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## Potential assessment of genome-wide association study and genomic selection in Japanese pear *Pyrus pyrifolia*

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Although the potential of marker-assisted selection (MAS) in fruit tree breeding has been reported, bi-parental QTL mapping before MAS has hindered the introduction of MAS to fruit tree breeding programs. Genome-wide association studies (GWAS) are an alternative to bi-parental QTL mapping in long-lived perennials. Selection based on genomic predictions of breeding values (genomic selection: GS) is another alternative for MAS. This study examined the potential of GWAS and GS in pear breeding with 76 Japanese pear cultivars to detect significant associations of 162 markers with nine agronomic traits. We applied multi-locus Bayesian models accounting for ordinal categorical phenotypes for GWAS and GS model training. Significant associations were detected at harvest time, black spot resistance and the number of spurs and two of the associations were closely linked to known loci. Genome-wide predictions for GS were accurate at the highest level (0.75) in harvest time, at medium levels (0.38–0.61) in resistance to black spot, firmness of flesh, fruit shape in longitudinal section, fruit size, acid content and number of spurs and at low levels (<0.2) in all soluble solid content and vigor of tree. Results suggest the potential of GWAS and GS for use in future breeding programs in Japanese pear.

**Key Words:** genome-wide association study (GWAS), genomic selection (GS), Japanese pear, ordinal categorical traits, harvest time, black spot resistance.

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### Introduction

Genetic improvement of fruit trees is strongly hindered by their long lifespan, large plant size, an extended juvenile phase for seedlings and a marketable product that cannot be assessed until a seedling is physiologically mature (Luby and Shaw 2001, Rikkerink *et al.* 2007). Multiple biotic and abiotic factors that affect both quality and quantity of products during preharvest and postharvest periods also add complications to the genetic improvement (Rikkerink *et al.* 2007). Marker-assisted selection (MAS) is one candidate technology to surmount fruit tree breeding problems because it enables selection without field testing and can accelerate the selection process and reduce the progeny sizes and the costs of carrying individuals to maturity in the fields (Luby and Shaw 2001). Attempts to use MAS in fruit tree breeding programs, however, are just beginning and remain limited to

the selection of a few simply inherited traits because marker development for MAS via bi-parental QTL mapping is also hindered by the same complications as described above.

High-throughput genotyping technologies such as DNA chips (Gupta *et al.* 2008) and genotyping using the next generation sequencing (Davey *et al.* 2011) have enabled new genomic-based strategies such as genome-wide association study (GWAS), which is a method for detecting causal genes or QTL based on the association between genome-wide markers and phenotypes caused by linkage disequilibrium (LD) between markers on one hand and causal genes or QTL on the other. Initially developed for detection of human disease genes, GWAS has emerged as a powerful tool also in plant species (Hamblin *et al.* 2011, Yu and Buckler 2006). GWAS, which requires line-crossing experiments, is suitable for QTL detection especially in long-lived perennials (Oraguzie *et al.* 2007). However, it is just beginning to be applied to fruit tree species and only in a few species (Myles *et al.* 2011).

Novel genotyping technologies also lead the way for genomic-based breeding strategies such as genomic selection

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(GS, Meuwissen *et al.* 2001). Similarly to GWAS, GS uses LD between markers on one hand and causal genes and QTL on the other. Unlike GWAS, however, it is designed to detect genes and QTL, GS aims to predict the genetic potential (e.g. breeding values) of breeding lines without locating genes and QTL. GS has been put to practical use in animal breeding (Hayes *et al.* 2009) and is gathering attention also in plant breeding (Grattapaglia *et al.* 2010, Heffner *et al.* 2009, Iwata *et al.* 2011, Jannink *et al.* 2010, Kumar *et al.* 2012, Lorenz *et al.* 2011). GS can avoid issues of uncertainty in QTL identification and effect estimation, which can be problematic in MAS, by estimating the effects of all marker loci simultaneously. The simultaneous estimation of genomic effects provides the further benefit that even effects that might be too small to be declared “statistically significant” can be captured by markers. Because of these characteristics, GS is expected to be efficient even for a low-heritability polygenic trait (Heffner *et al.* 2009, Jannink *et al.* 2010, Lorenz *et al.* 2011), whereas MAS is unsuitable for the improvement of such a trait.

Pear (*Pyrus* spp.), which belongs to the subfamily Spiraeoideae in the family Rosaceae, is an important fruit tree grown in Asia, Europe and all other temperate regions around the world. Approx. 22 million tons of pear fruit, accounting for 4% of all fruit production (FAOSTAT, <http://faostat.fao.org/>), are produced worldwide. Four major species (Japanese pear, *Pyrus pyrifolia* Nakai; European pear, *P. communis* L.; Chinese pear, *P. bretschneideri* Rehd. and *P. ussuriensis* Maxim.) are grown commercially in temperate regions (Bell 1990). In pear breeding, as in other fruit tree breeding, a long time is required. Japanese pear takes about 15–20 years from the crossing of parental lines to the release and registration of new cultivars. For example, ‘Hosui’ (syn. ‘Housui’) took 18 years (Kajiura *et al.* 1974) and ‘Shuurei’ 21 years (Kotobuki *et al.* 2004). During the long breeding process, large areas of land are necessary for plant maintenance and fruit evaluation. At the National Institute of Fruit Tree Science (NIFTS, Ibaraki, Japan), the first stage of selection programs of Japanese pear required 0.4 ha to test 1,000 trees in a field trial. The last stage required ca. 0.33 ha to test 100 trees. Intensive labor is also necessary for plant maintenance and fruit evaluation. At the end of the breeding process, however, a high proportion of inferior seedlings are destined for culling, as generally required for most fruit breeding programs (Rikkerink *et al.* 2007). In Japanese pear breeding, the current most important breeding targets are disease resistance and the rate of fruit set, in the short term and the restoration of vigor that has been reduced during unintentional inbreeding, in the long term. To investigate the inheritance of these economically important complex traits, numerous high-throughput SNP markers are currently under development in addition to hundreds of SSR markers that have been developed to date (Fernandez-Fernandez *et al.* 2006, Inoue *et al.* 2007, Nishitani *et al.* 2009, Sawamura *et al.* 2004, Yamamoto *et al.* 2002a, 2002b, 2002c, 2007). To improve the complex target traits described above while

maintaining the high-quality characteristics of current modern cultivars, it is necessary first to develop genomic-breeding approaches based on genomic studies and tools that have been conducted and developed. Pear is a long-lived perennial. Therefore, GWAS and GS will play a central role in the development and implementation of genomic-breeding approaches.

The object of this study is to evaluate the potential of GWAS and GS in future Japanese pear breeding. We conducted GWAS and validated the accuracy of GS with a dataset consisted of 162 markers and 76 Japanese pear cultivars. GWAS and GS are both LD-based approaches possibly affected by population stratification. Therefore, we evaluate the extent of LD and population structure in a population of the Japanese pear cultivars. For both GWAS and GS, we investigated nine agronomic traits that are important in Japanese pear production: harvest time, resistance to black spot, firmness of flesh, fruit size (fruit weight), fruit shape in longitudinal section, acid content, total soluble solid content (sugar content), number of spurs and tree vigor. In GWAS and GS model training, we applied multi-loci Bayesian models that were able to treat multi-allelic marker data and ordinal categorical trait data. Although the scale (i.e., the number of markers and cultivars) of this study might be small to evaluate the full potential of GWAS and GS, the study was undertaken to collect basic information about LD and population structure in a population of Japanese pear cultivars and to evaluate the future potential of GWAS and GS in pear breeding.

## Materials and Methods

### Plant materials

For this study, we used 76 Japanese pear varieties including 31 modern elite cultivars, 19 old cultivars, 17 indigenous cultivars, and 9 breeding lines (Table 1). Among them, 17 cultivars were bred and released by the National Institute of Fruit Tree Science (NIFTS, Ibaraki, Japan). All plant materials were maintained and collected at NIFTS. Genomic DNA was isolated from young leaves using a Genomic-tip 20/G (Qiagen Inc., Germany) as described by Yamamoto *et al.* (2006).

### Genetic markers

The 76 varieties were genotyped for 162 DNA markers, including 155 simple sequence repeats (SSRs) from pear and apple, four RAPD-STS, two ACC synthase genes and one locus encoding S-RNase gene, which are listed in the Appendix (Guilford *et al.* 1997, Ishimizu *et al.* 1999, Itai *et al.* 2003, Liebhard *et al.* 2002, Sawamura *et al.* 2004, Silfverberg-Dilworth *et al.* 2006, Terakami *et al.* 2006, Yamamoto *et al.* 2002b). Then SSR-PCR amplification was performed in a 10 µl reaction solution containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.001% gelatin, 0.2 mM each of dNTPs, 5 pmol of each forward primer labeled with fluorescent chemical (Fam/Vic/Ned)

**Table 1.** List of pear cultivars and breeding lines analyzed in this study. Asterisks after release years represent modern elite cultivars bred by the National Institute of Fruit Tree Science (NIFTS, Ibaraki, Japan)

Name	Type	Release year	Name	Type	Release year
Akiakari	Modern	2003*	Doitsu	Indigenous	
Akizuki	Modern	2001*	Natsuhikari	Modern	1995
Akibae	Modern	1997	Nangetsu	Modern	1997
Akemizu	Modern	1997	Niitaka	Old	
Asahi	Old		Nijisseiki	Indigenous	
Atago	Old		Nikkori	Modern	1996
Amanogawa	Indigenous		Hatsuaki	Old	
Ishii Wase	Indigenous		Hakko	Modern	1972*
Ichihara Wase	Indigenous		Hattatsu	Old	
Inagi	Old		Hayatama	Modern	1968*
Imamuraaki	Indigenous		Touno	Old	
Oshuu	Modern	2003*	Hiratsuka-10	Modern	
Okusankichi	Indigenous		Heiwa	Old	
Natsushizuku	Modern	2005*	Hougetsu	Modern	1994*
Gion	Old		Hosui	Modern	1972*
Kikusui	Old		Hokushin	Modern	1997
Kisui	Modern	1990	Mishirazu	Indigenous	
Kimitsuka Wase	Old		Meigetsu	Indigenous	
Kunitomi	Old		Yali	Indigenous	
Kumoi	Modern	1955*	Yasato	Modern	1990*
Kogetsu	Old		Yachiyo	Old	
Kosui	Modern	1959*	Yanaga	Old	
Sagami	Old		Laiyangcili	Indigenous	
Shuugyoku	Modern	1988*	Ri-14	Breeding line	
Shuurei	Modern	2003*	Wakahikari	Modern	1992
Shinkou	Old	1941	Waseaka	Indigenous	
Shinsui	Modern	1965*	Wase Kouzou	Indigenous	
Shinsei	Modern	1984*	162-29	Breeding line	
Shinseiki	Old		42-6	Breeding line	
Shinsetsu	Modern	1949	C2	Breeding line	
Suisei	Modern	1955*	266-27	Breeding line	
Seigyoku	Old		48-96	Breeding line	
Seiryuu	Indigenous		I-33	Breeding line	
Taihaku	Indigenous		O-9	Breeding line	
Tama	Modern	1971	Shinchuu	Indigenous	
Chikusui	Modern	1989*	92-7	Breeding line	
Choku	Modern	1973	Tsukuba52	Modern	
Chojuro	Indigenous		Tsukuba53	Modern	

and unlabelled reverse primer, 5 ng of genomic DNA and 0.25 unit of Taq polymerase (Invitrogen Corp., USA). Amplification was performed as follows: 35 cycles at 94°C for 1 min, 50–55°C for 1 min and 72°C for 2 min, for denaturation, annealing and primer extension, respectively. The PCR products were separated and detected using a DNA sequencer (Genetic Analyzer 3100, Applied Biosystems, USA). The amplified band size was determined based on an internal standard DNA (400HD-Rox, Applied Biosystems, USA) using GeneScan software (Applied Biosystems, USA).

Identification of *S1–S7* alleles was performed according to the protocol described by Ishimizu *et al.* (1999). A procedure for detecting the *S8* allele was reported by Castillo *et al.* (2001). Allele-specific PCR for detection of the *S9* allele was conducted by DNA sequencing, as described by Sawamura *et al.* (2002). Four RAPD-STS markers were

analyzed according to the method described in Terakami *et al.* (2006). Two ACC synthase genes, which were involved in the ethylene synthesis pathway, were genotyped using the method reported by Itai *et al.* (2003). Linkage groups and roughly estimated map positions of DNA markers in this study were identified using three genetic linkage maps of a Japanese pear variety ‘Hosui’ and European pear varieties ‘Bartlett’ and ‘La France’ (Nishitani *et al.* 2009, Terakami *et al.* 2009, Yamamoto *et al.* 2007). We combined the information of three linkage maps using ‘Bartlett’ map as a framework and locating markers that were specific to ‘Hosui’ and ‘La France’ maps at their relative positions to common markers. Correspondence between the pear maps to the saturated reference map of apple suggests that the combined information covered almost the entire genome, which consists of the 17 pairs of chromosomes of Japanese pear (Yamamoto *et al.* 2007).

#### Phenotypic records

Nine phenotypic traits were characterized for 76 cultivars based on the plant genetic resource criteria (Kotobuki 1999), including harvest time, resistance to black spot, firmness of flesh, fruit size (fruit weight), fruit shape in longitudinal section, acid content, total soluble solid content (sugar content), number of spurs and vigor of tree. The abbreviations, the number of rating scale categories and the number of observations in each category of traits analyzed in this study are presented in Table 2.

#### LD and population structure analysis

LD between pairs of markers was evaluated using the R package “genetics”. The function “LD” in the “genetics” package estimated unknown linkage phase using maximum likelihood and calculated LD statistics. In this study, we calculated the squared allele-frequency correlation ( $r^2$ ) between the most common alleles at both of markers located on the same chromosomes. The  $r^2$  values were shown against marker distance in cM. The relation between the  $r^2$  values and linkage map distance between the corresponding markers was further modeled by fitting local polynomials with the function “locpoly” in the R package “KernSmooth”.

The population structure of the 76 pear cultivars was estimated via hierarchical clustering and Bayesian model-based clustering. The hierarchical clustering was conducted based on the Ward method (Ward 1963) using the R function “hclust”. Simple allele-sharing distances (Bowcock *et al.* 1994) between the 76 cultivars were calculated based on the genotypes of the 162 markers and used in the clustering. Bayesian mode-based clustering (Pritchard *et al.* 2000) was conducted using the program Structure. Eighty markers were selected from the 162 markers so that each pair of adjacent markers was more than 5 cM apart. Markov chain Monte Carlo (MCMC) cycles were repeated  $1 \times 10^6$  times after  $1 \times 10^5$  cycles of a burn-in period. In the analyses, we tested the admixture models with 1–7 populations. Because the posterior probability of a model almost reached a plateau

**Table 2.** Summary of traits evaluated in this study

Trait	Abbr.	Number of levels	Rank or measurement unit	Number of observations in each category
Harvest time	HarT	4	very early, early, intermediate, late (observation)	10, 16, 32, 18
Resistance to black spot	BSR	2	resistance, susceptibility (observation)	11, 65
Firmness of flesh	FruH	3	soft (<4.9 lb), intermediate (5.0–6.9 lb), firm (>7.0 lb) (measurement using Magness-Taylor pressure tester)	45, 23, 6
Fruit size (fruit weight)	FruW	3	small (25–199 g), intermediate (200–399 g), large (≥400 g) (measurement of average weight of mature fruit)	3, 43, 28
Fruit shape in longitudinal section	FruS	5	oblate, round, broad elliptical, spindle, obovate (observation)	43, 19, 5, 3, 4
Acid content	Aci	3	low (<4.3), intermediate (4.4–4.6), high (>4.7) (measurement of pH of juice)	48, 18, 8
Total soluble solid content (sugar content)	SugC	3	low (<10.4%), intermediate (10.5–12.4%), high (>12.5%) (measurement of Brix of juice)	3, 52, 19
Number of spurs	SpuN	3	few, intermediate, many (observation)	14, 37, 25
Vigor of tree	TreV	3	weak, intermediate, strong (observation)	4, 57, 15

when the number of population ( $K$ ) was four, we chose  $K = 4$  and obtained estimates for the proportion of variety  $i$ 's genome that originated from population  $k$ ,  $q_{ik}$ . The Q matrix whose  $(i, k)$ -th element was  $q_{ik}$  was further incorporated into the Bayesian regression model for GWAS.

#### Bayesian regression in GWAS and GS prediction

In GWAS, we fit a linear model that includes the effects of population stratification as well as effects of multiple QTL (Iwata *et al.* 2007, 2009). The phenotypic records analyzed in this study were scored in several ordered categories. Therefore, we modeled observed ordinal data via an ordinal probit model (Iwata *et al.* 2009). The model presumed a latent (i.e., unobservable or unrecorded) continuous variable  $y_i^*$  that underlies the observable (or recorded) ordinal response  $y_i$  for the  $i$ th sample. When the phenotypic records were scored in  $M$  ordinal categories, the value of each  $y_i^*$  falls into one of  $M$  contiguous bins on the real line demarcated by the cut-points  $\kappa_0, \kappa_1, \dots, \kappa_M$  and the observed values of  $y_i$  are determined using the following relation:

$$y_i = m \quad \text{if } \kappa_{m-1} < y_i^* \leq \kappa_m \quad (m = 1, 2, \dots, M).$$

Because the cut-points are also unobservable, the values of  $\kappa_m$  were also estimated, but the first, second and last cut-points were fixed as  $\kappa_0 = -\infty$ ,  $\kappa_1 = 0$  and  $\kappa_M = \infty$ . The latent continuous variable  $y_i^*$  was then described as

$$y_i^* = \sum_k q_{ik} \alpha_k + \sum_j \sum_l \gamma_j (x_{ijl} + x'_{ijl}) \beta_{jl} + e_i. \quad (\text{Eq. 1})$$

In that equation,  $q_{ik}$  represents the estimated share of variety  $i$ 's genome that originated from population  $k$ .  $\alpha_k$  stands for the population effect associated with population  $k$  ( $k = 1, 2, \dots, K$ ).  $L_j$  represents the number of alleles of marker  $j$  ( $j = 1, 2, \dots, J$ ).  $x_{ijl}$  ( $x'_{ijl}$ ) denotes the maternal (paternal) allele of marker  $j$  for variety  $i$  and equals 1 if the maternal (paternal)

allele is the  $l$ th allele ( $l = 1, \dots, L_j$ ) and 0 otherwise.  $\gamma_j$  signifies the indicator variable and  $\gamma_j = 1$  corresponds to the case in which marker  $j$  is included in the model as a QTL representative and  $\gamma_j = 0$  implies exclusion.  $\beta_{jl}$  denotes the genetic effect associated with the allele  $l$  for marker  $j$ , which is assumed to follow  $N(0, \sigma_{\beta_j}^2)$ .  $e_i$  the residual error, which is assumed to follow  $N(0, \sigma_e^2)$ . The genetic variance of marker  $j$ ,  $\sigma_{\beta_j}^2$  and the error variance, i.e.,  $\sigma_e^2$  were assumed to follow scaled inverted chi-square distributions  $\text{Inv-}\chi^2(v_\beta, S_\beta)$  and  $\text{Inv-}\chi^2(v_e, S_e)$  as described in Iwata *et al.* (2009). A prior probability for  $\gamma_j$  is assumed to follow the Bernoulli distribution with probability  $\pi = \frac{\lambda}{J}$ , as described in Iwata *et al.* (2010).

In GS prediction, we used two different models for building prediction models. The first model is the same as that used in GWAS (i.e. Eq. 1), except that it did not involve the effects of population structure. We removed the effects of population structure from the GS prediction model, as is usually done, because spurious associations are not an important cause of loss of predictive ability (Lorenz *et al.* 2011). The second model assumed that all markers always have non-zero effects, aiming to capture polygenic effects and is a special case of the first, in which  $\gamma_j$  were fixed to 1. The model fixing  $\gamma_j$  to 1 corresponded to the model known as BayesA (Meuwissen *et al.* 2001) and Bayesian shrinkage regression (Xu 2003), whereas the model with variable  $\gamma_j$  corresponds to the model known as BayesB (Lorenz *et al.* 2011, Meuwissen *et al.* 2001) and stochastic search variable selection (George and McCulloch 1993, Kuo and Mallick 1998).

Bayesian estimation of parameters in the models was conducted as described in Iwata *et al.* (2009). MCMC sampling was used for Bayesian inference for each of the parameters. For each dataset, MCMC cycles were repeated

$13 \times 10^4$  times. The first  $3 \times 10^4$  (burn-in) cycles were not used for estimation of the parameter values. The sampling conducted every ten cycles to reduce serial correlation, so that the total number of samples we retained was  $1 \times 10^4$ . The hyperparameters of the models were set as  $\nu_\beta = 4$ ,  $S_\beta^2 = 0.04$ ,  $\nu_e = -2$ ,  $S_e^2 = 0$  and  $\pi = 0.06$  ( $\lambda = 10$ ) for BayesB. For BayesA, we set  $S_\beta^2 = 0.004$  instead to incorporate consideration of the fact that most markers have small genetic effects. This sampling scheme was based on the previously described evaluation of the convergence of MCMC cycles (Iwata *et al.* 2007, 2009).

In GWAS, we identified markers showing significant association with phenotype via the posterior mean of  $\gamma_j$ . Larger posterior mean of  $\gamma_j$  indicated that the  $k$ th marker was associated with phenotype more significantly. We used the phenotype permutation method (Churchill and Doerge 1994) to ascertain an appropriate threshold for the posterior mean of  $\gamma_j$ . We permuted the phenotypes randomly to eliminate the association between phenotypes and marker genotypes and fitted the Bayesian model to each of the permuted datasets in the same way as the original dataset. For each dataset, we monitored the largest posterior mean of  $\gamma_j$ , which was largest among all the markers, as a test statistic. An empirical distribution of the test statistic was obtained by repeating the permutation analysis 100 times. Finally, we chose the significance threshold based on the empirical distribution at the 90% percentile.

In GS prediction, we measured the accuracy of prediction via leave-one-out cross-validation (LOOCV). Using the Bayesian regression described above, we were able to predict the values ( $\hat{y}_i^*$ ) of latent continuous variable  $y_i^*$  for a left-out sample as

$$\hat{y}_i^* = \tilde{\alpha}_1 + \sum_j^L \tilde{\gamma}_j (x_{ijl} + x'_{ijl}) \tilde{\beta}_{jl}$$

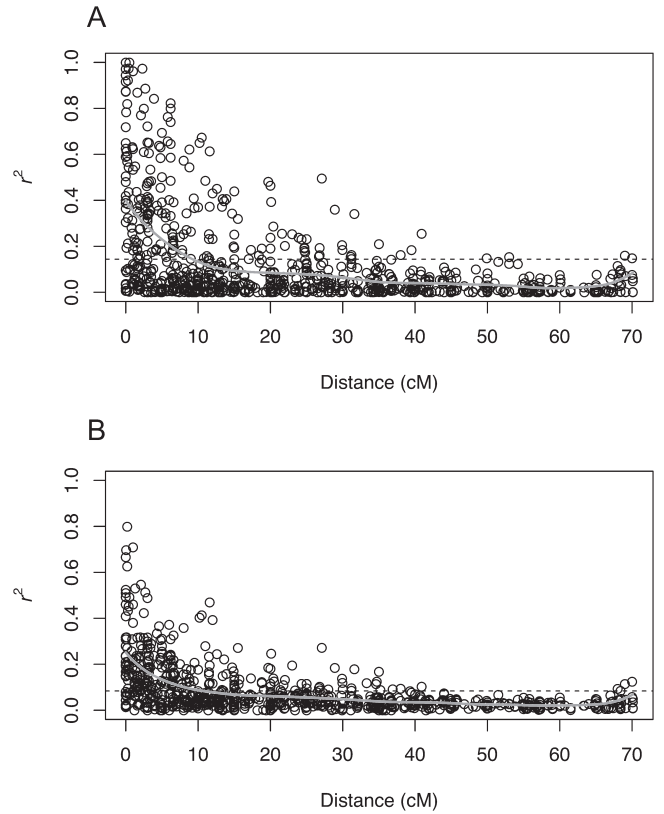
where  $\tilde{\alpha}_1$ ,  $\tilde{\beta}_{jk}$  and  $\tilde{\gamma}_j$  are the posterior means of the parameters estimated with non-left-out samples (i.e., samples for “training” a model). As described above, we removed the effects of population structure from the GS prediction model by setting  $q_{il} = 1$  and thus  $\tilde{\alpha}_1$  became the constant term. In BayesA,  $\tilde{\gamma}_j$  was always fixed to 1 as described above. We calculated a Pearson’s product moment correlation coefficient between  $y_i$  and  $y_i^*$  as an index of the accuracy of GS prediction.

To see the prediction accuracy based on markers that were significant in GWAS, we built GS prediction models that involved only the significant markers: the reduced model had the identical form as Eq. 1 except that it involved the effect of marker  $j$  only when the marker  $j$  was significant in GWAS. For the reduced model, the accuracy of the predictions was measured via LOOCV as for the full model (i.e., the model involving all markers).

## Results

### LD and population stratification

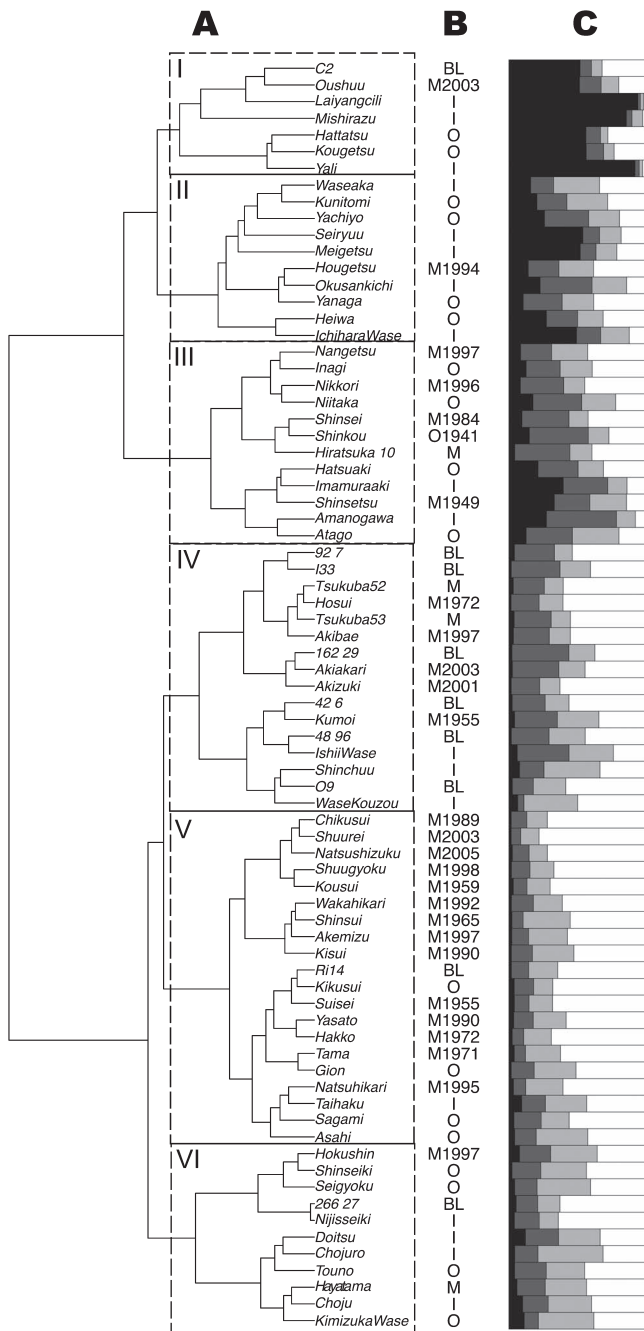
Seventy-six pear cultivars were used in this study



**Fig. 1.** Plots of linkage disequilibrium (LD) values ( $r^2$ ) against linkage map distance (cM) between pairs of markers linked on the same chromosome. The  $r^2$  values were calculated in two ways: calculated between the most major alleles at both markers (A), or calculated among all combinations of alleles at both markers and summed up with weight of allele frequencies (B). Gray curves show local polynomial fits using kernel smoothing regression. The horizontal dashed lines represent the baseline  $r^2$  values based on the 95<sup>th</sup> percentile of the distribution of  $r^2$  values between pairs of unlinked markers.

(Table 1). The 76 cultivars were genotyped with 155 SSRs, four RAPD-STS, two ACC synthase genes and the self-incompatibility (S-) locus. The LD statistics of two types were measured between pairs of markers linked on the same chromosome (Fig. 1). The  $r^2$  values based on the most major alleles were larger than those based on all allele combinations. Based on the intersections between the curves fitted to the relations between  $r^2$  values and map distance on one hand and the horizontal lines corresponding to the 95<sup>th</sup> percentile of the distribution of unlinked  $r^2$  values on the other, the short range of LD extended around 10 cM in a population of the 76 pear cultivars in both LD statistics. For the  $r^2$  values based on the most major alleles, averages were, respectively 0.35, 0.28 and 0.16 for markers <2, 2–5 and 5–10 cM apart. For the  $r^2$  values based on all allele combinations, average were, respectively 0.22, 0.16 and 0.10 for markers <2, 2–5 and 5–10 cM apart. Long range LD, i.e., LD between markers more than 10 cM apart, also existed between some marker combinations.

The population structure of the 76 pear cultivars was



**Fig. 2.** Estimated genetic structure among 76 pear varieties. The genetic structure was estimated using Ward clustering based on simple allele sharing distance (A) and by Bayesian clustering (C). Types and release years of the varieties are listed in B. The indices of variety types (O, old; I, indigenous; M, modern; BL, breeding line) are followed by the release years with four digits. In the Bayesian clustering, each cultivar is divided into four hypothetical sub-populations based on the population membership coefficients totaling 1 for the four sub-populations. Each subgroup is represented by a different color as listed: black (group 1), dark gray (group 2), light gray (group 3) and white (group 4).

estimated via hierarchical clustering and Bayesian clustering analyses (Fig. 2). In hierarchical clustering, clusters I and II mainly involved old and indigenous cultivars and the cluster

**Table 3.** Markers showing significant association with traits evaluated in GWAS. Linkage groups and positions of DNA markers were identified using three genetic linkage maps of pears (Nishitani *et al.* 2009, Terakami *et al.* 2009, Yamamoto *et al.* 2007)

Trait	Marker	LG <sup>a</sup>	Pos. <sup>b</sup>	Posterior average of $\gamma_j$	Allele size	Estimated effects
HarT	BGA35	3	70	0.47	129-bp	0.247
					130-bp	-0.030
					132-bp	-0.048
					134-bp	-0.026
					136-bp	-0.144
					136-bp	-0.144
BSR	CH04h02	15	13	0.39	245-bp	0.192
					253-bp	-0.068
					255-bp	-0.035
					263-bp	-0.089
					154-bp	4.845
					170-bp	1.251
SpuN	CH03g06	14	48.4	0.43	172-bp	-2.204
					182-bp	0.434
					184-bp	-4.360
					200-bp	0.034
					137-bp	0.228
					139-bp	-0.040
					144-bp	0.001
					146-bp	-0.026
					148-bp	-0.029
					155-bp	-0.055
					165-bp	-0.078

<sup>a</sup> Linkage group

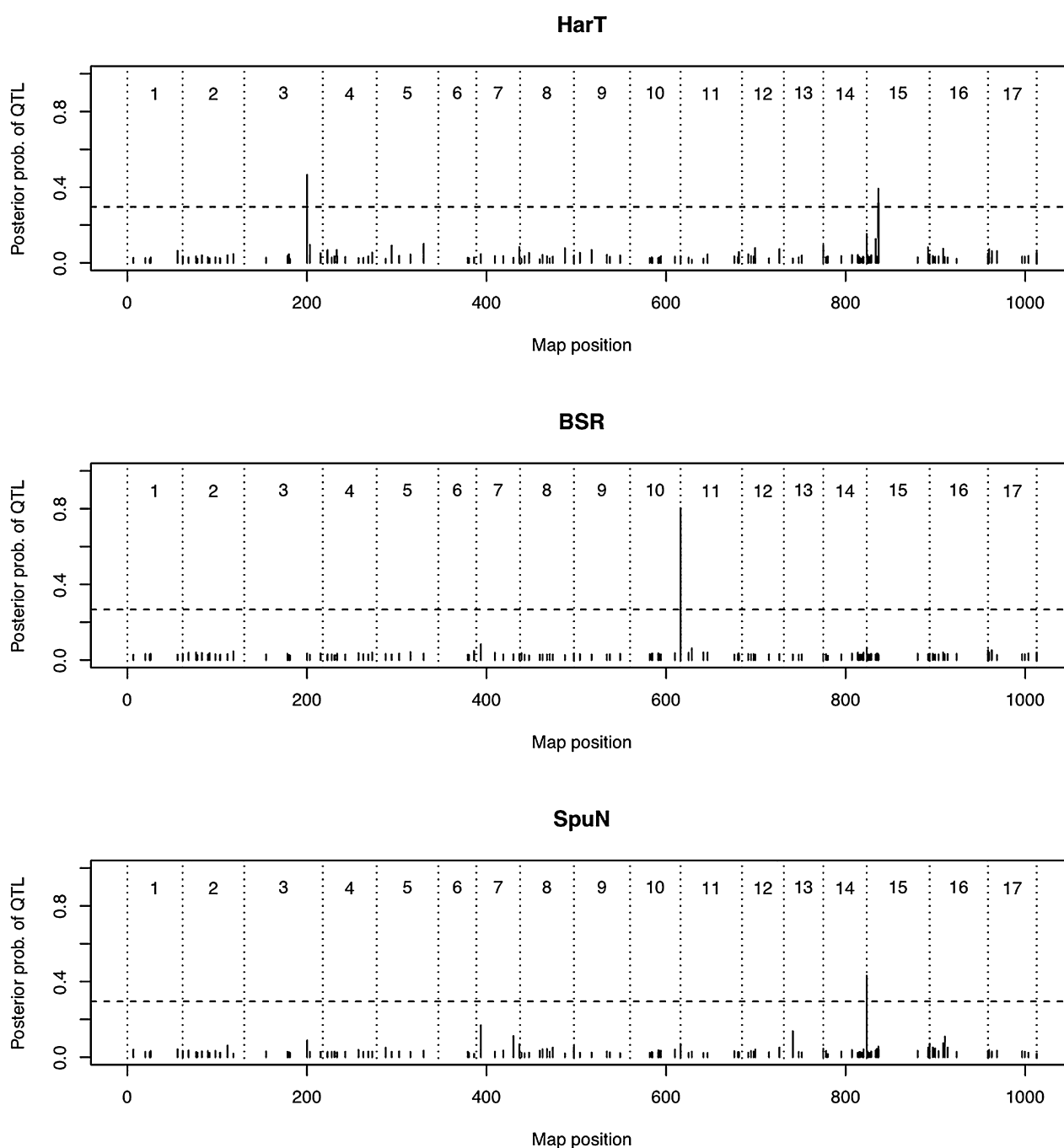
<sup>b</sup> Position (cM)

III old and modern cultivars (Fig. 2A and Table 1). The cluster IV mainly involved recent modern cultivars and current breeding lines. The cluster V mainly involved modern and old cultivars and the cluster VI old and indigenous cultivars (Fig. 2A, 2B). Correspondence between hierarchical clustering and Bayesian clustering analysis was not so clear (Fig. 2A, 2C). Varieties in cluster I had larger membership coefficients for group 1, whereas the varieties in the cluster V had larger membership coefficients for group 4. The difference in the membership coefficients between clusters II and III and also among the clusters IV, V and VI was not discrete but continuous.

### GWAS

We conducted GWAS to detect significant associations between the 162 markers (Table 2) and the nine agronomic traits (Table 3). Markers showing significant associations were detected in three traits (harvest time, resistance to black spot and number of spurs, Fig. 3 and Table 3), suggesting that these markers are linked to major QTL controlling these traits.

Two markers showed significant association with harvest time and one marker, BGA35, was located at the 70 cM position on the third chromosome. The other, PPACS2, was located at the 13 cM position on the fifteenth chromosome (Table 3). The 129-bp allele of BGA35 had a positive effect,



**Fig. 3.** Posterior probability of having a QTL (posterior average of  $\gamma_j$ ) at 162 marker positions, estimated for four agronomic traits for which significant associations were detected. Each marker position  $j$  ( $j = 1, 2, \dots, J$ ) has its own indicator variable  $\gamma_j$ , where the value one ( $\gamma_j = 1$ ) corresponds to the case in which the marker is included in the model as a QTL representative; the value zero ( $\gamma_j = 0$ ) implies exclusion. Horizontal broken lines correspond to the threshold obtained from the random permutation procedure.

which made harvest time later, while the remainder alleles of BGA35 had negative effects, which made harvest time earlier. The 245-bp allele of PPACS2 had a positive effect, whereas the other allele of PPACS2 had negative effects.

Regarding resistance to black spots, one marker, CH04h02, was located at the 0 cM position on the eleventh chromosome, showed significant association (Table 3). The 184-bp and 172-bp alleles of CH04h02 had negative effects,

suggesting that these two alleles linked to the susceptible alleles of a black spots gene.

In the number of spurs, one marker, CH03g06, which was located at the 48.4 cM position on the fourteenth chromosome, showed significant association (Table 3). The 137-bp allele of the CH03g06 had a positive effect, suggesting that this allele associated with a QTL allele increasing the number of spurs.

**Table 4.** Accuracy of genomic estimated breeding values (GEBVs) in traits evaluated in this study. Accuracy was evaluated as a Pearson's product-moment correlation coefficient between GEBVs and phenotypic values. Full models involved genotypes of all markers as explanatory variables, whereas reduced models involved genotypes of markers that were significant in GWAS as explanatory variables. Reduced models were built only for traits in which significant association(s) was (were) detected in GWAS.

	Full model		Reduced model	
	BayesA	BayesB	BayesA	BayesB
HarT	0.75	0.71	0.56	0.59
BSR	0.38	0.41	0.30	0.39
FruH	0.60	0.57	–	–
FruW	0.53	0.42	–	–
FruS	0.57	0.58	–	–
Aci	0.39	0.35	–	–
SugC	0.15	0.01	–	–
SpuN	0.61	0.43	0.15	0.04
TreV	–0.12	–0.45	–	–

#### Accuracy of GS predictions

The accuracy of GS predictions was evaluated through leave-one-out cross-validation of phenotype and marker genotype data of the 76 pear cultivars (Fig. 4 and Table 4). In the prediction, we applied two different models: BayesB, i.e., the model including the effects of a part of markers, while BayesA, i.e., the model including the effects of all markers. BayesB is the same as one used in the GWAS except that it did not have the effects of population structure. The accuracy of GS predictions was greater in BayesA than BayesB except for two traits, black spot resistance and fruit shape in longitudinal section (Table 4). With BayesA, the accuracy was highest (0.75) in harvest time, medium (0.38–0.61) in fruit size, firmness of flesh, number of spurs, fruit shape in longitudinal section, resistance to black spot, acid content and low (0.15, –0.12) in all soluble solid content and vigor of tree (Table 4).

The prediction accuracy described above was based on full prediction models, in which genotypes of all markers were included as explanatory variables. For traits in which significant association was detected in GWAS, we also built reduced prediction models, in which genotypes of markers found to be significant in GWAS were included as explanatory variables, to ascertain whether the accuracy of predictions based only on markers showing significant association with a target trait. In GWAS, significant associations were detected in harvest time, resistance to black spot and the number of spurs. The relative accuracy of the reduced model to the full model showed different patterns for different traits. In resistance to black spot, the reduced prediction model based on one significant marker showed a similar level of accuracy with the full prediction model based on all 162 markers. However, for harvest time and the number of spurs, the accuracy of the reduced prediction models was lower than the accuracy of the full prediction models. Espe-

cially for the number of spurs, the accuracy was markedly lower when we used only one significant marker for predictions.

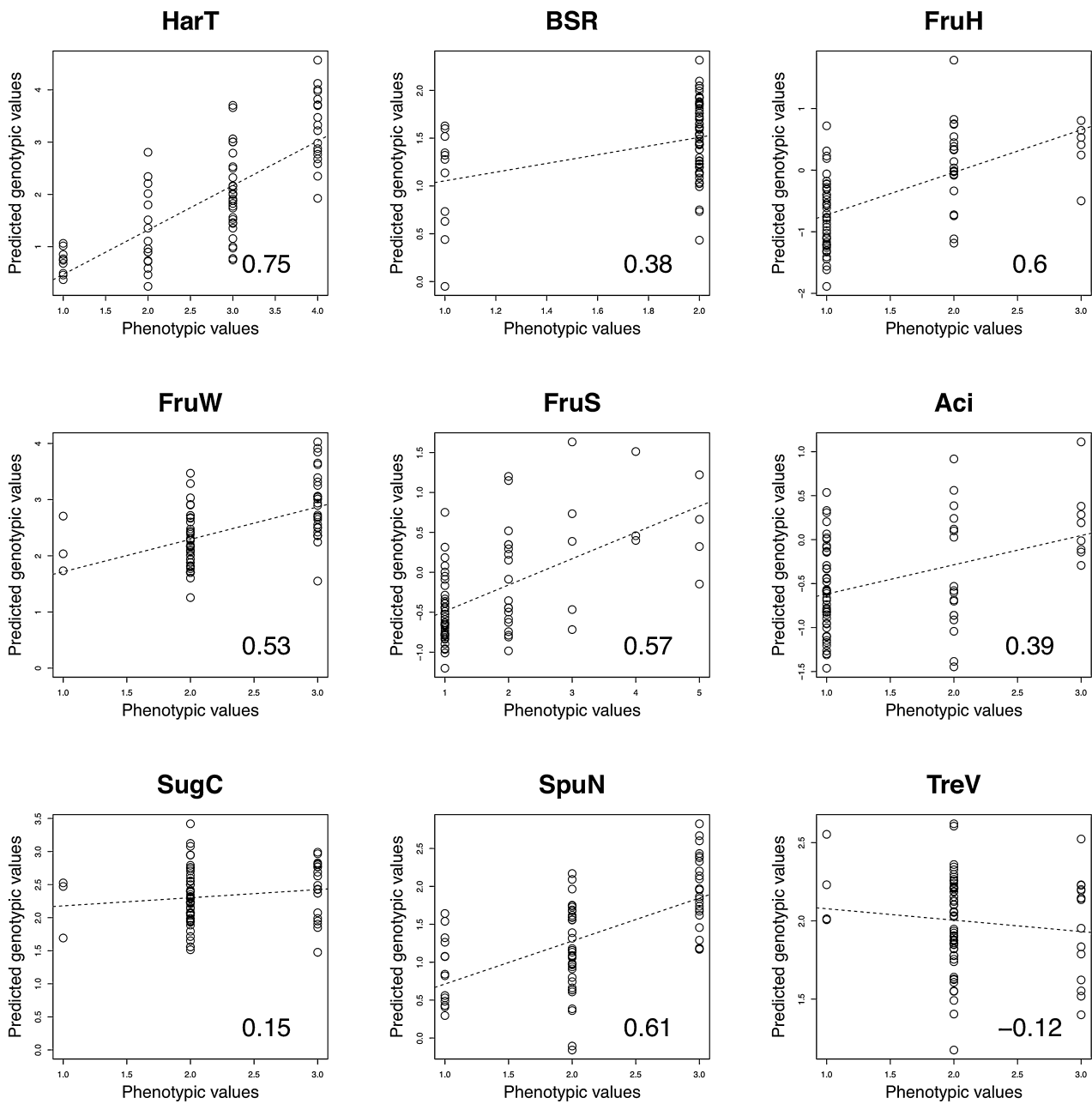
## Discussion

### *The pattern of LD in Japanese pear cultivars*

Detailed knowledge related to the extent of LD in a population of breeding lines and cultivars is basic information to consider the future potential of GWAS and GS in a target crop species because different levels of LD are expected in different species because the extent of LD and genetic stratification depend largely on their reproductive characteristics and their history and evolution as cultivated species (Gupta *et al.* 2005, Oraguzie *et al.* 2007). In the present study, we evaluated the extent of LD in 76 Japanese pear cultivars using 162 genome-wide markers. The extent of LD in the population of the 76 Japanese pear cultivars was higher than that expected from their cross-pollinating nature because of self-incompatibility, given that allogamous species have a low level of LD conservation in general (Gupta *et al.* 2005, Oraguzie *et al.* 2007). The high LD in the Japanese pear cultivars is regarded as the result of genetic bottlenecks during domestication and breeding of Japanese pear because a genetic bottleneck increases the extent of LD by eliminating recombinant lineages. Even when loci remain polymorphic during bottlenecks, the number of allelic combinations across loci can be much reduced, thereby leading to extensive haplotype structure (Hamblin *et al.* 2011). Vegetative reproduction, which is the most common way to propagate pear trees, conserves the LD caused by the bottlenecks because the genotype of each cultivar is fixed and the number of generations after the bottlenecks might be far fewer than in species with annual sexual reproduction (Rikkerrink *et al.* 2007). The common strategy in Japanese pear breeding, in which future cultivars are selected directly from F<sub>1</sub> progenies (i.e., are not selected from progenies in a later generation) derived from crosses between commercial cultivars, also makes the number of generations after the bottleneck small.

In Rosaceae crop species, the extent of LD has been reported in peach via genetic analysis with 50 SSR polymorphisms in 224 peach cultivars (Aranzana *et al.* 2010). In peach, LD was high and decreased with distance in all three cultivar groups: melting peaches, melting nectarines and non-melting peaches. In all groups, LD decayed at 13–15 cM with the threshold at the 5% level significant level. The extent of LD observed in the present study in pear cultivars is similar to that observed in the three groups in peach cultivars, despite their different reproductive characteristics: peach is self-fertile, whereas pear is self-incompatible because of its functional gametophytic self-compatibility. The extent of LD is influenced by various biological and historical factors of the species (Gupta *et al.* 2005, Oraguzie *et al.* 2007). Therefore, a careful comparison of the extent of LD among various Rosaceae species will be necessary to





**Fig. 4.** Correlations between phenotypic values and predicted genotypic values in nine agronomic traits. Predicted genotypic values were calculated via leave-one-out cross-validation. Each point corresponds to one cultivar. Dashed lines through the graphs are fitted regression lines of  $y$  and  $x$ .

elucidate the relation between the extent of LD and biological behavior of Rosaceae species.

#### Population structure in Japanese pear cultivars

Knowledge about genetic structure among breeding lines and cultivars is necessary for selecting promising parental lines for crossing and using efficiently genetic resources available for breeding. The population structure can also result in nonfunctional spurious associations in association studies (Lander and Schork 1994) and its information is expected to be involved in the statistical model of GWAS as a

factor affecting phenotypic variations (e.g., Iwata *et al.* 2007, Thornsberry *et al.* 2001, Yu *et al.* 2006). In this study, we evaluated the population structure among the 76 Japanese pear cultivars. In hierarchical clustering, five major clusters included cultivars of different types. For example, two clusters mainly involved old and indigenous cultivars, whereas three clusters mainly involved modern cultivars and breeding lines. Some exceptions (e.g., indigenous cultivars were also involved in the later type of clusters) were also observed. Bayesian clustering analysis revealed that the model dividing cultivars into four sub-populations was best. The

correspondence between the hierarchical clustering and Bayesian clustering analysis, however, was not clear but rather continuous. These results indicated that the population structure of Japanese pear cultivars is not distinct.

In Rosaceae crop species, the genetic structure was investigated in peach via analysis with 50 SSR polymorphisms in 224 peach cultivars (Aranzana *et al.* 2010). The 224 peach cultivars were divided into three main groups based mainly on major common commercial fruit characteristics: melting flesh peaches, melting flesh nectarines and non-melting varieties. The three main groups were obvious both in hierarchical clustering and Bayesian clustering analyses. Compared to the peach cultivars, Japanese pear cultivars had a weak and rather continuous population structure, except for the cluster I and group 1 (Fig. 2). This weakness might exist because a population of Japanese pear cultivars has no clear subdivision that is closely related to varietal characteristics. Self-compatibility in peach might also be a cause of a strong genetic structure in peach cultivars.

#### *GWAS in Japanese pear cultivars*

GWAS using the 76 Japanese pear cultivars detected four markers showing significant associations with three traits: resistance to black spot, harvest time and the number of spurs.

Black spot disease, caused by the Japanese pear pathotype of *Alternaria alternata* (Fr.) Keissler (previously *A. kikutiana* Tanaka), is a severe disease in Japanese pear cultivation. Susceptibility to the disease has been regarded as a simple trait, i.e., a trait controlled by a single dominant gene (Kozaki 1973). Map positions of genes controlling susceptibility have been estimated in linkage analyses with segregating families (Terakami *et al.* 2007). The marker CH04h02, which showed significant association with black spot resistance in the present study, was located at the top of linkage group (LG) 11 and was linked closely to the disease susceptibility genes *Ani* and *Ana*, which had been detected respectively in cultivars ‘Nijisseiki’ and ‘Nansui’ (Terakami *et al.* 2007). The 184-bp allele of CH04h02, which had the most negative estimated effects on the black spot resistance (Table 3), is known to be coupled with the susceptible allele *Ana* (Terakami *et al.* 2007). The 184-bp allele was harbored by 8 out of 11 susceptible cultivars and 3 out of 65 resistance cultivars in the present study. The 172-bp allele of CH04h02, which showed the second largest negative effects on the resistance (Table 3), is known to be coupled with the susceptible allele *Ani*.

Harvest time is a quantitative trait and an important characteristic of pear varieties because it differentiates varieties from other varieties and makes them competitive in the market. Two markers showed significant association with harvest time. One marker, PPACS2, was the marker for ACC synthase (1-aminocyclopropane-1-carboxylate synthase) gene. In Japanese pear, ripening and fruit storage potential are related to the amount of ethylene produced. Harvest time is related to the maximum ethylene production

during fruit ripening (Itai *et al.* 2003). ACC synthase (1-aminocyclopropane-1-carboxylate synthase) converts S-adenosyl methionine (SAM) to ACC. Then, ACC oxidase catalyzes the oxidative fragmentation of ACC to form ethylene. Itai *et al.* (2003) found that PPACS2 is associated with moderate ethylene production, suggesting that the association of PPACS2 observed in the present study is related to the ethylene production ability of Japanese pear cultivars. Although Itai *et al.* (2003) also found that another marker, PPACS1, was strongly associated with ethylene production, we found no significant association between PPACS1 and harvest time in the present analysis. This is probably because PPACS1 did not have causal alleles in the population of the 76 Japanese pear cultivars. Because PPACS1 is related to strong ethylene production, we speculate that the causal alleles of PPACS1 have been removed from a Japanese pear population via selection against extremely high ethylene production. In this study, we found significant association in the marker BGA35. This marker, probably unrelated to ethylene production, is a good candidate as a marker enabling us to select harvest time independently from ethylene production. Additional studies are expected to be fruitful for use of this marker effectively in Japanese pear breeding.

Spur number, a quantitative trait, has not been investigated for its mode of inheritance and QTL. Significant markers detected in this study might serve as clues for the concise genetic analysis of this trait, and be useful also for MAS of this trait in a pear breeding program.

In this study, significant associations were detected despite the few markers and few samples used for GWAS. In human GWAS, even when numerous samples and high-density markers are used, it is sometimes difficult to detect causal genes, and most genetic variation remains unexplained (e.g., Aulchenko *et al.* 2009). Recent research related to crop GWAS, however, has yielded results contrasting to human GWAS and are obtaining greater success with fewer markers and fewer samples (Hamblin *et al.* 2011). As Hamblin *et al.* (2011) suggested, genetic bottlenecks occurring during the histories of crop domestication and modern crop breeding have increased LD between markers and QTL and have also increased the minor allele frequency in both markers and QTL. A population of Japanese pear cultivars is thought to present similar circumstances to those of other crop species.

#### *GS prediction in Japanese pear cultivars*

Results of this study suggest that predictions based on genome-wide marker had high (0.75) or medium levels (0.38–0.61) of accuracy in seven out of nine traits. Even in traits for which no significant association was detected, GS predictions showed some levels of accuracy. Specifically, in fruit characteristics, i.e., firmness of flesh, fruit shape in longitudinal section, fruit size, acid content, total soluble solid content, which are important varietal characteristics in Japanese pear breeding, no significant association was detected in GWAS, but GS prediction showed medium levels

of accuracy except in all soluble solid content. In addition, in traits in which significant association was found in GWAS, GS predictions were more accurate than predictions based on significant markers. Specifically, in harvest time and the number of spurs, two and one significant markers were detected in GWAS, respectively, but the predictions based on significant markers was much less accurate than GS predictions, suggesting that the traits are determined by several minor and medium QTL as well as major QTL detected in GWAS. In resistance to black spot, predictions based on a significant marker detected in GWAS had comparable accuracy with GS prediction, indicating that the trait is controlled by a major gene and the significant marker closely linked to the major gene. Even in resistance to black spot, however, GS predictions were slightly better than predictions based on the significant marker, suggesting that some minor QTL might also be related to resistance to black spot.

For this study, we compared two Bayesian models—BayesA and BayesB—to determine the accuracy of GS predictions. BayesA, in which all marker effects are always included as explanatory variables, assumes that the trait is controlled by many loci, while BayesB, in which only markers selected via MCMC sampling process are included in the model, assumes that the trait is controlled by few loci (Lorenz *et al.* 2011). Results of the present study show that BayesA provides higher accuracy than BayesB in seven out of nine traits, suggesting that the assumption of BayesA is more plausible than the assumption of BayesB in these seven traits. At harvest time and the number of spurs, significant associations detected in GWAS suggest the existence of major QTL, but BayesA still showed higher accuracy than BayesB, which suggests that these traits are controlled by several minor and medium QTL as well as major QTL. In resistance to black spot, BayesB was more accurate than BayesA, which agrees with the result that predictions based on the significant marker had comparable accuracy with GS prediction. It is noteworthy that the advantage of BayesA over BayesB might depend not only on the number and size of QTL but also on LD between markers and QTL because genetic variation explained by each marker is determined by the level of LD between the marker and QTL linked to the markers as well as the size of the QTL (Jannink *et al.* 2010). Additional studies will be necessary to determine the relative advantage of the Bayesian models when the number of markers that is useful for genome-wide predictions is greatly increased in the future.

#### *Potential for GWAS and GS in Japanese pear breeding*

The results of this study suggest that GWAS and GS will be useful to accelerate genetic improvement of Japanese pear. Markers detected in GWAS are regarded as linked closely to causal genes and major QTL controlling important agronomic traits. Therefore, they will be useful for MAS. Even for traits for which no significant marker was detected via GWAS, we can predict their phenotypic values with GS prediction models at some level of accuracy, suggesting the

future potential of GS in the genetic improvement of complex traits controlled using a number of QTL, e.g., traits related to fruit quality and quantity. GS prediction models will be useful not only for selecting favorable seedlings but also for designing cross breeding and for selecting pairs of parental lines for crossing.

Neither the number of markers nor genotypes (i.e. cultivars) used in this study were sufficient to conduct full-scale GWAS and to train a prediction model for future GS. The power of GWAS and the accuracy of GS prediction can be enhanced by increasing the number of markers and genotypes. Recent advances in genotyping technology have made scoring genome-wide marker polymorphisms cost effective (Davey *et al.* 2011, Syvänen 2005). Therefore, the number of markers available for analyses can be increased considerably in the near future. A draft genome sequence of the domesticated apple has been published (Velasco *et al.* 2010). Approximately 2.1 million SNPs for the domesticated apple are now available on Genome Database for Rosaceae (GDR; <http://www.rosaceae.org/>). Given high collinearity of genomes and high transferability of genetic markers between *Malus* and *Pyrus* (Celton *et al.* 2009a, 2009b, Yamamoto *et al.* 2007), SNP information for domesticated apple will be useful for SNP marker development in *Pyrus* species. Genotyping using the next generation sequencer (Davey *et al.* 2011) will be useful to score a massive number of SNP markers for numerous genotypes. The number of genotypes can be increased by the inclusion of breeding lines that have been evaluated in local adaptability tests but which did not pass the tests. The phenotypes of these lines have been accumulated usually as breeding records. They are useful for GWAS and also for the training of GS prediction models.

A key advantage of the long-lived perennial trees might be the lasting presence of genotypes across years or centuries (Wilcox *et al.* 2007). The capability of asexual propagation is another advantage of most fruit trees that enables repeated measurements of trait phenotypes with clonally replicated genotypes. Actually, in Japanese pear, a promising breeding line is cloned and cloned genotypes are further tested for their local adaptability over multiple locations and multiple years. The long life and the ability of asexual propagation also enable us to use genotypes almost permanently as parental lines in cross-breeding programs. These advantages, however, might not be so prominent if we were forced to test every new genotype generated in a breeding program with cost-intensive field-testing. If we were able to build good prediction models using the field-test data over multiple locations and multiple years, then GS could reap a great harvest from the data and make the best use of the advantages of long-lived perennial trees.

In a breeding program, field test data are often collected as ordinal categorical scores rather than as continuous values to save labor for measuring traits. As demonstrated in this study, our Bayesian approach can accommodate ordinal categorical scores with modeling latent continuous variations behind the ordinal categorical scores both in GWAS and GS

prediction. The Bayesian approach is so flexible that it can be extended further to model genetic variations behind the data of various types (e.g., non-ordinal categorical data and count data). Flexibility will be necessary to use field-test data collected in breeding programs for conducting GWAS and building a GS prediction model. Even though the Bayesian modeling allows us to use ordinal categorical data, we should heed that ordinal categorical scoring might lose some information required for GWAS and GS. By simulation analysis, Iwata *et al.* (2009) suggested that GWAS in an ordinal trait could miss a large proportion of intermediate to minor QTL. Ordinal categorical scoring might also reduce the accuracy of GS prediction. It will be necessary to seek a balance between the benefits (e.g., increase in the power of GWAS and the accuracy of GS prediction) and costs (e.g., increase in time and labor, decrease in the number of measurable samples) from accurate phenotyping.

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### Literature Cited

- Aranzana, M.J., E.K. Abbassi, W. Howard and P. Arús (2010) Genetic variation, population structure and linkage disequilibrium in peach commercial varieties. *BMC Genetics* 11: 69.
- Aulchenko, Y.S., M.V. Struchalin, N.M. Belonogova, T.I. Axenovich, M.N. Weedon, A. Hofman, A.G. Uitterlinden, M. Kayser, B.A. Oostra, C.M. van Duijn *et al.* (2009) Predicting human height by Victorian and genomic methods. *Eur. J. Hum. Genet.* 17: 1070–1075.
- Bell, R.L. (1990) Pears (*Pyrus*). In: Moore, J.N. and J.R. Ballington Jr. (eds.) Genetic Resources of Temperate Fruit and Nut Crops I, International Society for Horticultural Science, Wageningen, The Netherlands, pp. 55–697.
- Bowcock, A.M., A. Ruiz-Linares, J. Tomfohrde, E. Minch, J.R. Kidd and L.L. Cavalli-Sforza (1994) High resolution of human evolutionary trees with polymorphic microsatellites. *Nature* 368: 455–457.
- Castillo, C., T. Takasaki, T. Saito, Y. Yoshimura, S. Norioka and T. Nakanishi (2001) Reconsideration of S-genotype assignments and discovery of a new allele based on S-RNase PCR-RFLP in Japanese pear cultivars. *Breed. Sci.* 51: 5–11.
- Celton, J.M., D.S. Tustin, D. Chagné and S.E. Gardiner (2009a) Construction of a dense genetic linkage map for apple rootstocks using SSRs developed from *Malus* ESTs and *Pyrus* genomic sequences. *Tree Genet. Genomes* 5: 93–107.
- Celton, J.M., D. Chagné, S.D. Tustin, S. Terakami, C. Nishitani, T. Yamamoto and S.E. Gardiner (2009b) Update on comparative genome mapping between *Malus* and *Pyrus*. *BMC Research Notes* 2: 182.
- Churchill, G.A. and R.W. Doerge (1994) Empirical threshold values for quantitative trait mapping. *Genetics* 138: 963–971.
- Davey, J.W., P.A. Hohenlohe, P.D. Etter, J.Q. Boone, J.M. Catchen and M.L. Blaxter (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nat. Rev. Genet.* 12: 499–510.
- Fernandez-Fernandez, F., N.G. Harvey and C.M. James (2006) Isolation and characterization of polymorphic microsatellite markers from European pear (*Pyrus communis* L.). *Mol. Ecol. Notes* 6: 1039–1041.
- George, E.I. and R.E. McCulloch (1993) Variable selection via Gibbs sampling. *J. Am. Stat. Assoc.* 88: 881–889.
- Grattapaglia, D. and M.D.B. Resende (2010) Genomic selection in forest tree breeding. *Tree Genet. Genomes* 7: 241–255.
- Guilford, P., S. Prakash, J.M. Zhu, E. Rikkerink, S. Gardiner, H. Bassett and R. Forster (1997) Microsatellites in *Malus X domestica* (apple): abundance, polymorphism and cultivar identification. *Theor. Appl. Genet.* 94: 249–254.
- Gupta, P.K., S. Rustgi and P.L. Kulwal (2005) Linkage disequilibrium and association studies in higher plants: present status and future prospects. *Plant Mol. Biol.* 57: 461–485.
- Gupta, P.K., S. Rustgi and R.R. Mir (2008) Array-based high-throughput DNA markers for crop improvement. *Heredity* 101: 5–18.
- Hamblin, M.T., E.S. Buckler and J.L. Jannink (2011) Population genetics of genomics-based crop improvement methods. *Trends Genet.* 27: 98–106.
- Hayes, J., P.J. Bowman, A.J. Chamberlain and M.E. Goddard (2009) Invited review: Genomic selection in dairy cattle: Progress and challenges. *J. Dairy Sci.* 92: 433–443.
- Heffner, E.L., M.E. Sorrells and J.L. Jannink (2009) Genomic selection for crop improvement. *Crop Sci.* 49: 1–12.
- Inoue, E., Y. Matsuki, H. Anzai and K. Evans (2007) Isolation and characterization of microsatellite markers in Japanese pear (*Pyrus pyrifolia* Nakai). *Mol. Ecol. Notes* 7: 445–447.
- Ishimizu, T., K. Inoue, M. Shimonaka, T. Saito, O. Terai and S. Norioka (1999) PCR-based method for identifying the S-genotypes of Japanese pear cultivars. *Theor. Appl. Genet.* 98: 961–967.
- Itai, A., T. Kotaki, K. Tanabe, F. Tamura, D. Kawaguchi and M. Fukuda (2003) Rapid identification of 1-aminocyclopropane-1-carboxylate (ACC) synthase genotypes in cultivars of Japanese pear (*Pyrus pyrifolia* Nakai) using CAPS markers. *Theor. Appl. Genet.* 106: 1266–1272.
- Iwata, H., Y. Uga, Y. Yoshioka, K. Ebana and T. Hayashi (2007) Bayesian association mapping of multiple quantitative trait loci and its application to the analysis of genetic variation among *Oryza sativa* L. germplasms. *Theor. Appl. Genet.* 114: 1437–1449.
- Iwata, H., K. Ebana, S. Fukuoka, J.L. Jannink and T. Hayashi (2009) Bayesian multilocus association mapping on ordinal and censored traits and its application to the analysis of genetic variation among *Oryza sativa* L. germplasms. *Theor. Appl. Genet.* 118: 865–880.
- Iwata, H., K. Ebana, Y. Uga, T. Hayashi and J.L. Jannink (2010) Genome-wide association study of grain shape variation among *Oryza sativa* L. germplasms based on elliptic Fourier analysis. *Mol. Breed.* 25: 203–215.
- Iwata, H., T. Hayashi and Y. Tsumura (2011) Prospects for genomic selection in conifer breeding: a simulation study of *Cryptomeria japonica*. *Tree Genet. Genomes* 7: 747–758.
- Jannink, J.L., A.J. Lorenz and H. Iwata (2010) Genomic selection in plant breeding: From theory to practice. *Brief. Funct. Genomics Proteomics* 9: 166–177.
- Kajiura, M., K. Kanato, Y. Machida, M. Maeda, I. Kozaki, T. Tashiro,

- O.Kishimoto and K.Seike (1974) New Japanese pear cultivar 'Hakko' and 'Hosui'. *Bull. Fruit Tree Res. Stn.* A1: 1–12.
- Kotobuki, K. (1999) Japanese pear. *In: Nagamine, T. and H. Takeda (eds.) The Descriptors for Characterization and Evaluation in Plant Genetic Resources (Volume 1)*. National Institute of Agrobiological Resources, Ministry of Agriculture, Forestry and Fisheries of Japan, pp. 340–345.
- Kotobuki, K., T. Saito, Y. Machida, I. Kajiura, Y. Sato, R. Masuda, K. Abe, A. Kurihara, T. Ogata, O. Terai *et al.* (2004) New Japanese pear cultivar 'Shuurei'. *Bull. Natl. Inst. Fruit Tree Sci.* 3: 31–40.
- Kozaki, I. (1973) Black spot disease resistance in Japanese pear. I. Heredity of the disease resistance. *Bull. Hort. Res. Stn.* A12: 17–27.
- Kumar, S., M.C.A.M. Bink, R.K. Volz, V.G.M. Bus and D. Chagné (2012) Towards genomic selection in apple (*Malus × domestica* Borkh.) breeding programmes: prospects, challenges and strategies. *Tree Genet. Genomes* 8: 1–14.
- Kuo, L. and B. Mallick (1998) Variable selection for regression models. *Sankhya Ser. B* 60: 65–81.
- Liebhart, R., L. Gianfranceschi, B. Koller, C.D. Ryder, R. Tarchini, E. Van de Weg and C. Gessler (2002) Development and characterization of 140 new microsatellites in apple (*Malus × domestica* Borkh.). *Mol. Breed.* 10: 217–241.
- Lander, E.S. and N.J. Schork (1994) Genetic dissection of complex traits. *Science* 265: 2037–2049.
- Lorenz, A.J., S. Chao, F.G. Asoro, E.L. Heffner, T. Hayashi, H. Iwata, K.P. Smith, M.E. Sorrells and J.L. Jannink (2011) Genomic selection in plant breeding: Knowledge and prospects. *Adv. Agron.* 110: 77–123.
- Luby, J.J. and D.V. Show (2001) Does marker-assisted selection make dollars and sense in a fruit breeding programme? *Hort. Sci.* 36: 872–879.
- Meuwissen, T.H.E., B.J. Hayes and M.E. Goddard (2001) Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157: 1819–1829.
- Myles, S., A.R. Boyko, C.L. Owens, P.J. Brown, F. Grassi, M.K. Aradhya, B. Prins, A. Reynolds, J.M. Chia, D. Ware *et al.* (2011) Genetic structure and domestication history of the grape. *Proc. Natl. Acad. Sci. USA* 108: 3530–3535.
- Nishitani, C., S. Terakami, Y. Sawamura, N. Takada and T. Yamamoto (2009) Development of novel EST-SSR markers derived from Japanese pear (*Pyrus pyrifolia*). *Breed. Sci.* 59: 391–400.
- Oraguzie, N.C., E.H.A. Rikkerink, S.E. Gardiner and H.N. De Silva (2007) Association mapping in plants. Springer, New York, p. 277.
- Pritchard, J.K., M. Stephens and P. Donnelly (2000) Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.
- Rikkerink, E.H.A., N.C. Oraguzie and S.E. Gardiner (2007) Prospects of association mapping in perennial horticultural crops. *In: Oraguzie, N.C., E.H.A. Rikkerink, S.E. Gardiner and H.N. De Silva (eds.) Association Mapping in Plants*. Springer, New York, pp. 249–269.
- Sawamura, Y., T. Saito, M. Shoda, T. Yamamoto, Y. Sato, T. Hayashi and K. Kotobuki (2002) A new self-incompatible allele in Japanese pear 'Shinsei' and 'Shinkou'. *J. Jpn. Soc. Hort. Sci.* 71: 342–347.
- Sawamura, Y., T. Saito, N. Takada, T. Yamamoto, T. Kimura, T. Hayashi and K. Kotobuki (2004) Identification of parentage of Japanese pear 'Housui'. *J. Jpn. Soc. Hort. Sci.* 73: 511–518.
- Silfverberg-Dilworth, E., C.L. Matasci, W.E. Van de Weg, M.P.W. Van Kaauwen, M. Walser, L.P. Kodde, V. Soglio, L. Gianfranceschi, C.E. Durel, F. Costa *et al.* (2006) Microsatellite markers spanning the apple (*Malus × domestica* Borkh.) genome. *Tree Genet. Genomes* 2: 202–224.
- Syvänen, A.C. (2005) Toward genome-wide SNP genotyping. *Nat. Genet.* 37: S5–S10.
- Terakami, S., M. Shoda, Y. Adachi, T. Gonai, M. Kasumi, Y. Sawamura, H. Iketani, K. Kotobuki, A. Patocchi, C. Gessler *et al.* (2006) Genetic mapping of the pear scab resistance gene Vnk of Japanese pear cultivar Kinchaku. *Theor. Appl. Genet.* 113: 743–752.
- Terakami, S., Y. Adachi, H. Iketani, Y. Sato, Y. Sawamura, N. Takada, C. Nishitani and T. Yamamoto (2007) Genetic mapping of genes for susceptibility to black spot disease in Japanese pears. *Genome* 50: 735–741.
- Terakami, S., T. Kimura, C. Nishitani, Y. Sawamura, T. Saito, T. Hirabayashi and T. Yamamoto (2009) Genetic linkage map of the Japanese pear 'Housui' identifying three homozygous genomic regions. *J. Jpn. Soc. Hort. Sci.* 78: 417–424.
- Thornberry, J.M., M.M. Goodman, J. Doebley, S. Kresovich, D. Nielsen and E.S. Buckler (2001) *Dwarf8* polymorphisms associate with variation with flowering time. *Nat. Genet.* 28: 286–289.
- Velasco, R., A. Zharkikh, J. Affourtit, A. Dhingra, A. Cestaro, A. Kalyanaraman, P. Fontana, S.K. Bhatnagar, M. Troggio, D. Pruss *et al.* (2010) The genome of the domesticated apple (*Malus × domestica* Borkh.). *Nat. Genet.* 42: 833–839.
- Ward, J.H. (1963) Hierarchical groupings to optimize an objective function. *J. Am. Stat. Assoc.* 58: 236–244.
- Wilcox, P.L., C.E. Echt and R.D. Burdon (2007) Gene-assisted selection: applications of association genetics for forest tree breeding. *In: Oraguzie, N.C., E.H.A. Rikkerink, S.E. Gardiner and H.N. De Silva (eds.) Association Mapping in Plants*, Springer, New York, pp. 211–247.
- Yamamoto, T., T. Kimura, Y. Sawamura, T. Manabe, K. Kotobuki, T. Hayashi, Y. Ban and N. Matsuta (2002a) Simple sequence repeats for genetic analysis in pear. *Euphytica* 124: 129–137.
- Yamamoto, T., T. Kimura, M. Shoda, T. Imai, T. Saito, Y. Sawamura, K. Kotobuki, T. Hayashi and N. Matsuta (2002b) Genetic linkage maps constructed by using an interspecific cross between Japanese and European pears. *Theor. Appl. Genet.* 106: 9–18.
- Yamamoto, T., T. Kimura, M. Shoda, Y. Ban, T. Hayashi and N. Matsuta (2002c) Development of microsatellite markers in Japanese pear (*Pyrus pyrifolia* Nakai). *Mol. Ecol. Notes* 2: 14–16.
- Yamamoto, T., T. Kimura, T. Hayashi and Y. Ban (2006) DNA profiling of fresh and processed fruits in pear. *Breed. Sci.* 56: 165–171.
- Yamamoto, T., T. Kimura, S. Terakami, C. Nishitani, Y. Sawamura, T. Saito, K. Kotobuki and T. Hayashi (2007) Integrated reference genetic linkage maps of pear based on SSR and AFLP markers. *Breed. Sci.* 57: 321–329.
- Xu, S. (2003) Estimating polygenic effects using markers of the entire genome. *Genetics* 163: 789–801.
- Yu, J. and E.S. Buckler (2006) Genetic association mapping and genome organization of maize. *Curr. Opin. Biotechnol.* 17: 155–160.
- Yu, J., G. Pressoir, W. Briggs, I. Vroh Bi, M. Yamasaki, J.F. Doebley, M.D. McMullen, B.S. Gaut, D.M. Nielsen, J.B. Holland *et al.* (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nat. Genet.* 38: 203–208.

**Appendix.** List of markers used in the present study. Linkage groups and positions of DNA markers were identified by three genetic linkage maps of pears (Nishitani *et al.* 2009, Terakami *et al.* 2009, Yamamoto *et al.* 2007)

Marker name	LG <sup>a</sup>	Pos. <sup>b</sup>	Origin	ID nos. or references
NH013a	1	6.6	pear SSR	AB061367
STS-OPW02	1	20.0	RAPD-STs	Terakami <i>et al.</i> (2006)
STS-OPAW13	1	24.8	RAPD-STs	Terakami <i>et al.</i> (2006)
STS-OPO09	1	26.0	RAPD-STs	Terakami <i>et al.</i> (2006)
KA4b	1	55.9	pear SSR	AB219793
TsuENH003	1	61.5	pear EST-SSR	AB450691
CN581493SSR	2	0.1	apple SSR	Silfverberg-Dilworth <i>et al.</i> (2006)
Hi22d06	2	0.1	apple SSR	Silfverberg-Dilworth <i>et al.</i> (2006)
TsuENH017	2	6.6	pear EST-SSR	AB450702
KU10	2	15.0	pear SSR	AB219798
TsuENH001	2	16.5	pear EST-SSR	AB450689
TsuENH062	2	21.5	pear EST-SSR	AB450733
CN444636SSR	2	21.5	apple SSR	Silfverberg-Dilworth <i>et al.</i> (2006)
BGT23b	2	28.1	pear SSR	AB219800
TsuENH045	2	30.0	pear EST-SSR	AB450721
CH03d10	2	36.5	apple SSR	Liebhards <i>et al.</i> (2002)
CH02b10	2	41.5	apple SSR	Liebhards <i>et al.</i> (2002)
CH02a04	2	42.0	apple SSR	Liebhards <i>et al.</i> (2002)
TsuENH087	2	50.0	pear EST-SSR	AB450752
NH002b	2	56.5	pear SSR	AB061359
NH023a	3	24.3	pear SSR	Yamamoto <i>et al.</i> (2002b)
NB113a	3	48.1	pear SSR	Yamamoto <i>et al.</i> (2002b)
NH203a	3	49.7	pear SSR	Sawamura <i>et al.</i> (2004)
TsuENH074	3	50.0	pear EST-SSR	AB450742
TsuENH023	3	51.0	pear EST-SSR	AB450706
BGA35	3	70.0	pear SSR	AB219799
NB109a	3	70.1	pear SSR	Yamamoto <i>et al.</i> (2002b)
MS14h03	3	73.0	apple SSR	Liebhards <i>et al.</i> (2002)
HGA8b	3	85.0	pear SSR	AB219801
NH209a	4	5.0	pear SSR	Sawamura <i>et al.</i> (2004)
NH011b	4	5.3	pear SSR	AB061365
TsuENH063	4	10.0	pear EST-SSR	AB450734
CH01d03	4	13.0	apple SSR	Liebhards <i>et al.</i> (2002)
TsuENH019	4	15.5	pear EST-SSR	AB450704
Hi23g08	4	16.0	apple SSR	Silfverberg-Dilworth <i>et al.</i> (2006)
CH02h11a	4	25.0	apple SSR	Liebhards <i>et al.</i> (2002)
NB141b	4	39.8	pear SSR	AB302443
TsuENH014	4	45.1	pear EST-SSR	AB450700
CH02c02b	4	50.7	apple SSR	Liebhards <i>et al.</i> (2002)
NB131a	4	55.0	pear SSR	AB302437
CH03a09	5	10.0	apple SSR	Liebhards <i>et al.</i> (2002)
EMPe106	5	16.7	pear SSR	AM182391
TsuENH086	5	25.0	pear EST-SSR	AB450751
NB103a	5	37.6	pear SSR	Yamamoto <i>et al.</i> (2002b)
CH02b12	5	52.3	apple SSR	Liebhards <i>et al.</i> (2002)
CH01b11	6	32.8	apple SSR	Liebhards <i>et al.</i> (2002)
TsuENH046	6	34.1	pear EST-SSR	AB450722
CH03d12	6	39.8	apple SSR	Liebhards <i>et al.</i> (2002)
TsuENH006	7	5.0	pear EST-SSR	AB450694
CH04e05	7	20.7	apple SSR	Liebhards <i>et al.</i> (2002)
NH019b	7	30.0	pear SSR	Yamamoto <i>et al.</i> (2002b)
EMPe117	7	41.3	pear SSR	AM182398
EMPe111	7	47.9	pear SSR	AM182394
NH036b	8	1.6	pear SSR	Sawamura <i>et al.</i> (2004)
EMPe116	8	5.0	pear SSR	AM182397
NB114a	8	10.0	pear SSR	AB302423
Hi20b03	8	22.0	apple SSR	Silfverberg-Dilworth <i>et al.</i> (2006)

## Appendix. (continued)

Marker name	LG <sup>a</sup>	Pos. <sup>b</sup>	Origin	ID nos. or references
OPH-19	8	25.0	RAPD-STS	Terakami <i>et al.</i> (2006)
TsuENH034	8	30.0	pear EST-SSR	AB450714
NH005b	8	33.0	pear SSR	AB061361
IPPN19	8	36.4	pear SSR	AB250799
CH01h10	8	50.0	apple SSR	Liebhards <i>et al.</i> (2002)
NH201a	8	60.0	pear SSR	Sawamura <i>et al.</i> (2004)
IPPN13	9	0.0	pear SSR	AB250792
NH029a	9	6.7	pear SSR	Yamamoto <i>et al.</i> (2002b)
TsuENH008	9	19.7	pear EST-SSR	AB450696
NB134a	9	36.7	pear SSR	AB302440
Hi04a05	9	40.0	apple SSR	Silfverberg-Dilworth <i>et al.</i> (2006)
CH05a03	9	51.5	apple SSR	Liebhards <i>et al.</i> (2002)
NH206a	10	22.0	pear SSR	Sawamura <i>et al.</i> (2004)
NH039a	10	24.1	pear SSR	Sawamura <i>et al.</i> (2004)
NH017a	10	25.0	pear SSR	AB061370
EMPc114	10	31.2	pear SSR	AM182395
Hi03f06	10	31.2	apple SSR	Silfverberg-Dilworth <i>et al.</i> (2006)
CH01f12	10	31.5	apple SSR	Liebhards <i>et al.</i> (2002)
NH045a	10	32.8	pear SSR	AB302421
EMPc105	10	34.4	pear SSR	AM182390
CH02b03b	10	50.0	apple SSR	Liebhards <i>et al.</i> (2002)
TsuENH029	10	56.2	pear EST-SSR	AB450710
TsuENH009	10	56.2	pear EST-SSR	AB450697
CH01f07a	10	56.2	apple SSR	Liebhards <i>et al.</i> (2002)
MS06g03	10	56.2	apple SSR	Liebhards <i>et al.</i> (2002)
CH04h02	11	0.0	apple SSR	Liebhards <i>et al.</i> (2002)
TsuENH083	11	9.0	pear EST-SSR	AB450749
CH03d02	11	12.6	apple SSR	Liebhards <i>et al.</i> (2002)
TsuENH044	11	25.5	pear EST-SSR	AB450720
IPPN14	11	30.1	pear SSR	AB250793
NB135a	11	60.0	pear SSR	AB302441
NB118a	11	64.0	pear SSR	AB302426
NB105a	11	64.8	pear SSR	Yamamoto <i>et al.</i> (2002b)
CH04g07	11	64.8	apple SSR	Liebhards <i>et al.</i> (2002)
CH04d08	11	65.0	apple SSR	Liebhards <i>et al.</i> (2002)
NH207a	12	7.0	pear SSR	Sawamura <i>et al.</i> (2004)
CH04g04	12	10.0	apple SSR	Liebhards <i>et al.</i> (2002)
CH05d11	12	12.7	apple SSR	Liebhards <i>et al.</i> (2002)
KA16	12	14.5	pear SSR	AB219796
NZ28f4	12	14.8	apple SSR	Guilford <i>et al.</i> (1997)
NB104a	12	30.0	pear SSR	Sawamura <i>et al.</i> (2004)
CH04d02	12	41.6	apple SSR	Liebhards <i>et al.</i> (2002)
CH02g01	13	10.0	apple SSR	Liebhards <i>et al.</i> (2002)
TsuENH025	13	16.6	pear EST-SSR	AB450707
NH009b	13	19.9	pear SSR	AB061364
NB120a	13	43.9	pear SSR	AB302428
TsuENH058	14	0.0	pear EST-SSR	AB450730
IPPN01	14	3.0	pear SSR	AB250786
NH004a	14	5.0	pear SSR	AB061360
CH01a09	14	20.1	apple SSR	Liebhards <i>et al.</i> (2002)
CH01g05	14	32.0	apple SSR	Liebhards <i>et al.</i> (2002)
CH04f06	14	32.0	apple SSR	Liebhards <i>et al.</i> (2002)
NH001c	14	38.5	pear SSR	AB061358
Hi02d11	14	40.1	apple SSR	Silfverberg-Dilworth <i>et al.</i> (2006)
CH04c07	14	41.7	apple SSR	Liebhards <i>et al.</i> (2002)
TsuENH032	14	44.0	pear EST-SSR	AB450712
NH035a	14	44.9	pear SSR	Sawamura <i>et al.</i> (2004)
TsuENH031	14	44.9	pear EST-SSR	AB450711

## Appendix. (continued)

Marker name	LG <sup>a</sup>	Pos. <sup>b</sup>	Origin	ID nos. or references
CH05d03	14	44.9	apple SSR	Liebhard <i>et al.</i> (2002)
CH03g06	14	48.4	apple SSR	Liebhard <i>et al.</i> (2002)
TsuENH093	15	0.0	pear EST-SSR	AB450757
NH027a	15	0.0	pear SSR	Yamamoto <i>et al.</i> (2002b)
CH02e12	15	1.0	apple SSR	Liebhard <i>et al.</i> (2002)
CH02d10b	15	1.6	apple SSR	Liebhard <i>et al.</i> (2002)
CH03b06	15	2.0	apple SSR	Liebhard <i>et al.</i> (2002)
Hi03g06	15	3.0	apple SSR	Silfverberg-Dilworth <i>et al.</i> (2006)
NH204a	15	5.0	pear SSR	Sawamura <i>et al.</i> (2004)
IPPN17	15	10.0	pear SSR	AB250796
NH025a	15	11.2	pear SSR	Yamamoto <i>et al.</i> (2002b)
IPPN08	15	12.8	pear SSR	AB250789
Hi02d02	15	12.8	apple SSR	Silfverberg-Dilworth <i>et al.</i> (2006)
PPACS2	15	13	ACC synthase	Itai <i>et al.</i> (2003)
Hi11a01	15	56.8	apple SSR	Silfverberg-Dilworth <i>et al.</i> (2006)
Hi09f01	15	56.8	apple SSR	Silfverberg-Dilworth <i>et al.</i> (2006)
TsuENH040	15	68.3	pear EST-SSR	AB450717
CH02c09	15	68.4	apple SSR	Liebhard <i>et al.</i> (2002)
TsuENH007	15	70.0	pear EST-SSR	AB450695
TsuENH035	15	70.1	pear EST-SSR	AB450715
TsuENH016	15	70.1	pear EST-SSR	AB450701
PPACS1	15	unknown	ACC synthase	Itai <i>et al.</i> (2003)
TsuENH079	16	0.0	pear EST-SSR	AB450745
TsuENH022	16	3.5	pear EST-SSR	AB450705
TsuENH036	16	3.5	pear EST-SSR	AB450716
NH026a	16	6.0	pear SSR	Yamamoto <i>et al.</i> (2002b)
KA14	16	10.0	pear SSR	AB219795
NH007b	16	15.0	pear SSR	AB061362
CH01f03a	16	15.0	apple SSR	Liebhard <i>et al.</i> (2002)
Hi04e04	16	17.0	apple SSR	Silfverberg-Dilworth <i>et al.</i> (2006)
AU301431SSR	16	20.0	apple SSR	Silfverberg-Dilworth <i>et al.</i> (2006)
CH05a04	16	30.0	apple SSR	Liebhard <i>et al.</i> (2002)
NB123a	16	65.0	pear SSR	AB302430
NB116b	16	65.0	pear SSR	AB302425
NH015a	17	0.0	pear SSR	AB061369
TsuENH026	17	1.2	pear EST-SSR	AB450708
AF527800SSR	17	1.2	apple SSR	Silfverberg-Dilworth <i>et al.</i> (2006)
AT000174SSR	17	1.2	apple SSR	Silfverberg-Dilworth <i>et al.</i> (2006)
CH01h01	17	3.8	apple SSR	Liebhard <i>et al.</i> (2002)
TsuENH033	17	4.2	pear EST-SSR	AB450713
NB125a	17	4.2	pear SSR	AB302432
CH05g03	17	10.0	apple SSR	Liebhard <i>et al.</i> (2002)
TsuENH071	17	38.0	pear EST-SSR	AB450740
NH014a	17	40.9	pear SSR	AB061368
NH008b	17	45.0	pear SSR	AB061363
Slocus	17	54.2	S-Rnase	Ishimizu <i>et al.</i> (1999)
CH01b12	unknown	unknown	pear SSR	Liebhard <i>et al.</i> (2002)

<sup>a</sup> Linkage group<sup>b</sup> Position (cM)