Population study

Kameyama Y. et al. (2001): Patterns and levels of gene flow in *Rhododendron metternichii* var. *hondoense* revealed by microsatellite analysis. *Molecular Ecology* 10:205-216

Reasons for the study

- detect pattern of gene flow within and among three rhododendron populations
- detect effect of tree height and distance to the relative fertility of adult trees
- detect pattern of pollen flow within populations

- direct estimation of gene flow – *parentage analysis*

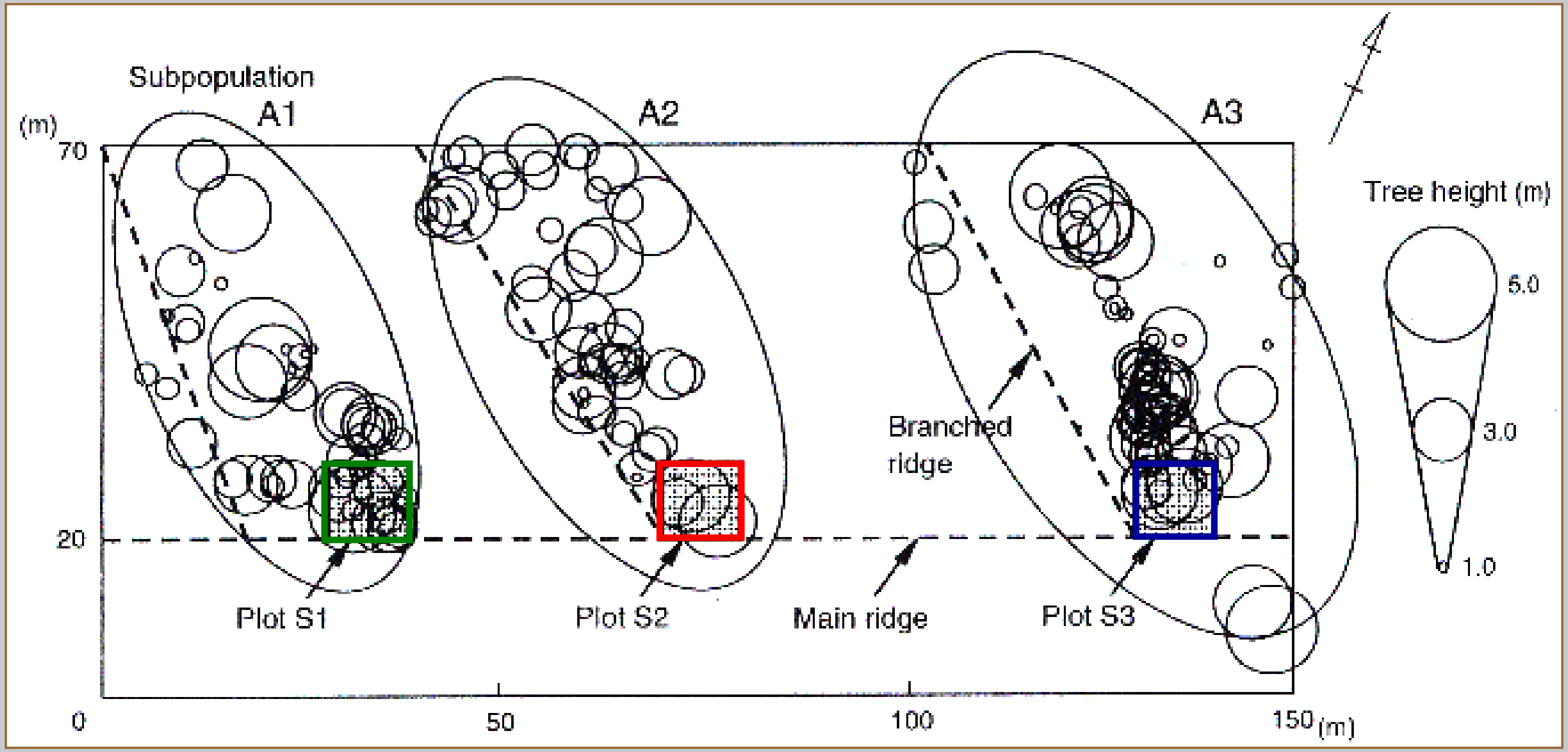
Study species

Rhododendron metternichii var. *hondoense*

- evergreen shrub up to 5m height
- montan region of western Japan
- pollinated mainly by bumble bees

Study populations

- 3 subpopulations (A1, A2, A3)
  - 49, 50 and 75 adult trees
- plot 10×10 m in each population (S1, S2, S3)
  - 70 randomly chosen seedlings from each plot



- 12 microsatellite loci

Parentage analysis

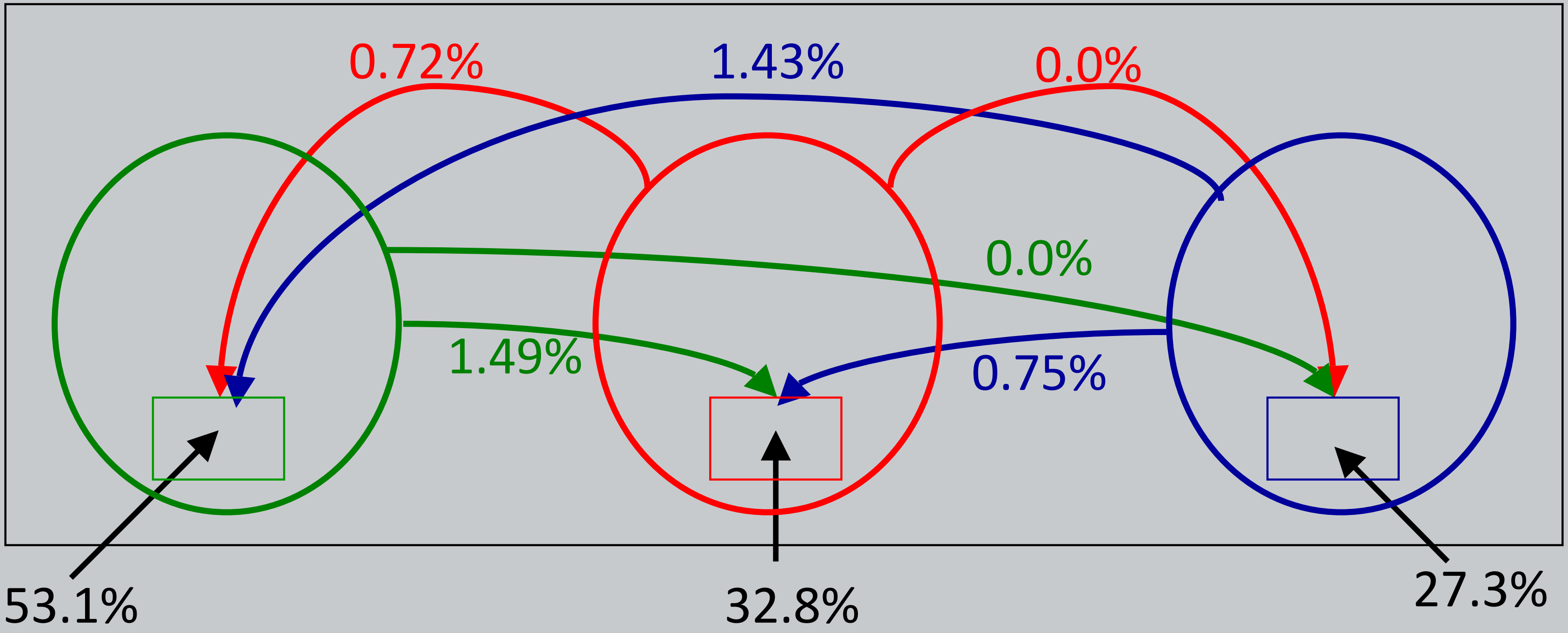
- genotype comparison of seedlings and all possible adult pairs – software CERVUS
- *exact matches* – combination of only two adult haplotypes could explain seedling genotype (× *multiple matches*)
- *null alleles* – homozygotes in loci with high frequency treated as heterozygotes for null alleles
- *cryptic gene flow* – the probability that an adult tree within the quadrat matched an unrelated seedling

Results

- 12 microsatelite markers – 6-33 alleles
- *multiple matches* – 6 seedlings in S1, 3 in S2, 4 in S3 – eliminated from further analyses
- number of seedlings produced by self-pollination – 2 in S1, 4 in S2, 4 in S3

Gene flow among populations

- number of parents within subpopulation (one, both)
- number of parents outside subpopulation (one, both)
- gene flow to the population – from another populations

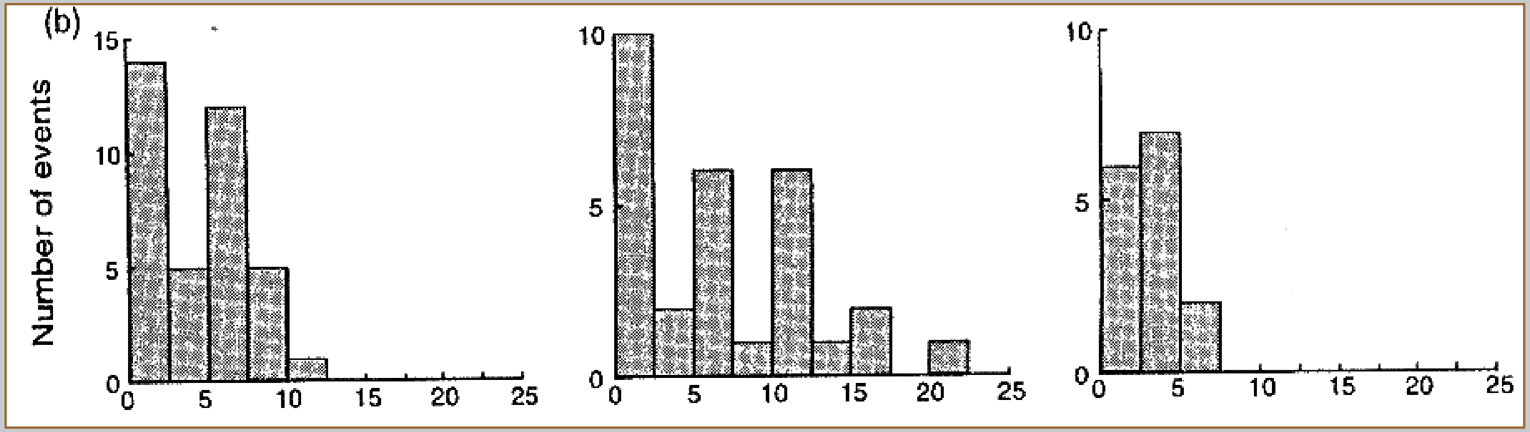


Effect of distance and tree height

- trees adjacent to the seedlings produced more seedlings (*Fig.2*)
- *long-distance gene flow* in population S2
- in A2 and A3 higher trees produced more seedlings (*Fig.3*)
- A1: 9 parental trees adjacent to seedlings
- A2: parental trees at relatively greater distances from seedlings
- A3: 13 parental trees, 4 higher than 3m produced 10 and more seedlings

Pollen flow in subpopulations

- estimated from positions of parental pairs (*Fig.5b*)



- distance is significantly greater than distance to the nearest neighbour (*Fig.5a*)
- … and significantly smaller than distance to the random neighbour (*Fig.5c*)

Discussion

- adult trees <5m from the seedlings produced extremely large amount of seeds
- no trees >25m produced seedlings
- tree height has an effect on the seed production if there are trees with different height adjacent to seedlings (*Fig.4c*)
- gene flow within subpopulation vary depending on population structure
- relative higher *pollen flow* in A2 due to low number of adults trees adjacent to the seedlings

Discussion II.

- why is gene flow within populations highly restricted but gene flow into the study area reaches 50%?
  - differences in phenology – asynchronous flowering
  - preference of pollen from further distance? *inbreeding depression*?
- why is gene flow among subpopulations extremely low (0-2%) but gene flow from neighbouring area is high?
  - ridges among subpopulations serve as barriers for seed dispersal?
  - pollinators preferentially moving along subpopulations?

Systematic study

Provan J. et al. (1999): Polymorphic chloroplast simple sequence repeat primers for systematic and population studies in the genus *Hordeum*. *Molecular Ecology* 8:505-511

Reasons for the study

- to design primers amplifying microsatellite regions
- to use the microsatellite polymorphism for study of relationships in the genus *Hordeum*

Material for the study

- 31 samples from 24 „wild“*Hordeum* species
- 51 samples *H*. *vulgare* ssp. *spontaneum*
- 125 samples *H*. *vulgare* ssp. *vulgare* („landraces“)
- 101 samples *H*. *vulgare* ssp. *vulgare* („cultivars“)

- diploids, tetraploids, hexaploids

Methods

- looking for mononucleotide repeats
    - searching in EMBL database (8 bp and more)
- primer design (software PRIMER)
- PCR amplification
- electrophoresis – denaturing polyacrylamide gel
- radioactive visualization of fragments

Evaluation

- cpSSR haplotypes definition
    - combination of allele presences
- genetic distance among individuals
    - proportion of shared alleles among cpSSR haplotypes
    - software MICROSAT
- neighbour-joining tree

Results

- design of seven primer pairs
- in total 25 haplotypes found (using combination of all loci)
- *Hordeum* spp.
  - 21 from 31 samples have unique haplotype
- *H. vulgare* ssp. *spontaneum*
  - 11 haplotypes found (from 51 samples)
  - 5 polymorphic loci – 2-3 alleles each

Results

- *H*. *vulgare* ssp. *vulgare* („landraces“)
  - only one polymorphic locus
  - 2 haplotypes (shared with *H*. *spontaneum*)
  - 1 haplotype in all European cultivars
  - bottleneck due to domestication

Phylogenetic relationships

- major difference between wild species and the other (*H*. ssp. *vulgare* and *spontaneum*) - *Fig.1*
- within *Hordeum* spp. – geographical pattern (Africa & Europe, America & Asie)
- *H*. *flexuosum*, *erectifolium*, *euclaston*, *stenostachys* – same haplotype × morphological differences ?
- different ploidy levels of *H. brachyantherum* – highly differing haplotypes – maybe an evidence of reticulate evolution and multiple cytoplasmic introgression
- contrarwise – 4n and 6n *H*. *murinum* ssp. *leporinum* – the same haplotype

Conclusions

- microsatellites have higher level of variation compared with RFLP
- ability to quantify intraspecific variability in chloroplast genome