# Parental participation in progeny and effective population sizes in experimental seed orchards of wild cherry Prunus avium L. (Batsch)

Stéphanie Mariette, Emilie Balsemin, Solenn Stoeckel, Muriel Tavaud, Hervé Le Bouler, Frédérique Santi, Michel Verger

Ann. For. Sci. 64 (2006) 533–539
© INRA, EDP Sciences, 2007
DOI: 10.1051/forest:2007030

Available online at:
www.afs-journal.org

**Original article**

# Parental participation in progeny and effective population sizes in experimental seed orchards of wild cherry *Prunus avium* L. (Batsch)

Stéphanie MARIETTE[a,b,c,g,*], Emilie BALSEMIN[a,b,g], Solenn STOECKEL[a,b], Muriel TAVAUD[d], Hervé LE BOULER[e], Frédérique SANTI[f], Michel VERGER[f]

[a] Unité de Recherches « Écosystèmes Forestiers », Domaine des Barres, 45290 Nogent-sur-Vernisson, France
[b] Université Paris XI, Laboratoire Écologie, Systématique et Évolution, Bât. 360, 91405 Orsay, France
[c] INIA, Unit of Forest Genetics, Center of Forest Research (CIFOR-INIA), Carretera de la Coruña Km 7.5, 28040 Madrid, Spain
[d] INRA , Domaine de Melgueil, 34130 Mauguio, France
[e] Pépinière Expérimentale, Route de Redon, 44290 Guémené-Penfao, France
[f] INRA, Unité Amélioration, Génétique et Physiologie Forestières, Avenue de la Pomme de Pin, 45166 Olivet, France
[g] New address: INRA UREFV, 71 avenue Edouard Bourlaux, BP 81, 33883 Villenave d'Ornon Cedex, France

(Received 23 August 2006; accepted 20 December 2006)

**Abstract –** Parents and progenies genetic diversity, and male and female contributions to the seed crop were assessed in three experimental *Prunus avium* seed orchards. Collected data were used to compare different effective population sizes, based on phenological, seed crop and paternity analysis. Our results did not show any difference of genetic diversity between parents and progenies. A limited pollen pollution was detected. We showed that distance and coflowering among clones had a significant effect on effective pollination, and a significant effect of the production of flowers was revealed in one of the seed orchards. Our study also revealed a quite low number of effective size of fathers per mother, but high effective sizes of mothers, fathers and parents at the level of the seed orchard. Finally, the calculation of effective size of mothers, fathers and parents was not highly modified when having the complete information based on the paternity analysis.

**seed orchard / genetic diversity / effective population size / parental participation / *Prunus avium***

**Résumé – Contributions mâle et femelle à la descendance et tailles efficaces de population dans des vergers à graines expérimentaux de merisier *Prunus avium* L. (Batsch).** La diversité génétique des parents et de leurs descendants, ainsi que les contributions mâle et femelle à la récolte de graines ont été estimées dans trois vergers à graines expérimentaux de *Prunus avium*. Les données collectées ont été utilisées pour comparer différentes tailles de population efficace, en se basant sur l'analyse de la phénologie, de la récolte de graines et de la paternité. Nos résultats n'ont pas montré de différence de diversité génétique entre les parents et les descendants. Une pollution pollinique limitée a été mise en évidence. Nous avons montré un effet significatif de la distance et de la cofloraison entre clones sur la pollinisation efficace, ainsi qu'un effet significatif de la production de fleurs dans l'un des vergers à graines. Notre étude a aussi révélé un faible nombre efficace de pères à l'échelle de chaque mère, et un nombre efficace de mères, de pères et de parents important à l'échelle du verger à graines. Enfin, le calcul des tailles efficaces de pères et de parents à l'échelle du verger à graines n'a pas été fortement influencé par le fait d'avoir l'information totale de contribution donnée par l'analyse de paternité.

**verger à graines / diversité génétique / taille efficace de population / participation parentale / *Prunus avium***

## 1. INTRODUCTION

Forest plantations are one of the impacts of human activities on forest tree spontaneous populations [13]. They are often made with plants grown from material collected in seed stands. For some species (*Populus*, *Eucalyptus*), breeding can lead to the plantations of clones selected for some particular traits. Seed orchards may provide interesting material, compared with seed stands, since their clonal components may be unrelated and genetically improved. Nevertheless, seed orchards can only be truely considered a better option than other material if their genetic base is large, and if the crossings in the seed orchard are effective, i.e. if the crossings occurring in reality are not very different from an ideal situation in which all clones contribute equally to the seed crop. Equal contribution of each clone would mean that all females have the same seed yield and that all males sire the same number of seeds. However, male and female reproductive successes are expected to show large variances in natural populations [6]. Several studies of coniferous seed orchards have indeed shown that the equal contribution of clones is not realised, mainly due to among-individual variation in fecundity and phenology [8,10]. Few studies on forest seed orchards have concerned animal-pollinated species so far.

* Corresponding author: smariett@bordeaux.inra.fr

The effective population number or status number [12,14] is often used to assess the levels of relatedness in seed orchards. However, this number is generally assessed in conifer species based on seed and pollen cones, assuming that they are an indication of gamete contribution [11]. Experimental comparisons among different assessments of effective population numbers are needed to test if this assumption can affect the estimators.

Wild cherry (*Prunus avium* L.) is a species from the Rosaceae family, native to Europe and western Asia. It is the species from which most sweet cherry cultivars are derived. Wild cherry is a scattered forest tree species producing entomophilous and hermaphroditic flowers. Its reproduction system is characterized by a gametophytic self-incompatible system which favours outcrossing. Wild cherry is a very valuable forest tree species. Foresters began to use wild cherry for reforestation and afforestation in the 1970's, using material from unknown origin. This material may originate not only from wild cherry but also from sweet cherry or even from sour cherry (*Prunus cerasus* L., the crossing between *Prunus avium* L. and *Prunus fruticosa* Pall.) [4]. In order to provide French foresters with adapted and identified material, a breeding programme, based on the phenotypic selection of some 400 "plus" trees in French forests [17], was initiated by the French National Institute for Agronomical Research (INRA). Further testing of these "plus" trees in clonal plantations has led to the selection of several clone cultivars based on their multisite adaptation, growth, form, resistance to disease. Seed orchards were constituted with selected clones to offer a more diversified material to forest managers, as an alternative to seed stand production.

In order to characterize parental contributions to the orchard seed crop, we analysed diversity, pollen flow among clones and seed production in three different seed orchards of *P. avium*. More precisely, our work aims at investigating three questions:

(1) Do flower production, co-flowering time and distance among clones influence effective pollination?
(2) Does genetic paternity analysis improve the effective population size estimates provided by flower production, co-flowering time, and seed production assessment?
(3) Is there a difference between effective numbers for each tree and effective numbers for the orchard?

## 2. MATERIALS AND METHODS

### 2.1. Seed orchards composition

The seed orchards analysed in this study were established at the experimental nursery of Guémené-Penfao (France). Three different experimental seed orchards were created in spring 2000. Two of them were installed inside greenhouses: (1) the first one with a simultaneous introduction of whole plants and (2) the second one with a delayed introduction of half of the individuals, the latest clones being installed before the earliest ones with the aim of increasing the co-flowering period among clones. The third orchard was installed outdoors, covered with a net to avoid the pillaging of cherries by birds. The two indoors orchards comprised the same 15 clones each, while the outdoors one comprised only 12 (all present in the orchards indoors). All clones were present twice in seed orchards #1 and #2. Ramets of clones were randomly distributed in each seed orchard. Clones were chosen from the *Prunus avium* breeding population created by INRA.

*Phenology, flowering and fruiting measures*

For each individual tree, the following measures were assessed in 2001: floral phenology was observed from the beginning of April to mid-May (one observation for each tree per week), the flowering start date (first flower) and the end of flowering date were inferred from the observations. The number of floral clumps (as a measure of flowering intensity) was counted (mid-April). Finally the number of produced cherries was counted at the beginning of July. All cherries were counted before they started falling.

### 2.2. Diversity and paternity analysis

#### *2.2.1. Genotyping of orchard clones and progenies*

All the parents and approximately 20 seeds per ramet were analysed with the same six microsatellites. Seeds were sampled at random from all fruits collected from each mother tree in 2001. Pits were extracted from cherries and broken to get almonds. The samples were then soaked in distilled water overnight and were dissected to extract the cotyledons and embryo (offspring tissue) from the endocarp (maternal tissue). Four microsatellite markers used in this study were initially developed in peach, and transferred to *P. avium* (Tavaud, unpublished results): UDP96001, UDP96005, PSCHGMS1 and BPPCT034. Other markers were identified in sour cherry (PCEGA34) or sweet cherry (PS12A02). Primers used in this study are given in Table I. DNA from leaves and from cotyledons plus embryo was extracted using the DNeasy 96 Plant Kit Qiagen method. Amplification reactions were performed using 2 μL of DNA (not diluted after the extraction), 0.2 μM of each primer: F (forward) and R (reverse), 0.1 mM of each dNTP, 1X buffer, 1.5mM of MgCl2 and 0.6 U of Taq DNA polymerase. A portion of each F primer (depending on the marker) contained fluorescence which was detected by the multiple capillary genetic analyser (ABI PRISM® 3100). The Genescan 3.7© software was used to genotype all the individuals.

#### *2.2.2. Diversity and paternity analysis*

Classical population genetics parameters were calculated on parents and progenies: number of alleles ($A$), observed heterozygosity ($H_O$) and expected heterozygosity ($H_E$). The genotypes of candidate fathers were compared against the offspring's genotype, and were excluded as fathers if a mismatch occurred at one or more loci. The Cervus 2.0© software [15] was used to make the analysis. Note that, for the two seed orchards in greenhouses, this analysis provided the identity of the clone but not of the ramet.

#### *2.2.3. Analysis of factors influencing effective pollination*

For each seed orchard, we made multiple linear regression analysis to detect the influences of the flower production displayed by the

**Table I.** Name, reference, name of the species for which the marker was developed, primer sequences, and expected PCR product size of the six microsatellites used in this study.

| SSR name | References | Species | Primer sequence (5'→ 3') | Length (bp) |
|---|---|---|---|---|
| UDP96001 | [3] and [20] | Peach *P. persica* | F : AGTTTGATTTTCTGATGCATCC | 120 |
| | | | R : TGCCATAAGGACCGGTATGT | |
| UDP96005 | [3] and [20] | Peach *P. persica* | F : GTAACGCTCGCTACCACAAA | 155 |
| | | | R : CACCCAGCTCATACACCTCA | |
| PS12A02 | [7] | Sweet cherry *P. avium* | F : GCCACCAATGGTTCTTCC | 200 |
| | | | R : AGCACCAGATGCACCTGA | (150–178) |
| PCHGMS1 | [20] | Peach *P. persica* | F : GGGTAAATATGCCCATTGTGCAATC | 194 |
| | | | R : GGATCATTGAACTACGTCAATCCTC | |
| PCEGA34 | [7] | Sour cherry *P. cerasus* | F : GAACATGTGGTGTGCTGGTT | 155 |
| | | | R : TCCACTAGGAGGTGCAAATG | (140–174) |
| BPPCT034 | [5] | Peach *P. persica* | F : CTACCTGAAATAAGCAGAGCCAT | 228 |
| | | | R : CAATGGAGAATGGGGTGC | |

mother ($F_m$), the flower production of each father ($F_f$), the coflowering period among clones $i$ and $j$ ($CF_{ij}$), and the distance among clones ($D_{ij}$) on the realised pollination among clones $i$ and $j$ ($P_{ij}$):

$$P_{ij} = a + \beta_1.CF_{ij} + \beta_2.D_{ij} + \beta_3.F_m + \beta_4.F_f + \varepsilon$$

where $a$ is a constant term, $\beta_i$ the regression coefficient of score values of the $i$th parameter explored and $\varepsilon$ the error term of the model.

The paternity analysis did not provide the identity of the ramet. Consequently, we chose to use the mean distance between the mother and the candidate father ramets as an estimator of the distance between the mother and the candidate father clone. The effect of compatibility among individuals on effective pollination was not examined in the present study, it will be analysed more precisely elsewhere (our unpublished results).

## 2.3. Definition of male and female gametic contributions according to available information

We defined as the relative gamete contribution of the [$i$th male–$j$th female] ramet pair to the total seed crop, $m_i$ and $m_I$ as the relative male gametic contribution of the $i$th ramet and the $I$th clone, respectively, and and as the relative female gametic contribution of the $j$th ramet and the $J$th clone, respectively. Note that $GC_{ij} = 0$ because *P. avium* is a self-incompatible species.

We used alternative estimators for $GC_{ij}$, $m_i$, $m_I$, $f_j$, and $f_J$, each of them defined to incorporate the different levels of information that may be available in practice at seed orchards: (1) measurements of flower production by counting floral clumps, (2) measurements of flower production by counting of floral clumps plus the estimation of co-flowering time, (3) a direct estimation of gametic contribution through the counting of seeds for the female contribution and the estimation of male success thanks to the paternity analysis for the male contribution. We wanted to evaluate the relative utility of each kind of measurement to estimate the effective parental size in seed orchards, providing useful criteria to optimise seed orchards management.

For each clone, $m_I$ was obtained summing $m_i$ on ramets of the $I$th clone (except in the case of the estimation of male success through the paternity analysis for which only can be obtained) and $f_J$ was obtained summing $f_j$ on ramets of the $J$th clone.

### *2.3.1. Male gametic contribution*

Assuming the information we have is:

(1) The estimation of flower productions:

$$GC_{ij1} = \frac{P_i O_j}{\sum_i \sum_j P_i O_j} \text{ and } m_{i1} = \sum_j GC_{ij1}$$

with $P_i$ being the relative contribution of flower clumps of the $i$th ramet and $O_j$ being the relative contribution of flower clumps of the $j$th ramet.

(2) The estimation of flower productions plus the estimation of co-flowering periods:

$$GC_{ij2} = \frac{P_i O_j CF_{ij}}{\sum_i \sum_j P_i O_j CF_{ij}} \text{ and } m_{i2} = \sum_j GC_{ij2}$$

with $CF_{ij}$ being the length of the co-flowering time between the $i$th ramet and the $j$th ramet.

(3) The proportion of the $I$th clone having sired the $j$th mother, available from the paternity analysis.

Based on the unambiguous results from the paternity analysis, i.e. based on the seedlings for which only one father was found in the seed orchard, we calculated the proportion of times that the $I$th clone pollinated the $j$th female, that we called $EP_{Ij}$, and $EP_{IJ}$ is the proportion of times that the $I$th clone pollinated the $J$th clone. This proportion is relative to the total number of seeds analysed in the paternity analysis if we assume that the number of seeds is not known.

The male gametic contribution of the $I$th clone was then assessed as $m_{I3} = \sum_J EP_{IJ}$.

It was also calculated relatively to the total number of seed of the seed crop when it is known.

The male gametic contribution of the $I$th clone taking account the total number of seed of the seed crop was then assessed as $m_{I3tot} = \sum_J EP_{IJ}$.

**Table II.** Genetic composition of the parents and progenies in the seed orchards.

| | Parents Orchards 1 and 2 | | | Progenies Orchard 1 | | | Progenies Orchard 2 | | | Parents Orchard 3 | | | Progenies Orchard 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $A$ | $H_o$ | $H_E$ | $A$ | $H_o$ | $H_E$ | $A$ | $H_o$ | $H_E$ | $A$ | $H_o$ | $H_E$ | $A$ | $H_o$ | $H_E$ |
| UDP96001 | 4 | 0.533 | 0.609 | 5 | 0.523 | 0.572 | 4 | 0.605 | 0.561 | 4 | 0.583 | 0.627 | 3 | 0.497 | 0.540 |
| UDP96005 | 5 | 0.467 | 0.766 | 8 | 0.661 | 0.761 | 7 | 0.704 | 0.769 | 5 | 0.500 | 0.750 | 5 | 0.790 | 0.751 |
| PS12A02 | 8 | 0.800 | 0.782 | 8 | 0.738 | 0.731 | 9 | 0.782 | 0.752 | 6 | 0.750 | 0.757 | 7 | 0.737 | 0.736 |
| PCHGMS1-1 | 6 | 0.733 | 0.708 | 6 | 0.681 | 0.632 | 6 | 0.706 | 0.664 | 6 | 0.750 | 0.717 | 6 | 0.711 | 0.642 |
| PCHGMS1-2 | 4 | 0.400 | 0.444 | 4 | 0.468 | 0.430 | 4 | 0.425 | 0.417 | 4 | 0.417 | 0.482 | 5 | 0.413 | 0.381 |
| PCEGA34 | 9 | 0.933 | 0.892 | 10 | 0.898 | 0.842 | 9 | 0.874 | 0.844 | 9 | 0.917 | 0.895 | 11 | 0.880 | 0.827 |
| BPPCT034 | 7 | 0.867 | 0.811 | 8 | 0.807 | 0.792 | 7 | 0.850 | 0.808 | 6 | 0.833 | 0.804 | 8 | 0.817 | 0.787 |
| Mean | 6.14 | 0.681 | 0.716 | 7.00 | 0.682 | 0.680 | 6.57 | 0.707 | 0.688 | 5.71 | 0.679 | 0.719 | 6.43 | 0.692 | 0.666 |

#### *2.3.2. Female gametic contribution*

Assuming the information we have:

(1) The estimation of flower productions
With only the number of floral clumps as information, the simpler assumption is that all the ovules are transformed into seeds. In this first case, we assume that only pollen represents a limitation and we assume that all the flowers give a seed which simply means: $f_{j1} = O_j$.

(2) The estimation of flower productions plus the estimation of co-flowering periods:

$$f_{j2} = \sum_i GC_{ij2}$$

(3) The seed crop per tree and the the total seed crop:
With the number of seeds as information, the total female gametic contribution of the $j$th ramet is $f_{j3}$, which is simply the proportion of seeds from the $j$th ramet in the seed production.

#### *2.3.3. Effective population sizes in seed orchards*

(1) Based on the paternity analysis, we calculated in each seed orchard the effective number of fathers per mother tree:

$$N_{emj} = \frac{1}{\sum_I EP_{Ij}^2}$$

Based on the different estimations of and , we calculated in each seed orchard:

(2) The effective paternal size at the level of the seed orchard as:

$$N_{em} = \frac{1}{\sum_I m_I^2}$$

(3) The effective maternal size at the level of the seed orchard as:

$$N_{ef} = \frac{1}{\sum_J f_J^2}$$

(4) The effective size of the parental population at the level of the seed orchard as:

$$N_{ep} = \frac{1}{\sum_P r_P^2}$$

where $r_P$ is the average gametic contribution of the $P$th parental individual ($r_P = \frac{m_P + f_P}{2}$). For this estimation, we used seven different combinations of the estimations of $m_I$ and $f_J$.

We took no account of pollen contamination when we assessed the different effective population sizes.

## 3. RESULTS

### 3.1. Parents and progenies showed similar levels of genetic diversity, and new alleles were detected in the progenies

PCR products with the PCHGMS1 marker revealed two segregating locus (PCHGMS1-1 and PCHGMS1-2) that were included in the analyses.

Results of the genetic analysis of parents and progenies are given in Table II.

The seven loci showed average and respectively of 0.681 and 0.716 for the 15 clones in orchards #1 and #2, and 0.679 and 0.719 for the 12 clones in orchard #3. These values were not significantly different from the values calculated for progenies: and were 0.682 and 0.680 for the progenies of orchard #1, 0.707 and 0.688 for progenies of orchard #2, and finally 0.692 and 0.666 for progenies of orchard #3.

The average allelic richness was 6.14 for the 15 clones present in seed orchards #1 and #2 (5.71 for the 12 clones available in orchard #3). These values were higher for progenies. The average allelic richness was 7.57 for the 495 progenies analysed in the first orchard. In comparison with the clones, one more allele was found for UDP96001, for PCEGA34 and BPPCT034, and three more alleles for UDP96005. $A$ was 6.57 for the 491 individuals analysed in the second orchard. In comparison with the clones, one more allele was found for PS12A02, and two more alleles for UDP96005 (also detected in orchard #1). $A$ was 6.43 for the 190 individuals analysed in the third orchard. In comparison with the clones in the seed orchard, one allele was lost for UDP96001, PCHGMS1-1 and PCHGMS1-2, one more allele was found for PS12A02, PCHGMS1-1 and two more alleles were found for PCHGMS1-2, PCEGA34 and BPPCT034.

### 3.2. Reduced level of pollen contamination in greenhouse seed-orchards, and significant effects of the co-flowering time and of the distance among clones on effective pollination were detected

The total exclusionary power for the second parent was 0.9929 in the first and second seed orchard (15 clones) and 0.9916 in the third one (12 clones).

**Table III.** Summarized results of the paternity analysis.

| | Seed orchard #1 | Seed orchard #2 | Seed orchard #3 |
|---|---|---|---|
| Progenies | 495 | 491 | 190 |
| One father identified | 365 (73.7%) | 362 (73.8%) | 137 (72.1%) |
| More than one father | 9 (1.8%) | 10 (2%) | 3 (1.6%) |
| No father | 42 (8.5%) | 59 (12%) | 33 (16.8%) |
| Genotyping problem | 79 (16%) | 60 (12.2%) | 17 (9.5%) |

Summarized results of the paternity analysis are given in Table III. For the three seed orchards, one father could be found in the clones of the orchards for more than 70% of the analysed seedlings, and more than one father was detected for about 2% of the seedlings. The percentage of seedlings for which no father was found in the clones of the seed orchard reached 16.8% in the third one (outside one), being 8.5% in the first one and 12% in the second one. In the first orchard, 5 (11.9%) out of the 42 seedlings for which no father was found showed at least one new allele (4 at one locus and 1 at two locus). In the second orchard, a similar result was found, i.e. 5 (8.5%) out of the 59 seedlings showed one new allele at one locus. However, in the third one, 18 (56%) out of the 32 seedlings for which no father was found showed one new allele at one or more locus (17 at one and 1 at two locus). Besides, negative correlations were detected between the number of seedlings for which no father was found and the mean co-flowering time of clones in orchards #1 and #2. More particularly the clone showing the highest number of seedlings with no detected father (respectively 12 and 14 out of 42 and 59 seedlings in orchards #1 and #2, the clone was not present in orchard #3) showed the lowest co-flowering time with other clones in the seed orchards.

In the three seed orchards, regression analyses revealed a significant effect ($p < 0.01$) of the co-flowering period (positive relationship) and of the distance among clones (negative relationship), except in the third seed orchard for which the influence of the distance was not found significant and for which the coflowering was only significant at the 5% level. The production of flowers was found significant at the 5% level in the seed orchard #2 but not in the two others (data not shown).

### 3.3. The quite low number of effective size of fathers per mother contrasts with high effective sizes of mothers, fathers and parents at the level of the seed orchard

Summarized data of effective sizes of fathers per mother are given in Table IV. In the seed orchard #1, the effective size of fathers per mother varied between 1 (6.7% of the total size of the seed orchard) and 6.8 (45.3%), the mean value being 3.7 (24.7%) and the standard deviation being 1.5. In the seed orchard #2, the effective size varied between 1.4 (9.3%) and 6.4 (42.7%), the mean value being 3.4 (22.7%) and the standard deviation being 1.4. In the seed orchard #3, the effective size varied between 2.5 (20.8%) and 4.6 (38.3%) in the seed orchard #2, the mean value being 4.4 (36,7%) and the standard deviation being 0.9.

**Table IV.** Effective sizes of fathers per mother tree in the three seed orchards.

| | Seed orchard #1 | Seed orchard #2 | Seed orchard #3 |
|---|---|---|---|
| Lower observed effective size | 1 | 1.4 | 2.5 |
| Higher observed effective size | 6.8 | 6.4 | 4.6 |
| Mean effective size (standard deviation) | 3.7 (1.5) | 3.4 (1.4) | 4.4 (0.9) |

Effective sizes of mothers and fathers, based on different levels of information, are given in Table V.

For the female function, considering the information provided by co-flowering time on top of the number of flowers led to observe an increase of the effective size of 0.4 for seed orchard #2 and a decrease of 0.7 for seed orchard#3. The two estimations were the same for seed orchard#1. Considering the number of seeds harvested for each ramet instead of the number of flowers led to an observation of a reduction in the effective size of 1.2 and 1 respectively in seed orchard #1 and #3 and the effective size remained stable in seed orchard #2.

For the male function, considering the information provided by co-flowering time on top of the number of flowers led to observe a diminution of the effective size of 0.5, 0.1 and 1.3 respectively in seed orchards #1, #2 and #3. Considering the pollination success obtained with the paternity analysis instead of the number of flowers led to observe a diminution of the effective size of 1.5, 1.5 and 1.2 respectively in seed orchard #1, #2 and #3. Considering the pollination success obtained with the paternity analysis, calculated based on the seed crop, instead of the number of flowers led to observe a diminution of the effective size of 1.8, 3.6 and 1.4 respectively in seed orchard #1, #2 and #3.

Effective sizes of parents, based on different levels of information, are given in Table VI.

For the seed orchard #1, the effective size varied between 11.4, obtained with counting of flowers for females and males, and 12.3, obtained with counting of flowers for females and paternity test for males. The value obtained with counting seeds for females and with the paternity analysis for males was 11.5, and only low differences of values were obtained with other estimators. Very similar results were obtained in seed orchard #2, except that the highest values were obtained with the counting of seeds and the counting of flowers, and with the counting of seeds and the counting of flowers plus taking account the coflowering time (12.1).

The results obtained on the seed orchard #3 were different: the lower value was obtained with counting counting flowers and taking account the coflowering time (7.5) and the highest value was obtained with counting seeds and counting flowers (10.3). The value obtained with counting seeds for females and with the paternity analysis for males was 8.3 and the closest value was obtained with counting flowers for males and females (8.5).

**Table V.** Effective sizes of mothers and fathers in the seed orchards.

| | Female function | | | Male function | | | |
|---|---|---|---|---|---|---|---|
| | Flowers $N_{ef1}$ | Flowers + CF $N_{ef2}$ | Seeds $N_{ef3}$ | Flowers $N_{em1}$ | Flowers + CF $N_{em2}$ | Paternity $N_{em3}$ | Paternity + Seeds $N_{em3tot}$ |
| Seed orchard #1 | 11.2 | 11.2 | 10 | 11.7 | 11.2 | 10.2 | 9.9 |
| Seed orchard #2 | 10.2 | 10.6 | 10.5 | 10.7 | 10.6 | 9.2 | 7.1 |
| Seed orchard #3 | 8.2 | 7.5 | 7.2 | 8.8 | 7.5 | 7.6 | 7.4 |

**Table VI.** Effective sizes of parents in the seed orchards.

| Information on female contribution | Information on male contribution | Effective size | Seed orchard #1 | Seed orchard #2 | Seed orchard #3 |
|---|---|---|---|---|---|
| Flowers | Flowers | $N_{ep11}$ | 11.4 | 10.5 | 8.5 |
| Flowers | Paternity | $N_{ep13}$ | 12.3 | 11 | 9.1 |
| Flowers + CF | Flowers + CF | $N_{ep22}$ | 11.2 | 10.6 | 7.5 |
| Flowers + CF | Paternity | $N_{ep23}$ | 12.1 | 11.1 | 8.4 |
| Seeds | Flowers | $N_{ep31}$ | 12.1 | 12.1 | 10.3 |
| Seeds | Flowers + CF | $N_{ep32}$ | 11.8 | 12.1 | 7.6 |
| Seeds | Paternity | $N_{ep33}$ | 11.9 | 11.9 | 8.5 |
| Seeds | Paternity + Seeds | $N_{ep33tot}$ | 11.5 | 11.3 | 8.3 |

## 4. DISCUSSION

The first aim of this work was to analyse whether significant deviations from panmixia like pollination can be detected in the experimental seed orchards of *P. avium* that we studied. No difference in levels of diversity, measured with observed and expected genetic diversities, were found between the parents of the seed orchards and the progenies. Moreover, at the level of seed orchards, the effective size of fathers, measured by the paternity analysis, was between 7.6 and 10.6, respectively 63% and 68% of the population size. The effective size of mothers was between 7.2 and 10.5, i.e. 60% and 70% of the sizes of populations. The effective size of parents was between 8.5 and 11.9, that are 70% and 80% of the sizes of populations. These results would allow us to conclude that "panmixia-like pollination" occurred in the seed orchards. However, the estimates of effective sizes at the level of the whole seed orchard contrasted with the average effective size of fathers per mother, that were respectively 24.7%, 22.7% and 36.7% of the total sizes of seed orchards, respectively. We also demonstrated that effective pollination depends significantly on the distance and on the phenological overlap among clones. We also gave evidence that the clonal production of flowers contributed to explain the male success of clones in the seed orchard #2. These figures show that the seeds derived from particular crossings among trees. The progenies represent only a portion of the possible crossings from the seed orchards and panmixia is not reached. Deviations from panmixia caused by a difference in the distance between clones, variation in the amounts of pollen produced, and phenological synchrony between clones have already been suspected or demonstrated in seed orchards to explain pollination patterns (study on *Pinus thunbergii* [9] and study on *Cryptomeria japonica* [16]). On the contrary, the effective number of males that mate with each female was found to be large in a study of a *Eucalyptus regnans* seed orchard using the neighbourhood model [1]. Panmixia-like pollination was also found in a *Eucalyptus grandis* seed orchard [2]. More generally, distance-dependent pollination is expected in forest tree species populations, though large distances events can be observed with a significant proportion [19]. Finally, the effective sizes of fathers per mother that we measured in our study (neglecting pollen contamination) are close to the low values that are expected in animal-pollinated species: generally less than 10 [19].

The second aim of this work was to compare the information given by phenological and molecular data with the information provided by molecular data to assess effective population size in seed orchards, and assess the quality of gene flow in seed orchards. Genetic diversity and relatedness is often assessed using the concept of effective population number or status number [12, 14]. In coniferous species, the calculation of the effective population number is very generally based on the counting of male and female flowers, assuming implicitly that female and male production of flowers is a good representative of their gametic contribution [11]. The effective number of parents that we calculated in this study can be considered equivalent to the status number if the seed orchard parents are non-inbred and unrelated. In the present study, we estimated the effective number of parents calculated using different levels of information: production of flowers, production of flowers and co-flowering time, production of seeds and effective pollination measured through a paternity analysis. Our results showed that, for the whole parent contribution, few differences were found between those estimates of the effective size made respectively with the data of the production of flowers, with the production of flowers and the co-flowering time, and with the effective pollination or seed data. The differences

were higher when the male or female contribution was considered. Then, in our study, molecular analyses and phenological data gave similar information regarding effective population size. Those results are surprising since, in our experimental seed orchards, the production of flowers was not found to be a good predictor of the production of seeds (data not shown). Moreover, the paternity analysis showed that distance and co-flowering time strongly influenced the pattern of effective pollination. However, if those results were confirmed in other species cultivated in seed orchards, using phenological data (production of flowers) could provide a sufficient information to assess effective population numbers in seed orchards.

Finally, the use of the effective population number only to analyse the genetic diversity and relatedness in seed orchards populations can be questioned. In our study, the effective number of parents represented up to 80% of the total population size. However, according to the paternity analysis made with molecular markers, the calculation of the effective size of fathers per mother showed that few males contributed to the pollination of a given mother. A close neighbor may dominate among fathers to a mother tree in a seed orchard, and thus the effective number of fathers can be low (see [18] for a pioneer study on the topic and [21] for a recent study). The effective numbers of males, females and parents that are calculated at the level of the whole seed orchard do not reflect this result. Those observations may appear contradictory. In fact, the effective number of males and females at the level of the seed orchards compare the contribution of each clone to the seed crop, but does not allow us to know if seeds harvested on a given mother results from crossings realised with a limited number of fathers. A high effective number of parents at the level of the seed orchard is not incompatible with high correlated paternity within a mother. This result indicates that it is necessary to mix seeds harvested on different mothers in a seed orchard to make sure that resulting plantations will not be realised with a limited number of genotypes.

**Acknowledgements:** We would like to extend our warmest thanks to Marie-Claude Lesage-Descauses for the inestimable help of running microsatellites on the capillary sequencer at INRA Orléans. We thank Bernard Héois, Patrick Baldet and Gwenaël Philippe for correcting an early version of the manuscript. Dušan Gömöry is acknowledged for clarifying some formulas for us. Juan José Robledo-Arnuncio is acknowledged for useful discussion on an early draft of the manuscript. This work was financially supported by the French « Ministère de l'Agriculture, de l'Alimentation, de la Pêche et des Affaires Rurales » (Direction de l'Espace Rural et de la Forêt) ». In Spain, Stéphanie Mariette was supported by the « Ramón y Cajal » program.

## REFERENCES

[1] Burczyk J., Adams W.T., Moran G.F., Griffins A.R., Complex patterns of mating revealed in a *Eucalyptus regnans* seed orchard using allozyme markers and the neighbourhood model, Mol. Ecol. 11 (2002) 2379–2391.

[2] Chaix G., Gerber S., Razafimaharo V., Vigneron P., Verhaegen D., Hamon S., Gene flow estimation with microsatellites in a Malagasy seed orchard of *Eucalyptus grandis*, Theor. Appl. Genet. 107 (2003) 705–712.

[3] Cipriani G., Lot G., Huang W.-G., Marrazzo M.T., Peterlunger E., Testolin R., AC/GT and AG/CT microsatellite repeats in peach (*Prunus persica* (L.) Batsch): isolation, characterization and cross-species amplification in *Prunus*, Theor. Appl. Genet. 99 (1999) 65–72.

[4] Crave M.F., Le groupe de travail merisier-chêne rouge de l'IDF, Forêt-Entreprise 46 (1987) 10–28.

[5] Dirlewanger E., Cosson P., Tavaud M., Aranzana M.J., Poizat C., Zanetto A., Arus P., Laigret F., Development of microsatellite markers in peach (*Prunus persica* (L.) Batsch) and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.), Theor. Appl. Genet. 105 (2002) 127–138.

[6] Devlin B., Clegg J., Ellstrand N.C., The effect of flower production on male reproductive success in wild radish populations, Evolution 46 (1992) 1030–1042.

[7] Downey S.L., Iezzoni A.F., Polymorphic DNA markers in black cherry (*Prunus serotina*) are identified using sequences from sweet cherry, peach, and sour cherry, J. Am. Soc. Hortic. Sci. 125 (2000) 76–80.

[8] Gömöry D., Bruchanik R., Longauer R., Fertility variation and flowering asynchrony in *Pinus sylvestris*: Consequences for the genetic structure of progeny in seed orchards, For. Ecol. Manage. 174 (2003) 117–126.

[9] Goto S., Miyahara F., Ide Y., Identification of the male parents of half-sib progeny from Japanese black pine (*Pinus thunbergii* Parl.) clonal seed orchard using RAPD markers, Breed. Sci. 52 (2002) 71–77.

[10] Jonsson A., Ekberg I., Eriksson G., Flowering in a seed orchard of *Pinus sylvestris* L., Studia Forestalia Suecica 135 (1976) 38 p.

[11] Kang K.S., El-Kassaby Y.A., Considerations of correlated fertility between genders on genetic diversity: the *Pinus densiflora* seed orchard as a model, Theor. Appl. Genet. 105 (2002) 1183–1189.

[12] Kjær E.D., Wellendorf H., Variation in flowering and reproductive success in a Danish *Picea abies* (Karst.) seed orchard, For. Genet. 4 (1997) 181–188.

[13] Lefèvre F., Human impacts on forest genetic resources in the temperate zone: an updated review, For. Ecol. Manage. 197 (2004) 257–271.

[14] Lindgren D., Mullin T.J., Relatedness and status number is seed orchard crops, Can. J. For. Res. 28 (1998) 276–283.

[15] Marshall T.C., Slate J., Kruuk L.E.B., Pemberton J.M., Statistical confidence for likelihood-based paternity inference in natural populations, Mol. Ecol. 7 (1998) 639–655.

[16] Moriguchi Y., Taira H., Tani N., Tsumura Y., Variation of paternal contribution in a seed orchard of *Cryptomeria japonica* determined using microsatellite markers, Can. J. For. Res. 34 (2004) 1683–1690.

[17] Santi F., Variabilité génétique intra et interpopulations chez le merisier (*Prunus avium* L.), Thèse de Doctorat de l'Institut National Agronomique de Paris Grignon, 1988.

[18] Shen H.H., Rudin D., Lindgren D., Study of the pollination pattern in a Scots pine seed orchard by means of isozyme analysis, Silvae Genet. 30 (1981) 7–15.

[19] Smouse P., Sork V.L., Measuring pollen flow in forest trees : an exposition of alternative approaches, For. Ecol. Manage. 197 (2004) 21–38.

[20] Sosinski B., Gannavarapu M., Hager L.D., Beck L.E., King G.J., Ryder C.D., Rajapakse S., Baird W.V., Ballard R.E., Abbott A.G., Characterization of microsatellite markers in peach (*Prunus persica* (L.) Batsch), Theor. Appl. Genet. 101 (2000) 421–428.

[21] Vanden Broeck A., Cottrell J., Quataert P., Breyne P., Storme V., Boerjan W., Van Slycken J., Paternity analysis of *Populus nigra* L. offspring in a Belgian plantation of native and exotic poplars, Ann. For. Sci. 63 (2006) 783–790.