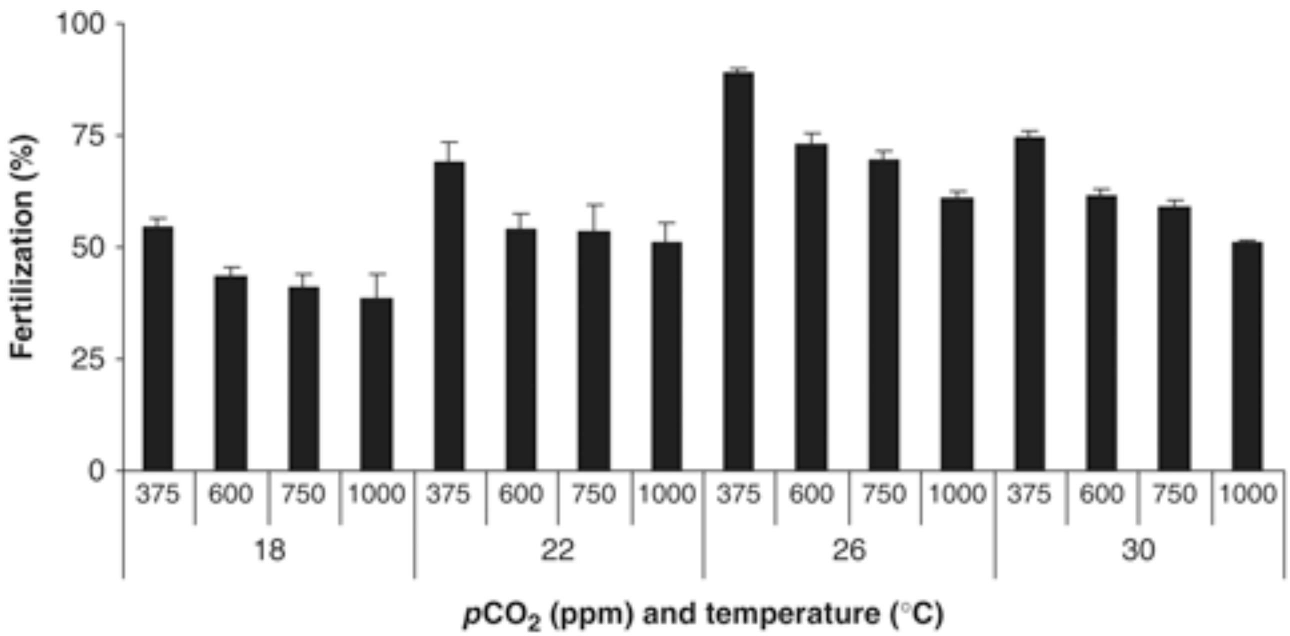
Issue

Article first published online: 17 FEB 2009

DOI: 10.1111/j.1365-2486.2009.01895.x

Global Change Biology

Volume 15, Issue 9, pages 2123–2136, September 2009



Significant effects on fertilization… before calcification

Elevated CO_2 and ocean acidification are among the many environmental challenges faced by marine organisms

> temperature
> anoxia
> salinity
> pressure
> metals
> nutrients
> predators
> competitors
> prey availability
> … combinations of the above

Response to environmental changes

- Behavioral adaptations
- Structural adaptations
- Physiological adaptations
  - Gene regulation
  - Post-translational (Lars Tomanek, proteomics)
- Evolutionary adaptations

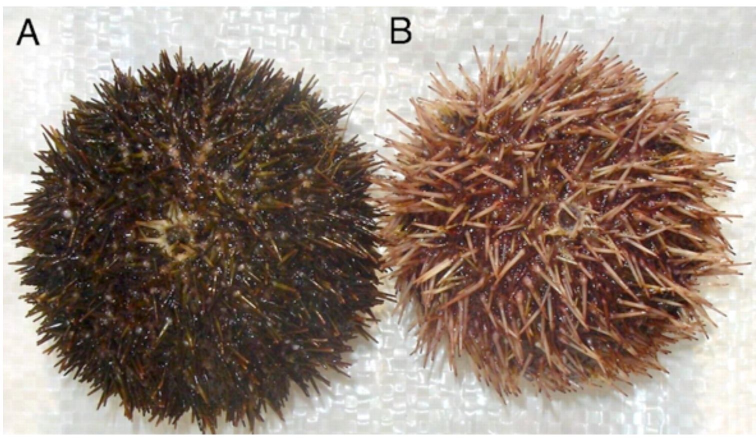
focus today {

“Thermal stability of proteins... Over evolutionary timescales, amino acid substitutions have resulted in adaptive changes in stability and kinetic properties of enzymes to yield orthologous enzymes that function efficiently under specific habitat temperatures… however, the time required for these types of amino acid substitutions is at a scale that dwarfs the decades that represent committed climate change.”

Common view showing lack of integration between evolutionary biologist and physiologists… MUCH VARIATION EXISTS WITHIN SPECIES and selection experiments show rapid responses.

Natural populations harbor extensive genetic variation – DNA to morphology

The U (A) and G (B) morphological forms of Strongylocentrotus intermedius.



Balakirev E S et al. PNAS 2008;105:16218-16223

Juvenile colour polymorphism in the red rock crab, Cancer productus

J. Krause-Nehring et al. / Zoology 113 (2010) 131–139 133

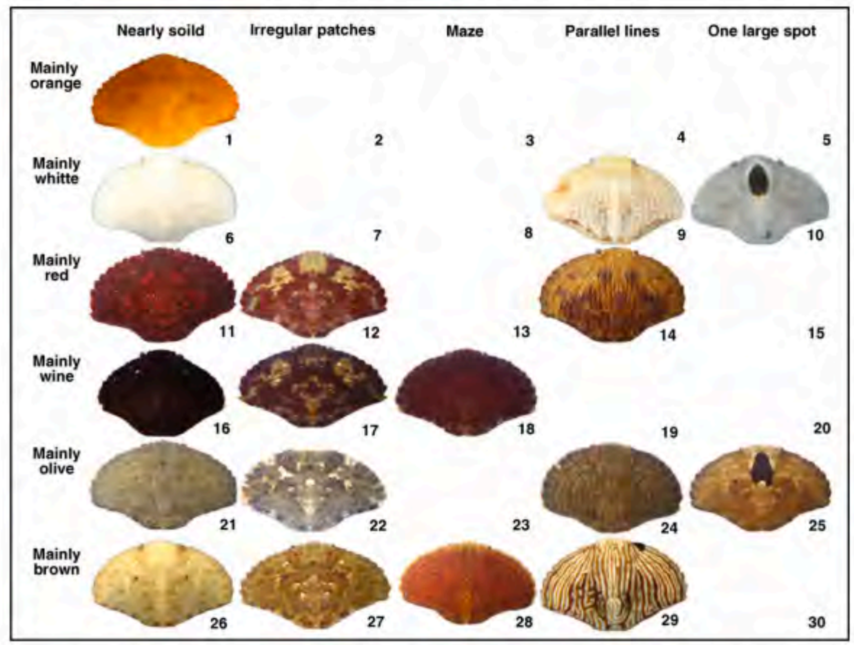


Fig. 1. Representative images of all morph categories observed among 163 *C. productus* juveniles. The combination of six colour types (=rows) and five pattern types (=columns) yielded 30 morph categories. Some boxes do not contain a picture either because no sampled crab fit into the specific category or because the category was too diverse (="other" as an extra category, see Section 2.1).

Thanks for pointing that out, Charles…

Evolution can be rapid due to natural selection on existing variation and recombinants

Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 6897–6901, September 1986
Evolution

Intense natural selection caused a rapid morphological transition in a living marine snail

(predation/morphological variation/evolution)

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Department of Biology, Yale University, P.O. Box 6666, New Haven, CT 06511

Communicated by G. Evelyn Hutchinson, May 12, 1986

ABSTRACT Shell shape and shell thickness of the intertidal snail *Littorina obtusata* changed markedly between 1871 and 1984 in northern New England. Shells collected prior to 1900 were high-spired with thin walls, whereas shells collected in 1982–84 were low-spired with thick walls. An intertidal crab (*Carcinus maenas*) which preys on *L. obtusata* expanded its range into northern New England around 1900. This suggests that the change in snail shell form was a response to predation by *Carcinus*. Field and laboratory experiments demonstrated that the high-spired form of *L. obtusata*, which can still be found in some Maine localities, is more vulnerable to predation by *Carcinus* than is the low-spired form of *L. obtusata*. Electrophoretic comparisons of high- and low-spired populations of *L. obtusata* confirmed that these populations represent different morphological forms of *L. obtusata* rather than different species [Nei's *D* (unbiased measure of genetic distance) = 0.003]. These data demonstrate that classical Darwinian selection can produce a rapid morphological tran-

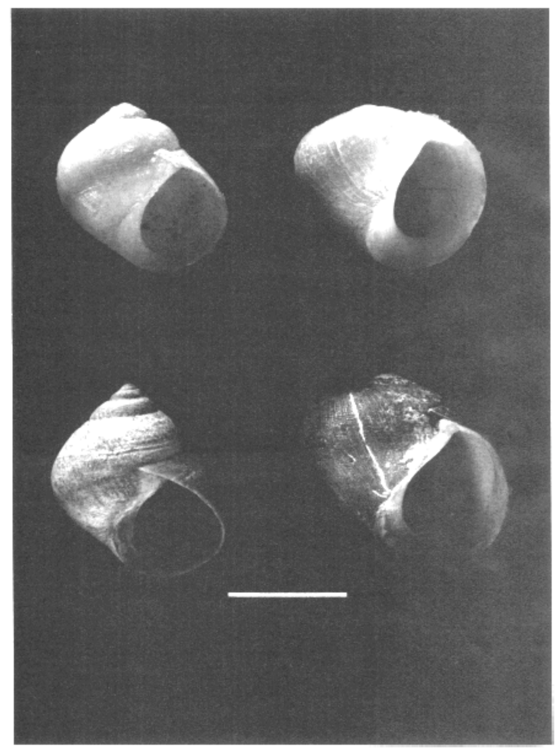
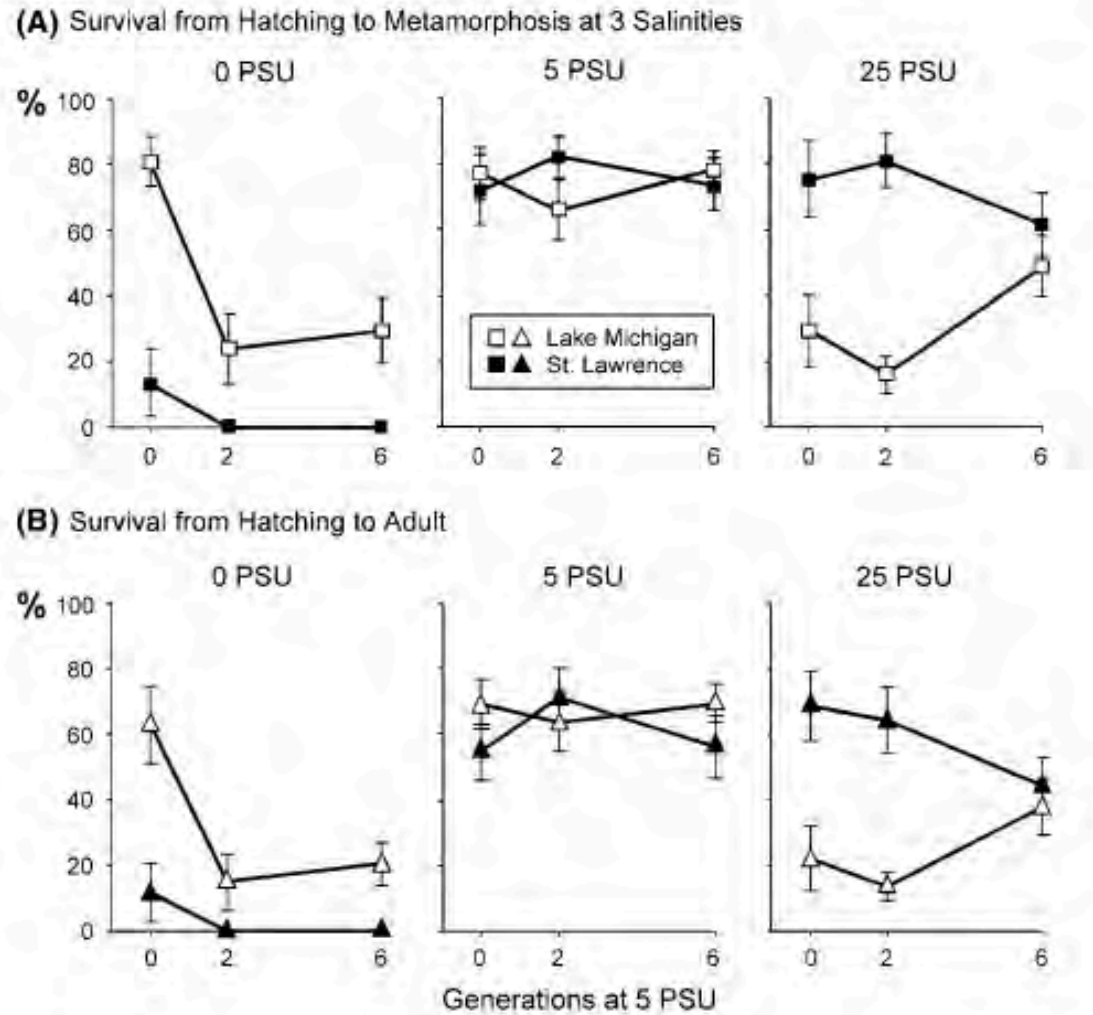


FIG. 1. Historical and geographic variation in *L. obtusata* (L.) from Maine (USA). (*Upper Left*) Appledore Island, 1871 (YPM 19351). (*Upper Right*) Appledore Island, 1982 (YPM 19349). (*Lower Left*) Sipp Bay, Perry, 1984 (YPM 19359). (*Lower Right*) Gleason Point, Perry, 1984 (YPM 19360). (Bar = 5 mm.)

Response to selection and evolvability of invasive populations

Carol Eunmi Lee · Jane Louise Remfert · Yu-Mei Chang

Fig. 4 Shifts in tolerance at three salinities (0, 5, 25 PSU) in response to selection at 5 PSU. Graph shows mean survival (%survival within clutch) for 8 to 14 clutches ± SE for each salinity treatment. Survival was measured for populations from saline St. Lawrence marsh and freshwater Lake Michigan after they were reared at 5 PSU for 0, 2, and 6 generations. (**A**) Survival from hatching to metamorphosis. (**B**) Survival from hatching to adult



Genetica 2007 129:179-192

At the risk of being offensive… Biology 1

- DNA – the "genome" - encodes proteins and their expression

transcription

RNA - the "transcriptome" –what genes are currently active?

translation

Proteins – the "proteome" - the actual enzymes and structural proteins

At the level of DNA variation…

-structural gene variation

> mutations change protein structure

-regulatory variation

> mutations change where and when proteins are produced

Humans:

3 billion bp genome

average ~1 million differences between individuals

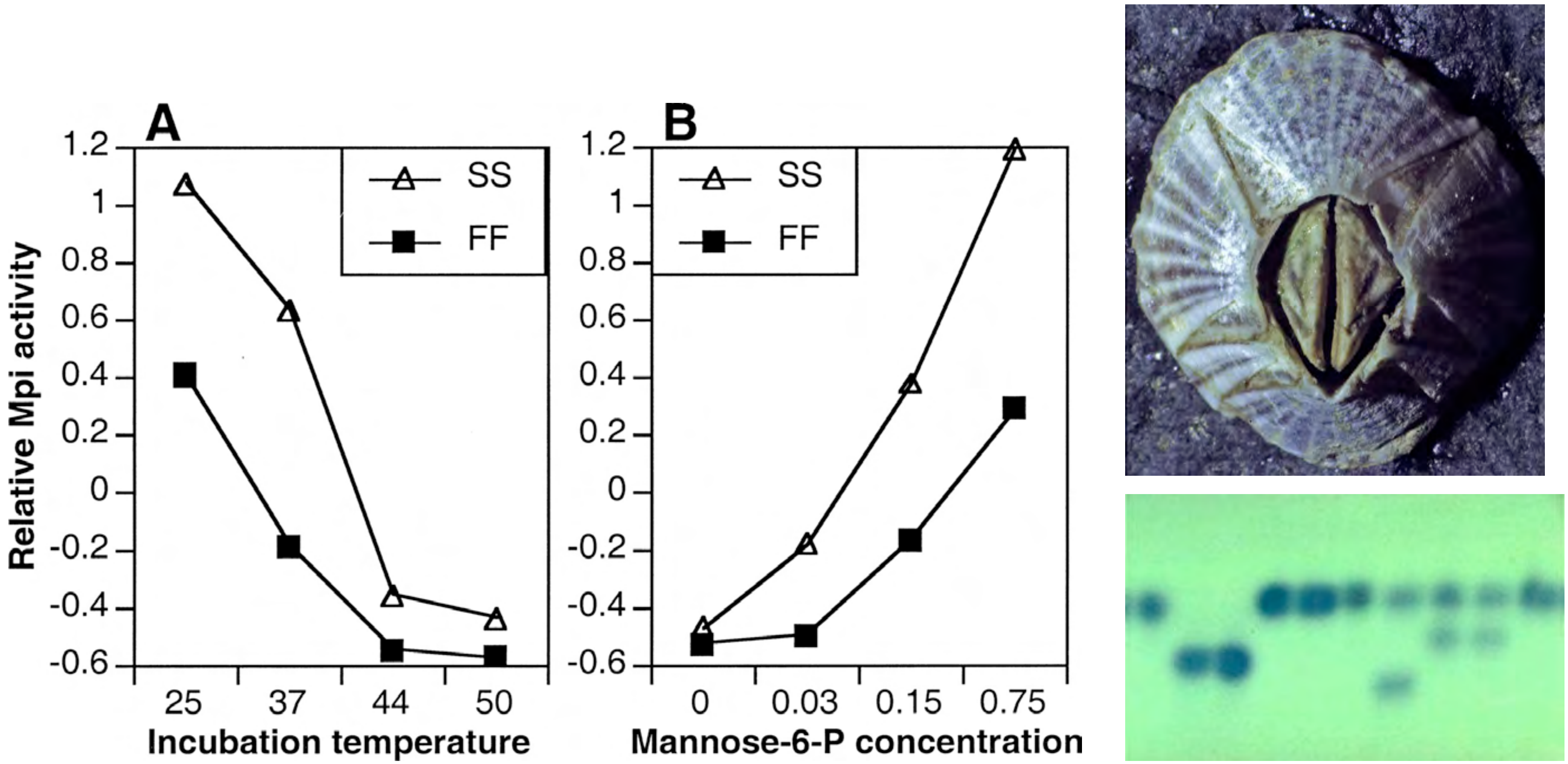


Fig. 4. Enzyme activities of Mpi-SS and –FF genotypes. A. Effect of temperature on enzyme activity. Barnacle homogenate was incubated for one hour at the temperature specified on the X-axis prior to performing the assay. B. Effect of substrate (mannose-6-phosphate) concentration on enzyme activity. Substrate was added to the homogenate at four different final concentrations as specified on the X-axis. Differences between genotypes, temperatures and substrate concentrations are highly significant.

Rand D M et al. Integr. Comp. Biol. 2002;42:825-836

“Genetic structure” refers to the distribution of genetic variation within and among populations

Molecular Ecology (2008) **17**, 4222–4232 doi: 10.1111/j.1365-294X.2008.03905.x

Range-wide genetic homogeneity in the California sea mussel (*Mytilus californianus*): a comparison of allozymes, nuclear DNA markers, and mitochondrial DNA sequences

JASON A. ADDISON, BRIAN S. ORT,* KATHRYN A. MESA and GRANT H. POGSON
Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, CA 95064, USA

Abstract

… Despite our extensive sampling and genotyping efforts, we detected no significant differences among localities and no signal of isolation by distance suggesting that *M. californianus* is genetically homogeneous throughout its range…

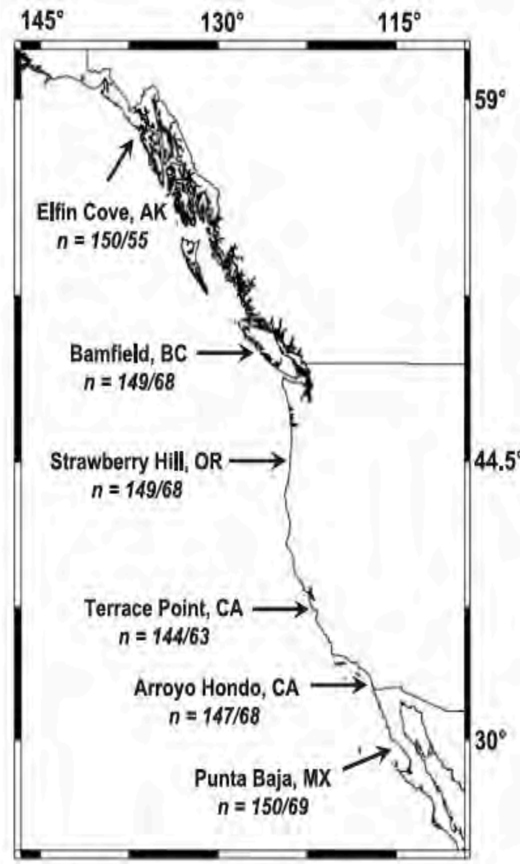
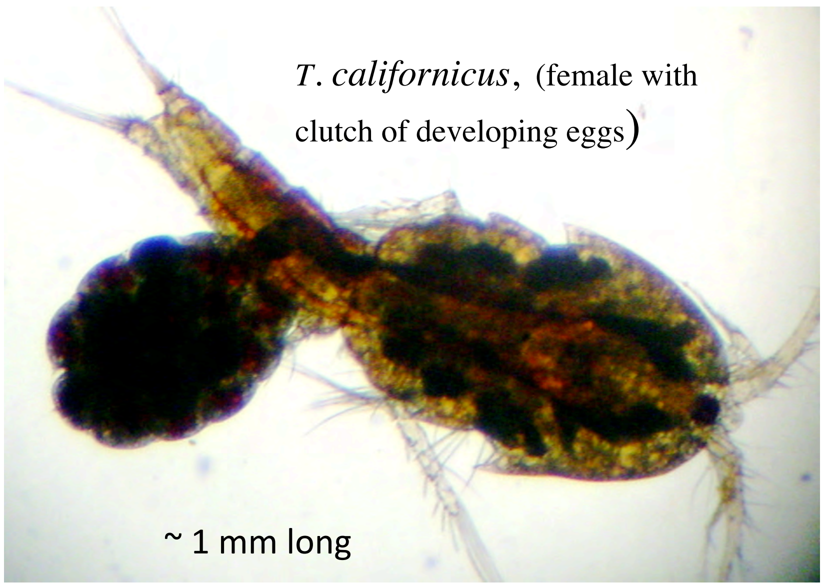


Fig. 1 *Mytilus californianus* collection sites with the average samples sizes (*n*) for the nuclear markers (allozyme/scnDNA).

A model system for analysis of adaptation and differentiation: the intertidal copepod *Tigriopus californicus*



- Easy to collect from the field
- 3-4 week generation time
- Easy to rear in lab
- Broad physiological tolerances
- Strong geographic population structure

Male clasped to juvenile female

Nauplius larva

T. californicus habitat is patchy along the coast and populations are isolated by stretches of sandy beach.

T. californicus ranges from Alaska to Baja

Populations of *T. californicus* show strong geographic divergence even on local scales

PGI polymorphism shows sharp local differentiation (1979): 3 alleles, two are "private"

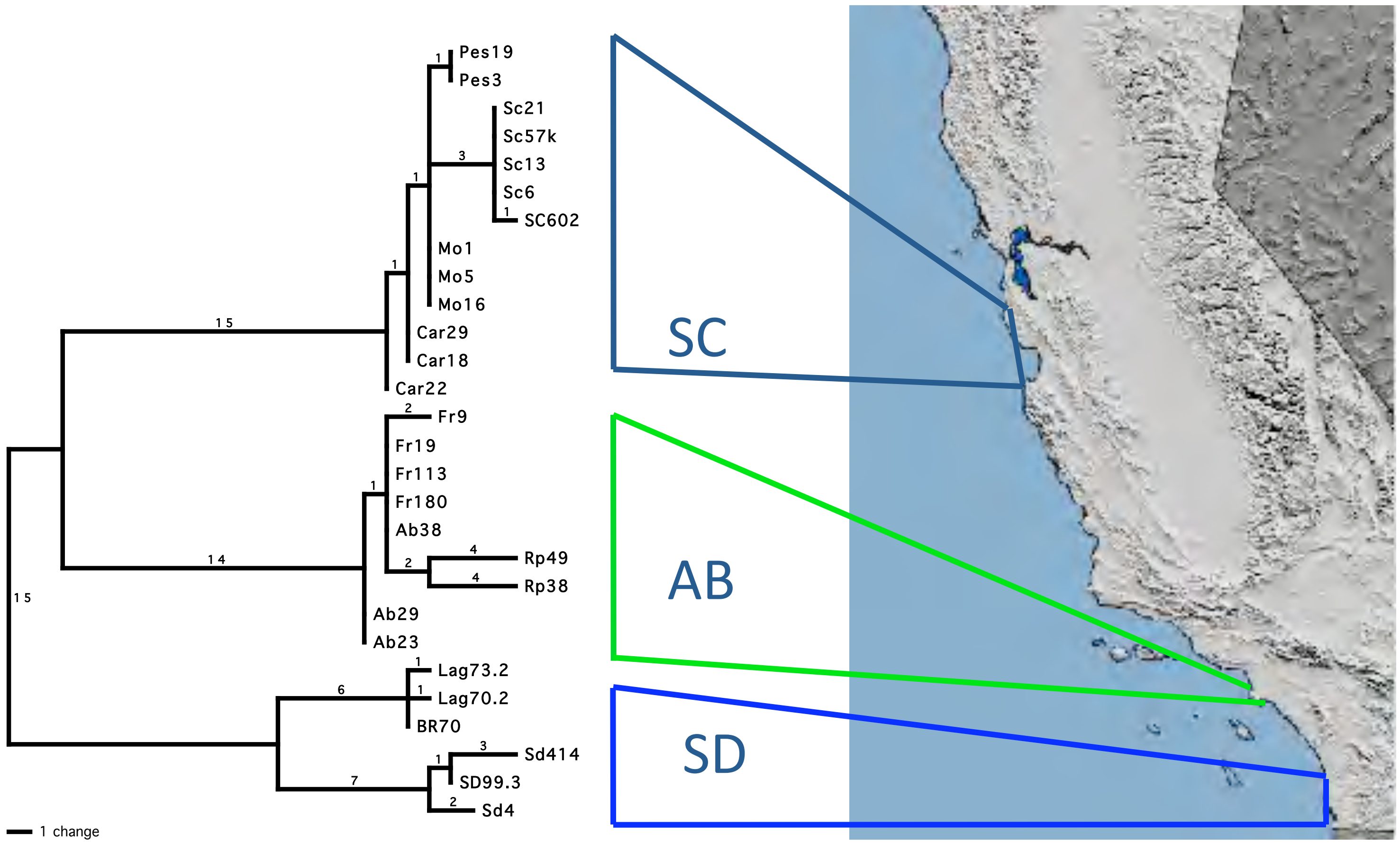
Genetic divergence is stable over time

(so far, 27 years of sampling)

Burton et al. 1979, MEPS
Burton 1997, Evolution
Flowers and Burton, in prep.

Deep divergence between regional mtDNA clades:

1) Long history of population isolation
2) Little contemporary gene flow



> 15% nucleotide divergence among clades at COI

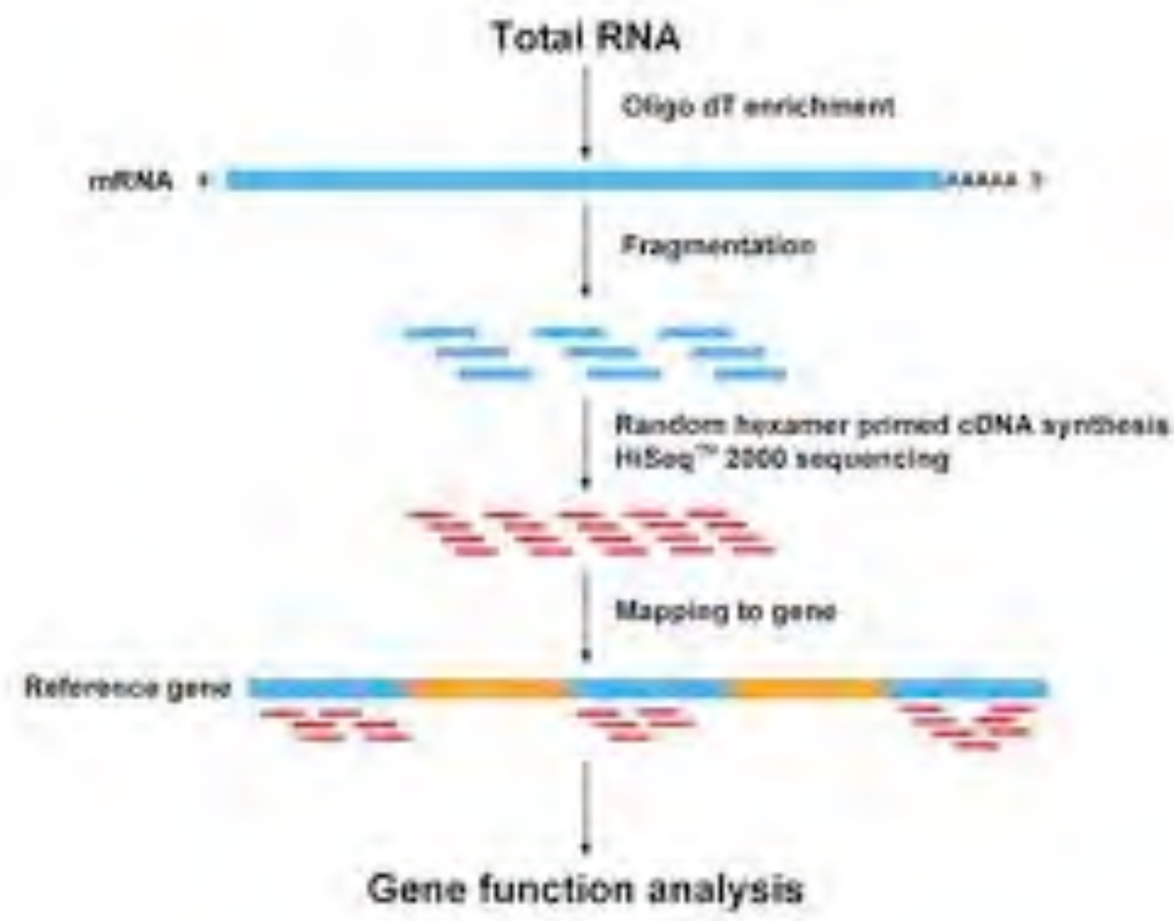
We have (amazing) tools to observe *and manipulate* genetic variation and gene expression

(You used to have to be smart… or work on a “model” organism)

Ocean acidification alters skeletogenesis and gene expression in larval sea urchins. O'Donnell et al. 2010 MEPS

Gene name	Gene ID	Fold change	p	Protein information
Metabolism				
Suclg1	SPU_025397	–1.79 → –1.25	0.0247	Succinyl-CoA synthetase
Atp5b	SPU_005296	–1.78 → –1.24	0.0256	ATP synthase, F1 complex, beta subunit
Slc25a4	SPU_014660	–1.78 → –1.24	0.0274	ADP,ATP carrier protein 1
Biomineralization				
SM30-like	SPU_027906	–2.01 → –1.41	0.0036	Spicule matrix protein
Osteonectin	SPU_028275	–1.97 → –1.38	0.0050	Mammalian SPARC-related gene
Cellular stress & defensome				
HIP1	SPU_010488	–1.72 → –1.20	0.0439	Chaperone cofactor
Fmo5	SPU_028586	–1.97 → –1.37	0.0056	Flavoprotein monooxygenase
Keap1	SPU_011306	–2.08 → –1.45	0.0021	Oxidative stress transcription
Aldh1-like1	SPU_002650	–2.46 → –1.72	0.0001	Oxidoreductase
Calcineurin b	SPU_017036	–1.55 → –1.08	0.0044	Activates transcription of IL-
Acid-base & ion regulation				
Slco3a1	SPU_005806	–2.33 → –1.62	0.0002	Organic anion transporter
Atp8a1	SPU_019141	–1.73 → –1.21	0.0387	Class I, type 8 ATPase
Atp2a1	SPU_006779	+1.81 → +1.26	0.0210	Ca2+ ATPas
Protein synthesis—translational control				
eEF3e	SPU_007226	–1.63 → –1.14	0.0827	Translation initiation factor
eEF1Bδ	SPU_000960	–1.79 → –1.25	0.0255	Translation elongation factor
eEF1Bγ	SPU_002587	–1.70 → –1.18	0.0520	Translation elongation factor

RNA-seq -- high throughput sequencing of the entire transcriptome



Compare populations --
levels of divergence across all transcribed genes

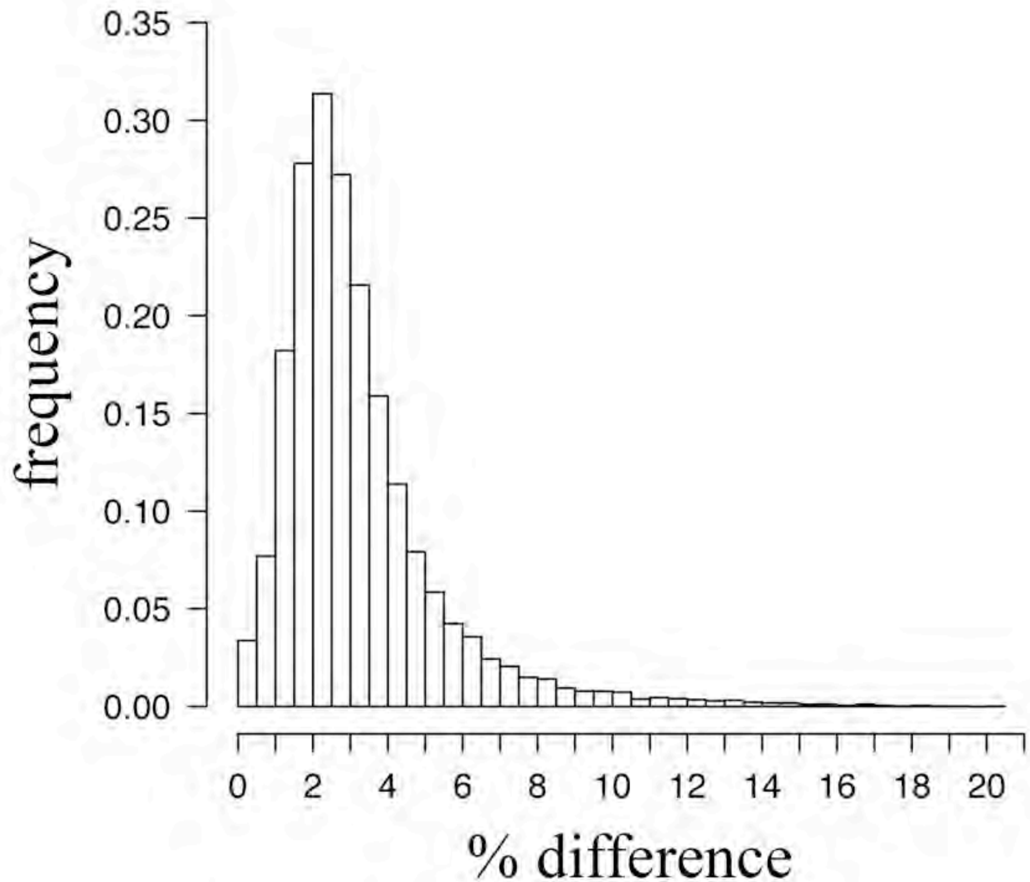
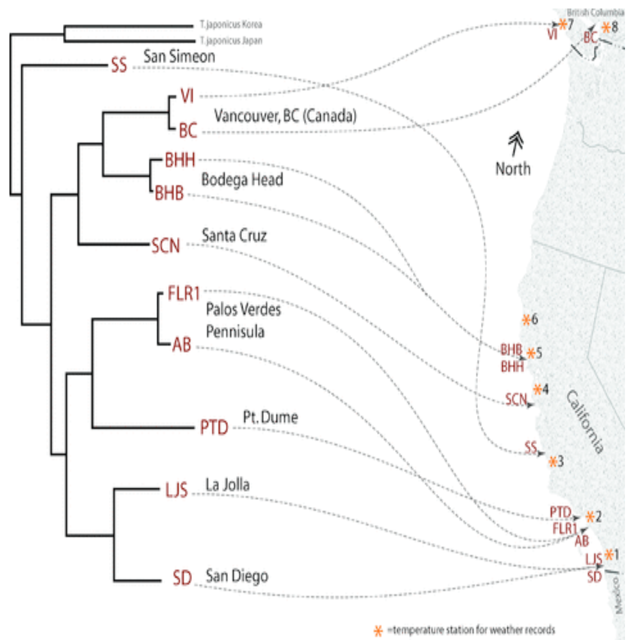
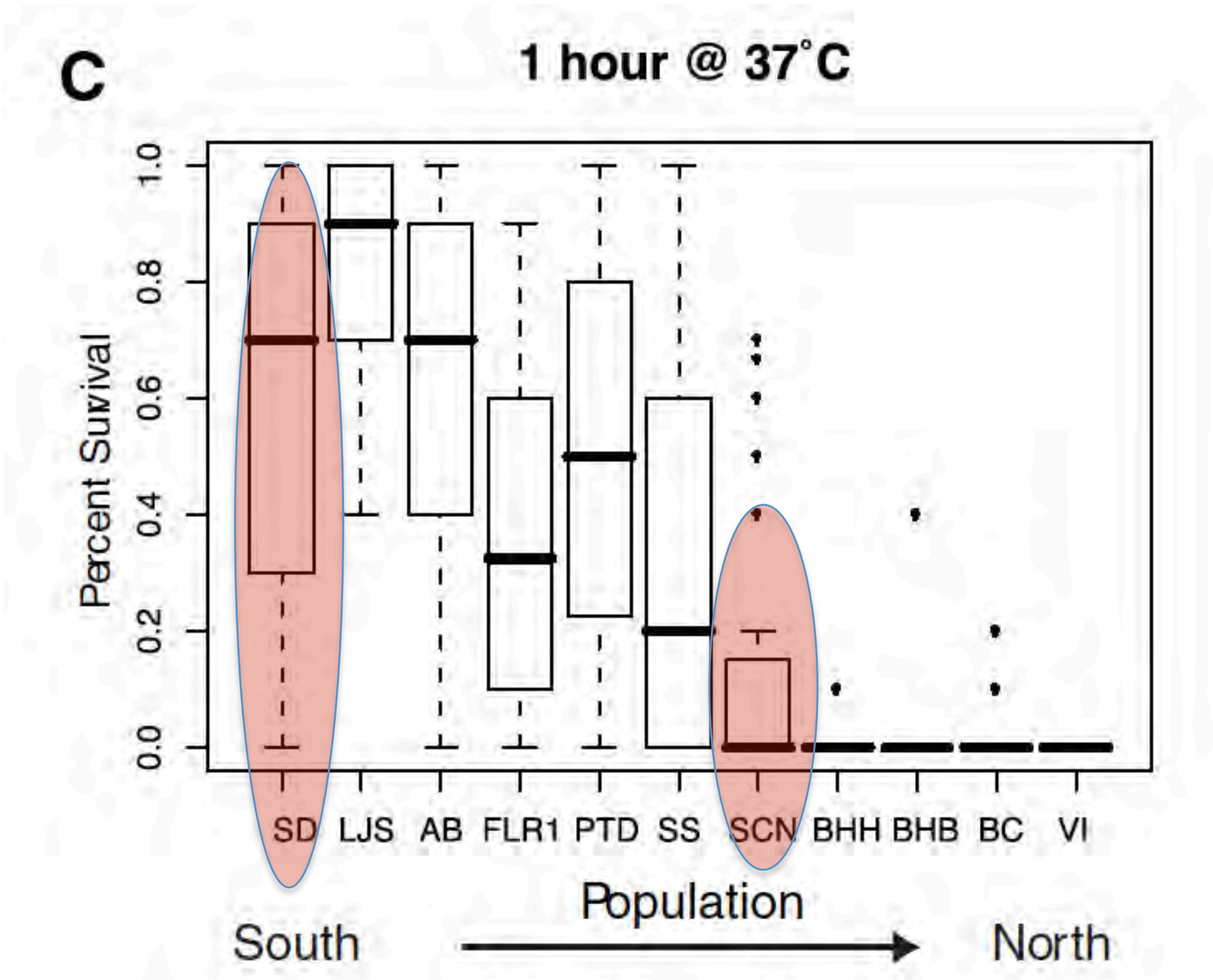


Fig. 3 Nucleotide sequence divergence between SD and SC orthologous unigenes. Alignments were based on reciprocal BLASTN searches.

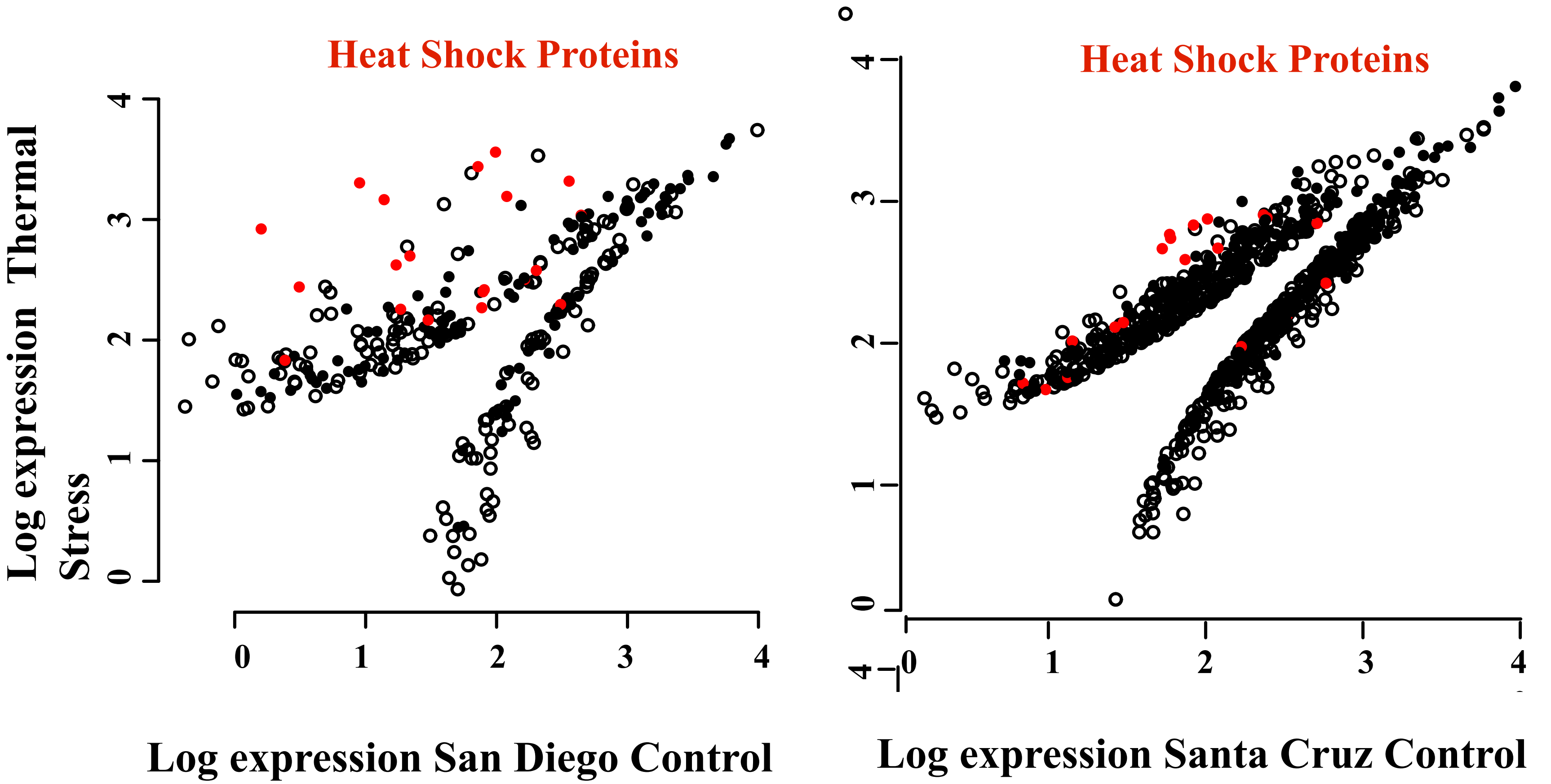




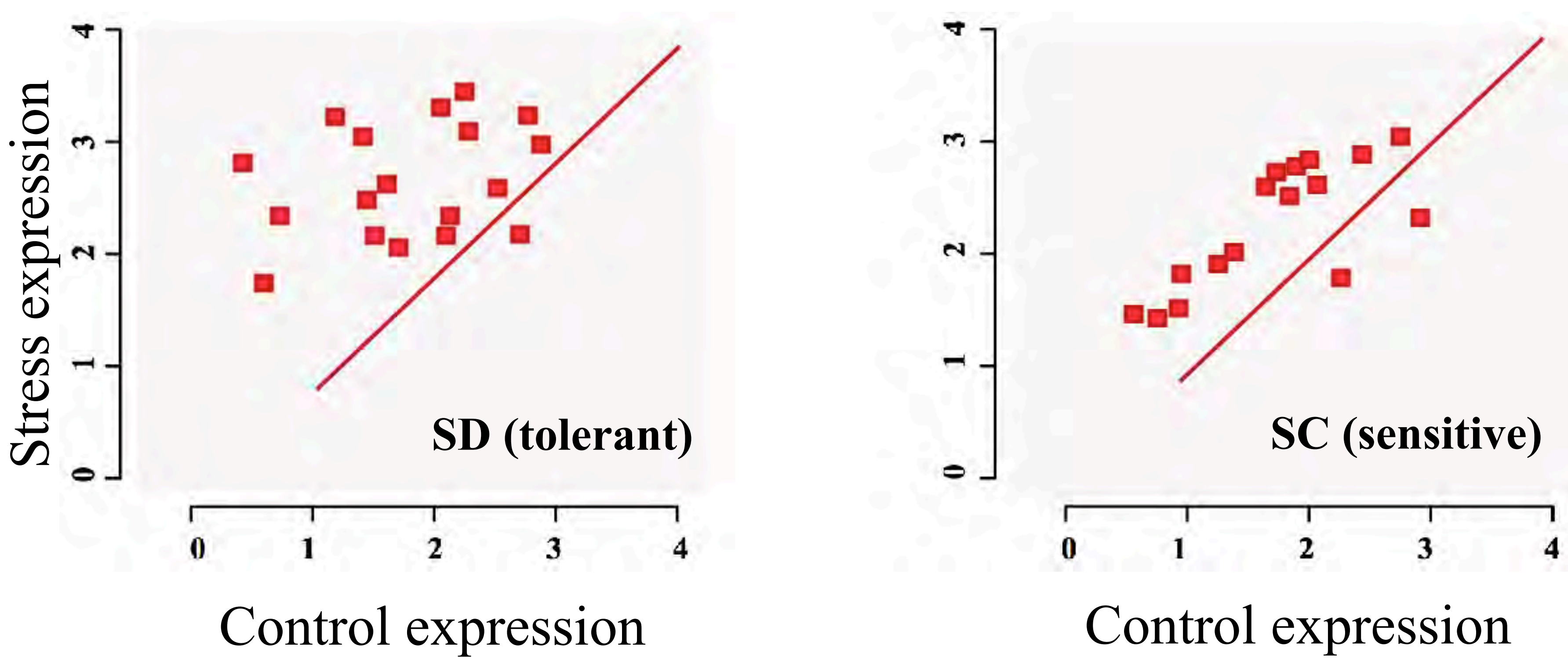
POTENTIAL FITNESS TRADE-OFFS FOR THERMAL TOLERANCE IN THE INTERTIDAL COPEPOD *TIGRIOPUS CALIFORNICUS*

Christopher S. Willett, *Evolution* 2010.

SAN DIEGO RNA-Seq Thermal Stress
(significantly up- and down regulated genes)



Population differences in physiological response – gene expression

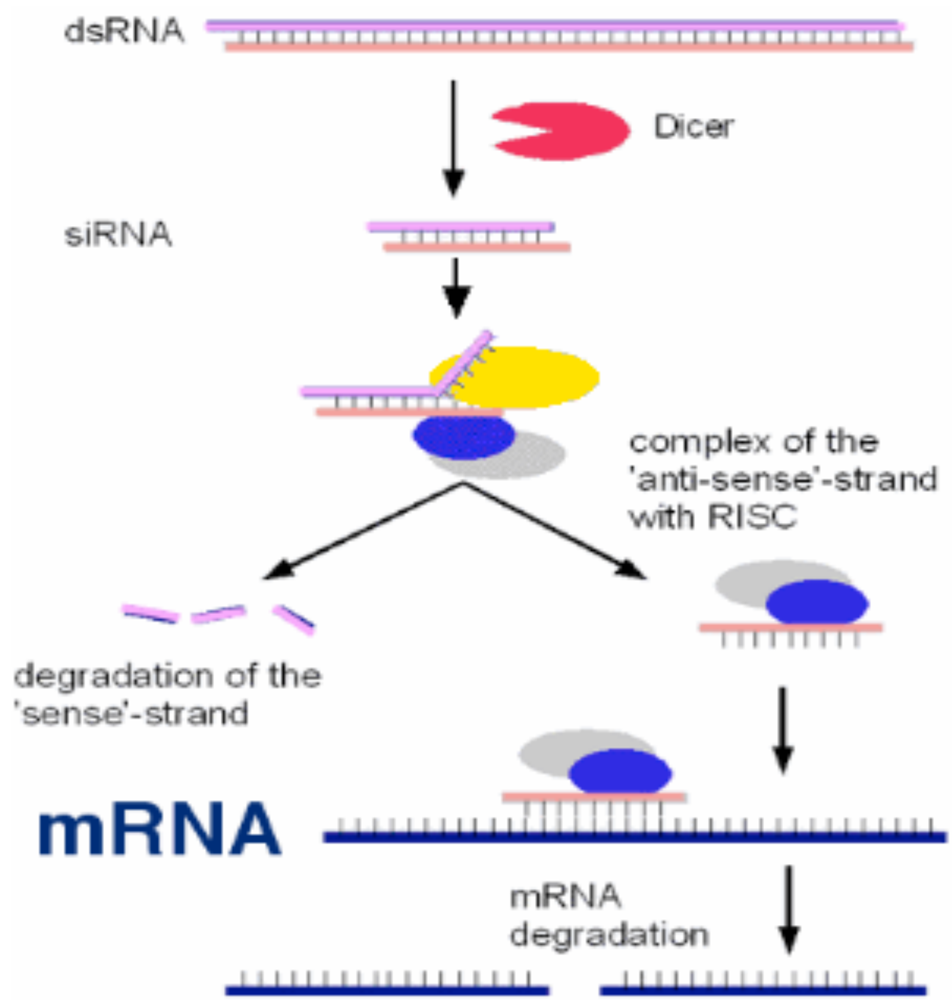


Next step…

- Selection experiment –
  - does SC evolve tolerance (Morgan Kelly, grad student at UC Davis found a positive response)
  - Does SD evolve increased tolerance (Morgan found negative response)

Mix populations (simulating gene flow) and select – look at changes in expression and SC/SD mix

Another trick…
Suppression of gene expression with RNAi



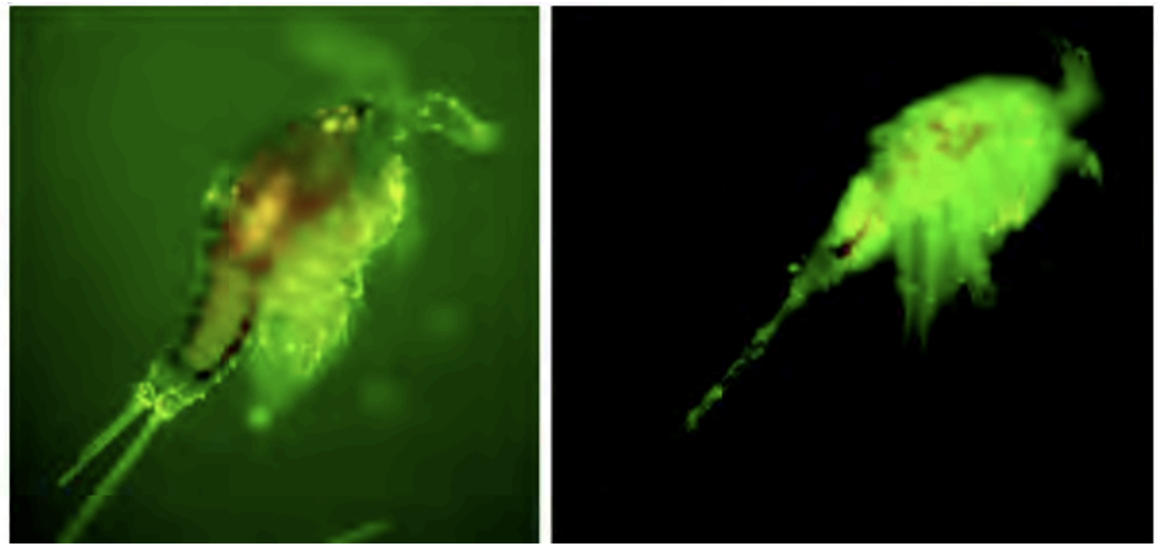
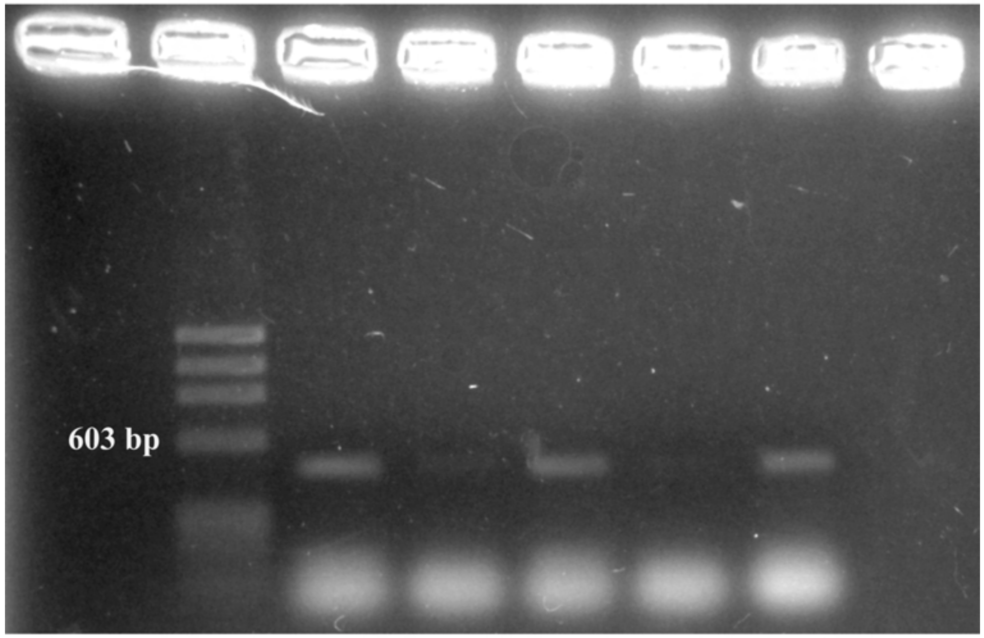


Figure 4. Introduction of dsRNA by electroporation.
Left: Untreated adult *T. californicus*.
Right: RNAi treatment of adult *T. californicus* using fluorescein-labeled HSP70 dsRNA.

RNA interference

PCR of HSP70 gene fragment in *T. californicus* showing allele-specific knockdown using RNAi



Ladder | Control | San Diego treated with SD HSP70 | San Diego treated with SC HSP70 | Santa Cruz treated with SC HSP70 | Santa Cruz treated with SD HSP70

24 hr post electroporation; Heat-shock 35C 90min

	Elution Buffer	Lac-Z (0.1 uM)	HSP70 (0.1uM)
Survivors	17	25	0
Dead	12	17	72
% Survival	59%	60%	0%

PHYSIOLOGICAL RESPONSES

Ronald S Burton

Scripps Institution of Oceanography

March, 2010

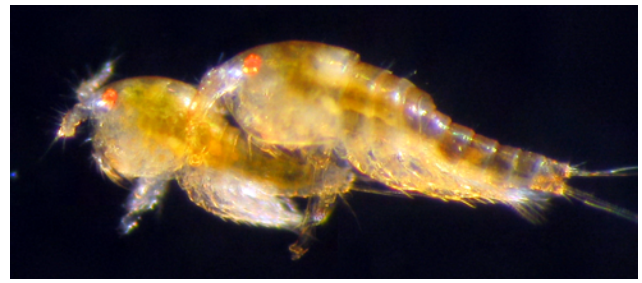
Genomic and Evolutionary Approaches to Understanding Physiological Response to Ocean Acidification

Where we are:

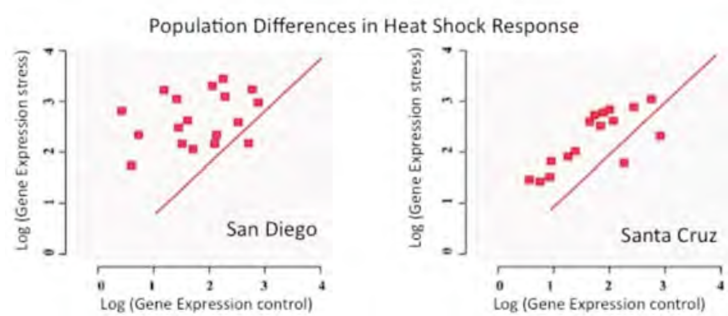
- Essentially all physiological OA studies in metazoans involve only acute stress responses
- Extensive genetic variation within and between populations is largely ignored
- Strong focus on relatively few species

Where we probably should go:

- ***Experimental evolution studies***, covering multiple generations, to assess potential for adaptation to OA and other stresses
- ***Population genomic studies*** indicate the potential for local adaptation
- Broaden the range of species studied
- Employ new technologies and examine both *gene expression* ***and*** *protein diversity*



The intertidal copepod *Tigriopus californicus: a model system for genomic and evolutionary* responses to environmental change



The more pronounced up-regulation of heat shock genes in the San Diego population may explain its higher stress tolerance compared to the Santa Cruz population.

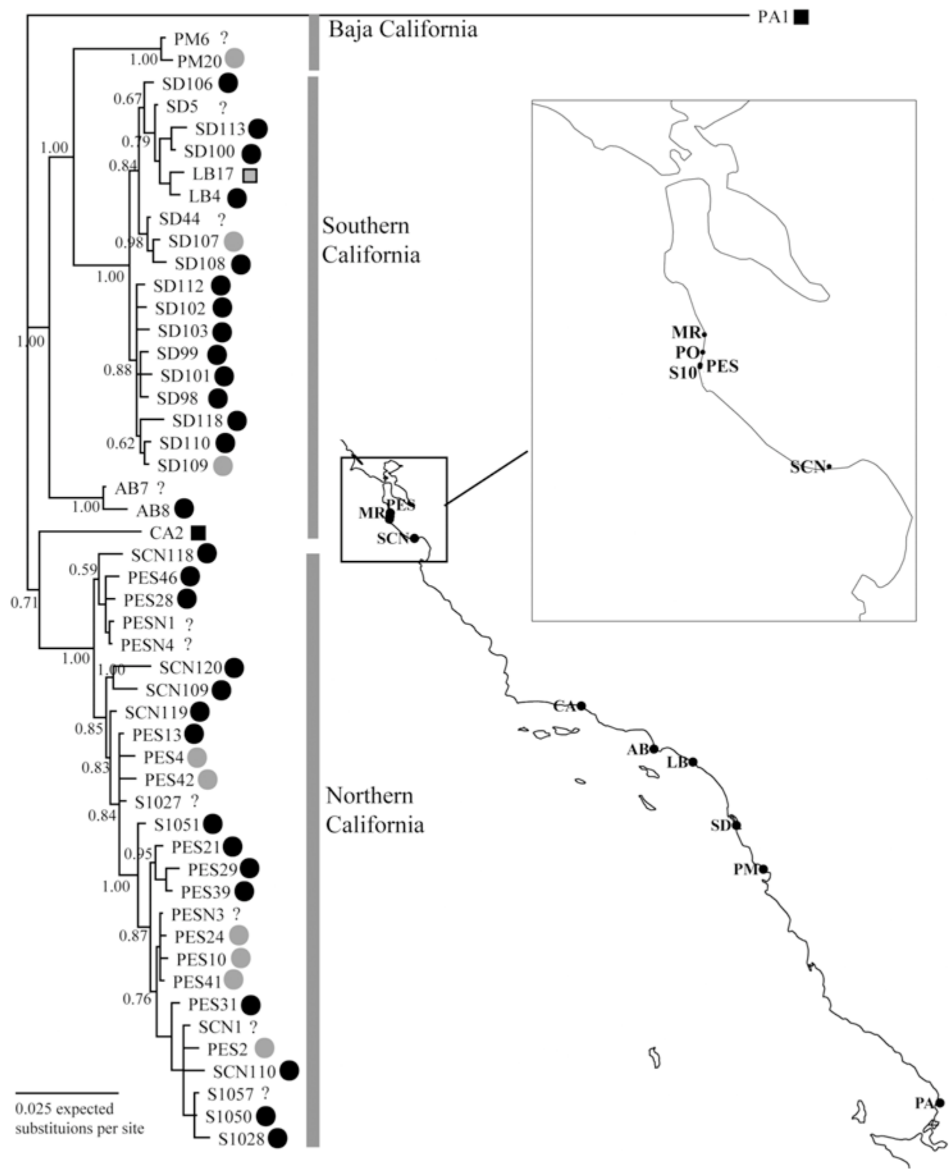
Individuals may acclimate to environmental change

- survival (not enough!)
- reproduction
- life and death

Populations may evolve in response to environmental change

- survival (until better times)
- reproduction
- persistence and extirpation

Variation in a protein (PGI) in *Tigriopus californicus*



What are the most rapidly evolving genes?

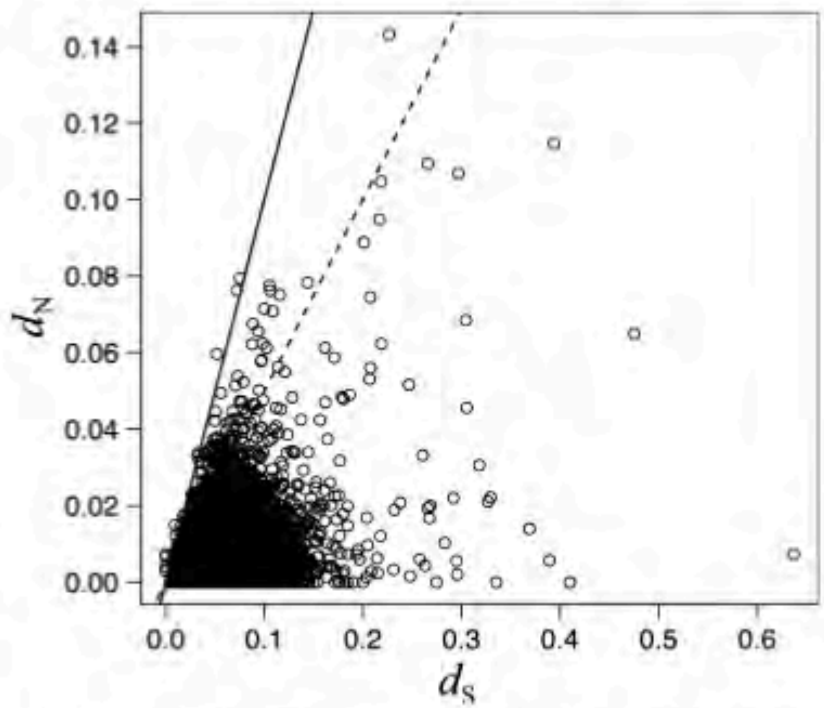


Fig. 4 Relationship between the number of nonsynonymous substitutions per nonsynonymous site (d_N) and the number of synonymous substitutions per synonymous site (d_S) for 5897 protein-coding regions of *Tigriopus californicus*. Analysis was performed on alignments of San Diego and Santa Cruz orthologous sequences, using the method of Yang & Nielsen (2000). The solid line shows the threshold of $d_N/d_S = 1$, while the dashed line marks the less conservative threshold of $d_N/d_S = 0.5$.

Substantial "control region" variation between populations

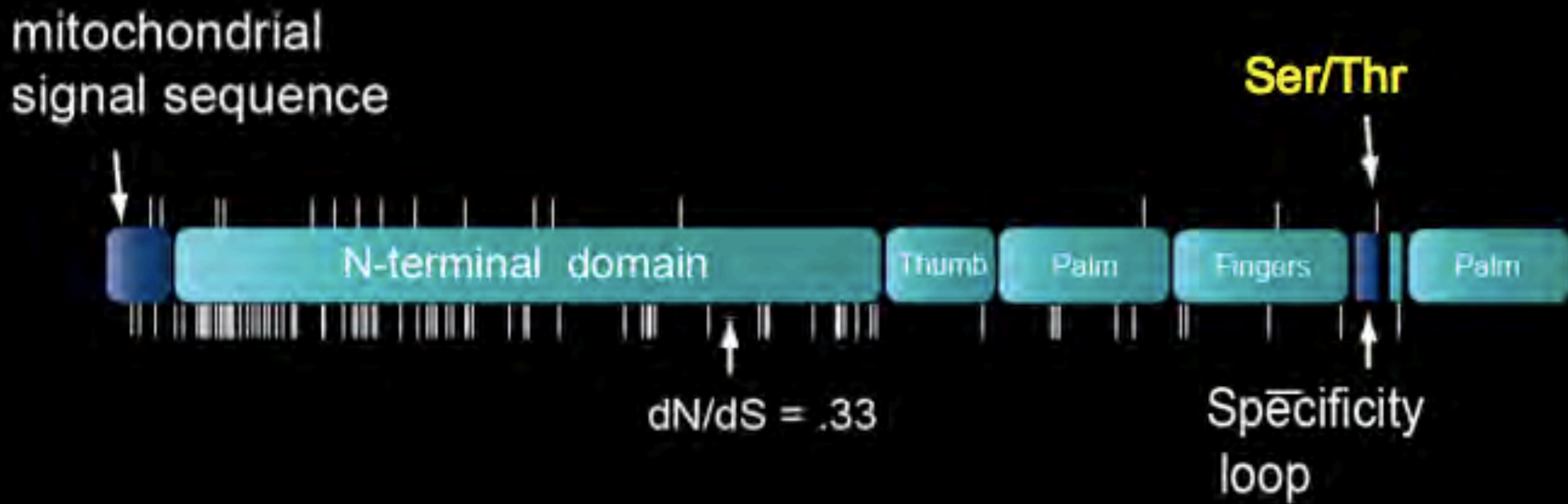
```
SD    GGCCATTTGGACGTTTGCGTGTAATTAATTTTTGAAGATTAAATGGGCG
      |||| |  ||||   ||||||| ||||||||||||||||||||||||||
SC    GGCCCTCCGGACAGGTGCGTGTCATTAATTTTTGAAGATTAAATGGGCG

SD    CCGATCCGCGGTCGGGGGATGTTTAGA*COI CODING......
      | || ||| ||||  |||||||| |||
SC    CTGAGCCGTGGTC--GGGATGTTAAGA*COI CODING......
```

mtRPOL binding   mtDNA transcription initiation

So necessary variation in mtRPOL and mtDNA exists…

Extensive amino acid divergence in mitochondrial RNA polymerase (mtRPOL) in *Tigriopus*, but no evidence of positive selection



Flowers and Burton, in prep

Gene expression analysis

- Small number of genes –qPCR
- Genome-scale
  - Microarrays
    - Oligo arrays
    - Spotted cDNA arrays
  - RNA-seq