DNA and Floristics

‘DNA Barcoding’

or

the future of Botany 401?
“DNA Barcoding”

The use of DNA sequences to identify specimens across all of life in a manner analogous to the commercially ubiquitous Universal Product Codes (UPC)
DNA Barcoding

- Use 1-4 DNA sequences to unambiguously “identify” a species
DNA Barcoding - the History

• Paul Hebert, University of Guelph, Ontario, suggests in 2003 that all species could be identified by a unique and short molecular sequence - a ‘DNA barcode’

• E.O. Wilson, Harvard University, argues in 2003 for the Encyclopedia of Life - a page of information for every species in world
DNA Barcoding - the History

• Paul Hebert, University of Guelph, Ontario, suggests in 2003 that all species could be identified by a unique and short molecular sequence - a ‘DNA barcode’

• Dan Janzen, University of Pennsylvania & Guanacaste, Costa Rica, approached Paul Hebert at a meeting with suitcase containing 16 skipper butterflies, and a proposal . . .
DNA Barcoding - the History

... could the ‘DNA barcode’ method solve what was going on with a species of skippers in Costa Rica that looked essentially identical as adults, but ...
DNA Barcoding - the History

• The caterpillars of this one species differ in appearance and eat different plants.

• The one species turned out to be 10 different species when Hebert looked at the DNA barcodes of 500 individuals!

• The Hebert et al. 2004 paper in PNAS was the first DNA barcode paper.
DNA Barcoding - the History

• The Consortium for the Barcode of Life (CBOL) was launched in May, 2004

• Over 125 international organizations are now included

• The Barcode of Life Data System (BOLD) was initiated in 2007
Plant Working Group / CBOL

UNITED KINGDOM
Kew Gardens => Conostylis, Pinus, Equisetum, Dactylorhiza
Natural History Museum, London => Tortella, Ptychomniaceae, Asplenium
Edinburgh => Podocarpus, Araucaria, Asterella, Anastrophyllum

DENMARK
Natural History Museum, Denmark => Hordeum, Scalesia, Crocus

SOUTH AFRICA
University of Cape Town => Anastrophyllum/Barbilophozia, Bryum
South African National Biodiversity Institute => Encephalartos, Mimetes

COLOMBIA
Universidad de los Andes => Lauraceae

MEXICO
Instituto de Biologia UNAM => Agave

BRAZIL
Universidade Estadual de Feira de Santana => Laelia, Cattleya

UNITED STATES OF AMERICA
The New York Botanical Garden => Elaphoglossum, Cupressus, Labordia
DNA Barcoding - What is it?

- At the far end of the spectrum from genomics

Extract DNA

PCR amplify a barcode gene locus with universal primers

Sequence gene locus

Query a computer database & / or build a tree

Make identification
DNA Barcoding - What is it?

- DNA barcodes are color coded by base for each position in the sequence - differences are highlighted
DNA Barcoding - What is it?

**BOLD system**

1. Taxonomic assignment of unknown
2. Sequence similarity between unknown and reference sequences
3. Link to species page
4. ID phylogenetic tree showing 100 nearest taxa
DNA Barcoding - What is it?

BOLD system

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Differences in DNA barcodes help create phylogenetic trees showing genetic distances between species
Barcoding Life: Ten Reasons

1. Works with fragments. Barcoding can identify a species from bits and pieces. When established, barcoding will quickly identify undesirable animal or plant material in processed foodstuffs and detect commercial products derived from regulated species. Barcoding will help reconstruct food cycles by identifying fragments in stomachs and assist plant science by identifying roots sampled from soil layers.
2. **Works for all stages of life.** Barcoding can identify a species in its many forms, from eggs and seed, through larvae and seedlings, to adults and flowers.
• DNA barcodes useful when traditional keying does not work

• What species is this?
Fraxinus L. "Ash"

1. Samaras differentiated into a flat wing and a terete or subterete body
   2. Leaves papillose beneath; wing of the samara only shortly decurrent into the upper third of the body
   2. Leaves not papillose; wing of the samara decurrent to the middle of the body, or beyond

1. Samaras flat or nearly so, winged at the base
   3. Twigs terete; flowers all or partly unisexual
   3. Twigs sharply quadrangular; flowers perfect

. . . you could use a dichotomous key after you have taken Botany 401 - but this specimen has no sexual parts!
. . . or you could use your iPod & *Key to Wisconsin Woody Plants* for this unknown
. . . or you could snap an image of it and use your iPhone & leafsnap app to id this unknown
or you could resort to a ‘DNA barcode’ for this unknown

and compare to a library of DNA barcodes of all species of ashes (and all other ca. 275,000 flowering plants)
The point with all these “keys” is to get the right species identification – no “key” is more inherently “legitimate” or “scientific”

**Fraxinus americana** – white ash
Tiny, vegetative shoot of “shagbark hickory” id’ed by leading authority on Wisconsin plants . . .

. . . is really an ash based on DNA barcode!
Barcoding Life: Ten Reasons

3. **Unmask look-alikes.** Barcoding can distinguish among species that look alike, uncovering dangerous organisms masquerading as harmless ones and enabling a more accurate view of biodiversity.
Barcoding Life: Ten Reasons

4. **Reduces ambiguity.** Written as a sequence of four discrete nucleotides - CATG - along a uniform locality on genomes, a barcode of life provides a digital identifying feature, supplementing the more analog gradations of words, shapes and colors. A library of digital barcodes will provide an unambiguous reference that will facilitate identifying species invading and retreating across the globe and through centuries.

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Mitochondrial gene COI nucleotide sequence  Honey Bee
       American Robin
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C = blue
A = green
T = red
G = black
Barcoding Life: Ten Reasons

5. **Makes expertise go further.** The bewildering diversity of about 2 million species already known confines even an expert to morphological identification of only a small part of the plant and animal kingdoms. Foreseeing millions more species to go, scientists can equip themselves with barcoding to speed identification of known organisms and facilitate rapid recognition of new species.

Known species:
- >150,000 flies, mosquitoes
- >250,000 flowering plants
- >300,000 beetles
- >30,000 crabs, lobsters
Barcoding Life: Ten Reasons

6. **Democratizes access.** A standardized library of barcodes will empower many more people to call by name the species around them. It will make possible identification of species whether abundant or rare, native or invasive, engendering appreciation of biodiversity locally and globally.

Instead of saying “this is some type of red oak,” anyone could say “this is *Quercus ellipsoidalis*, the Northern pin oak”
Barcoding Life: Ten Reasons

7. **Opens the way for an electronic handheld field guide, the Life Barcoder.**
Barcoding links biological identification to advancing frontiers in DNA sequencing, miniaturization in electronics, and computerized information storage. Integrating those links will lead to portable desktop devices and ultimately to hand-held barcoders.
8. Sprouts new leaves on the tree of life.
Since Darwin, biologists seeking a natural system of classification have drawn genealogical trees to represent evolutionary history. Barcoding the similarities and differences among the nearly 2 million species already named will provide a wealth of genetic detail, helping to draw the tree of life on Earth. Barcoding newly discovered species will help show where they belong among known species, sprouting new leaves on the tree of life.
Compiling the library of barcodes begins with the multimillions of specimens in museums, herbaria, zoos and gardens, and other biological repositories. The spotlight that barcoding shines on these institutions and their collections will strengthen their ongoing efforts to preserve Earth's biodiversity.
Barcoding Life: Ten Reasons

One can envision that in the next half century during what will probably be the final great age of biological exploration the electronic naturalist will be equipped with new technologies to enhance and accelerate his/her work, including micro-global positioning systems, palm-top computers, web-based satellite communication, and mini-DNA samplers and sequencers. [John Kress, 2004]
10. **Speeds writing the encyclopedia of life.** Compiling a library of barcodes linked to vouchered specimens and their binomial names will enhance public access to biological knowledge, helping to create an on-line encyclopedia of life on Earth, with a web page for every species of plant and animal.

First suggested by E.O. Wilson in 2003 - and in book form - the EOL has gone electronic
DNA Barcoding - Is it working?

- Will be a boon for fungal identification - a group which has a ‘messy’ taxonomy because of sexual and asexual forms, or are soil microbes only
• All animals based on 648 bp of cytochrome oxidase I gene in the mitochondrial genome
DNA Barcoding - Is it working?

- a universal barcode for plants has been contentious

“It’s a very contentious issue,” says Kenneth Cameron, director of the Wisconsin State Herbarium at the University of Wisconsin-Madison. “There are a lot of politics and personalities involved.”

Botanical identities

finally consensus! (?)  

2 gene barcode  

*Fritillaria meleagris* in English wet meadows & its *rbcL* barcode
DNA Barcoding – WIS Flora

- Waller, Givnish, Cameron & Sytsma – awarded an NSF Biodiversity grant to in part (1) barcode the entire Wisconsin Flora and (2) use the resulting phylogenetic tree to access community assemblage, phylogenetic signal in species losers and winners over the last 60 years

with the help of Alyssa et al.
DNA Barcoding – WIS Flora

Barcode and produce a molecular phylogeny for the entire vascular flora (1889 natives + 683 introduced = 2572 species) using *rbcL* and *matK* sequences
Ordinal Tree of WIS

- Ordinal relationships are “known”

- Do “Barcodes” recover these relationships?
Wisconsin Vascular Flora –

They do!
DNA Barcoding – WIS Flora

Using the phylogenetic tree

- Phylogenetic analysis of C-values, correlated evolution with habit, latitude, functional traits, etc.
- Phylogenetic analysis of functional traits, correlated evolution, etc.
- Community assembly and disassembly in phylogenetic framework
- Patterns of species richness vs. phylogenetic diversity across the WI landscape
- Niche (Bioclim & soil) evolution in selected lineages
Assembly of WI Sedges

1. Shade
   - Yellow: Intolerant
   - Gray: Tolerant

2. Wetness
   - Blue: Wet
   - Brown: Dry

3. C-value
   - Green: High
   - White: Intermediate
   - Yellow: Low
• $H_0$: trait is randomly occurring

• $H_A$: trait exhibits phylogenetic signal

• All three traits exhibit significant phylogenetic signal

• Closely related species are ecologically similar