Mating pattern and empty seed formation in relation to crown level of *Larix decidua* (Mill.) clones

Jarosław Burczyk, Grzegorz Kosiński & Andrzej Lewandowski

TIIVISTELMÄ: PÖLYTYSSUHTEET JA TYHJIEN SIEMENTEN OSUUS EUROOPANLEHTIKUUSEN VARTTEIDEN YLÄ- JA ALAOSASSA

Burczyk, J., Kosiński, G., Lewandowski, A. 1991. Mating pattern and empty seed formation in relation to crown level of *Larix decidua* (Mill.) clones. Tiivistelmä: Pölytyssuhteet ja tyhjien siementen osuus euroopanlehtikuusen vartteiden ylä- ja alaosassa. Silva Fennica 25(4): 201–205.

The mating system was analyzed in the upper and lower crown of two groups of European larch clones divided according to the percentage of full seeds in the upper and lower crown parts. The overall multilocus estimate of outcrossing rate (t) was calculated to be 0.929. The differences of outcrossing rates between crown levels and clonal groups respectively were not statistically significant. The t estimates were greater for the upper level and for clones with higher percentage of full seeds in the upper crown level. However, among all observations there was no correlation between outcrossing rates and percentages of full seeds for particular crown levels and groups of clone. Observed similarity of outcrossing among grafts of the same clones may indicate genetic control of self-fertilization rate in individual European larches.

Risteytymistapaa tutkittiin 22 vartteen latvusten ylä- ja alaosissa kahdesssa klooniryhmässä. Kohteena olleet neljä kloonia ryhmiteltiin latvuksen osien täysisiemenprosenttien mukaan. Keskimääräiseksi ristisiitosasteeksi (t) esti-moitiin multilokus-menetelmällä 0,929. Ristisiitosasteen erot latvuksen osien ja klooniryhmien välillä eivät olleet tilastollisesti merkitseviä. Ristisiitosaste oli korkein latvuksen yläosassa silloin, kun samalla täysisiemenprosentti oli ylä-osassa korkea. Havaittu samankaltaisuus saman kloonin eri vartteiden risti-siitososuudessa viittaa geneettiseen kontrolliin euroopanlehtikuusen itse-fertiilisyydessä.

Authors' address: Polish Academy of Sciences, Institute of Dendrology, 62-035 Kórnik, Poland.

1 Introduction

European larch (*Larix decidua* Mill.) is one of the most important native coniferous species in Central Europe, besides Scots pine and Norway spruce. Many countries that regard European larch as a promising species include the establishment of new seed orchards and the management of older but not always natural populations for seed production in their management plans.

The mating system of larches was studied occasionally (Knowles et al. 1987, Lewandowski et al. 1991) but there is no information about mating processes occurring within seed orchards. Because of heavy pollen grains, which have no air sacs, and frequent deficiency of pollen, the mating system in larch orchards may differ from that of other conifers.

Knowledge about conditions affecting the mating processes and consequently suitable pollen management would perhaps allow breeders to decrease empty seed formation, a serious problem in European larch (Kosiński 1986). The aim of this study is to measure the rate of outcrossing versus inbreeding in relation to empty seed percentage. Furthermore, variation among clones and the relationship between the lower and the upper part of the crown is studied.

2 Materials and methods

Cones were collected from several clones growing in the clonal archive of the Institute of Dendrology in Kórnik, Poland. Crown of each sampled tree was divided into two crown levels, lower and upper. The lower level was defined as up to 2 m from the ground and the upper level as above 2 m. The cones were collected as far from the division zone (2 m from the ground) as it was possible for a picker. There were 22 grafts from which a sufficient number of seeds was harvested for the lower and upper crowns. Additionally some trees were sampled only for one of the levels.

The proportion of full seeds was studied by dissecting 1000 seeds per sample. The proportion of full seeds was calculated as the percentage of all analyzed seeds.

The seeds were kept separate for maternal parent and stored dry below 0 °C until laboratory analysis. Electrophoresis and staining were performed according to Lewandowski and Mejnartowicz (1990). At least three loci: Mdh-1, Mdh-2 and Mdh-3 were used for the estimation of the mating system parameters.

Multilocus rates of outcrossing were calculated based on the maximum-likelihood procedures of Ritland and El-Kassaby (1985) that were developed for conifers, using an MLTF computer program. Homogeneity of the outcrossed pollen pool was analyzed by the method of Brown et al. (1975). Wright's fixation indices were calculated according to Nei (1975).

3 Results

Based on the percentage of full seeds at a particular crown level the 22 sample trees were scored into two groups: 14 grafts with higher percentage of full seeds in the upper level (clone group A) and 8 grafts with higher percentage of full seeds in the lower crown level (clone group B). Thus there were four analytical samples: two groups of trees with two crown levels. Overall the percentage of full seeds was higher for the upper crown portion and the clone group A (Table 1).

The overall outcrossing rate (t) estimated on the basis of 33 sample grafts was 0.929. Multilocus estimates of t made using seeds collected from the upper and lower portion of the crown of two clone groups are given in Table 2. Estimates of t were higher for the upper crown samples in both cases. The mean outcrossing for upper-crown samples is 0.961 while that for lower-crown samples is 0.907. The lowest t estimate was observed for lower crown portion of clone group B and the highest one for the upper crown portion of the same group. Thus the difference between upper and lower crown levels was expressed distinctly within clone group B. The differences of outcrossing rates were not statistically significant among clone groups and crown levels. There was no correlation between outcrossing rate and the percentage of full seeds either.

It was possible to estimate the individual outcrossing rates (t_i) for 22 of the 33 examined grafts. The average number of analyzed seeds sampled per family was about 44 (from 30 to 68). Individual t estimates ranged from 0.707 to 1.249, with a mean of 0.908 and there were significant differences among sampled trees ($p < 0.022$, $\chi^2 = 36.01$). However, the outcrossing pollen pool was homogeneous across sampled trees indicating that the variation of the mating system among grafts is not due to spatial variation in the pollen dispersal. Estimates for three grafts, which belong to clone group B, were significantly different from that expected under full outcrossing.

Among 22 trees sampled for individual t estimates there were four clones represented by more than two grafts, which enabled comparison within and among clones in outcrossing rate (Table 3). One-way ANOVA indicated significant variation among clones ($p < 0.005$, F =

10.3), but this is mainly due to the difference between clone 10-08 and the others.

The level of inbreeding as measured by Wright's fixation index was calculated to be –0.084 and +0.035 in parental and filial generations respectively indicating small but not significant ($p > 0.05$) increase of homozygosity in the progeny.

Table 1. Percentage of full seeds in particular crown levels and groups of clones (n = number of sampled grafts).

Crown level	A Clones with higher percent. of full seeds in upper level	B Clones with higher percent. of full seeds in lower level	Mean
Upper level	57.1 % (n = 14)	34.0 % (n = 8)	48.7 % (n = 22)
Lower level	40.6 % (n = 14)	42.4 % (n = 8)	41.2 % (n = 22)
Mean	48.8 % (n = 14)	38.2 % (n = 8)	44.9 % (n = 22)

Table 2. Outcrossing rates (t) for particular crown levels and groups of clones, SD in brackets (n = number of sampled grafts).

Crown level	A Clones with higher percent. of full seeds in upper level	B Clones with higher percent. of full seeds in lower level	Pooled
Upper level	0.943 (0.062) n = 14	0.955 (0.065) n = 8	0.961 (0.045) n = 22
Lower level	0.927 (0.056) n = 14	0.845 (0.085) n = 8	0.907 (0.049) n = 22
Pooled	0.934 (0.041) n = 14	0.895 (0.055) n = 8	0.933 (0.033) n = 22

All studied grafts = 0.929 (0.020) n = 33

Table 3. Individual outcrossing rates for selected grafts.

Clone/graft number	Outcrossing rate (SE)	Sample size
10-08/da	1.078 (0.005)	30
10-08/db	1.170 (0.005)	30
10-17/da	0.803 (0.003)	30
10-17/db	0.777 (0.003)	30
10-17/dn	0.932 (0.113)	50
10-27/gb	0.929 (0.138)	50
10-27/d	0.783 (0.073)	66
10-27/d1	0.878 (0.112)	50
10-27/d2	0.824 (0.005)	50
10-27/d3	0.925 (0.112)	50
15-64/d	0.750 (0.004)	66
15-64/n	0.821 (0.082)	50

4 Discussion

The observed overall outcrossing rate suggests that only a small proportion of the viable seed from a clonal archive is due to selfing (7 %, according to s = 1 – t). This is in line with results previously reported for an old stand of European larch (t = 0.943) (Lewandowski et al. 1991), however, in contrast Knowles et al. (1987) found in tamarack (*Larix laricina*) a much lower outcrossing rate (t = 0.730).

Equilibrium populations exhibit a relationship between Wright's fixation index (F) and outcrossing rate (t) according to formula F = (1 – t)/(1 + t) (Allard et al. 1968). Estimates of t calculated on the basis of the F index observed in the progeny was calculated to be 0.932, which is very close to that observed (t = 0.929), indicating that inbreeding is mainly due to selfing.

Although there was no significant difference between crown levels, the outcrossing rates were in general higher in the upper crown part. If we would consider selfing instead of outcrossing (s = 1 – t) it is almost three times higher in the lower crown portion than in the upper one. This phenomenon is common for most of conifers (Shen et al. 1981, Shaw and Allard 1982, El-Kassaby et al. 1986, Omi and Adams 1986).

Omi and Adams (1986) observed that the significant difference in outcrossing between the upper and lower parts of crown in Douglas-fir was due to large differences in two of the five studied clones. It was suggested that most of the selfed offspring originate from a few highly self-fertile clones (Shaw and Allard 1982). The very low t estimates for lower level of clone group B could be due to high self-fertility of some clones in this group. Kosiński found that the number of embryonic lethals was only 2 for clones 10-27 and 15-63, which are included in clone group B (Kosiński 1986), while the average number of embryonic lethals was about 5.3 for remaining nine clones analyzed in that study. Thus someone could expect that the difference between upper and lower crown portion could be much greater in self fertile clones when selfing could be important. Elimination of such well known self fertile grafts when seeds are collected would decrease the inbreeding level due to selfing, which often results in reduced viability and bad growth characteristics in progeny.

In this study there was no relationships between outcrossing and percentage of full seeds. The relation would probably be more clear, when genetic load is the main factor affecting the mating system (Omi and Adams 1986).

The differences of outcrossing rates among clones, especially distinct, when clone 10-08 was compared to the others, may indicate the genetic control of outcrossing. But grafts of clone 10-08 expressed t estimates greater than unity which may be a symptom of negative assortative mating due to differences in male and female flowering phenology within a tree and between a tree and its surrounding grafts (Burczyk 1991). The investigated clonal archive was designed in a systematic layout (Giertych 1971), thus each graft of a clone has the same neighbours. Mating processes occurring among ramets in different part of the archive may cause similarity of the outcrossing rate among grafts of the same clone.

References

Allard, B.W., Jain, S.K. & Workman, P.L. 1968. The genetics of inbreeding populations. Advanced Genetics 14: 55–131.

Brown, A.H.D., Matheson, A.C. & Eldridge, K.G. 1975. Estimation of the mating system of Eucalyptus obliqua L'Herit. by using allozyme polymorphisms. Australian Journal of Botany 23: 931–949.

Burczyk, J. 1991. The mating system in a Scots pine clonal seed orchard in Poland. Annales des Sciences Forestiers. (in press).

Giertych, M. 1971. Systematic lay-outs for seed orchards. Silvae Genetica 20: 137–138.

El-Kassaby, Y.A., Parkinson, J. & Devitt, W.J.B. 1986. The effect of crown segment on the mating system in a Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seed orchard. Silvae Genetica 35(4): 149–155.

Knowles, P., Furnier, G.R., Aleksiuk, M.A. & Perry, D.J. 1987. Significant level of self-fertilization in natural populations of tamarack. Canadian Journal of Botany 65(6): 1087–1091.

Kosiński, G. 1986. Przyczyny powstawania pustych nasion u modrzewia europejskiego (Larix decidua Mill.). Summary: Causes of empty seed formation in European larch. Arboretum Kórnickie 31: 107–182.

Lewandowski, A. & Mejnartowicz, L. 1990. Genetic control of Polish larch (Larix decidua subsp. polonica (Racib.) Domin) malate dehydrogenase (EC 1.1.1.37). Genetica Polonica 31(3–4): 217–221.

— , Burczyk, L. & Mejnartowicz, L. 1991. Genetic structure and the mating system in an old stand of Polish larch. Silvae Genetica 40(2): 75–79.

Nei, M. 1975. Molecular population genetics and evolution. North-Holland Publishing Inc., Amsterdam. 288 p.

Omi, S.K. & Adams, W. T. 1986. Variation in seed set and proportions of outcrossed progeny with clones, crown position, and top pruning in a Douglas-fir seed orchard. Canadian Journal of Forest Research 16: 502–507.

Ritland, K. & El-Kassaby, Y.A. 1985. The nature of inbreeding in a seed orchard of Douglas fir as shown by an efficient multilocus model. Theoretical and Applied Genetics 71: 375–384.

Shaw, D. V. & Allard, R. W. 1982. Estimation of outcrossing rates in Douglas-fir using isozyme markers. Theoretical and Applied Genetics 62: 113–120.

Shen, H. H., Rudin, D. & Lindgren, D. 1981. Study of the pollination pattern in a Scots pine seed orchard by means of isozyme analysis. Silvae Genetica 30(1): 7–15.

Total of 14 references