

Evolution of hotolyases in Kinetoplastid protists Howard Hughes Medical Institute

Hypothesis

We hypothesize that some of the fascinating abilities of Kinetoplastida, including their unique mDNA organisation and RNA modification mechanisms, can be explained by an evolutionary scenario that involves UV as a strong selection factor. That predicts positive selection being present on the photolyase sequences, which can be tested using bioinformatic algorithms.

Methods

Photolyase-encoding sequences were retrieved from TriTrypDB and EUKProt database (for Euglenids), from PhycoCosm (for Chlorophyta). Orthology was verified by building a protein phylogeny. Codon-wise alignment with MACSE was performed for the test set of 23 orthologous photolyase genes from kinetoplastids and control set of 16 photolyase genes from Chlorophytes; alignments were manually trimmed. Dn/ds was calculated using Datamonkey package.

1632



Results & conclusions



Chlorophyte photolyases dn/ds (control)

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partitions

partitions

3 classes

N/A:N/A

synonymous rate variation

0.0 0.20.40.6.8.0.2

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Update

Tested ω

Mean = 0.06127, CoV = 3.997

Update

1046

codon sites in the alignment

1621

codon sites in the alignment

non-synonymous rate variation

3 classes



EP01010 Urceolus cornutus P002560 1-453 EP00759 Apiculatamorpha spiralis P047414 1-514 EP00759 Apiculatamorpha spiralis P047418 1-514 EP00759 Apiculatamorpha spiralis P047419 1-514 EP01061 Rhynchopus euleeides P032070 1-122 EP01012 Entosiphon sulcatum P057300 1-399 EP01013 Petalomonas cantuscygni P043737 1-612 EP01079 Euglenida sp SAG-EU17-18 P003235 1-558 EP00667 Euglena gracilis P005117 1-609

EP01065 Artemidia motanka P009491 1-459 EP01065 Artemidia motanka P009492 1-317 EP01062 Namystynia karyoxenos P047519 1-368 EP01062 Namystynia karyoxenos P047518 1-500 EP00669 Euglena mutabilis P000618 1-159 EP00669 Euglena mutabilis P003809 1-242 EP00667 Euglena gracilis P030965 1-129 EP00667 Euglena gracilis P006230 1-559 EP00756 Hemistasia phaeocysticola P003073 1-486 EP01063 Lacrimia lanifica P001176 1-348 EP01063 Lacrimia lanifica P001177 1-344 EP00755 Sulcionema specki P034618 1-531 EP00906 Rhabdomonas costata P013808 1-555 EP00996 Jenningsia fusiforme P001827 1-341 EP01006 Ploeotia vitrea P019562 1-560 EP00667 Euglena gracilis P007566 1-443 EP01004 Peranema trichophorum P009353 1-188 EP01004 Peranema trichophorum P002961 1-513 EP00670 Eutreptiella braarudii P011835 1-671 EP00669 Euglena mutabilis P008424 1-250 EP00667 Euglena gracilis P007028 1-529 EP00668 Euglena longa P017138 1-533 EP00668 Euglena longa P017139 1-533 EP00659 Diplonema papillatum P012231 1-589 EP01063 Lacrimia Ianifica P018553 1-611 EP00755 Sulcionema specki P024470 1-304 EP00667 Euglena gracilis P004345 1-586 EP00669 Euglena mutabilis P013467 1-575 EP00667 Euglena gracilis P032454 1-131 EP00667 Euglena gracilis P017323 1-304 EP00759 Apiculatamorpha spiralis P027976 1-520 EP00906 Rhabdomonas costata P001454 1-226 P00906 Rhabdomonas costata P001456 1-498 EP01063 Lacrimia lanifica P020413 1-549 EP00996 Jenningsia fusiforme P007189 1-532 EP00756 Hemistasia phaeocysticola P022951 1-537 EP01013 Petalomonas cantuscygni P003445 1-529 EP00906 Rhabdomonas costata P009816 1-206 EP00760 Papus ankaliazontas P014172 1-504

EP00754 Rhynchopus humris P020700 1-531 EP01063 Lacrimia lanifica P018491 1-535 EP01061 Rhynchopus euleeides P037928 1-519 EP00659 Diplonema papillatum P003872 1-481 EP01059 Diplonema ambulator P008050 1-445 EP01001 Neometanema parovale P009050 1-431 EP01013 Petalomonas cantuscygni P005647 1-465 EP01013 Petalomonas cantuscygni P025481 1-317 EP01013 Petalomonas cantuscygni P036024 1-475 EP00998 Keelungia sp KM082 P007727 1-279 EP00659 Diplonema papillatum P011460 1-538 EP00758 Cryptobia borreli P009757 1-466 EP00758 Cryptobia borreli P009758 1-466 P00667 Euglena gracilis P016791 1-287 EP00667 Euglena gracilis P004696 1-628 EP00670 Eutreptiella braarudii P020567 1-98





Automated quantification of DNA content in nuclei and mitochondria of

Kinetoplastid protists Vsevolod Kuksin (image analysis), Sofiia Porkhun, Sevinch Zaripova (image acquisition), Borys Olifirov (primary script for image analysis), Daryna Zavadska (slide stainings)

Methods

DNA of kinetoplastids was stained by DAPI and DRAQ5. The preferential binding of DAPI with AT pairs and the fact that kDNA is usually AT-rich allowed to differentiate kDNA from nuclear DNA based on the fluorescence intensity of both structures in DAPI channel. Python script was used for automated quantification of nuclear and kDNA content.



Results & conclusions





The script is capable of automatically detecting cells based on the brightfield image; differentiating 3 different species of kinetoplastids (Cruzella marina and 2 distantly related isolates from *Neobodo designis* species complex) based on the estimated cell volume and DNA content; The preliminary estimates from a few draft microphotographs suggest that the amount of mt and nuclear DNA is lower in 2 representatives of *Neobodo* designis species complex if compared to Cruzella *marina*; moreover, the ratio of nuclear DNA content to mtDNA content consistently differs across species.