

# Seed dispersal abilities of forest orchids

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## Introduction

The minute orchid seeds are often thought to be practically unlimited in their dispersal. However, our present knowledge on orchid seed dispersal shows a substantial decrease of seed rain with the distance from the mother plant. This conclusion based on few studies of meadow species suggests that impressive reports of long distance dispersal need to be regarded as rare cases. In forest habitats, the dispersal efficiency of windborne seeds might be even lower due to restricted air movement.

## Data collection

### Seed traps:

We investigated seed dispersal of *Epipactis atrorubens* and *Cephalanthera damasonium* in two forest types - beech and pine forests (each in two replicates). The seed traps (sticky Petri dishes, 140 mm) were regularly spaced in a 20x20 m network laid around investigated plants (3 plots per site). The seed traps were exposed for four weeks and then scored under stereomicroscope. Position of each trap was geo-referenced. Differences in seed dispersal curves between forest types were compared using the Mann-Whitney-Wilcoxon test.

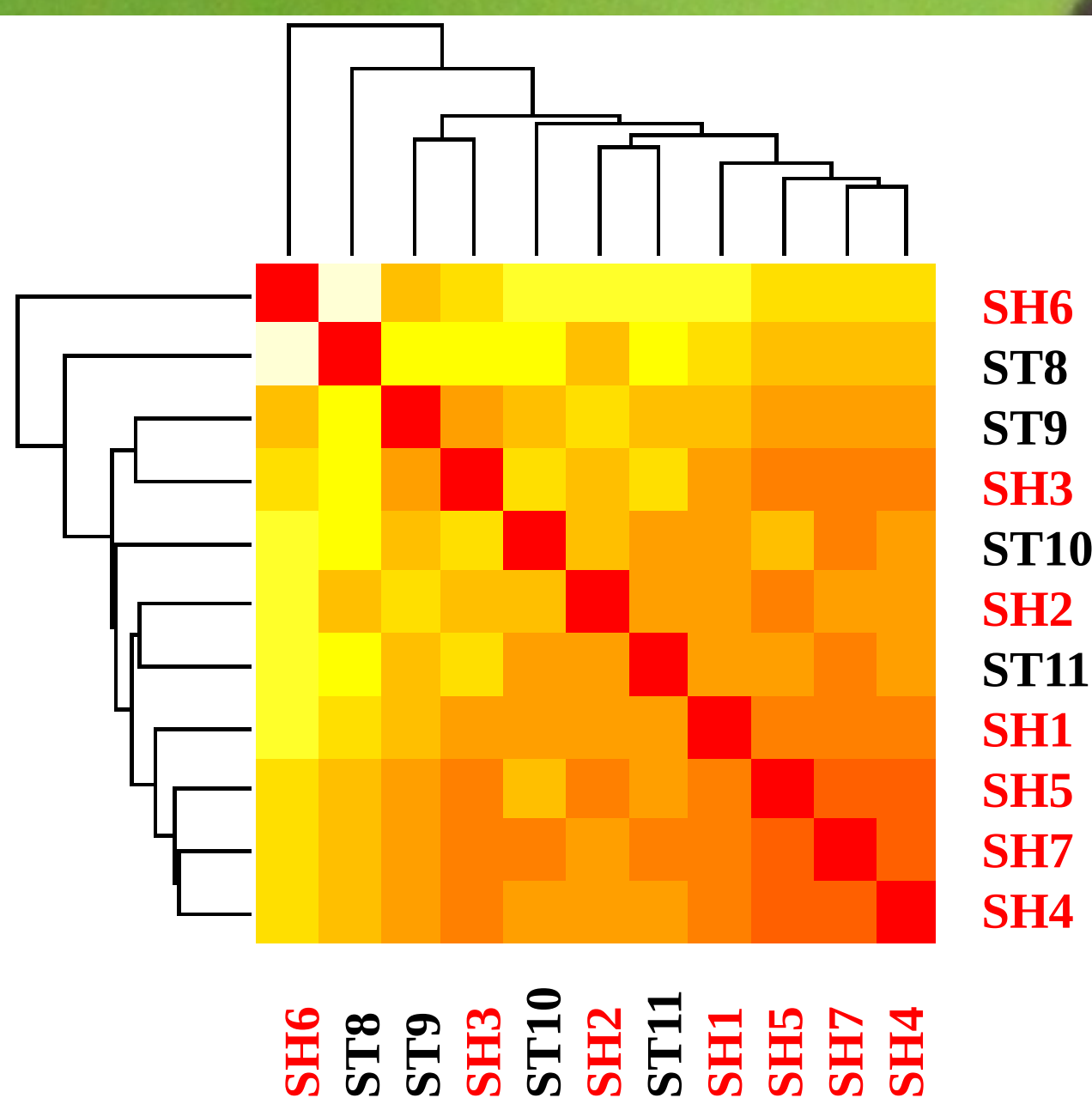
### Microsatellites:

Our sampling covers 11 populations of *Cephalanthera rubra* (167 individuals) in two areas and 23 populations of *Epipactis atrorubens* (321 individuals) in four areas. Extracted DNA was used for amplification of 12 microsatellite loci for *C. rubra* and 10 microsatellite loci for *E. atrorubens*. Data were processed by Arlequin 3.5, MSA 4.05 and ape, ade4 and adegenet packages in R 3.2 software.

## Results

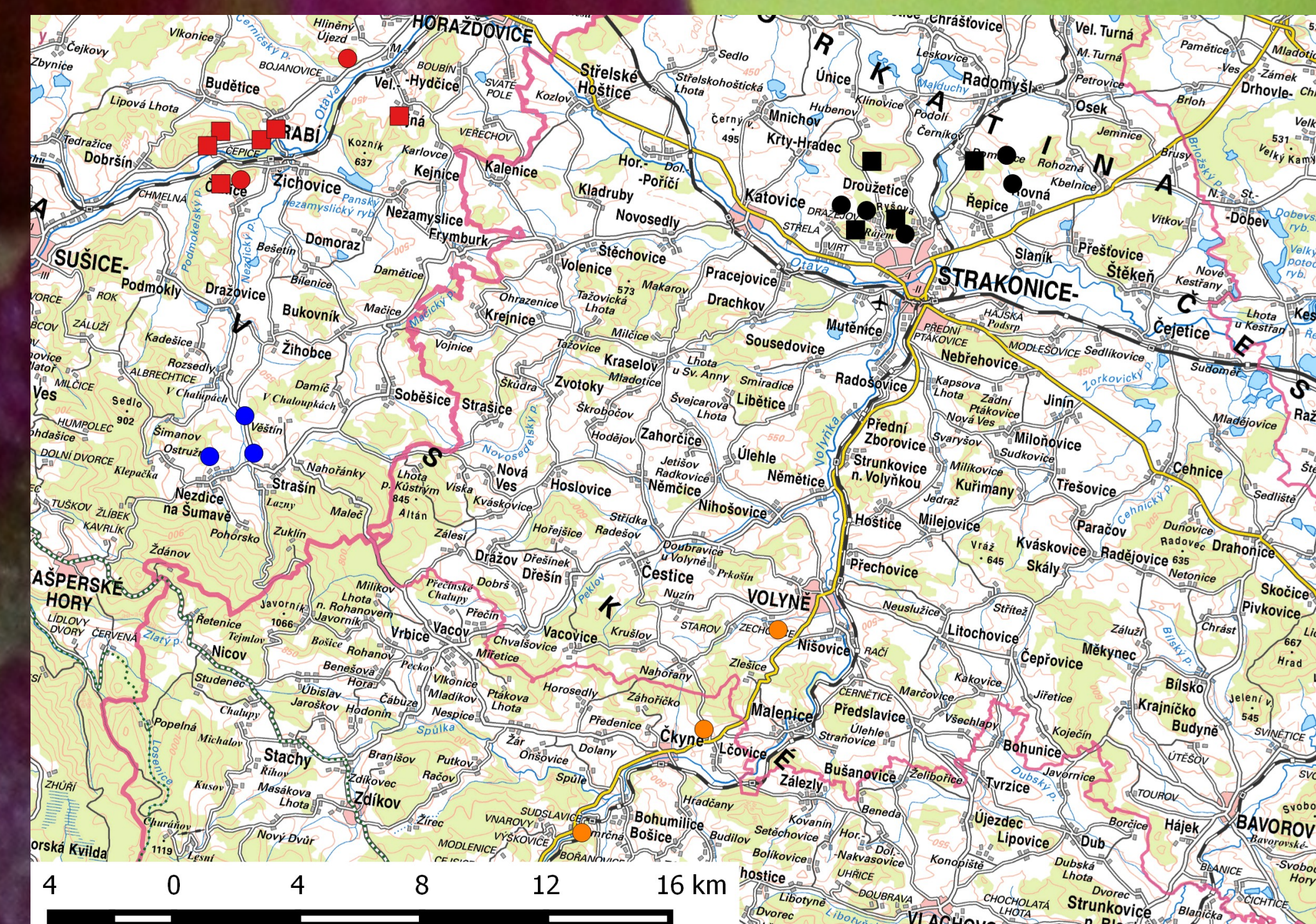
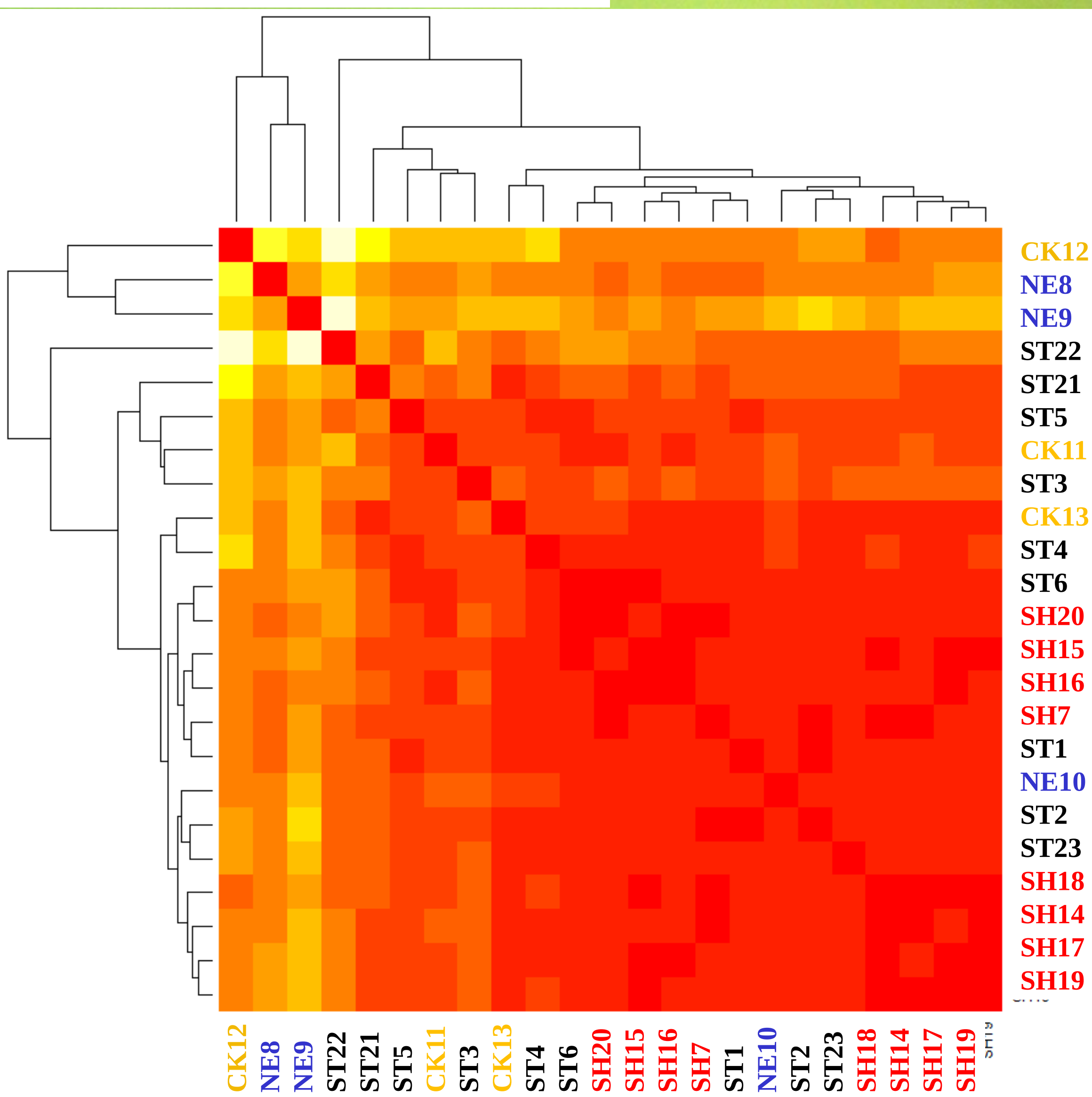
The majority of seeds of both study species were found up to 6 or 7 m from the mother plants. Comparison of *E. atrorubens* seed dispersal curves between forest types suggested significantly wider dispersal ranges in the beech than pine forest. In distance up to 6 m from the mother plant, we found 53 % of all seeds in the beech forest, whereas in the pine forest, it was 85 % of all seeds. By contrast, there was no significant difference in seed dispersal curves of *C. damasonium*. AMOVA indicated a significant genetic differentiation between and within population, however not between areas. The low genetic differentiation can be explained by high levels of gene flow and low levels of drift. The global Mantel test across all population pairs did not show significant effect of isolation by geographical distance.

Source	df	SS	Est. Var.	%	P
<i>Cephalanthera rubra</i>					
Among areas	1	13,2	0,035	1,3	-
Among populations	9	72,1	0,185	6,7	>0.001
Within populations	323	825,2	2,775	92,1	>0.001
<i>Epipactis atrorubens</i>					
Among areas	3	37,4	0,032	0,8	-
Among populations	19	138,9	0,139	3,7	>0.001
Within populations	619	2222,6	3,590	95,5	>0.001



Results of analysis of molecular variance (AMOVA).

Heatmap with NJ tree based on Nei's distance shows relationships between populations of *C. rubra* (left) and *E. atrorubens* (down). Colors of population names correspond with colors of areas in map.



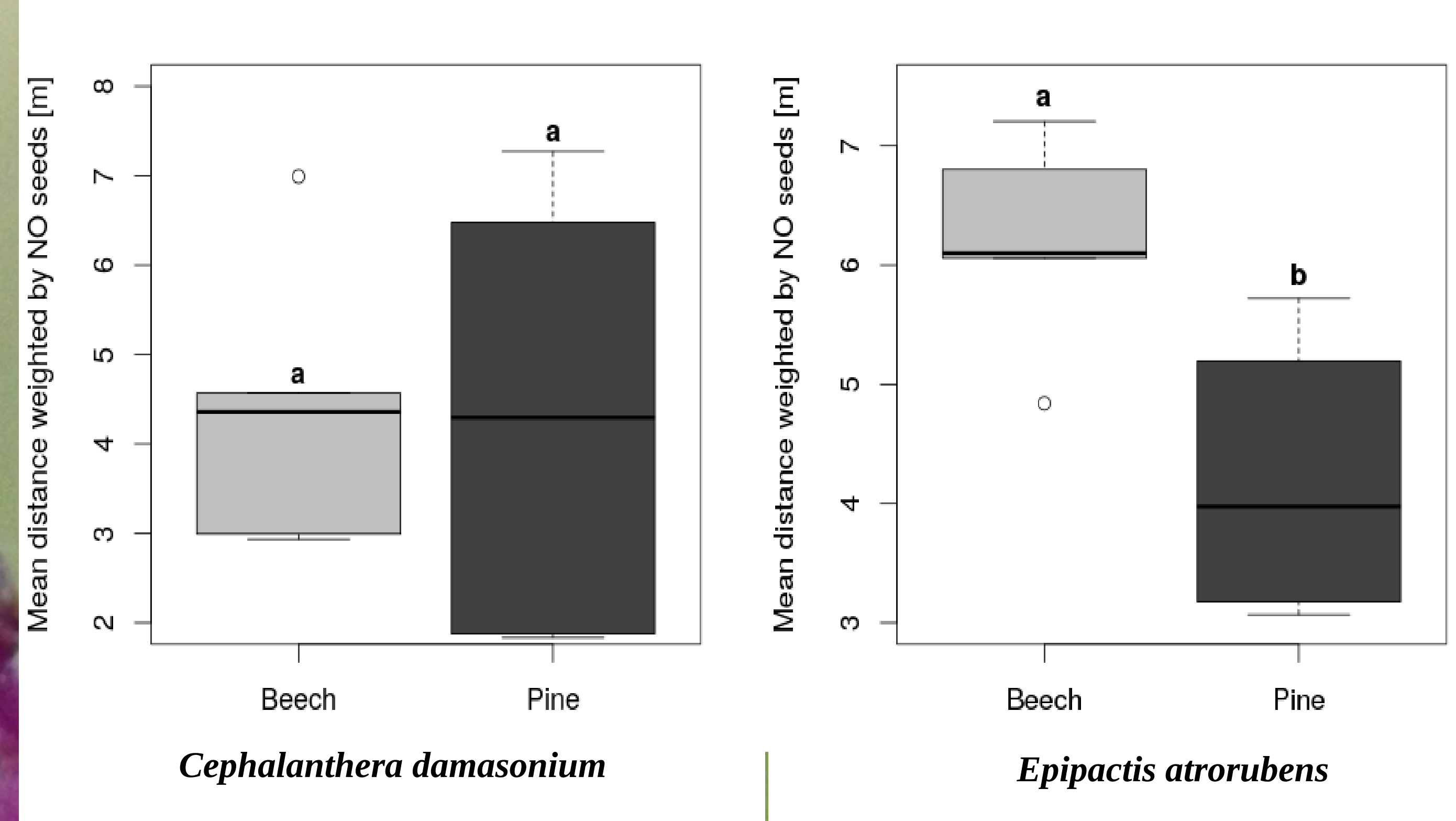
Map of studied populations divided into four areas (indicated by color). Symbols: square – site with both studied species, circle – *E. atrorubens* site.

### Genetic variation and genotypic diversity in populations of *E. atrorubens* (left) and *C. rubra* (right).

N – number of ramets sampled, % – percent of polymorphic loci, Al – mean number of alleles per locus, Ho – observed heterozygosity, He – expected heterozygosity, G – number of genotypes, S – Simpson's diversity index

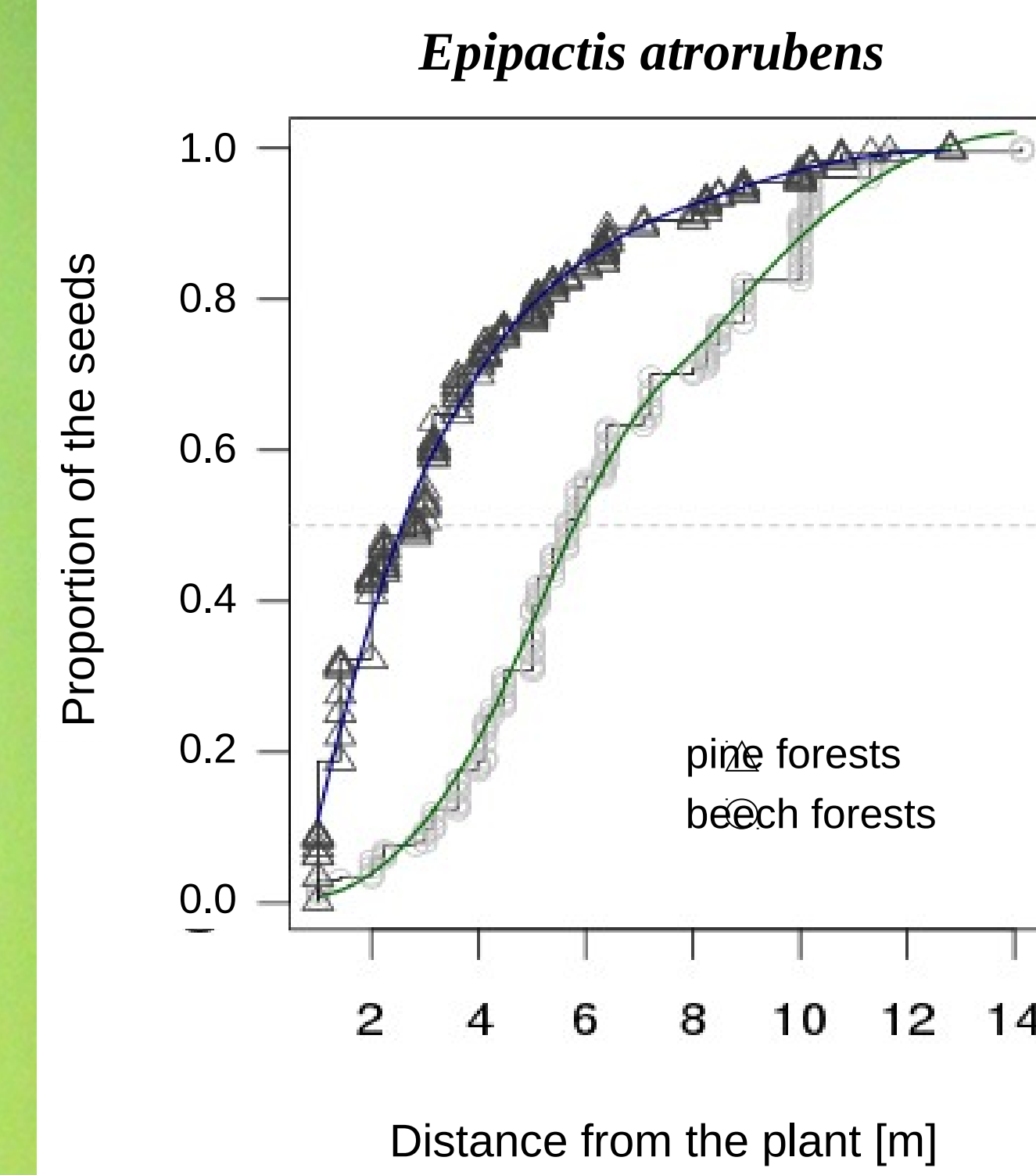
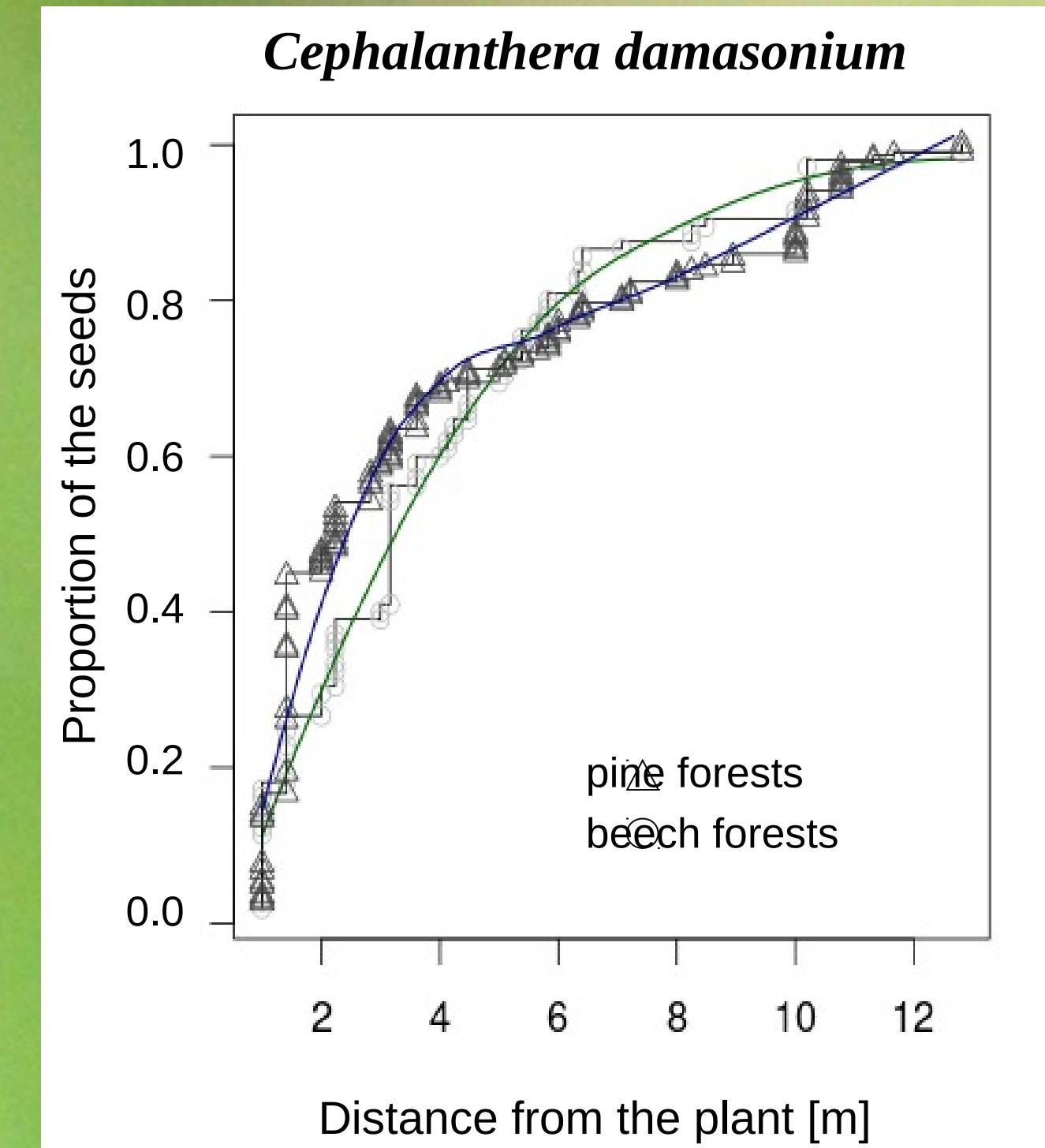
	N	%	Al	Ho	He	G	S
ST1	19	100	7,5	0,65	0,74	19	0,95
ST2	20	90	7,6	0,70	0,74	20	0,95
ST3	10	100	4,8	0,63	0,66	10	0,90
ST4	10	80	6,0	0,69	0,73	10	0,90
ST5	16	100	6,9	0,66	0,70	15	0,93
ST6	10	100	5,8	0,64	0,74	10	0,90
SH7	20	90	7,5	0,70	0,75	20	0,95
NE8	3	90	3,0	0,74	0,70	3	0,68
NE9	1	60	2,0	1,00	1,00	1	0,00
NE10	19	80	7,3	0,65	0,75	19	0,95
CK11	6	90	3,8	0,63	0,68	6	0,83
CK12	2	70	3,0	0,86	0,83	2	0,50
CK13	20	100	7,8	0,68	0,76	20	0,95
SH14	19	50	7,4	0,69	0,75	19	0,95
SH15	20	100	7,5	0,66	0,73	20	0,95
SH16	18	90	7,2	0,67	0,72	18	0,94
SH17	20	100	7,6	0,60	0,74	20	0,95
SH18	20	90	7,3	0,70	0,76	20	0,95
SH19	20	100	7,2	0,66	0,74	20	0,95
SH20	19	90	7,1	0,62	0,71	19	0,95
ST21	4	100	3,7	0,73	0,71	3	0,63
ST22	5	90	3,8	0,64	0,69	5	0,80
ST23	20	100	6,5	0,69	0,73	20	0,95

	N	%	Al	Ho	He	G	Sim
SH1	19	75	3,9	0,50	0,53	15	0,92
SH2	20	75	4,4	0,44	0,59	16	0,93
SH3	20	83	4,1	0,51	0,51	10	0,78
SH4	20	83	5,3	0,46	0,54	17	0,94
SH5	20	83	5,0	0,45	0,55	18	0,94
SH6	4	75	2,8	0,69	0,58	3	0,63
SH7	20	83	5,5	0,50	0,63	20	0,95
ST8	2	75	2,6	0,61	0,67	2	0,50
ST9	15	92	4,5	0,51	0,58	12	0,90
ST10	20	83	3,8	0,56	0,57	8	0,83
ST11	7	75	3,3	0,53	0,57	7	0,78



Box and whisker plots of the mean distances weighted by the number of seeds. Letters denote significant differences (W=19, p=0.032) between forests by *E. atrorubens* and non-significant by *C. damasonium*.

Cumulative distribution curves of dispersed seeds in two forest types. Lines were fitted by loess smoother.



## Summary

- There is significant difference in *E. atrorubens* seed dispersion curves between beech and pine forests.
- The majority of seeds of both study species were found up to 6 or 7 m from the mother plants
- Results of microsatellites analyses suggest, that seeds are rarely but regularly transferred on long distance (over 15 kilometers) between populations
- Transfers of seeds from large populations (hundreds or thousands of individuals) enable to preserve genetic diversity of smaller populations (dozens of individuals) and surviving of small (up to twenty individuals) populations