Ranavirus Evolution

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Acknowledgements

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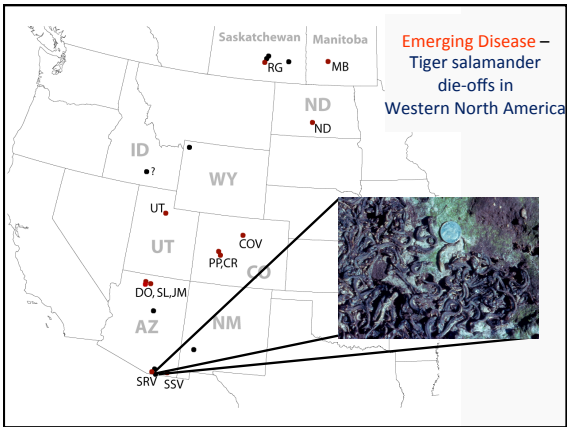


Emerging Disease – Tiger salamander die-offs in Western North America

ATV- *Ambystoma tigrinum* virus

- Koch's postulates satisfied
- Large dsDNA virus (~106 kb)
- Easily transmitted
- Host range: most vectors eliminated
  - Salamanders primary vector
- Epizootics: some to all salamanders in a given year
  - Some populations not recovered

Emerging infectious diseases: A leading threat to public and wildlife health

Big questions:

1) Why do diseases [ranaviruses] emerge?
2) What is their capacity to spread?

1) Why did ATV emerge?
[Why are ranaviruses emerging?]

Alternative Hypotheses:

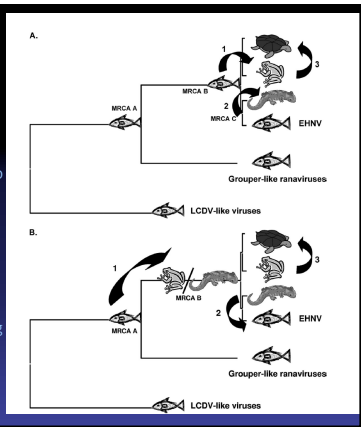
a) Because disease has expanded its range ("novel")

Or

b) Environment has changed (disease is old; "endemic")

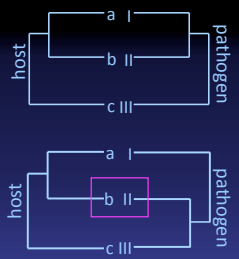
Iridovirus phylogeny
(from Jancovich et al. 2010)

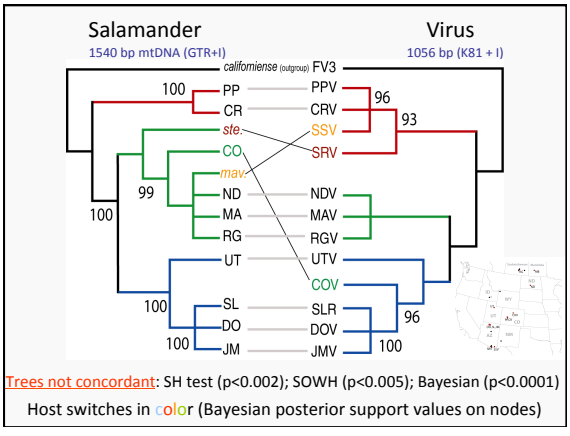
- Phylogeny suggests that ATV arose as a host switch from fish to salamanders
- Closely related strain – EHNV – came from rainbow trout
  - Rainbow trout introduced for fishing



Is disease new or old? A test using phylogenetic concordance.

- If old, expect:
  - virus to be coevolved with host and thus phylogenetic concordance
- If new, expect:
  - lack of coevolution and thus non-concordance



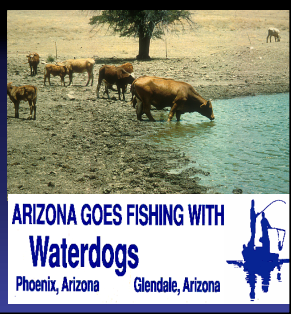


Trees not concordant: SH test ($p<0.002$); SOWH ($p<0.005$); Bayesian ($p<0.0001$)

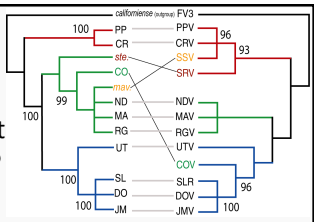
Host switches in color (Bayesian posterior support values on nodes)

Are host switching events caused by human movement of salamanders?

- Ecological evidence:
  - Human-dominated landscape (cattle tanks)
  - Bait salamanders moved throughout W.US
    - AZ/NM/TX bait shops positive for virus (Picco & Collins 2008)

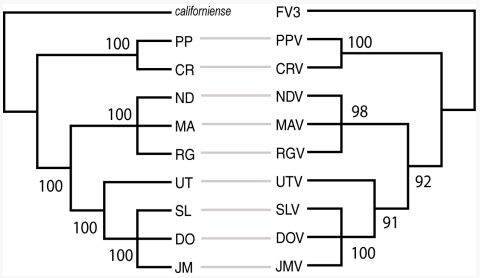


Are host switching events caused by human movement of bait salamanders?



- SC AZ (SSV) salamanders introduced (Collins 1981)
- SC AZ (ATV):
  - hybridization between native subspecies (*stebbinsi* endangered) & introduced (bait – *mavortium)* (Storfer et al., *Copeia* 2004)
- W. CO. (MUD) strain nearly identical to bait shop strain (Jancovich…Storfer, *Mol. Ecol.* 2005)

Salamander | Virus



Topology: $p < 0.0001$
Coalescent time concordance: $r^2 = 0.859$
Evidence of coevolution

Storfer et al. 2007, *Ecology Letters*

1) Why did ATV emerge?

a) ~~Because disease has expanded its range ("novel") or~~

b) Environment has changed (disease is old; "endemic")

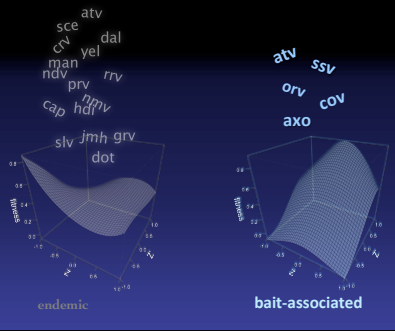
- Concordance suggests evidence for coevolution

1) Why do diseases emerge?

✓ Environment has changed

- How?
  - Hypothesis: Bait environment high density – could artificially select for high virulence
    - Classic virulence-transmission tradeoff
  - Prediction: Introduced strains should be more virulent than coevolved strains
  - Test: Cross-infection experiment of coevolved versus introduced virus strain

Does selection differ between bait and endemic strains?



ATV Genomic Sequencing

Sequenced 9 ORFs associated with host immune evasion/ virulence.
4 showed evidence of positive selection (ω>1) among viral strains:
βOH – host steroid upregulation
DNK/RDR – mobilization of host resources for viral replication
DMT – genome methylation, TLR-9 downregulation

locus	BEB	p_{purSel}	p_{posSel}	$-lnL(m0)$	$-lnL(m1)$	$-lnL(m2)$	interpretation
beta-hydroxy oxidoreductase (BOH)	24 (0.50); 34 (0.85); 39 (0.85); 43 (0.85)	0.2923	0.0147	265.3	266.1	259.8	positive selection
caspase recruitment domain (CRD)	35 (0.60)	0.6662	0.8262	481.4	481.0	480.8	no departure from neutrality
cytosine DNA-methyltransferase (DMT)	81 (0.84); 153 (0.84)	0.0000	0.1981	1034.2	1023.2	1021.6	purifying & weak positive selection
deoxynucleotide kinase (DNK)	44 (0.96); 45 (0.98)	0.0000	0.0182	1062.6	1043.8	1041.8	purifying & positive selection
eukaryotic initiation factor (EIF)		0.0000	0.9990	1165.6	1153.1	1153.1	purifying selection
ribonucleoside-diphosphate reductase (RDR)	204 (1.81); 243 (0.80); 381 (0.79); 443 (0.79); 552 (0.94)	0.0000	0.0900	2826.1	2814.8	2812.5	purifying & weak positive selection
ribonuclease III (RNA)		0.0000	0.9998	1714.6	1704.5	1704.5	purifying selection
tumor necrosis factor receptor (TNF)		0.1562	1.0000	372.9	371.0	371.0	no departure from neutrality
deoxyuridine triphosphatase (UTP)	34 (0.69)	0.2390	0.4728	676.7	675.3	674.5	no departure from neutrality

Ridenhour & Storfer, 2008; *JEB*
Storfer & Eastman, unpubl.

Molecular evolution: **POSITIVE** selection

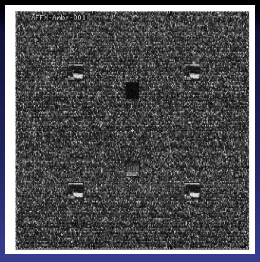
endemic versus **bait**-associated strains for genes **globally** under **positive** selection

Amino acid sites 'typically' under positive selection are more strongly selected in bait-shops (p = 0.007)

1) Why is ATV emerging?

Jennifer Stewart, (MS 2008)

Hypothesis: Weak host immune response
Approach: Microarray analysis



- Compare gene expression (mRNA transcript) levels in infected vs uninfected animals
- *A. mexicanum/ tigrinum* oligo (Affymetrix) chip with:
  - 4844 EST-based features
  - ~250 controls/replicates

Molecular Evolution: Host response

- Microarray study supports potential role of these viral genes:
  - Upregulation of genes in steroid pathway (*βOH)*
  - Downregulation of genes associated with:
    - Protein destruction (ubiqiutins)
    - Apoptosis (caspases; CRD)
    - Interferon pathway (VIF2-α; DMT)
    - Tumor recognition
  - limited adaptive immune response in host

Cotter, Storfer et al., 2008, *BMC Genomics*

1) Why do diseases emerge?

- ✓ Environment has changed
- How?
  - ✓ Bait strains under stronger selection at putative host immune evasion genes than native strains
  - Hypothesis: Bait environment artificially high density – could select for high virulence
    - Classic virulence-transmission tradeoff

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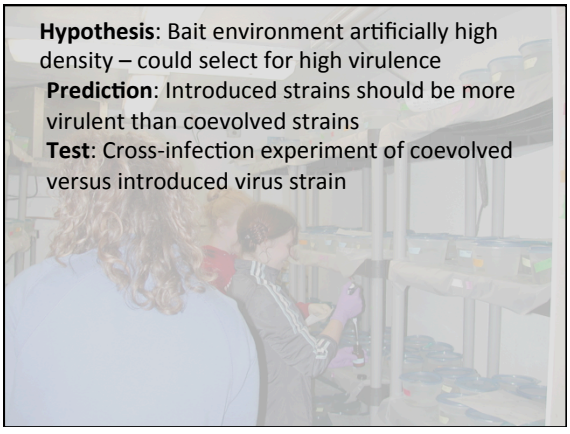
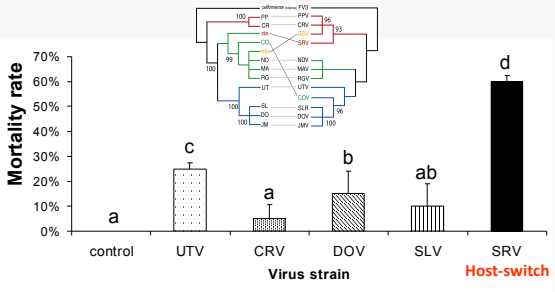


Virulence experiment results



Overall ANOVA ($F_{5,18} = 17.116$, $p < 0.0001$)

Storfer et al. 2007, *Ecology Letters*

Why is ATV emerging?

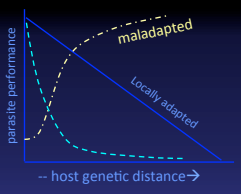
- Viral evolution
  - Artificial selection on bait strains
    - Particularly genes associated with host immune evasion
    - Genes do downregulate host defenses
  - Evolution of higher virulence in bait strains
    - Virulence-resistance trade-off relaxed
  - Movement of higher virulence strains through bait trade
    - OIE reportable pathogens

2) What is the capacity of ATV to spread?

- Concern: introduction and spread of more virulent bait strains
- Test: Local adaptation experiment
- Hypothesis: Virus strains should be locally adapted
  - Higher mutation rates, reproductive rates and shorter generation time than hosts

Measures of local adaptation

Home vs. away

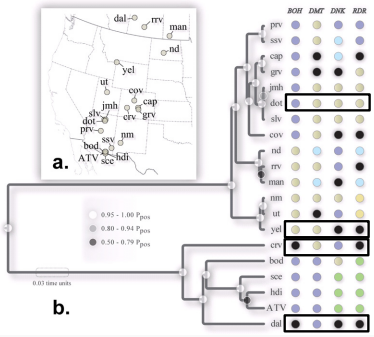


Practical application:
Can think of h v a as a measure of a pathogen's *invasiveness*

Local vs. foreign

Practical application:
Can think of l v f as a measure of a pathogen's *invasibility*

Geographic Pattern of Molecular Selection



4 ORFs associated with host immune evasion/ virulence.

Pattern not associated with phylogeny

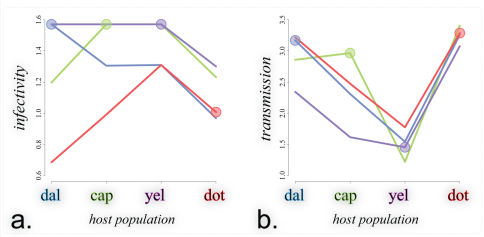
Suggests ATV strains undergo local selection

Some strains may be better adapted than others

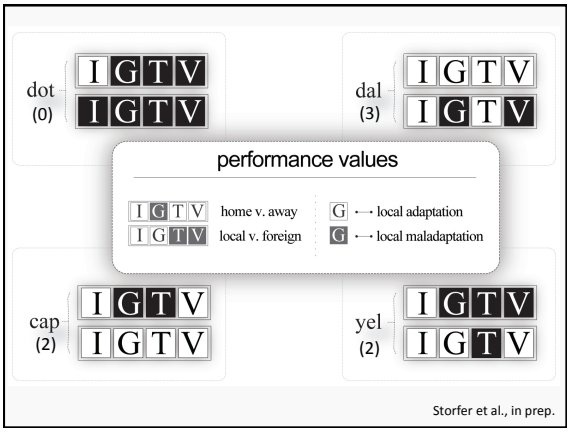
Methods:

- 4 x 5 factorial infection experiment
  - 4 host strains (DAL, CRV, YEL, DOT)
  - 5 virus treatments (DAL, CRV, YEL, DOT, control)
    - $10^{3.5}$ p.f.u./ ml
  - 30 animals per treatment
    - 600 animals total
- Response variables:
  - Infectivity, virulence (mortality), within-host growth (qPCR), transmission (qPCR)

Results: Infectivity vs Transmission

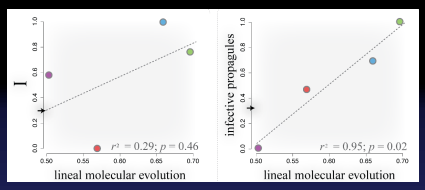


Storfer et al., in prep.



Storfer et al., in prep.

Viral fitness and molecular evolution



- Viral performance multivariate
  - Infectivity often sole measure
  - Can be misleading
- Performance strongly correlated with molecular evolutionary rate

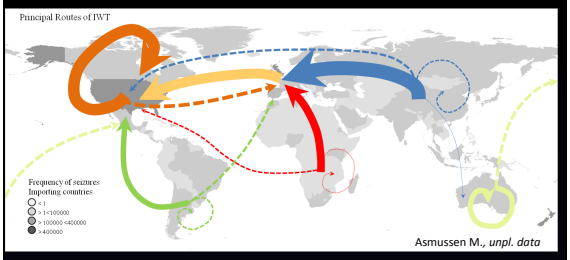
Conclusions: capacity to spread

- Two strains (DAL, CAP) appear locally adapted with host shifts potentially less likely
- One strain appears maladapted (DOT) with host shifts potentially more likely
- One strain (YEL) equivocal

Remaining questions about ATV/ ranaviruses in general?

- Evolution for higher virulence in captivity a general phenomenon?
- Evolution of hosts?

Global wildlife trade



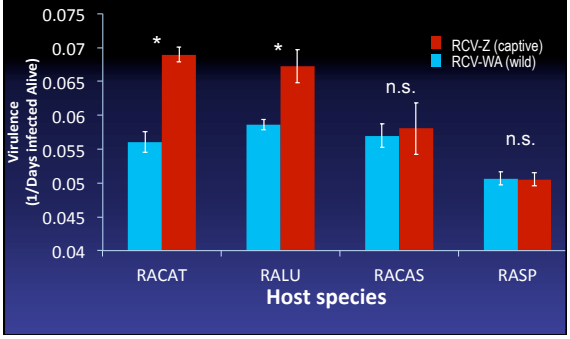
28 million amphibians imported into US between 2000-2005 with 8.5% prevalence of ranavirus infection

Bullfrogs are a problem

Bullfrogs (*Rana catesbeiana*)

Cross-infection experiment: farmed bullfrogs and their ranavirus vs. introduced bullfrogs & their virus

Bullfrog ranavirus virulence



Host evolution

- Common frogs infected for several generations showed selection for certain MHC alleles than uninfected populations (Teacher et al. 2009; *PLoS One*)
- Possible behavioral change (assortative mating) in common frogs after selection by disease (Teacher et al. 2009; *Mol Ecol*)
  - Maybe selectively mating with resistant individuals?
- Agile frog susceptibility related to background genetic diversity (Garner et al. 2005)

Emerging infectious diseases

1) Why is ATV emerging?

Phylogenetic concordance suggests virus is endemic (old); emerging because of:

✓ human artificial selection for, and introduction of, virulent bait strains

2) What is the capacity of ATV to spread?

a) Spatially variable selection on virus virulence genes

b) Local adaptation results mixed
   - Some strains may spread; others less likely

c) Future: experiments to test bait strain invasibility of naïve hosts and hosts with resident strains
   - Some strains may be invaded; others less likely

More studies needed on:

- Ranavirus evolution (in general)
- Host immunity/ response to RV infection
  - RNA work
- Spatial variation in die-offs among host population
- Genetic/ genomic causes for variability in host susceptibility