Differential Expression of a Heat Shock Protein in a Skipper Butterfly, *Erynnis Propertius*

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Why Butterflies?

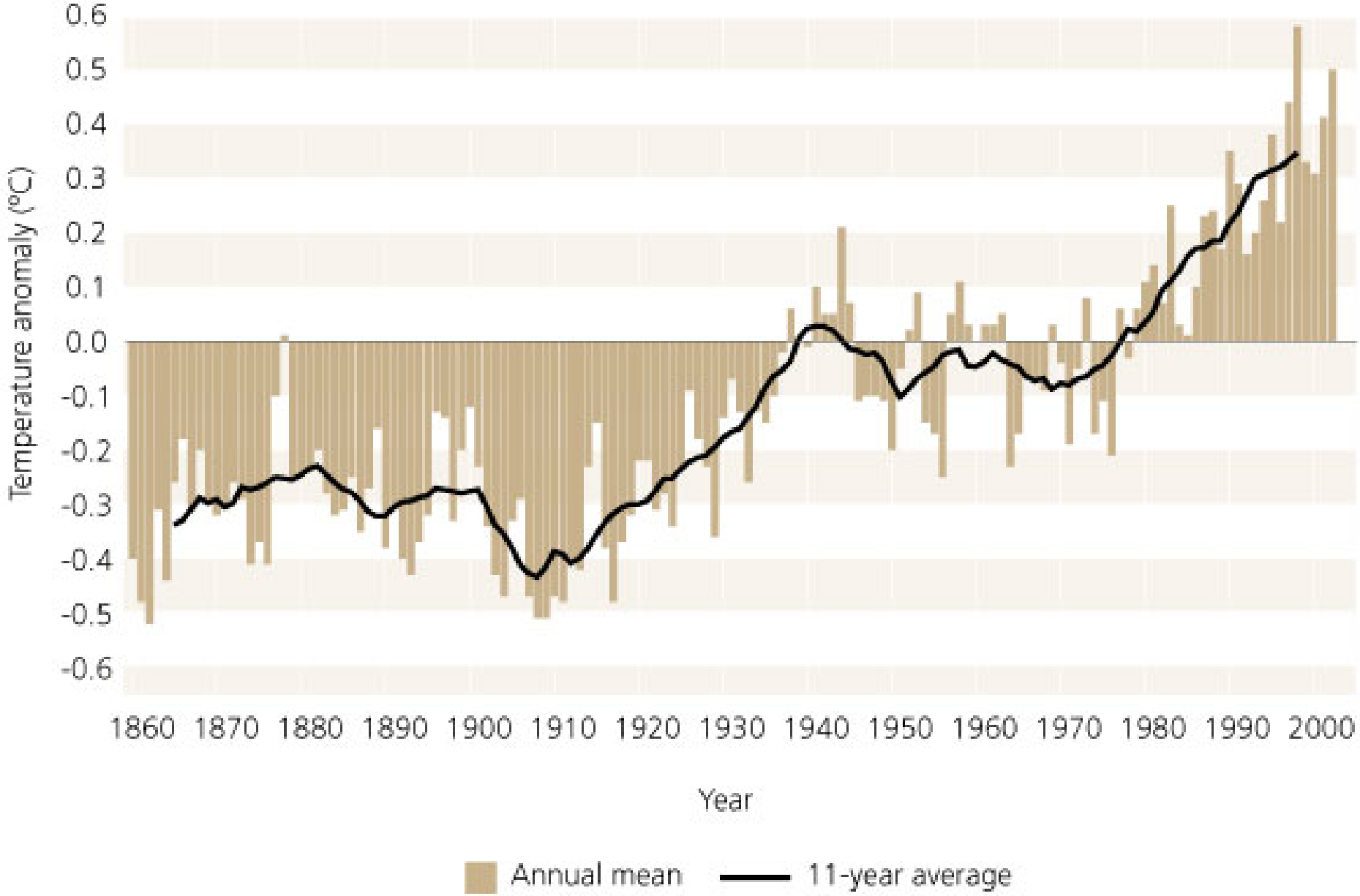
- Sensitive to environmental change
- Predictable and short reproductive cycles
- Easy to spot and catch
- We know a lot about them
- Good model organisms

Previously documented butterfly range shift (Pararge aegeria)

1915-1939 (black) 1940-1969 (red) 1970-1997 (blue)

QuickTime™ and a decompressor are needed to see this picture.

Parmesan et al., 1999



http://www.soe.wa.gov.au/report/fundamental-pressures/climate-change.html

- “In a sample of 35 non-migratory butterflies, 63% have ranges that have shifted north by 35-240 km during this century, and only 3% have shifted to the south.” (Parmesan et al., 1999).

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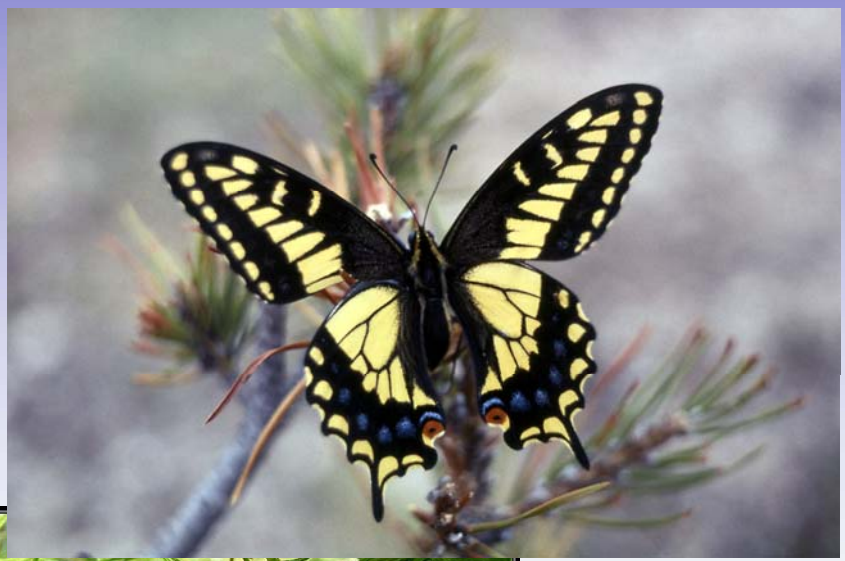
decompressor
are needed to see this picture.

Parmesan et al., 1999

Our Study Organisms



Erynnis Propertius

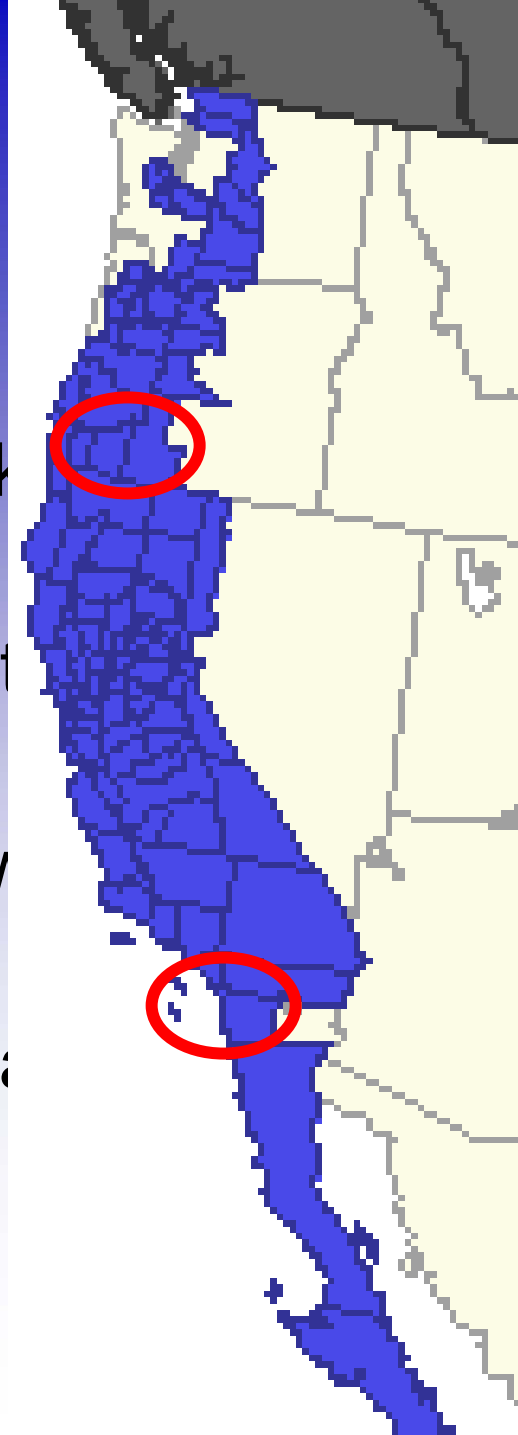
Papilio Zelicaon

Heat shock Proteins (Hsps)

- Protein “chaperones”
- Upregulated in response to a variety of different environmental stresses
- Variety of sizes (110, 100, 90, **70**, 60, 40, and 10 kilodaltons and smaller)

My Experiment

- Identify the 70-kilodalton heat shock
  in Erynnis propertius
- Determine conditions under which it
  expressed
- Look for differential expression betw
  Oregon and California populations
- Improve our understanding of the na
  climate-forced insect range shift



My Methods

- Two treatments:
  - Heat shocked at 41º C
    - Six Oregon bugs
    - Five California Bugs
  - Not heat shocked:
    - Five Oregon bugs

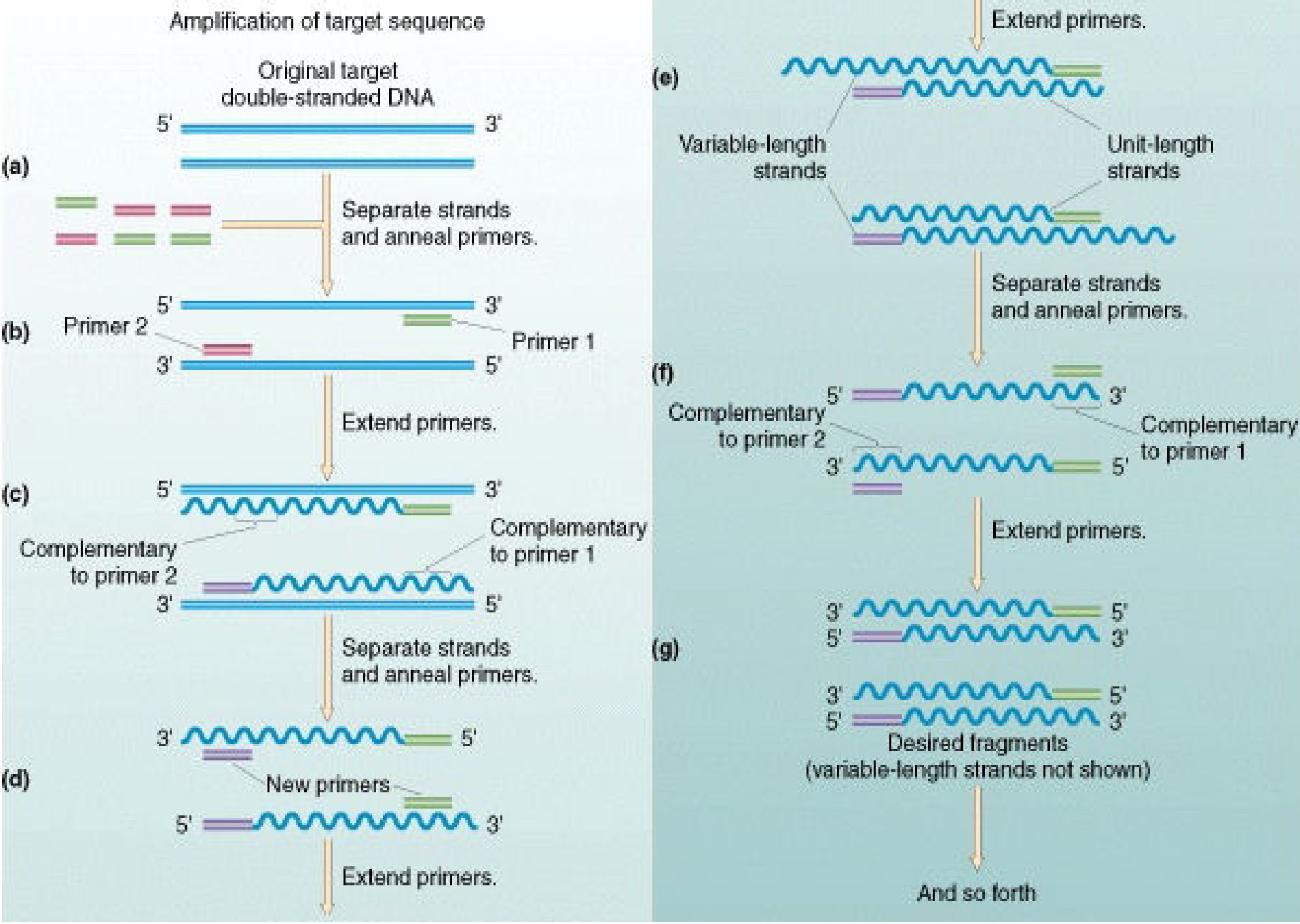
My Methods

- Isolate RNA from treated caterpillars
- RT-PCR: Convert RNA to cDNA and pick out gene using personally designed primers
- Quantify expression using gel electrophoresis

Reverse Transcriptase Polymerase Chain Reaction

- Within a PCR reaction:
  - Use random primers and reverse transcriptase enzyme to convert mRNA to cDNA
  - Use gene-specific primers to amplify gene of interest from cDNA
  - Also amplify universally expressed housekeeping gene for normalization (18S)

http://www.mun.ca/biology/scarr/Gr12-26.html


Amplification of target sequence
Original target double-stranded DNA
5'
3'
(a)
Separate strands and anneal primers.
(b)
Primer 2
Primer 1
Extend primers.
(c)
Complementary to primer 2
Complementary to primer 1
Separate strands and anneal primers.
(d)
New primers
Extend primers.
(e)
Extend primers.
Variable-length strands
Unit-length strands
Separate strands and anneal primers.
(f)
Complementary to primer 2
Complementary to primer 1
Extend primers.
(g)
Desired fragments (variable-length strands not shown)
And so forth

Primers for Hsp70

- Forward Primer: GTGGAGATCATCGCGAACGA
- Reverse Primer: AAGGGCCAGTGCTTCATGTC

ClustalW:

QuickTime™ and a
decompressor
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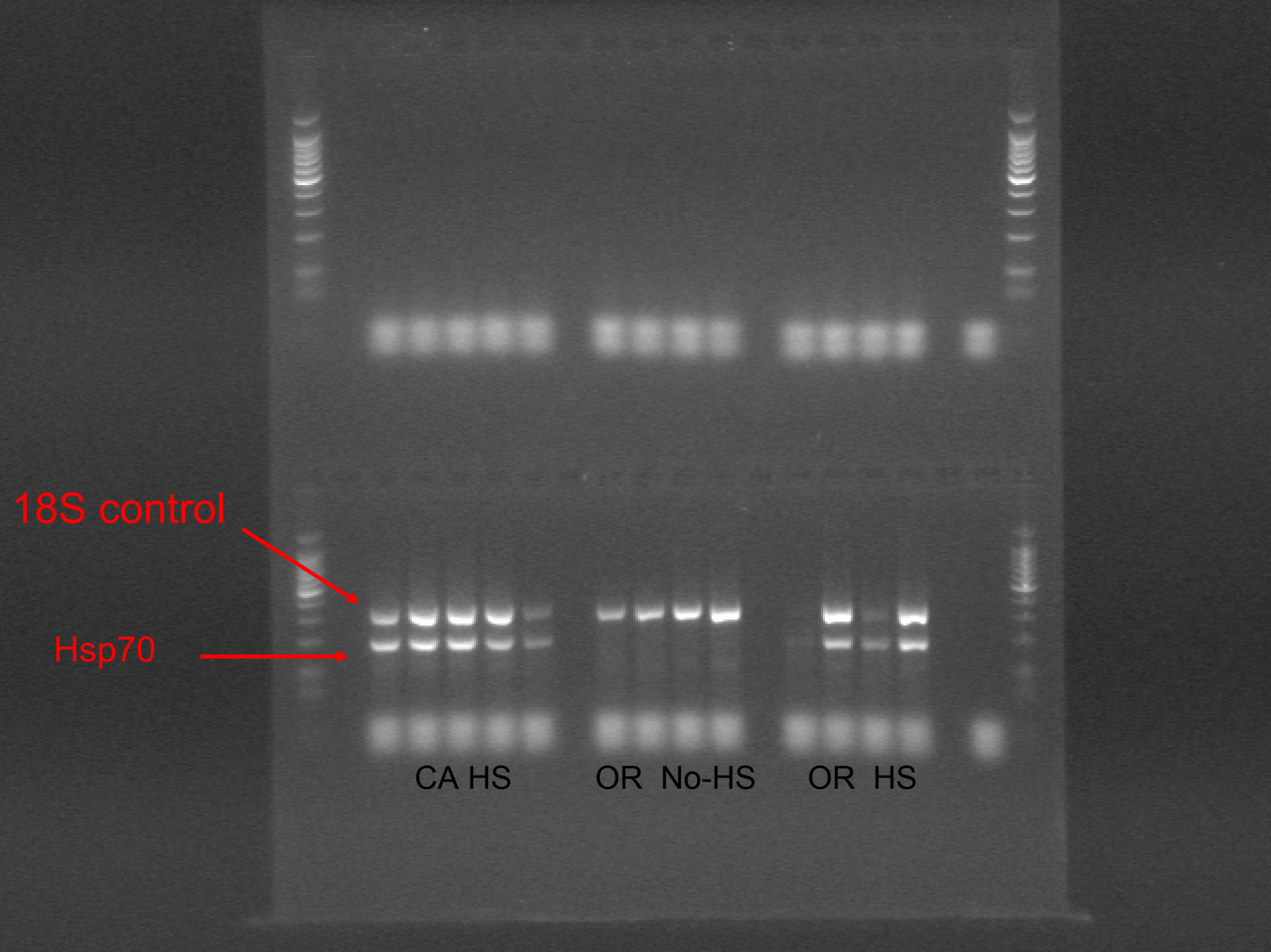
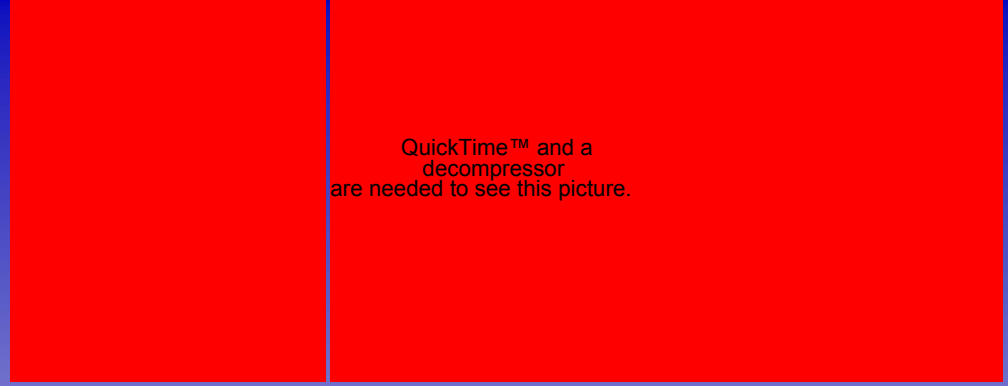
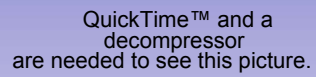
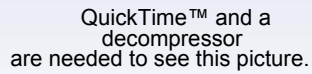

18S control
Hsp70
CA HS
OR No-HS
OR HS



California Heat Shocked



Oregon Non-Heat Shocked



Oregon Heat Shocked

Future Work

- Utilize microarray data
  - Is 18S really equally expressed?
  - Other factors? Cold stress? Desiccation?
  - Catalogue expression of other HSPs, Hsp70 in different instars

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