Clonal Variation in Acorn Production and its Effect on the Effective Population Size in a *Quercus acutissima* Seed Orchard

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Abstract

Acorn production was surveyed for eight consecutive years (2000–2007) in a 94-clone Sawtooth oak (*Quercus acutissima*) seed orchard established in 1992. Acorn production commenced in 2000 and peaked in 2005 and was characterized by a 3–4 years interval. Sixty out of the orchard’s 94 clones were consistent producers across the study period. Acorn production’s Pearson product-moment and Spearman rank correlation coefficients were significant and consistently positive over the eight years study period. Parental cumulative reproductive output, represented by parental balance curves, slightly varied among mast years and showed steady improvement (less distortion) over years. Effective population size (\(N_p\)) was high in moderate and good acorn production years; however, departure from clonal equal contribution was observed throughout the study period.  

Parental effective population size was estimated under various scenarios of male fecundity (pollen production is: 1) proportional to clone size, 2) equal to female contribution, and 3) equal across all clones) resulted in high \(N_p\) and low group co-ancestry under equal male fecundity scenario while moderate \(N_p\) size and group co-ancestry were observed when male fecundity was assumed to be proportional to clone size (i.e., ramet number).

Key words: *Quercus acutissima*, contribution, parental balance, fertility variation, gene diversity, effective number, co-ancestry.

Introduction

*Quercus acutissima*, Sawtooth oak, is native to Korea, China, Japan and the Himalayas with adult trees reaching 15–20 meters in height. The species is deciduous, with monoecious trees that are widely planted and frequently used as a viable source for structure wood. Due to its high strength and density as well as its attractive color and grain pattern it is being extensively used in veneer and furniture production as well as tool handles. Sawtooth oak is also used as an ornamental tree and valued for its acorn production as a wildlife food source (LEE, 1979; KANG et al., 2005). The species female flowers are small and inconspicuous while male flowers are pendulous, messy with yellow-green catkins. Fruits are large acorns that are enclosed in involucres formed by the male flowers. The species is deciduous, with monoecious trees that are widely planted and frequently used as a viable source for structure wood. Due to its high strength and density as well as its attractive color and grain pattern it is being extensively used in veneer and furniture production as well as tool handles.

The standing volume of *Q. acutissima* is estimated to be close to 27% of the total forest trees inventory in Korea, highlighting the species ecological importance to the Korean forest ecosystem. The Korean *Q. acutissima* breeding program started during the 1950s with plus-tree selection from natural stands, production of wind-pollinated seedling for progeny testing, and the production of grafted scion material for Sawtooth oak seed orchards establishment. Following orchards establishment, high root stock-scion incompatibility was observed and new orchards were exclusively established with seedling rather than clonal material. At present, most of the *Q. acutissima* seed orchards have been established from seedlings following the late Richard Barnes’ (BARNES, 1995) breeding seed orchard concept (KANG et al., 2005).

In general, seed orchards constitute the predominant source of genetically improved seeds production for reforestation programs. The superiority of orchards’ seed depends primarily on the genetic quality of the selected parents as well as the orchards’ fulfillment of Hardy-Weinberg Expectations that require: a) random mating system, b) limited relatedness among orchard’s parents, c) reproductive phenology synchrony, d) reproductive output equality, e) minimum gene flow (i.e., pollen contamination), and f) minimum selfing (ERIKSSON et al., 1973).

Fecundity is important for population survival and resilience and is measured by individuals’ capacity to reproduce through the production of viable gametes (KREBS, 2008). Fecundity of trees within populations fluctuates according to environmental and biotic conditions such as site or climate conditions, nutrition competition and tree age (SORK et al., 1993; MASAKA and SATO, 2002). In seed orchards, fecundity variation describes the extent of reproductive output variability among clones, and thus is used to estimate the differences in their gametic contributions to offspring and the expected effective population sizes (genetic diversity) of their seedling crops.

The objectives of this study are to: 1) estimate the impact of female fecundity variation, as determined by clonal annual acorn production, on genetic diversity, 2) invoke three different male fecundity scenarios and evaluate their impact on genetic diversity assessment, and 3) discuss the results role on Sawtooth oak seed orchard management.

Methods and Materials

Seed orchard and acorn production survey

The studied Sawtooth oak seed orchard was established 1992 by clonal material (grafts) planted at 5 x 5 m
spacing and covering a 2 hectares. The orchard is located at Balan, Hwasung district, Northwest South Korea (lat. 37°08’N, long. 126°55’E, elevation 50 m). The orchard’s original intent was to provide dual function as a clonal archive for breeding and as an interim acorn production. Originally the seed orchard consisted of 113 clones arranged in a random single tree mix; however, due to mortality and scion-rootstock incompatibility this number was reduced to 94 clones (70.1% survival rate). Clone size average was 4.1 ramets (range: 1 to 17). Actual acorn production count was conducted on every individual tree over 8 consecutive years (2000–2007) using ground nets placed under each tree. Acorn count was conducted daily throughout the autumn season.

**Correlation and parental balance**

Pearson’s product-moment and Spearman’s rank correlations among years were calculated using the proc corr function of SAS program (1990). Polynomial regression curve with a degree of five was drawn based on the averages of acorns per year to depict acorn production over time and to predict the expected acorn production in future years using the plot function in Microsoft® Excel, 2000.

Parental balance was assessed by the cumulative curve of acorn production following the method of GRIFFIN (1982). Simply clones’ acorn production is ranked in ascending order and cumulative percentages over clones are estimated and plotted against the number of clones.

**Effective population size estimation and male reproductive assessment**

Clonal fecundity is defined as the potential reproductive capacity of the clone, measured by the number of acorns (for female) and estimate of male gametes under the three scenarios used (see below). Fecundity is known to be under both genetic and environmental control, and is the major measure of fitness (SAVOLAINEN et al., 1993; KJÆR and WELLENDORF, 1998).

In the present study, the effective number of parent (\( N_p \)) was used as the representative of effective population size, which is calculated solely based on fecundity variation (KANG and LINDGREN, 1999; BILIR et al. 2005; PRESCHER, 2007) as:

\[
N_p = \left( \sum_{i=1}^{N} f_i^2 \right)^{-1}
\]  

[1]

where \( f_i \) is the female fecundity of \( i \)th clone in the seed orchard.

We used clonal acorn production of the 8 assessed years, as a representative of female fecundity and male fecundity was estimated using the three following scenarios representing the range of male reproductive output options and in turn were included in turn were included in the estimation of clonal \( N_p \) as follows:

**Scenario A**: male fecundity is assumed to proportional to the number of ramet per clone (i.e., clone size),

\[
N_p = 1 / \sum_{i=1}^{N} p_i^2 = 1 / \sum_{i=1}^{N} \left( \frac{f_i + r_i}{2} \right)^2
\]  

[2]

**Scenario B**: male fecundity is assumed to be equal to female fecundity (i.e., sexual symmetry), and

\[
N_p = 1 / \sum_{i=1}^{N} p_i^2 = 1 / \sum_{i=1}^{N} \left( \frac{f_i + f_i}{2} \right)^2
\]  

[3]

**Scenario C**: male fecundity is assumed to be equal among clones and therefore equals the reverse of clone number (i.e., \( 1/N = 1/94 \)).

\[
N_p = 1 / \sum_{i=1}^{N} p_i^2 = 1 / \sum_{i=1}^{N} \left( \frac{f_i + 1/N}{2} \right)^2
\]  

[4]

where \( p_i \) is the total contribution of \( i \)th clone, \( f_i \) is the female contribution of \( i \)th clone (acorn production) and male contribution in the Scenario B, \( r_i \) is the ramet proportion of \( i \)th clone, and \( N \) is the census number of clone in the seed orchard. Assuming that clones were non-inbred and nor related, then group co-ancestry (\( \Theta \)) was calculated as:

\[
\Theta = 0.5 \sum p_i^2
\]  

[5]

and relative effective population size (\( N_r \)) was calculated as:

\[
N_r = N_p / N
\]  

[6]

**Results**

Acorn production fluctuated over years and reached its peak during 2005 with a clone average of 327.5 (± 496.5) and was lowest in 2000 with an average of 5.2 (± 11.9) acorns per clone (Table 1). In general, approximately 60 out of the 94 clones were consistent producers and yielded an acorn crop almost every year (Table 1).

Acorn production fluctuated and followed a 3 or 4-year interval period with the first cycle encompassed 2001 to 2005 while the second covered 2005 to 2008 (Fig. 1). All 94 clones produced acorns at least once during the eight-year assessment period. Polynomial regression depicted these cycles and predicted an increased acorn production in 2008 (\( R^2 = 0.64 \)) (Fig. 1). It should be stated that extrapolation outside of the polynomial regression range

<table>
<thead>
<tr>
<th>Year</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N )</td>
<td>22</td>
<td>56</td>
<td>64</td>
<td>60</td>
<td>61</td>
<td>63</td>
<td>61</td>
<td>58</td>
</tr>
<tr>
<td>Acorn</td>
<td>485</td>
<td>22,574</td>
<td>9,767</td>
<td>7,401</td>
<td>2,059</td>
<td>30,782</td>
<td>8,102</td>
<td>7,561</td>
</tr>
<tr>
<td>Average</td>
<td>5.2</td>
<td>240.1</td>
<td>103.9</td>
<td>78.7</td>
<td>21.9</td>
<td>327.5</td>
<td>86.2</td>
<td>80.4</td>
</tr>
<tr>
<td>( SD )</td>
<td>11.9</td>
<td>383.6</td>
<td>156.7</td>
<td>128.3</td>
<td>37.0</td>
<td>496.5</td>
<td>109.1</td>
<td>144.6</td>
</tr>
</tbody>
</table>

Table 1. Number of acorn producing clones (\( N \)), total acorn production, average acorn production per clone, and standard deviation of acorn production (\( SD \)) over 8 years in a clonal Q. acutissima seed orchard.
is unreliable and should be viewed with caution; however, the 2008 acorn production assessment supported this prediction (K.K-S., personal observation). This polynomial regression was exclusively based on the acorn production of only two cycles, however, the addition of other information such as orchard’s ambient precipitation and temperature and the extent of pest damage might have contributed to more insight into the production cycle of this species in this site.

With the exception of 2000 acorn production (very poor year), all Pearson’s product-moment and Spearman’s rank correlation coefficients were positive and highly significant (Table 2). The observed significant correlations indicate that the acorn production of any specific year is greatly influenced by the production of other years and also indicate that the production rank of individual clone is maintained its order over years (Table 2).

Parental balance curves expressed by the cumulative contribution of clonal acorn production over the 8 studied years showed pronounced level of distortion, indicating that the orchard production is far from the ideal situation of clonal equal contribution (Fig. 2; straight-line). While the 8 year curves showed a large distortion (80% of the acorn crop was contributed by 20–30% of the orchard’s clones), a steady improvement over time (decrease of the distortion level as the orchard ages) was found.

Effective population size ($N_p$) and relative effective population size ($N_r$) for the acorn crops did not reach their theoretical maximums of 94 and 100, respectively (Table 3). High $N_p$ values (> 26.5) were observed for four out of the studied 8 years (2001-02, 2005-06); however, these values were far from equal acorn contribution among clones but were independent of crop size (i.e., relatively high $N_p$ values were obtained during high and

![Figure 1. – Average acorn production per clone in the clonal Quercus acutissima seed orchard and the polynomial regression function depicting and predicting acorn production over the studied 8 year.](image)

![Table 2. – Acorn production Pearson’s (upper diagonal) and Spearman’s rank (lower diagonal) correlation coefficients among 8 assessment years.](image)

<table>
<thead>
<tr>
<th>Correlation</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>-</td>
<td>0.304</td>
<td>0.357*</td>
<td>0.220</td>
<td>0.293</td>
<td>0.104</td>
<td>0.192</td>
<td>0.264</td>
</tr>
<tr>
<td>2001</td>
<td>0.280</td>
<td>-</td>
<td>0.510**</td>
<td>0.412**</td>
<td>0.581**</td>
<td>0.494**</td>
<td>0.483**</td>
<td>0.558**</td>
</tr>
<tr>
<td>2002</td>
<td>0.303</td>
<td>0.530**</td>
<td>-</td>
<td>0.614**</td>
<td>0.559**</td>
<td>0.520**</td>
<td>0.613**</td>
<td>0.641**</td>
</tr>
<tr>
<td>2003</td>
<td>0.278</td>
<td>0.386**</td>
<td>0.468**</td>
<td>-</td>
<td>0.609**</td>
<td>0.613**</td>
<td>0.596**</td>
<td>0.739**</td>
</tr>
<tr>
<td>2004</td>
<td>0.273</td>
<td>0.349*</td>
<td>0.515**</td>
<td>0.527**</td>
<td>-</td>
<td>0.501**</td>
<td>0.543**</td>
<td>0.754**</td>
</tr>
<tr>
<td>2005</td>
<td>0.182</td>
<td>0.470**</td>
<td>0.564**</td>
<td>0.684**</td>
<td>0.617**</td>
<td>-</td>
<td>0.709**</td>
<td>0.681**</td>
</tr>
<tr>
<td>2006</td>
<td>0.199</td>
<td>0.428**</td>
<td>0.552**</td>
<td>0.521**</td>
<td>0.616**</td>
<td>0.717**</td>
<td>-</td>
<td>0.710**</td>
</tr>
<tr>
<td>2007</td>
<td>0.133</td>
<td>0.538**</td>
<td>0.588**</td>
<td>0.708**</td>
<td>0.629**</td>
<td>0.777**</td>
<td>0.690**</td>
<td>-</td>
</tr>
</tbody>
</table>

* and ** represent significant at the 5% and 1% probability level, respectively.
low acorn production years) (Table 3). Similarly, \( N_r \) mirrored the trend observed for \( N_p \) (Table 3). These observations were supported by the high coefficient of variation (CV) values observed for acorn production in years of low crops \( N_p \) and \( N_r \) (Table 3). In other words, the greater the acorn production disparity among clones (i.e., high CV), the greater the deviation from equal contribution and hence the lower genetic diversity estimates. When all acorn production data from the 8 years were pooled, the meta-population estimates of CV, \( N_p \),

![Graph showing clonal cumulative acorn production curves over 8 years represented by the 94 studied clones (straight line represents equal contribution).](image)

**Table 3.** – Coefficient of variation (CV), group coancestry (\( \Theta \)), effective population size (\( N_p \)) and relative effective population size (\( N_r \)) for female fecundity (acorn production).

<table>
<thead>
<tr>
<th>Year</th>
<th>CV</th>
<th>( \Theta )</th>
<th>( N_p )</th>
<th>( N_r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>2.297</td>
<td>0.033</td>
<td>15.0</td>
<td>15.9</td>
</tr>
<tr>
<td>2001</td>
<td>1.957</td>
<td>0.019</td>
<td>26.5</td>
<td>28.2</td>
</tr>
<tr>
<td>2002</td>
<td>1.508</td>
<td>0.017</td>
<td>28.7</td>
<td>30.5</td>
</tr>
<tr>
<td>2003</td>
<td>1.629</td>
<td>0.019</td>
<td>25.7</td>
<td>27.4</td>
</tr>
<tr>
<td>2004</td>
<td>1.675</td>
<td>0.020</td>
<td>24.4</td>
<td>26.0</td>
</tr>
<tr>
<td>2005</td>
<td>1.516</td>
<td>0.018</td>
<td>28.5</td>
<td>30.3</td>
</tr>
<tr>
<td>2006</td>
<td>1.266</td>
<td>0.014</td>
<td>36.1</td>
<td>38.4</td>
</tr>
<tr>
<td>2007</td>
<td>1.797</td>
<td>0.023</td>
<td>22.2</td>
<td>23.6</td>
</tr>
<tr>
<td>Pooled</td>
<td>1.758</td>
<td>0.014</td>
<td>36.4</td>
<td>38.7</td>
</tr>
</tbody>
</table>

**Table 4.** – Group coancestry (\( \Theta \)), effective population size (\( N_p \)) and relative effective population size (\( N_r \)) under various scenarios with different male fecundity conditions.

<table>
<thead>
<tr>
<th>Scenario A*</th>
<th>Scenario B</th>
<th>Scenario C</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Theta )</td>
<td>( N_p )</td>
<td>( N_r )</td>
</tr>
<tr>
<td>2000</td>
<td>0.016</td>
<td>32.2</td>
</tr>
<tr>
<td>2001</td>
<td>0.011</td>
<td>44.2</td>
</tr>
<tr>
<td>2002</td>
<td>0.011</td>
<td>44.3</td>
</tr>
<tr>
<td>2003</td>
<td>0.012</td>
<td>42.8</td>
</tr>
<tr>
<td>2004</td>
<td>0.012</td>
<td>40.7</td>
</tr>
<tr>
<td>2005</td>
<td>0.011</td>
<td>46.5</td>
</tr>
<tr>
<td>2006</td>
<td>0.010</td>
<td>50.4</td>
</tr>
<tr>
<td>2007</td>
<td>0.013</td>
<td>38.2</td>
</tr>
<tr>
<td>Pooled</td>
<td>0.010</td>
<td>49.6</td>
</tr>
</tbody>
</table>

*Scenario A: male fecundity is proportional to the number of ramet per clone (clone size), Scenario B: male fecundity is the same as female fecundity (sexual symmetry), and Scenario C: male fecundity is equal among clones (1/N).*
and $N_p$ indicate higher collective genetic diversity over the Quercus acutissima plantations as compared to that produced from any individual year separately (Table 3).

Effective population size ($N_e$ and $N_c$) estimates and group co-ancestry ($\Theta$) of Scenario A (male fecundity was proportional to clone size) produced intermediate values to those of Scenarios B (equal male and female fecundity) and C (even male fecundity) (Table 4). We do not have an exact estimate of male reproductive output; however, Scenario A’s estimate seem to present a reasonable approximation of the actual gamete production (K.K-S., personal observations), yielding better genetic diversity parameters compared to that obtained using female (acorn) production alone (Table 4).

Discussion

A three to four years acorn production interval was observed in the studied orchard. Based on eight years observations, 2, 3, and 4 years acorn production intervals were reported for Q. alba, Q. rubra, and Q. velutina, respectively (SÖRBJ et al., 1993). While no significant fluctuation in acorn production was for several oak species (Q. lobata, Q. douglasii, Q. agrifolia, Q. chrysolepis, and Q. kelloggii); however, considerable among years variation was also observed (KOENIG et al., 1994). Among years and individuals within year variation in acorn production was reported for red (Q. rubra) and black (Q. velutina) oak and their hybrid (HEALY et al., 1999). Among clones variation in acorn production was also observed in a Q. alba seed orchard (FARMER, 1981) while MASAKA and SATO (2002) reported noticeable decrease in acorn production in Q. dentate associated with cool conditions during flowering period.

With the exception of 2000, and in spite of the steady increase in acorn production over years, clonal cumulative contribution curves were somewhat similar and equal contribution among clones was not reached (Fig. 3), resulting into the observed reduction in all genetic diversity parameters (Table 3). Reproductive output equality among orchard’s clones is a major factor contributing to increased orchard’s genetic efficiency (EL-KASSABY and REYNOLDS, 1990; EL-KASSABY, 1992; KANG and LINDGREN, 1999) and deviation from this situation leads to genetic erosion (BURCZYK and CHALUPKA, 1997; KANG et al., 2005) that is proportionate to the observed deviation.

While pollen contamination is known to reduce the genetic gain in tested seed orchards and the present orchard is under testing and harbours a large number of clones, thus at this stage we view the role of contamination as a genetic diversity source. Notwithstanding the role of pollen contamination, in most cases female fecundity is easier to assess than that of males. In the present study, we used a range of options to evaluate the combined female-male reproductive output on genetic diversity. Based on our observation of the orchard’s reproductive output, Scenario A produced a reasonable approximation for a rather difficult to assess parameter (e.g., male reproductive output). Accurate assessment of male fertility needs to be evaluated using either systematic reproductive output surveys or molecular genetic markers to select the closest scenario and eventually the selection of a reasonable approximation (FUNDA et al., 2008).

Under idealized situation of equal contribution $N_p$ is expected to be equal to census number ($N=94$), our meta-population estimate is 36.4 indicate that the collective diversity of the 8 years is low. LINDGREN et al. (1996) introduced the status number concept as a measure of genetic diversity: $N_S = 0.5/\Theta$, where $\Theta$ is the group co-ancestry as $\Theta = 1/2N_e$ and $N_e$ is the effective population number. In the present orchard, loss of diversity is evident from the observed fecundity variation and additional loss by the build-up of inbreeding caused by selfing should also be considered (LINDGREN and KANG, 1997).

Accumulation of co-ancestry (COCKERHAM, 1967) and/or fecundity variation often result in loss of genetic diversity. The combined effect of these two factors has played a role in the study orchard’s crop. Relative loss of genetic diversity due to: a) fecundity variation is equal to 0.014 (pooled $\Theta$; Table 3) and b) to co-ancestry (i.e., selfing in this case) can be estimated using average of all co-ancestry pairs that is equal to 0.5 (self co-ancestry) x the number of clones ($N$) / the total number of crosses ($N^2$) = 0.5/94, which is 0.0053. The diversity loss difference between these two causes (fecundity variation and selfing) indicates that in first generation seed orchards where parents are unrelated, most of the genetic diversity loss is caused by fecundity variation rather than inbreeding. Thus fecundity variation management is of vital concern, and optimization techniques that maximize the genetic gain at a desired diversity level are recommended (FUNDA et al., 2009).

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References


Karyotypic Studies in Ecotypes of Hippophaë rhamnoides L. from Romania

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Abstract

Sea buckthorn is a dioecious Eurasian shrub or small tree with large morphological, biochemical and physiological variability, evidenced by the great number of studies. Cytogenetically, uncertainties exist on species basic number, ploidy level, and sex chromosomes. In this study, detailed cytogenetic measurements were carried out on six Romanian ecotypes belonging to Hippophaë rhamnoides L. ssp. carpatica Rousi, in order to establish the features and the symmetry degree of karyotypes, to evidence the sex chromosomes, and to construct the idogram. The ecotypes have 2n = 24 metacentric and submetacentric chromosomes. An intraspecific variation exists concerning the proportion of these two morphotypes. The karyotypes have similar symmetry patterns (R = 2.57–2.89; TF% = 38.54–42.70; AsI% = 57.99–61.41; A1 = 0.27–0.35; A2 = 0.26–0.36) and belong to 1B and 2B classes, being relatively high symmetric. Based on obtained results, we presume that the male sex chromosomes are heteromorphic, while in female plants are homomorphic. The Y chromosome is larger than X chromosome.

Key words: Hippophaë rhamnoides L. ssp. carpatica Rousi, heterosomes, idogram, karyotype.

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