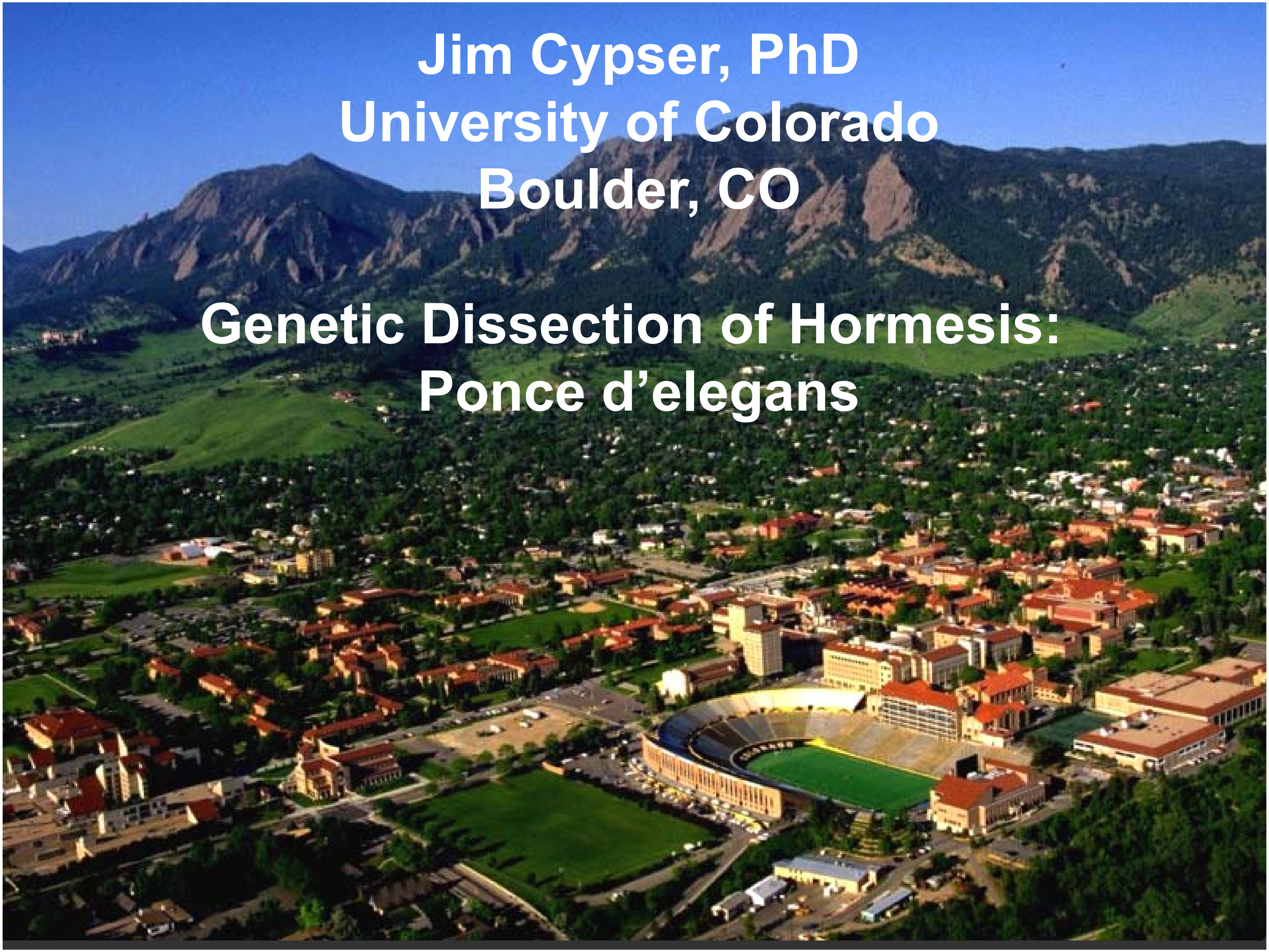



Jim Cypser, PhD
University of Colorado
Boulder, CO


# Genetic Dissection of Hormesis: Ponce d’elegans

# OVERVIEW

## How can an invertebrate be used to study hormesis?

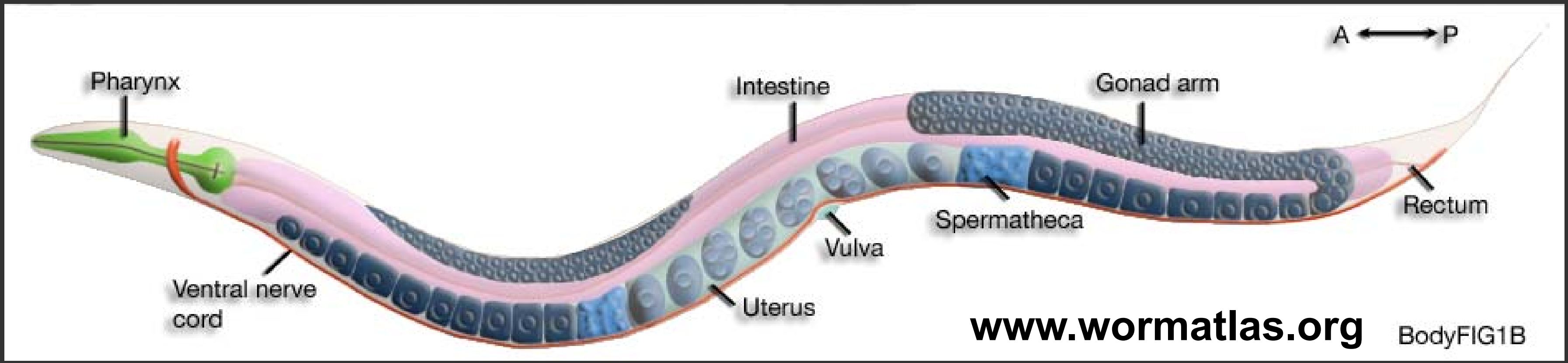
## Introduction to hormesis in the worm *C. elegans*

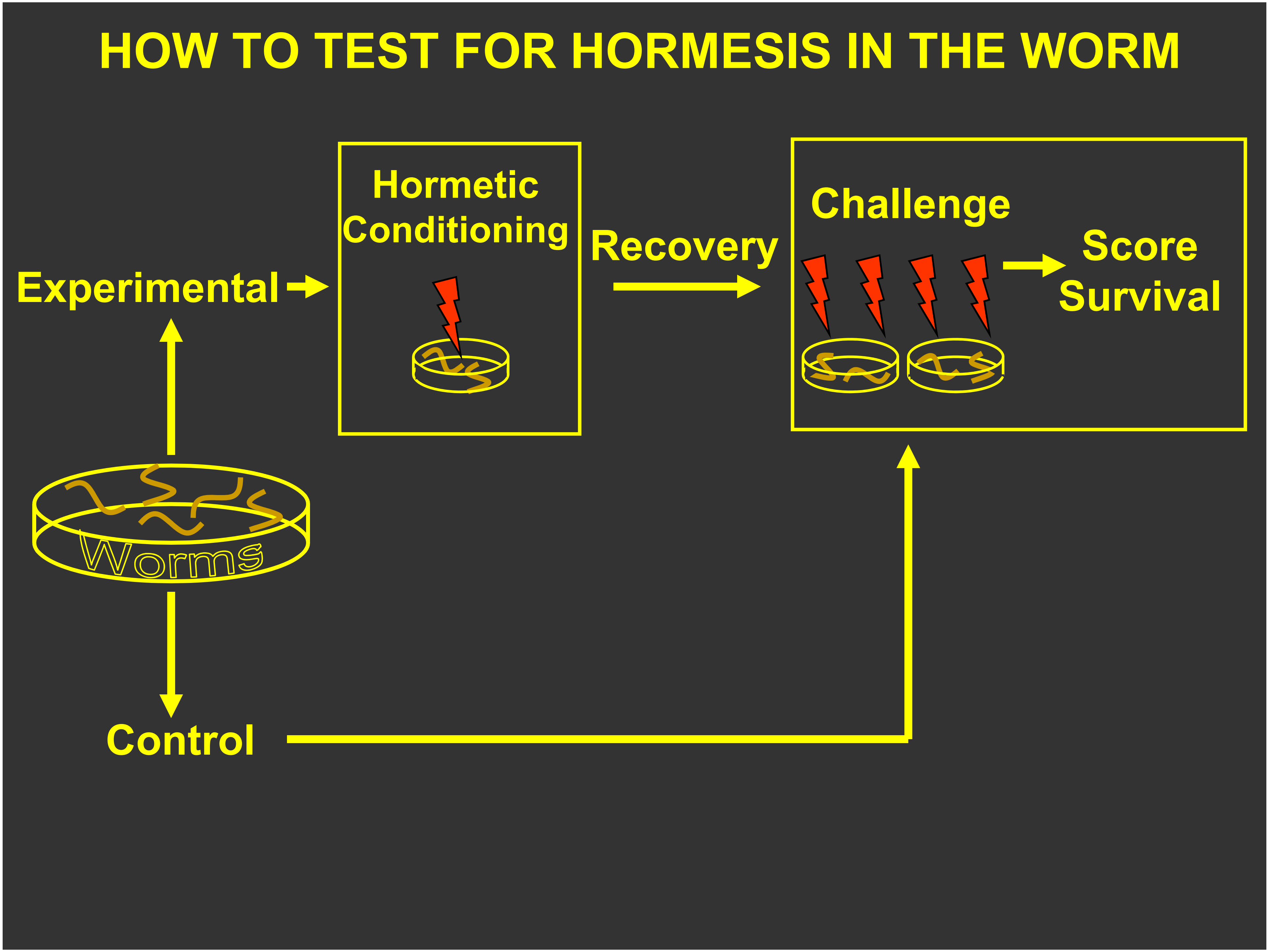
- Genetic dissection of heat hormesis
- Individual worm response to heat as a predictor of longevity
- Heritability of worms' response to heat hormesis

# •THE NEMATODE WORM MODEL SYSTEM: *C. elegans*

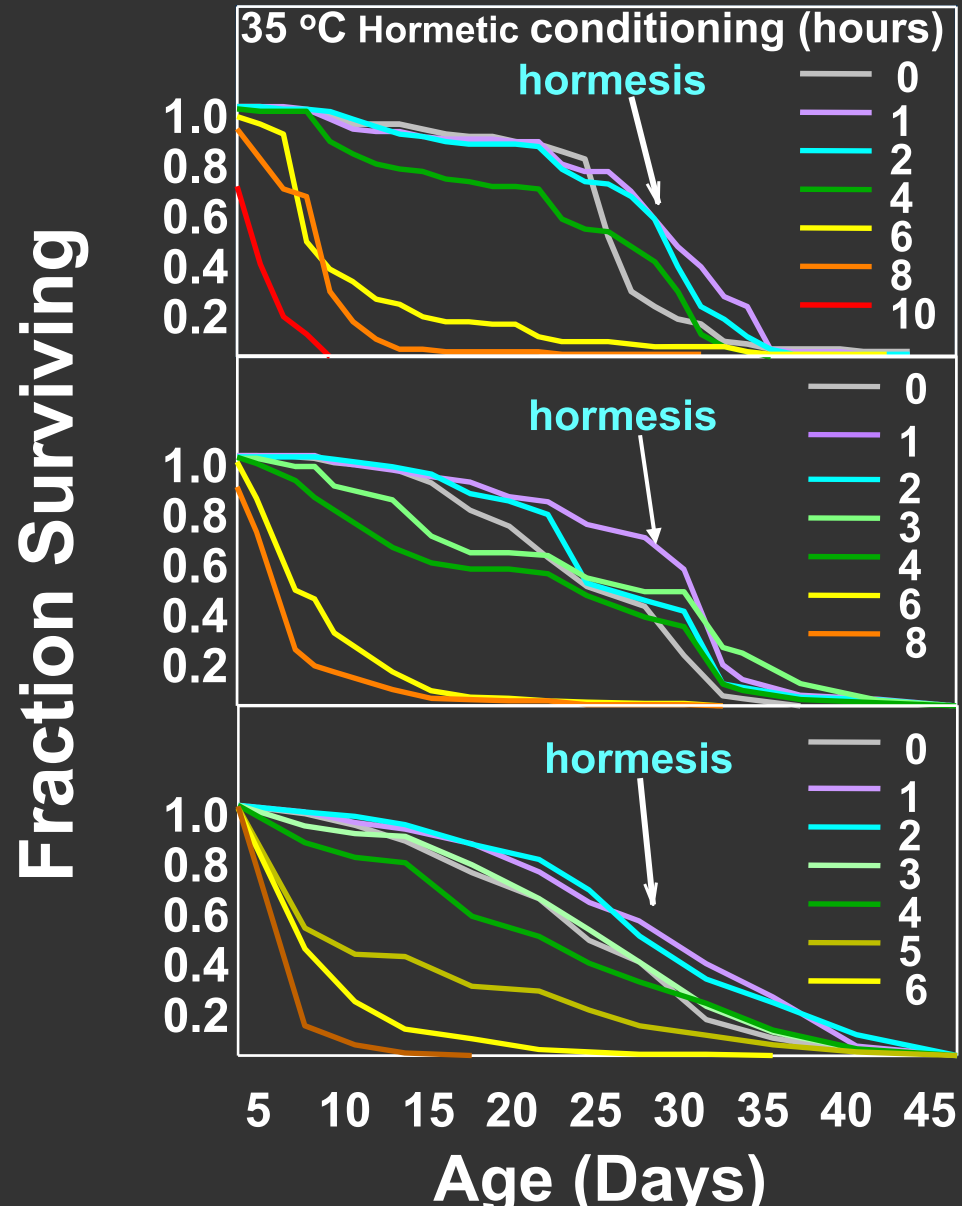
**Anatomy:**

- Only 959 somatic cells in hermaphrodites (self-fertilizing!)
- Major tissues: muscle, hypodermis, neurons, gonads, gut
- Can visualize changes in tissues after stress / aging





HOW TO TEST FOR HORMESIS IN THE WORM
Hormetic Conditioning
Recovery
Challenge
Score Survival
Experimental
Worms
Control

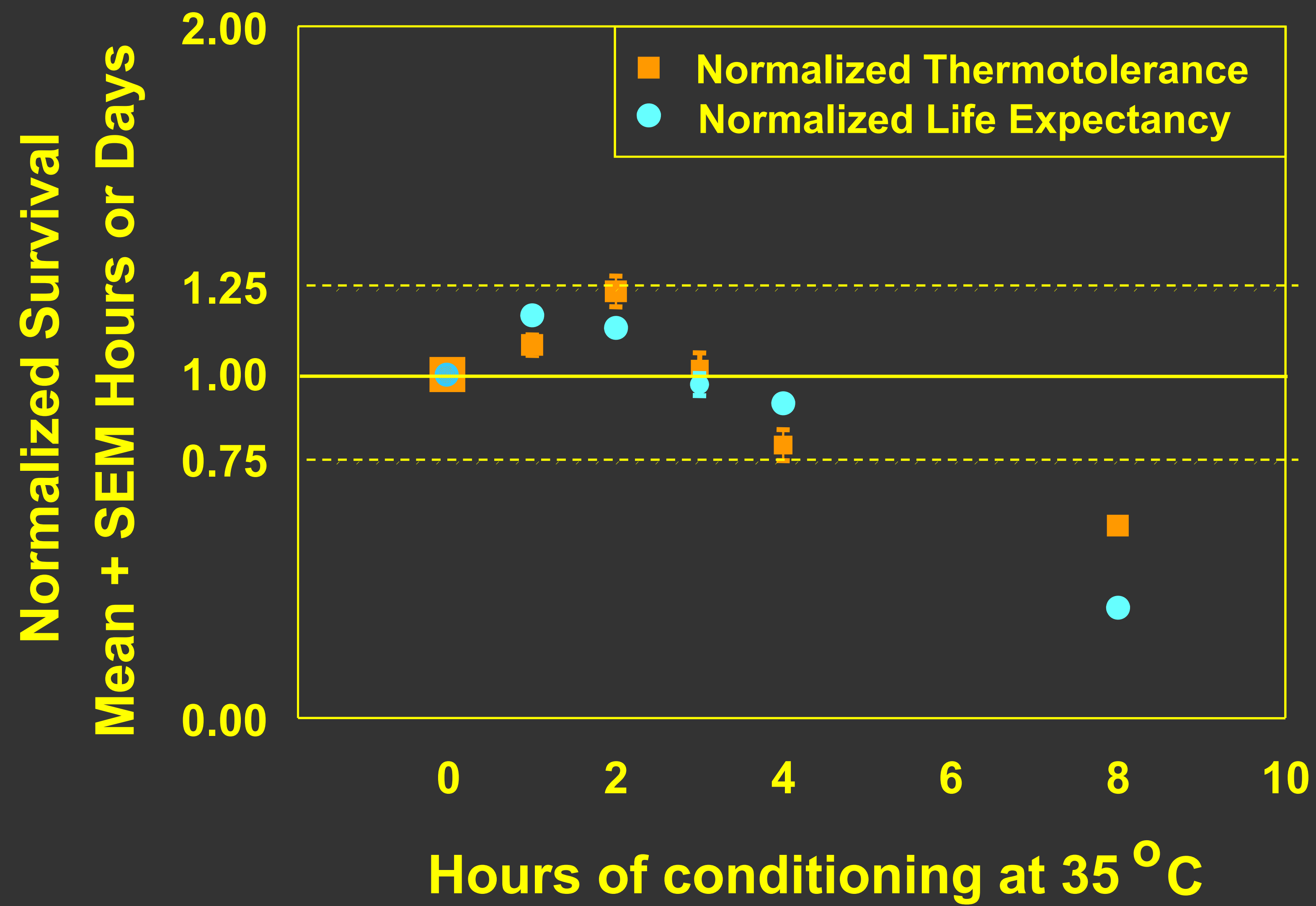
# SURVIVAL OF WORMS AFTER MILD HEAT STRESS




## HORMESIS CAN BE OBSERVED REPRODUCIBLY IN THE WORM

*Michalski et al, Biogerontol*. 2001

# DOSE-RESPONSE CURVES OF HEAT HORMESIS IN THE WORM

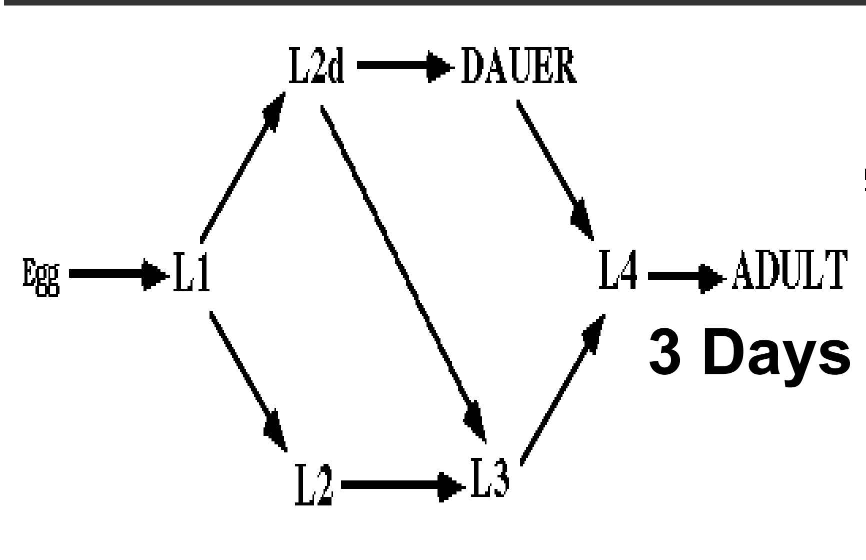



THE WORM DISPLAYS PARALLEL
HORMETIC THERMOTOLERANCE AND HORMETIC SLOWED AGING

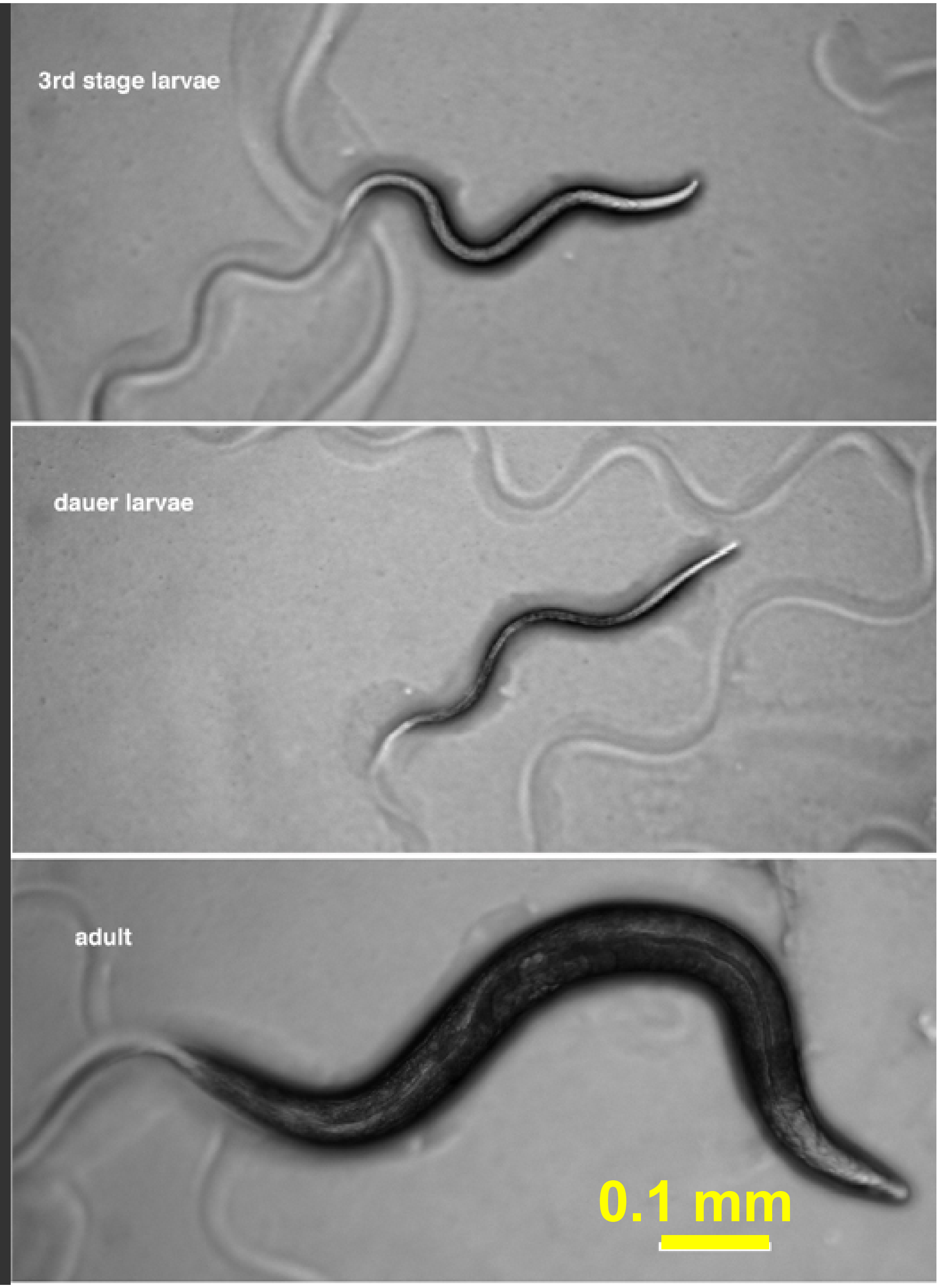
*Cypser et al, J. Gerontol*. 2002

# *Caenorhabditis elegans:* **life cycle**

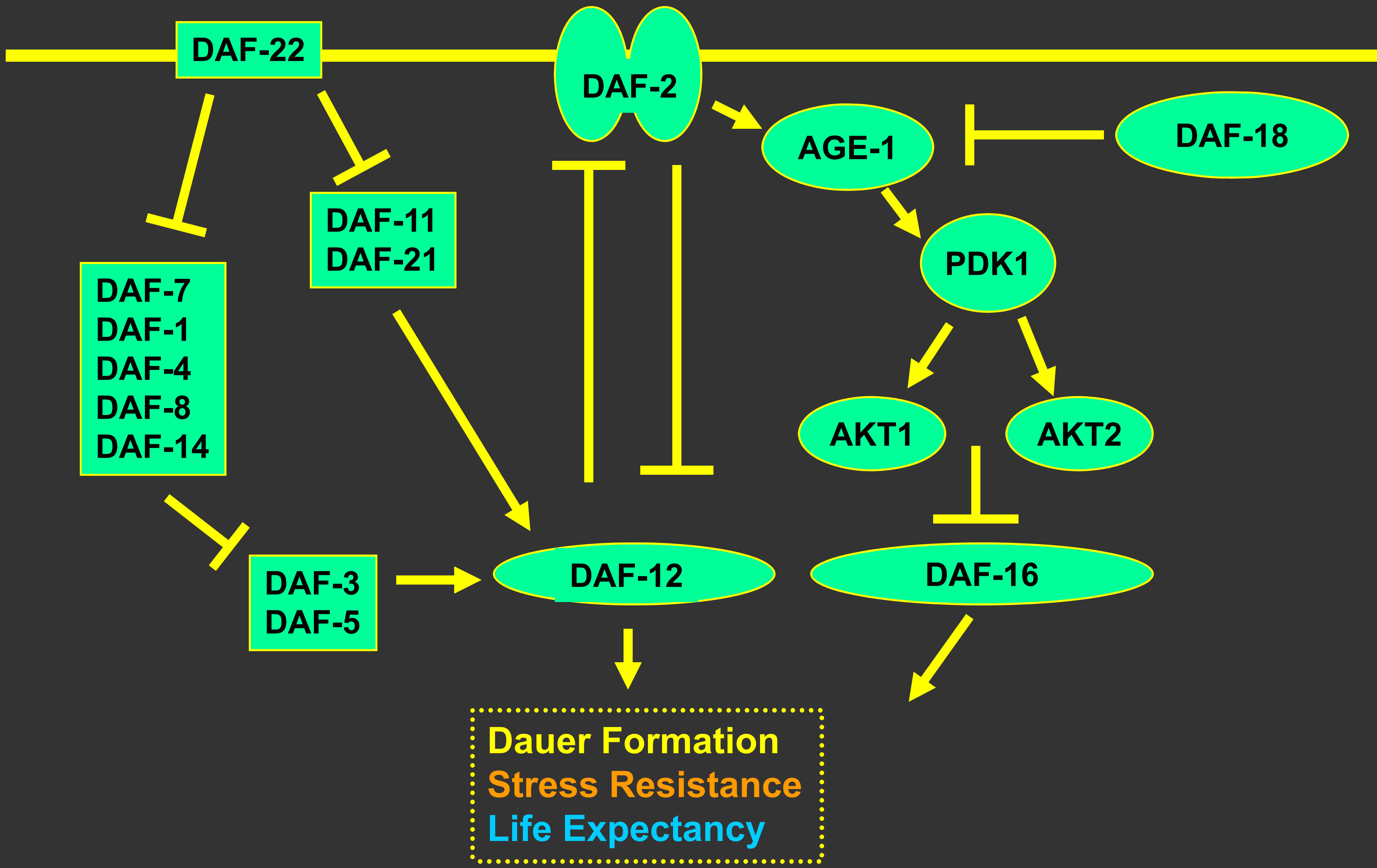
**Migratory (dauer) phase**




**Reproductive phase**

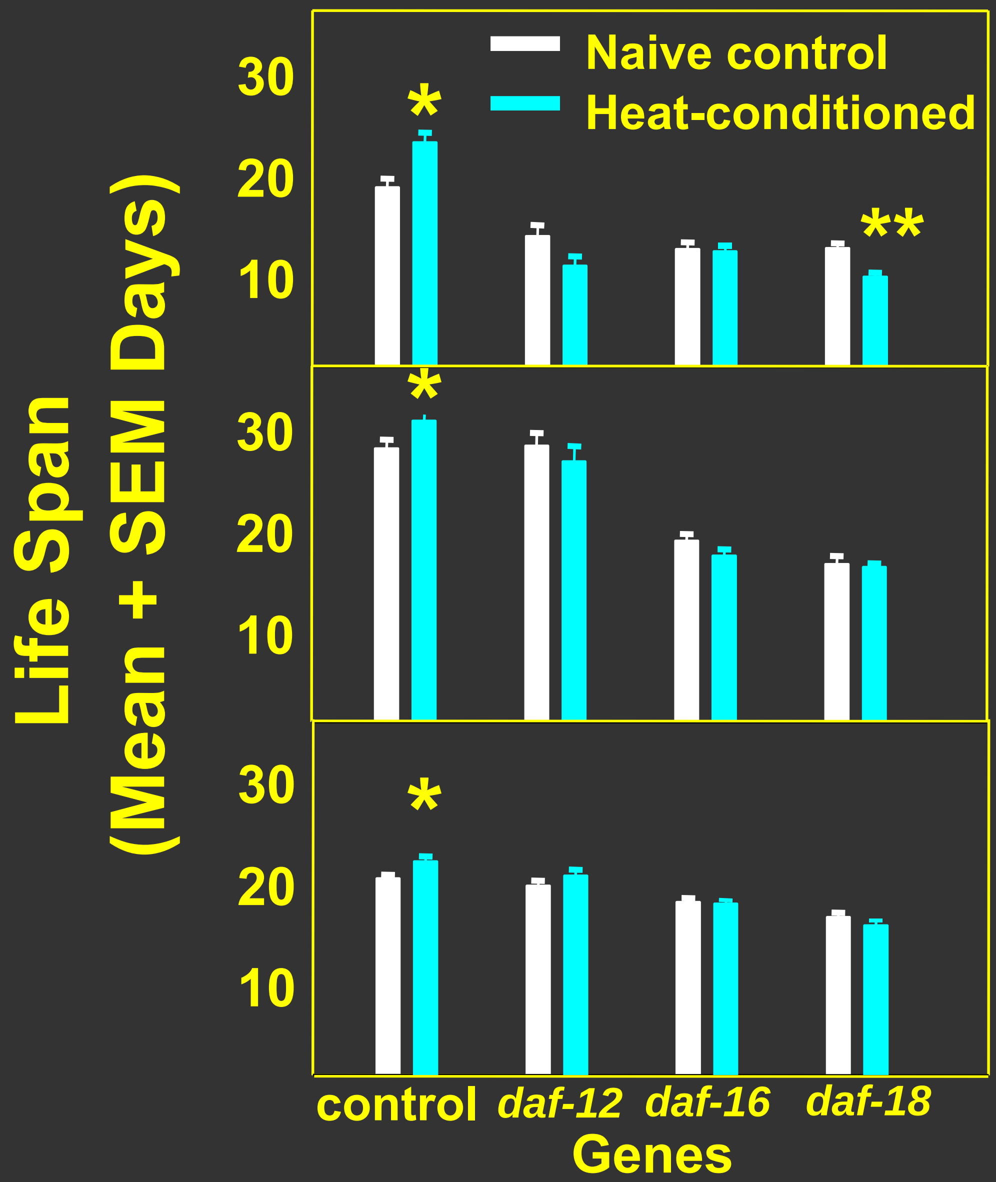


# DAUER FORMATION REQUIRES THE INSULIN-LIKE PATHWAY



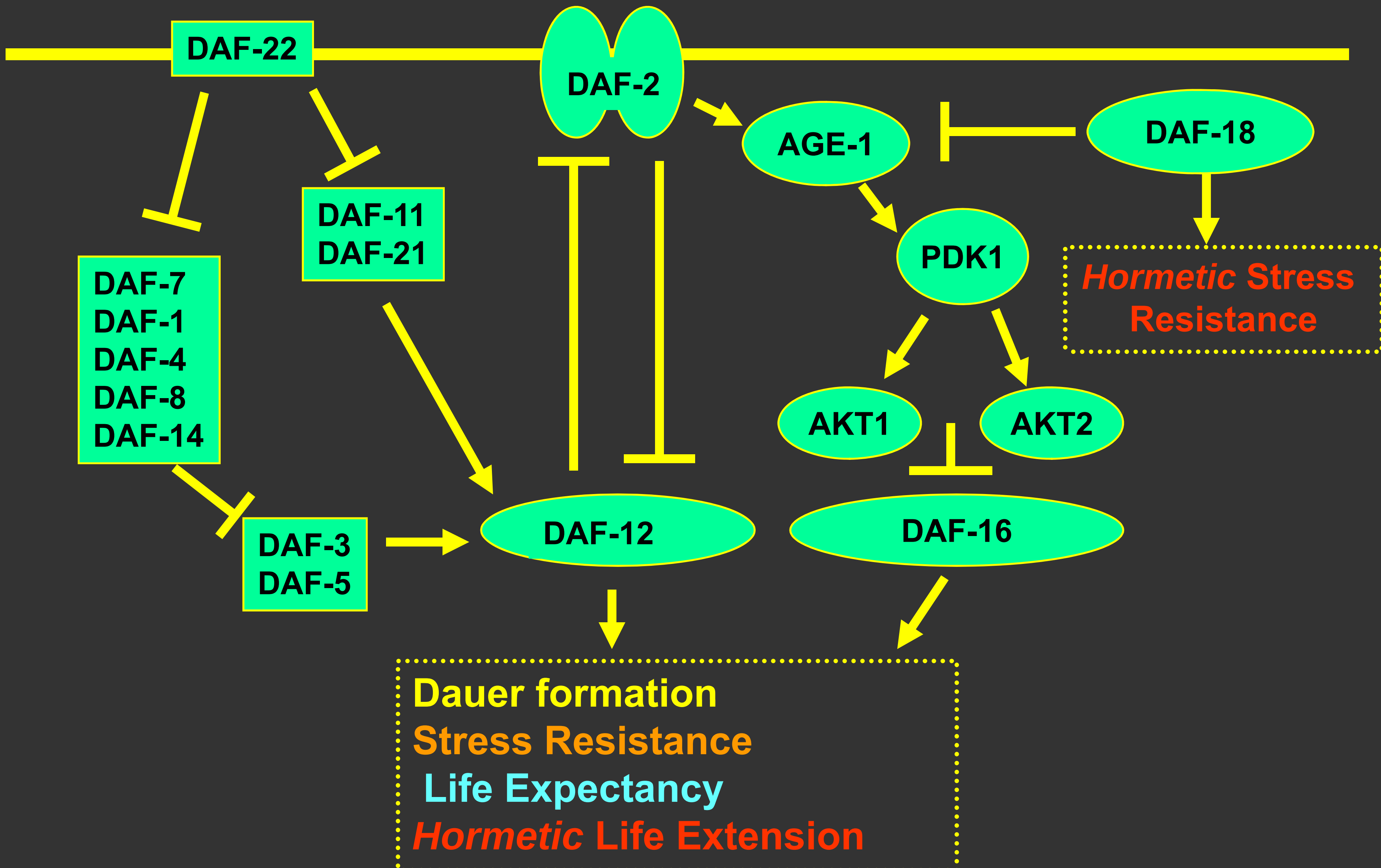

## THE DAUER / INSULIN PATHWAY ALSO MEDIATES STRESS RESISTANCE AND LIFE EXPECTANCY IN WORMS

# *daf-12, daf-16 & daf-18* REQUIRED FOR HORMETIC LIFE EXTENSION




**WORMS CARRYING MUTATIONS OF THE INSULIN PATHWAY DISPLAY DEFECTS IN HORMETIC LIFE EXTENSION** *Cypser et al, Biogerontol*. 2003

# DAUER FORMATION / INSULIN-LIKE PATHWAY OF THE WORM




**GENES OF THE INSULIN PATHWAY ARE REQUIRED FOR HORMESIS IN THE WORM**

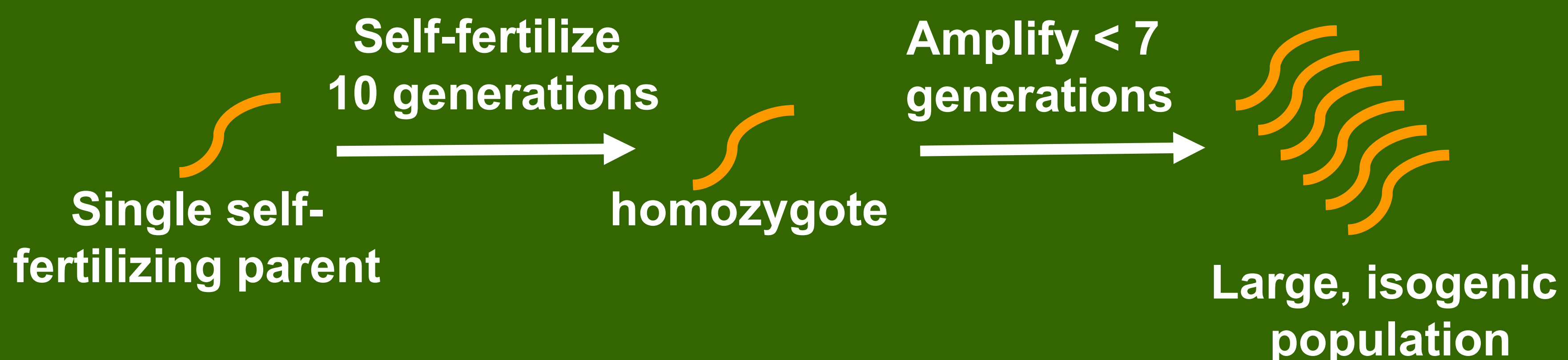
*Cypser et al, Biogerontol*, 2003

## SUMMARY OF WORM GENETICS AND HORMESIS

Genes (***daf-16, daf-12, daf-18***) of the insulin response pathway are required for extended life span after hormetic conditioning in the worm

The ***daf-18*** gene is required for a full hormetic response of (thermotolerance) in the worm

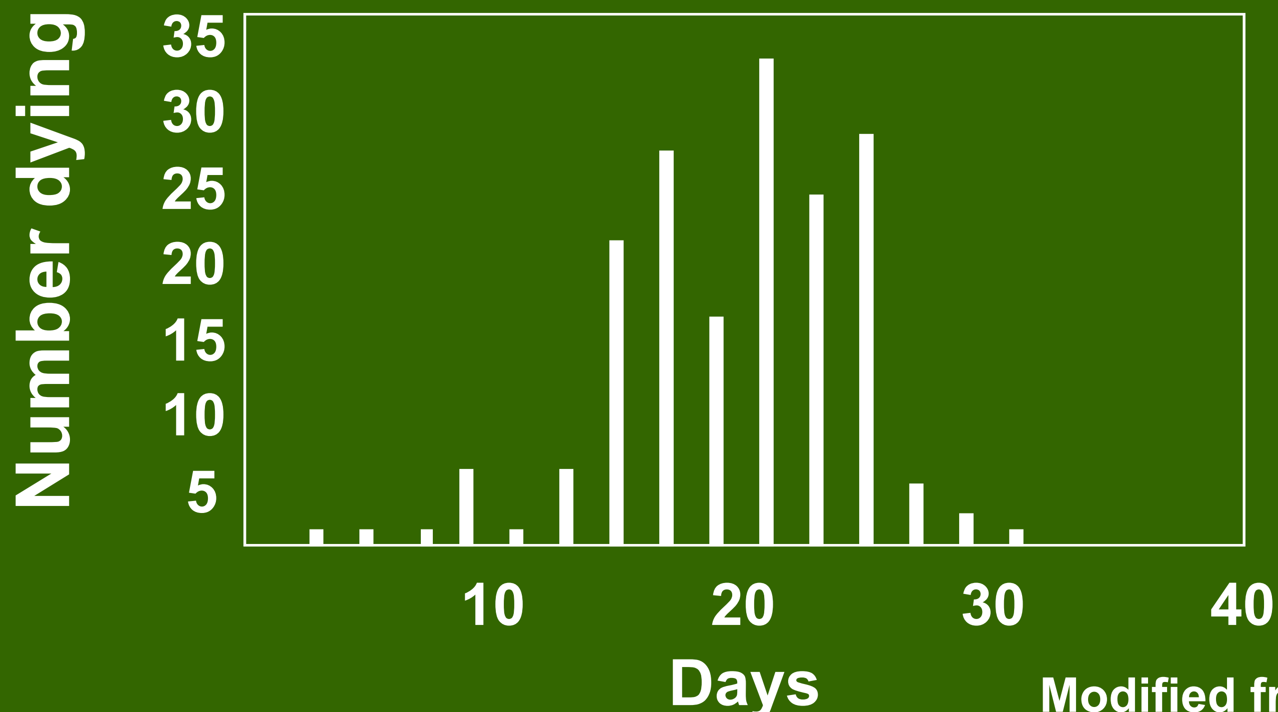
## CAN WE STUDY HORMESIS IN ISOGENIC POPULATIONS OF WORMS?




EASY TO MAKE

LARGE, SAME-AGED, ISOGENIC POPULATIONS OF WORMS

# Ages at death of wild-type, isogenic worms




Modified from Kirkwood, *Nature* 2002

**Variation in life span exists even in isogenic populations of worms: Variation in gene expression should also occur.**

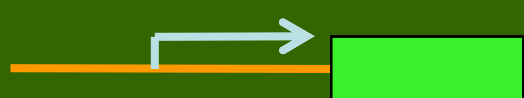
# Heat Shock Protein-16 genes (*hsp-16*)

**Specifically, *hsp-16.2* (family of four genes)**

- **Encodes a 16-kD heat shock protein**
- **Expression mediated by DAF-16 & Heat Shock Factor 1**
- **Induced in response to heat shock, hypoxia, other environmental stresses**
- **Up regulated in long-lived mutants**
- **Over expression has modest life extension**

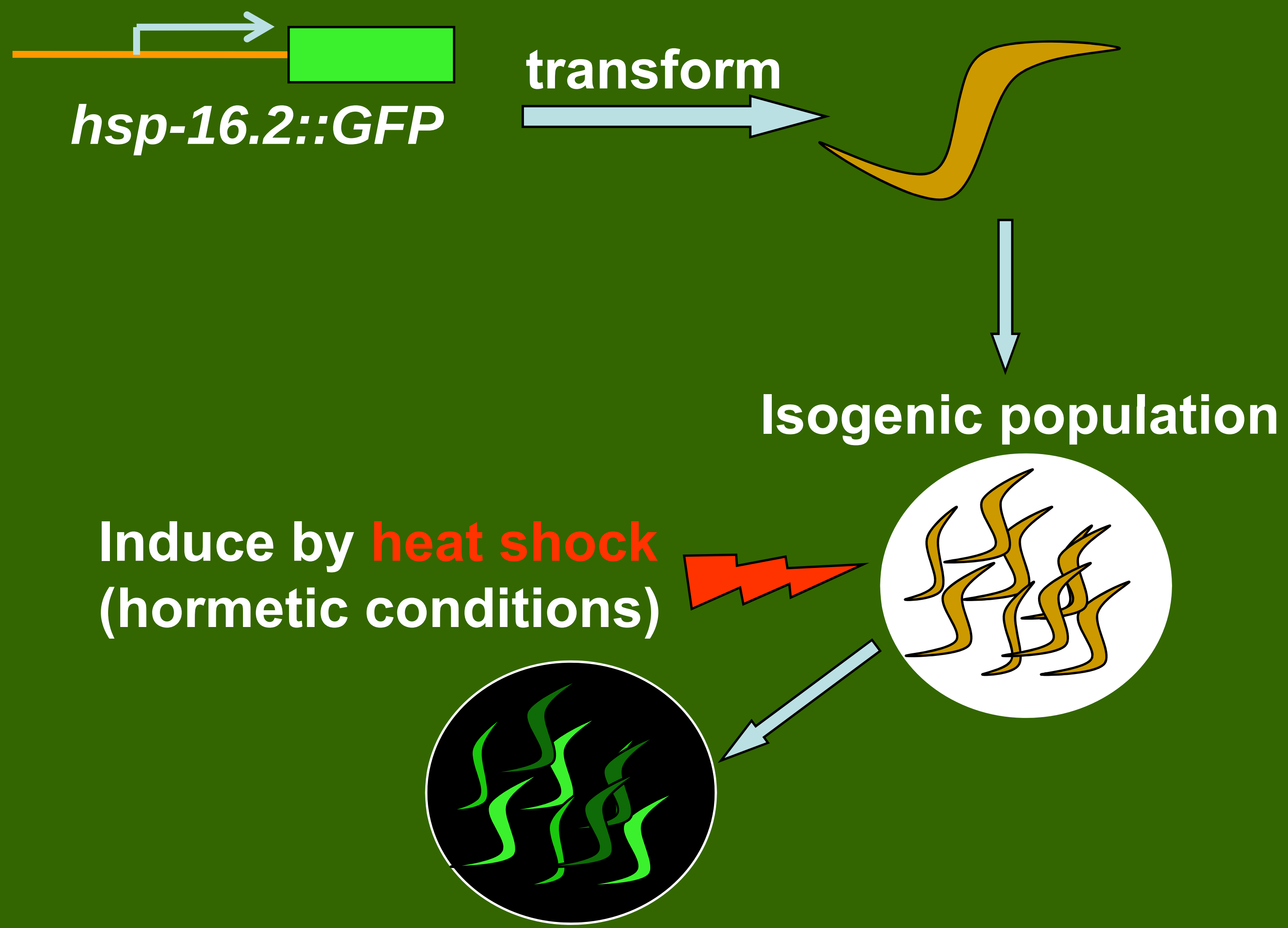
## Can create a transgene

- **Promoter: worm *hsp-16.2* gene**
- **Coding: jellyfish Green Fluorescent Protein (*GFP*) gene**



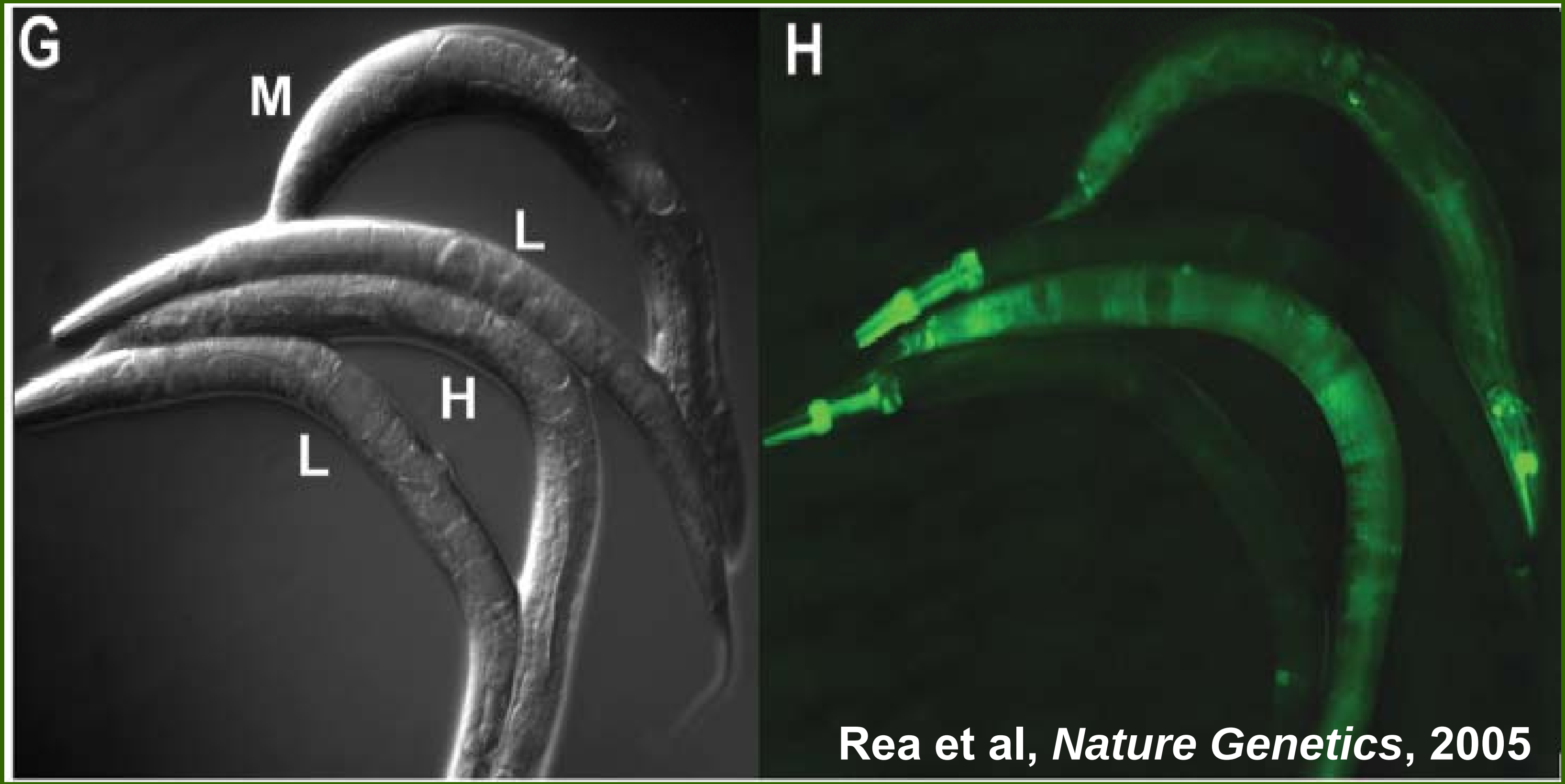
***hsp-16.2::GFP***

# CREATING A TRANSGENIC WORM STRAIN



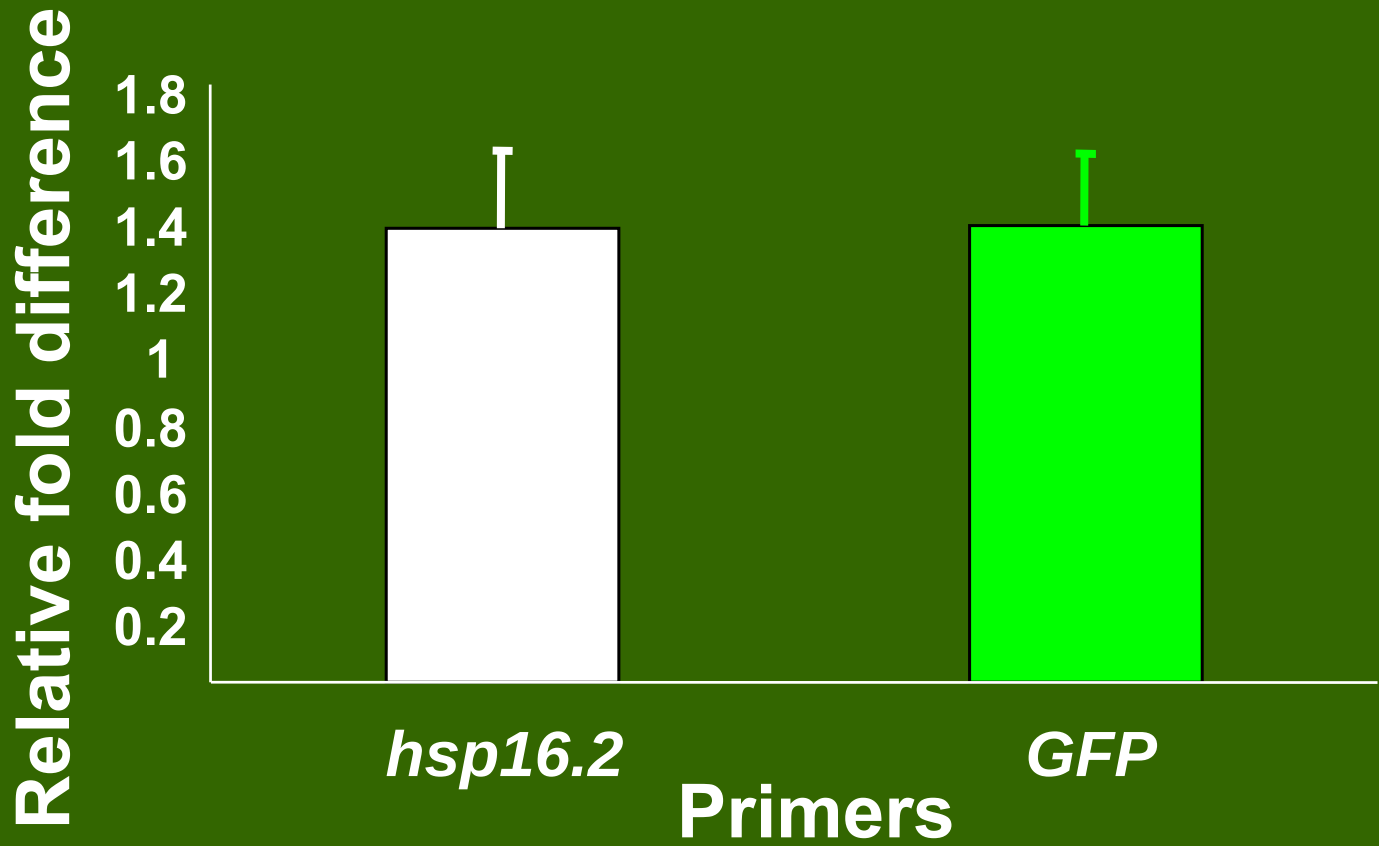

**Green Fluorescent Protein (GFP) is a “reporter” for a single, stress-sensitive gene: *hsp-16.2***

# EXPRESSION OF *hsp-16.2::GFP*



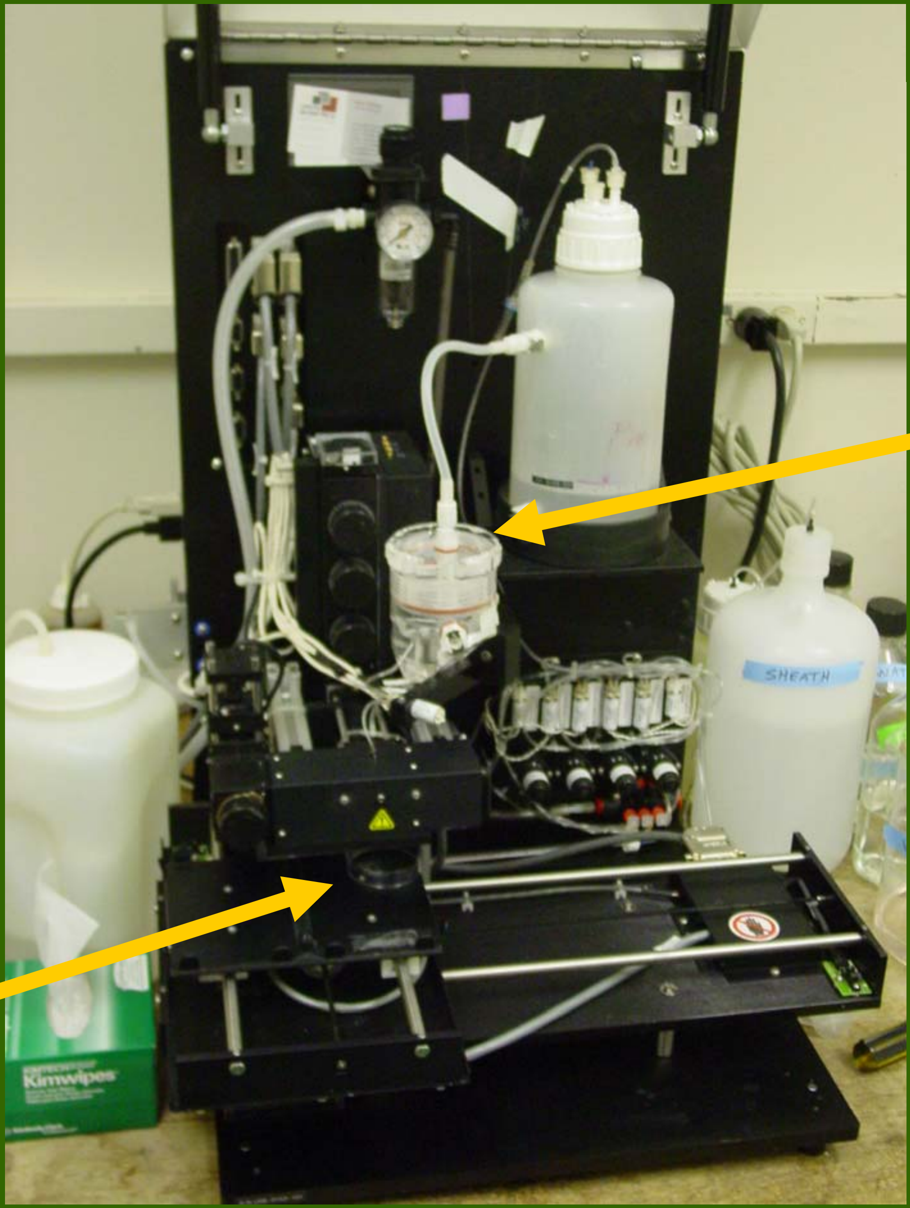

**Worms respond differently to heat shock.**
**Due to differences in transgene copy number?**

# QPCR on genomic DNA of *hsp16.2::GFP*-expressing animals




**Individual variation NOT due to differences in copy number of transgene or endogenous *hsp-16.2* gene** (P. Tedesco, unpublished)

# GFP brightness is measured using a worm sorter




Worms go in here

Sorted by GFP expression (bright v. dim) at ~$10^4$ / hr

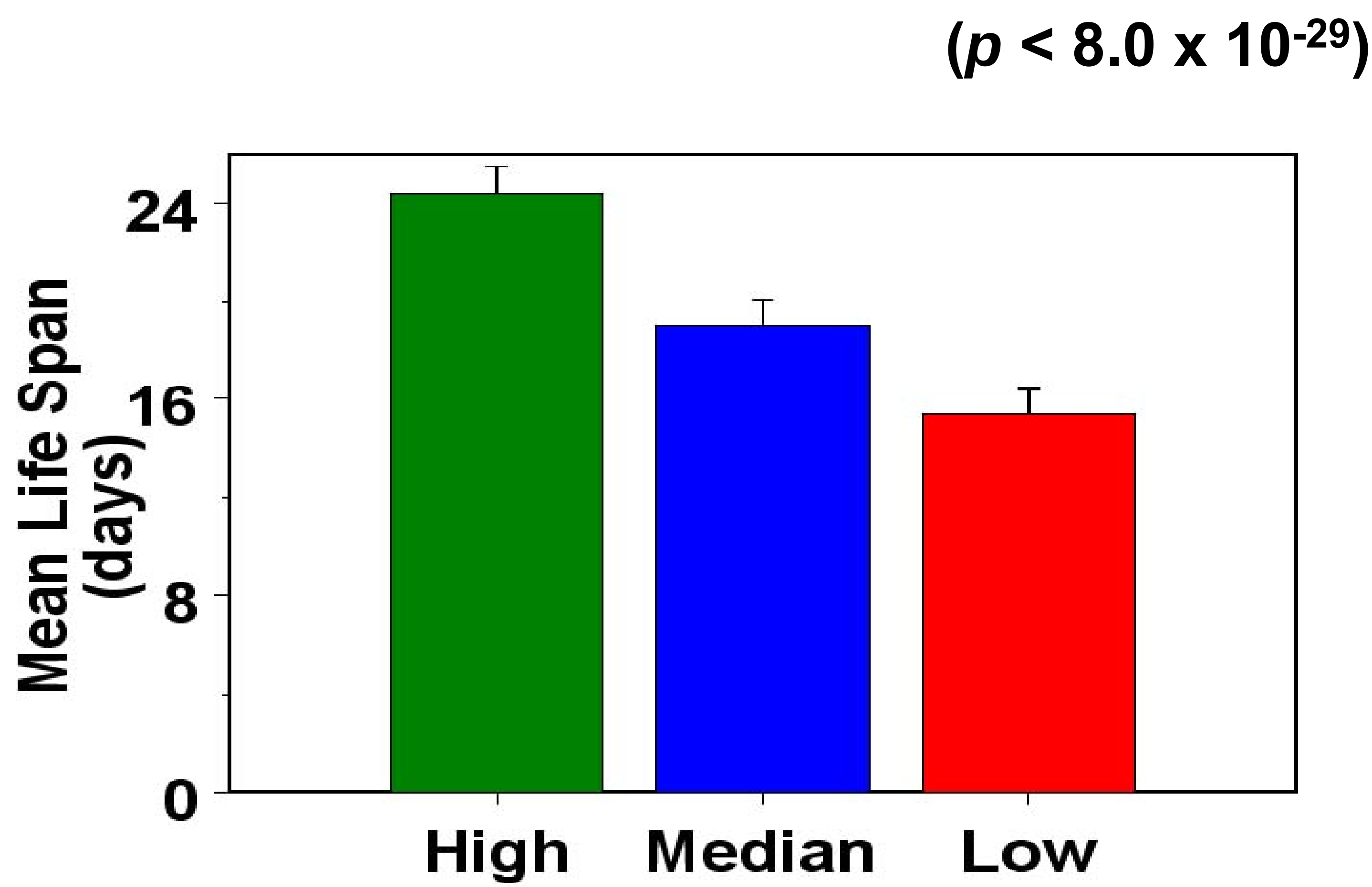
Union Biometrica, Holliston, MA

# Questions about individual variation and hormesis

Does *hsp-16.2* expression after hormetic conditioning correlate with:

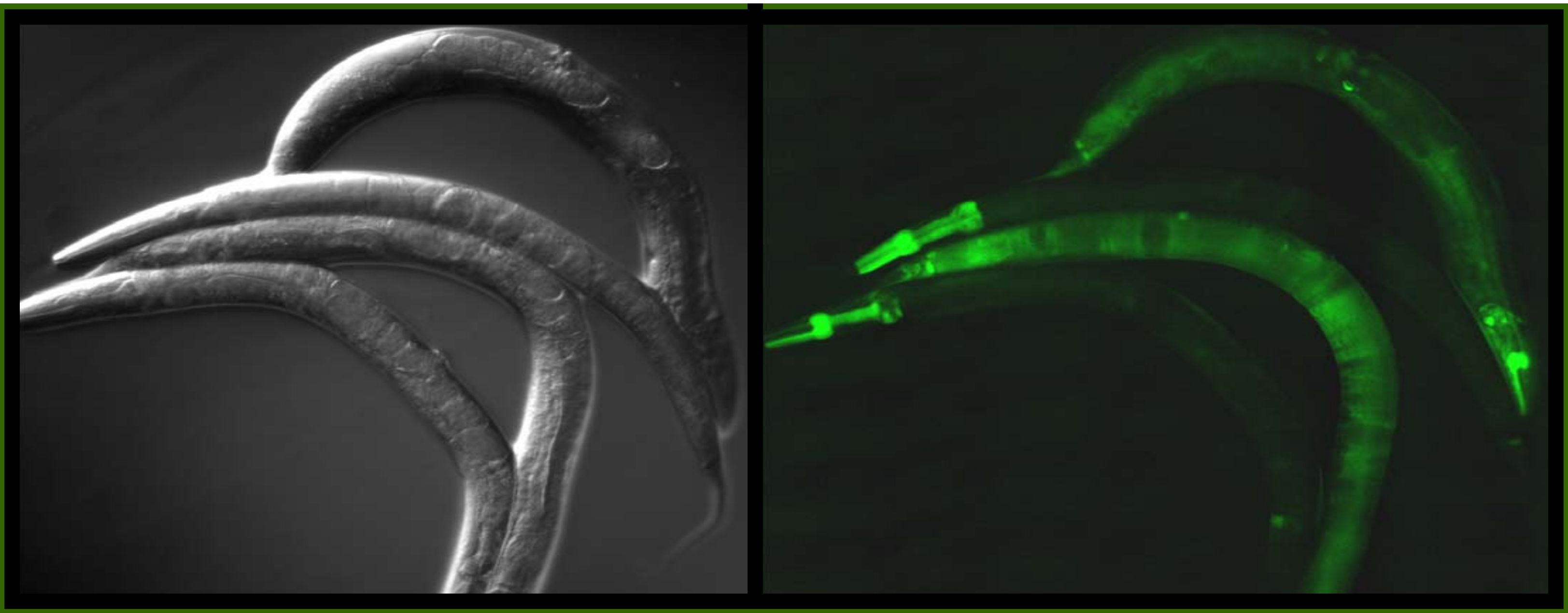
- Differential thermotolerance?
- With differential lifespan?

# COMBINED LONGEVITY EXPERIMENTS




Rea et al, NATURE GENETICS August, 2005

**Expression of *hsp-16.2::GFP* predicts longevity of genetically identical individuals**



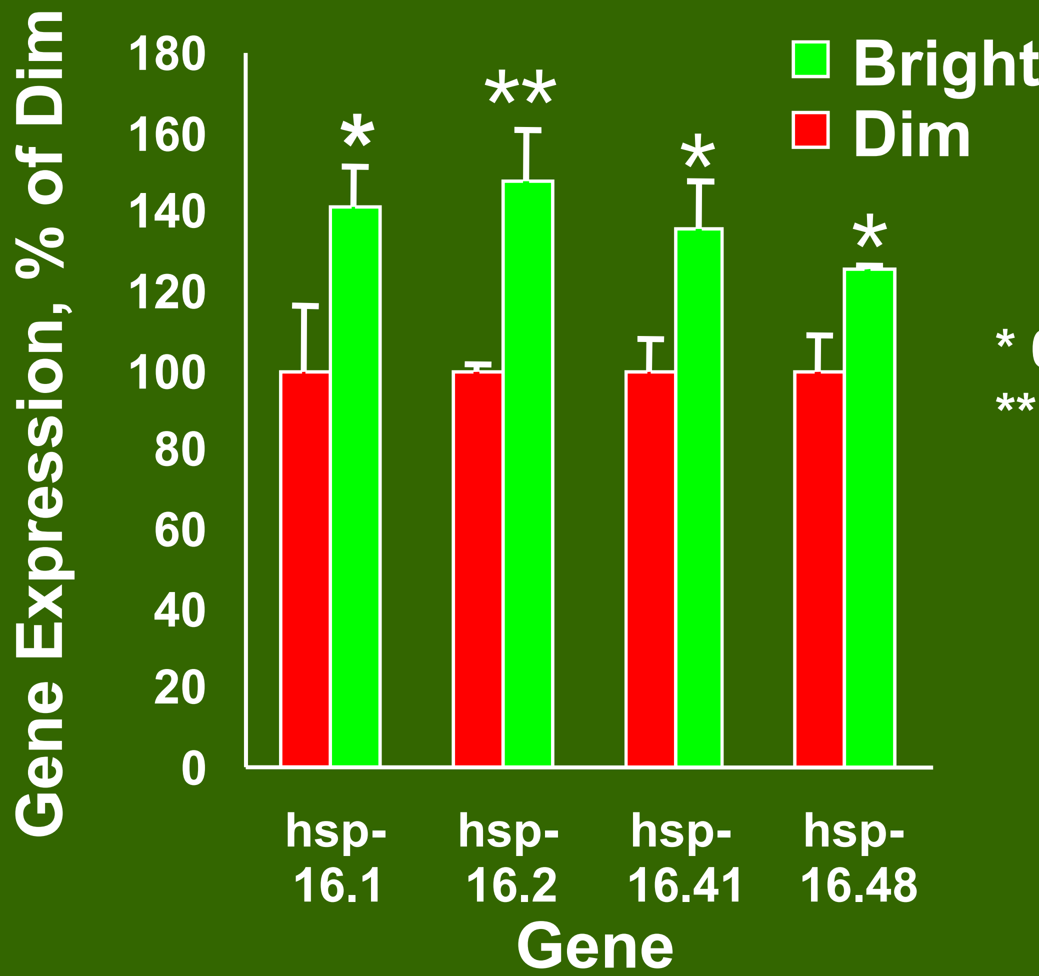
# CONCLUSIONS:

- Isogenic worms display variance in life span and thermotolerance
- Response to hormetic conditioning predicts life span
- Not due to genetic differences
- "Physiologic state" on first day of adulthood predicts life expectancy after hormetic conditioning

# QUESTIONS ABOUT PHYSIOLOGIC STATE AFTER HORMETIC CONDITIONING

- **Are genes besides *hsp-16.2* differentially expressed?**
- **Is physiologic state heritable?**
- **Are there epigenetic influences?**

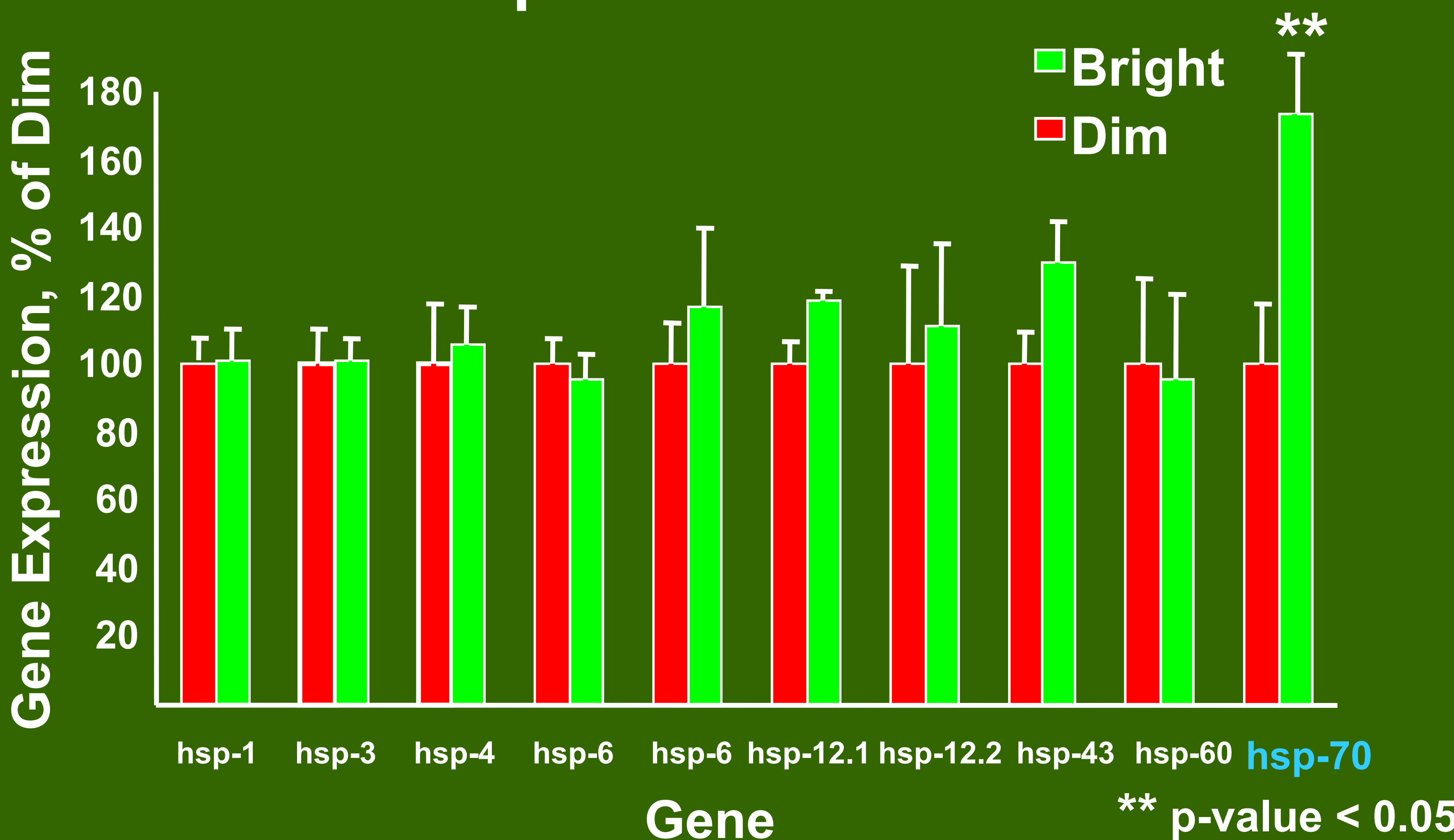
# MICRO-ARRAY ANALYSIS:
# INDUCTION OF *HSP-16* CLASS IN BRIGHT WORMS




* 0.05 < p-value < 0.1
** p-value < 0.05

**MULTIPLE GENES OF THE HEAT-SHOCK PROTEIN-16 CLASS ARE UPREGULATED IN BRIGHT WORMS (S.-K. Park, unpublished)**

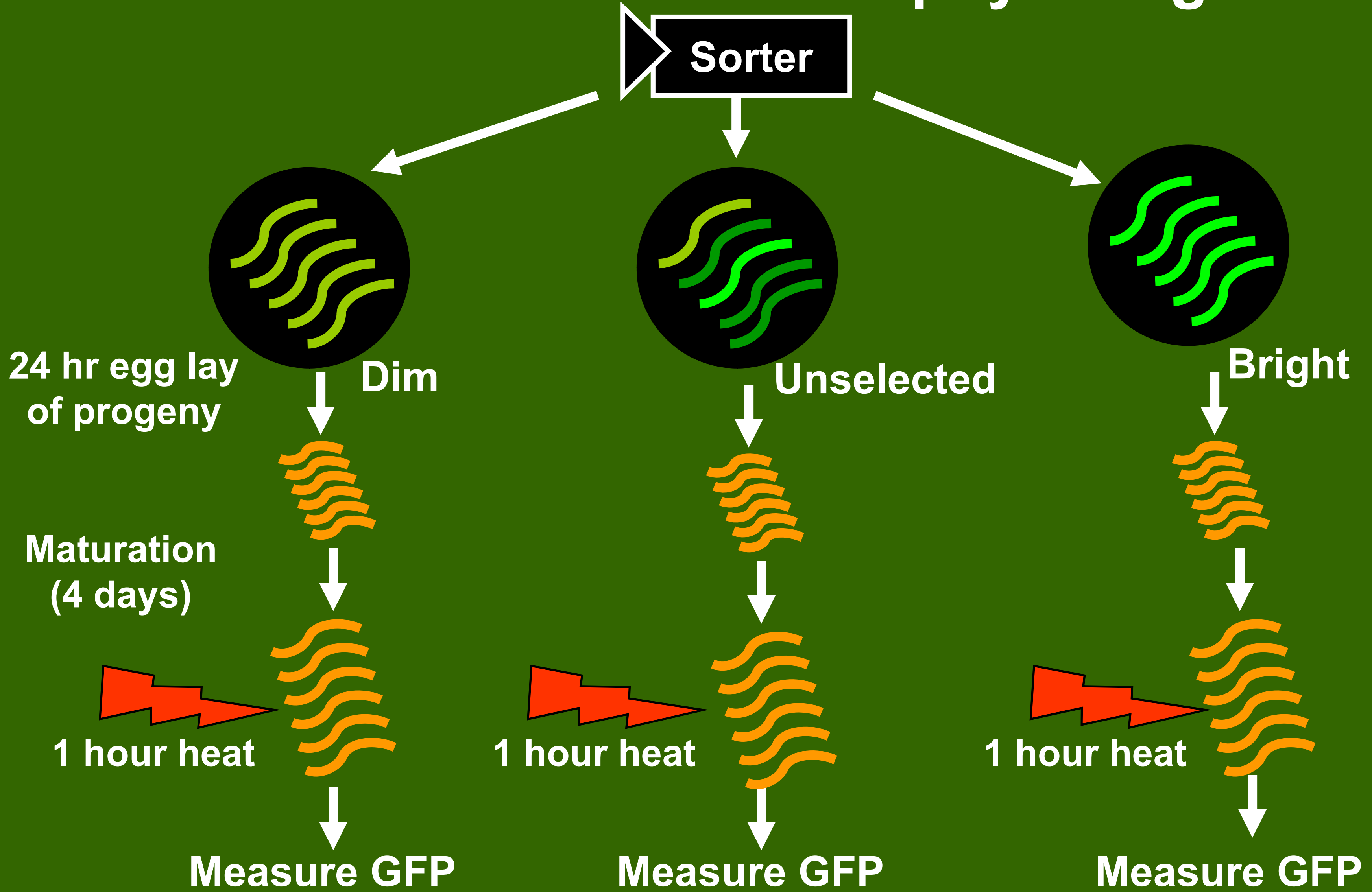
# Micro array analysis:
# Other heat shock proteins in conditioned worms




***hsp-70* is up-regulated in bright, longer-lived worms**

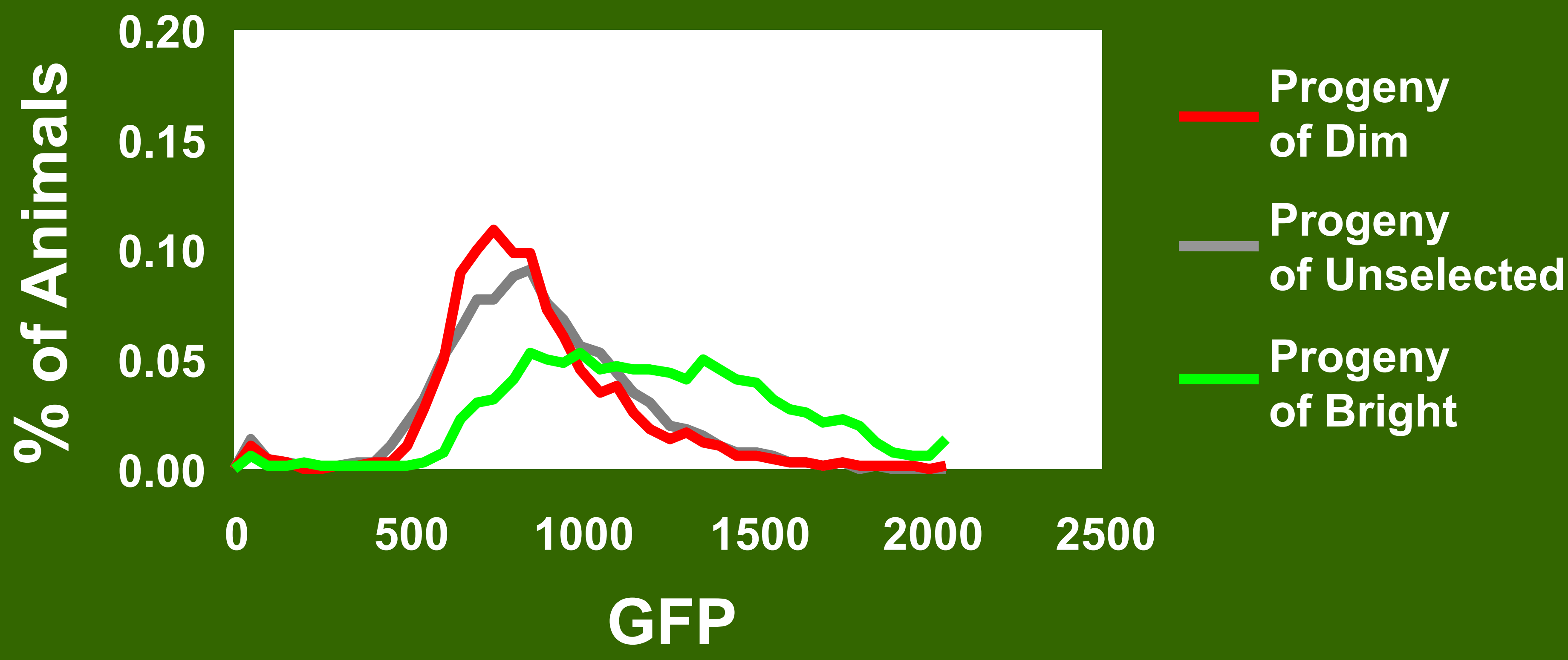
(S.-K. Park, unpublished)

# How to test for inheritance of physiologic state




**Expectation: All groups of progeny *should* display similar GFP expression**

# *hsp-16.2::GFP* expression of progeny




n > 3000 / group
Dim vs. Bright:
$p \ll 10^{-17}$ (chi-square test)

**PROGENY RESPONSE TO CONDITIONING SIMILAR TO PARENTS EVEN IN ISOGENIC POPULATIONS**

(J.R. Cypser, unpublished)

# OVERALL SUMMARY

**Insulin pathway genes required for hormetic heat conditioning in the worm**
- **For life expectancy: *daf-12, daf-16, daf-18***
- **For thermotolerance: *daf-18***

**Individual variation in individual gene expression after hormetic heat conditioning predicts subsequent thermotolerance and life expectancy.**
- **For life expectancy OR thermotolerance: *hsp-16.2 predictive***

**Physiologic state of worms after heat conditioning may be heritable even in an isogenic population**
- ***hsp-16.2::GFP* expression after heat conditioning of progeny reflects expression displayed by parents.**
- **Epigenetic effects may act in response to heat conditioning; *sir-2.1* required for sustained conditioning**

# Future Directions

**Test inheritance of *hsp-16.2::GFP* response further**

- **Variation in promoter region of transgene?**
- **Inheritance of thermotolerance / life span?**
- **Difference observable at protein level?**

**Are there epigenetic influences on hormesis?**

- **Test histone-modifying genes**
- **Pharmacologic interventions**

**Does expression of *hsp-16.2::GFP* in specific tissues predict hormetic survival?**

# Thanks to:

Deqing Wu
Pat Tedesco
Sang-Kyu Park
Shane Rea

Tom Johnson

International Dose-Response Society

# Heat Shock Protein-16 gene (*hsp-16*)

- *hsp-16.2* encodes a 16-kD heat shock protein (HSP)
- Member of the hsp16/hsp20/alphaB-crystallin (HSP16) family of heat shock proteins; 6 near identical homologs
- *hsp-16.2* expression, strongest in intestine and pharynx
- Under Daf-16 & HSF-1
- Induced in response to heat shock, hypoxia, or other environmental stresses
- Mount 36; other HSPs
- Up regulated in Age mutants
- Over expression has modest life extension
- Interacts with intracellular human beta amyloid peptide, a primary component of plaques in Alzheimer's disease
- HSP-16.2 is likely to function as a passive ligand temporarily preventing unfolded proteins from aggregating

# Summary:
# Physiologic state after conditioning

**Physiologic state associated with induction of:**

- **multiple heat shock protein-16 class genes**
- **heat shock protein-70**

**Degree of expression of GFP driven by *hsp-16.2* is heritable even in populations of isogenic animals.**

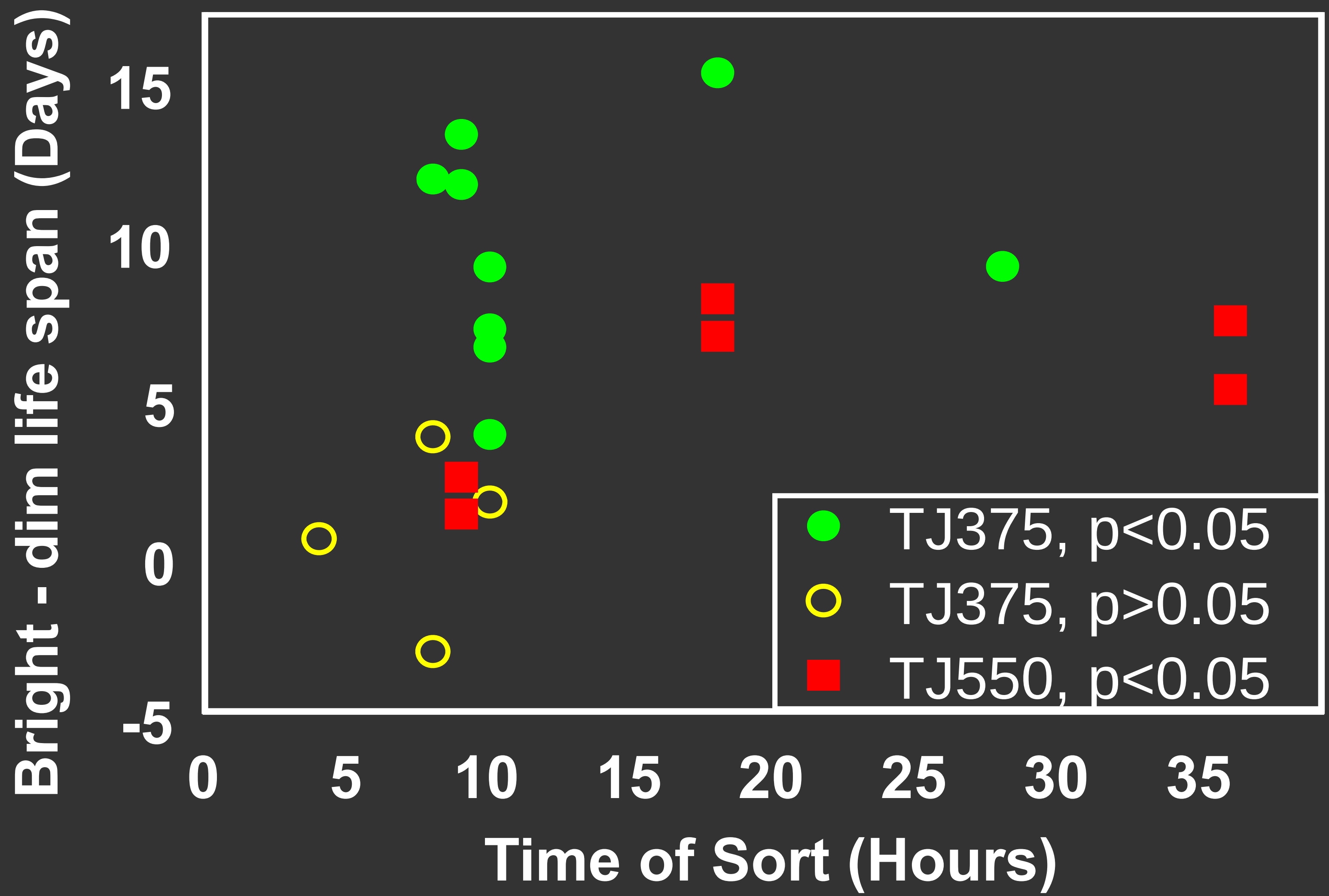
**Models:**

**Duplications / deletions of the *hsp-16.2::GFP* transgene occur rapidly, creating subpopulations with different copy numbers: NO**

**Differences in promoter sequence? NOT TESTED**

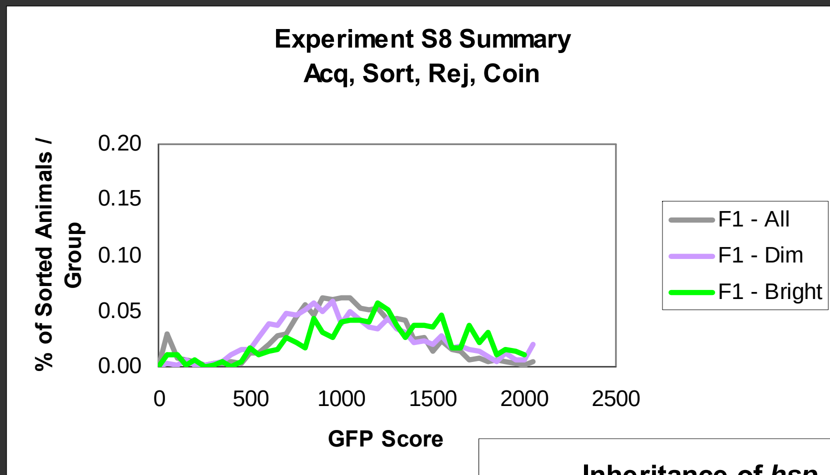
**Differences in epigenetic state? MAYBE; *sir-2.1* role in heat conditioning**

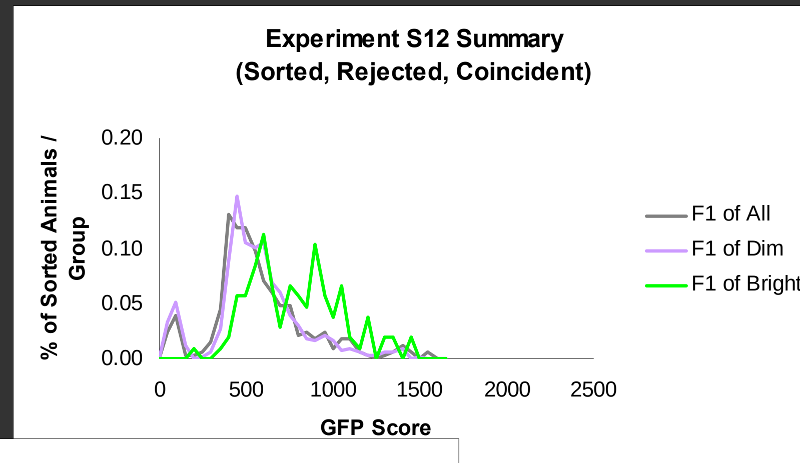
## Differential Longevity: All Individual Experiments

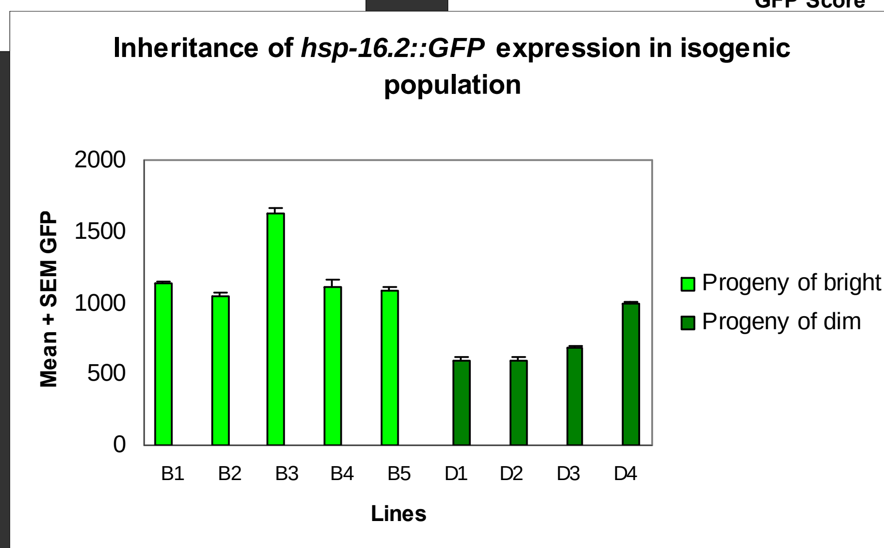



Rea et al, NATURE GENETICS, 2005

# Expression of hsp-16.2::GFP in progeny of dim vs. bright worms



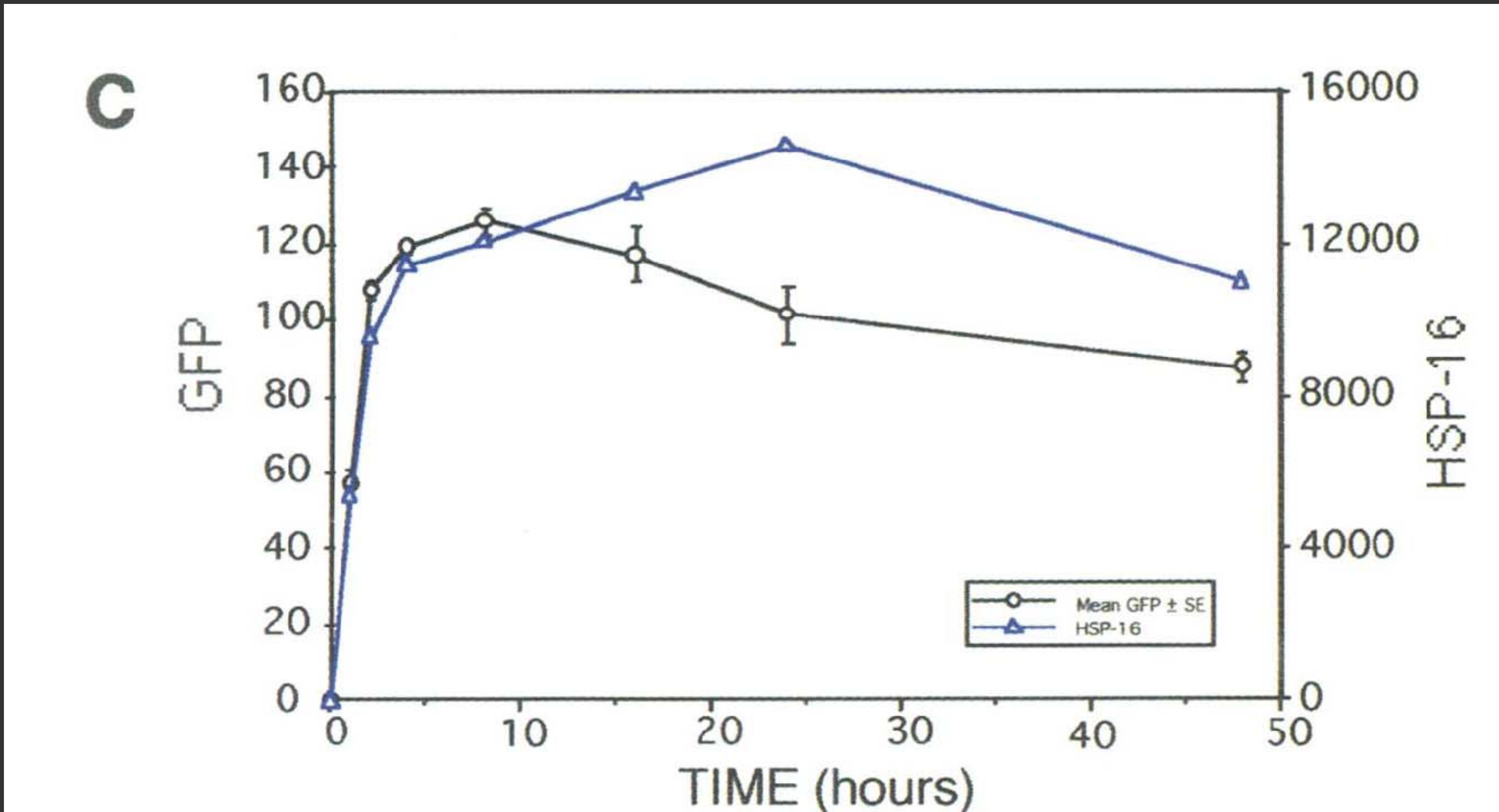




**Progeny reliably reflect expression of GFP even in isogenic populations**

**J. R. Cypser, unpublished**

# Endogenous HSP-16.2 expression tracks HSP-16.2::GFP expression




**hsp-16.2::GFP accurately reports expression of the native HSP-16.2 protein**

**Link et al, *Cell Stress & Chaperones*, 1999**

**Direct Observation of Stress Response in Caenorhabditis elegans Using a Reporter Transgene.** Christopher D. Link, James R. Cypser, Carolyn J. Johnson and Thomas E. Johnson

*Cell Stress & Chaperones*, Vol. 4, No. 4 (Dec., 1999), pp. 235-242

***Michalski et al, Biogerontol*. 2001**

***Cypser et al, J. Gerontol*. 2002**

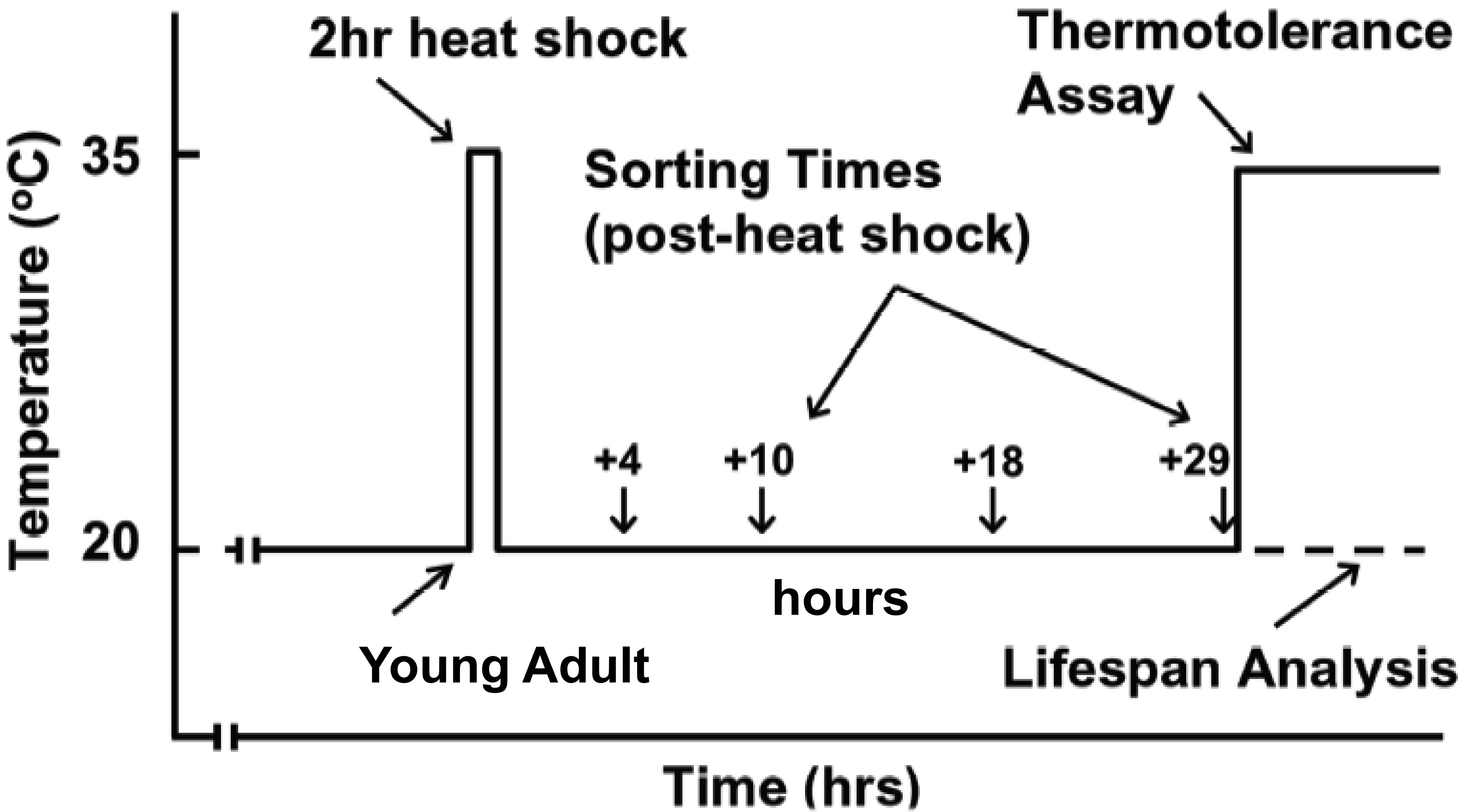
***Cypser et al, Biogerontol*, 2003**

**Modified from Kirkwood,**
***Nature* 2002**

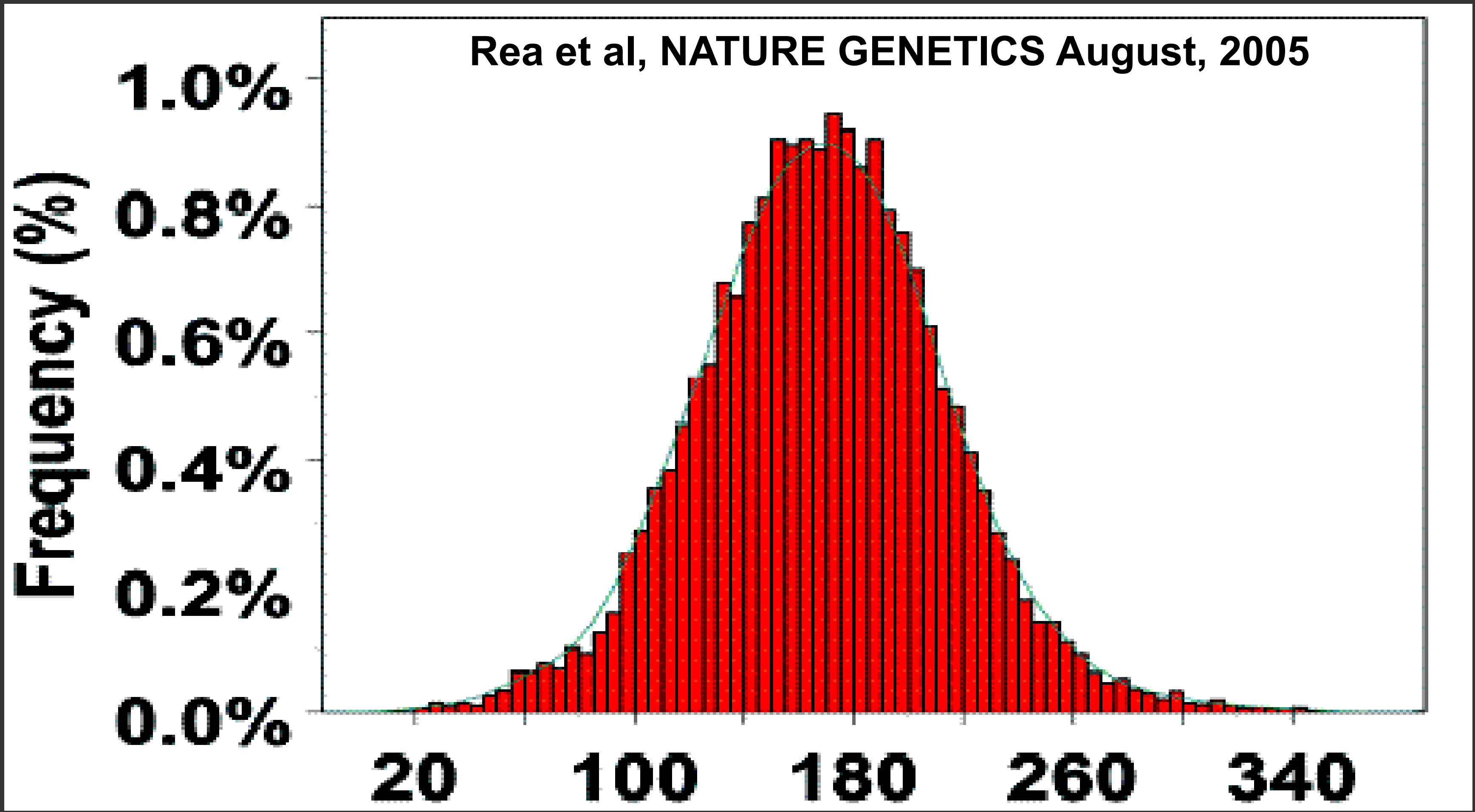
**Rea et al, NATURE GENETICS August, 2005**

# Induction Methods

**Rea et al, NATURE GENETICS August, 2005**



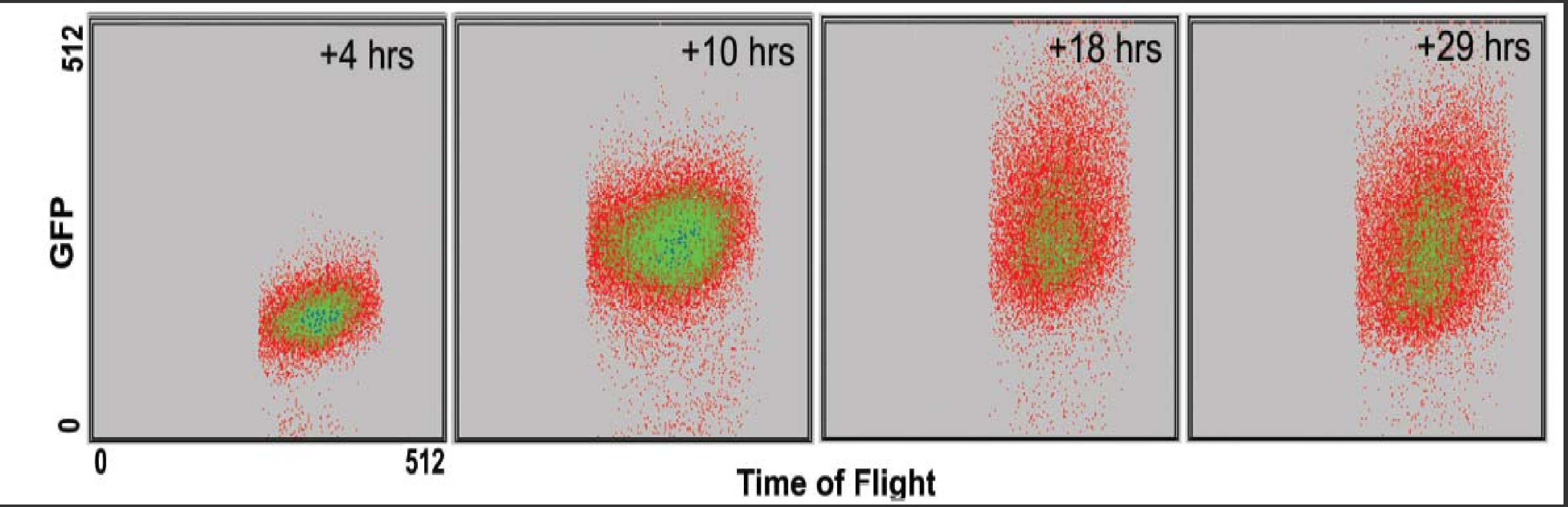
# GFP Is Normally Distributed



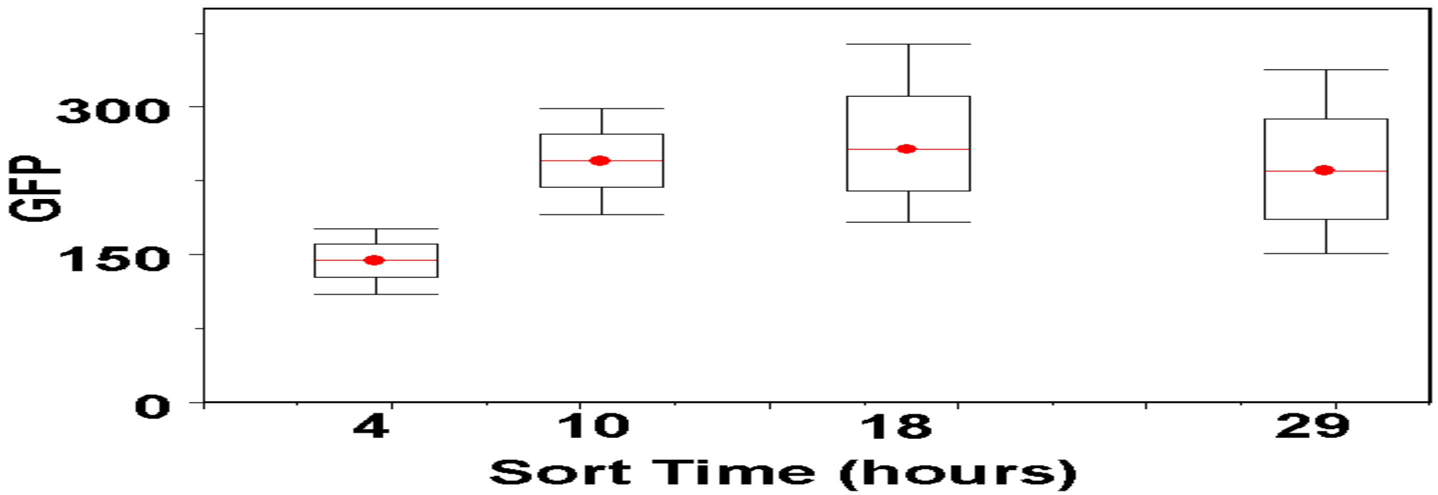

GFP Level (Arbitrary Units)

2 hour conditioning, 19 hour sort, 60,000 worms

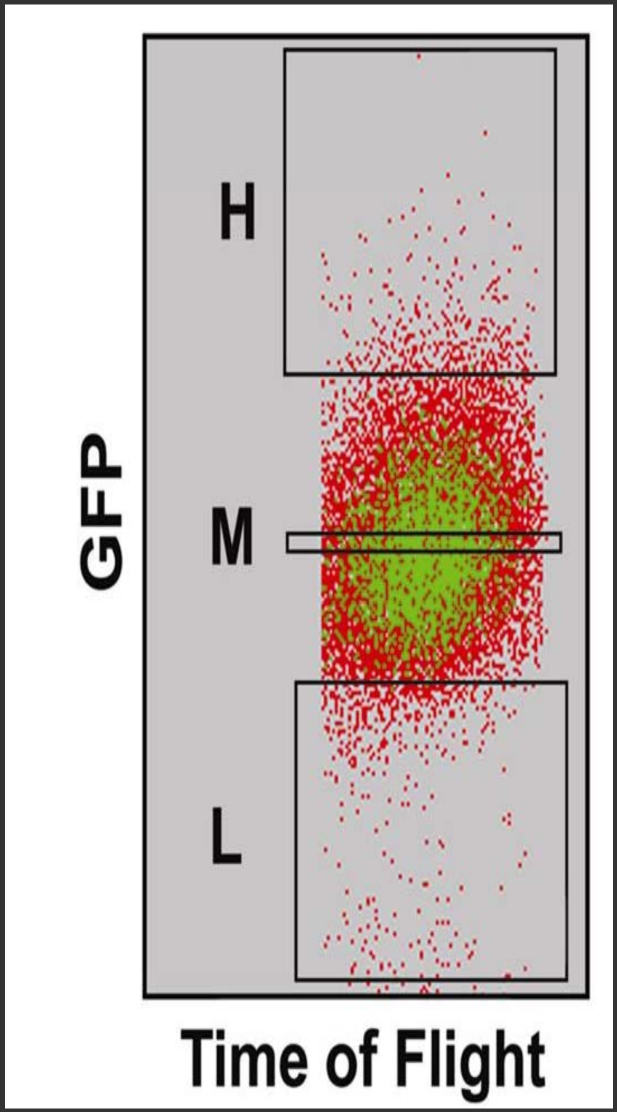
# Variance Increases with Time after Sort



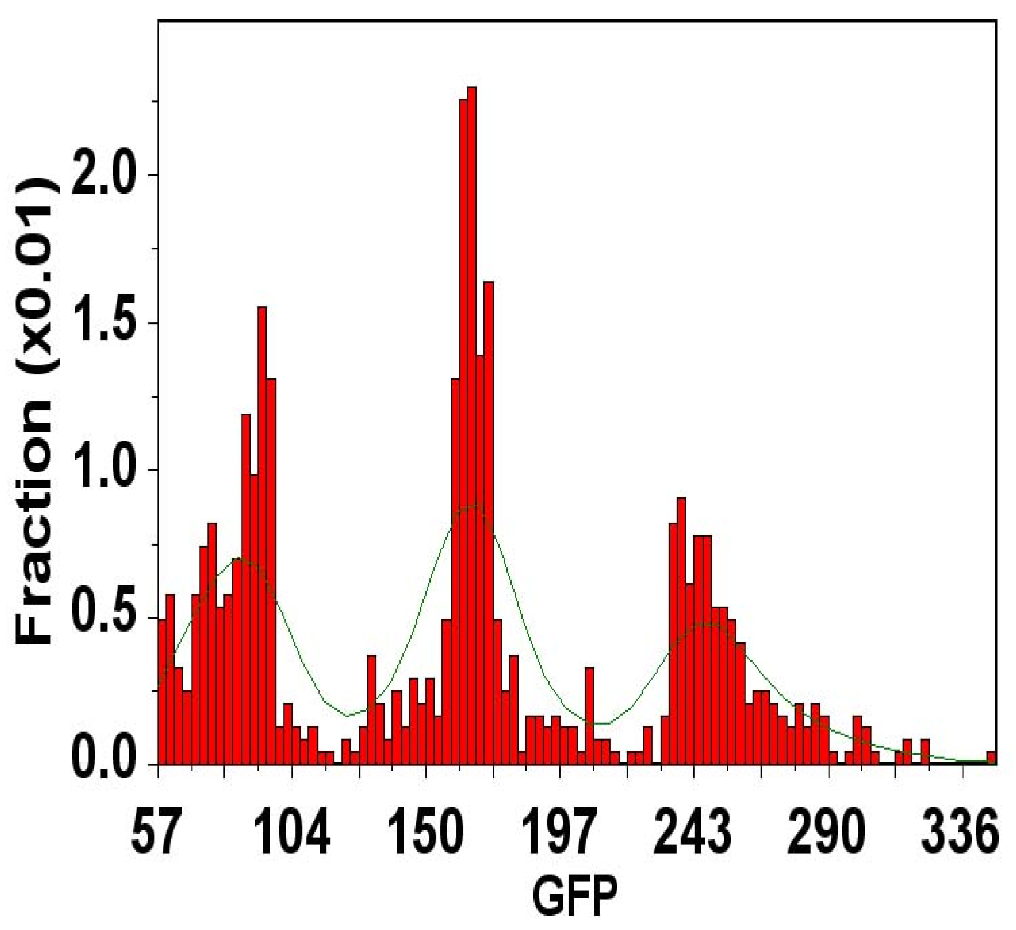

Rea et al, NATURE GENETICS August, 2005

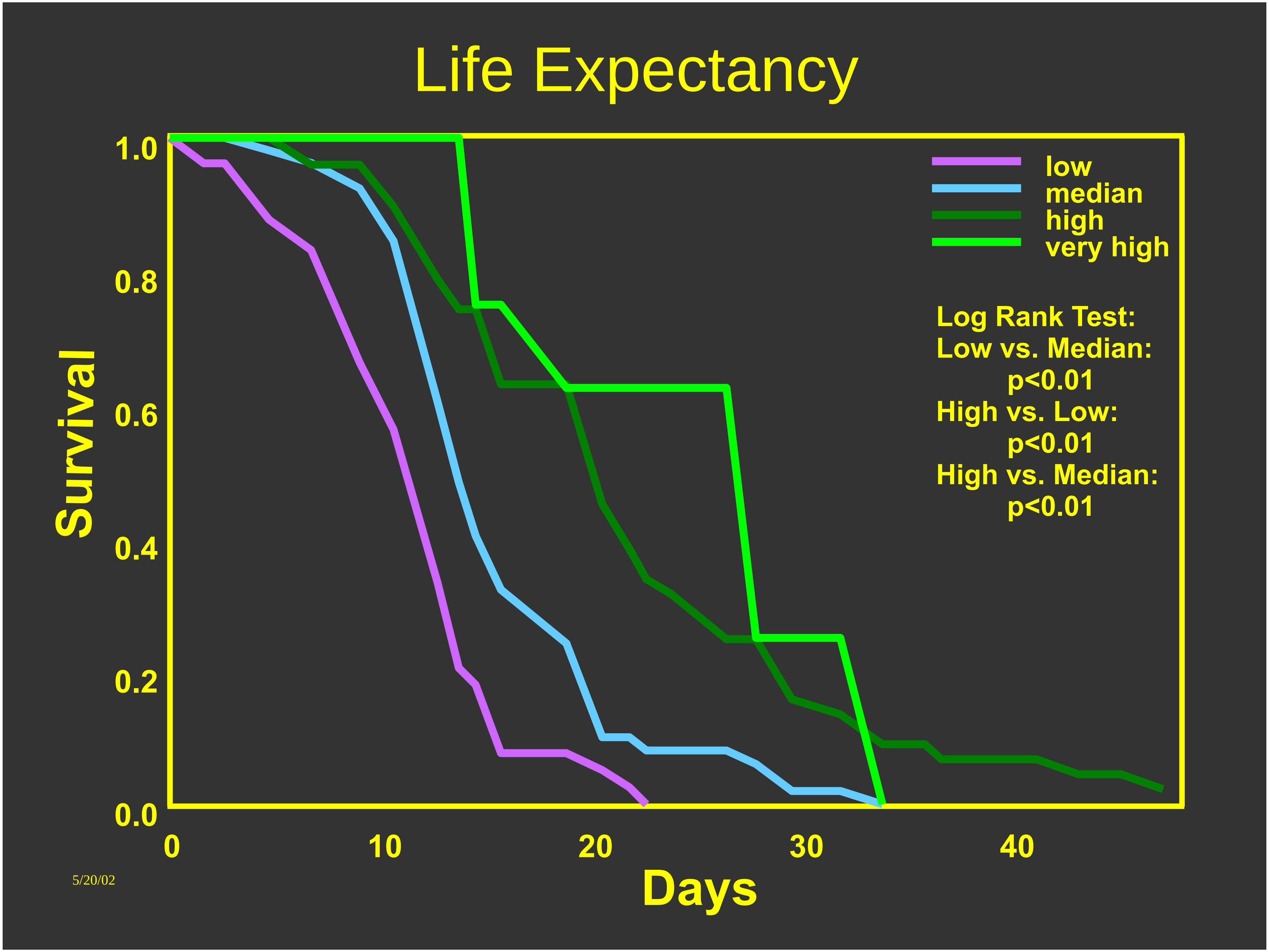


# Sorting for Differential Brightness




Rea et al, NATURE GENETICS August, 2005




Life Expectancy
Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
Days
low
median
high
very high
Log Rank Test:
Low vs. Median:
p<0.01
High vs. Low:
p<0.01
High vs. Median:
p<0.01
5/20/02

# Microarray Data: control vs. hyperbaric $O_2$ (effect of reduced skn-1)

22,548 transcripts

*normalization by GCOS*

*statistical analysis by SAM*

**Up-regulation**

948

*under SKN-1 Control*

119 (12.6%)

**Down-regulation**

392

92 (23.5%)

*functional classification by DAVID*

Antioxidant response

ET/Detoxification

Homeostasis

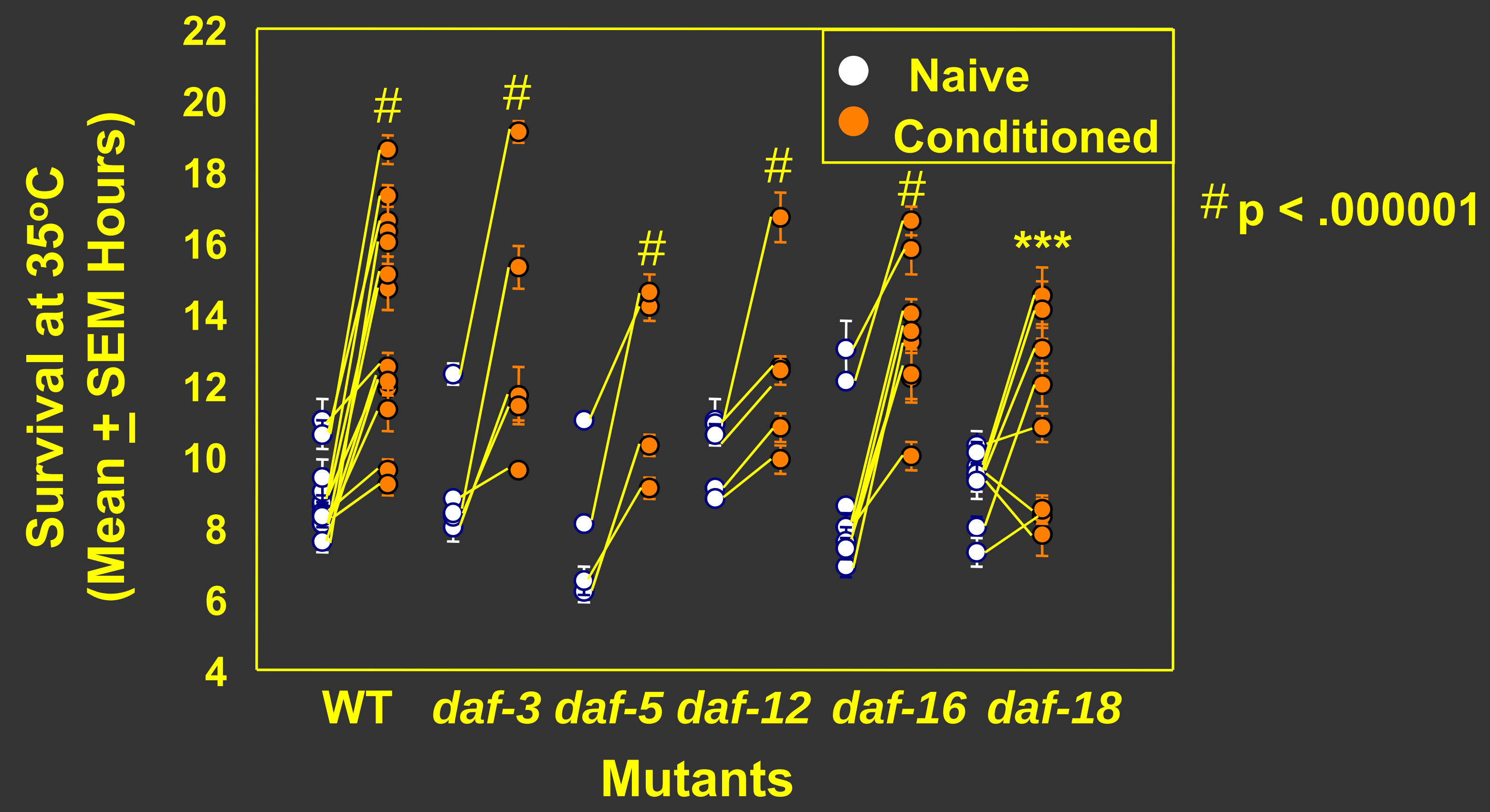
Reproduction

Analysis by S.-K. Park

**Juan Ponce de León** (ca. 1471 – July 1521) was a Spanish conquistador. He was born in Santervás de Campos (Valladolid). Ponce de León accompanied Christopher Columbus on the latter's second voyage to the New World. He became the first Governor of Puerto Rico by appointment of the Spanish Crown. He is also notable for his voyage to Florida, the first known European excursion there, as well as for being associated with the legend of the Fountain of Youth, which was said to be in Florida.

- Wikipedia

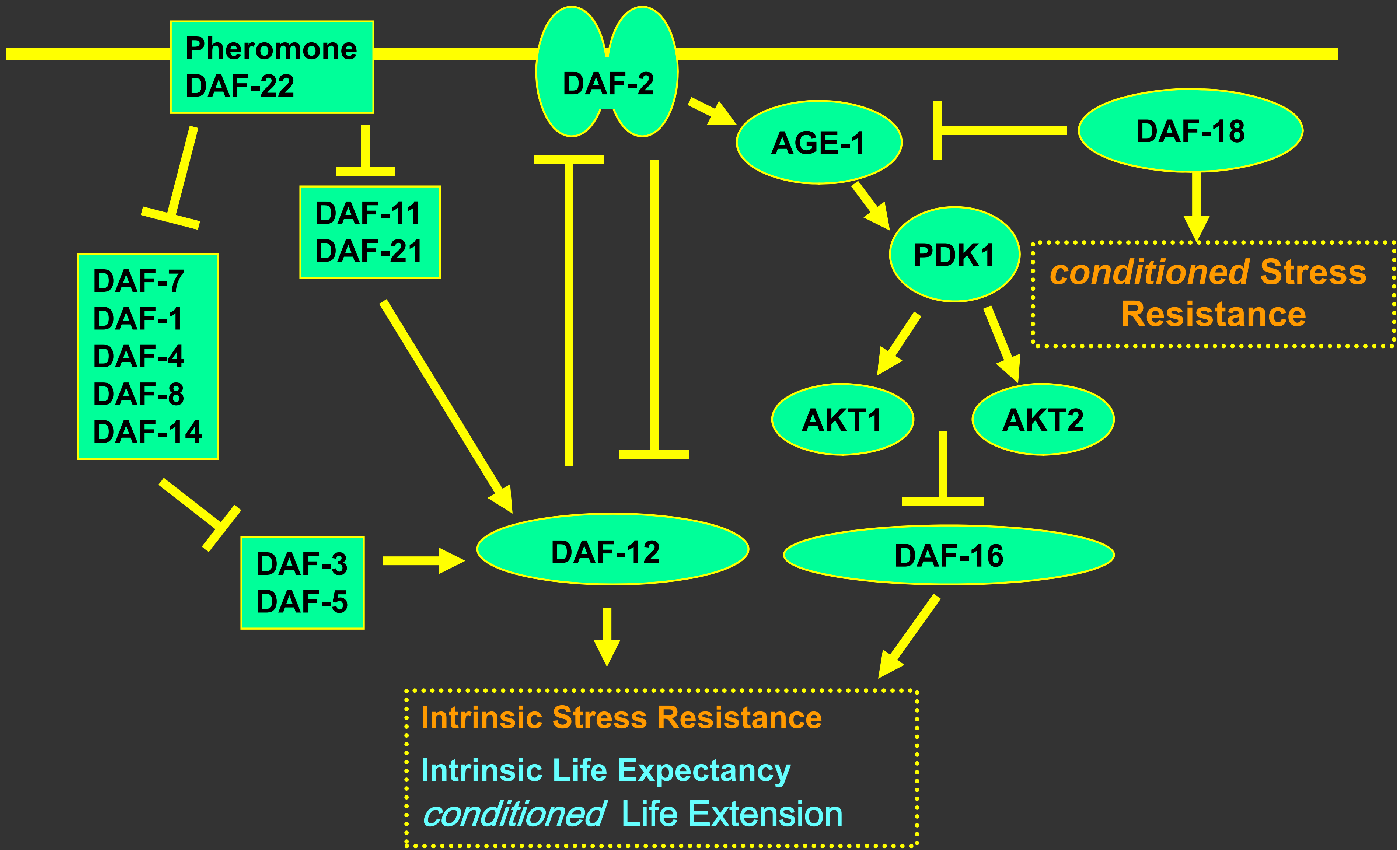
# GENETIC DISSECTION OF HEAT HORMESIS




**MUTANT WORMS THAT CANNOT FORM DAUERS STILL DISPLAY HORMETIC THERMOTOLERANCE**

*Cypser et al, Biogerontol*. 2003

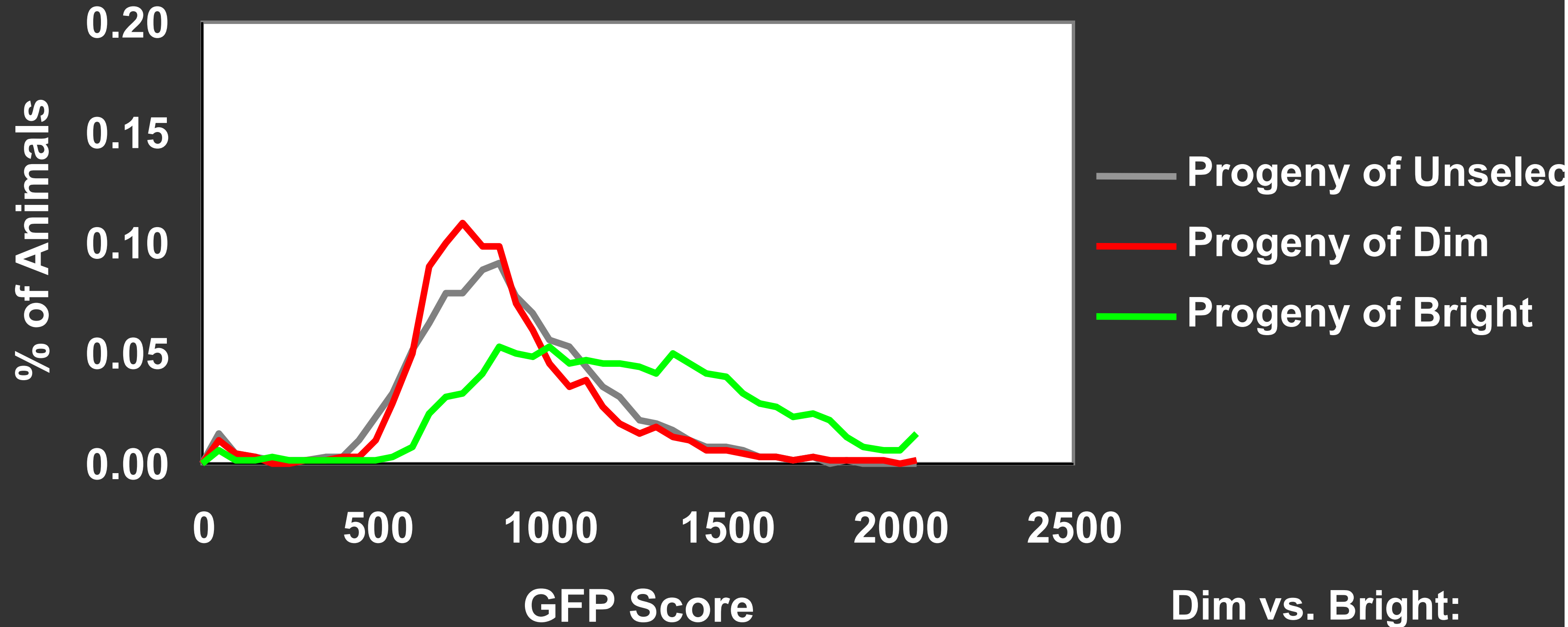
## Dauer formation requires the insulin-like response pathway




Elements of this pathway are required for hormesis in the worm

*Biogerontology*, 2003

# hsp-16::GFP expression of progeny




**Dim vs. Bright:**
**$p << 10^{-17}$ (א$^2$ test)**

| | Mean ± SD | ~n |
|---|---|---|
| F1 of All | 847 ± 273 | 6300 |
| F1 of Dim | 835 ± 271 | 3300 |
| F1 of Bright | 1167 ± 371 | 3100 |

rogeny tend to display same response to preconditioning as parents
in isogenic populations (replicated five times; J.R. Cypser, unpublish

Differentiate daf-16 from hsp-16
Use notes features at bottom
Fold diff in life span (red-blue-green bars) twin analogy
As usual more ideas than funding
Check no slides are hidden
Emphasize HEAT?
Printouts of slides?
Other replicates of heritability
Italics
Reporter tracking endogenous HSP16 slide
Slide with references