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To cite this version:

Jean-Baptiste Durand, Baptiste Guitton, Jean Peyhardi, Yan Holtz, Yann Guédon, et al.. New insights for estimating the genetic value of F1 apple progenies for irregular bearing during the first years of tree production. Journal of Experimental Botany, 2013, 64 (16), pp.5099-5113. 10.1093/jxb/ert297 . hal-00845353

HAL Id: hal-00845353

https://hal.science/hal-00845353v1

Submitted on 7 Jan 2015

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New insights for estimating the genetic value of F1 apple progenies for irregular bearing during first years of tree production

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Running title: Early quantification of alternation in flowering for F1 apple progenies

Submitted: 02-18-2013

Word counts: 7,986 words

6 Figures (colour online-only),

5 Tables

Supporting information: 1,362 words, 7 Figures, 6 Tables

- *Background and Aims* Because irregular bearing generates major agronomic issues in fruit-tree species, particularly in apple, the selection of regular cultivars is desirable. Herein, we aimed at defining methods and descriptors allowing an early diagnostic in population segregating for bearing behaviour.
- *Methods* Flowering occurrences were collected at whole tree and annual shoot (AS) scales on a F1 apple population. At both scales, the number of inflorescences over years was modelled. Two descriptors were derived from model residuals: a new biennial bearing index, based on deviation around yield trend over years, and an autoregressive coefficient, which represents dependency between consecutive yields. At shoot scale, entropy was also considered to represent the within-tree flowering synchronism. Clusters of genotypes with similar bearing behaviours were built and compared between scales.
- *Key Results* Both annual shoot and whole tree scale descriptors were consistent for most genotypes, and Quantitative Trait Loci were detected for the new biennial bearing index. Biennial bearing was characterised by the conjunction in AS flowering synchronism in a given year and AS alternation between consecutive years.
- *Conclusions* The proposed methods and indices open an avenue to quantify bearing behaviour during early stage of tree production and to capture genetic variations. Their extension to other progenies and species, possible variants of descriptors and their use in breeding programmes are discussed.

Key words: alternation indices, biennial bearing, cultivar breeding, linear mixed models, *Malus x domestica*, multiscale model, QTL detection, synchronism.

1/ Introduction

Numerous economically important fruit-tree species, including apple, are prone to irregular bearing, i.e. the irregular fruit load of a tree over consecutive years, characterised by numerous small sized and low quality fruits during years of high production ("ON" years) and by few oversized fruits during years of low yields ("OFF" years) (Monselise & Goldschmidt, 1982). Alternate bearing denotes a specific case of irregular bearing for which fruiting pattern is biennial, this case being predominant in apple even though more complex patterns are possible. In addition to the fluctuation of production, this alternation generates major agronomic issues such as an increased demand for labour and chemical products to regulate the crop load (Jonkers, 1979). However, because of their impact on the environment, less chemical solutions are available nowadays for growers. This implies the need for innovating solutions to attenuate irregular bearing. The main hypothesis behind biennial bearing phenomenon is that fruit yield in one year has a negative effect on floral formation for the subsequent year (Jonkers, 1979; Monselise & Goldschmidt, 1982; Neilsen & Dennis, 2000). A previous study showed that biennial bearing is inheritable and segregates in an apple progeny ("Starkrimson"×"Granny Smith" population) (Guitton *et al.*, 2012), suggesting that selecting new varieties with intrinsic regular bearing is a possible strategy. However, breeding programs for fruit-tree species do not consider this trait yet, because it expresses lately, i.e. exclusively during tree mature phase and requires observations over several years before its value for a given genotype can be assessed. Development of methods and tools for a faster diagnostic of the bearing tendency of a genotype during its first years of production is thus highly desirable.

Several parameters have been proposed to quantify biennial bearing. These parameters are based on either: (1) indices quantifying the stability of the production through time or (2) repeated measure analysis to understand the relationships between successive yields. The first

approach, based on indices, has been developed to assess bienniality and intensity of alternation, as well as its synchronicity in different locations. Hoblyn *et al.* (1936) have proposed an index to estimate the intensity of deviation in yield during successive years, which has been renamed by Wilcox (1944) as Biennial Bearing Index (BBI). BBI has become the accepted standard to describe biennial bearing and has been applied to yield (mass of fruit) at different scales: whole areas, individual trees or branches - on apple and other fruit tree species (Wilcox, 1944; Singh, 1948; Pearce & Dobersek-Urbane, 1967; Jonkers, 1979; Reddy *et al.*, 2003; Smith *et al.*, 2004; Rosenstock *et al.*, 2010). Huff (2001) highlighted that the distribution of BBI strongly depends on the mean and variance of yields, under the hypothesis that they are a random sample. Therefore, the accepted interpretation of BBI as a measure of the magnitude of irregular bearing is questionable. To address this issue, Huff (2001) proposed a significance test of BBI but it has been used only once, on citrus (Smith *et al.*, 2004). Pearce & Dobersek-Urbane (1967) investigated empirical properties of indices for bienniality in practical situations, using data simulated with several models of patterns. In particular, they highlighted that simulated trended series without alternation had positive BBI values. As a result, using BBI on trended series may lead to confound alternation and trend.

Indices for alternation have also been proposed by analysing succession of shoot types (including vegetative, flowering but not fruiting, and fruiting) along branches over consecutive years (Lauri *et al.*, 1995 and 1997). Two indices have been proposed, a local one reflecting the ability to produce bourse-over-bourse, and a global one quantifying the alternation synchronism within the tree. While characterising genotypic variations in fruiting patterns in various apple cultivars, Lauri *et al.* (1995) noticed that alternation-to-fruit patterns can be completely hidden when alternating sequences are desynchronized. The index developed to assess this phenomenon (named *alternation synchronism*) quantifies the balance

between the number of two-years-long sequences with fruiting alternation, depending on whether fruiting occurs even or odd years.

The second approach, referred to as *longitudinal data analysis*, relies on mixed models to analyse multi-year datasets (Cnaan *et al.*, 1997; Verbeke & Molenberghs, 2000). Their main strength is to provide a model for the serial correlation between successive observations for an individual (Diggle & Kenward, 1994; Hand & Crowder, 1996). Diggle & Kenward (1994) identified three sources of variation in longitudinal data: serial correlations, random effects, and measurement error. As a consequence, such models have been used to decompose the global variability between these sources.

Recently, an experiment studying yield stability over time on *Coffea canephora* considered both strategies, i.e. longitudinal data analysis and indices (Cilas *et al.*, 2011). Longitudinal data analysis was used to model the residual correlation structure between tree yields over time, so as to estimate genotypic values associated with yield as accurately as possible. Independently from this model, classical indices (BBI and the number of sign changes in the difference between consecutive yields) were computed on yield directly, in the prospect of deriving heritability values and correlations between traits.

In contrast, our analysis is based on an integrated model that combines correlations between annual yields together with their genetic variability. It focuses on the estimation of genotype value to flower regularly and relies on flowering occurrences collected through several consecutive years during the beginning of the mature phase of an apple tree progeny, at both the whole tree and the annual shoot (AS) scales. The first objective of the present study was the early identification of irregular genotypes during the first years of tree production. Since tree production at the beginning of the mature phase is affected by a trend (i.e. a gradual increase in the mean level of yield over years), quantification of biennial bearing at whole tree scale relied on mixed models to separate this trend from the alternating pattern related to

biennial bearing. Quantification of alternation resulted from a dependency model of the successive deviations of yields from the trend. Thus, the intensity of biennial bearing was assessed using either the model parameters or indices measuring the relative amplitudes of the deviations. Our second objective was to investigate the possibility of early identification of irregular genotypes using annual shoot samples in individuals. The use of such samples, consisting in a limited number of sequences, turned phenotyping easier and faster than exhaustive measurements. Thus, we established and validated a methodology to predict bearing behaviour from annual shoot samples. Moreover, combining descriptors of alternation computed at axis scale on the one hand and of synchronisation between axes on the other hand, brought new (but partial) insight on how regularity at tree scale may result from different strategies of synchronisation in flowering at local scale.

2/ Materials and methods

2.1/ Plant material

A segregating population described in Guitton *et al.* (2012) was obtained from a cross between 'Starkrimson' and 'Granny Smith', and was used to study the bearing behaviour of genotypes. Two tree replicates (or just one in rare cases) were available for each genotype.

The STK×GS progeny composed of 123 genotypes showed segregation of both tree architecture during the juvenile phase (Segura *et al.*, 2006; 2007 and 2009) and biennial bearing during the beginning of the mature phase (Guitton *et al.*, 2012). Most of the trees of the population (87.6%) were able to set flower during their third year after grafting (2006). Contrasted bearing behaviours were observed within the population, ranging from regular to strongly biennial bearing with some genotypes exhibiting irregular patterns (Guitton *et al.*, 2012).

2.2/ Phenotyping

Flowering recurrence was measured at two different scales: whole tree and annual shoot scales. At the whole tree scale, the total number of inflorescences was observed during six consecutive years, from their second to their seventh year after grafting.

At the annual shoot scale, the succession of vegetative v. floral annual shoots (defined as the portion of axes developed during the same year) were observed over the same consecutive years along different types of shoots: trunk, long and short axillary shoots (LAS and SAS respectively) (Fig. S1 **[Supplementary Information]**). Axillary shoots were classified depending on their AS length, with all AS length < 5cm in SAS, with AS length between 5 and 20 cm in brindles and at least one AS with length ≥ 20 cm in LAS (see Segura *et al.*, 2006). Within LAS, proleptic and sylleptic branches were distinguished according to whether the meristem outgrowth occurred after a dormant period or not (Long Proleptic Axillary Shoot, LPAS and Long Sylleptic Axillary Shoot, LSAS respectively). Vegetative and floral AS were distinguished according to the presence/absence of an inflorescence. Floral AS were composed of a "bourse" (leafy basal part ending with an inflorescence) and a "bourse-shoot" (vegetative growth units originating from a lateral meristem of the bourse).

Flowering occurrence was observed along the trunk of each tree, as well as along two LAS sampled along first trunk's AS (AS 2004): one LSAS and one LPAS (Fig. S1). For each LAS, two short axillary shoots (SAS) per annual AS were also sampled and phenotyped the same way. As a consequence, 10 SAS of 5 to 1 years were recorded on LSAS, and 8 SAS of 4 to 1 years were recorded on LPAS. The flowering pattern was described by recording the presence/absence of flowering event on AS (6 possible flowering occurrences on the trunk and LSAS, 5 on LPAS). The data thus consisted in vegetative vs. floral AS in 6 to 1 year sequences.

<u>2.3/ Statistical modelling</u>

A two-step modelling was used to quantify biennial bearing at the whole tree scale. To dissociate increase in the number of inflorescences per tree from biennial bearing, a trend model based on a linear mixed model was applied first. Irregular bearing was quantified afterwards using the deviations around the trend (model residuals) and combining two approaches: (i) a new index was proposed to quantify the amplitude of residuals, taking account of their order; and (ii) an auto-regressive model was estimated to characterise the dependencies between successive residuals and distinguish biennial patterns specifically from other irregular patterns, using an autocorrelation parameter. Then, clusters of genotypes that have similar patterns of annual yields were identified using the two descriptors jointly.

To quantify biennial bearing using only a limited number of AS sequences, the descriptors described hereabove were computed using the same procedure, except that the annual amount of inflorescences in the sequences was used instead of the annual amount of inflorescences at whole tree scale.

An analysis of the relation between alternation in flowering at whole tree and AS scales was achieved, based on two strategies. The first one consists in predicting either the classes, or the descriptors that quantify irregular bearing of genotypes at tree scale, from the local descriptors (i.e. those computed using subsamples). The second strategy relies on including an additional local descriptor, to improve prediction of the classes. This descriptor is related to synchronism in flowering between all AS of a same year in a given tree.

2.3.1/ Modelling biennial bearing at the whole tree scale

Trend models

Two linear mixed models (referred to as A and B) were considered. Their slope and intercept can be decomposed into three terms: mean, genotype and tree replication effects. Both models can be generically written as:

$$Y_{g,r,t} = \underbrace{\beta + \beta_g + \zeta_{g,r}}_{\text{Intercept}} + \underbrace{(\alpha + \alpha_g + \xi_{g,r})}_{\text{Slope}} t + \varepsilon_{g,r,t}$$

where $Y_{g,r,t}$ is the production of tree replicate *r* of genotype *g* at year *t*, $\beta + \alpha\ t$ represents the mean trend (treated as fixed effect), $\beta_g + \alpha_g t$ the trend deviation for genotype *g*, $\zeta_{g,r} + \xi_{g,r} t$ the trend deviation for replicate *r* of genotype *g*, and where $\varepsilon_{g,r,t}$ is the Gaussian residual of tree replicate *r* of genotype *g* at time *t*. Genotype effects β_g and α_g are considered as fixed effects in model A and as Gaussian random effects in model B, with variances τ_β^2 and τ_α^2, respectively. Replication-specific parameters $\zeta_{g,r}$ and $\xi_{g,r}$ were not directly of interest since we aimed at quantifying irregular bearing at the genotype scale. Therefore, they were treated as random effects, with variances τ_ζ^2 and τ_ξ^2, respectively. Our interest being to characterise each genotype for its bearing behaviour, modelling genotype effect as fixed (model A) rather than as random effect (model B) was preferred a priori. Selection of the most suitable model among A and B was achieved using minimisation of AIC and BIC criteria (Verbeke & Molenberghs, 2000).

Trees for which less than two years of substantial yield were available were discarded. The first years such that flowering did not occur for any tree were also discarded.

Quantifying deviation around trend

a) Construction of indices inspired from BBI

Following Huff's (2001) suggestion that yields should be detrended in BBI calculation, we propose to characterise irregular bearing in the context of trended yield by incorporating the empirical residuals $\hat{\varepsilon}_{g,r,t}$ of trend models A or B into BBI-related indices.

Let us recall that BBI is defined for a sample by:

$$\mathrm{BBI} = \frac{2}{\sum_r (T_{g,r} - 1)} \sum_r \sum_{t=2}^{T_{g,r}} \frac{\left| Y_{g,r,t} - Y_{g,r,t-1} \right|}{Y_{g,r,t-1} + Y_{g,r,t}}$$

where $T_{g,r}$ denotes the number of measurements for replication *r* of genotype *g*. Compared to the usual presentation of BBI, a multiplying factor of value 2 is introduced to make BBI

comparable in scale to the indices introduced below. The justification is that, in this way, the elementary terms which are averaged take the form of a ratio between an absolute difference $\left|Y_{g,r,t} - Y_{g,r,t-1}\right|$ and a mean $\left(Y_{g,r,t-1} + Y_{g,r,t}\right)/2$.

The BBI suffers from shortcomings mentioned in Introduction. A detailed mathematical analysis of these shortcomings is given in **[Supplementary Information]** (section A). This analysis led us to propose the following variants of BBI, called BBI_norm and BBI_res_norm, which consist in a normalisation of the residual fluctuations by total yield.

$$\text{BBI_norm} = \frac{\sum_r \sum_{t=2}^{T_{g,r}} \left|Y_{g,r,t} - Y_{g,r,t-1}\right| / \sum_r (T_{g,r} - 1)}{\sum_r \sum_{t=1}^{T_{g,r}} Y_{g,r,t} / \sum_r T_{g,r}}.$$

$$\text{BBI_res_norm} = \frac{\sum_r \sum_{t=2}^{T_{g,r}} \left|\hat{\varepsilon}_{g,r,t} - \hat{\varepsilon}_{g,r,t-1}\right| / \sum_r (T_{g,r} - 1)}{\sum_r \sum_{t=1}^{T_{g,r}} Y_{g,r,t} / \sum_r T_{g,r}}.$$

BBI_norm is the ratio between the mean absolute difference between successive yields and the average yield. In contrast to BBI where each absolute difference is given a different weight, they are simply summed and normalized in BBI_norm. This normalization scheme is expected to render BBI less sensible to deviation of the assumption of alternating residual with amplitudes roughly proportional to the corresponding trend level (see proposition P2 in **[supplementary information]**). If the trend variation between *t* - 1 and *t* is small with respect to $\hat{\varepsilon}_{g,r,t-1}$ and $\hat{\varepsilon}_{g,r,t}$, BBI_res_norm is expected to be a close approximation of BBI_norm (see proposition P3 in **[supplementary information]**). The role of BBI_res_norm is to measure the amplitude of successive variations of yield around the trend, relative to total yield. Although this is a measure of yield irregularity, biennial or irregular series with same BBI_res_norm could be built, showing that it is not sufficient to discriminate between these patterns. The distinction between both requires modelling the dependencies between successive residuals.

b) Modelling residual structure

The hypothesis of regular bearing for a given genotype *g* is associated with the assumption of independent residuals $(\varepsilon_{g,r,t})_{0\le t\le T}$ with small BBI_res_norm. On the contrary, the hypothesis of biennial bearing is associated with dependent residuals, negative correlation between successive residuals, since they are expected to have opposite signs, and high BBI_res_norm. The individuals combining independent residuals and high BBI_res_norm are expected to be associated with irregular bearing.

Dependency between successive residuals can be characterised using first-order autoregressive (AR1) models. Two models (referred to as (I) and (II)) were considered; they can be generically written as:

$$\varepsilon_{g,r,t} = (\gamma + \gamma_g + \gamma_{g,r})\varepsilon_{g,r,t-1} + u_{g,r,t}$$

where $\varepsilon_{g,r,t}$ is the same residual as in trend model A with fixed effects, γ is the mean AR1 coefficient (common to every genotype and replicate), γ_g the fixed deviation from mean AR1 coefficient for genotype *g*, $\gamma_{g,r}$ the random deviation from AR1 coefficient $\gamma + \gamma_g$ for tree replicate *r* of genotype *g* and $u_{g,r,t}$ the residual of residual $\varepsilon_{g,r,t}$ of tree replicate *r* of genotype *g* at time *t*. Let τ_1^2 and ρ^2 denote the variance of $\gamma_{g,r}$ and $u_{g,r,t}$, respectively. The models do not have intercepts, since by hypothesis the $\varepsilon_{g,r,t}$ have zero mean. It is expected that regular genotypes will have values of $\gamma + \gamma_g$ close to zero, whereas biennial bearing genotypes will have values close to -1. Model (II) is the model defined by the above equation, whereas model (I) is obtained by letting the variance τ_1^2 of $\gamma_{g,r}$ being null (thus removing these effects from the model).

In the analysis, for similar reasons as those detailed above for trend models, model (I) is a priori preferred to model (II). Practically, to estimate these models, the empirical residuals $\hat{\varepsilon}_{g,r,t}$ were used, rather than the actual (unknown) residuals $\varepsilon_{g,r,t}$ of trend model A with fixed

effects. The model parameters were estimated by maximum likelihood and the Best Linear Unbiased Predictors (BLUPs) for the random effects were computed using the R software (R Development Core Team, 2008), and specifically the function *lmer* of package *lme4* (Bates *et al.*, 2011).

2.3.2/ Prediction of biennial bearing using subsamples of annual shoot sequences

Biennial bearing at AS scale was characterised using 1 to 6 year sequences of vegetative v. flowering AS $(F_{g,r,t,\ell})_{\ell \geq 0}$, ($F_{g,r,t,\ell} = 0$) denoting the absence and ($F_{g,r,t,\ell} = 1$) the presence of flower for replication r of genotype g at year t, at location (or AS) ℓ in the tree. Information contained in this subsample of sequences was used to predict both indices developed in Section 2.3.1.

Firstly, for each replication r of each genotype g, the total number of inflorescences

$$Y'_{g,r,t} = \sum_l F_{g,r,t,l}$$

contained in the subsample of sequences was computed. These quantities were used as in Section 2.3.1 to compute both indices BBI_res_norm and the genotype AR coefficient γ_g, replacing $Y_{g,r,t}$ by $Y'_{g,r,t}$ in the above formulas. It is hypothesised that the indices obtained using the subsample of sequences are approximations of the same indices computed using the data at whole tree scale.

Secondly, we developed a third index dedicated to the quantification of synchronism in flowering between all AS of a same year. This index is based on the entropy of a random variable, which is a measure of uncertainty concerning its values. In the case of a binary variable F with Bernoulli distribution with parameter p, the entropy is $-p\log p-(1-p)\log(1-p)$. Its minimum 0 is reached when $p = 0$ or $p = 1$ (no uncertainty on F and perfect synchronism in flowering), and its maximum log 2 is reached when $p = 0.5$ (maximal uncertainty on F and perfectly erratic flowering). The entropy of $F_{g,r,t,m,\ell}$ averaged over years t is given by

$$Ent_{g,r} = -\frac{1}{\sum_t n_{g,r,t}} \sum_t \left(n_{g,r,t,0} \log \frac{n_{g,r,t,0}}{n_{g,r,t}} + n_{g,r,t,1} \log \frac{n_{g,r,t,1}}{n_{g,r,t}} \right)$$

where $n_{g,r,t} = n_{g,r,t,0} + n_{g,r,t,1}$ is the number of AS for replication *r* of genotype *g* at year *t*, $n_{g,r,t,0} / n_{g,r,t}$ the estimated probability of vegetative AS and $n_{g,r,t,1} / n_{g,r,t}$ the estimated probability of flowering AS. The signification and behaviour of $Ent_{g,r}$ are illustrated in Table T1 **[Supplementary Information]** on replications of regular, irregular and biennial bearing genotypes. This index can be directly extended to characterise synchronism at the genotype scale, replacing $n_{g,r,t}$, by the number $n_{g,t}$ of AS for genotype *g* at year *t*, and $n_{g,r,t,v}$ by $n_{g,t,v}$ accordingly. In practice, we chose to characterise synchronism at the genotype scale by computing the mean value $\overline{Ent}_g$ of value $Ent_{g,r}$ averaged over *r*, to favour synchronism within replications rather than between replications.

Alternation in yield for each genotype was characterised using two indices computed at whole tree scale and resulting from trend model A with fixed effects and residual AR1 model (I) with fixed effects: Biennial bearing index using the estimated residuals with normalisation by average yields (BBI_res_norm) and the genotype-related component in the AR1 model of the residual structure (γ_g, referred to in brief as *genotype AR coefficient* in the sequel). These are referred to as *global indices*. The same indices computed from the subsamples of sequences (denoted respectively by B^{loc} and γ^{loc}), plus the entropy index, are referred to as *local indices*.

2.3.3/ Clustering of genotypes from global indices

In order to identify groups of genotypes with similar bearing behaviours, a clustering has been achieved, using the vector of global indices for each genotype. The model used for clustering (into *K* clusters) is a Gaussian mixture model with *K* components (McLachlan & Peel, 2000) assuming that within each cluster, BBI_res_norm and γ_g are independent

Gaussian random variables which means and variances depend on the cluster. The detailed variant of the model is described formally in **[Supplementary Information]** (section M1).

2.3.4/ Prediction of classes and global indices from local indices

In order to characterise the relation between alternation in flowering at both AS and whole tree scale, two kinds of statistical methods have been combined. The first one consists in predicting the global indices of alternation I_g from the local indices B^{loc}, γ^{loc} and $\overline{Ent}_g$. This has been achieved using Gaussian linear models, written generically as

$$I_g = a_0 + a_1 B_g^{loc} + a_2 \gamma_g^{loc} + a_3 \overline{Ent}_g + \eta_g ,$$

where I_g denotes either BBI_res_norm or γ_g and where η_g is a Gaussian residual. P-values of regressions and (multiple) correlation coefficients were computed, with confidence intervals.

The second one relies in the prediction of the classes yielded by Gaussian mixture clustering. The aim of this approach is to predict (i.e. to retrieve) the class of each genotype, using information contained in the subsamples of sequences only. This issue corresponds to the statistical framework of supervised classification. The three local indices were used (simultaneously) as predictors. Thus, in the case of K classes, a classification provides a partition of $\mathbb{R}^3$ where each of the K components of the partition is associated with one given class. Supervised classification was achieved using feed-forward neural networks (NNs in short), which essentially are non-linear multivariate regression models (Bishop, 2006, Chapter 5). These models provide probabilities for each genotype to belong to every possible class. The R-based implementation *nnet* was used (Venables & Ripley, 2002).

Support Vector Machines (SVMs) were also considered (Bishop, 2006, Chapter 7). SVMs provide partitions determined by optimizing geometric criteria, so as to maximise the distance between the boundaries and the genotypes closest to these boundaries. The R-based implementation *kernlab* was used (Karatzoglou *et al*., 2004). Further precisions on parameter

estimation or model selection and validation are given in **[Supplementary Information]** (section M2).

2.3.5/ Model validation: prediction of future yields at tree scale from past yields

The statistical trended AR model (A-I) can be used to predict future yields at tree scale. The predictions have been validated, using separate learning and test samples. Six years of production were available, but model (A-I) has been re-estimated using the first five years of production only (i.e. production between 2005 and 2009 as learning sample), leaving 2010 data in as test sample. The conditional distribution of $Y_{g,r,t}$ at year 2010 (corresponding to t = 5) given the data up to 2009 has been computed. This conditional distribution is Gaussian with mean $\beta + \beta_g + (\alpha + \alpha_g + E[\xi_{g,r}|\boldsymbol{Y}])t + \gamma_g \varepsilon_{g,r,t-1}$ and variance $t^2 \operatorname{var}[\xi_{g,r}|\boldsymbol{Y}] + \rho^2$, where the BLUP $E[\xi_{g,r}|\boldsymbol{Y}])$ and the conditional variance $\operatorname{var}[\xi_{g,r}|\boldsymbol{Y}]$ can be computed directly, using *lme4* package of the R software. Then a prediction interval that contains $Y_{g,r,t}$ with probability 1 - p (p = 0.05) has been deduced, and the actual frequency of $Y_{g,r,t}$ in year 2010 that actually belonged to this interval of prediction has been computed.

The clusters based on the new estimates of BBI_res_norm and γ_g (using the first five years of production) have been updated for each genotype. The comparison between these clusters and those using six years of production (see Section 2.3.3) has been achieved through a contingency table.

<u>2.4/ QTL mapping</u>

At the term of model definition and index construction, six descriptors were used for QTL detection. Two groups of descriptors were distinguished: (i) those linked to biennial bearing at the whole tree scale (global descriptors): these are the Biennial Bearing Index using residuals of trend model (BBI_norm) with normalisation by total yields (BBI_res_norm) and

the genotype AR coefficient (γ_g); (ii) and those linked to the local scale (local descriptors), which are B^{loc}, γ^{loc} and the entropy (linked to the synchronism of flowering).

QTL analyses were performed using the STK × GS consensus genetic map (Guitton *et al.*, 2012). QTL mapping was carried out using MapQTL® 5.0. (Van Ooijen, 2004) through a classical analysis including a permutation test to determine the LOD threshold (genome-wide error rate of 0.05), an interval mapping (IM) analysis to detect potential genomic regions associated to the trait, a cofactor selection and a multiple QTL mapping (MQM) analysis.

3/ Results

3.1/ Quantification of alternation at tree scale

The models presented hereafter were fitted using the whole dataset, i.e. including year 2010 (except in the validation step). The random effect $\zeta_{g,r}$ (replication-specific random intercept) was assessed as non-significant in trend models (A) and (B) (AIC=16,902; BIC=18,208 in model (A)) and new models without $\zeta_{g,r}$ were finally estimated (AIC = 16,845; BIC=18,138 in model (A)). Selection of a trend model among fixed-effects model (A) and random-effect model (B) was strongly in favour of (A) rather than (B) (AIC = 18,972; BIC = 18,998). The random effect $\gamma_{g,r}$ (replication-specific random deviation from AR coefficient) was assessed as non-significant in residual AR1 model (II) (BIC=16,058), compared to residual AR1 model (I) without random effect $\gamma_{g,r}$ (BIC=16,036). These results appeared as an a posteriori confirmation of our preference of modelling genotypes as fixed effects. The estimates of fixed effects and variances are given in Table 1. Confidence intervals are also given at level 95%; they were computed using a bootstrap approach (Baayen *et al.*, 2008).

Values fitted for trended AR model (A-I) are given by $\hat{Y}_{g,r,t} = \beta + \beta_g + (\alpha + \alpha_g + E[\xi_{g,r} | \boldsymbol{Y} = \boldsymbol{y}])t$, where $\boldsymbol{Y}$ denotes the full vector of measurements $(Y_{g,r,t})_{g,r,t}$. The estimated model is illustrated in Fig. 1 to 3 for genotypes with contrasted

bearing habits. Fig. 1 represents the fitted values in trend model (A) (Fig. 1a) and fitted residuals in residual AR1 model (I) (Fig. 1b) for genotype 85 representative of regular bearing. The residuals as a function of year are depicted in Fig. S3 **[Supplementary Information]**. The indices and coefficients are BBI = 1.14, BBI_res_norm = 0.45 and γ_g = -0.07, meaning that the residuals can be considered as independent.

Fig. 2 represents the fitted values and residuals for genotype 107 representative of a biennial bearing. The residuals as a function of year are depicted in Fig. S4 **[Supplementary Information]**. The indices and coefficients are BBI = 1.43, BBI_res_norm = 1.21 and γ_g = -0.88, this indicating that the residuals alternate over time.

Fig. 3 represents the fitted values and residuals for genotype 108 which is representative of irregular bearing. The residuals as a function of year are depicted in Fig. S5 **[Supplementary Information]**. The indices and coefficients are BBI = 1.10, BBI_res_norm =1.17 and γ_g = -0.28. These values of BBI_res_norm and γ_g are characteristic of unstructured residuals, which variability is high with regard to production. Genotypes 85 and 108 are quite similar from the point of view of BBI, though. This highlights that BBI is not able to discriminate regular from irregular genotypes, and confirms that this index should not be used to quantify irregularity of production in our setting. Genotypes 107 and 108 are also quite similar from the point of view of BBI_res_norm, which highlights that the AR coefficient is also necessary to discriminate biennial bearing from irregular genotypes.

<u>3.2/ Clusters of genotypes</u>

Using BIC as a criterion to select the number of clusters led us to consider models with two to four clusters, which fitted the data equally well. However, the 3-cluster model was far more easily interpretable than the 2- and 4-cluster models, and had a slightly lower BIC value. The 3-cluster model was thus selected (Fig. 4) and can be interpreted as follows:

- Cluster 1 contains 36 regular bearing genotypes. These genotypes mostly have values of genotype AR coefficient γ_g either positive or close to 0 (0.08 on average), and small values of BBI_res_norm (0.56 on average). Genotype 85 belongs to this cluster.
- Cluster 2 contains 31 biennial bearing genotypes. These genotypes mostly have negative values of γ_g (-0.82 on average), and high values of BBI_res_norm (1.47 on average). Genotype 107 belongs to this cluster. Note that in absolute terms, 1.47 should be considered as a rather high value of BBI_res_norm. It is shown in proposition P4 in **[supplementary information]** that if yield is linearly growing with biennial alternation, BBI_res_norm tends towards 2 as the length of the time series increases.
- Cluster 3 contains the other 55 irregular bearing genotypes. These genotypes mostly have intermediate values of γ_g (-0.48 on average), and intermediate values of BBI_res_norm (0.95 on average). Genotype 108 belongs to this cluster.

Consequently, the 3-cluster model allows regular, irregular and biennial bearing genotypes to be clearly discriminated, whereas in the 2-cluster model, the irregular genotypes would be distributed between the irregular and biennial bearing genotypes. The 4-cluster model would comprise an additional cluster containing the 2 genotypes with highest values of γ_g, which would not substantially improve the interpretation of the data.

3.3/ Model validation

The estimates for the fixed effects and variances of the model estimated on the first 5 years of production only (referred to as prediction model) are given in Table 2. It can be noted that the estimates are in the confidence intervals given in Table 1 (estimates using the whole dataset). Prediction intervals for the 6th year yield (2010) were computed for each replication of each genotype, at level 0.95. The frequency of actual yields in 2010 that were in the prediction interval was 0.74. This shows that the variance of the number of flowers in 2010 tends to be underestimated by the models. This is consistent with the comparison between Tables 1 and

2, which shows that the variance parameters σ^2 and ρ^2 increase if year 2010 is considered in the analysis. As expected, the percentage of observations within the prediction interval is higher in regular or biennial bearing genotypes than in irregular genotypes. This results from trended AR model (A-I) being essentially dedicated to modelling regular and biennial flowering. In contrast, irregular bearing can be seen as poor adequacy to this model (i.e. low autocorrelation but high residuals). An illustration of the predicted number of flowers in 2010 for genotype 107 (biennial bearing) is provided in Fig. 5. The predictions associated with genotypes 85 (regular) and 108 (irregular) are provided in Fig. S6 **[Supplementary Information]**.

Changes in the estimates in the model parameters and random effects caused switches in the clusters of several genotypes. Taking clusters obtained using the 6 years of production as a reference (Fig. 4), 5 genotypes switched from irregular to regular bearing (among which 2 were not significant since they lay at the boundary of both clusters), 5 switched from irregular to biennial bearing, 6 switched from biennial bearing to irregular. Only 1 regular genotype switched to biennial bearing, and 7 switched to irregular (among which 2 were not significant). No biennial bearing genotype was assessed as regular. The class switches can be summed up by a contingency table (Table T2 in **[Supplementary Information]**). Such switches result from the small number of years available, which make the last three years of great importance in the model (the first three years are associated with low yield for every genotype, and thus have no significant discriminating power). As an example, the clusters of the three genotypes illustrated in Fig. 1 to 3 did not change.

<u>3.4/ Quantification of alternation using local indices</u>

Gaussian linear models with predictors B^{loc}, γ^{loc} and $\overline{Ent}_g$ were estimated to assess their correlations with BBI_res_norm and γ_g. The predictor $\overline{Ent}_g$ was not assessed as significant to predict neither BBI_res_norm (p-value of the t-test on nullity of coefficient: 0.98)

nor γ_g (p-value of the t-test: 0.37). Excluding $\overline{Ent}_g$ from the set of predictors, the multiple coefficient of correlation was 0.74 in predicting BBI_res_norm and -0.65 in predicting γ_g (with p-values of the overall F-test for regression less than 1e-13 in both cases). The correlations of each predictor considered separately with BBI_res_norm and γ_g are given in Table 3. Every correlation is significantly positive at level 0.05.

The correlations of mean entropy $\overline{Ent}_g$ with the quantities characterising regularity of flowering at whole tree scale (BBI_res_norm and γ_g) suggest that the genotypes with highest synchronism (lowest value of mean entropy) tend to be of biennial bearing type (high values of BBI_res_norm and low values of γ_g). These genotypes also tend to show alternation at axis scale (B^{loc} and γ^{loc}). An analysis of variance (ANOVA) performed on $\overline{Ent}_g$ to assess its separation on the three clusters provides a p-value of 1e-6, which proves a significant separation of $\overline{Ent}_g$ with respect to clusters. Considering the means, the biennial bearing genotypes (mean entropy: 0.23) can be discriminated from the irregular (mean entropy: 0.38) and the regular bearing genotypes (mean entropy: 0.38). However, the two latter categories cannot be separated by their mean entropies.

During the global model validation step (Section 3.3), the correlation matrix between the indices at both scales (Table 3) was updated. The updated matrix is given in Table T3 **[Supplementary Information]**. The correlations are within the confidence intervals given in Table 3, which shows that the above conclusions do not qualitatively change if year 2010 is included or excluded from the analysis.

A Gaussian mixture clustering was used to identify clusters from the local indices, as in Section 3.3. This approach did not take benefit from the knowledge of the clusters obtained using the global indices, nor from the relations between the global indices and these clusters. Hence, its performance was expected to be representative of using the local indices on new

F1 progenies, with different parents than ‘Starkrimson’ and ‘Granny Smith’, to infer the bearing behaviour of genotypes. The error rate was 0.4; it is upper-bounded by that of the worst possible classifier (best random classifier independent on the three indices), i.e. 0.56. The confusion between clusters is illustrated by the contingency Table 4.

In contrast, the statistical properties of the global and local indices and the clusters of future genotypes resulting from a cross between the same ‘Starkrimson’ and ‘Granny Smith’ parents as the genotypes used in this study, are expected to be comparable with the statistical properties of these genotypes. In this case, the ability of the three local indices to predict accurately the bearing behaviour of these future genotypes can be assessed by the cross-validated error rate of supervised classification. SVMs achieved an optimal cross-validated error rate of 0.38, which is only slightly better than unsupervised classification (i.e. Gaussian mixture clustering). This should be considered of a quantification of the accuracy of the best possible predictor of the bearing behaviour from local indices. The cross-validated error rate provided by NNs was 0.41. The errors systematically involved confusion between irregular bearing genotypes on the one hand, and either regular or biennial bearing genotypes on the other hand, as illustrated by the contingency Table T4 **[Supplementary Information]**. This is consistent with the results in Section 3.3 (model validation using the first 5 years of production only), and confirms that the overlap of irregular bearing genotypes with the other classes is strong.

A visual representation of such overlap of the classes of genotypes, as characterised by the three local indices, is provided in Fig. S7 **[Supplementary Information]** by a factorial discriminant analysis (FDA, see Tabachnick & Fidell, 2007). FDA provides a dimensionality reduction described in **[Supplementary Information]** (section M3), such that the classes are as separated as possible. Here, even with such optimal representation, the irregular genotypes

seem to be uniformly distributed between both other classes. Hence they cannot be discriminated using the local indices.

3.5/ QTL mapping

Concerning the descriptors at whole tree scale, four QTLs altogether were identified, for BBI_norm and BBI_res_norm, in two separated genomic regions (Fig. 6), on LG1 and LG8 respectively. By contrast, no significant QTL was detected for genotype AR coefficient γ_g. QTLs for BBI_norm and BBI_res_norm were characterised by high LOD scores, from 5.27 to 6.19 (Table 5). The same cofactors were used for both variables, MdGA20ox1a_S and MdEFL3a_G respectively for LG1 and 8 (Table 5). The trait variability explained by these QTLs ranged from 17.7 to 20%

Concerning the descriptors at AS scale, one QTL was mapped for B^{loc} on LG8, with a LOD score of 4.32 (Table 5) with the cofactor CH01c06_G. This QTL is located in the same region than the QTL mapped for BBI indexes at the global scale, since the cofactor CH01c06_G is located less than 1 cM away from the marker MdEFL3a_G. Two QTLs were also detected for genotype AR coefficient at the local scale γ^{loc}. These QTL, located on LG11 and LG14, had high LOD score (7.24 and 4.55), and explained 22.5 and 13.5% of the total variability, respectively. By contrast, no QTL was detected for entropy.

4/ Discussion

4.1/ Possibility of applying the phenotyping method to other species

In the present study, we investigated biennial bearing using the number of inflorescences even though previous studies, on line with pip fruit growers and breeders concerns, have been focused on the number or the mass of harvested fruits (Cilas *et al.*, 2011). This choice results from the fact that the number of inflorescences is less subject to environmental variation than variables related to fruits, e.g. fruit-set, fruit drop caused by climatic conditions or pests and

diseases. Consistently, when the same models, as for the number of inflorescences, were applied to harvested fruit variables, the tree replicate and the residual variability was higher than the genetic variability (data not shown). We can thus conclude that phenotyping the number of inflorescences per tree rather than the number of harvested fruits is more efficient for quantifying the genetic parameters of biennial bearing in apple.

Phenotyping at local scale constitutes a faster strategy and is made easier by the retrospective observation of flowering occurrences in the apple, due to the terminal position of flowering and the possibility to distinguish bourse from vegetative growth units. Similar phenotyping could be applied to other fruit-tree species with terminal flowering and an easy observation of flowering sites, such as pear (Huet, 1972) or walnut (Sabatier *et al.*, 1998). However, it may not be applicable on species with lateral flowering such as *Prunus* species (Fournier *et al.*, 1994; Costes *et al.*, 2006), due to the lack of morphological markers for a retrospective observation of flowering occurrences.

4.2/ New descriptors: use and possible extensions or variants

We proposed three descriptors having a biological meaning to quantify biennial bearing that are defined at either whole tree or AS scale, and are deduced from models, either directly (parameter γ_g of a linear mixed model) or indirectly (using estimated residuals in the case of BBI_res_norm). In the same way, the positive intercept on x-axis of the trend model could be used to predict precocity. The main challenge is to model jointly dependencies between successive years, between shoots of a same tree, and replications of a same genotype.

At whole tree scale, Cilas *et al.* (2011) have estimated the genotypic values associated with yield by linear mixed models with residual correlations having genotype-invariant structure. A Compound Symmetry model (i.e. with constant correlation between years) with heterogeneous variances was assessed to achieve the best fit to the data. To assess regularity of flowering in the prospect of deriving heritability values and correlations between traits,

classical indices (BBI and the number of sign changes in the difference between consecutive yields) were computed on yield directly, independently from the linear mixed model. Their approach is justified by the particular context of their study, where yield was not affected by a trend. In contrast, our approach aims at estimating the bearing behaviour of new genotypes during their first years of fruiting while yield increases, and each genotype has a specific correlation structure between consecutive years. This led us to estimate residuals from linear mixed models and incorporate them into a modified version of the BBI (BBI_res_norm). However, it must be noticed that like BBI, BBI_res_norm is sensitive to the residual variance under the hypothesis of random fluctuations. Thus generally, its value for a given individual cannot be interpreted directly, and it must be either compared with the values for other individuals of similar nature (as it has been the case in our work), or compared to its expected value under some given hypothesis - for example using a resampling approach, as in Huff (2001).

At AS scale, an entropy index has been proposed to characterise synchronism in flowering. This proposal was initially inspired from Lauri *et al.* (1995), who summarized the information of five to six years long sequences by an alternation synchronism index that characterised synchronous patterns of alternation over two successive years. We rather propose to study full-length sequences and to use repetition of sequences within the tree in order to estimate genetic parameters related to alternation. As a complement, the new entropy index aims at characterising how frequently the AS are either simultaneously flowering the same year, or stay vegetative the same year. Entropy indices could be computed at tree scale, but this would require measurement of the annual total number of vegetative AS per tree.

It can be noted that the subsamples of sequences contain structural information that is not fully exploited by the local indices. Successive AS along sequences are subject to dependencies (which depend a priori on the bearing behaviour) that could be taken benefit

from. Markov models could achieve this goal, as illustrated by Costes & Guédon (2012), still in the case of apple trees, but for sequences of growth units. Their approach may be adapted to let the parameters of this statistical model depend on the genotypes, and to predict classes of bearing behaviour using a different Markov model for each class.

The analysis of correlations between global and local descriptors of alternation and synchronism leads us to hypothesise that biennial bearing at tree scale results from the conjunction of two phenomena: synchronism in flowering at tree scale between AS in a given year and biennial alternation at AS scale between consecutive years. On the contrary, regular bearing genotypes at tree scale result in either asynchronous alternating flowering or regular flowering at AS scale. Irregular genotypes exhibit intermediate values for each of the five descriptors, related to bienniality and synchronism. Thus, it can be hypothesised that these genotypes are characterised by partial biennial alternation at AS scale and partial synchronism. However, more complex within-tree organisation of synchronisms could exist, especially at branch scale, as previously proposed for a range of fruit species (Couranjou, 1978; Monselise & Goldschmidt, 1982), and has not been considered in the present study.

4.3/ Genetic determinism of biennial bearing and practical use of descriptors in selection

QTL detection is the first step in the development of tools for marker assisted selection for biennial bearing. In the present study, QTL detection was undertaken on the descriptors we defined to quantify biennial bearing in apple. We showed that these descriptors are usable as quantitative traits for QTL detection and we identified genomic regions involved in biennial bearing.

QTL were detected in two genomic regions for BBI_norm and BBI_res_norm, which corroborate zones that have been previously identified in a previous study (Guitton *et al.*, 2012). The QTL cluster on LG1 for BBI_norm, and BBI_res_norm (global and local scale) also co-located with QTLs for inflorescence yield of a given year and fruit yield QTLs for the

year before in Guitton *et al.* (2012). This LG1 QTL seems to be linked to the antagonist relationship of fruit production of the current year and inflorescence development for the year after that has been reported by Banghert (2009).

The two QTL revealed on LG11 and LG14 for the autocorrelation index at the local scale are located on zones that were not previously associated to flowering or bearing traits in this progeny, even though QTL were detected in the same regions for branching and internode length respectively (Segura *et al.*, 2009). Further exploration of these genomic regions is required to further interpret which mechanism could underline these associations. The absence of QTL detection for the same descriptor, at tree scale and for the entropy may be due to the population size. Indeed, 120 individuals can be limiting to detect QTL with small effects (Bernardo, 2004) and only major QTL were confirmed in the present study. For instance, a LOD pick (3.04) was observed for the autocorrelation index on the global scale on LG8 at the same position as detected for BBI_res and BBI_res_norm, but was lower than the threshold at 95% (3.7) and thus was not considered in our results.

The absence of QTL detection on the LG1 concerning the BBI_res_norm at local scale can be explained by the same reason. Indeed, a QTL was found, but under the threshold at 95%.

The practical application of the proposed method in breeding programmes is still challenging because quantifying irregular bearing implies the collection of data during tree's mature phase over consecutive years. However, an estimation of fruiting behaviour of genotypes could be performed in the last steps of selection scheme, on pre-selected genotypes (i.e. genotypes that have overcome the first individual selections) (Laurens *et al.*, 2000). The methods proposed herein could accelerate and make the estimation process more robust, through a first diagnostic made on a limited number of years. From our results, it can be suspected that after a regular increase in yield, some genotypes would start exhibiting irregular or biennial bearing at some age (unknown in advance). This hypothesis is supported

by Smith *et al.* (2004), who noticed while studying biennial bearing on citrus that there was a clear increase in the intensity of the biennial bearing pattern as the trees aged. Such behaviours were highlighted in our study through the changes in clusters after models considering or not the year 2010. As a consequence, we can propose that breeders (i) progressively suppress biennial or irregular genotypes after the first observation of a large decrease in flowering during the beginning of mature phase (ii) confirm the regular fruiting behaviour of the pre-selected genotypes during stable mature phase. It is likely that such confirmation based on BBI_res_norm and the genotype AR coefficient directly could not be performed by breeders, because it would require the number of inflorescences to be measured each year. But measures performed at AS scale only are more likely to be performed, and the results in Section 3.4 show that supervised classification based on the local indices allows practically every biennial bearing and part of the irregular genotypes to be discarded.

Supervised classification raises the issue of the statistical properties of indices obtained on new F1 progenies obtained using different families than 'Starkrimson' and 'Granny Smith'. Indeed, it is not garanteed that classification rules built using 'Starkrimson' and 'Granny Smith' genotypes may perform well if applied to other families, which indices could have significantly different statistical behaviour. In such cases, the results obtained by unsupervised clustering from local indices show that good discrimination between regular and biennial bearing genotypes remains possible if no a priori information related to the clusters is available. Alternatively, a ranking of the genotypes could be achieved using their coordinates on the first FDA axis (see Fig. S7 in **[Supplementary Information]**).

Since the trend model presented in this paper is dedicated to a period beginning with the mature phase and ending just before stability of this mature phase is reached, it should not include the stable mature period which length could bias the quantification of biennial bearing using our methodology. To address both issues, a possibility would be to resort to a

regime switching model, including two stationary regimes (juvenile and stable mature, without trend) and one regime with trend (yield growth). Each regime would be associated with the state of a Markovian model, which would model the times of switches between regimes, as previously proposed by Chaubert-Pereira *et al.* (2009).

Conclusion

In the present study, we revisited the indices and descriptors available for estimating the bearing behaviour of fruit trees in a breeding context, i.e. at genotype scale. Using innovative modelling approach, we proposed new descriptors at both whole tree and annual shoot scales. This approach opens new possibilities to accelerate evaluation process and allow breeders to perform a first diagnostic during the first years of tree production when yield is still increasing. Further investigations could contribute to test multivariate classifying methods and to extend the estimation at genotype scale to the mature phase of trees.

Acknowledgements

The authors thank Christian Lavergne, Christian Cilas and Pierre Éric Lauri for fruitful discussions and David Chagné for critical reading of the manuscript. Baptiste Guitton PhD scholarship was supported by the Plant Breeding Department of the National Institute of Agronomic Research of France (INRA), by Plant & Food Research (Pipfruit Internal Investment Project), and by the New Zealand Ministry of Science and Innovation [Horticultural Genomics program (CO6X0810)]. Jean Peyhardi's PhD was founded by University of Montpellier 2 (UM2).

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Table 1. Estimates of fixed effects and variances (with 95% confidence intervals) of the trended AR model. Intercept β, residual standard deviations σ of the residuals ε and ρ of the AR model are expressed in number of flowers. Slope α and random effect standard deviation τ_{ξ} are given in number of flowers per year.

Parameter	β	α	τ_{ξ}	σ	ρ
Estimate	43.1 (-108,194)	90.7 (36,145)	20.9 (13.7,24.0)	178 (172,185)	150 (134,172)

Table 2. Estimates of fixed effects and variances of the trended AR model for prediction (with 95% confidence intervals. Intercept β, residual standard deviations σ of the residuals ε and ρ of the AR model. Slope α and random effect standard deviation τ_{ξ} are given in number of flowers per year.

Parameter	β	α	τ_{ξ}	σ	ρ
Estimate	44.2 (-101,188)	90.0 (29,154)	23.0 (10.9,25.8)	160.4 (155,168)	138.7 (123,159)

Table 3. Correlation coefficient between indices at whole tree and AS scales, with 95% confidence intervals.

	Genotype AR coefficient γ_g	Local BBI_res_norm B_g^{loc}	Local genotype AR coefficient γ_g^{loc}	Mean entropy $\overline{Ent}_g$
BBI_res_norm	-0.66 (-0.75;-0.55)	0.72 (0.61;0.80)	-0.58 (-0.69;-0.45)	-0.49 (-0.62;-0.34)
γ_g	1	-0.61 (-0.71;-0.49)	0.55 (0.41;0.67)	0.33 (0.16;0.48)
B_g^{loc}		1	-0.63 (-0.73;-0.51)	-0.66 (-0.75;-0.54)
γ_g^{loc}			1	0.33 (0.15;0.48)

Table 4. Contingency table for the number of genotypes assigned to class c2 by Gaussian Mixture Clustering on local indices and assigned to class c1 by Gaussian Mixture Clustering on global indices. Cluster 1 corresponds to regular genotypes, cluster 2 to biennial bearing and cluster 3 to irregular genotypes.

		Cluster c1 on global indices		
		1	**2**	**3**
Cluster c2 on local indices	**1**	33	2	29
	2	0	18	4
	3	2	9	18

Table 5. QTLs detected on the consensus STK×GS genetic map by MQM mapping for variables related biennial bearing at whole tree scale (BBI_norm and BBI_res_norm) and AS scale (B^{loc} and γ^{loc}). The table displays the linkage group (LG) the QTL has been detected on, the LOD score (LOD), the proportion of genetic variability (R^2) explained by the QTL and the co-factor used for the QTL mapping.

Variable	**LG**	**LOD**	**R^2**	**Co-factor**
BBI_norm	1	6.69	0.201	MdGA20ox1a_S
	8	5.82	0.186	MdEFL3a_G
BBI_res_norm	1	5.91	0.177	MdGA20ox1a_S
	8	5.74	0.186	MdEFL3a_G
B^{loc}	8	4.32	0.154	CH01c06_G
γ^{loc}	11	7.24	0.225	CH04h02_SG
	14	4.55	0.134	CH05g07z_SG

Fig. 1. Measurements and fitted values of yield $Y_{g,r,t}$ of replication r of genotype g at year t (a), empirical and predicted residuals as a function of previous residual (b) for regular bearing genotype g = 85.

Fig. 2. Measurements and fitted values of $Y_{g,r,t}$ of replication r of genotype g at year t (a), empirical and predicted residuals as a function of previous residual (b) for biennial bearing genotype g = 107.

Fig. 3. Measurements and fitted values of $Y_{g,r,t}$ of replication r of genotype g at year t (a), empirical and predicted residuals as a function of previous residual (b) for (non-biennial) irregular bearing genotype g = 108.

Fig. 4. Clustering obtained using a 3-component mixture of Gaussian distributions of BBI_res_norm (x-axis) and genotype AR coefficient γ_g (y-axis named *auto.cov*). Cluster 1 can be interpreted as regular bearing genotypes, cluster 2 as biennial bearing genotypes and cluster 3 as irregular bearing genotypes.

Fig. 5. Measurements and predicted yield values in 2010 (year number 5) for irregular genotype g = 107. Circles are the measured values; triangles are the predicted values, located in the middle of prediction intervals (dotted segments).

Fig. 6. Genomic positions of the QTLs detected on the consensus 'Starkrimson'×'Granny Smith' (STK×GS) genetic map. QTLs are represented by boxes, which length represents the LOD–1 confidence interval. Parameters at whole tree scale are BBI_norm (Biennial Bearing Index normalized by total yield) and BBI_res_norm (using residuals of trend model).

Parameters at AS scale are B^{loc} and γ^{loc} (resp. BBI_res_norm and genotype AR coefficient computed from data at AS scale). Underlined genetic markers correspond to co-factors used for QTL mapping.