A Step Toward Barcoding Life:A Model-Based, Decision-Theoretic Method to Assign Genes to Preexisting Species Groups

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The Gist

- Objective: Assign newly sequenced individuals to existing, pre-identified groups
- Associate based on statistical evidence of similarity

What Has Been Done?

- DNA barcoding is already heavily utilized
  - Unacceptable Error rates in some groups such as cowries (snails)
- Discrepancy of effectiveness of existing methods attributed to different levels of variation between each species
- Sampled sequences must exactly match a known sequence, else inference must be made

What We Need

- Statistical method to determine appropriate assignment
  - Would give us a degree of confidence of our classification
- Assign new individuals to existing groups, previously established by taxonomists
- Taking a sequence and determining appropriate group of origin is:
  - A clustering problem (unsupervised), not tackled in paper
  - An assignment problem (supervised), assumes pre-existing groups defined elsewhere

The Method

- Assuming a correct group k, assigning individual to wrong group j results in error utilized to reflect assignment uncertainty
- Loss function $L(k, j)$ quantifies error
- k is likely not known
- Instead, find group i that minimizes posterior risk of assigning to a group

$$R_i = E[L(k, i)] = \sum_k L(k, i) Pr(x \epsilon k | x)$$

Independence and Transformation

$$R_i = E[L(k,i)] = \sum_k L(k,i) Pr(x \epsilon k | x)$$

...assumes that individuals within a group are $i.i.d$
This is not the case when they share a common history, so we write:

$$R_i = E[L(k,i)] = \sum_k L(k,i) Pr(x \epsilon k | x, D)$$

...where D represents preexisting data.
Also, using Bayes rule, we can calculate the posterior with:

$$Pr(x \epsilon k | x) = \frac{Pr(x | x \epsilon k) Pr(x \epsilon k)}{\sum_j Pr(x | x \epsilon j) Pr(x \epsilon j)}$$

The Coalescent

- Describes the behavior of a sample of individuals, backward in time, until they find their most recent common ancestor (MRCA)
- Eliminates need to consider unobserved and extinct individuals
- Model to be used combines aforementioned classifier and coalescent to form the “Coalescent Assigner”

The Coalescent Assigner

- Avoids increased complexity that comes with considering phylogenetic history (pre-existing data in past equation)
- We assume that the ancestors of each population have been evolving long enough that it is now evolving independent of other groups
  - The stationarity of the nucelotide substitution process has been attained.
- Assume: Different groups that are potential targets of assignment are fully defined and prespecified
- Assume: Each group forms a panmictic population that follows Wright-Fisher neutral model
  - i.e. does not allow recpmbination, selection or migration
- $\therefore$ Evolutionary process within each group is governed by one parameter
  - θ - expected number of mutational steps between any two individuals within that group
- Because groups are fully defined, θ known with certainty

Formalizing the Loss Function

$$L(k,i) = \begin{cases} LS - dist[x - consensus(d_k)] & : k \neq i \\ 0 & : k = i \end{cases}$$

LS = length of sequence under study
$dist()$ = # nucleotides diff betw. individual and consensus of potential group

- This function is not unique, any function can be created to reflect importance of other factors.

Implicit Doubt

- We set a limit above which assigning an individual to the group with minimal risk is questionable
  - Highlights need to evaluate origin of that individual and possibility of it being misplaced
- Proportion of nucleotides that match by change $= 0.25$
- Each group is likely to obtain assignment
- Doubt threshold calculated as $\frac{0.25}{M}$ where M is the total $\#$ of available groups

Posterior Probability of Membership

$$Pr(x \epsilon k | k, D, \theta) = \frac{Pr(x, x \epsilon k | D, \theta)}{Pr(x | D, \theta)}$$

. . . where θ is a known vector of parameters $\theta_1, \theta_2, \ldots, \theta_k, \ldots, \theta_M$
Using Bayes rule:

$$Pr(x \epsilon k | x, D, \theta) = \frac{Pr(x | x \epsilon k, D, \theta) Pr(x \epsilon k | D, \theta)}{\sum_j Pr(x | x \epsilon j, D, \theta) Pr(x \epsilon j | D, \theta)}$$

. . . where $P(x | x \epsilon k, D, \theta) =$ likelihood of newly added sequence, x given that:

- It originated from group k
- The known, preassigned data D
- The known evolutionary param θ

Posterior Probability of Membership (continued)

The likelihood $Pr(x|x\epsilon k, D, \theta)$ can be rewritten as...

$$Pr(x|x\epsilon k, D, \theta) = \frac{Pr(x, D_k|x\epsilon k, D_1, ..., D_{k-1}, D_{k+1}, ..., D_M, \theta_1, ..., \theta_k, ..., \theta_M)}{Pr(D_k|x\epsilon k, D_1, ..., D_{k-1}, D_{k+1}, ..., D_M, \theta_1, ..., \theta_k, ..., \theta_M)}$$

...assuming independence:

$$Pr(x|x\epsilon k, D, \theta) = \frac{Pr(x, D_k|x\epsilon k, \theta_k)}{Pr(D_k|x\epsilon k, \theta_k)}$$

...assuming x belonging to group k will not signif. alter likelihood of data D_k given θ_k:

$$Pr(x\epsilon k|x, D, \theta) = \frac{\frac{Pr(x, D_k|x\epsilon k, \theta_k)}{Pr(D_k|x\epsilon k, \theta_k)} Pr(x\epsilon k|D, \theta)}{\sum_j \frac{Pr(x, D_j|x\epsilon k, \theta_j)}{Pr(D_j|x\epsilon k, \theta_j)} Pr(x\epsilon j|D, \theta)}$$

Posterior Probability of Membership (continued)

. . . assuming presampled D and true parameter vector θ will not where assigned new individual and **assume** a uniform prior on where the new individual might belong:

$$Pr(x \epsilon k | x, D\theta) = \frac{\frac{Pr(x, D_k | x \epsilon k, \theta_k)}{Pr(D_k | \theta_k)}}{\sum_j \frac{Pr(x, D_j | x \epsilon k, \theta_j)}{Pr(D_j | \theta_j)}}$$

Above has calculated likelihood of an individual belonging to a certain group rather than calculating the likelihood of the group once the individual is added!

We can put the result into the risk equation from before

$$R_i = \sum_k L(k, i) Pr(x \epsilon k | x, D, \theta) = \sum_k L(k, i) \frac{\frac{Pr(x, D_k | x \epsilon k, \theta_k)}{Pr(D_k | \theta_k)}}{\sum_j \frac{Pr(x, D_j | x \epsilon k, \theta_j)}{Pr(D_j | \theta_j)}}$$

Implementing the Coalescent Assigner

1. Establish true parameter θ for each target group
   - This calculates the likelihood of the presampled data given its group of origin
   - Not possible in practice, we instead estimate the parameter for each group using available data
   - Produces Maximum Likelihood Estaimates (MLEs), which are "close to the truth"
2. Use MLEs to calculate likelihood of data after adding individual to each available group.
   - No direct method exists to evaluate likelihood after adding individual but new likelihood function would be $Pr(x, D_k|x\epsilon k, \theta_k) = \sum_G Pr(x, D_k|x\epsilon k, G)Pr(G|\theta_k)$ . . . where G is all possible genealogies.
   - Both a naïve method (sampling from posterior $Pr(G|\theta_k)$ and a Markov Chain Monte Carlo approach were implemented to calculate the sum with an adjustment that allowed the direct calculation of the likelihood
   - Remember: This assumes full knowledge of θ_k.
   - Naïve method works best for small within group sample sizes - used to show effectiveness vs. distance method

Implementing the Coalescent Assigner (cont)

3. Finding the consensus sequence
   - Used a 50% majority rule
4. Evaluate the risk using the risk formula
   - Straightforward once all likelihoods are evaluated

Validation

- Simulated and real data used to evaluate performance of Coalescent Assigner
- Compared against distance approach that does not take understanding of evolutionary process into account.
- The "correct" group of assignment, k was one with min $dist[x - consensus(D_k)]$
- Assignment is doubtful if $\exists$ Group j where $j \neq k$ with $dist[x - consensus(D_k)]/LS < 3\%^*$ or equal distance from the newly sampled individual

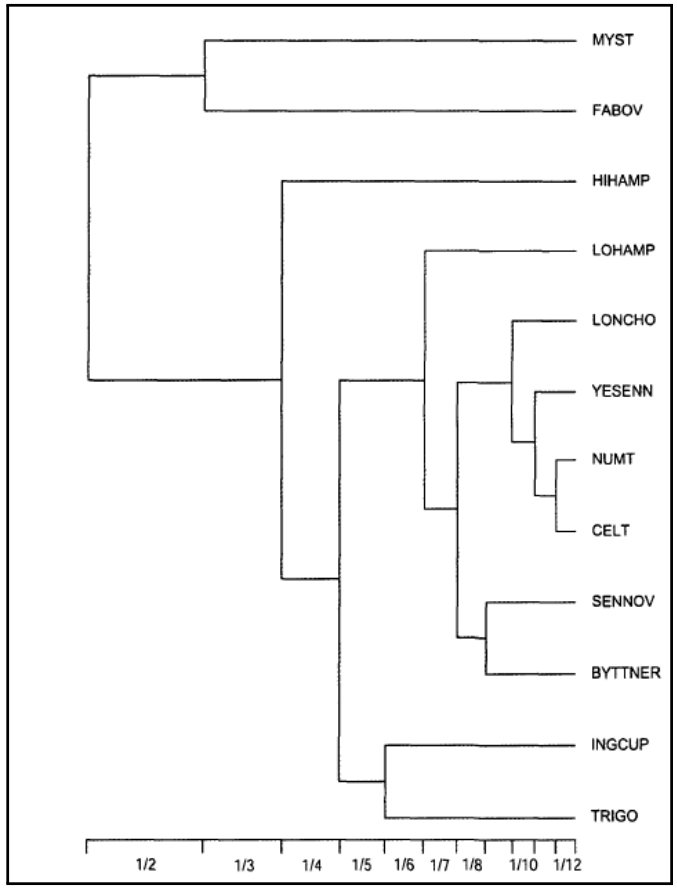
* This threshold is derived from Herbert et al (2003b) and was shown to highlight the range of appropriate use of the distance measure

Simulated Data

- We want to evaluate ability of proposed method to assign an individual to its true group of origin under various degrees of divergence and overlap
- Utilized *Asraptes flugerator* (neotropical skipper butterfly) data, assuming existence of 12 predefined groups
- Using PAUP*4b10, we estimate phylogeny and parameters of model based on consensus sequences
  - Model of choice was General Time-Reversible (GTR) model
  - Unresolved nodes were resolved arbitrarily to create bifuracting tree
  - Only topology of tree and est. params of model were used for next steps of simulation

A Generated Tree Example

- Branch lengths represent the expected number of subtitutions, on average, base on Yule model with parameter $\frac{1}{v}$
- Depth varied based on rate of substitution
  - $v = 1$ divergent groups
  - $v = 0.1$ moderately divergent groups
  - $v = 0.01$ short tree with moderate group overlap
  - $v = 0.001$ short tree with much overlap
- Utilized neutral coalescent to model within-group evolution
- An extra individual was generated within each group, 1/12 reassigned.
  - Done to maintain equal group sizes prior to the assignment
- In the case of no within group variation, assume the group's diversity was very small and set θ to 10^{-8}



Tree used to simulate DNS sequences of ancestors (MRCAs) of each of the 12 groups

Applying the Method

Name	Size
Astraptes-MYST	3
Astraptes-FABOV	31
Astraptes-YESENN	78
Astraptes-SENNOV	103
Astraptes-BYTTNER	4
Astraptes-INGCUP	65
Astrnptes-TRIGO	51
Astraptes-NUMT	4
Astraptes-HIHAMP	16
Astraptes-LOHAMF	47
Astraptes-CELT	23
Astrnptes-LONCHO	41

- Neotropical skipper butterflies have traditionally been classified as a single species
- On the basis of barcode data, class represents 12 sympatric species
- In evaluating method, draw one query sequence at random from all sequences
- Resonstruct groups without this sequence
- Perform assignment on excluded sequence

Performance Evaluation

- Evaluated change in power of coalescent assigner when used to assign presampled individual to groups with
  - high overlap (short phylogeny, high θ)
  - high divergence and group delimitation (deep phylogeny, low θ)
  - other scenarios between these 2 extremes
- Compared to change in power with scenarios using simple distance method

Coalescent Assigner vs. Distance method

	Coalescent assigner Distance					Distance			
		θ					θ		
	V	0.001	0.01	0.1		V	0.001	0.01	0.1
n = 5	0.001	93%	89%	82%	n = 5	0.001	92%	61%	56%
	0.01	99%	97%	92%		0.01	99%	93%	60%
	0.1	100%	100%	99%		0.1	100%	100%	92%
	1	100%	100%	100%		1	100%	100%	99%
n = 10	0.001	99%	93%	82%	n = 10	0.001	88%	59%	55%
	0.01	99%	93%	82%		0.01	95%	96%	71%
	0.1	100%	100%	95%		0.1	100%	100%	97%
	1	100%	100%	100%		1	100%	100%	100%
n = 25	0.001	98%	89%	52%	n = 25	0.001	86%	78%	72%
	0.01	100%	95%	68%		0.01	98%	90%	79%
	0.1	100%	100%	91%		0.1	100%	100%	94%
	1	100%	100%	97%		1	100%	100%	100%

Table: % Correct with 50,000 chain length. Divergence v measured in substitutions/site; θ is exp. # evolutionary steps between two individuals; n is group size

- Table shows clear improvement of methods as divergence of phylogeny, v, increases compared to within group evolution
- The more divergent the groups are, the greater the distinction between their MRCAs and the more likely that the withing group evolutionary process are independent and easier to distinguish
- Coalescent assigner is more poweful than distance method in distinguishing newly assigned samples

Conclusions

- Evolutionary history is critical in accurately assigning individuals to the most approprirate group
- Large # of taxa combined with larger # of nucleotide patterns can result in an extensive search requiring a much larger Markov Chain to guarantee a good sample, which would be drawn from the tail of the genealogical distribution.
- The coalescent assigner is much more powerful than the distance method when overlap might mask the correct assignment.
- Coalescent Assigner is simpler, faster than fully incorporating phylogenetic and population genetic history.

References

- Z. Abdo and G.B. Golding, A step toward barcoding life: a model-based, decision theoretic method to assign genes to pre-existing species groups. Systematic Biology, 56 (2007), pp. 113.