

ARTICLE

Genotyping-by-sequencing of pear (*Pyrus* spp.) accessions unravels novel patterns of genetic diversity and selection footprints

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Understanding of genetic diversity and marker-trait relationships in pears (*Pyrus* spp.) forms an important part of gene conservation and cultivar breeding. Accessions of Asian and European pear species, and interspecific hybrids were planted in a common garden experiment. Genotyping-by-sequencing (GBS) was used to genotype 214 accessions, which were also phenotyped for fruit quality traits. A combination of selection scans and association analyses were used to identify signatures of selection. Patterns of genetic diversity, population structure and introgression were also investigated. About 15 000 high-quality SNP markers were identified from the GBS data, of which 25% and 11% harboured private alleles for European and Asian species, respectively. Bayesian clustering analysis suggested negligible gene flow, resulting in highly significant population differentiation ($F_{st} = 0.45$) between Asian and European pears. Interspecific hybrids displayed an average of 55% and 45% introgression from their Asian and European ancestors, respectively. Phenotypic (firmness, acidity, shape and so on) variation between accessions was significantly associated with genetic differentiation. Allele frequencies at large-effect SNP loci were significantly different between genetic groups, suggesting footprints of directional selection. Selection scan analyses identified over 20 outlier SNP loci with substantial statistical support, likely to be subject to directional selection or closely linked to loci under selection.

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INTRODUCTION

Pear belongs to the genus *Pyrus* in the family Rosaceae, and has a basic chromosome number of $x = 17$. The number of catalogued species, most of which are diploid ($2n = 34$), in the genus *Pyrus* vary according to different studies, but there could be as many as 75 species.¹ It is believed that genus *Pyrus* originated during the Tertiary period (65 to 55 million years ago) in the mountainous regions of western China. Evidence suggests that pear dispersion and speciation followed the mountain ranges to both the east and the west.² The ancient Romans made a great contribution to pear domestication by developing methods of propagation, grafting and caring for fruit. There were reported to be more than 40 cultivars existing in the 1st century B.C.¹ Pear has been cultivated for at least 2000–3000 years, and is currently grown commercially in > 50 countries in Europe, Northern Africa, Asia, Australasia and North America.³ One of the main reasons breeding programmes are present in almost every continent is because it is important to have cultivars adapted to their growing environment. In spite of the wide geographical distribution of the genus, there are no major incompatibility barriers to interspecific hybridisation. Interspecific hybrids are sometimes developed in pear breeding programmes to produce new cultivars with novel combinations of texture and flavour, and to improve resistance to pests and diseases.^{4,5}

Molecular markers have become the preferred tools for characterising genetic diversity. The most frequently used method to assess population differentiation is the calculation of F_{st} , a summary statistic that quantifies the variation in marker allele

frequencies between populations.⁶ Genetic diversity and genetic relatedness studies within and between species in Asian pears identified markers specific to species, and the clustering of species was largely in agreement with their geographic distribution.^{7–9} Genetic analysis of 145 wild and cultivated accessions of *P. communis* clearly separated accessions native to the Caucasus Mountains from those native to Eastern European countries.¹⁰ Clustering patterns corresponding with geographic origin were also observed among *P. communis* accessions collected from 12 provenances in Northern Spain.¹¹

Studies on genetic diversity among Asiatic and European pears revealed three genetic groups, with the primary division between occidental (Europe and Central Asia) and oriental (East Asia) pears, followed by division of Japanese and Chinese accessions.^{7,12,13} Artificial as well as natural interspecific hybridisation have resulted in complex population structures of pear accessions. Bayesian inference of population structures showed that Japanese *P. ussuriensis* was genetically admixed with two genetic clusters: true native *P. ussuriensis* var. *ussuriensis* and prehistorically introduced *P. pyrifolia*.¹⁴ Clustering patterns of some *P. communis* accessions from Turkey and Macedonia indicated gene flow and introgression resulting from co-occurring congeneric subspecies.¹⁰ Some earlier studies using dominant markers revealed that the Chinese sand pear (*P. pyrifolia*) and the white pear (*P. x bretschneideri*) might share a common ancestor.⁸

Pyrus diversity studies to date have relied on a limited number of markers (< 150). Using a small number of markers can only detect genetic diversity of limited regions of the genome, and

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