

Gene flow in poplar

experiments, analysis and modelling to prevent transgene
outcrossing

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Outline

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- 2 Gene flow I
- 3 Results
- 4 Ecology
- 5 Genetics
- 6 Establishment
- 7 Gene flow II
- 8 Introgression
- 9 Summary



Projects

Modelling pollen mediated gene flow in transgenic trees (UBA)



Aims:

- Development of a simulation model to analyse the dispersal probability of transgenic pollen within a real landscape
- for a specific tree species (Poplar)
- usage for risk assessment and generation of so called “worst case”- scenarios

Projects

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Modelling gene flow in Poplar within a real landscape (BMBF)



Aims:

- risk assessment of seed and pollen dispersal in the context of vertical gene flow (Introgression)
- modelling vertical gene transfer between different poplar species
- with special emphasis on the long living organisms and long distance dispersal

The species (*P. × canadensis*)



Figure: A typical female hybrid clone (Leipzig) located in the nature reserve Ederau (tree number E083).

mostly 'artificial hybrid'
derived from crossing the
American Black Poplar
(*P. deltoides* ♀)
and the European Black Poplar
(*P. nigra* ♂).

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Important tree for silviculture

- cultivation as plantation
but often also
- along streets, lanes, sports
grounds and rivers
and nowadays
- short rotation plantations,
energy plantations

Investigation areas (Germany)

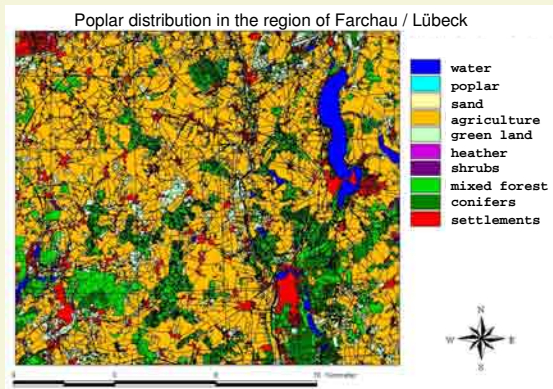


- three different investigation areas
 - 1 Farchau-Lübeck (Schleswig-Holstein)
 - 2 NSG Ederau (Hesse)
 - 3 NSG Kühkopf (Hesse)
- average about $30 \times 30 \text{ km}^2$
- selection criterion: presence of local Poplar stands

Real landscape



- plot: Farchau-Lübeck (Schleswig-Holstein)

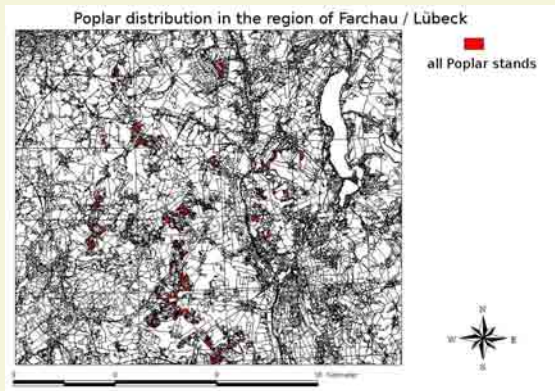


- mesoscale meteorological model METRAS (Prof. Schlünzen, Uni Hamburg)
- different forest, water and agricultural areas (Elbe-Lübeck-Chanel / Baltic Sea)
- GIS maps were available (ATKIS, Forest Offices)

Real landscape

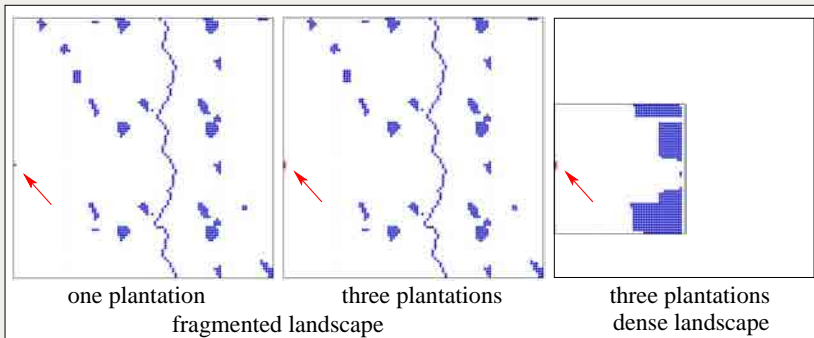


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Setup of artificial landscapes



- **red arrows** ⇒ plantations in the landscapes (west side)
- **blue squares** ⇒ natural poplar stands
- **left landscapes** ⇒ elements of the real landscape
- **right landscape** ⇒ dense arrangement of real populations

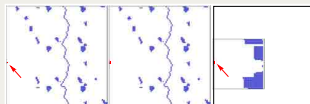
Scenarios

parameter combinations

No.	density [trees/cell]	size [no. of cells]	landscape type
1	40	1	1
2	40	3	1
3	40	3	2
4	110	1	1
5	110	3	1
6	110	3	2
7	330	1	1
8	330	3	1
9	330	3	2
10	990	1	1
11	990	3	1
12	990	3	2

We varied:

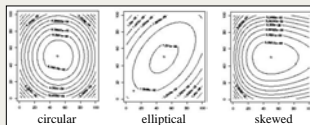
1 landscape



2 density of GM plantation

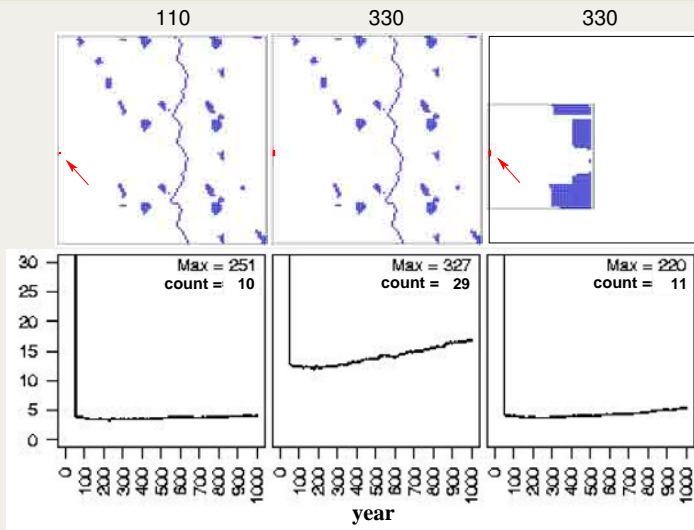
3 size of GM plantation

4 pollen kernel



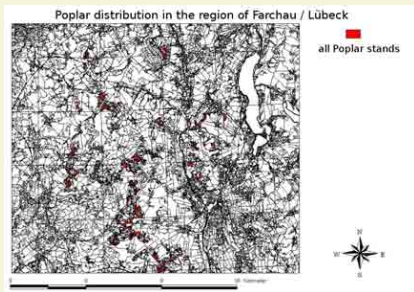
Results of fragmentation

amount of transgenic trees and density of recipients

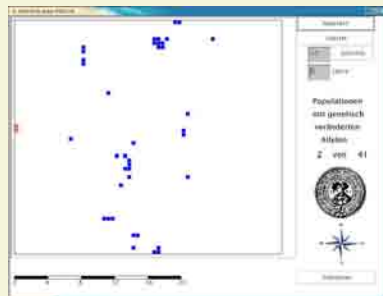


Pilot study – Setup

- For this study a region between Lübeck and Farchau had been selected. This region was chosen because data for meteorological simulations (METRAS) were already available.



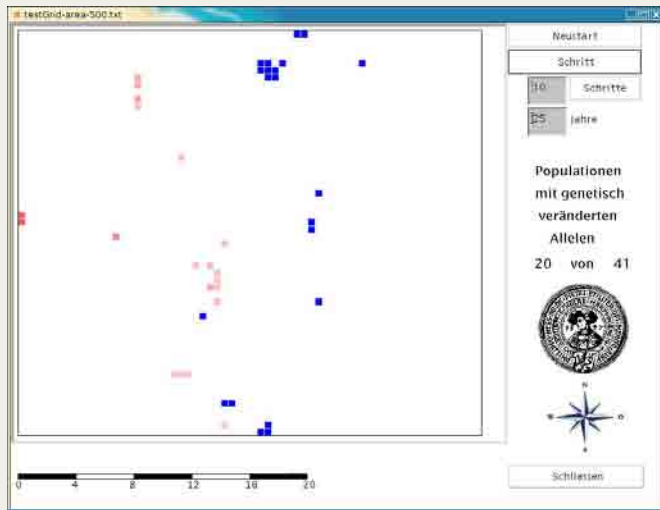
- ArcView project – all Poplar populations within the selected region (red)



- Representation of the selected region within the program INTROGRESSION[©]

INTROGRESSION[©]

running program



real
landscape

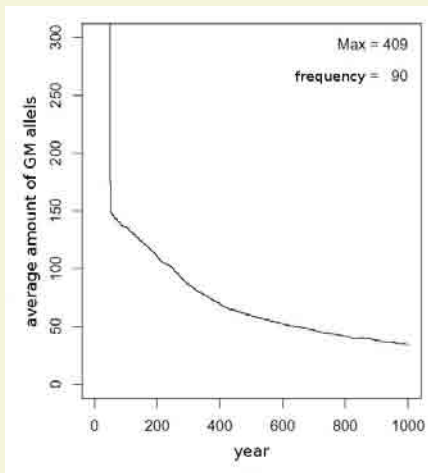
with 980
GM-tress

after
25 years

Legend:

- Plantation resp. > 45 GM allels
- 0 – 45 GM allels
- only WT allels

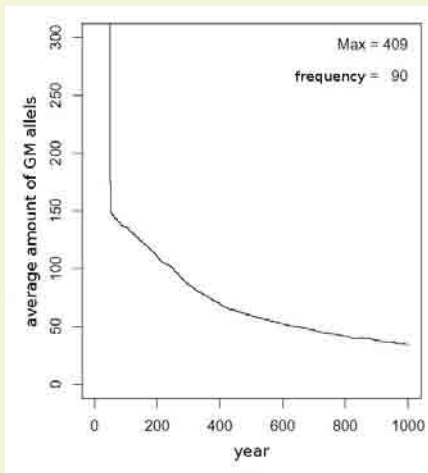
Pilot study – Results



- Plantations with 1000 trees exist for 50 years.

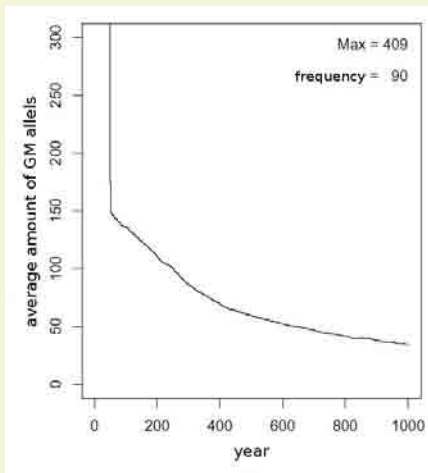
- Chronological sequence of the amount of GM alleles within the real landscape.

Pilot study – Results



- Plantations with 1000 trees exist for 50 years.
- Running this simulation for 1000 years we recorded in 90% of all cases that GM alleles are still present in the landscape.
- Chronological sequence of the amount of GM alleles within the real landscape.

Pilot study – Results



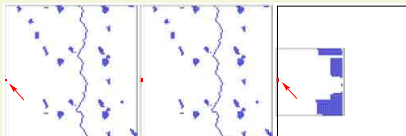
- Plantations with 1000 trees exist for 50 years.
 - Running this simulation for 1000 years we recorded in 90% of all cases that GM alleles are still present in the landscape.
 - The “worst case” (maximal amount) of GM alleles after 1000 years had been 409 alleles.
- Chronological sequence of the amount of GM alleles within the real landscape.

Conclusions (first part)

- Because of the long ranging dispersal and wide geographic distribution, the application of simulation models is the only safe way to gain more insights into outcrossing of GM alleles.

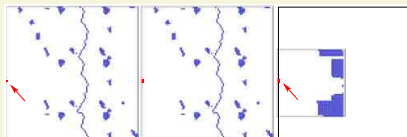
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- Because of the long ranging dispersal and wide geographic distribution, the application of simulation models is the only safe way to gain more insights into outcrossing of GM alleles.
- One important result is the fact that the risk of outcrossing is reduced by an increasing distance from the source population but also by an increasing size of the sink population.



Conclusions (first part)

- Because of the long ranging dispersal and wide geographic distribution, the application of simulation models is the only safe way to gain more insights into outcrossing of GM alleles.
- One important result is the fact that the risk of outcrossing is reduced by an increasing distance from the source population but also by an increasing size of the sink population.



- For a safe application of transgenic trees in plantations it is necessary to evaluate the risk of outcrossing of these species.

The Poplar complex

(*P. nigra* & *P. × canadensis*)

Populus nigra (Black poplar)

- character tree of the European floodplain

endangered because of:

- 1 river regulations & grazing
- 2 remaining relict populations suffer genetic erosion
and furthermore
- 3 introgressive gene flow with its hybrid (*P. × canadensis*)

- Both species exist in the same regions in Europe.



Figure: *P. nigra* (left) and *P. × canadensis* (right) live in the same habitat.

- they are able to mate with each other
- good system to investigate introgression
- risk assessment without real GM alleles in the landscape

Investigation areas (Germany)



- three different investigation areas
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- average about $30 \times 30 \text{ km}^2$
- selection criterion: presence of local Poplar stands

Investigation areas (Hesse)



“NSG K uhkopf”

- samples from 59 trees
- large amount of juveniles are present
- flowering phenology in 2006



“NSG Ederau”

- > 600 samples of trees within the nature reserve and surrounding
- *P. nigra* also outside the nature reserve detected
- conduct of parentage analysis of seeds and juveniles of this region

Flowering phenology

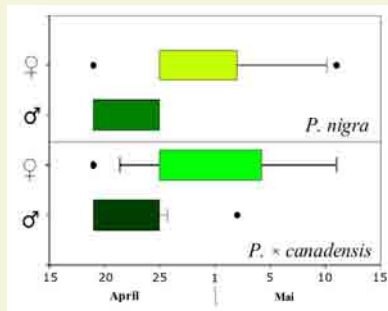
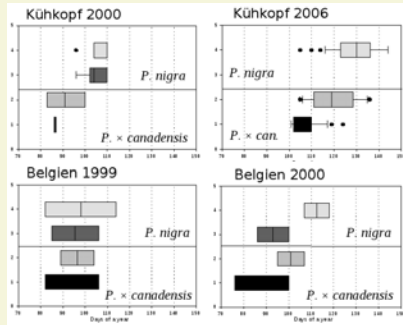


Figure: Time span of flowering of *P. nigra* and *P. x canadensis* in 2006

- in 2006 – almost 100% overlapping of *P. nigra* and *P. x canadensis* because of an extreme long winter



- *P. x can.* ahead of *P. nigra*
- male trees are always ahead of female trees
- ⇒ higher probability of pollination of *P. x can.* (♀) by *P. nigra* (♂)

Seed collection



Figure: Seed collection directly at the branches of the mother trees using a “Ruthmann Steiger” of the public services of Fritzlar.

- collection of seeds from 15 mother trees
- 2006 – removal of branches and afterripening in the green house
- 2007 – collection of seeds directly from the trees at the time the capsule burst open

Germination experiments



Figure: Germination of seeds within a Petri dish using Vermiculite

Setup

- 15 mother trees
- 50 seeds per Petri dish
- 4 repetitions per tree
- control and collection after 2-4 days

Germination experiments



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Results

- no differences between species ($\approx 90\%$)

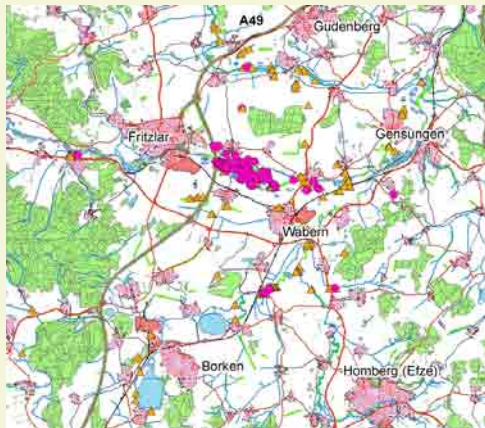
Genetic marker system

Locus	WPMS 05	WPMS 09	WPMS 14	WPMS 18	WPMS 20	PMGC 14	PMGC 2163
Na	14	12	13	8	57	9	11
Ne	6,968	3,547	6,989	3,385	2,734	5,310	3,598
PI pro Locus	0,036	0,104	0,037	0,125	0,19	0,061	0,103
Allele mit p<5%	8	8	7	4	4	4	6
Allele mit p>10%	4	4	5	3	3	4	3
Chromosom- Nr., Species	XII D,T	VI N,T	V T	? T	XIII P. tr.	XIII D, N, T	X T
Amplifikation	+	+	(+)	+	+	+	+
Verlässlichkeit	+	+	+	+	+	+	+

Figure: The marker systems used to determine the species affiliation, the diversity and parentage analysis.

- 7 nuclear mikrosatellites permit the differentiation between individuals with an accuracy of 10^{-9}

Species determination



By usage of 4 diagnostic marker specific for the hybrid ...

Figure: Distribution of *P. nigra* and *P. x can.* within the investigation area according to our genetic marker system.

Species determination

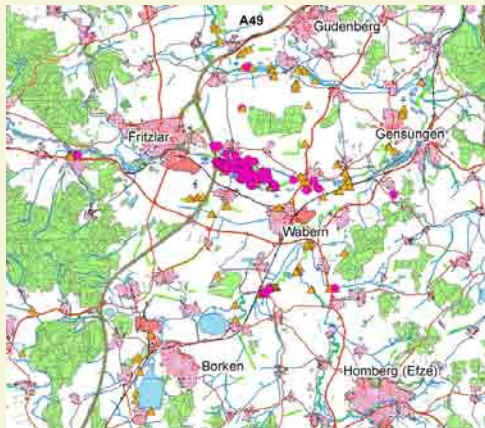


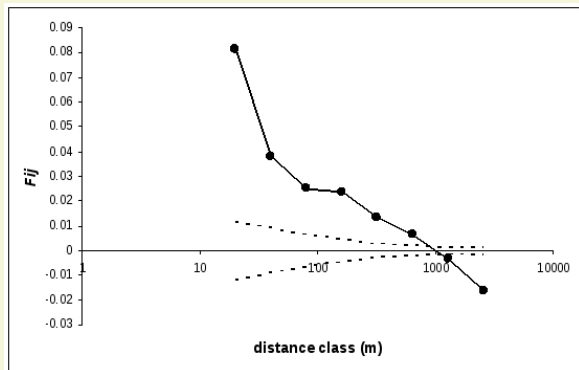
Figure: Distribution of *P. nigra* and *P. x canad.* within the investigation area according to our genetic marker system.

By usage of 4 diagnostic marker specific for the hybrid ...

We found:

- 320 × *P. nigra* and 246 × *P. x canad.* trees.
- the sex ratio in *P. nigra* is 1:0.83 (146 ♀ and 124 ♂)
- the sex division in *P. x canadensis* is 95 ♀ and 143 ♂

Spatial genetic structure (SGS)



- strong degree of kinship within the adult population
- indication for spatial restricted gene flow
- but ... similar age of all adult trees

Figure: Correlogram of the degree of kinschhip between all adult trees using the software “SPAGeDi 1.2” (Hardy & Vekemans 2002).

Establishment



Figure: Sowing of seeds under three different competition scenarios using grass.

- fitness parameter:
 - 1 establishment rate
 - 2 sprout length
 - 3 amount of leaves

Setup

- 200 seeds per tree,
- 3 different scenarios
 - Null – bare soil
 - Eins – 60% grass
 - Zwei – 80% grass
- 4 repetitions per competition scenario
 - total 14.400 seeds
- averaging over one box the fitness parameter:
 - sprout length and
 - amount of leaves

Interaction analysis

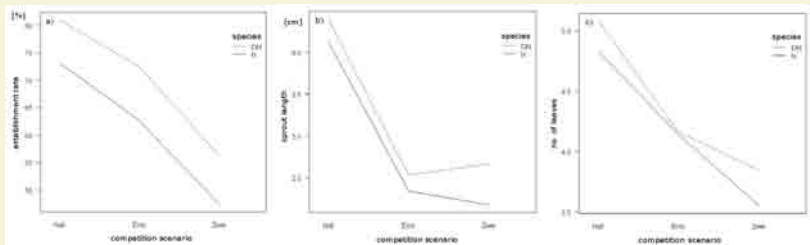


Figure: Interaction plot of fitness parameter comparing all three competition scenarios (Null, Eins, Zwei – no competition, 60% grass, 80% grass).

- no interaction between the factor species and competition scenario
- hybrid seeds are marginally better (**BUT**: not significant)
- only maternal parts are analysed (both fathers are possible)

Parenthood of seeds

- 1032 seedlings
- each 129 from the competition scenario Null and Zwei of *P. nigra* mothers (E017 and E001) as well as
- each 129 for scenario Null and Zwei of *P. × canadensis* mothers (E146 and E083)

status including the father assignment (4 groups)

DN/DN: mother *P. × canadensis*, father *P. × canadensis*;

DN/N: mother *P. × canadensis*, father *P. nigra*;

N/DN: mother *P. nigra*, father *P. × canadensis*

N/N: mother *P. nigra*, father *P. nigra*

- a seedling got assigned to the group DN/DN if it got at least at one locus the species specific allele for *P. deltoides* homozygot

Fatherhood – conclusion

Hypotheses

H₀: The amount of Hybrid and *P. nigra* fathers is the same between the competition treatments.

H_A: The amount of Hybrid and *P. nigra* fathers is different between the competition treatments.

- tested using a contingency table ($\chi^2 = 18,77$)
- critical value for 95% is 3,84 \Rightarrow H₀ is rejected \rightarrow H_A is true

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Conclusion

The offspring of Hybrid fathers establish more often under strong competing condition than the offspring of *P. nigra* fathers.

Gene flow analysis

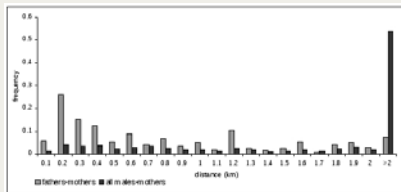
Parantage analysis

We collected and analysed leave material from:

- 566 adult trees
- 2839 seedlings
- 380 juveniles

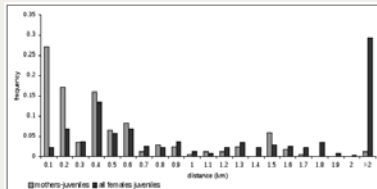
Parenthood could be identified for more than the half of the samples.

Results for pollen



Generated on the basis of the single tree progenies (seedlings) and the results from paternity analysis using the software CERVUS.

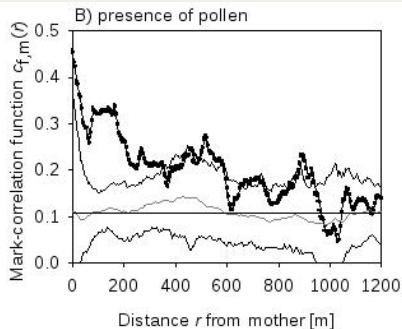
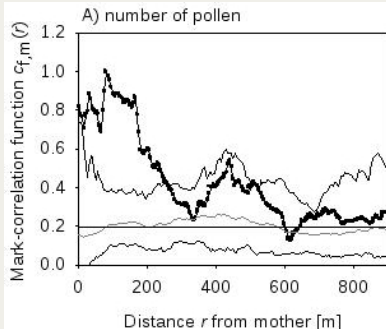
Results for seeds



Generated on the basis of the juvenile population and the results from maternity analysis using the software CERVUS.

“Point Pattern” – Results

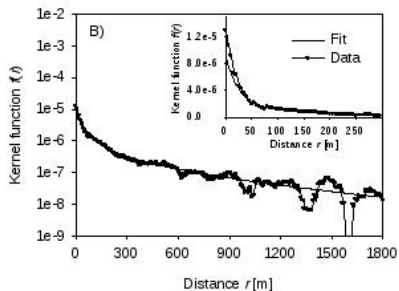
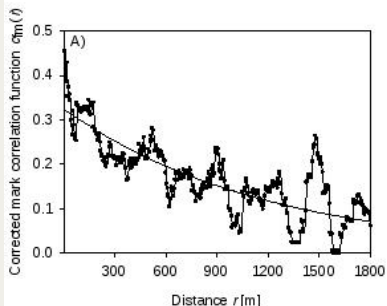
Test of deviation from a random assignment of fathers



- “Goodness of Fit” test shows a clear correlation at distances up to 2100 m
- significant deviation from a random assignment up to a distance of 300 m
- differences between the two years are most probably due to differences in the amount of samples available

Dispersal kernel

Fitting of the kernel to the statistics – not to the count data



- correction with the area size of the rings in the 'O-ring statistics'
(Wiegand & Moloney, 2004)
- fitting to different functions (exponential, exponential power, ...)
- usage of these functions for further simulation studies

Introgression

What do we mean by introgression?

- Introgression is the establishment of alleles from one species within the genome of a different species.
- Previous hybridisations (*P. × canadensis*) aid and enhance introgression.
- Backcrossing of hybrids into one of the parents introduce new genes permanently into the parental population.

Poplar complex:

- There are specific alleles, which are only present in *P. deltoides*.
- These alleles can function as diagnostic marker for such backcrossings.



Introgression of *P. × canadensis* pollen

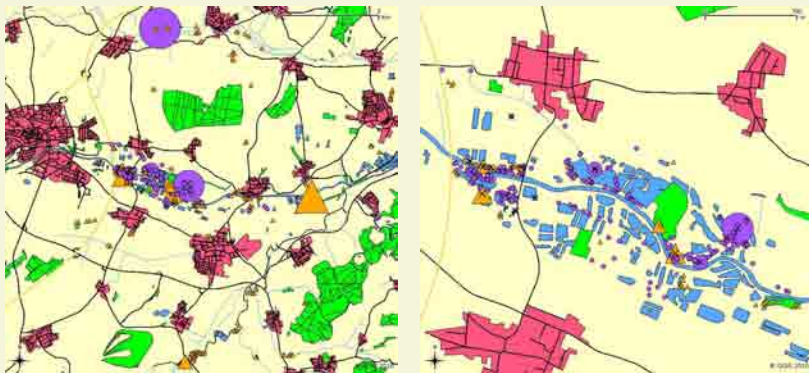
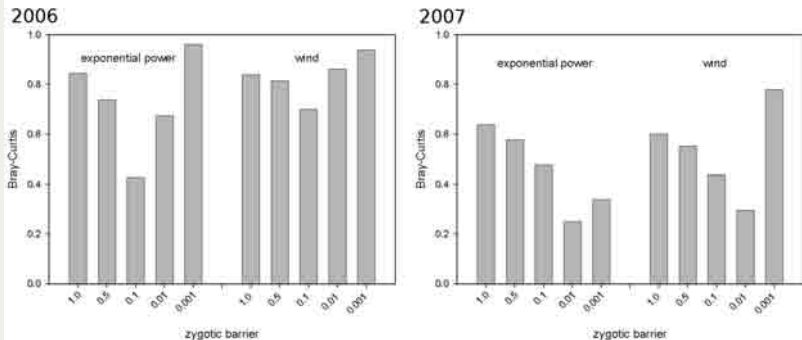


Figure: The pictures show the amount of pollen from *P. × canadensis* fertilising the mother trees. The size of the symbol indicates the amount.

- generally only low introgression rates in *P. nigra*
- but also low introgression rates in *P. × canadensis*
- rate is dependent on location and surrounding of mother trees

Results for simulation of a zygotic barrier

Simulation with two different dispersal functions



- difference in the zygotic barrier because of different flowering phenology

- exponential power: $p(x) = \frac{1}{2a\Gamma(1+1/b)} e^{-\left[\left|\frac{x}{a}\right|^b\right]}$

- wind: $p(x, y, z) = \frac{E}{2 \cdot \pi \cdot \sigma_2(x) \cdot \sigma_3(x) \cdot u} e^{-\left[\frac{y^2}{2\sigma_2^2(x)} + \frac{(z-h)^2}{2\sigma_3^2(x)}\right]}$

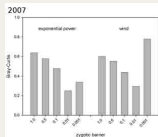
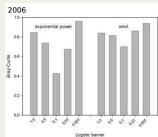
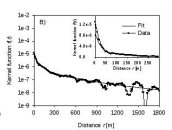
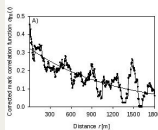
Summary

Take Home Messages:

- identification of species using molecular marker



	80 Null	80 Zwi
Value N	479	457
Value DN	24	66
	909	523



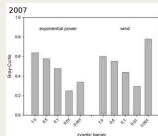
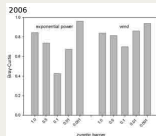
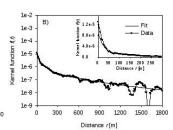
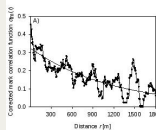
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Value DN	24	66	90
	503	523	1026



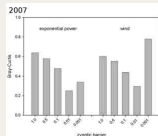
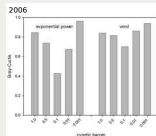
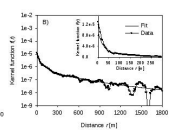
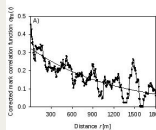
Summary

Take Home Messages:

- identification of species using molecular marker
- germination and establishment show an **advantage for hybrids** under competition scenarios
- **general more “*P. nigra*” pollen in seeds from single trees**



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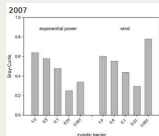
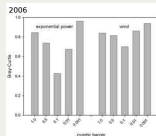
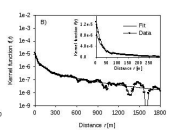
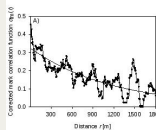
Summary

Take Home Messages:

- identification of species using molecular marker
- germination and establishment show an **advantage for hybrids** under competition scenarios
- general **more “*P. nigra*” pollen** in seeds from single trees
- **this is a hint for the existence of a zygotic barrier**



	<i>P. nigra</i>	<i>P. avicularis</i>	Total
Value N	479	457	936
Value DN	24	66	90
	503	523	1026



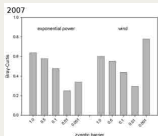
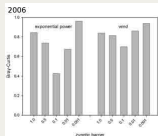
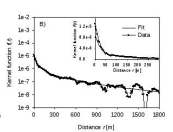
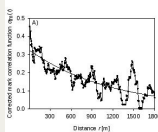
Summary

Take Home Messages:

- identification of species using molecular marker
- germination and establishment show an **advantage for hybrids** under competition scenarios
- general **more “*P. nigra*” pollen** in seeds from single trees
- this is a hint for the existence of a **zygotic barrier**
- **simulation of empirical introgression rates**



	80 Null	80 Zwi	936
Value N	479	457	936
Value DN	24	66	90
	503	523	926



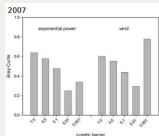
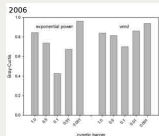
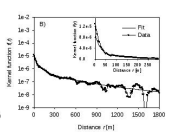
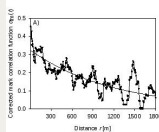
Summary

Take Home Messages:

- identification of species using molecular marker
- germination and establishment show an **advantage for hybrids** under competition scenarios
- general **more “*P. nigra*” pollen** in seeds from single trees
- this is a hint for the existence of a **zygotic barrier**
- simulation of empirical introgression rates
- **different introgression rates because of different flowering phenology**



	80 Nubi	80 Zwi	95k
Value N	479	457	936
Value DN	24	66	90
	503	523	1026



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- PhD students:
 - Marc Niggemann
 - Georg Rathmacher
- Diploma students:
 - Melanie Köhnen
- Assistance:
 - Oliver Jakoby
 - Sarah Wanning
 - Hania Wypukol
 - Nina Holstein
 - Katharina Budde
 - Dagmar Kudernatsch

- Dr. habil. M. Fladung (vTI)
- Prof. Dr. B. Ziegenhagen
- Dr. J. Arndt (NW-FVA)
- Dr. K. Gebhardt (NW-FVA)
- Dr. T. Wiegand (UFZ)
- Dr. J. Vollmer (MPI)
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- Dr. J. Robledo (Madrid)



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... and to you ... for your attention!