Alternative splicing of β-carbonic anhydrase genes and implications for the evolution of C_4 photosynthesis in the grass subtribe Neurachninae

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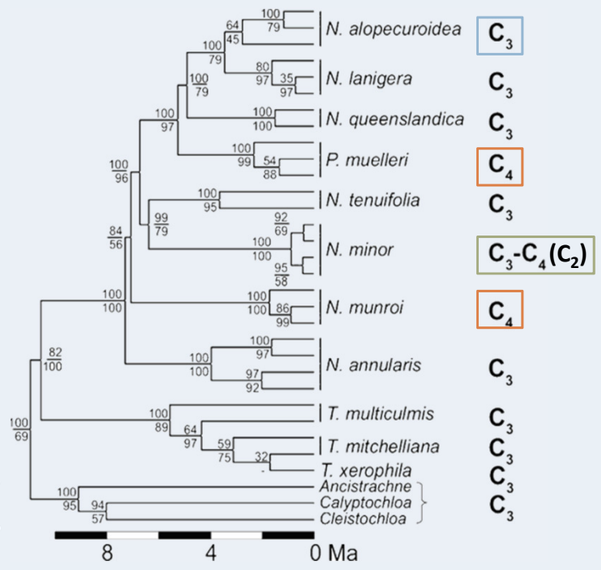
BACKGROUND

Neurachninae

- Subtribe of Australian native grasses
- Three genera: *Neurachne*, *Paraneurachne* and *Thyridolepis*
- Among grasses, this is an ideal lineage for studying key steps in molecular evolution of C_4 photosynthesis
- Molecular phylogeny suggests two C_4 origins, one C_2 origin
- Four species selected for study: *Neurachne alopecuroidea* (C_3), *N. minor* (C_2), *N. munroi* (C_4), *and Paraneurachne muelleri* (C_4)



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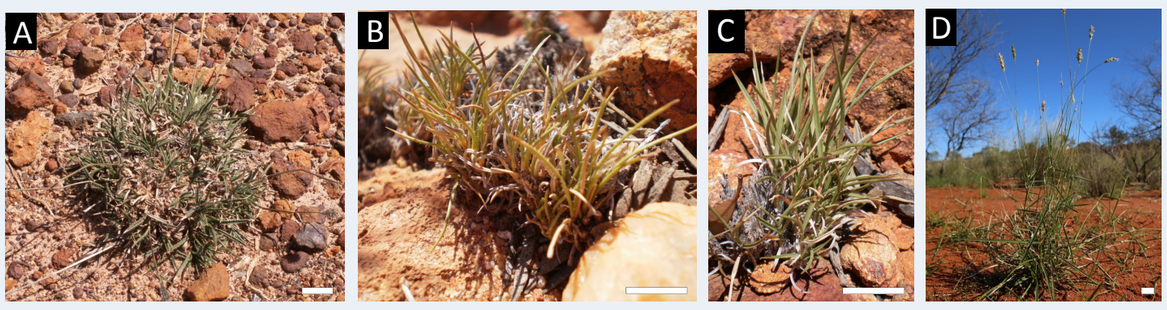


Photographs of Neurachninae plants growing at collection sites in Western Australia. **A)** *N. alopecuroidea*, C_3 **B)** *N. minor*, C_2 **C)** *N. munroi*, C_4 **D)** *P. muelleri*, C_4, photograph by Rowan Sage. Scale bar ≈ 2 cm

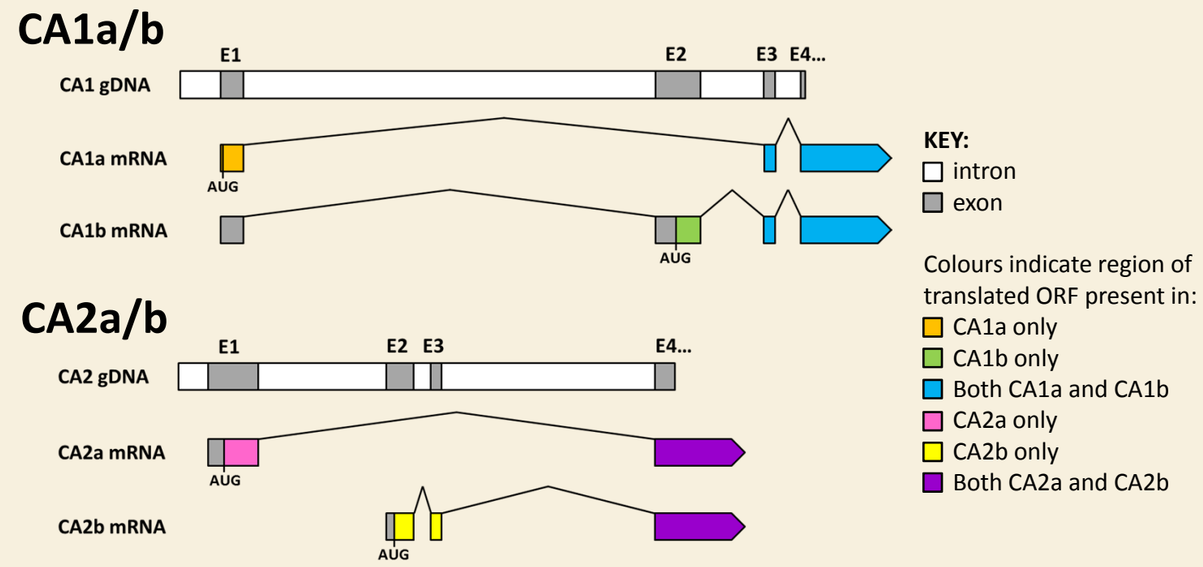
β-carbonic anhydrase (CA)

- Catalyses : $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$
- Small gene family
- Many roles in plants
- C_4 plants – provides HCO_3^- to PEPC in the mesophyll cell cytosol

RESULTS/DISCUSSION

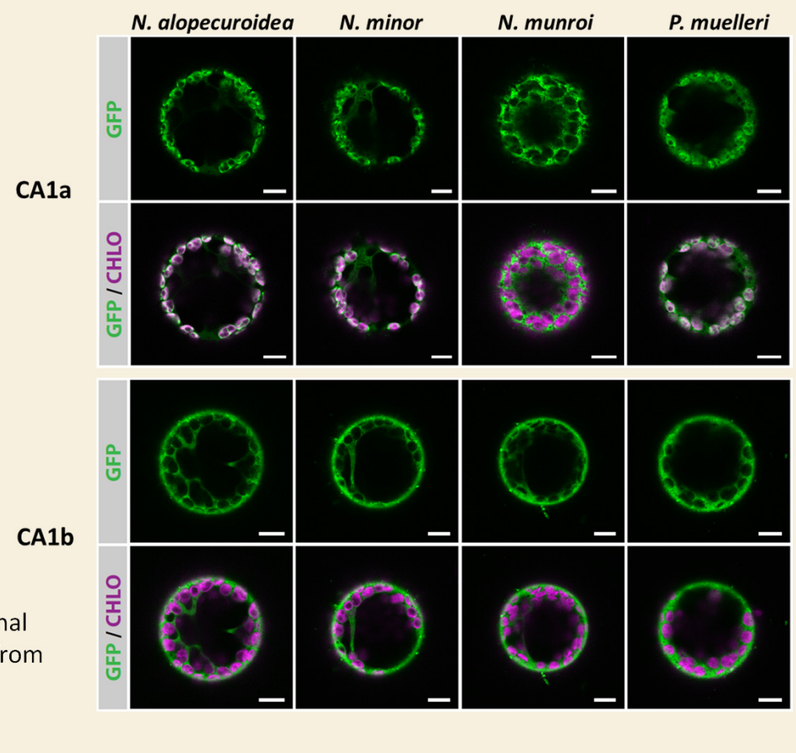
Alternative splicing of β-CA transcripts in Neurachninae

- Identified mRNAs encoding four distinct β-CA isoforms (CA1a, CA1b, CA2a, CA2b) from *N. alopecuroidea*, *N. minor*, *N. munroi*, and *P. muelleri*
- Noted identity in 3'-region of transcripts
- Sequence data from genomic DNA fragments showed the four transcripts are generated from only two genes via alternative splicing
- First experimental evidence of alternative splicing of plant β-CA transcripts



Protein products of alternative splice forms show different subcellular locations

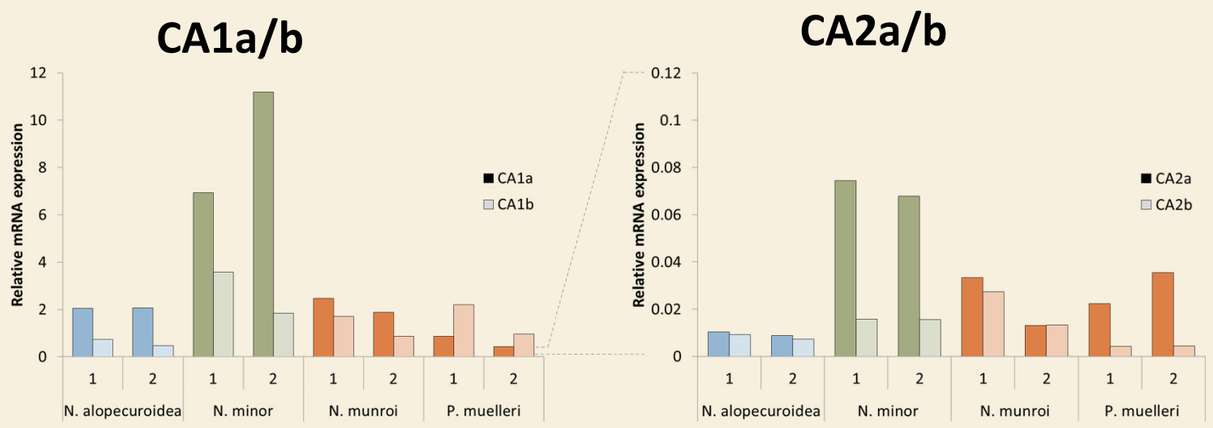
- GFP fusion constructs show Neurachninae CA1b is targeted to the cytosol whereas CA1a shows species-specific localisation
- CA1a from *N. alopecuroidea* (C_3), *N. minor* (C_2), and *P. muelleri* (C_4) is targeted to the chloroplast
- CA1a from *N. munroi* (C_4) is cytosolic
- Multiple sequence alignment indicates 11 amino acids in N-terminal region of the CA1a isoforms are not present in *N. munroi* CA1a → non-functional chloroplast transit peptide



GFP = green fluorescent protein signal from fusion protein, **CHLO** = signal from chlorophyll autofluorescence

Differential expression of splice forms at the transcript level

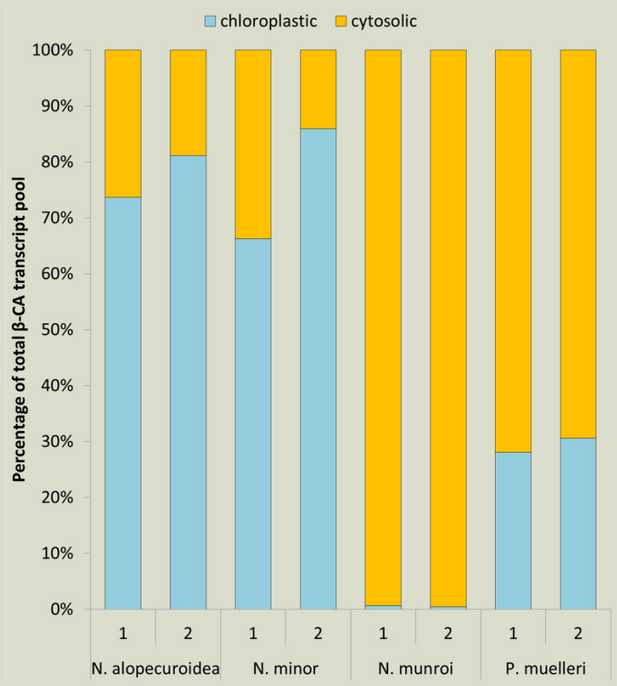
- Transcripts of CA1a and CA1b at least ten times more abundant than CA2a or CA2b transcripts in all species tested. Low expression in leaves suggests non-photosynthetic function.
- CA1a transcripts more abundant than CA1b in *N. alopecuroidea* (C_3), *N. minor* (C_2), and *N. munroi* (C_4), **but not** in *P. muelleri* (C_4)



CONCLUSION

Two molecular mechanisms for increasing abundance of mRNAs encoding cytosolic β-CAs in C_4 Neurachninae species

- C_4 species have more transcripts encoding cytosolic isoforms than chloroplastic isoforms. The opposite is the case in *N. alopecuroidea* (C_3) and *N. minor* (C_2).
- Two distinct evolutionary origins of C_4 photosynthesis in Neurachninae
  - path appears different
  - *N. munroi* – elimination of the CA1a chloroplast transit peptide
  - *P. muelleri* – increased abundance of transcripts encoding the cytosolic CA1b



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REFERENCES

Christin PA, Wallace MJ, Clayton H, Edwards EJ, Furbank RT, Hattersley PW, Sage RF, Macfarlane T, Ludwig M (2012) Multiple photosynthetic transitions, polyploidy, and lateral gene transfer in the grass subtribe Neurachninae. J Exp Bot **63:** 6297-6308