Population study

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 ($\times$ *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 microsatellite 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

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53.1%  32.8%  27.3%

0.72%  1.43%  0.0%

1.49%  0.0%  0.75%
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

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