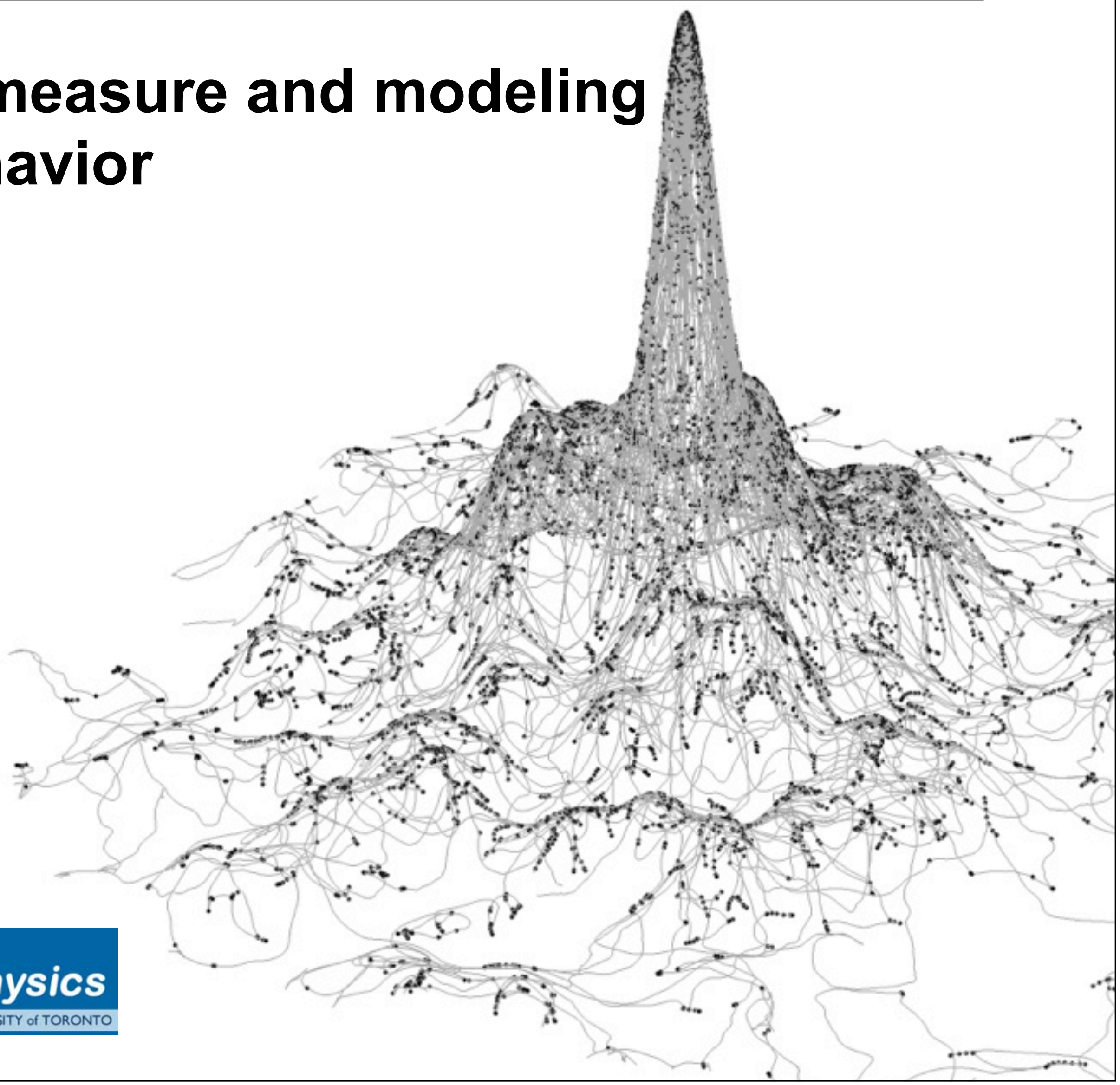
UNIVERSITY OF TORONTO
Dept. of Physics & Donnelly Centre for Cellular and Biomolecular Research

Comprehensive measure and modeling of *C. elegans* behavior

William Ryu

COMBINE 2012



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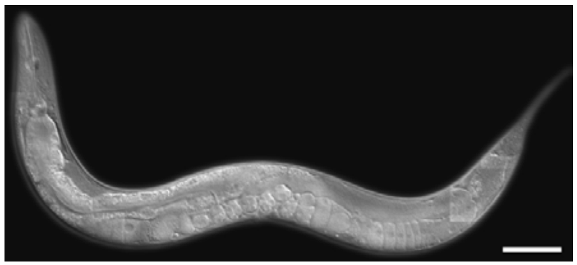
C. elegans – model organism



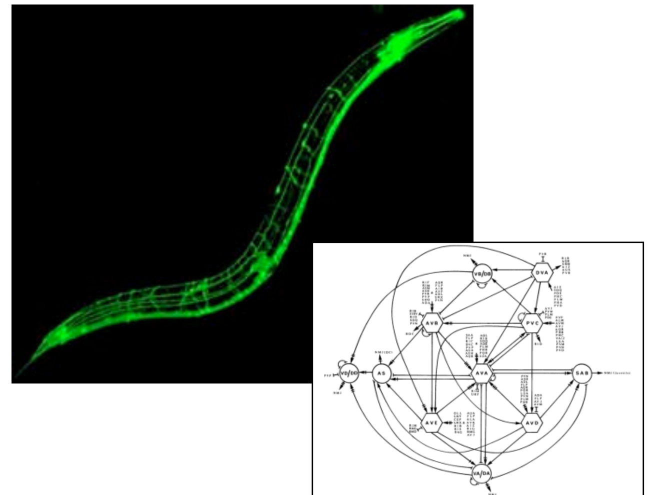
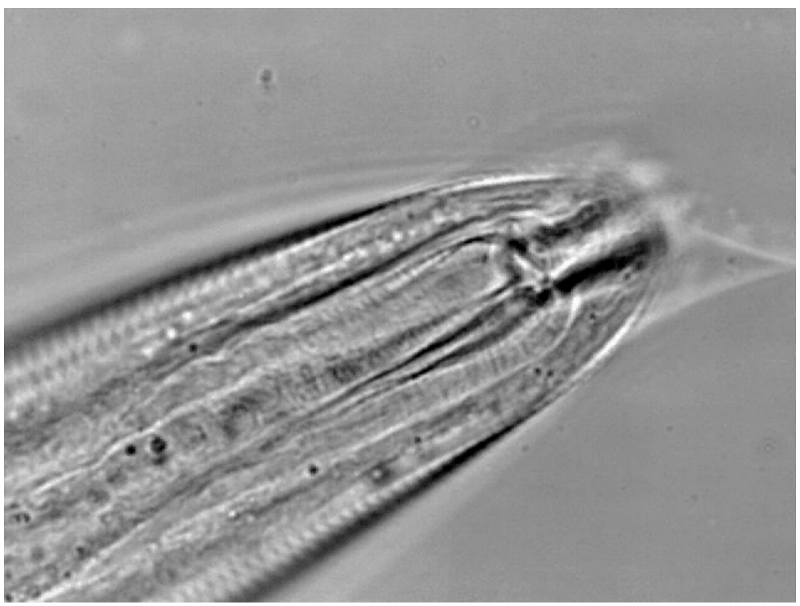


FIGURE 21. (d) Circuitry associated with the motoneurons of the ventral cord.

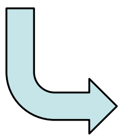


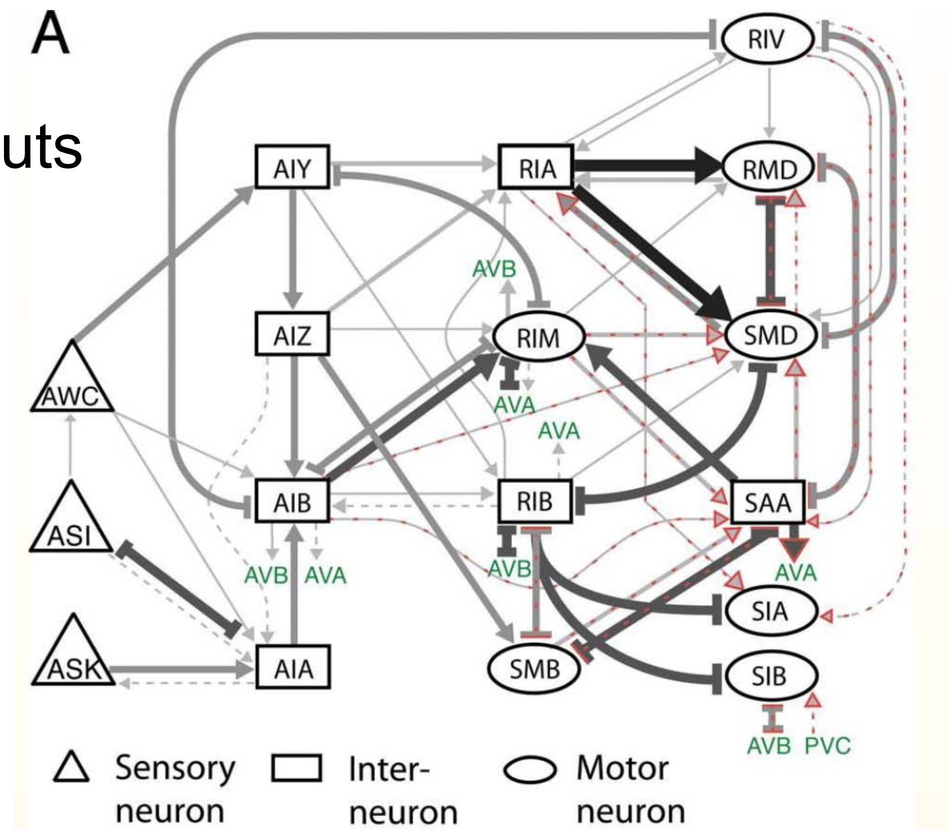
"Simple" multicellular organism: 959 cells, 302 neurons

Perturbations: genetic, environmental, neural ablation

Sensorimotor behavior

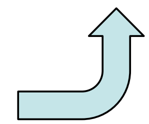
Sensory inputs





Gray+etal (2005)

Motor output



Multi-dimensional

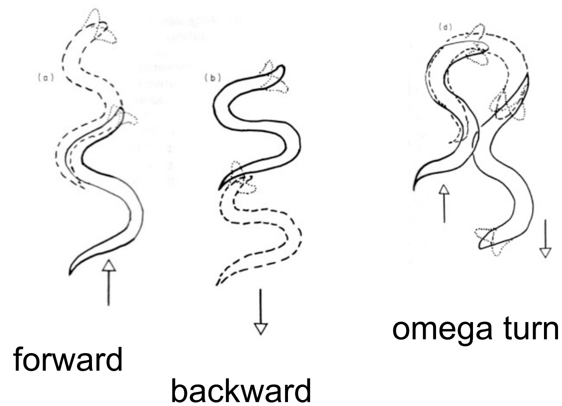
Stochastic

Subjective description

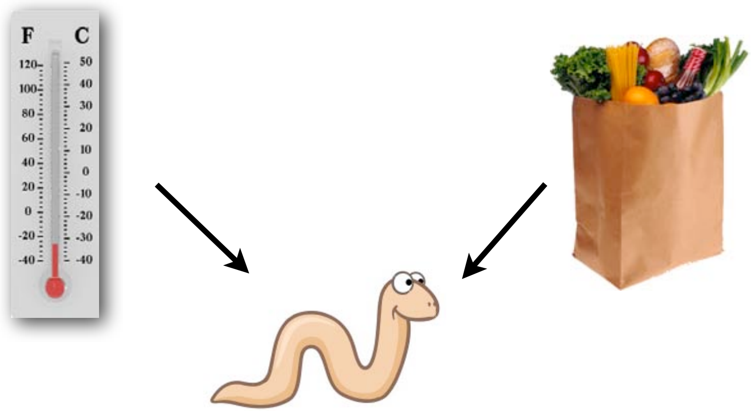
Stochastic and deterministic motor behavior

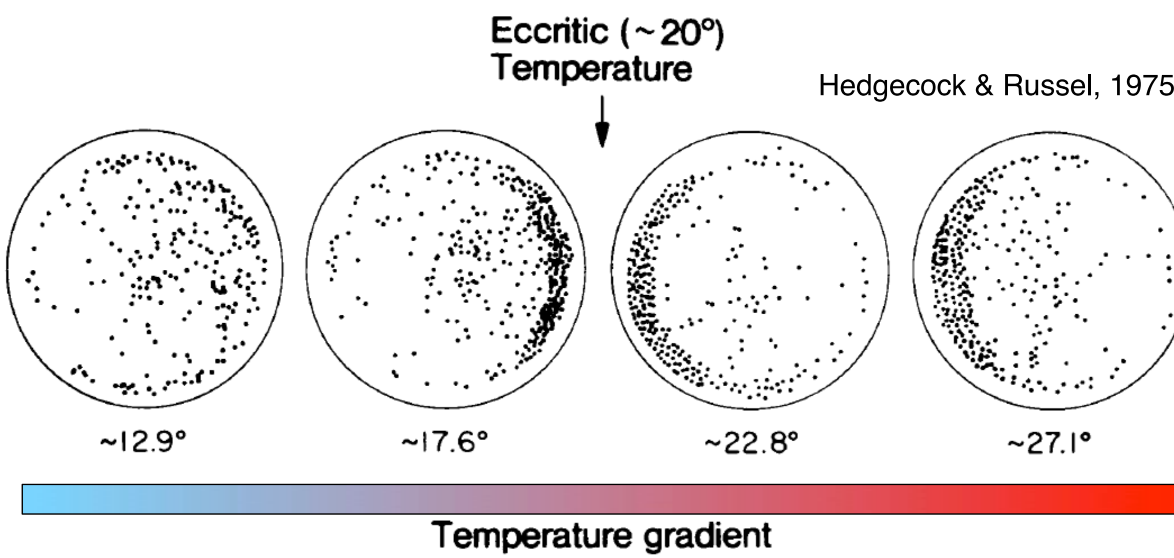
Random Search

30x real speed



Thermotaxis

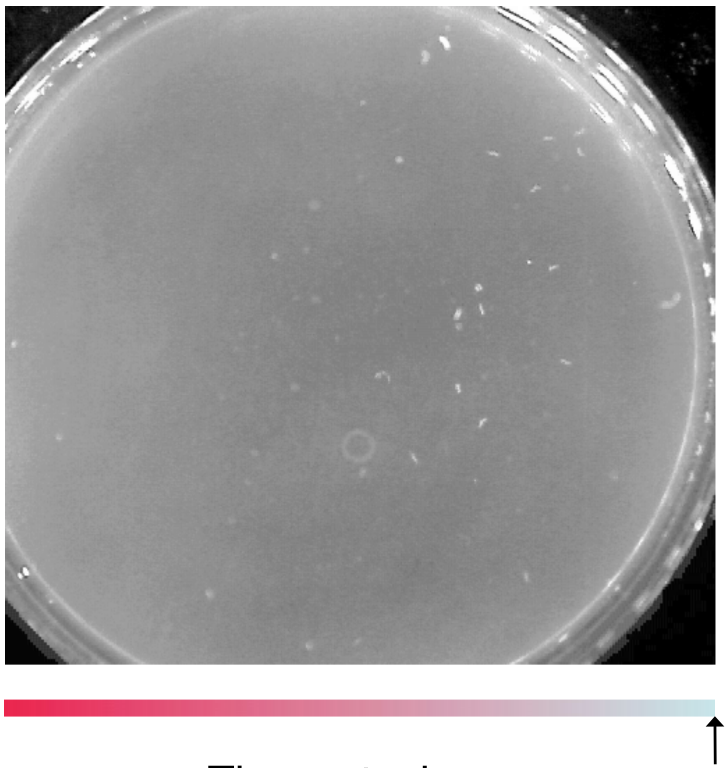




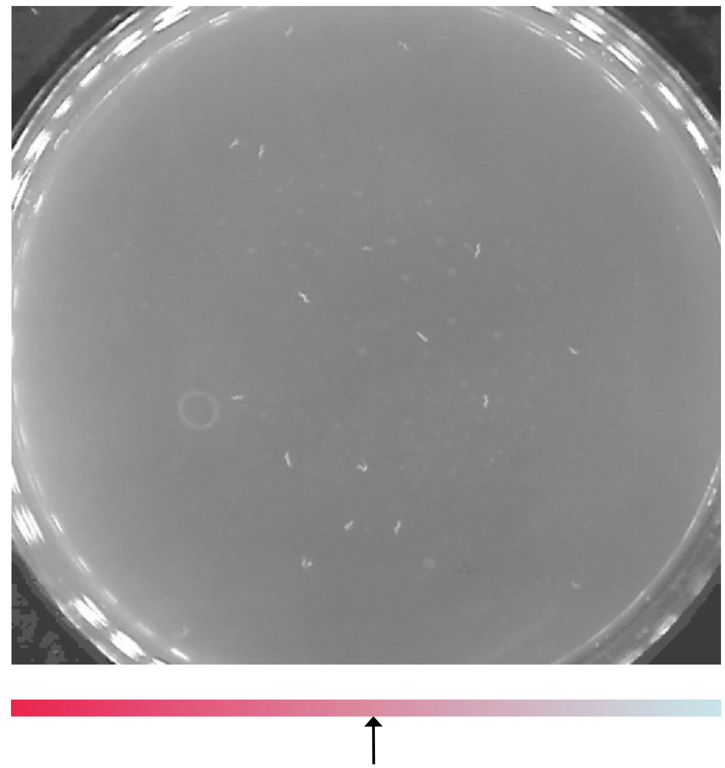
Hedgecock & Russel, 1975

Thermotaxis - *C. elegans* on spatial gradients

1 frame / sec

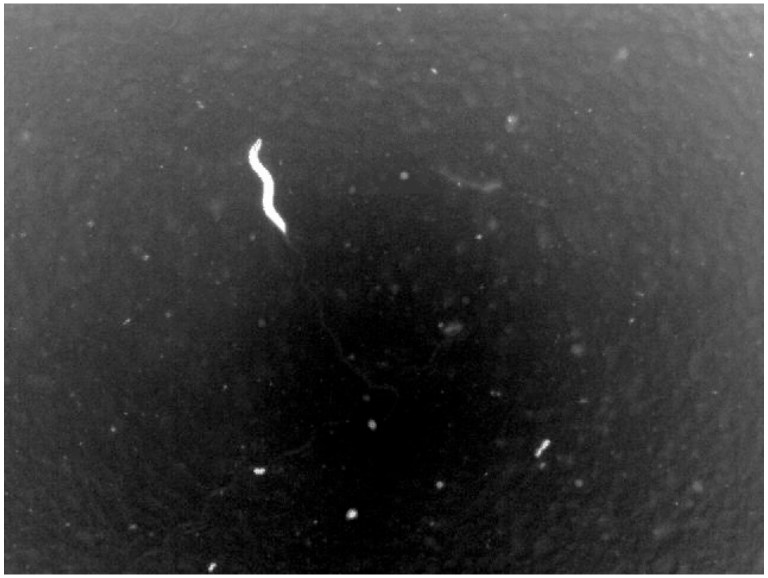


Thermotaxis
set point - right of the plate

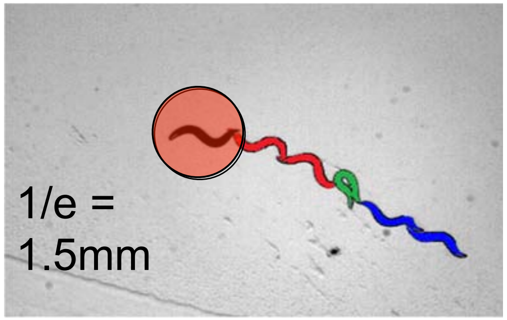


Isothermal tracking
set point - middle of plate

Thermal nociception

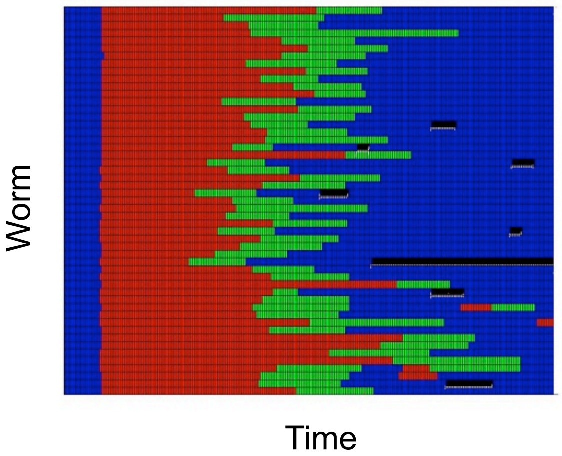


800 mA, 0.5 sec laser pulse (1440nm)



Thermal impulse response

Ethogram

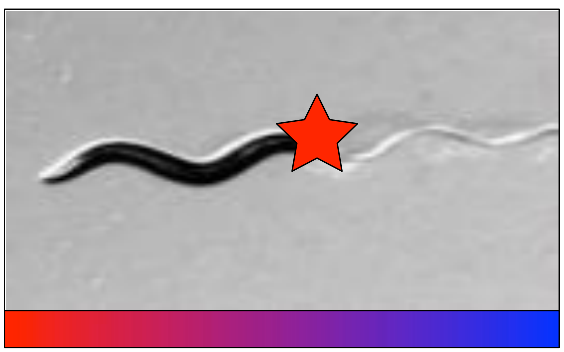


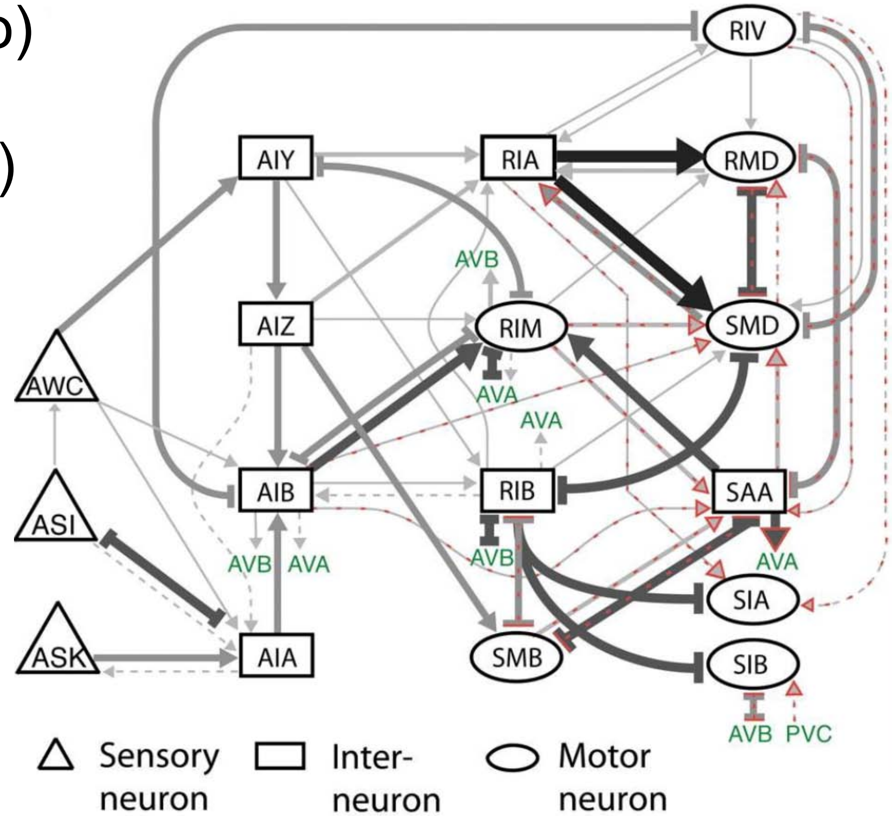
C. elegans exploration

1) Searching (no sensory info)

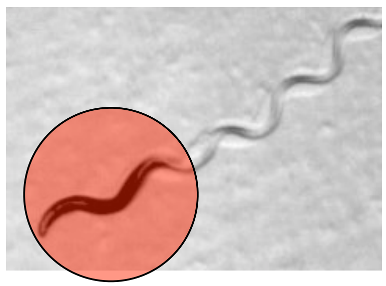
2) Taxis (sensory information)

3) Escape (nociception)

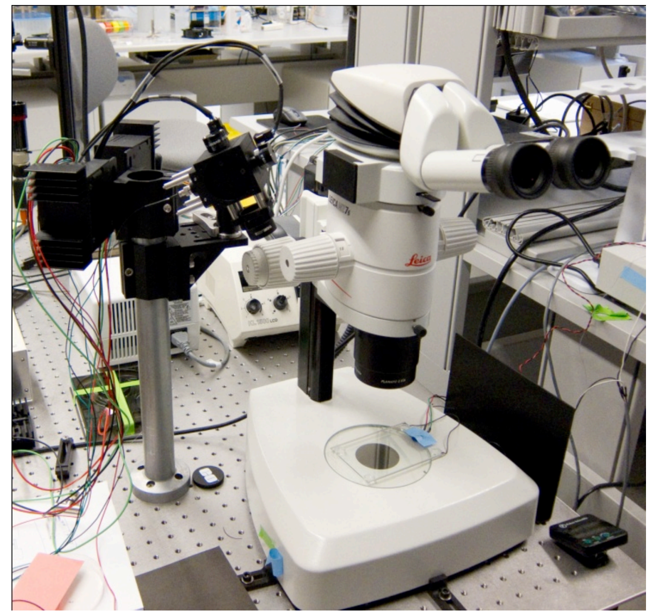




Laser heating, multi-parameter phenotyping



Thermal impulse response

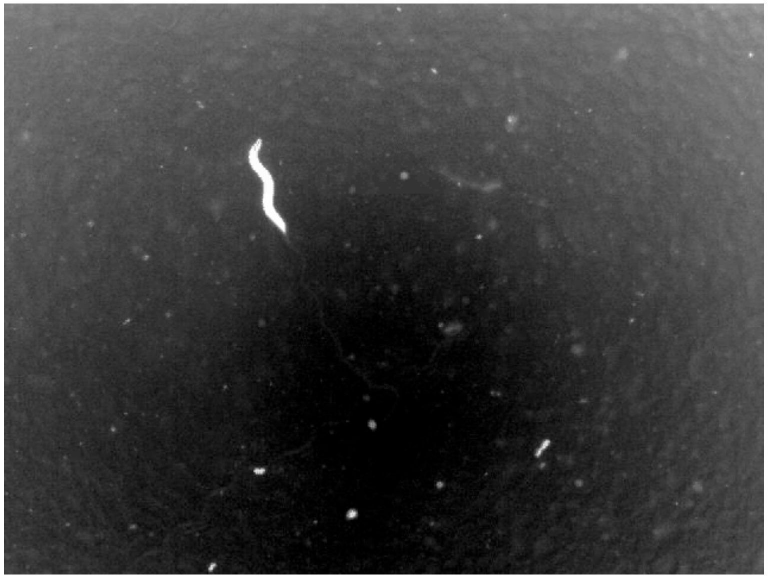


Laser zapper 1.0

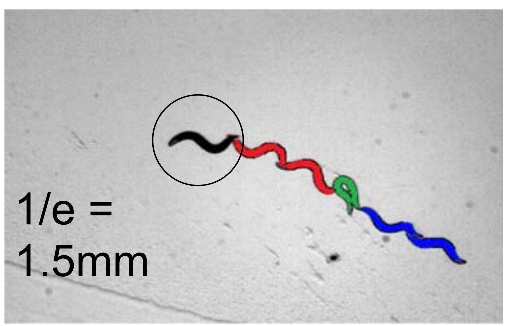
Raj Ghosh - Experiments, Data analysis
Aylia Mohammadi - Data analysis

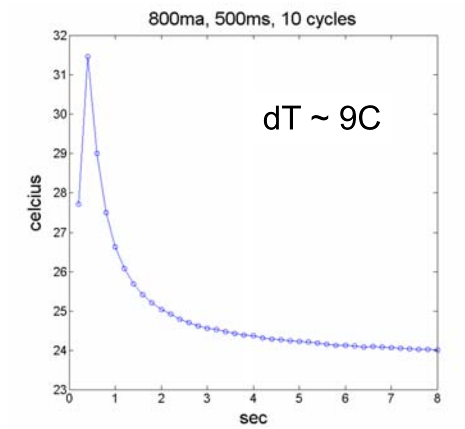
How do you deal with “complex” behavior?
What if that behavior changes as a function of stimulus amplitude?

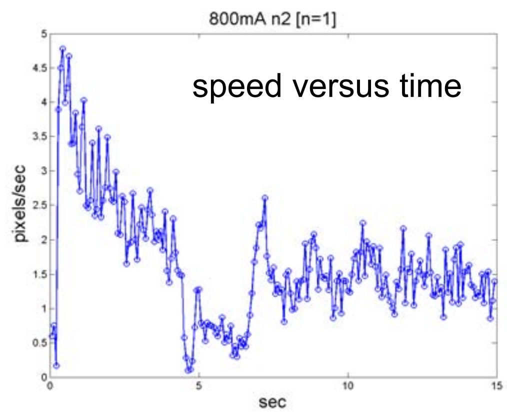
Thermal stimulus / response example



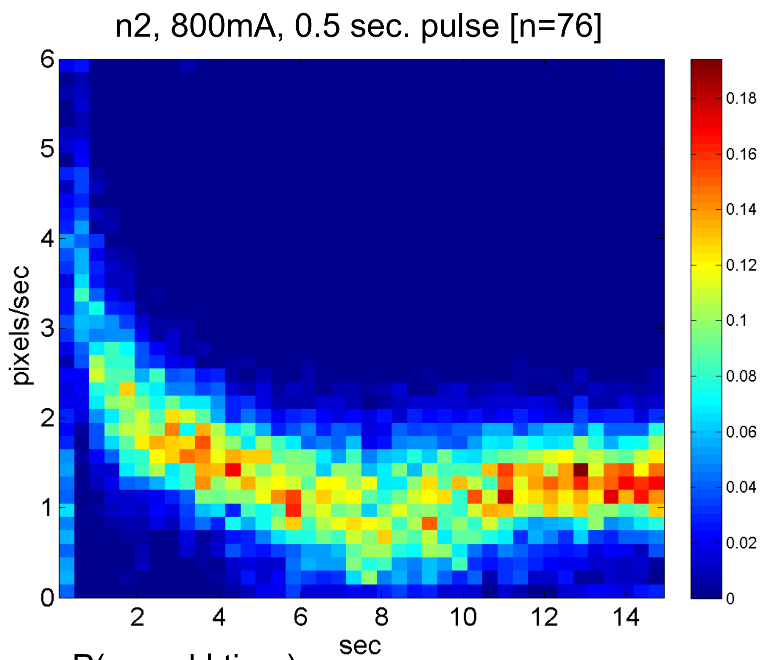
800 mA, 0.5 sec laser pulse (1440nm)



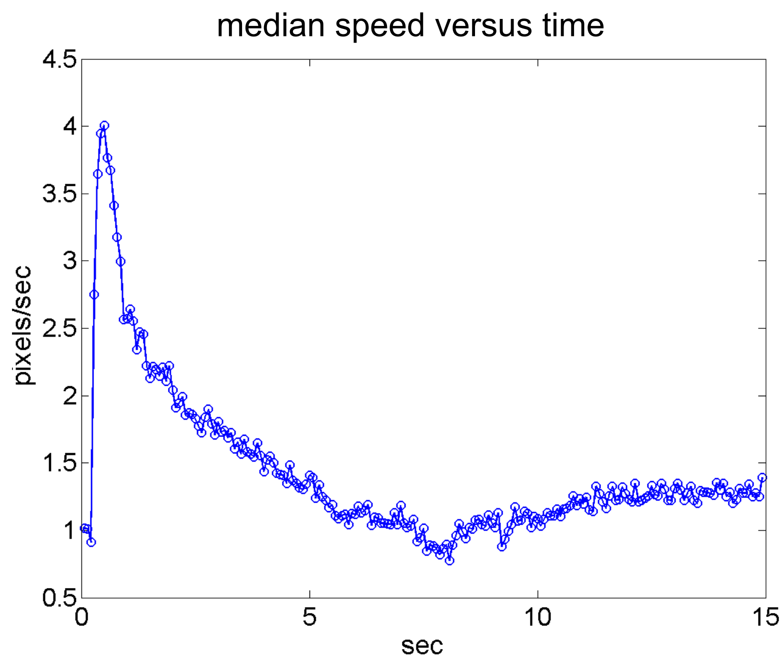




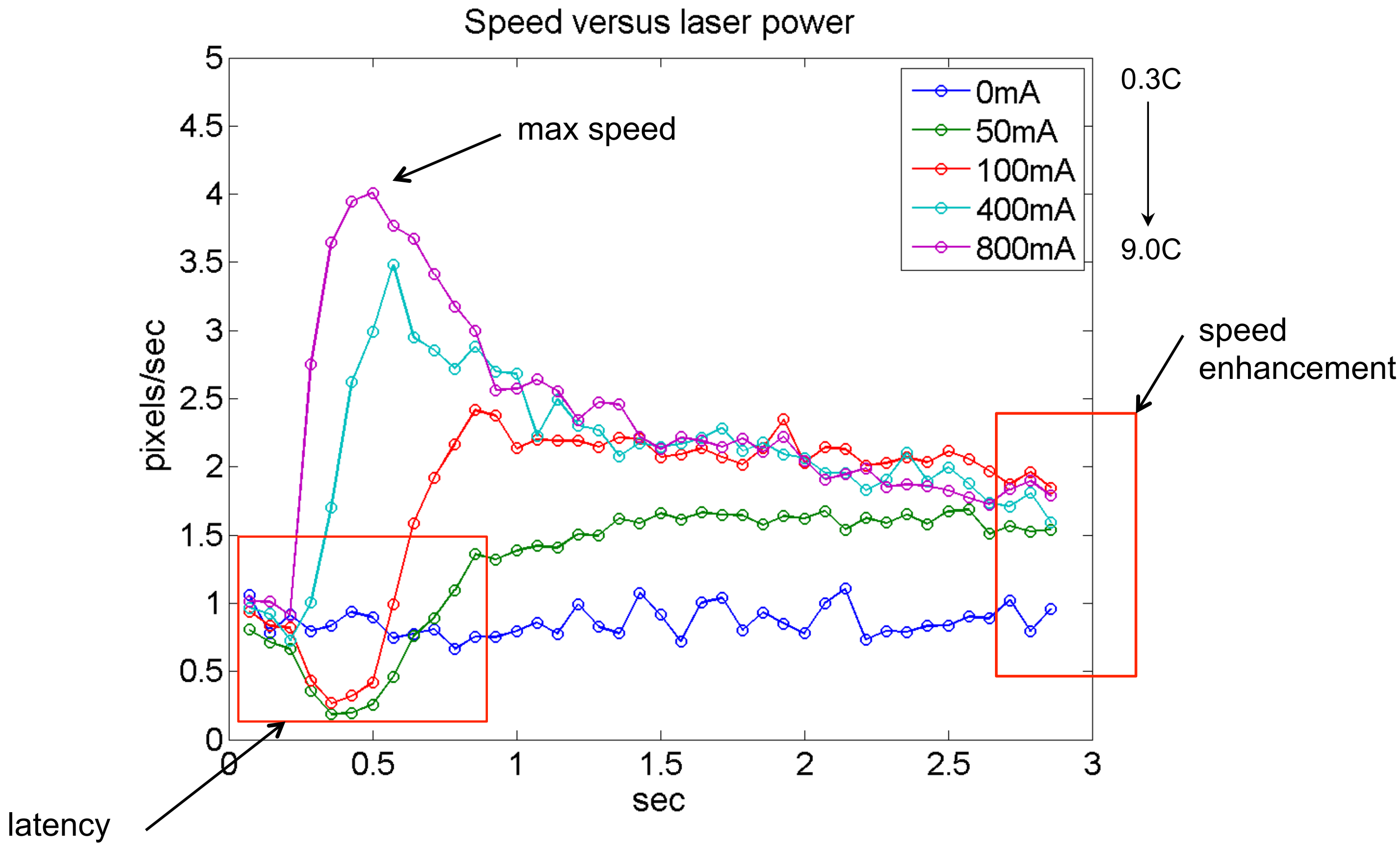
Speed versus time shows stereotyped behavior



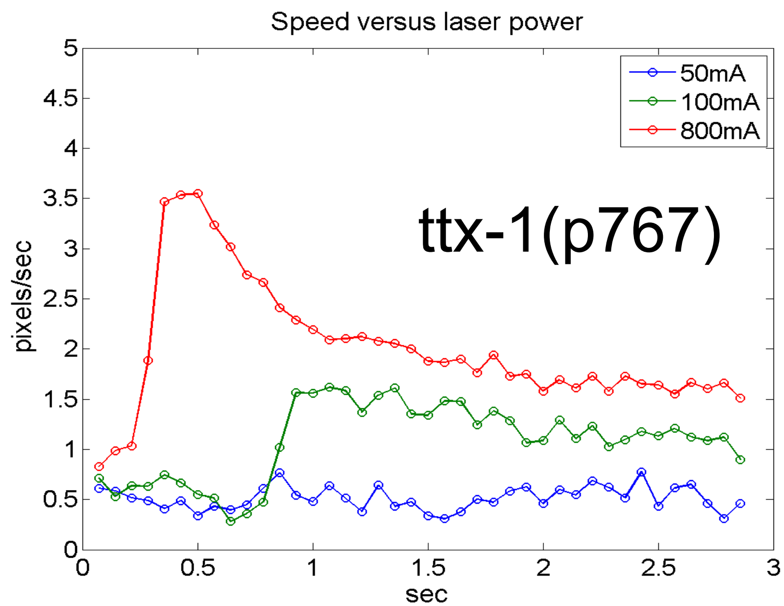
P(speed | time)



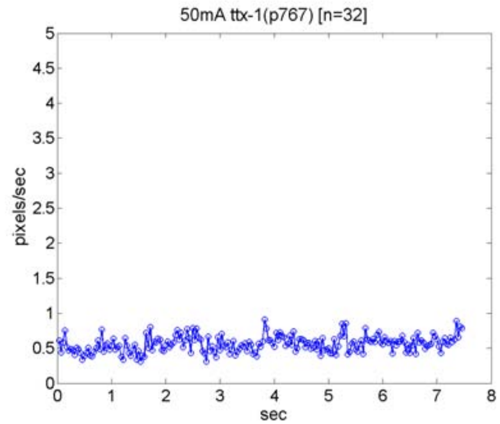
Speed versus laser power



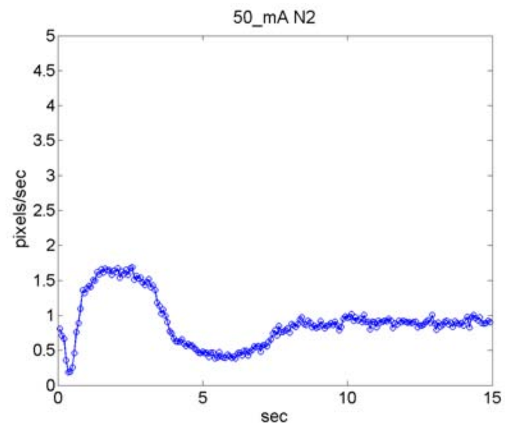
Thermal response or pain response?



ttx-1(p767)

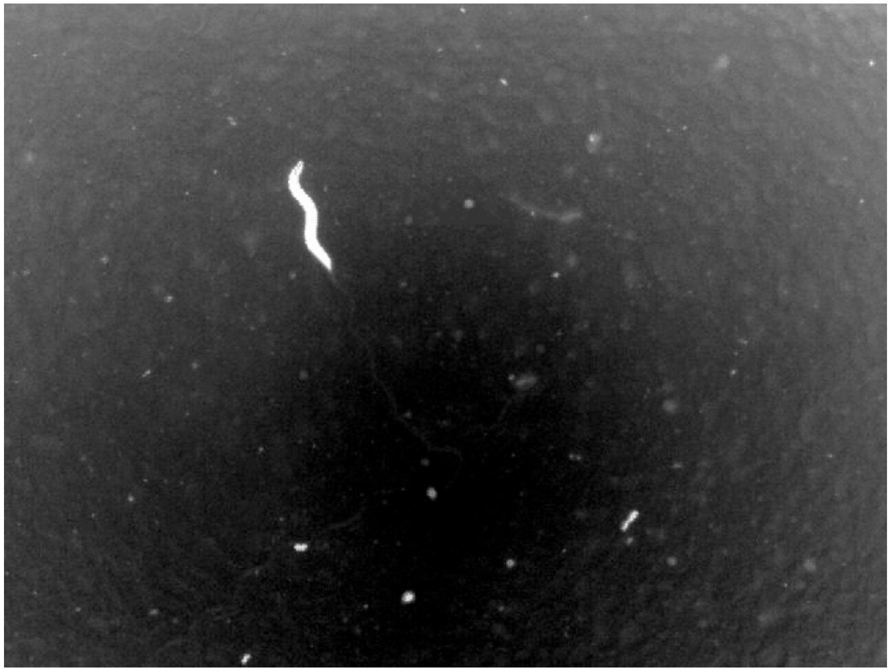


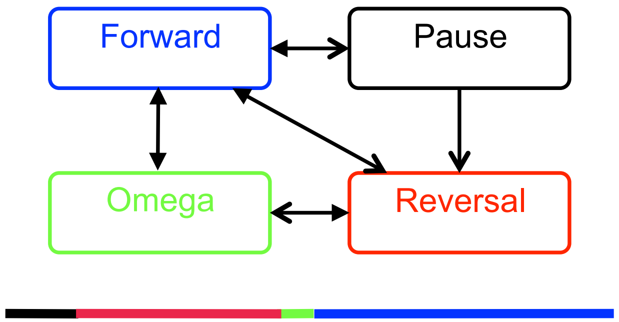
N2 - wildtype

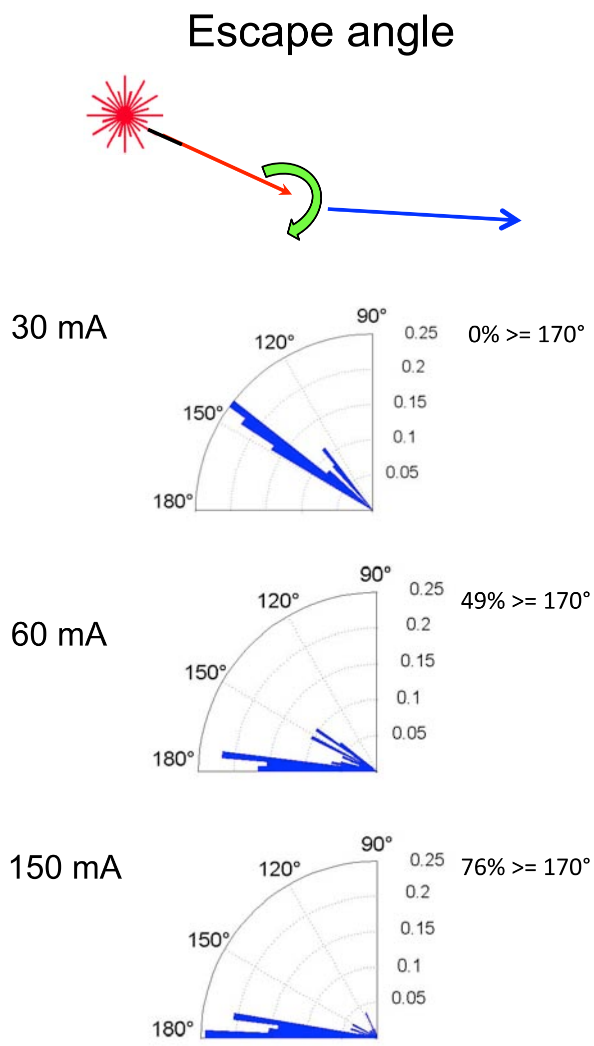


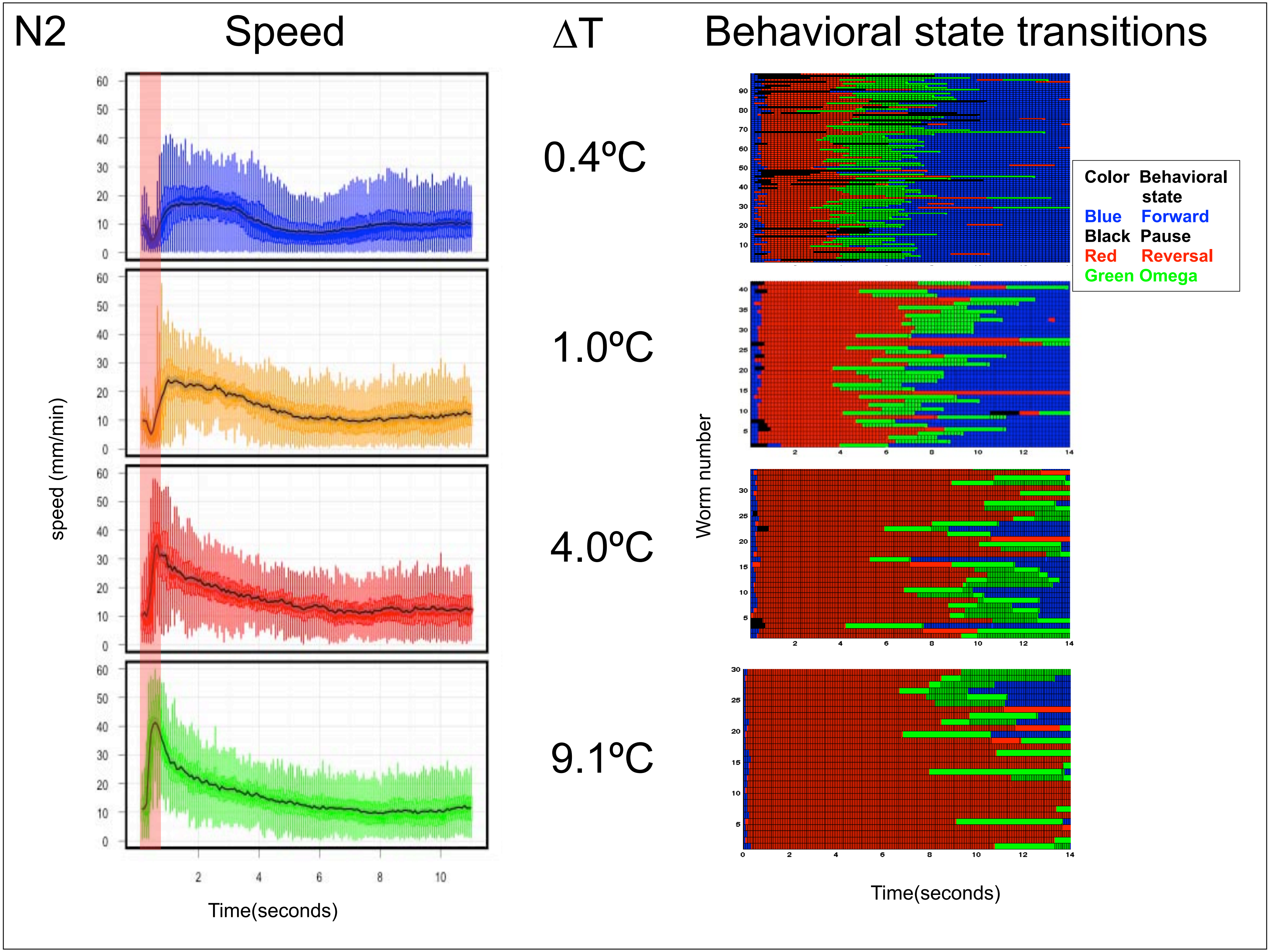
ttx-1: thermosensory neuron defect (afd)

Behavioral states & decision making



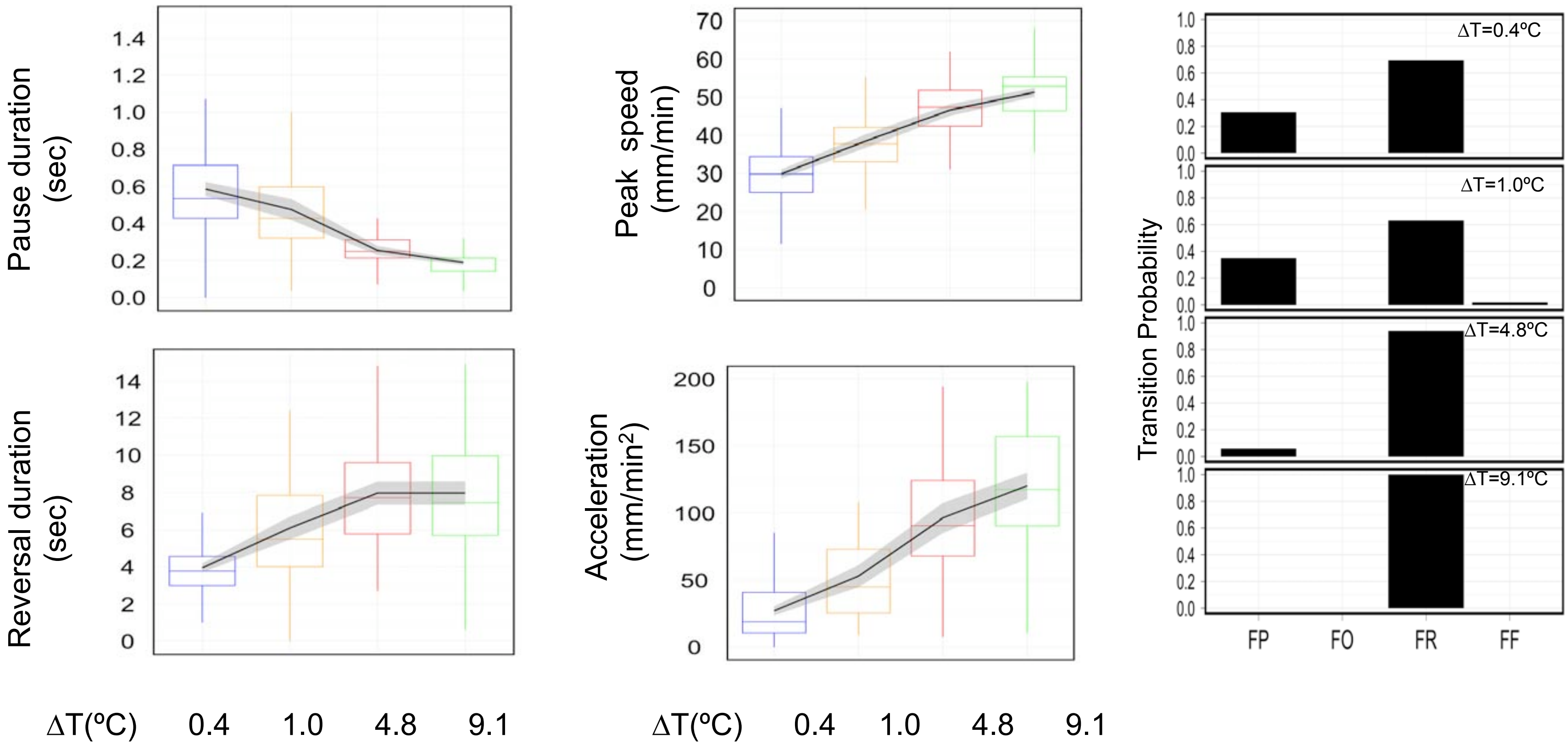




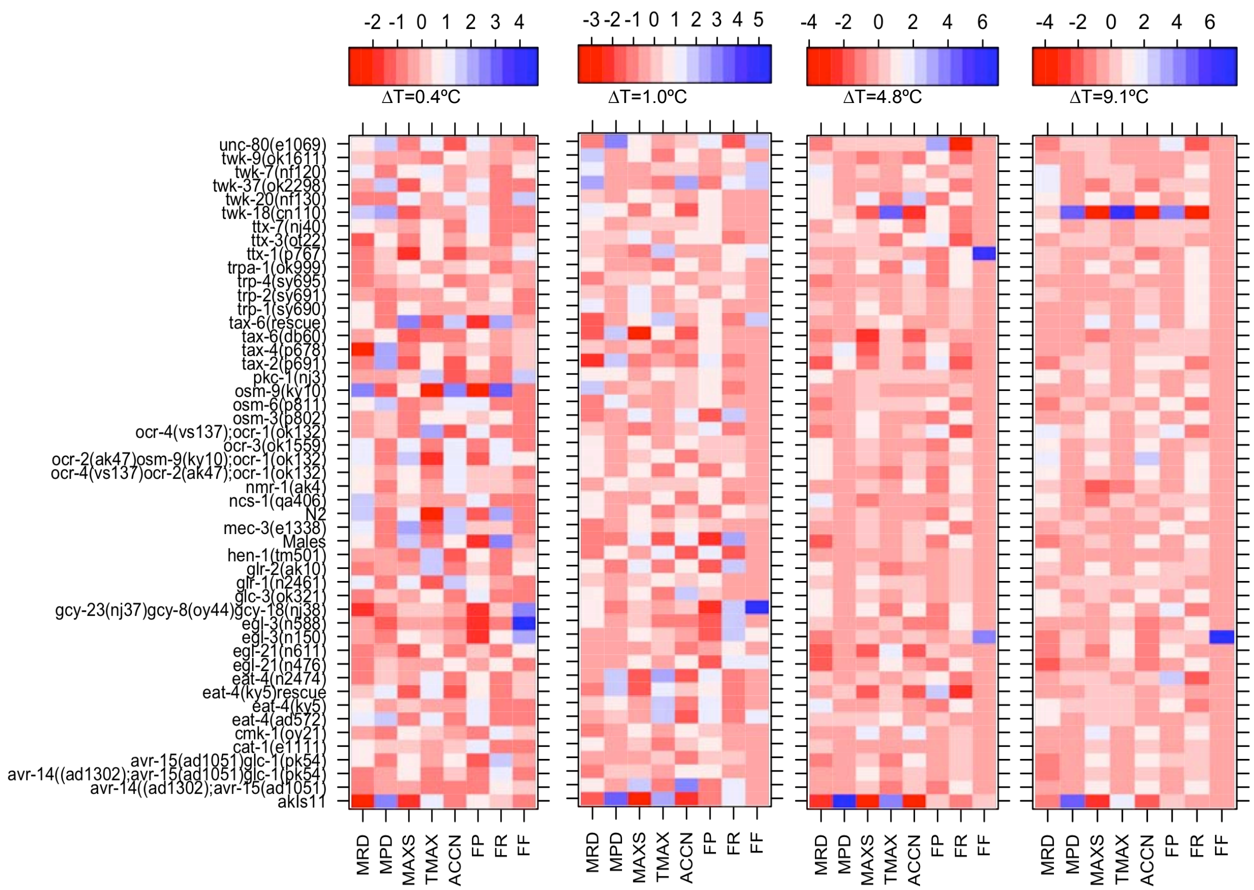


N2
Speed
ΔT
Behavioral state transitions
0.4ºC
1.0ºC
4.0ºC
9.1ºC
speed (mm/min)
Time(seconds)
Worm number
Time(seconds)
Color Behavioral state
Blue Forward
Black Pause
Red Reversal
Green Omega

Choosing behavioural measurements

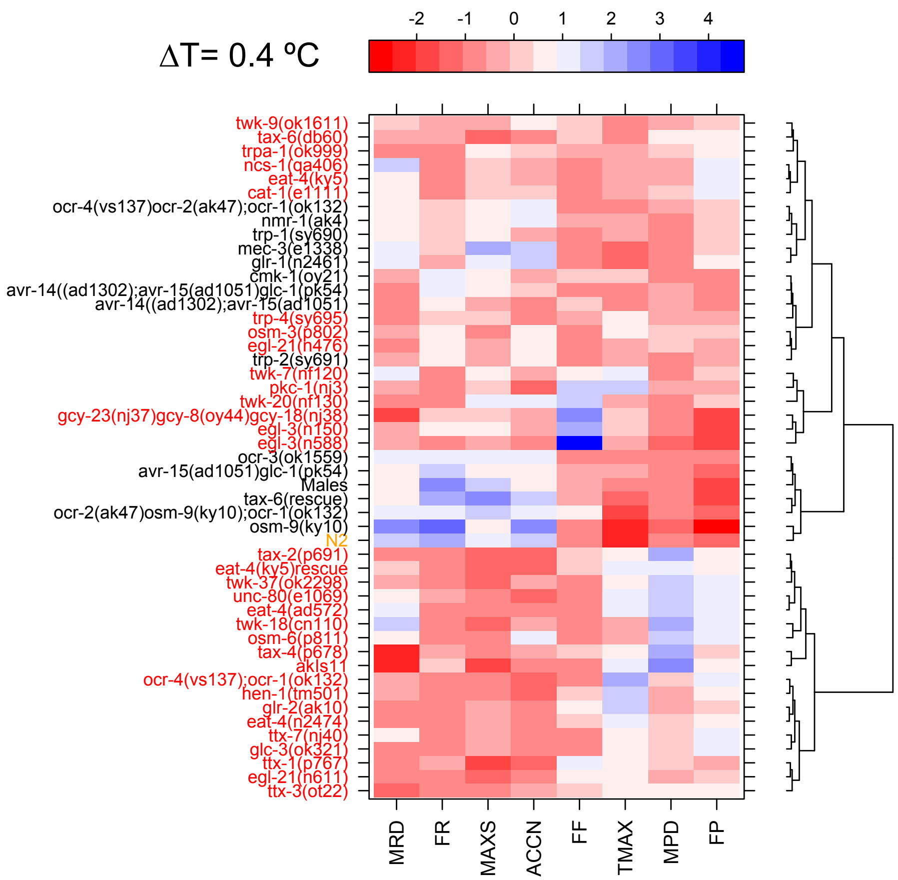


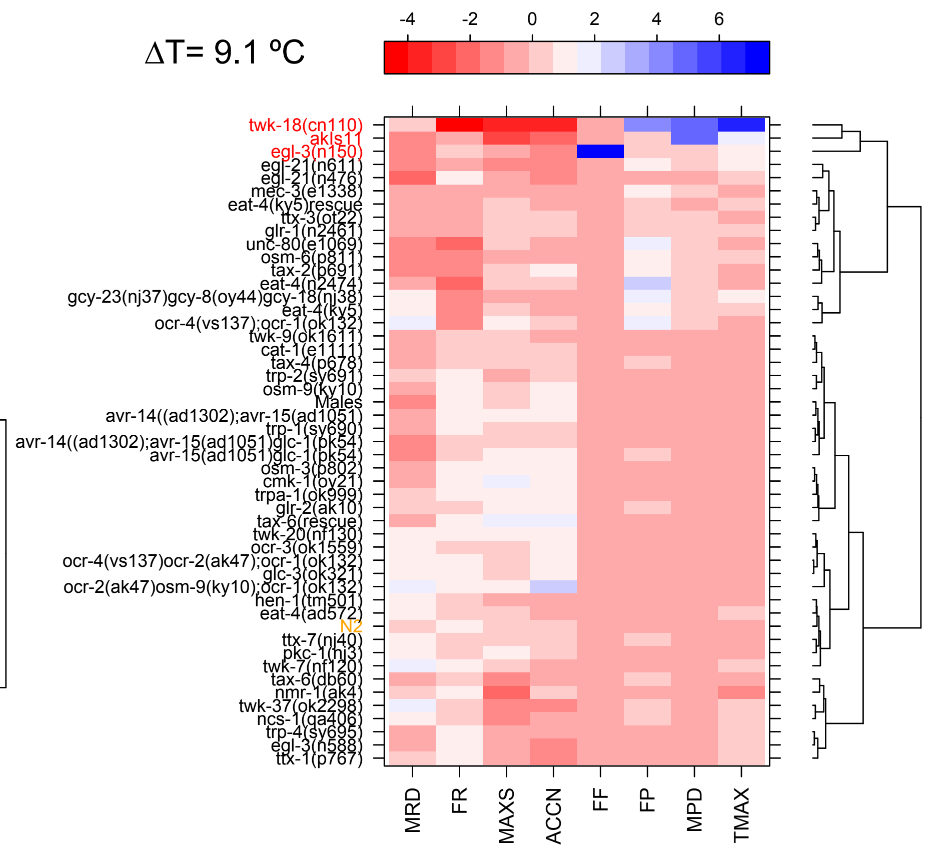
Behavioral barcoding



The behavioral features: **MRD**: Mean reversal duration; **MPD**: Mean Pause duration; **MAXS**: Maximum speed after laser pulse; **TMAX**: time to reach MAXS; **ACCN**: Acceleration; **FP**: Forward to pause transition probability ;**FR**: Forward to reversal transition probability; **FF**: forward to forward transition probability

Behavioral barcode clustering

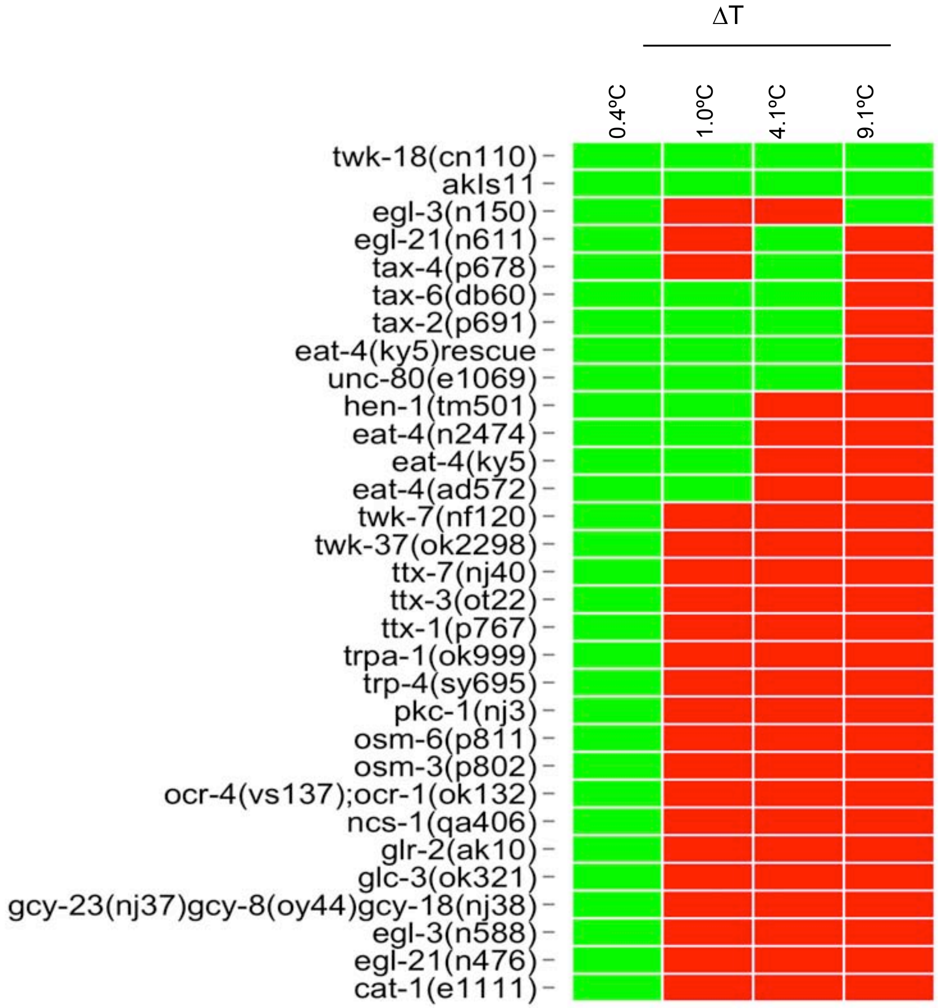




RED: $p < 0.1$, via bootstrapping, 4-cluster

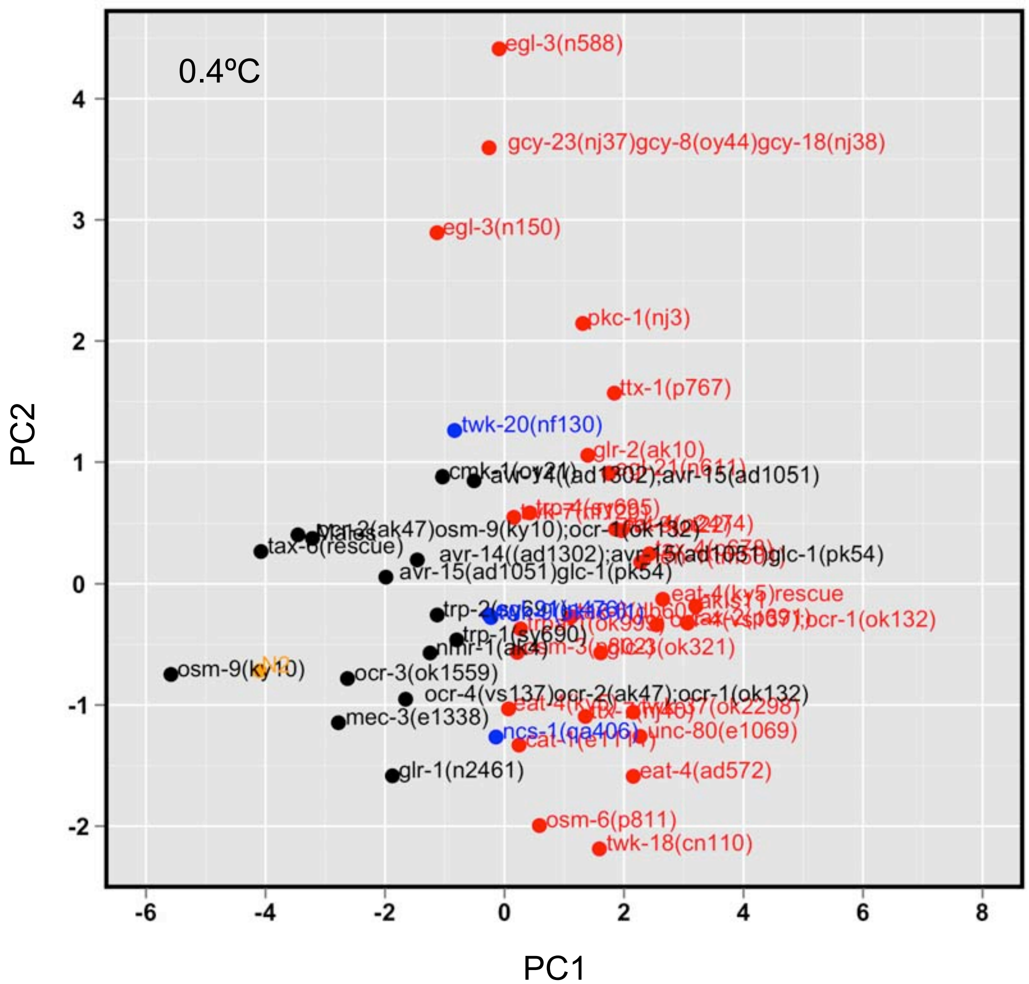
The behavioral features: **MRD**: Mean reversal duration; **MPD**: Mean Pause duration; **MAXS**: Maximum speed after laser pulse; **TMAX**: time to reach MAXS; **ACCN**: Acceleration; **FP**: Forward to pause transition probability ;**FR**: Forward to reversal transition probability; **FF**: forward to forward transition probability

Barcode clustering



green: different from N2
red: similar to N2

PCA phenotyping



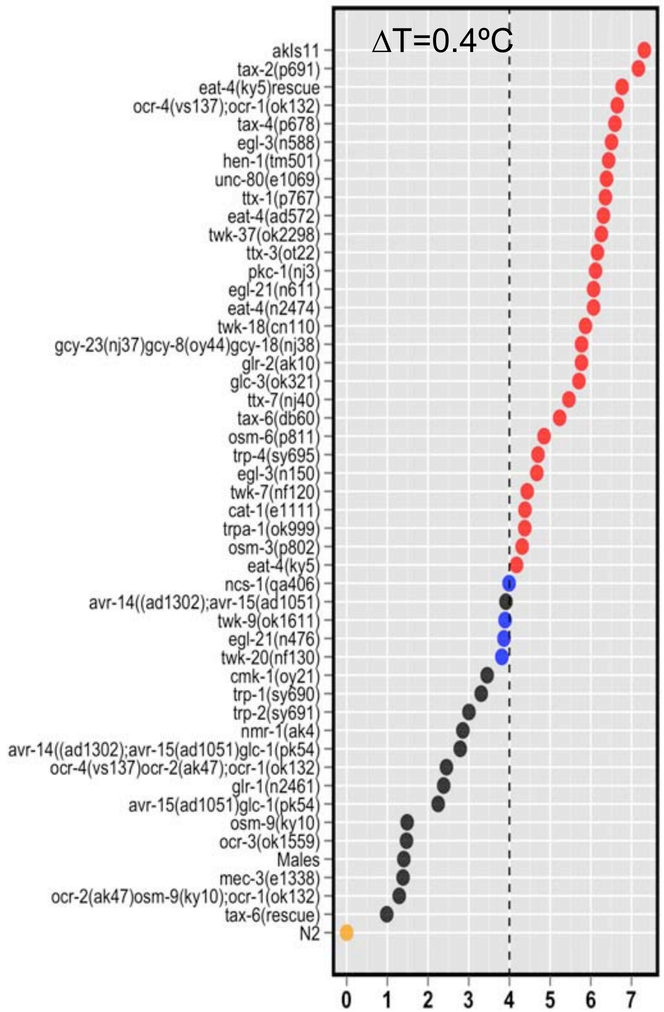
For each ΔT retained PC's capture >94% of variance

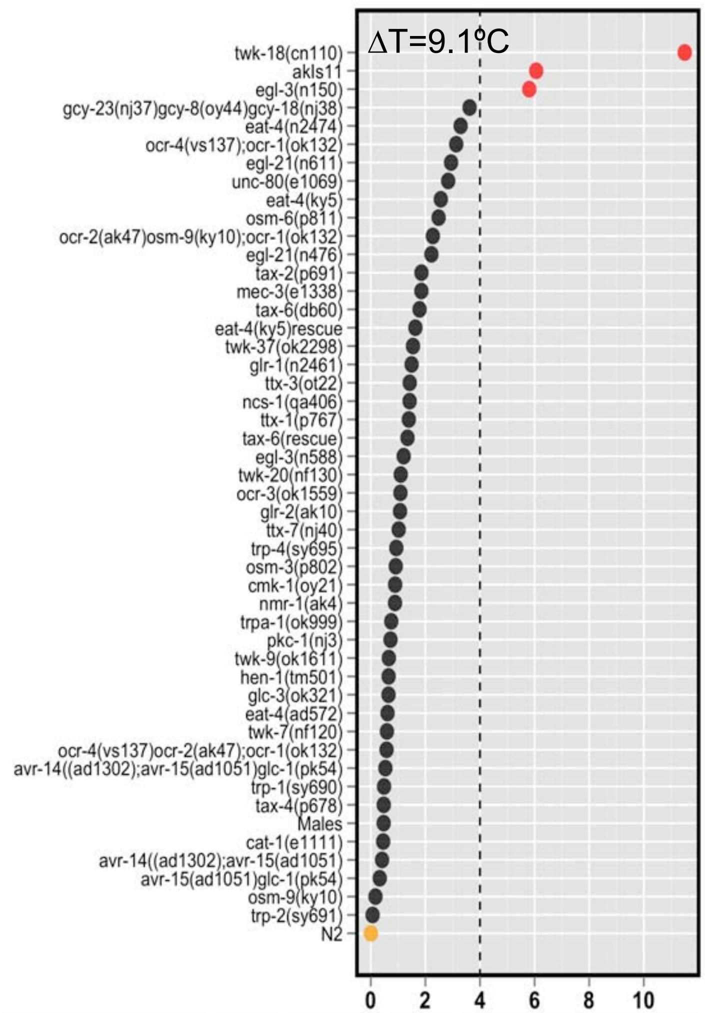
Proportion of variance explained

	PC1	PC2	PC3	PC4	PC5	PC6
0.4ºC	0.54	0.21	0.1	0.06	0.05	0.02
1.0ºC	0.48	0.18	0.12	0.1	0.06	0.04
4.8ºC	0.47	0.19	0.13	0.09	0.06	0.05
9.1ºC	0.52	0.16	0.13	0.1	0.06	0.03

Red: Different from N2 > 4 units
Blue: Bootstrapping but <4 units
Black: < 4 units

PCA - ordered by distance from N2





Defective thermal sensory mutants

Strains	ΔT= 0.4ºC			ΔT= 1.0ºC			ΔT= 4.8ºC			ΔT= 9.1ºC		
	B	D	A	B	D	A	B	D	A	B	D	A
akIs11	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
cat-1(e1111)	Y	Y	Y	N	N	N	N	N	N	N	N	N
eat-4(ad572)	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N
eat-4(ky5)	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N
eat-4(ky5)rescue	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N
eat-4(n2474)	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N
egl-21(n476)	Y	N	Y	N	N	N	Y	Y	Y	N	N	N
egl-21(n611)	Y	Y	Y	N	N	N	N	N	N	N	N	N
egl-3(n150)	Y	Y	Y	N	N	N	N	N	N	Y	Y	Y
egl-3(n588)	Y	Y	Y	N	N	N	N	N	N	N	N	N
gcy-23(nj37)gcy-8(oy44)gcy-18(nj38)	Y	Y	Y	N	N	N	N	N	N	N	N	N
glc-3(ok321)	Y	Y	Y	N	N	N	N	N	N	N	N	N
glr-2(ak10)	Y	Y	Y	N	N	N	N	N	N	N	N	N
hen-1(tm501)	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N
ncs-1(qa406)	Y	N	Y	Y	N	N	N	N	N	N	N	N
nmr-1(ak4)	N	N	N	Y	N	N	N	N	N	N	N	N
ocr-4(vs137);ocr-1(ok132)	Y	Y	Y	N	N	N	N	N	N	N	N	N
osm-3(p802)	Y	Y	Y	N	N	N	N	N	N	N	N	N
osm-6(p811)	Y	Y	Y	Y	Y	N	N	N	N	N	N	N
pkc-1(nj3)	Y	Y	Y	Y	N	N	N	N	N	N	N	N
tax-2(p691)	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N
tax-4(p678)	Y	Y	Y	N	N	N	Y	Y	Y	N	N	N
tax-6(db60)	Y	Y	Y	Y	Y	N	Y	Y	Y	N	N	N
tax-6 rescue	N	N	N	Y	N	N	N	N	N	N	N	N
trp-4(sy695)	Y	Y	Y	Y	N	N	N	N	N	N	N	N
trpa-1(ok999)	Y	Y	Y	N	N	N	N	N	N	N	N	N
ttx-1(p767)	Y	Y	Y	N	N	N	N	N	N	N	N	N
ttx-3(ot22)	Y	Y	Y	N	N	N	N	N	N	N	N	N
ttx-7(nj40)	Y	Y	Y	N	N	N	Y	Y	N	N	N	N
twk-18(cn110)	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
twk-20(nf130)	Y	N	N	N	N	N	N	N	N	N	N	N
twk-37(ok2298)	Y	Y	Y	N	N	N	N	N	N	N	N	N
twk-7(nf120)	Y	Y	Y	N	N	N	N	N	N	N	N	N
twk-9(ok1611)	Y	N	N	N	N	N	N	N	N	N	N	N
unc-80(e1069)	Y	Y	Y	Y	N	Y	N	N	N	N	N	N

Mutants identified by 3 different methods: 1) Wards, 2) PCA distance, 3) ANOVA

Summary

“High-content” phenotyping reveal differences in a broad qualitatively similar response

Different thermal sensory ranges depend on different molecules:e.g: glutamatergic and peptidergic neurotransmission

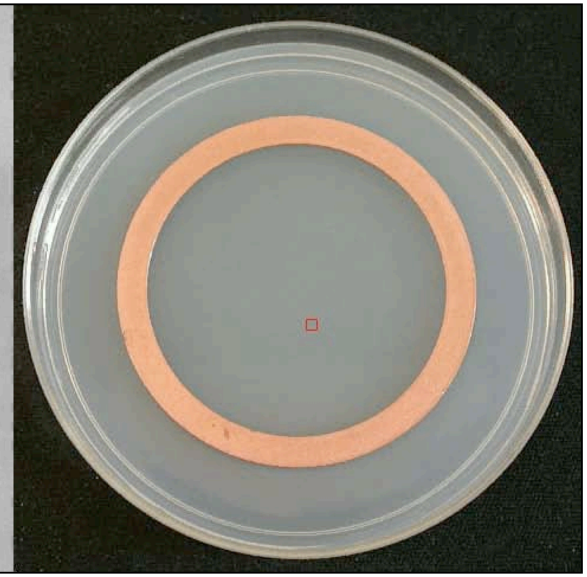
New molecules for low temperature escape response implicated: TRP-4, TRPA-1, TWK-7, TWK-37

Assay has been used for QTL analysis (RIL) of thermal sensory measurement

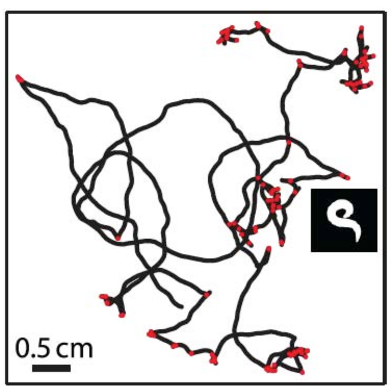
Challenging to derive objective measures of behavior useful for genetic screening in a comprehensive way

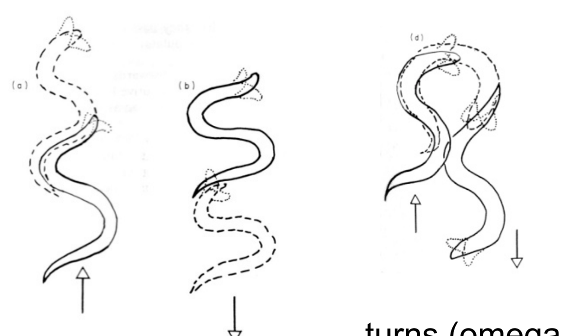
C. elegans searching behavior

length ~ 1 mm, 2.5x real speed



6 cm dia. copper ring corral



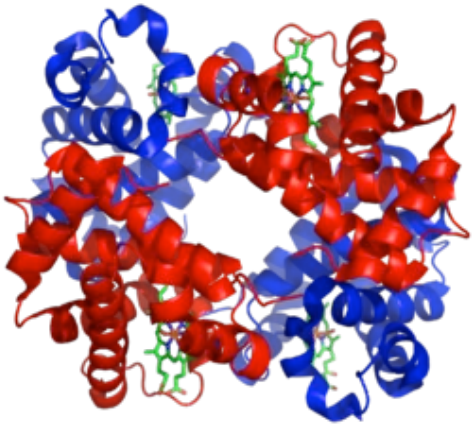


forward

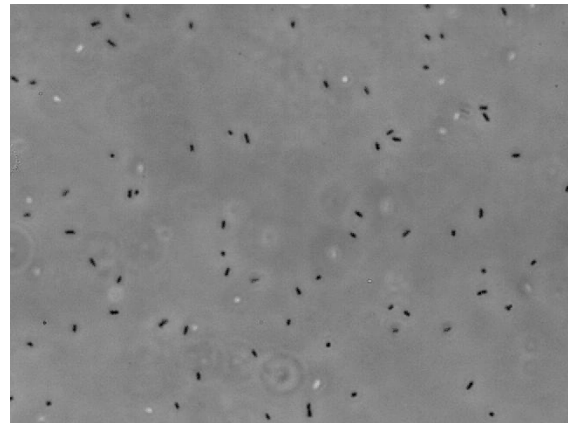
backward

turns (omega)

Diffusive searching: from molecules to cells

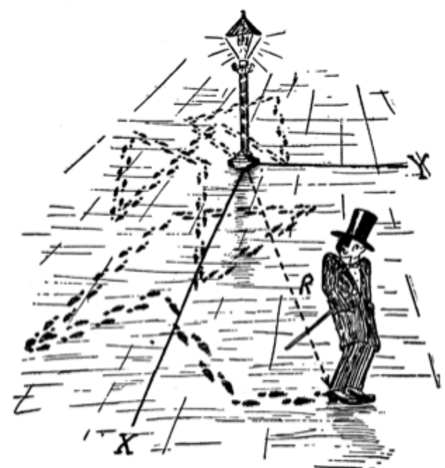


Proteins



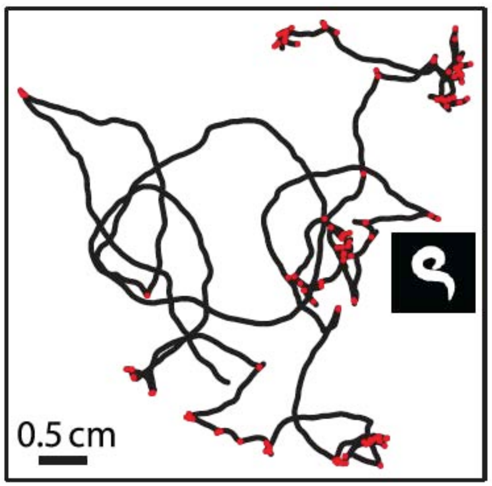
Bacteria

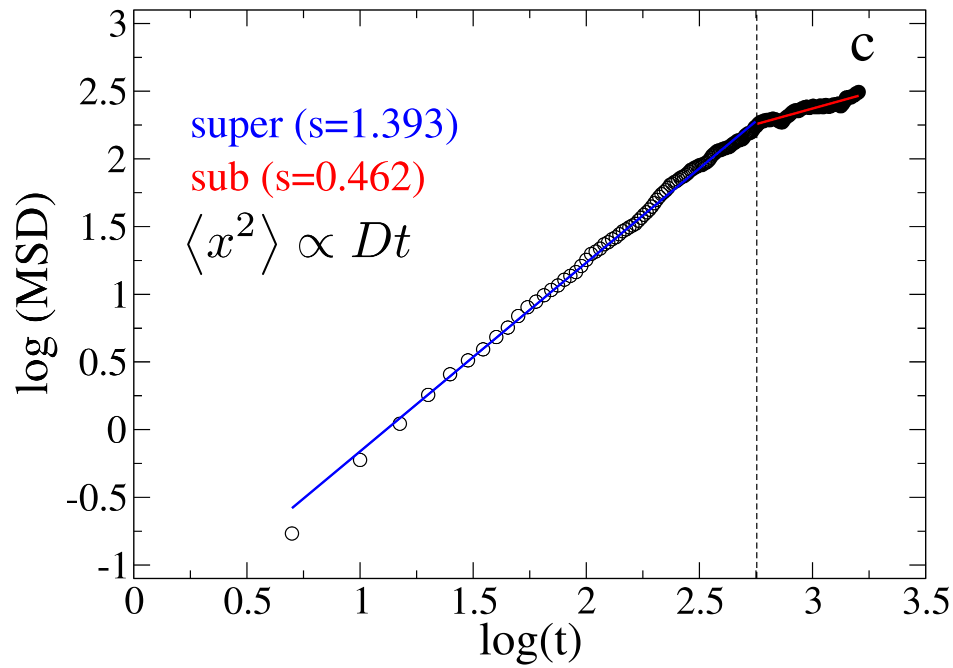
Nematodes



Drunkard's walk

Measuring searching – anomalous diffusion

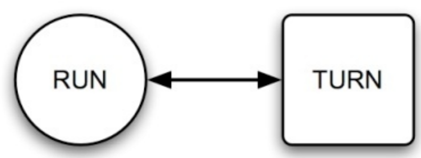




Statistical behavioral strategy?

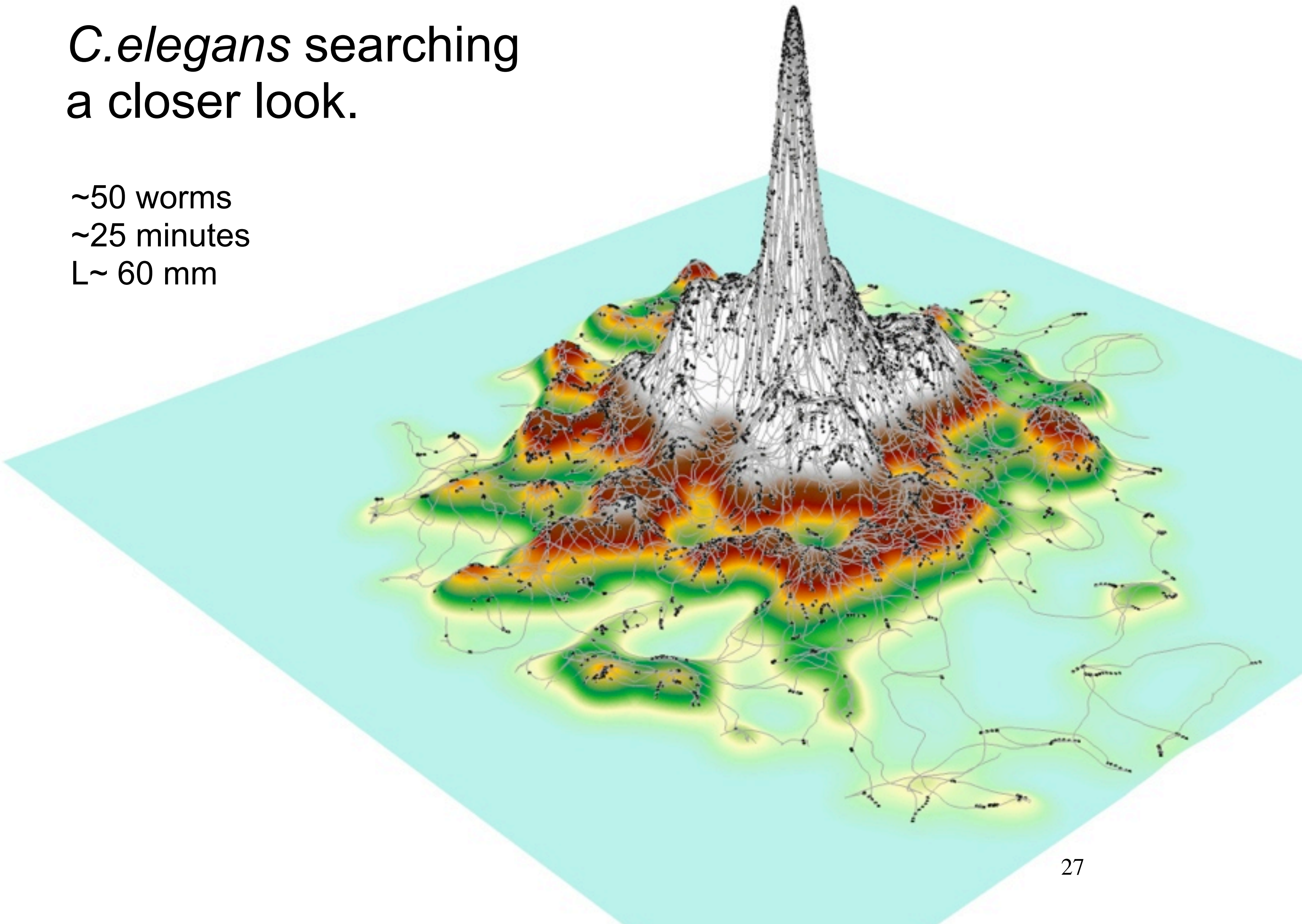
Tension between intrinsic (local) vs. extrinsic (global) search

$$P(l) = \exp(-l/\tau)^{\beta}$$

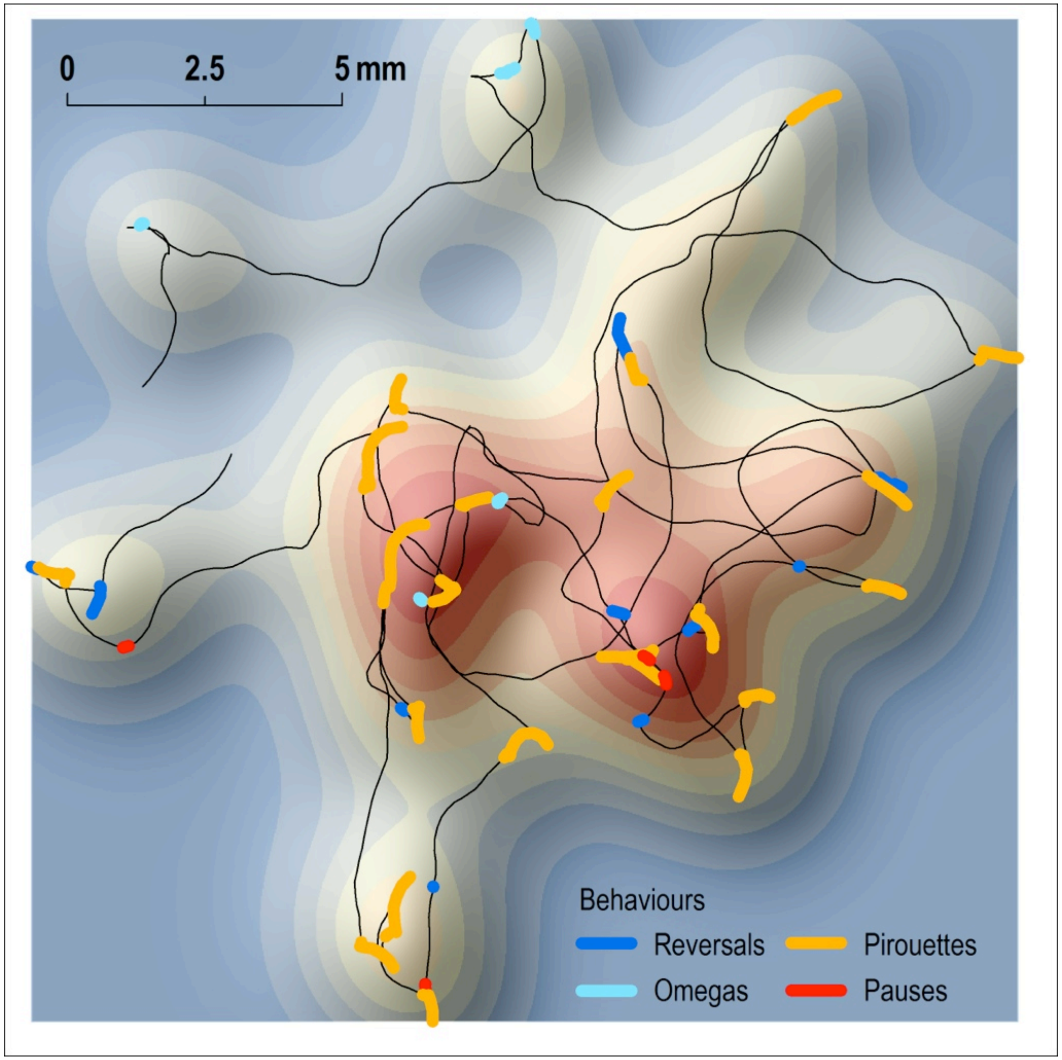


C.elegans searching a closer look.

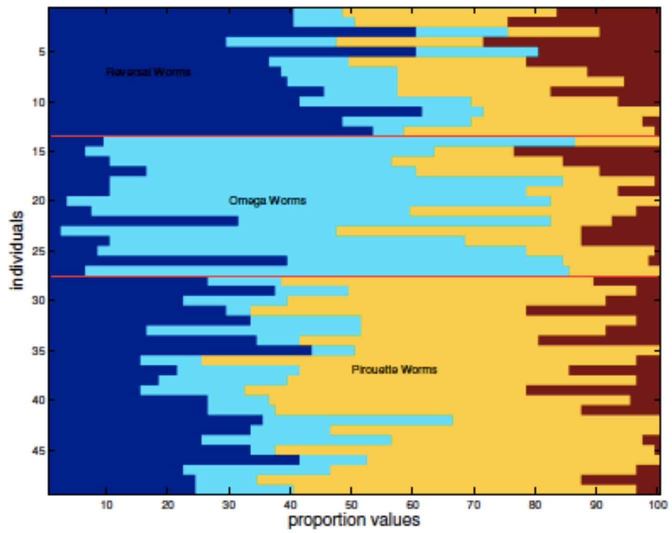
~50 worms
~25 minutes
L~ 60 mm



C. elegans searching - reorientation events



Cluster by maximum proportion

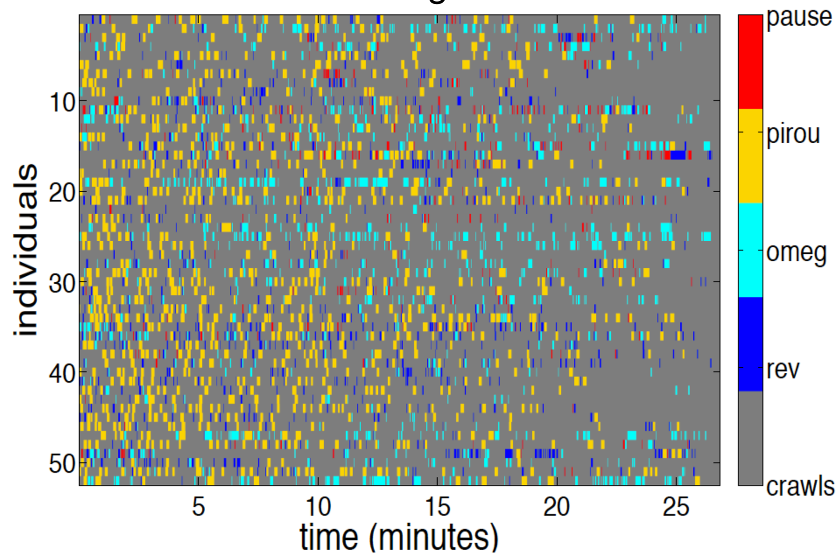


Worm types: reversing, omega, pirouette'ing

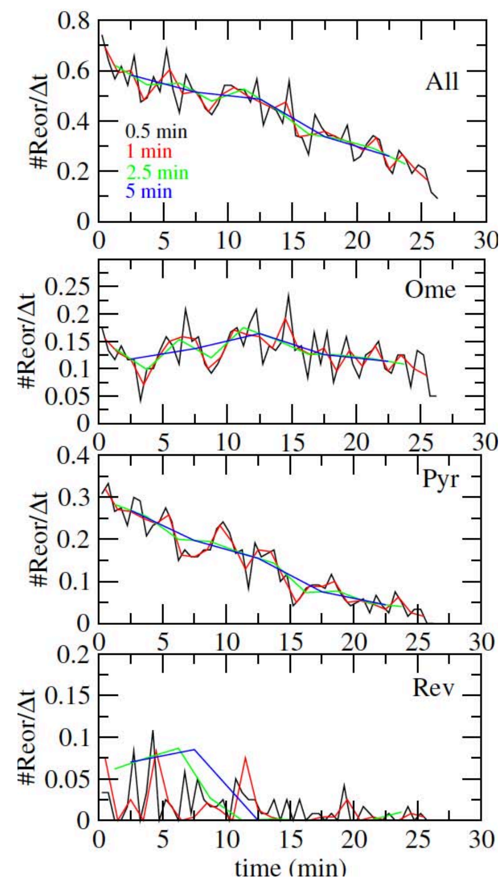
Worm have different searching personality

Searching statistics

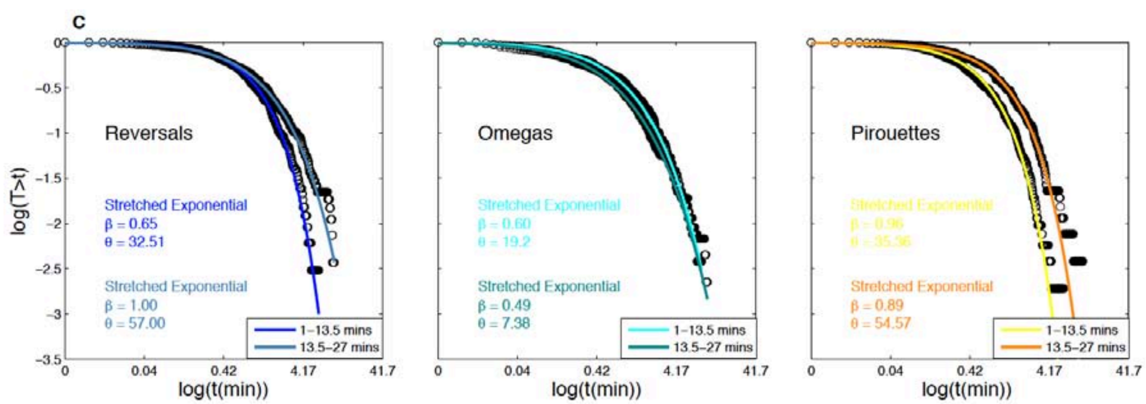
Ethogram



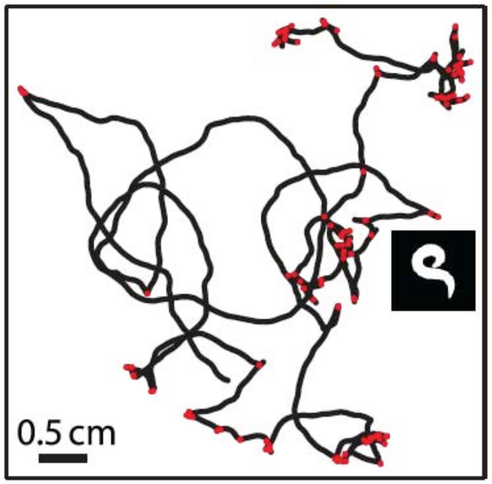
Reorientation events vs time



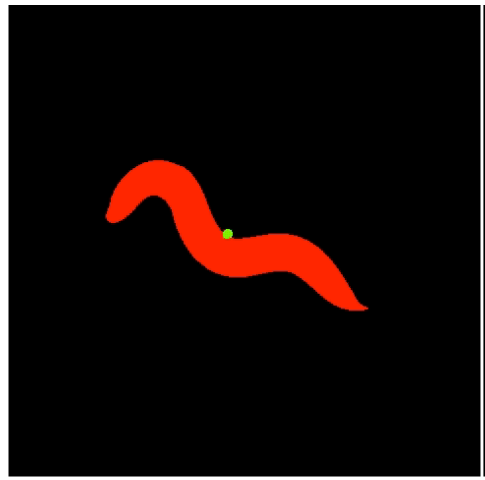
Reorientation event distribution



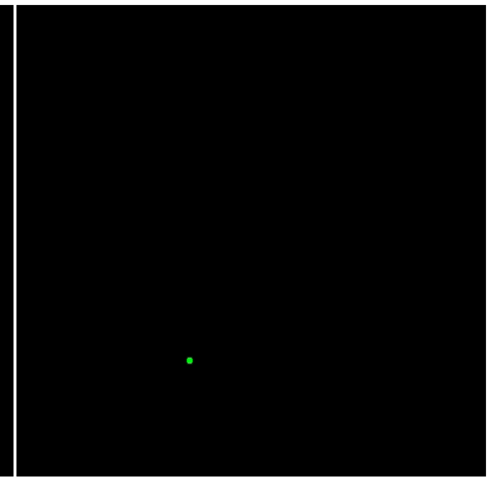
Dwell time in the forward moving state



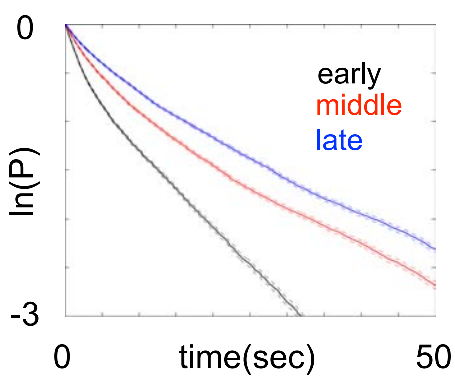
Center of mass trajectory



Pirouette



Center of mass position

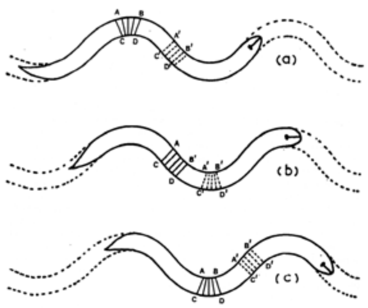


Survival probability of forward state before reversing depends on feeding state

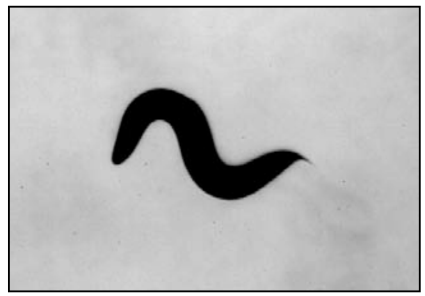
Tau ~ 15 secs

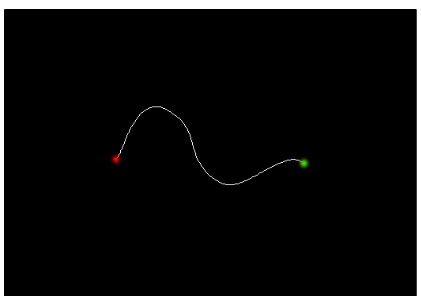
How do worms set this time constant?

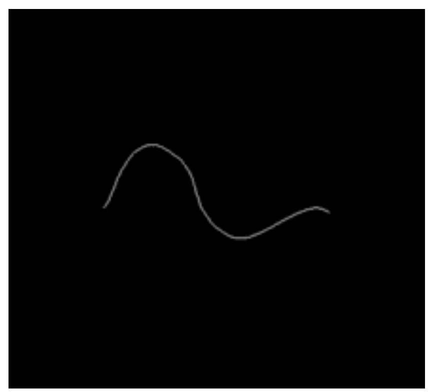
Parameterization – worm curvature

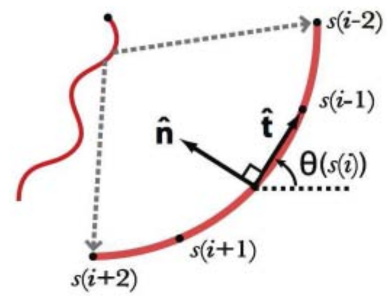


Wallace,1972



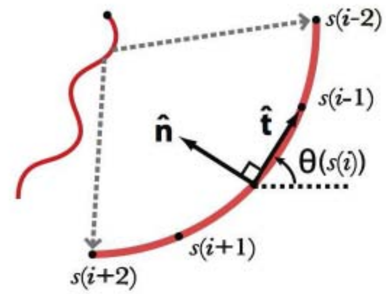




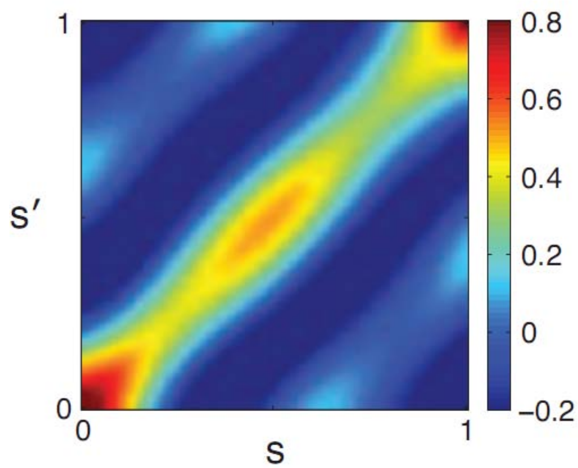


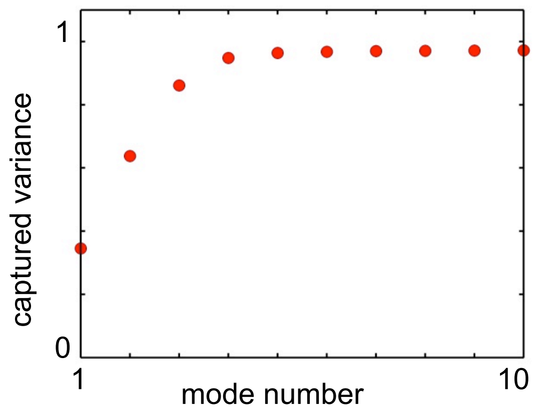
Reducing dimensionality – PCA

$$\vec{f}(\theta) = [\theta_1, \theta_2, \theta_3, \ldots, \theta_{100}]$$



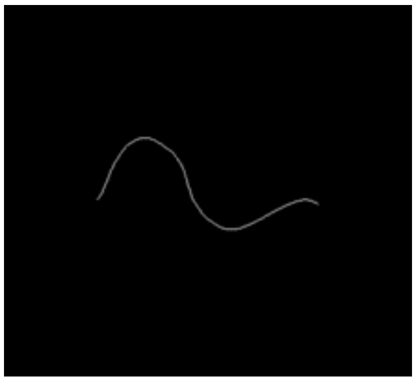
Covariance matrix of angles

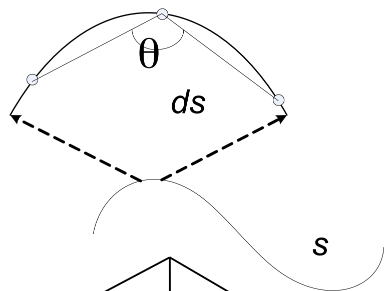




Only a small number of modes (~ 4) needed to capture the majority of the variance

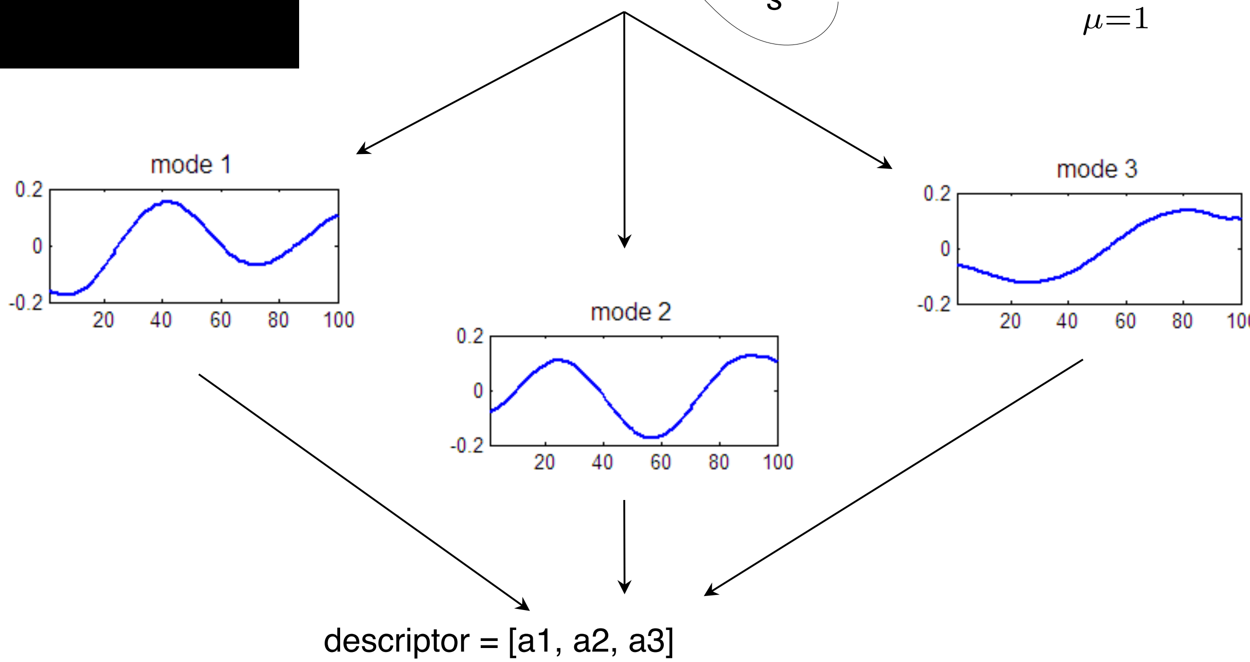
Eigenworms as a behavioral coordinate system



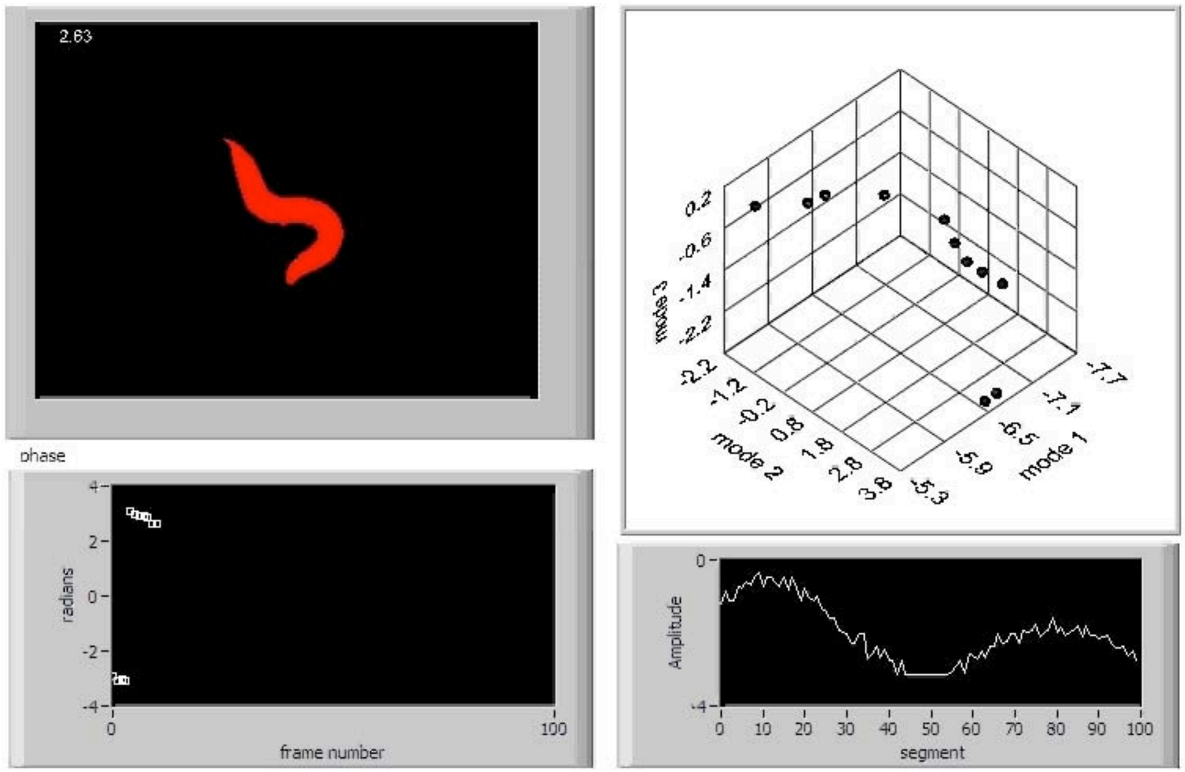


Project shapes onto eigenworms to capture behavioral description

$$\Theta(s) = \sum_{\mu=1}^{K} a_\mu u_\mu(s)$$



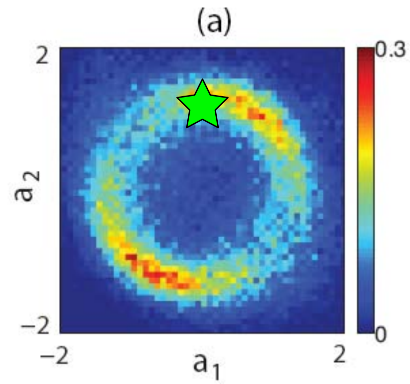
Eigenmode dynamics while tracking



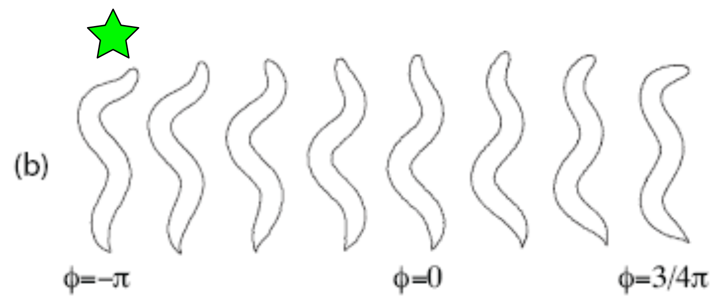
New behavioral description allows us to track worm and calculate eigenvalues

Stephens G, Bialek W, and Ryu WS, "Dimensionality and Dynamics in the Behavior of C. elegans." PLoS Comput Biol 2008

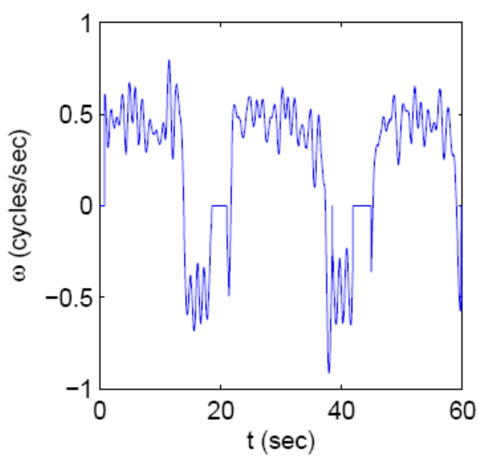
Modes 1 & 2 – phase oscillator



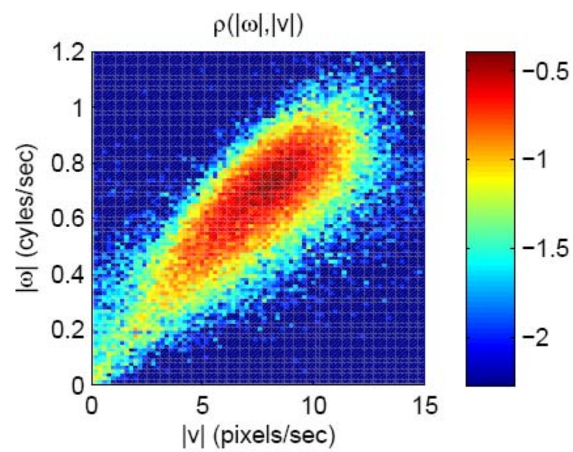
Joint distribution of mode 1 & 2



Worm shape versus phase



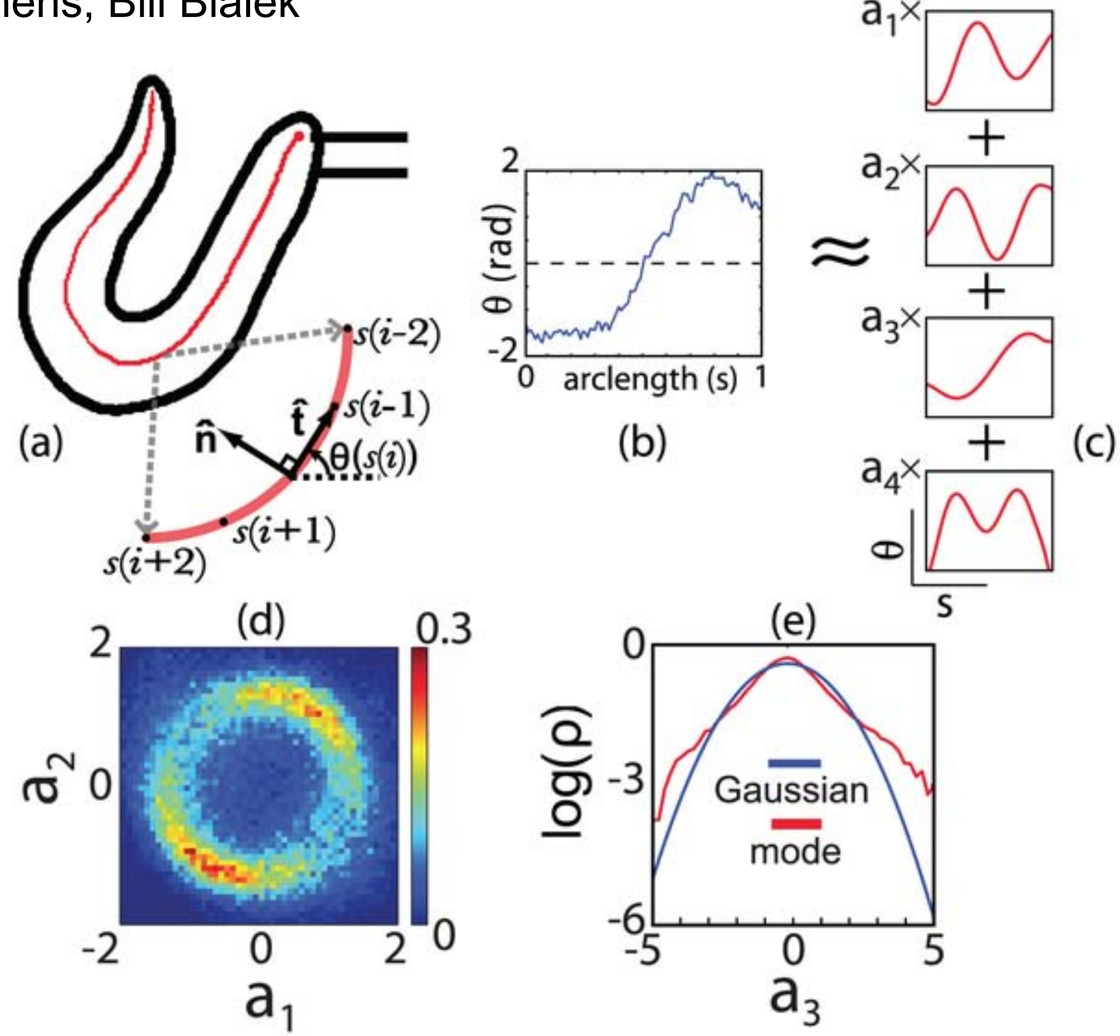
Phase velocity is a measure of worm velocity



Phase velocity matches real velocity

Representation of behaviour

Greg Stephens, Bill Bialek

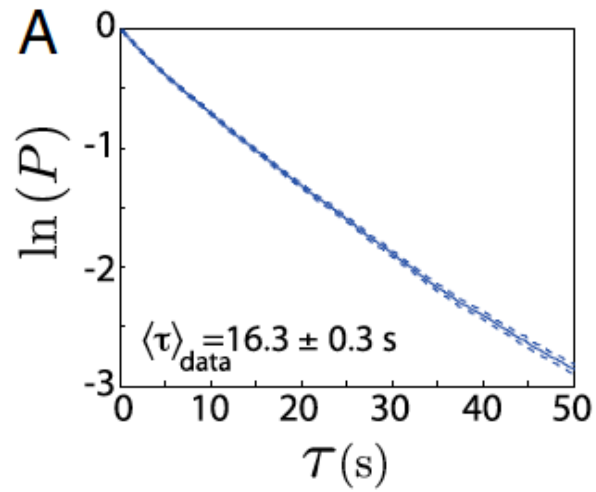


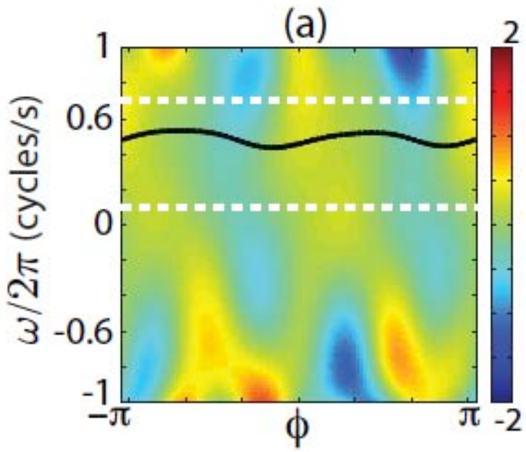
Equations of motion and generating timescales

$$\frac{d\phi}{dt} = \omega$$ Phase velocity

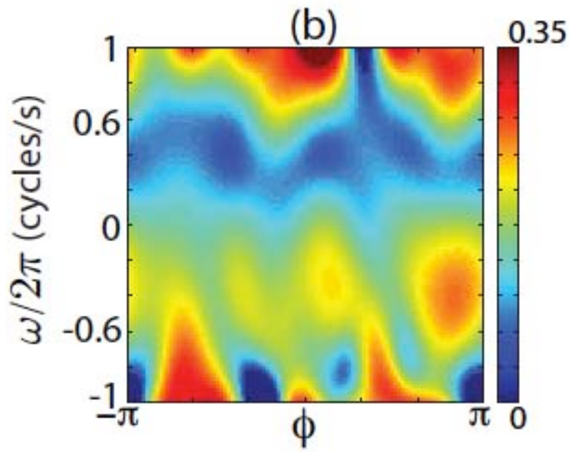
Effective force — Noise (state dependent)

$$\frac{d\omega}{dt} = F(\omega, \phi) + \sigma(\omega, \phi)\eta(t)$$

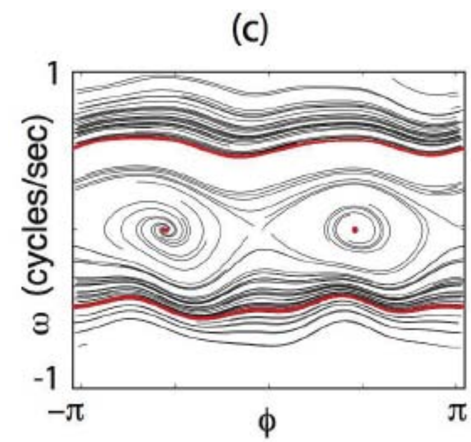




$F(\omega, \phi)$

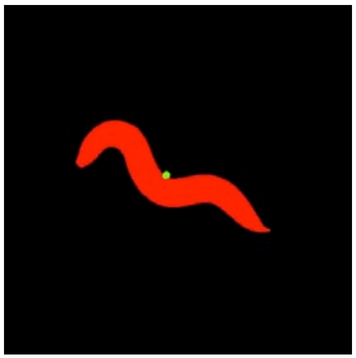


$\sigma(\omega, \phi)$



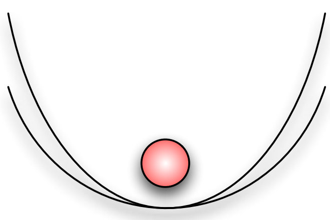
Sample trajectories no noise

Equations of motion: an analogy



$$\frac{d\phi}{dt} = \omega$$

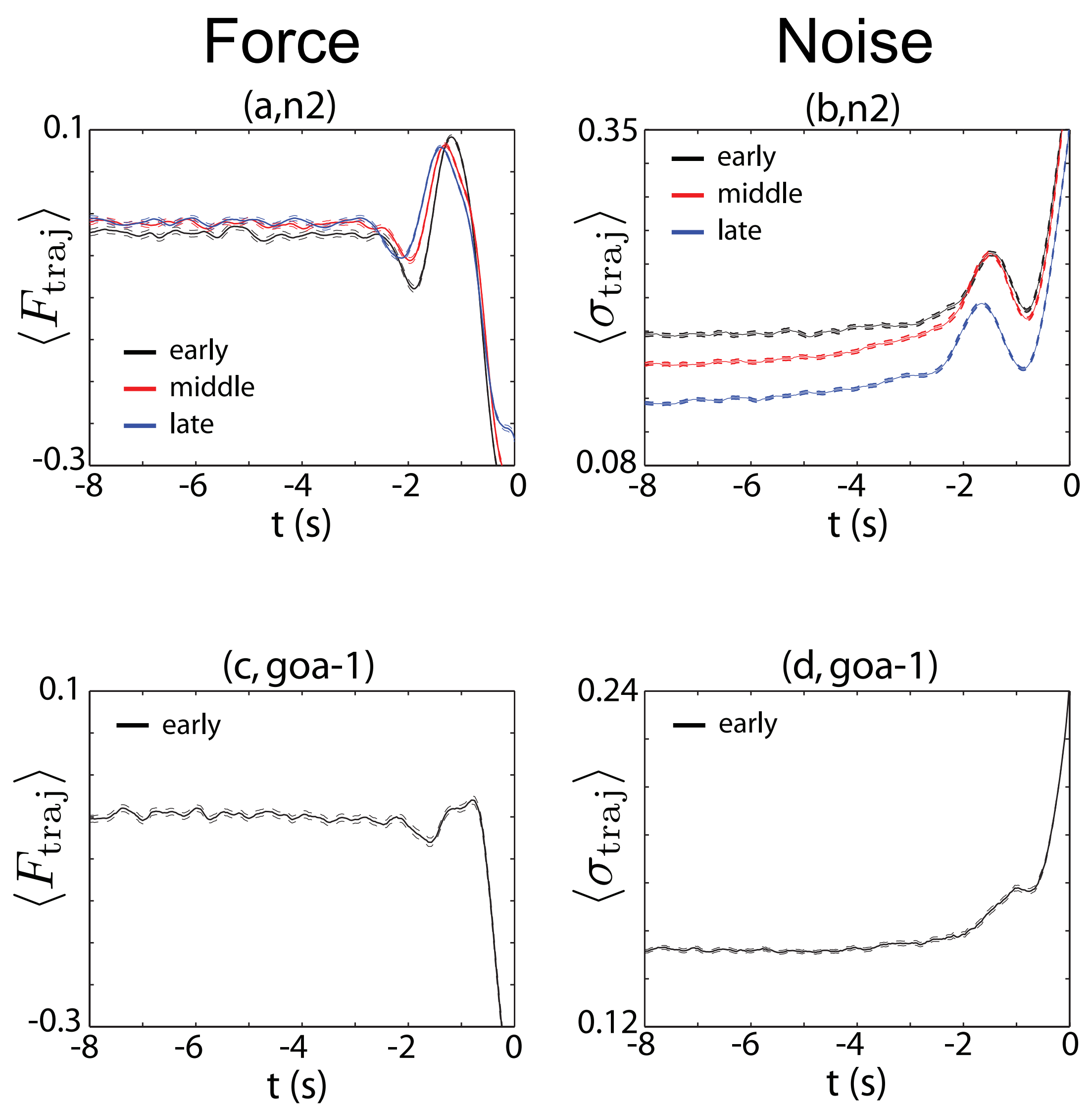
$$\frac{d\omega}{dt} = F(\omega, \phi) + \sigma(\omega, \phi)\eta(t)$$



$$\frac{dx}{dt} = v$$

$$m\frac{dv}{dt} = f(x, y) + \eta(t)$$

Forces and noise



Summary & Acknowledgements

Stochasticity in search may be modelled as noisy escape from a potential landscape

Simplicity in complex systems underlying motor control may be discoverable by course grain modeling (requires lots of data)

Matt Bueno de Mesquita - data collection

Greg Stephens, Bill Bialek - (Princeton University)

Fred Bartumeus, Ilya Nemenman

Raj Ghosh, Leonid Kruglyak, (Princeton University)