

Population study

Sanz M. et al. (2014): Southern isolation and northern long-distance dispersal shaped the phylogeography of the widespread, but highly disjunct, European high mountain plant *Artemisia eriantha* (Asteraceae). *Botanical Journal of the Linnean Society* 174: 214–226.



Reason for the study

- explore range dynamics in European Mountain System
- contribution of old vicariance and recent long-distance dispersal (LDD) to range formation of highly disjunct species
 - vicariance – reciprocally monophyletic lineages
 - recent LDD – closely related to their source, certain degree of nestedness

Study species

- rare herbaceous perennial mountain plant
- small isolated populations
- at elevations between 1500 and 3400 m
- wind pollinated, self-incompatible?
- diploid
- morphologically and cytologically homogenous

- 17 populations, 100 individuals
- 5-10 plants/population

Methods

- AFLP – 3 selective primer combinations (12 tested)
 - $f_{\text{tot}}, f_{\text{poly}}, f_{\text{pp}}, f_{\text{fp}}$
 - NJ, 1000 bootstrap replicates (TreeCon)
 - NeighbourNet (SplitsTree)
 - BAPS2, STRUCTURE (+ Structure-Sum, CLUMPP)
 - AMOVA (Arlequin)
- plastid sequencing – 3 regions (*ycf3-trnS*, *trnG* intron, *rpl16* intron) – 2 to 4 individuals/population
 - statistical parsimony network (TCS)
 - phylogenetic tree (MrBayes + MrModelTest)
 - maximum parsimony tree (PAUP)
 - allopatric differentiation testing – approximate Bayes factor

Results

- AFLP
 - 229 fragments, 185 polymorphic, error rate 1.3%
 - Pyrenees, Alps, the Balkans – highest f_{pp}
 - NJ+NN – the Balkans, western Pyrenees, Apennines
 - STRUCTURE – K=5 – Pyrenees, the Balkans, Galibier, Widespread, the Apennines
 - AMOVA – 58% among pops, 20.4% among mountain ranges
- cpDNA
 - 39 variable characters (including indels)
 - 14 haplotypes (out of 36 individuals)
 - 5 lineages – Alpine-Pyrenean, Widespread, Apennine, S Carpathians, the Balkan – confirmed by high BS and PP
 - hypothesis of vicariance among ranges – strongly rejected

Discussion

- historical range dynamics shaped by both vicariance and extensive LDD
- earliest phase of range expansion – westward migration from the Balkans
- subsequent isolation and diversification in different refugia
 - each peninsula – specific haplotype lineage and AFLP gene pool (southern refugia concept) – private markers, AMOVA
- not strictly allopatric – widespread lineage highly disjunct – secondary eastward (re)colonization
- several LDD events

Discussion II.

- discrepancies between AFLP and cpDNA
 - differences in evolutionary dynamics
 - plastid – readily fixed in isolated and small populations
 - AFLP – gene flow, replacement by immigrants
- Alpine population 5
 - separate AFLP cluster, high f_{poly} and f_{fp}
 - introgression by *A. genipi* (evidence from morphology and genome size data)

Systematic study

Renner S.S. (2004): A chloroplast phylogeny of *Arisaema* (Araceae) illustrates Tertiary floristic links between Asia, North America, and East Africa.
American Journal of Botany 91(6): 881–888



Reasons for the study

- testing the monophylly of *Arisaema*
- better understand relationships of Asian species to African and North America species
- gain insight into the evolutionary plasticity of sex change strategy
- congruence of molecular data with leaf and inflorescence morphology

Study species

- 150 species, 5-6 in Africa, 3 in North America
- interannual „sex change“ – sex determined by environment, reversals possible
- *A. flavum* subsp. *flavum* and *A.f.* subsp. *abbreviatum* – non-sex-changing, tetraploids

Methods

- 81 accessions of *Arisaema*, 5 of *Pinellia*, 9 of *Typhonium*
- cpDNA sequencing – *trnL-trnF*, *trnL* intron, *rpl20-rps12*
- phylogenetic analysis – MP (PAUP, 200 bootstrap replicates), ML (GTR+G+I model), BI (GTR+G) – MrBayes
- molecular clock dating – likelihood ratio test (LRT)
 - no clock rejection → use of ultrametric (clock-enforced) tree
 - cross-validated *penalized likelihood* (software r8s) – different substitution rates between ancestral and descendent branches, penalty – increases with the abruptness of rate change between adjacent branches
 - *A. triphyllum* infrutescence fossil – 18-16 million years ago

Results

- concatenated sequences – 2048 nucleotides, 274 eliminated (poly-T runs or TA tandem array)
- *trnL*-F spacer – too little signal but many species group according to section (Fig. 2)
- combined *trnL*-F and *rpl20-rps12* spacer (Fig. 3)
 - strong support for *Arisaema* monophyly
 - limited support for intrageneric relationships
 - section *Tortuosa* is polyphyletic
- addition of *trnL* intron – greatly improved support, North American species do not form a clade

Dating results

- LRT – no rejection of clock model
 - data could be modeled clocklike
 - *A. triphyllum*-*A. amurense* (18 my old – calibration)
 - Asian *A. costatum* vs African *A. schimperianum* (31±10)
 - Asian *A. heterophyllum* and N American *A. dracontium* (49±12)
- penalized likelihood (*A. triph.*-*A. amur.* min 18 my; root of the genus max 60 my)
 - Asian *A. costatum* vs African *A. schimperianum* (19)
 - Asian *A. heteroph.* and N American *A. drac.* (32)
 - *A. flavum* subsp. *flavum* from *A.f.* tetraploids – 13/8 my

Discussion

- *Arisaema* is monophyletic
- sex change evolved once in the common ancestor
- reversion from sex-change to bisexuals only – *A. flavum* subsp. *flavum*, *A.f.* subsp. *abbreviatum* – due to spread into drier habitats?
- relationships to *Pinellia* and *Typhonium* remain unresolved (trichotomy)
- molecular data support newly circumscribed sections (but without statistical support)
- sect. *Arisaema* – morphologically and geographically coherent
 - close ties between some African (*A. mildbraedii*, *A. schimperianum*) and Asian species (*A. costatum*)

Discussion II.

- age estimates – influenced by taxon sampling (lowest in India and Africa)
- three American species in three different Asian clades
 - support for repeated Beringia crossing (similar pattern reported in *Magnolia*, *Trillium* etc.)
- future – addition of sequences from fast-evolving nuclear gene
 - ITS – problematic – multiple paralogous copies – result of slow concerted evolution