ESPM
Forensic Genetic Analysis
19 March, 2015

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Who done it?

The case of Colin Pitchfork

- Narborough England 1983 and Leicestershire England 1987: 2 brutal rapes/murders of 15-year-old girls unsolved. Cases so closely matched that police strongly believed a single suspect committed both.
- A 17-year-old suspect, Richard John Buckland, first denied involvement, but under extensive questioning admitted to the second but not the first murder
- 1984, Leicester University: Professor Alec Jeffreys develops techniques for DNA fingerprinting
- Genetic comparison of crime scene and suspect's blood samples showed he was not responsible for either murder.

- **Richard John Buckland was the first person exonerated of a crime by DNA evidence.**

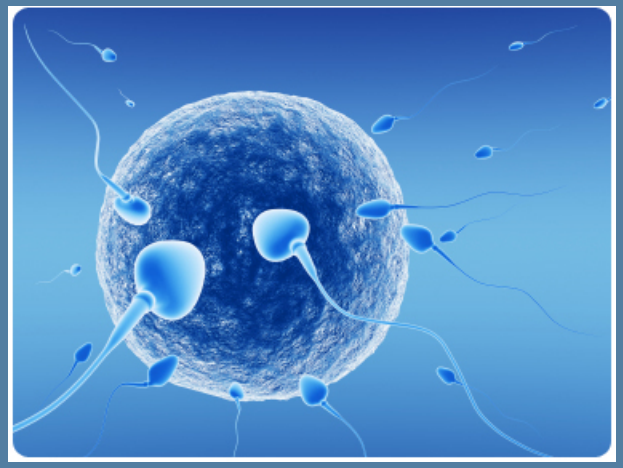
The case of Colin Pitchfork

- Police subsequently took blood samples from every 13-30-year-old man in 3 local villages
- A local bakery owner overheard a conversation where one man bragged about paying someone else to provide a sample on his behalf, reported him to police, and man was apprehended
- DNA evidence implicated the man, Colin Pitchfork, in the crimes -- the first person to be convicted based on genetic fingerprinting

How can we assess the relatedness of individuals?

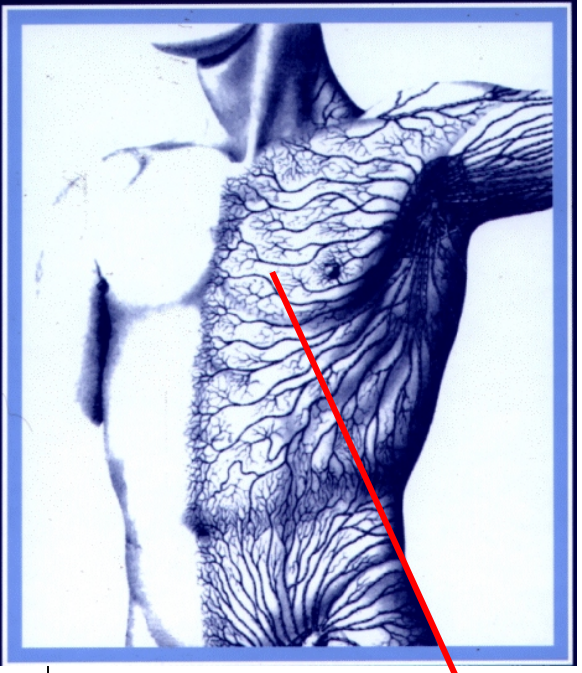
Inheritance = Passing of genetic material (DNA) from parent to offspring

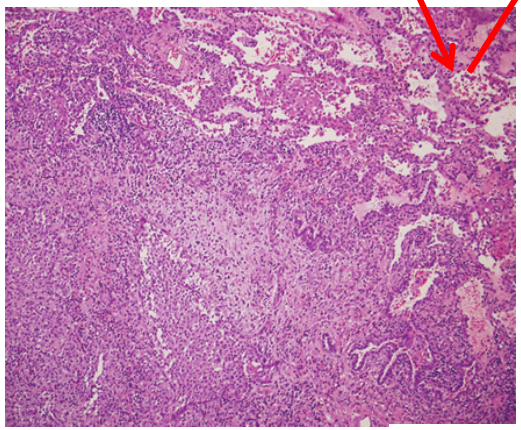
Relatedness = proportion of genetic material shared between individuals

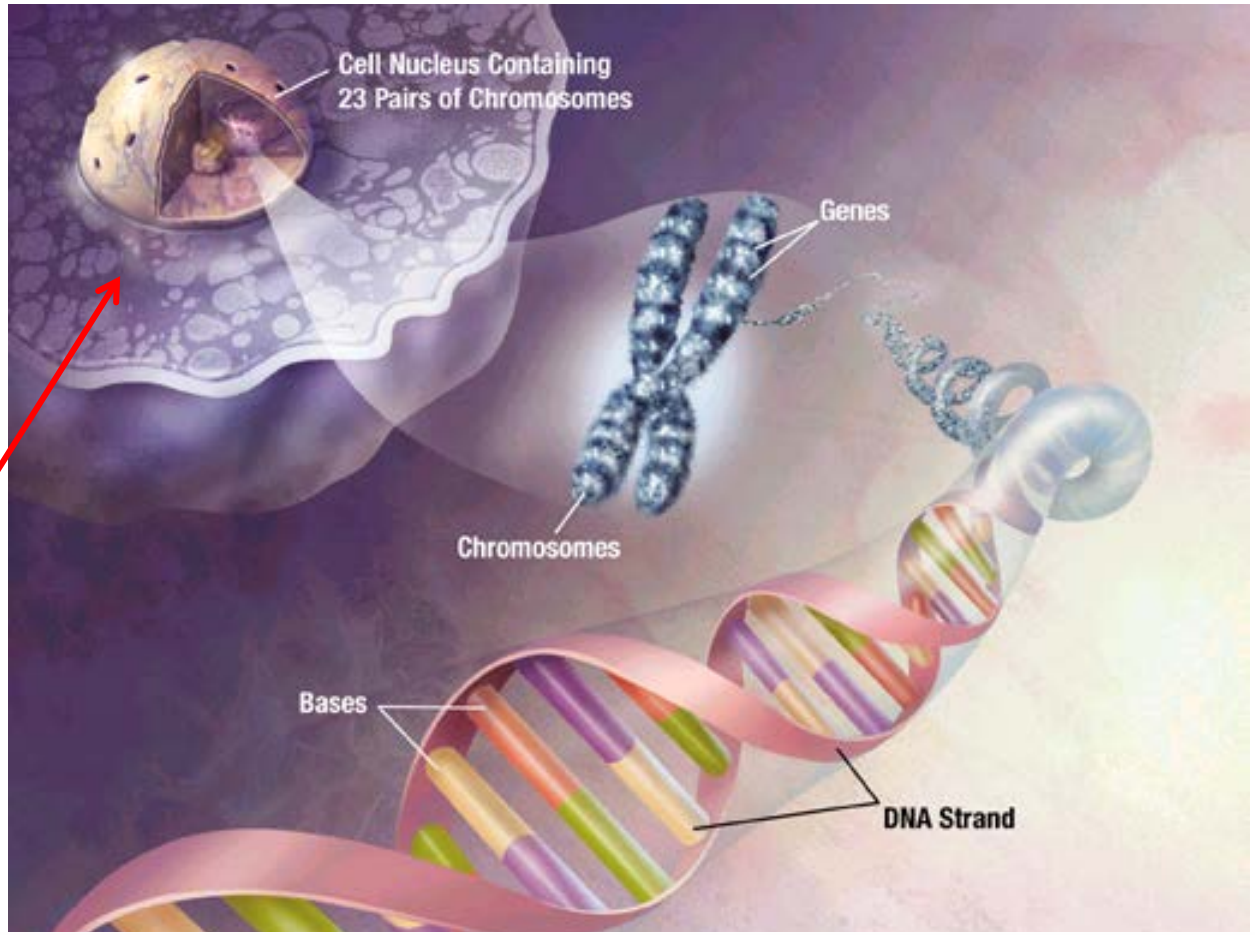


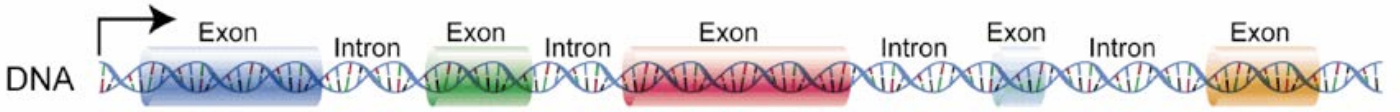
I. A Brief Genetics Primer

From Tissues to Cells to Genes

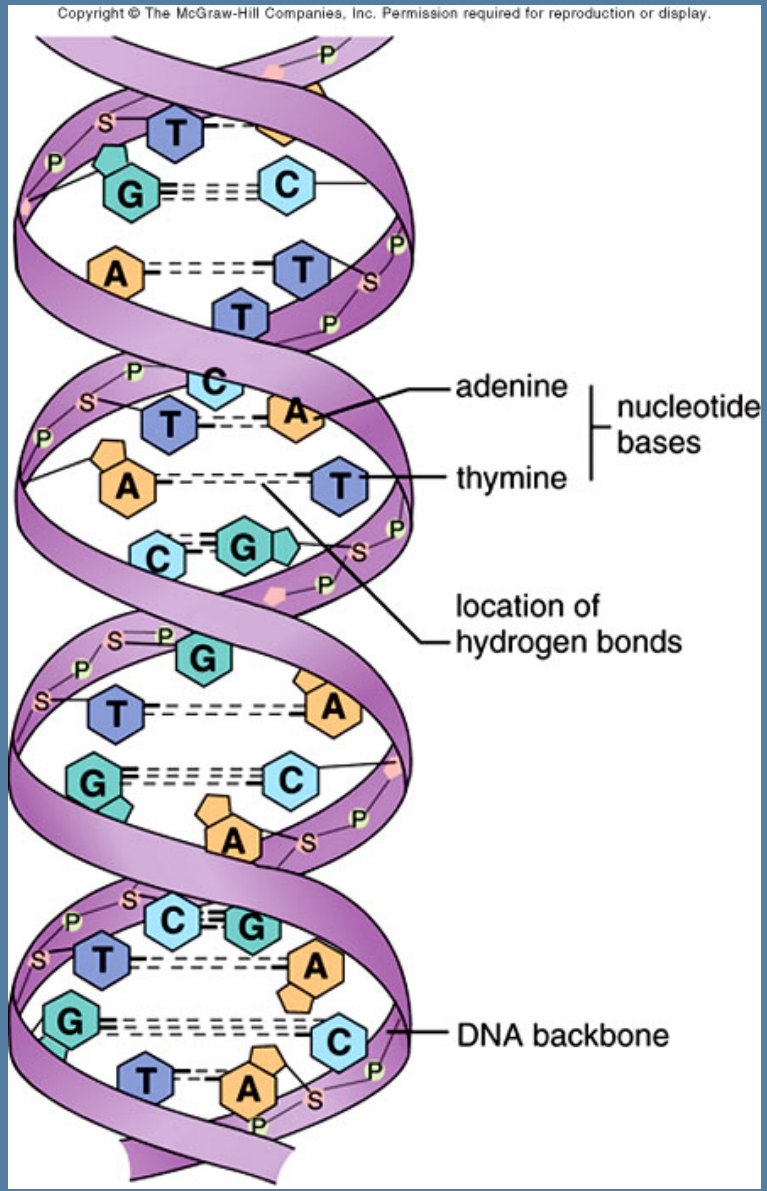








Sources: (clockwise from upper left: http://www.healthinmotion.net/HIM/HTM/LS.html; http://www.alzheimers.org/rmedia/IMAGES/LOW/Dna_low.jpg; http://radiographics.rsna.org



DNA Structure

• DNA is the genetic information-carrying molecule in a cell

• 4 building blocks (bases): **A**denine, **C**ytosine, **G**uanine, and **T**hymine

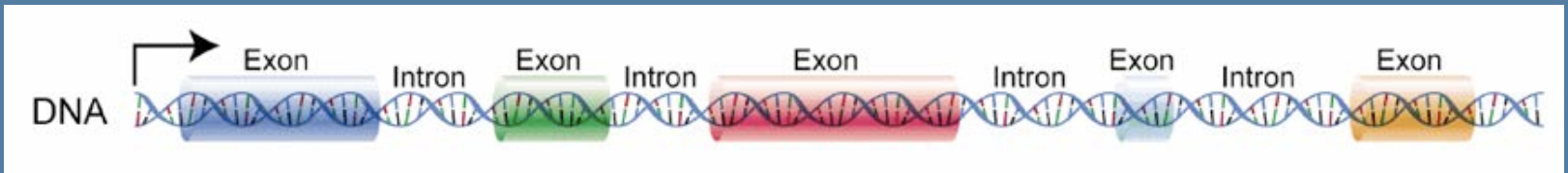
A and **T** bind together

G and **C** bind together

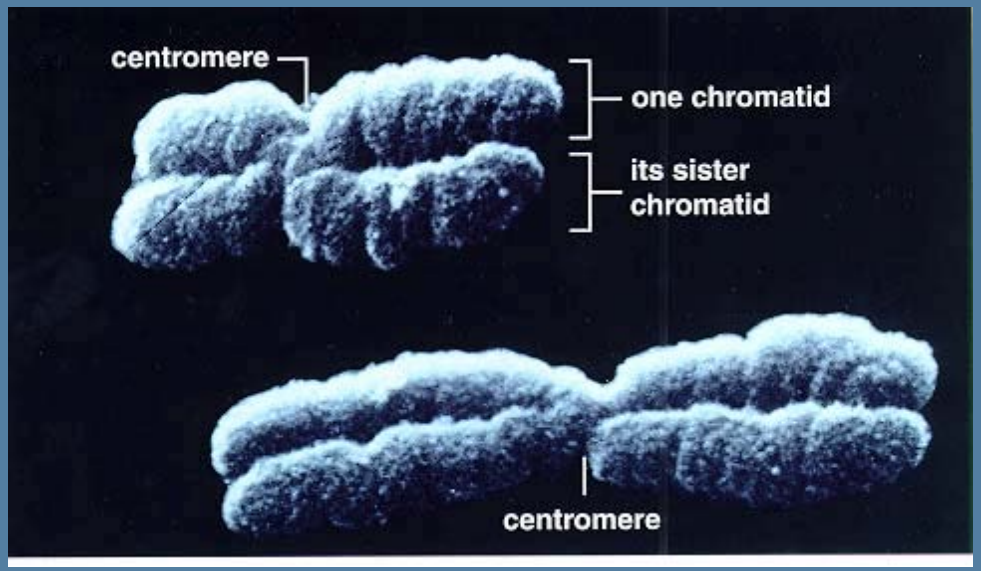
• 2 strands arranged in a double helix

• The **sequence** of a piece of DNA is the order of its bases, depicted as a string of letters (e.g., TGCATTACTACGTG)

• Because of the predictable pattern of **complementary binding** (A +T, G + C), if we determine the sequence of one strand, we automatically know the sequence of the other strand

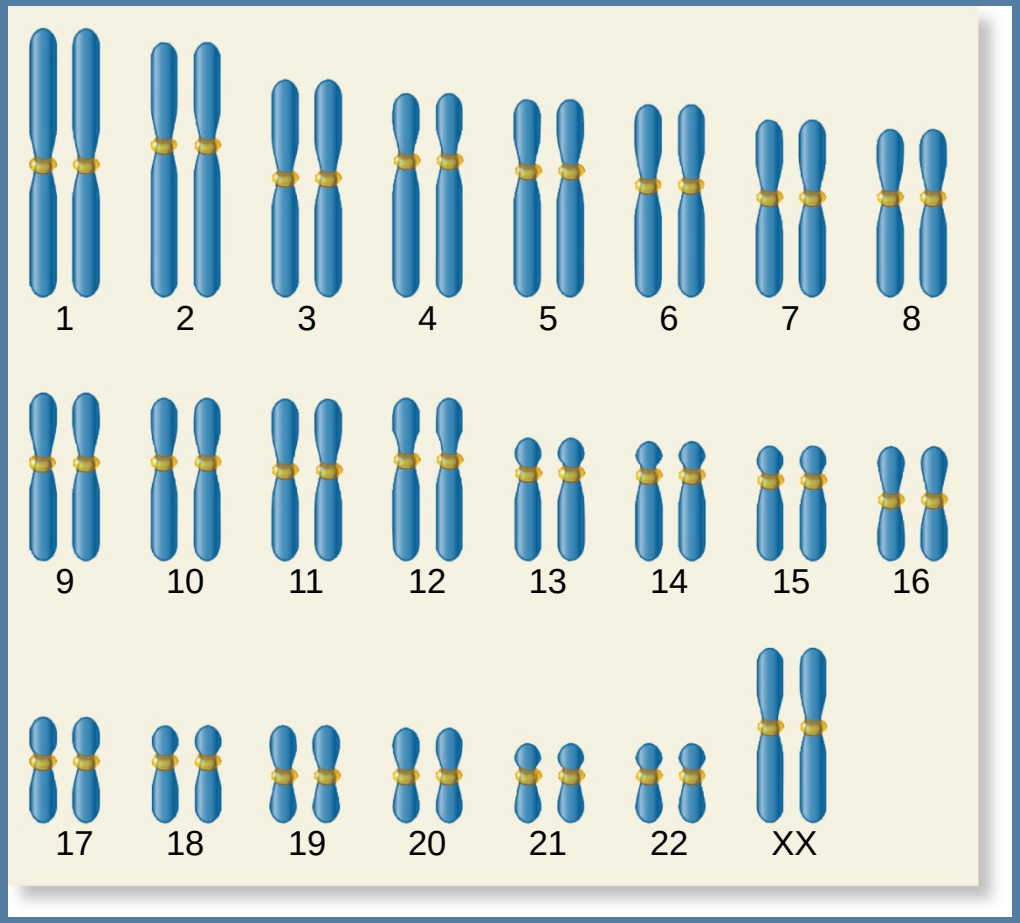


In Eukaryotes DNA is Arranged into Chromosomes

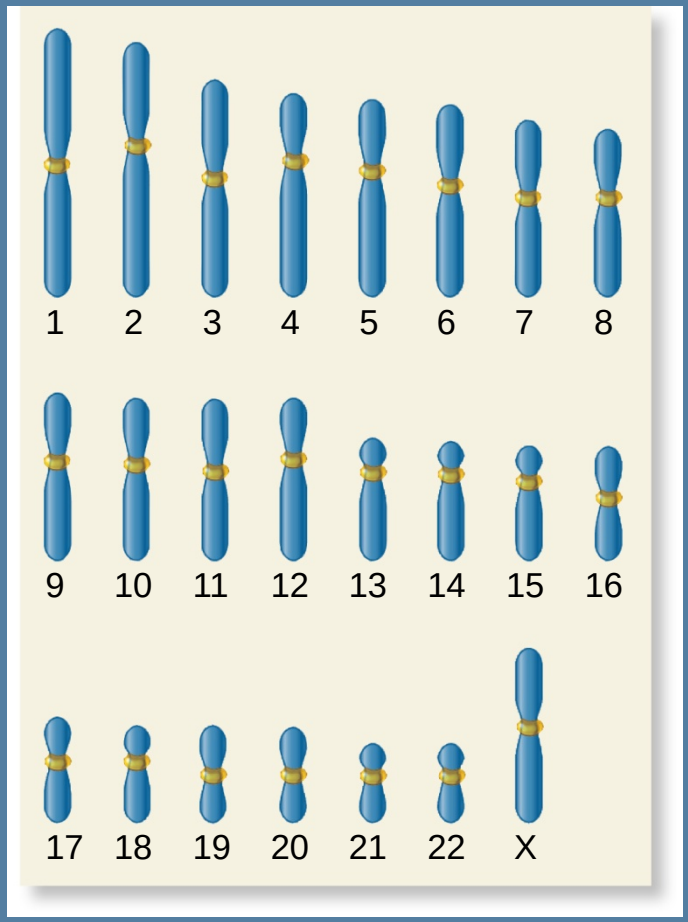


http://www.uic.edu/classes/bios/bios100/lecturesf04am/realchromo.jpg

Our chromosomes come in pairs: one from our mother, one from our father.

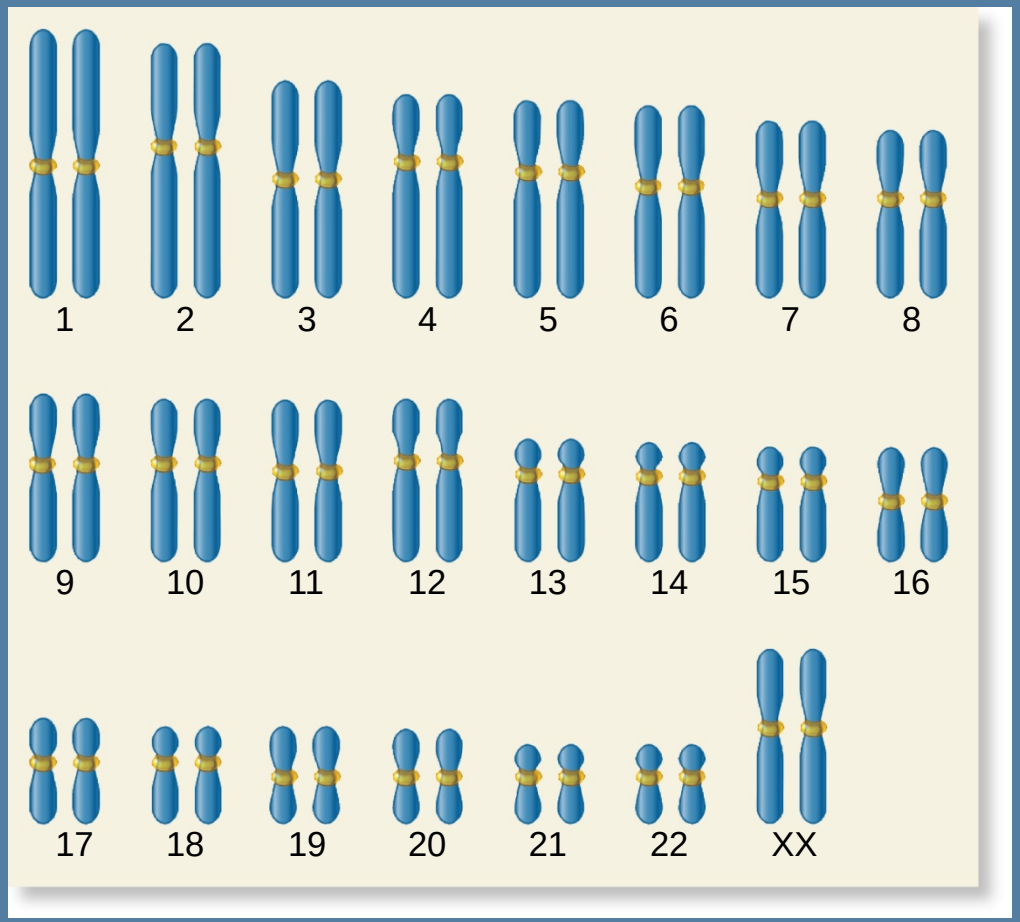


(a) Chromosomal composition found in human cells (46 chromosomes / 23 pairs)

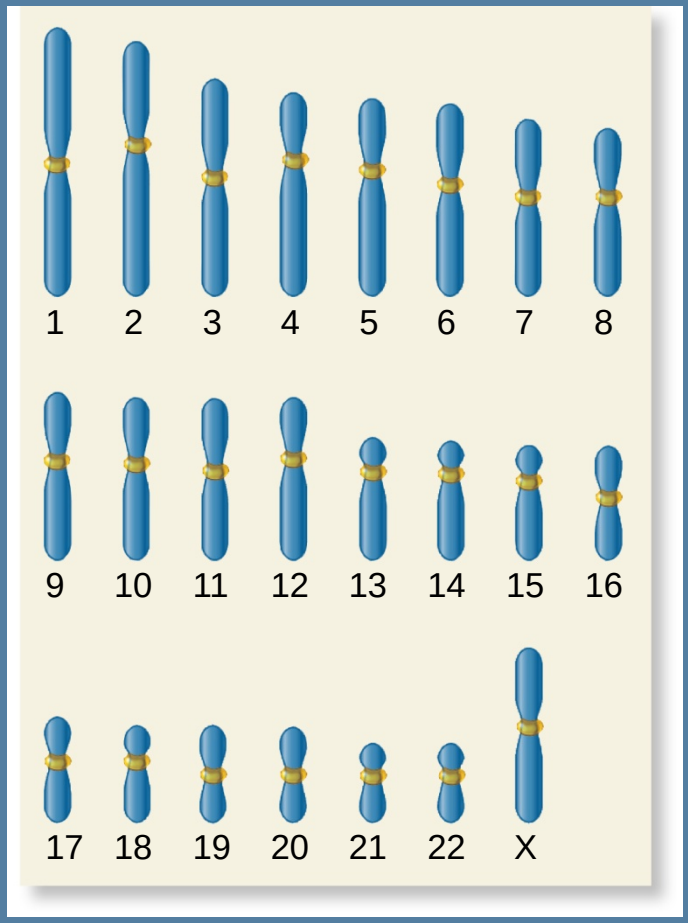


(b) Chromosomal composition found in a human gamete (23 chromosomes)

Gametes (egg, sperm) have half of the normal number of chromosomes (otherwise, offspring would have 2X our number of chromosomes and would be tertraploid)



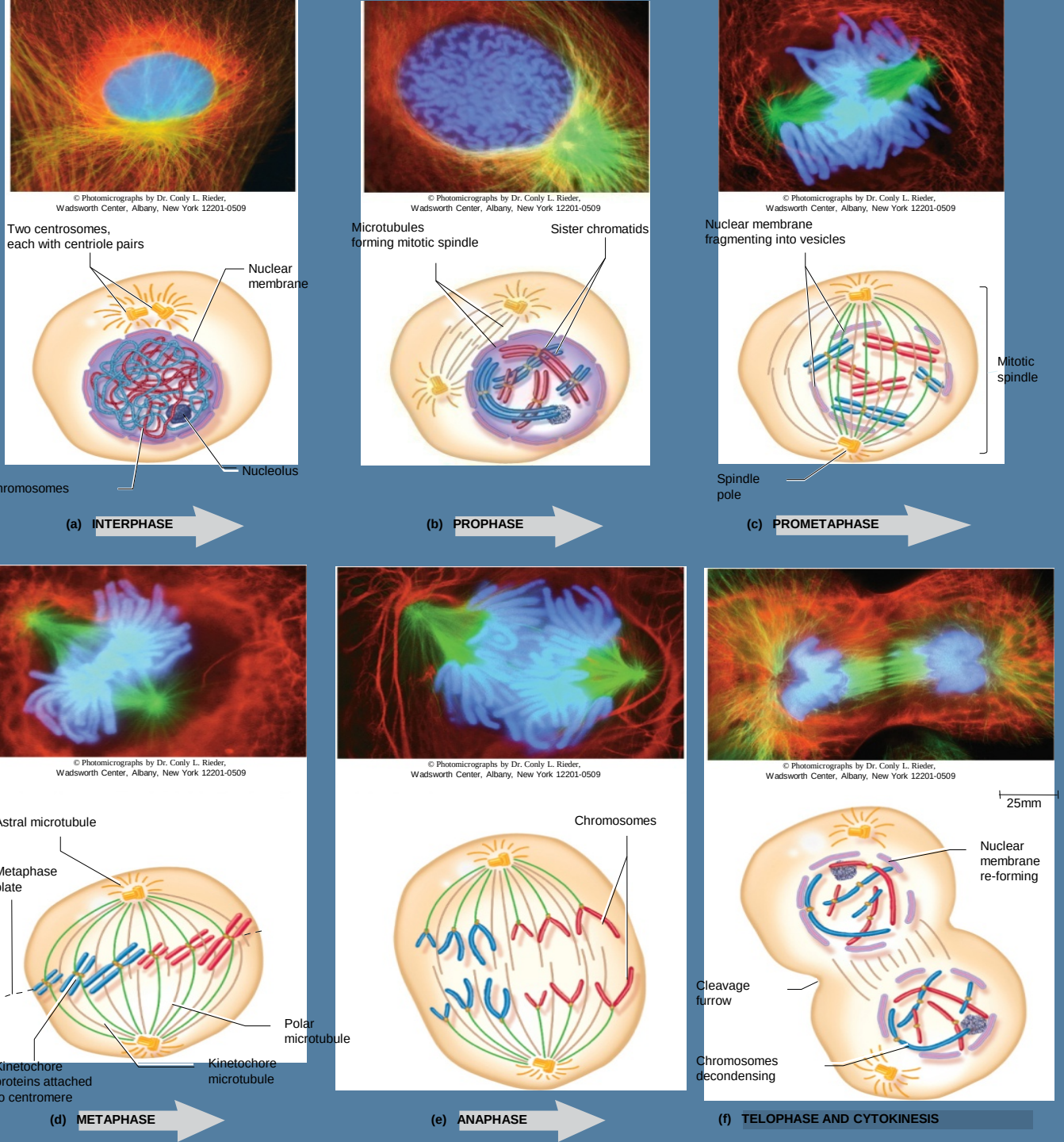
(a) Chromosomal composition found in most human cells (46 chromosomes)



(b) Chromosomal composition found in a human gamete (23 chromosomes)

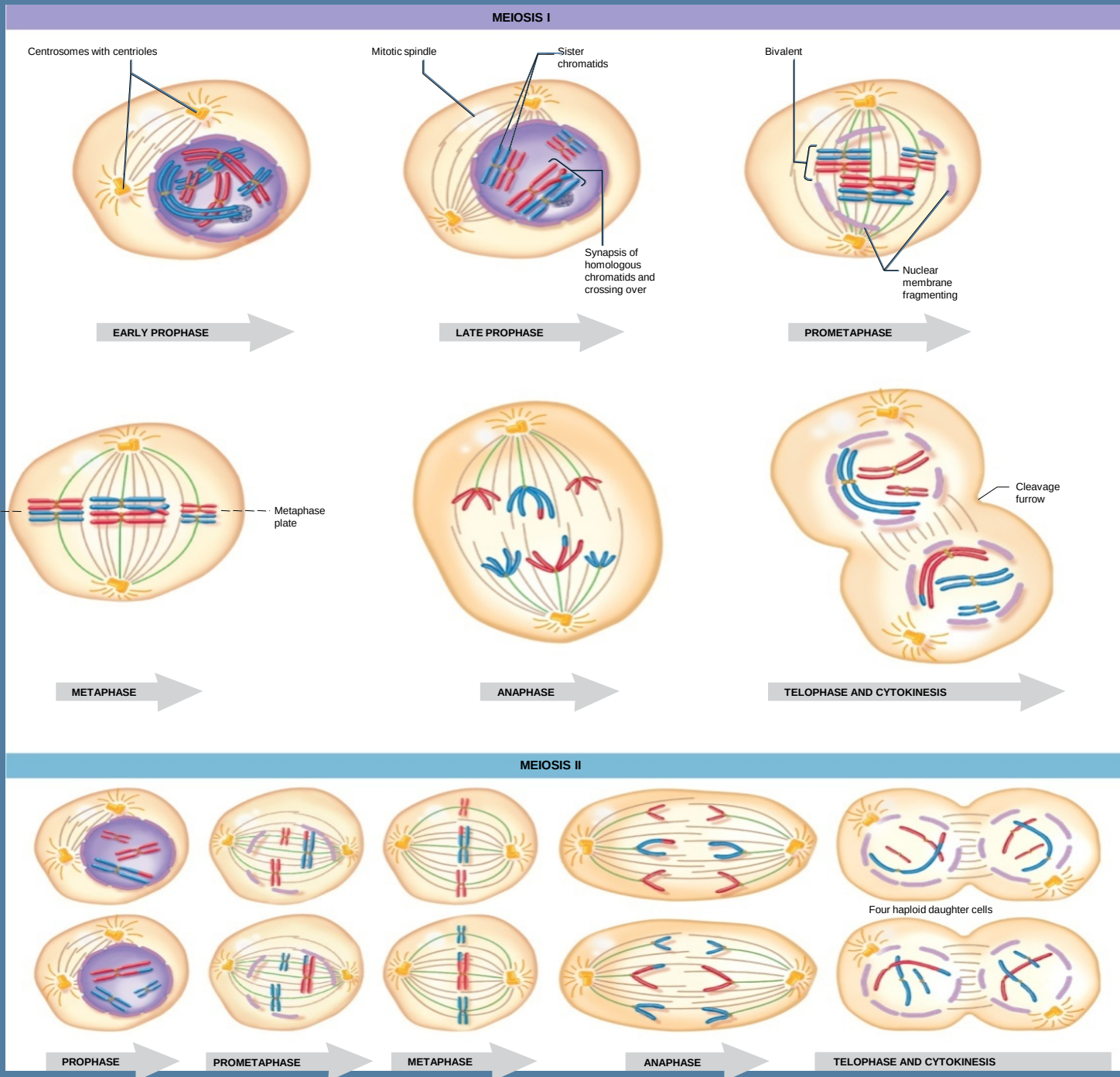
Mitosis

- Cell division that creates genetically identical “daughter” cells
- Normal growth
- Asexual reproduction

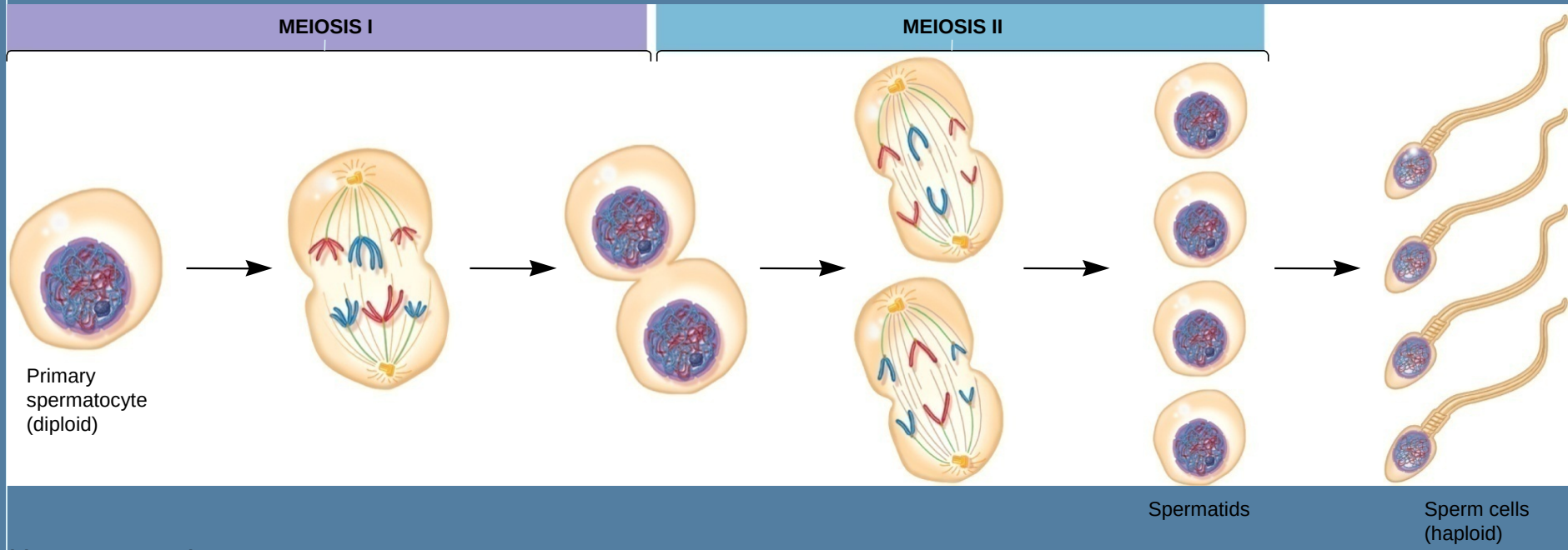


Meiosis

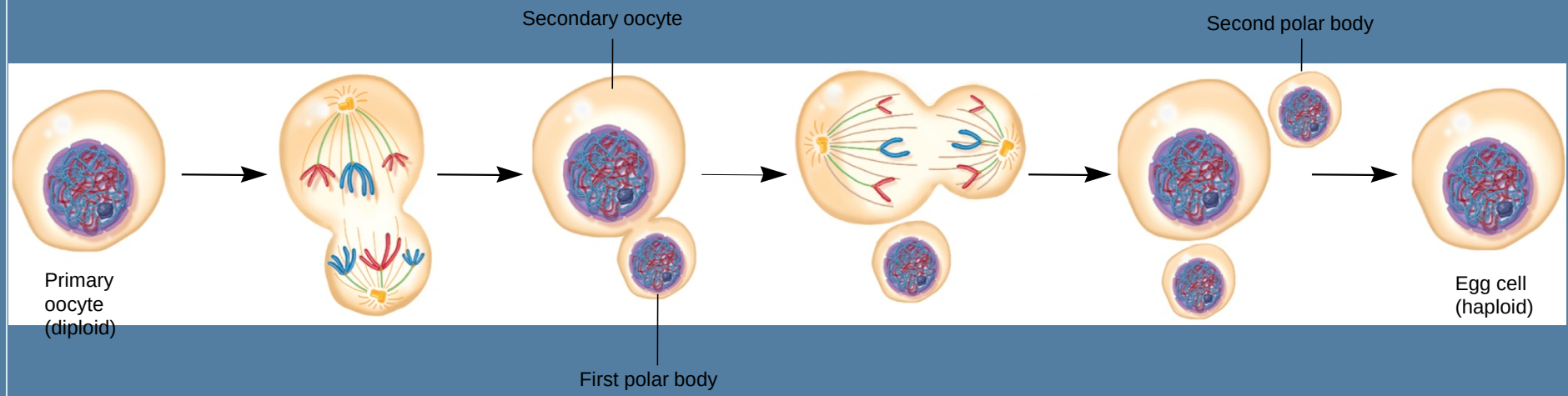
- Cell division that creates gametes with ½ the normal number of chromosomes
- Sexual reproduction



Animal Gametogenesis



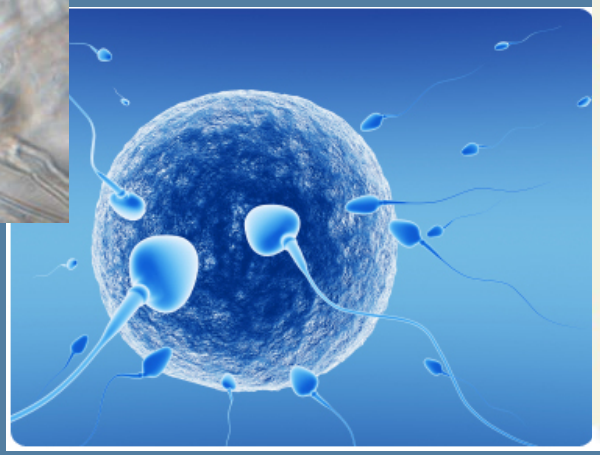
(a) Spermatogenesis



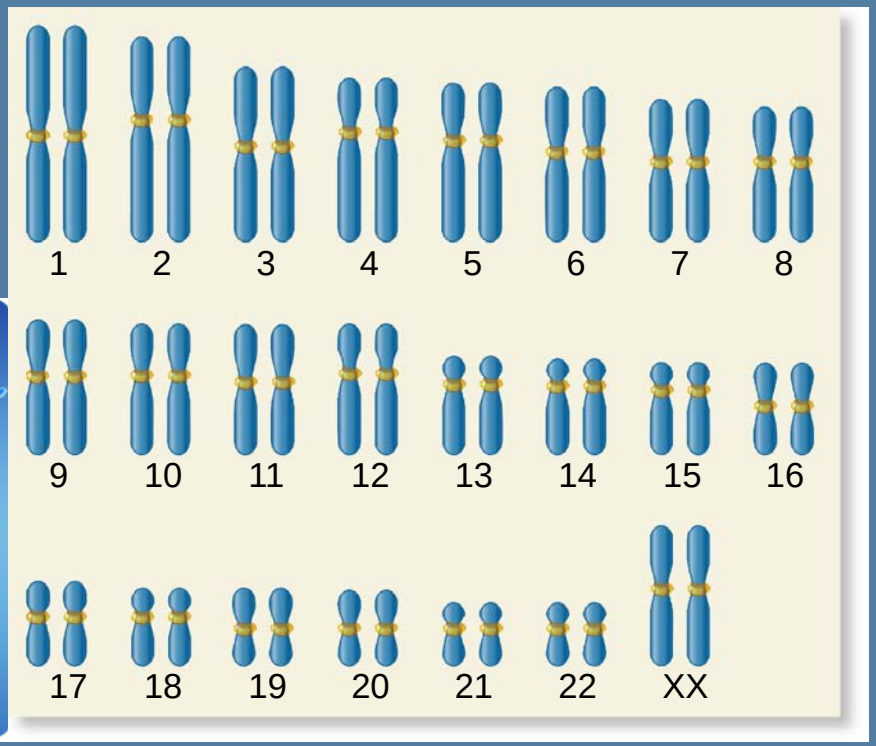
(b) Oogenesis

Fertilization

- Chromosomes from the 2 parents join during fertilization
- Paired chromosomes (one from each parent) are similar but not identical
- Different forms of a gene are known as alleles

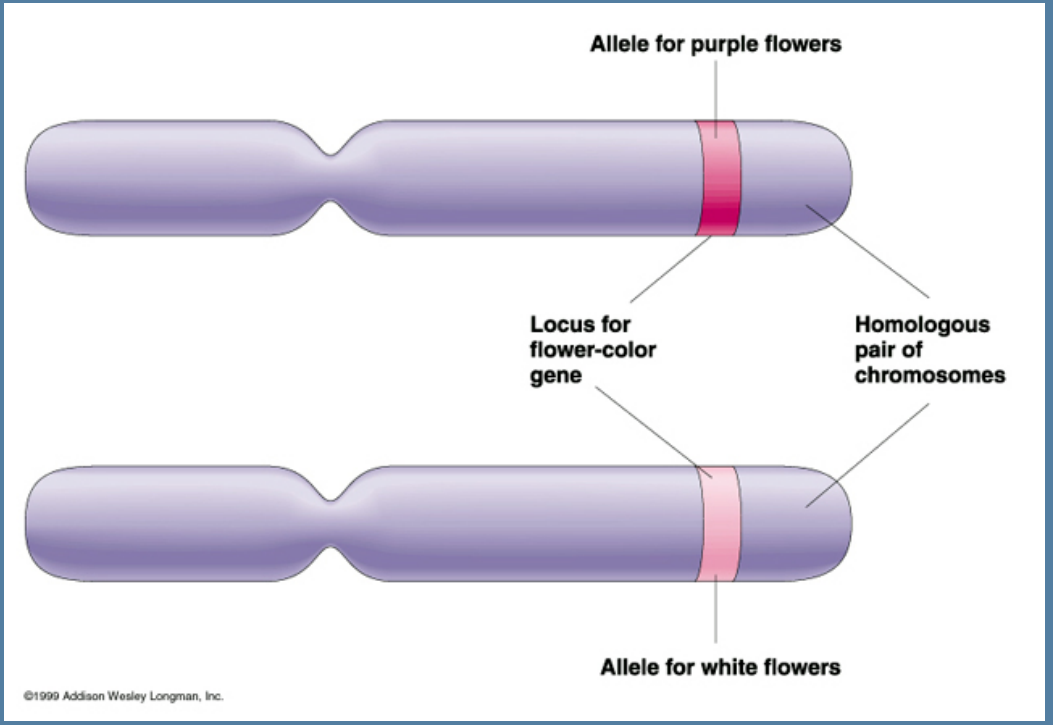


www.center4fertility.com



Loci and Alleles

- Locus (plural: loci): location of a gene on a chromosome (i.e., gene that controls flower color by encoding a protein involved in pigment synthesis)
- Allele: specific form of a gene encodes for a particular character

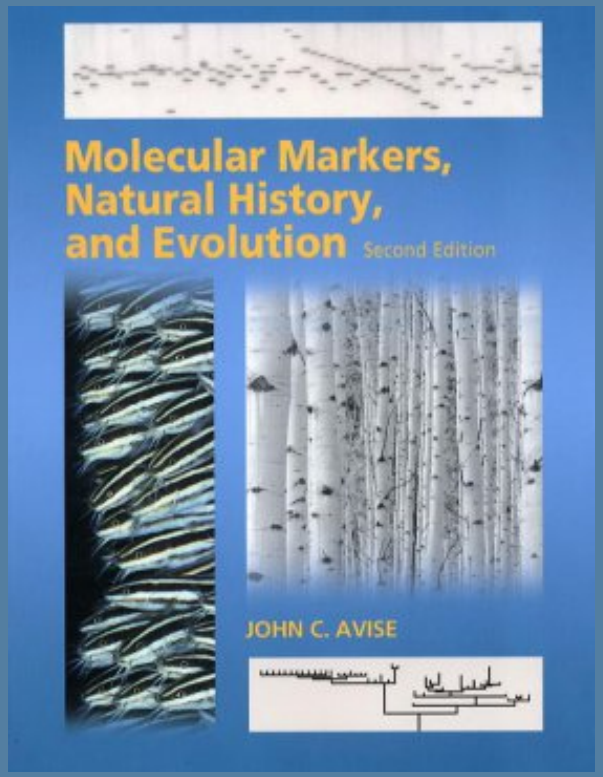


II. Molecular Markers

Molecular Markers

- We can look at the DNA sequence directly (for single genes up to the whole genome), or we can use 'proxy' methods that examine genotype more indirectly.

- Genotype differences that we can examine are molecular markers.

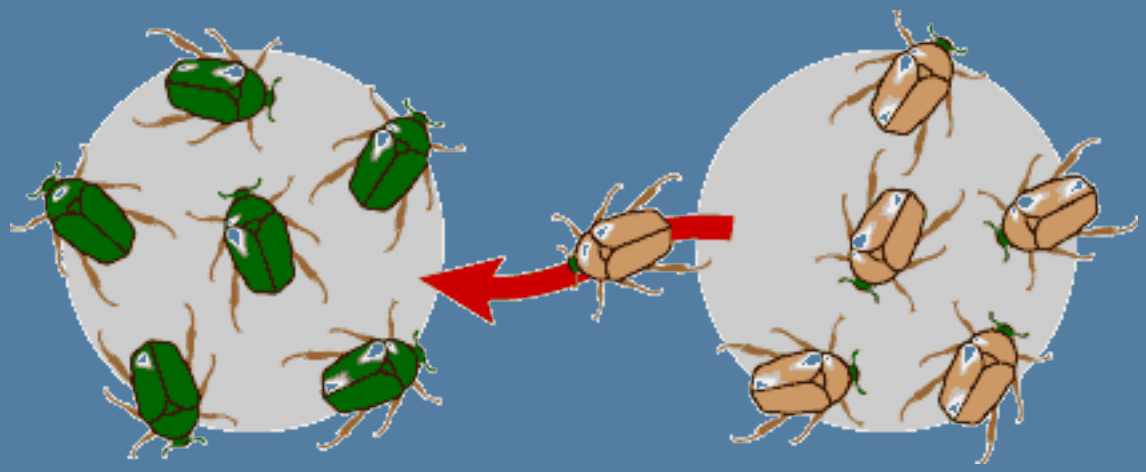


Molecular Markers

- Within sexually-reproducing species, individuals are related by heredity
  - Offspring are genetically similar to parents, but differ genetically due to 3 phenomena:
    - Meiotic segregation and independent assortment: Gametes receive only one chromosome from each pair
    - Crossing over during Meiosis I
    - Combination of 2 parental gametes during fertilization results in new genetic combinations
- Additional genetic differences result due to mutation

Molecular Markers

- Sexual reproduction is pasing on alleles to offspring, not genotypes, within the population. Sharing of DNA within a population (via mating) is known as gene flow.
- Geographically isolated populations may experience limited or no gene flow. Migration of individuals between populations, followed by mating, allows gene flow between populations.



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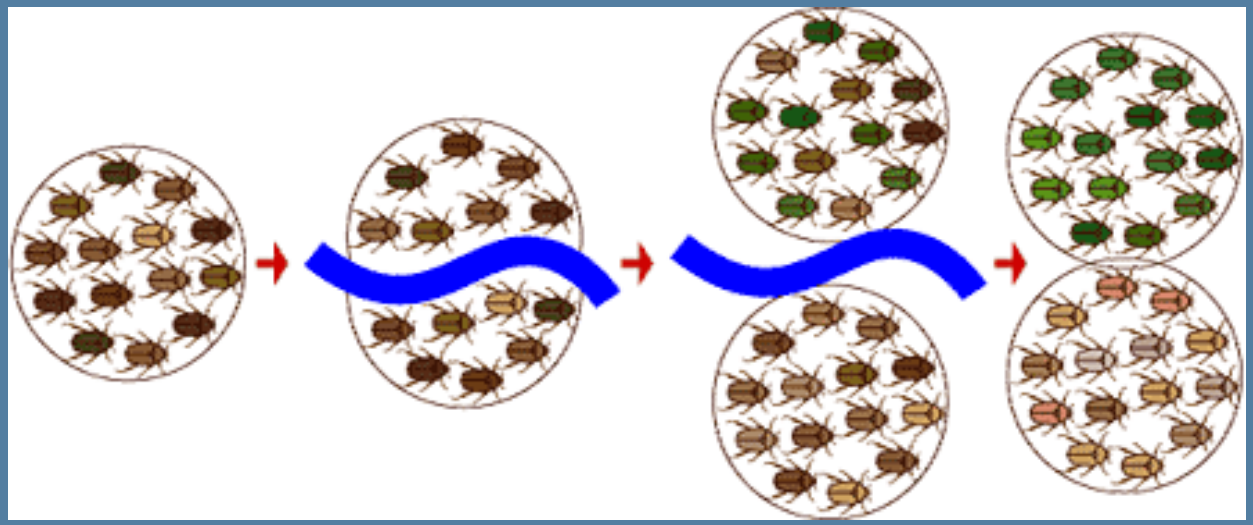
Molecular Markers

- Sexual reproduction (interbreeding across subpopulations within a population) tends to homogenize (spread) the alleles present within a population.
- Conversely, when sexual reproduction is reduced or inhibited, different (sub)populations can form accumulate differences; can lead to speciation



Speciation

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Molecular Markers

- Would you expect sexual or asexual organisms to have higher population-level genetic diversity?
- In asexually-reproducing organisms, new individuals are produced by mitosis (or similar mechanisms such as fission or budding)
- Therefore, offspring are genetically identical to parent
- Genetic differences between individuals result due to mutation

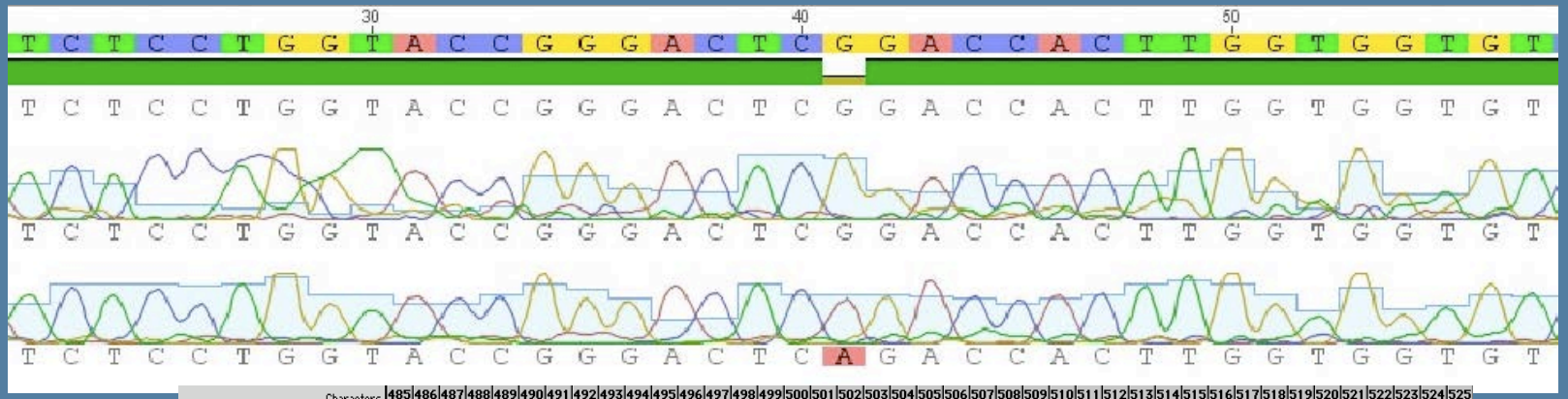
Molecular Markers

- Because related individuals share genetic (DNA) similarities, we can examine DNA to infer many things about an organism, such as:
  - What species does it belong to?
  - Origin and movement: Where did it come from? What are its patterns of migration? What is its natural dispersal capacity? Has it moved as a result of human activity?
  - Does it reproduce sexually or asexually?

III. Major Types of Molecular Markers

DNA Sequences

Sequence: The order of nucleotides in a particular region of DNA. Order matters, like letters in a word: RATS ≠ TARS ≠ ARTS ≠ STAR ≠ TRAS ≠ RTSA



Taxa	Characters 485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525
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2	L. amethyst-occ TENN42526	C	G	G	G	C	T	T	C	A	T	T	A	-	-	-	-	A	T	G	A	G	G	T	T	G	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
3	L. laccata CLC1370	C	A	G	G	C	T	T	C	A	T	T	T	G	A	C	T	A	T	G	A	G	G	T	C	A	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
4	L. bicolor TENN42529	C	G	G	G	C	T	T	C	A	T	T	A	-	-	-	-	A	T	G	A	G	G	T	C	G	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
5	Tricholoma portentosum	C	G	G	G	C	T	T	T	-	-	-	-	-	-	-	T	A	A	G	A	A	G	T	C	G	G	C	T	C	T	C	C	T	T	A	A	A	T	T	C	A
6	L. laccata CLC1603	C	A	G	G	C	T	T	C	A	T	T	T	G	A	C	T	A	T	G	A	G	G	T	C	A	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
7	L. proxima TENN42922	C	G	G	G	C	T	T	C	A	T	T	A	-	-	-	-	A	C	G	A	G	G	T	C	G	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
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15	L. bicolor CLC1304	C	G	G	G	C	T	T	C	A	-	-	-	-	T	C	A	C	T	G	A	G	G	T	C	G	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
16	L. montana TENN42882	T	G	G	G	C	T	T	C	A	T	T	A	-	-	-	-	A	T	G	A	G	G	T	C	A	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
17	L. nobilis TENN42527	C	G	G	G	C	T	T	C	A	T	C	A	-	-	-	-	C	T	G	A	G	G	T	C	G	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
18	L. montana TW0319	T	G	G	G	C	T	T	C	A	-	-	-	-	T	T	A	A	T	G	A	G	G	T	C	A	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
19	L. bicolor CLC1445	C	G	G	G	C	T	T	C	A	-	-	-	-	T	C	A	C	T	G	A	G	G	T	C	G	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
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21	L. montana TW0591	T	G	G	G	C	T	T	C	A	-	-	-	-	T	T	A	A	T	G	A	G	G	T	C	A	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
22	L. montana TENN42885	T	G	G	G	C	T	T	C	A	T	T	A	-	-	-	-	A	T	G	A	G	G	T	C	A	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
23	L. bicolor CLC1672	C	G	G	G	C	T	T	C	A	-	-	-	-	T	C	A	A	T	G	A	G	G	T	C	G	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
24	L. trichoderm. TENN42523	C	A	G	G	C	T	T	C	A	T	C	A	-	-	-	-	C	T	G	A	G	G	T	C	G	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
25	L. bicolor TW0752	C	G	G	G	C	T	T	C	A	-	-	-	-	T	T	A	A	T	G	A	G	G	T	C	G	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
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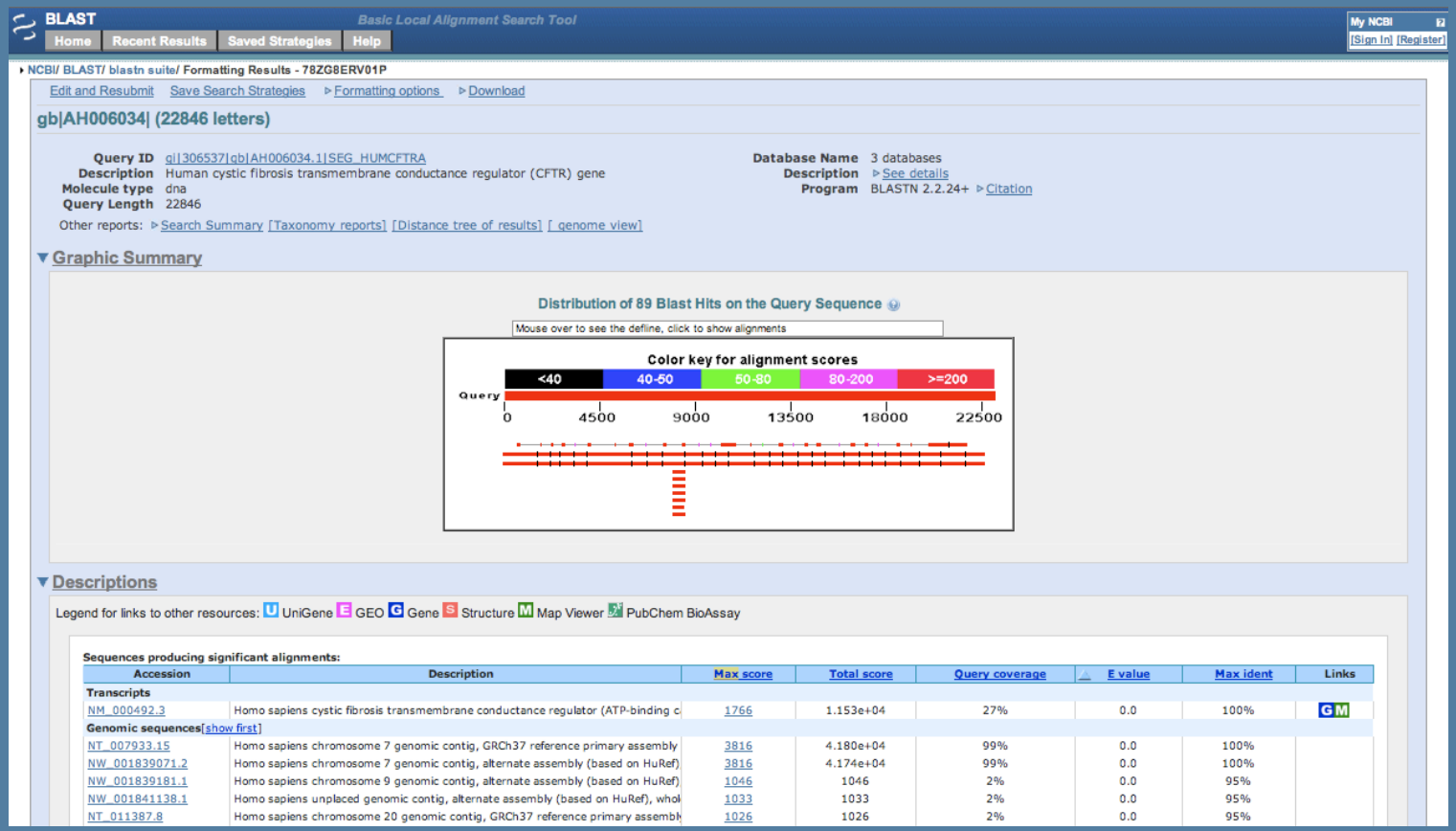
27	L. pseudomontana CLC1625	C	G	G	G	C	T	T	C	A	-	-	-	-	T	C	A	A	T	G	A	G	G	T	C	G	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
28	L. tortilis DBGH20904	T	G	G	G	C	T	T	C	A	-	-	-	-	T	T	A	A	T	G	A	G	G	T	C	G	A	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A
29	L. pumila TW0501	T	G	G	G	C	T	T	C	A	-	-	-	-	T	T	A	A	T	G	A	G	G	T	C	A	G	C	T	C	T	C	C	T	T	A	A	A	T	G	C	A

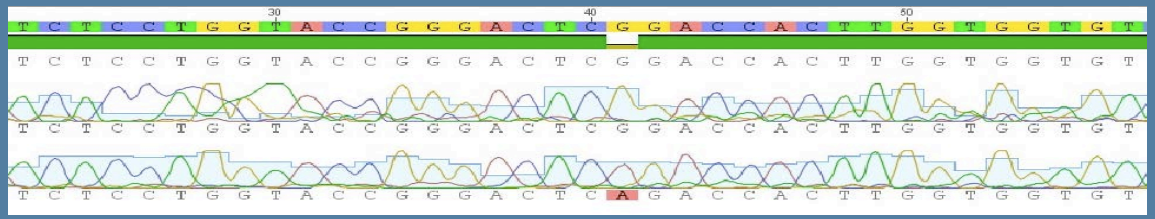
DNA Sequence Identification

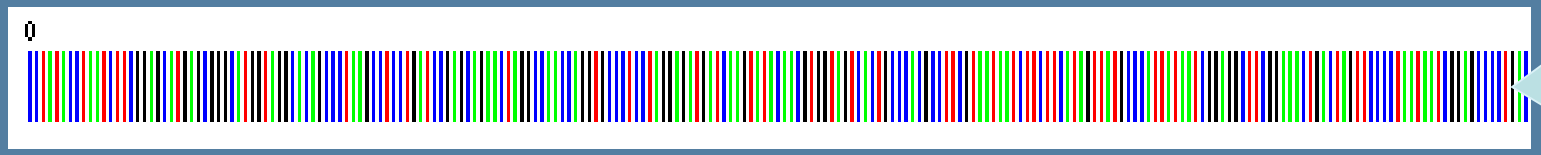
» **Example:** BLAST querying the GenBank database to determine the identity or putative function of an unknown DNA sequence by comparing it to the sequence of known genes.



DNA Barcoding

- Identification of species by sequencing an agreed-upon gene (cytochrome oxidase 1 for most animals; rDNA internal transcribed spacer for fungi)
- Assuming that each species differs in the sequence of this gene (and that the gene sequence is constant **within** a species), each species will have a unique genetic code, analogous to the supermarket UPC code.





DNA Barcoding: Uses

- Identifying cryptic species (different species that cannot be distinguished just by looking at them)
- Matching different life stages (e.g., larvae and adults)
- Identifying species when distinguishing characteristics are missing (e.g., plants lacking flowers; fungi lacking spores)

DNA Barcoding: Uses

- Identifying species in processed products
  - Sushi, caviar
  - Teas
  - Herbal medicines

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Commercial Teas Highlight Plant DNA Barcode Identification Successes and Obstacles

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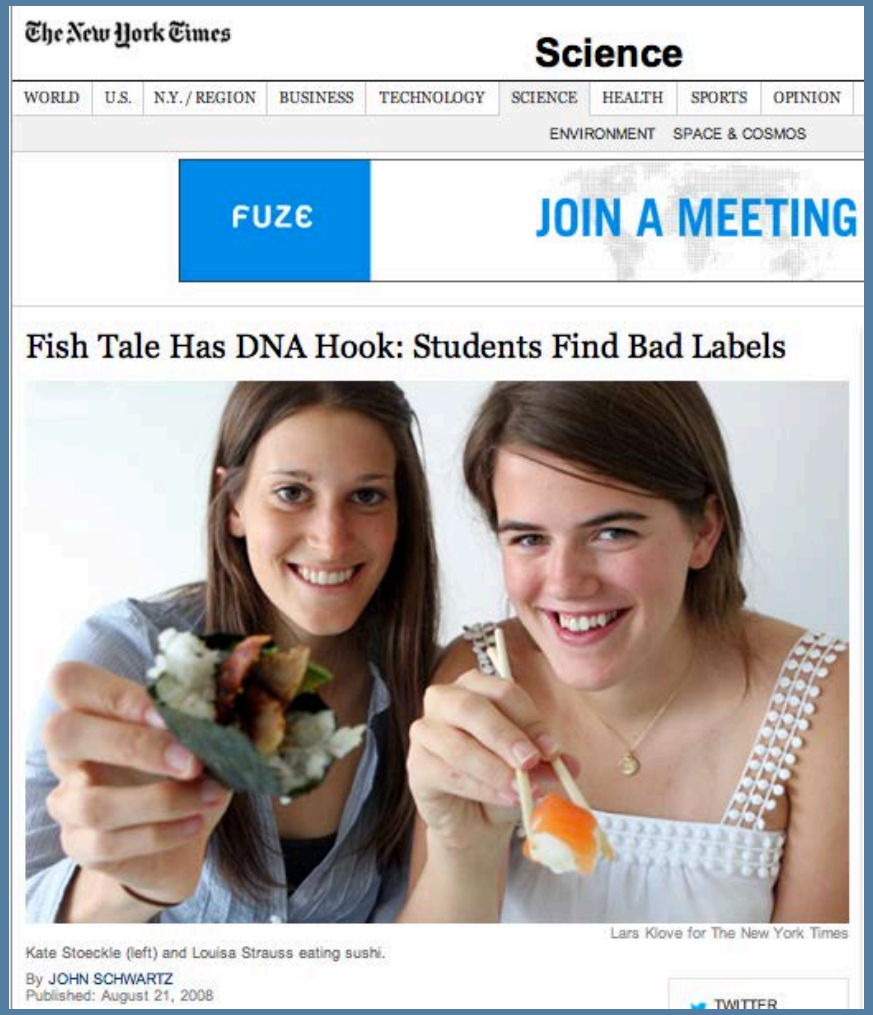

The New York Times

Science

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Fish Tale Has DNA Hook: Students Find Bad Labels

Lars Klove for The New York Times

Kate Stoeckle (left) and Louisa Strauss eating sushi.

By JOHN SCHWARTZ
Published: August 21, 2008

TWITTER

DECEMBER 4, 2009

DNA BARCODING AND SUSHI

What's Really on the Menu

Whistleblower: 'White tuna' on menu can be gut-wrenching escolar

In a new DNA test, researchers at the American Museum of Natural History discovered that fish you ordered from the menu may not always be the species served.
Using an experimental genetic identification technique called DNA barcoding on fish identified as tuna on the menu at 31 restaurants in New York and Denver, the researchers found that customers were sometimes served a cheaper substitute, an endangered species or a fish banned in several countries as a health hazard. See examples below. (See related article.)

The menu says	The waiter says	Price	The DNA shows	What it means
White Tuna	Tuna	$3.00	Escolar	Health hazard. Contains gempylotoxin, which may lead to intestinal cramping and diarrhea.
Tuna	Blue fin tuna	$2.75	Yellow fin tuna	Cheaper substitute
Toro	Blue fin tuna	$8.00	Bigeye tuna	Cheaper substitute
Belly tuna	Blue fin tuna	$5.50	Southern bluefish tuna	Has critically endangered species status
Albacore tuna	--	$2.25	Escolar	Health hazard. Contains gempylotoxin, which may lead to intestinal cramping and diarrhea.
Toro	Yellow fin tuna	$5.50	Bigeye tuna	Mislabeled
Tuna	Bigeye tuna	$2.90	Yellow fin tuna	Mislabeled
Super White Tuna	White tuna	$8.95	Escolar	Health hazard. Contains gempylotoxin, which may lead to intestinal cramping and diarrhea.
Mid fat tuna	Blue fin tuna	$6.50	Northern blue fin	Has been overfished. Is proposed for endangered species status

Sources: PLoS One, American Museum of Natural History; Photo: iStockphoto

DNA Barcoding: Uses

- Finding species in the environment: Tracking Bears!

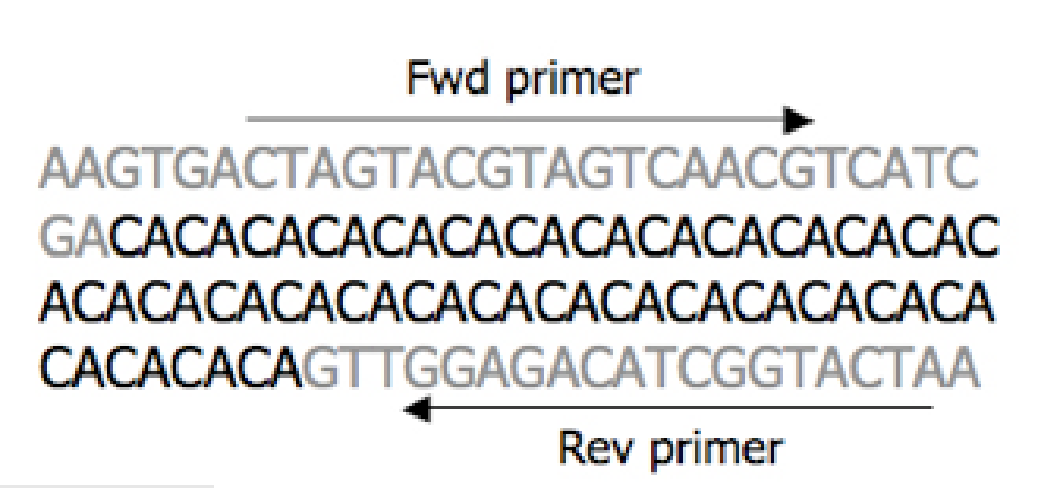
Multilocus Genotyping

- What if we want to compare 2 closely-related individuals?
  - Will their genotypes be similar?
  - When trying to distinguish closely-related (compared to distantly-related) individuals, would you have to look at more, or fewer, parts of the genome?

Microsatellites (Simple Tandem Repeats)

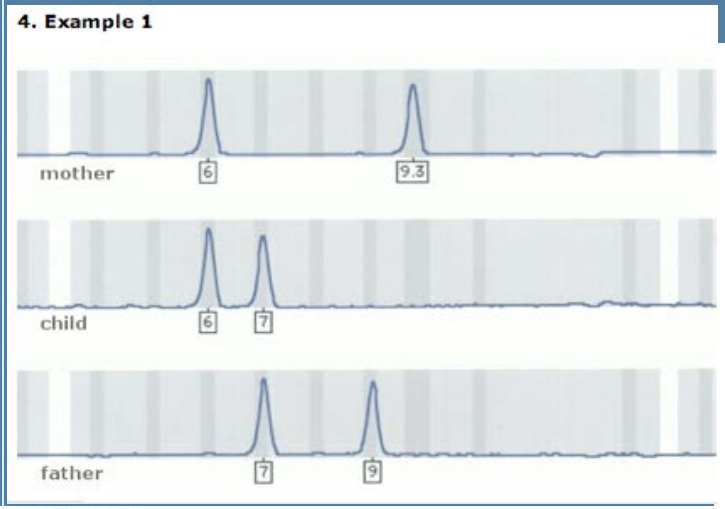
- Regions where a small motif of nucleotides (CA in the example below) is repeated multiple times. Can occur in coding or noncoding regions of the genome.The number of repeats, like other aspects of DNA, is heritable.

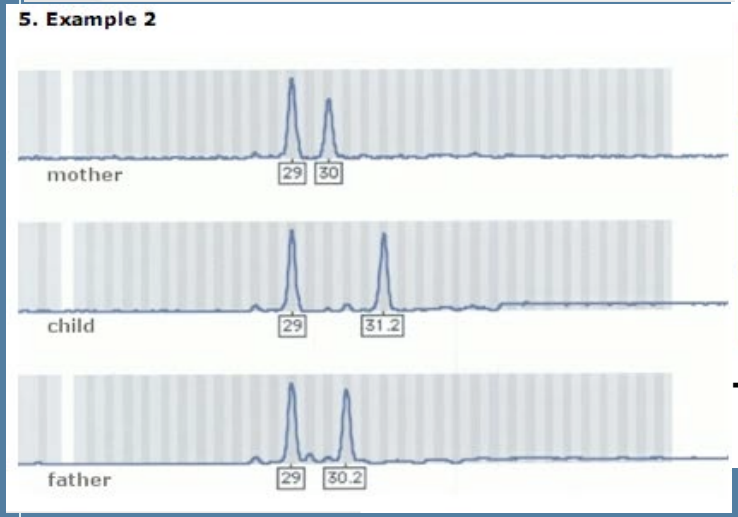
- In a breeding population microsatellites are recombined during sexual reproduction and the population maintains a variety of microsatellites **characteristic to that population**



Microsatellites (Simple Tandem Repeats)

- Frequent addition or subtraction of the number of repeats means that individuals can often be distinguished using STR loci





This photo shows Earl Washington just before his release in 2001, after 17 years in prison.

Source of sample	STR marker 1	STR marker 2	STR marker 3
Semen on victim	17, 19	13, 16	12, 12
Earl Washington	16, 18	14, 15	11, 12
Kenneth Tinsley	17, 19	13, 16	12, 12

These and other STR data exonerated Washington and led Tinsley to plead guilty to the murder.

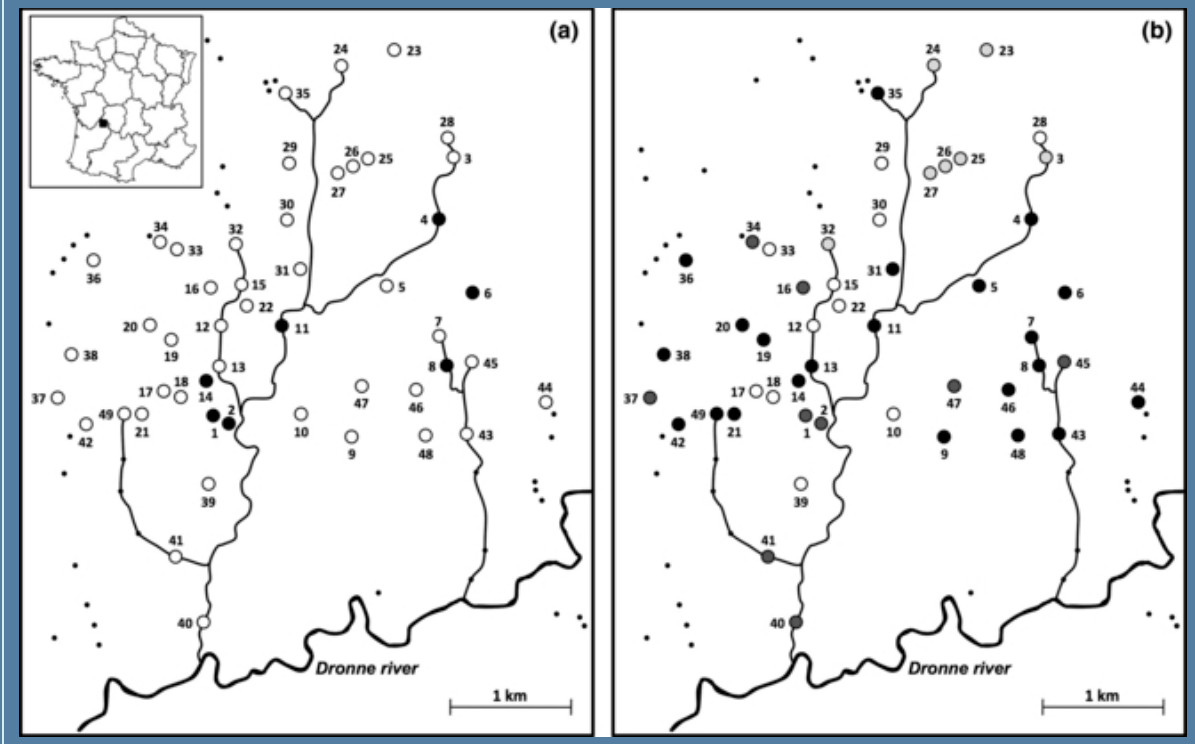
Left Top: http://kingsley.stanford.edu

Bottom: http://www.paternity.be/information_EN.html

IV. DNA as a Tool for Understanding Biological Invasions

A. Detection

Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus* [in France]



www.hylawerkgroep.be

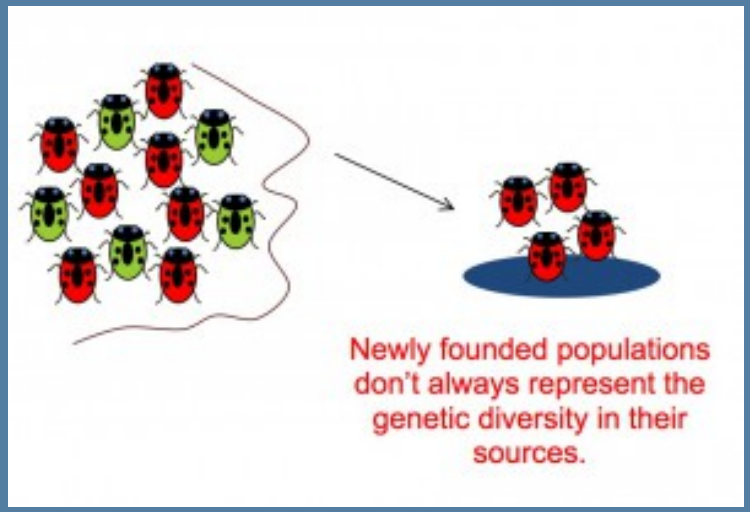
(a) Traditional surveys. Filled circles: ponds where the American bullfrog was detected; Open circles: not detected. (b) DNA surveys. Detected in 1 (light gray), 2 (dark gray), 3 (black) samples; open symbols: not detected.

Journal of Applied Ecology
Vol. 49, Issue 4, pages 953-959, 5 JUL 2012 DOI: 10.1111/j.1365-2664.2012.02171.x
http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2664.2012.02171.x/full#f2

B. Tracking Invasion History

Genetic Clues to Invasion History

- Because geography structures allele distributions within populations, genetic signatures (e.g., specific alleles or level of genetic similarity) can indicate origin.
- **Founder events** (colonization by limited number of individuals) generally limits the degree of genetic variability within the invasive population; genetic variability is generally higher in area of origin, lower in areas of recent colonization.



http://beacon-center.org

Invasion History of the Black Rat (*Rattus rattus*)

Photo: Encyclopedia of life (www.eol.org)

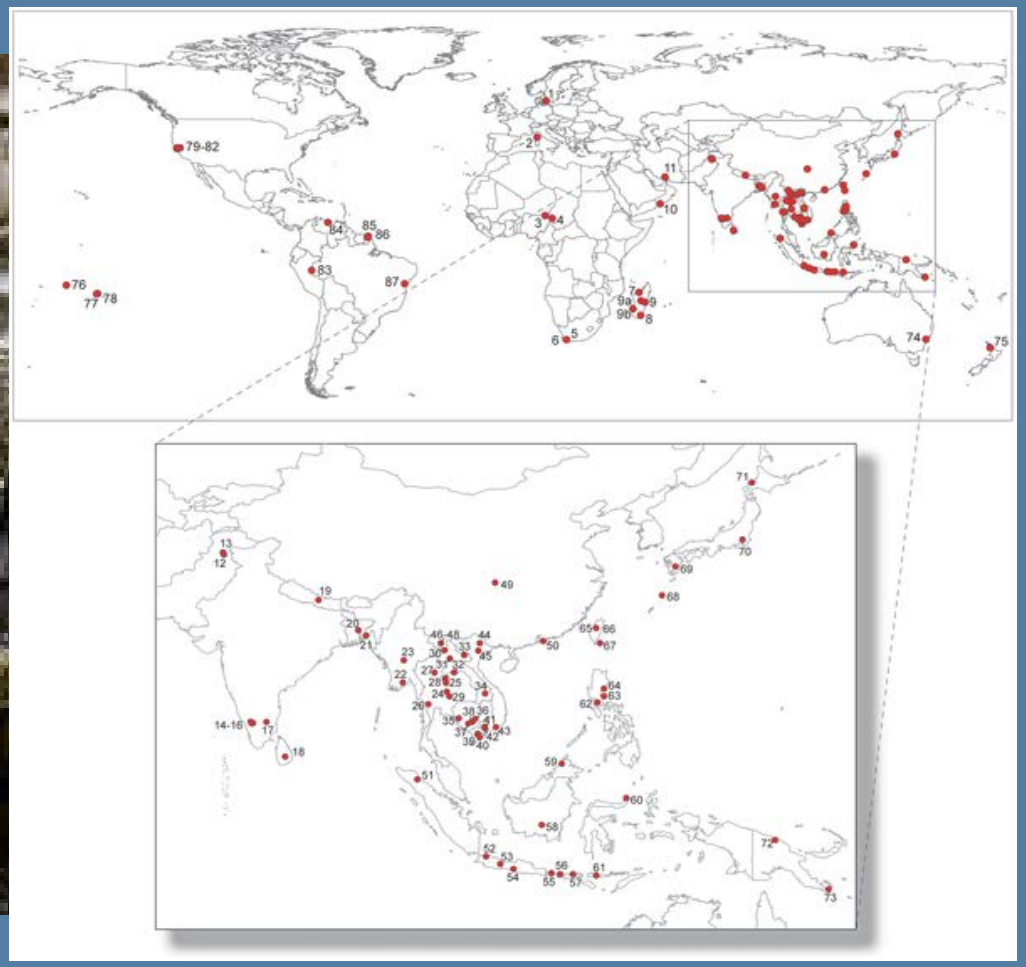


Figure 1. Maps showing the global distribution of sampling localities.

Sampling map from: Aplin KP, Suzuki H, Chinen AA, Chesser RT, et al. (2011) Multiple Geographic Origins of Commensalism and Complex Dispersal History of Black Rats. PLoS ONE 6(11): e26357. doi:10.1371/journal.pone.0026357
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0026357

PLOS | ONE

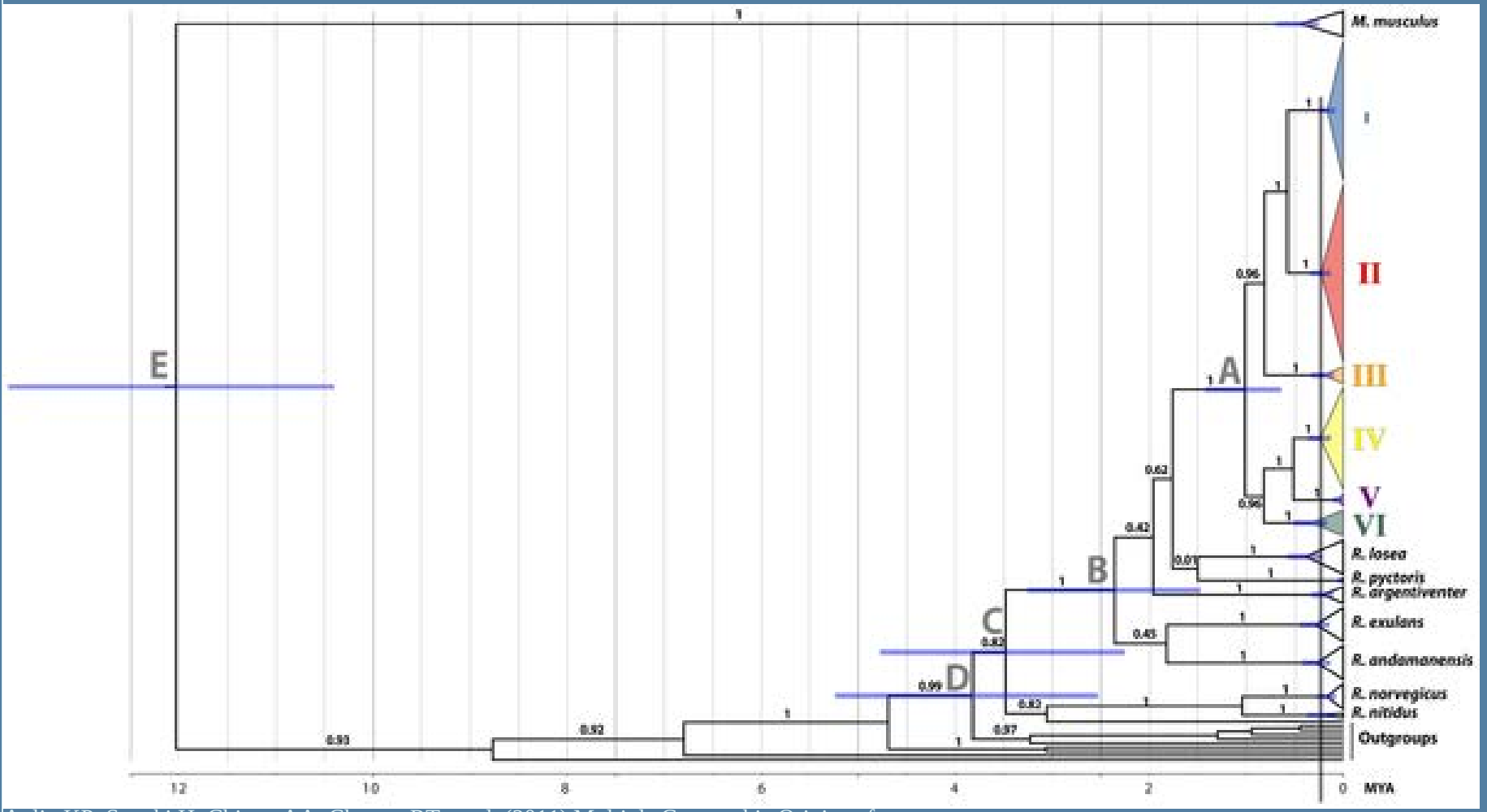
Invasion History of the Black Rat (*Rattus rattus*)

- Phylogenetic analysis recognizes 6 species in the *R. rattus* species complex
- Genotypes examined throughout much of the known geographic range to infer extent of migration and patterns of spread

»Identical genotypes found in distant geographical areas can suggest (i) slow evolutionary rate or (ii) recent dispersal. In this case, genotype analysis is consistent with an explanation of widespread human-mediated dispersal

Divergence time estimates for key diversification events in the Rattini and the *Rattus rattus* complex.

6 major evolutionary lineages



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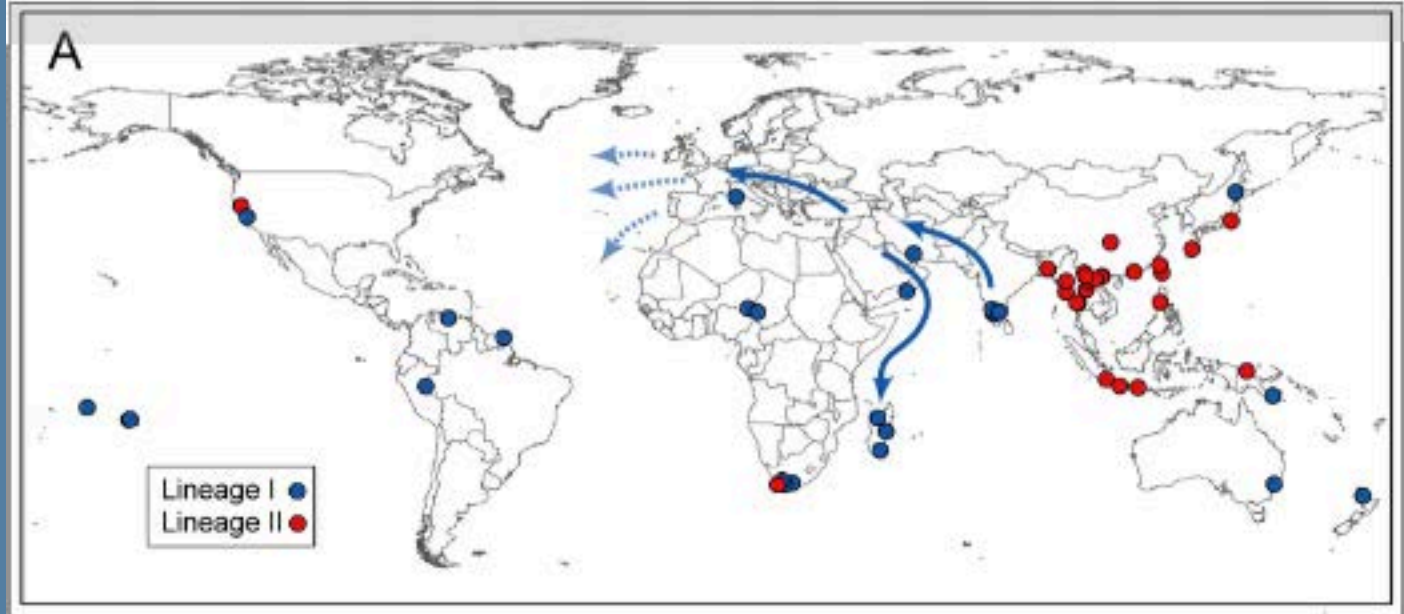
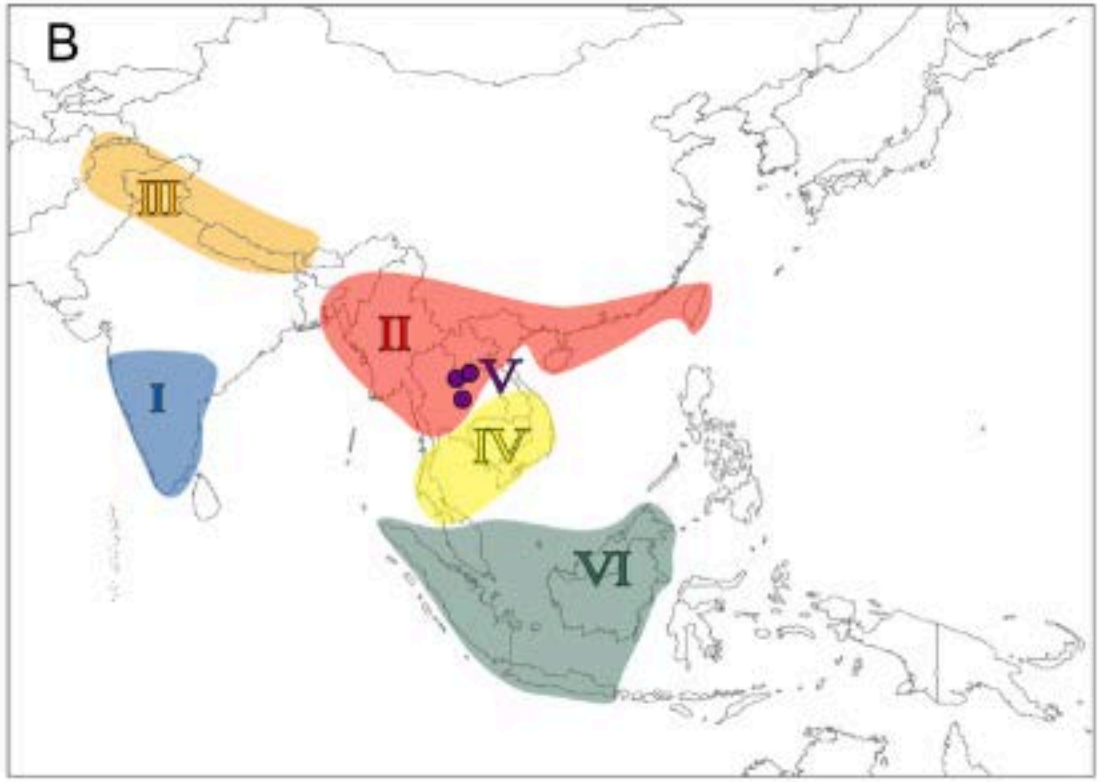
Geographic distribution and inferred dispersal episodes of the 6 lineages of the *R. rattus* complex





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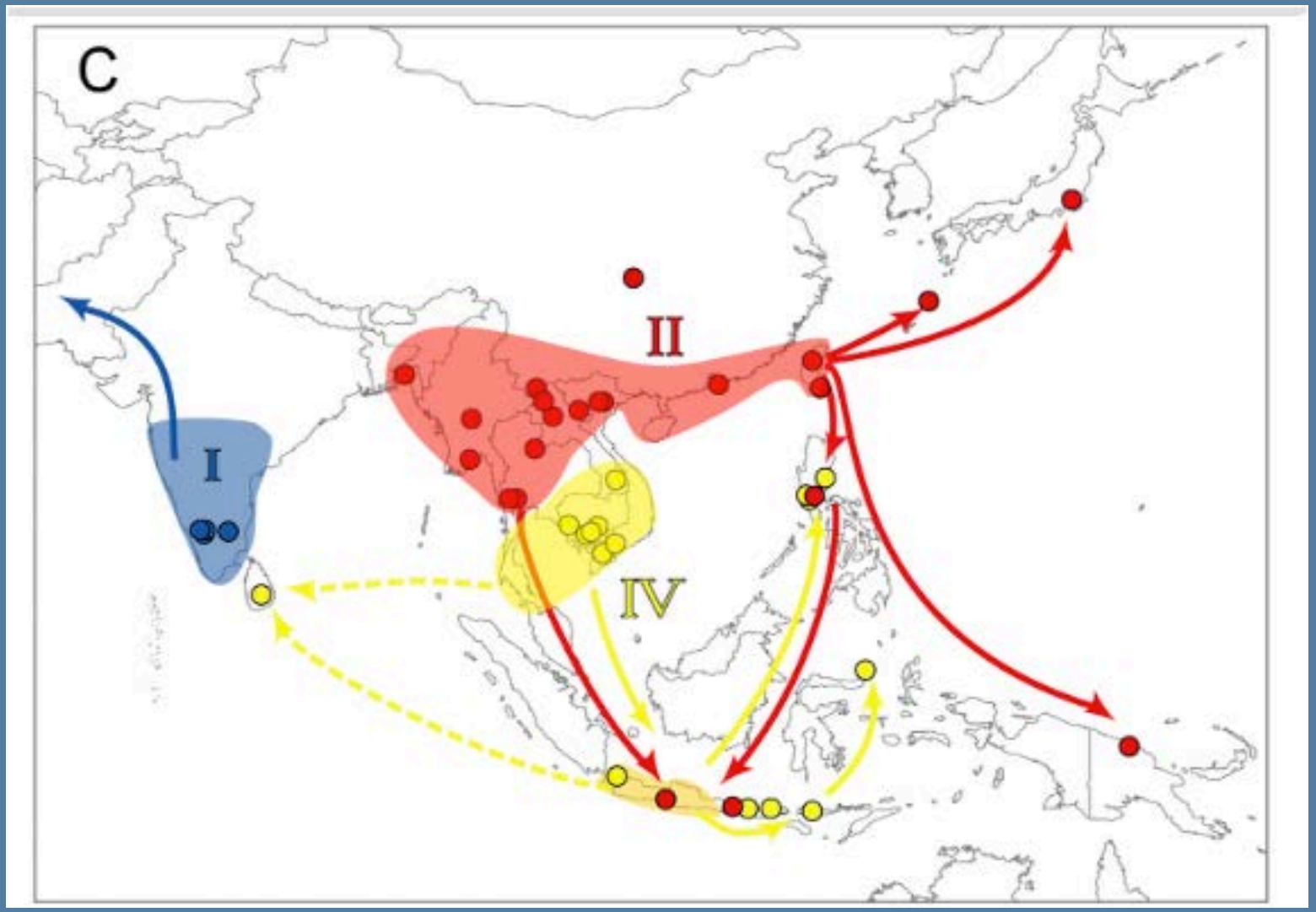

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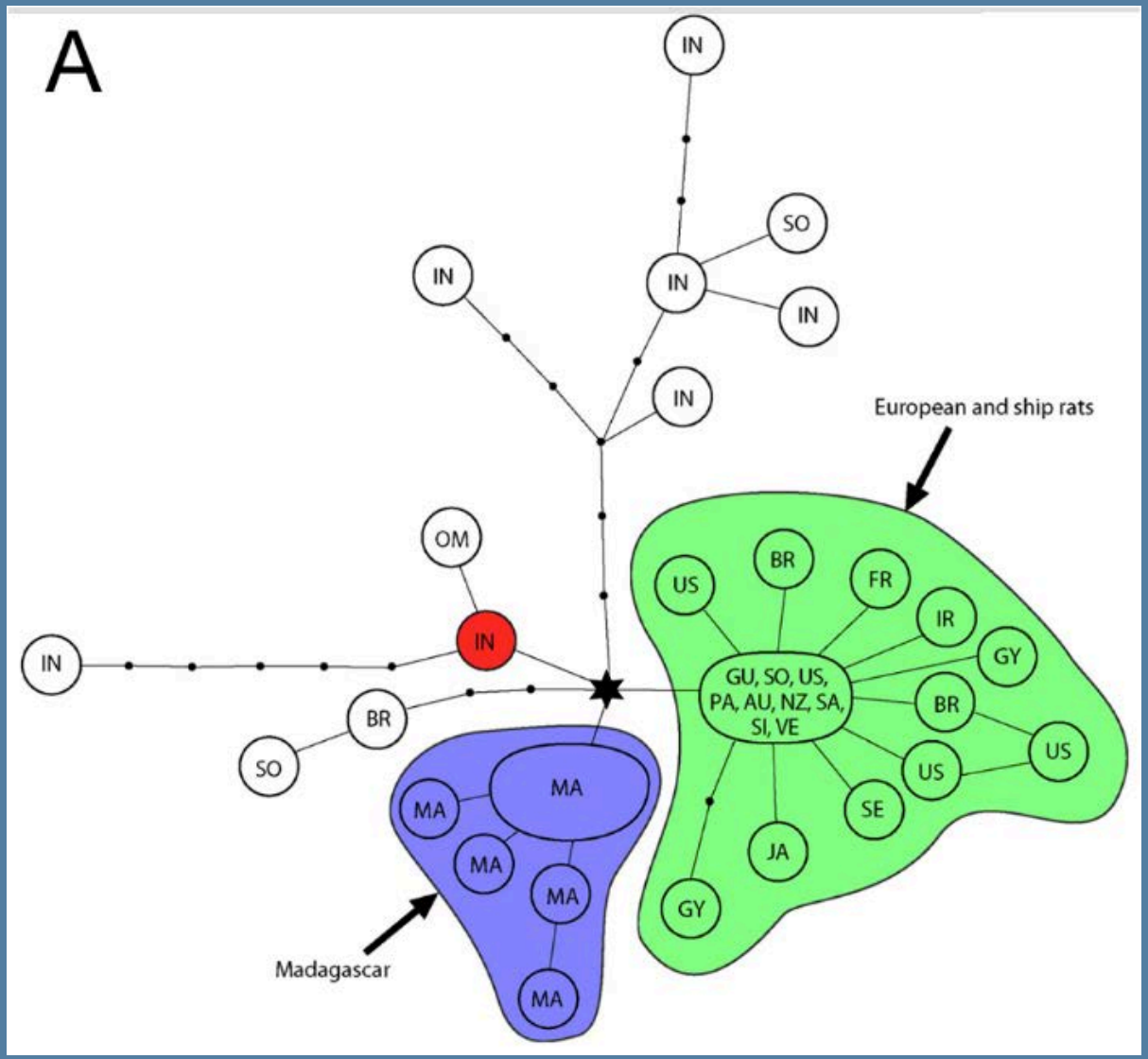
Higher genetic diversity in India suggests geographical origin there, with founder effects in "ship rat" populations

Lineage	N	Nucleotide diversity, *Pi* ± s.d.
All Lineage I	35	0.0052±0.00054
Lineage I Indian	9	0.0062±0.00143
Lineage I ship-rat	21	0.0026±0.00035

Aplin KP, Suzuki H, Chinen AA, Chesser RT, et al. (2011) Multiple Geographic Origins of Commensalism and Complex Dispersal History of Black Rats. PLoS ONE 6(11): e26357. doi:10.1371/journal.pone.0026357

Close genetic relatedness over large geographic area is typical of human-mediated transport

(Country codes : AU: Australia; BR: Brazil: FR: France; GU: Guinea; GY: Guyana; IN: India; IR: Iran; JA: Japan; MA: Madagascar; NZ: New Zealand; OM: Oman; PA: Papua New Guinea; SA: Samoa; SE: Senegal; SI: Society Islands; SO: South Africa; US: United States of America; VE: Venezuela)



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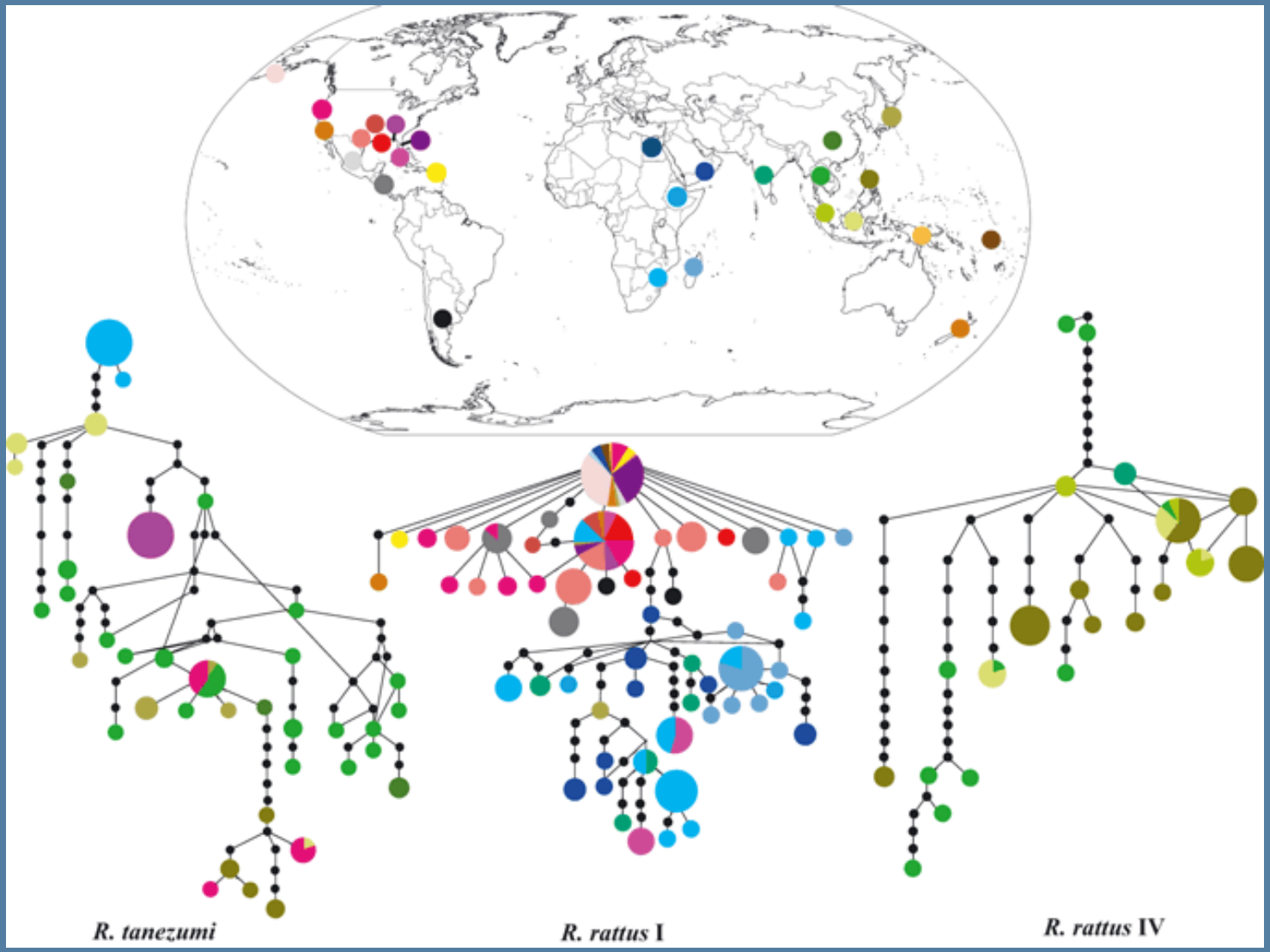
Haplotype networks indicate that multiple Black Rat species have undergone migrations

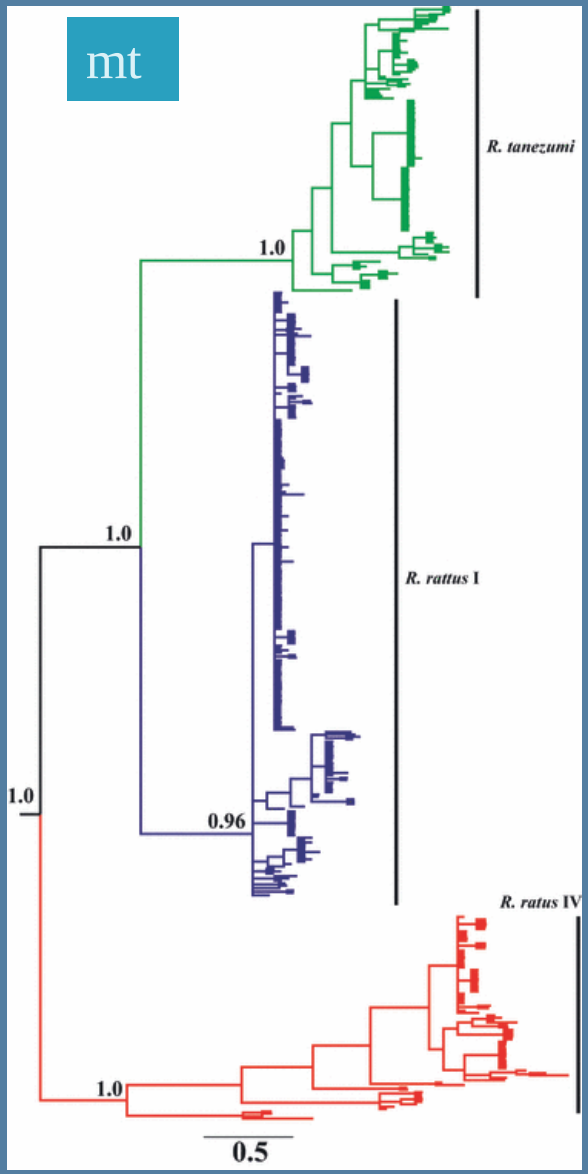


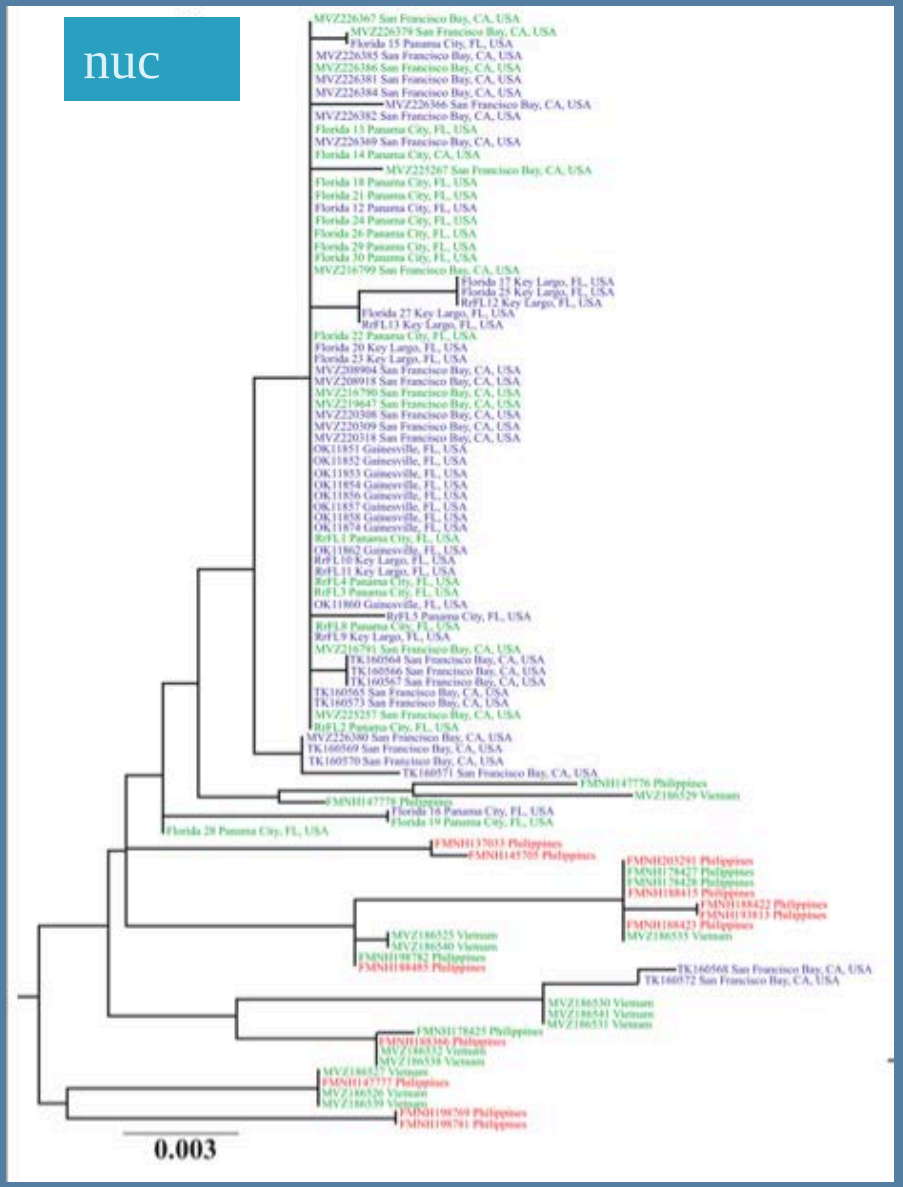
Invasion facilitates hybridization with introgression in the *Rattus rattus* species complex. Molecular Ecology, Volume 21, Issue 14, pages 3545-3561, 18 MAY 2012 DOI: 10.1111/j.1365-294X.2012.05620.x, http://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2012.05620.x/full#f2

Genetic Admixture

- Invasive species may interbreed with native species; leading to genetic introgression.
- Introgression is a concern because it can:
  - Increase the fitness and invasion potential of the invader
  - Dilute the genome of the native species ("genetic swamping")

Conflict between mitochondrial and nuclear gene trees provides evidence of hybridization in *Rattus rattus*





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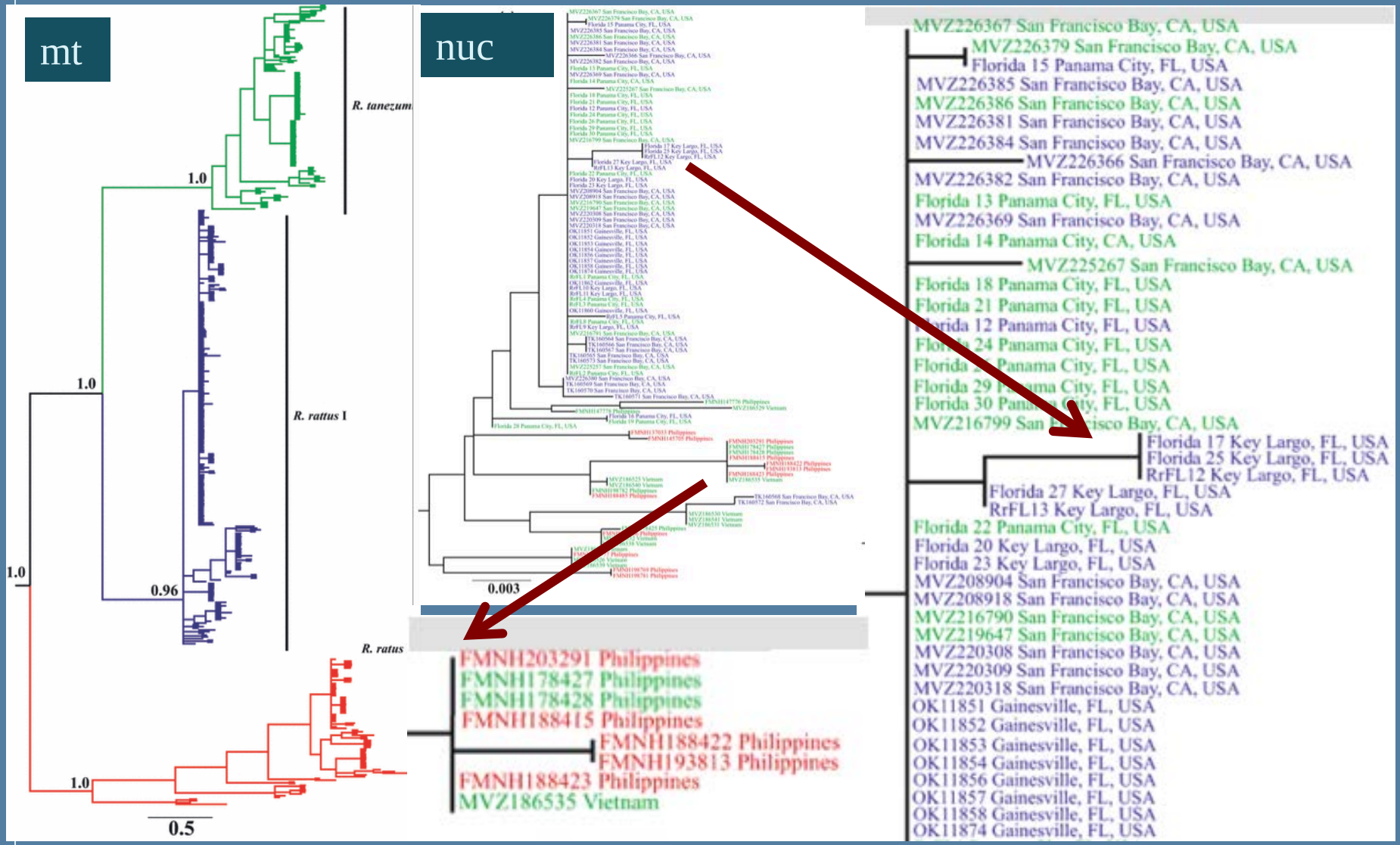

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C. Disease Control

Reproductive Mode and the Importance of Sexual Recombination

- Sexual reproduction => much more genetic variability than asexual reproduction
- If only a single gender or mating type is introduced, capacity for genetic variability is relatively low (mutation only); if second gender/mating type arrives, genetic variability can skyrocket
- Evolution of pesticide resistance, attacks on host defenses, etc.
- Sexual reproduction can be detected by mating type gene presence, evidence of recombining population structure, and/or high genetic variability

Detection of sexual reproduction in *Phytophthora infestans* in N. America

- Cause of potato late blight
- Increased frequency of A2 mating type in early 1990s
- Evidence of both mating types, and of likely recombinant (sexually-produced) offspring in fields in British Columbia

Goodwin et al., *Phytopathology* 85(4): 1995.

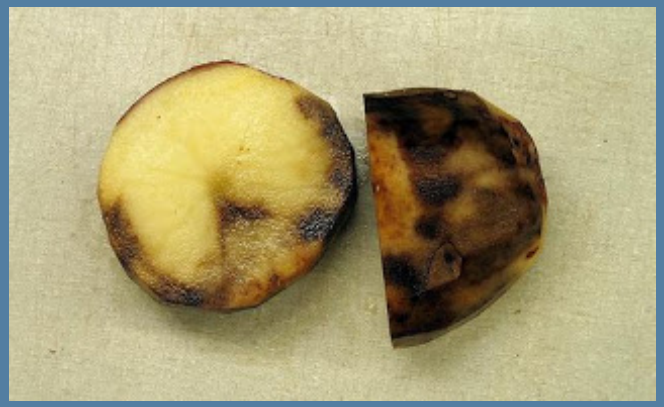
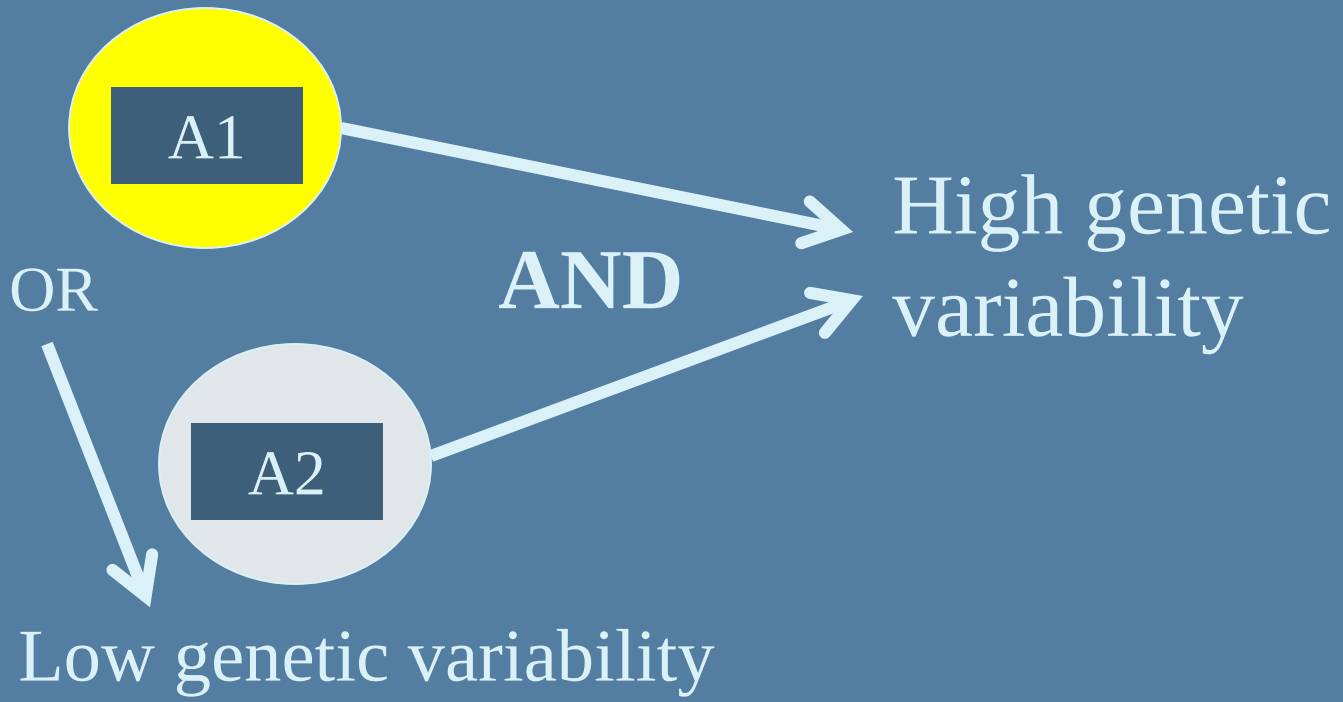


Fig. 1. States and provinces in which A2 mating type isolates were detected during 1979–1991 and 1992–1993. **A,** Only four A2 mating type isolates were detected among 191 isolates analyzed during 1979–1991 (4,10): one A2 mating type isolate was detected in Pennsylvania in 1987 (4), one in British Columbia in 1989 (4), and two in British Columbia in 1991 (10). **B,** A2 mating type isolates were common (61% of all isolates tested) and widely distributed among 384 isolates sampled during 1992–1993.

476 PHYTOPATHOLOGY

Reproductive mode and disease control

- In cases where only one mating type has invaded, it is very important to prevent invasion of a sexually compatible mating type.



Garbelotto (2008) Review article: Molecular analysis to study invasions by forest pathogens: examples from Mediterranean ecosystems

Phytopathol. Mediterr. (2008) 47, 183–203

REVIEW

Molecular analysis to study invasions by forest pathogens: examples from Mediterranean ecosystems

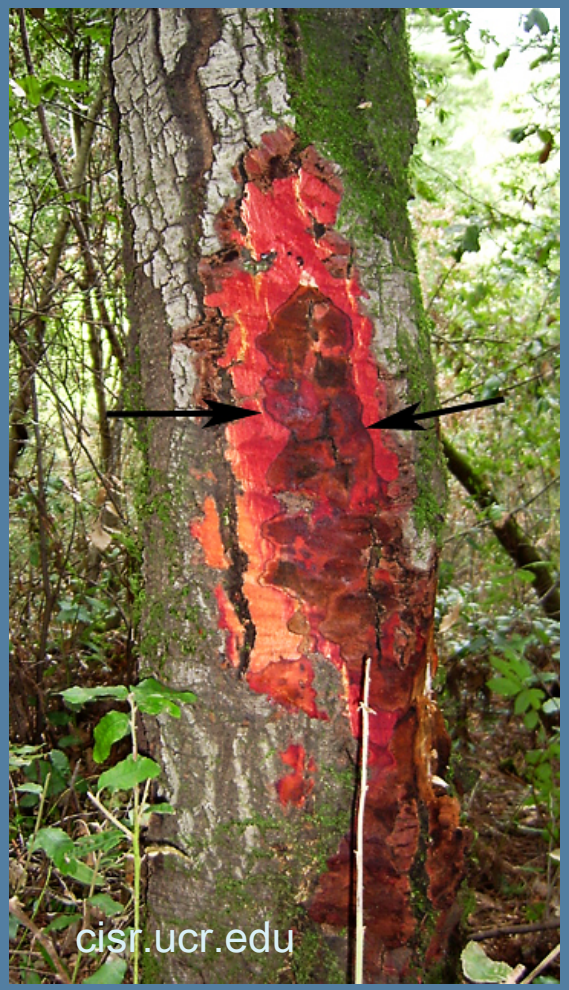
MATTEO GARBELOTTO

Department of Environmental Science, Policy and Management, 137 Mulford Hall, University of California, Berkeley, CA 94720, USA

Summary. Biological invasions by plants and animals have been the subject of several review papers, but invasions by plant pathogens have only occasionally been described and reviewed. The present paper discusses exotic plant diseases whose epidemiology has been clarified using molecular analysis. Because the list of all exotic plant diseases is quite large, this review focuses on forest diseases caused by exotic microbes in Mediterranean ecosystems. In particular, the contribution of molecular studies on exotic forest diseases in favor of or against general invasion biology theory is highlighted. The review follows different phases of the invasion process, giving examples of particular diseases/pathogens for which characteristics have been analyzed.

Garbelotto (2008):

- 3 components for emergent exotic disease:
- 1. Pathogen imported into new system
- 2. Pathogen finds new, suitable, susceptible host
- 3. Ecological/biological conditions favor disease spread



Garbelotto (2008)

- **Phase I: Introduction**
- Pathogen migrates to (in most or all cases, is brought to) a new geographic location
  - From where?
  - How was it introduced?
  - How many introductions?

Phylogeographic or population genetic approaches can be informative for understanding introduction pathways.

Garbelotto (2008)

- Modes of introduction:
- 1. Direct introduction from native source
- 2. Short-term indirect
- 3. Long term indirect (routine)

Garbelotto (2008)

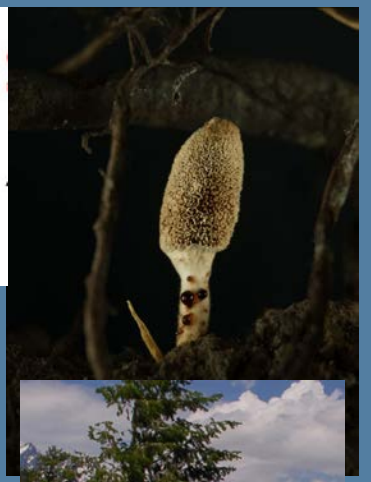
- **Phase II: Establishment**
- Pathogen invades suitable host(s); population size begins to increase
- Symptoms on host are often the first clue that the disease is present; epidemics often first spotted in establishment (not introduction) phase
- Characteristics:
  - Genetic bottlenecks
  - Disequilibrium
  - Strong genetic structuring
- Sexual vs. asexual reproductive modes

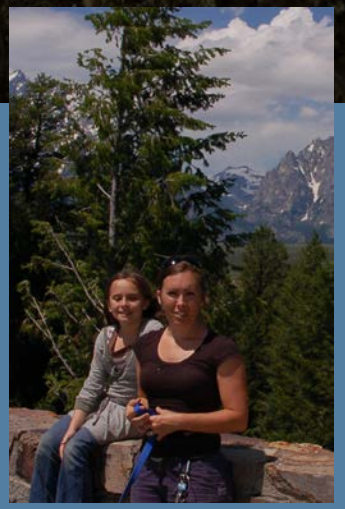
Garbelotto (2008)

- **Phase III: Invasion**
- Spread of the exotic pathogen into new habitats and host populations
- Potential for further genetic bottlenecks because of the loss of alleles by genetic drift in small new populations.
- 2 elements may be required for a successful invasion: population sizes need to increase in order to limit the effects of genetic drift, and new genetic variability needs to arise, either through mutation, recombination, or interspecific gene flow.

In Conclusion-

Forensic Genetic Analysis can help us answer questions such as:





- Who done it?
- How are individuals related?
- What species is it?
- Where does this species come from?
- What are the patterns of migration?
- What is the dispersal capacity?
- Does it reproduce sexually or asexually?
- Has it moved as a result of human activity?