Investigating the mechanisms implicated in the maintenance of photosynthetic endosymbiosis between *Paramecium bursaria* and *Chlorella*

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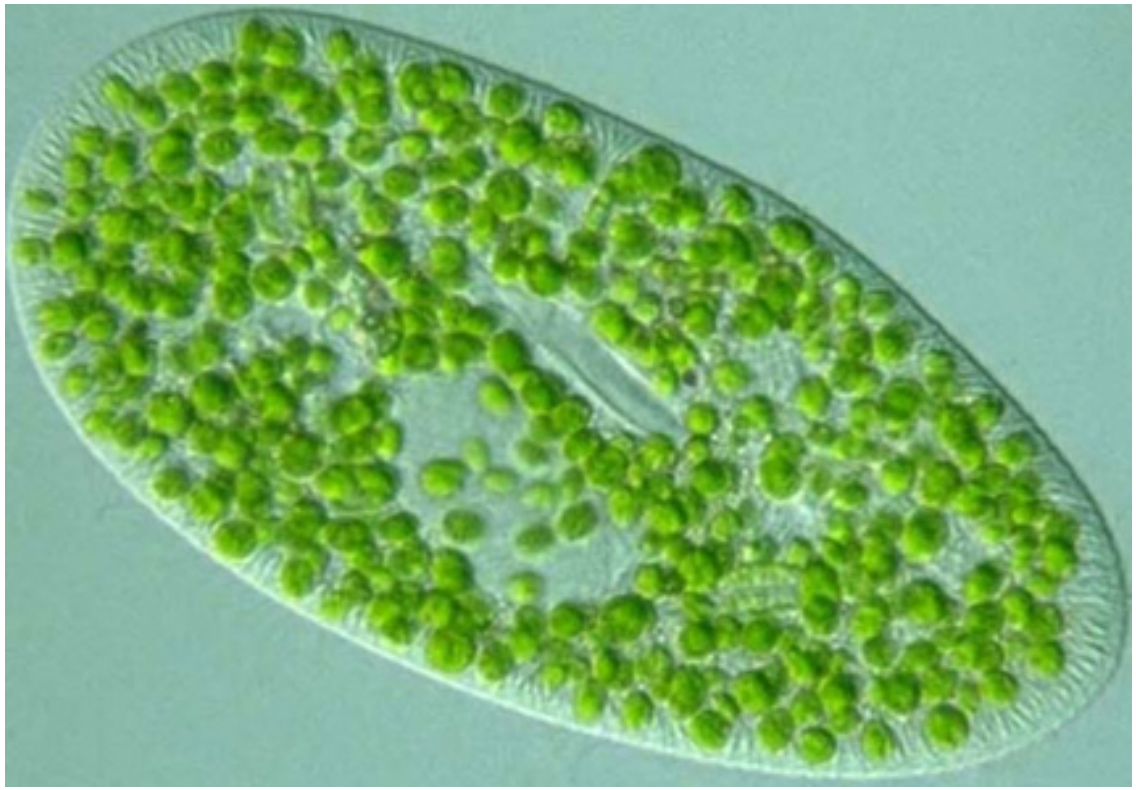
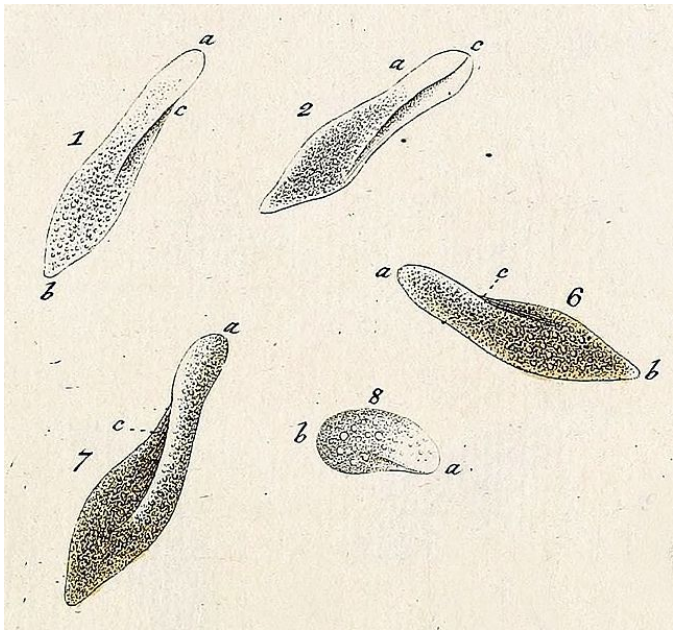


Background Biology

- Putatively facultative photosynthetic endosymbiosis between *Paramecium bursaria*, a ciliate, and *Chlorella*, a green algae
- One of the earliest studied micro-organisms (figure illustrated by Otto Muller in 1773)
- Complex, multi-factor relationship (on top of pure energetics: predation, photoprotection, thermotolerance, exploitation of low oxygen environments etc.)
- Theoretically forms and interesting and tractable system to study endosymbiosis before metabolic co-dependence becomes fixed



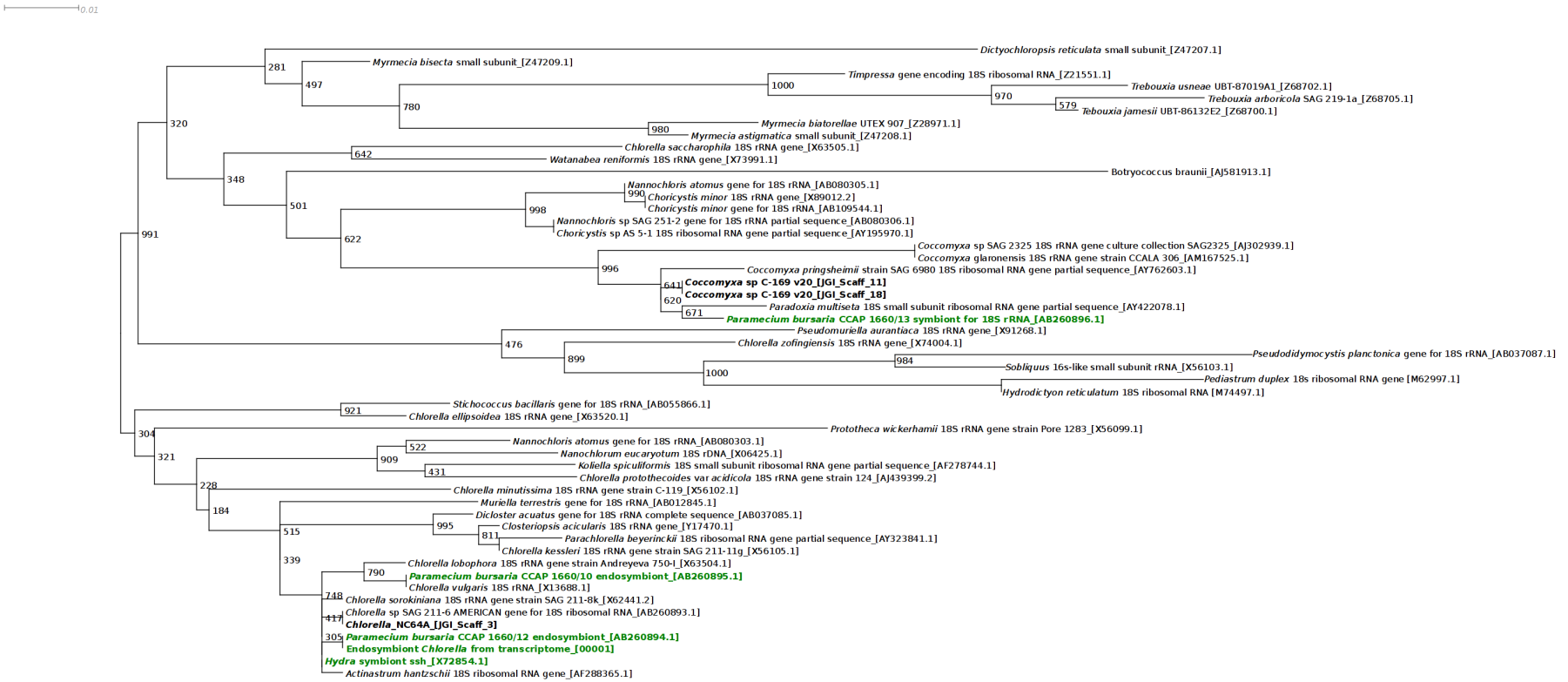
Transcriptomics on the system

- Day and night bulk RNA-Seq
- De-novo total assembly (pooled reads followed by remapping)
- Multiple assemblers and parameters used
- Referenced assemblies (*Coccomyxa*) but applicability of references requires fine-scale endosymbiont and host identification

Assembly Metric	Oases Assembly	Trinity Assembly
Min Contig Length:	100	201
Max Contig Length:	16,202	17,729
Mean Contig Length:	648.90	959.32
Standard Deviation of Contig Length:	939.04	1080
N50 Contig Length:	1,368	1,621
Number of Contigs:	117,570	48,003
Number of Contigs $\geq$1kb:	22,225	14,774
Number of Contigs in N50:	14,977	8,060
Number of Bases in All Contigs:	76,290,606	46,050,097
Number of Bases in All Contigs $\geq$1kb:	46,695,005	31,602,626
GC Content of Contigs:	28.99%	30.97%

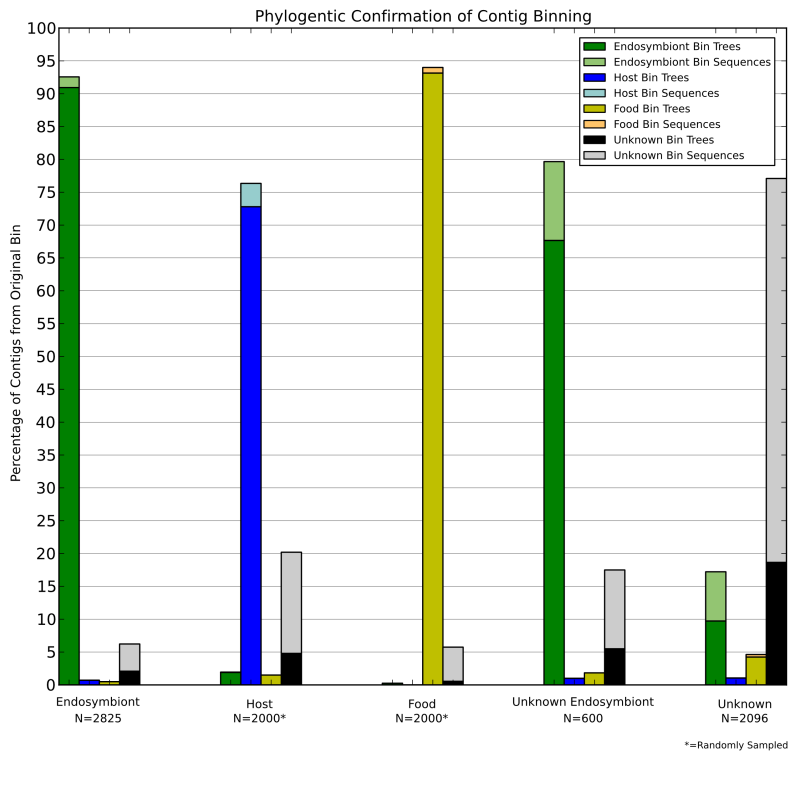
Confirming the identity of the host/endosymbiont

- rRNA fragments from within the transcriptome
- ITS2 sequencing
- ML and Bayesian phylogenetics
- Concluding: Referenced host assembly not applicable (not shown) but host (*Paramecium bursaria*) relatively distance, including 2 whole genome duplications from closest genome (*Paramecium tetraurelia*)

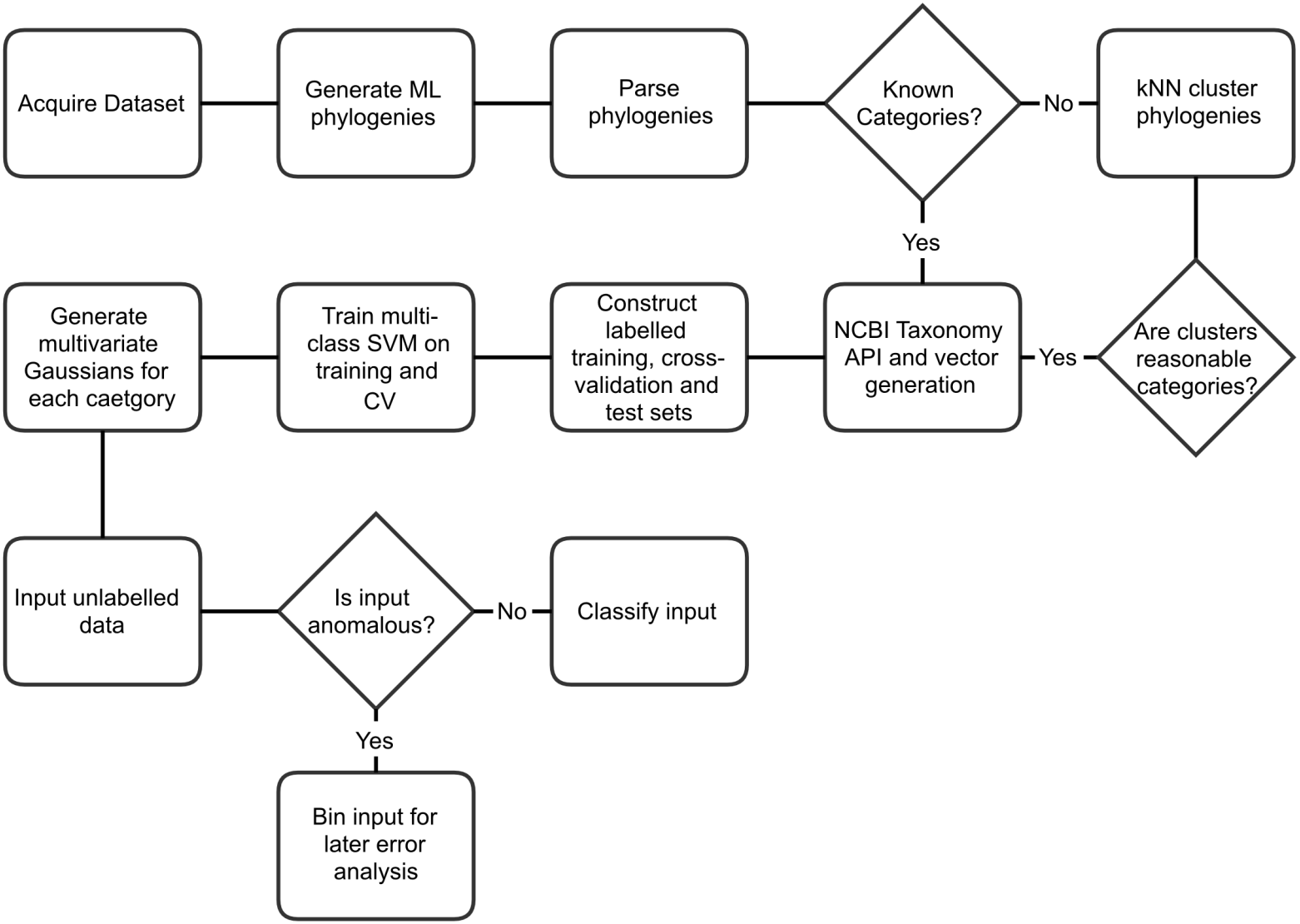


Identifying transcript origin: problem formulation

- Metatranscriptome problem - most solutions geared towards environmental studies
- Diverse transcript origins (e.g. bacterial food sequences, other potential contaminants, as well as host and endosymbiont)
- Existing small-scale methods use relatively crude measures e.g. CDS calling, GC%, BLAST
- We tested how well these type of measures perform compared to manually evaluated phylogenies



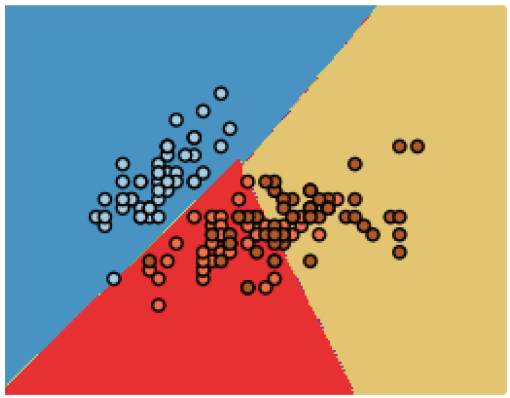
Automated high-throughput transcript identification tool



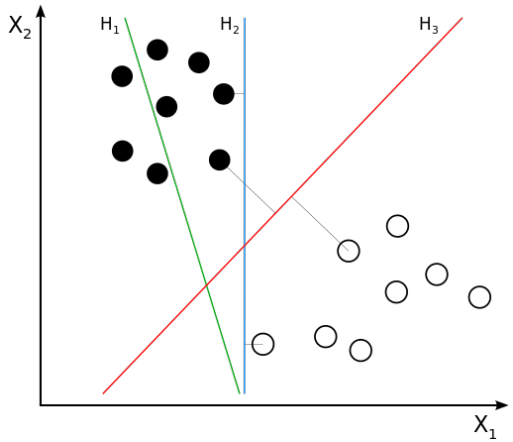
Parallelised automated phylogeny generation and parsing

- Running using coarse parallelism (each transcript being processed using an individual node not requiring shared memory) - 'supermarket queue'
- Approximately 35% faster than serial multi-threaded execution of each step
- For each transcript:
  - BLAST against curated database of 900 genomes
  - Align recovered sequences using MUSCLE
  - Automatically mask using TrimAL
  - Generate rapid maximum-likelihood phylogenies using FastTree2
- Once each phylogeny has been generated they can be parsed
- If categories have been decided vectors can be generated:
  - Parse each phylogeny using ETE2 and recover N-nearest neighbours to transcript in phylogeny
  - Using the NCBI taxonomy API determine taxonomy and categorisation of these neighbours
  - Sum the reciprocal total distance for each category within the N-neighbours
  - i.e. For the i-th phylogeny the j-th parameter in its feature vector will be $\frac{1}{\sum_{p=1}^{n} X_p}$ where X_p corresponds to the tree distance between the transcript and the p-th neighbour (for the $n \subseteq N$ neighbours s.t. $n \in$ to the appropriate category).

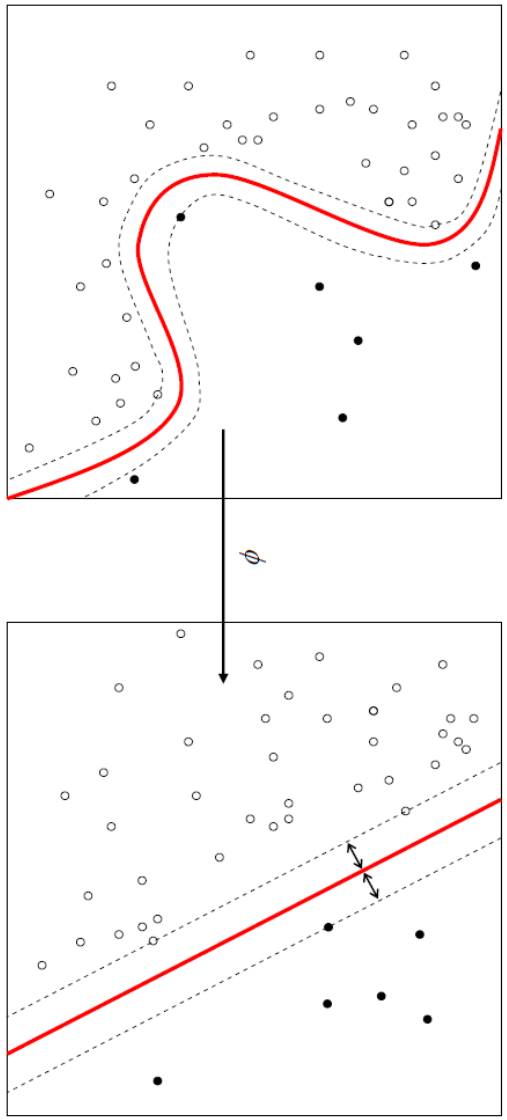
Support Vector Machines



- Linear classification:
  - Maximum margin solution
    +
    regularisation

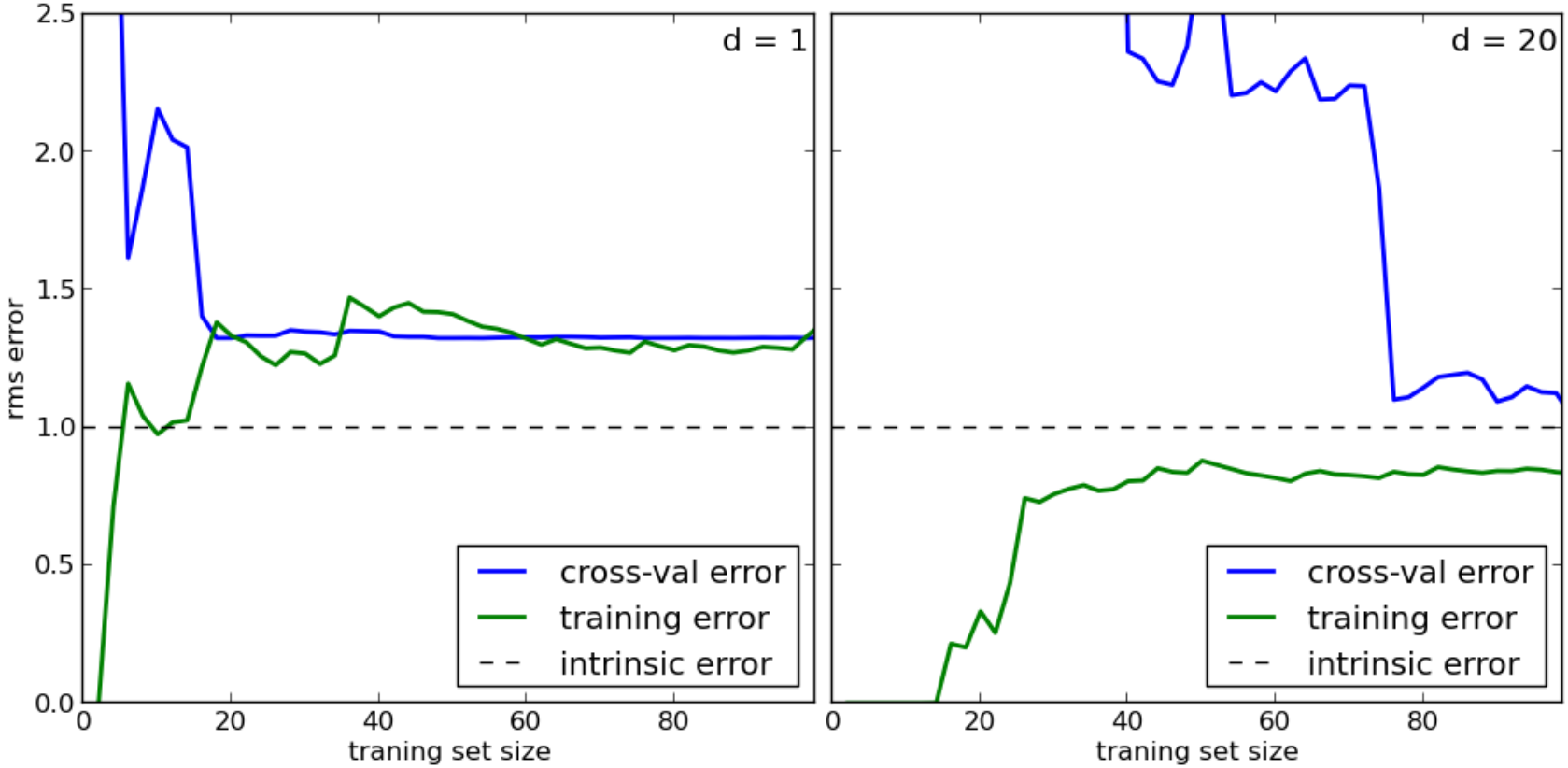


- Non-linear classification:
  - Kernel functions (map to feature space)



- Multi-class classification (e.g. 'Endosymbiont', 'Host', 'Food', 'Unknown'):
  - One-vs-all
  - In-built

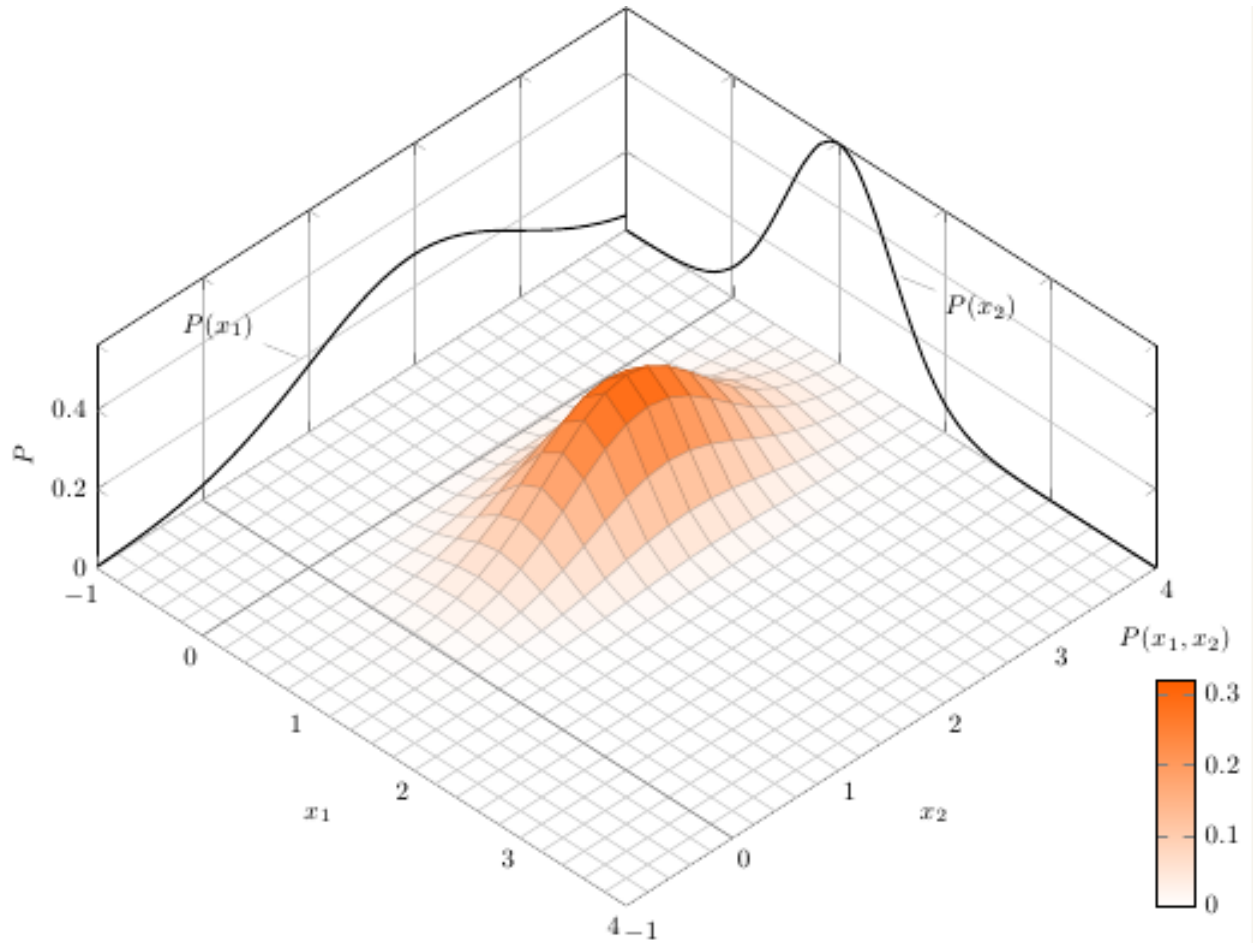
Assessing SVM function



- Optimise C and θ
- Error analysis
- Learning curves (Variance vs Bias)
- Precision (proportion of returned results that are relevant) / Recall (proportion of relevant results returned) (F_1 Score)

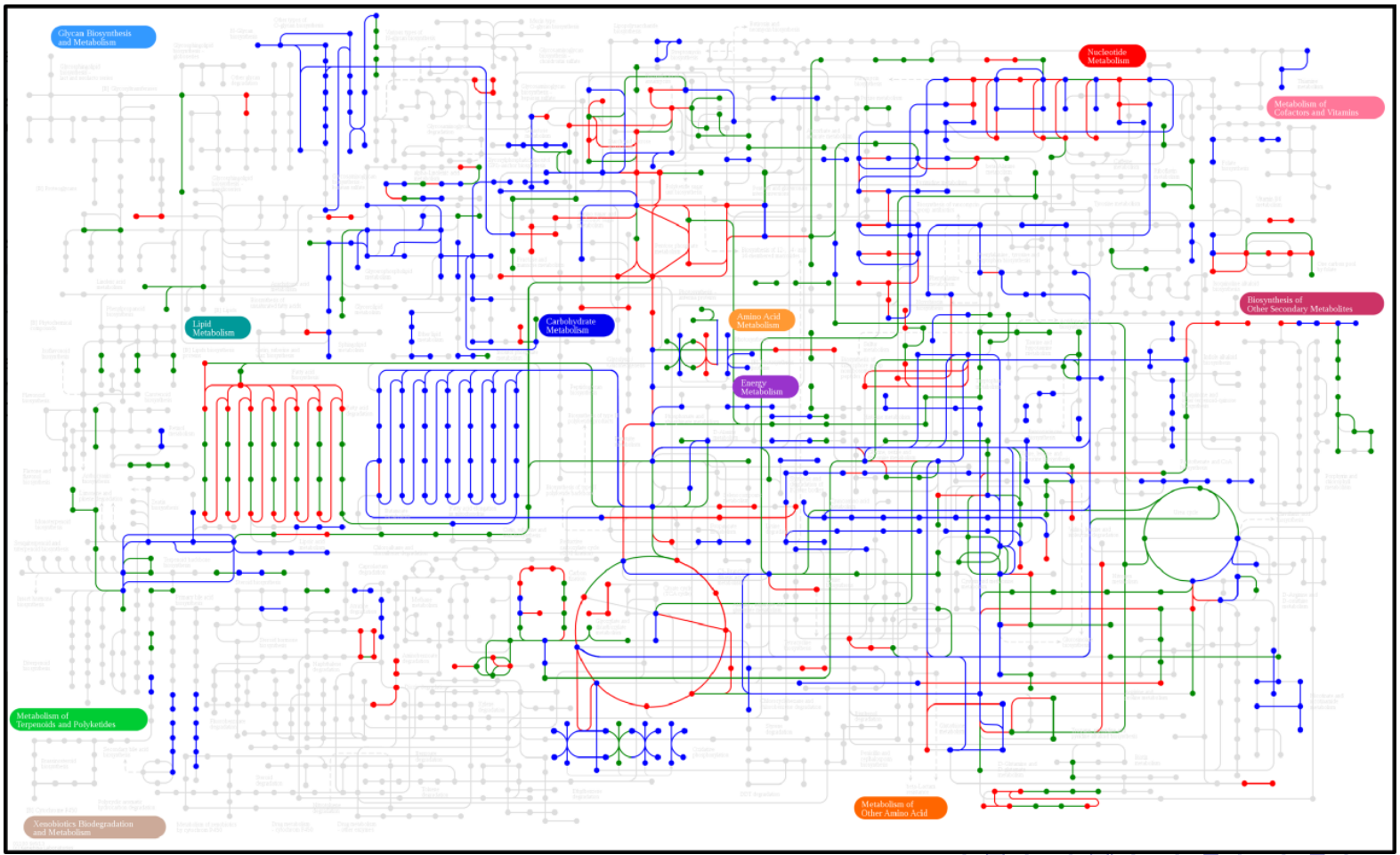
Anomaly detection

- Generate multivariate Gaussians for each category (using labelled data)
- Assign a threshold ϵ
- If $P(X) \leq \epsilon$ for each Gaussian then flag input at potentially anomalous
- Manually investigate the anomalies
- Tweak ϵ to maximise TP while secondarily minimising FP



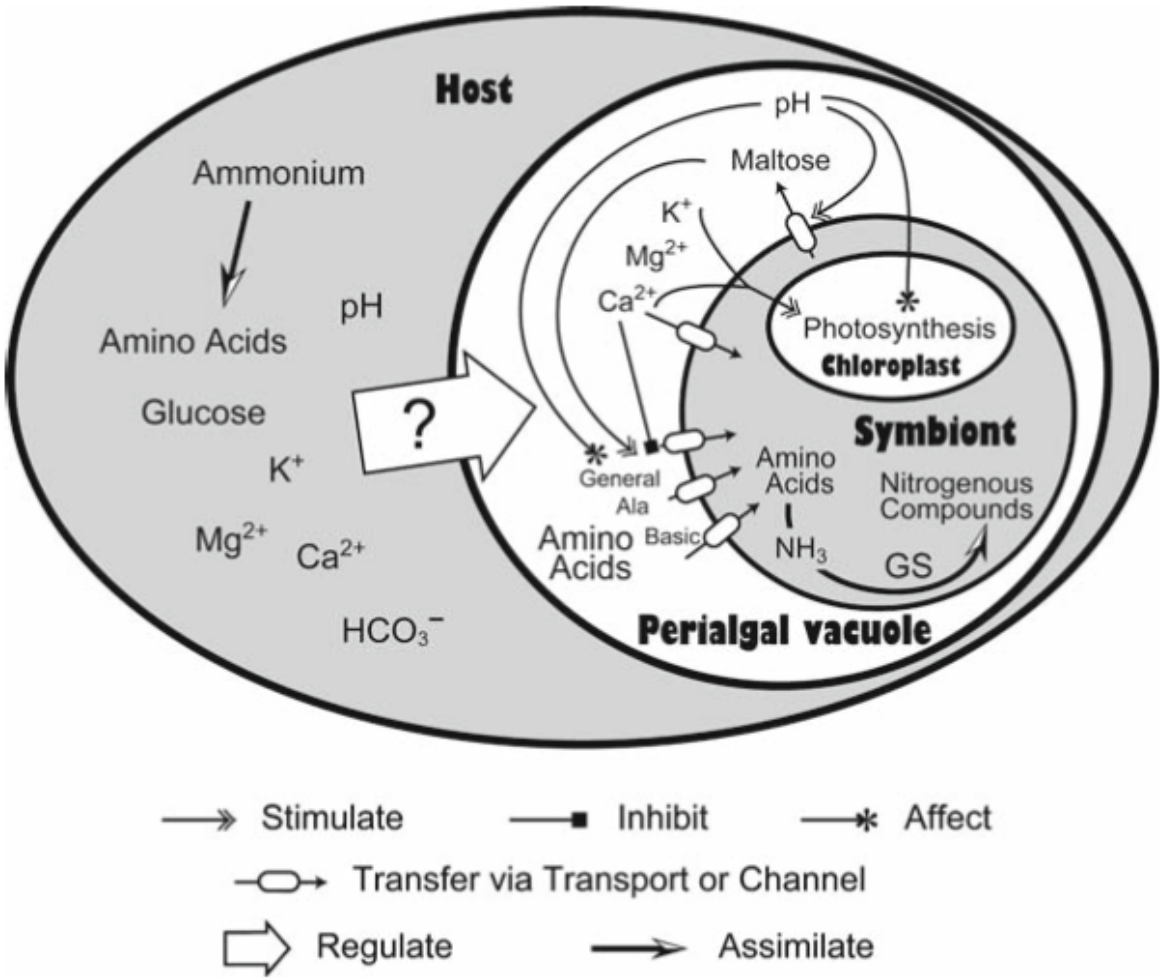
Beginning metabolic reconstruction

- Use the transcripts as partitioned into host and endosymbiont origin to map onto KEGG metabolic networks
- GO and KO annotation of transcripts
- Combine KEGG modelling with differential expression data and known literature to identify putative candidates involved in the maintenance of the endosymbiosis



Evidence supporting theoretical model

- Figure adapted from [Kato & Imamura, 2009]
- Putatively differentially expressed
  - 6 endosymbiont sugar transporters putatively differentially up-regulated
  - 4 host cation transporters (K^+, Ca^{2+}, Mg^{2+})
  - 2 endosymbiont cation transporters (Ca^{2+}, K^+)



Summary

- Creation of an effective tool in resolving a key problem in multi-member transcriptome analyses
- Mapping and evaluating a complex data source in exploratory analysis
- Make predictions of key candidates for further investigation (still improving)
- Molecular validation of models and candidate proteins (in progress):
  - Validate these predictions as having a role via RNAi
  - System tested using Bug22 marker with mixed success
  - Confirm differential expression (single cell transcriptomes/qPCR)