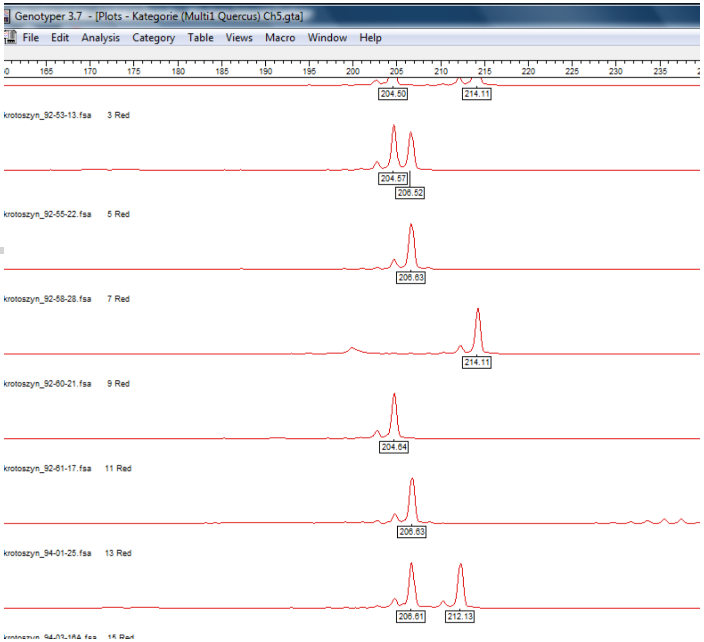
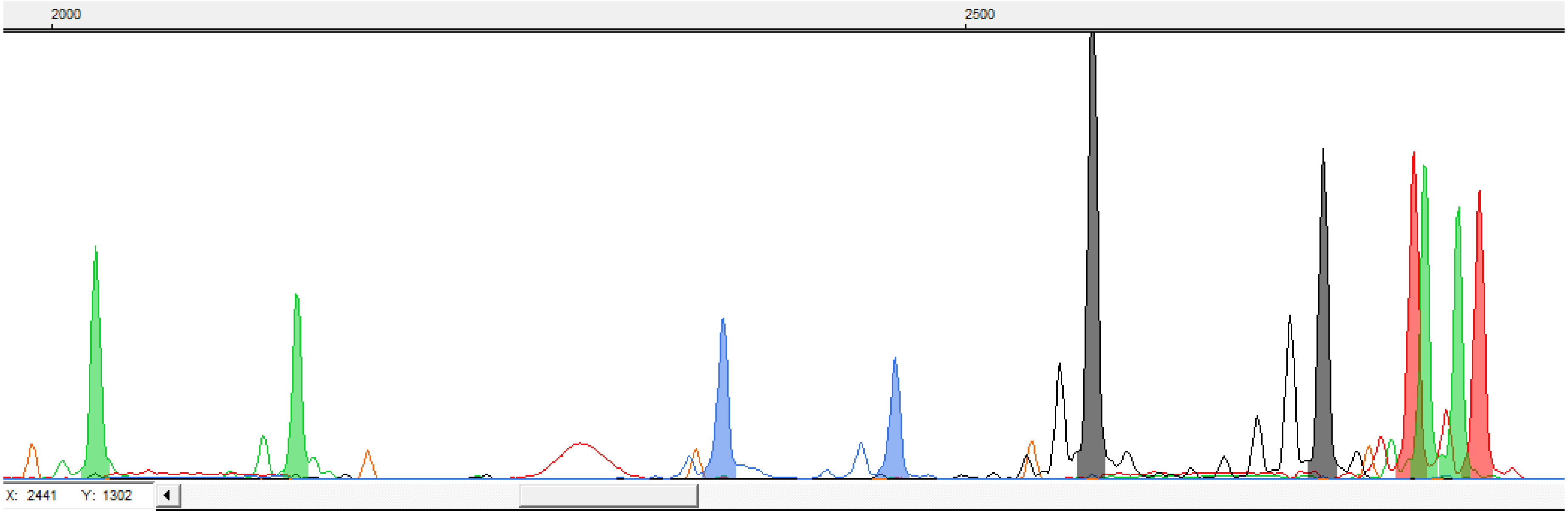
UNIWERSYTET
KAZIMIERZA WIELKIEGO
W BYDGOSZCZY

Microsatellites and genetic diversity in seed orchard and provenance test

Magdalena Trojankiewicz

Microsatellites



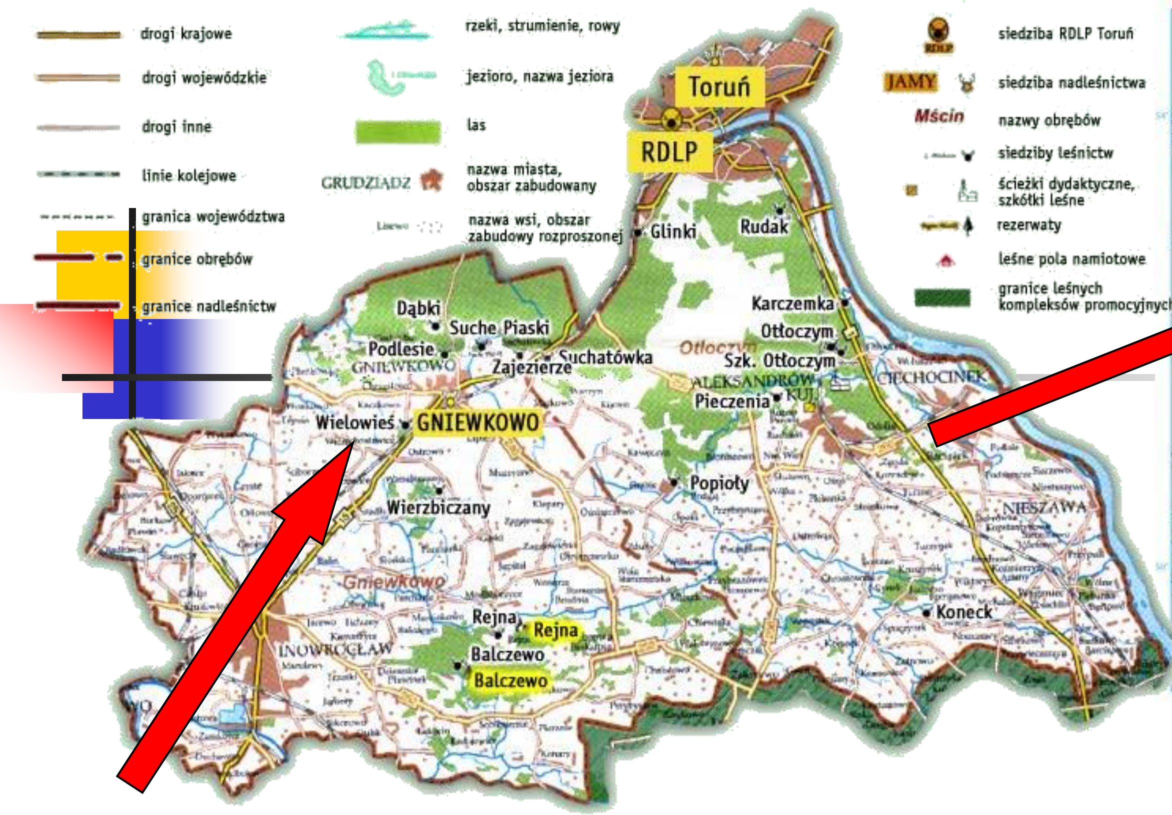


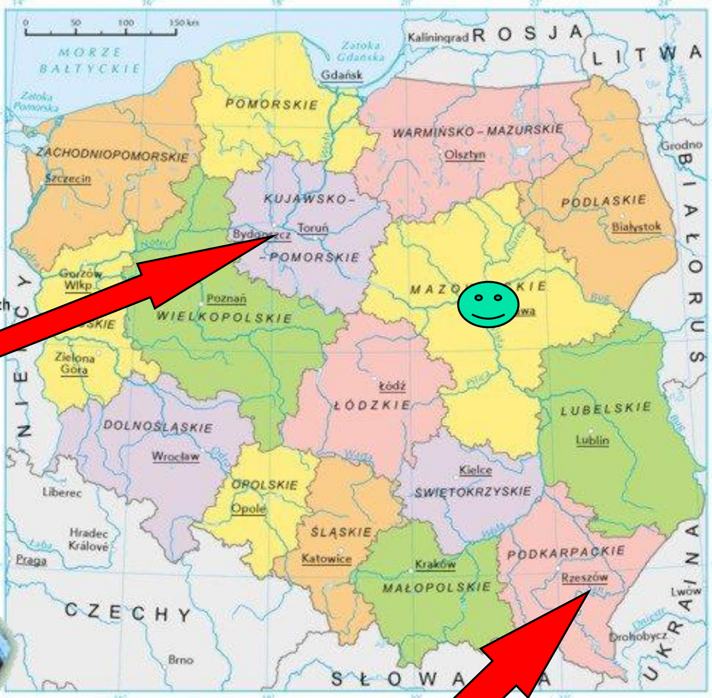
Seed orchard

Pinus silvestris

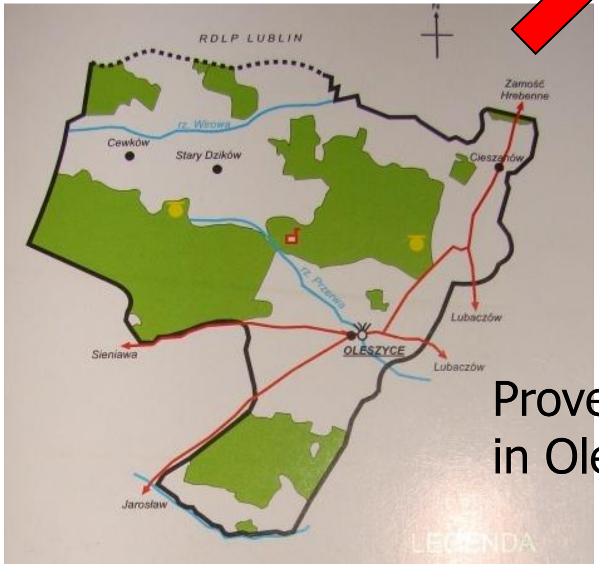
Provenance test

Quercus robur





Seed orchard in Gniewkowo Forestry



Provenance test
in Oleszyce Forestry

Seed orchard in Gniewkowo forestry

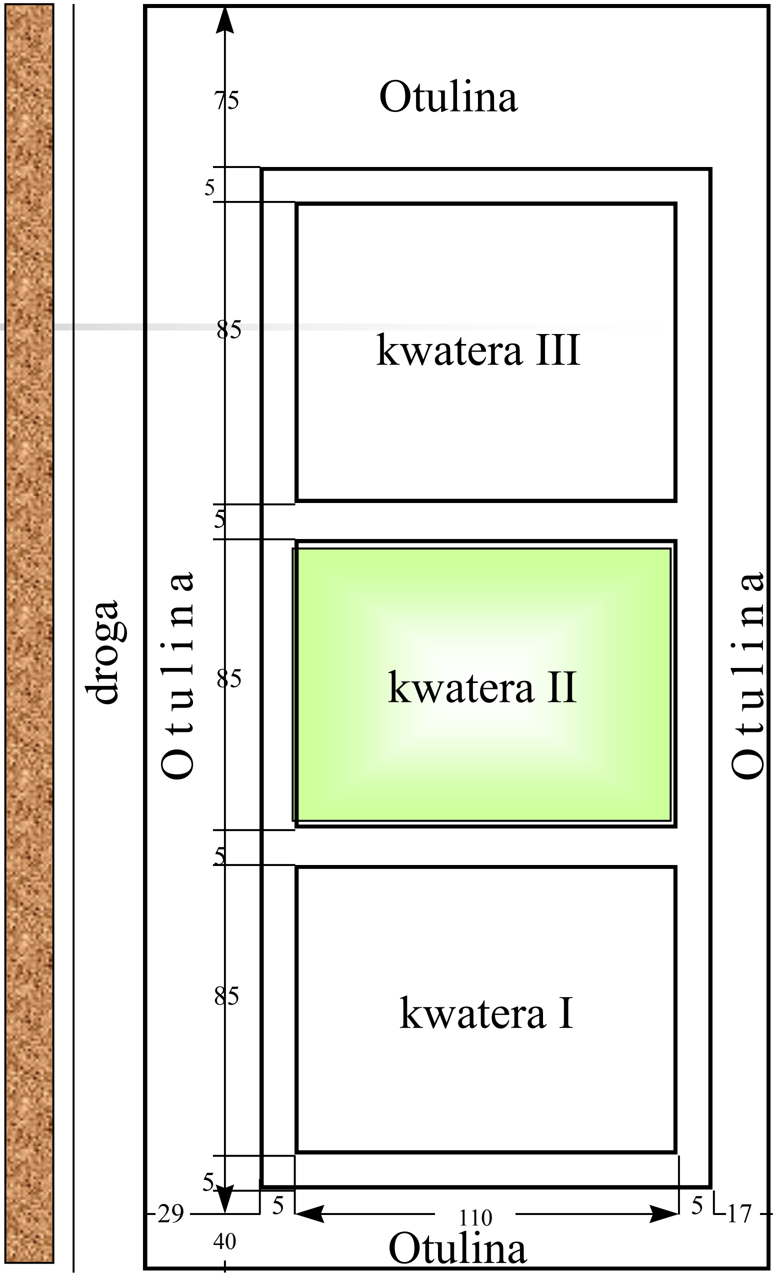
The aim of this study was to investigate reproductive processes in seed orchard

- Genetic diversity of parental and progeny population
- Mating system and pollen dispersal
- Effective population size of male paterns

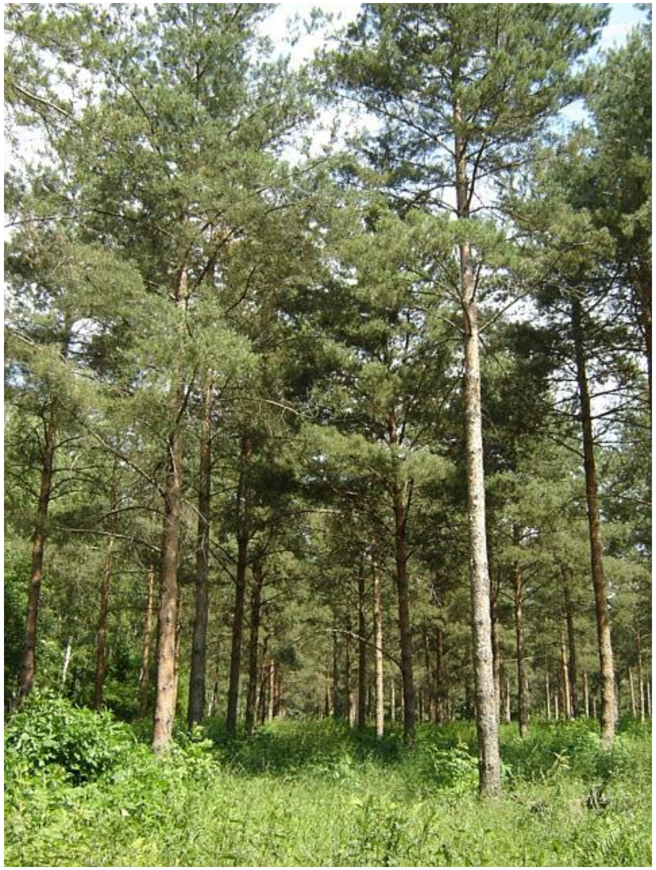
32 clones **1122** ramets

29 clones **187** ramets

1972-73 **3.3** ha



Location of clones in seed orchard



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2	229				223	220		214		214	228		229	225		231	223
3					228		213		225		235		211				228
4					235	217	232		231	212			240	230		237	235
5	227										223				236		238
6	233	219			230		233		221	218	**230**		233		221		
7	229	214		218		220					223		229		225		
8			224	217			**239**			220		236			225	215	
9	220				235		240		212		234		232				232
10			236	225		232		236	**219**	223			218		236		
11	233		221				233		239		230		233		221		230
12	229		233		223		219	236	213	213	228	231		217	238		223
13					228		231		**225**		235		234			220	
14	212	220			235	216	236		231		225		240		212		219
15	233		236		234		215		240		**232**	220			234		
16	233	225			230		233	230	235	230	230		233			239	230
17	229		233				229		229		223				238		228
18	234	232	224		219		234	231		217	228		234			223	235
19	220						240		212		237		232		212		
20	218		236		237	220		224	235	239	240		220	224		240	214
21	233		226		229		233		226				233		226		233
22		219	238	216	217			230			217	223		225	238		217

Characteristics of nuclear microsatellites
(*Pinus sylvestris*, seed orchard)

Locus		Sequence 5’ – 3’
PtTx3025	**(CAA)$_{10}$**	**F: TTC TAT ATT CGC TTT TAG TTT C** **R: CTA TTT GAG TTA AGA AGG GAG TC**
PtTx3107	**(CAT)$_{14}$**	**F: AAA CAA GCC CAC ATC GTC AAT C** **R: TCC CCT GGA TCT GAG GA**
PtTx 3116	**(TTG)$_{7}$(TTG)$_{5}$**	**F: CCT CCC AAA GCC TAA AGA AT** **R: CAT ACA AGG CCT TAT CTT ACA GAA**
PtTx4001	**(GT)$_{15}$**	F: CTA TTT GAG TTA AGA AGG GAG TC R: CTG TGG GTA GCA TCA TC
Spag7.14	**(AT)$_{5}$(GT)$_{19}$**	**F: TCA CAA AAC ACG TGA TTC ACA** **R: GAA AAT AGC CCT GTG TGA GAC A**
Spac12.5	**(GT)$_{20}$(GA)$_{10}$**	F: CTT CCT CAC TAG TTT CCT TTG G R: TTG GTT ATA GGC ATA GAT TGC

Localtion of clones in seed orchard after corrections

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1		211	221	*214*			*233*		221		234	220	233		221		234
2	*236*				223	220		214		214	228		*310*	225		231	223
3					228		213		*221*		235		211				*228*
4					235	*220*	*213*		*301*	212			240	230		237	235
5	227										223				236		*213*
6	233	219			230		*232*		*302*	218	230		233		221		
7	*309*	214		218		220					223		229		225		
8			224	217			*221*			*307*		236			225	215	
9	*229*				235		240		212		234		232				232
10			236	225		*220*		*315*	219	223			218		236		
11	233		221				*240*		*221*		230		233		221		230
12	*235*		233		223		*306*	236	*233*	213	228	231		*305*	238		223
13					228		*238*		225		235		234			220	
14	*219*	220			235	216	236		*301*		225		240		212		219
15	*219*		236		234		*303*		240		232	220			234		
16	*313*	225			230		*312*	230	235	*236*	230		233			*216*	230
17	*304*		233				229		229		*230*				238		228
18	234	232	224		219		*233*	*232*		217	228		234			*308*	235
19	*302*						*234*		212		237		*231*		212		
20	218		236		237	220		224	235	*314*	240		220	224		*216*	*211*
21	233		226		*219*		*240*		226				233		226		233
22		*212*	*236*	216	*229*			*224*			217	223		225	238		*234*

Genetic diversity

Genetic diversity of parental population

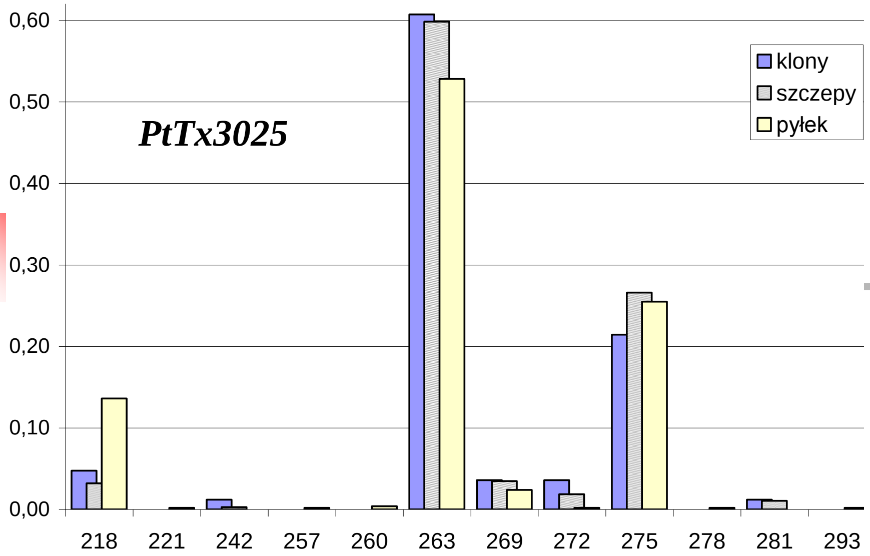
Locus	***A***	A_e	H_e	H_o	***PE(1)***	***HW***	***Null***	***F***
PtTx3025	8	2.38	0.581	0.619	0.187	NS	-0.07	-0.07
PtTx3107	10	6.41	0.844	0.667	0.501	NS	0.12	0.21
PtTx3116	9	4.18	0.761	0.810	0.350	NS	-0.05	-0.06
PtTx4001	9	3.67	0.728	0.762	0.318	NS	-0.04	-0.05
Spag7.14	26	23.8	0.958	0.905	0.805	NS	0.02	0.06
Spac12.5	24	19.23	0.948	0.952	0.772	NS	-0.01	0.00
average	14.33	9.94	0.803	0.785	0.992		0.005	0.015

A – number of allels. A_e – effectiv number of allels. H_o H_e – observed and expected heterozygosity. *PE(1)* - exclusion probability. F = 1 – (Ho/He)

Genetic diversity of offspring population

Locus	A	A_e	H_e	H_o	$PE(1)$	HW	Null	F
PtTx3025	11	2.56	0.609	0.736	0.204	**	-0.109	-0.21
PtTx3107	10	3.23	0.690	0.829	0.284	**	-0.111	-0.20
PtTx3116	14	4.18	0.761	0.915	0.366	**	-0.099	-0.20
PtTx4001	11	3.26	0.693	0.797	0.291	**	-0.081	-0.15
Spag7.14	32	8.85	0.887	0.855	0.633	NS	0.0169	0.04
Spac12.5	33	11.49	0.913	0.952	0.703	NS	-0.022	-0.04
average	19.33	5.9	0.759	0.847	0.972		-0.068	-0.127

A – number of allels. A_e – effectiv number of allels. H_o H_e – observed and expected heterozygosity.
PE(1) - exclusion probability. F = 1 – (Ho/He)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klony
szczepy
pyłek
0,60
0,50
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0,30
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0,10
0,00
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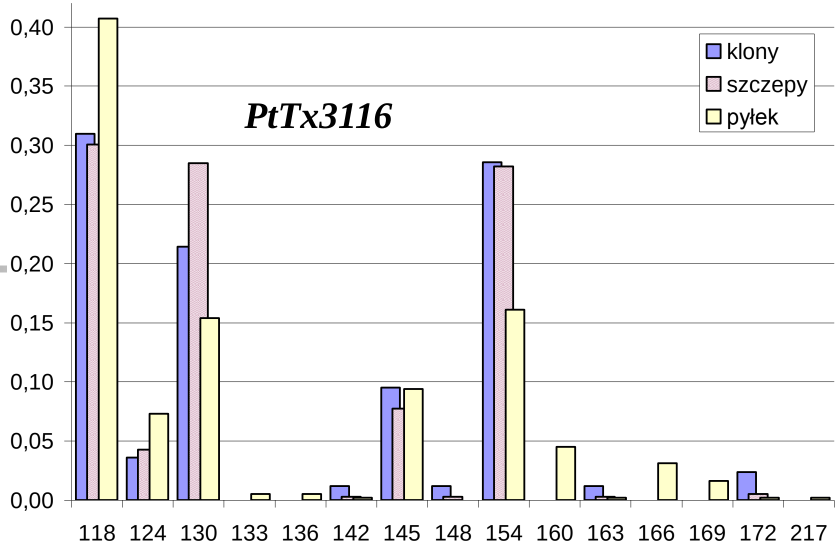
221
242
257
260
263
269
272
275
278
281
293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szczepy
pyłek
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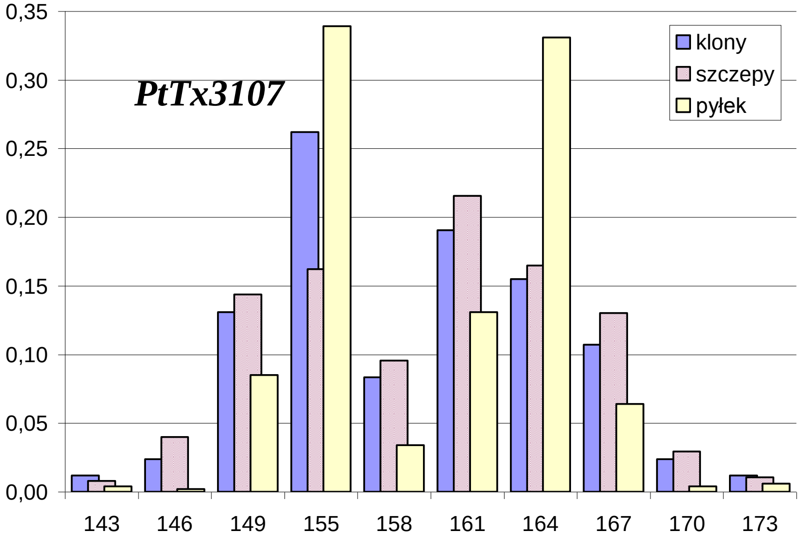
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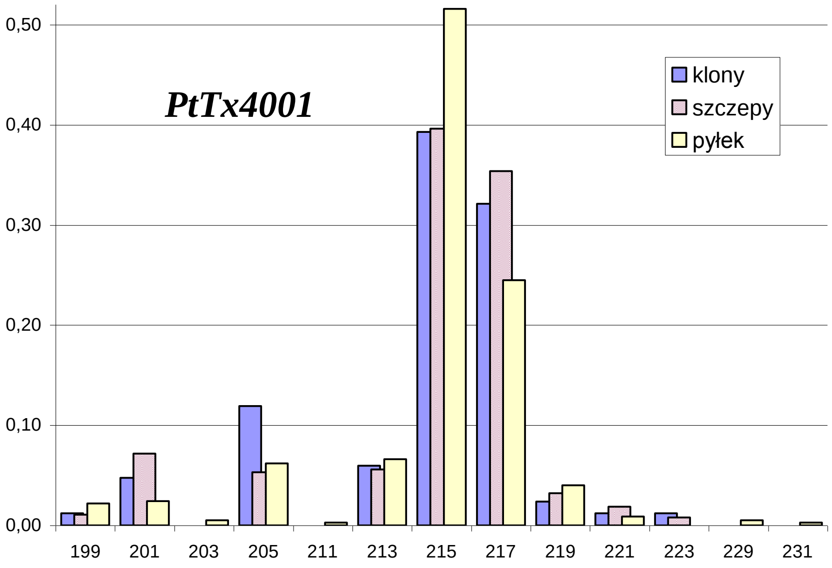
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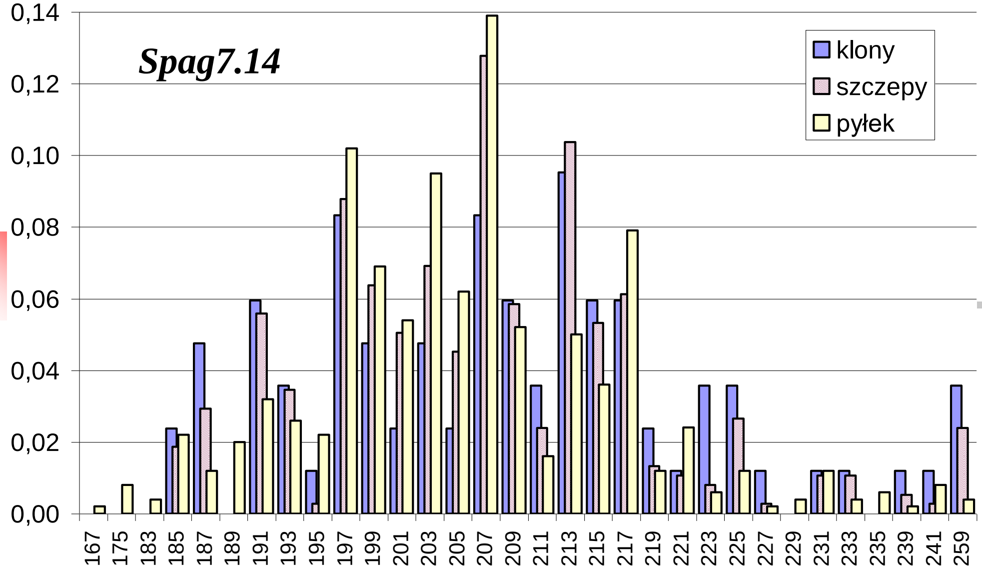
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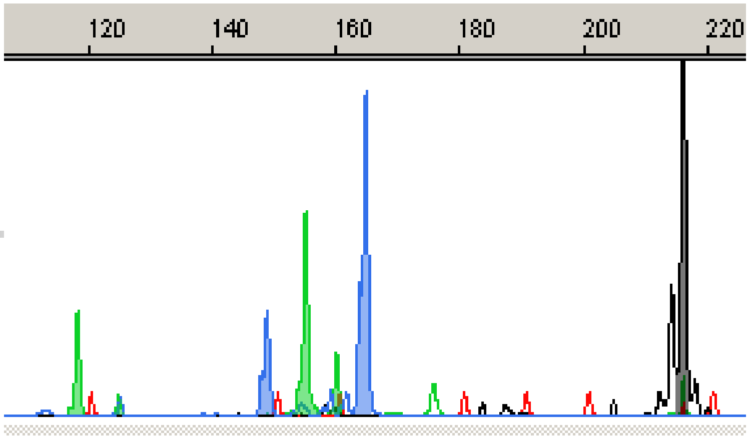
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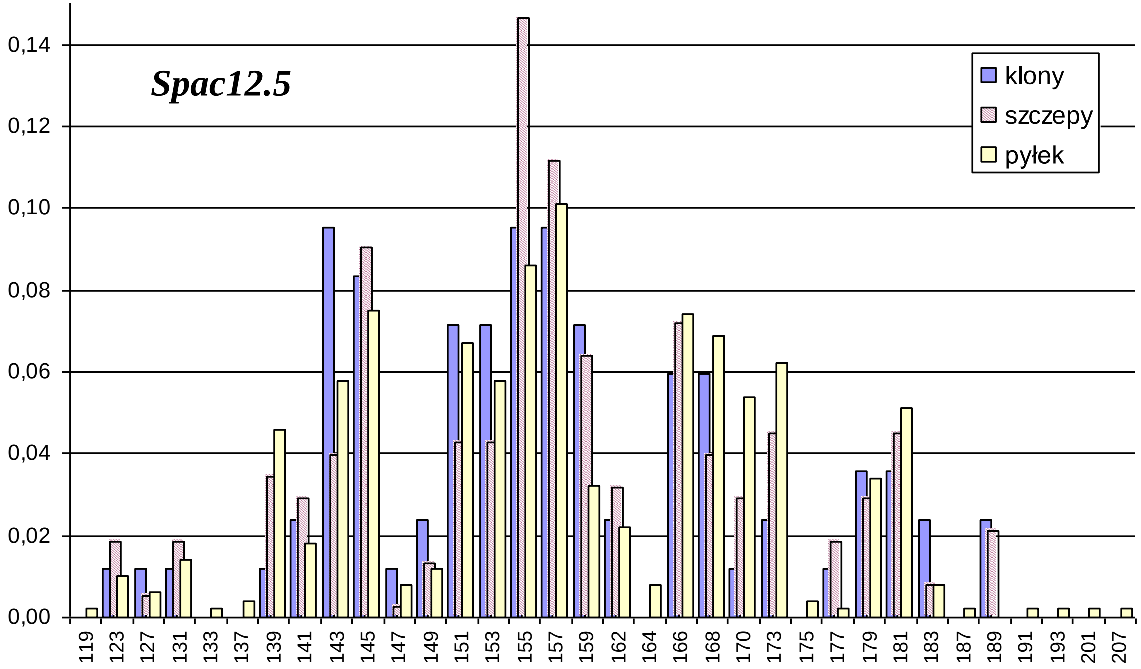
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klony
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pyłek
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0,08
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0,02
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127
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187
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191
193
201
207

Effective population size

Effective population size of male parents calculated based on different methods

Methods used to calculate effective population size	***Ne***
Variance of allele frequencies – $N_{e(v)}$ Wariancja częstości alleli	24.80
Correlation of paternity analysis – $N_{e(r)}$ Analiza korelacji ojcostwa	21.74
Genetic structure of pollen pool TWOGENER - $N_{e(p)}$	52.57
Paternity analysis – reproductive success $N_{e(f)}$ Analiza ojcostwa – sukces kojarzenia -	17.14

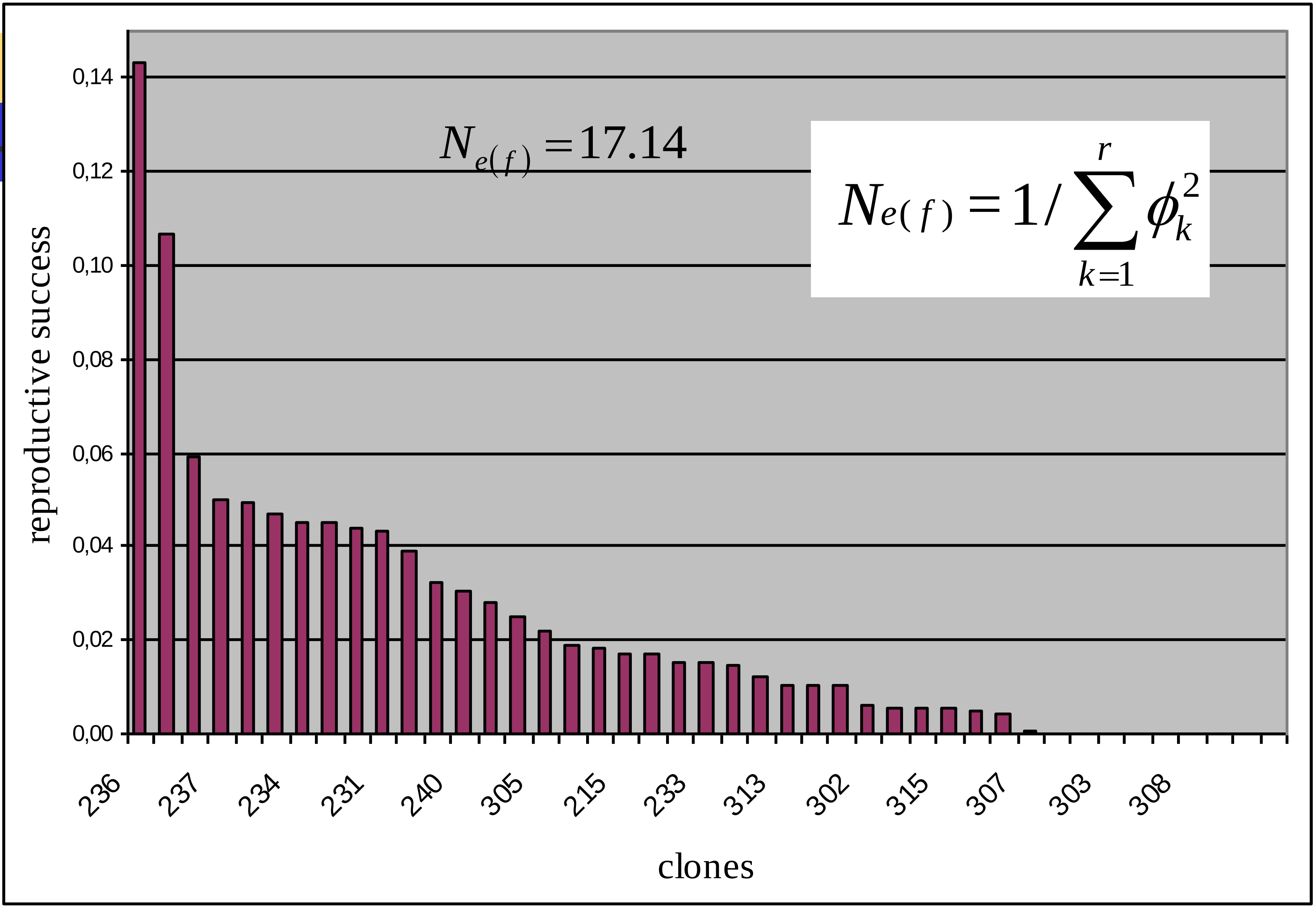
Variance effective population size

Locus	**$N_{e(v)}$**
PtTx3025	10.96
PtTx3107	8.02
PtTx3116	20.07
PtTx4001	28.25
Spag7.14	54.58
Spac12.5	77.00
	24.80

$$N_{e(v)} = \frac{(n-1)}{2(f_{(p)}n-1)}$$

Roberds et al. 1991, Burczyk 1996,

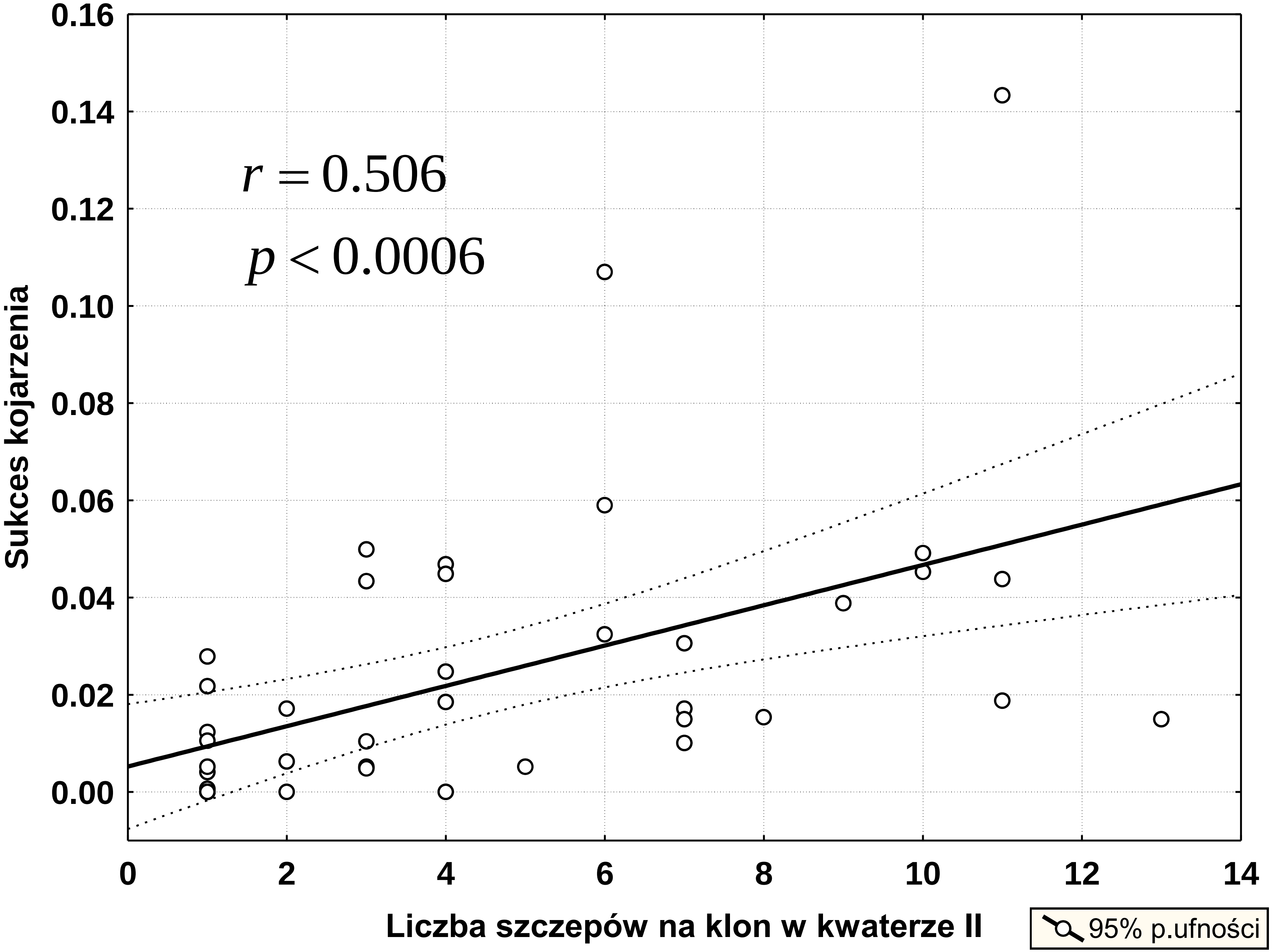
Effective population size - paternity analysis



$$N_{e(f)} = 17.14$$

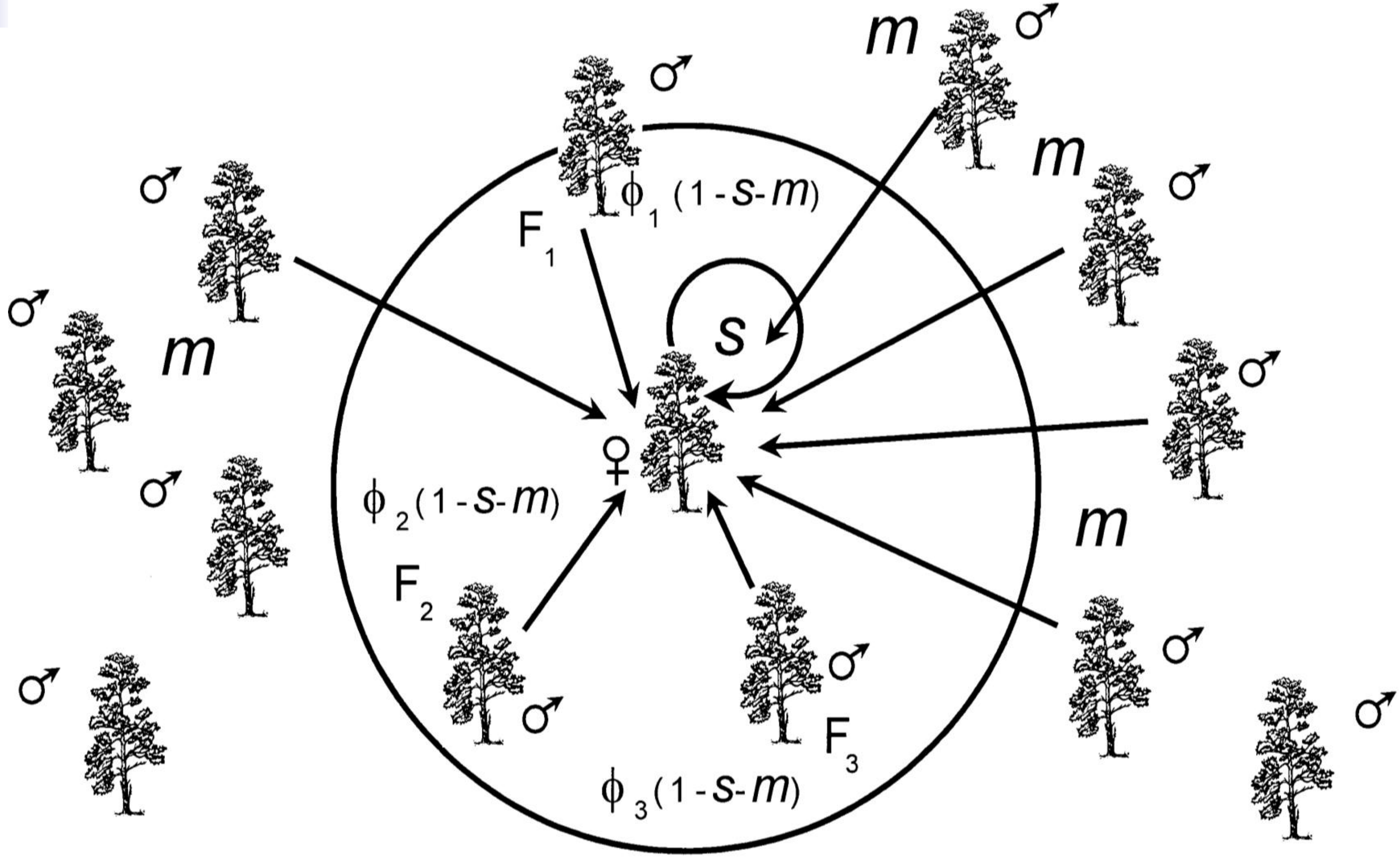
$$N_{e(f)} = 1 / \sum_{k=1}^{r} \phi_k^2$$

Highly variable contribution of individual clones in production of progeny population



Relationship between reproductive success of clons and the number of ramets per clone

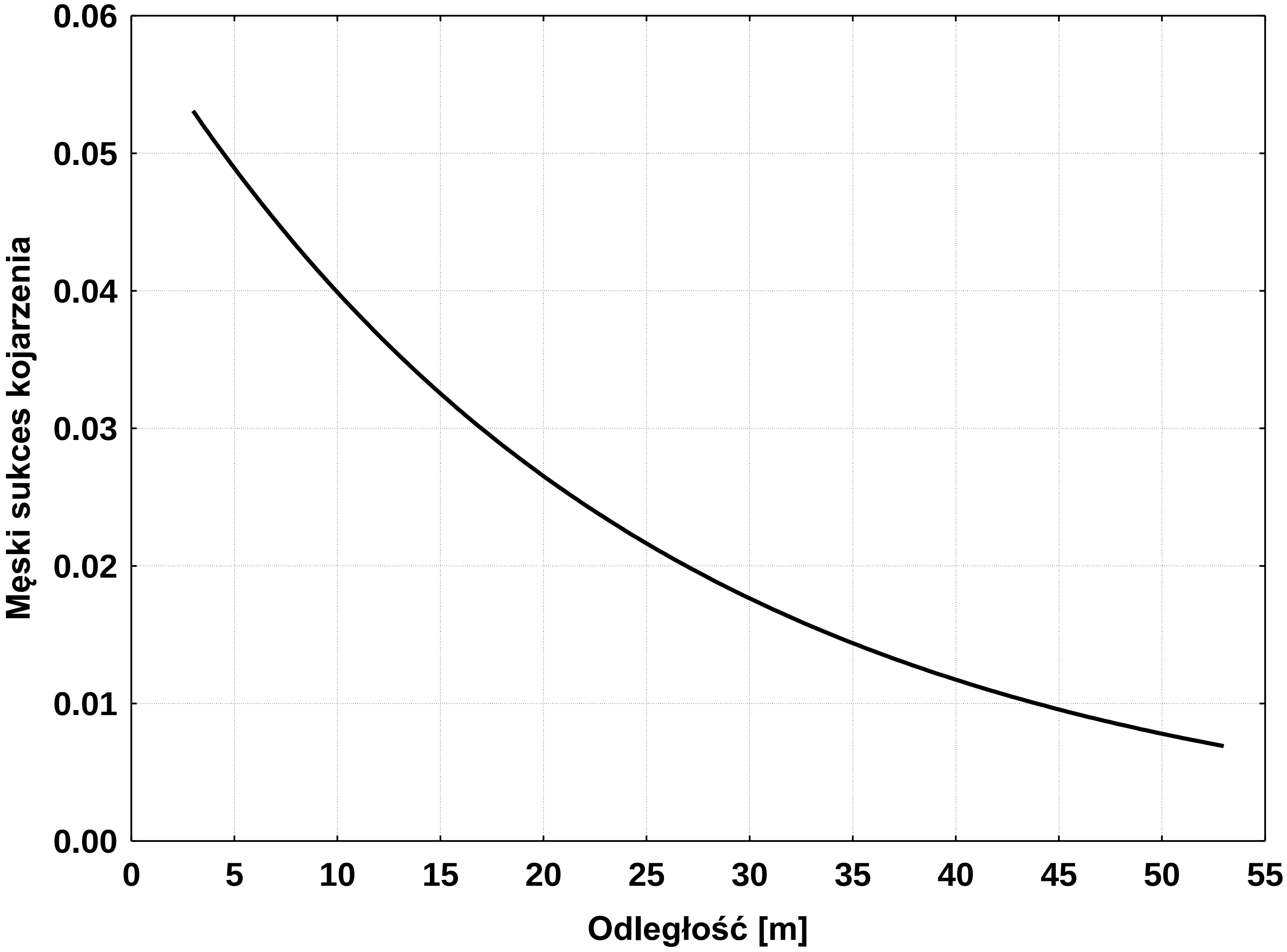
Determinants of male reproductive success



Determinants of male reproductive success

Model	Imigration (m)	Determinants of miting success			$N_{e(s)}$ ($\%N_{e(s)}/N$)
		Distance (β)	Fecundity (γ)	Diameter (δ)	
$m\ \beta$	**0.6034 (0.0326)**	**-0.0408 (0.0137)**	-	-	124.2 (66.4%)
$m\ \gamma$	**0.6118 (0.0329)**	-	**0.2885 (0.0960)**	-	139.9 (74.7%)
$m\ \delta$	**0.5966 (0.0326)**	-	-	**0.1868 (0.0564)**	150.9 (80.7%)
$m\ \beta\ \gamma\ \delta$	**0.5899 (0.0323)**	**-0.0302 (0.0114)**	**0.2785 (0.0762)**	**0.1643 (0.0594)**	92.7 (49.6%)

$N_{e(s)}$ – effective number of ramets.
$\%N_{e(s)}/N$ – percet effevtive number of ramets to number of ramets



Relationship between mating success and distance from maternal trees.

Results

1. **Genetic diversity and genetic structure :**

- Genetic diversity of offspring population is similar to genetic diversity paternal population.

- Inbreding level is similar in both ppulation.

2. Mating system and level of pollen imigration:

- Level of self-fertilization is low (close to 0)

- Level of pollen immigration is large (60%)

3. Effective number of male paterns:

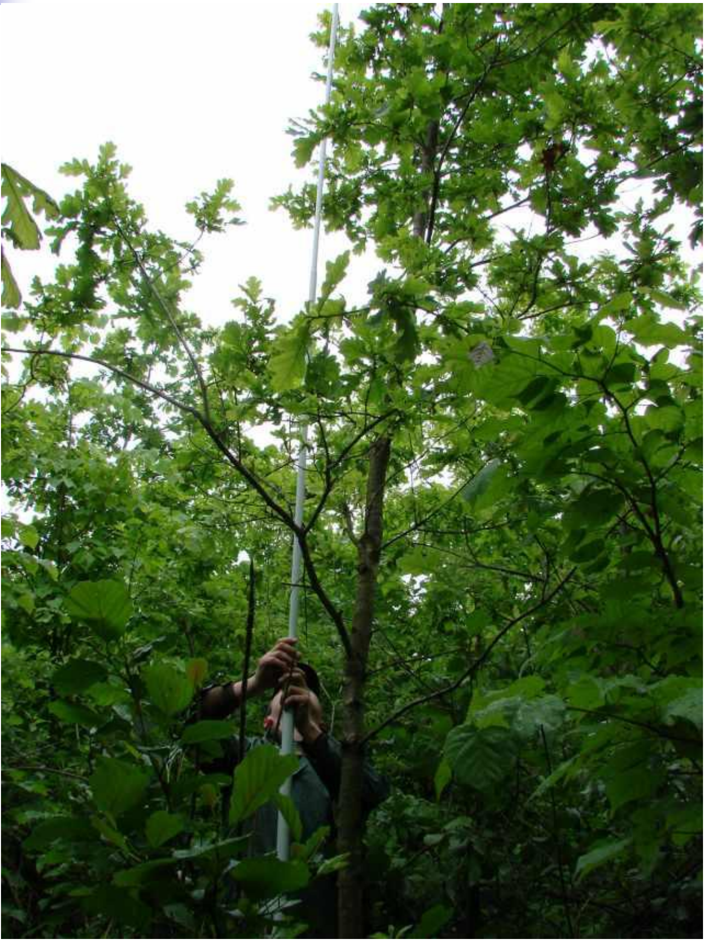
- Effective population size of male patrens is extensive (17 – 52, pollen pool imigration 22-79)

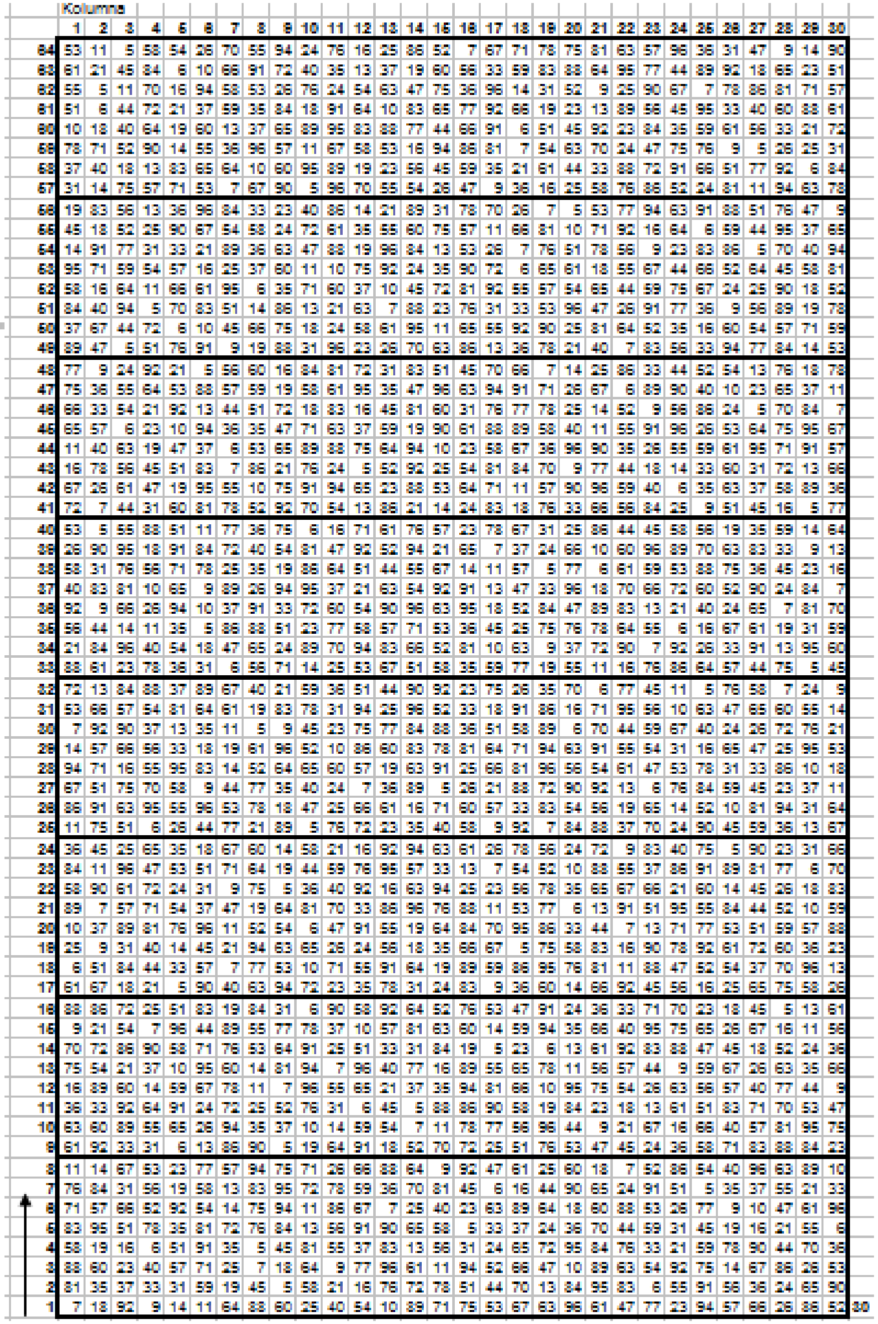
  and comparable among different methods

4. Male mating success of individual ramets depends on:

- Distance to sampled mothers
- Flowering intensity and tree diameter

Provenance test in Oleszyce Forestry





	Kolumna																													
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64	53	11	5	58	54	26	70	55	94	24	76	16	25	86	52	7	67	71	78	75	81	63	57	96	36	31	47	9	14	90
63	61	21	45	84	6	10	66	91	72	40	35	13	37	19	60	56	33	59	83	88	64	95	77	44	89	92	18	65	23	51
62	55	5	11	70	16	94	58	53	26	76	24	54	63	47	75	36	96	14	31	52	9	25	90	67	7	78	86	81	71	57
61	51	6	44	72	21	37	59	35	84	18	91	64	10	83	65	77	92	66	19	23	13	89	56	45	95	33	40	60	88	61
60	10	18	40	64	19	60	13	37	65	89	95	83	88	77	44	66	91	6	51	45	92	23	84	35	59	61	56	33	21	72
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56	19	83	56	13	36	96	84	33	23	40	86	14	21	89	31	78	70	26	7	5	53	77	94	63	91	88	51	76	47	9
55	45	18	52	25	90	67	54	58	24	72	61	35	55	60	75	57	11	66	81	10	71	92	16	64	6	59	44	95	37	65
54	14	91	77	31	33	21	89	36	63	47	88	19	96	84	13	53	26	7	76	51	78	56	9	23	83	86	5	70	40	94
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49	89	47	5	51	76	91	9	19	88	31	96	23	26	70	63	86	13	36	78	21	40	7	83	56	33	94	77	84	14	53
48	77	9	24	92	21	5	56	60	16	84	81	72	31	83	51	45	70	66	7	14	25	86	33	44	52	54	13	76	18	78
47	75	36	55	64	53	88	57	59	19	58	61	95	35	47	96	63	94	91	71	26	67	6	89	90	40	10	23	65	37	11
46	66	33	54	21	92	13	44	51	72	18	83	16	45	81	60	31	76	77	78	25	14	52	9	56	86	24	5	70	84	7
45	65	57	6	23	10	94	36	35	47	71	63	37	59	19	90	61	88	89	58	40	11	55	91	96	26	53	64	75	95	67
44	11	40	63	19	47	37	6	53	65	89	88	75	64	94	10	23	58	67	36	96	90	35	26	55	59	61	95	71	91	57
43	16	78	56	45	51	83	7	86	21	76	24	5	52	92	25	54	81	84	70	9	77	44	18	14	33	60	31	72	13	66
42	67	26	61	47	19	95	55	10	75	91	94	65	23	88	53	64	71	11	57	90	96	59	40	6	35	63	37	58	89	36
41	72	7	44	31	60	81	78	52	92	70	54	13	86	21	14	24	83	18	76	33	66	56	84	25	9	51	45	16	5	77
40	53	5	55	88	51	11	77	36	75	6	16	71	61	76	57	23	78	67	31	25	86	44	45	58	56	19	35	59	14	64
39	26	90	95	18	91	84	72	40	54	81	47	92	52	94	21	65	7	37	24	66	10	60	96	89	70	63	83	33	9	13
38	58	31	76	56	71	78	25	35	19	86	64	51	44	55	67	14	11	57	5	77	6	61	59	53	88	75	36	45	23	16
37	40	83	81	10	65	9	89	26	94	95	37	21	63	54	92	91	13	47	33	96	18	70	66	72	60	52	90	24	84	7
36	92	9	66	26	94	10	37	91	33	72	60	54	90	96	63	95	18	52	84	47	89	83	13	21	40	24	65	7	81	70
35	56	44	14	11	35	5	86	88	51	23	77	58	57	71	53	36	45	25	75	76	78	64	55	6	16	67	61	19	31	59
34	21	84	96	40	54	18	47	65	24	89	70	94	83	66	52	81	10	63	9	37	72	90	7	92	26	33	91	13	95	60
33	88	61	23	78	36	31	6	56	71	14	25	53	67	51	58	35	59	77	19	55	11	16	76	86	64	57	44	75	5	45
32	72	13	84	88	37	89	67	40	21	59	36	51	44	90	92	23	75	26	35	70	6	77	45	11	5	76	58	7	24	9
31	53	66	57	54	81	64	61	19	83	78	31	94	25	96	52	33	18	91	86	16	71	95	56	10	63	47	65	60	55	14
30	7	92	90	37	13	35	11	5	9	45	23	75	77	84	88	36	51	58	89	6	70	44	59	67	40	24	26	72	76	21
29	14	57	66	56	33	18	19	61	96	52	10	86	60	83	78	81	64	71	94	63	91	55	54	31	16	65	47	25	95	53
28	94	71	16	55	95	83	14	52	64	65	60	57	19	63	91	25	66	81	96	56	54	61	47	53	78	31	33	86	10	18
27	67	51	75	70	58	9	44	77	35	40	24	7	36	89	5	26	21	88	72	90	92	13	6	76	84	59	45	23	37	11
26	86	91	63	95	55	96	53	78	18	47	25	66	61	16	71	60	57	33	83	54	56	19	65	14	52	10	81	94	31	64
25	11	75	51	6	26	44	77	21	89	5	76	72	23	35	40	58	9	92	7	84	88	37	70	24	90	45	59	36	13	67
24	36	45	25	65	35	18	67	60	14	58	21	16	92	94	63	61	26	78	56	24	72	9	83	40	75	5	90	23	31	66
23	84	11	96	47	53	51	71	64	19	44	59	76	95	57	33	13	7	54	52	10	88	55	37	86	91	89	81	77	6	70
22	58	90	61	72	24	31	9	75	5	36	40	92	16	63	94	25	23	56	78	35	65	67	66	21	60	14	45	26	18	83
21	89	7	57	71	54	37	47	19	64	81	70	33	86	96	76	88	11	53	77	6	13	91	51	95	55	84	44	52	10	59
20	10	37	89	81	76	96	11	52	54	6	47	91	55	19	64	84	70	95	86	33	44	7	13	71	77	53	51	59	57	88
19	25	9	31	40	14	45	21	94	63	65	26	24	56	18	35	66	67	5	75	58	83	16	90	78	92	61	72	60	36	23
18	6	51	84	44	33	57	7	77	53	10	71	55	91	64	19	89	59	86	95	76	81	11	88	47	52	54	37	70	96	13
17	61	67	18	21	5	90	40	63	94	72	23	35	78	31	24	83	9	36	60	14	66	92	45	56	16	25	65	75	58	26
16	88	86	72	25	51	83	19	84	31	6	90	58	92	64	52	76	53	47	91	24	36	33	71	70	23	18	45	5	13	61
15	9	21	54	7	96	44	89	55	77	78	37	10	57	81	63	60	14	59	94	35	66	40	95	75	65	26	67	16	11	56
14	70	72	86	90	58	71	76	53	64	91	25	51	33	31	84	19	5	23	6	13	61	92	83	88	47	45	18	52	24	36
13	75	54	21	37	10	95	60	14	81	94	7	96	40	77	16	89	55	65	78	11	56	57	44	9	59	67	26	63	35	66
12	16	89	60	14	59	67	78	11	7	96	55	65	21	37	35	94	81	66	10	95	75	54	26	63	56	57	40	77	44	9
11	36	33	92	64	91	24	72	25	52	76	31	6	45	5	88	86	90	58	19	84	23	18	13	61	51	83	71	70	53	47
10	63	60	89	55	65	26	94	35	37	10	14	59	54	7	11	78	77	56	96	44	9	21	67	16	66	40	57	81	95	75
9	61	92	33	31	6	13	86	90	5	19	64	91	18	52	70	72	25	51	76	53	47	45	24	36	58	71	83	88	84	23
8	11	14	67	53	23	77	57	94	75	71	26	66	88	64	9	92	47	61	25	60	18	7	52	86	54	40	96	63	89	10
7	76	84	31	56	19	58	13	83	95	72	78	59	36	70	81	45	6	16	44	90	65	24	91	51	5	35	37	55	21	33
6	71	57	66	52	92	54	14	75	94	11	86	67	7	25	40	23	63	89	64	18	60	88	53	26	77	9	10	47	61	96
5	83	95	51	78	35	81	72	76	84	13	56	91	90	65	58	5	33	37	24	36	70	44	59	31	45	19	16	21	55	6
4	58	19	16	6	51	91	35	5	45	81	55	37	83	13	56	31	24	65	72	95	84	76	33	21	59	78	90	44	70	36
3	88	60	23	40	57	71	25	7	18	64	9	77	96	61	11	94	52	66	47	10	89	63	54	92	75	14	67	86	26	53
2	81	35	37	33	31	59	19	45	5	58	21	16	76	72	78	51	44	70	13	84	95	83	6	55	91	56	36	24	65	90
1	7	18	92	9	14	11	64	88	60	25	40	54	10	89	71	75	53	67	63	96	61	47	77	23	94	57	66	26	86	52

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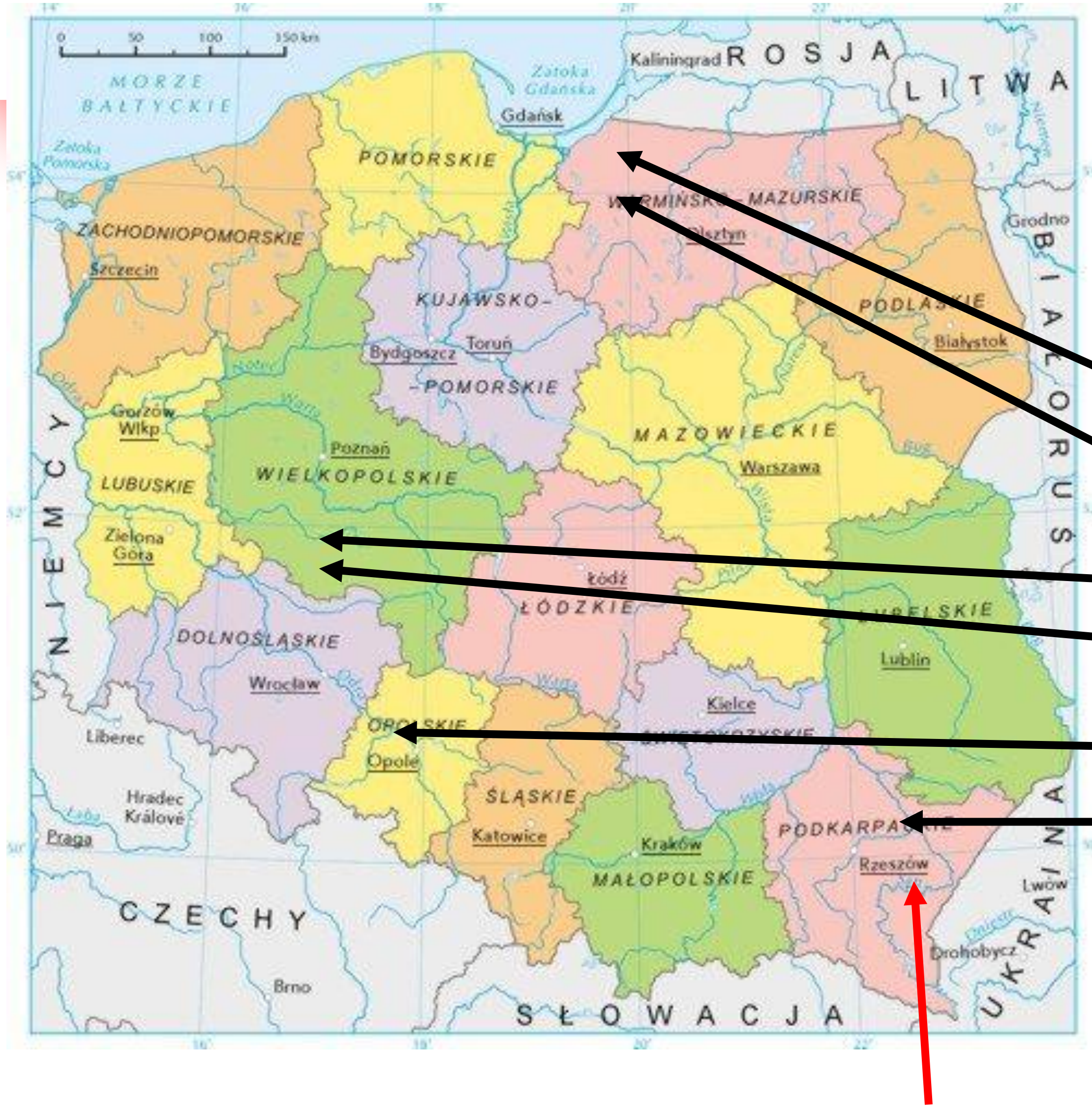

Provienience	**Number of half-sibs**
Młynary II	8
Młynary I	19
Milicz	9
Krotoszyn	8
Opole	8
Sieniawa	8
total	60

Provenence test in Oleszyce forestry

Microsatellites

Locus		Average size (Bp)	Sequence
ssrQrZAG 7	$(TC)_n$	150	5'-gca att aca ggc tag gct gg -3' 5'-gtc tgg acc tag ccc tca tg -3'
ssrQrZAG 20	$(TC)_n$	178	5'-cca tta aaa gaa gca gta ttt tgt -3' 5'-gca aca ctc agc cta tat cta gaa -3'
ssrQpZAG 9	$(AG)_n$	196	5'-gca att aca ggc tag gct gg -3' 5'-gtc tgg acc tag ccc tca tg -3'
ssrQpZAG 110	$(AG)_n$	234	5'-gga ggc ttc ctt caa cct act -3' 5'-gat ctc ttg tgt gct gta ttt -3'
MSQ 4	$(GA)_n$	219	5'-tct cct ctc ccc ata aac agg -3' 5'-gtt cct cta tcc aat cag tag tga g -3'

Aims of the study

1. To verify the composition of individual half-sibs (identify individuals that do not belong to particular half-sibs due to contamination at the time of trial establishment).

   Such contamination may inflate the variance of quantitative traits within ‘half-sibs’
2. To investigate effective number of males contributing to each half-sib.

   Low effective number of males may narrow the variance of quantitative traits within ‘half-sibs’

Aims of the study

1. Quantitative genetic analyses will be done based on initial (original) and corrected data to see the differences.

2. We will test if genetic markers can be efficiently used for verification of family trials.

Work done so far…

- Phenotypic traits are measured (tree diameter and height)

- All individuals are sampled and DNA is being isolated
- SSR analyses started…