

Fungal Diseases

SOME EXISTENTIAL QUESTIONS

- WHY ARE WE HERE?

FUNGI

- WHY ARE WE SO HOT?

FUNGI

- WHY DO WE EAT SO MUCH?

FUNGI

- WHY ARE MAMMALS THE DOMINANT LARGE ANIMALS?

FUNGI

Miasmas are still with us



People worry about disease from dead bodies after natural disasters

Germ Theory of Disease (1850-1880)



Critic

15
RUDOLF VIRCHOW
DEUTSCHE POST
BERLIN

alamy stock photo

'Germs seek their natural habitat in disease tissue'

Believed in a social cause to Infectious diseases



GERM THEORY OF DISEASE WAS A REVOLUTION IN THOUGHT

- CAUSAL ASSOCIATION BETWEEN CERTAIN MICROBES AND DISEASE
- PROVIDED ACTIONABLE INFORMATION TO REDUCE MORTALITY FROM INFECTIOUS DISEASES



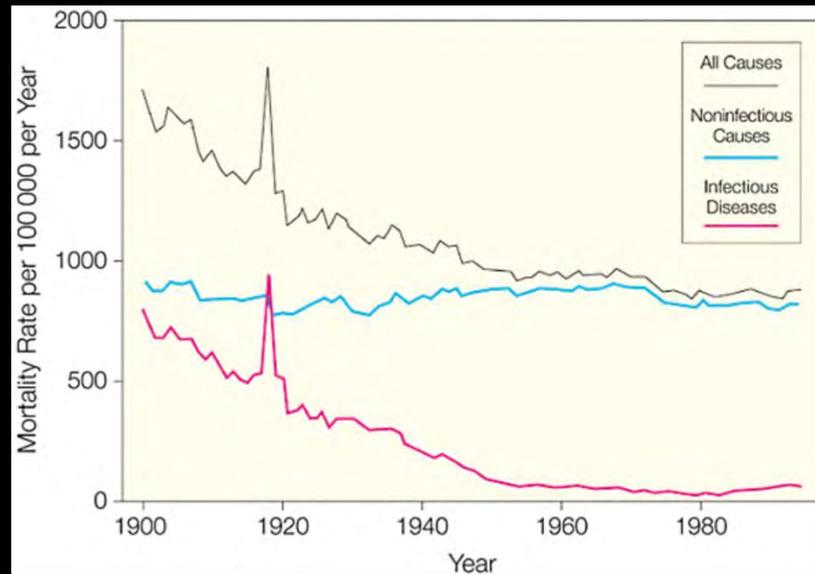
SANITATION



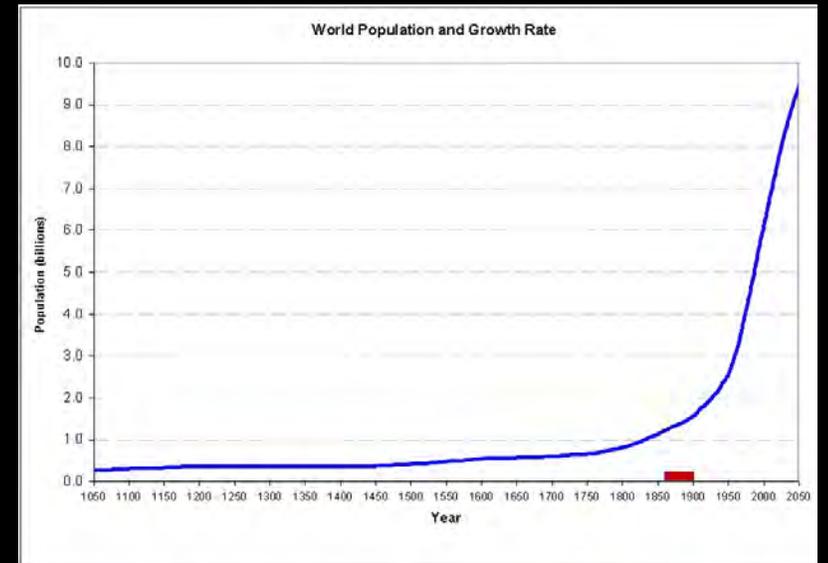
SERUM THERAPY



VACCINES



JAMA 1999;281(1):61-66



Forgotten facts: causality between germs and disease was established first with fungi



Agostino Bassi
Silkworm Disease



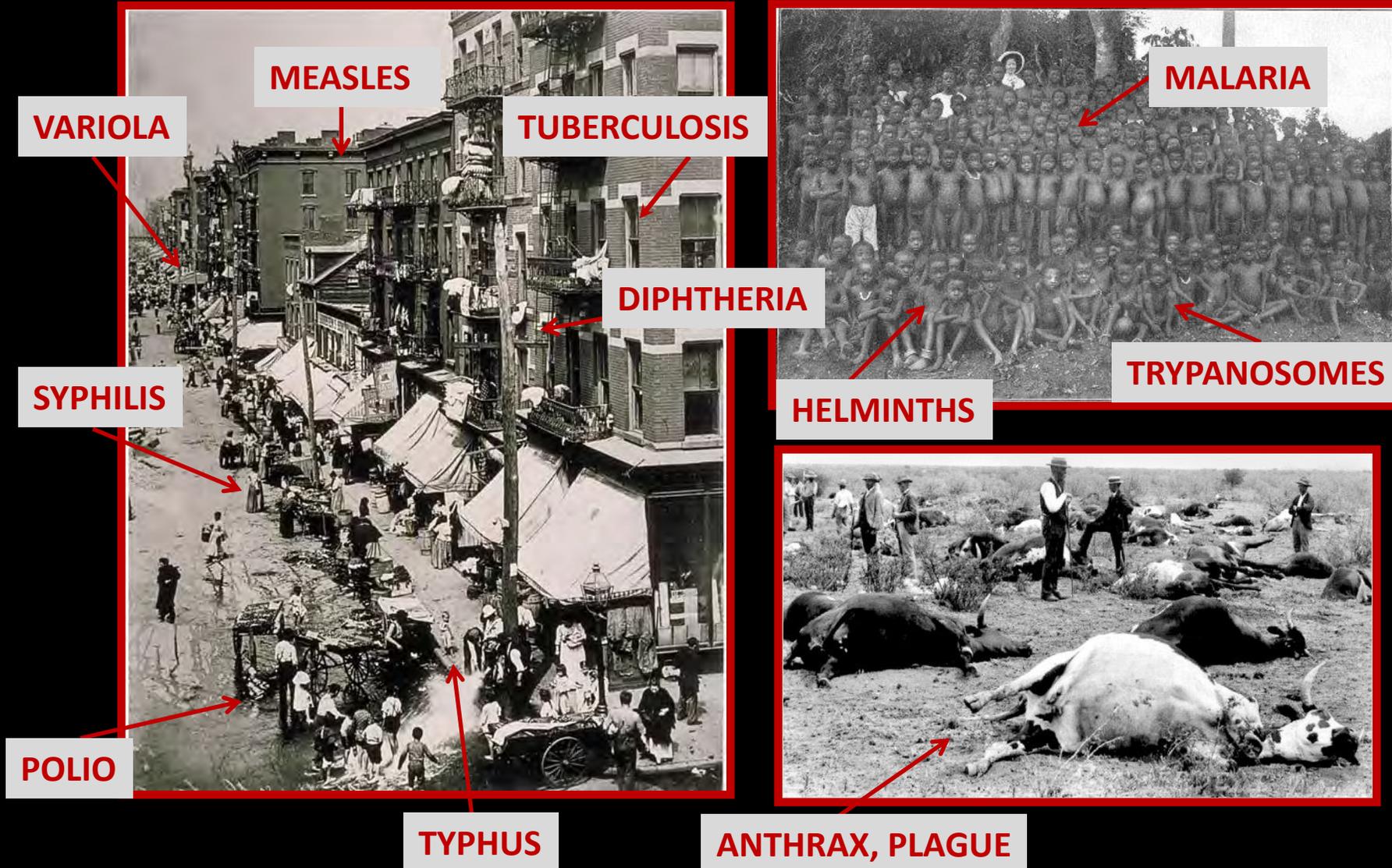
Beauveria bassiana



David Gruby
Ringworm

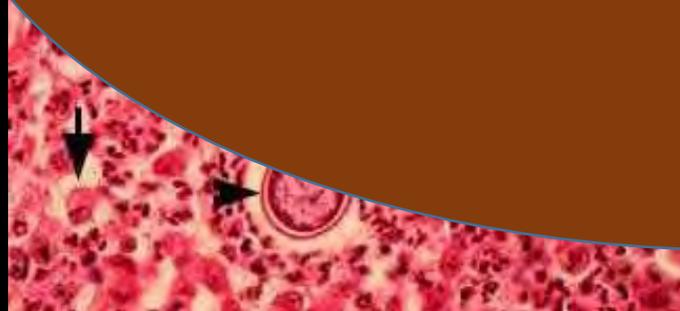


1899 *FIN DE SIECLE*: FUNGAL DISEASES EXTREMELY RARE

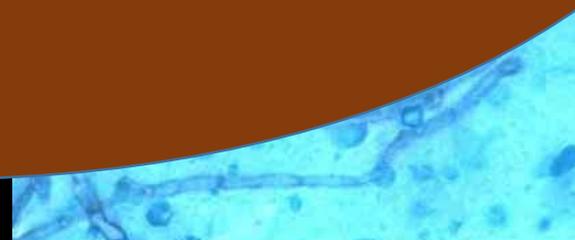


1999 *FIN DE SIECLE*: FUNGAL DISEASES DISTRESSINGLY COMMON

WHAT HAPPENED? HOSTS
CHANGED...MICROBE-FOCUSED VIEWS OF
MICROBIAL PATHOGENESIS COULD NOT
COPE WITH THE CHANGE



Coccidioides immitis



Aspergillus spp.

Table 1. Statistics of the 10 most significant invasive fungal infections.

Disease (most common species)	Location	Estimated life-threatening infections/ year at that location*	Mortality rates (% in infected populations)*
Fungal Diseases are: Not Reportable Underestimated Poorly studied Neglected Not Priorities WHY????			
Opportunistic invasive mycoses			
Aspergillosis (<i>Aspergillus fumigatus</i>)			30–95
Candidiasis (<i>Candida albicans</i>)			46–75
Cryptococcosis (<i>Cryptococcus neoformans</i>)			20–70
Mucormycosis (<i>Rhizopus oryzae</i>)			30–90
Pneumocystis (<i>Pneumocystis jirovecii</i>)			20–80
Endemic dimorphic mycoses*†			
Blastomycosis (<i>Blastomyces dermatitidis</i>)			<2–68
Coccidioidomycosis (<i>Coccidioides immitis</i>)			<1–70
Histoplasmosis (<i>Histoplasma capsulatum</i>)			28–50
Paracoccidioidomycosis (<i>Paracoccidioides brasiliensis</i>)			5–27
Penicilliosis (<i>Penicillium marneffeii</i>)			2–75

*Most of these figures are estimates based on available data, and the logic behind these estimates can be found in the text and in the Supplementary Materials. †Endemic dimorphic mycoses can occur at many locations throughout the world. However, data for most of those locations are severely limited. For these mycoses, we have estimated the infections per year and the mortality at a specific location, where the most data are available.

The late 20th Century Crisis

1900: Two types of microbes: pathogens & non-pathogens **(fungal diseases rare)**

1950: 'Non-pathogens' begin to be associated with disease in setting of steroid use, antimicrobial use and early chemotherapy **(fungal diseases emerge)**

1970: Concept of 'opportunism' emerges to explain 'non-pathogen' causing disease **(fungal diseases seen as opportunistic)**

1990: 'non-pathogens' have become pathogens **(fungal diseases common).**

Microbe-centric theories of pathogenesis cannot explain changes

Crisis leads to a messy lexicon - examples

Candida albicans

'commensal' in asymptomatic humans

'primary pathogen' in vaginal candidiasis

'opportunistic pathogen' in AIDS

Pneumococcus

'colonizer' in asymptomatic humans

'primary pathogen' in elderly pneumonia

'opportunistic pathogen' in AIDS pneumonia

Aspergillus spp.

'saprophyte' in asymptomatic humans

'primary pathogen' in allergic aspergillosis

'opportunistic pathogen' in transplant patients

1995-1999

Liise-anne Pirofski and I are asked to create a new graduate course at the Albert Einstein College of Medicine on Microbial Pathogenesis

Designing a course on an overarching concept of microbial virulence was impossible since none was available

Began to experiment with various concepts, which led to many discussions.

By 1999 class notes had converged into a new framework and we realized that we had something new.

1999 – First Publication

INFECTION AND IMMUNITY, Aug. 1999, p. 3703–3713
0019-9567/99/\$04.00+0
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MINIREVIEW

Host-Pathogen Interactions: Redefining the Basic Concepts of Virulence and Pathogenicity

ARTURO CASADEVALL* AND LIISE-ANNE PIROFSKI

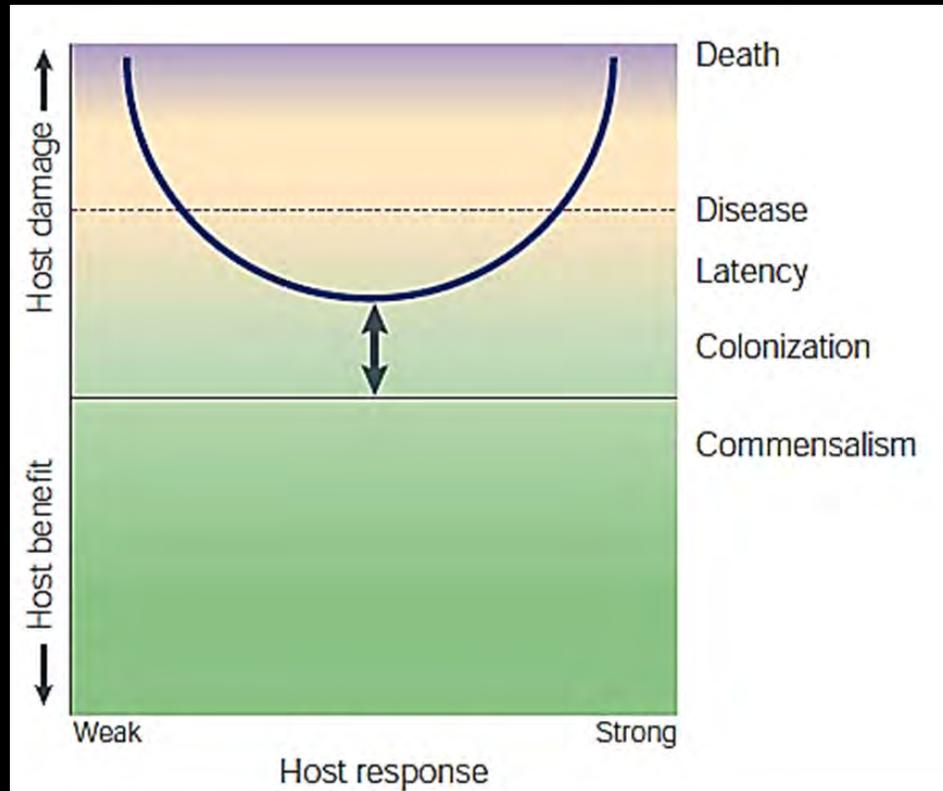
*Division of Infectious Diseases, Department of Medicine, and Department of Microbiology and Immunology,
Albert Einstein College of Medicine, Bronx, New York 10461*

Thought and Theory are not highly valued in current biological sciences. Only way to get this published was to hide it inside a review

Lexicon of microbial pathogenesis was a mess

Virulence	Degree of pathogenicity (33, 34)	The relative capacity of a microbe to cause damage in a host
	Virulence \propto 1/resistance (8)	
	Strength of the pathogenic activity (12)	
	Relative capacity to overcome available defenses (31)	
	Disease severity as assessed by reductions in host fitness following infection (24)	
	Percent of death per infection (7)	
	A synonym for pathogenicity (34)	
	Property of invasive power (35)	
	Measure of the capacity of a microorganism to infect or damage a host (21)	
	Relative capacity to enter and multiply in a given host (29)	

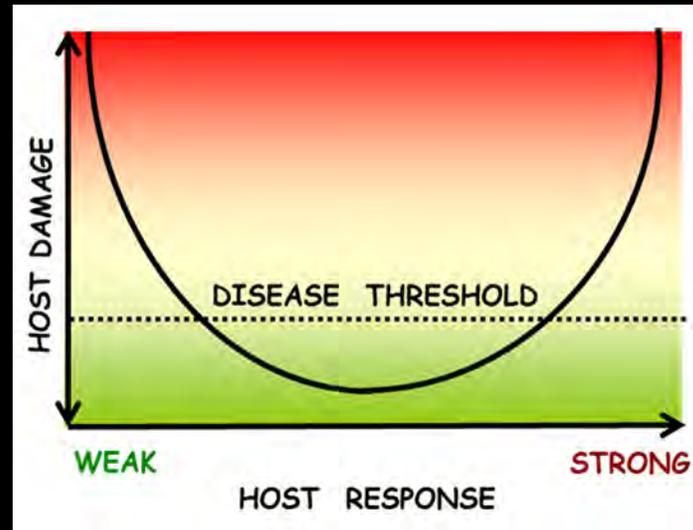
The 'Damage-Response Framework' of Microbial Pathogenesis



- Disease is (only) *one outcome* of an *interaction* between a *microbe* and a *susceptible host*.
- The relevant measure of host-microbe interaction is *host damage* due to host or microbial factors, or both.
- Microbes are defined by their *state in a host*, not by their traits or what they 'do'.

Fungal Diseases occur at both horns of the parabola

Cryptococcosis
Disseminated aspergillosis
Disseminated Candidiasis



Fungal asthma
Allergic pulmonary aspergillosis
Vaginal candidiasis

**DISSEMINATED
HISTOPLASMOSIS**



H. capsulatum



**MEDIASTINAL
FIBROSIS**

'Damage-Response Framework': Five Consequences

1. SHIFTS DEBATE FROM 'PATHOGENS' vs 'NON-PATHOGENS' TO OUTCOME OF THE INTERACTION
2. THERE ARE NO 'PATHOGENS', 'COMMENSALS', 'OPPPORTUNITISTS', 'SAPROPHYTES': THERE ARE ONLY *MICROBES AND HOSTS*
3. VIRULENCE IS ONE OUTCOME OF THE INTERACTION OF MICROBE AND HOSTS (OTHER OUTCOMES ARE COMMENSALISM, SYMBIOSIS, INDIFERENCE, ETC)
4. OUTCOMES OR STATES ARE CONTINUOUS ARE DIFFER ONLY IN THE AMOUNT OF DAMAGE INCURRED BY THE HOST
5. SEES VIRULENCE AS AN EMERGENT PROPERTY

WHAT IS A HOST?



A HOST IS AN ENTITY THAT HOUSES AN ASSOCIATED MICROBITA/MICROBIOME AND INTERACTS WITH MICROBES SUCH THAT THE OUTCOME RESULTS IN DAMAGE, BENEFIT OR INDIFFERENCE THUS RESULTING IN THE STATES OF SYMBIOSIS, COLONIZATION, COMMENSALISM, LATENCY OR DISEASE



NOT A HOST



MINIREVIEW

What Is a Host? Incorporating the Microbiota into the Damage-Response Framework

Arturo Casadevall, Lilse-anne Pirofski

Departments of Microbiology and Immunology and Medicine (Division of Infectious Diseases), Albert Einstein College of Medicine, Bronx, New York, USA

COMMENT

CHEMISTRY Why did Goethe write a scandalous novel about bonding? **p.168**

FILM Biopic *The Fly Room* centres on the crucible of early genetics **p.169**

BIODIVERSITY Call for conflict-of-interest rules for panel-report authors **p.170**

CONSERVATION Manage military training grounds for environmental value too **p.170**

EYE OF SCIENCE/SPL



Could a mathematical formalism be developed that would account for both host + microbe that also illustrated the continuity of interactions?



The often harmless fungus *Aspergillus fumigatus* can cause severe pulmonary disease in people with leukaemia.

Ditch the term pathogen

Disease is as much about the host as it is the infectious agent — the focus on microbes is hindering research into treatments, say **Arturo Casadevall** and **Liise-anne Pirofski**.

PATHOGENIC POTENTIAL OF A MICROBE

- **NEW CONCEPT: THE 'PATHOGENIC POTENTIAL'**
- **DEVELOPED A FORMALISM TO QUANTIFY IT**
- **$PP=(F_s/I)(10^M)$**
- **F_s IS THE FRACTION SYMPTOMATIC, I IS THE INOCULUM AND M IS MORTALITY FRACTION**

The Pathogenic Potential of a Microbe

Arturo Casadevall

Microbe	Mortality	Innoculum	PP	Reference
Theoretical Max.	1.0	1	1.0×10^1	This work
<i>Francisella tularensis</i>	0.5	1-2	2.5×10^0	(13)
<i>Bacillus anthracis</i>	0.5	2.6	1.2×10^0	(14)
<i>Brucella suis</i>	0.5	3.8	8.3×10^{-1}	(15)
<i>Toxoplasma gondii</i>	0.5	15	2.1×10^{-1}	(16)
<i>Coccidioides immitis</i>	0.5	16.7	1.9×10^{-1}	(17)
<i>Klebsiella pneumoniae</i>	0.5	17.9	1.8×10^{-1}	(18)
<i>Streptococcus pneumoniae</i>	0.5	30	1.1×10^{-1}	(19)
<i>Yersinia pestis</i>	0.5	37	8.5×10^{-2}	(20)
<i>Cryptococcus neoformans</i>	0.5	51	6.2×10^{-2}	(21)
<i>Vibrio vulnificus</i>	0.5	75	4.2×10^{-2}	(22)
Herpes Simplex Virus	0.5	219	1.4×10^{-2}	(23)
<i>Escherichia coli</i>	0.5	1×10^3	3.2×10^{-3}	(24)
<i>Candida albicans</i>	0.5	6.6×10^3	4.5×10^{-4}	(25)
Murine Cytomegalovirus	0.5	5.0×10^4	6.3×10^{-5}	(23)
<i>Aspergillus fumigatus</i>	0.25	6.0×10^4	3.0×10^{-5}	(26)
Group B Streptococcus	0.5	6.3×10^4	5.0×10^{-5}	(27)
Murine adenovirus	0.5	1.0×10^5	3.2×10^{-5}	(28)
<i>Listeria monocytogenes</i>	0.5	2.4×10^5	1.3×10^{-5}	(29)
<i>Nocardia asteroides</i>	0.5	8.5×10^5	3.7×10^{-6}	(30)
<i>Shigella sonnei</i>	0.5	1.6×10^6	2.0×10^{-6}	(31)
<i>Naegleria fowleri</i>	0.75	5.0×10^6	1.1×10^{-6}	(32)
<i>Bacillus cereus</i>	0.5	1.0×10^7	3.1×10^{-7}	(14)
<i>Staphylococcus saprophyticus</i>	0.5	2.7×10^7	1.2×10^{-7}	(33)
<i>Bacillus thuringiensis</i>	0.5	1.1×10^7	2.9×10^{-7}	(14)
<i>Pseudomonas aeruginosa</i>	0.5	5.0×10^7	6.3×10^{-8}	(34)
<i>Legionella pneumophila</i>	0.5	6.7×10^7	4.7×10^{-8}	(35)
<i>Staphylococcus epidermitis</i>	0.5	6.0×10^7	5.3×10^{-8}	(33)
<i>Staphylococcus aureus</i>	0.5	1.0×10^8	3.1×10^{-8}	(36)
Hemophilus influenza b	0.5	2.0×10^8	1.6×10^{-8}	(37)
+ mucin ²	0.5	3.4×10^4	9.3×10^{-5}	(37)
<i>Enterococcus faecalis</i>	0.5	2.6×10^8	1.2×10^{-8}	(38)

PATHOGENIC POTENTIAL IS A FUNCTION OF THE HOST

Microbe	Mouse Strain	Mortality	Inoculum	PP
<i>L. monocytogenes</i>	C57B1/6	0.5	9.0×10^5	3.5×10^{-6}
	B10.D2	0.5	2.2×10^5	1.4×10^{-5}
	B10.A	0.5	2.2×10^5	1.4×10^{-5}
	BALB/c	0.5	3.9×10^3	8.8×10^{-3}
	CBA	0.5	5.0×10^3	6.3×10^{-4}
	A/WySn	0.5	8.0×10^3	4.0×10^{-4}
	(C57Bl/6 x BALB/c)F1	0.5	3.4×10^4	9.3×10^{-5}
<i>B. anthracis</i>	A/J	0.5	2.6×10^0	1.2×10^0
	C3H/HeJ	0.5	5.6×10^0	5.6×10^{-1}
	BALB/cJ	0.5	6.6×10^0	4.8×10^{-1}
	C58J	0.5	9.0×10^0	3.5×10^{-1}
	C57BL/6J	0.5	1.4×10^1	2.3×10^{-1}
	C57L/J	0.5	2.2×10^1	1.4×10^{-1}
Sendai virus	129/ReJ 1	0.5	3.2×10^0	1.0×10^{-1}
	SWR/J	0.5	5.0×10^2	6.3×10^{-3}
	C58/J	0.5	1.5×10^3	2.1×10^{-3}
	C57BL/6J	0.5	2.5×10^4	1.3×10^{-4}
	SJL/J	0.5	1.0×10^5	3.2×10^{-5}
<i>C. immitis</i>	BALB/cAnN	0.5	4.6×10^1	6.9×10^{-2}
	C57BL/10N	0.5	5.9×10^2	5.4×10^{-3}
	C57BL/6N	0.5	6.8×10^2	4.7×10^{-3}
	DBA/2NX1	0.5	1.8×10^5	1.8×10^{-5}

Concept of 'pathogenic potential' beginning to be used instead of 'virulence'

Metabolite Transporter PEG344 Is Required for Full Virulence of Hypervirulent *Klebsiella pneumoniae* Strain hvKP1 after Pulmonary but Not Subcutaneous Challenge

Jeffrey Bulger,^a Ulrike MacDonald,^{b,e} Ruth Olson,^{b,e} Janet Beanan,^{b,e} Thomas A. Russo^{b,c,d,e}



TABLE 1 Analysis of the pathogenic potential (PP) of hvKP1 and hvKP1 Δ peg-344 after pulmonary challenge with or without the element of time

Strain	Inoculum (CFU)	M ^a	PP ^b	PP1/PP2 ^c	PP ₁ ^d	PP ₁ /PP ₂ ^e
hvKP1 (1)	3.0 × 10 ³	0.570	12.4		2.34	
hvKP1 Δ peg-344 (2)	2.8 × 10 ³	0.125	4.75	2.6	0.59	3.97
hvKP1	3.0 × 10 ⁴	1.000	3.33		0.833	
hvKP1 Δ peg-344	2.8 × 10 ⁴	0.375	0.85	3.9	0.122	6.83
hvKP1	3.0 × 10 ⁵	0.86	0.24		0.046	
hvKP1 Δ peg-344	2.8 × 10 ⁵	0.875	0.27	0.88	0.046	1.0
hvKP1	3.0 × 10 ⁶	0.860	0.024		0.008	
hvKP1 Δ peg-344	2.8 × 10 ⁶	0.875	0.027	0.88	0.005	1.6

^aM, mortality fraction.

^bPP was calculated as 1/inoculum × 10^M × 10,000.

^cPP1/PP2, PP of hvKP1/PP of hvKP1 Δ peg-344.

^dPP₁, pathogenic potential incorporating the element of time (PP × 1/T, where T is the mean number of days to death).

^ePP₁/PP₂, PP₁ of hvKP1/PP₂ of hvKP1 Δ peg-344.

RESEARCH ARTICLE

Infection history of the blood-meal host dictates pathogenic potential of the Lyme disease spirochete within the feeding tick vector

Bharti Bhatia, Chad Hillman, Valentina Carracci, Britney N. Cheff, Kir Tilly, Patricia A. Rosa*

Ingestion of host blood by infected ticks stimulates spirochete replication and induces changes that are critical for transmission of *B. burgdorferi* s.l. to the vertebrate host and survival in this disparate environment [6–10]. In contrast to the highly infectious phenotype of spirochetes in replete ticks, a recent study from our lab demonstrated that spirochetes colonizing unfed ticks are viable, but essentially non-infectious [11]. We will use the term “pathogenic potential” rather than “virulence”, as proposed by Casadevall [12], to describe the infectious phenotype of wild-type *B. burgdorferi* s.l. in an experimental mouse-tick cycle because infection does not cause disease in these rodent hosts. We conclude that in addition to stimulating spirochete replication, exposure to vertebrate blood during tick feeding also induces phenotypic changes that conditionally prime *B. burgdorferi* s.l. for subsequent infection of a vertebrate host, thereby dramatically enhancing the pathogenic potential of tick-borne spirochetes.

The Fungal Kingdom

- > 6 million species
- Includes major pathogens of plants, insects, invertebrates and ectothermic vertebrates
- Fungi currently devastating major ecosystems
 - Bats devastated by 'white nose syndrome'
 - Catastrophic amphibian declines from *Batrachochytrium dendrobatidis*
 - Salamanders declines in Europe from *Batrachochytrium salamandrivorans*
 - Snakes in North America
- Mammals are remarkably resistant!

Few fungal species are pathogenic for humans

Host Associated



Candida spp.



Dermatophytes



Pneumocystis spp.



Histoplasma spp.



Aspergillus spp.

Environment



Cryptococcus spp.



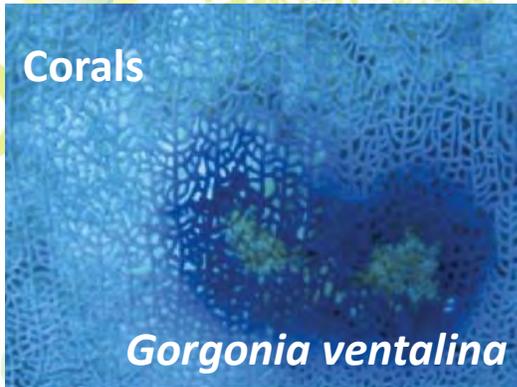
Coccidioides spp.



Blastomyces spp.

Fungi are devastating entire ecosystems

Corals



Gorgonia ventalina

Frogs



B. dendrobatidis

Rice



Magnaporthe oryzae

Salamanders



B. salmandrivorans

Turtles



Fusarium solani

Wheat



Puccinia graminis

Snakes



O. ophiodiicola

Emerging fungal threats to animal, plant and ecosystem health

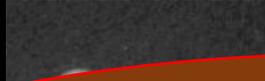
Matthew C. Fisher¹, Daniel A. Henk¹, Cheryl J. Briggs², John S. Brownstein³, Lawrence C. Madoff⁴, Sarah L. McCraw⁵ & Sarah J. Gurr⁵

Rabbit Experiments Suggest that Remarkable Resistance of Mammals is a Result of Adaptive Immunity PLUS High Temperature (J. Perfect and others)

40-41



C. neoformans

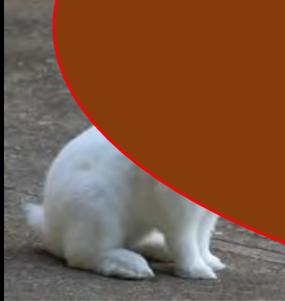


SYSTEMIC

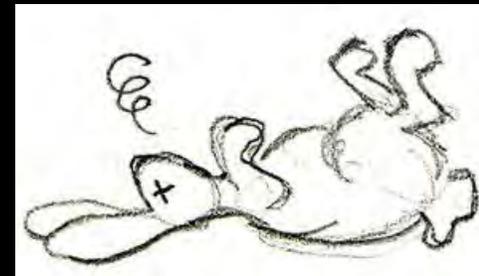


EMERGING PATTERN:

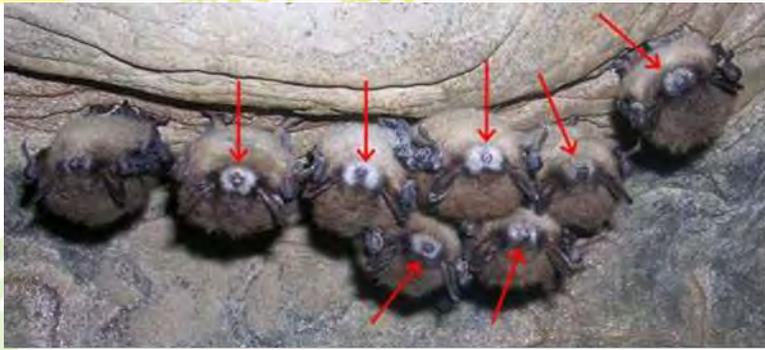
ADAPTIVE IMMUNITY + ENDOTHERMY =
RESISTANCE TO FUNGAL DISEASES



SYSTEMIC
INFECTION +
STERIODS



White Nose Syndrome in Bats

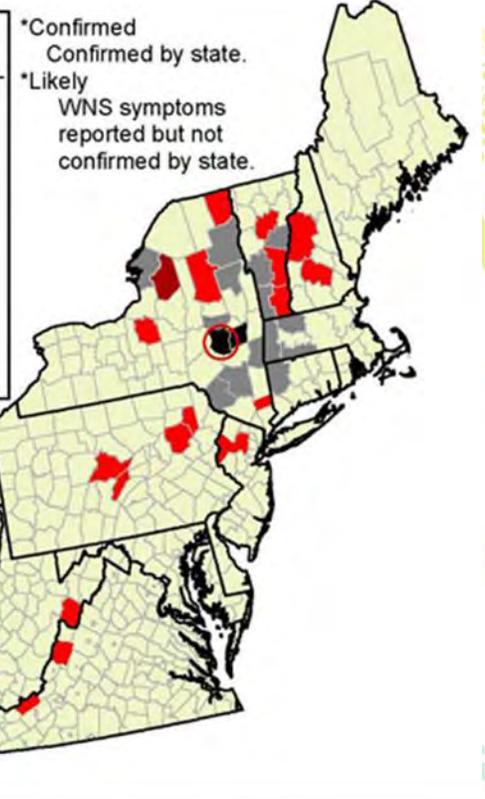


04/07/09
Bat White Nose Syndrome (WNS)
Occurrence by County*

- Feb. 2006: 1st detected in Schoharie Co., NY
- Mortality- Winter 2006/07
- Confirmed in 2007/08

Fall/Winter/Spring 2008/09

- Confirmed
- Likely but not confirmed



*Confirmed
Confirmed by state.
*Likely
WNS symptoms reported but not confirmed by state.

AS BATS HIBERNATE TEMPERATURE DROPS TO 10-12 C AND THEY BECOME SUSCEPTIBLE TO GEOMYCES spp.

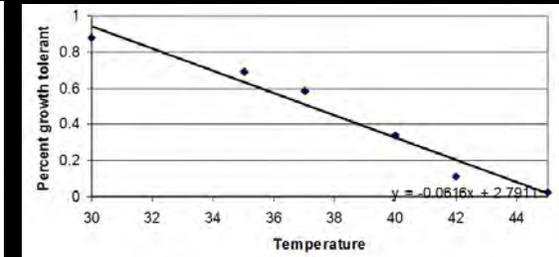
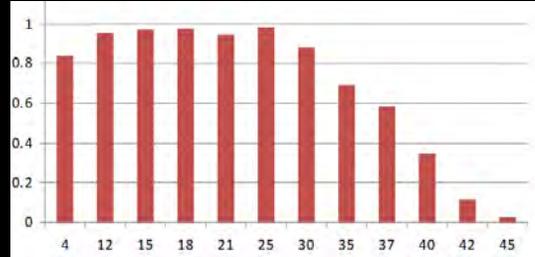
What makes mammals so special?

- Adaptive Immunity
- Endothermy
- Being 'hot' is a tradeoff
- Birds also resistant to fungal diseases
- Primitive mammals (e.g. platypus) susceptible

Cost $B(T) \propto M^{3/4} e^{-E_i/kT}$

Benefit $F(T) \propto F_0 [1 - (1 - s)^T]$

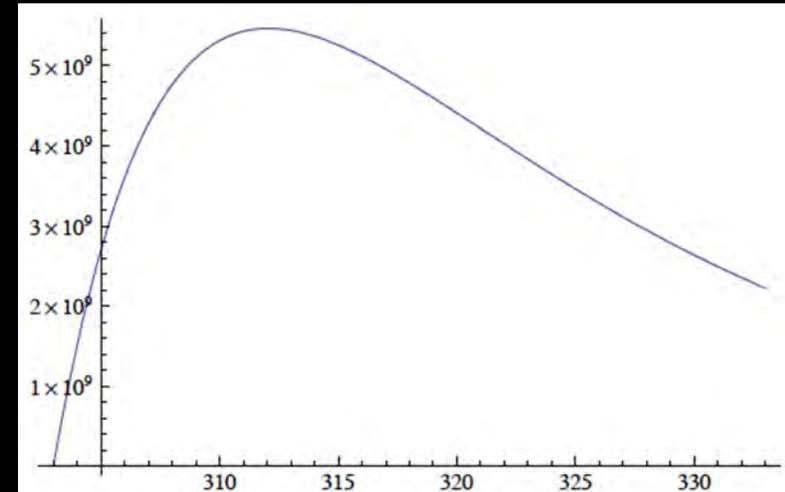
Maxima: 36.7 C



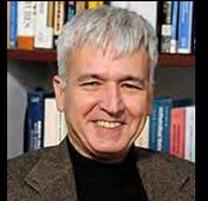
Robert & Casadevall, JID 2009



Vicent Robert



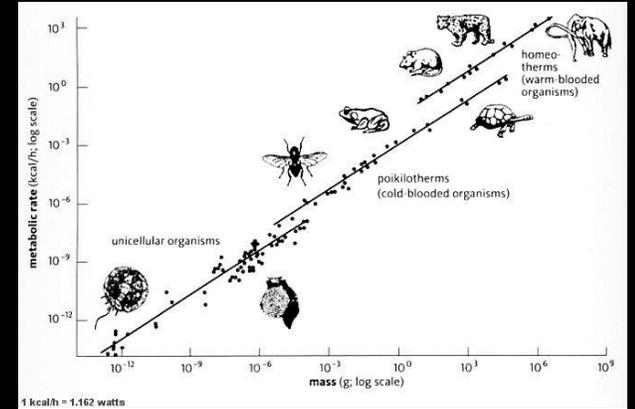
Bergman & Casadevall, mBio 2010



Aviv Bergman

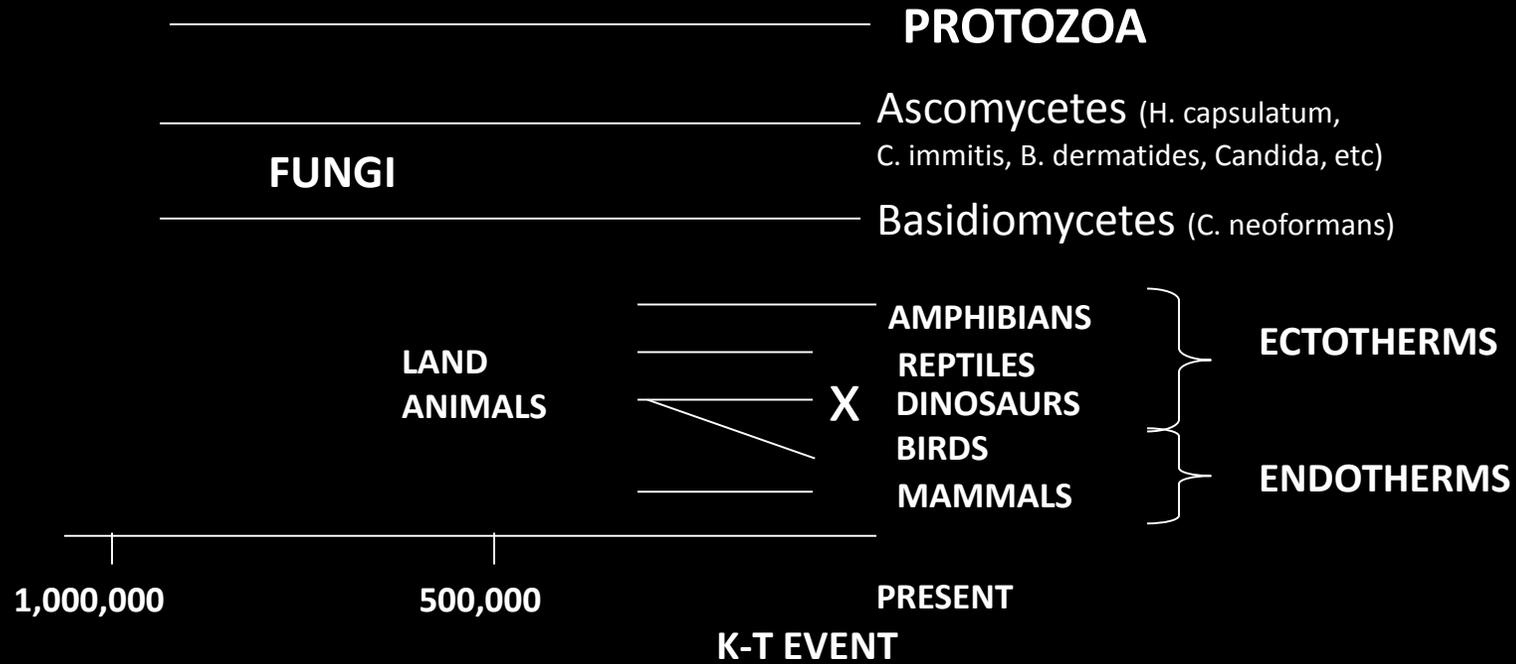
The cost of the mammalian lifestyle

- Mammalian lifestyle is very expensive
- Mammals very minor class until 65 mY ago
- Great mammalian radiation after K/T event
- How did this unfavorable lifestyle become dominant?



Could the explanation be in the distant past? When mammals emerged?

AGE OF MAMMALS BEGINS AFTER THE K-T EVENT

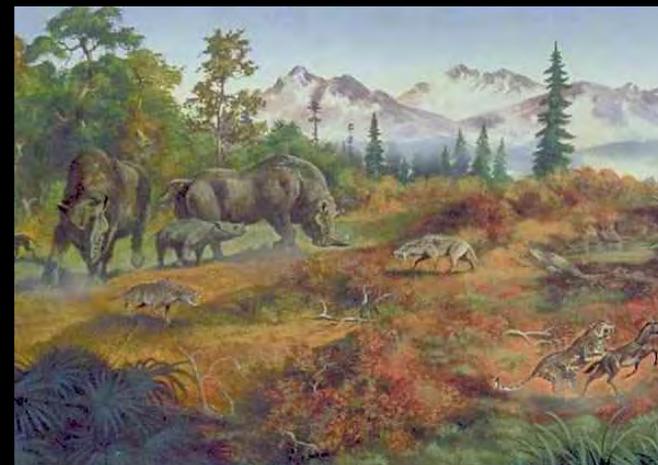


CRETACEOUS



REPTILIAN MEGAFAUNA

TERTIARY

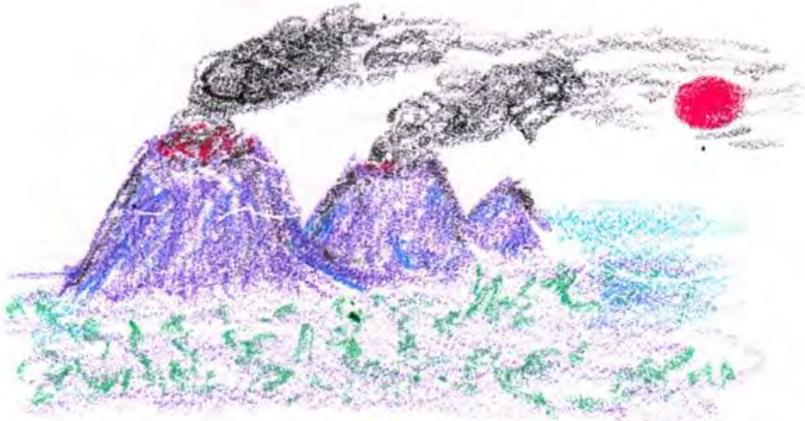


MAMMALIAN MEGAFAUNA



THE CRETACEOUS WORLD AND ITS END

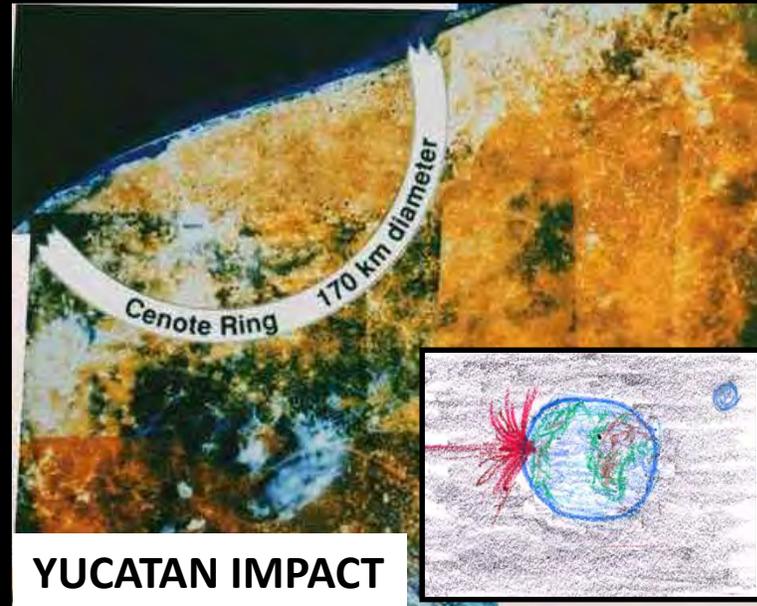
A WARM, VOLCANIC AND FORESTED PLANET



THE ATLANTIC WAS TINY...



REPTILIAN MEGAFUNA



YUCATAN IMPACT

THE POST-IMPACT WORLD

Indication of Global Deforestation at the Cretaceous-Tertiary Boundary by New Zealand Fern Spike

Vivi Vajda,^{1*} J. Ian Raine,² Christopher J. Hollis²

The devastating effect on terrestrial plant communities of a bolide impact at the Cretaceous-Tertiary boundary is shown in fossil pollen and spore assemblages by a diverse flora being abruptly replaced by one dominated by a few species of fern. Well documented in North America, this fern spike signals widespread deforestation due to an impact winter or massive wildfires. A Southern Hemisphere record of a fern spike, together with a large iridium anomaly, indicates that the devastation was truly global. Recovery of New Zealand plant communities followed a pattern consistent with major climatic perturbations occurring after an impact winter that was possibly preceded by global wildfires.



- FIRES, SMOKE, DUST OBSCURES SUN
- PHOTOSYNTHESIS SHUT DOWN FOR > 6 MONTHS
- GLOBAL TEMPERATURES DROP

KT EVENT CATASTROPE = GLOBAL FUNGAL COMPOST

Fungal Proliferation at the Cretaceous-Tertiary Boundary

Vivi Vajda¹ and Stephen McLoughlin²

The evolution of life on Earth has been interrupted by several mass extinction events. The Cretaceous-Tertiary (K-T) extinction [65 million years ago (Ma)] is associated with the impact of a large bolide (1). On the basis of extensive data (2-4), the K-T boundary is characterized by a palynological extinction horizon coincident with a geochemical marker bed commonly succeeded by a bed rich in fern spores (2-5).

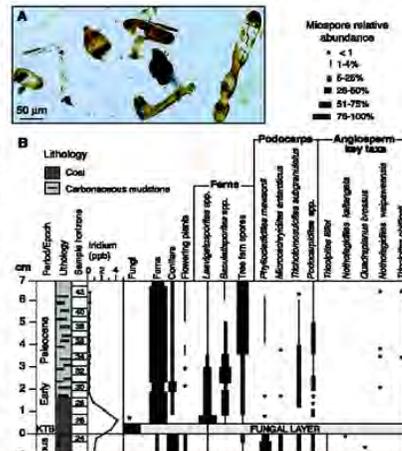
We have found that a fungal spike occurs between the diverse Late Cretaceous palynoflora and the low-diversity fern-dominated early Paleocene assemblages in a New Zealand section. The fungal layer is coincident with the Ir anomaly marking the extinction event.

The studied K-T boundary occurs within a 10-cm-thick coal seam bounded by mudstones of the nonmarine Revanui Coal Measures Member (upper Paparoa Coal Measures) at Moody Creek Mine, Greymouth Coalfield, New Zealand (171°16'40"E, 42°23'18"S). We identified palynomorph assemblages from consecutive 3- to 5-mm laminae through 20-cm coal and mudstone succession. One half of each sample was analyzed for elemental abundance (6) (table S1).

The basal 8 cm of coal hosts Late Cretaceous spore-pollen assemblages incorporating around 80 conifer,

undescribed species of fungi (7) (Fig. 1A). Overlying laminae contain low-diversity, fern-dominated assemblages. No significant macroscopic changes or clastic partings occur within the coal to suggest a change in sedimentation rate or depositional setting across the K-T boundary.

Analysis of a nearby section revealed an identical pattern of floristic turnover, indicating that the fungi-rich interval characterizes a distinct layer in the coal. The fungal acme is coincident with extinction of several microspore index taxa and an iridium abundance of ~4 parts per billion (ppb) (Fig. 1B; table S1).



This fungi-rich interval implies a catastrophic dieback of photosynthetic vegetation at the K-T boundary in this region. The peak is interpreted to represent a sharp increase in the available substrate for saprophytic organisms (which are dependent on photosynthesis) provided by forest dieback after the Chicxulub impact (5). Post-impact conditions of high humidity and reduced solar insolation due to atmospheric sulfur aerosols and would have favored saprophyte assemblages. This interval would have been short-lived because of rapid atmospheric settling.

Fungal dominance would have lasted only a few years at most, because the reestablishment of a maximum biomass layer (Fig. 1B). This suggests a rapid reestablishment of pteridophyte communities following the impact event.

A global fungal or algal (*Redonites*) spike followed by a pteridophyte recovery has also been reported from the Permian-Triassic boundary (9). The K-T and P-Tr recoveries represent similar terrestrial ecosystem destabilization and collapse, although the P-Tr crisis was more prolonged (9).

References and Notes
 1. L. W. Alvarez et al., *Science* **208**, 1095 (1985).
 2. D. J. Nichols, K. H. Johnson, *Geol. Soc. Am. Bull.* **114**, 95 (2002).
 3. A. R. Sweet et al., *Can. J. Earth Sci.* **36**, 1095 (1998).
 4. J. A. Wolfe, D. A. Sussall, in *Paleobotany*, P. R. Crowther, Eds. (Blackwell, Oxford, 2001), pp. 232-234.
 5. V. Vajda et al., *Science* **294**, 1700 (2001).
 6. Material and methods are available as supplementary material on Science Online.
 7. W. Eick, personal communication.
 8. K. O. Pece et al., *Geophys. Res. Lett.* **102**, 10,301 (1995).
 9. M. J. Benton, R. J. Twitchett, *Trends Ecol. Evol.* **18**, 184 (2003).
 10. We thank L. Raine for collaboration in the field, F. Asaro for geochemistry, and E. Anders, A. Odompo, R. Spicar, B. Trenarain for comments and technical assistance. R. Boyd and C. Helles assisted in the field. This work was supported by the Swedish research council for Environment and Nature (grant 20020547) and the New Zealand Foundation grant CTS-02-201 (V.V.).

VEGETATION DIEOFF = FUNGAL PROLIFERATION
FUNGAL PROLIFERATION = SPORE PROLIFERATION
SPORE PROLIFERATION = LARGE INOCULA
ENDOTHERMY = RESISTANCE TO FUNGAL DISEASE



Fungal virulence, vertebrate endothermy, and dinosaur extinction: is there a connection?

Arturo Casadevall*

FUNGAL GEN BIOL 2005



THE POST IMPACT WORLD



REPTILIAN MEGAFUNA KILLED BY:

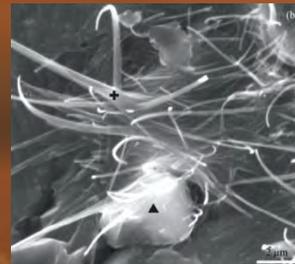
- IMPACT BLAST
- DISRUPTION IN FOOD SOURCES
- CHILLING OF THE PLANET
- FUNGAL DISEASES?



REPTILES FIGHT OFF FUNGAL INFECTIONS WITH INDUCED FEVERS BUT THERE WAS NO SUN



REPTILIAN EGGS SUSCEPTIBLE TO FUNGAL DISEASES

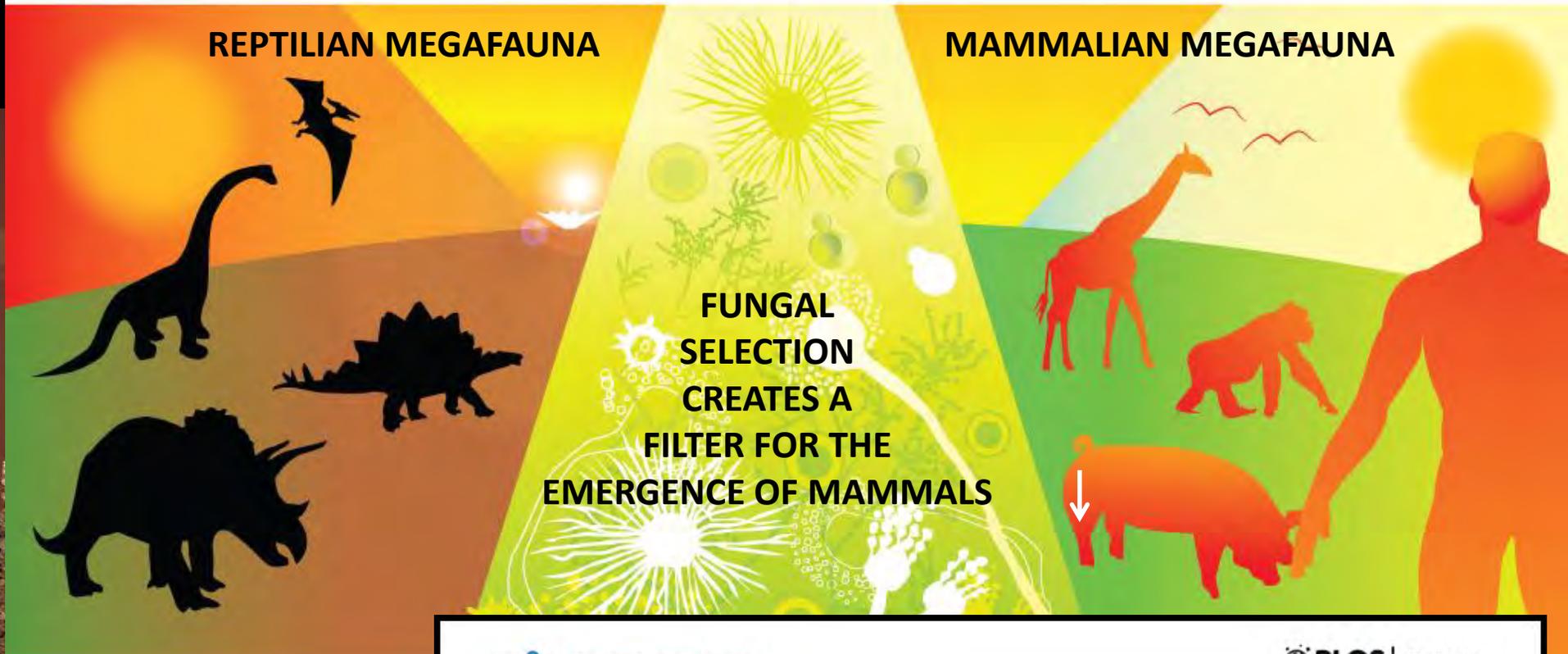


Endolithic fungi: A possible killer for the mass extinction of Cretaceous dinosaurs

GONG YiMing^{1,2†}, XU Ran¹ & HU Bi²

CHINESE EARTH SCIENCES 2008

**SOME SMALL REPTILES ONES ALSO SURVIVED
IF THE REPTILES WERE SO FIT: HOW COME WE DID NOT HAVE A SECOND REPTILIAN AGE?**



REPTILIAN MEGAFUNA

MAMMALIAN MEGAFUNA

FUNGAL SELECTION CREATES A FILTER FOR THE EMERGENCE OF MAMMALS

OPEN ACCESS Freely available online

PLOS PATHOGENS

Pearls

Fungi and the Rise of Mammals

Arturo Casadevall*

Departments of Microbiology and Immunology and Medicine, Division of Infectious Diseases, Albert Einstein College of Medicine, Bronx, New York, United States of America

Courtesy of NIAID

How do we know that there were fungal pathogens in the distant past?

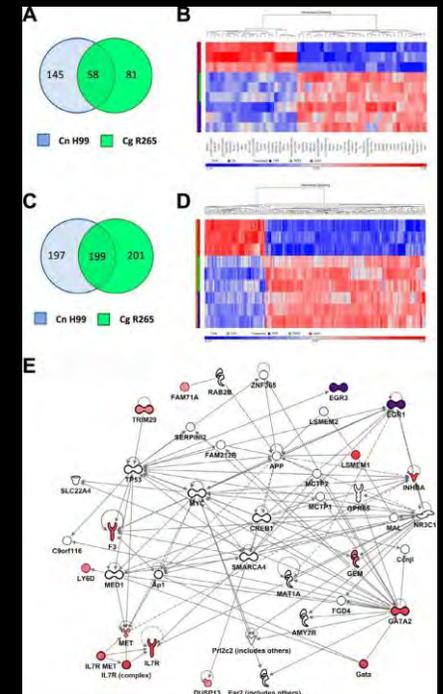
The Cryptococcal Intracellular Pathogenic Strategy dates to Cretaceous

C. neoformans Strains

H99 (VN1)
 WM179 (VG1)
 R265 (VG2a)
 WM161 (VG3)

Diverged
 50-100 mY
 Ago

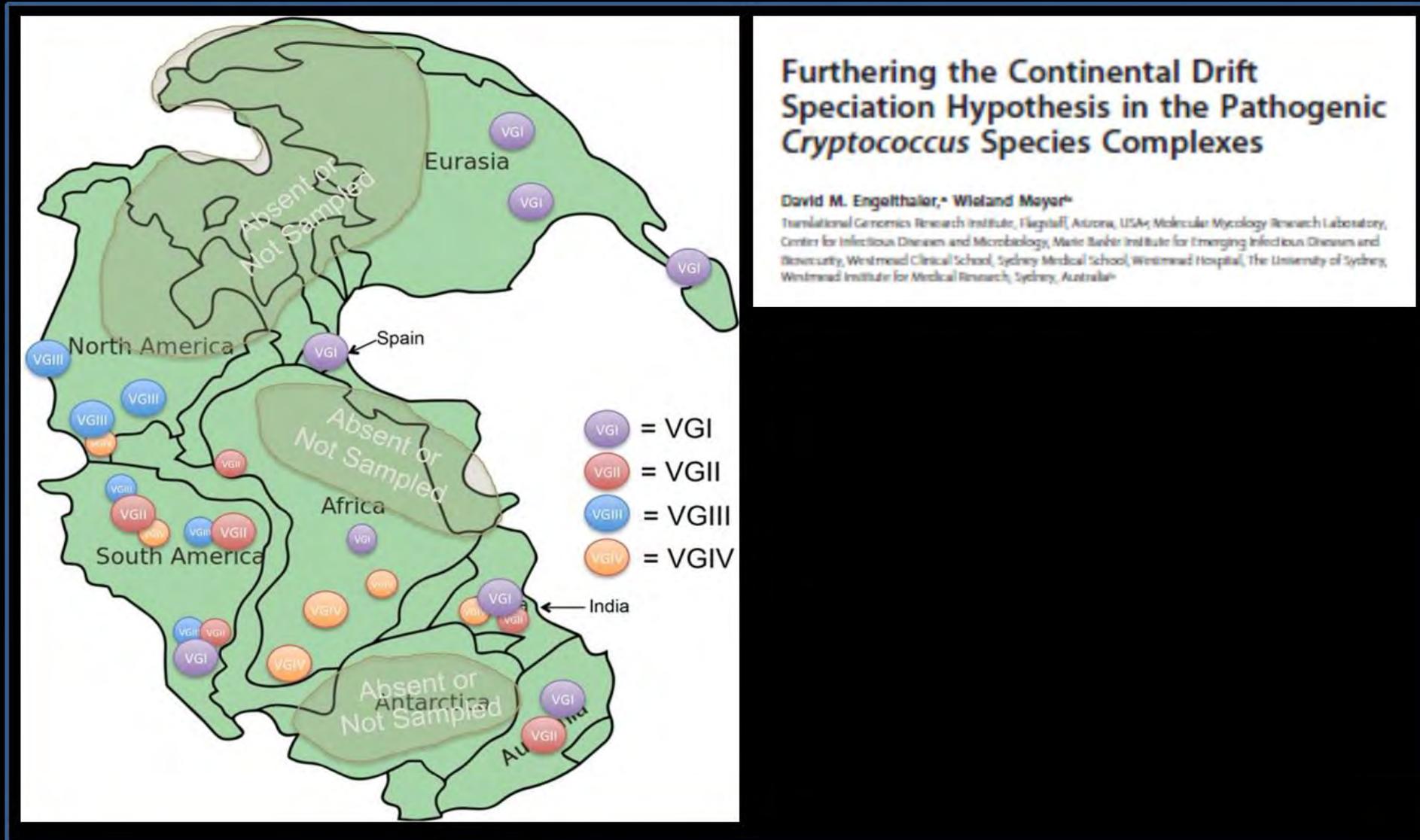
Intracellular replication
 Intracellular capsular enlargement
 Phagosomal acidification
 Phagosomal leakage
 Non-lytic exocytosis
 Macrophage transcriptional profile



Conservation of Intracellular Pathogenic Strategy among Distantly Related Cryptococcal Species

Joudah B. Freij,^{a*} Man Shun Fu,^{a*} Carlos M. De Leon Rodriguez,^{a*} Amanda Dziedzic,^{a*} Anna E. Jedlicka,^{a*} Quigly Dragotakes,^{a*} Diego C. P. Bossi,^{a*} Eric H. Jung,^{a,b*} Carolina Coelho,^{a*} Arturo Casadevall^{a*}

A Cryptococcus Pangean Ancestor had pathogenic potential



Requirements for Fungal Human Pathogenicity

Thermotolerance

- Host associated such as *Candida* spp. already thermotolerant
- Only 6% of species in environment can tolerate > 37 °C (Robert & Casadevall JID 2009)
- Only a few 'major' pathogenic fungi (*Aspergillus*, *Cryptococcus*, *Histoplasma*, *Sporothrix*, *Coccidioides* spp.)

Survival in host and replication

- "Virulence factors"
- Survive, replicate and evade immune mechanisms
- Highly varied...

Capsules

Toxins

Antioxidant systems

Intracellular replication

Stress resistance

etc., etc., etc.

Cryptococcus neoformans



INHALATION



NORMAL HOST

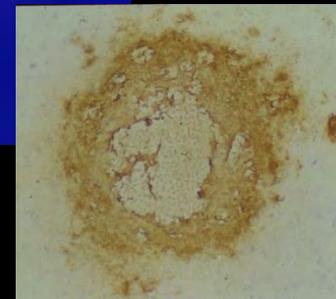
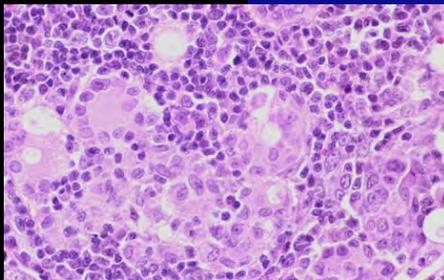
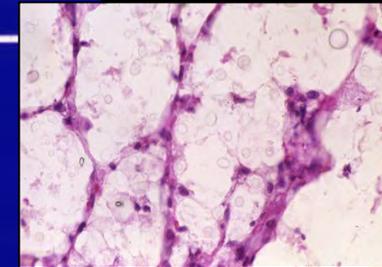
IMMUNOCOMPROMISED HOST

RESOLUTION

LATENT INFECTION

DISSEMINATION INFECTION

MENINGITIS



For *C. neoformans* capsule and melanin make largest contribution to virulence composite

INFECTION AND IMMUNITY, Mar. 2006, p. 1500–1504
 0019-9567/06/\$08.00+0 doi:10.1128/IAI.74.3.1500-1504.2006
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Vol. 74, No. 3

Estimating the Relative Contributions of Virulence Factors for Pathogenic Microbes†

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 and ²Department of Psychology, Westminster College, 1840 South 1300 East, Salt Lake City, Utah 84105

Received 6 November 2005/Returned for modification 23 November 2005/Accepted 18 December 2005

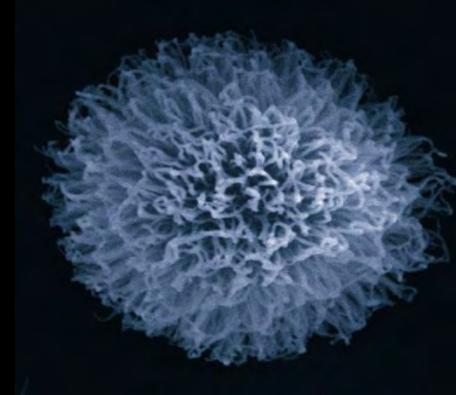
Many pathogenic microbes have multiple virulence factors that can cause damage to the host and thus contribute to an overall virulence phenotype for that organism. Although current techniques are suitable for demonstrating that a particular microbial characteristic contributes to virulence, no formal approach for defining the relative contributions of multiple virulence factors to overall virulence has been proposed. This paper describes the use of multivariate linear regression to estimate the relative contributions of virulence factors to the overall phenomenon of virulence. The approach is illustrated here with sample calculations of the relative contributions of individual *Cryptococcus neoformans* and *Bacillus anthracis* virulence factors to the overall virulence phenotype. These calculations were derived from a small underpowered experimental data set for the fungus and two larger sets of randomly generated data for both microbes. The major limitation of this method is a requirement for large data sets of microbial strains that differ in virulence and virulence factor expression. Multivariate linear regression can be used to identify the relative levels of importance of virulence factors in virulence studies, and this information can be used to prioritize antigen identification for vaccine development and the design of antimicrobial strategies that target virulence mechanisms.

TABLE 4. Hierarchical regression analysis results for *C. neoformans*, with low correlation between predictors

Virulence factor	Correlation with time to death (r)	R^2 change	df	F value	P value ^a
Capsule size	0.643	0.414	1, 37	26.10	<0.001
Melanin	0.571	0.162	1, 36	13.76	0.001
GXM release	-0.323	0.002	1, 35	0.15	0.70 (NS)
Urease	0.344	0.005	1, 34	0.38	0.54 (NS)
Doubling time	0.075	0.038	1, 33	3.33	0.08 (NS)

^a NS, not significant.

POLYSACCHARIDE CAPSULE



~26%

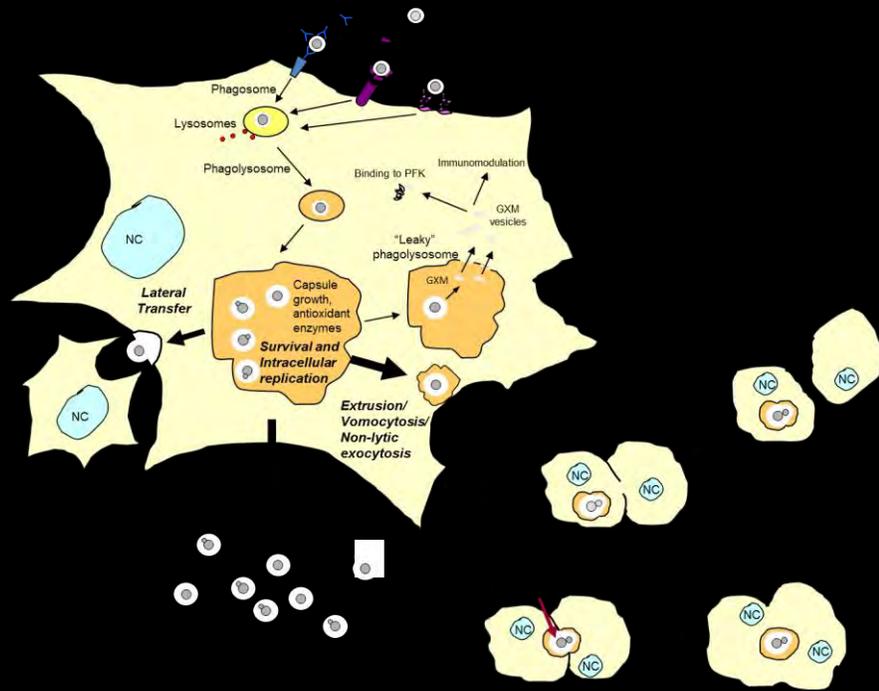
MELANIN IN CELL WALL



~14%

CONUNDRUM: C. NEOFORMANS IS A NON-SPECIFIC PATHOGEN WITH A VERY SOPHISTICATED CELLULAR PATHOGENIC STRATEGY..WHAT IS GOING ON HERE?

SOPHISTICATION



MANY HOSTS - NONSPECIFICITY



PLANTS

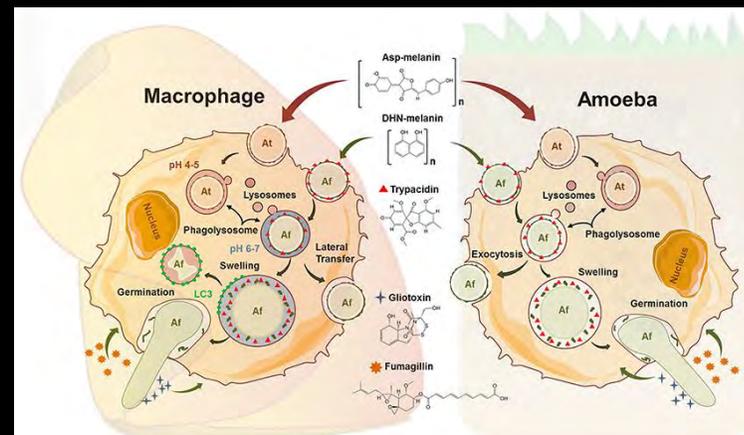
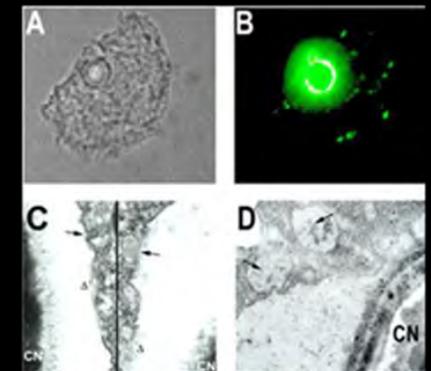
WHY DOES A SOIL ORGANISM WITH NO NEED FOR AN ANIMAL HOST HAVE A SOPHISTICATED VIRULENCE STRATEGY?

HOW DOES A SOIL ORGANISM WITH NO NEED FOR ANY HOST MANAGES TO SUBVERT SUCH DIVERSE HOST DEFENSES?

Why do soil organisms have mammalian virulence factors?

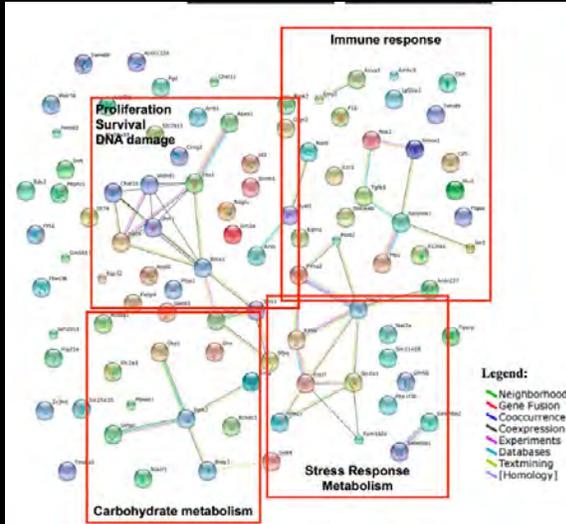
- *C. neoformans* virulence factors capsule, melanin and phospholipase allow survival in confrontation with amoeba
- Similar findings with other pathogenic fungi
- ‘Ameboid predator hypothesis’
Capacity for virulence is accidental (Casadevall & Pirofski 2007)

Cryptococcus neoformans interactions with amoebae suggest an explanation for its virulence and intracellular pathogenic strategy in macrophages
J. N. Steenbergen*, H. A. Shuman*, and A. Casadevall**†
PNAS 2001



Front. Cell. Infect. Microbiol 2017

Macrophages sustain damage across many critical cellular systems



**CRISPR-CAS9 SCREEN FOR RESISTANCE GENES
NETWORK ANALYSIS OF MACROPHAGE GENES
SHOW MITOCHONDRIAL AND RIBOSOMAL
GENES ARE DISPROPORTIONALLY AFFECTED BY
C. NEOFORMANS INFECTION**

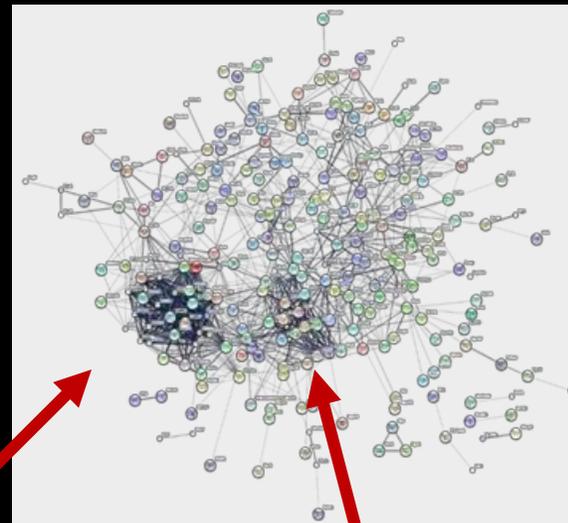
Macrophage Mitochondrial and Stress Response to Ingestion of *Cryptococcus neoformans*

Carolina Coelho,^{*,†} Ana Camila Oliveira Souza,[‡] Lorena da Silveira Derengowski,[‡] Carlos de Leon-Rodriguez,^{*} Bo Wang,^{*,§} Rosiris Leon-Rivera,^{¶,||} Anamelia Lorenzetti Bocca,[‡] Teresa Gonçalves,[†] and Arturo Casadevall^{*}

Table 1. Summary of findings

	J774.16 Macrophage-like Cells	BMDM	Peritoneal Macrophages
Fungal control	++	++	+
ATP levels	↓	↓ ↓	↓ ↓
PCD pathways			
LDH release	Yes	No	Yes
Annexin V binding	Yes	"	Yes
Activation of Caspases	-8	-1, -3, -8	-3
RIP	Yes	No	h
AIF	Yes	Yes	h
PARP	No	No	h
Release of cytochrome c	No	Yes	h
Predicted PCD activated	Necrosis	Apoptosis	Apoptosis/Necrosis
Gene expression	Stress pathways activated		
Mitochondrial characteristics			
Depolarization	++	+	++
Morphology	Fragmented	Unchanged	Fragmented
ROS production	No	No	Yes
Mitochondrial-derived ROS	No	No	No
NO dependence	Yes	"	Yes
Protein translation	↓	"	↓

^{*}Not pertinent for conclusions of study.
[†]Not performed owing to technical limitations.
PCD, protein cell death.



MITOCHONDRIA

RIBOSOMAL



Carolina Coelho

UNPUBLISHED

New Roles for Old Cryptococcal Virulence Factors

Virulence factor

Known Role

New Role

→ Urease

Brain invasion

Intracellular pathogenesis

Nutrient acquisition

Polysaccharide Capsule

Antiphagocytic

Phagosome pH Regulation

Antioxidant

Flotation Device

→ Melanin

Antioxidant

Heat capture

C. neoformans Urease

Mnemonics to remember Urease Positive Organisms

"PUNCH"

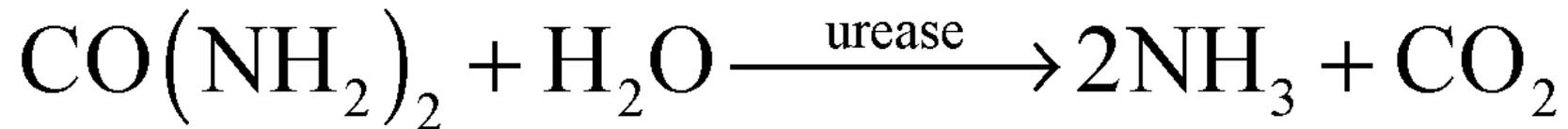
P: *Proteus*
U: *Ureaplasma*
N: *Nocardia*
C: *Cryptococcus/Corynebacteria*
H: *Helicobacter pylori*



Urease as a Virulence Factor in Experimental Cryptococcosis

GARY M. COX,^{1*} JEAN MUKHERJEE,² GARRY T. COLE,³ ARTURO CASADEVALL,²
AND JOHN R. PERFECT¹

Division of Infectious Disease, Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710¹; Division of Infectious Diseases, Department of Medicine, and Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461²; and Department of Microbiology and Immunology, Medical College of Ohio, Toledo, Ohio 43614³



Urease Expression by *Cryptococcus neoformans* Promotes Microvascular Sequestration, Thereby Enhancing Central Nervous System Invasion

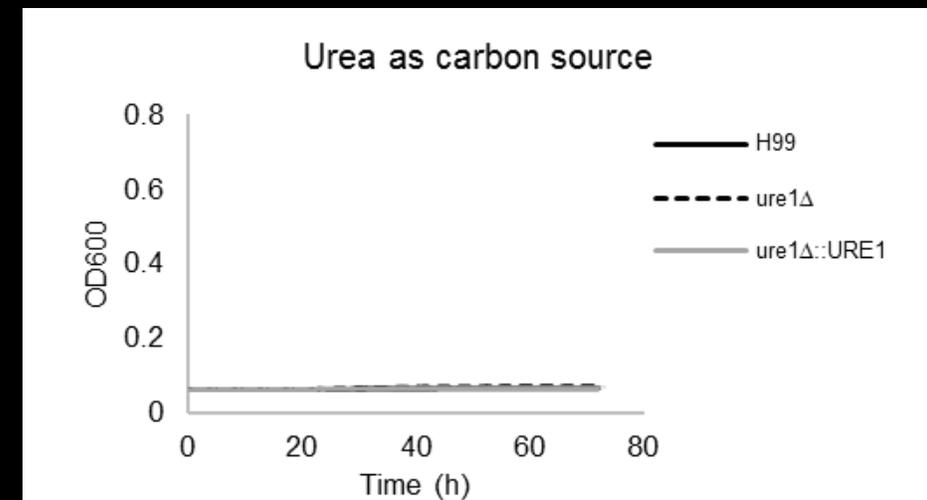
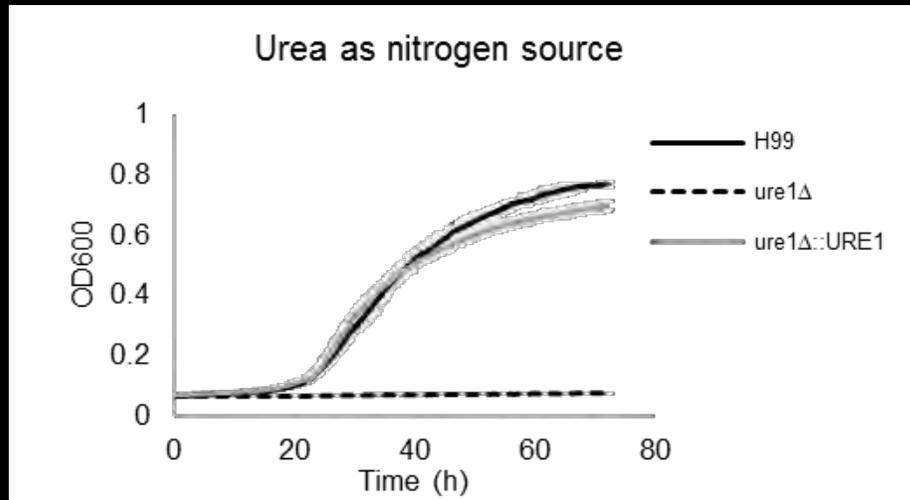
Michal A. Olszewski,^{1†} Mairi C. Nowert,¹
Gwo-Hsiiao Chen,¹ Galen B. Toews,^{1†}
Gary M. Cox,² John R. Perfect,² and
Gary B. Huffnagle¹

Real-time imaging of trapping and urease- dependent transmigration of *Cryptococcus* *neoformans* in mouse brain

Meiqing Shi, ... , Paul Kubes, Christopher H. Mody

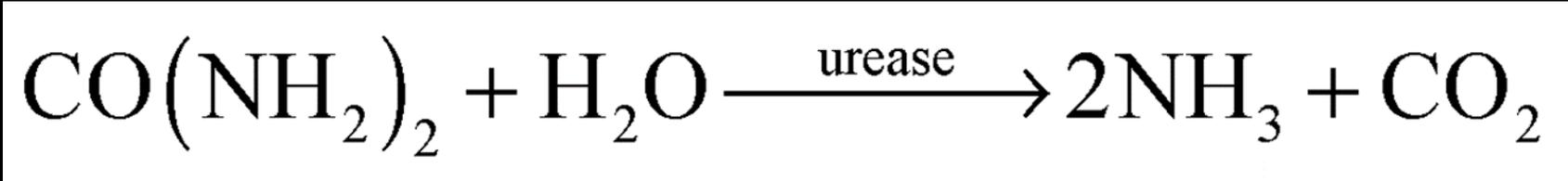
J Clin Invest. 2010;120(5):1683-1693. <https://doi.org/10.1172/JCI41963>.

Urease role is as a nutritional enzyme

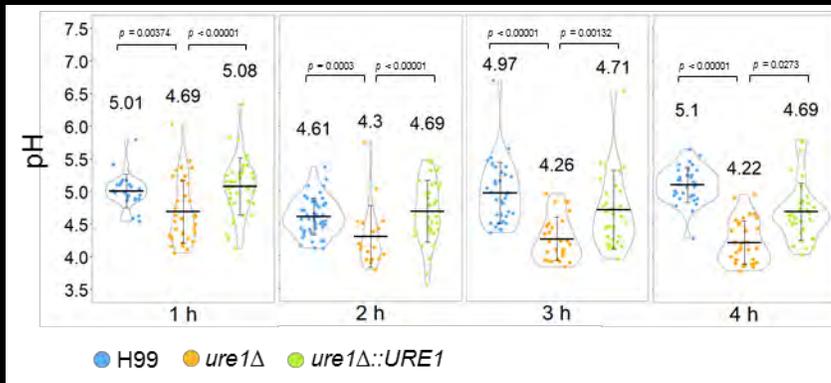


An example of a nutritional enzyme affecting virulence

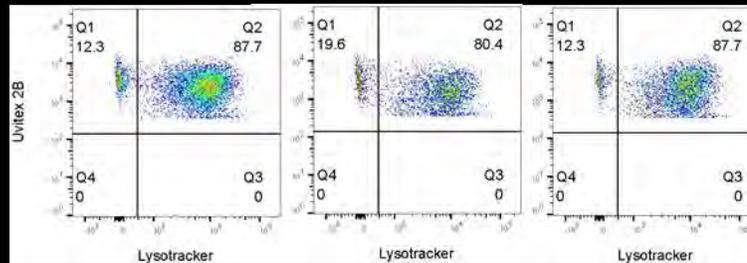
Mechanism of urease action intracellularly



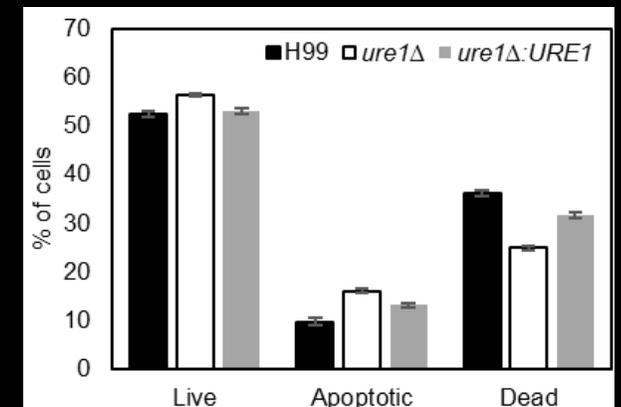
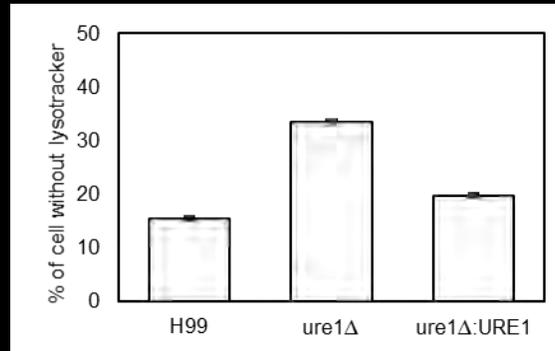
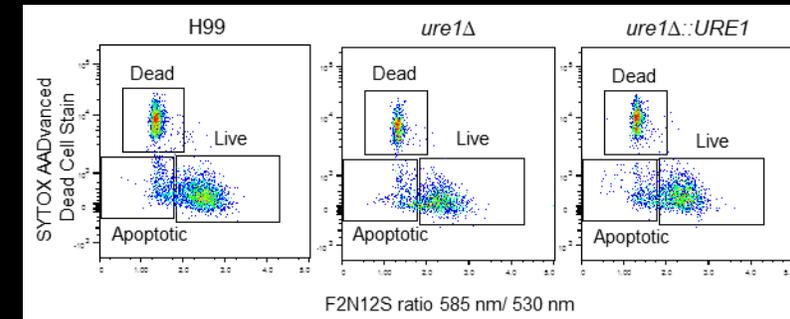
Urease changes phagosomal pH



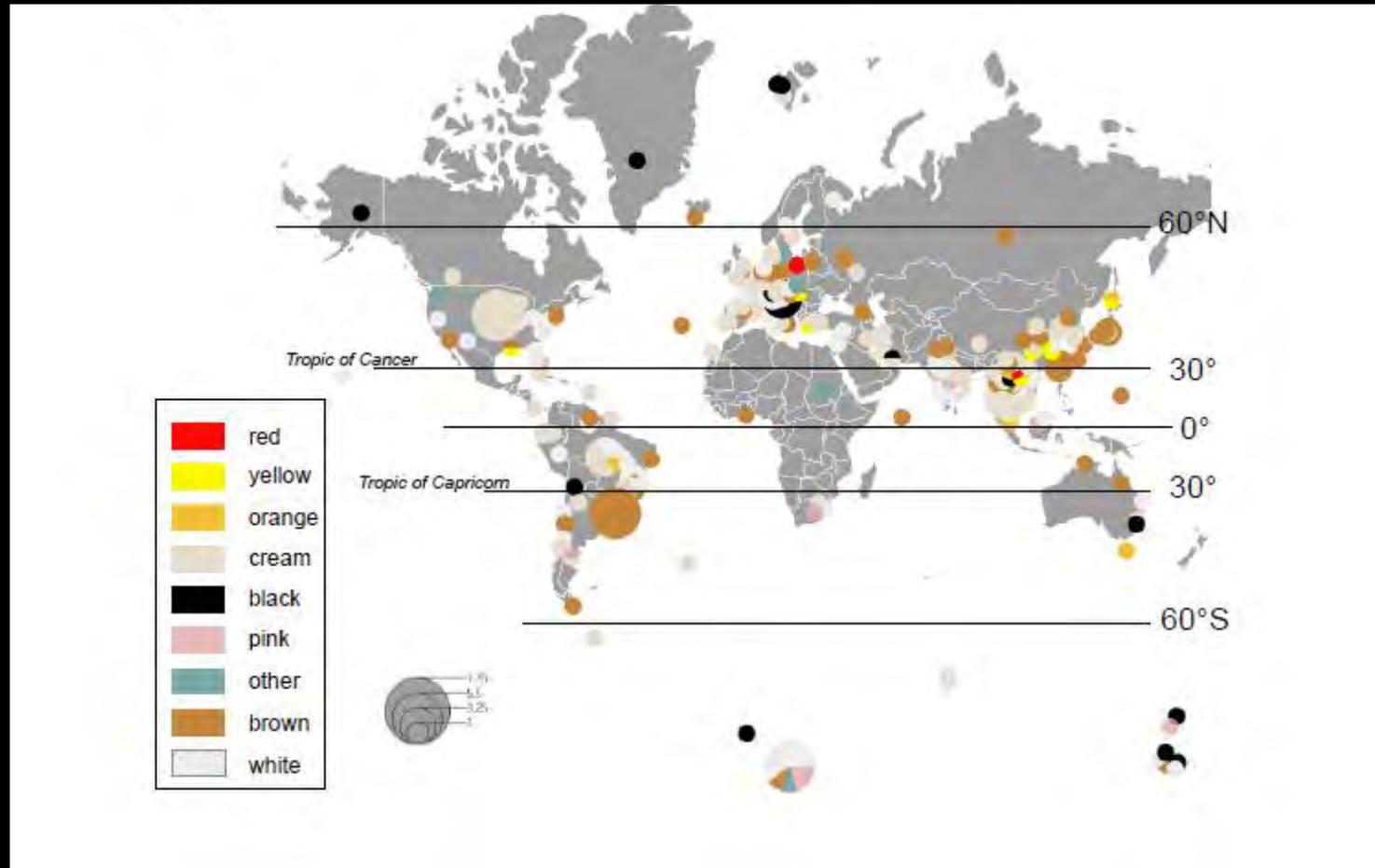
...which affects phagosomal leakage



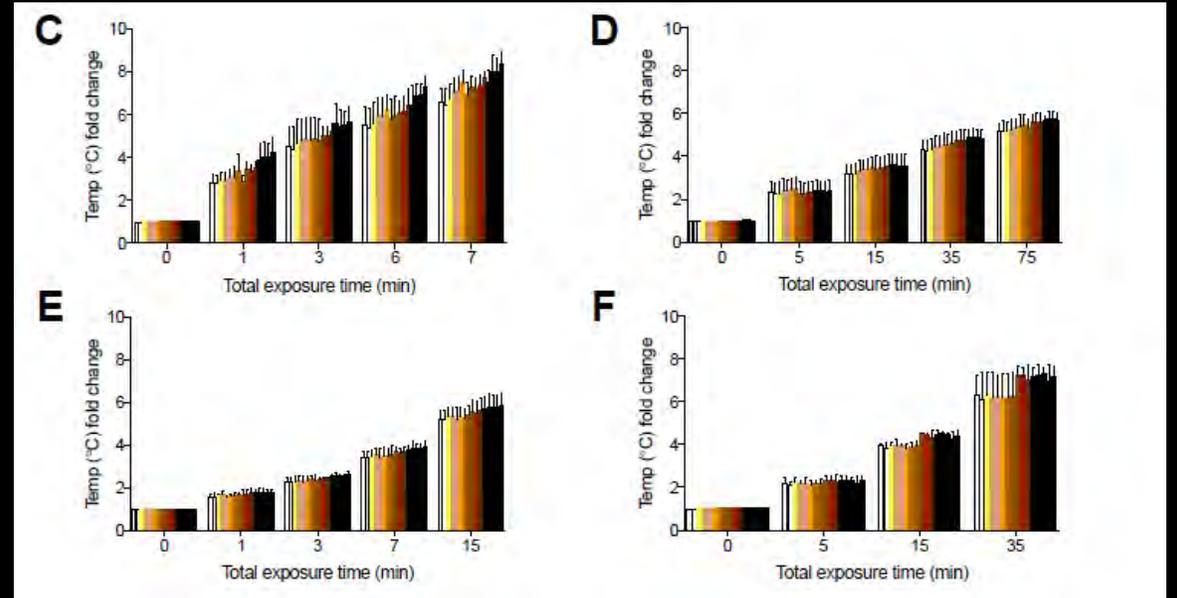
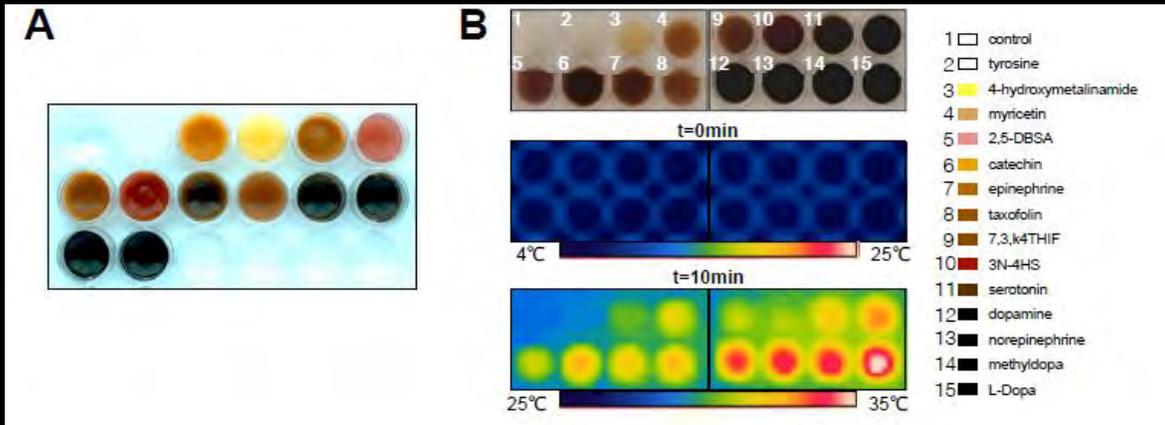
...which affects mode of death



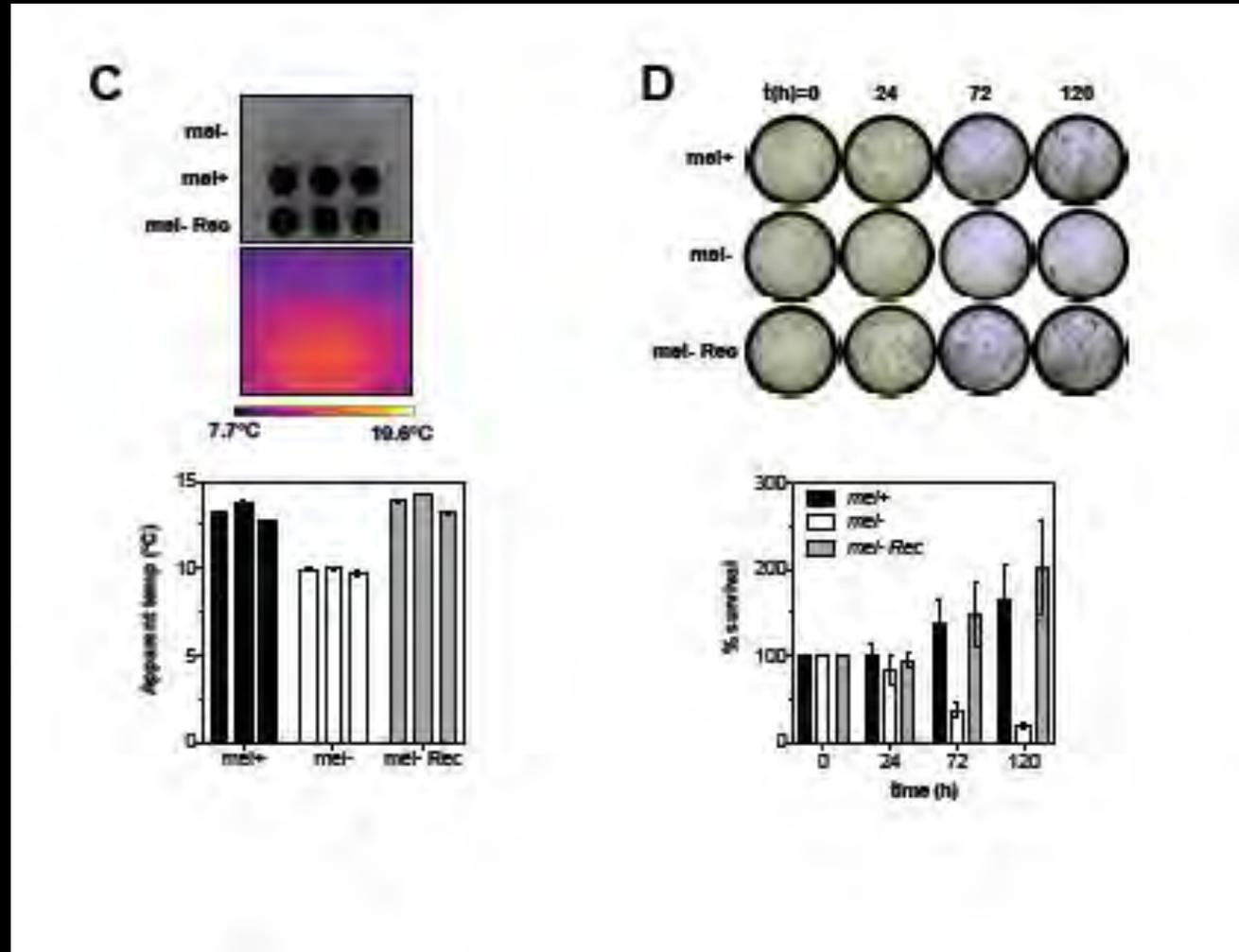
A New Role for Melanin



Laccase catalyzes synthesis of pigments with different colors in *C. neoformans*



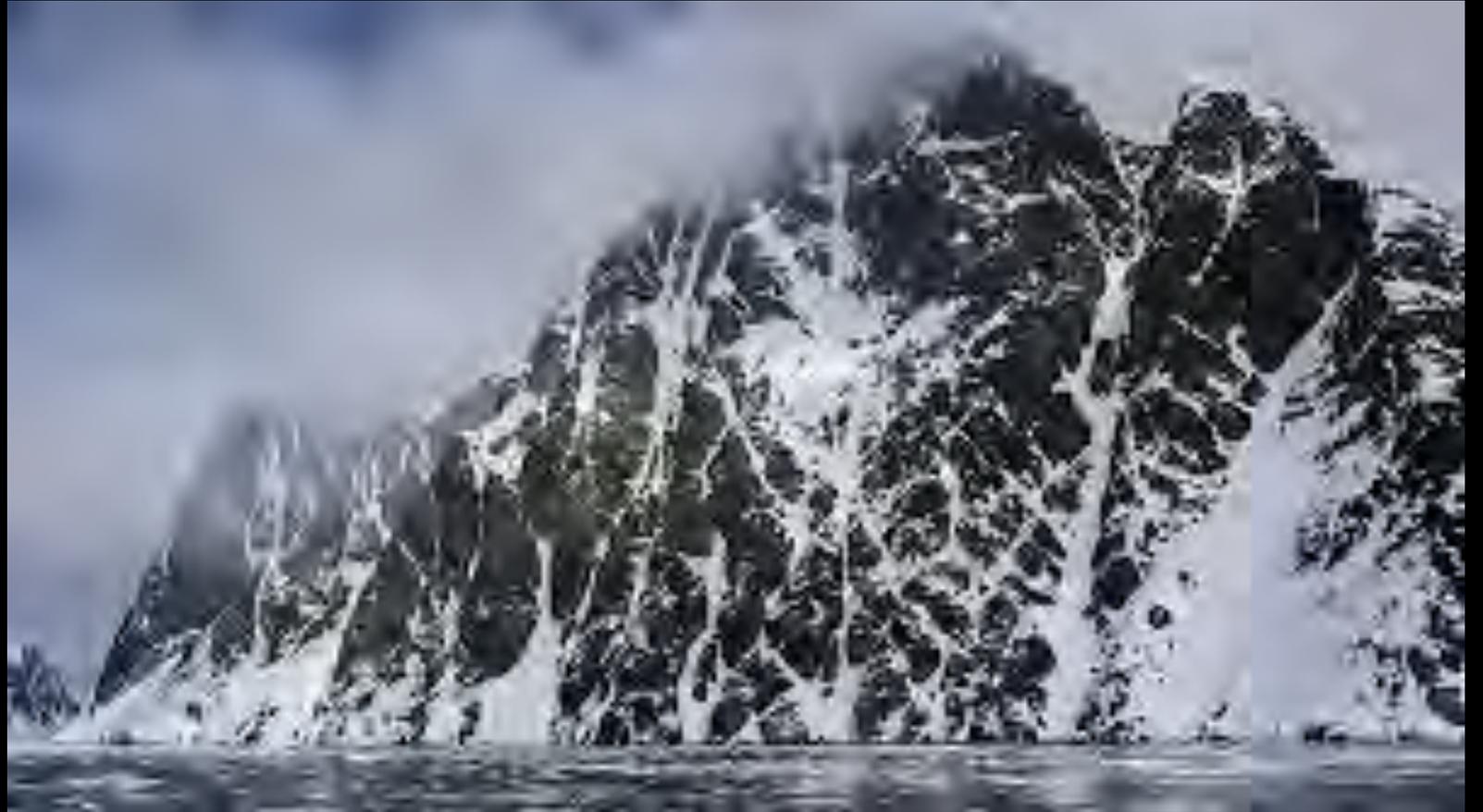
Pigmented *C. neoformans* harvest energy from light in cold room to grow faster



Microbial Pigments in Energy Harvest and Global Warming

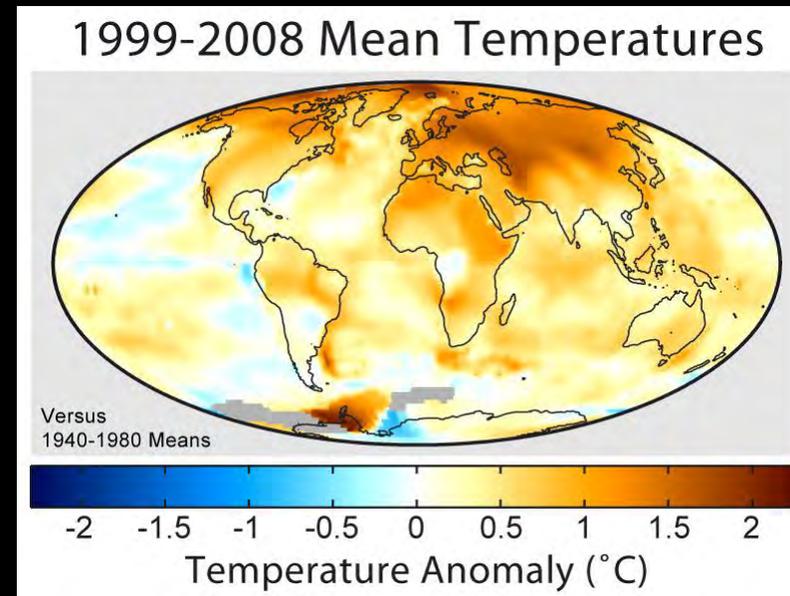
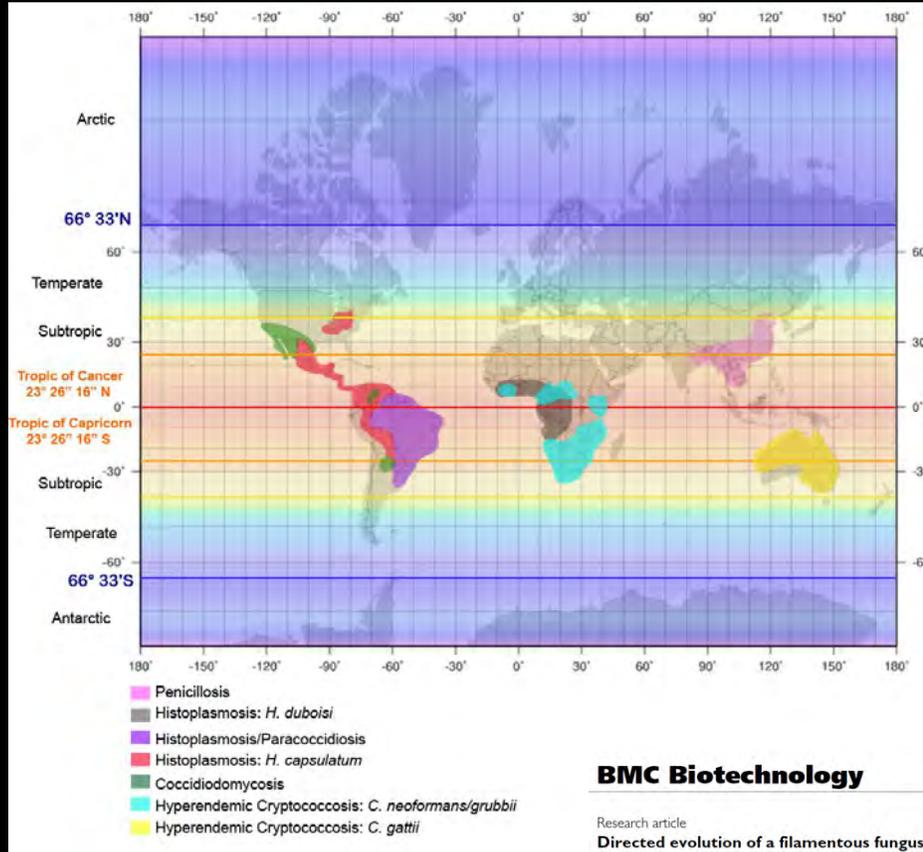


Pigmented microbes colonize Meltwater and enhance melting



Black Mountains of Antarctica where pigment comes from microbes

GEOGRAPHIC FACTS: MOST ENDEMIC MYCOSES OCCUR IN TROPICAL AND SUB-TROPICAL REGIONS



BMC Biotechnology

Research article

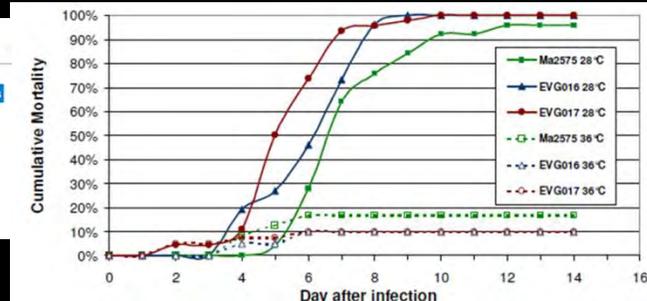
Directed evolution of a filamentous fungus for thermotolerance
Eudes de Crecy¹, Stefan Jaronski², Benjamin Lyons¹, Thomas J Lyons¹ and Nemat O Keyhani^{1*3}

Address: ¹Evolugate LLC, 2153 SE Hawthorne Road, 15 Gainesville, FL 32641, USA; ²USDA ARS NPARE, 1500 N. Central Ave., Sidney MT 59270, USA and ³Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611, USA
Email: Eudes de Crecy - edc@evolugate.com; Stefan Jaronski - SJaronski@ARS.USDA.GOV; Benjamin Lyons - bjlyons@evolugate.com; Thomas J Lyons - tjlyons@evolugate.com; Nemat O Keyhani^{*} - keyhani@ufl.edu

* Corresponding author



Open Access



AVERAGE FUNGAL THERMAL TOLERANCES OVER PAST 30 YEARS

DEADLY GERMS, LOST CURES

A Mysterious Infection, Spanning the Globe in a Climate of Secrecy

The rise of *Candida auris* embodies a serious and growing public health threat: drug-resistant germs.

mammalian temperatures create a thermal exclusionary zone

capacity to grow at 37 °C

GLOBAL TEMPERATURE

Summary

Fungal diseases cause tremendous suffering for humanity

Fungal diseases are neglected, understudied, underfunded...

Most humans do not fear fungal diseases

Remarkable mammalian resistance to fungi due to endothermy + acquired immunity

Late 20th century emergence of fungi as major pathogens reflect changes that undermined immunity

Global warming is next big threat as it will reduce protective thermal gradient and new fungal diseases are likely to occur

Some Answers to Existential Questions from a Fungal Point of View

- **WHY ARE WE HERE?**
KT EVENT AND FUNGAL SELECTION
- **WHY ARE WE SO HOT?**
TO KEEP THE FUNGI AWAY
- **WHY DO WE EAT SO MUCH?**
TO MAINTAIN HIGH TEMPERATURE
- **WHY ARE MAMMALS THE DOMINANT ANIMALS?**
FUNGAL SELECTION KEPT DOWN THE REPTILES
NO SECOND AGE OF REPTILES!