# Differences Between Georgian and French Wild Cherry Populations and Consequence for Wild Cherry Breeding Programmes

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**Abstract**

For the first time, the diversity of wild cherry in Caucasia was sampled: 5 populations of Georgia, together with 11 French populations. 23 alleles from 7 isozyme loci were scored, among them 6 new alleles in Georgia. Though the total number of alleles was higher in Georgia ($A = 2.4$) than in France ($A = 2.0$), the diversity was higher in France ($H_e = 0.324$) than in Georgia ($H_e = 0.284$).

A higher level of differentiation was found in France ($F_{st} = 0.094$) than in Georgia ($F_{st} = 0.057$), and the total $F_{st}$ was even higher (0.108). Mean pairwise distances inside the French group, the Georgian group and between the two groups were 0.054, 0.037 and 0.094, respectively. The Pearson's correlation coefficient between genetic and geographical distances was 0.58 ($p = 0.014$) between France and Georgia, which indicated a moderate pattern of isolation by distance. The number of migrants after correction of size was high among the French populations ($Nm = 7.6$) and even higher among the four nearby Georgian populations ($Nm = 32$), but it was very low between the pooled French populations and the pooled Georgian populations ($Nm = 0.33$).

Georgia in Caucasia, as an extreme country in the distribution area, can be considered as a source of neutral gene diversity for wild cherry, and thus may also be one for adaptative gene diversity we could use to increase the genetic base of our western country wild cherry breeding populations.

*Key words: Prunus avium*, isozyme, diversity, breeding, France, Georgia.

## Introduction

Wild cherry wood has appreciated industrial properties and a high commercial value, as it is used for cabinet making and panelling. Wild cherry cultivation is profitable for foresters when trees have undergone intensive sylviculture. Breeding programmes aim to provide fast-growing varieties, well-adapted to most conditions, with a good form and a broad genetic base (RASSE *et al.,* 2005). In France, we have tried to enlarge this base by crossing our selected trees with material coming from breeding programmes of other western countries bordering France. Pedoclimatic conditions are there partly similar to France: introduced material may involve potentially useful adaptive traits. After the last glaciation, during the expansion of the species, selection and genetic drift may lead to differentiation of alleles of genes implied in adaptation. To enlarge the diversity of adaptative genes (resistance to pathogens and insects, ability of growing in diverse environments), we could also collect material far away from France but in similar climatic conditions. Evaluating directly the differentiation of populations for adaptative genes is very difficult. Though, if we find a differentiation for neutral genes between France and the target region, this may also imply differentiation for adaptative genes.

Wild cherry spreads widely from North Africa to Southern Sweden, from Ireland to Caucasia. France and Georgia are among extreme countries in this distribution pattern (*Figure 1*). The pollen is dispersed by insects and seeds are dispersed by birds, small mammals and humans (RUSSELL, 2003). The gametophytic incompatibility system leads to a strict outcrossing mating system (DE CUYPER *et al.,* 2005; VAUGHAN *et al.,*

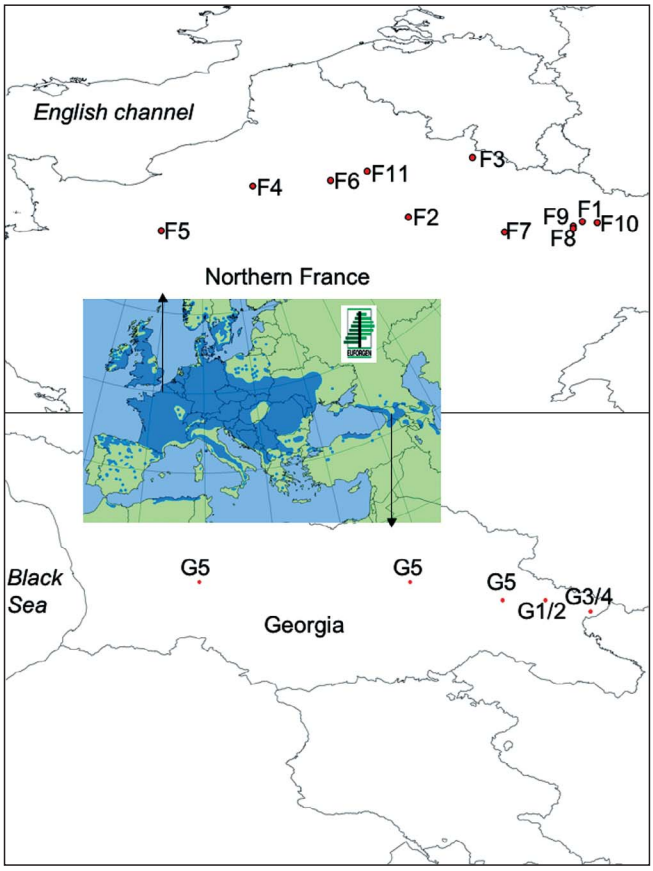


*Figure 1.* – Repartition of populations in France and Georgia. G5 is a miscellaneous "population" composed of seeds sampled in several stands. Source of the distribution map: EUFORGEN.

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2006). Wild cherries grow mainly as single trees or small clumps. The species is even more scattered in southern regions under Mediterranean influence, even if it grows in altitude: this is the case in Italy and Georgia. The number of different genotypes in stands is limited by the frequent occurrence of natural suckering (FRASCARIA *et al.,* 1993; DUCCI and SANTI, 1998; GÖMÖRY and PAULE, 2001; GÖMÖRY, 2004; SCHUELER *et al.,* 2006; VAUGHAN *et al.,* 2007a, 2007b).

Diversity in eight French wild cherry populations were analysed with isozymes (FRASCARIA *et al.,* 1993; MARIETTE *et al.,* 1997; DUCCI and SANTI, 1998). Fourteen populations were also studied with isozymes in Italy (DUCCI and PROIETTI, 1997), and one in Slovakia (GÖMÖRY and PAULE, 2001; GÖMÖRY, 2004). SSRs have been used to explore wild cherry intrapopulation diversity or gene flows (DE CUYPER *et al.,* 2005; SCHUELER *et al.,* 2006; VAUGHAN *et al.,* 2007). MOHANTY *et al.* (2001) explored the cpDNA haplotype differentiation of 23 European wild cherry populations.

For all European species, the last glaciation episode was a major event which affects the distribution of variability. The diversity inside a large part of the distribution area including eventually the history after the last glaciation have been described for numerous wind-pollinated species as for example *Quercus ilex* (MICHAUD *et al.,* 1995), *Fagus sylvatica* (COMBS *et al.,* 2001), *Betula pendula* (PALME *et al.,* 2003b), *Fraxinus angustifolia* (POSTOLACHE *et al.,* 2005), *Ilex aquilofium* (RENDELL and ENNOS, 2003), *Pinus nigra* (AFZAL-RAFII and DODD, 2007), *Picea abies* (VENDRAMIN *et al.,* 2000), but insect-pollinated forest trees have very rarely been studied, and at a limited scale: *Sorbus aucuparia* (RASPÉ and JACQUEMART, 1998), *Prunus spinosa* (MOHANTY *et al.,* 2002).

We aimed to investigate the diversity present in wild cherry populations from countries placed inside the Western and Eastern extremes of the distribution: France and Georgia. It is likely that Georgia represents a centre of origin for the species and therefore may be a source of high adaptive potential. We used isozyme markers: we can compare our results with most other multi-population studies made on wild cherry nucleus neutral variation.

## Material and Methods

*Sampled populations*

We sampled 11 populations in the north of France, collected for commercial purposes (*Tables 1* and *2*, *Figure 1*). The maximum distance between two populations was 442 km, and the minimal distance was three km. Seeds were typically harvested from 10–20 mother trees and pooled into commercial seed lots. Approximately 60 seed were then collected for further analysis in this study. As a consequence of the sampling strategy the parental population structure is unknown and the likely proportion of sibling progeny in each lot is also unknown. In Georgia, as seeds were most often collected under the trees they may originate from more than one mother tree. In order to spare material for future use, we raised plants. Collected seeds germinated poorly, a lot of progenies were lost. We collected buds from 378 plants raised in a nursery from 2 to 21 progenies in eight populations (*Tables 1* and *2, Figure 1*). Five to 21 samples (which each may contain more than one half-sib progeny) inside

*Table 1.* – Collected populations in France and in Asian Georgia.

| Population name | Lat. N | Long. E | Collected by | Mother trees |
|---|---|---|---|---|
| *French populations* | | | | |
| F1 = Bouxières | 48°45' | 6°25' | Office National des Forêts | 10 to 20 |
| F2 = Congy | 48°49' | 3°53' | Vilmorin | 10 to 20 |
| F3 = Damouzy | 49°41' | 4°49' | Vilmorin | 10 to 20 |
| F4 = Dangu | 49°16' | 1°37' | Office National des Forêts | 10 to 20 |
| F5 = La Genevraie | 48°37' | 0°17' | Vilmorin | 10 to 20 |
| F6 = Le Fayel | 49°21' | 2°45' | Vilmorin | 10 to 20 |
| F7 = Montiers | 48°36' | 5°17' | Office National des Forêts | 10 to 20 |
| F8 = Pulnoy | 48°41' | 6°17' | Office National des Forêts | 10 to 20 |
| F9 = Saulxures | 48°39' | 6°17' | Vilmorin | 10 to 20 |
| F10 = Sorneville | 48°44' | 6°38' | Office National des Forêts | 10 to 20 |
| F11 = Saint Gobain | 49°29' | 3°17' | Office National des Forêts | 10 to 20 |
| *Georgian populations* | | | | |
| G1 = Kvareli-A | 41°55' | 45°51' | Raoul Mille, Tbilissi For. Res. Inst. | 6 |
| G2 = Kvareli-B | 41°55' | 45°51' | Raoul Mille, Tbilissi For. Res. Inst. | 5 |
| G3 = Lagodechi-A | 41°49' | 46°15' | Raoul Mille, Tbilissi For. Res. Inst. | 21 |
| G4 = Lagodechi-B | 41°49' | 46°15' | Raoul Mille, Tbilissi For. Res. Inst. | 9 |
| G5 = miscellaneous, included: | | | | 10 |
| *Duseti-A* | *42°05'* | *44°39'* | *Raoul Mille, Tbilissi For. Res. Inst.* | *2* |
| *Duseti-B* | *42°05'* | *44°39'* | *Raoul Mille, Tbilissi For. Res. Inst.* | *3* |
| *Majakovskij* | *42°05'* | *42°47'* | *Raoul Mille, Tbilissi For. Res. Inst.* | *3* |
| *Telavi* | *41°55'* | *45°28'* | *Raoul Mille, Tbilissi For. Res. Inst.* | *2* |

Lat.: latitude, Long.: longitude, For. Res. Inst.: Forest Research Institute
«Mother trees»: in France, putative number of different half-sib progenies sampled on trees; in Georgia, number of seed lots collected under the trees (a lot may contain more than one half-sib progeny).



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*Table 2.* – Genetic diversity estimates for all loci and inbreeding parameters for Sdh-1.

| Populations | Size | P | A | He | | Fis and the result of HW test | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | value | SD | Sdh-1 | Got-1 | Pgi-2 | Idh-1 | Mdh-1 | Pgd-1 | Lap-1 |
| France: | | | | | | | | | | | | |
| F1 | 60 | 86 | 2.1 | 0.333 | *0.011* | **-0.528 *** | -0.063 | 0.027 | 0.322 | -0.017 | 0.190 | -0.175 |
| F2 | 61 | 100 | 2 | 0.371 | *0.013* | -0.321 | 0.051 | -0.055 | **-0.495 *** | -0.297 | -0.181 | -0.071 |
| F3 | 59 | 86 | 1.9 | 0.308 | *0.012* | -0.147 | -0.163 | | 0.269 | 0.166 | -0.237 | -0.150 |
| F4 | 46 | 57 | 2.1 | 0.248 | *0.013* | -0.324 | -0.034 | -0.011 | -0.055 | -0.034 | -0.059 | 0.011 |
| F5 | 59 | 100 | 2 | 0.296 | *0.012* | -0.022 | -0.056 | -0.074 | 0.038 | -0.055 | 0.051 | 0.057 |
| F6 | 60 | 71 | 1.9 | 0.231 | *0.015* | -0.119 | -0.180 | | 0.027 | 0.654 | -0.152 | -0.162 |
| F7 | 59 | 86 | 1.9 | 0.285 | *0.013* | -0.105 | 0.045 | | -0.043 | 0.016 | -0.126 | -0.177 |
| F8 | 62 | 71 | 2 | 0.320 | *0.012* | -0.085 | 0.019 | -0.017 | -0.008 | -0.176 | -0.043 | 0.024 |
| F9 | 60 | 100 | 2 | 0.304 | *0.015* | 0.094 | 0.048 | 0.108 | 0.088 | 0.307 | 0.113 | 0.077 |
| F10 | 57 | 71 | 2 | 0.284 | *0.012* | -0.041 | -0.020 | | 0.328 | 0.300 | 0.077 | 0.003 |
| F11 | 58 | 71 | 2 | 0.242 | *0.013* | -0.152 | -0.027 | -0.018 | 0.209 | -0.046 | **-0.382 *** | -0.113 |
| Pooled | 641 | 100 | 2.1 | 0.324 | *0.005* | | | | | | | |
| Georgia: | | | | | | | | | | | | |
| G1 | 59 | 86 | 2.1 | 0.246 | *0.015* | -0.149 | -0.040 | 0.281 | 0.048 | | 0.016 | 0.101 |
| G2 | 60 | 57 | 2.6 | 0.254 | *0.012* | -0.063 | 0.123 | -0.023 | -0.089 | | -0.009 | -0.228 |
| G3 | 102 | 71 | 2.4 | 0.247 | *0.010* | -0.049 | 0.141 | -0.125 | -0.009 | | -0.041 | 0.151 |
| G4 | 50 | 86 | 2.3 | 0.280 | *0.015* | 0.173 | -0.147 | -0.127 | -0.030 | | -0.086 | -0.227 |
| G5 | 107 | 100 | 2.4 | 0.318 | *0.011* | | | | | | | |
| Pooled | *378* | 86 | 2.3 | 0.284 | *0.006* | | | | | | | |
| Pooled regions | 1019 | 100 | 3.3 | 0.318 | *0.004* | | | | | | | |

*P*: percentage of polymorphic loci, *A*: mean number of alleles over loci, $H_e$: expected heterozygosity and SD: its standard deviation, $F_{is}$: inbreeding coefficient and the result of the Hardy-weinberg test **(* P-value < 0.003)**.

each of two populations roughly 30 km apart at Lagodechi and two populations roughly 10 km apart at Kvareli were collected. Roughly 20% to 50% of the adult wild cherry trees were sampled. We could collect only one or two progenies inside four other populations, which we gathered into one "miscellaneous population" G5 to include them in analyses for which its composite origin did not cause problem. A maximum of 12 plants (6 plants for Lagodechi) per collected mother-tree were used, in order to compare results with French populations without too much imbalance between samples.

*Electrophoretic analyses*

Seven polymorphic loci were studied after protein extraction (Ducci and Santi, 1998) of the germinating seeds or buds. Idh-1 (isocitrate dehydrogenase, E.C. 1.1.1.42), Mdh-1 (malate dehydrogenase, E.C. 1.1.1.37), Pgd-1 (6-phosphogluconate dehydrogenase, E.C. 1.1.1.43) were stained after migration on a morpholine starch system (Clayton and Tretiak, 1972). Got-1 (glutamate oxaloacetate transaminase, E.C. 2.6.1.1), Pgi-2 (phosphoglucoisomerase, E.C. 5.3.1.9) were stained after migration on lithium-borate starch system (Scandalios 1969, modified). Lap-1 (leucine aminopeptidase, E.C. 3.4.11.1) and Sdh-1 (shikimate dehydrogenase, E.C. 1.1.1.25) were stained after isoelectrofocusing (Santi and Lemoine, 1990). Staining procedures and patterns were described in Kaurisch *et al.* (1988) and Santi and Lemoine (1990). The genetic analysis of Idh-1, Mdh-1, Got-1, Lap-1, Sdh-1 was in accordance with a single locus codominant mode of inheritance (Santi and Lemoine, 1990).

*Statistical analyses*

Except when otherwise stated, computations and statistical tests were performed using GENETIX 4.01 (Belkhir Biosoft). Estimates of genetic variation were computed within each of the 16 populations and within the "pooled regions" (all populations inside the region are pooled): percentage of loci polymorphic at the 95% level (*P*), mean number of alleles per locus (*A*), expected heterozygosity ($H_e$) corrected for small sample sizes (Nei, 1978) and its Standard Deviation, computed with 1000 permutations on alleles. For each locus in each population (except G5) the inbreeding coefficient ($F_{is}$) was computed following Weir and Cockerham (1984), and an exact test for departure from Hardy-Weinberg equilibrium was performed using GENEPOP 3.2 (Rousset and Raymond, 1995). The latter software was also used to test the genotypic desequibrium between two loci inside each population and across all populations (except G5). The individual rejection level α' was adjusted for $k = 315$ tests by the Dunn-Sidak method (Sokal and Rohlf, 1995): we choose $\alpha = 5\%$ thus $\alpha' = 1-(1-\alpha)^{1/k} = 0.00016$. Past gene flow, expressed as number of migrants per generation (*Nm*), was calculated using GENEPOP 3.2: a corrected multilocus estimate was calculated using the values from the closest regression line, as published in Barton and Slatkin (1986). Genetic distances of Cavalli-Sforza and Edwards (1967) between



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population pairs were tested based on 10 000 random permutations of individuals. We computed the $G_{st}$ of NEI (1977), the $F_{st}$ of WEIR and COCKERHAM (1984) and *RH'*, an unbiased estimator with minimal variance replacing advantageously $F_{st}$ for $F_{st} < 0.05$ and more than two alleles (RAUFASTE and BONHOMME, 2000). The correlation between genetic and geographical distances between the populations was tested by the Pearson's coefficient of correlation *r*. The true *r* was compared to 1000 pseudo *r* values obtained by random permutations of populations in one of the two matrixes.

## Results

*Allozyme diversity*

A total of 23 alleles (21 in Georgia, 16 in France) were scored at the seven loci across the 16 populations analysed (*Table 3*). The number of alleles detected at each locus ranged from two (Pgd-1, Mdh-1) to four (Lap-1, Pgi-2). Compared with previous studies on wild cherry, we found seven new alleles. We observed only one, very rare, and different from the rare allele found by GÖMÖRY and PAULE (2001), in a French population (Idh-1 allele c in F4, frequency 0.033), others concerned Georgian populations. We detected four very rare alleles (Got-1 alleles d and e, Lap-1 alleles c and d, frequencies from 0.005 to 0.030) in one to three Georgian populations. The two other alleles (Pgi-2 allele d and Sdh-1 allele c) were present in all Georgian populations with frequencies from 0.025 to 0.266 and in four out of five populations with frequencies from 0.026 to 0.067, respectively. Pgi-2 allele d was different from the rare allele detected by GÖMÖRY and PAULE (2001). Got-1 allele c was absent in all populations of this study. It was a very rare allele recorded previously in a French population (FRASCARIA *et al.,* 1993). The range of percentage of polymorphic loci (*P*), mean number of alleles per locus (*A*) and expected heterozygosity ($H_e$) were 57–100%, 1.9–2.6 and 0.231–0.371, respectively (*Table 2*). *P* and *A* were roughly the same (100 and 86%, 2.1 and 2.3) when we pooled French or Georgian populations, but the mean *A* was higher in the Georgian group (2.4) than in the French group (2.0). $H_e$ varied sometimes significantly among populations, and also between the pooled French and the Georgian groups: $H_e = 0.324$ (C.I. = 0.314–0.334) and 0.284 (C.I. = 0.272–0.296), though the mean $H_e$ inside the French and the Georgian groups were not different (0.293 and 0.270). A significant genotypic disequilibrium was found between Got-1 and Lap-1 at multipopulation level, even if it was significant only in F9 at population level.

*Hardy-Weinberg equilibrium tests and $F_{is}$*

A significant multipopulation heterozygote excess was found for Sdh-1 over the 11 French populations, and over all populations, G5 excluded ($F_{is} = -0.181$ and $-0.152$, *Table 3*). Heterozygote excesses for Sdh-1 were scored in ten French populations and in three Georgian populations but only one (F1) is significant ($\alpha = 0.05$, $k = 15$ thus $\alpha' = 0.003$, *Table 2*) at population level. A multilocus significant heterozygote excess was found only in F2 population ($F_{is} = -0.229$, *Table 3*): heterozygote excesses were scored for six loci out of seven and were significant for four of them (*Table 3*).

*Spatial structure of variation and gene flow*

All $F_{st}$ values were significant (*Table 3*). The total multilocus differentiation among 15 populations was 11%. A higher level of differentiation was found inside the French group ($F_{st} = 0.094$) than inside the Georgian group ($F_{st} = 0.057$). Genetic pairwise distances varied from 0.015 to 0.202 (*Table 4*) and the distance between pooled French populations and pooled Georgian populations was 0.061. With the exception of F9–F11 distance ($p = 0.002$), all were significantly ($p < 0.0005$) different from zero. Mean pairwise distances inside the French group, the Georgian group (G5 removed) and between the two groups were 0.054, 0.037 and 0.094, respectively. The Pearson's correlation coefficient between genetic and geographical distances, tested with 1000 permutations of the 15 (G5 removed) populations was 0.580 ($p = 0.014$), which indicated a moderate pattern of isolation by distance. The number of migrants after correction of size was high among the French populations

*Table 3.* – Genetic parameters.

| Parameter | Populations | Got-1 | Idh-1 | Lap-1 | Mdh-1 | Pgd-1 | Pgi-2 | Sdh-1 | all |
|---|---|---|---|---|---|---|---|---|---|
| Number | over 16 populations | 4 | 3 | 4 | 2 | 2 | 4 | 3 | 23 |
| of | *French group* | 2 | 3 | 2 | 2 | 2 | 3 | 2 | 16 |
| alleles | *Georgian group* | 4 | 2 | 4 | 2 | 2 | 4 | 3 | 21 |
| $G_{st}$ | over 15 populations | 0.035 | 0.102 | 0.094 | 0.096 | 0.136 | 0.174 | 0.124 | 0.108 |
| $F_{st}$ | over 15 populations | **0,021*** | **0.098** | **0.091** | **0.099** | **0.145** | **0.174** | **0.133** | **0,108** |
| | *French group* | **0.042** | **0.074*** | **0.094** | **0.064** | **0.133** | **0.242** | **0.115** | **0.094** |
| | *Georgian group* | **0,002*** | **0.037*** | **0.098** | - | **0.096** | **0.047*** | **0.075** | **0.057** |
| $F_{is}$ | over 15 populations | 0.004 | 0.036 | -0.049 | 0.018 | -0.068 | -0.023 | **-0.152** | -0.037 |
| | *French group* | 0.006 | 0.062 | -0.062 | 0.018 | -0.032 | -0.013 | **-0.181** | -0.045 |
| | *F2* | 0.051 | **-0.495** | -0.071 | **-0.297** | **-0.181** | -0.055 | **-0.181** | **-0.229** |
| | *Georgian group* | 0.058 | -0.021 | -0,020 | - | -0.043 | -0.035 | -0.048 | -0.016 |

$G_{st}$, $F_{st}$: differentiation parameters, $F_{is}$: inbreeding coefficient, **Bold values**: significantly different from 0 ($\alpha = 0.05$, $\alpha' = 0.001$), *: RH' replaced $F_{st}$ (RH' is an unbiased estimator with minimal variance replacing advantageously $F_{st}$ for $F_{st} < 0.05$ and more than two alleles). "over 15 populations": the miscellaneous G5 population is excluded.



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*Table 4.* – Genetic distances (CAVALLI-SFORZA and EDWARDS, 1967).

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| F2 | 0.14 | | | | | | | | | | | | | |
| F3 | 0.04 | 0.11 | | | | | | | | | | | | |
| F4 | 0.05 | 0.11 | 0.06 | | | | | | | | | | | |
| F5 | 0.04 | 0.11 | 0.04 | 0.06 | | | | | | | | | | |
| F6 | 0.06 | 0.11 | 0.02 | 0.04 | 0.06 | | | | | | | | | |
| F7 | 0.06 | 0.11 | 0.03 | 0.05 | 0.03 | 0.02 | | | | | | | | |
| F8 | 0.06 | 0.11 | 0.04 | 0.04 | 0.09 | 0.03 | 0.04 | | | | | | | |
| F9 | 0.05 | 0.05 | 0.03 | 0.04 | 0.03 | 0.02 | 0.02 | 0.05 | | | | | | |
| F10 | 0.06 | 0.09 | 0.04 | 0.04 | 0.09 | 0.06 | 0.08 | 0.04 | 0.06 | | | | | |
| F11 | 0.05 | 0.08 | 0.04 | 0.04 | 0.03 | 0.03 | 0.03 | 0.07 | 0.02* | 0.05 | | | | |
| G1 | **0.07** | **0.20** | **0.11** | **0.05** | **0.09** | **0.09** | **0.09** | **0.08** | **0.10** | **0.11** | **0.08** | | | |
| G2 | **0.09** | **0.14** | **0.09** | **0.04** | **0.11** | **0.06** | **0.09** | **0.06** | **0.07** | **0.08** | **0.07** | 0.05 | | |
| G3 | **0.12** | **0.18** | **0.12** | **0.07** | **0.11** | **0.07** | **0.09** | **0.09** | **0.09** | **0.13** | **0.07** | 0.04 | 0.03 | |
| G4 | **0.09** | **0.19** | **0.12** | **0.08** | **0.08** | **0.09** | **0.08** | **0.11** | **0.09** | **0.14** | **0.07** | 0.03 | 0.05 | 0.03 |
| G5 | **0.09** | **0.16** | **0.10** | **0.06** | **0.09** | **0.07** | **0.07** | **0.07** | **0.08** | **0.10** | **0.08** | - | - | - |
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | G1 | G2 | G3 |

*All distances are significantly different from zero except this one.
**Bold**: Georgian x French populations distances.

($Nm = 7.6$) and even higher among the four nearby Georgian populations ($Nm = 32$), but it was very low between the pooled French populations and the pooled Georgian populations ($Nm = 0.33$). There is no relation between geographical and genetic distances inside the French group, and inside the Georgian group.

## Discussion

The sampling in Georgian and French populations was different. In France, the sampling was first done by a commercial firm on an unknown number of mother trees, and a subsequent re-sampling was done inside the lot resulting in unknown number of half-sib progeny and unknown number of individuals by progeny. In Georgia, sampling points were known, even with possibly more than one half-sib progeny within each, and subsequent re-sampling was done with precise number of individuals by sampling point. In all these populations, the genetic structure of the sampled mother trees was unknown: relative importance of suckering, possible familial structure. VAUGHAN *et al.* (2007b) concluded their detailed genetic analysis of a wild cherry stand by recommending that minimum distances of at least 100 m should be imposed between trees selected in seed stands to promote genotypic diversity. No such recommendation existed for French wild cherry seed stands and those have a limited surface size. Sampled trees may have had identical genotypes or be closely related full-siblings, but the proportion of these cases, which is likely to be highly variable between populations, is unknown. Due to the sampling, a French population may be represented by only around 5 half-sib progenies, but also to more than 20. The number of analysed individuals by population is quite homogeneous (46 to 102, most around 60) and the number and variance of half-sib progenies collected inside population may be not that different in France and Georgia with such sampling strategies (more trees collected in France, but the possible suckering may lead to fewer progenies, less trees collected in Georgia, but probably several progenies collected under some trees). Anyhow, the sampling method implies some heterogeneity in number of half-sib progenies even between populations inside each zone, and not less that between zones.

We found a multilocus excess of heterozygotes in one population (F2). It cannot be due to selection for heterozygotes during forest life as seeds were under analysis. The effect of self-incompatibility system would be significant only at closely linked loci (GLÉMIN *et al.,* 2001). STOECKEL *et al.* (2006) associated heterozygote excess with clonality but here only unique genotypes were under analyse. In small populations a missing proportion of homozygotes may appear in offsprings, as allele frequencies between mating types can differ by chance alone when few breeders participate in mating (STOECKEL *et al.,* 2006): the lack of homozygotes in F2 could be explained if the parental population was composed of only several clonal patches. In a Slovakian population, the mating system analysis revealed a high role of mating among relatives: the multilocus effective selfing rate (a measure of consanguinity) was found to be over 5% and very variable among trees, and a high proportion of individuals within single tree's progenies could be full-sibs (GÖMÖRY and PAULE, 2001).

We know that the gametophytic incompatibility locus S and Sdh-1 were linked in the intra-specific cross F1/3A x Charger (JOOBEUR *et al.,* 1998) or the inter-specific cross Napoleon x *P. nipponica* (estimated distance: 32.8 cM, CLARKE *et al.,* 2009): linkage disequilibrium in populations could also explain the observed excess of heterozygotes for Sdh-1. Such excesses were also observed by STOECKEL *et al.* (2006) in three wild cherry populations for UDP98-412, SSR marker very close to the S locus. The linkage disequilibrium between Got-1 and Lap-1 is also explained by the physical linkage between the two loci (SANTI and LEMOINE, 1990).



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The nearby four Georgian populations G1 to G4 were not well differentiated ($F_{st}$ = 0.058, mean genetic distance = 0.037, $Nm$ = 32). French populations, which cover the north of France, were better differentiated ($F_{st}$ = 0.094, mean genetic distance = 0.054, $Nm$ = 7.6). The 14 Italian populations described in DUCCI and PROIETTI (1997) covered Italy from north to south and the $F_{st}$ was the highest found for wild cherry (0.115). In DUCCI and SANTI (1998) the number of different genotypes (2, 6, 7, 7 and 33) found after clone determination in four French and one Italian populations was so low that $F_{st}$ computations were not realistic. With three French and one German populations, a low $F_{st}$ (0.049) and low Gst (0.065) was found in FRASCARIA *et al.* (1993). With one true population and five "populations" composed with French plus-trees, $G_{st}$ was estimated and was also low (0.052) in MARIETTE *et al.* (1997). With SSRs markers, STOECKEL *et al.* (2006) estimated a slightly higher $F_{st}$ (0.074) between three French populations. Western Europe populations appeared not well differentiated.

The $G_{st}$ estimations for wild cherry were comparable with the mean $G_{st}$ of 0.051 HAMRICK *et al.* (1992) reported for species which seeds are dispersed by animal ingestion rather than other dispersion methods, or for species implying asexual as well as sexual rather than only sexual reproduction. Though, in these two cases, the $G_{st}$ differences between species were not significant, which means that other factors affect too much $G_{st}$ variations. A rather similar estimate of $G_{st}$ (0.060) was found for *Sorbus aucuparia* (RASPÉ and JACQUEMART, 1998). Three populations of *Prunus cerasoides* in northern Thailand (PAKKAD *et al.,* 2004) were more distinct ($G_{st}$ = 0.115). Three wild populations of *Prunus armeniaca* in west China revealed also more differentiated ($G_{st}$ = 0.137), and the genetic distance was correlated to the geographical distance; the authors (HE *et al.,* 2007) think that long-distance dispersal of pollen limited the differentiation. Even more differentiation between populations was found for *Sorbus torminalis*: $F_{st}$ = 0.150, as reported in France by DEMESURE *et al.* (2000). A long-distance dispersal of seeds by birds was expected as fruit maturity occurs in autumn rather than in early summer. For this scattered species, the authors suggested the influence of periodical extinction and creation of new populations in a metapopulation model to increase the differentiation of populations. Such an explanation could also be applied for wild cherry considering the large, continuous wild cherry distribution pattern in Northern France and the scattered and better differentiated populations of Italy. Another explanation could be that the landscape is more structured (mountains) in whole Italy compared to the open landscape of Northern France: SU *et al.* (2003) found that for four insect pollinated species (*Prunus armeniaca* included) populations were more differentiated when sampled on both sides of the Great Wall anthropoid structure rather than on the control side. This could occur even if scattered trees have a wider range of pollen donors than clustered trees (insect-pollinated *Enterolobium cyclocarpum*, HAMRICK, 2004), and thus may disseminate largely.

Differentiation was low inside the French and Georgian groups, and was higher between French and Georgian populations, according to several parameters. The mean number of migrants $Nm$ was very low (less than one) between the two pooled groups, compared to the $Nm$ estimates inside French and Georgian groups (23 fold and 97 fold higher). With SSRs, STOECKEL *et al.* (2006) estimated $Nm$ = 3.4 between three French wild cherry populations, which was comparable to our estimate (7.6). The mean genetic distance was larger between French and Georgian populations than inside those groups and we found a significant correlation between geographical and genetic distances only between the French and Georgian groups. Using SSR markers, VAUGHAN *et al.* (2007b) showed that both pollen and seed dispersal were limited in a British wild cherry stand. Though, the animals "in charge" of the dispersal of pollen (mainly bumblebees) and seeds (small mammals, birds and humans) can potentially cover kilometric distances, which explains such small regional differentiations. Diversity based on $H_e$ indices (on pooled populations and means over populations) was smaller in Georgia as new alleles were rare ones, some alleles were less frequent and the sampled population size was smaller. The main difference between the two areas was the six new alleles we discovered in Georgia, despite the populations analysed there were very close one to another, and the number of analysed individuals far lower (1/2 of French sample within this study, and 1/3 of genets analysed with isozymes in France). Moreover, the selection of enzymatic systems was based on previous studies where only French trees were analysed: the monomorphic systems were then excluded in following studies. This prevented us to discover more new alleles in Georgia. SSRs would have been more efficient to more completely reveal new diversity, without such a bias, as those markers are more polymorphic. Central Europe revealed also original diversity, compared to France, Georgia and Italy, as GÖMÖRY and PAULE (2001) found three new rare alleles in one population of Slovakia.

European species found refugia in the south during the last glaciation, except for species able to survive at higher latitudes (e.g. *Betula pendula*, PALME *et al.,* 2003b). Specific diversity is thus expected in southern European areas, though most total diversity could be expected higher, north of the main mountain ranges, due to the mixing of colonization routes (PETIT *et al.,* 2003). Previous studies on wild cherry in Italy (DUCCI and PROIETTI, 1997, DUCCI and SANTI, 1998) did not reveal any new isozyme alleles, when compared with studies on French populations. Alps and the Pyrenees have been identified as suture zones for some species, but in some species there was no differentiation between Italy and northern areas (PALME *et al.,* 2003b). For the larger study on wild cherry, using cpDNA (a marker which reveals the influence of seed dispersal gene flow, as it is maternally-inherited in angiosperms), the 23 populations were sampled from Great Britain to Greece and from Sweden to Italy, and their analyses revealed an efficient cytoplasmic gene flow when compared to other European, animal-dispersed seed species: due to the edible flesh of wild cherry, long distance seed dispersal by humans appeared as a source of gene flow among populations. The authors (MOHANTY *et al.,* 2001) could



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even not precise the history of colonization routes: wild cherry history after the last glaciation remains unknown, on the contrary of some species for which gene pools have been identified (*Pinus nigra*, Afzal-Rafii and Dodd, 2007); though, a sharp decrease of haplotype diversity was nevertheless observed from south to north of Europe. Unfortunately, palynological records lack on Prunus. For the shrub *Prunus spinosa*, intensive gene flow also probably follow recolonization routes: haplotype diversity was higher in southern Europe than in northern Europe, indicating probable localization of glacial refugia in southern Europe, but there was incongruency between the phylogeny of haplotypes and their geographic locations (25 populations analysed by Mohanty *et al.,* 2002). For *Salix caprea*, the lack of phylogeographic structure (even lower than for wild cherry) could include the consequence of the presence of intermediate latitude refugia with large population sizes and a high speed of recolonization and dispersal ability (Palme *et al*., 2003a). We cannot exclude intermediate latitude refugia for wild cherry. For *Quercus petraea*, recent gene flow and diversifying selection erased progressively the initial structure established at the end of the glaciation and maintained during the colonization (Kremer *et al.,* 1999). For wild cherry, long distance gene flow but diversifying selection on selected genes may also provide populations differentiated for their adaptative values.

For the first time, the diversity of wild cherry in Caucasia was sampled, in a small region of Georgia. The markers used revealed original diversity there, though the intrinsic within-population diversity was lower ($H_e = 0.284$) than in France ($H_e = 0.324$). As reviewed by Hamrick (2004), tree species life characteristics (individual longevity, high intra-population genetic diversity, high rates of pollen flow) make them especially resistant to the loss of genetic diversity during changing environmental conditions. Though, when possible we ought to take into account the potential of populations when managing breeding programmes or conservation programmes. As stated by Petit *et al.* (2003) about a large panel of species, the genetic uniqueness of south eastern European populations should largely outweigh their low diversity for long-term conservation purposes. Georgia in Caucasia, as an extreme country in the distribution area, can be considered as a source of neutral gene diversity for wild cherry, and thus may be one for adaptative gene diversity also. Between extreme regions isolated by distance, interesting alleles may have been lost by drift: as stated by Gömöry and Paule (2001), the lower genetic variation observed in wild cherry, compared to other tree species, wind-pollinated, with large populations and no vegetative propagation could be related to allele loss through genetic drift. We could use the eastern source of diversity to increase the genetic base of our western countries wild cherry breeding populations, which is a necessary goal with a challenging climate change.

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