

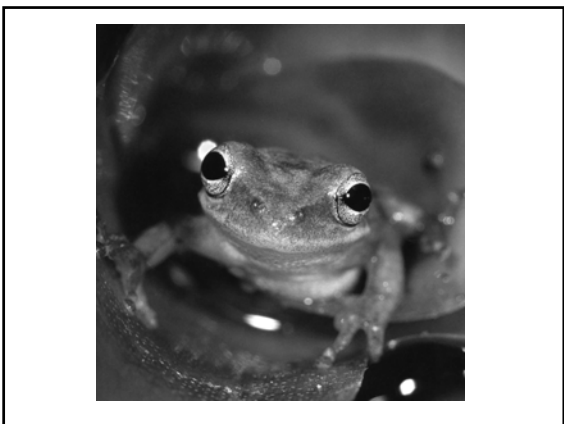
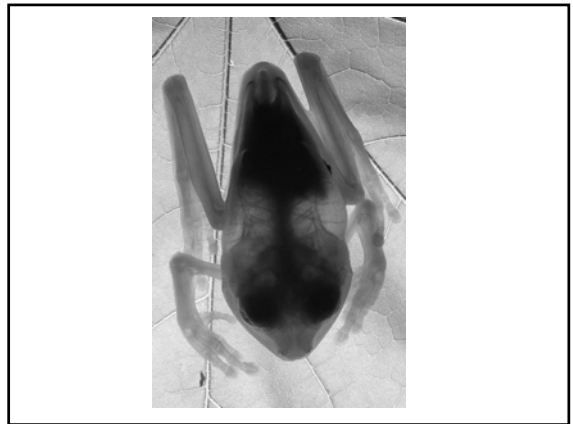
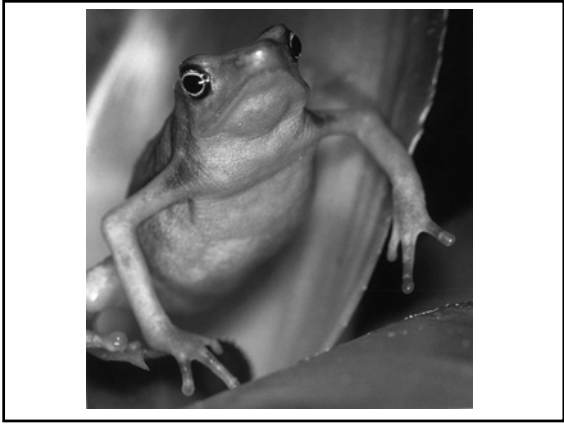
*There Are Approximately 4,000 Species of Frogs*

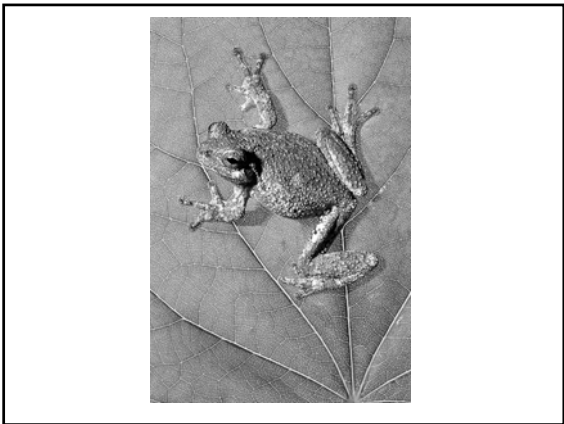


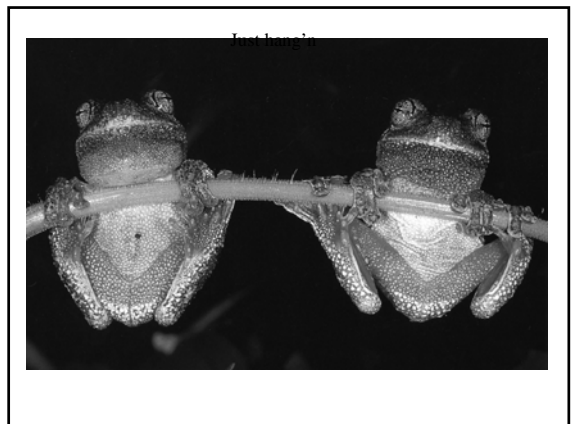
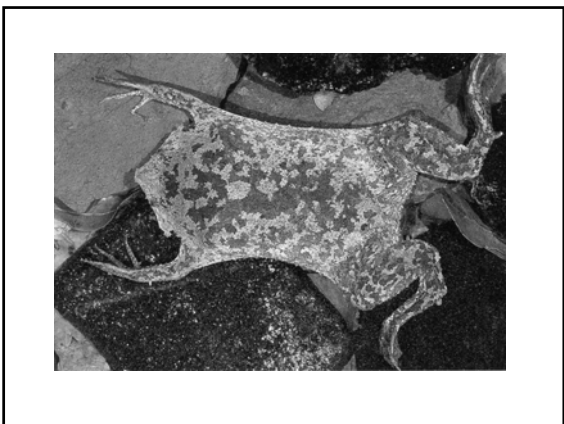
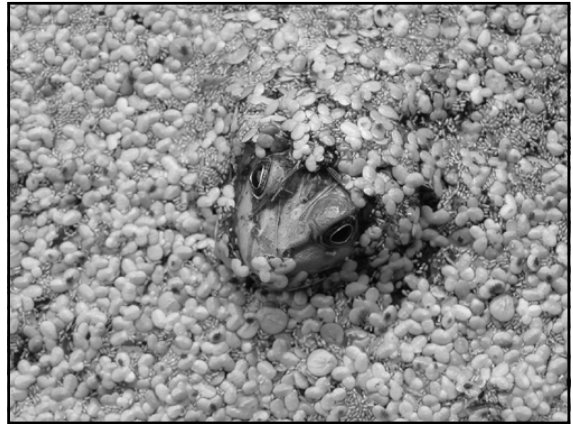
Here are a few of them

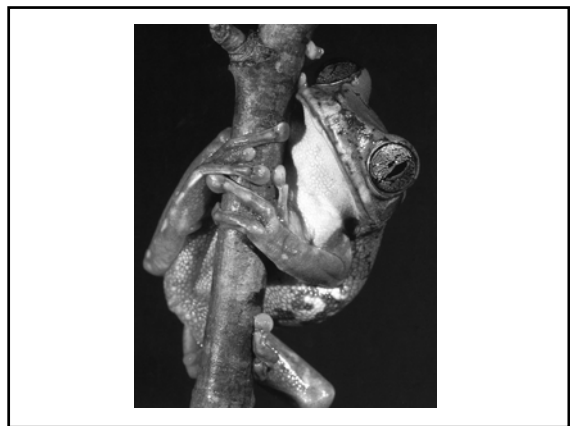
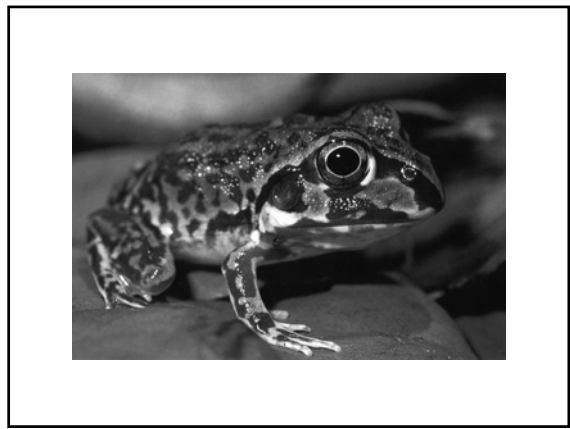
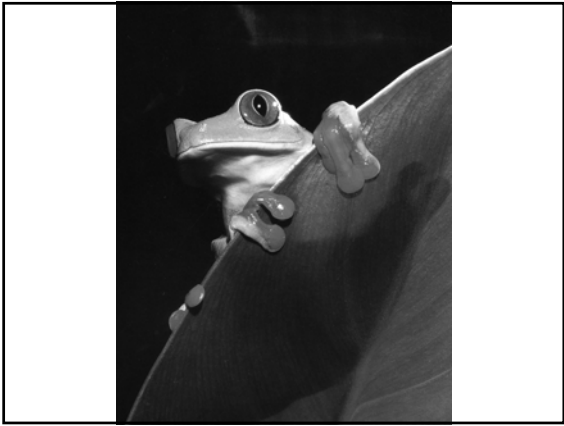
## **The Frog Gallery**

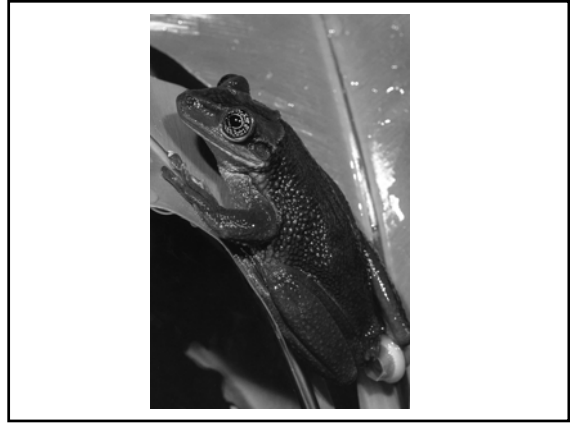


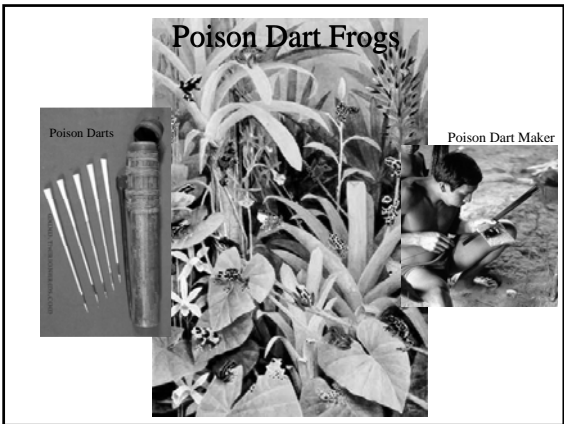
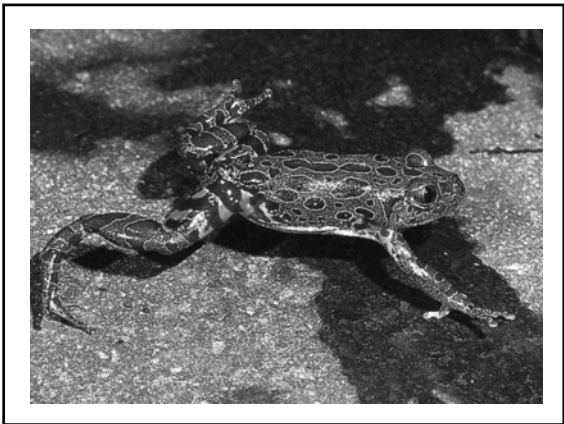


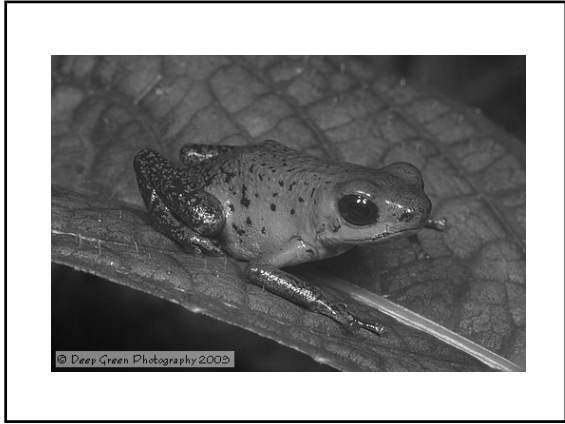






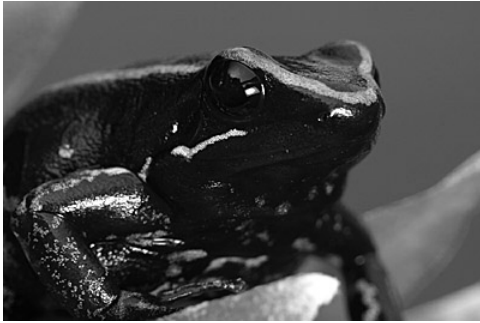








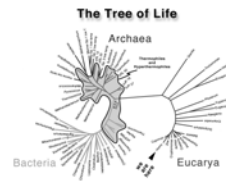
Or Blue



Interesting. Very, very interesting!

Biologically active peptides, amines, steroidal bufadienolides, and samandarine alkaloids represent some of the classes of compounds that amphibians elaborate and store in granular skin glands, apparently for chemical defense against microbial skin infections and/or predators. However, the wide variety of lipophilic alkaloids, discovered in skin of dendrobatid frogs, do not appear to be synthesized by such frogs, since captive-raised dendrobatid frogs completely lacked such alkaloids, but could sequester alkaloids unchanged into skin. Ants, beetles, and millipedes were found to represent the dietary source for many of the "dendrobatid alkaloids".

### Chopping Down The Tree Of Life



The frogs of the world  
have a few health problems

INTEGRATED RESEARCH CHALLENGES IN ENVIRONMENTAL BIOLOGY  
*The Global Decline of Amphibians*  
OVERVIEW OBJECTIVES MEMBERS PUBLICATIONS PROTOCOLS LINKS NEWS  
HOST-PATHOGEN BIOLOGY AND THE GLOBAL DECLINE OF AMPHIBIANS



**USGS**  
science for a changing world  
Biological Resources

**Evaluation of Solar Ultraviolet Radiation as a Factor in Amphibian Decline in Montane Habitats**

Duration: February 1999 - September 2003

Principal Contact: Dr. Edward E. Little, USGS  
Columbia Environmental Research Center

**The Volunteer Monitor**

The National Newsletter of Volunteer Water Quality Monitoring  
Vol. 10, No. 1, Spring 1998

Note: This information is provided for reference purposes only. Although the information provided here was accurate and current when first created, it is now outdated.

**Amphibian Decline: Monitors Search for Answers**

**NATIONAL WILDLIFE FEDERATION**

IN-DEPTH RESOURCES: OUR PROGRAMS / WHERE WE WORK

Home Wildlife Your Yard Take Action

**Frogwatch USA**  
Help scientists conserve frogs and toads

**Endangered Frog Research Centre**

Training

**Griffith UNIVERSITY**

1. Declining populations
2. Increased incidence of deformities

**IN SEARCH OF THE GOLDEN FROG**

MARTY CRUMP

## US Endangered Species: Amphibians

Amphibians	Species Name
Status	Species Name
T	Coqui, golden ( <i>Eleutherodactylus jasperi</i> )
T	Frog, California red-legged ( <i>Rana aurora draytoni</i> )
T	Frog, Chiricahua leopard ( <i>Rana chiricahuensis</i> )
E	Frog, Mississippi gopher ( <i>Rana capito swissa</i> )
T	Frog, mountain yellow-legged ( <i>Rana muscosa</i> )
E	Guaion ( <i>Eleutherodactylus cooki</i> )
E	Salamander, Barton Springs ( <i>Eurycea sosorum</i> )
E	Salamander, California tiger ( <i>Ambystoma californiense</i> )
T	Salamander, Cheat Mountain ( <i>Plethodon nettingi</i> )
E	Salamander, desert slender ( <i>Batrachoseps aridus</i> )
T	Salamander, flatwoods ( <i>Ambystoma cingulatum</i> )
T	Salamander, Red Hills ( <i>Phaeognathus hubrichti</i> )
T	Salamander, San Marcos ( <i>Eurycea nana</i> )
E	Salamander, Santa Cruz long-toed ( <i>Ambystoma macrodactylum croceum</i> )
E	Salamander, Shenandoah ( <i>Plethodon shenandoah</i> )
E	Salamander, Sonora tiger ( <i>Ambystoma tigrinum stebbinsi</i> )
E	Salamander, Texas blind ( <i>Typhlonothus californicus</i> )
E	Toad, arroyo (arroyo southwestern) ( <i>Bufo californicus (emicroscaphus)</i> )
E	Toad, Houston ( <i>Bufo houstonensis</i> )
E	Toad, Puerto Rican crested ( <i>Peltophryne lemuir</i> )
E	Toad, Wyoming ( <i>Bufo baxteri (hemiophrys)</i> )

## AUSTRALIA'S DECLINING FROGS

### Missing

- Armoured Mist Frog, *Litoria lorica*
- Mountain Mist Frog, *L. nyakalensis*
- Southern Gastric-brooding Frog, *Rheobatrachus silus*
- Northern Gastric-brooding Frog, *R. vitellinus*
- Sharp-knouted Day Frog, *Taudactylus acutirostris*
- Southern Day Frog, *T. diurnus*
- Northern Tinker Frog, *T. rheophilus*

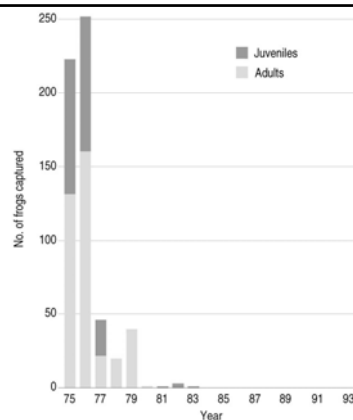
### Declining

- Green and Golden Bell Frog, *L. aurea*
- Yellow-Spotted Tree Frog, *Litoria castanea*
- Freyinger's Frog, *L. freyingeri*
- Green-eyed Tree Frog, *L. genimaculata*
- Waterfall Frog, *L. ranmolle*
- Wallum Sedge Frog, *L. olongburenensis*
- Cascade Tree Frog, *L. pearsoniana*
- Common Mist Frog, *L. rheocola*
- Spotted Tree Frog, *Litoria spenceri*
- Australian Lace-lid, *Nyctimystes dayi*
- Eungella Day Frog, *T. eungellensis*
- Wallum Froglet, *Cinia cinerea*
- Fleay's Barred Frog, *Mixophyes fleayi*
- Giant Barred Frog, *M. iteratus*
- Southern Corroboree Frog, *Pseudophryne corroboree*

## Tarahumara frog (*Rana tarahumarae*) in Mexico.



Fig. 1. Range of the Tarahumara frog, *Rana tarahumarae*. Copper smelters are at Douglas, AZ (now closed), and Cananea and Nacoziari, Sonora. Historical locations include both surveyed populations that appeared stable, and unvisited historical localities (Campbell 1931; Little 1940; Williams 1960; Hale et al. 1977; Hale and May 1983; Hale and Jarchow 1988).



We conclude that the Tarahumara frog is not threatened with extinction throughout its range at this time, although the sudden declines and local extirpations in northern populations, coincident with declines of leopard frogs, are a serious concern.

## Over 100 Frog Species Discovered in Sri Lanka

John Pickrell  
for National Geographic News  
October 16, 2002



Declines in the US are particularly serious in:

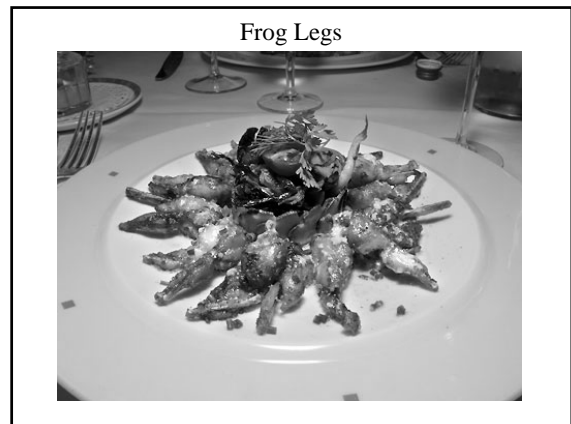
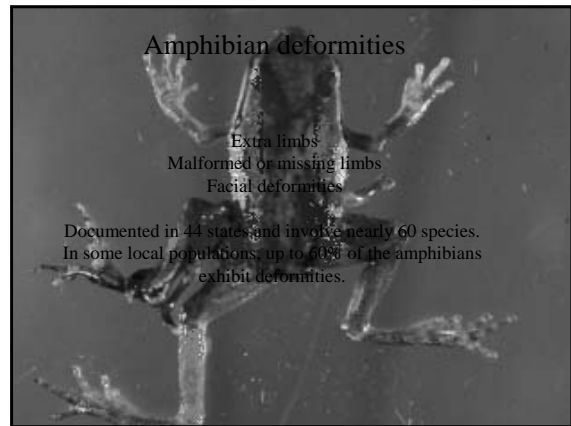
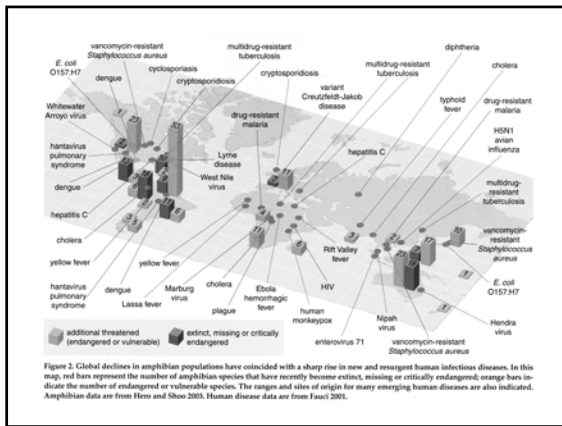
California

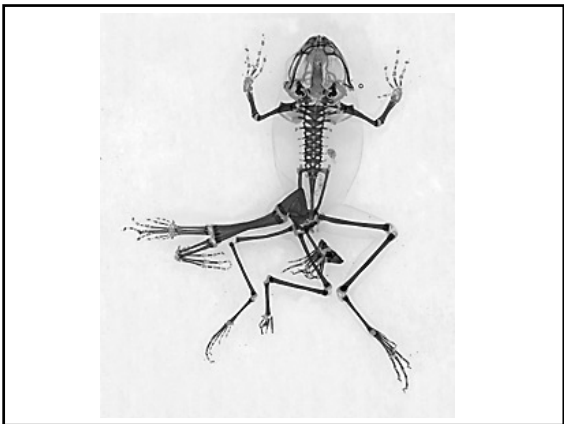
The Rocky Mountains

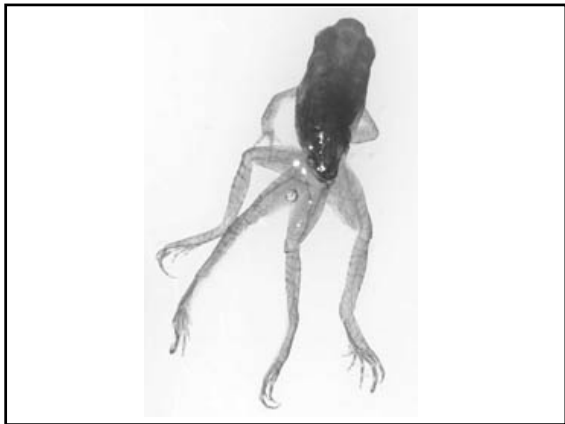
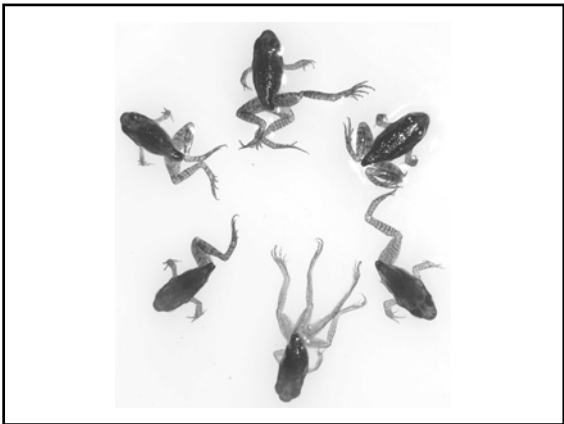
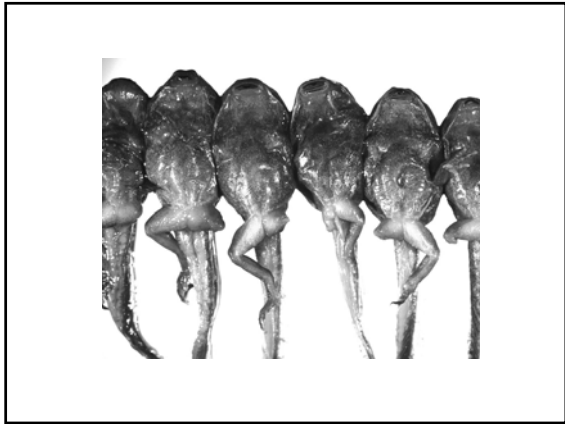
The Southwest

Puerto Rico.

Worldwide, decline "hot spots" also include Australia and Central America.





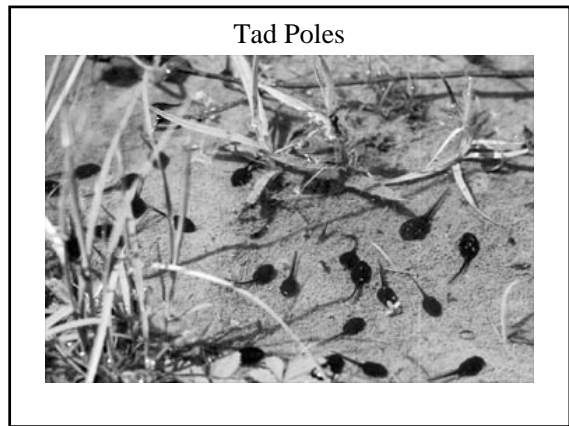
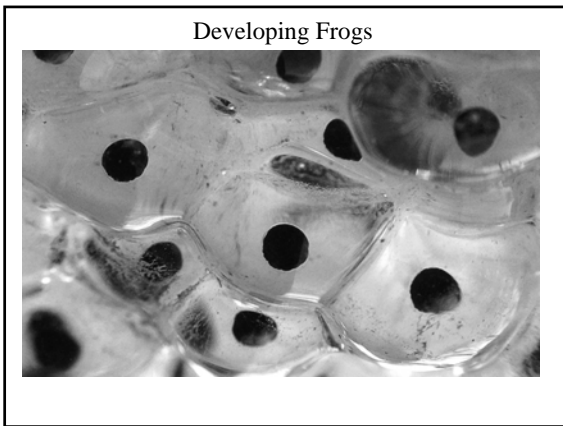
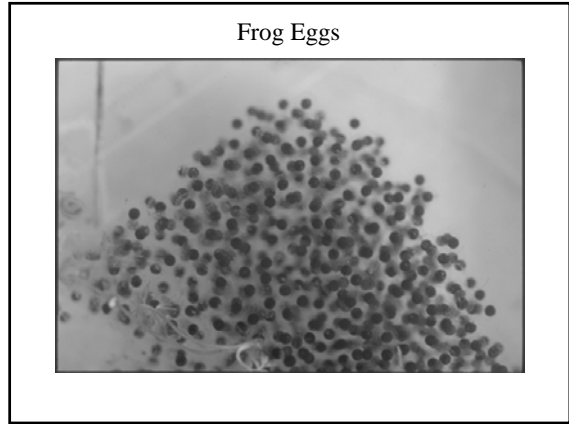
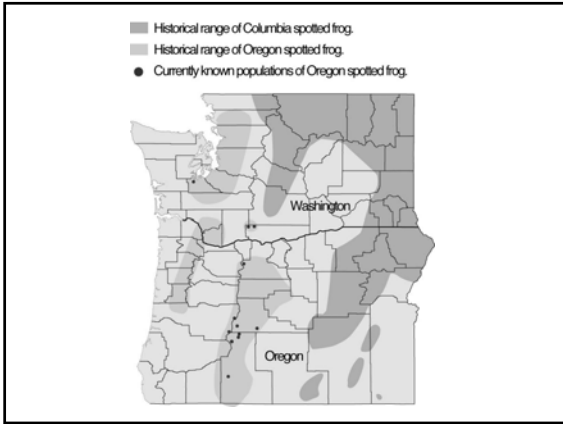


Andrew Blaustein  
and friends



Andrew Blaustein at work





### MORE UV = TROUBLE FOR FROGS

The most dangerous kind of UV rays is UV-B. When they get past the ozone layer - frogs look out! Fur, feathers, clothes or sunscreen can stop UV-B. Frogs don't have any of these things to protect themselves.

UV-B rays are even more dangerous to frogs' eggs. The eggs don't have a hard shell to protect them. Most eggs are laid in shallow water, close to land. The UV-B rays have no problem getting to the eggs. UV-B rays can stop frog eggs from hatching.

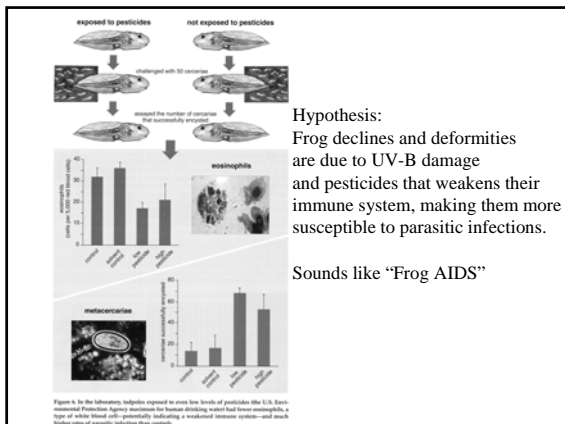
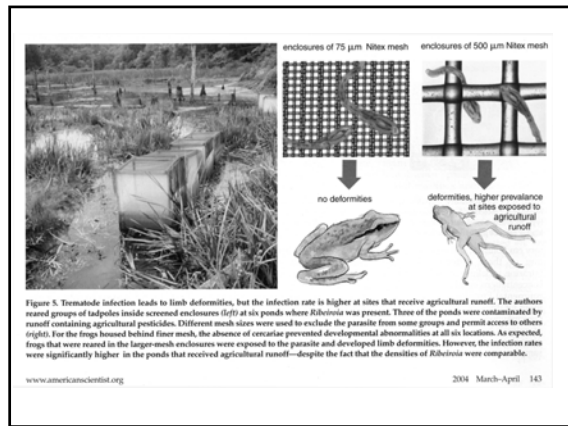
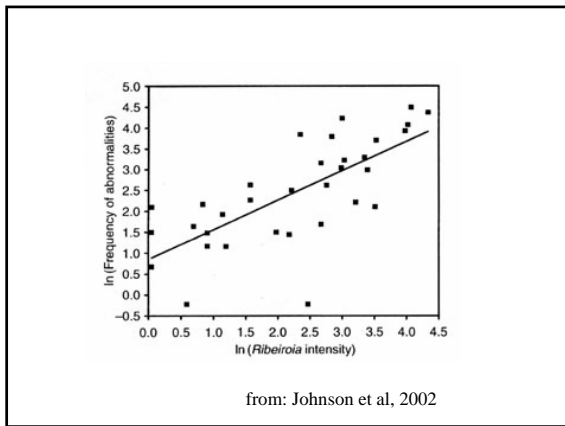
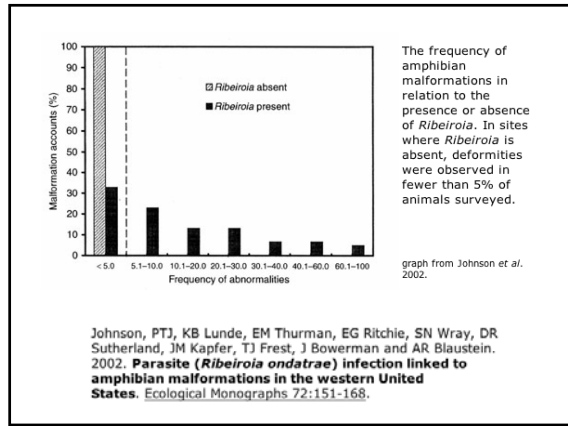
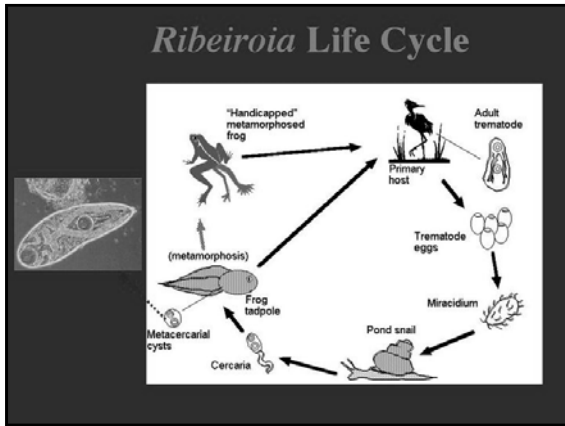
Scientists also think UV-B rays can cause frogs to be born with extra legs or make other weird things happen to their bodies.

UV                      Ozone                      Trouble

### Possible causes of declines and malformations

1. Increased levels of UV-B radiation
2. Chemical agents (pesticides, herbicides, fertilizers)
3. Parasites - Ribeiroia, a trematode

A black and white photograph of a single Ribeiroia trematode parasite. It is an elongated, oval-shaped organism with a complex internal structure, including what appears to be a central body and several branching or internal organs.



### Blaustein References

Blaustein, A.R., Kiesecker, J.M., Chivers, D.P., and R.G. Anthony. 1997. Ambient UV-B radiation causes deformities in amphibian embryos. *Proceedings of the National Academy of Sciences*, 94:1375-1377.

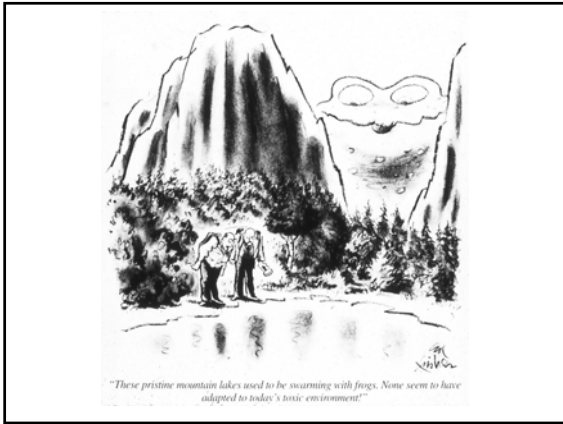
Blaustein, A.R., Hoffman, P.D., Kiesecker, J.M. and J.B. Hays. 1996. DNA repair and resistance to solar UV-B radiation in eggs of the red-legged frog. *Conservation Biology*, 10:1396-1402.

Blaustein, A.R. and D.B. Wake. 1995. The puzzle of declining amphibian populations. *Scientific American*, 272:52-57.

Hokit, D.G. and A.R. Blaustein. 1997. The effects of kinship on interactions between tadpoles of *Rana cascadae*. *Ecology*, 78:1722-1735.

Hokit, D.G. Walls, S.C., and A.R. Blaustein. 1996. Context-dependent kin discrimination in larvae of the marbled salamander. *Animal Behaviour*, 52:17-31.

Kiesecker, D.G. and A.R. Blaustein. 1997. Population differences in responses of red-legged frogs (*Rana aurora*) to introduced bullfrogs (*Rana catesbeiana*). *Ecology*, 78:1752-1760.



Q: Who allows this?

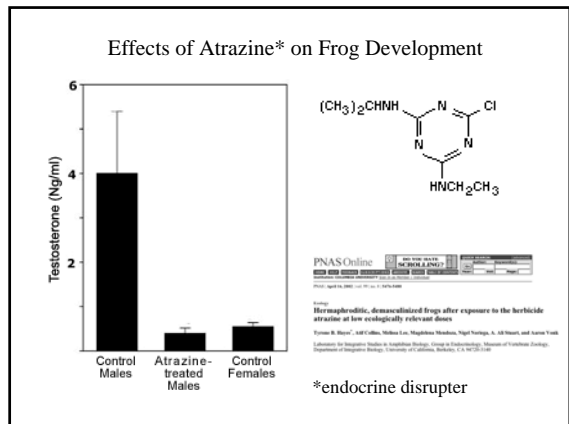
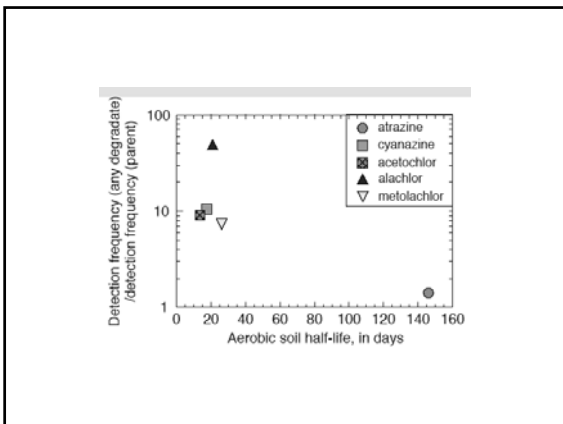
A: The EPA:  
Agricultural runoff is exempt from  
The Clean Water Act


See: <http://www.epa.gov/region5/water/cwa.htm>

Herbicide:  
Atrazine Usage: 2001

State	Pounds of Atrazine
CO, GA, KY, NC, ND, NY, PA, SD, TX, WI	Between 166,000 and 1,915,000
MI, MO, MN	Between 1,915,000 and 3,664,000
KS, OH	Between 3,664,000 and 5,413,000
NE	Between 5,413,000 and 7,162,000
IA, IN	Between 7,162,000 and 8,911,000
IL	Between 12,409,000 and 14,198,000

No data or very little data:  
AL, AR, AZ, CA, CT, DC, DE, FL, ID,  
LA, MA, MD, ME, MS, MT, NH, NJ,  
NM, NV, OK, OR, RI, SC, TN, VA, VT,  
WA, WV, WY

CN(C)C1=NC=NC(Cl)=N1


earth observatory 

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glossary on/off

**News**

**MEDIA ALERTS ARCHIVE**

July 8, 2002

**DEFORMED FROGS FORM WHEN PARASITES AND PESTICIDES COMBINE**

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**Pesticides, Parasite May Cause Frog Deformities**

Stentor Danielson  
National Geographic News  
July 9, 2002

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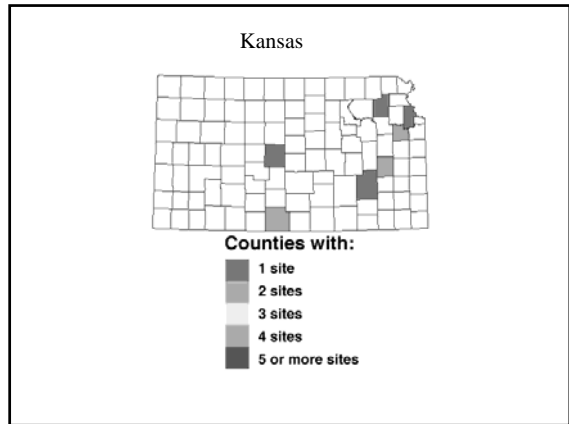
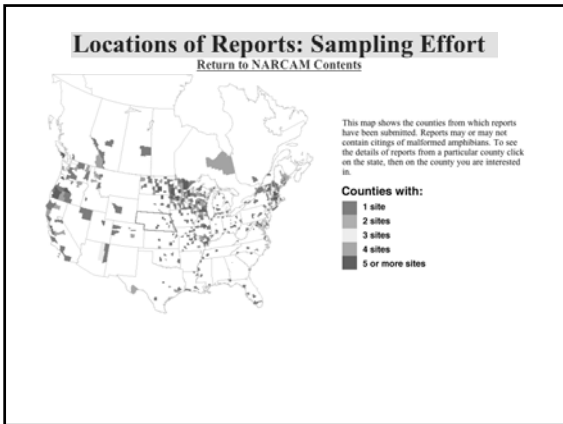
**Hermaphrodite Frogs Caused By Popular Weed Killer?**

By Rigel P. Driscoll  
National Geographic Today  
April 16, 2002

USGS  
ADVANCED INFORMATION SYSTEMS  
OMB 1028-0056, Expiration: 9/31/04

**NORTH AMERICAN REPORTING CENTER FOR AMPHIBIAN MALFORMATIONS**

Northern Prairie  
Wildlife Research Center  
Biological Resources Division  
U.S. Geological Survey  
Jamestown, North Dakota  
<http://www.npwrc.usgs.gov/narcam>





Some background first.

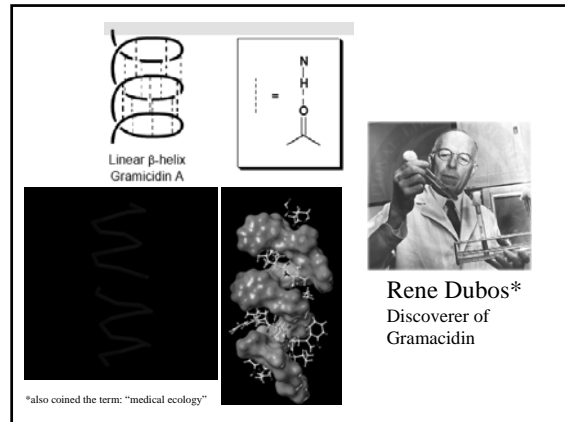
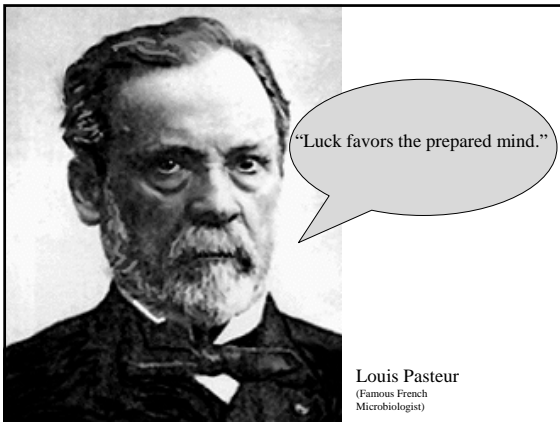
## A 21st Century Dilemma: Microbial Drug Resistance\*

\*See: <http://www.fda.gov/cvm/antimicrobial/022300slides/White/tdm004.htm>

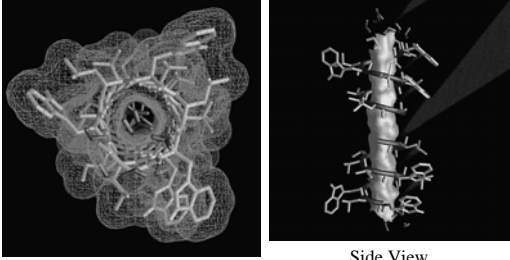
### Top Ten Drug-resistant Microbes

Microbe	Disease	Resistance
Enterobacteriaceae	bacteremia, pneumonia wound infections	aminoglycosides β-lactams chloramphenicol trimethoprim FQs
Enterococcus	bacteremia wound infections	aminoglycosides β-lactams erythromycin glycopeptides
<i>Haemophilus influenzae</i>	meningitis, otitis media pneumonia	β-lactams tetracycline chloramphenicol trimethoprim
<i>Mycobacterium tuberculosis</i>	tuberculosis	aminoglycosides ethambutol isoniazid rifampin
<i>Neisseria gonorrhoea</i>	gonorrhea	β-lactams tetracycline FQs

Microbe	Disease	Resistance
<i>Plasmodium falciparum</i>	malaria	chloroquine
<i>Pseudomonas aeruginosa</i>	bacteremia, pneumonia UTIs	aminoglycosides β-lactams tetracycline TMX
<i>Shigella dysenteriae</i>	severe dysentery	ampicillin chloramphenicol tetracycline TMX
<i>Staphylococcus aureus</i>	bacteremia, pneumonia wound infections	chloramphenicol macrolides trimethoprim FQs β-lactams tetracycline glycopeptides?
<i>Streptococcus pneumoniae</i>	meningitis, pneumonia	aminoglycosides chloramphenicol macrolides penicillin



## Gramicidin



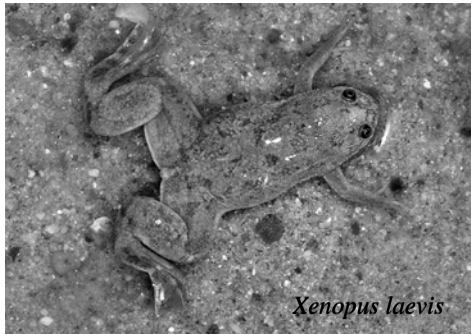
Side View

Gramicidin is a much studied channel "protein". This structure, obtained by molecular dynamics (Jacobs and Brennaman), is shown with the surface in mesh form. The backbone bonds are in gold, the side chains in white. In the channel itself are waters; the channel first appears in surface when a probe radius of 1.0 angstrom is used, but not before, making it quite narrow for the water column.

## Michael Zasloff

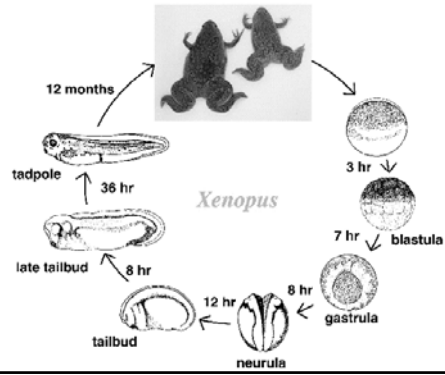


## The African Clawed Frog



*Xenopus laevis*

## Life Cycle of *Xenopus laevis*

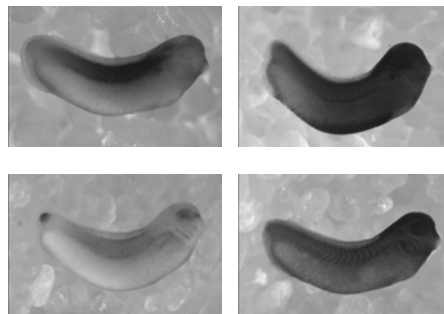


## Transgenic frogs

**A binary transgenic system used often in flies has been successfully adapted to drive transgene expression in *Xenopus*.** | By Jonathan B Weitzman

The binary Gal4-UAS system has been used to drive the tissue-specific expression of transgenes in a number of animal models. In the February 5 issue of Proceedings of the National Academy of Sciences, Katharine Hartley and colleagues at the Wellcome/CRC Institute Cambridge, UK, report application of the Gal4-UAS system to create transgenic *Xenopus* (*Proc Natl Acad Sci USA* 2002, 99:1377-1382).

## Examples of *in situ* hybridization in *Xenopus laevis* tad poles



### Selected References

1. Kroll, K. and E. Amaya, Transgenic *Xenopus* embryos from sperm nuclear transplantations reveal FGF signaling requirements during gastrulation. *Development*, 1996, **122**(10): p. 3173-3183.
2. Amaya, E. and K.K. Kroll, A method for generating transgenic frog embryos. in *Methods in Molecular Biology*. P. Sharpe and I. Mason, Editors. 1996, Humana Press: Totowa, N.J.
3. Gerhart, J., et al., Localization and induction in early development of *Xenopus*. *Phil. Trans. R. Soc. Lond. B*, 1984, **307**: p. 319-330.
4. Keller, R., Early embryonic development of *Xenopus laevis*. *Methods Cell Biol*, 1991, **36**: p. 61-113.
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6. Lallier, T., C. Whitaker, and D. DeSimone, Integrin  $\alpha 5$  is required for early nervous system development in *Xenopus laevis*. *Development*, 1998, **122**.
7. Smith, J.C., A mesoderm inducing factor is produced by a *Xenopus* cell line. *Development*, 1987, **99**: p. 3-14.
8. Smith, W.C. and R.M. Harland, Injected *Xant-8* RNA acts early in *Xenopus* embryos to promote formation of a vegetal dorsaling center. *Cell*, 1991, **67**: p. 753-765.
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In most experiments in which *Xenopus laevis* was used as an egg donor, a 20% mortality rate was encountered. Michael Zaslloff asked why so many *survived*, given the unsanitary conditions of their "recovery room."

#### Cleaning\*

All frog tanks are cleaned 3 times per week (usually Tu, Th, Sat). Tanks are drained, leaving an inch or two of water in the bottom, and uneaten food is swept down the drain. The tank walls and floor are gently scrubbed and rinsed down and then the tank is refilled. The tank scrubbers are treated with ethanol and rinsed between each tank. We use drain covers

\*current situation

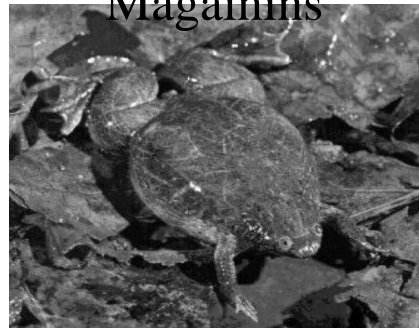
### Michael Zaslloff References\*

Matsuzaki K, Mitani Y, Akada KY, Murase O, Yonevama S, Zaslloff M, Miyajima K.  
Mechanism of synergism between antimicrobial peptides magainin 2 and PGLa.  
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Tubian JA, Drinkard L, Zaslloff M.  
tRNA nuclear transport: defining the critical regions of human tRNAmet by point mutagenesis.  
*Cell*. 1985, **43**:415-22.  
PMID: 3852693

\* note the shift in research interests

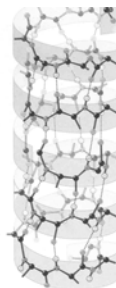
## Magainins



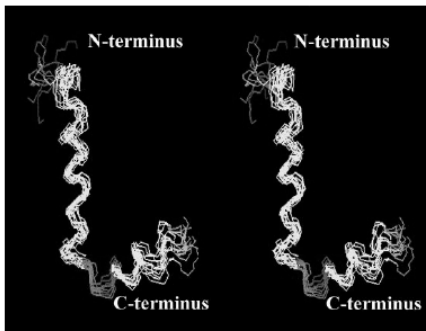
### Magainins:

1. Amphipathic peptides (21-26 AA residues)
2. Found in frog skin
3. Form right handed helixes
4. Have anti-bacterial (both gram + and gram -), anti-fungal, and anti-protozal activity
5. They form pores in lipid bi-layers

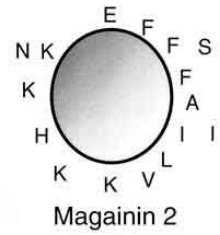
### Structure Of Magainins



### 3-D Structure Of Prototypical Magainin

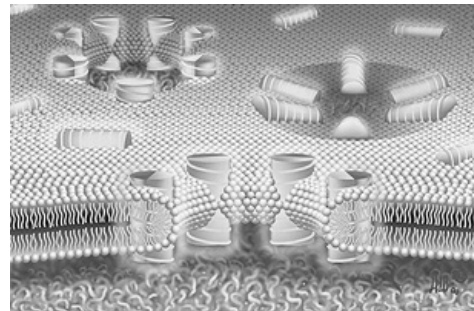


### Wheel Structure of Magainin 2

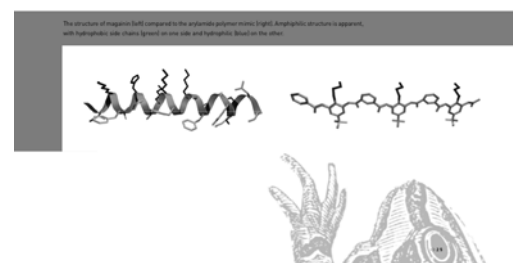
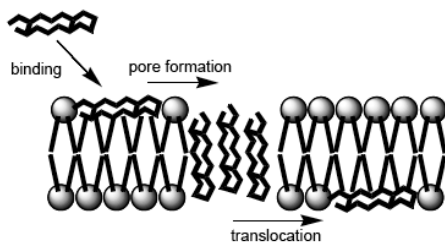


Behaves chemically like Gramicidin

### Mode Of Action Of Magainin



### Mechanism of action of magainin



Each species of frog produces between 8-10 different kinds of magainin. Since there are approximately 4,000 frog species still out there, some of these magainins may prove useful for each of our own antibiotic needs.

*Sometimes Its Easy Being Green!*



Sometimes its not!



\* You never know when you might need one!