Rethinking the biological function of small RNAs in animals

Hervé Seitz

University of Massachusetts Medical School (Worcester, MA, USA);
LBME (CNRS and Université Paul Sabatier, Toulouse, France)

May 19, 2009

Introduction

Introduction

- Biological function of miRNAs?

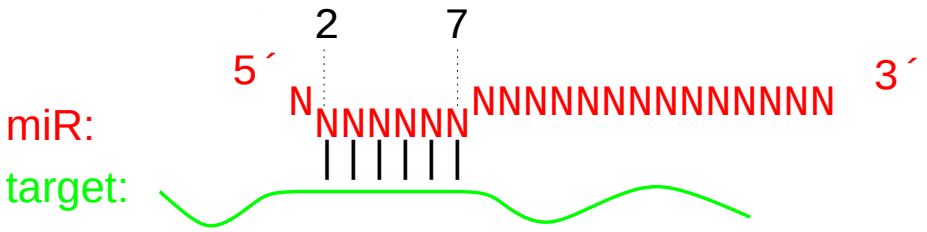
Introduction

- Biological function of miRNAs? What is a miRNA target – in a physiological sense?

Introduction

- Biological function of miRNAs? What is a miRNA target – in a physiological sense?
- Biological function of piRNAs?

microRNA targets



$\longrightarrow$ the seed

microRNA targets

Target prediction algorithms: find evolutionarily conserved seed matches in 3´ UTRs.

microRNA targets

Target prediction algorithms: find evolutionarily conserved seed matches in 3´ UTRs.

Such short complementarities are very frequent (60 % of human genes are targeted by miRNAs: Friedman *et al.*, 2009).

microRNA targets

Target prediction algorithms: find evolutionarily conserved seed matches in 3´ UTRs.

Such short complementarities are very frequent (60 % of human genes are targeted by miRNAs: Friedman *et al.*, 2009).

$\Longrightarrow$ miRNAs are implicated in every biological process in animals

microRNA targets

Target prediction algorithms: find evolutionarily conserved seed matches in 3´ UTRs.

Such short complementarities are very frequent (60 % of human genes are targeted by miRNAs: Friedman *et al.*, 2009).

$\Longrightarrow$ miRNAs are implicated in every biological process in animals; "miRNA X regulates gene Y, which is involved in disease Z" (varying the values of X, Y and Z...).

Weaknesses in the dogma

Weaknesses in the dogma

- Non-conserved sites are functional too

Weaknesses in the dogma

- Non-conserved sites are functional too (Grimson *et al*, 2007).

Weaknesses in the dogma

- Non-conserved sites are functional too (Grimson *et al*, 2007).
- Binding sites outside the 3´ UTR are functional too (several known examples *in vivo* + computational analyses).

Weaknesses in the dogma

- Non-conserved sites are functional too (Grimson *et al*, 2007).
- Binding sites outside the 3´ UTR are functional too (several known examples *in vivo* + computational analyses).
- Three paradoxes.

First paradox

Computational programs identify tens or hundreds of targets for each miRNA, yet genetic studies identify a single one.

First paradox

Computational programs identify tens or hundreds of targets for each miRNA, yet genetic studies identify a single one.

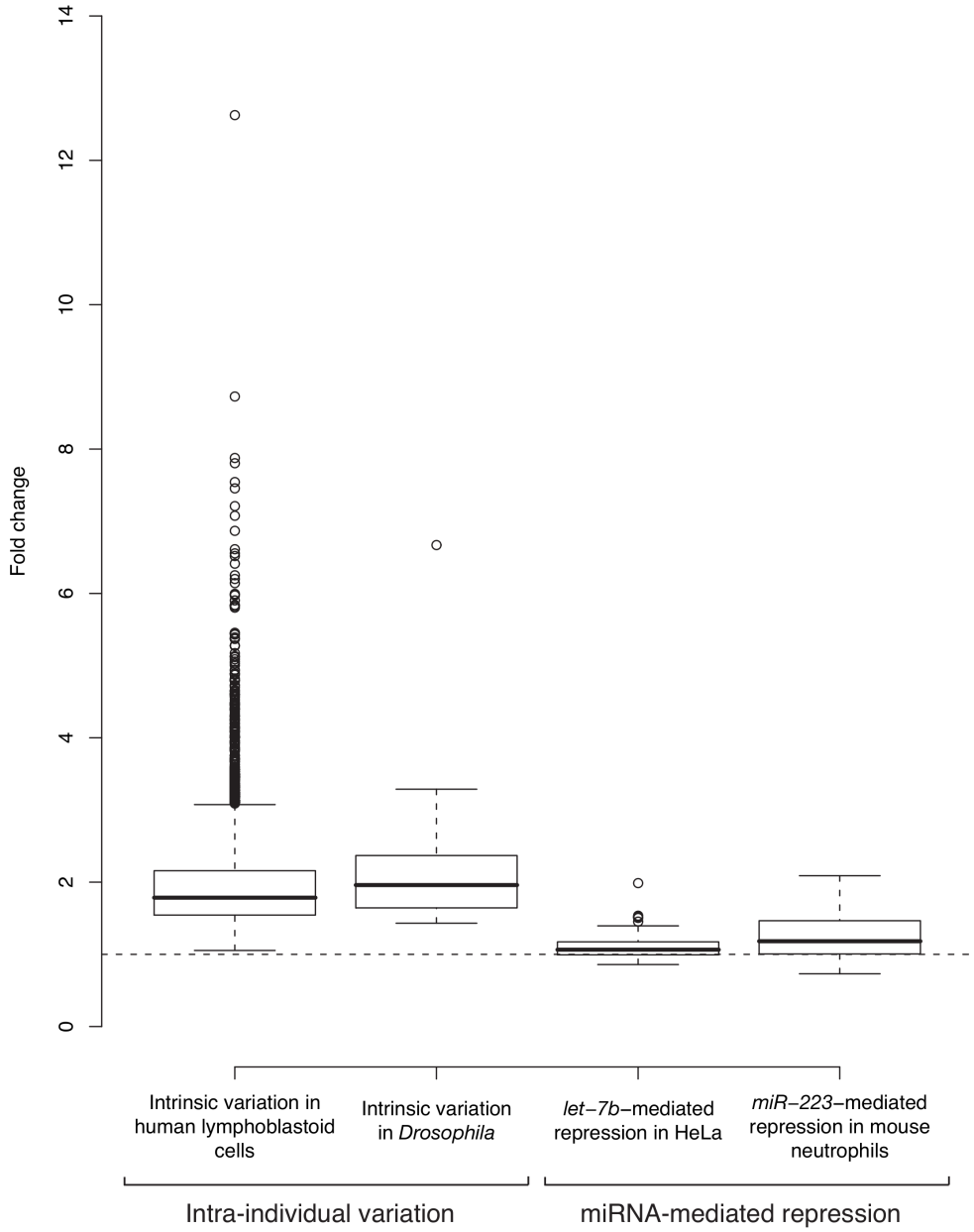
miRNA	Genetic experiments		Number of computationally predicted targets	
	Target	Reference	TargetScan	PicTar
worm *let-7*	*lin-41*	Slack *et al.* (2000)	65	89
worm *lin-4*	*lin-14*	Ambros (1989)	22	32
fly *let-7*	*abrupt*	Ambros and Chen (2007)	50	68

Second paradox

MiRNA-mediated repression is much smaller than intrinsic, well-tolerated variations in gene expression.

▸ Supplementary data

Second paradox

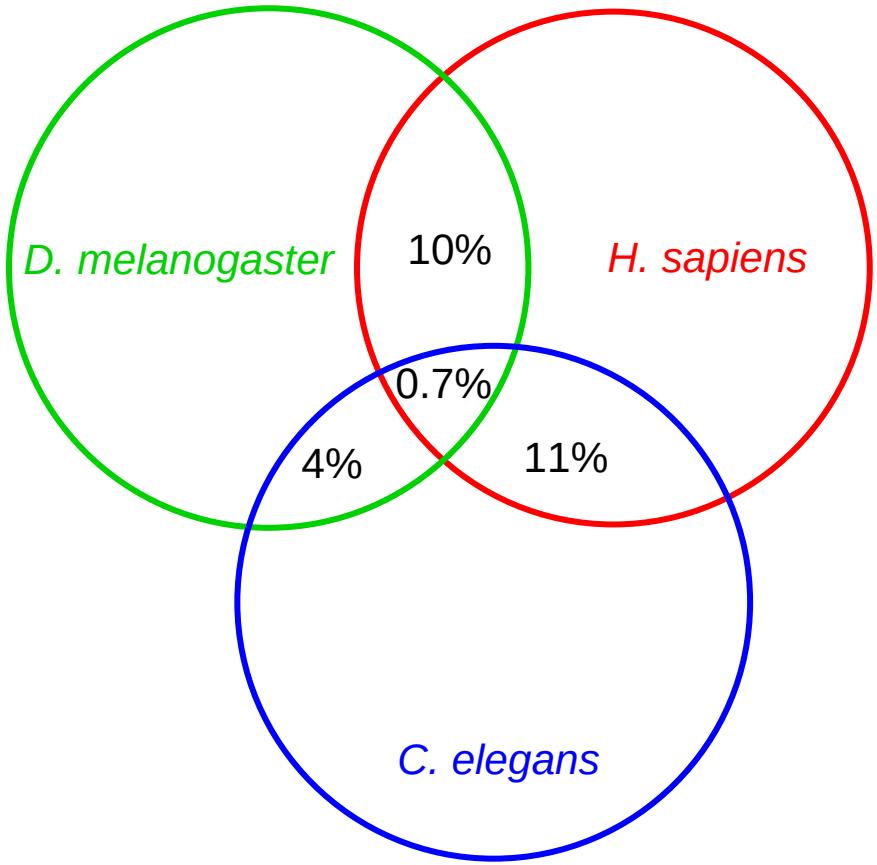


Third paradox

Computationnally-identified miRNA targets are poorly conserved.

Third paradox

Computationnally-identified miRNA targets are poorly conserved.



(Data from Chen and Rajewsky, 2006)

An alternative hypothesis

An alternative hypothesis

- Most computationnally-predicted targets are not functionally targeted by miRNAs (not repressed enough).

An alternative hypothesis

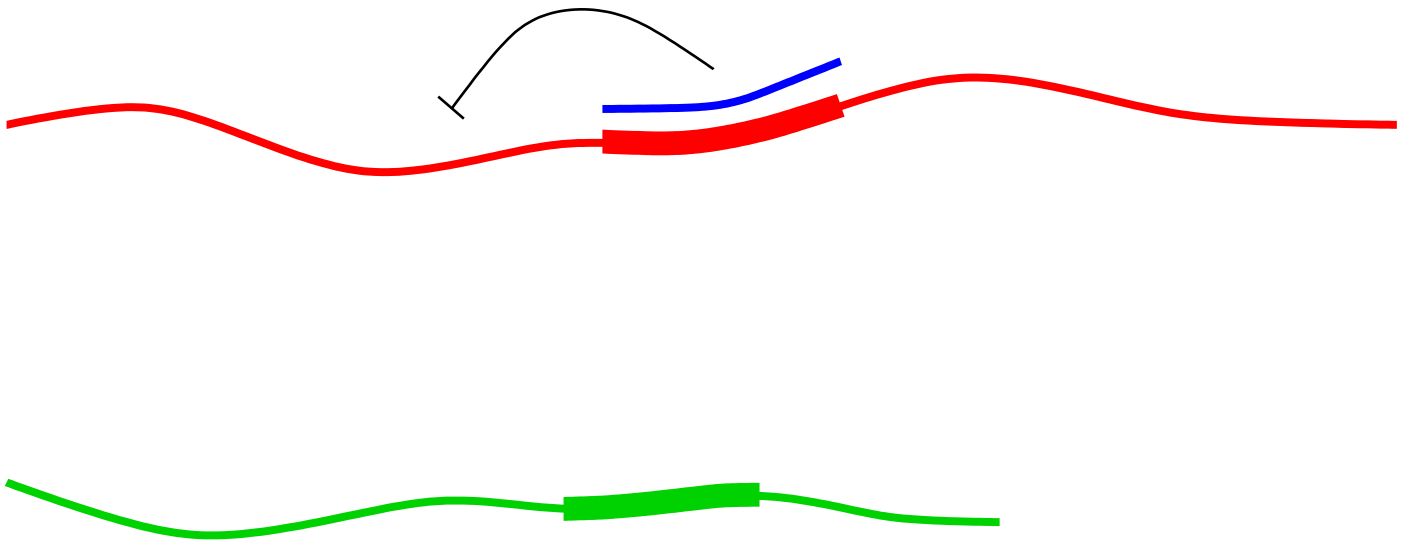
- Most computationnally-predicted targets are not functionally targeted by miRNAs (not repressed enough).
- Their phylogenetic conservation means that these miRNA binding sites have a function.

An alternative hypothesis

- Most computationnally-predicted targets are not functionally targeted by miRNAs (not repressed enough).
- Their phylogenetic conservation means that these miRNA binding sites have a function.
- This function could be: preventing the miRNA from repressing its real targets, by sequestering it.

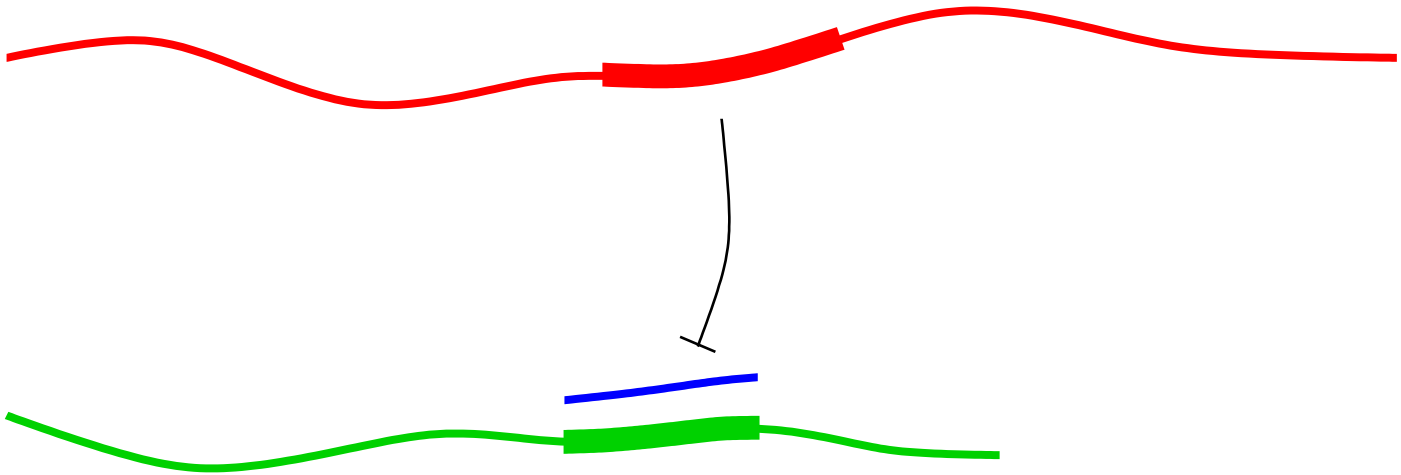
An alternative hypothesis

- Most computationnally-predicted targets are not functionally targeted by miRNAs (not repressed enough).
- Their phylogenetic conservation means that these miRNA binding sites have a function.
- This function could be: preventing the miRNA from repressing its real targets, by sequestering it.



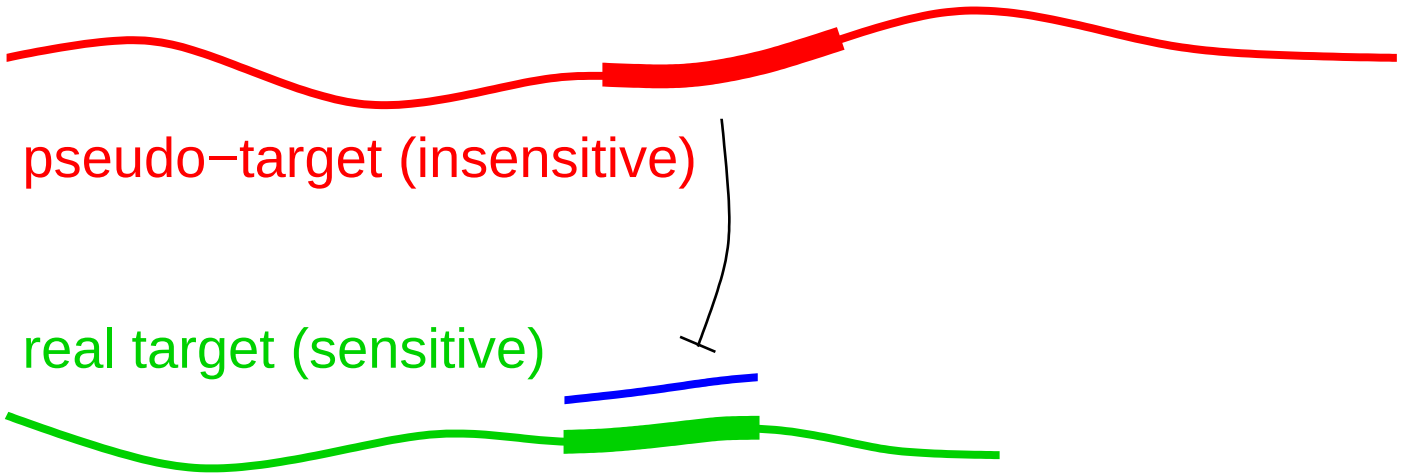
An alternative hypothesis

- Most computationnally-predicted targets are not functionally targeted by miRNAs (not repressed enough).
- Their phylogenetic conservation means that these miRNA binding sites have a function.
- This function could be: preventing the miRNA from repressing its real targets, by sequestering it.



An alternative hypothesis

- Most computationnally-predicted targets are not functionally targeted by miRNAs (not repressed enough).
- Their phylogenetic conservation means that these miRNA binding sites have a function.
- This function could be: preventing the miRNA from repressing its real targets, by sequestering it.



An alternative hypothesis

An alternative hypothesis

- First paradox:

An alternative hypothesis

- First paradox: computational searches find both pseudo-targets and real targets, genetics find only the real targets.

An alternative hypothesis

- First paradox: computational searches find both pseudo-targets and real targets, genetics find only the real targets.
- Second paradox:

An alternative hypothesis

- First paradox: computational searches find both pseudo-targets and real targets, genetics find only the real targets.
- Second paradox: pseudo-targets are not sensitive to a 1.1 – 2-fold change, real targets are.

An alternative hypothesis

- First paradox: computational searches find both pseudo-targets and real targets, genetics find only the real targets.
- Second paradox: pseudo-targets are not sensitive to a 1.1 – 2-fold change, real targets are.
- Third paradox:

An alternative hypothesis

- First paradox: computational searches find both pseudo-targets and real targets, genetics find only the real targets.
- Second paradox: pseudo-targets are not sensitive to a 1.1 – 2-fold change, real targets are.
- Third paradox: set of pseudo-targets can fluctuate during evolution (universally-conserved targets would be enriched in real targets).

An alternative hypothesis

- First paradox: computational searches find both pseudo-targets and real targets, genetics find only the real targets.
- Second paradox: pseudo-targets are not sensitive to a 1.1 – 2-fold change, real targets are.
- Third paradox: set of pseudo-targets can fluctuate during evolution (universally-conserved targets would be enriched in real targets).

Prediction: only dose-sensitive genes can be targeted by miRNAs.

An alternative hypothesis

Gene	Target for
worm *lin-14*	*lin-4*
worm *lin-41*	*let-7*
fly *abrupt*	*let-7*

An alternative hypothesis

Gene	Target for	Indication for dosage sensitivity
worm *lin-14*	*lin-4*	Formation of lateral alae at L4 molt is sensitive to *lin-14* dosage
worm *lin-41*	*let-7*	Worm viability is sensitive to *lin-41* dosage
fly *abrupt*	*let-7*	Dendritic branching in multiple dendritic sensory neurons is sensitive to *abrupt* dosage

An alternative hypothesis

Gene	Target for	Indication for dosage sensitivity
worm *lin-14*	*lin-4*	Formation of lateral alae at L4 molt is sensitive to *lin-14* dosage
worm *lin-41*	*let-7*	Worm viability is sensitive to *lin-41* dosage
fly *abrupt*	*let-7*	Dendritic branching in multiple dendritic sensory neurons is sensitive to *abrupt* dosage
worm *cog-1*	*lsy-6*	Left/right ASE neuron asymmetry is sensitive to *cog-1* dosage
fly *hid*	*bantam*	Survival of eye bristle cells is sensitive to *hid* dosage
Mammalian myostatin	*miR-1* and *miR-206* in mutant sheep	Muscle mass and racing performance correlate with gene dosage in dogs
mouse *Ptbp1*	*miR-124*	Alternative splicing is sensitive to *Ptbp1* expression level

What is a microRNA target?

An mRNA that is repressed by a miRNA.

What is a microRNA target?

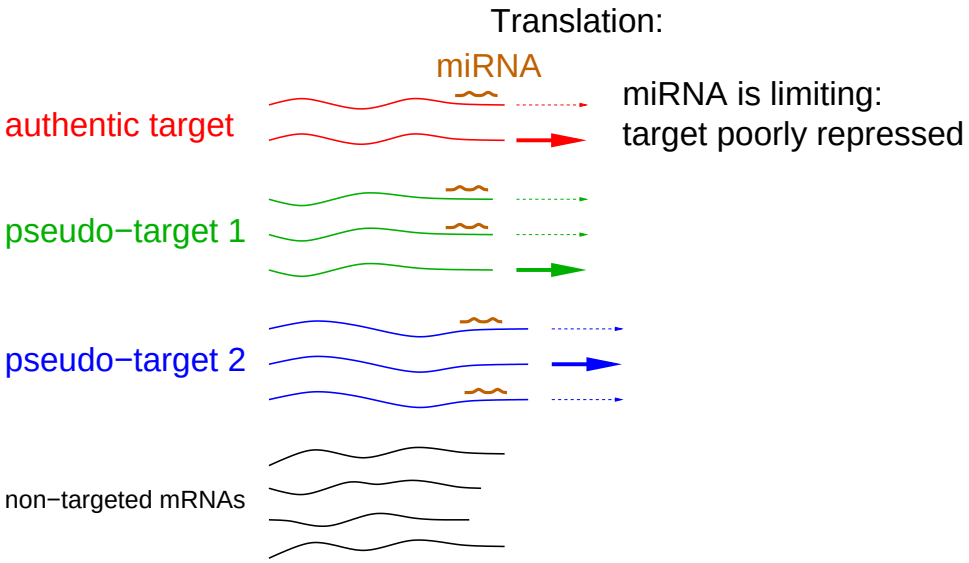
An mRNA that is repressed enough by a miRNA.

What is a microRNA target?

An mRNA that is repressed enough by a miRNA.

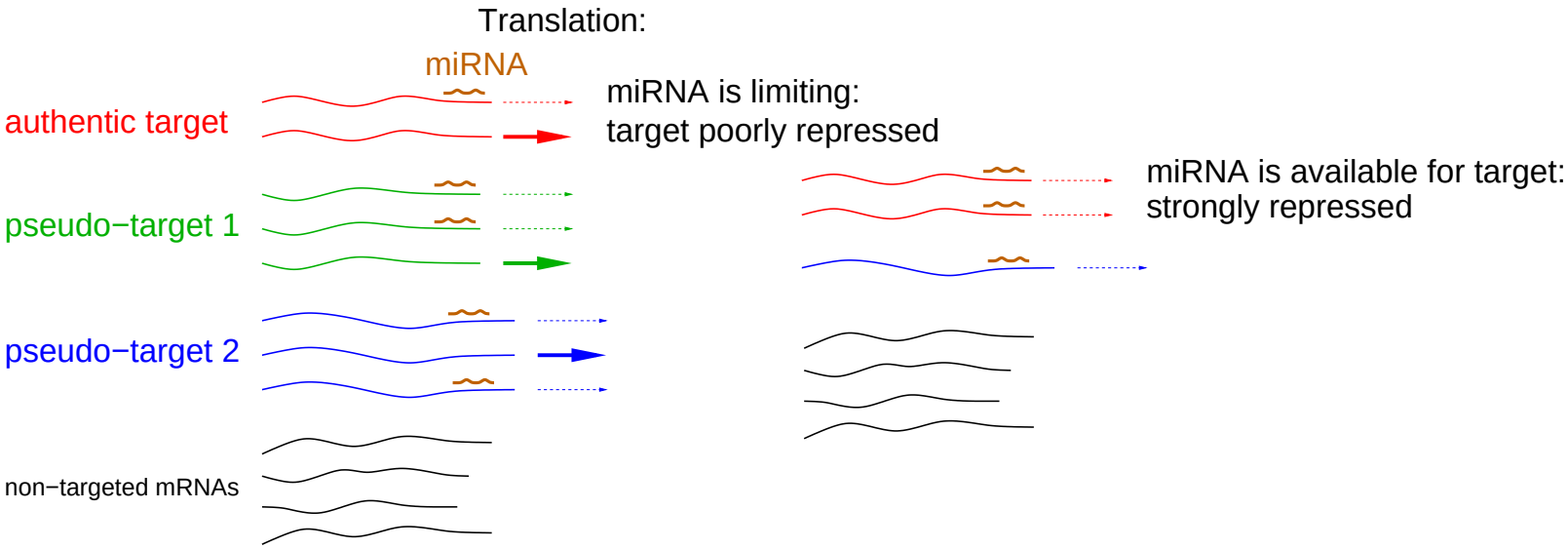
A "systems biology" conception of miRNA-target interactions: functional output depends on complex interaction networks.

What is a microRNA target?

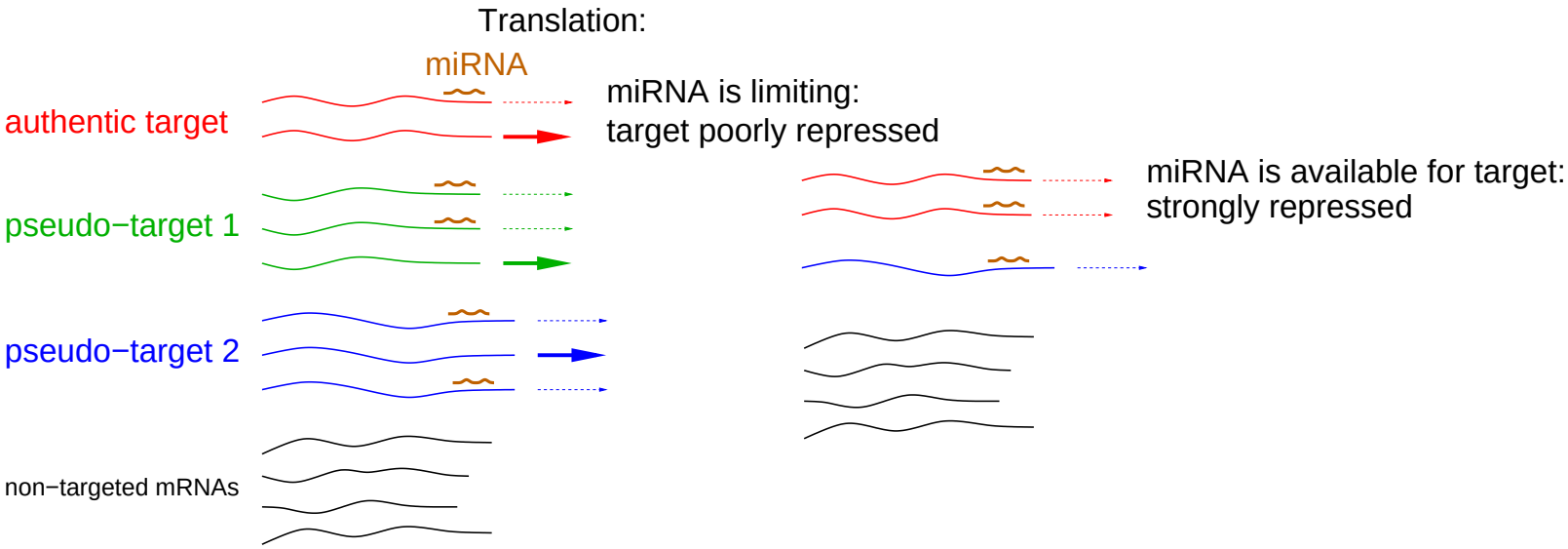


Cell type 1 (transcriptome composition 1)

What is a microRNA target?

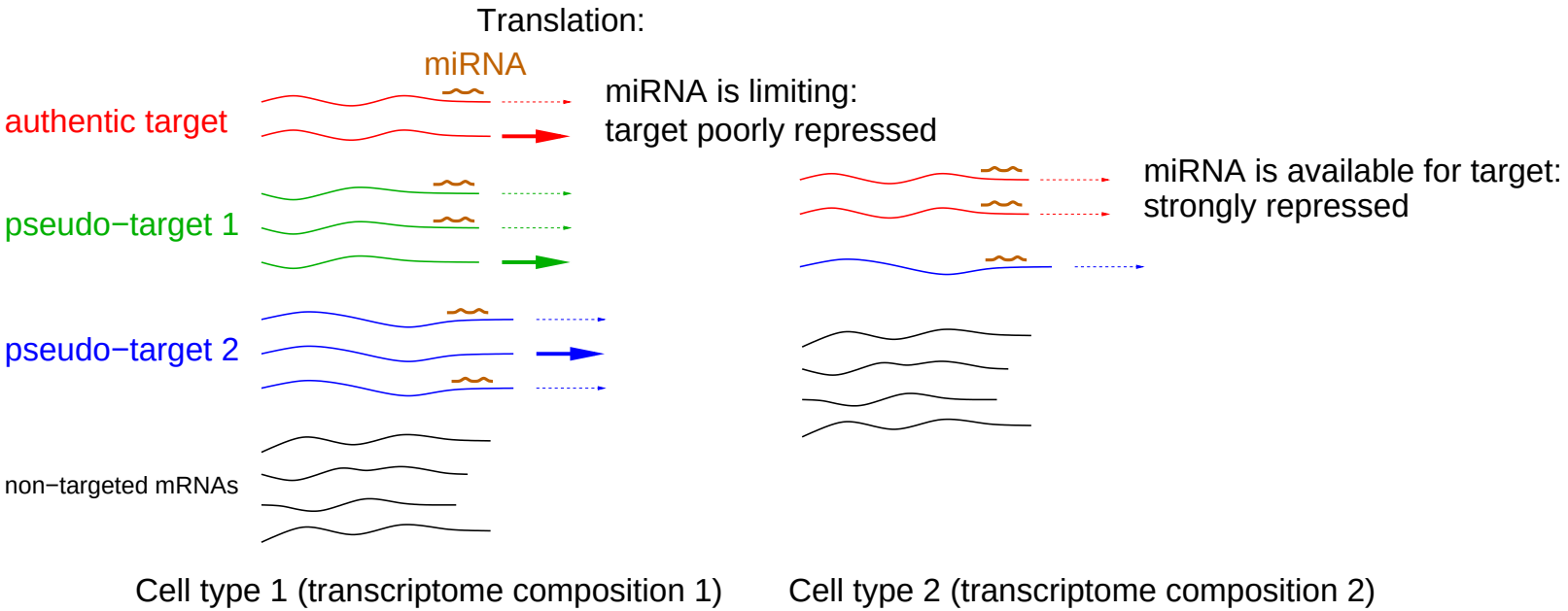


What is a microRNA target?



Cells modulate miRNA activity in a cell type-specific manner.

What is a microRNA target?



Cells modulate miRNA activity in a cell type-specific manner.

Integrating expression information of hundreds of genes on a single regulator.

piRNAs

piRNAs

- piRNAs: 23 to 30 nt; repress repeated sequences in germ line;

piRNAs

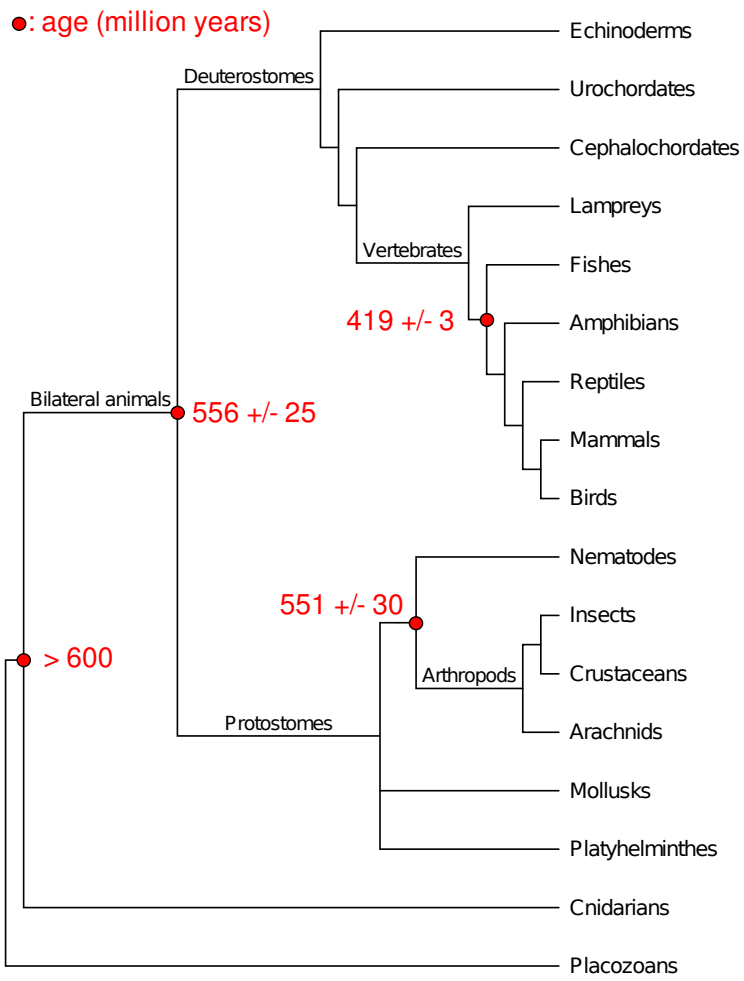
- piRNAs: 23 to 30 nt; repress repeated sequences in germ line;
- siRNAs: $\approx$ 20 bp; repress repeated sequences and other genes (in soma and germ line).

piRNAs

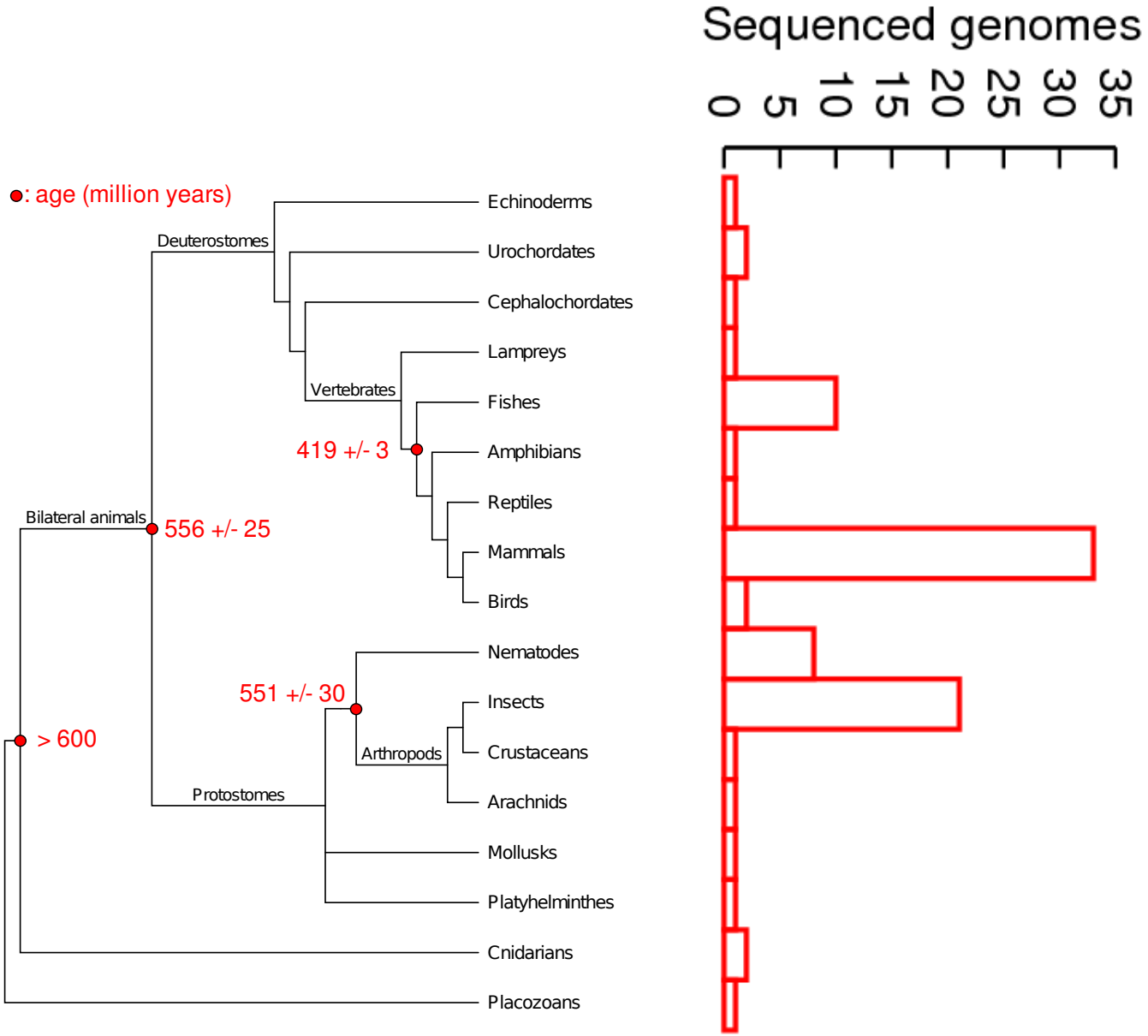
- piRNAs: 23 to 30 nt; repress repeated sequences in germ line;
- siRNAs: $\approx$ 20 bp; repress repeated sequences and other genes (in soma and germ line).

Could siRNAs replace piRNAs? Or do piRNAs have additional, unknown functions?

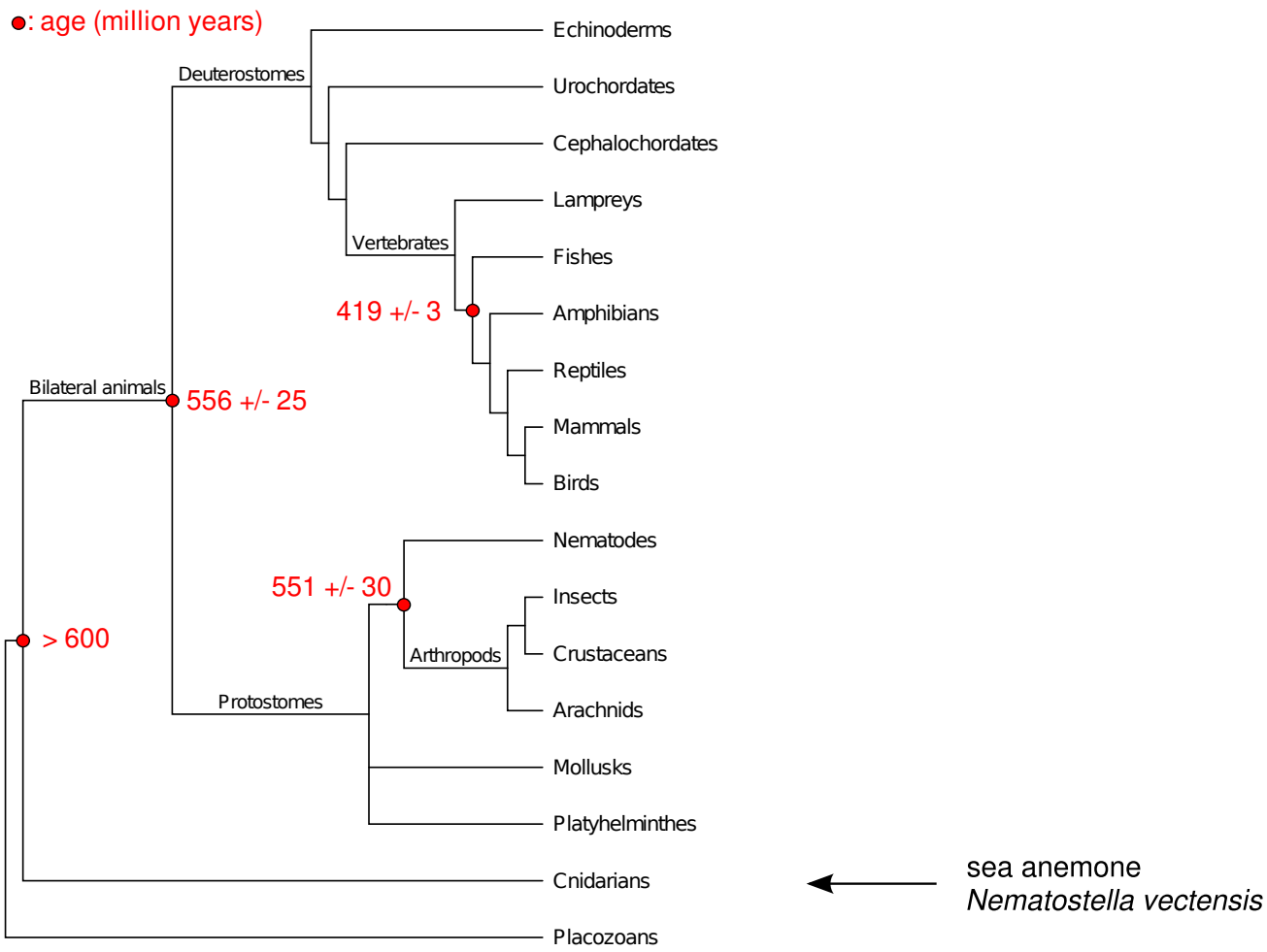
Metazoan phylogenetic tree



Metazoan phylogenetic tree



Metazoan phylogenetic tree

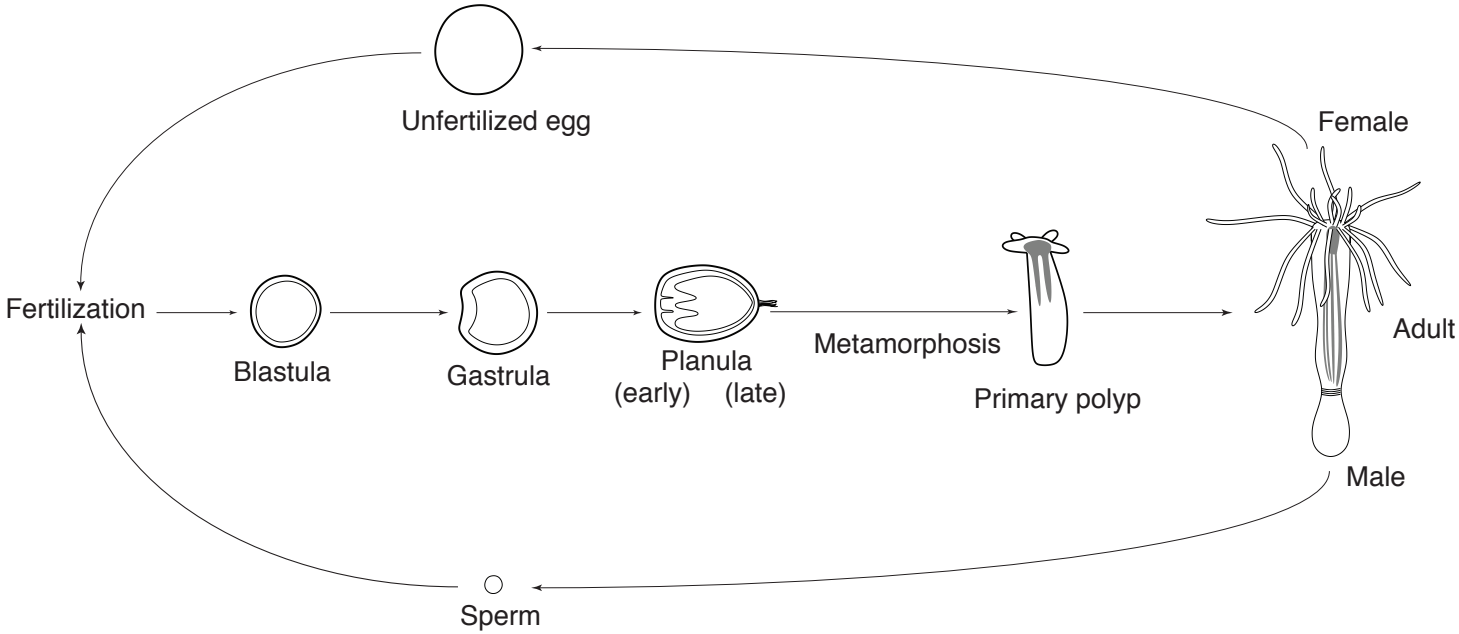


Small RNA-associated proteins in *Nematostella*

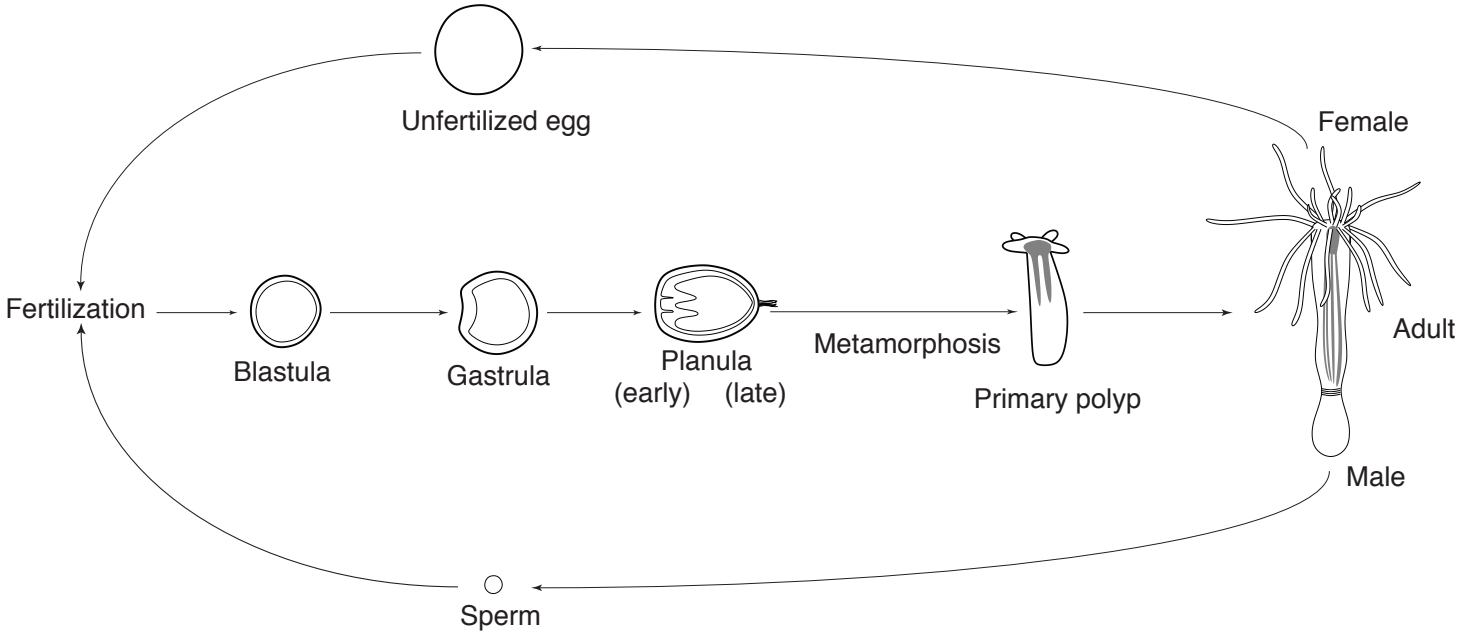
Protein	Number in *Nematostella*	Function
AGO	2	Binds siRNAs and miRNAs
Piwi	3	Binds piRNAs
Dicer	2	Last cleavage of miRNA precursors; siRNA biogenesis
Drosha	1	First cleavage of miRNA precursors
Hen1	1	Methylation of piRNAs and siRNAs (some miRNAs)

▸ Supplementary data

Nematostella life cycle

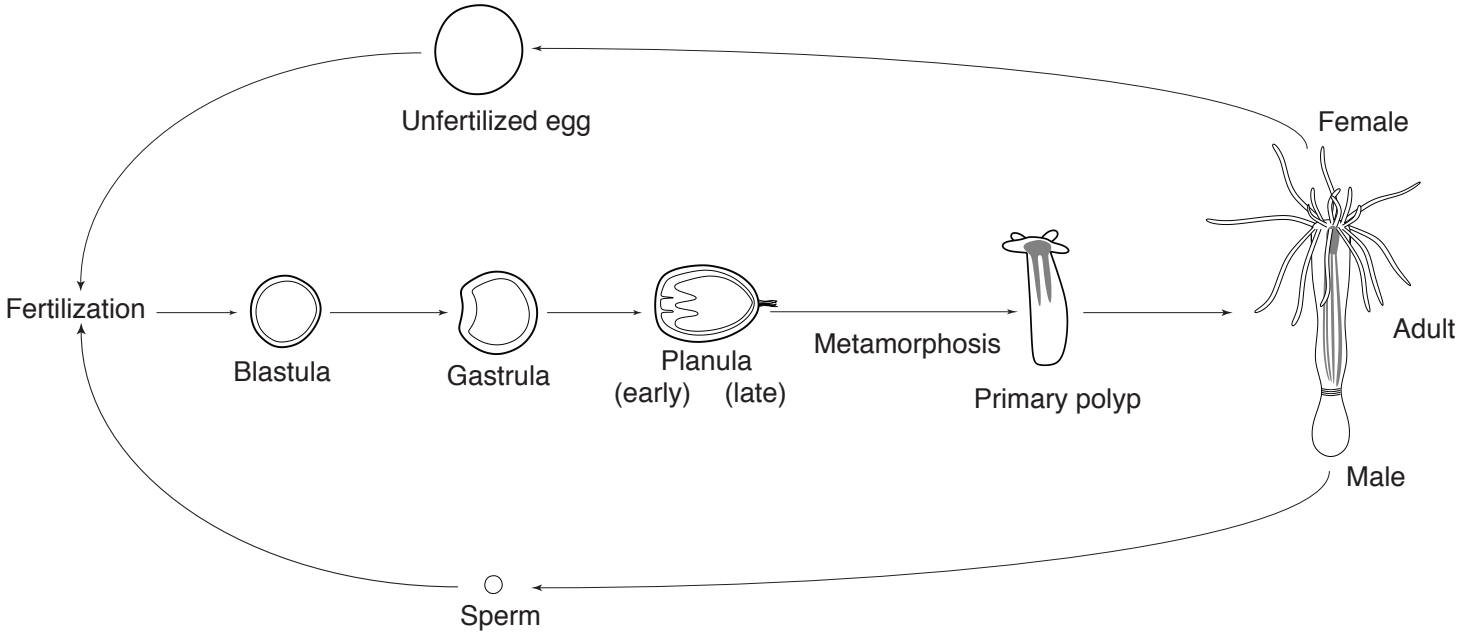


Nematostella life cycle



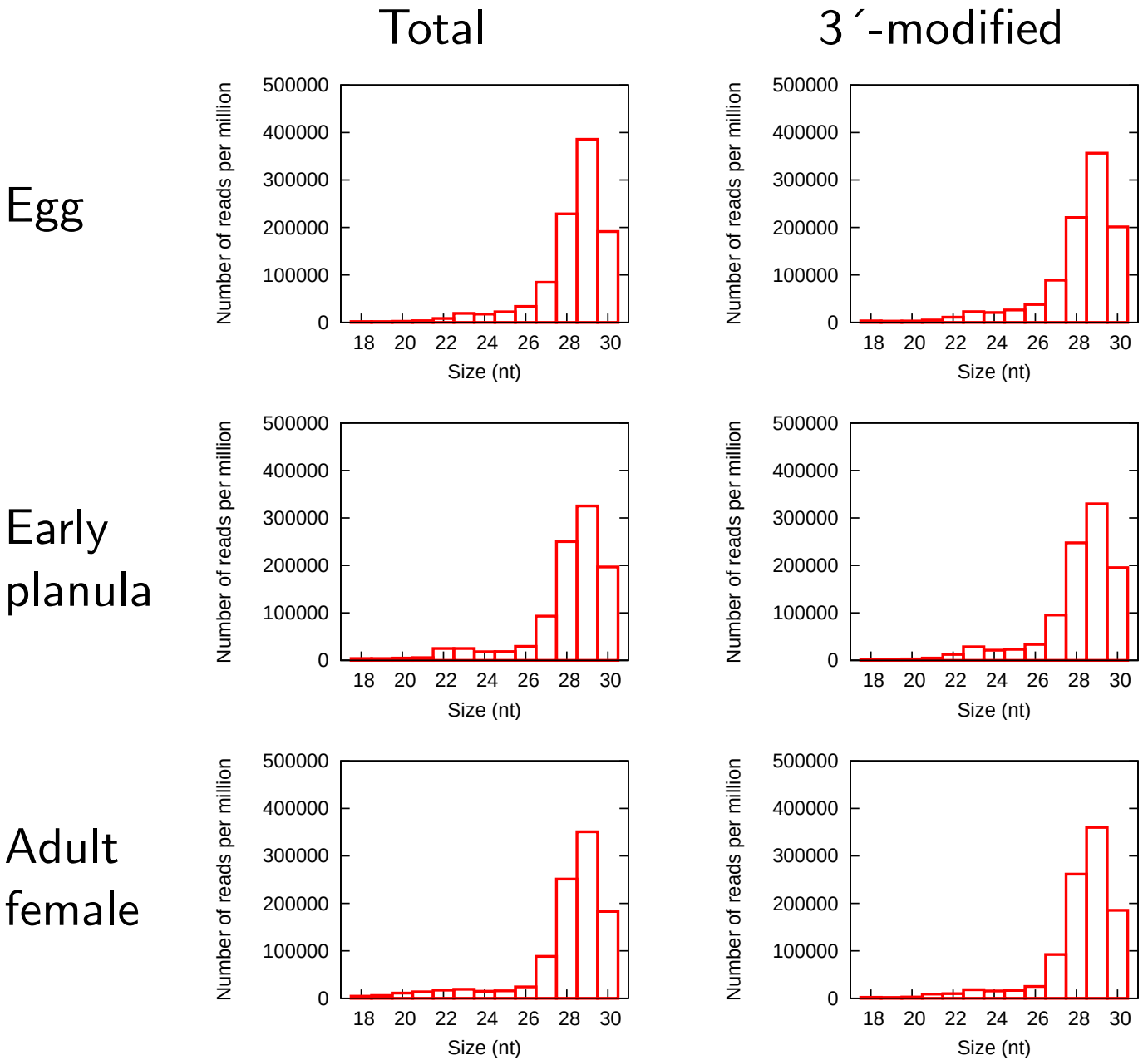
- Fabian Rentzsch (Sars Centre for Marine Molecular Biology, Bergen, Norway)

Nematostella life cycle



- Fabian Rentzsch (Sars Centre for Marine Molecular Biology, Bergen, Norway)
- 9 developmental stages, 2 libraries for each ("total RNAs" and "3´-modified RNAs")

Deep sequencing of *Nematostella* small RNAs



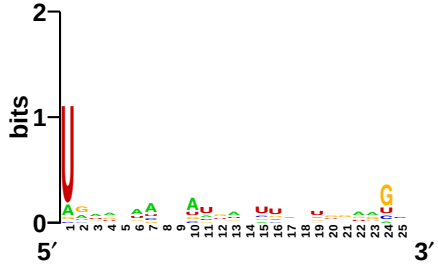
Nematostella piRNAs

Adult females, 3´-modified RNAs:

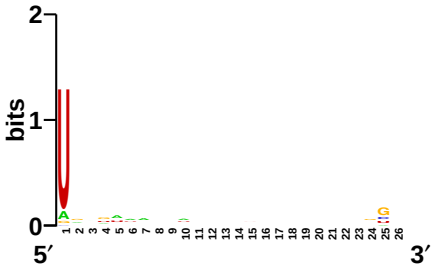
23-mers:

24-mers:

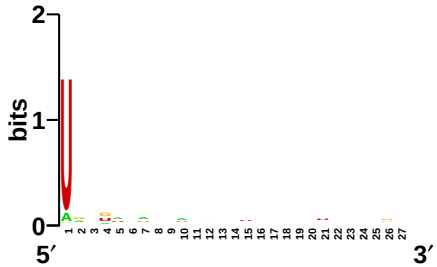
25-mers:



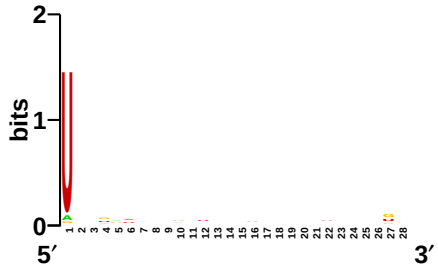
26-mers:



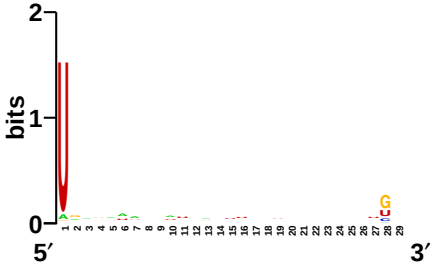
27-mers:



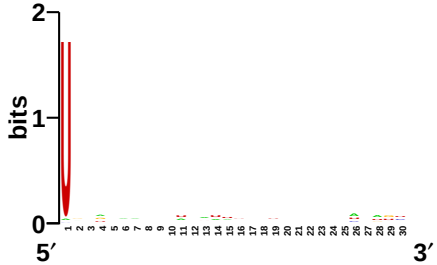
28-mers:



29-mers:



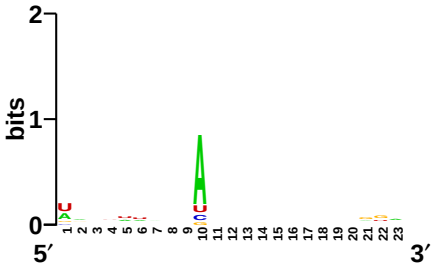
30-mers:



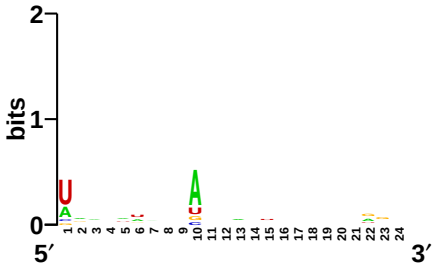
Nematostella piRNAs

Adult females, 3´-modified RNAs:

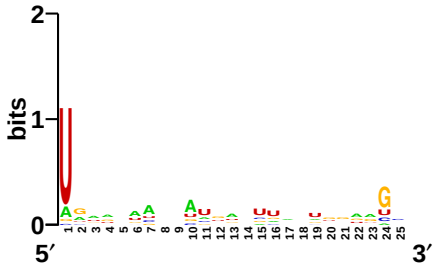
23-mers:



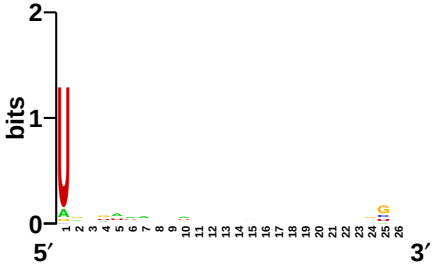
24-mers:



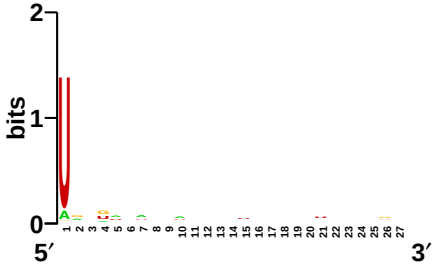
25-mers:



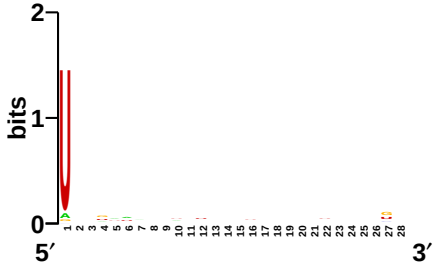
26-mers:



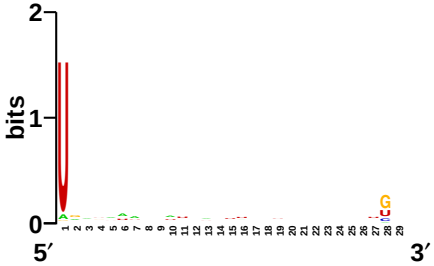
27-mers:



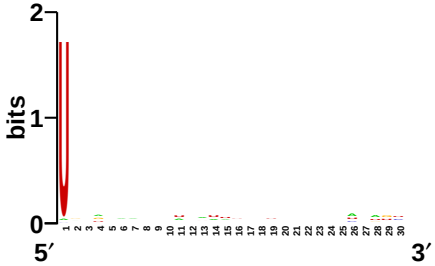
28-mers:



29-mers:



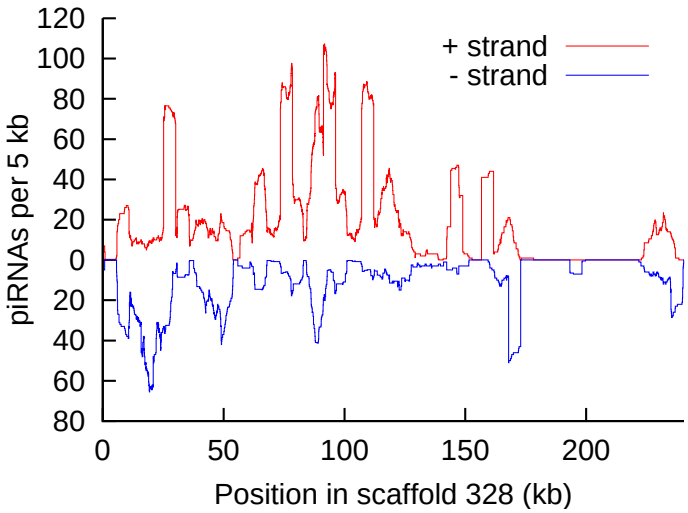
30-mers:



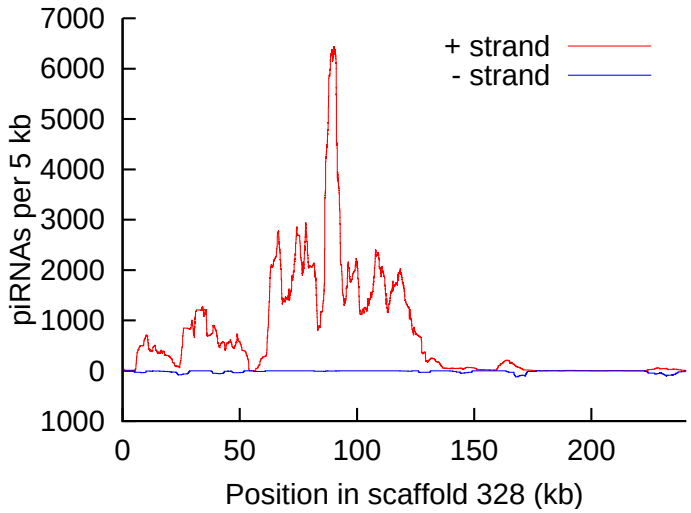
Nematostella piRNAs

Adult females, 3´-modified RNAs:

23-mers:



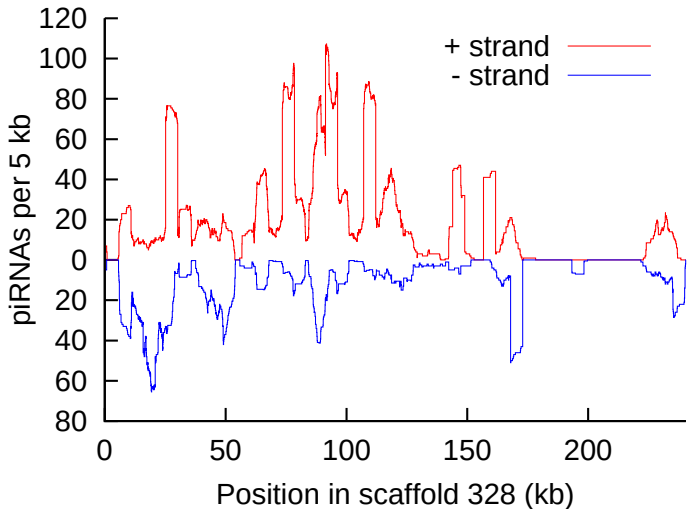
29-mers:



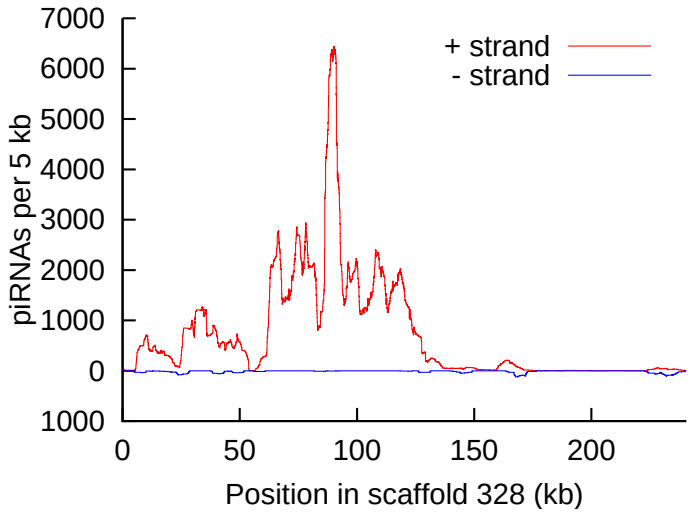
Nematostella piRNAs

Adult females, 3´-modified RNAs:

23-mers:

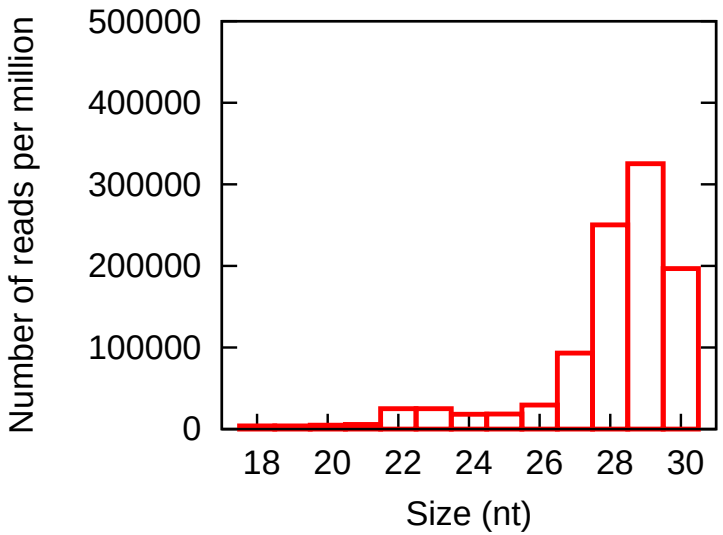


29-mers:



30 % of piRNAs map on transposons (transposons constitute only 10 % of the *Nematostella* genome).

Nematostella miRNAs

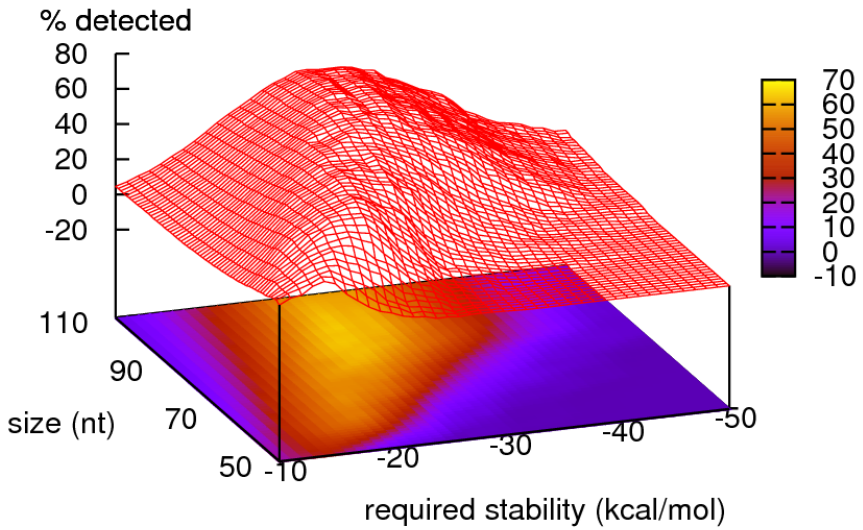


Nematostella miRNAs

Optimization of structural parameters.

Nematostella miRNAs

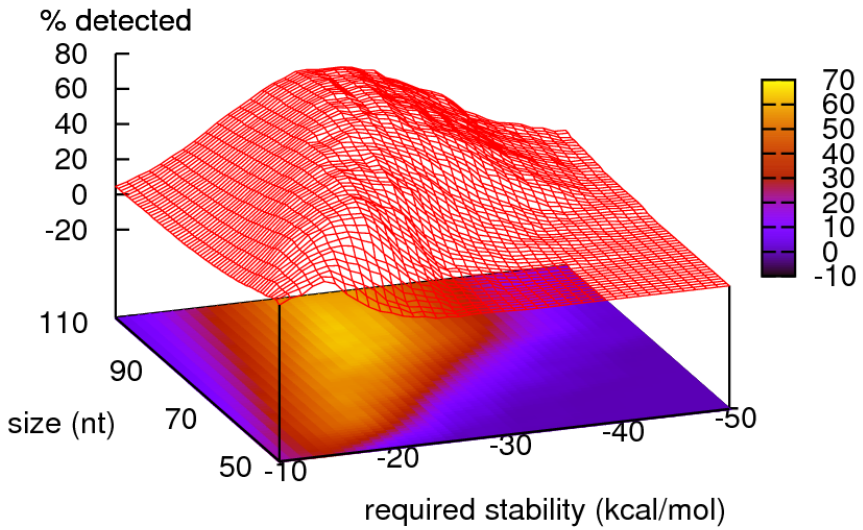
Optimization of structural parameters.



▸ Supplementary data

Nematostella miRNAs

Optimization of structural parameters.

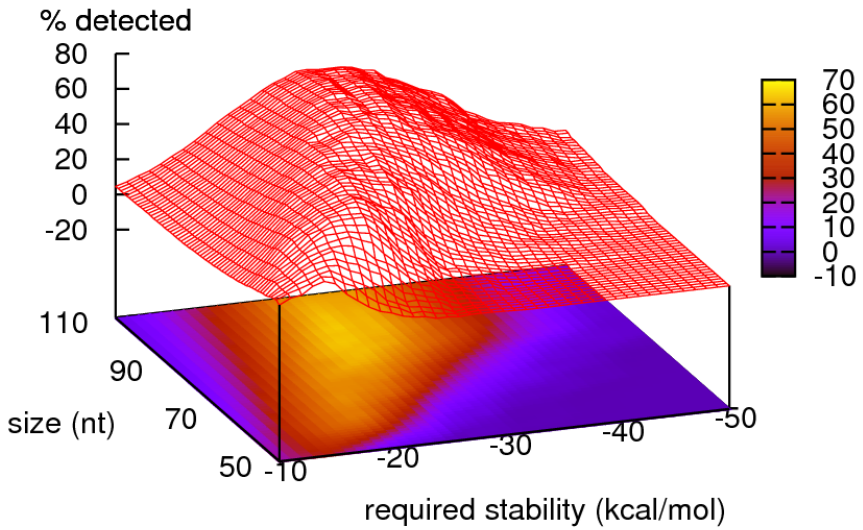


- Fold 96 nt-long sequences; stability cutoff: $\Delta G \leqslant -26\ kcal.mol^{-1}$;

▸ Supplementary data

Nematostella miRNAs

Optimization of structural parameters.



- Fold 96 nt-long sequences; stability cutoff: $\Delta G \leqslant -26\ kcal.mol^{-1}$;
- Detect both miRNA and miRNA*; miRNA must have homogeneous 5´ end.

▸ Supplementary data

Nematostella miRNAs

Grimson *et al.* (2008): identified 40 miRNAs in *N. vectensis*.

Nematostella miRNAs

Grimson *et al.* (2008): identified 40 miRNAs in *N. vectensis*.

Actually many more: 265.

Nematostella miRNAs

Grimson *et al.* (2008): identified 40 miRNAs in *N. vectensis*.

Actually many more: 265.

More miRNA genes than some vertebrates.

▸ Supplementary data

Nematostella miRNAs

Grimson *et al.* (2008): identified 40 miRNAs in *N. vectensis*.

Actually many more: 265.

More miRNA genes than some vertebrates: if the miRNA repertoire correlates with complexity, then *Nematostella* is complex.

▸ Supplementary data

Nematostella miRNAs

Grimson *et al.* (2008): identified 40 miRNAs in *N. vectensis*.

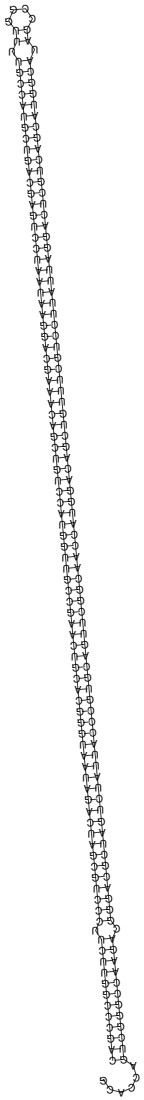
Actually many more: 265.

More miRNA genes than some vertebrates: if the miRNA repertoire correlates with complexity, then *Nematostella* is complex (but does this question make sense?).

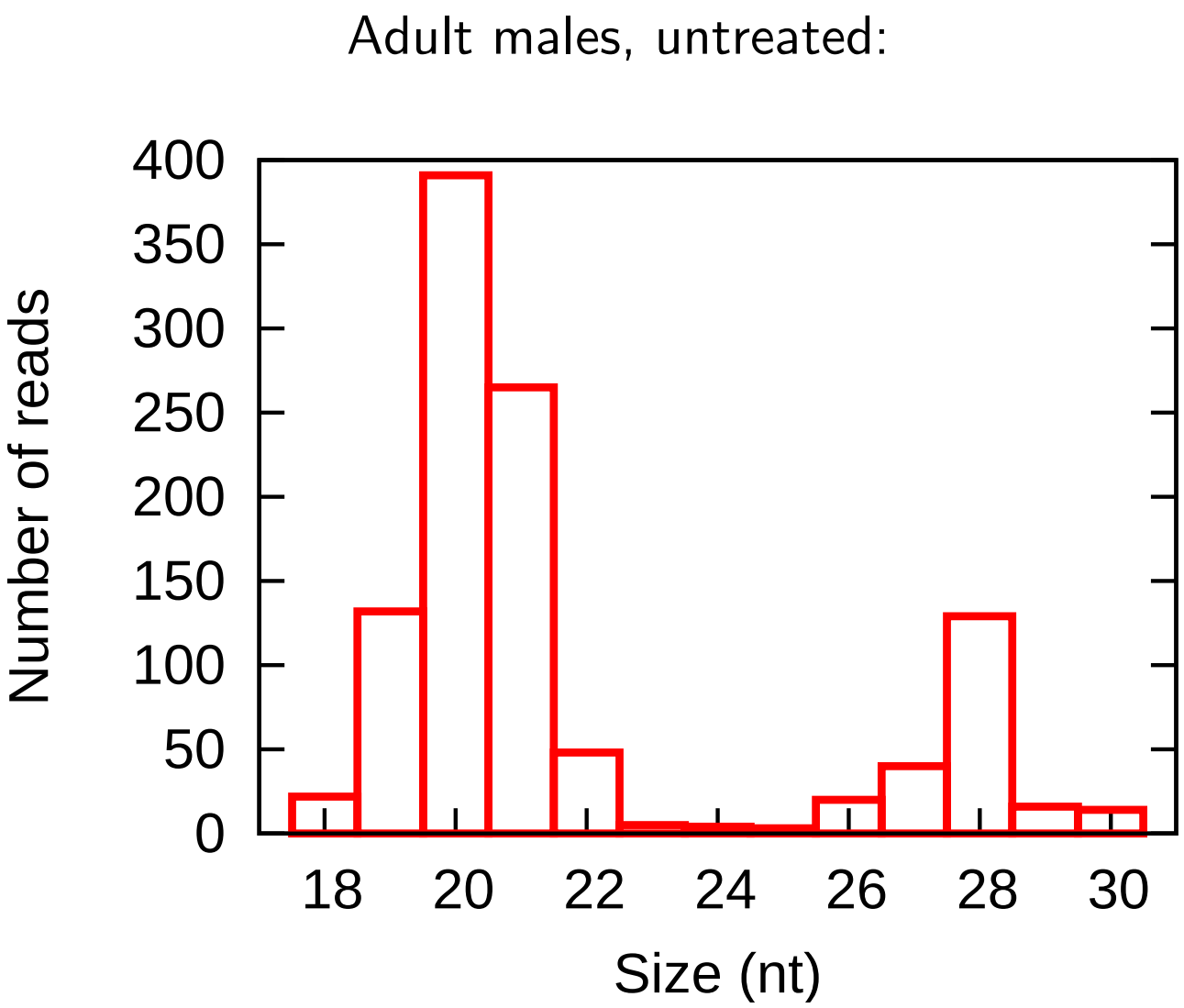
▸ Supplementary data

Nematostella siRNAs

Nematostella siRNAs

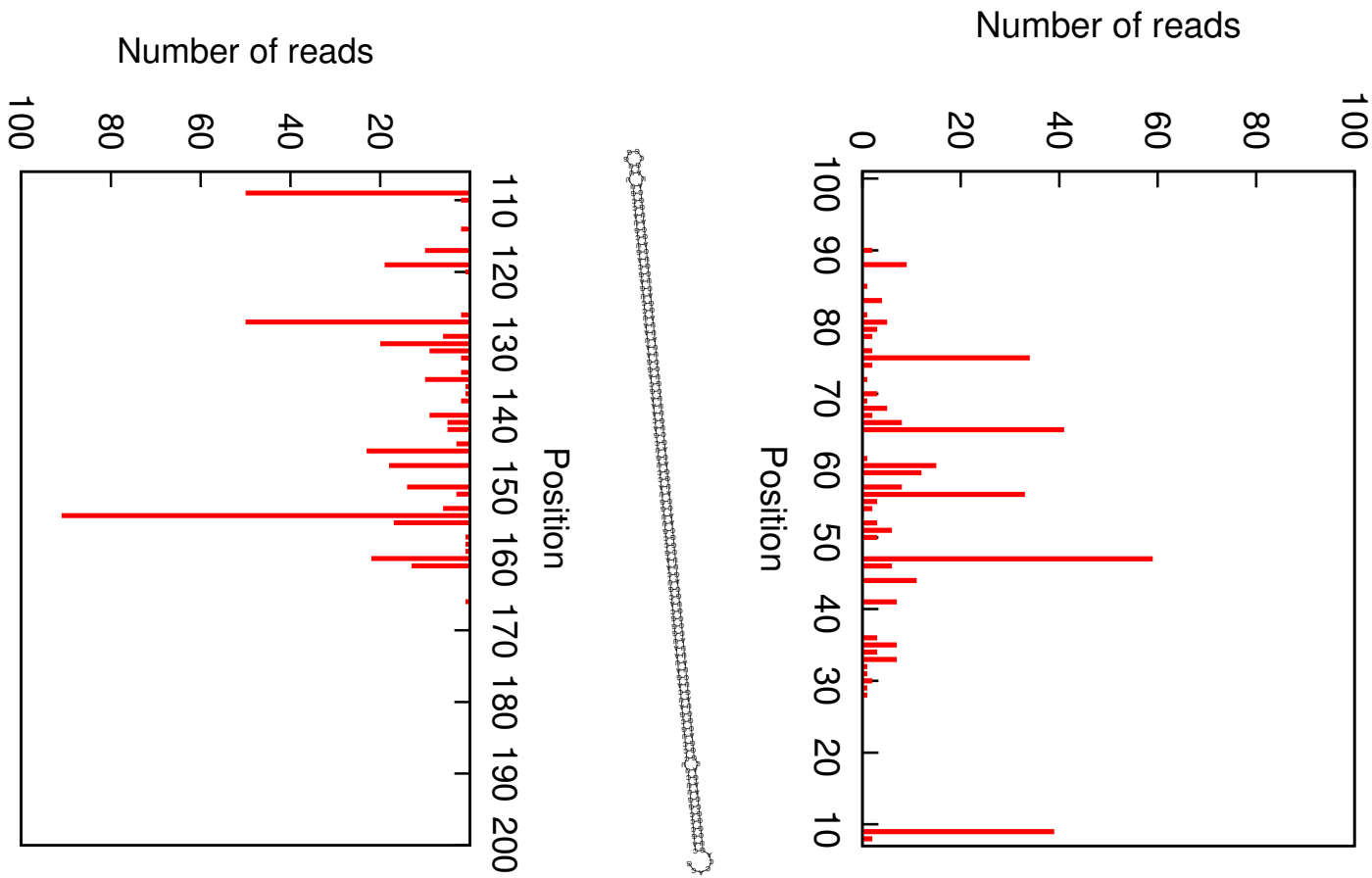


Nematostella siRNAs



Nematostella siRNAs

Adult males, untreated:



Nematostella siRNAs

Many siRNA-expressing hairpins map on repeated sequences (transposons *Penelope-4*, *Harbinger-N7*, *Polinton-2_NV*, ...).

Conclusion

Conclusion

- MiRNAs repress mRNAs, but mRNAs could repress miRNAs too.

Conclusion

- MiRNAs repress mRNAs, but mRNAs could repress miRNAs too.
- Target prediction algorithms may have missed an essential feature of miRNA target repression.

Conclusion

- MiRNAs repress mRNAs, but mRNAs could repress miRNAs too.
- Target prediction algorithms may have missed an essential feature of miRNA target repression.
- Sea anemones have as many miRNAs as vertebrates.

Conclusion

- MiRNAs repress mRNAs, but mRNAs could repress miRNAs too.
- Target prediction algorithms may have missed an essential feature of miRNA target repression.
- Sea anemones have as many miRNAs as vertebrates.
- PiRNAs and siRNAs have repressed transposons concomitantly for at least 600 million years.

▸ Supplementary data

Conclusion

- MiRNAs repress mRNAs, but mRNAs could repress miRNAs too.
- Target prediction algorithms may have missed an essential feature of miRNA target repression.
- Sea anemones have as many miRNAs as vertebrates.
- PiRNAs and siRNAs have repressed transposons concomitantly for at least 600 million years.
- What is the additional function of piRNAs?

▸ Supplementary data

Conclusion

- MiRNAs repress mRNAs, but mRNAs could repress miRNAs too.
- Target prediction algorithms may have missed an essential feature of miRNA target repression.
- Sea anemones have as many miRNAs as vertebrates.
- PiRNAs and siRNAs have repressed transposons concomitantly for at least 600 million years.
- What is the additional function of piRNAs?

Nematostella undergoes sexual and asexual reproduction

▸ Supplementary data

Conclusion

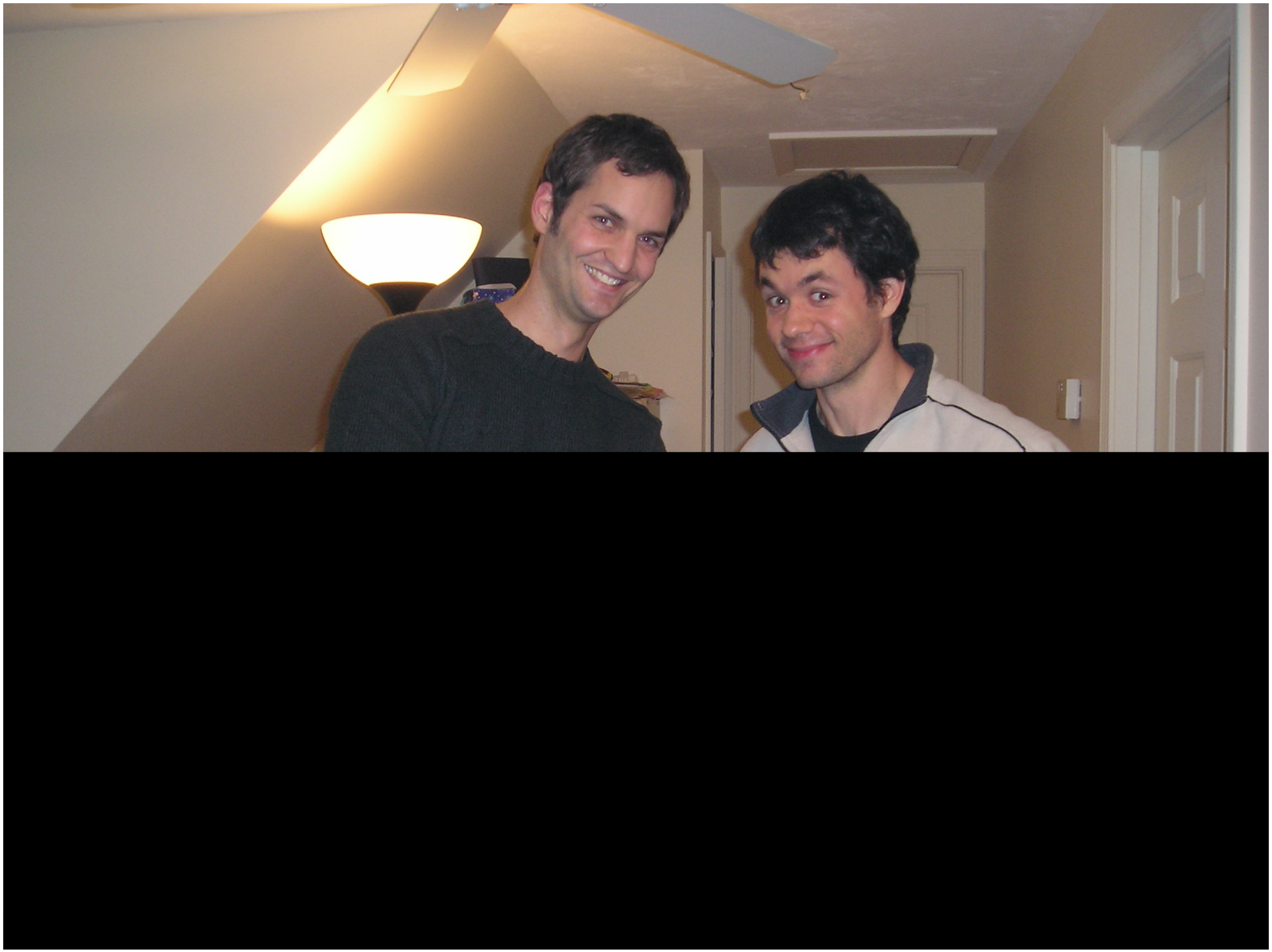
- MiRNAs repress mRNAs, but mRNAs could repress miRNAs too.
- Target prediction algorithms may have missed an essential feature of miRNA target repression.
- Sea anemones have as many miRNAs as vertebrates.
- PiRNAs and siRNAs have repressed transposons concomitantly for at least 600 million years.
- What is the additional function of piRNAs?

Nematostella undergoes sexual and asexual reproduction: are piRNAs required to make a progeny by asexual reproduction?

▸ Supplementary data

Acknowledgements

Acknowledgements



Acknowledgements

Acknowledgements

UMass Medical School (Worcester, MA, USA):
Phil Zamore, Liang Meng Wee, Zamore lab members
Ellen Kittler and Maria Zapp (deep-seq. facility)

Sars Center (Bergen, Norway):
Fabian Rentzsch (*Nematostella* RNA samples)

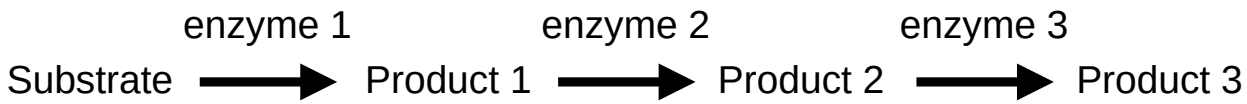
Marine Biological Laboratory (Woods Hole, MA, USA):
Rhys Probyn and Ed Enos (live specimens of *Nematostella*)

Acknowledgements

Supplementary data

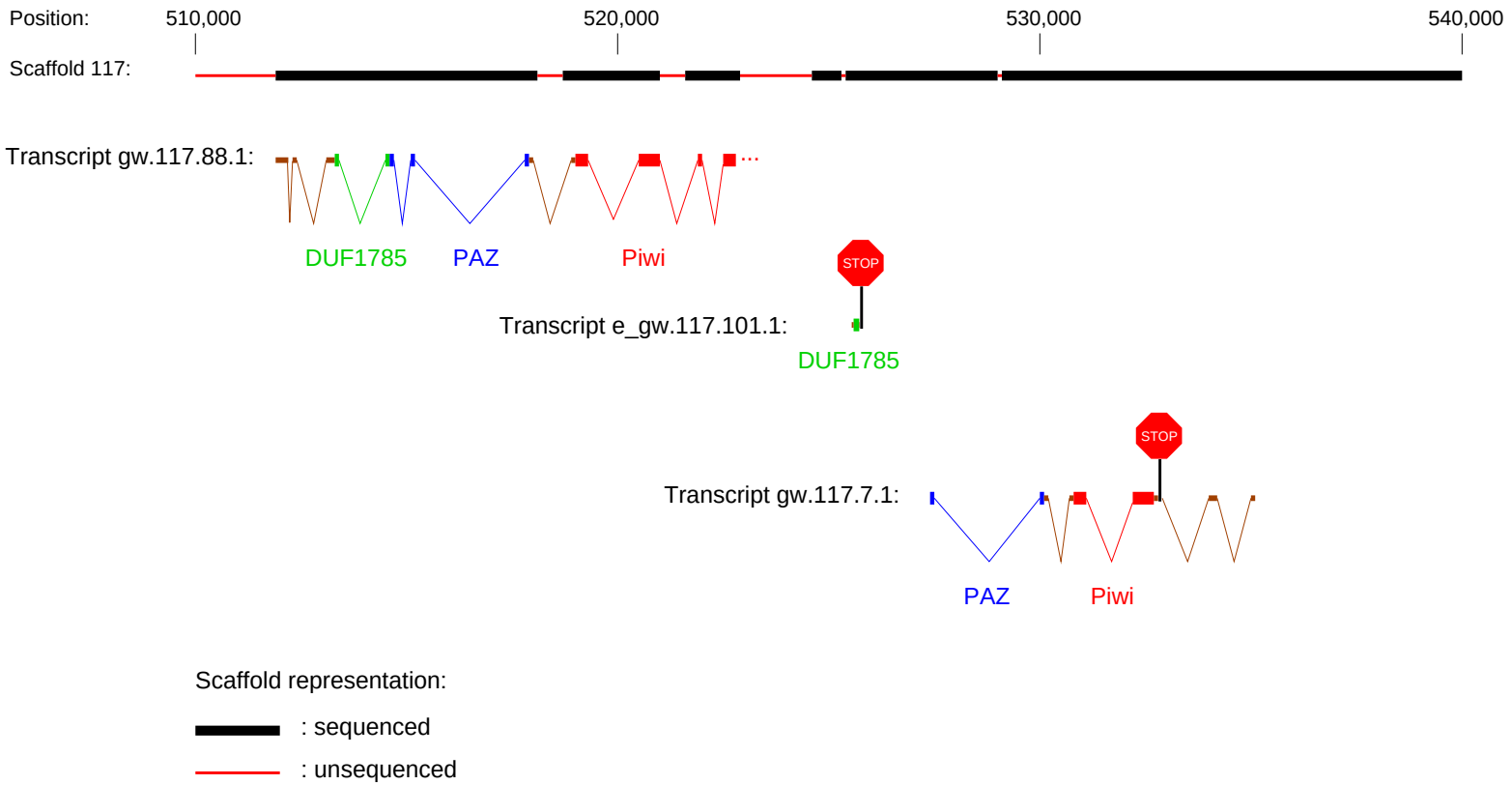
- Robustness of biological pathways
- *Nematostella* AGO2 locus
- Size distribution histograms
- Pre-miRNA folding parameters
- RNA profile on pre-miRNAs
- *Nematostella* and vertebrate miRNAs
- Known miRNAs vs. article number
- siRNA chemical structure
- Geographic distribution

Second paradox



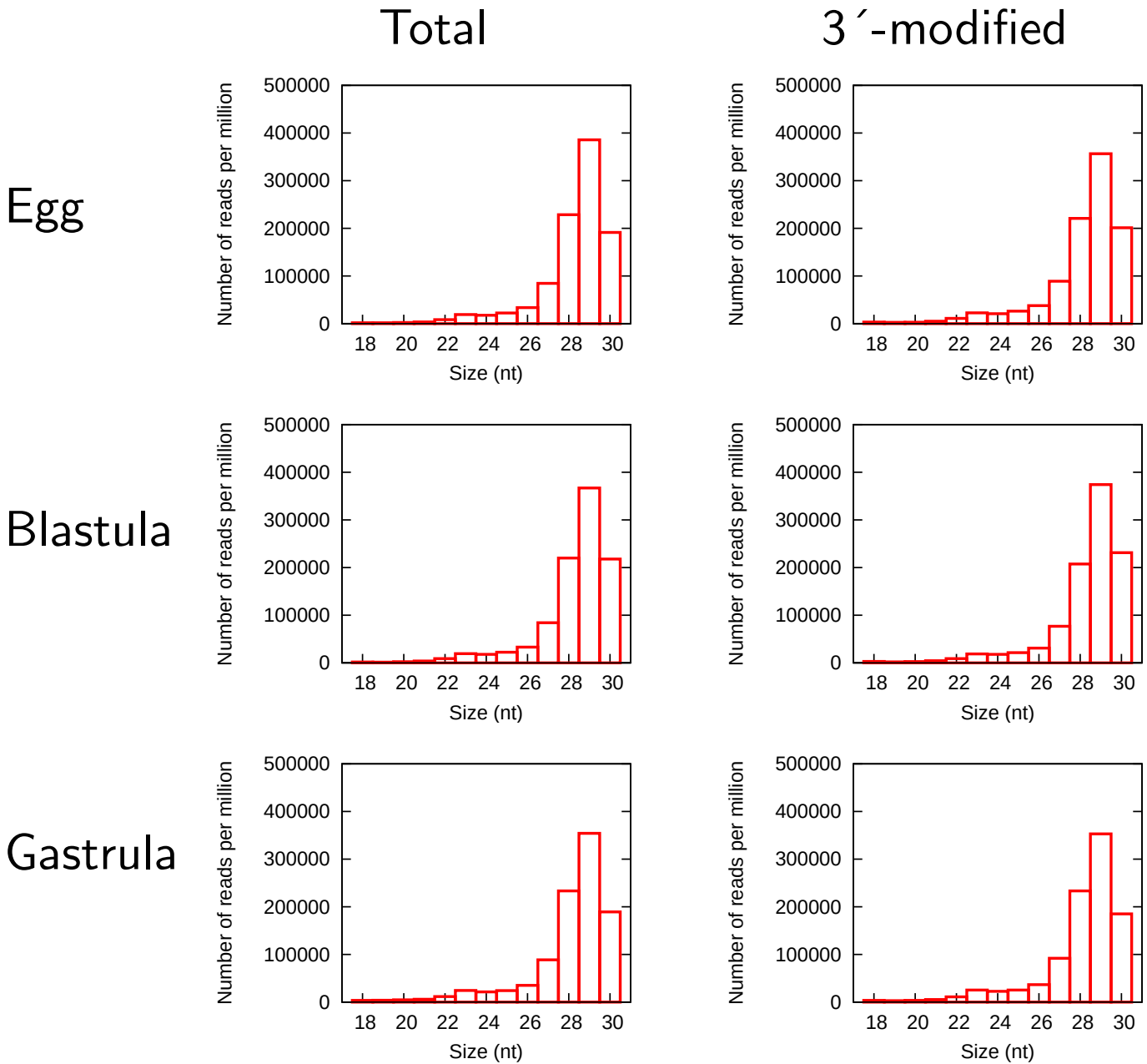
Return

Small RNA-associated proteins in *Nematostella*

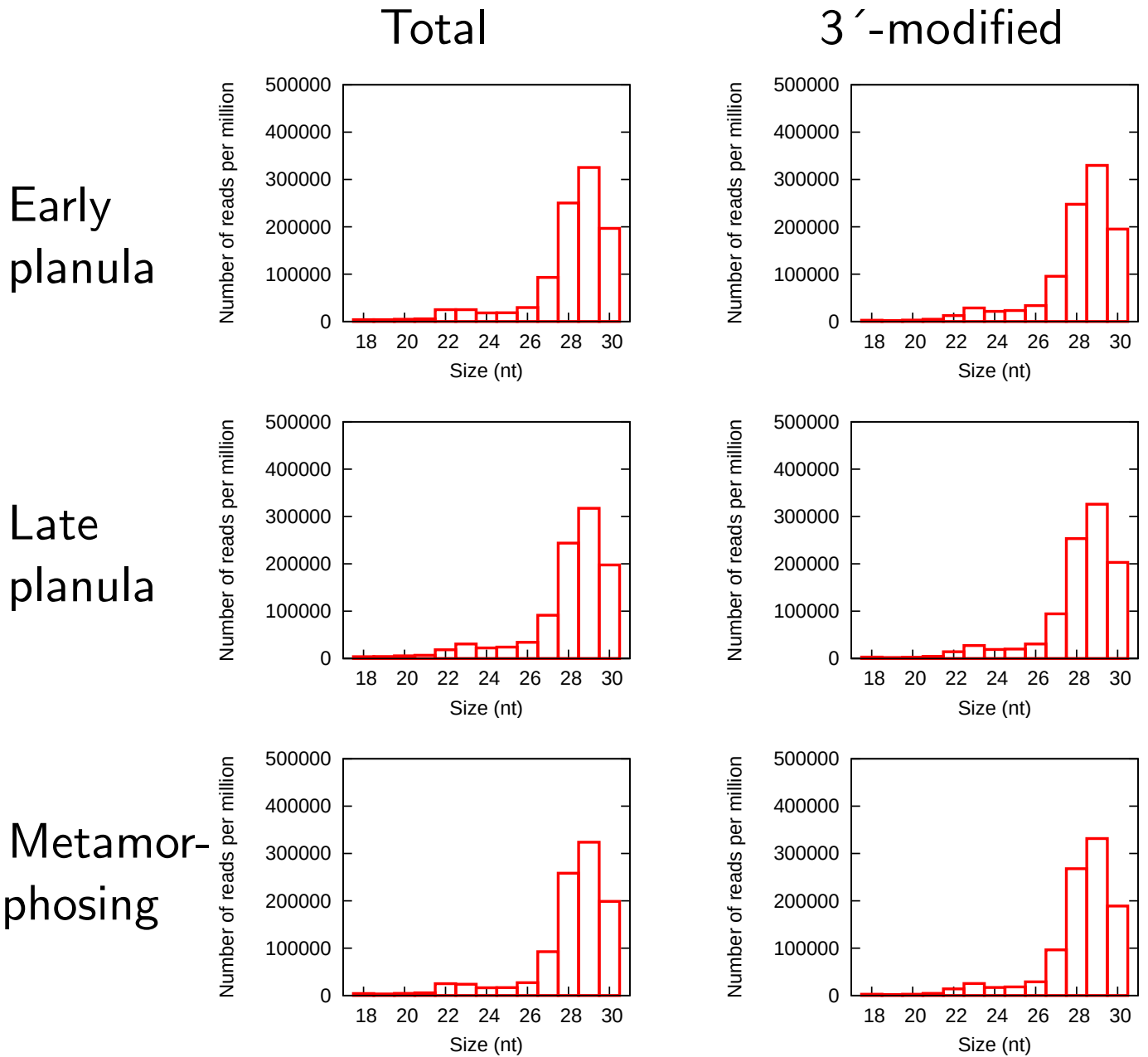


◂ Return

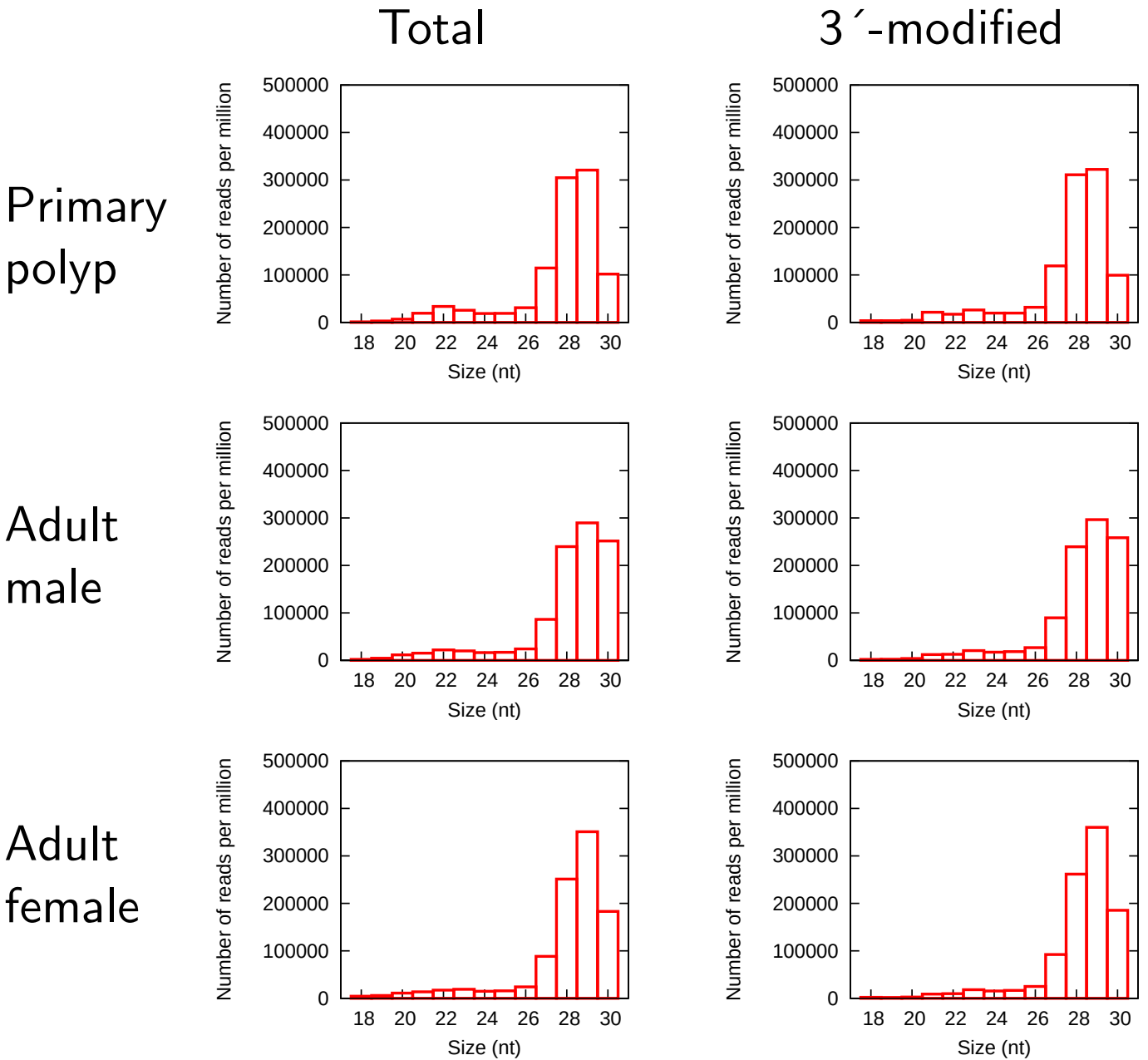
Deep sequencing of *Nematostella* small RNAs



Deep sequencing of *Nematostella* small RNAs

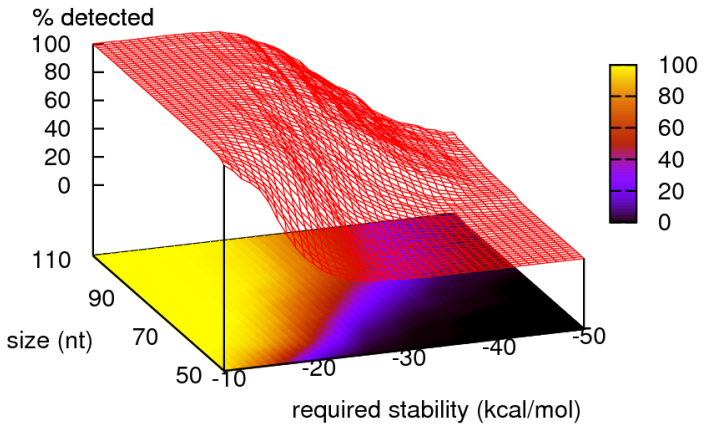


Deep sequencing of *Nematostella* small RNAs



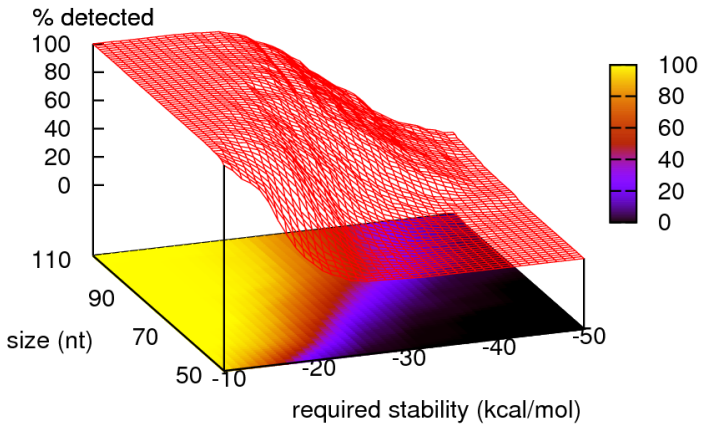
Nematostella miRNAs

Pre-miRNAs:

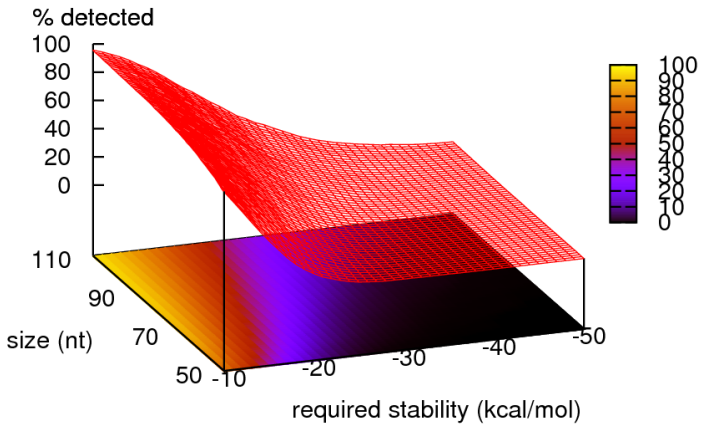


Nematostella miRNAs

Pre-miRNAs:

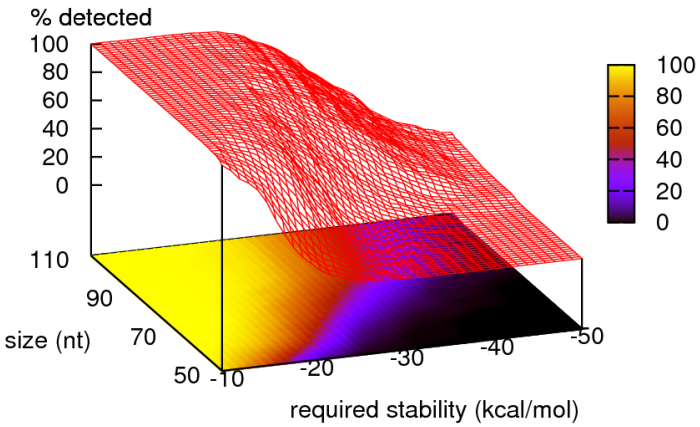


Negative control:

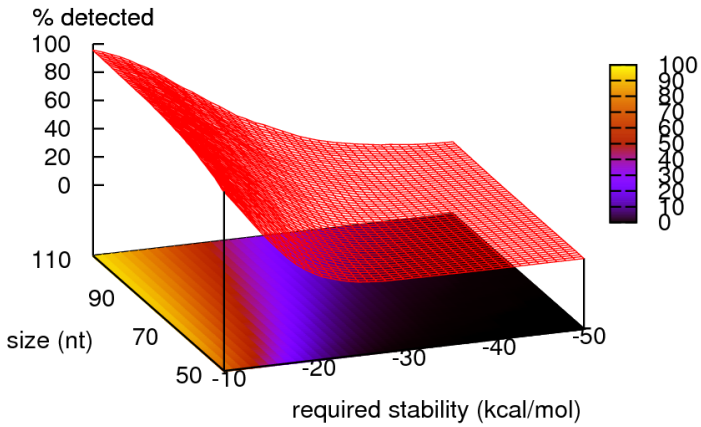


Nematostella miRNAs

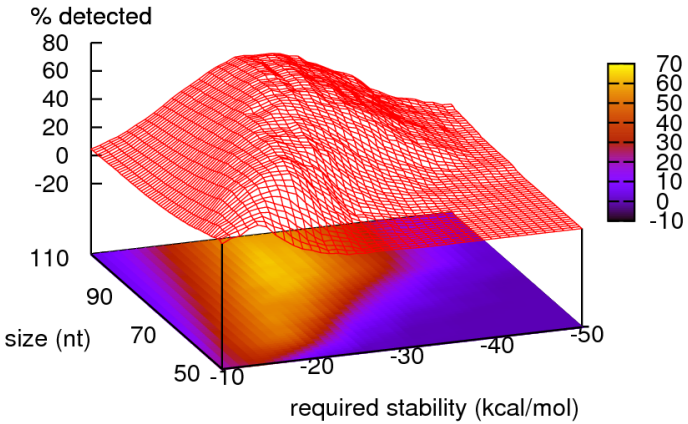
Pre-miRNAs:



Negative control:

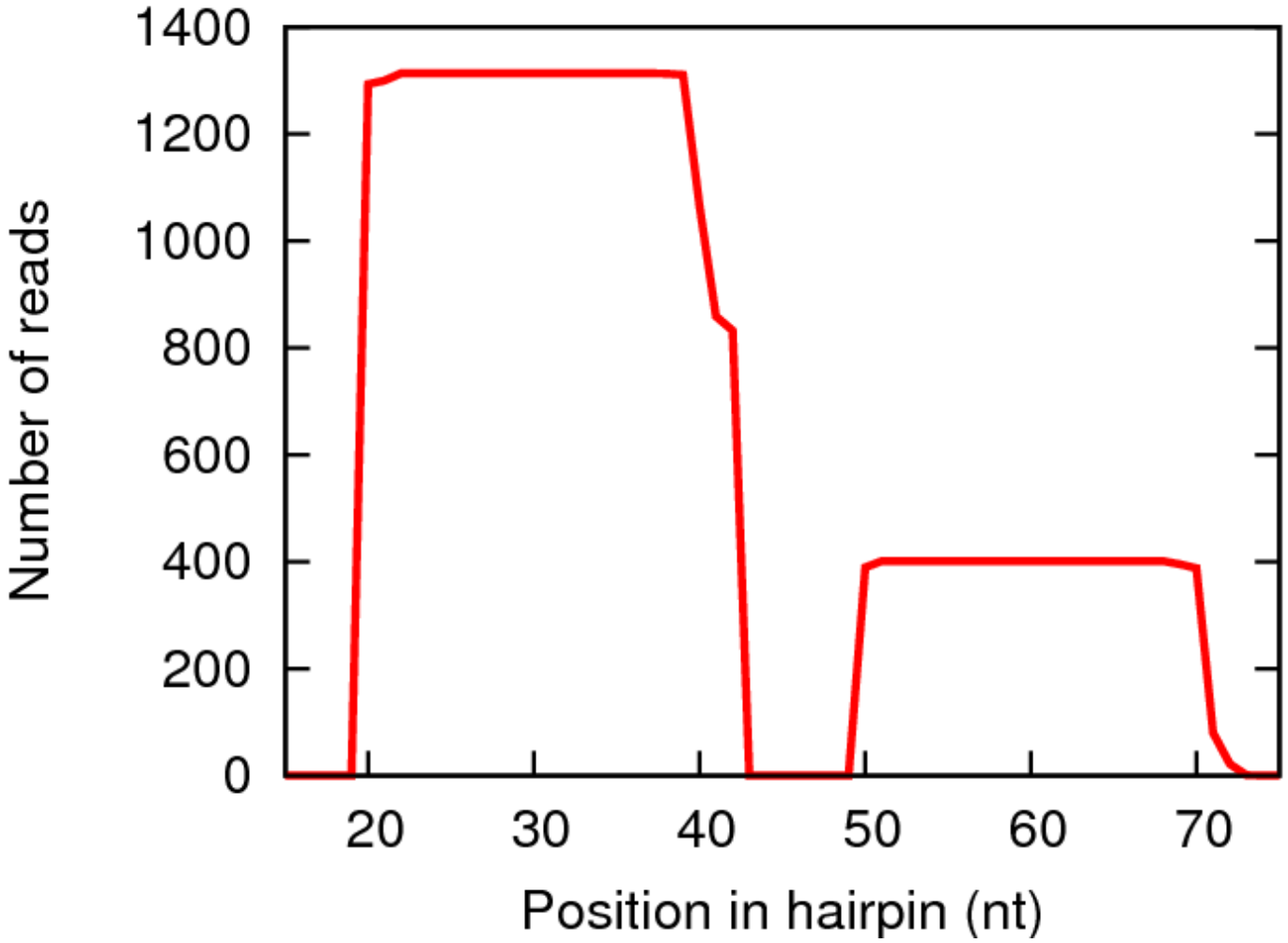


Difference:



Return

Nematostella miRNAs



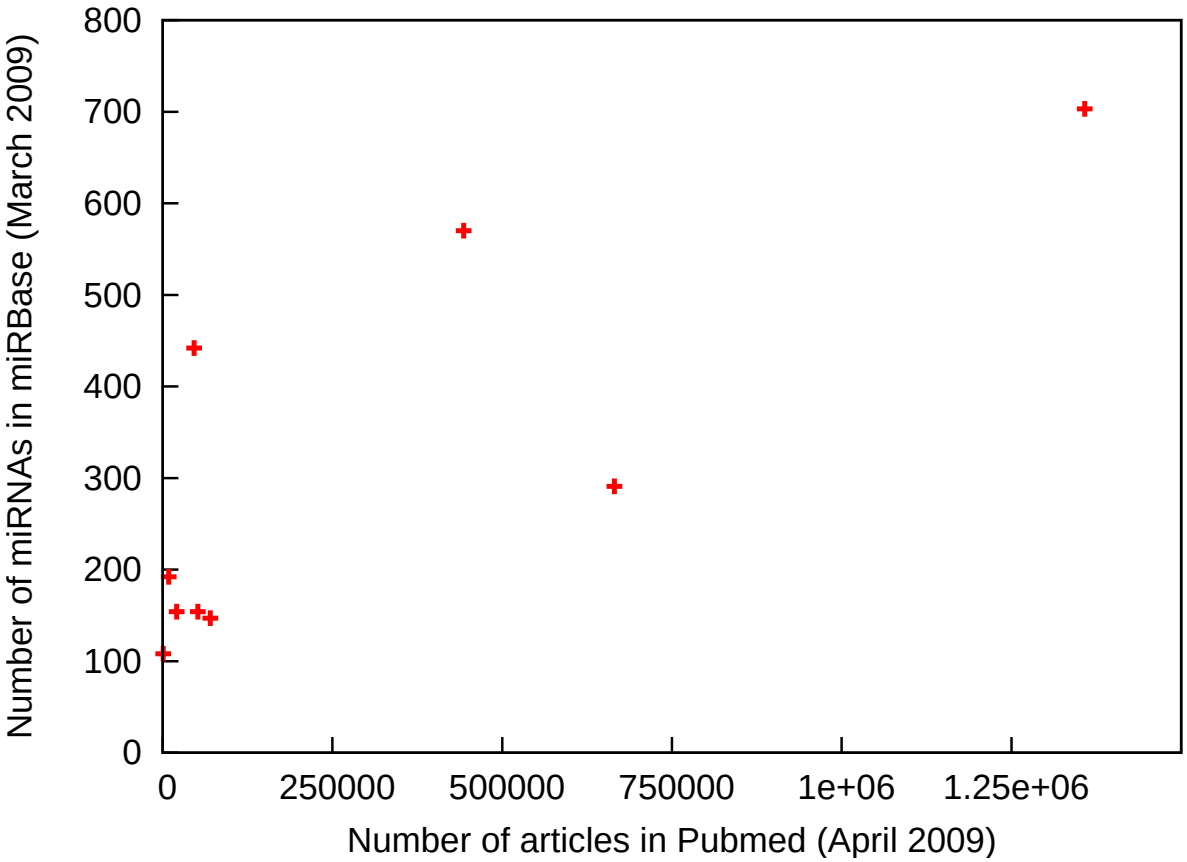

```
AGGCUUGCUUGUUGGUAAUUUUGCAUCUGUUGCACAUGCGAUUUUACCAAAAUGCAAUUCU

((..((((...(((((((..((((((.((...)).))))))..)))))))...)))))..))
```

Nematostella miRNAs

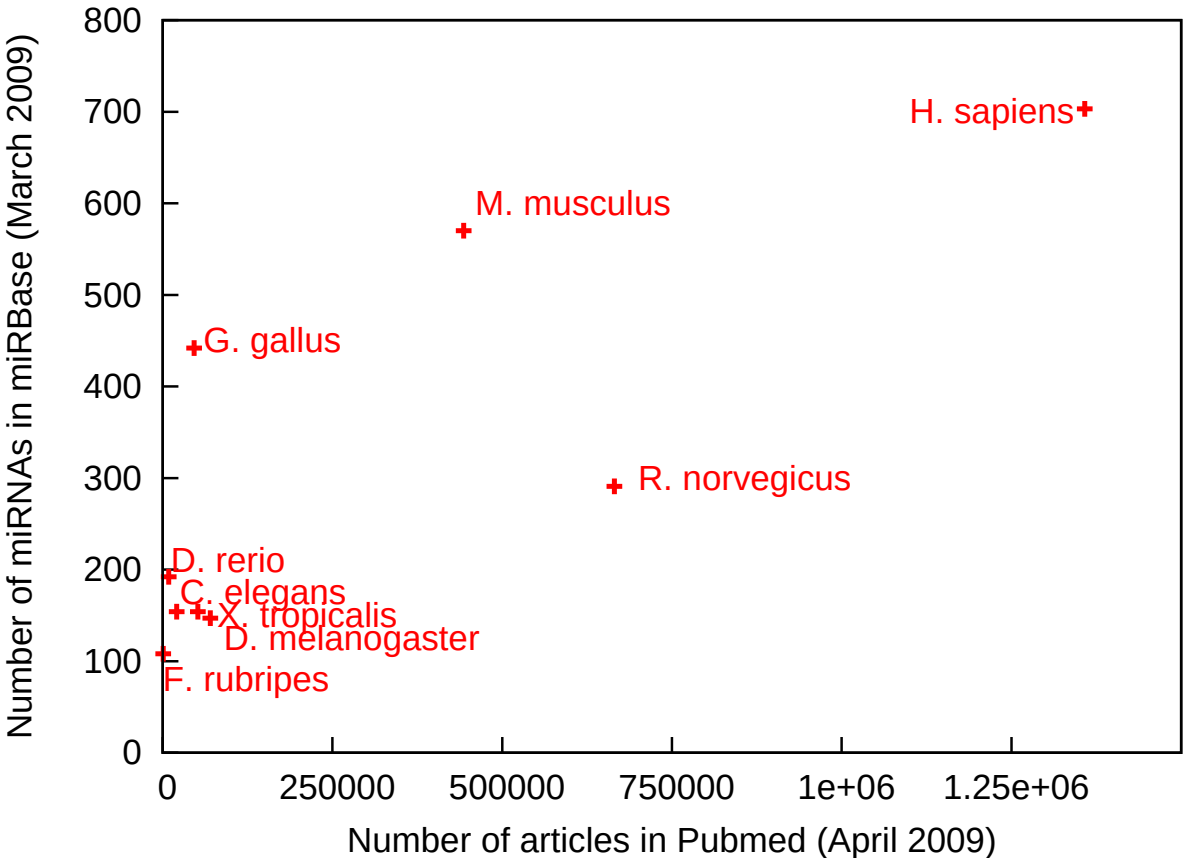
Species	Number of miRNAs (March 2009)
Homo sapiens	703
Mus musculus	570
Gallus gallus	442
Rattus norvegicus	291
Nematostella vectensis	265+18
Danio rerio	192
Xenopus tropicalis	154
Monodelphis domestica	109
Tetraodon nigroviridis	108
Fugu rubripes	108

◂ Return

Nematostella miRNAs

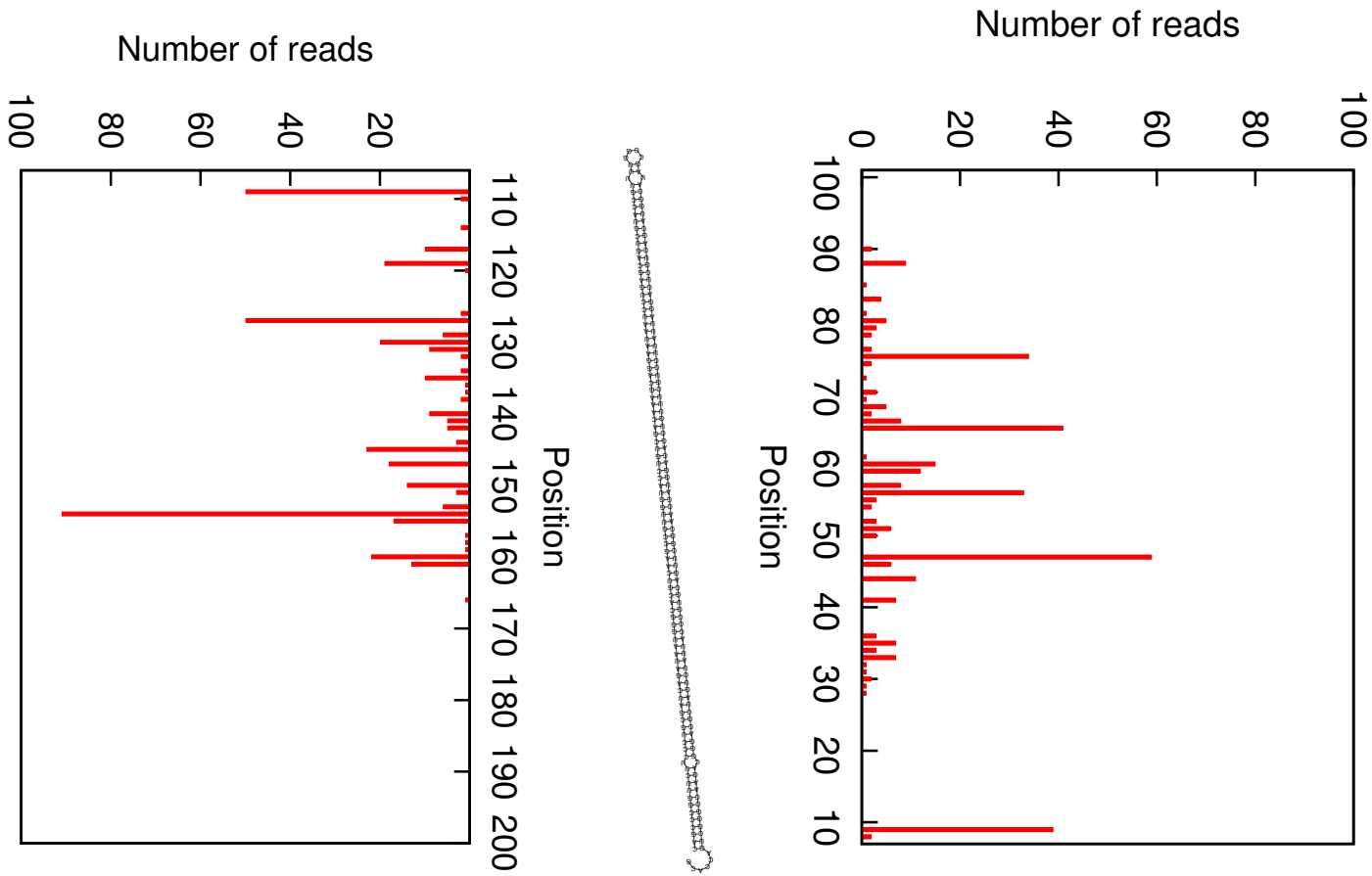


Nematostella miRNAs



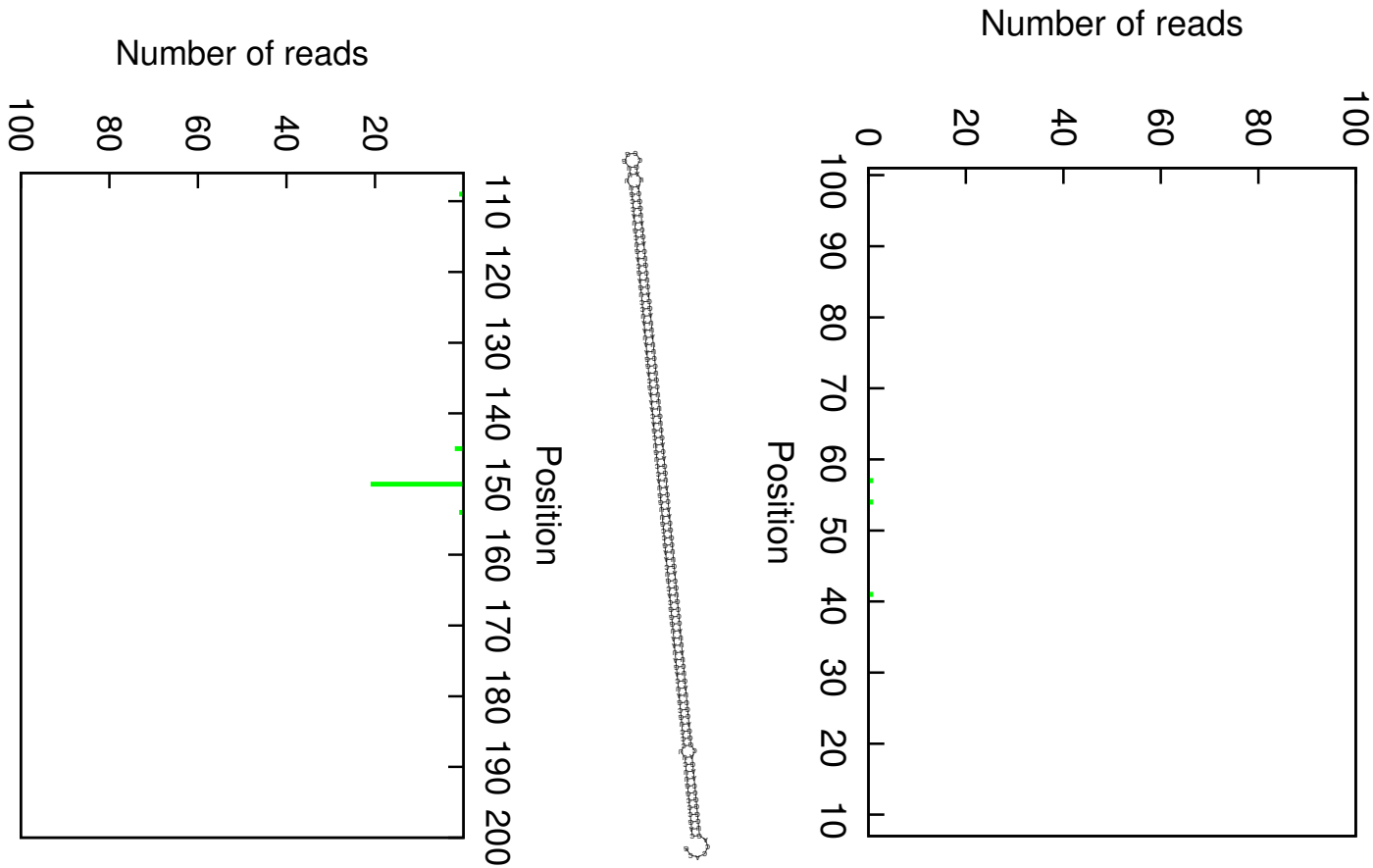
Nematostella siRNAs

Adult males, untreated:

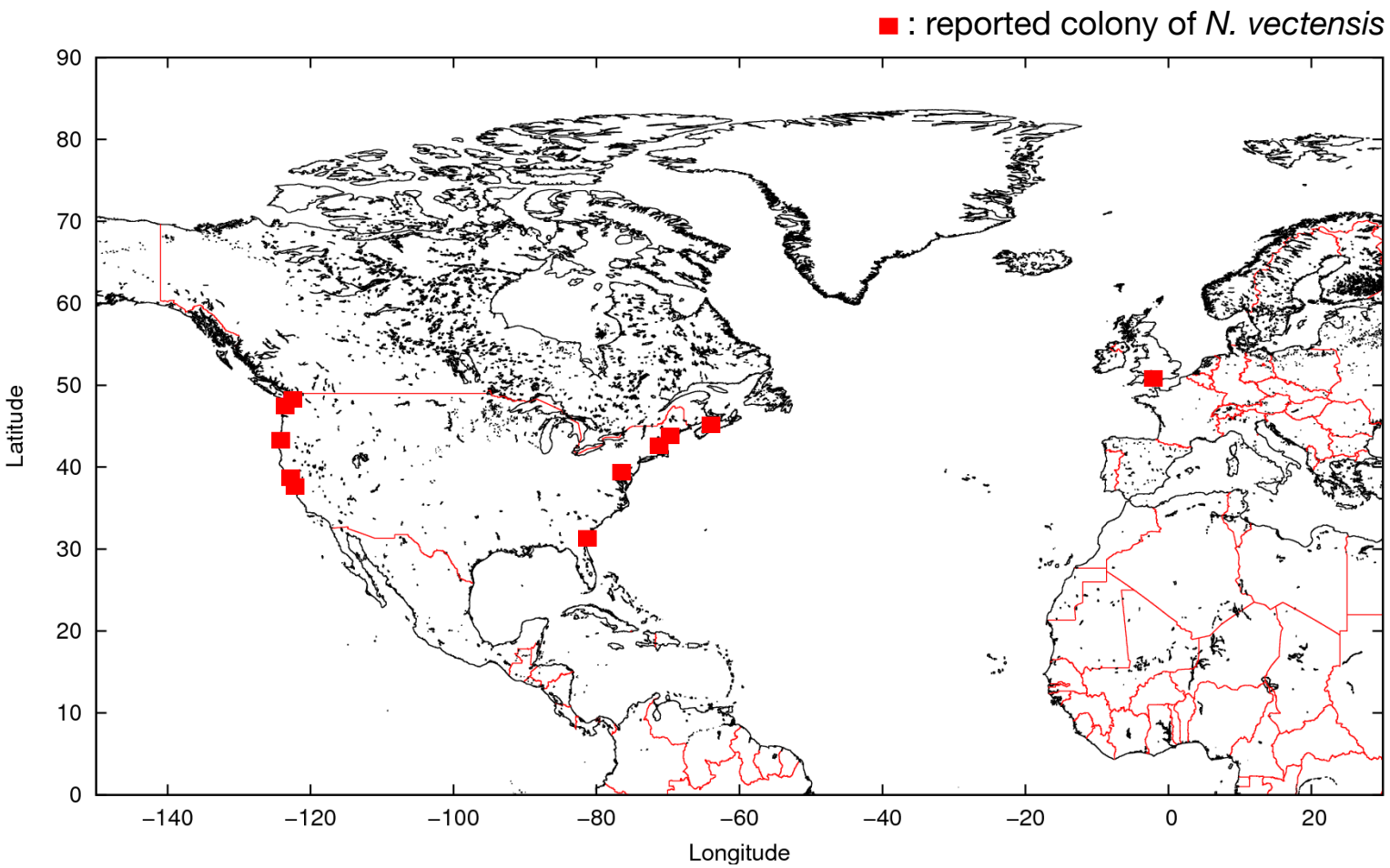


Nematostella siRNAs

Adult males, oxidized:



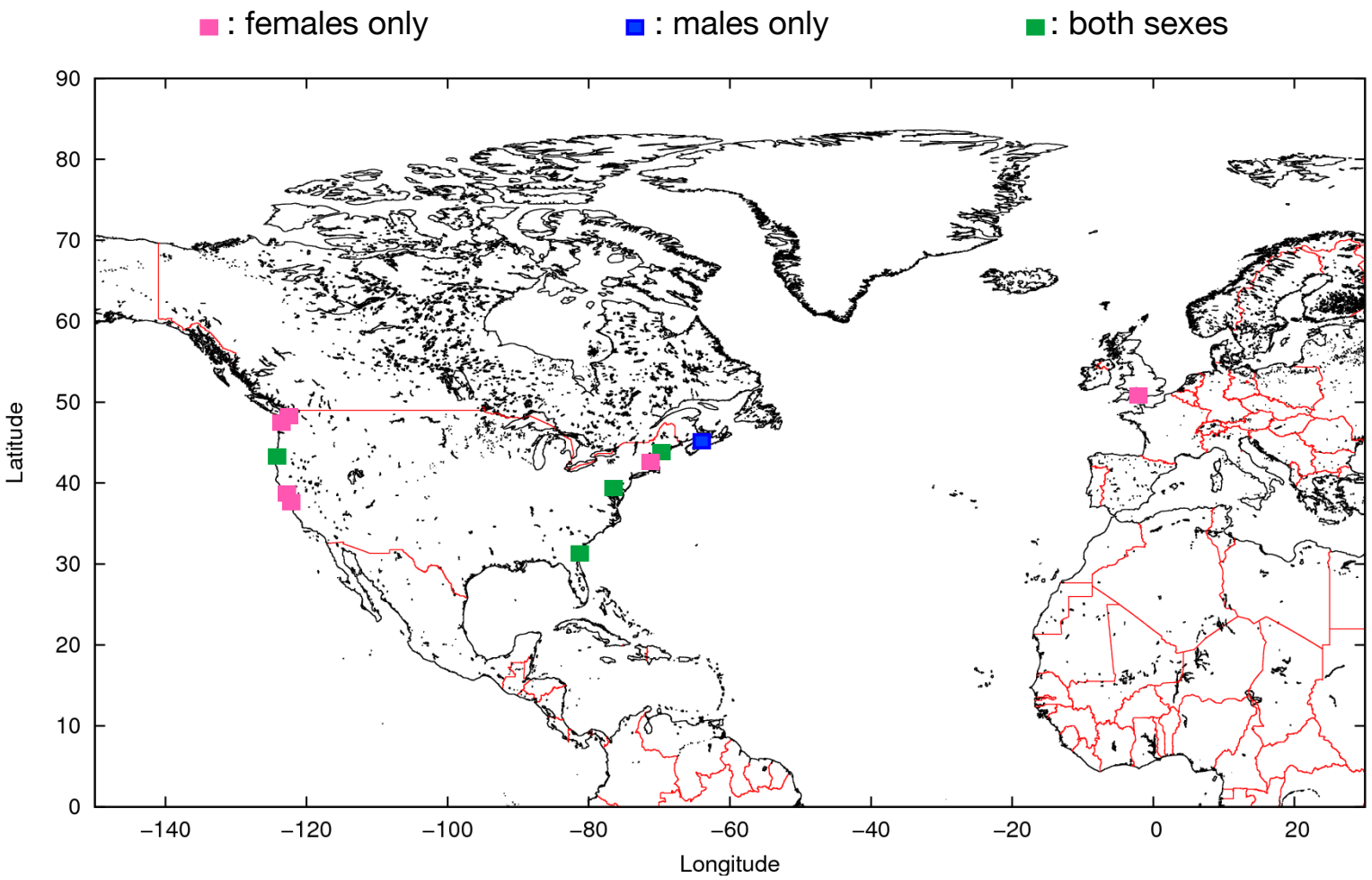
Conclusion



data from Hand and Uhlinger (1994)

◂ Return

Conclusion



data from Hand and Uhlinger (1994)

◂ Return