

Conservation and the threat of hybridization and introgression: *Populus nigra* and *Taxus baccata* in Central Europe

Berthold Heinze

Federal Research Centre for Forests
(BFW), Department of Genetics,
Hauptstrasse 7, A-1140 Vienna



AUSTRIA - EUROPE



Plantations can have an impact on native tree species

- replacement:
- direct loss of habitat (forests) for plantations
- genetic effects:
- hybridization and interbreeding
- genetic "swamping"
- changing the gene pool



Examples from Europe

- black poplar - *Populus nigra* L.
- karakavak
-
- common yew - *Taxus baccata*
- porsuk ağacı

- Experience and research needs

Populus nigra

- a floodplain forest species
- depends on “wild” big rivers
- pioneer on open soil
- old paintings show the former landscape
- old maps show the meandering of rivers in past times





Old trees are now rare in floodplain forests



Seedlings germinate on river margins

"pioneer species" that colonizes raw soil



often restricted to secondary, disturbed sites



threats for black poplar

- affected by cultivation of clones
- "hybrid poplars"
- in great numbers
- has become an endangered species



Hybrid poplars and their genetic effects on native poplars

- hybrids from crossing different poplar species
- clones in Central Europe:
- *P. deltoides* x *P. nigra* = *P. x canadensis*
- balsam poplars (sect. *Tacamahaca*) and hybrids
- Lombardy poplar – columnar male clone of *P. nigra*
 - possibly originating in Western or Central Asia
- cultivated in clonal blocks, rows, ...



Hybrid poplars and their genetic effects on native poplars

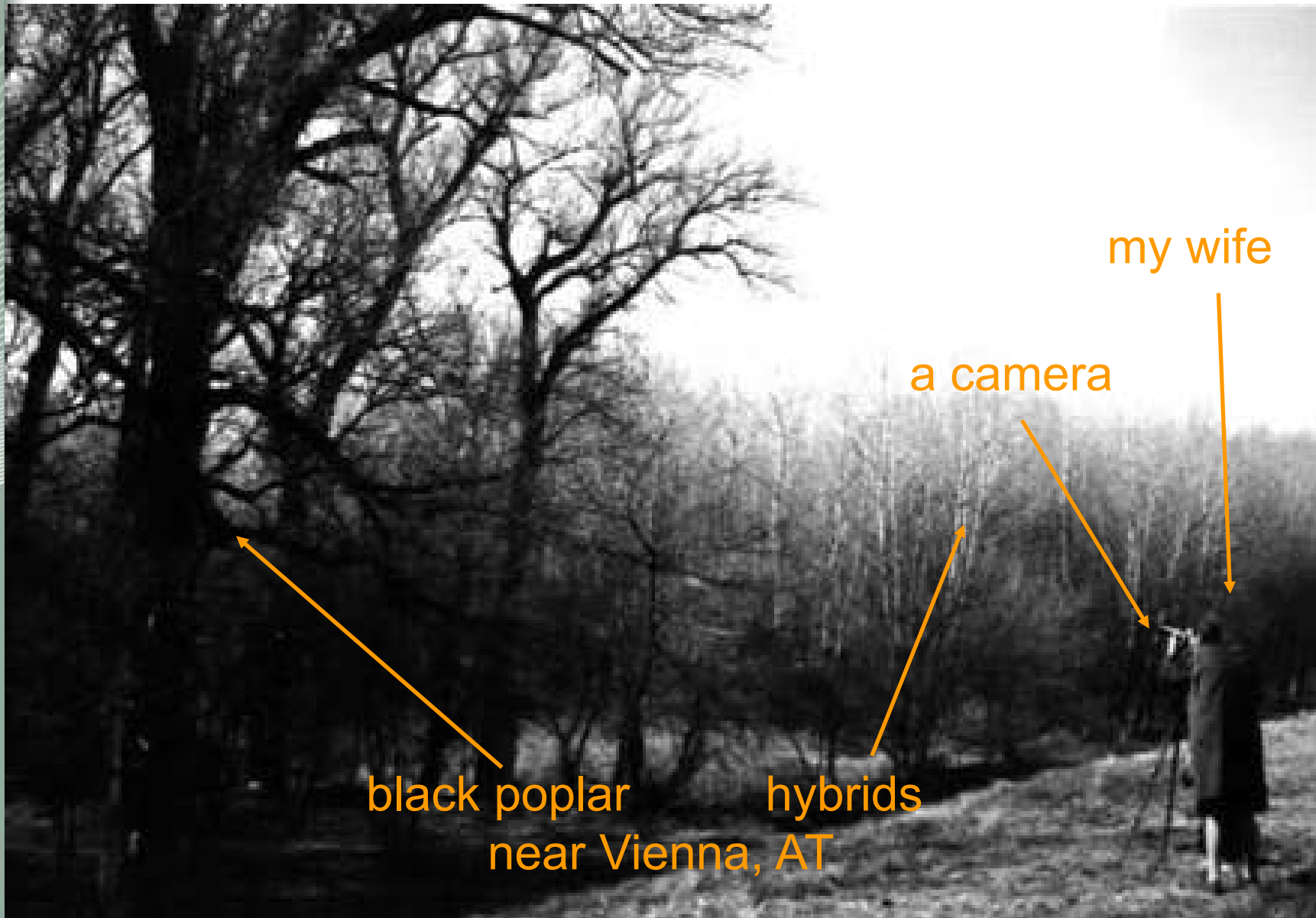
- genetic cross-breeding with *P. nigra* expected – "introgression"
- may have bad effect on native species:
- introduction of genes with negative effect on fitness
- "swamping" with identical genes (narrow gene pool of hybrids)
- inbreeding in further generations



**Remaining
specimen
closely mixed
with
cultivated
clones**



Flower phenology overlaps

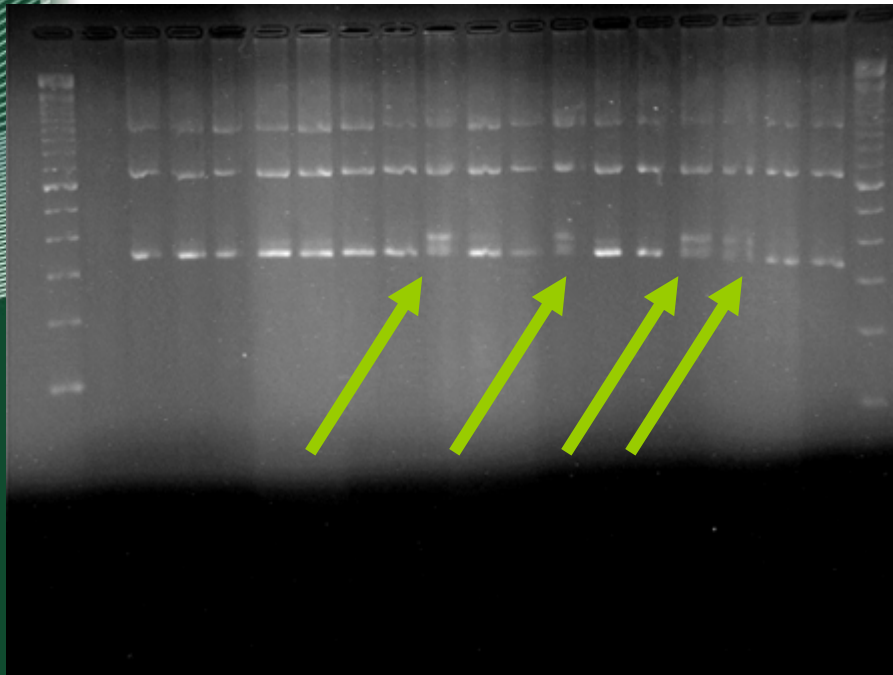


Investigations of crossing and introgression

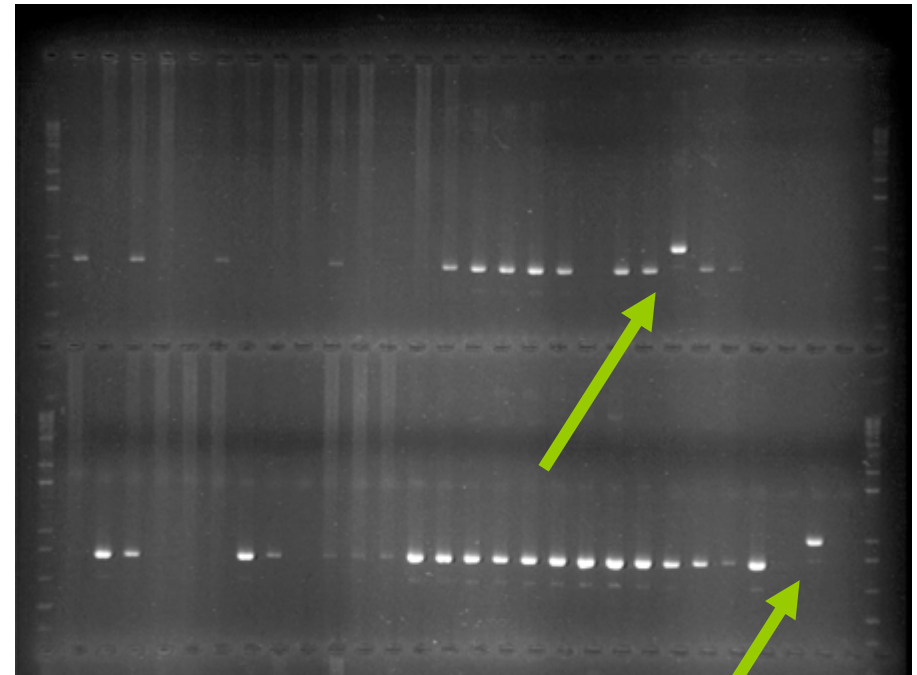
- research project in Austria 1996-1997
- seeds, seedlings and young trees were tested
- molecular genetic testing methods (DNA)
- these methods can easily detect genes of other species
- in parallel, investigations also started in other countries in Europe

Results from molecular studies

- genes of other species are sometimes present, but ...



nuclear gene PPAL



chloroplast marker trnT-trnD

introgression detectable with simple molecular markers in chloroplasts and nuclear genes

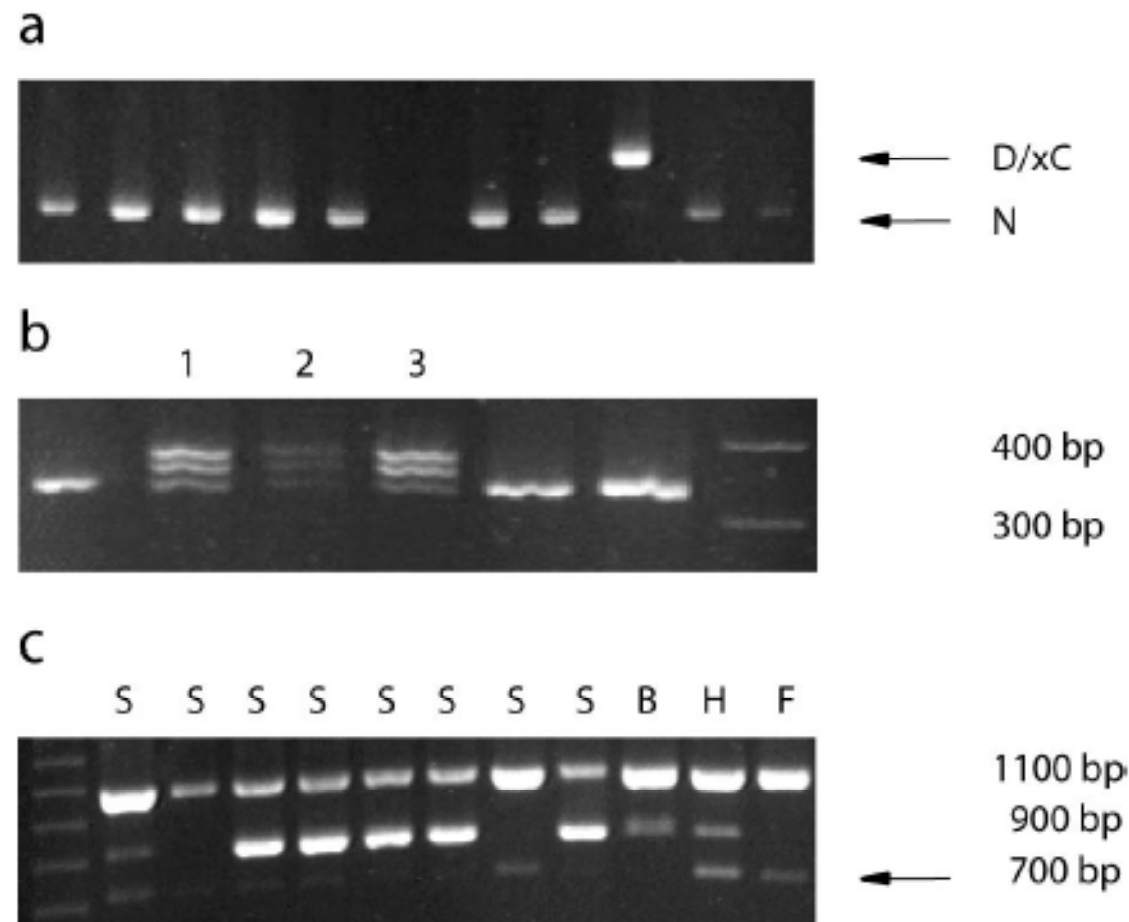


Fig. 1. – Hybrid poplar genes introgressed into black poplar plants: (a) tree Klosterneuburg 14 with *Populus deltoides*/*P. × canadensis* chloroplast DNA type (D/xC, approx. 800 basepairs (bp)), among *P. nigra* trees (N, approx. 1000 bp). (b) Three trees (Grafenegg 4–6, lanes 1–3) with one *P. nigra* and presumably one *P. deltoides* PPAL/Hae III allele (and one heteroduplex DNA band, see Heinze 1997), among true-breeding *P. nigra*. (c) Balsam poplar POPX/MspI alleles (arrow, approx. 700 bp) introgressed in a female (F) and some seedlings (S), at Heiligenstadt. H, a *P. nigra* × *P. maximowiczii* hybrid growing close by, is the possible source of these alleles. B, *P. laurifolia* × *P. nigra* hybrid 'Berolinen sis', also present at this site in large numbers, is a less likely pollen donor.

publications:

- Heinze 1997, 1998a,b
- Heinze and Lickl 2002
- Pspiskova and Salkova 2006
- Vanden Broeck et al. 2003-2006
- Heinze 2008
- Ziegenhagen et al. 2008
- ... and others across Europe and North America ...

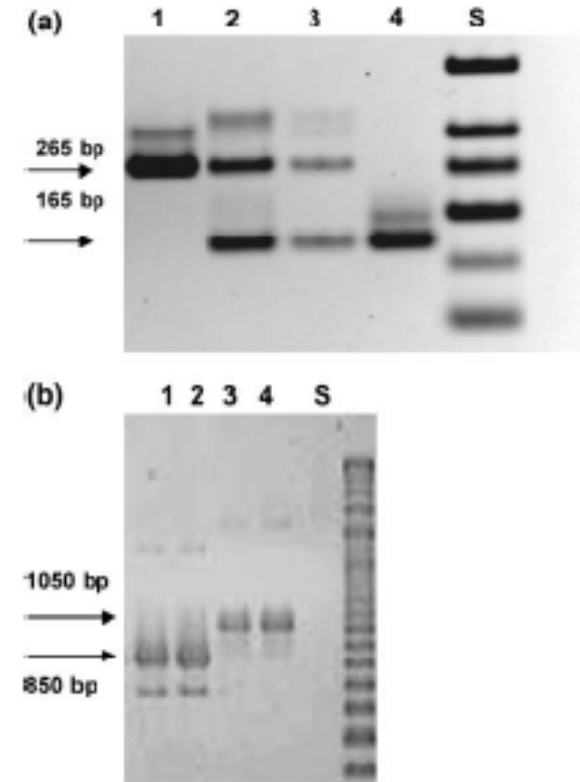
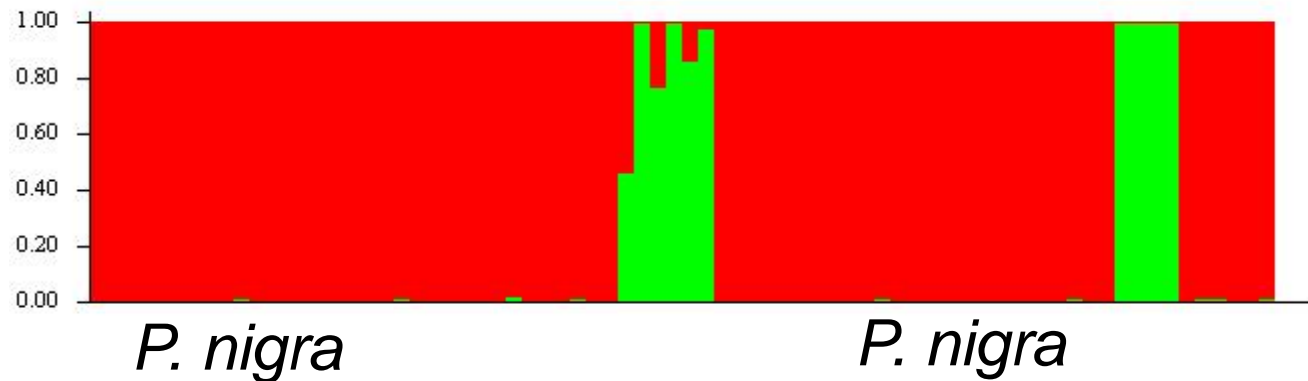


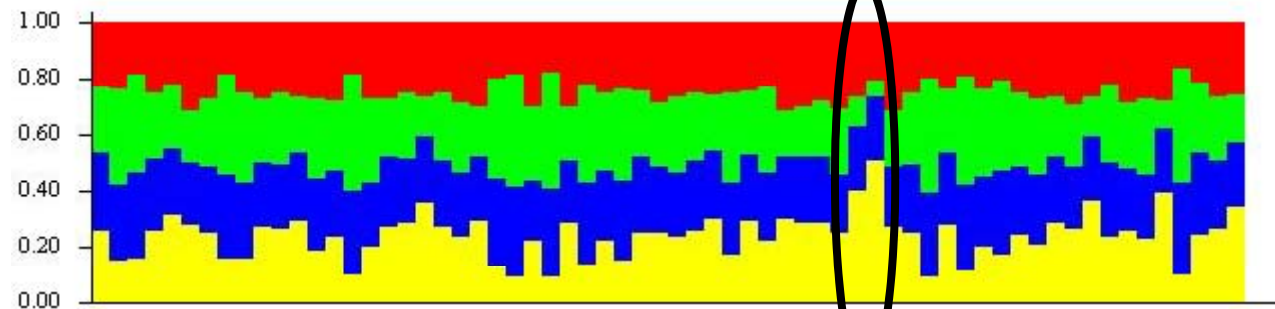
Fig. 2 Diagnostic genotypes and haplotypes. (a) Agarose gel electrophoresis at the nuclear DNA STS locus WIN3 based on poplars of the clone collection. The arrows mark the *P. nigra* diagnostic allele (165 bp) and the *P. deltoidea* diagnostic allele (265 bp). Other bands are unspecific amplifications. Lane 1: Homozygous genotype diagnostic for *P. deltoidea* (clone from the clone collection Hamoversch-Mürden); lane 2 and 3: heterozygous genotypes diagnostic for F1 *P. x canadensis* hybrids (clones from the clone collection Hamoversch-Mürden); lane 4: homozygous genotype diagnostic for *P. nigra* (clone from the clone collection Hamoversch-Mürden); S = molecular size standard (Gene Ruler 50-bp DNA Laddler, Fermentas, St. Leon-Rot, Germany). (b) Agarose gel electrophoresis at chloroplast DNA intergenic region *trnD-trnT* based on the poplars of the study site. The arrows mark the *P. nigra* diagnostic allele (850 bp) and the *P. deltoidea* diagnostic allele (1050 bp). Other bands are unspecific amplifications. Lanes 1 and 2 *P. nigra* diagnostic haplotype (Paschenwerler); lanes 3 and 4 Maternal haplotype of *P. deltoidea* in this case in a *P. x canadensis* hybrid (confirmed by the WIN3 marker, data not shown; Paschenwerler); S = molecular size standard (DNA Laddler Mix 100-5000, AppliChem, Darmstadt, Germany)

Microsatellites + STRUCTURE now confirm older results

- whole data set
- cultivated clones *P. deltooides*



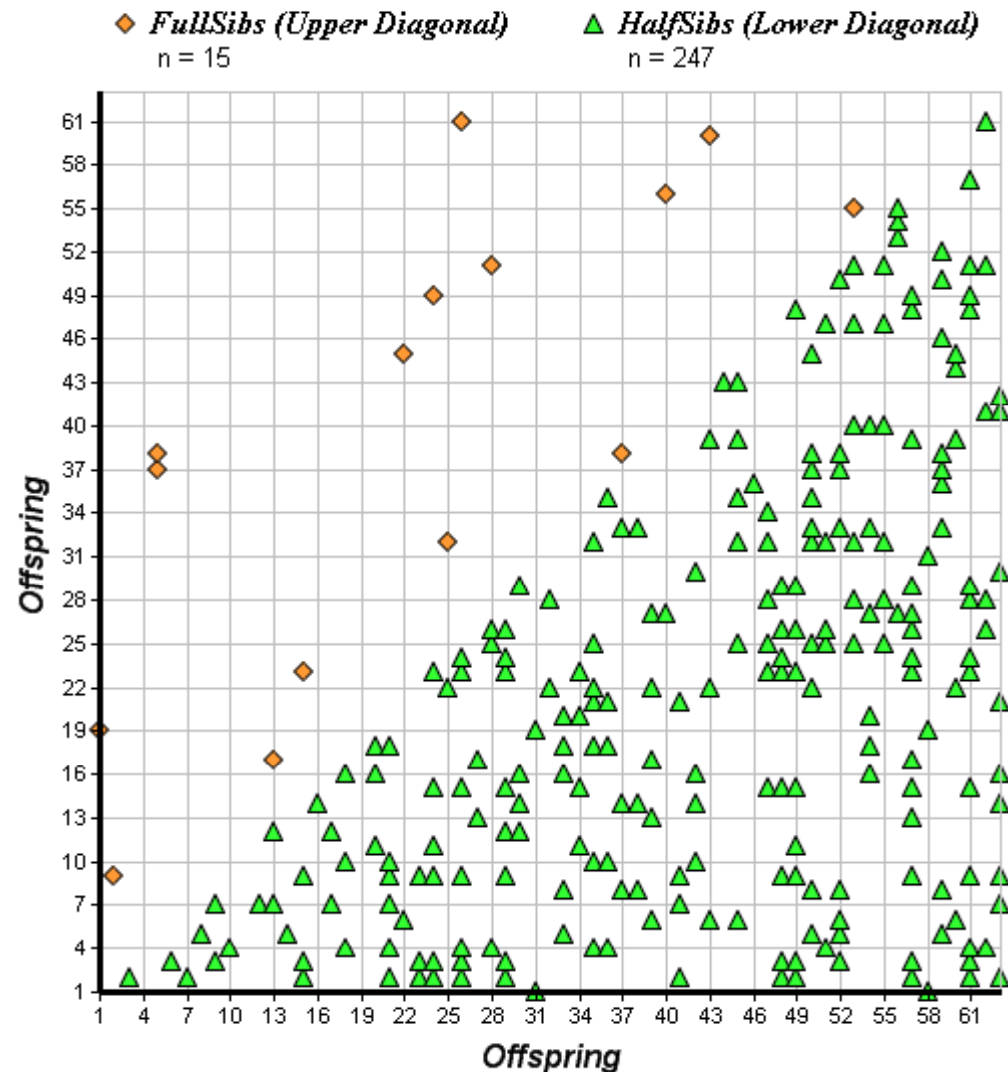
- only local *P. nigra*



two possible later generation introgressants

Identifying siblings with CERVUS and COLONY programmes

- programmes are possibly too „permissive“ for technical errors / mutations
- raw data quality is most important



Rate of introgression depends on specific circumstances

- if several male and female *P. nigra* trees are present, practically no introgression can be found in their offspring
- initial crossing barriers between species break down in hybrids
- "pollen competition" (An Vanden Broeck)
- "lonely females" may be susceptible to pollination by hybrids (G. Rathmacher)
- but female hybrid clones can produce seed & seedlings
- overall, 1-10% of seedlings may carry introgressed genes

Overall introgression ratios

- five to ten percent introgression of *Populus deltoides* (N-Am) genes into native European endangered *P. nigra* via *P. x canadensis* hybrids
- in several studies across Europe
- depending on local conditions
- female hybrid trees produce viable offspring
 - often by backcrossing to native *P. nigra*

Evolutionary consequences?

- 'lonely female trees' may get pollinated by 'alien' pollen
- seeds compete for regeneration niches with direct hybrid offspring
 - in space and time
 - bigger problem now, as National Parks are established along big rivers?
 - and advanced generation hybrids are used for cultivation?



Considerations for conserving the native gene pool

- conservation stands have to be large enough
- they must offer raw soils for regeneration
- (female) hybrids should not be present in the vicinity
- if native trees are planted, there should be many different clones (genotypes)

- a Network of experts was established 1994: EUFORGEN – European Forest Genetic Resources Programme
- for coordination and exchange of experience



Taxus baccata

- occurs as understory in e.g. beech forests
- tolerates shade
- low competitiveness
- toxic

- native stands are rare in Europe
- reasons:
 - past overexploitation
 - for archery bows in the Middle Ages
 - heavy browsing by deer
 - rareness of suitable dark forests for establishment of seedlings



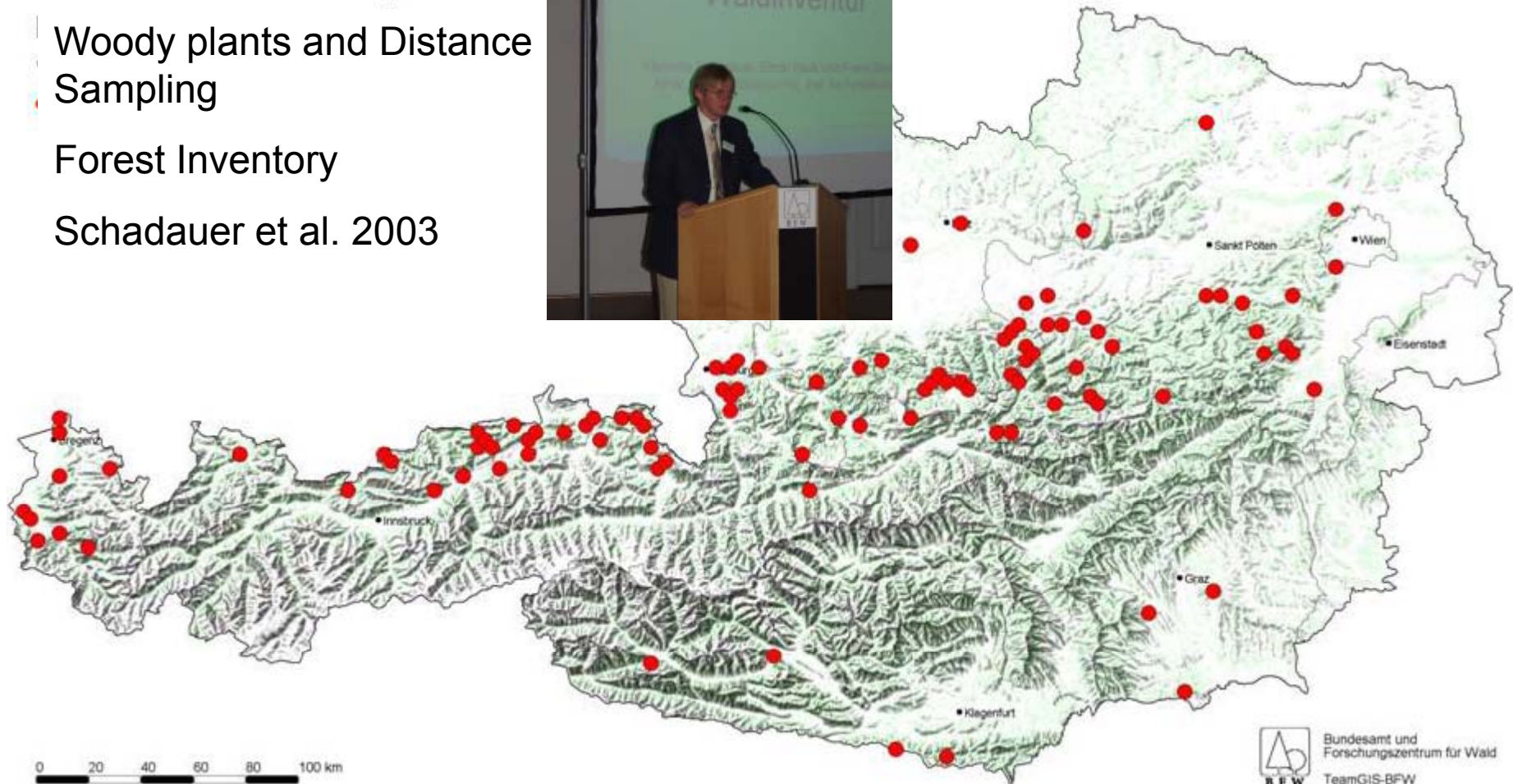


Yew stands in Austria

Woody plants and Distance Sampling

Forest Inventory

Schadauer et al. 2003



Bundesamt und
Forschungszentrum für Wald
TeamGIS-BFW



Department of Genetics



History of conservation measures

- one of the first tree species regarded by the nature conservation movement (1920ies, 1930ies)
- "total protection" of stands
- little success in stopping the decline
- remaining stands are often remote
 - steep slopes, rocks, canyons, ...



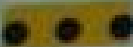
Yew in horticulture

- planted in gardens and parks
- can spread into adjacent woodlands
- pollen may spread even further
 - very light-weight
- but no investigations of this topic to date

- hybrids are possible (*T. x media*)
- is this all a threat in Europe?
- we do not know !



DEHNEPARK

-  Kernzone - Hundefrei (Hunde nur am Korridorweg!)
-  Außenzone (Hunde an der Leine!)
-  Wege als Korridor durch die Kernzone (Hunde an der Leine!)



Horticultural yew



An investigation of chloroplast DNA polymorphisms in European *Taxus*

- together with Norbert FRANK, University of West Hungary, Sopron
- amplified and analysed (also sequenced) several chloroplast fragments
- no variation detectable
- independently confirmed by Santiago Gonzalez-Martinez et al. (Spain), Ladislav Paule et al. (Slovakia)





Nuclear microsatellites

- investigation of the last remaining Hungarian stand in progress
- high variability
 - as often in conifers
- need to check suitability for detecting „park yew“ genes

Conservation measures

- „total protection“ sites
- raising awareness
- gene conservation forests
 - silvicultural intervention to ensure yew regeneration
- cautious „conservation by utilization“ approach
- *ex situ* measures and restoration projects



Saving old
“monuments”
with
conservation
orders

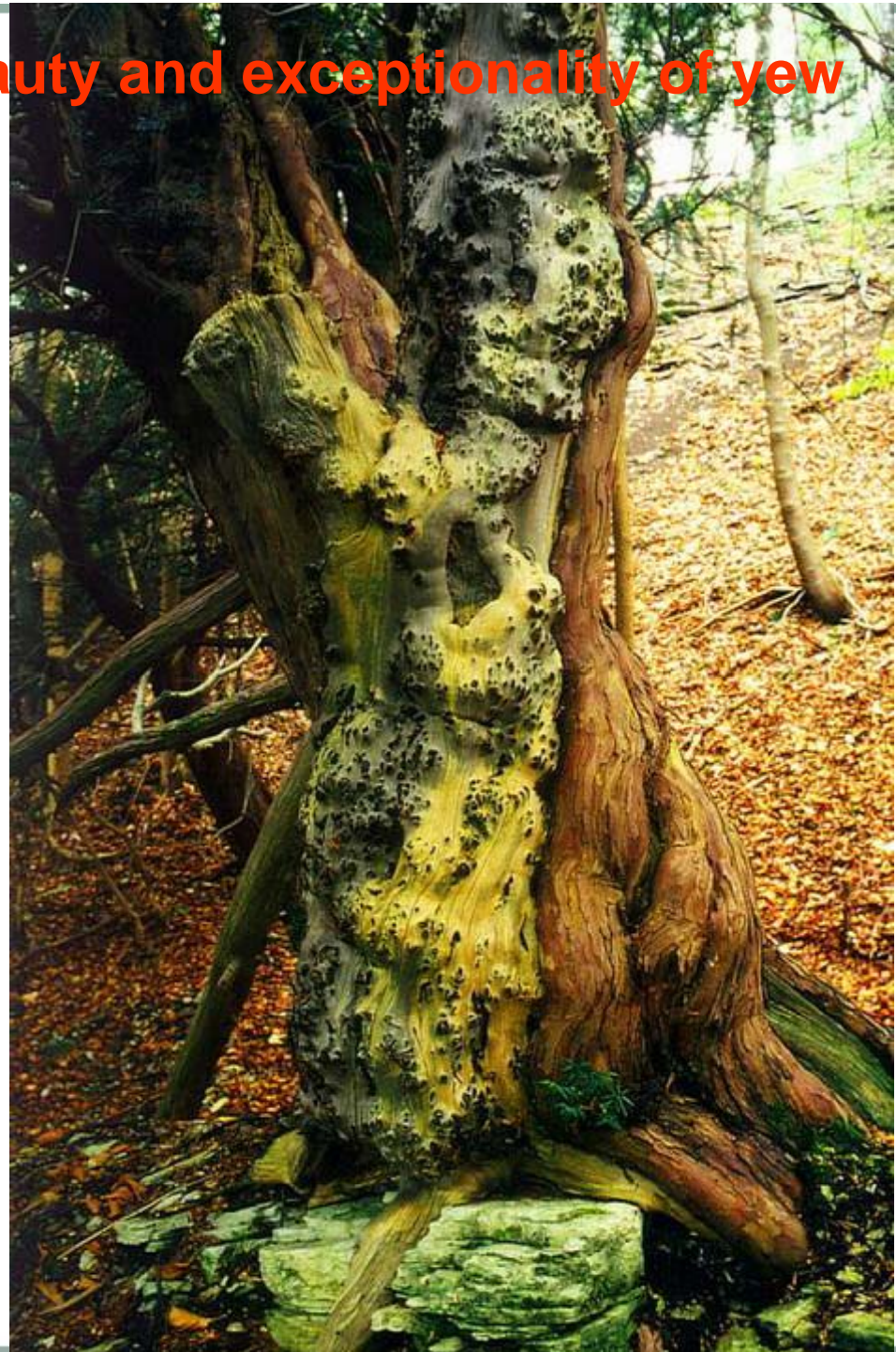
hollow yew in
Vienna





totally protected yew stand in Lower Austria

Raising awareness for the beauty and exceptionality of yew





Fotos: Christian Wolf

Organising meetings for the interested public



Yew Meeting 2003 in Vienna





Excursions



**Gene conservation project J. Lechner,
Thernberg (Lower Austria)**



**Gene conservation project Faistenau,
Salzburg**











Yew project Stiwollgraben, Styria



- felling of overstorey trees in order to enhance yew growth
- in 3 variants
- severly challenged by storm event

**Conservation by utilization:
conserve valuable
resources ...**



**... by sustainable
utilization**





ex situ conservation –
vegetative propagation



Conclusions

- biology and ecology of the species must be carefully considered
- geographical proximity vs. potential for spreading pollen and seed
- in *Populus nigra*, genetic distance may prevent overall collapse of species barriers
- in *Taxus baccata*, geographic distance may prevent massive introgression at the moment



Precautionary principle:

**what is gone cannot be
brought back –
this is also true for genes –
a careful plan should be
present before changing things
irreversibly**

Thank you for your attention !

