

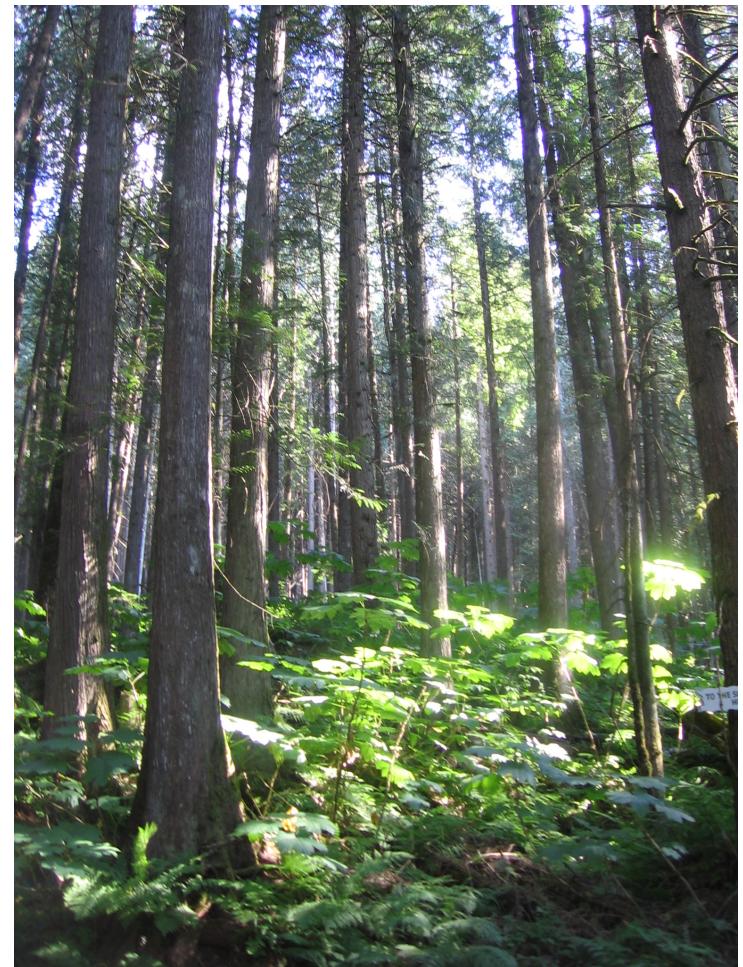


Barcode-assisted Assessments of Lepidopteran Diversity in British Columbia

Jeremy deWaard, Jean-François Landry and Leland Humble

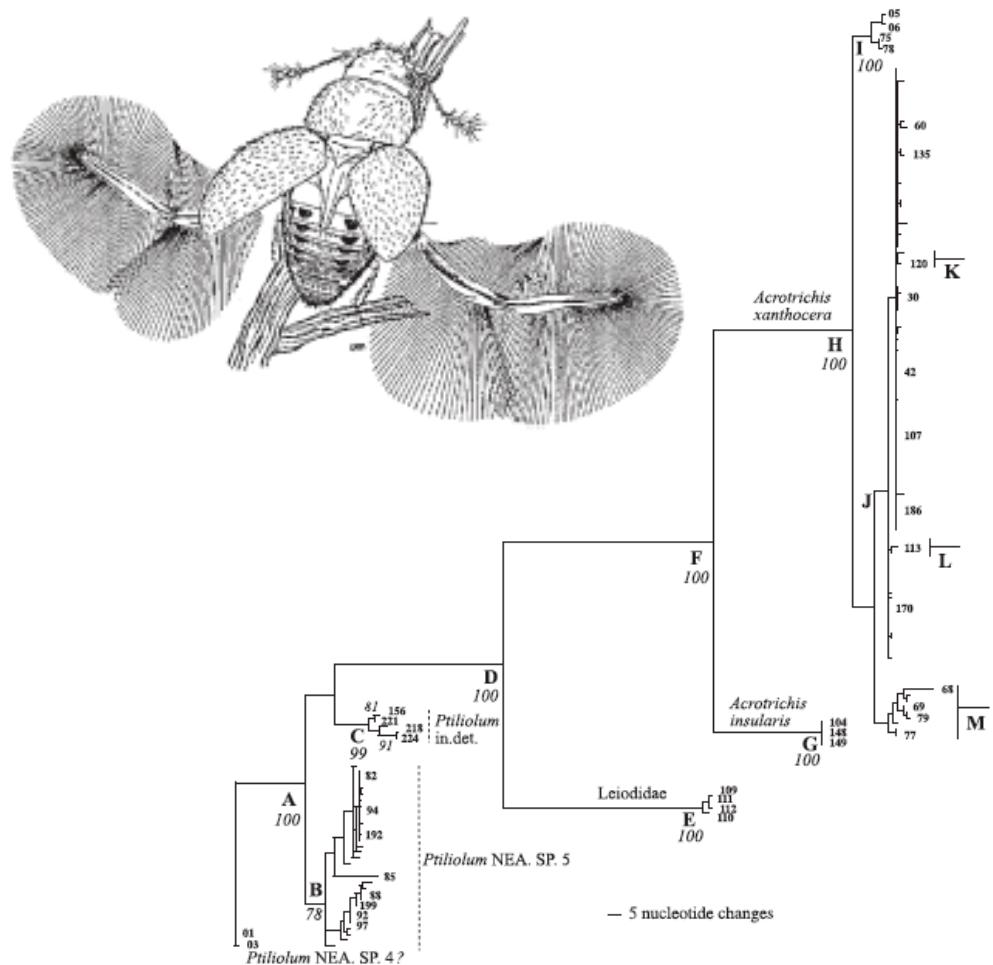
Inventories & assessments

- formidable task for terrestrial arthropods
 - sorting/prep time 40X longer than sampling time (Marshall *et al.* 1994)
 - identification time usually longer
 - large samples, damaged specimens, many species at low density
 - lack of trained taxonomists => lack of usable keys



Genetic techniques: sorting

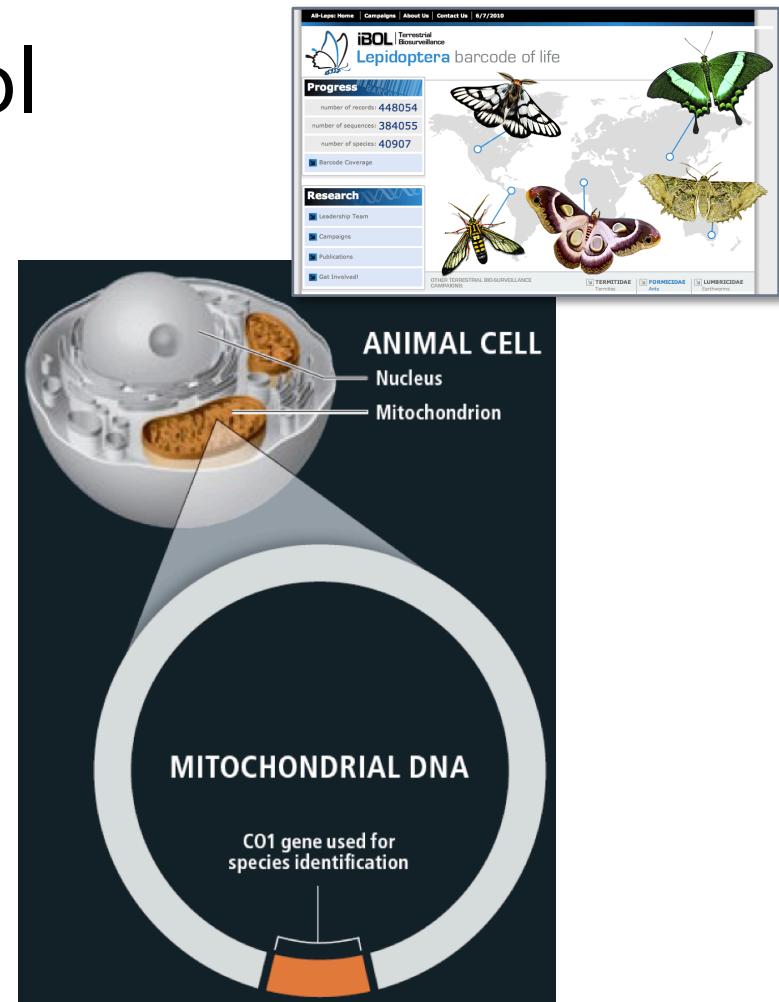
- Caesar *et al.* 2006
 - feather-wing beetles (Ptiliidae)
 - informed sort
 - only 25% of specimens required examination by specialist



COI tree (Caesar et al 2006)

DNA barcoding: sorting & IDs

- DNA barcoding as a tool for inventories (e.g. Janzen *et al.* 2005)
 - informed sorting and tentative species identification
 - minimize specialist time



Urban park moth inventory

- Stanley Park,
Vancouver in
2007

Biodivers Conserv (2009) 18:3825–3839
DOI 10.1007/s10531-009-9682-7

ORIGINAL PAPER

In the dark in a large urban park: DNA barcodes illuminate cryptic and introduced moth species

**Jeremy R. deWaard · Jean-François Landry · B. Christian Schmidt ·
Jennifer Derhousoff · John A. McLean · Leland M. Humble**

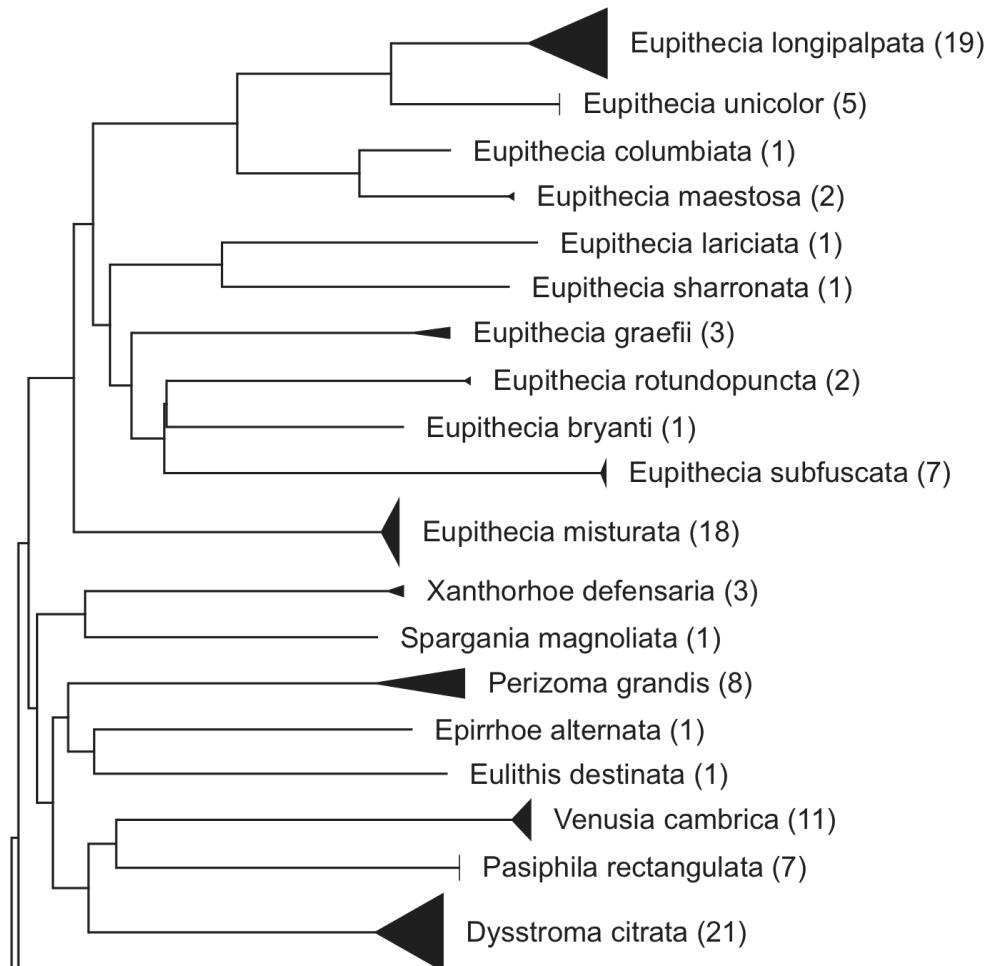


Methods: collection & analysis

- MV light / sheet collecting
- synoptic each collection night
- DNA barcoded all 925 specimens
- BOLD-ID for IDs (where possible)
- confirmed morphologically



Results: species assignment



- 190 clusters, 124 assigned to species, 61 to genus using BOLD-ID
- 66 remaining clusters identified
 - 25 with brief comparison
 - 41 with genitalic dissections
- * first (and only) step for specialist; 7% of specimens

Results: highlights of inventory



Prays fraxinella



Dichelia histrionana



Paraswammerdamia lutarea

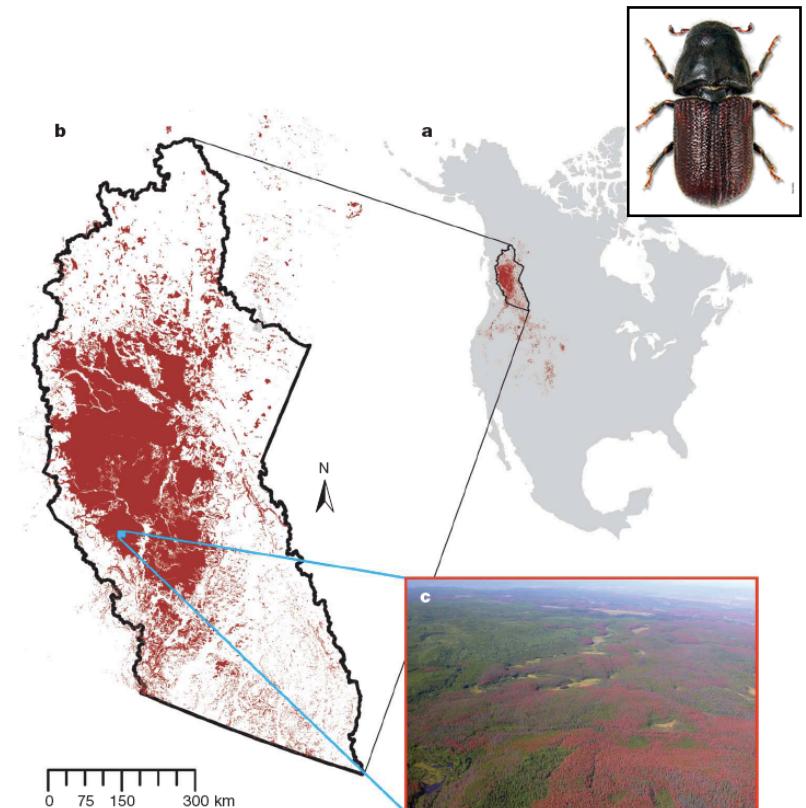


Argyresthia pruniella

- 190 species
- 21 families
- 71 singletons
- * taxonomically diverse; many at low density
- * 4 non-indigenous
- * not complete, but solid start

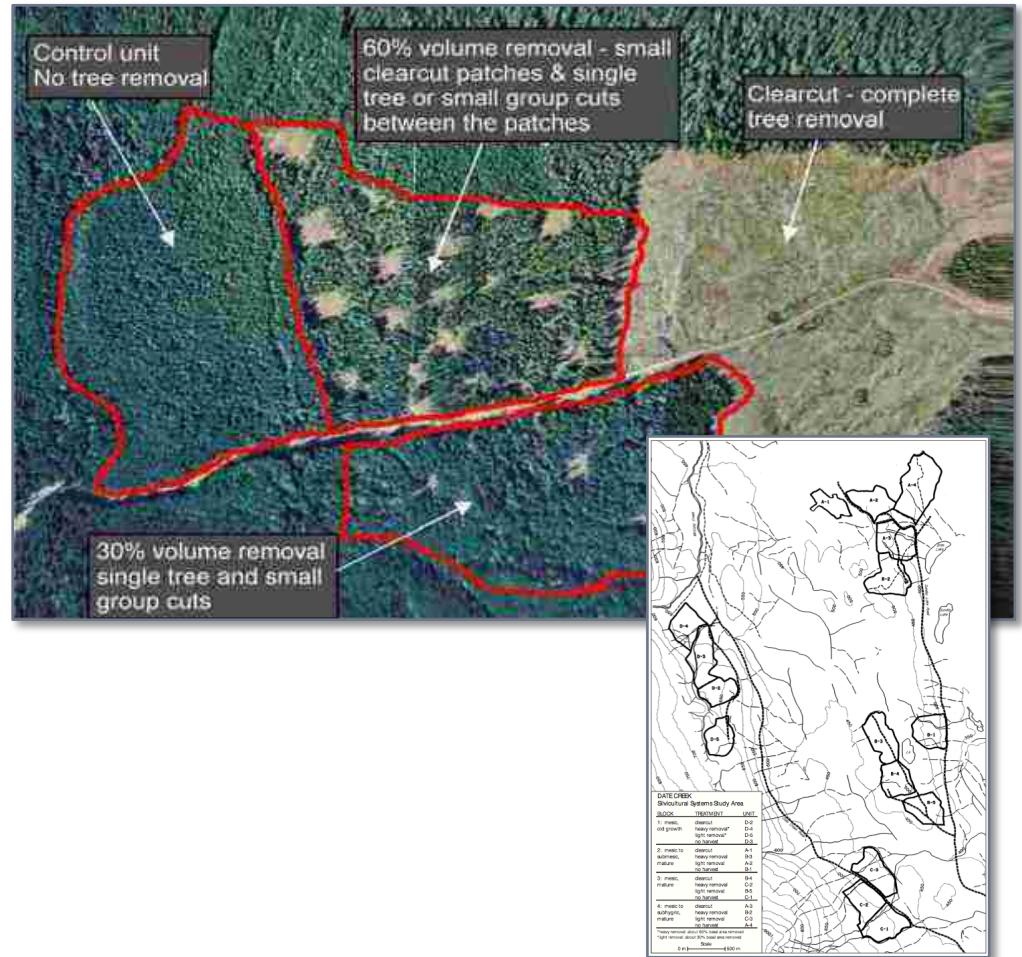
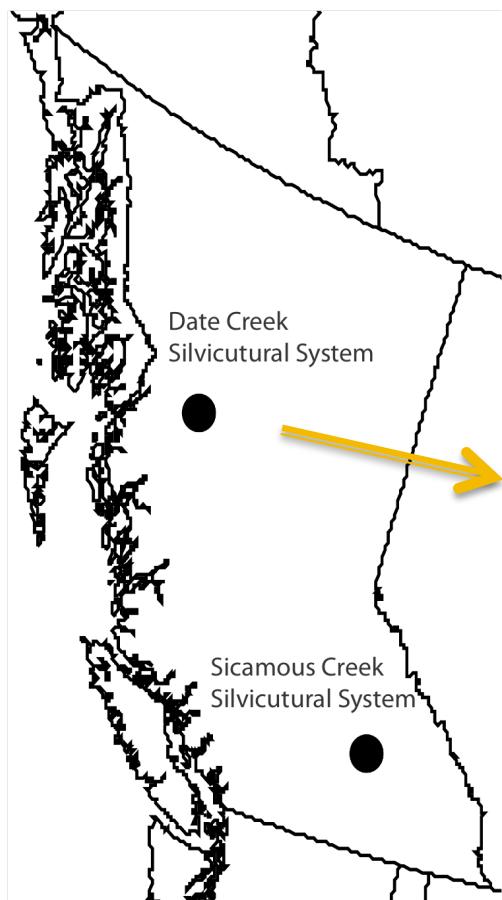
Diversity effects of disturbance

- how do anthropogenic and natural disturbances affect forest biodiversity?
 - we can now rapidly assess diversity changes in hyper-diverse assemblages



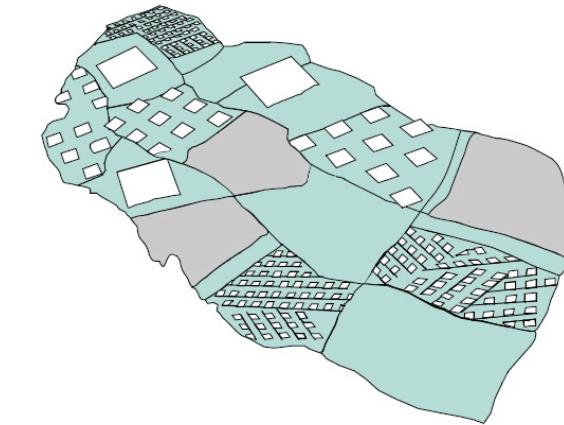
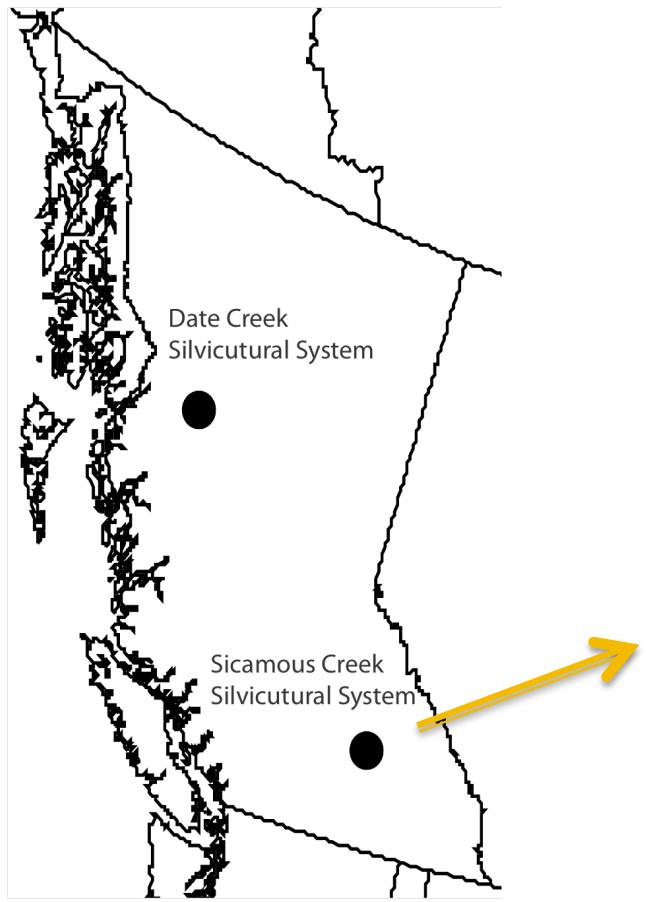
Natural disturbance example: native pest outbreak of mountain pine beetle (Kurz et al 2008; NRC)

The effect of harvest types on lepidopteran diversity



Date Creek Silvicultural System (Coates et al 1997): Interior Cedar–Hemlock, ~450m

The effect of harvest types on lepidopteran diversity



Sicamous Creek S.S. (Huggard & Vyse 2002): Engelmann Spruce - Subalpine Fir, ~1650m

Harvest treatments

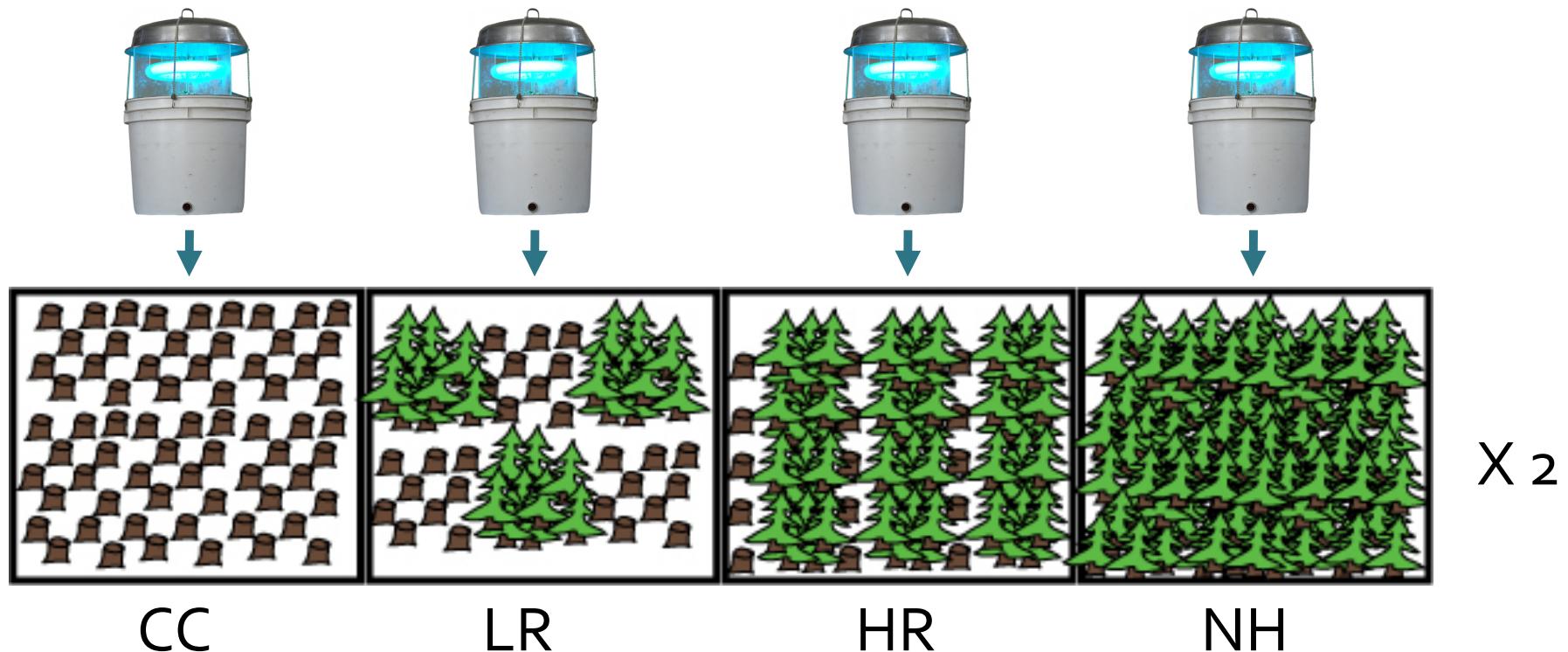
- 3 treatments, 1 control
 - CC – clear-cut
 - HR – heavy removal
(patch cut)
 - LR – light removal
(individual tree selection)
 - NH – no harvest (350 year old stand)



Date Creek treatments

Methods: trapping

- UV light traps allow standardized effort
- single trap per treatment, far from edges
- 8 traps per night; ~tri-weekly through flight season



Methods: sorting

- 27 complete comparisons
(CC vs HR vs LR vs NH)
 - 108 traps
 - 10,904 specimens
 - field-pinned nearly all
 - sorted to morphospecies



Methods: sampling

- for barcoding, we analyzed a synoptic of each trap
- analyzed all specimens not confidently placed to single morphospecies (e.g. rubbed specimens; tough genera)
- imaged, sampled and vouchered 6117 specimens



Methods: lab

- completed at Canadian Centre for DNA Barcoding in Guelph, ON
- amplified and sequenced COI using standard protocols
- all sequence data, images, and collection data stored on BOLD



The screenshot shows the homepage of the Barcode of Life Data Systems (BOLD) website. The header includes the BOLD logo and navigation links for "Barcode of Life Data Systems v7.5", "About BOLD", and "Contact Us". Below the header, there are sections for "BARCODE OF LIFE DATA SYSTEMS", "MANAGEMENT & ANALYSIS", and "IDENTIFICATION ENGINE". The "MANAGEMENT & ANALYSIS" section displays barcode counts and a user login form. The "IDENTIFICATION ENGINE" section features a "SPECIES IDENTIFICATION" tool with a butterfly image. The footer contains copyright information and a note about the version 7.5 release.

Barcode of Life Data Systems v7.5

About BOLD Contact Us

Barcode of Life Data Systems (BOLD) is an online workbench that aids collection, management, analysis, and use of DNA barcodes. It consists of 3 components (MAS, MAS, and ECS) that each address the needs of various groups in the barcoding community.

MANAGEMENT & ANALYSIS

BOLD-MAS provides a repository for barcode records coupled with a management and analysis online workbench for the DNA barcode community.

IDENTIFICATION ENGINE

BOLD-IDB provides a species identification tool that accepts a barcode image or a sequence region and returns a taxonomic assignment to the species level when possible.

BOLDSYSTEMS BOLD 2.5 Release

Version 2.5, released on Nov 11th 2009 at the Third International Barcoding of life conference in Mexico City, provides new core functionality including support for multiple sequence markers per specimen and more complex workflow. It also includes a management system for barcode records and markers, comparative analytics, web services and a variety of convenience upgrades. A few are highlighted below.

Barcode diversity of species and sequences by site or higher level taxonomy

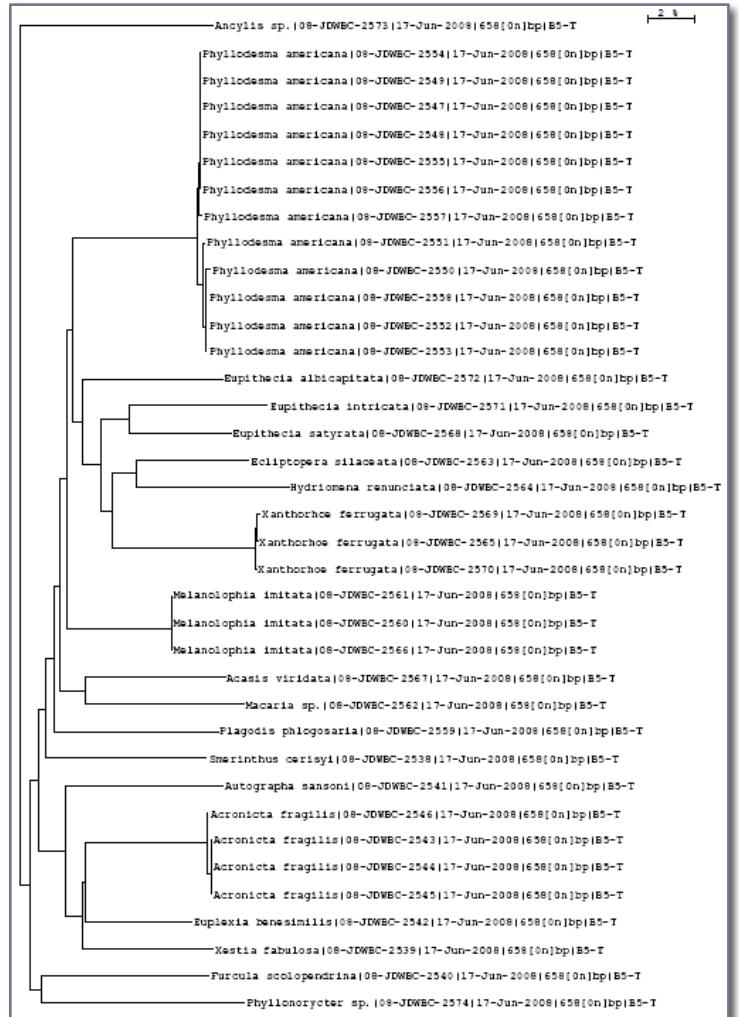
Marker curves

Multi-marker

All analytical tools have been upgraded to support

Results: barcoding

- 6101/6117 successfully barcoded
 - remaining 16 were easily assigned to species
- most* matched single species / monophyletic cluster in BOLD
- * confirmation/identification ongoing



BOLD TaxonID tree for trap LR5

Results: 2 inventories

- Date Creek (DC)
 - 7978 specimens
 - 340 spp.

- Sicamous Creek (SC)
 - 2926 specimens
 - 211 spp.



Parornix DRD30



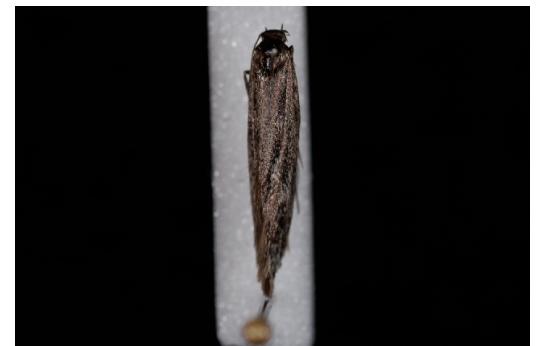
Hyles gallii

Neoarctia beanii



Highlights of inventories

- high diversity – 26 families, 219 genera
- e.g.
 - *Walshia miscecolorella* (Cosmopterigidae)
 - *Batrachedra praeangusta* (Batrachedridae)
 - *Scythris noricella* (Xyloryctidae)
- single trap at Date Ck – 100 spp.



Highlights of inventories

- several difficult genera
 - *Euxoa* spp. (*divergens*)
 - 8 spp. in just 11 individuals at SC
 - *Eupithecia* spp. (*gelidata*)
 - 12 spp. at DC, 13 spp. at SC
 - *Epinotia* spp. (*plumbolineana*)
 - 16 spp. at DC



Highlights of inventories

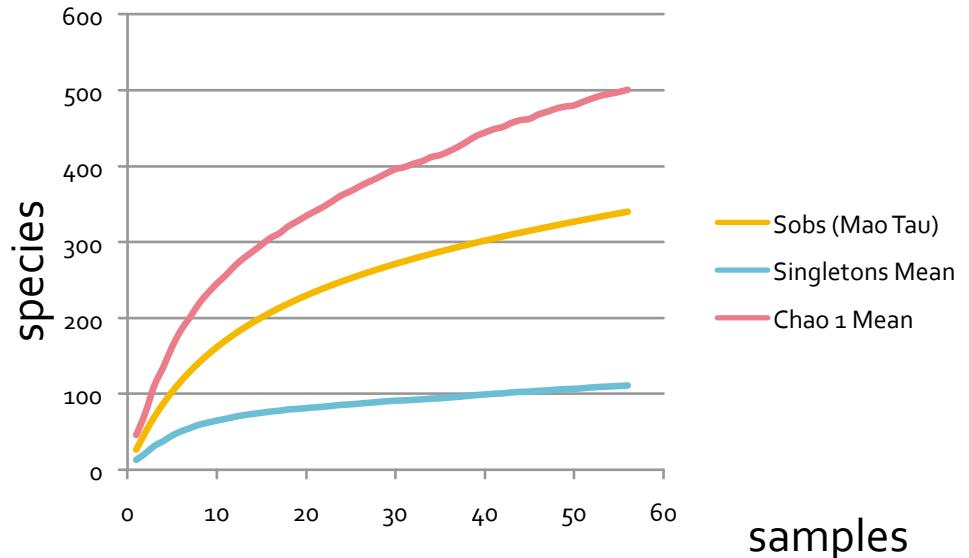
- many pest species in moderate abundance
 - hemlock looper (*Lambdina fiscellaria*)
 - aspen leaf miner (*Phyllocnistis populiella*) (* 481 in 1 trap at DC)
 - western spruce budworm (*Choristoneura occidentalis*)



Completeness of inventories

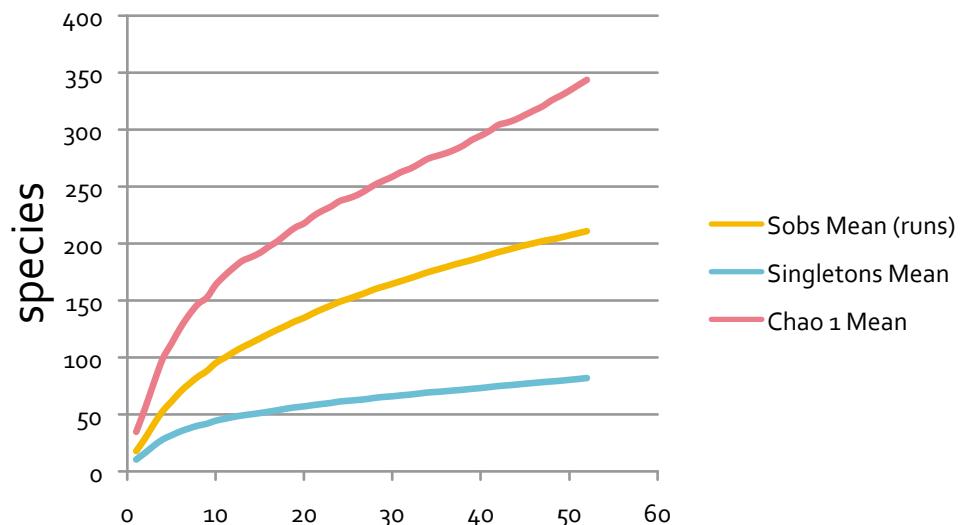
Date Creek

- 501 spp. estimated
- $340/501 = 68\%$
- 111 singletons



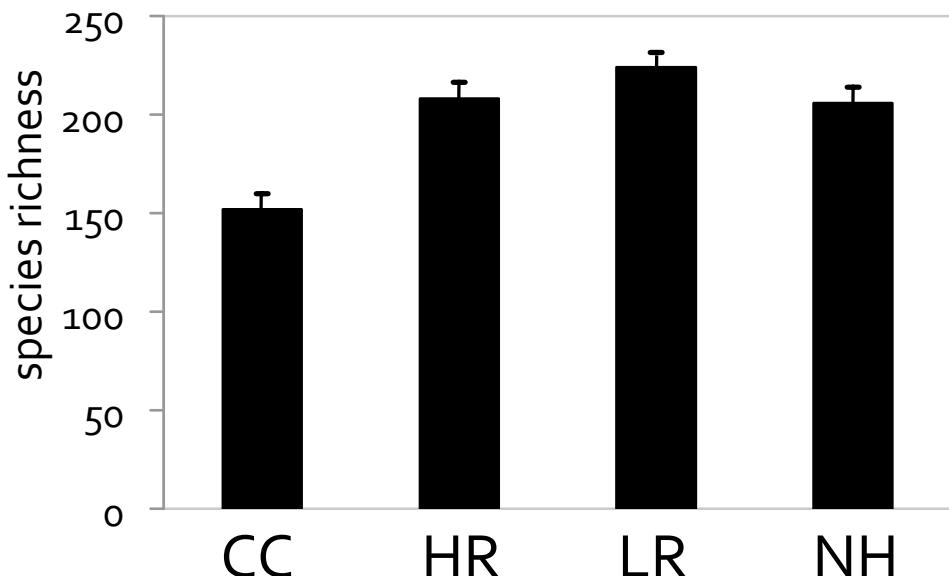
Sicamous Creek

- 344 spp. estimated
- $211/344 = 61\%$
- 82 singletons

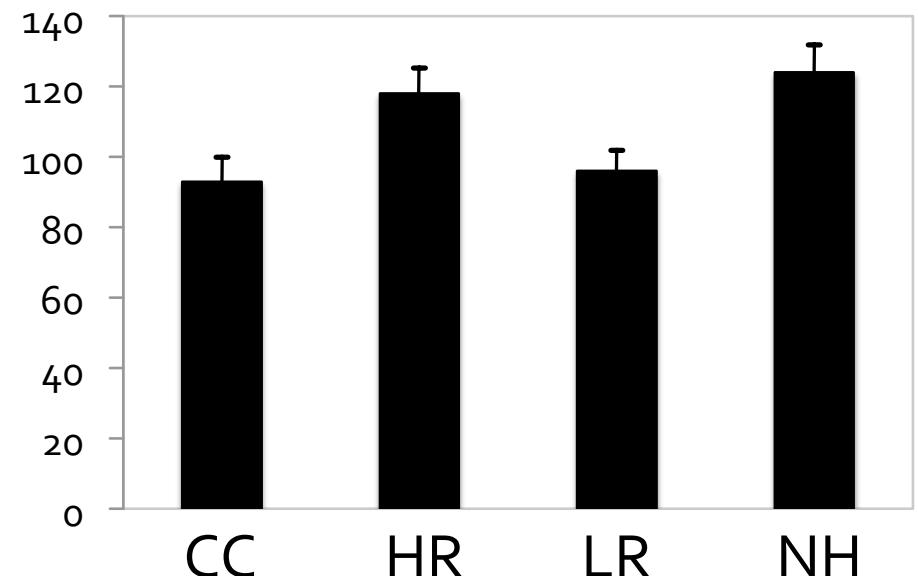


Harvest effects on species richness

Date Creek:



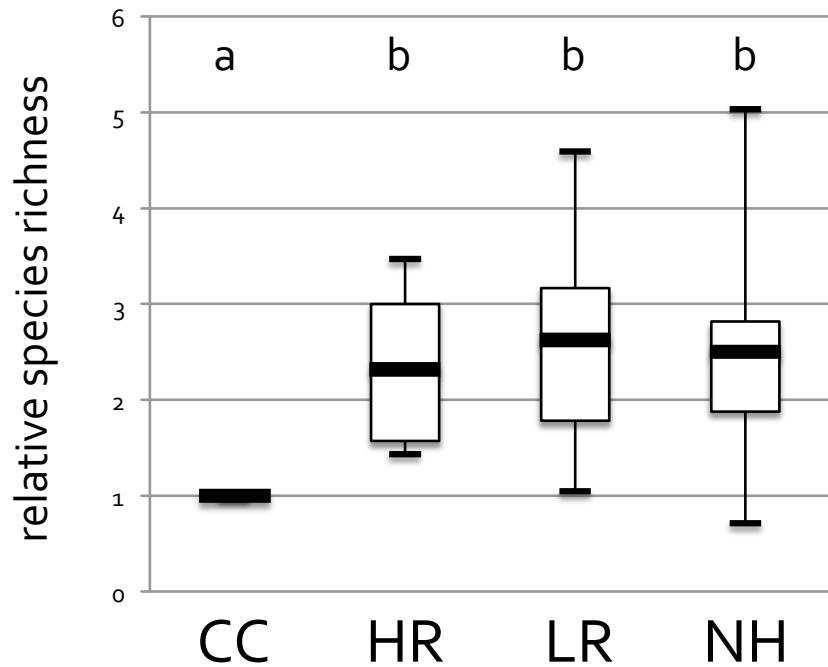
Sicamous Creek:



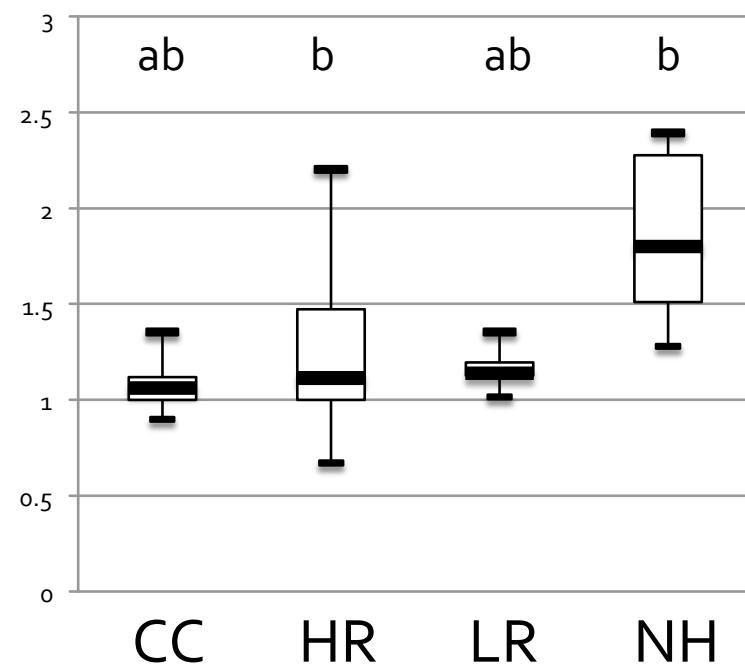
- rarified species richness per treatment

Results: relative comparisons

Date Creek:



Sicamous Creek:

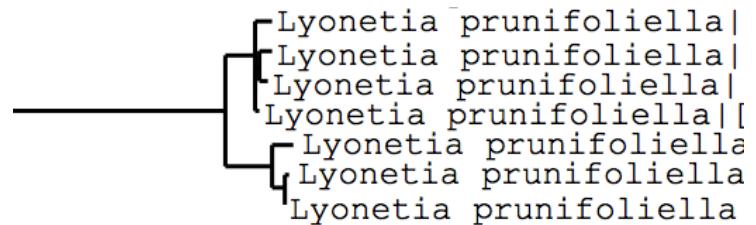


- relative species richness (same collection event)

Additional levels of diversity

- genetic diversity (GD)
 - e.g. haplotype diversity
 - Stanley Park Leps – mean of 3.4 haplotypes/spp., or one new haplotype every 3.9 individuals

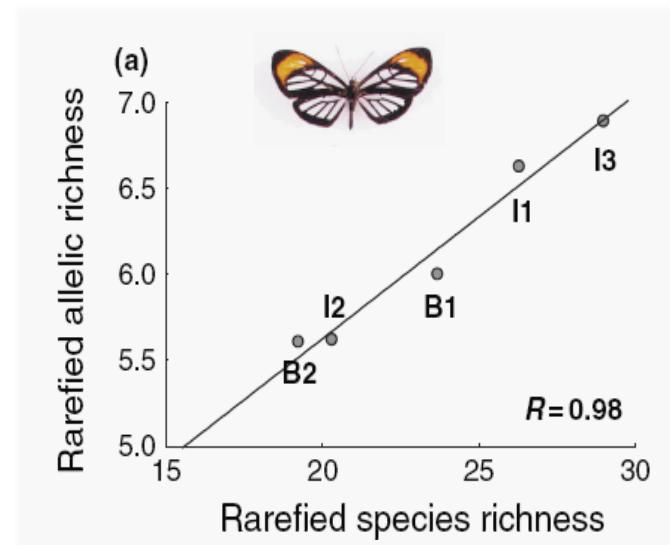
- phylogenetic diversity (PD)
 - variation in evolutionary history
 - can add phylogeny backbone to primary barcode, or not (see Faith 2007)



Lyonetia prunifoliella (Lyonetiidae) contributes highly to both GD and PD

More to do...

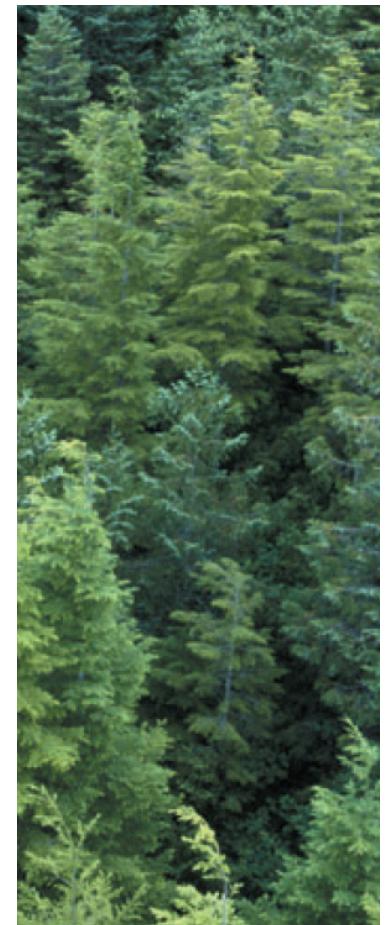
- interplay between 3 levels of diversity
 - can 1 or 2 be a proxy for all?
- family and feeding guild responses
- interesting hypotheses to address
 - e.g. species/genetic diversity correlation (SGDC) (Vellend 2003)



Post-disturbance SGDC in butterflies (Cleary et al 2006).

Conclusions

- DNA barcoding has proven valuable for forest inventory work and looks promising for post-disturbance assessments
- DNA barcoding provides informed sorting, reduction of specialist time, enhanced sensitivity at low density, and estimates of multiple levels of diversity



Acknowledgements

■ People:

- Sujeevan Ratnasingham, Stephanie Kirk, Paul Hebert & staff of the CCDB (U. of Guelph)
- Doug Steventon and Alan Vyse (BC Forest Service)

■ Funding:

- Canadian Barcode of Life Network [Genome Canada, Ontario Genomics Institute, NSERC]
- NSERC Graduate Scholarship Program
- Canadian Food Inspection Agency
- BC Ministry of Forests and Range
- Canadian Forest Service – Pacific
- Forest Investment Account – FSP





Thank you.

Hup Holland Hup!

