

# RNAi pathway components and function in *Paramecium bursaria*

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June 2, 2016

# Overview

## Motivation

RNAi in ciliates

Experimental RNAi induction in *P. bursaria*

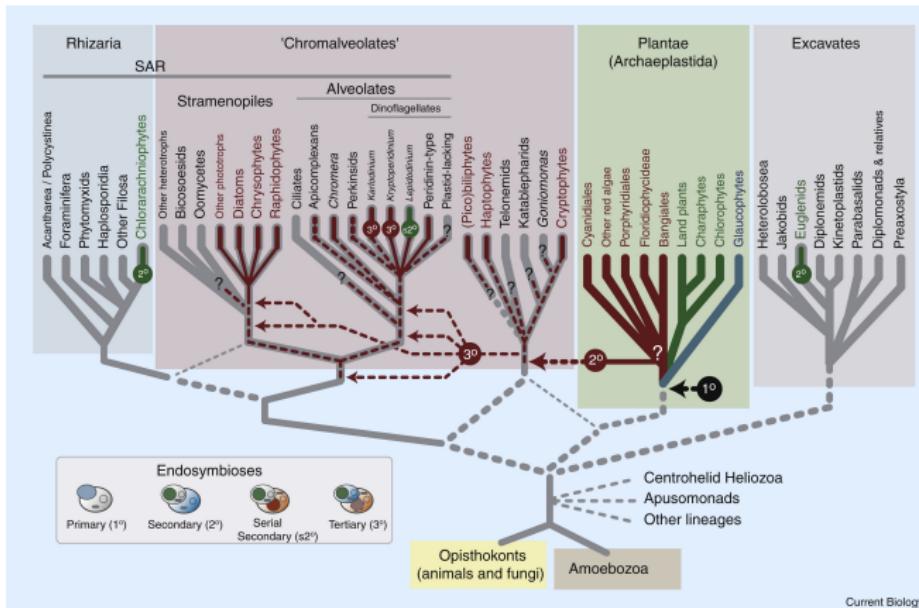
RNAi pathway components in active *P. bursaria* transcriptome(s)

*In-silico* analysis of potential endosymbiont 'cross-talk'

## Conclusions

Why is *Paramecium bursaria*  
potentially a good model for  
(secondary photosynthetic)  
endosymbiosis?

# Broad diversity of second order plastid endosymbioses



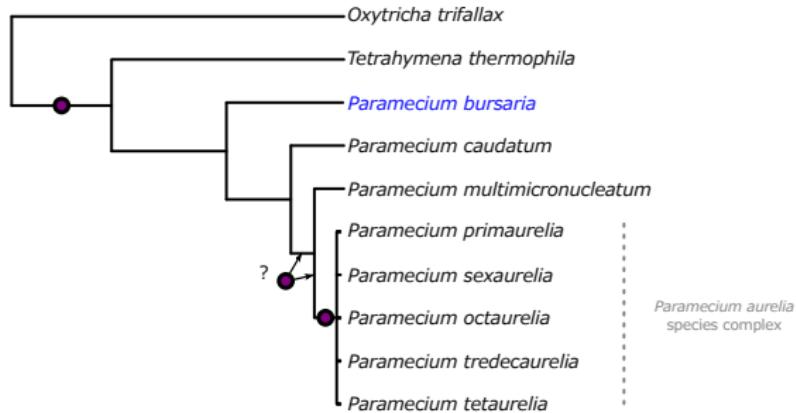
Reproduced from [Arc09]

## *Paramecium bursaria* and its green algal endosymbionts



- ▶ 100 µm to 160 µm serial phagotrophic ciliate (nuclear dimorphism).
- ▶ ~ 300 endosymbiotic algae in stable heritable facultative(?) endosymbiosis.
- ▶ Multiple independent origins of these endosymbioses.
- ▶ Single cell transcriptome and genome of *P. bursaria-Micractinium reisseri* CCAP 1660/12.
- ▶ *P. bursaria* bulk transcriptome [KSD<sup>+</sup>14].

# RNAi pathways in the ciliates



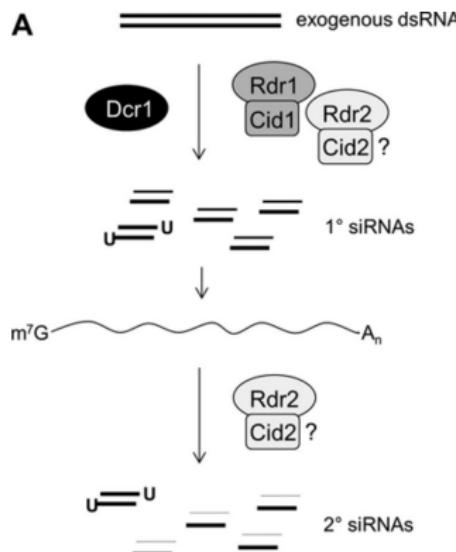
A good model needs a means to test hypotheses:

- ▶ Ciliate specific scnRNA system [MG04, CMM13].
- ▶ siRNA pathways present in *Paramecium tetaurelia* [GS01, GS02] (and *Tetrahymena thermophila* [CL06, YC05]):
  1. Transgene inducible pathway [GS01].
  2. Exogenous dsRNA inducible pathway (feeding or injection) [GS02].

## Transgene pathway

- ▶ Microinjection and transformation of MAC with high-copy transgenes lacking 3' UTR [GS01]
  1. 23nt siRNA generated from transgene transcripts (Dcr1, Rdr2, Rdr) and Cid2) [LNS<sup>+09</sup>, MCT<sup>+14</sup>].
  2. mRNA cleavage (Ptiwi13 and Ptiwi14)

# Exogenous dsRNA pathway

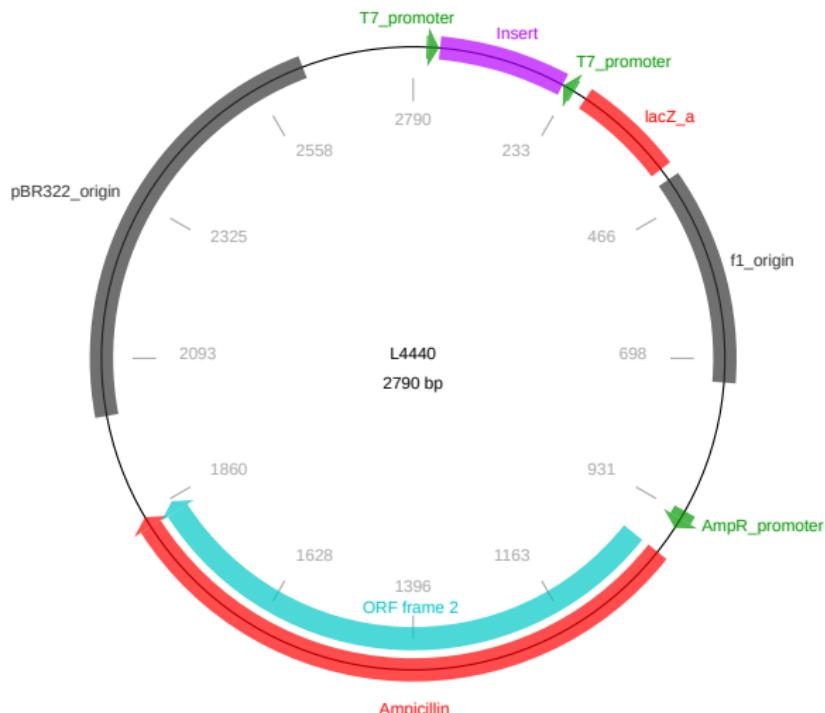


Reproduced from [CGA<sup>+</sup>15]

- ▶ Exogenous dsRNA via feeding (or microinjection) [GS02].
- ▶ 1° siRNA targeted cleavage (Ptwi13) [BGK<sup>+</sup>11].
- ▶ Undefined role in MAC for 2° siRNA (Ptwi12, Ptwi15) [MCT<sup>+</sup>14, CGA<sup>+</sup>15, BGK<sup>+</sup>11]
- ▶ Pds1 involved in uptake of dsRNA from vacuole? [CGA<sup>+</sup>15].
- ▶ Activated at low levels by ssRNA from normal food bacteria [CGA<sup>+</sup>15].

So, can we experimentally induce  
RNAi in *P. bursaria*?

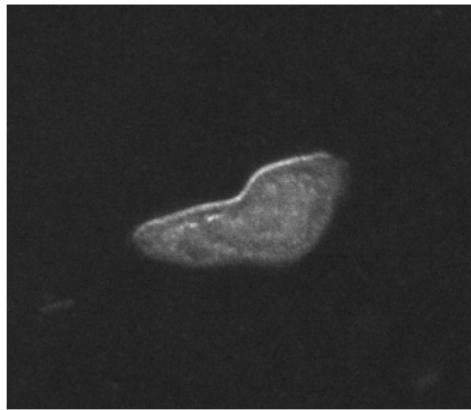
# Experimental feeding vector



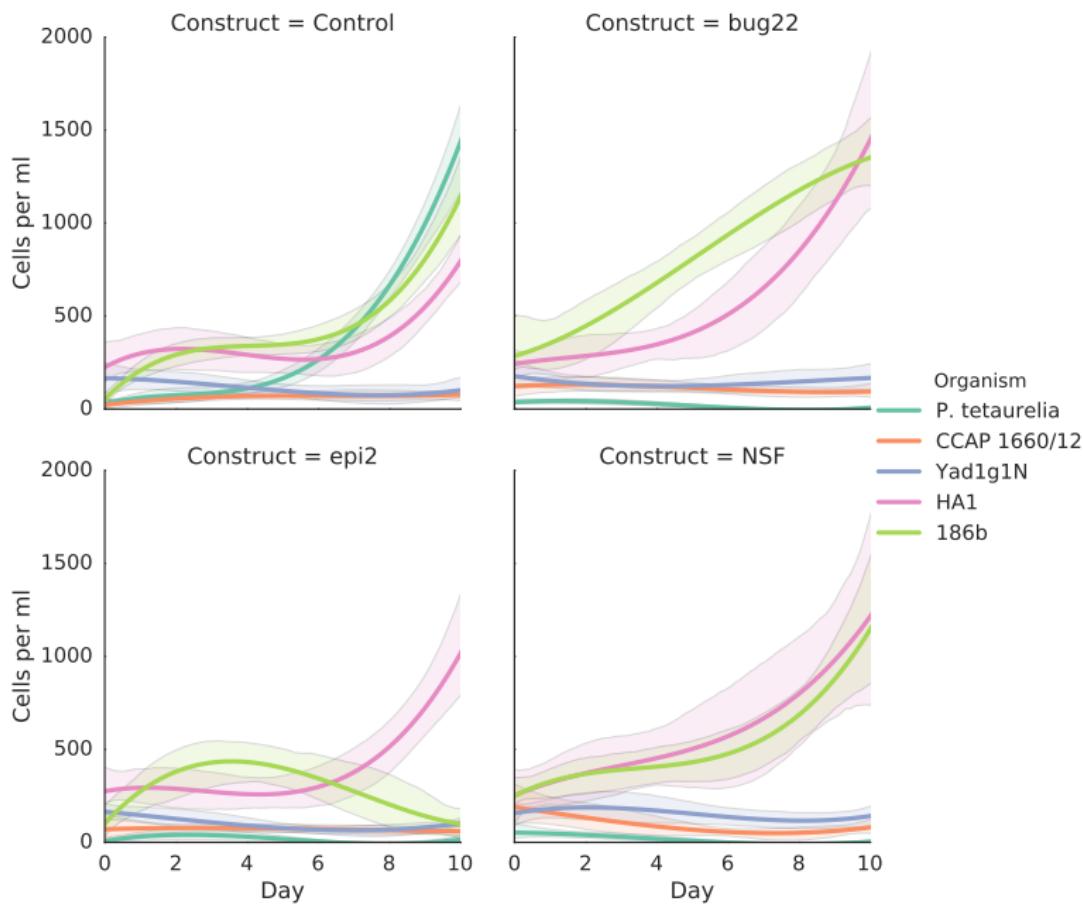
Transformed into *E. coli* with IPTG-inducible T7 polymerase and RNAse III deficiency.

## Construct inserts

Gene	Function	RNAi phenotype in <i>P. tetraurelia</i>
<i>epi2</i>	Epiplasmin	"Monstrous" cells
NSF	Membrane fusion factor	Lethal
<i>bug22</i>	Basal body/ciliary protein	Slow swimming and death



# RNAi feeding had mixed results

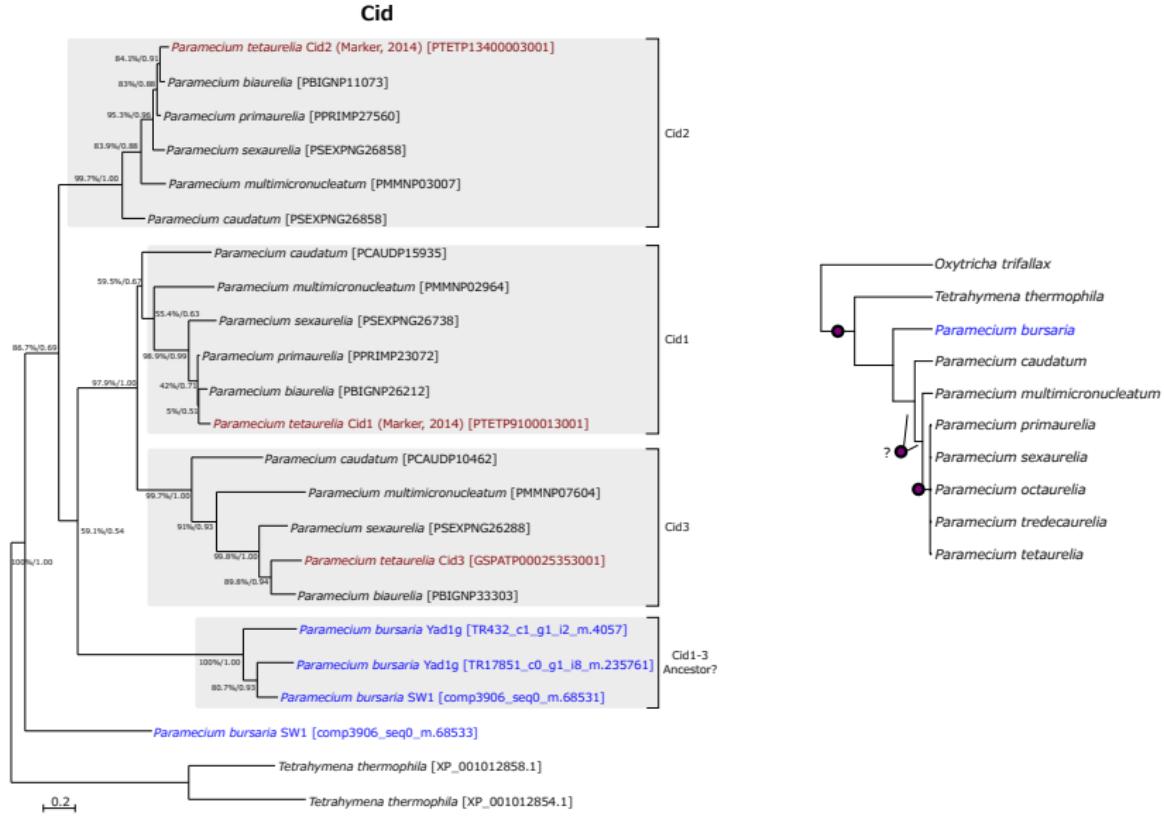


Are all the known RNAi pathway components present in the active transcriptome((s))?

## Summary of known RNAi components

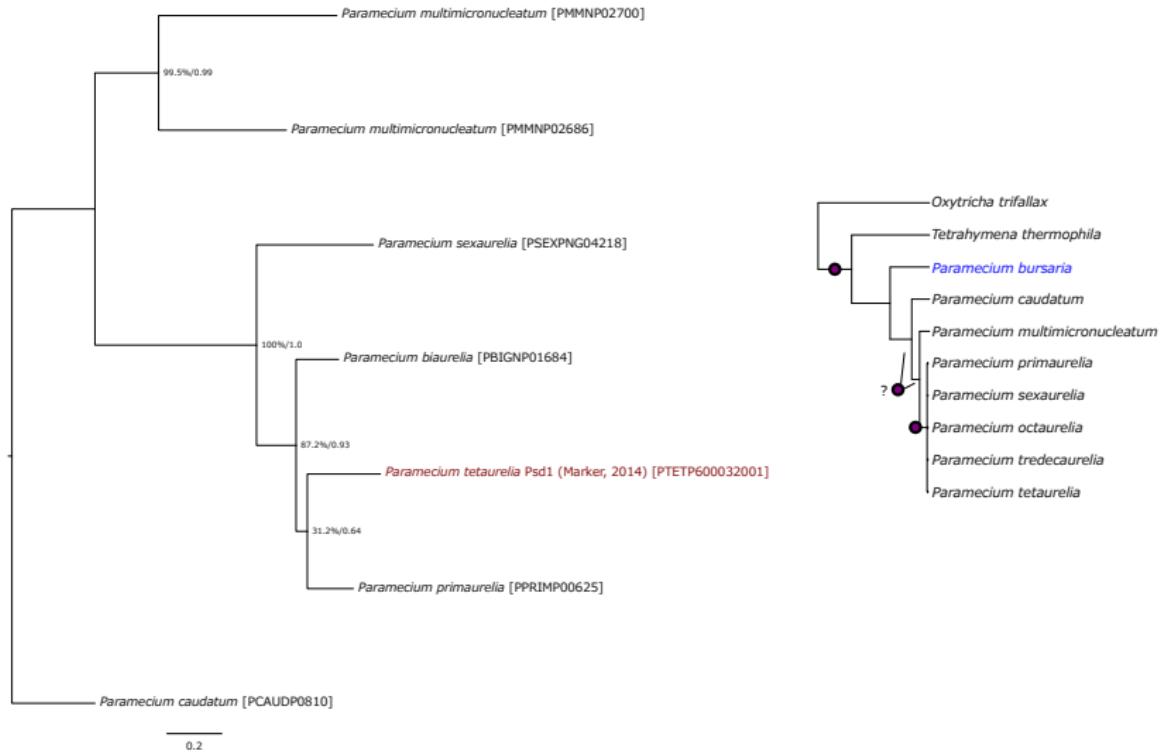
Pathway	Component	Function
transgene-induced siRNA	Rdr3 Ptwi14	RdRP Piwi
both pathways	Rdr2 Dcr1 Ptwi13 Cid2	RdRP Dicer Piwi Nucleotidyl transferase
exogenous dsRNA-induced siRNA	Rdr1 Cid1 Ptwi12 Ptwi15 Pds1	RdRP Nucleotidyl transferase Piwi Piwi Import of dsRNA?

# Cid ancestor

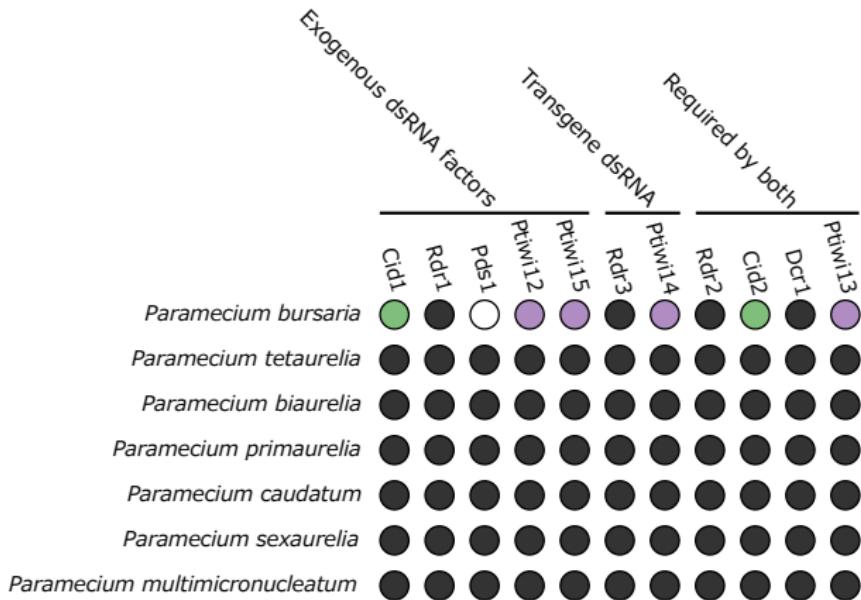


# Pds1 absent

## Pds1

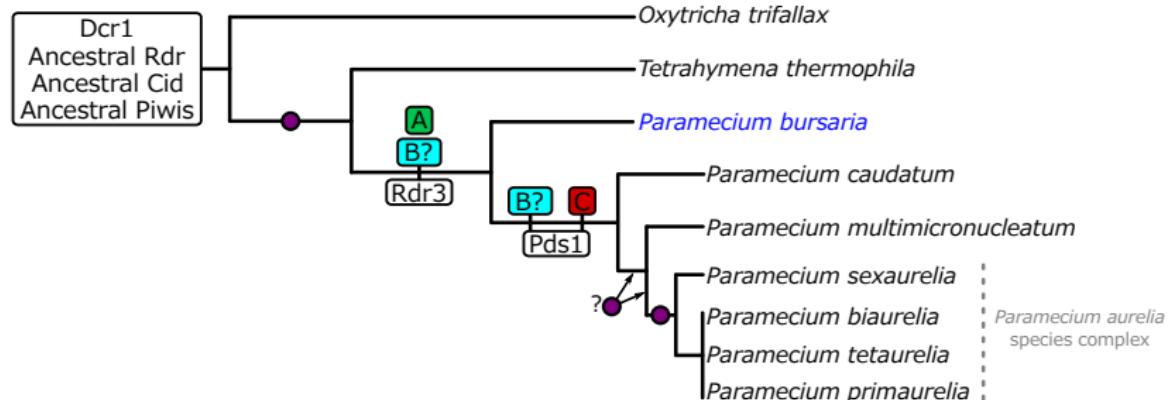


# Presence/absence of known pathway components



- Absence of homologue
- Presence of homologue
- Unresolved
- Putative unduplicated ancestral orthologue

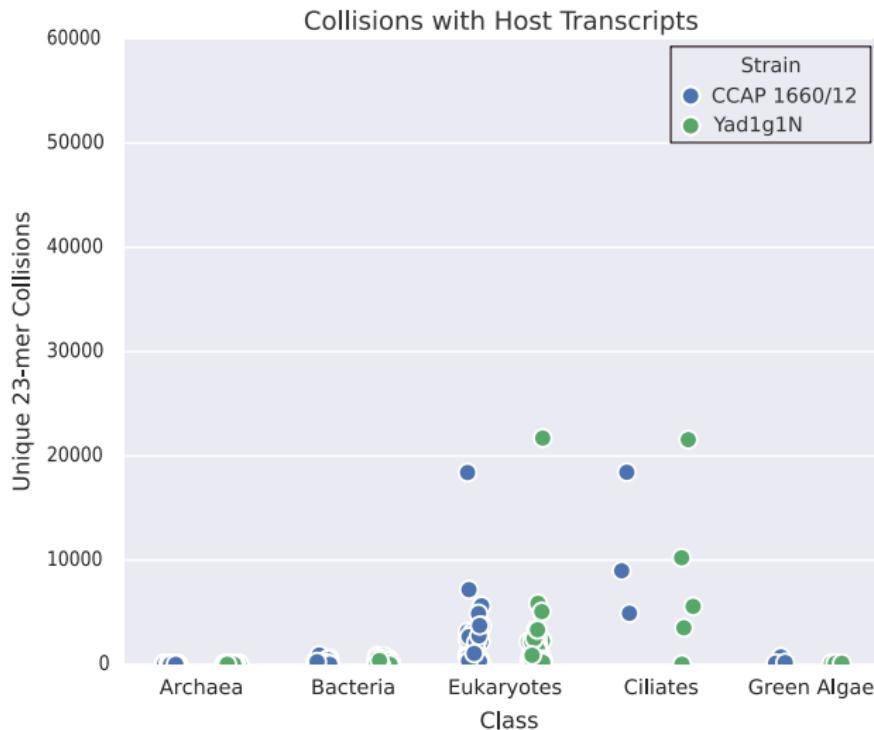
# Putative RNAi component evolution scenario



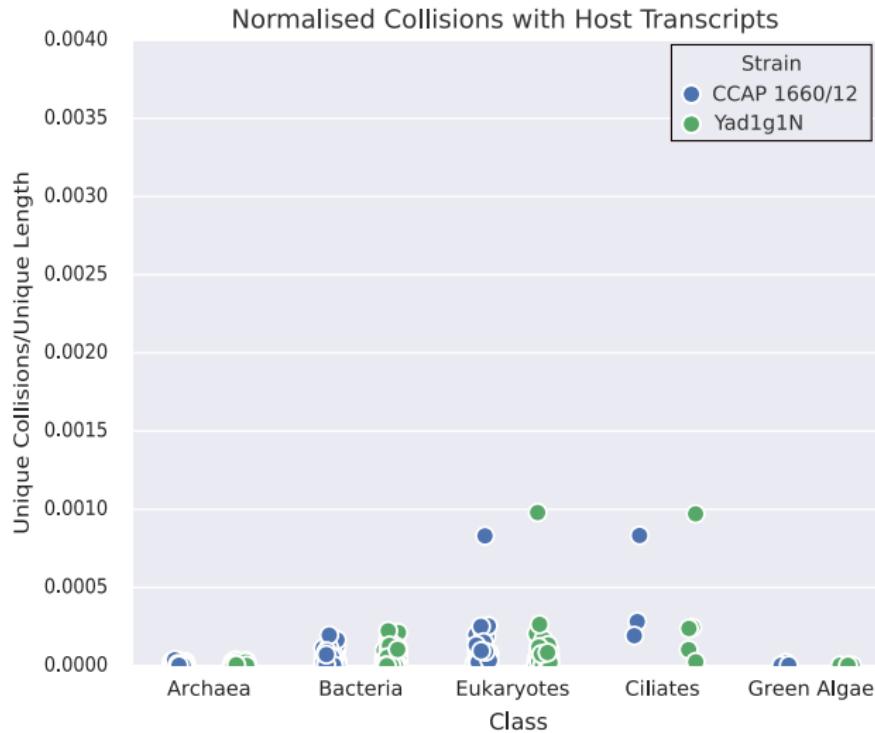
- [A] Duplication of ancestral Rdr into Rdr1 and Rdr2
- [B?] Duplication of ancestral Cid into Cid2 and Cid1-Cid3 ancestor
- [C] Duplication of Cid1-Cid3 ancestor into Cid1 and Cid3
- Whole genome duplication

Could having a eukaryotic endosymbiont and RNAi activated by dsRNA in vacuoles be deleterious?

# Higher level of collisions with eukaryotes



# Collisions are a function of transcriptome size



## Conclusions

- ▶ RNAi phenotypes not inducible in most *P. bursaria* strains via feeding.
- ▶ *P. bursaria* lacks Pds1 (in active transcriptome) thus may be unable to take up RNA from digestive vacuoles.
- ▶ High levels of 23-mer collisions between *P. bursaria* and eukaryotic endosymbiont transcriptomes may lead to deactivation of dsRNA uptake from vacuoles.
- ▶ Presence of other factors in active transcriptomes of *P. bursaria* indicate transgene and microinjected exogenous dsRNA pathways may function.

# Acknowledgements

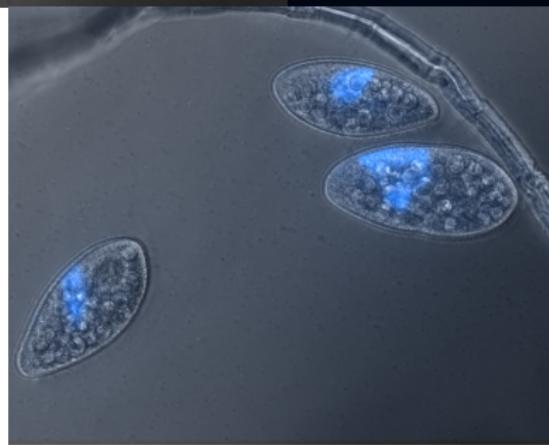
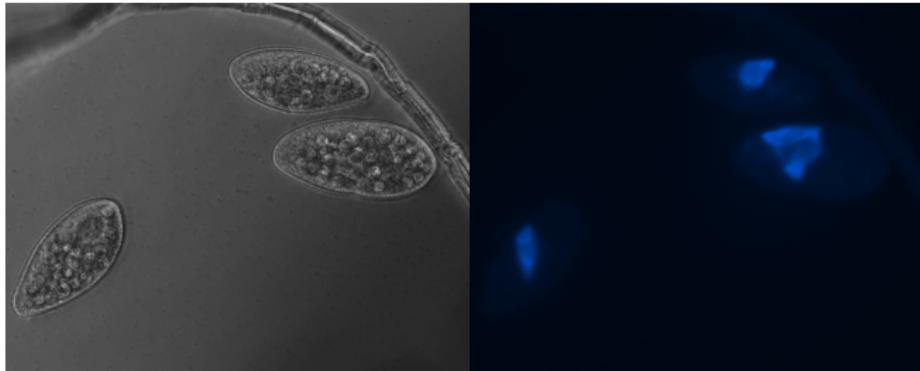
- ▶ Ben Jenkins (feeding experiments)
- ▶ David Milner (labwork)
- ▶ Tom Richards (PI)
- ▶ NHM-UCL PhD Studentship (main funding)



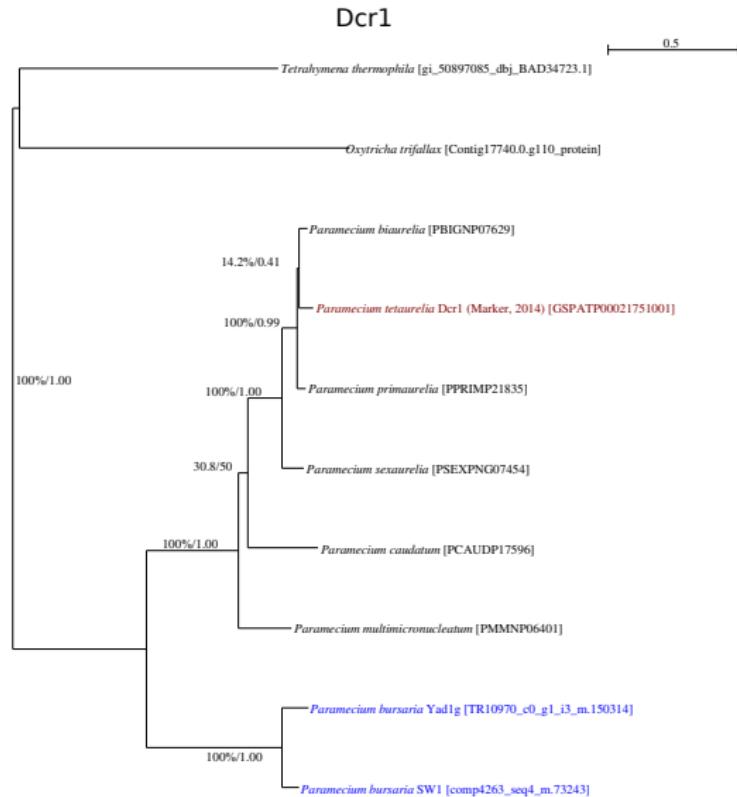
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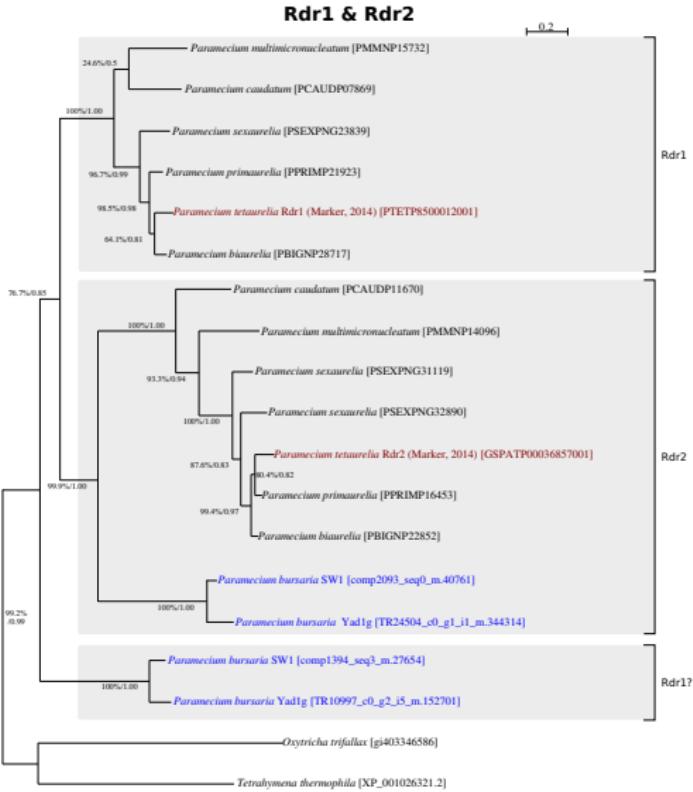
# Microinjection proved difficult



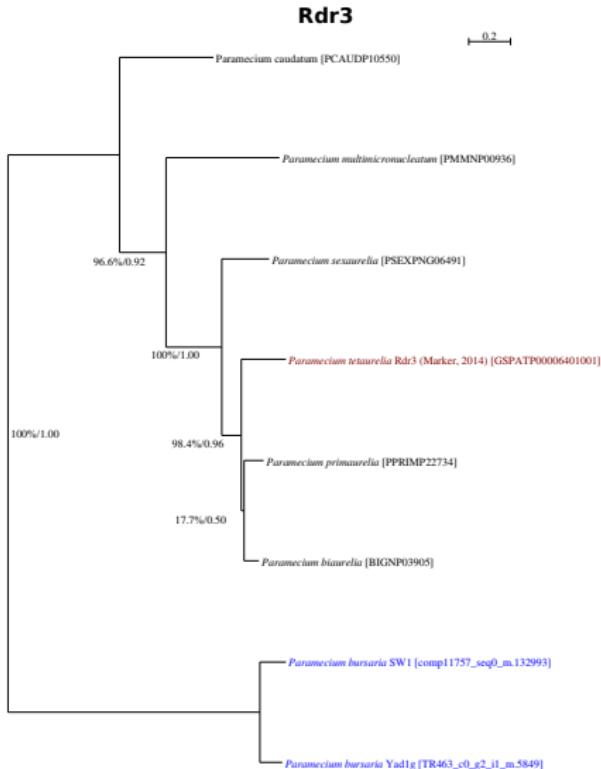
# Dcr1



# Rdr1 Rdr2



# Rdr3



# Psd1 Structure

