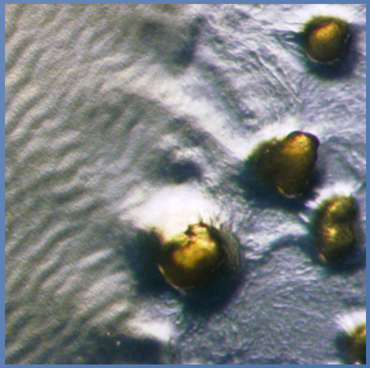
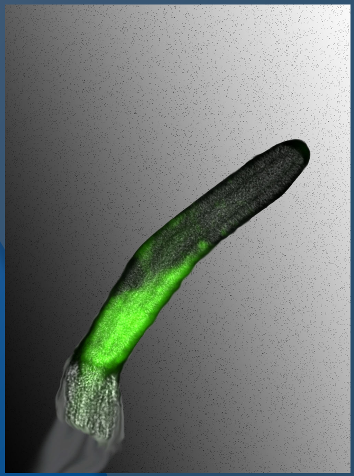


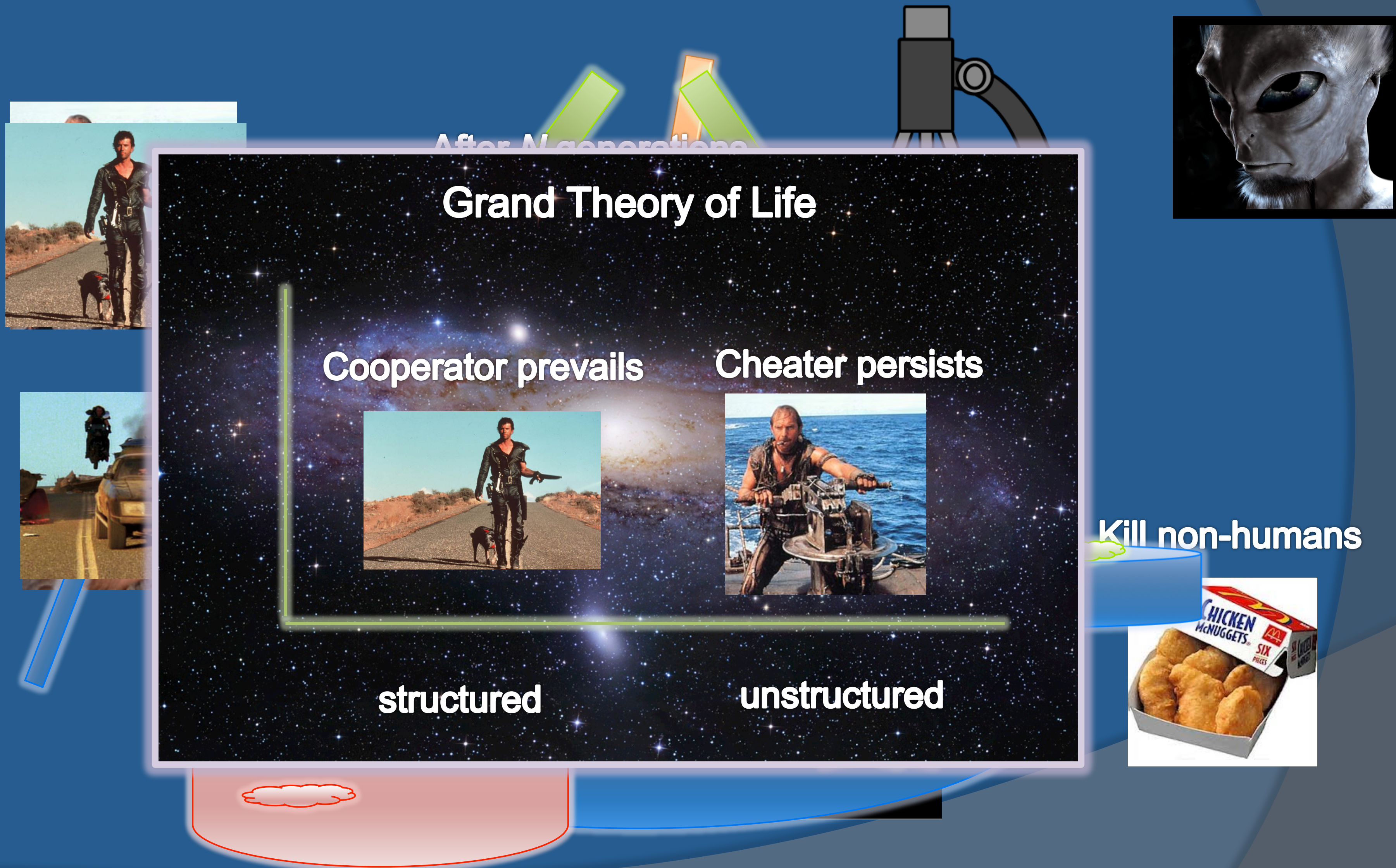


Owen Gilbert, Independent and KITP

A critical look at the field of microbial social evolution

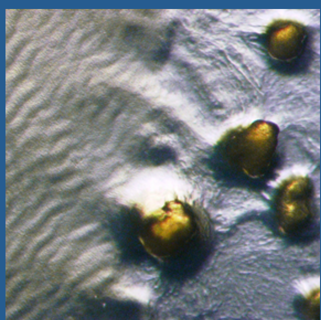
22 January 2013

A caricature of the field



Experimental manipulations of population structure

Myxobacteria



Developmental cheating in the social bacterium *Myxococcus xanthus*

Gregory J. Velicer*†, Lee Kroos* & Richard E. Lenski†

* Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824, USA
† Center for Microbial Ecology, Michigan State University, East Lansing,

Competitive fates of bacterial social parasites: persistence and self-induced extinction of *Myxococcus xanthus* cheaters

Francesca Fiegna and Gregory J. Velicer*

Endemic social diversity within natural kin groups of a cooperative bacterium

Susanne A. Kraemer[1] and Gregory J. Velicer

Department of Biology, Indiana University, Bloomington, IN 47405

Edited by John C. Avise, University of California, Irvine, CA, and approved May 18, 2011 (received for review February 22, 2011)

Pseudomonas


Figure 1. Pseudomonas aeruginosa Provided by Julia Plotnikov

Cooperation and competition in pathogenic bacteria

Ashleigh S. Griffin[1], Stuart A. West[1] & Angus Buckling[2]

[1]Institute of Cell, Animal & Population Biology, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh EH9 3JT, UK
[2]Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, UK

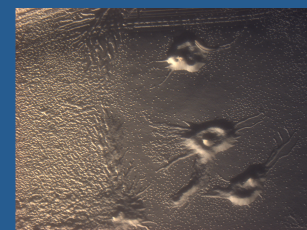
Viscous medium promotes cooperation in the pathogenic bacterium *Pseudomonas aeruginosa*

Rolf Kümmerli[1], Ashleigh S. Griffin[1,2], Stuart A. West[1,2], Angus Buckling[2] and Freya Harrison[2,3,*]

[1]Institute of Evolutionary Biology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK
[2]Department of Zoology, Oxford University, South Parks Road, Oxford OX1 3PS, UK
[3]Biodiversity Lab, Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, UK

There has been extensive theoretical debate over whether population viscosity (limited dispersal) can favour cooperation. While limited dispersal increases the probability of interactions occurring between relatives, which can favour cooperation, it can also lead to an increase in competition between relatives

Dictyostelium



***Dictyostelium* amoebae lacking an F-box protein form spores rather than stalk in chimeras with wild type**

Herbert L. Ennis*†, Dee N. Dao*†, Stefan U. Pukatzki, and Richard H. Kessin

Department of Anatomy and Cell Biology, Columbia University, 630 West 168th Street, New York, NY 10032

Communicated by J. T. Bonner, Princeton University, Princeton, NJ, January 6, 2000 (received for review November 10, 1999)

High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants

Owen M. Gilbert*, Kevin R. Foster†, Natasha J. Mehdiabadi, Joan E. Strassmann, and David C. Queller

Department of Ecology and Evolutionary Biology, Rice University, MS 170, 6100 Main Street, Houston, TX 77005

Edited by Gene E. Robinson, University of Illinois at Urbana–Champaign, Urbana, IL, and approved April 10, 2007 (received for review March 23, 2007)

The control of cheating is important for understanding major transitions in evolution, from the simplest genes to the most complex societies. Cooperative systems can be ruined if cheaters that lower group productivity are able to spread. Kin-selection theory predicts that high genetic relatedness can limit cheating, because separation of cheaters and cooperators limits opportuni-

The best known social amoeba, *Dictyostelium discoideum*, is a model organism that, unusually, allows both estimation of relatedness in the field and the study of cheater mutants. Relatedness of vegetative *D. discoideum* cells naturally cooccurring in very small soil samples (0.2 g) has been estimated as 0.52 (26), but relatedness in actual fruiting bodies has not been estimated.

High Relatedness Is Necessary and Sufficient to Maintain Multicellularity in *Dictyostelium*

Jennie J. Kuzdzal-Fick,[1,2] Sara A. Fox,[1] Joan E. Strassmann,[1,3] David C. Queller[1,3]*

Most complex multicellular organisms develop clonally from a single cell. This should limit conflicts between cell lineages that could threaten the extensive cooperation of cells within multicellular

A Shakespearean concern

Hamlet

"There are more things in heaven and earth, Alien, than are dreamt of in your petri dish."

❉✰✪❏❏❀❊❏!!!

Hayek

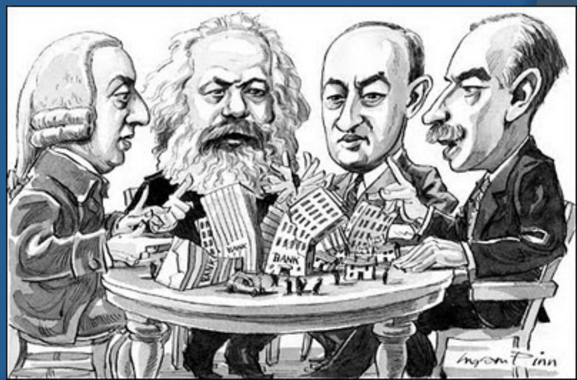
Complex phenomena

"…in the social sciences often **that is treated as important which happens to be accessible to measurement**."

"…It sometimes almost seems as if the techniques of science were more easily learnt than **the thinking that shows us what the problems are and how to approach them.**"

2008

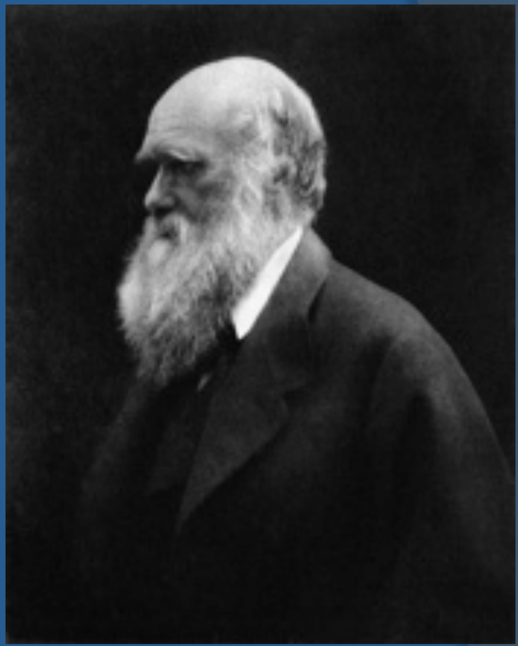
Stock Market



Hayek, 1974 *Nobel Lecture*

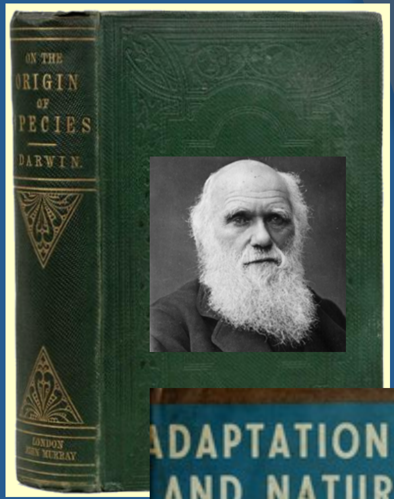
The Darwinian Approach to Biology

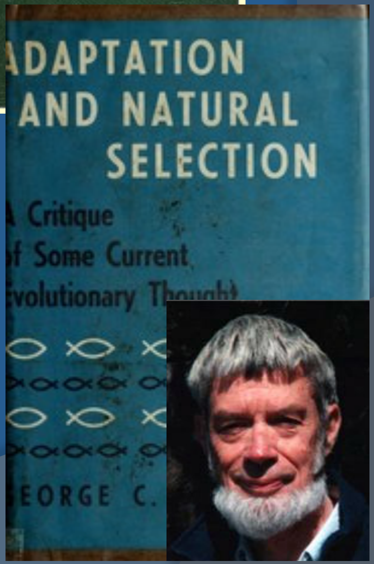
- Understand the organism in its natural environment.
- Use comparative evidence to formulate adaptive hypotheses.
- Entertain alternative hypotheses if biologically plausible.



Withering scrutiny

- Treat each adaptive hypothesis with contempt.
- Be willing to give up favored hypotheses.
- Be imaginative when thinking of alternatives.
- Use abductive reasoning.

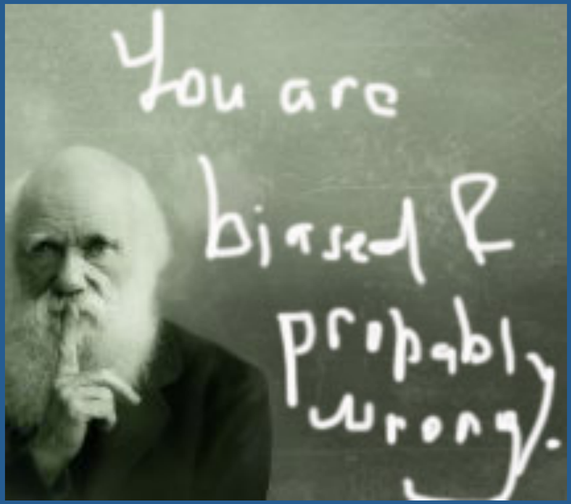




Darwin (1859); Darwin (1887); G. C. Williams (1966); Harmon (1965)

Of course

- Most *darwinists* are biased in favor of pet theories.
- Their friends do not share the same commitments.
- Through discourse, *darwinists* formulate and test hypotheses.



Structure for talk

- Introduction to the problem.
- Review of means that it has been addressed.
- Future work.

Structure for talk

- Introduction to the problem.
- Review of means that it has been addressed.
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Why study microbial social evolution?

- Understand human diseases
- Genetic basis of behavior
- The evolution of cooperation
- The evolution of multicellularity
- The origin of organisms

Why study microbial social evolution?

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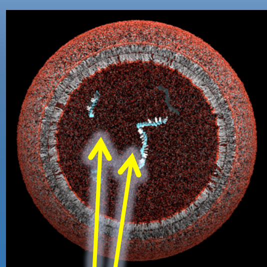
- The origin of organisms

The Origin of Organisms

Cellular organisms

Protocell

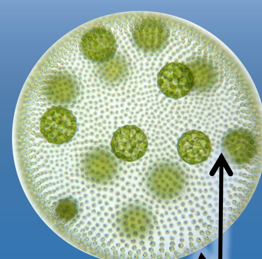


Credit: J. Iwasa

Cooperative replicators

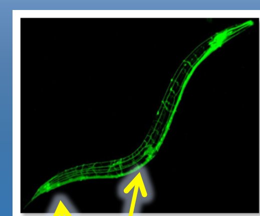
Multicellular organisms

V. carteri



Credit: A. Nedelcu

C. elegans

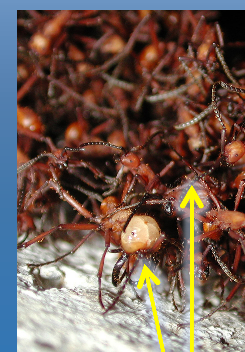


Credit: J. Bessereau

Cooperative cells

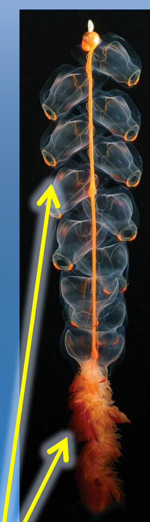
Colonial organisms

E. hamatum



Credit: L. Gilbert

Siphonophore

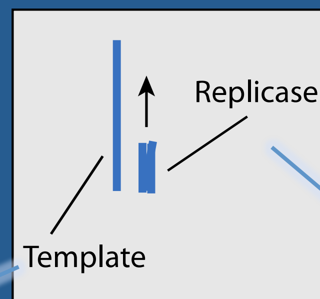


Credit: K. Raskoff

Cooperative Individuals

"*Multiple levels of organization have emerged in the history of life, and each such emergence raises the same existence problem as does life itself.*" –Fontana & Buss (1994)

Origin of life




Volume 69, Number 16 PHYSICAL REVIEW LETTERS 19 October 1992

Nucleation and Transients at the Onset of Vortex Turbulence

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Preben Alstrøm
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Tomas Bohr
The Niels Bohr Institute, 2100 Copenhagen Ø, Denmark
(Received 28 February 1992)

We present analytical and numerical results that explain the transient turbulent dynamics observed in the complex Ginzburg-Landau equation. Just below the transition to turbulence, we observe that metastable turbulent states break down by the nucleation and growth of single-vortex droplets, leading to a "frozen" state with a low (but finite) density of stationary vortices. We derive the relation between nucleation time and radius, and determine their dependence on the distance to the turbulence transition line.

PACS numbers: 64.60.My, 47.25.Ae, 64.60.Qb

The appearance of turbulence (or "spatiotemporal chaos") in extended dynamical systems is not well understood. On the one hand, one would like to understand in what respects these systems behave like their low-dimensional, chaotic counterparts; on the other hand, it is obvious that, in some respects, they behave like stochastic systems, e.g., systems in thermal equilibrium. The onset of turbulence in media possessing a stable, time-periodic, global state has proven interesting since there one finds that the turbulence is associated with the appearance of "defects" (vortices or spiral waves) and thus one knows the important "elementary excitations." Vortex turbulence arises in oscillatory chemical media, and is characterized by a spatiotemporal chaotic dynamics, governed by the creation and annihilation of moving vortices [1-5]. A prototype for modeling this dynamics is the complex Ginzburg-Landau equation [6], which describes an oscillatory chemical medium in the vicinity of a Hopf bifurcation,

$$\dot{A}(\mathbf{x},t) = \mu A(\mathbf{x},t) - (1+i\alpha)|A(\mathbf{x},t)|^2 A(\mathbf{x},t) + (1+i\beta)\nabla^2 A(\mathbf{x},t) . \quad (1)$$

Here, $A(\mathbf{x},t)$, the complex amplitude at point $\mathbf{x}$ and time t, is related to the chemical mode $c(\mathbf{x},t)$ that develops the instability,

$$c(\mathbf{x},t) = c_0 + [A(\mathbf{x},t)e^{i\omega_0 t} + \text{c.c.}] , \quad (2)$$

where c_0 is a constant, ω_0 is the oscillation frequency, and c.c. denotes the complex conjugate.

The parameters μ, α, and β in (1) are real numbers. For $\mu \le 0$, $A(\mathbf{x},t) = 0$; for $\mu > 0$, $A(\mathbf{x},t)$ is nonzero. In the latter case, a simple, homogeneous solution exists, $A(t) = \sqrt{\mu}e^{-i\alpha\mu t}$. Linear stability analysis shows that the homogeneous solution is stable when $\alpha\beta + 1 > 0$. However, in general the system does not end up in the homogeneous state; rather it finds a "vortex state," i.e., one containing vortices and antivortices (spiral waves) with the property that the angle field changes by $\pm 2\pi n$ along any contour containing them. This state can be a "frozen" state of stationary vortices [2,3], or it can be a highly turbulent state with vortex-antivortex pair creation and annihilation.

Of fundamental interest is the occurrence of transient turbulent states near the transition line (in α-β space) that separates the frozen states from the turbulent states [2]. In this Letter, we present new analytical and numerical results on these transient states, which we identify as "metastable" states having a well-defined vortex density. We find that the metastable states break down through the nucleation and growth of single vortices, ending up in frozen states, which, due to the large system sizes in our simulations, we can identify as states with a low but finite density of stationary vortices. The lifetime of the metastable states depends on the distance to the transition line. We present arguments giving the analytical form of this dependence, and test it numerically.

As first noted by Oono and Puri [7], an efficient way to study Ginzburg-Landau models numerically is to construct a coupled-map lattice with a similar spatiotemporal dynamics. Such a construction has also been made for the complex Ginzburg-Landau model [2,5]. The idea is to integrate (1) in two steps by (i) solving the complex heat equation (taking only the Laplacian term into account), and (ii) integrating the nonlinear part neglecting the Laplacian. Here, we use the coupled-map lattice of Bohr *et al.* [2] adapted to the parallel architecture of the Boston University Connection Machine 2.

Figure 1 shows the phase diagram found for the complex Ginzburg-Landau model with $0 \le \alpha \le 2$ and $-2 \le \beta \le 0$, and solved on a square lattice of size 512×512. In the discrete model used, the (Benjamin-Feir) stability line for the homogeneous solution is

+

Template (what is copied)

Replicase (copier)

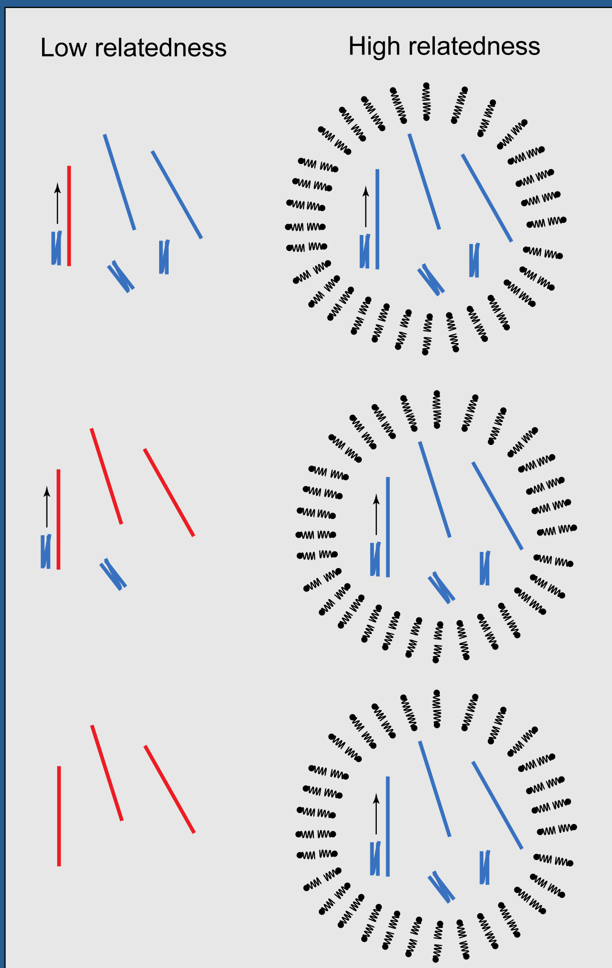
Szostak *et al.* (2001); Szathmary (2006); Chen (2006)

Population structure

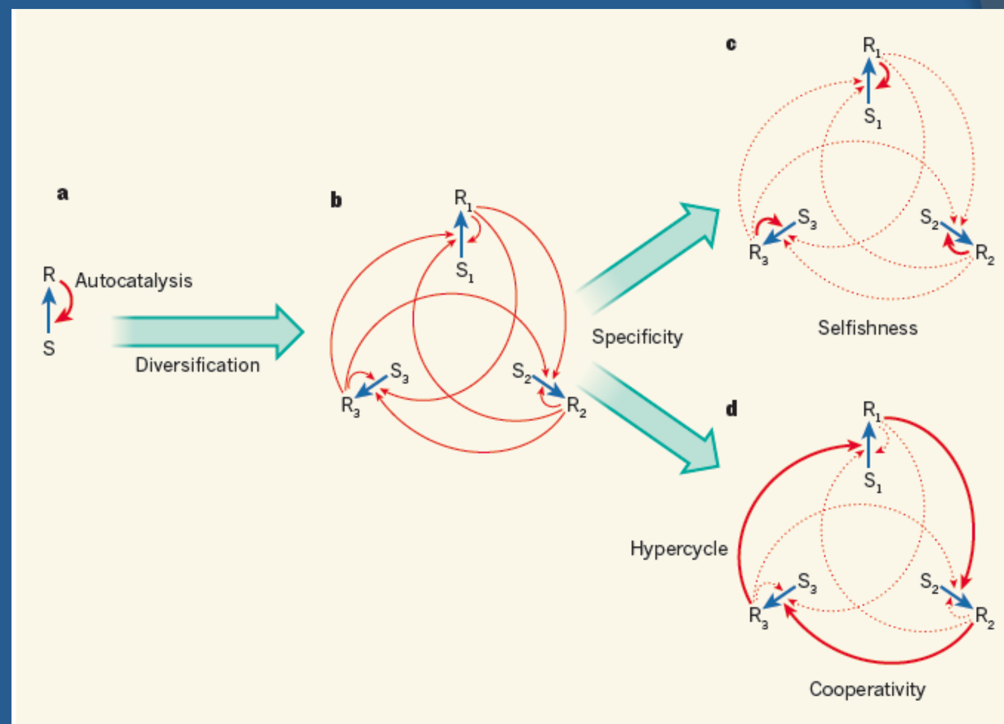
Selfish replicator (produces template only)

Altruistic replicator (folds to also produce replicase)

Protocell



Hypercycles

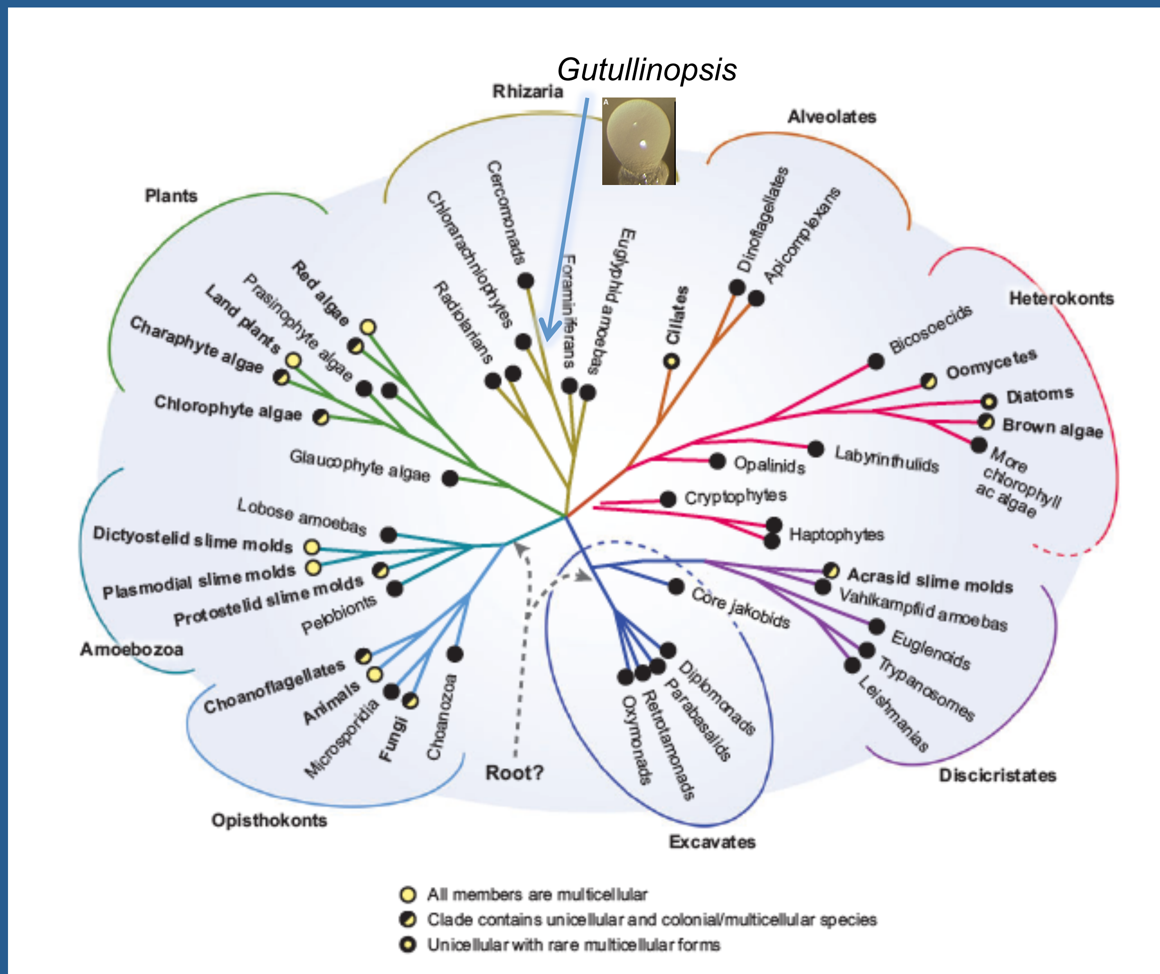


Attwater & Holliger (2012); Vaidya *et al.* (2012)

The problem of information loss

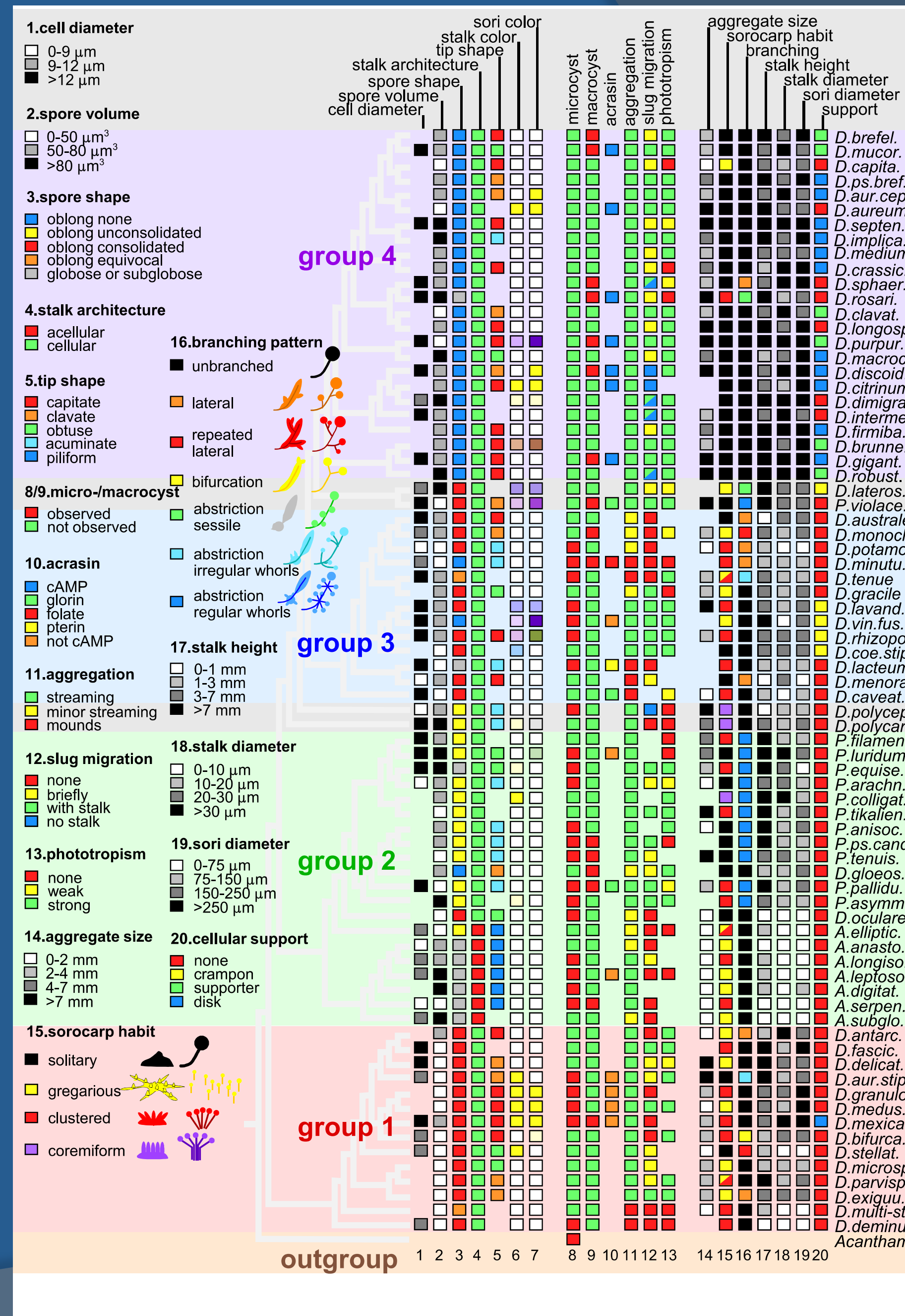
Tough to know what happened 3.8 bya

The relatively “recent” past



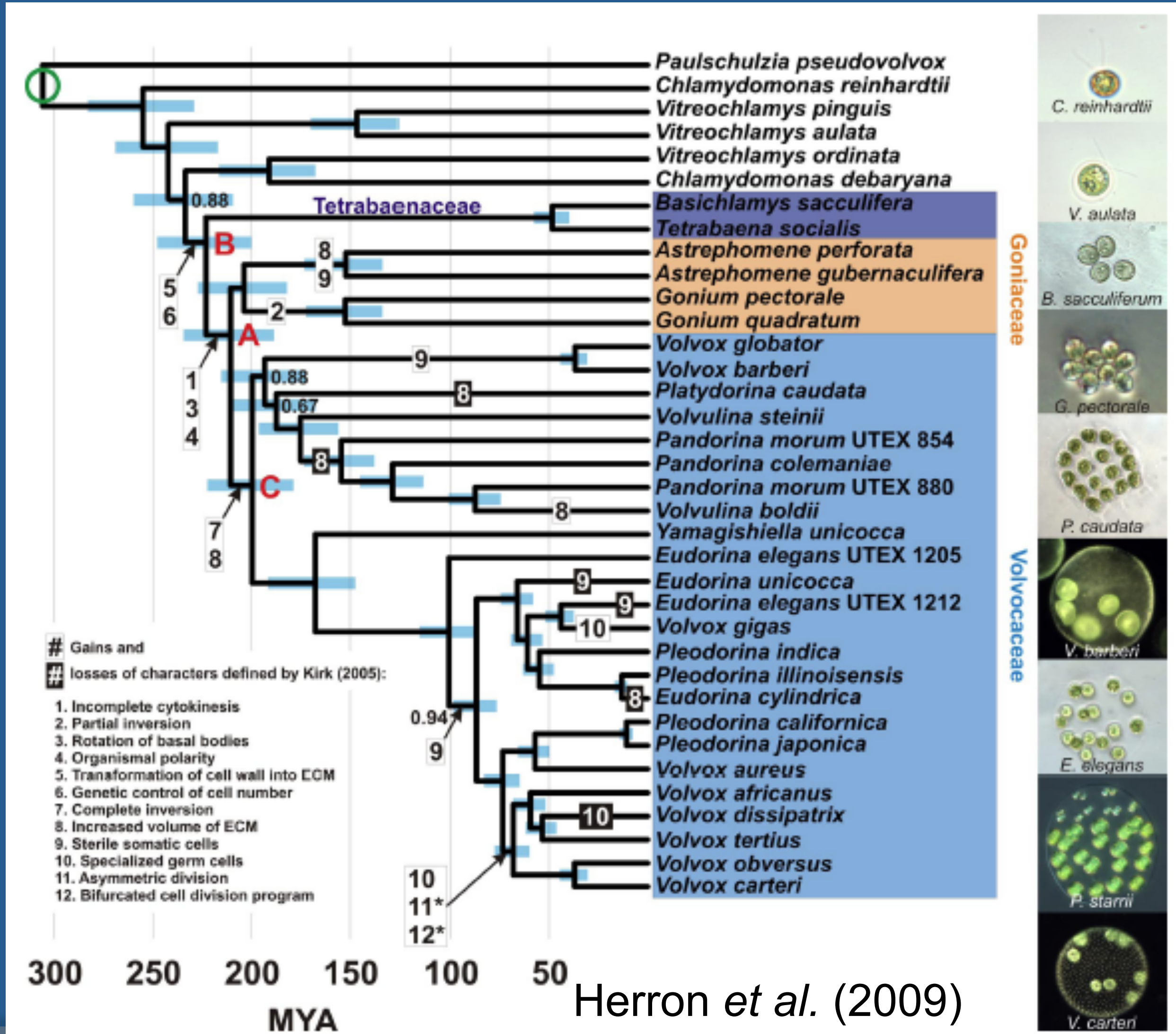
Grosberg & Strathmann (2007)

Social amoebae



Schaap *et al.* (2009)

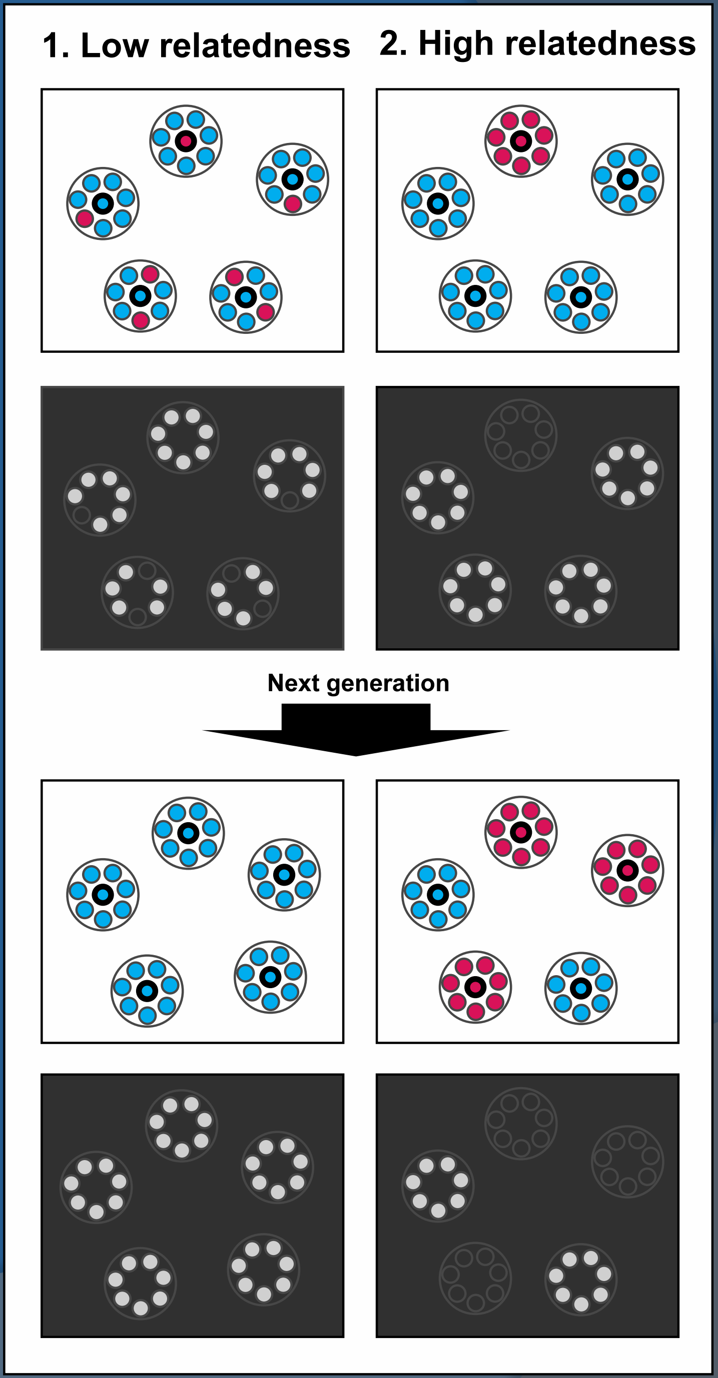
Volvocine green algae



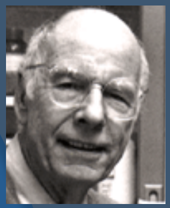
Herron *et al.* (2009)

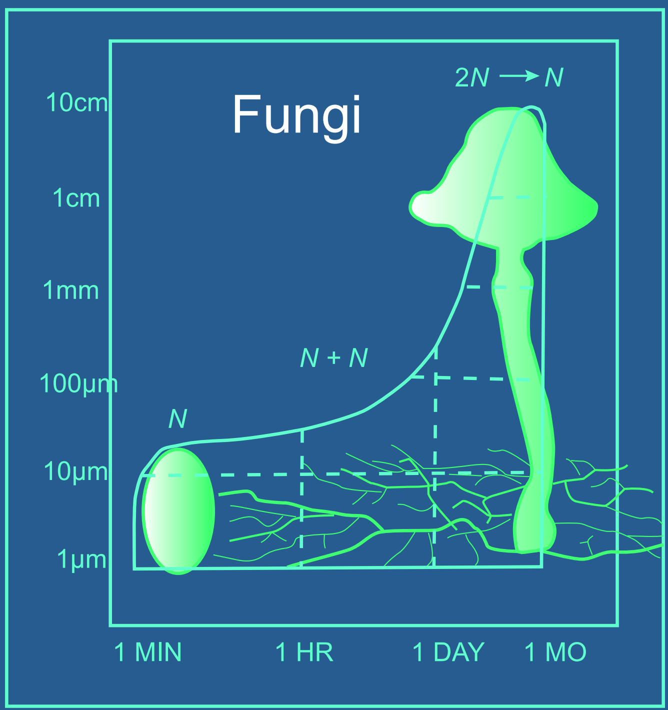
Evolvability

- Imaginary planktonic multicellular alga with sterile soma.
- Red mutation conveys camouflage in deep water.

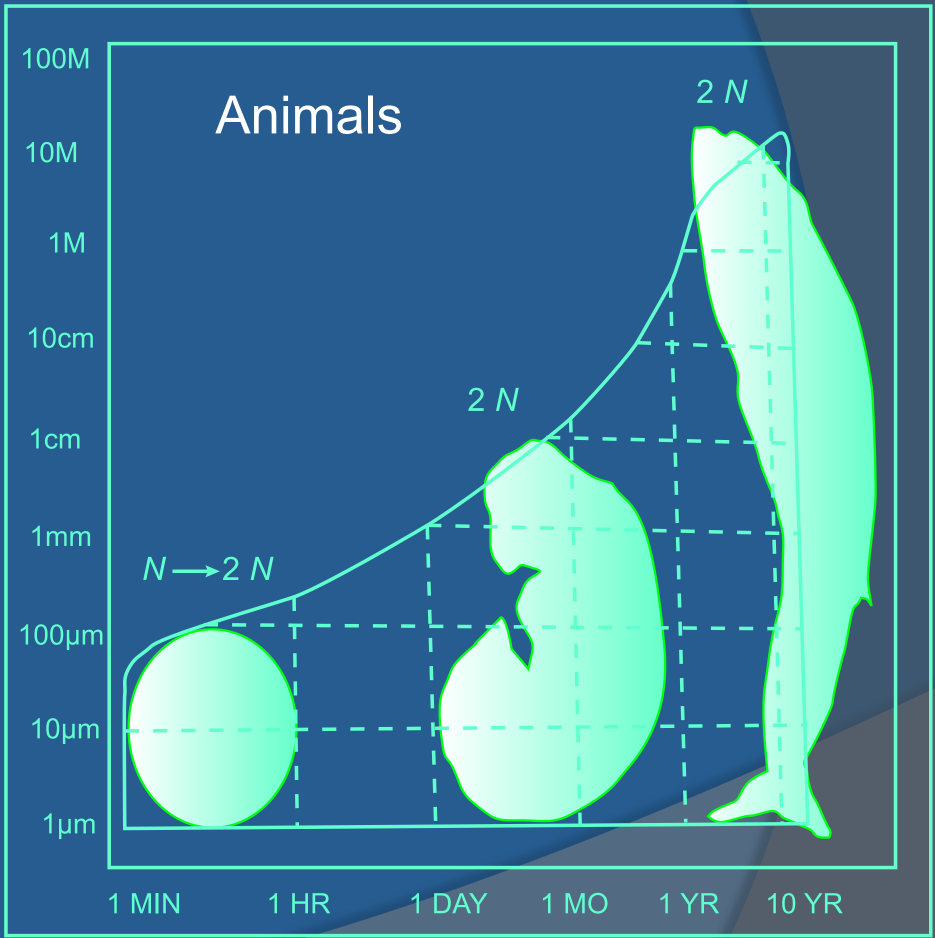


Bottlenecked life cycles





Coprinus sterquilinus



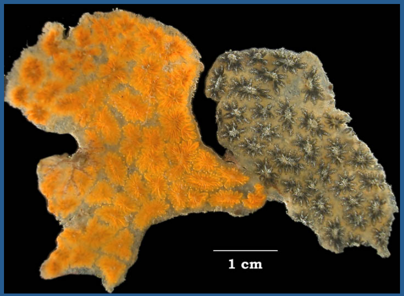
Balaenoptera musculus

Bonner (1966) *Size and Cycle*

Fusion compatibility systems

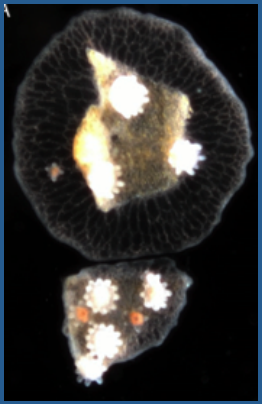
Multicellular organisms that fuse somatic tissue have somatic compatibility systems

Urochordates



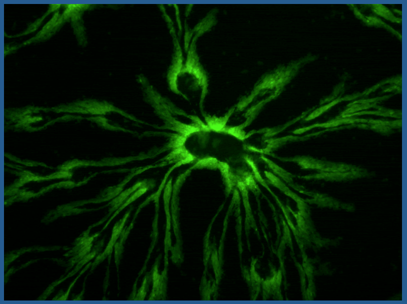
Credit: California Academy of Sciences

Cnidarians



Credit: A. Powell

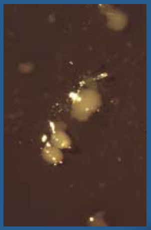
Social amoebae



Tasmanian devil tumor

Credit: M. Jones

Dicty chtA

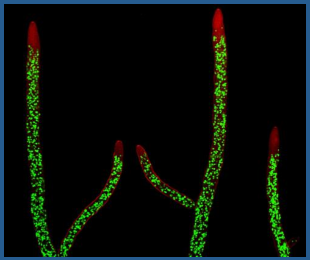


Credit: K. Foster

Myxomycetes

Credit: K. simmons

Fungi



Credit: P. Hickey & N. Read

May help prevent spread of somatic parasites

Buss (1982) *P. Natl. Acad. Sci. USA*; Pearse & Swift (2006) *Nature*

Structure for talk

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- Review of means that it has been addressed.
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A tale of two unicellular organisms…

Myxococcus xanthus

Gram negative, gliding *Protobacteria*

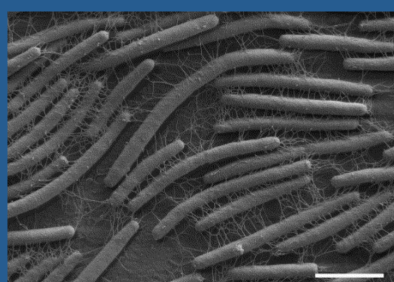


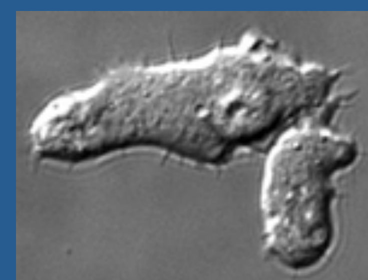
Image credit: Kearns & Shumkets (2001)

"Wolf pack" hunting behaviors

Secrete digestive enzymes

Dictyostelium discoideum

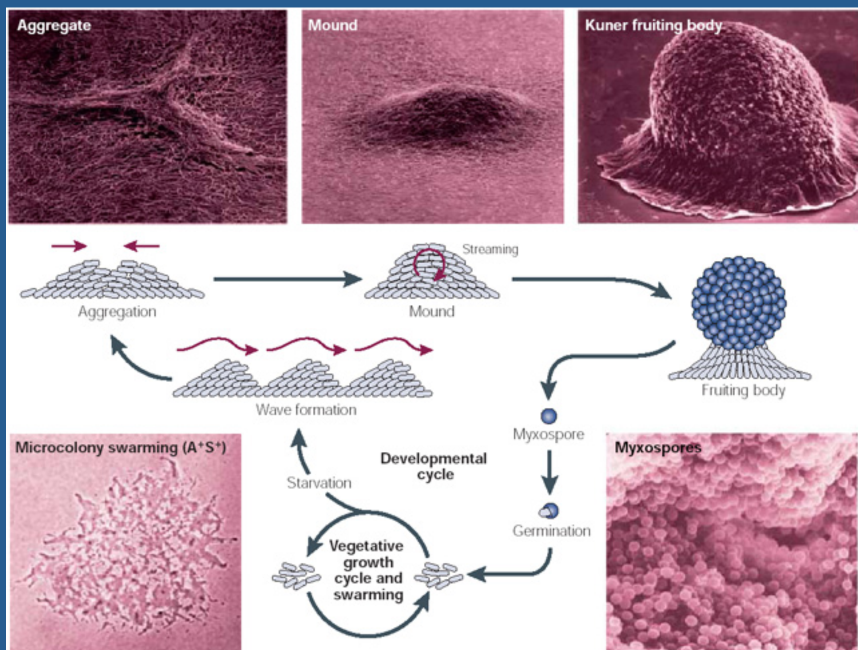
Cellular slime molds, "social amoebae"



Individual amoebae capable of feeding independently

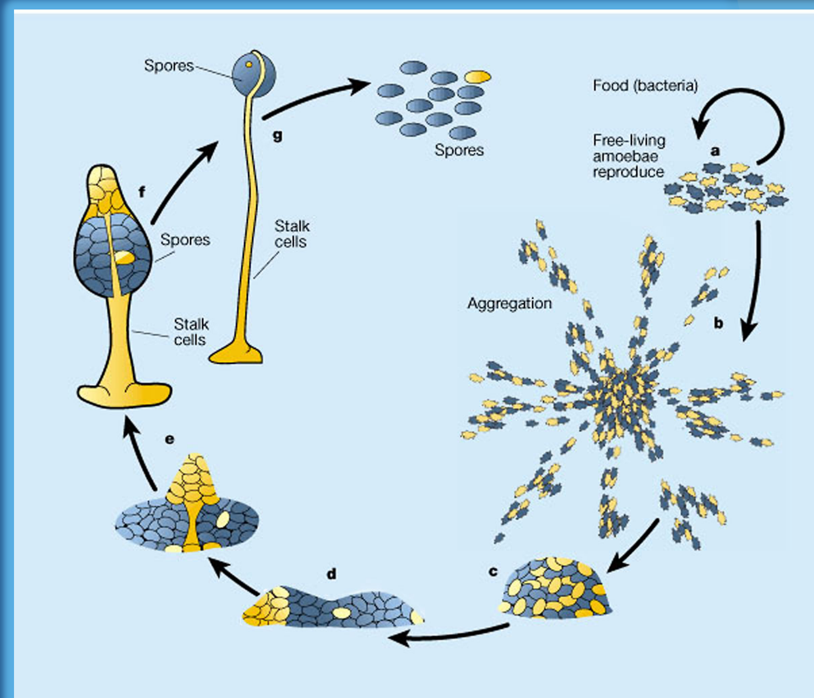
...also multicellular

Myxococcus xanthus



Credit: D. Kaiser

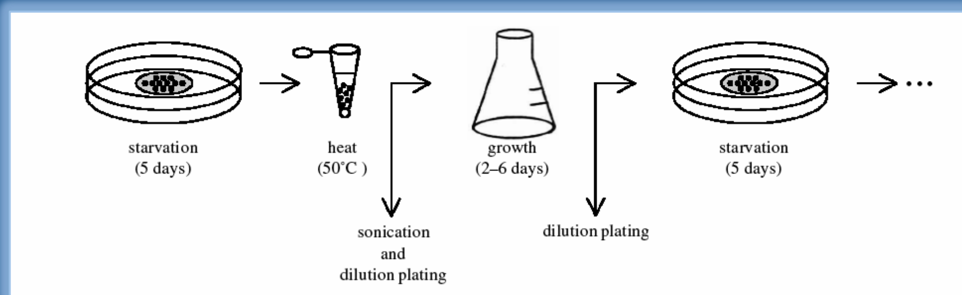
Dictyostelium discoideum



Credit: Kessin (2000) Nature

Myxo populations susceptible to cheating under well-mixed conditions

D1 D2



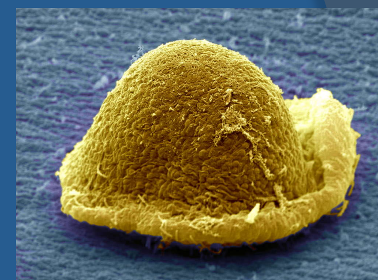
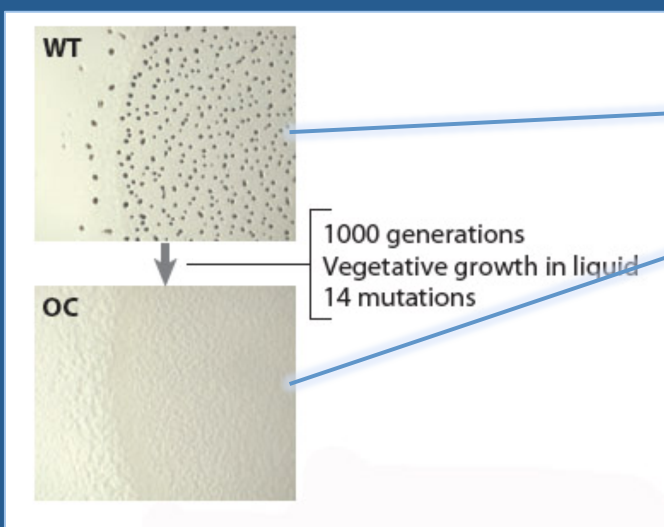
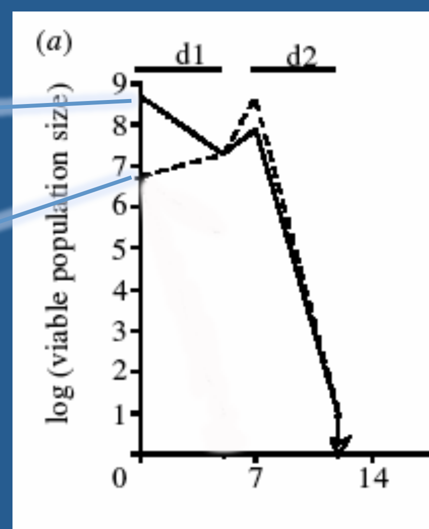


Image credit: G. Velicer





- 8 / 16 replicates of OC / WT incurred cheater-induced extinction

Fiegna & Velicer (2003)

Preliminary data on natural structure

What is minimum average relatedness in sample?

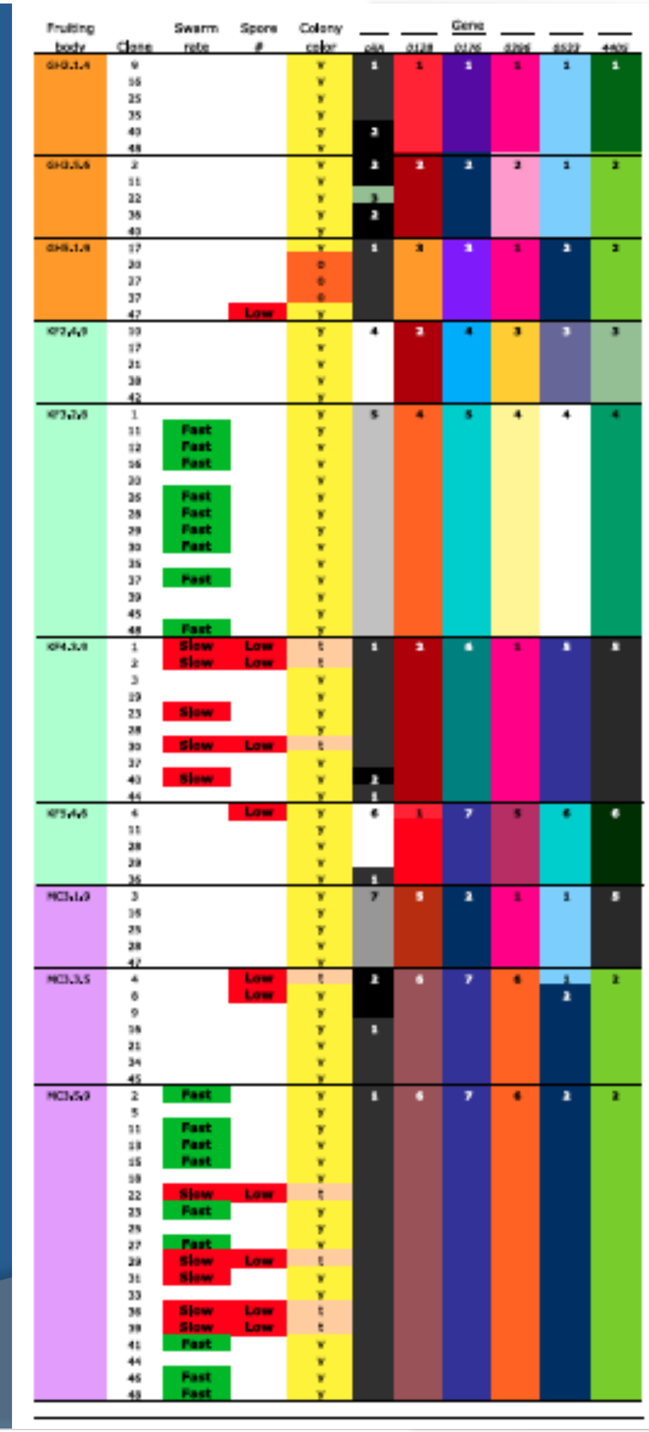
Assume
1. Any allelic difference = different clone
2. Different clones unrelated

$$R = \sum_i^n (p_i^2 + q_i^2 + (1 - p_i - q_i)^2) / n$$

N = 10

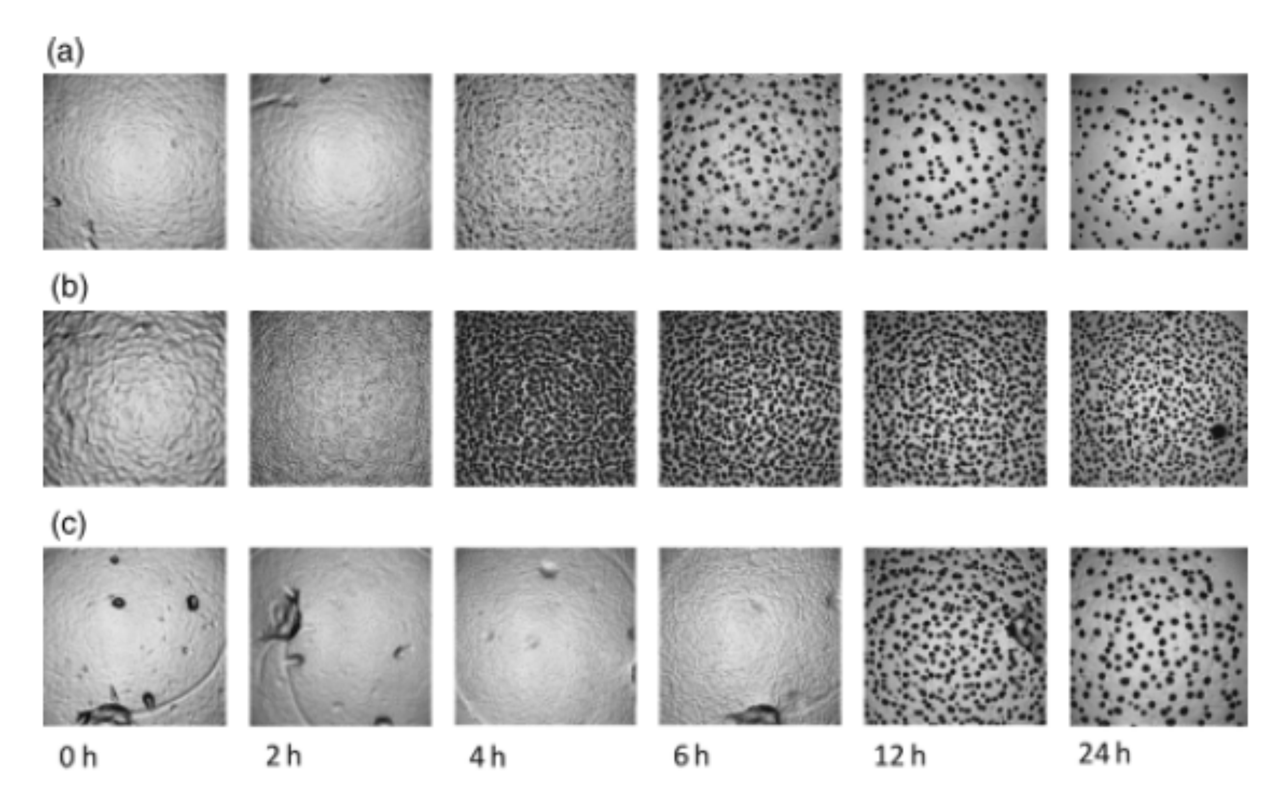
FB	# spores/clone			clone proportion			R_i
	Cl 1	Cl 2	Cl 3	p	q	$1 - p - q$	
GH2.1.4	4	2	0	0.67	0.33	0.00	0.56
GH3.5.6	5	1	0	0.83	0.17	0.00	0.72
GH5.1.9	6	0	0	1.00	0.00	0.00	1.00
KF2.4.9	6	0	0	1.00	0.00	0.00	1.00
KF3.2.8	14	0	0	1.00	0.00	0.00	1.00
KF4.3.9	9	1	0	0.90	0.10	0.00	0.82
KF5.4.6	5	1	0	0.83	0.17	0.00	0.72
MC3.1.9	6	0	0	1.00	0.00	0.00	1.00
MC3.3.5	4	2	1	0.67	0.33	0.14	0.58
MC3.5.9	19	0	0	1.00	0.00	0.00	1.00

$R = 0.84$



Kraemer & Velicer (2011)

Are there obligate cheaters in nature?



Development time

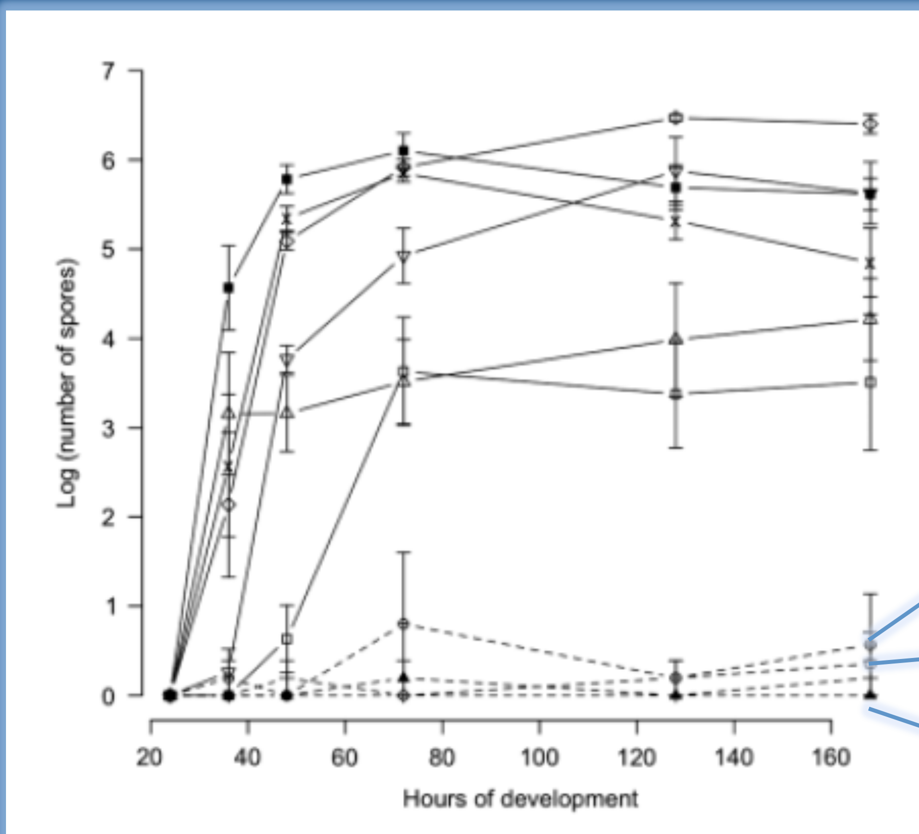
(a) Moderate (clone A30)

(b) Fast (clone A 98)

(c) Slow (clone A 94)

Kraemer *et al.* (2010)

Are there obligate cheaters in nature?



Vos & Velicer (2009)

Clone	Competitiveness
A9	Victim
A41	Average
A66	Not tested

Clone A41

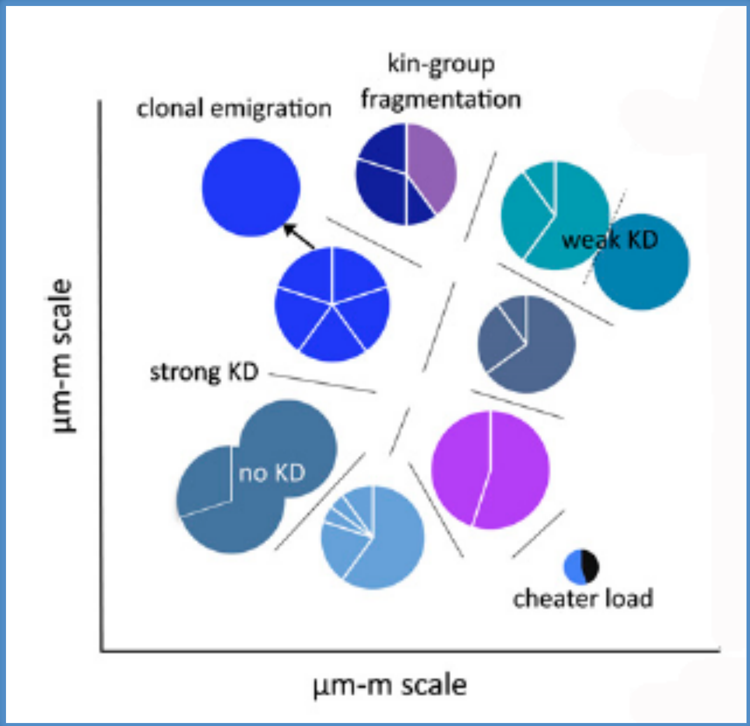
Clone A66

Clone A9

} Delayed development like OC

Kraemer *et al.* (2010)

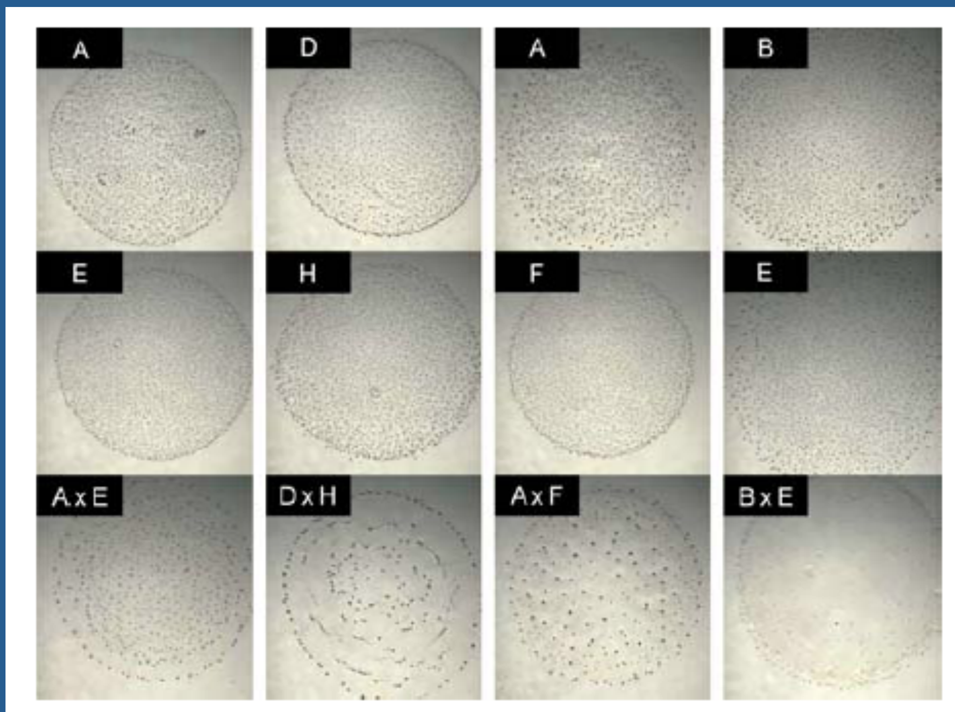
What are population dynamics?



- Do obligate cheaters build up?
- What is the role of kin discrimination?
- Do patches go extinct because of starvation / predation / environmental insult?

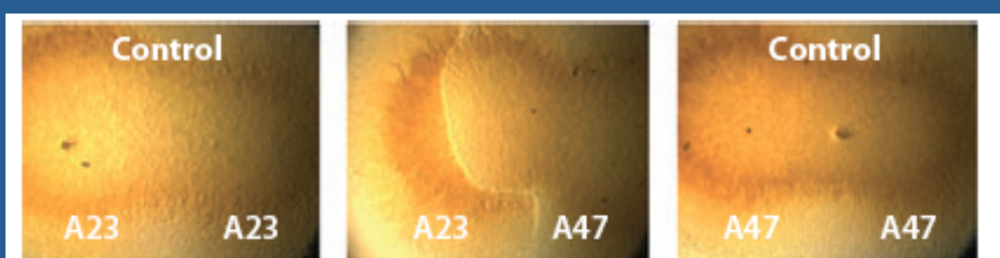
Kraemer & Velicer (2011)

Kin discrimination



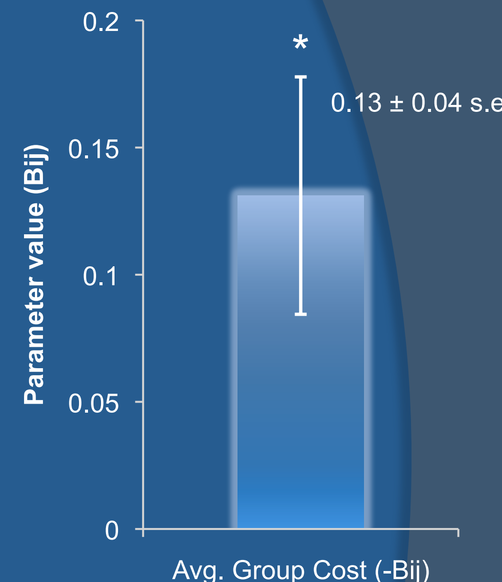
} Pure Clones

} Mixes



45 allorecognition types in 78 local isolates

Avg. group cost in co-occurring clones



*Two-tailed t-test
P < 0.02

Not diff. from Normal
Shapiro-Wilks p =0.78

Fiegna & Velicer (2005); Vos & Velicer (2009); Grosberg & Quinn (1988)

Origin of genetic polymorphism

R. Grosberg

J. Quinn

$$W_i = 1 + P_i\,(b_f - c_f)$$

Fitness of cue allele

Frequency cue allele

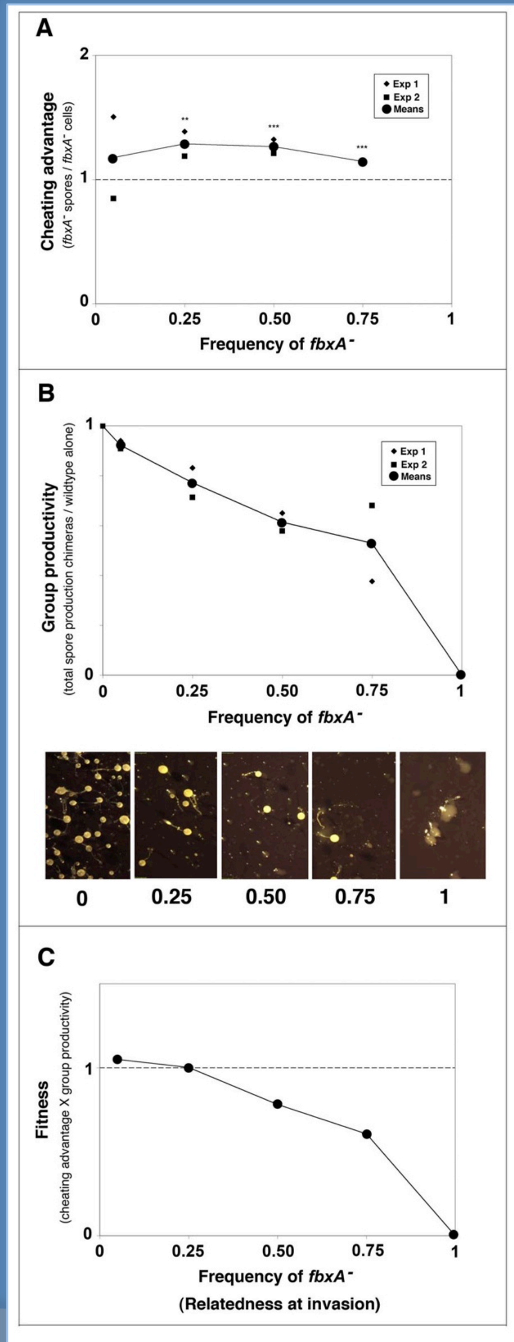
Benefit of fusion

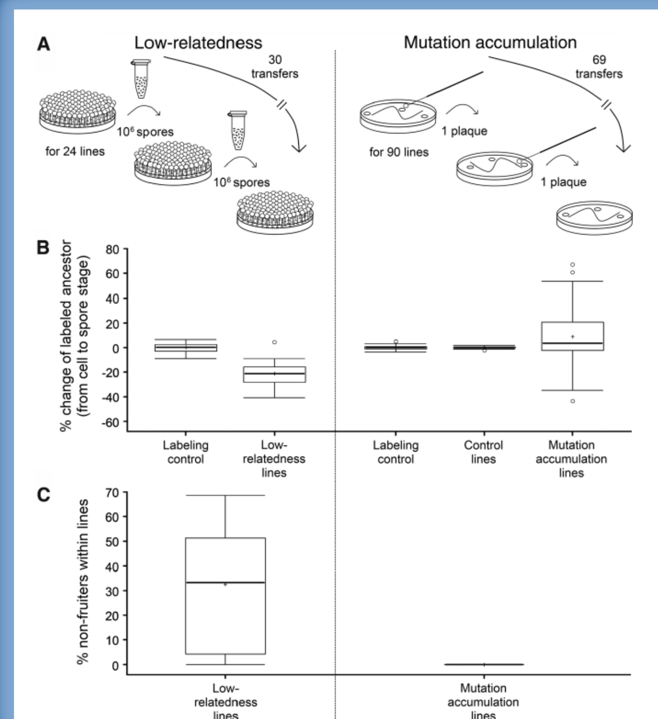
Cost of fusion

if $c_f > b_f$ then W_i increases with decreasing P_i

Grosberg & Quinn (1988) in *Invertebrate Historecognition;* Grosberg (1988) *Q. Rev. Biol.*

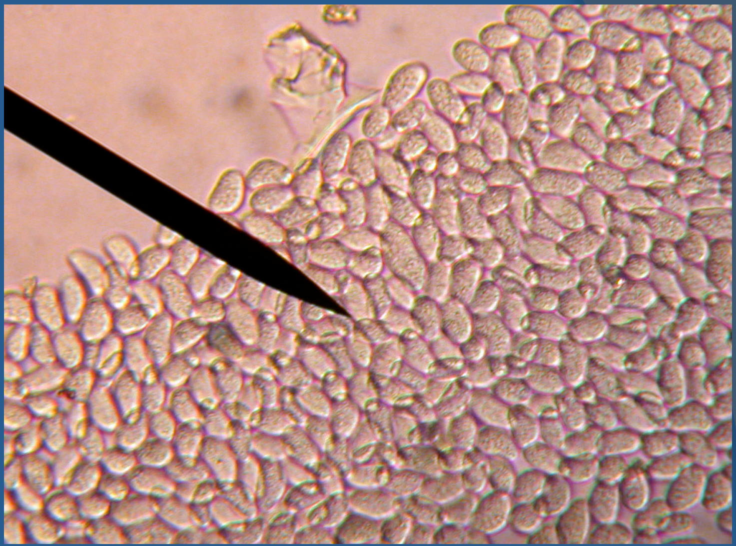
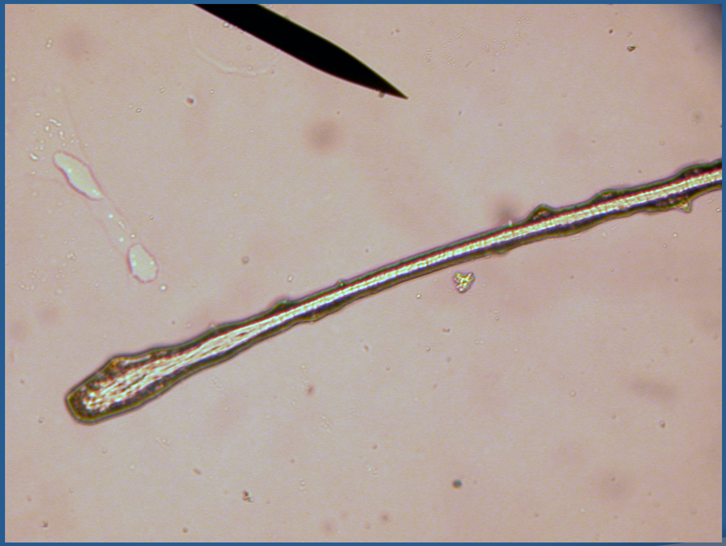
Dicty populations susceptible to obligate cheating under well-mixed conditions





Gilbert *et* al. (2007); Kuzdzal Fick *et al.* (2011)

What is relatedness in natural populations of *Dicty*?

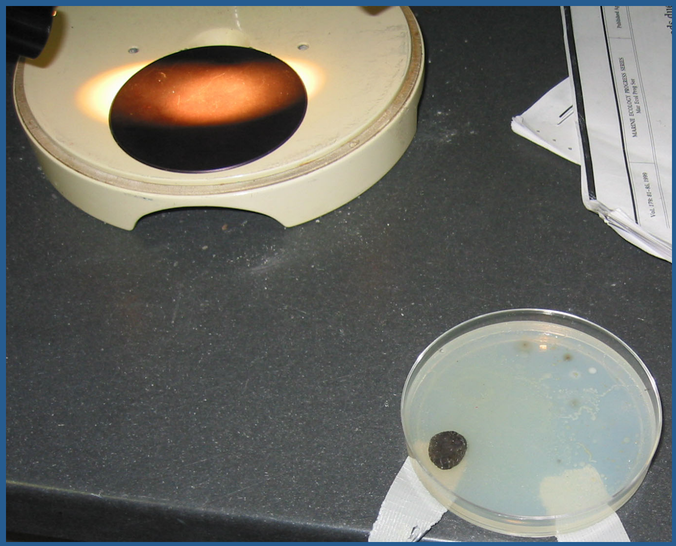




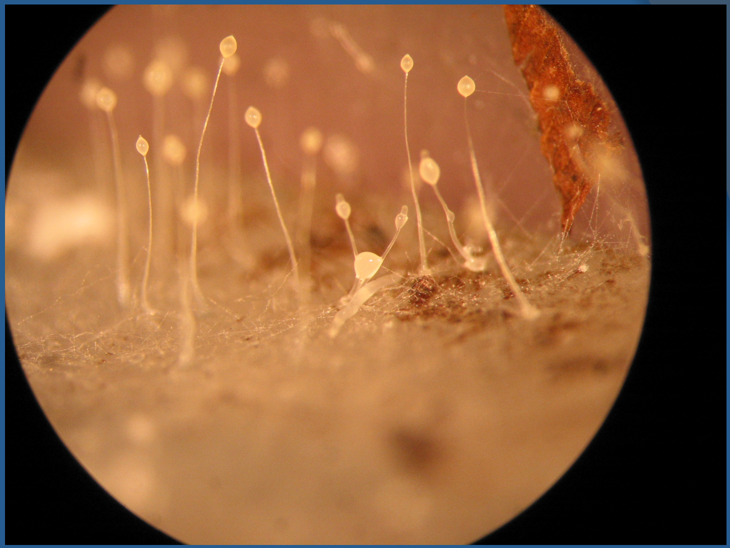


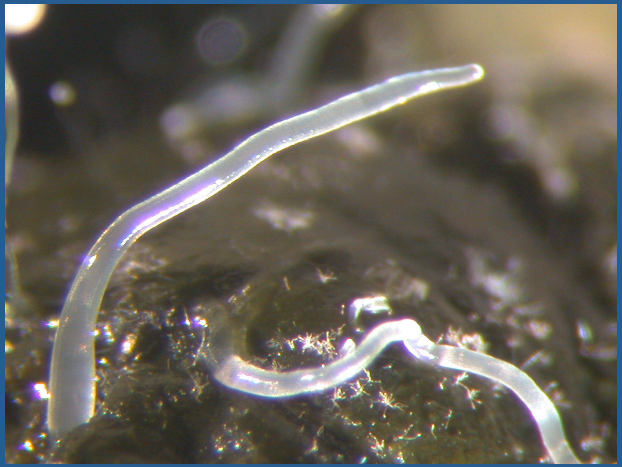
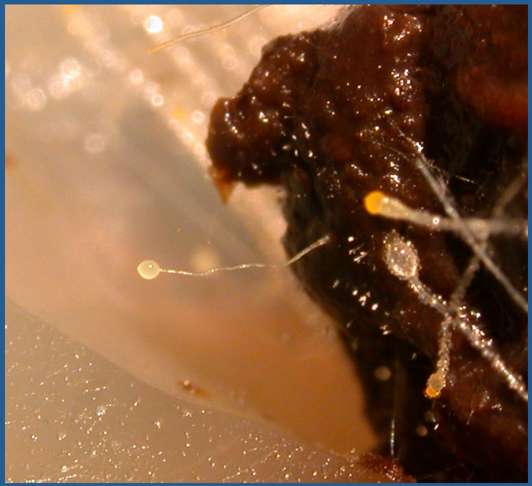
10 cm

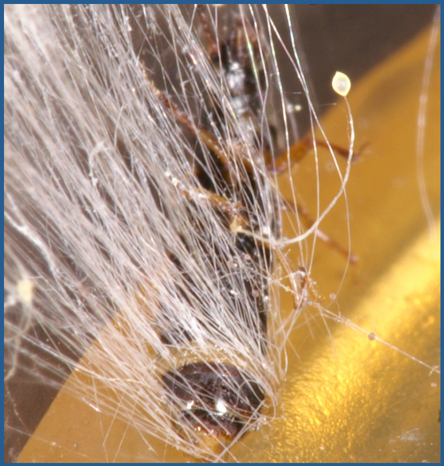

5 mm


1 mm

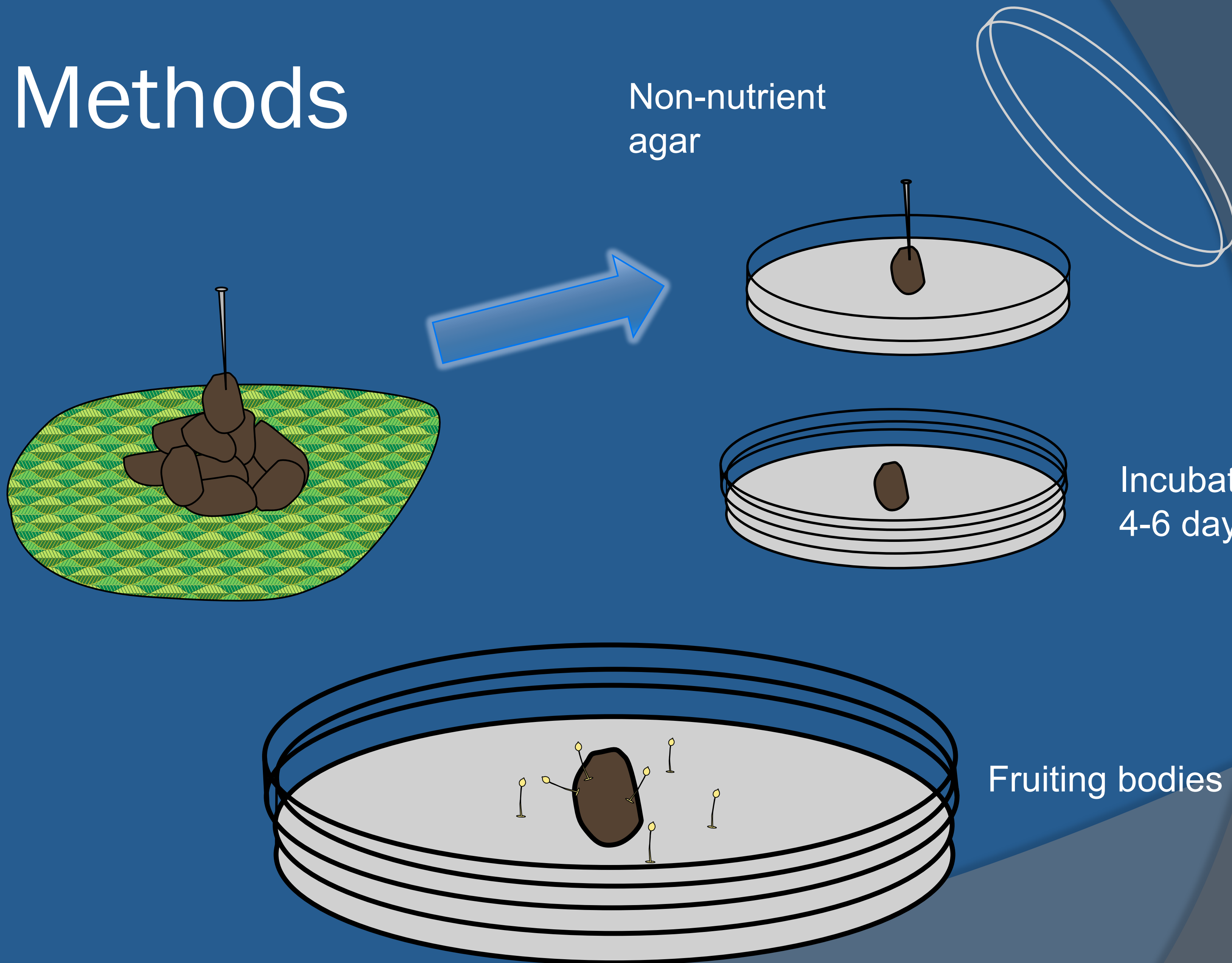




Methods



Microsatellite genotyping

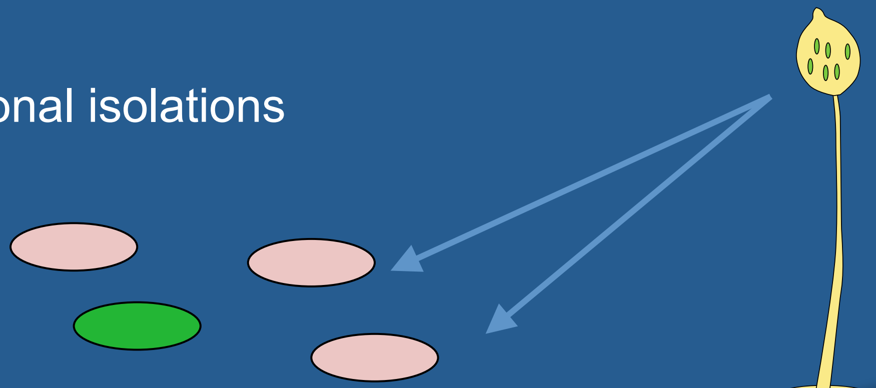
Microsatellites are DNA sequence repeats present in high copy number

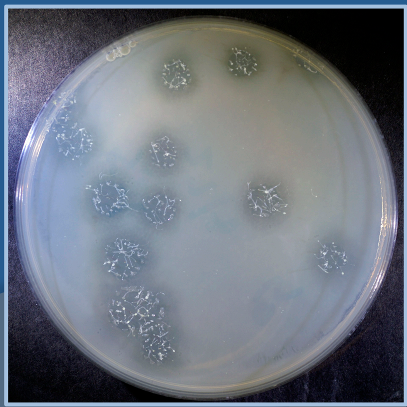
High mutation rates – good for distinguishing closely related individuals

Two Methods of Genotyping

1. Whole fruiting bodies

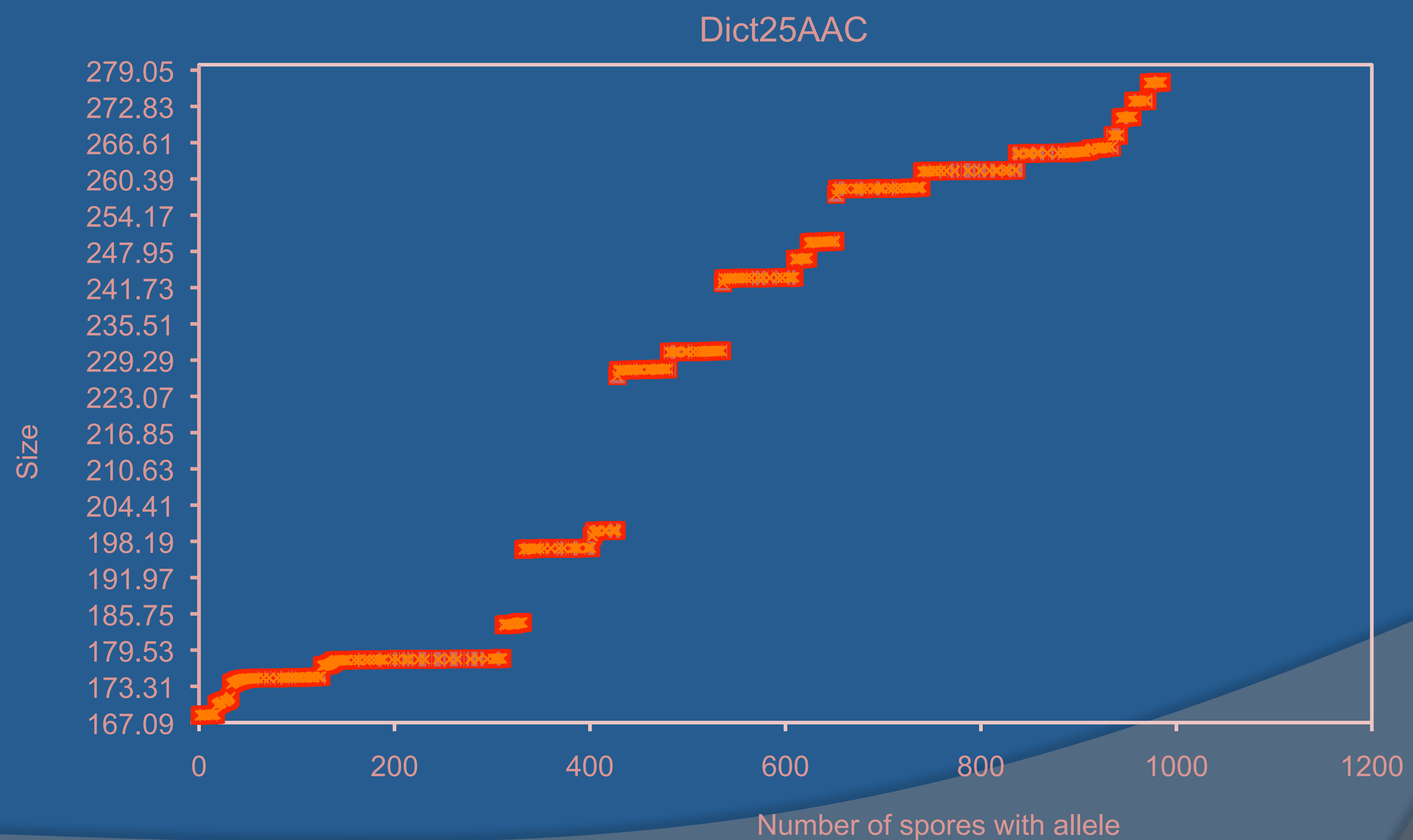
2. Clonal isolations



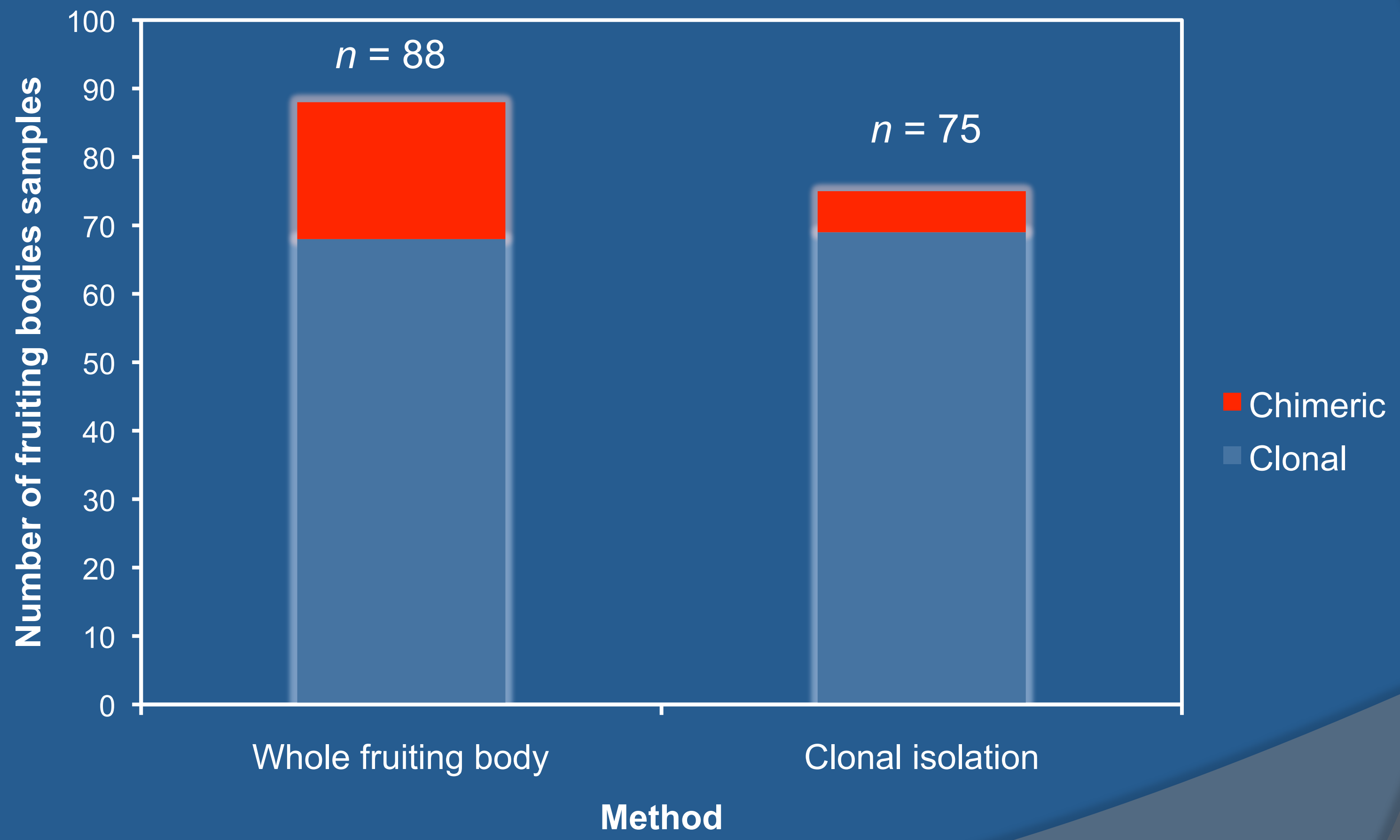


High variability of microsatellite loci

< 1 % probability of sharing 3 alleles by chance



Most fruiting bodies clonal



25 dung piles,
1 time of year

50 dung piles,
3 times of year

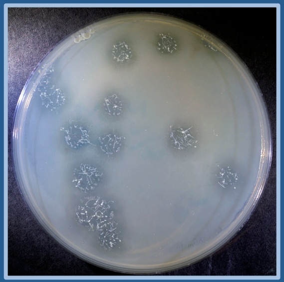
Gilbert *et al*. (2007)

Relatedness very high in nature

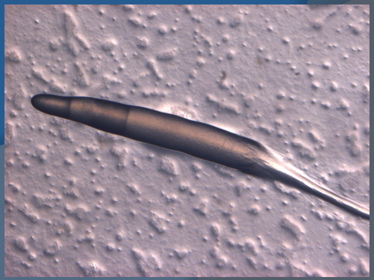
Whole fruiting bodies

0.86 ± 0.03 SE

Clonal isolations

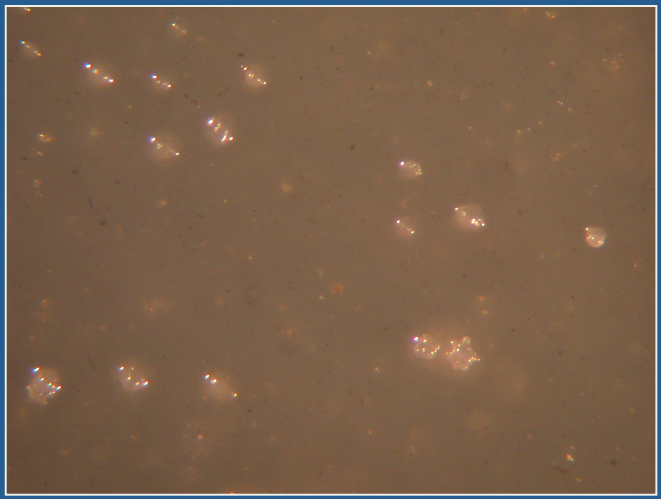


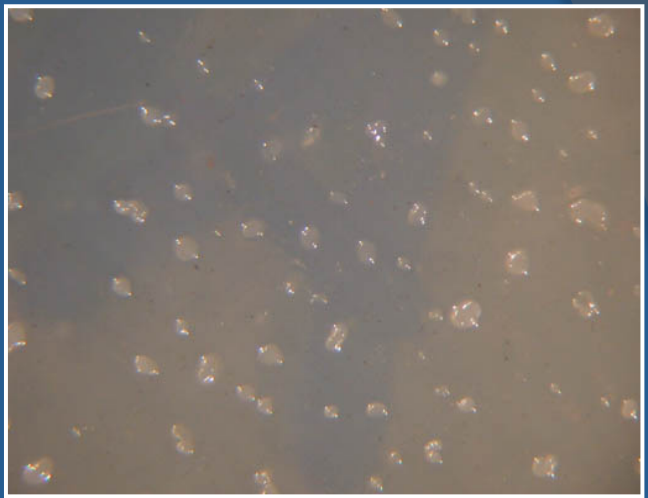
0.98 ± 0.01 SE*

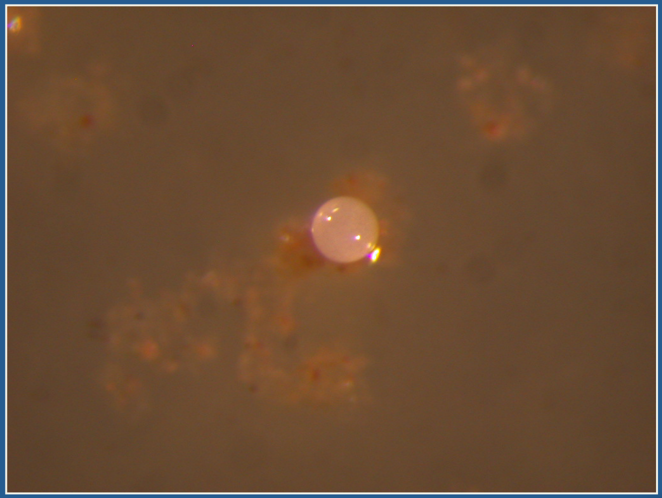


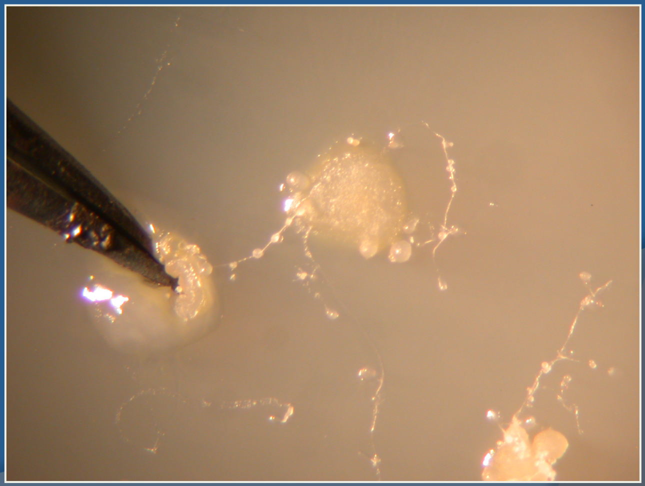
*measured using Relatedness 5.0 (Goodnight Software)

How to isolate cheaters in nature?





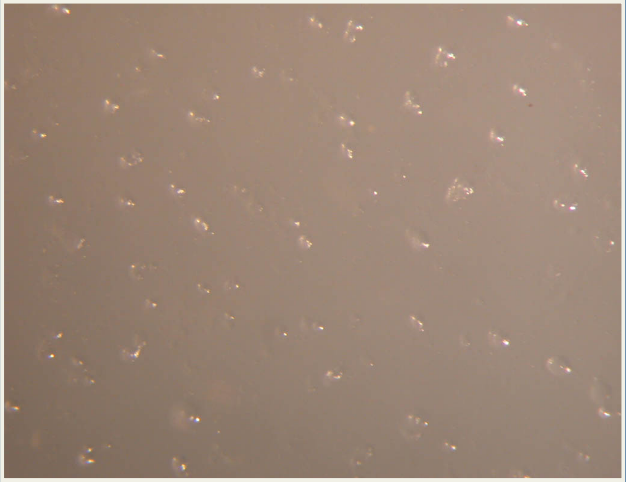




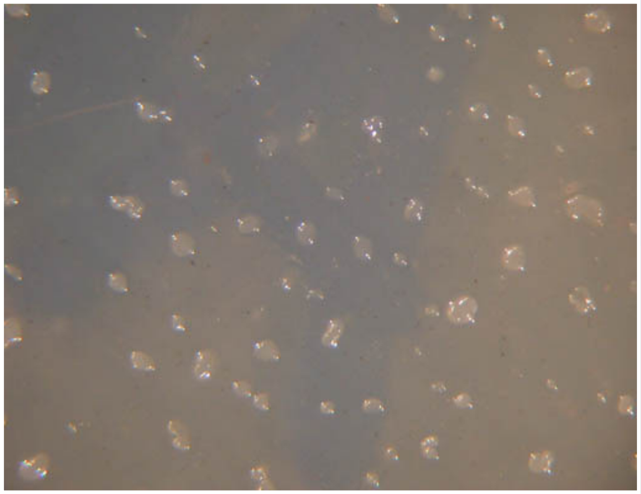
Normal methods do not see mutants

ChtA looks like primitive social amoeba

D. discoideum chtA

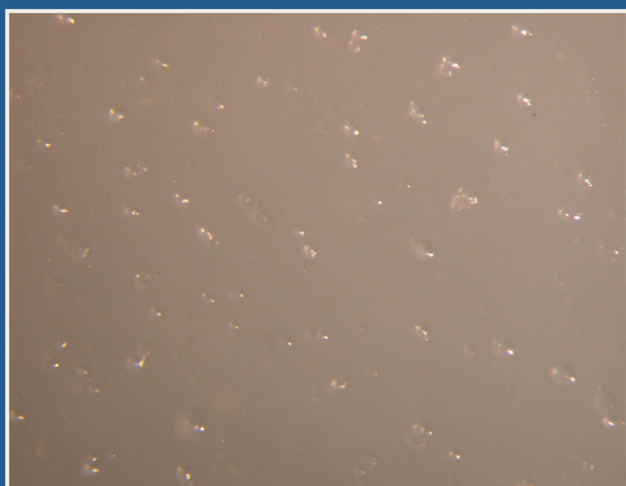


Guttulina sp.

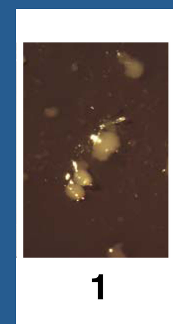


ChtA cannot be re-plated

D. discoideum chtA



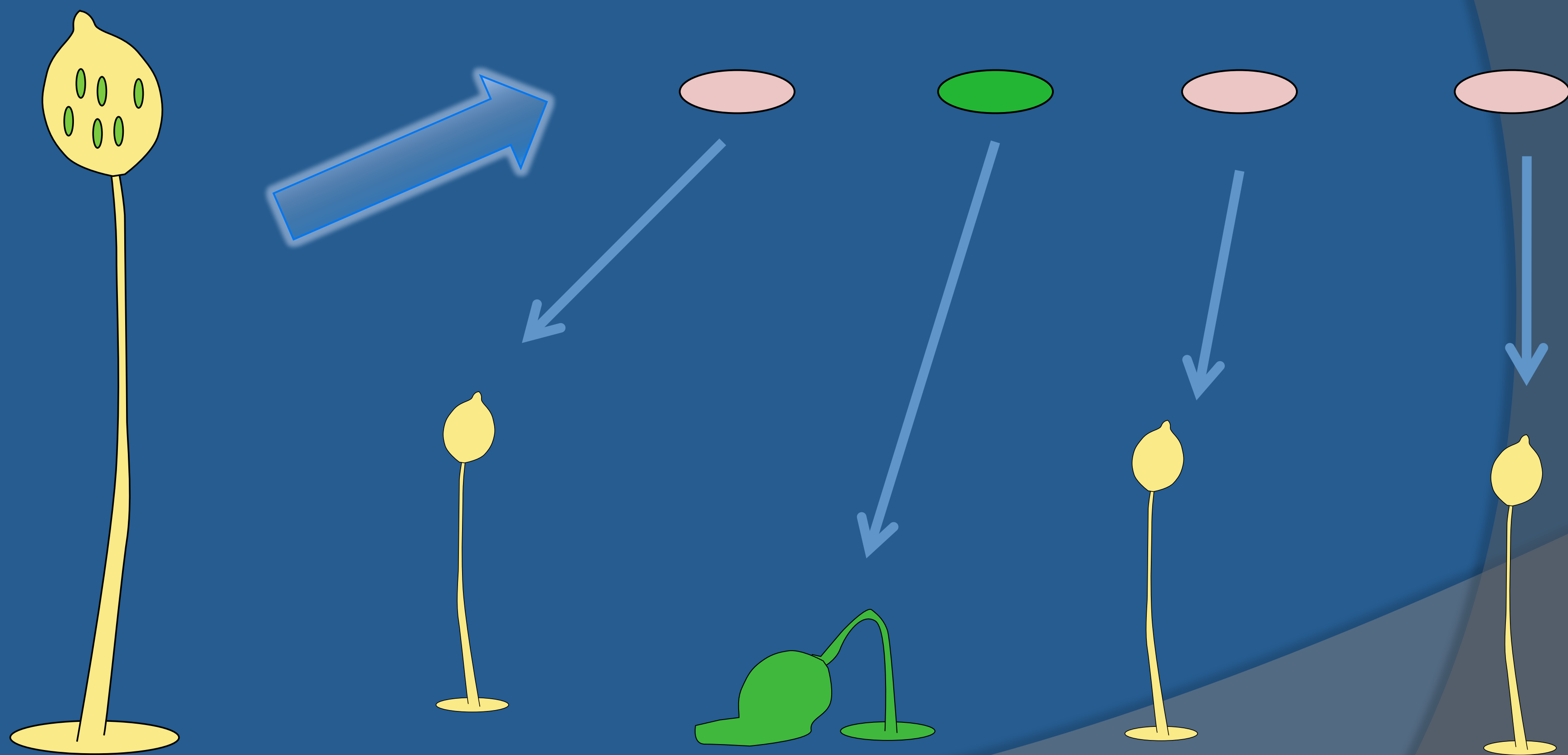
chtA



When clonal does not sporulate

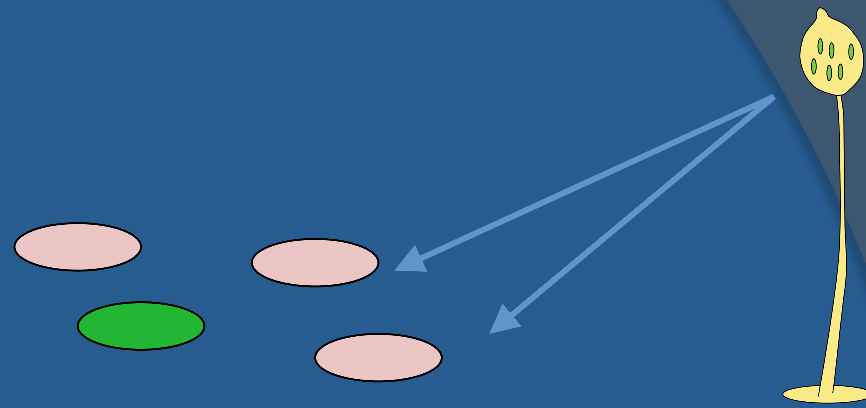
Ennis et al. (2000) *P. Natl. Acad. Sci. USA;* Gilbert et al. (2007) *P. Natl. Acad. Sci. USA*

How to look for cheater mutants

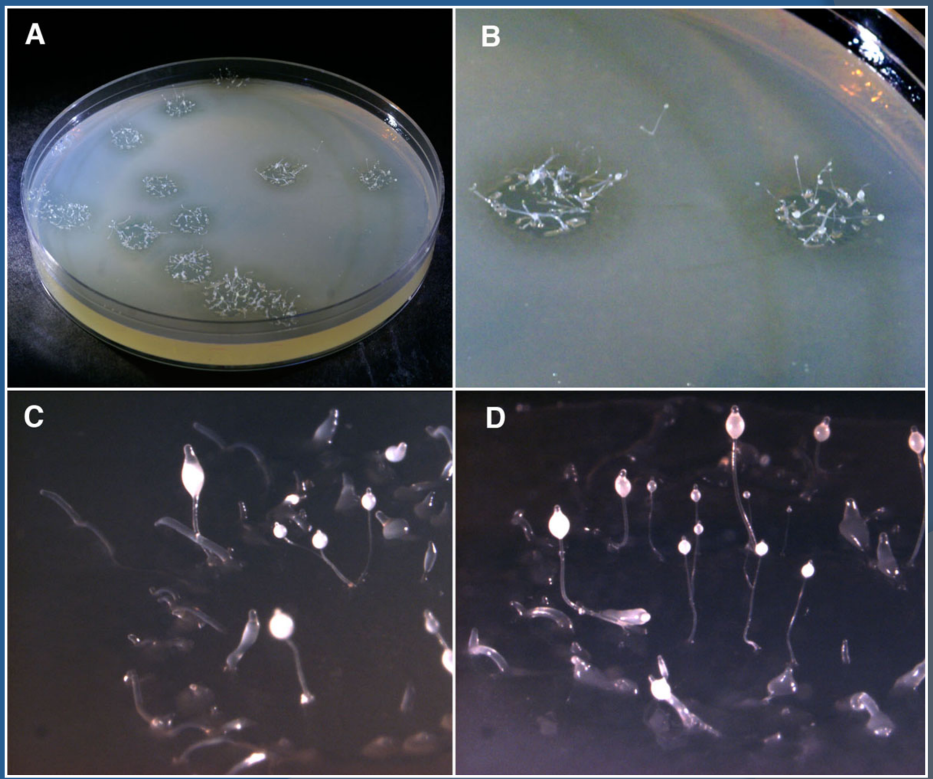


Gilbert *et al. PNAS* (2007)

Results



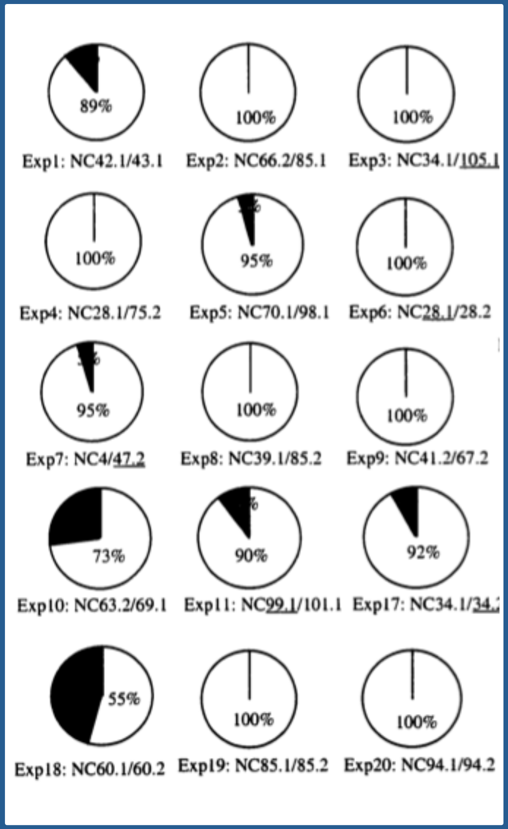
- 95 wild fruiting bodies.
- 63 locations.
- 4 times of year.
- 3316 spores.

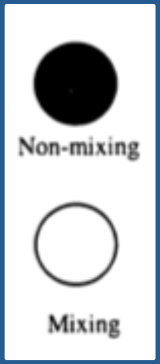


Gilbert *et al. PNAS* (2007)

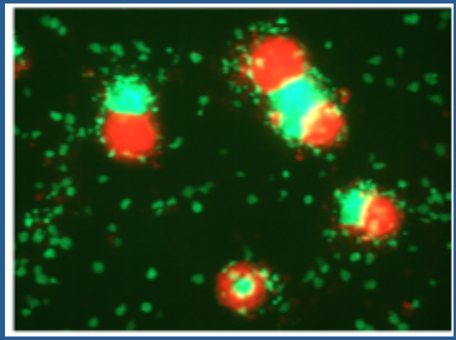
Does kin discrimination explain high relatedness in nature?

Unclear effect on relatedness

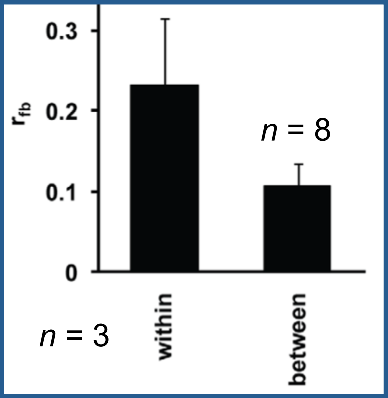




Clones from different geographic regions



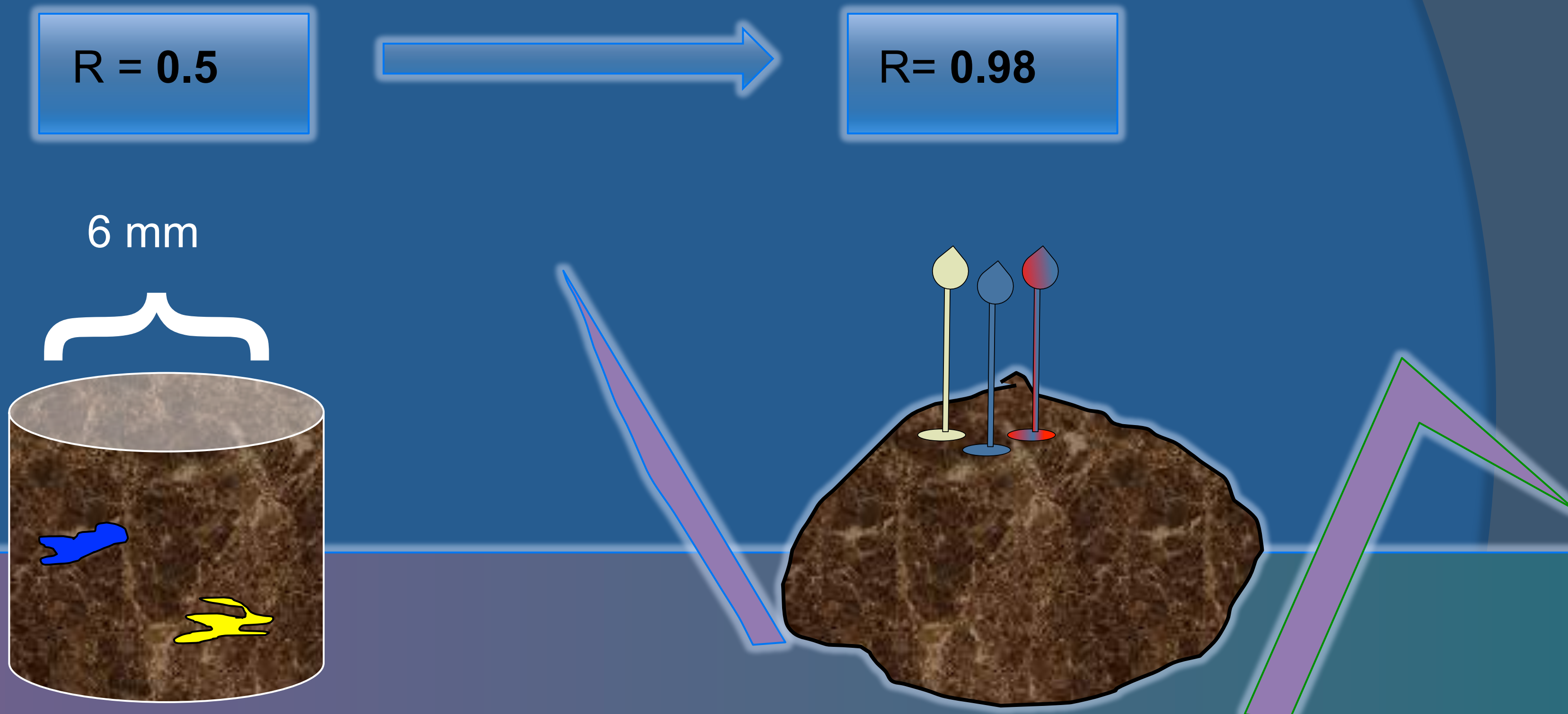
Ostrowski *et al.* (2008)



Flowers *et al.* (2010)

Strassmann *et al.* (2000) *Nature;* Ostrowski *et al.* (2008) *PLoS Bio.;* Flowers *et al.* (2010) *PLoS Gen.*

Hypothesized role for kin discrimination



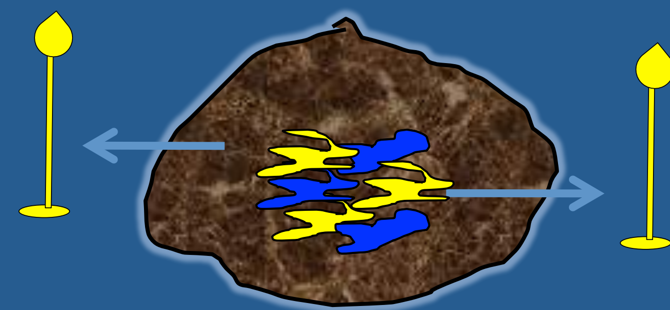
6-mm scale
population structure

Actual fruiting bodies

Fortunato *et al.* (2003); Gilbert *et al.* (2007) *PNAS*

Possible factors

Within 6-mm pop. structure



Competition / domination

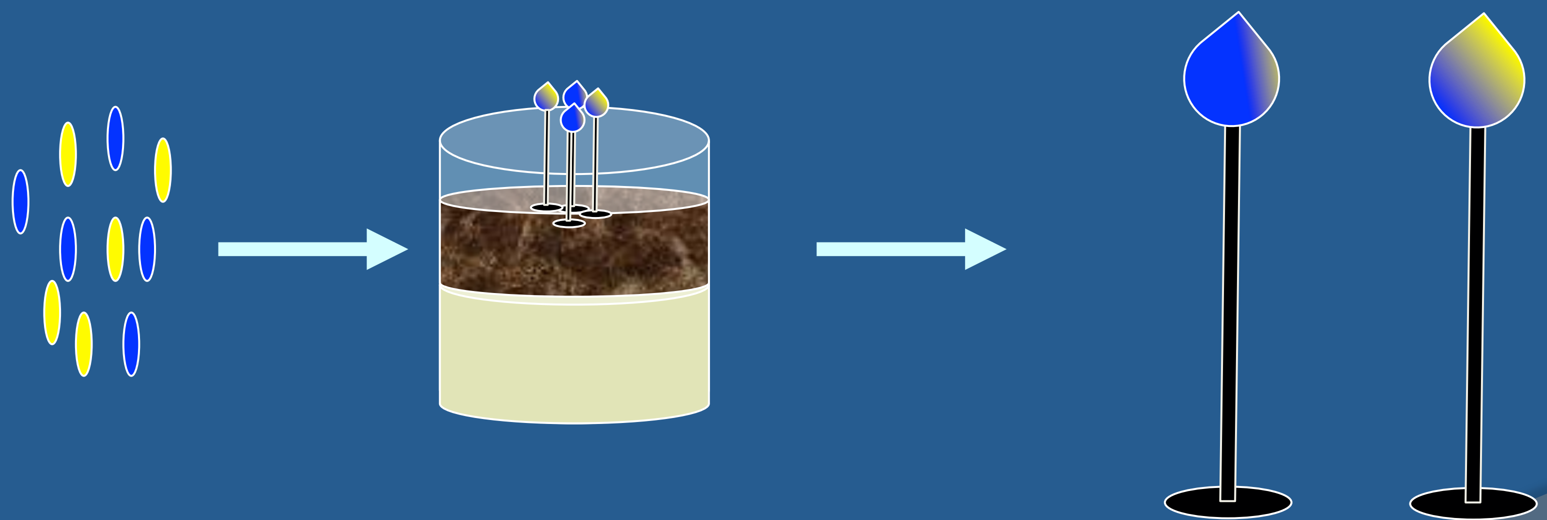
Kin discrimination / segregation

Gilbert *et al.* (2012)

Protocol

Mix Spores at High Density

Genotype Individual Fruiting Bodies



50:50

Gilbert *et al.* (2012)

Methods

- 18 pairwise mixes
- 3 independent trials
- 16 fruiting bodies per mix per trial
- 1 control experiment
- 1047 fbs genotyped (14.8 ± 0.02 fbs per mix)

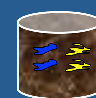
Gilbert *et al.* (2012)

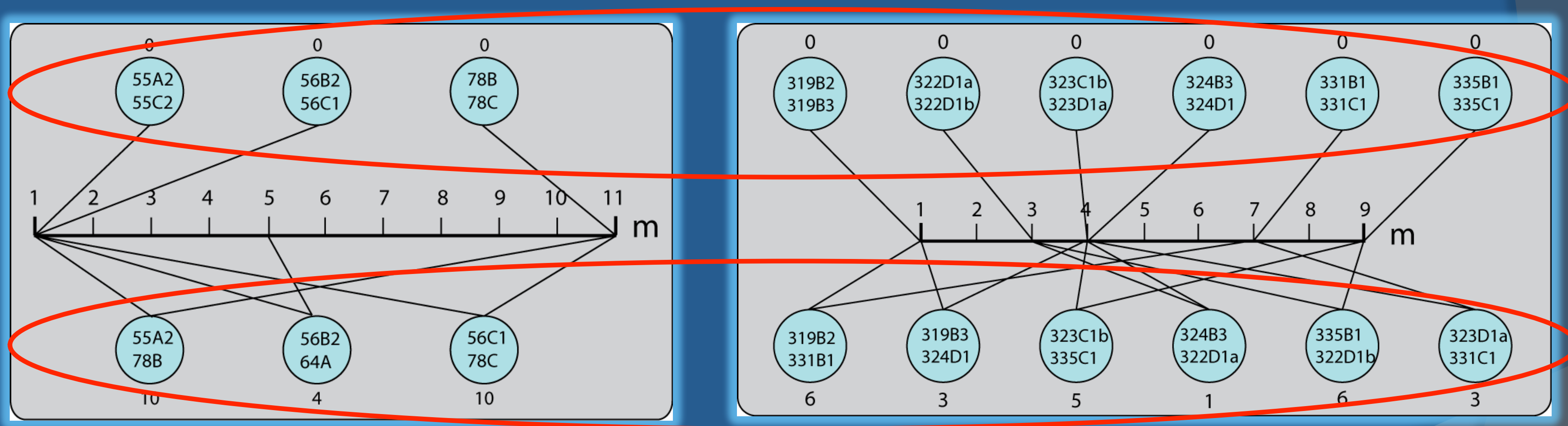
Clones used from Bald Knob, VA

Transect 1 : Sep 25, 2000

Transect 2: Oct 15, 2000

mm-scale mixes





meter-scale mixes

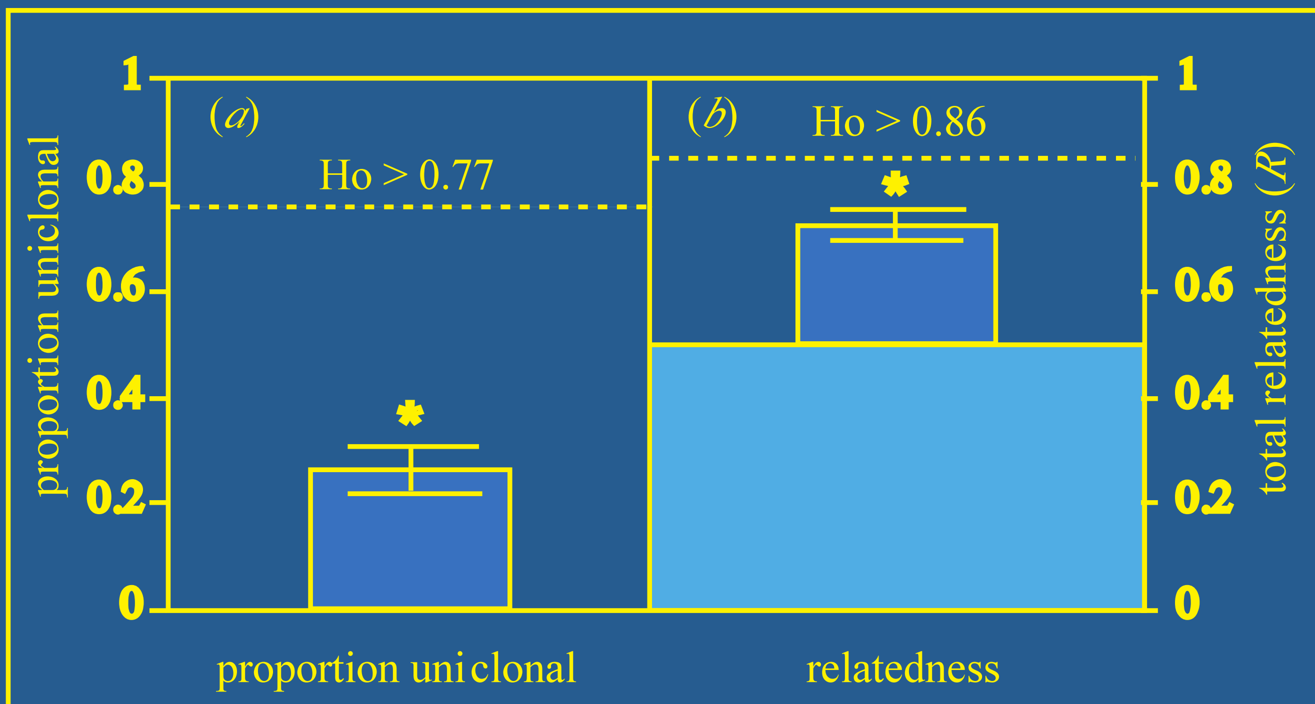
Relatedness between clones

Locus	Chromosome	No. of alleles here
Dict398a.AAT	1	9
Dict404.AAT	1	6
Dict25.AAC	1	9
Dict505e.AAT	2	6
Dict506e.AAT	2	9
Dict511.AAT	3	8
Dict513e.TAA	3	6
Dict518.AAT	3	7
Dict604.AAT	4	6
Dict13.CAT	4	7
Dict19.AAC	4	6
Dict406a.AAT	5	7
Dict414'.TTA	5	11
Dict414a'.TTA	5	4
Dict417.AAT	6	5
Dict418.TTA	6	6

- Clones genotyped for 16 polymorphic microsatellite loci
- All 6 chromosomes represented
- Relatedness estimated using *Relatedness 5.0.8*
- Avg. *R* not sig. different from zero.

Goodnight software; Queller & Goodnight (1989) *Evolution*

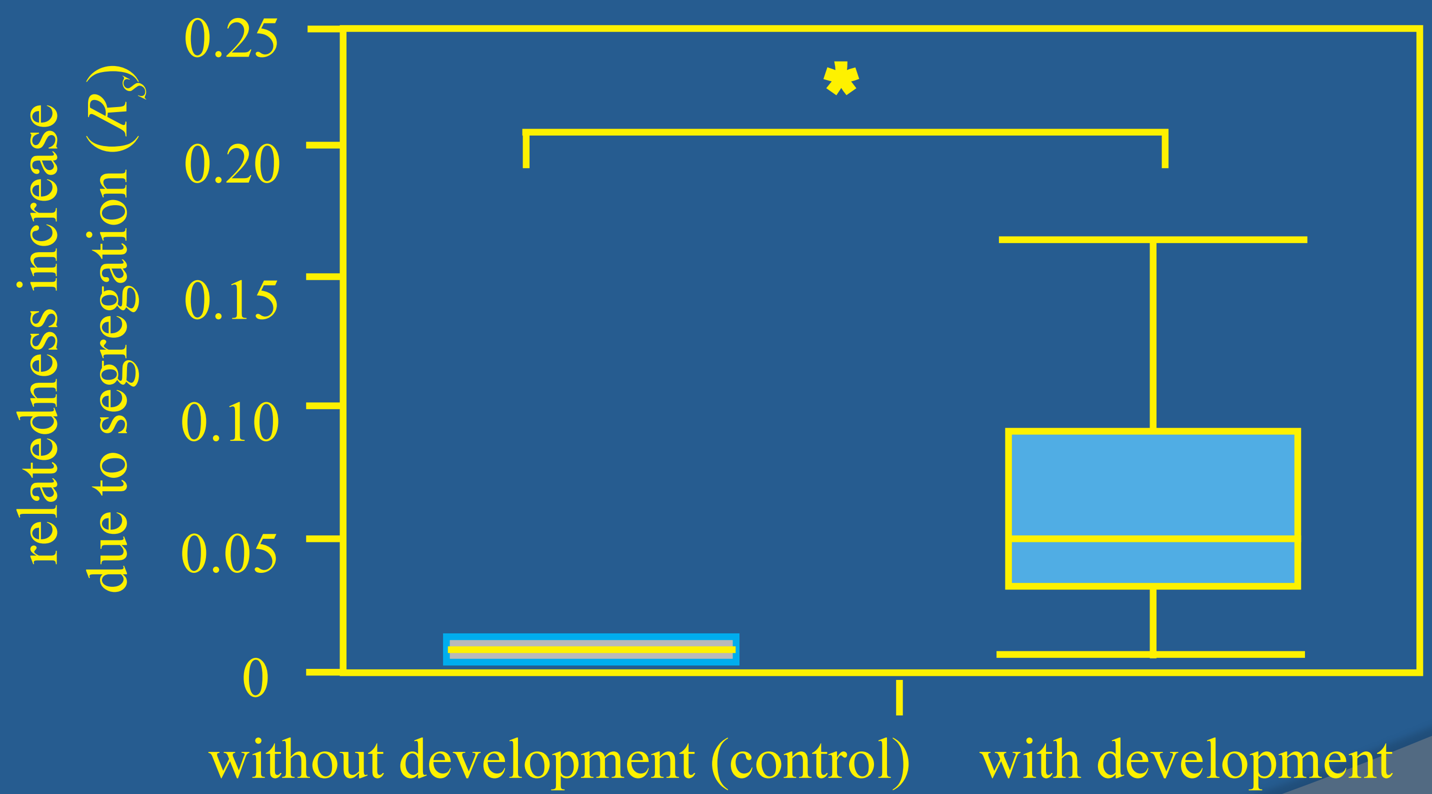
Results



*one-tailed *t*-test, p < 0.001; comparison to lower estimate from nature

Gilbert *et al.* (2012)

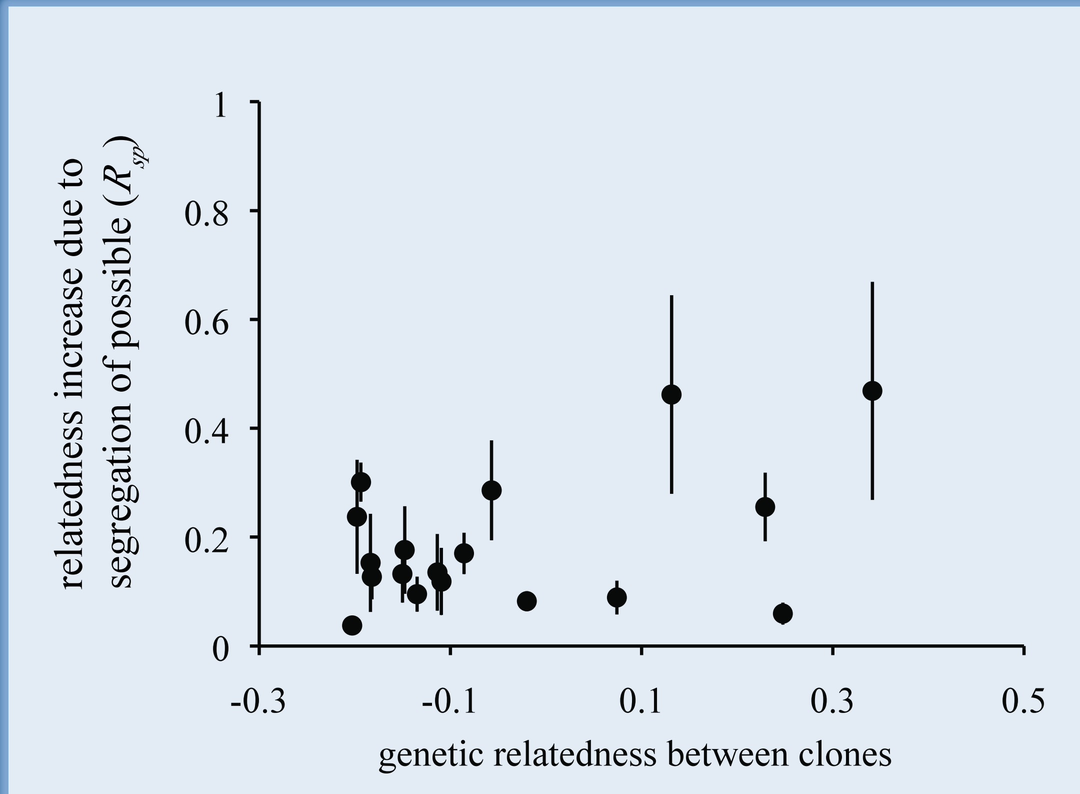
Control shows some segregation



* Wilcoxon rank-sum, $P < 0.0001$

Gilbert *et al.* (2012)

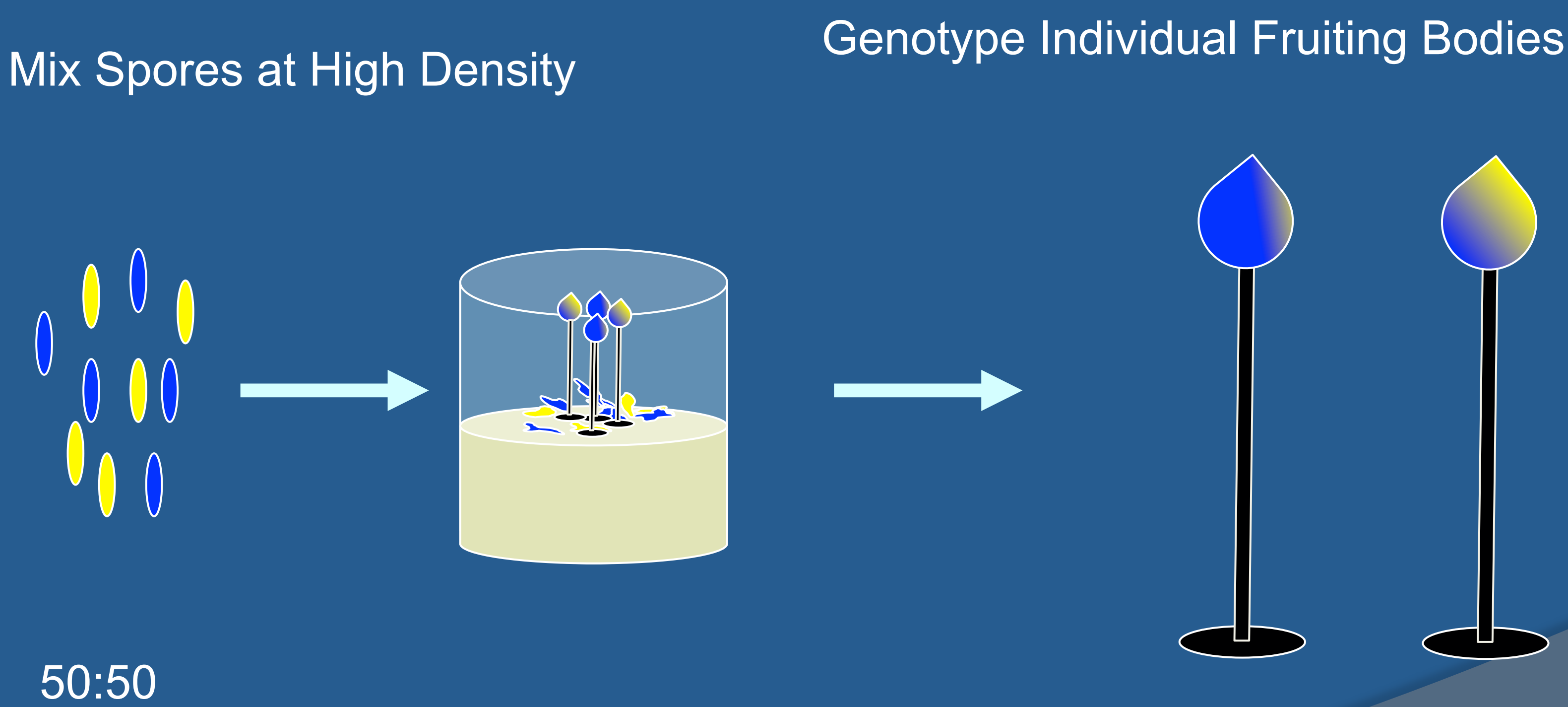
No correlation with *R*



ANCOVA: Effect of *R*, *P* = 0.22

Gilbert *et al.* (2012)

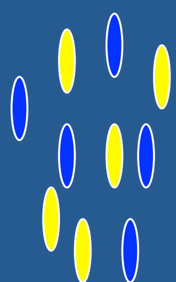
Control 1: Experiment also without soil on second day



n = 18 mixes

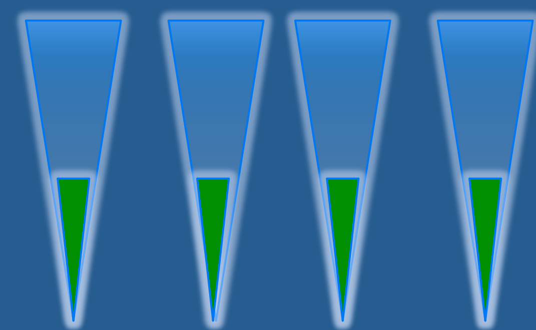
Control 2: Genotyping method

Mix Spores at known proportions



05:95, 10:90, 25:75, 50:50, 75:25, 90:10, 95:05

Genotype 4 aliquots of each proportion

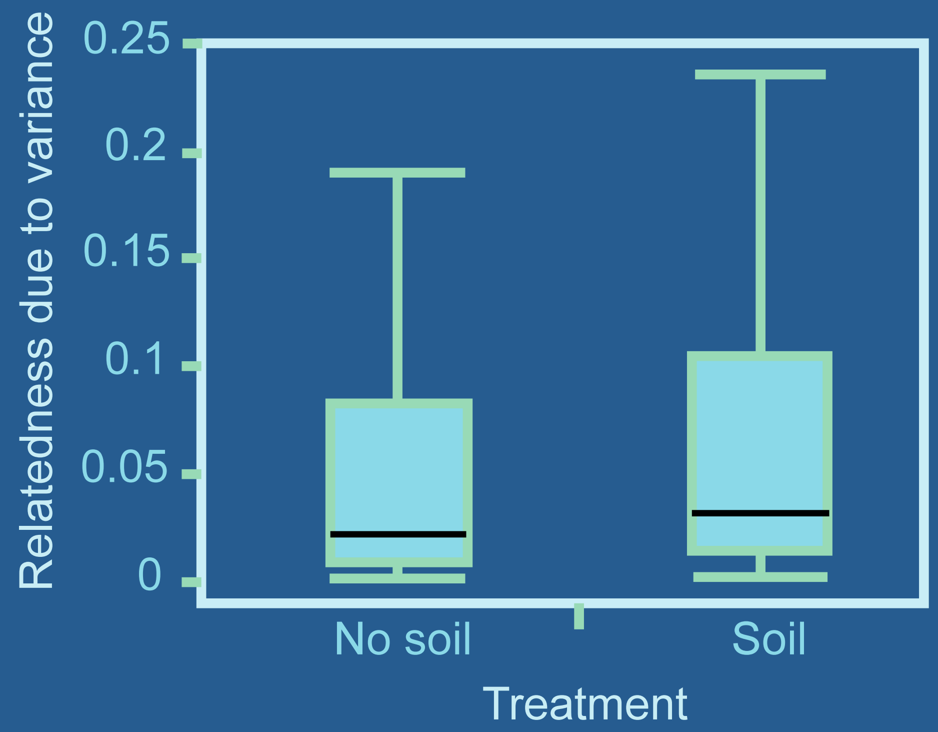


1a. Control for random variation between genotyping wells

1b. Measure sensitivity to rare clones

1c. Control for PCR bias

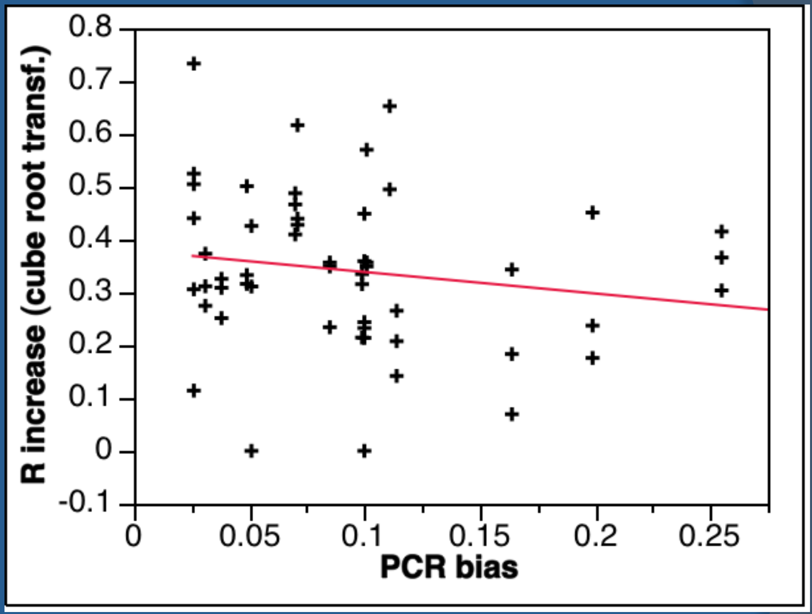
Control 1: Experiment without soil



**Ho not rejected, Wilcoxon test, *P* = 0.44, *n* = 17

Control 2: PCR bias

- Rare clone detectable @ 5%
- PCR bias not predictor of relatedness increase due to variance



(R square= 0.027, P = 0.24)

Overall contribution of kin discrimination

Structure for talk

- Introduction to the problem.
- Review of means that it has been addressed.
- Future work.

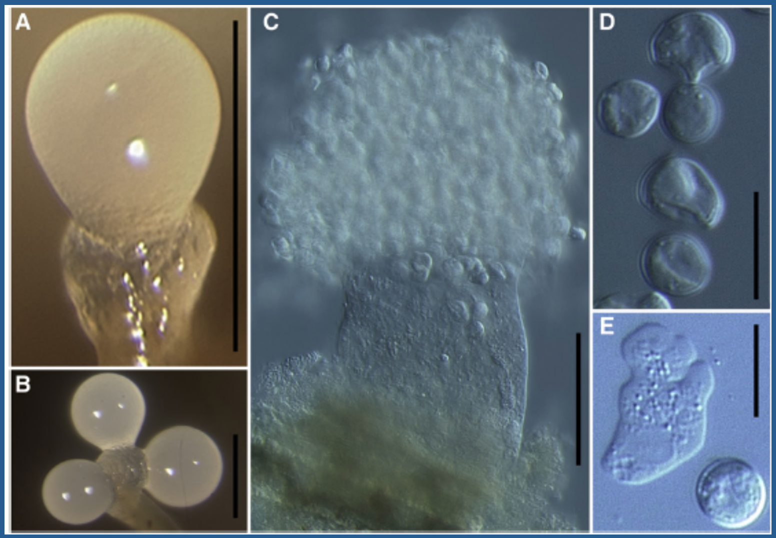
Structure for talk

- Introduction to the problem.
- Review of means that it has been addressed.
- Future work.

Questions

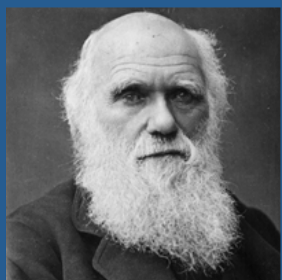
- What structures populations?
- How important is kin discrimination?
- How important are different types of cheating (obligate vs. facultative)?
- How do traits of microbes map to habitats and functions?

Much remains to be discovered



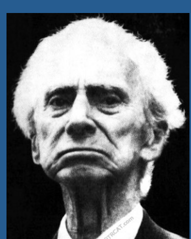
Brown *et al.* (2012)

Last thoughts



"Looking back, I think it was more difficult to see what the problems were than to solve them."

"We are apt to fall into the error of thinking that the facts are simple because simplicity is the goal of our quest."

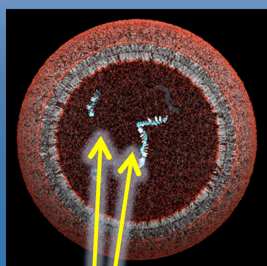


"When a man tells you that he knows the exact truth about anything, you are safe in inferring that he is an inexact man."

The Origin of Organisms

Cellular organisms

Protocell

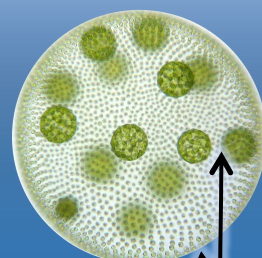


Credit: J. Iwasa

Cooperative replicators

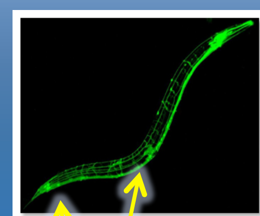
Multicellular organisms

V. carteri



Credit: A. Nedelcu

C. elegans

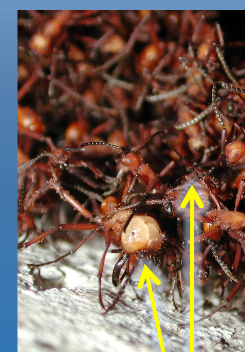


Credit: J. Bessereau

Cooperative cells

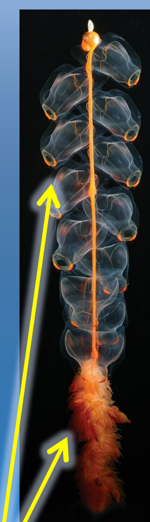
Colonial organisms

E. hamatum



Credit: L. Gilbert

Siphonophore



Credit: K. Raskoff

Cooperative Individuals

Thank You!

- Wray-Todd graduate fellowship
- NSF

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