# Molecular interactions of orthologues of floral homeotic proteins from the gymnosperm Gnetum gnemon provide a clue to the evolutionary origin of 'floral quartets'.

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### Abstract

Several lines of evidence suggest that the identity of floral organs in angiosperms is specified by multimeric transcription factor complexes composed of MADS-domain proteins. These bind to specific cis-regulatory elements ('CArG-boxes') of their target genes involving DNA-loop formation, thus constituting 'floral quartets'. Gymnosperms, angiosperms' closest relatives, contain orthologues of floral homeotic genes, but when and how the interactions constituting floral quartets were established during evolution has remained unknown. We have comprehensively studied the dimerization and DNA-binding of several classes of MADS-domain proteins from the gymnosperm Gnetum gnemon. Determination of protein-protein and protein-DNA interactions by yeast two-hybrid, in vitro pull-down and electrophoretic mobility shift assays revealed complex patterns of homo- and heterodimerization among orthologues of floral homeotic class B, class C and class E proteins and B(sister) proteins. Using DNase I footprint assays we demonstrate that both orthologues of class B with C proteins, and orthologues of class C proteins alone, but not orthologues of class B proteins alone can loop DNA in floral quartet-like complexes. This is in contrast to class B and class C proteins from angiosperms, which require other factors such as class E floral homeotic proteins to 'glue' them together in multimeric complexes. Our findings suggest that the evolutionary origin of floral quartet formation is based on the interaction of different DNA-bound homodimers, does not depend on class E proteins, and predates the origin of angiosperms.

# Identifier

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