# Evolutionary Biology, Quantitative Genetics, and (maybe) Biophysics of Cell Division


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**in collaboration with: E. Anderson, C.F. Baer, M. Delattre, G. Fabig, T. Mueller-Reichert**



**Funding: HFSP, BSF, NSF, NIH**

# Talk Overview:

1) Introduction

2) Investigating Evolutionary Basis of Spindle Diversity

3) Using Quantitative Genetics to Study Biophysics of Cell Division

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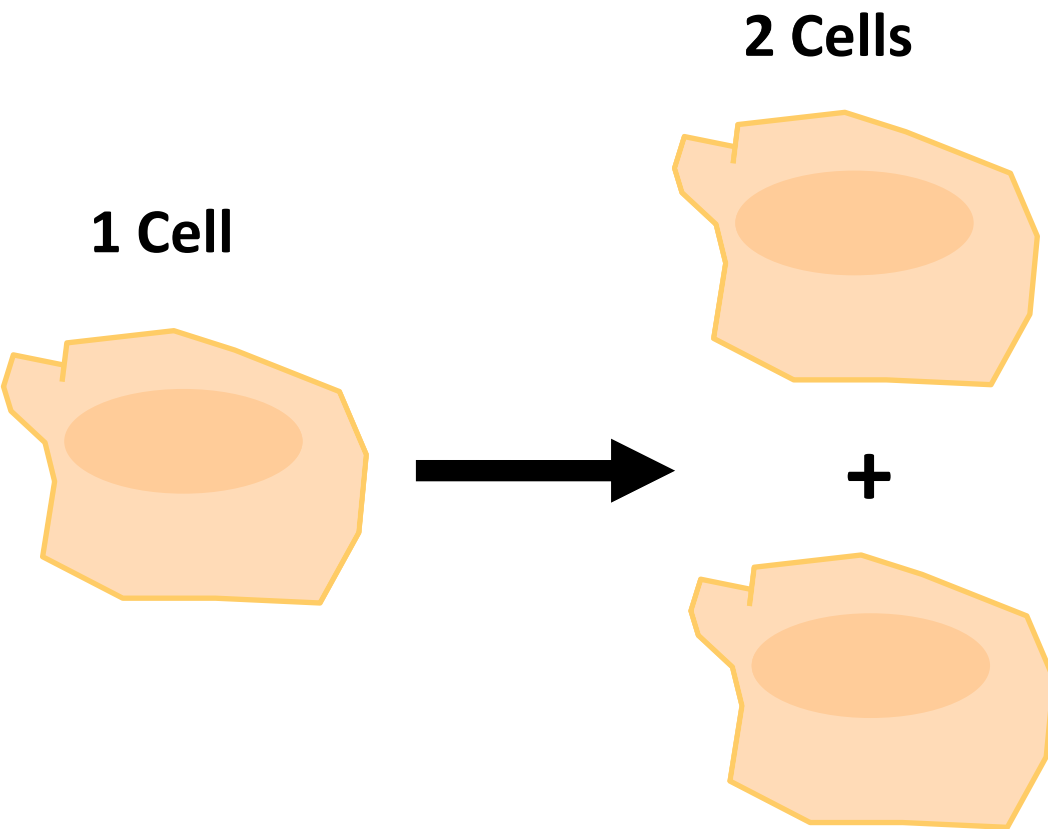
# Cell Division is Essential For All Life




## Required For. . .

- Reproduction
- Development
- Growth

$\sim 10^{13}$ cells per adult human, all originated from a single cell

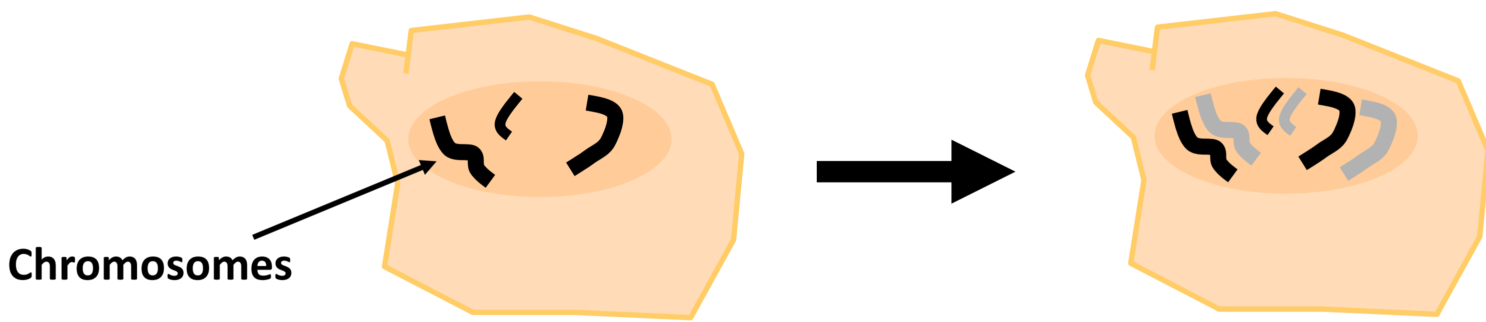
- Stasis

$\sim 10^{7}$ cells/second die and are replaced by division

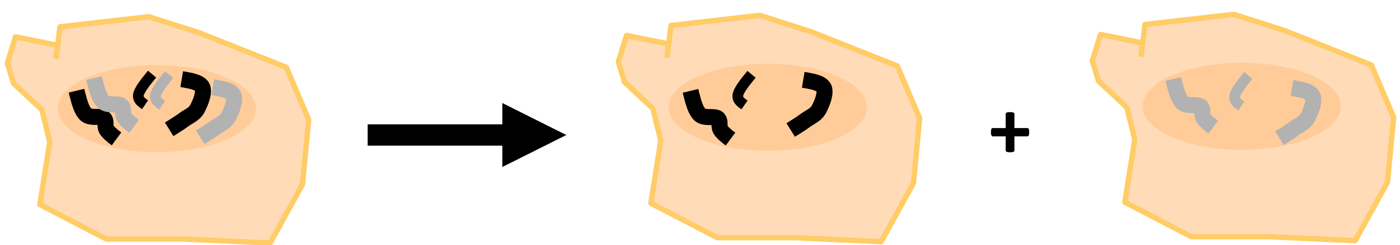
## . . .In Multicellular Organisms

# Cell Division Requires The Duplication And Segregation Of The Genetic Material

## Duplication

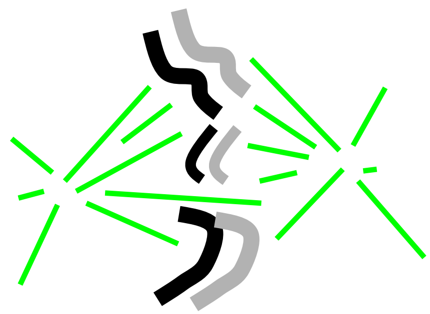



## Segregation

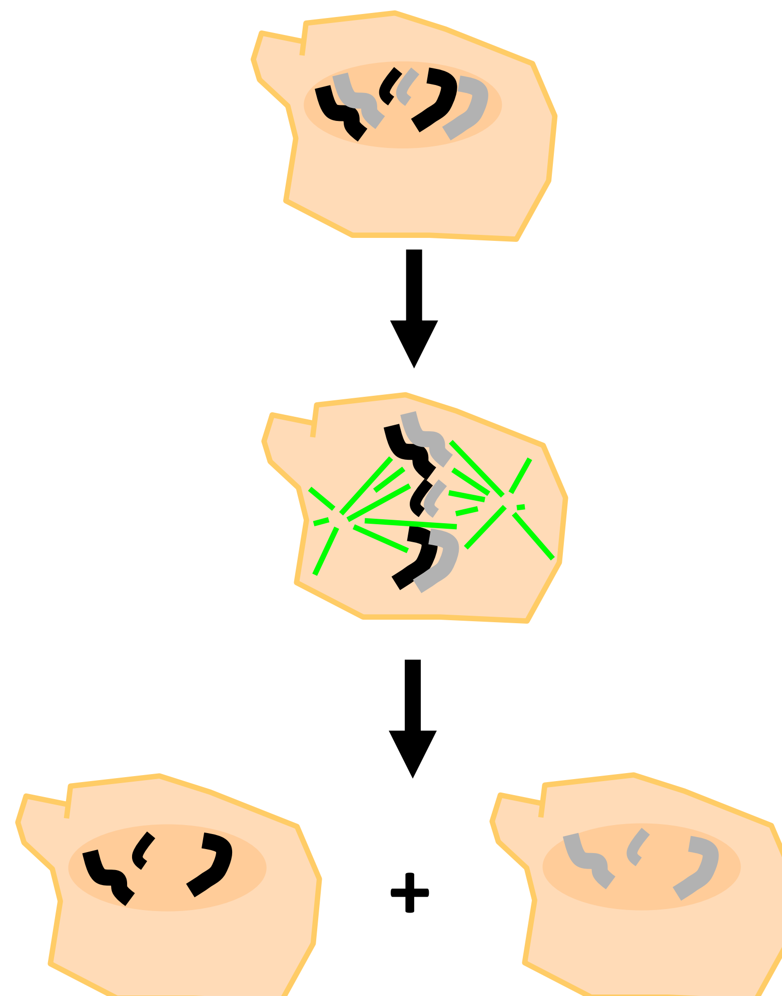


# The Spindle Segregates Chromosomes In Eukaryotes

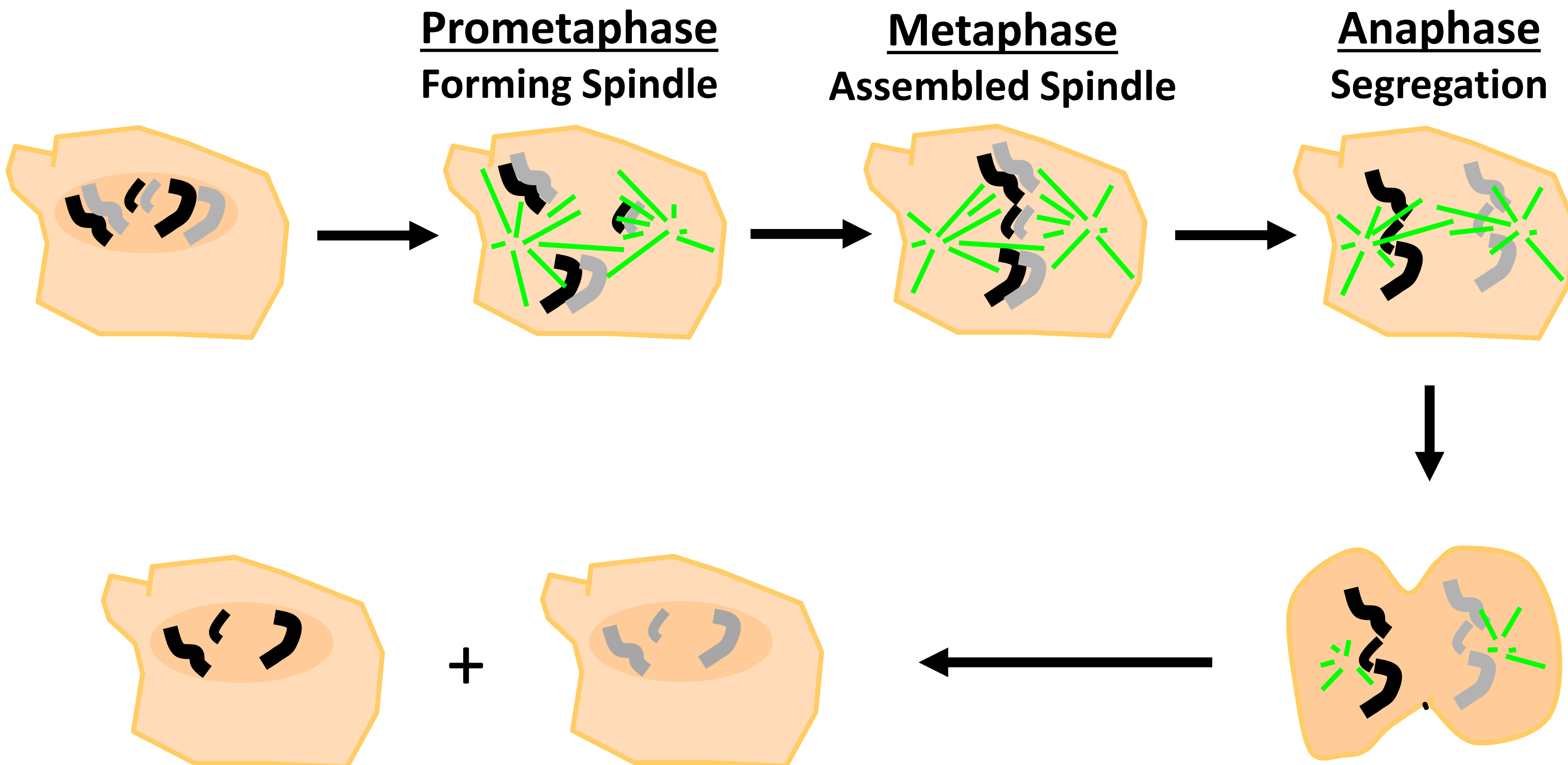
## The Spindle:



- A Dynamic Protein Assembly
- Assembles, Segregates Chromosomes, Then Disassembles



# Cell Division and The Spindle




## Fundamental Importance

Biology

Physics

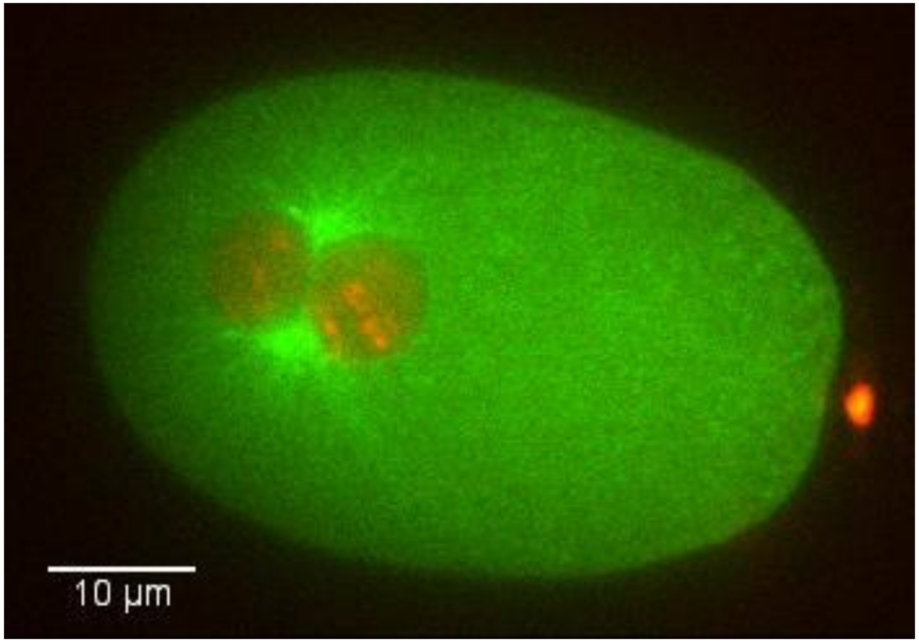
## Medical Importance

Cancer

Infertility

# Cell Division and The Spindle

**First Mitotic Division in *C. elegans***




Tubulin (Microtubules)
Histones (Chromosomes)

## Approach:

- Quantitative Measurements
- Technique Development
- Comparison with Theory

## How Does The Spindle

### Assemble?

Peter Foster, Bryan Kaye, Doogie Oh, Sebastian Furthauer, Jan Brugues (now at MPI)

### Position Itself?

Hai-Yin Wu, Manqi Deng

### Elongate?

Che-Hang Yu

### Segregate Chromosomes?

Tae Yeon Yoo

## Consequences For

**Medicine:** Tim Sanchez
infertility and assisted reproductive technologies

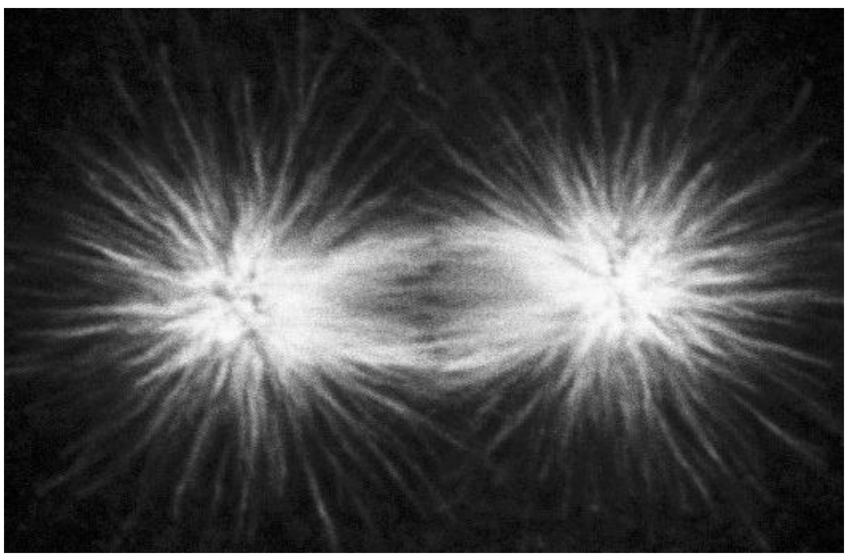
**Evolution:** Reza Farhadifar
micro and macro evolution of cell division

# Some reasons I initially became interested in evolutionary cell biology of spindles:

**1) What aspects of the spindle are "important"?**

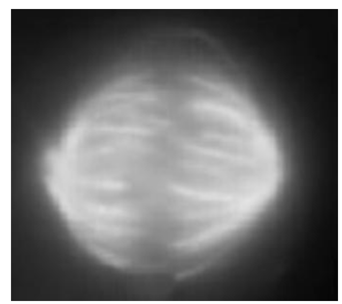
**2) Why are spindles in different organisms different?**

**S. Purpuratus "Purple Urchin"**



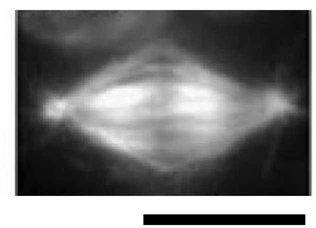
Victoria Foe, Center for Cell Cycle Dynamics

**Human Tissue Culture**



Sauer, et al, Mol. Cel. Prot. 4.1,35, 2005

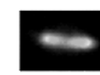
**Drosophila Tissue Culture**



~10 mm

Goshima, et al, Curr. Bio. 15:1979, 2005

**Yeast**



Pearson, et al, Mol. Bio. Cell. 17:4069, 2006

**Variation in:** Centrosomes Nuclear Envelope Breakdown

Spindle Size, Shape, Movements Microtubule Dynamics . . .

# Some reasons I initially became interested in evolutionary cell biology of spindles:

1) What aspects of the spindle are "important"?

2) Why are spindles in different organisms different?

3) How to combine cellular biophysics
with evolutionary cell biology?

**Brian Charlesworth**
**in a book review of The Plausibility of Life by Kirschner and Gehart**

**. . .they argue that the basic properties of cells and their interactions during development have profound consequences for the properties of the variability available for use by selection.**

**. . .Until we have a predictive theory of developmental genetics, our understanding of the molecular basis of development —however fascinating. . .**
**sheds little light on what variation is potentially available for the use of selection.**

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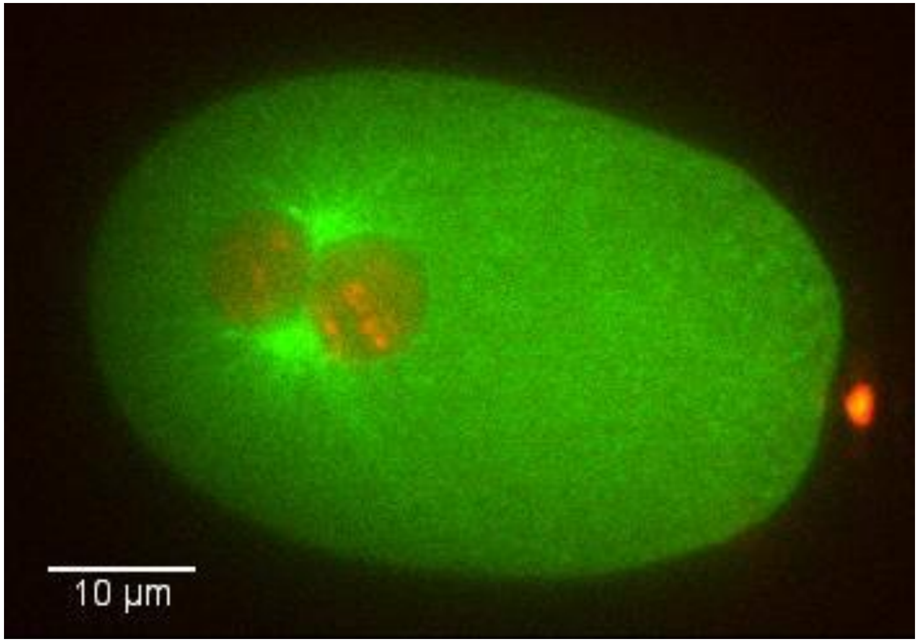
# Spindles in Different Species Are Different

## First Mitotic Division in *C. elegans*




Tubulin (Microtubules)
Histones (Chromosomes)

## How Does The Spindle

**Assemble?**
Peter Foster, Bryan Kaye, Doogie Oh, Sebastian Furthauer, Jan Brugues (now at MPI)

**Position Itself?**
Hai-Yin Wu, Manqi Deng

**Elongate?**
Che-Hang Yu

**Segregate Chromosomes?**
Tae Yeon Yoo

## Why are there differences in spindle

**Assemble?**

**Positioning?**

**Elongation?**

**Chromosome Segregation?**

**etc…**

## Consequences For

**Medicine:** Tim Sanchez
infertility and assisted reproductive technologies

**Evolution:** Reza Farhadifar
micro and macro evolution of cell division

# Spindles in Different Species Are Different

## Maybe. . .

**Why are there differences in spindle**

**Assemble?**

**Positioning?**

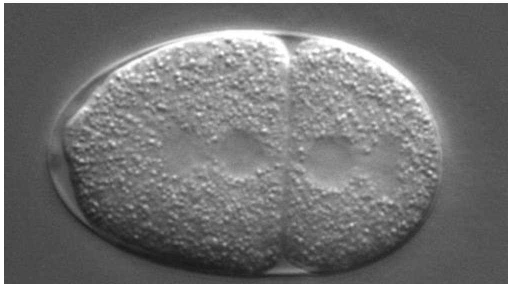
**Elongation?**

**Chromosome Segregation?**

**etc. . .**

**Division Plane Position is Important for Development Differences?**

**Asymmetric Division**



*C. kamaaina*

**Symmetric Division**

*P. pacificus*

# Spindles in Different Species Are Different

## Maybe. . .

### Adaptive

Division Plane Position is Important for Development Differences?

Tradeoff Between Speed and Accuracy of Cell Division?

. . .

### Nonadaptive

Self-Organization and Biophysics?

Neutral?

. . .

## Why are there differences in spindle

Assemble?

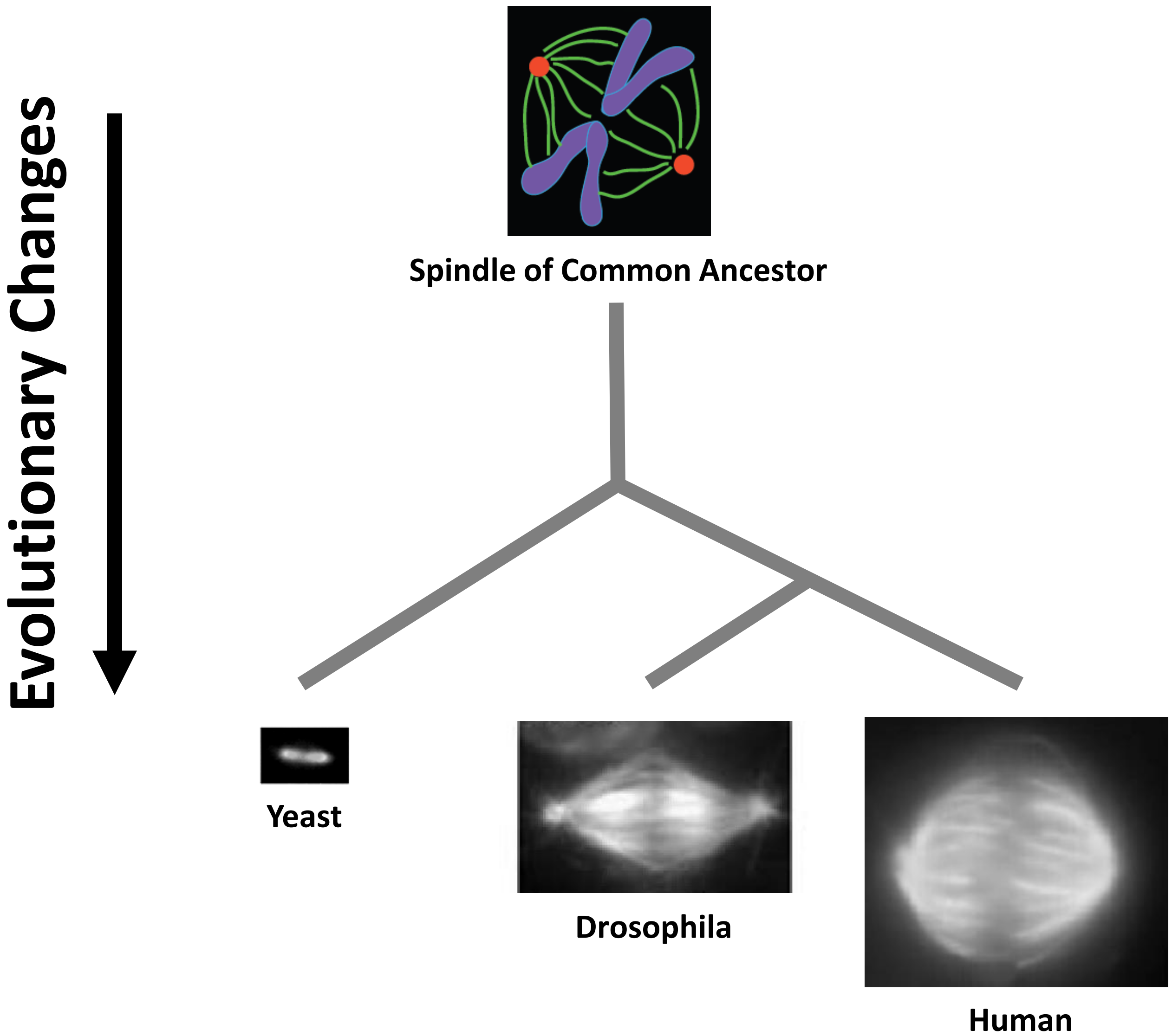
Positioning?

Elongation?

Chromosome Segregation?

etc. . .

# Evolutionary Basis of Spindle Diversity?




**Microscopy from:**

Pearson, et al, Mol. Bio. Cell. 17:4069, 2006

Goshima, et al, Curr. Bio. 15:1979, 2005

Sauer, et al, Mol. Cel. Prot. 4.1,35, 2005

# Evolutionary Basis of Spindle Diversity

**spontaneous mutation is the ultimate source of variation**

↓

**selection, population dynamics, genetic drift, . . .**

↓

**within species variations of the spindle**

↓

**between species variations of the spindle**

# Evolutionary Basis of Spindle Diversity

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selection, population dynamics, genetic drift, . . .

↓

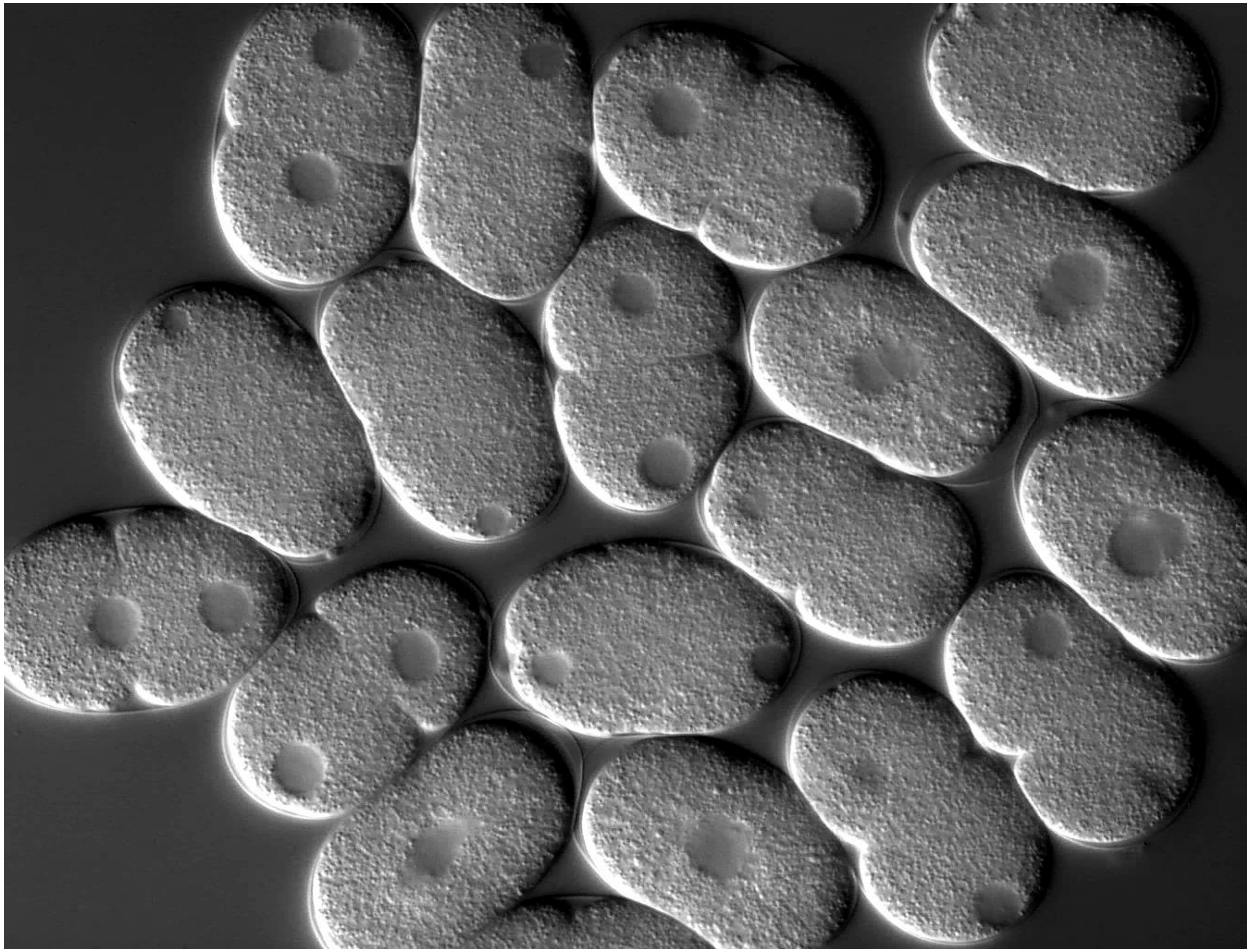
within species variations of the spindle

↓

between species variations of the spindle

Study within species variations of the first mitotic spindle in *C. elegan*

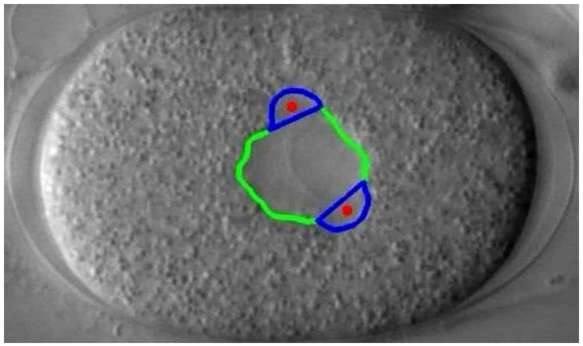
# High Throughput Imaging of the First Mitotic Spindle in *C. elegans*



Imaged more than 20,000 embryos

# Automated Segmentation and Tracking of the First Mitotic Spindle in *C. elegans*

## Tracking

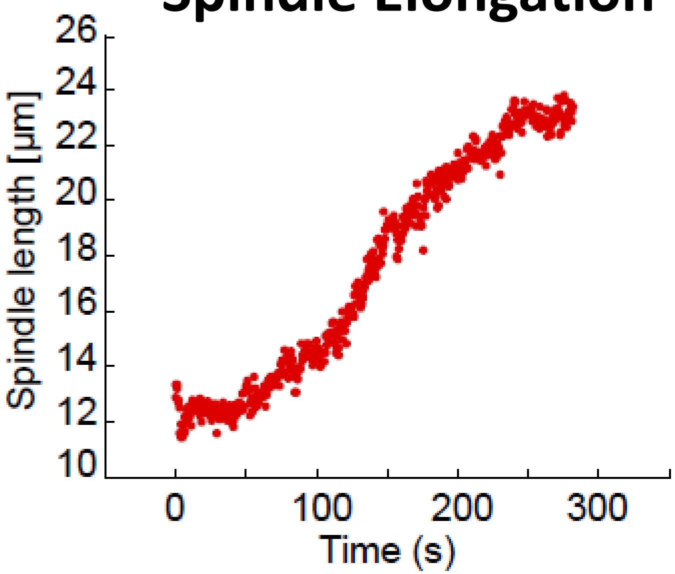


## Quantify

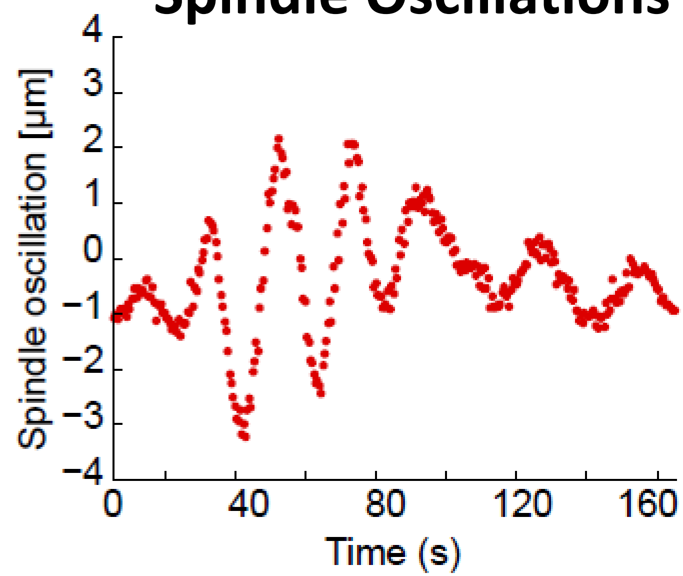
- Spindle Elongation
- Oscillations
- Centrosome Size
- Division Plane

etc. . .

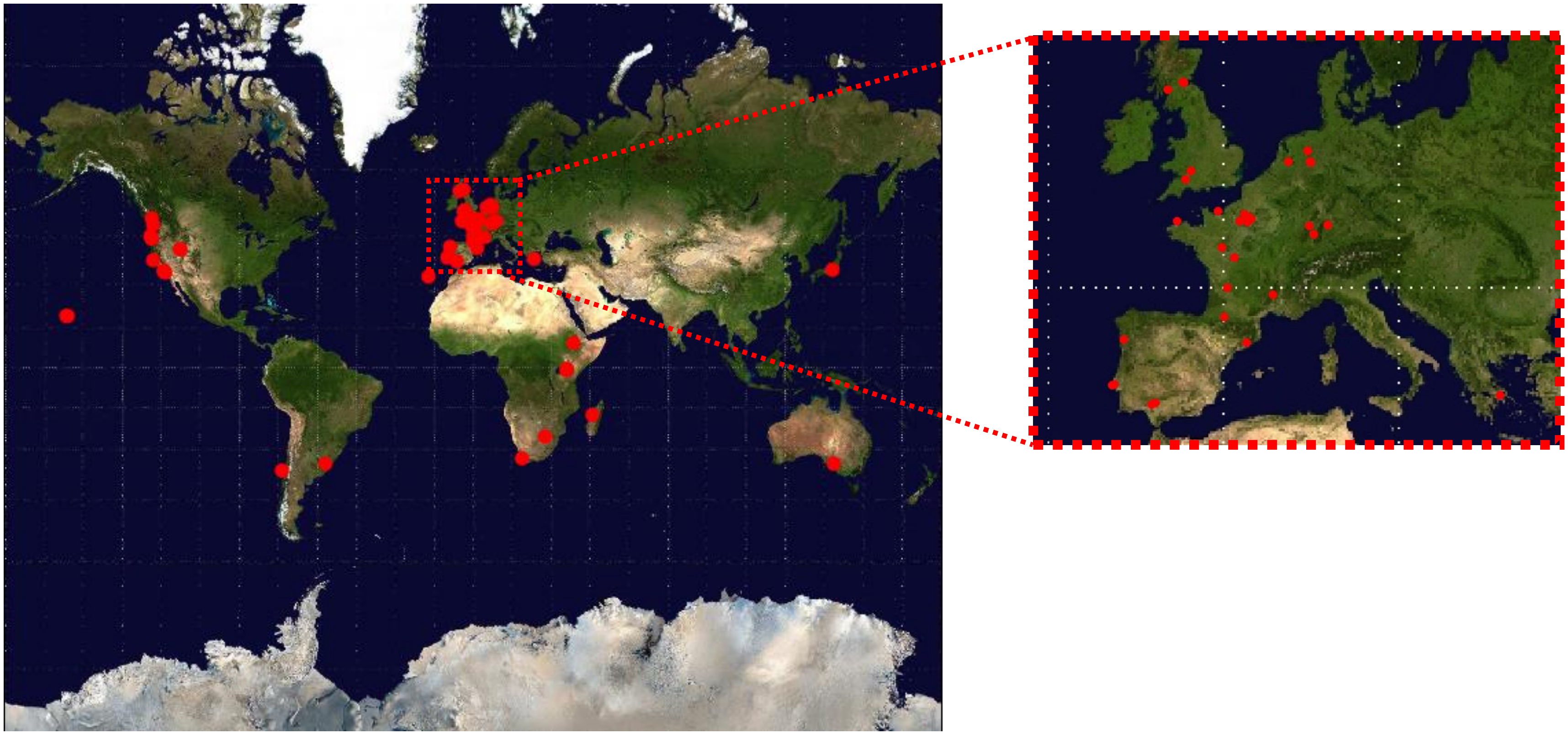
### Spindle Elongation




### Spindle Oscillations



# Studied the First Mitotic Spindle in Many *C. elegans* Wild Isolates From Around the World

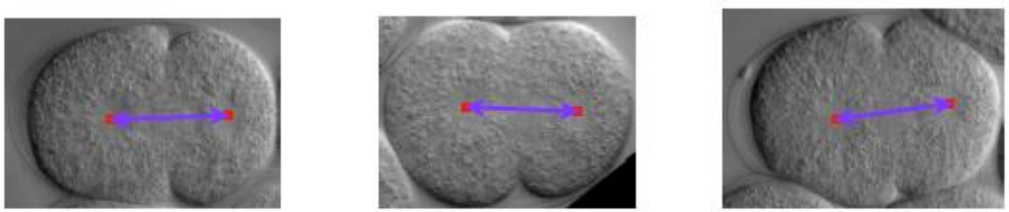


Isolated collected by Marie-Anne Felix, Erik Andersen, and many others

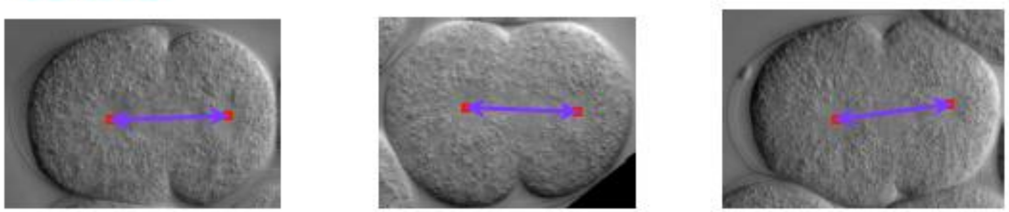
- ~100 *C. elegans* wild isolates collected from around the globe
- wild isolates sequenced by Erik Anderson and Leonid Kruglyak

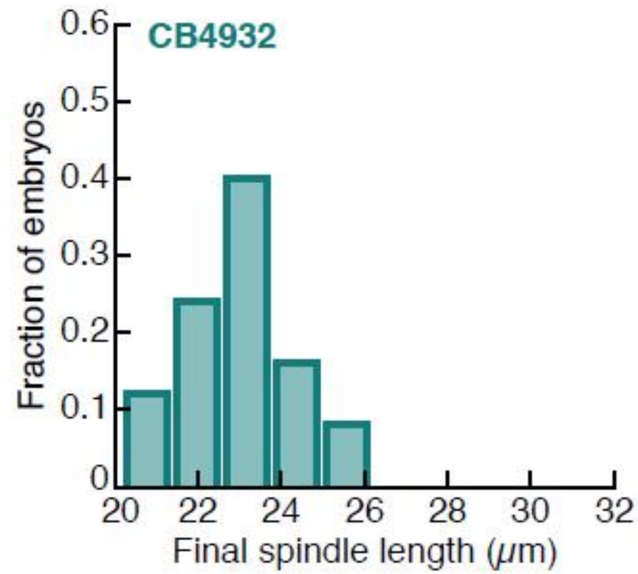
# Extensive Standing GENETIC Variation For Spindle Dynamics and Morphology In *C. elegans* Wild Isolates

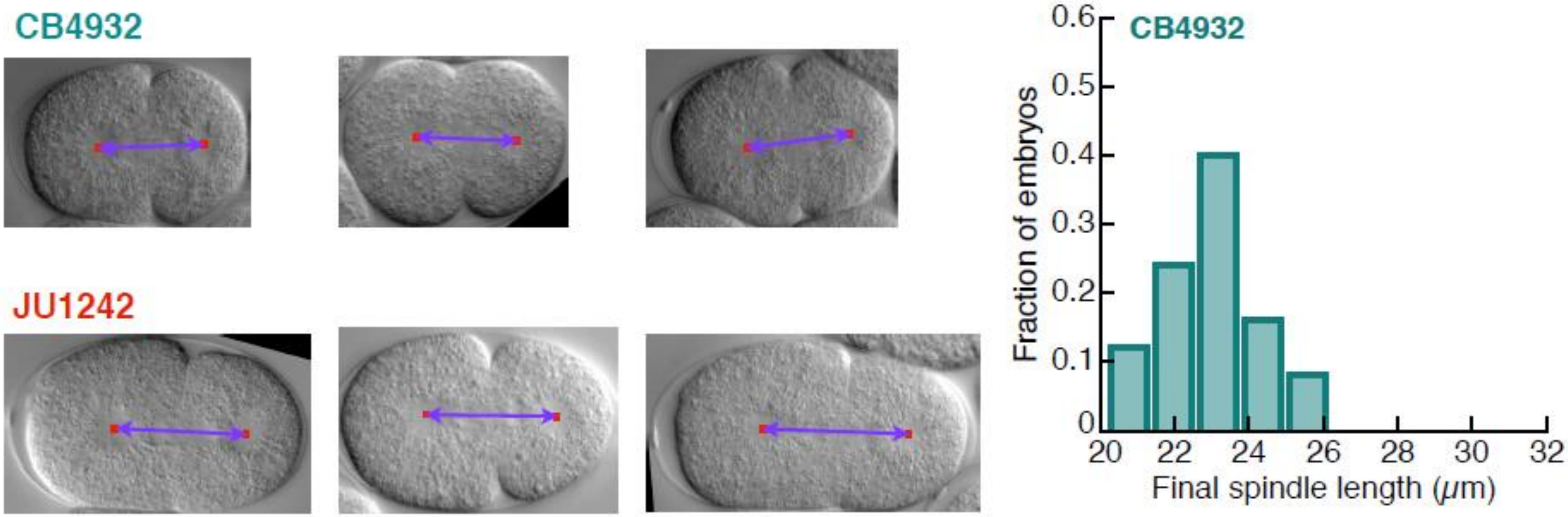
CB4932



CB4932




CB4932
0.6
0.5
0.4
0.3
0.2
0.1
0
Fraction of embryos
20
22
24
26
28
30
32
Final spindle length (μm)

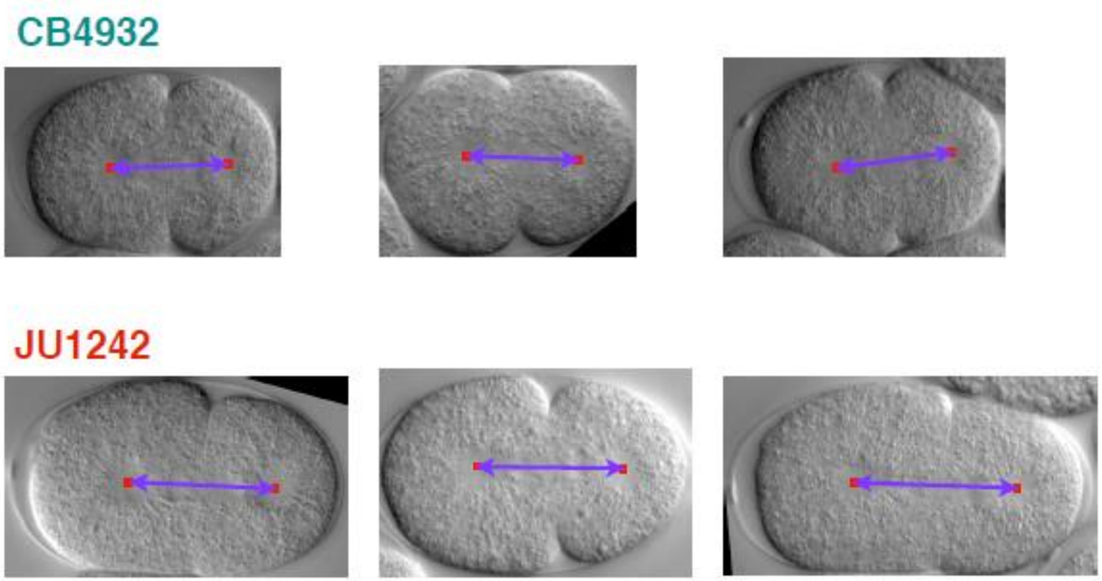


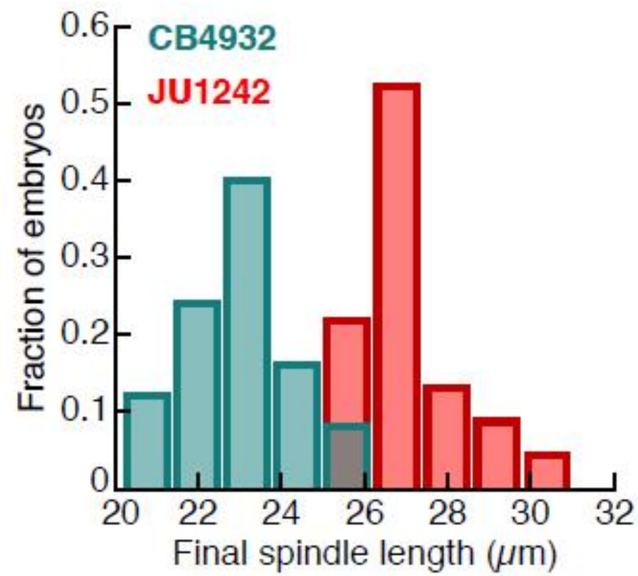
CB4932
JU1242
CB4932
Fraction of embryos
0.6
0.5
0.4
0.3
0.2
0.1
0
20
22
24
26
28
30
32
Final spindle length (μm)

**Final spindle length differs in different wild isolates**

↓

**There is GENETIC variation for final spindle length in *C. elegans***

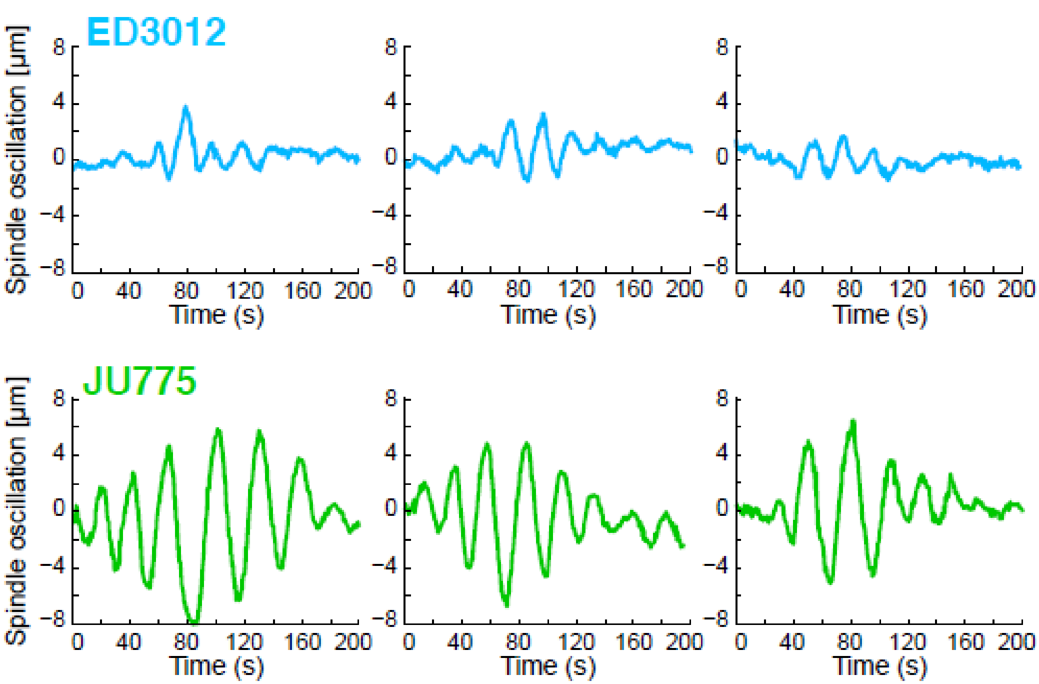


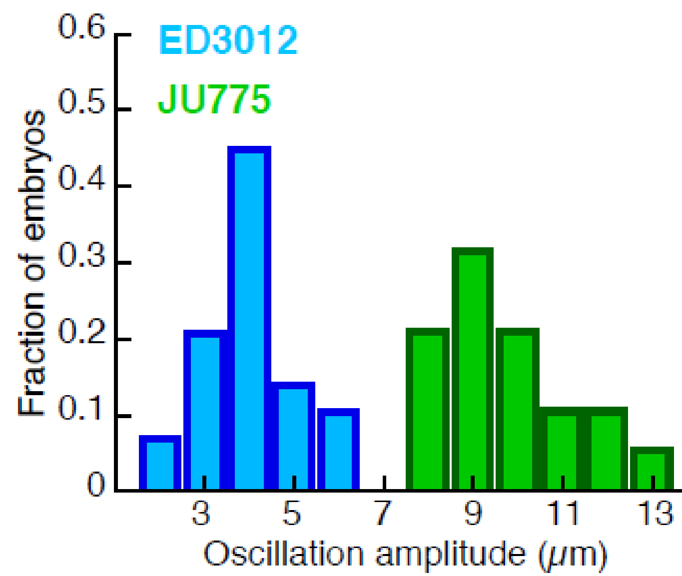



# Spindles oscillate differently in different wild isolates

↓

# There is GENETIC variation for spindle oscillations in *C. elegans*

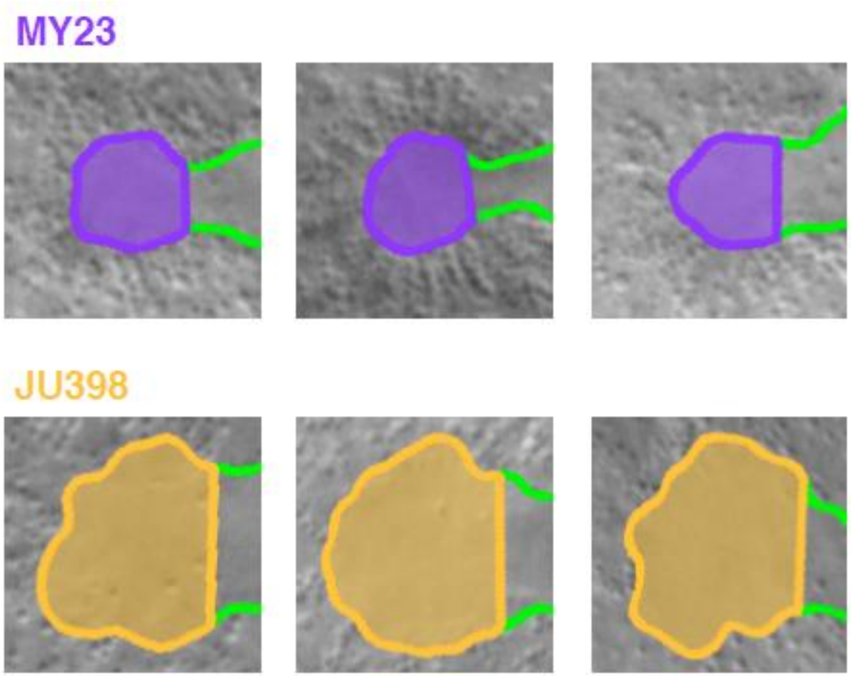


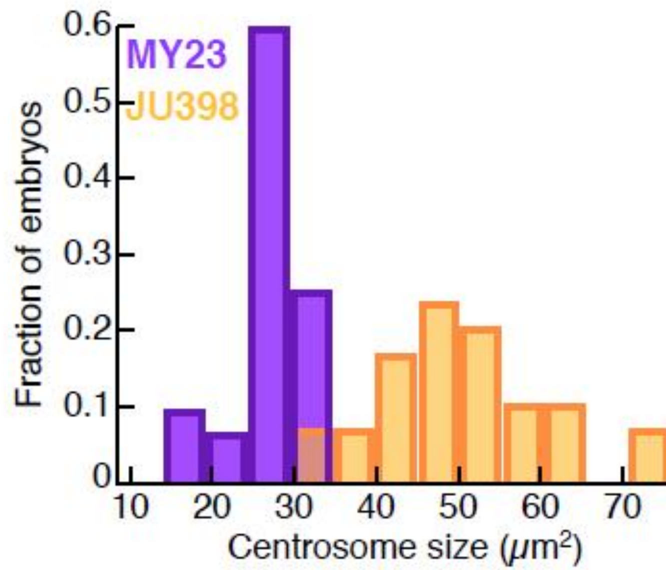



**Centrosome size differs in different wild isolates**

↓

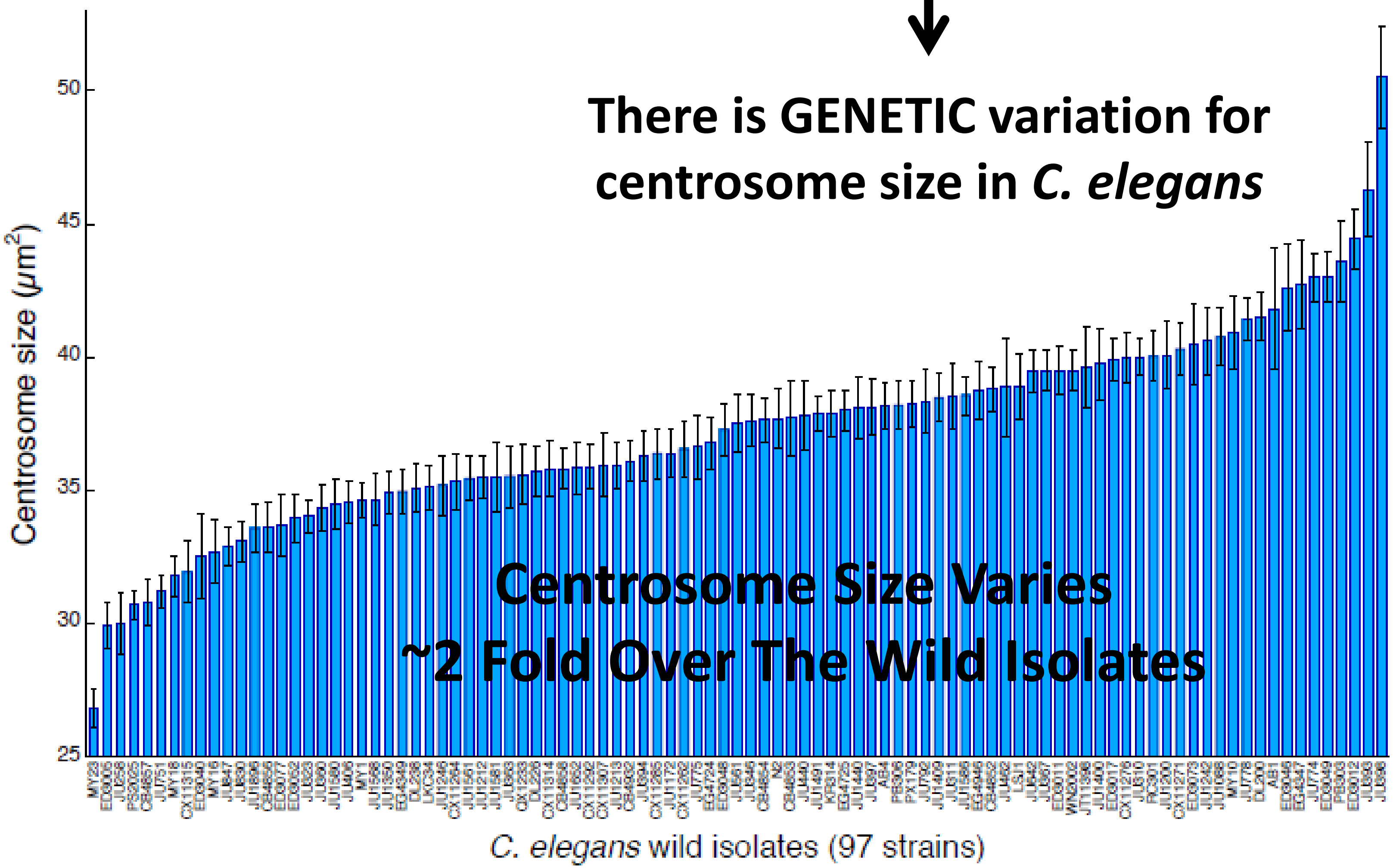
**There is GENETIC variation for centrosome size in *C. elegans***



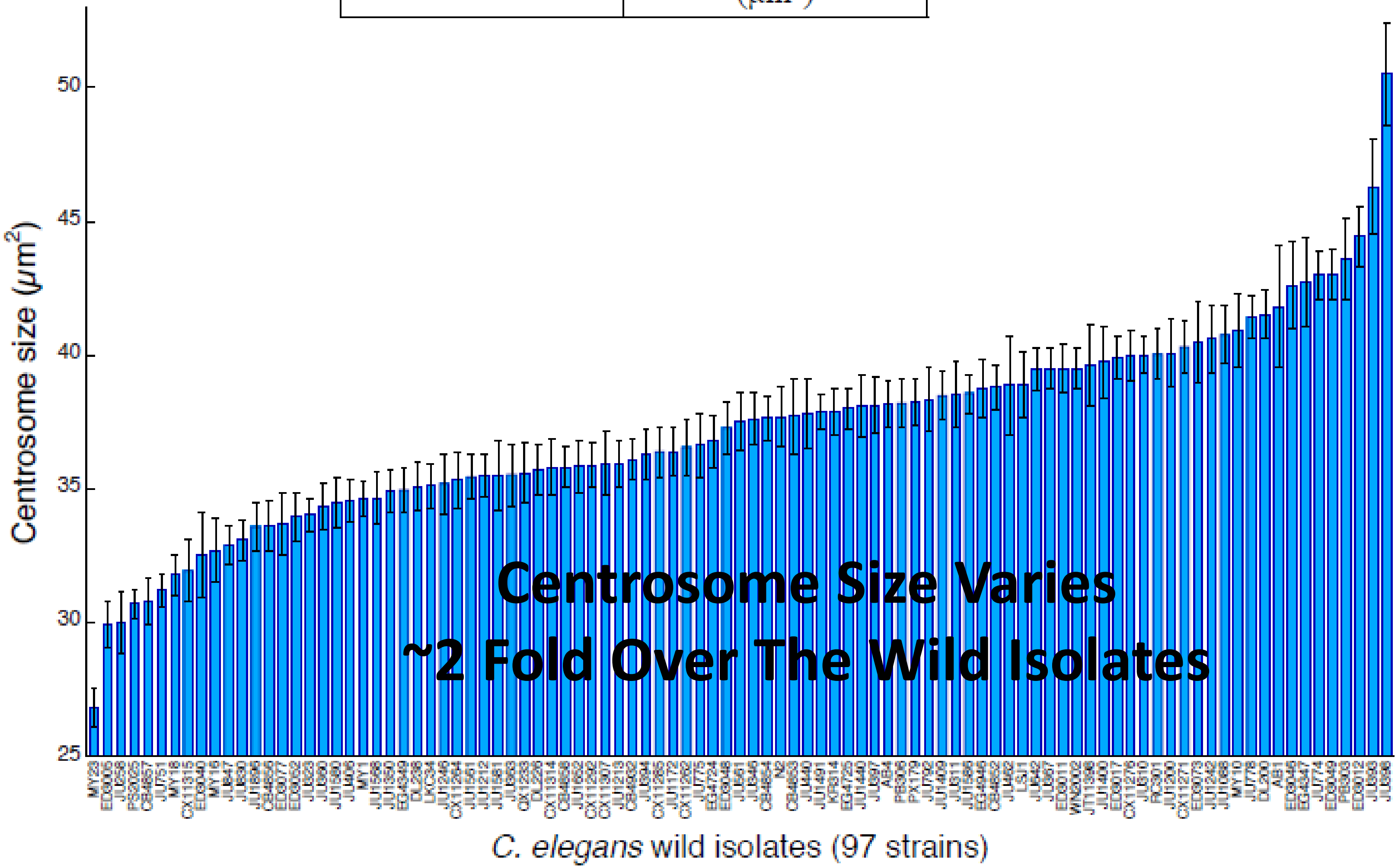
# Centrosome size differs in different wild isolates

**There is GENETIC variation for centrosome size in *C. elegans***



# GENETIC variance in centrosome size
# = variance associated with differences between isolates

| | |
|---|---|
| Centrosome Size | $6.52 \pm 1.37$ ($\mu m^4$) |

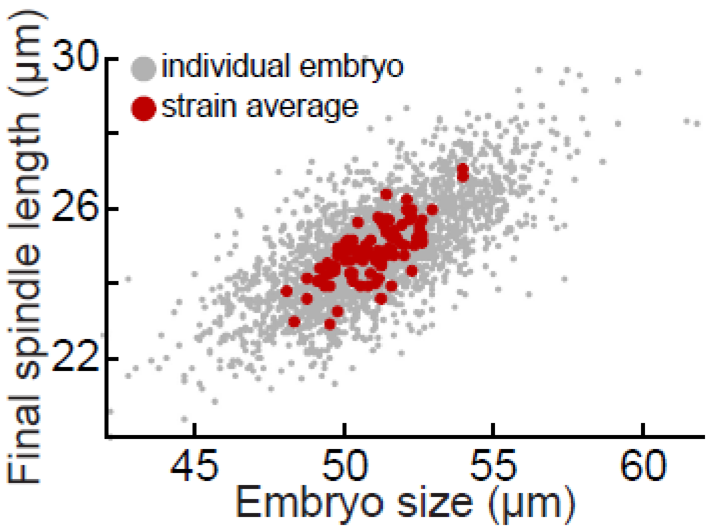


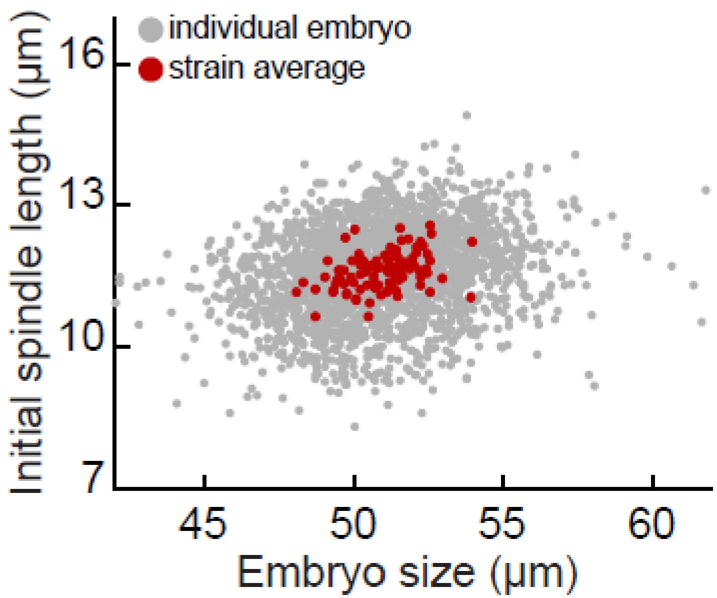
# GENETIC variance in the spindle and cell division

# = variance associated with differences between isolates

| | |
|---|---|
| Centrosome Size | $6.52 \pm 1.37$ ($\mu m^4$) |
| Initial Length | $0.06 \pm 0.01$ ($\mu m^2$) |
| Final Length | $0.24 \pm 0.04$ ($\mu m^2$) |
| Elongation Rate | $0.20 \pm 0.04$ ($\mu m^2/min^2$) |
| Division Plane | $0.15 \pm 0.03$ ($\mu m^2$) |
| Embryo Size | $0.55 \pm 0.09$ ($\mu m^2$) |

# Aspects of the Spindle and Cell are Correlated




## All Traits are Correlated With Embryo Size/Cell Size

| | Final Length | Elongation Rate | Centrosome Size | Division Plane | Embryo Size |
|---|---|---|---|---|---|
| Initial Length | (nonsignificant) | 0.27 | (nonsignificant) | 0.17 | 0.22 |
| Final Length | | 0.31 | 0.25 | 0.40 | 0.70 |
| Elongation Rate | | | -0.20 | 0.28 | 0.45 |
| Centrosome Size | | | | (nonsignificant) | 0.24 |
| Division Plane | | | | | 0.71 |

Significant correlation (p<0.001)

Nonsignificant

**Some correlations with cell size are weak**

**Cell size is not the sole determinant of these traits**

# Our Approach to Understanding Spindle Evolution

spontaneous mutation is the ultimate source of variation

↓

selection, population dynamics, genetic drift, . . .

↓

within species variations of the spindle

↓

between species variations of the spindle

need to characterize effects of spontaneous mutations on cell division traits

how to do this?

extensive GENETIC variation for cell division traits in *C. elegan*

what determines the level of variation of cell division traits?

# Mutation Accumulation to Measure the Spectrum of Spontaneous Mutations

## Standard Propagation Protocol

competition between individuals imposes selection in lab

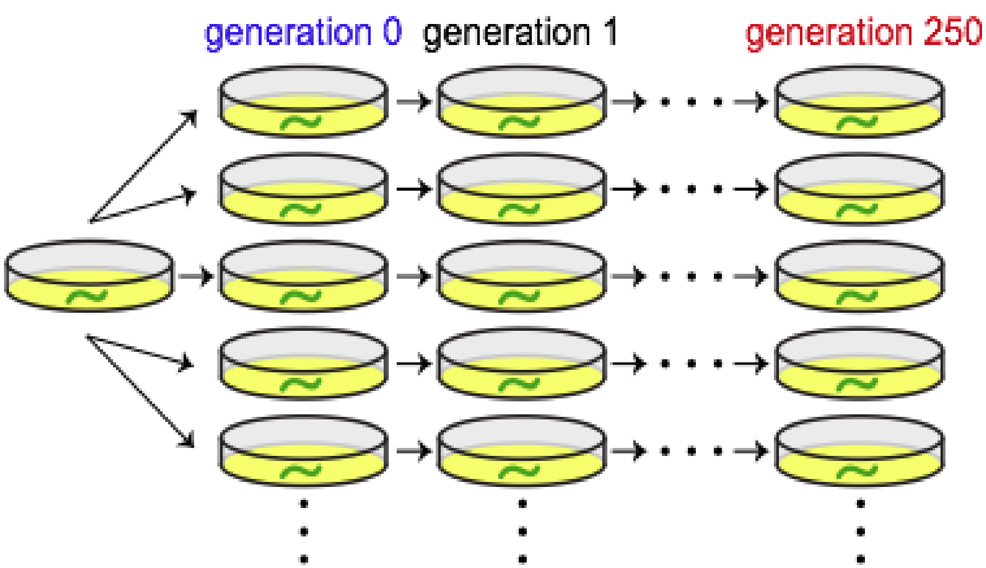
## Mutation Accumulation Propagation Protocol

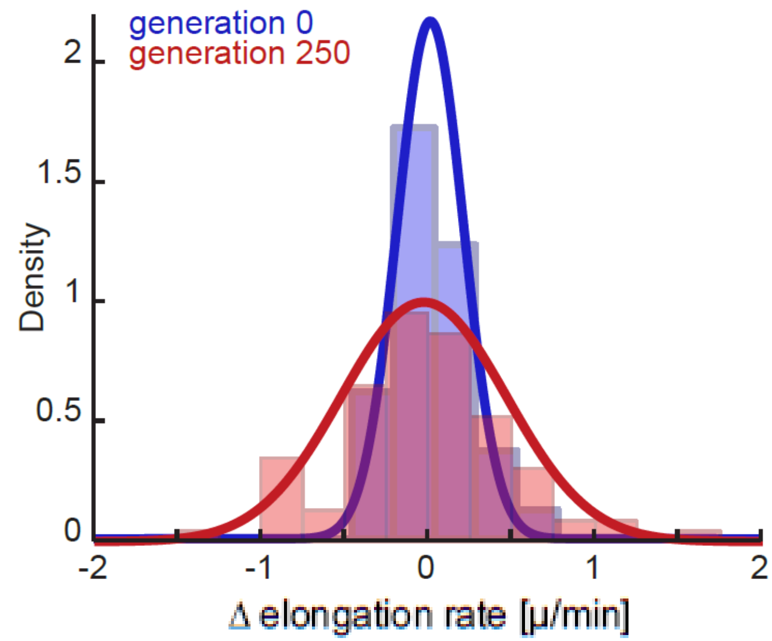
negligible competition between individuals imposes negligible selection in lab

mutations not resulting in sterility and lethality are maintained

# Mutation Accumulation to Measure the Spectrum of Spontaneous Mutations

## spontaneous mutations modify cell division traits



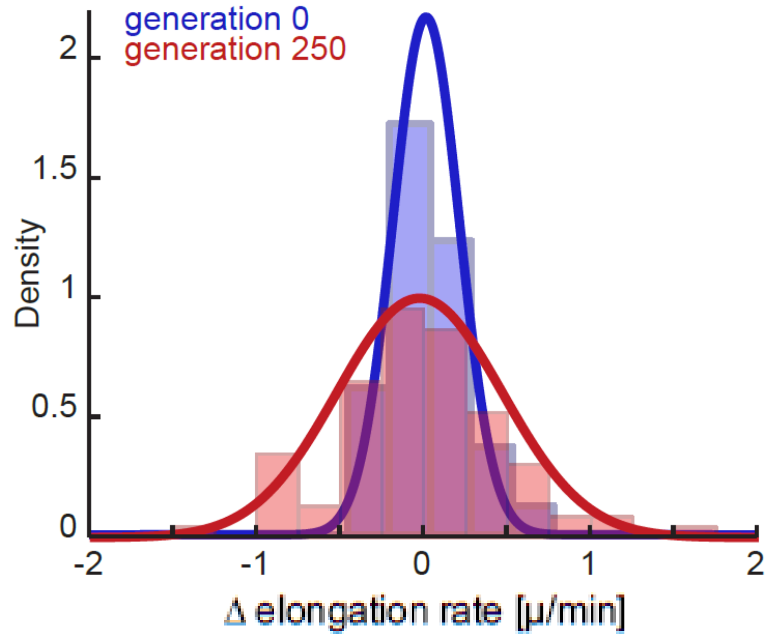

**Propagated for 250 generations (created by Charlie Baer)**
**Imaged ~100 MA lines in two different genetic backgrounds**
**Imaged ~20-40 embryos per line**

# Mutation Accumulation to Measure the Spectrum of Spontaneous Mutations

## spontaneous mutations modify cell division traits

**Variance in elongation rate generated by mutations per generation**

| | |
|---|---|
| Elongation Rate | 3.37E-4 ± 7.46E-5 ($\mu m^2/min^2$) |

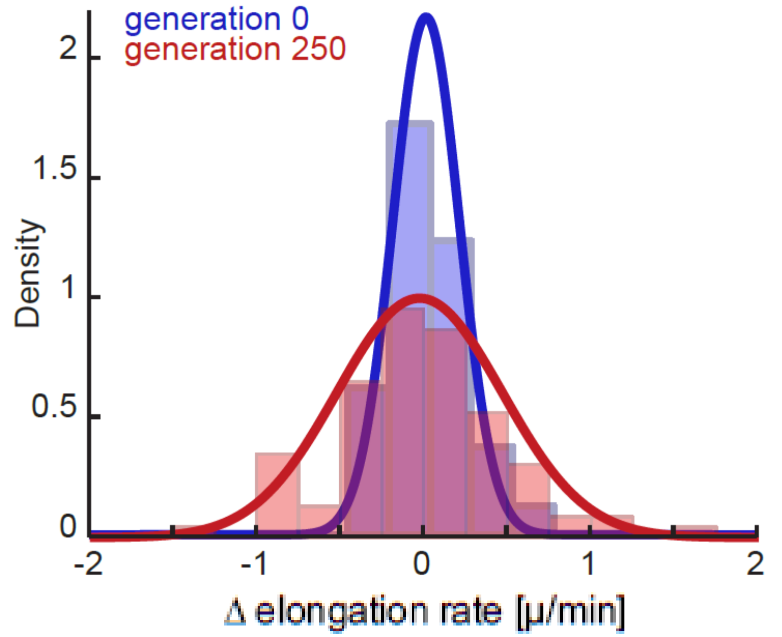


# Mutation Accumulation to Measure the Spectrum of Spontaneous Mutations

## spontaneous mutations modify cell division traits

**Variance in the spindle and cell division generated by mutations per generation**

| | |
|---|---|
| Elongation Rate | 3.37E-4 ± 7.46E-5 ($\mu m^2/min^2$) |
| Initial Length | 7.06E-5 ± 1.07E-4 ($\mu m^2$) |
| Final Length | 4.20E-4 ± 1.72E-4 ($\mu m^2$) |
| Centrosome Size | 4.70E-3 ± 2.50E-3 ($\mu m^4$) |
| Division Plane | 4.11E-4 ± 1.21E-4 ($\mu m^2$) |
| Embryo Size | 2.17E-3 ± 8.32E-4 ($\mu m^2$) |




**also measured mutational covariances between traits**

# Our Approach to Understanding Spindle Evolution

spontaneous mutation is the ultimate source of variation

↓

selection, population dynamics, genetic drift, . . .

↓

within species variations of the spindle

↓

between species variations of the spindle

characterized effects of spontaneous mutations on cell division traits

?

extensive GENETIC variation for cell division traits in *C. elegan*

what determines the level of variation of cell division traits?

# What determines the level of variation of cell division traits?

## Are cell division traits neutral?

## For a neutral trait, governed by drift:

$$V_g / V_m = 4\ N_e = \sim 40{,}000$$

Genetic variance of a trait in nature

Measured in Wild Isolates

Variation generated by mutation per generation

Measured in Mutation Accumulation Lines

effective population size

Determined from sequencing

Andersen et al, Nat Genet. 44, 285-290, 2012

# What determines the level of variation of cell division traits?

## Are cell division traits neutral?

**For a neutral trait, governed by drift:**

$$V_g / V_m = 4\ N_e = \sim 40{,}000$$

## Measurements:

for embryo size $V_g / V_m = \sim 250$

for other cell division traits $V_g / V_m = \sim 400 - 1{,}400$

**Cell Division Traits Are NOT Neutral**

**Can Also Argue Against Other Possibilities:**

NOT Local Adaptation, NOT Linked Selection

**What determines the level of variation of cell division traits?**

**Perhaps there is one optimal set of cell division traits in *C. elegans*?**

**i.e. level of variation produced by a balance between mutation and selection**

**If so, which of these traits is selection acting on?**

**Embryo size shows greatest deviation from neutral model**

**Embryo size is correlated with all other cell division traits**

**Maybe selection only acts on embryo size?**

# Stabilizing Selection on Embryo Size and Variation in Cell Division Traits Between Isolates

## Prediction of quantitative genetics theory of mutation-selection balance:

$$M = GWG$$

**M**: matrix of variation/covariation of cell division traits from mutation

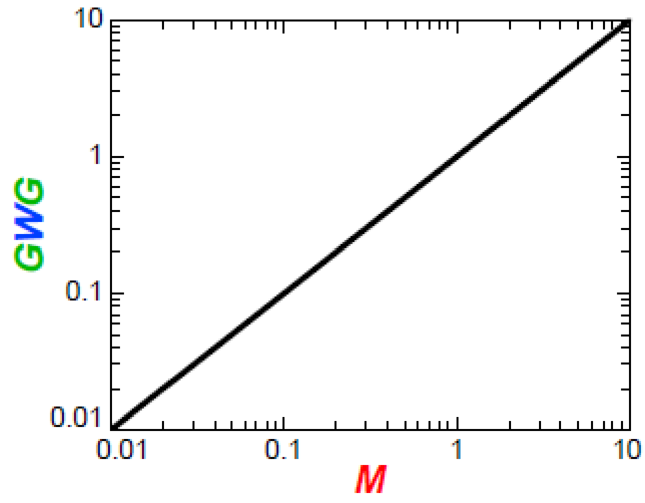
**W**: matrix of selection coefficients

**G**: matrix of variation/covariation of cell division traits in nature

Measured in Mutation Accumulation Lines

Postulated Stabilizing Selection on Embryo Size

Measured in Wild Isolates



# Stabilizing Selection on Embryo Size and Variation in Cell Division Traits Between Isolates

**Prediction of quantitative genetics theory of mutation-selection balance:**

**M = GWG**

matrix of variation/covariation of cell division traits from mutation

matrix of selection coefficients

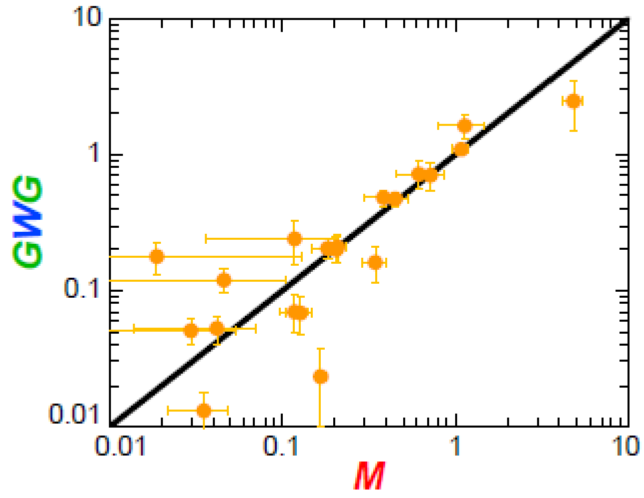
matrix of variation/covariation of cell division traits in nature

Measured in Mutation Accumulation Lines

Postulated Stabilizing Selection on Embryo Size

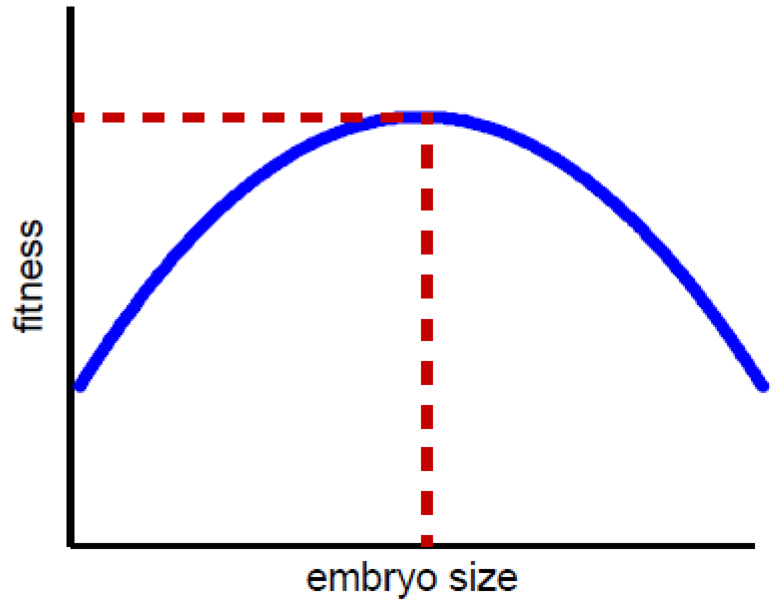
Measured in Wild Isolates

**Stabilizing selection on embryo size explains variations in all other cell division traits!**



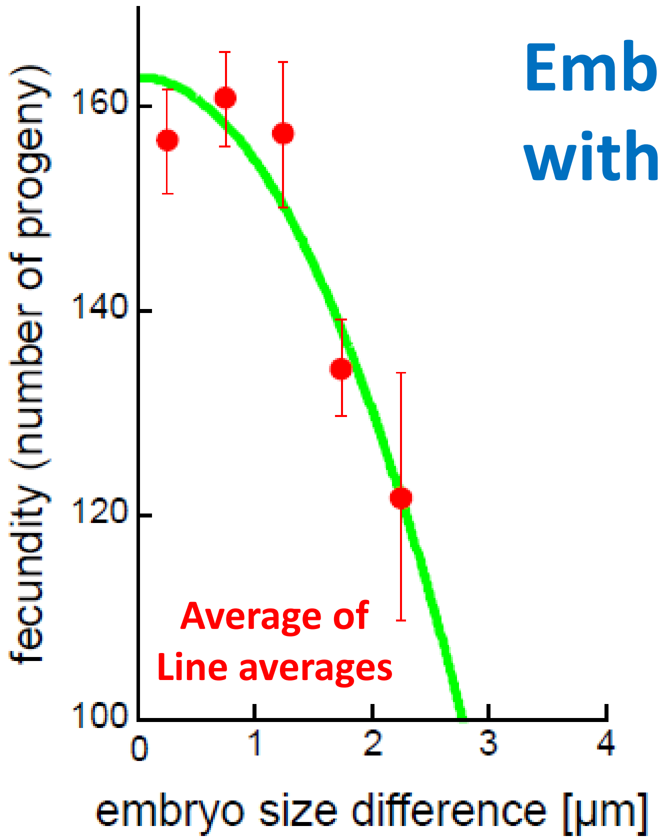
# Further Test of Stabilizing Selection on Embryo Size

- **optimal embryo size ⟶ highest fitness**
- **further from optimal embryo size ⟶ lower fitness**



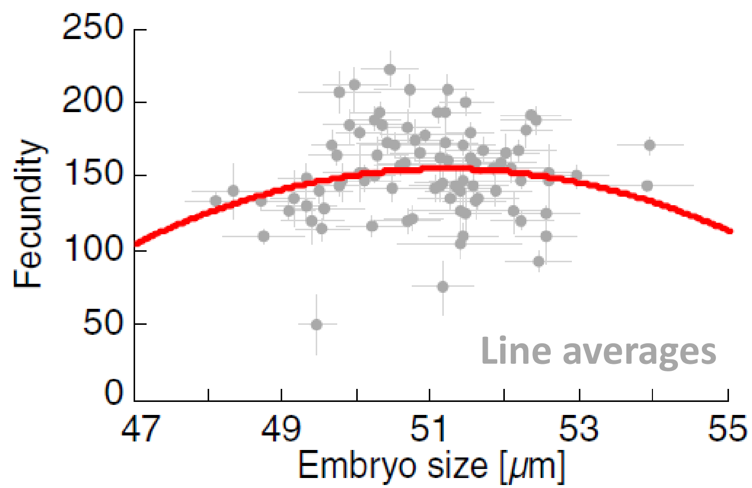
# Further Test of Stabilizing Selection on Embryo Size

- **optimal embryo size ⟶ highest fitness**
- **further from optimal embryo size ⟶ lower fitness**
- **measured lifetime fecundity (number of progeny)**




**Embryo size most strongly associated with fecundity, of all cell division traits**

**optimal embryo size ~51 μm**




**Fecundity measurements by Eric Andersen (Northwestern)**

# Further Test of Stabilizing Selection on Embryo Size

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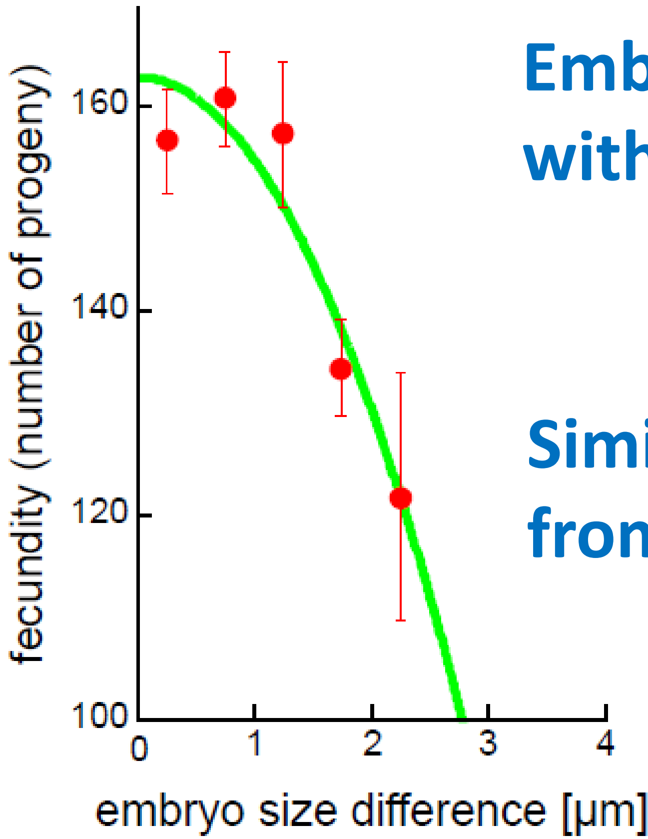



**Embryo size most strongly associated with fecundity, of all cell division traits**

**optimal embryo size ~51 μm**

**Similar strength of selection as inferred from mutation accumulation study**

**Fecundity measurements by Eric Andersen (Northwestern)**

# Our Approach to Understanding Spindle Evolution

spontaneous mutation is the ultimate source of variation

↓

selection, population dynamics, genetic drift, . . .

↓

within species variations of the spindle

↓

between species variations of the spindle

characterized effects of spontaneous mutations on cell division traits

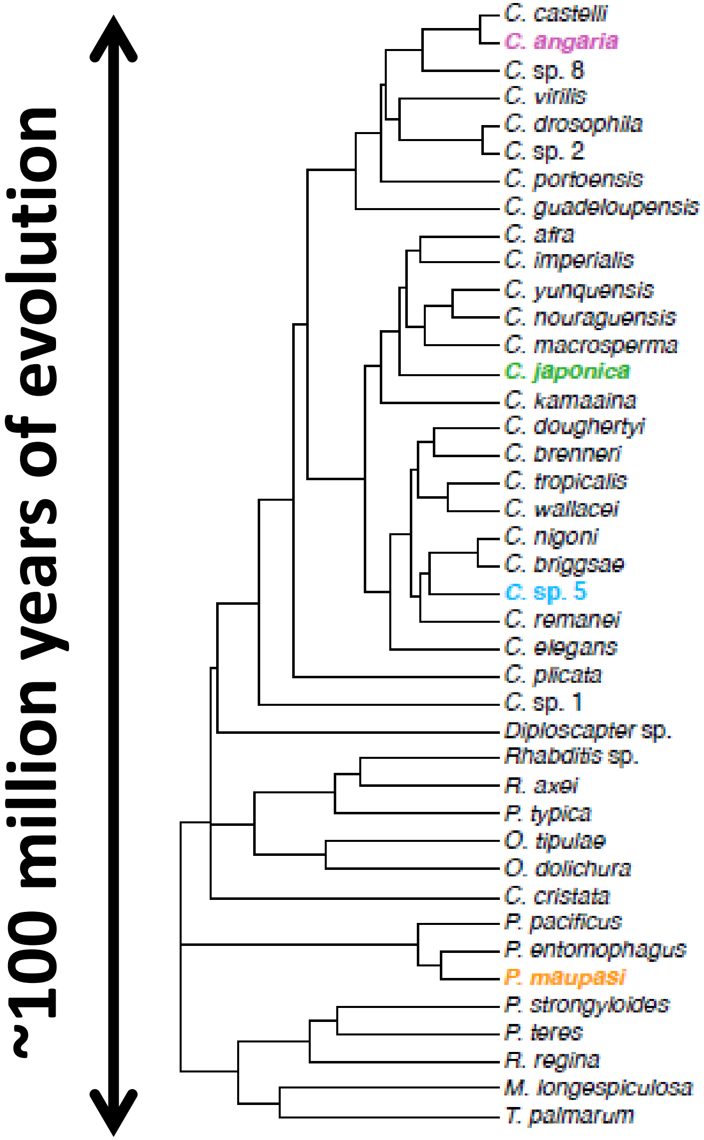
balance between mutations and stabilizing selection on embryo size explains variation of cell division traits

extensive GENETIC variation for cell division traits in *C. elegan*

what determines the level of variation of cell division traits?

?

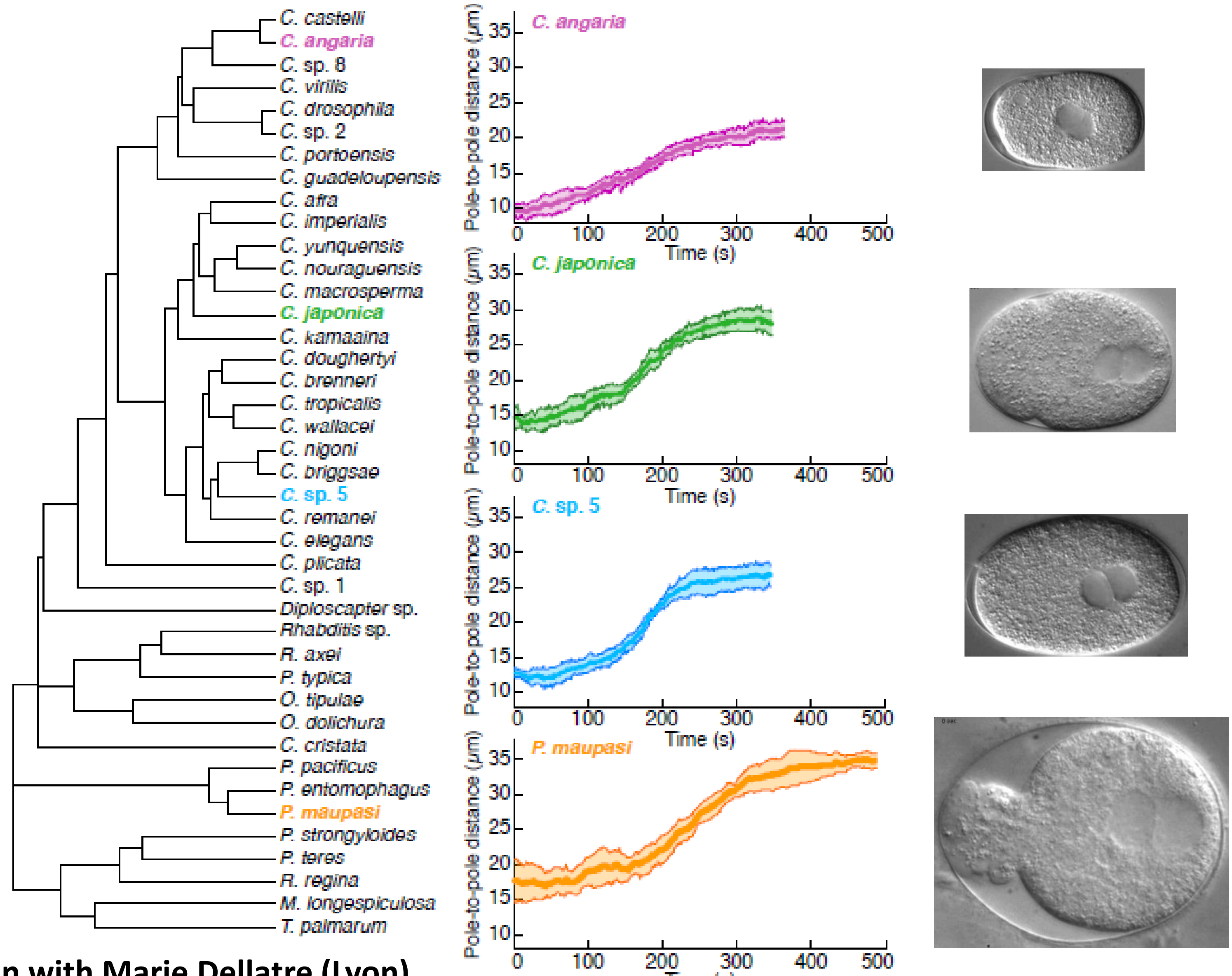
# What determines between species variations of cell division traits?




**Studied first mitotic division in ~40 species nematodes of known phylogeny**

**In collaboration with Marie Dellatre (Lyon)**

# What determines between species variations of cell division traits?

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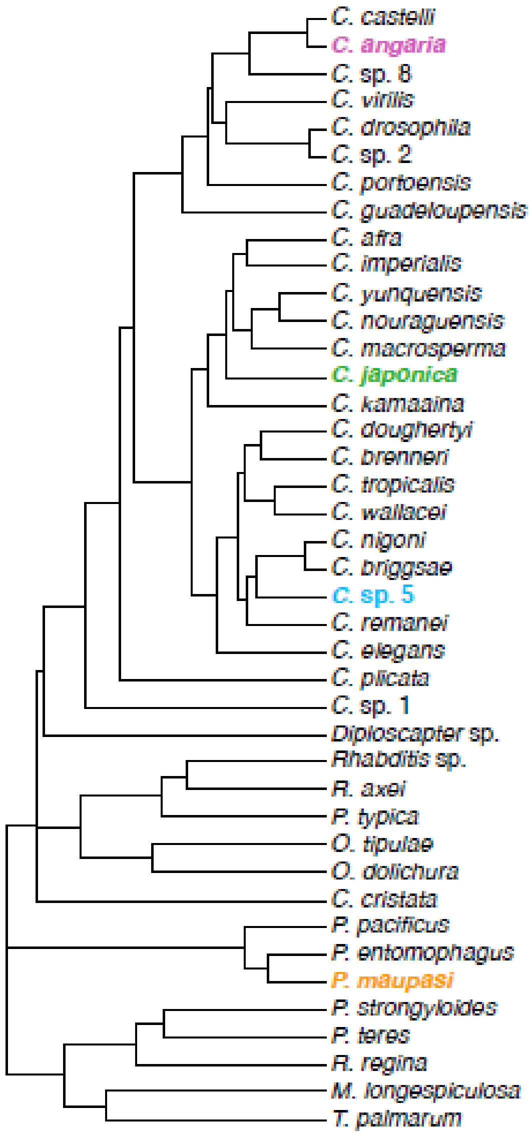
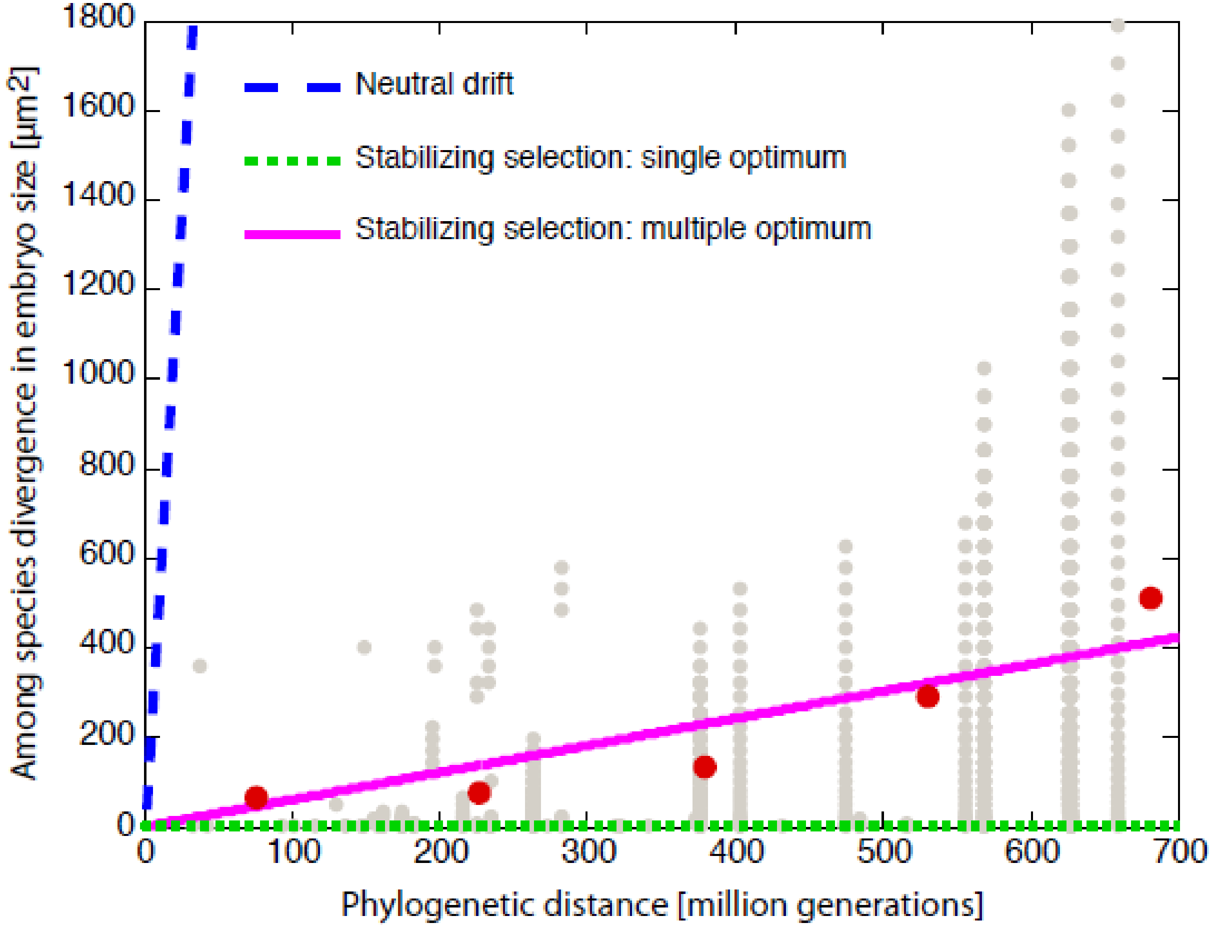



## Embryo Size

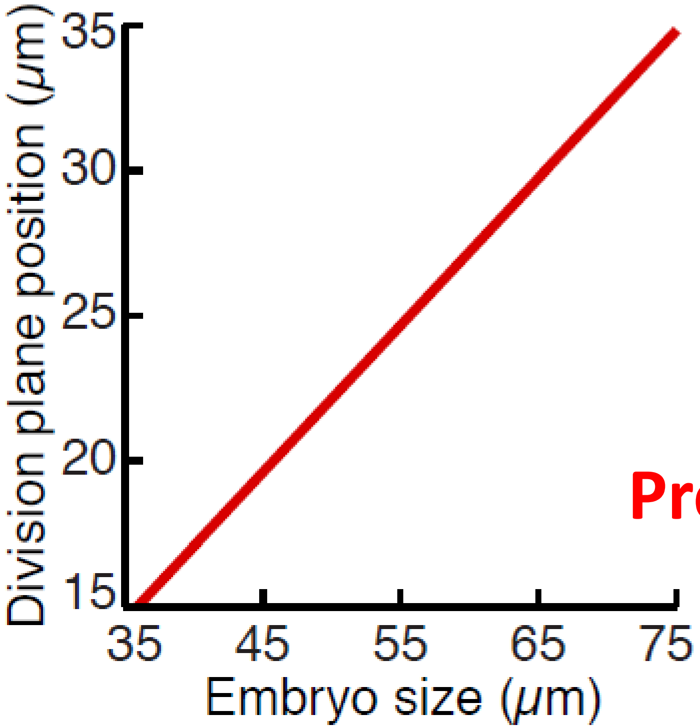
### variation with phylogenetic distance



# What determines between species variations of cell division traits?

If each species has it's own optimal embryo size. . .

. . . and the statistics of mutational effects are the same across species. . .




**quantitative genetic theory predicts scaling with embryo size**

**Lande, Evolution (1976) 33, 402**

**Prediction: No Adjustable Parameters**

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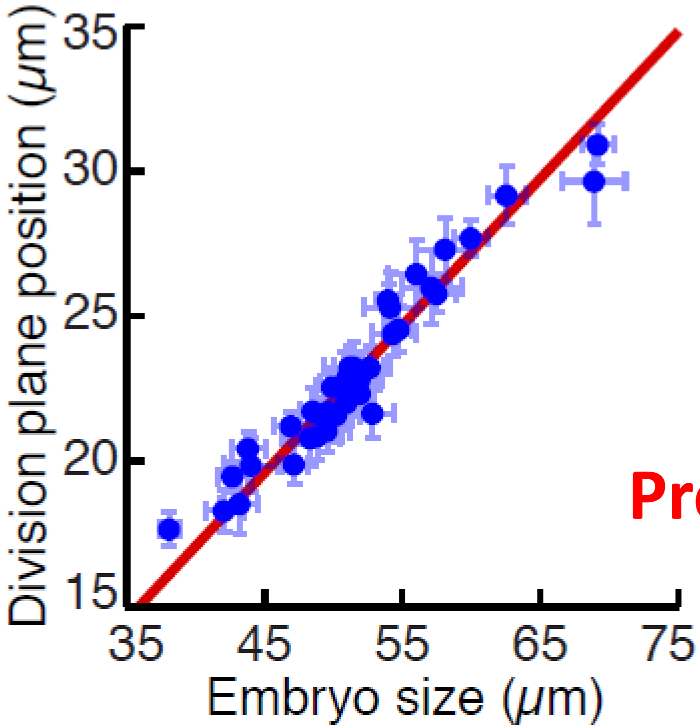


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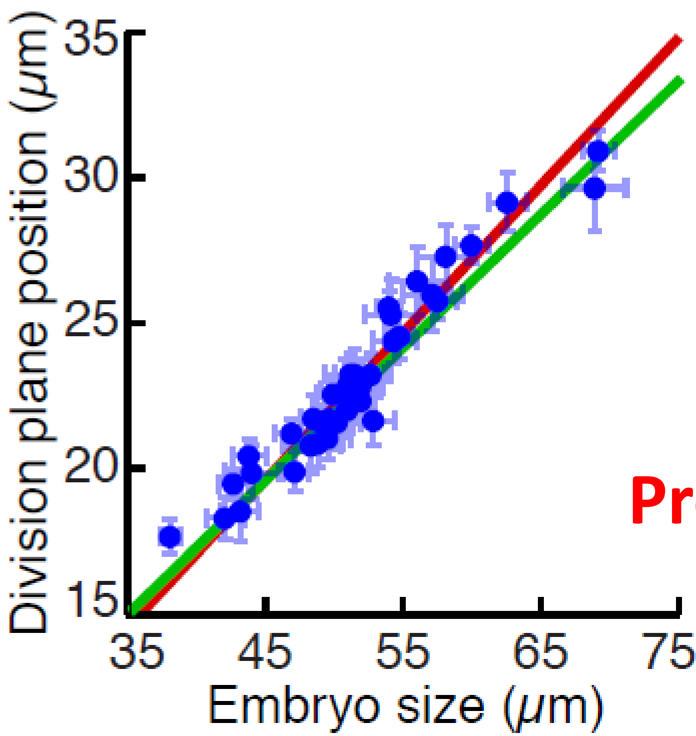


| | predicted | measured |
|---|---|---|
| Initial length | 0.14 ± 0.04 | 0.20 ± 0.06 |
| Final length | 0.55 ± 0.05 | 0.57 ± 0.10 |
| Elongation rate | 0.21 ± 0.05 | 0.15 ± 0.08 |
| Centrosome size | 1.67 ± 0.49 | 1.55 ± 0.64 |
| Division plane | 0.51 ± 0.04 | 0.46 ± 0.04 |

**pre ... size**

Lande, Evolution (1976) 33, 402

**Prediction: No Adjustable Parameters**

**Best Fit**

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. . . and the statistics of mutational effects are the same across species. . .

**Stabilizing selection on embryo size explains variations in spindles across species!**

| | predicted | measured |
|---|---|---|
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Prediction: No Adjustable Parameters

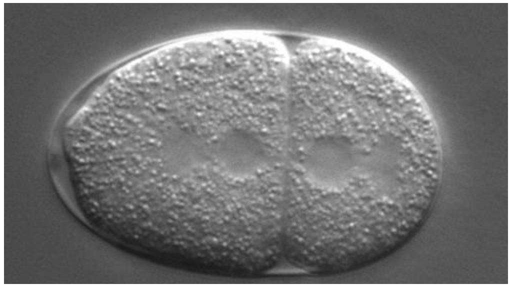
Best Fit

# Extent of Asymmetry?

**EARLY, I ASKED IF**

**Differences in Division Plane Position Are Due to Development Differences?**

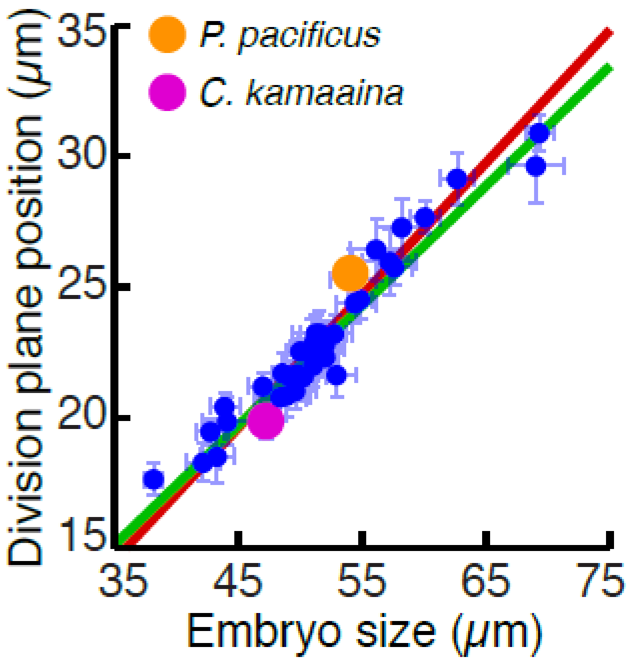
**Asymmetric Division**



*C. kamaaina*

**Symmetric Division**

*P. pacificus*




**Prediction: No Adjustable Parameters**

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# Stabilizing selection on embryo size explains extent of asymmetry

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between species variations of the spindle

characterized effects of spontaneous mutations on cell division traits

stabilizing selection on embryo size

explains within species variation for cell division traits in *C. elegan*

explains between species variation for cell division traits in nematodes

# Talk Overview:

1) Introduction

2) Investigating Evolutionary Basis of Spindle Diversity

3) Using Quantitative Genetics to Study Biophysics of Cell Division

# Conclusions

1) There is extensive within species genetic variation for all aspects of cell division

2) Balance between mutations and stabilizing selection on embryo size explains within and between species variations in cell division

3) Dissecting within species variation may provide novel mechanistic insights into cell biology

# Variances and Correlations for Cell Division Traits are the Same for *C. remani*, *C. briggsae*, *C. brenneri*, and *C. elegans*