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A new integrative indicator to assess crop genetic diversity

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ABSTRACT

Various international initiatives have emerged to develop new sets of biodiversity indicators for wild biodiversity but little progress has been made for crop diversity. This study proposes a new methodology to assess the diversity of cultivated species. We developed a new integrative indicator $H_T{}^*$ that takes account of varietal richness, spatial evenness, between-variety genetic diversity, and within-variety genetic diversity. This index is compared to existing indexes using a comprehensive historical dataset from a French territory. The study reveals that more varieties (the varietal richness factor) can mean less diversity when (i) their genetic structure is more similar (the effect of between-variety genetic diversity), or (ii) when more diverse landraces are replaced by many homogeneous lines (the effect of within-variety genetic diversity) or (iii) when one or a few varieties become hegemonic in the landscape (the spatial evenness effect). Furthermore, an increased evenness in variety distribution (varietal evenness) can also mean less diversity when varieties are genetically related. This is why indicators which do not take into account the full range of factors (varietal richness, between-variety genetic diversity, spatial evenness and within-variety genetic diversity) may provide a misleading assessment of the state and trend of crop biodiversity. New integrative indicators such as $H_T{}^*$ are critically needed. We discuss potential further development such as the use of this indicator for monitoring and the perspective of a broader indicator to assess genetic diversity at the multi-species scale and landscape level. In the future, this indicator could be used to guide managerial decisions to prevent the erosion of crop genetic diversity in agricultural landscapes.

1. Introduction

The conservation of crop biodiversity is increasingly recognized as a key issue both for biodiversity at large and for the resilience of agricultural systems (Folke et al., 2004; Heal et al., 2004; Finckh, 2008; Østergård et al., 2009; Frison et al., 2011). To address higher climatic instability and new pest dynamics at regional levels that result from global environmental changes, many have stressed the strategic importance of maintaining (*in situ* conservation) and developping (plant breeding) a wider genetic basis for cultivated plants (Wolfe et al., 2008; Østergård et al., 2009; Hajjar et al., 2008; Jarvis et al., 2008). Future food safety relies on the ability of plants to adapt to changes such as climate change or pest and disease variations which occur in the field.

Biological diversity is the variability among all living organisms; this includes diversity within species, between species and of ecosystems. A component of biodiversity at large, agrobiodiversity, plays an indispensable role in human provision of food and renewable primary products. Agrobiodiversity includes the variability of agro-ecosystems, between and within non-crop species present in these agro-ecosystems, and between and within crop species (crop biodiversity). More specifically, crop biodiversity involves a specific level and an intraspecific level.

At the specific level, it is widely acknowledged that the range of species that underpin human livelihoods has decreased in the last century. It is estimated that currently only 30 crops provide 95 percent of human food energy needs and just four of them – rice, wheat, maize and potatoes – provide more than 60 percent. In addition, an estimated 75 percent of crop diversity was lost between 1900 and 2000, and that genetic erosion is still continuing (FAO, 2010).

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But at the within-species level of diversity, the loss of genetic diversity for given crops is rather poorly documented. The second Global Biodiversity Outlook states that “loss of genetic diversity through the disappearance of locally adapted varieties and landraces of crops and livestock breeds is widely reported but difficult to quantify” (Global Biodiversity Outlook 2, 2006, chap. 2, p. 27). As a consequence, although the last Global Biodiversity Outlook concluded that “crop and livestock genetic diversity continues to decline in agricultural systems” (Global Biodiversity Outlook 3, 2010, p. 9), it does not provide any indicator of crop diversity and published studies still disagree over the severity of genetic erosion that may have occurred, as well as over the present trends. While some studies did not detect any erosion of crop genetic diversity in the past century, or even a recent increase in developed countries, (Christiansen et al., 2002; Le Clerc et al., 2006; De Wouw et al., 2010), other publications report a clear loss of crop genetic diversity in recent decades (Reif et al., 2005; Le Clerc et al., 2005; Hammer and Laghetti, 2005; Roussel et al., 2004).

These contradictory results, stemming from varying methodologies and fragmentary data from different crops and regions, call for new and comprehensive indicators to assess crop biodiversity, that need to be harmonized and based on the latest advances in ecology and genetics. Various international initiatives have emerged to develop new sets of biodiversity indicators but little progress has been made for crop biodiversity. The Pan European initiative, SEBI2010 (Streamlining European 2010 Biodiversity Indicators) which began in 2004, has developed a set of 26 biodiversity indicators, but none of these was related to crop diversity (EEA, 2007; Mace and Baillie, 2007). The OECD proposed two indicators (and provided data for several countries) for crop diversity: (a) the total number of varieties registered and certified for marketing and (b) the share of key crop varieties in total marketed production for individual crops (OECD, 2001, pp. 320–322). However, indicators based upon the number of registered varieties are only a partial hint to assess the state and trend of crop genetic diversity in agricultural landscapes. An increase of the number of registered varieties can hide a decrease of genetic diversity in agricultural ecosystems, if farmers on a given territory – at local, regional or national level – effectively grow only a small and fluctuating number of varieties. Measuring the share of key varieties in total crop production is an easy proxy to take this *in situ* evenness aspect into account. But even this second indicator has its limits as the OECD acknowledges: “the genetic structure of the varieties in current use is likely to be similar, independent of the number of varieties grown (. . .). In other words, the twenty principal varieties in 1998, for example, may not have more genetic diversity than the two principal varieties grown in 1985” (OECD, 2001, p. 304). This means that a more realistic and comprehensive indicator should also quantify the genetic diversity between varieties and within varieties (as we shall see).

The Conference of Parties of the Convention of Biological Diversity (CBD) has adopted a set of headline biodiversity indicators in 2004. Acknowledging that indicators based on the diversity present in *ex situ* gene banks do not measure the *state* (nor the *trend*) of crop biodiversity, but rather the institutional *response* to its erosion, the CBD proposed that additional “information on *in situ* conservation of genetic resources should be incorporated (. . .) once data and a methodology for calculating such an indicator become available” (CBD, 2004, p. 8).

In summary, we currently lack relevant indicators to realistically assess crop species biodiversity trends. This paper intends to bridge this gap. We first review existing studies and indicators and discuss their methodological limits. We then propose a new composite indicator which assesses on-farm crop biodiversity in a comprehensive way, integrating genetic and landscape levels. Finally, we provide a comparison of the insights given by the various indicators to date, including ours, using a rich dataset that starts as early as 1912, from a major agricultural region of France. We conclude with perspectives towards a more scientific and systematic monitoring of crop genetic diversity.

2. Existing methods and indicators

We have reviewed 162 published studies assessing bread wheat diversity as well a range of general studies on the assessment of crop genetic diversity (see list in Goffaux et al., 2011). They can be classified according to the level of genetic diversity they target (*i.e.* varietal or allelic) and in which pool it is measured (*i.e. ex situ* collections, registered varieties, or *in situ* (on-farm)) (Suppl. Table 1).

Most studies (Ortiz et al., 2003; Roussel et al., 2004; Donini et al., 2000; Christiansen et al., 2002; Huang et al., 2007; Le Clerc et al., 2006) assess diversity within registered varieties or genebanks, while only a few consider the diversity actually cultivated (Table 1). In the studies addressing *in situ* (on farm) diversity, we have identified several indexes that have been used: (i) the number of varieties; (ii) the Shannon index (SW), the Simpson index (Es, Simpson, 1949) and the Piélou’s hierarchical diversity index (J, Pielou, 1966), and (iii) the Nei index (H). The indexes in (ii) all account for the evenness of varieties spatial distribution. While SW is sensitive to rare varieties, Es is mainly sensitive to lead varieties’ dominance and J is corrected for the number of varieties and depends on the gap between the acreage share of lead varieties and minor ones. Nei’s index accounts for allelic diversity across a pool of varieties (Table 1). To date no study has combined the study of allelic diversity and a concern on evenness of the spatial distribution of alleles. Furthermore, while the Nei index can be used to account for between-variety allelic diversity (two genetically different varieties may have more genetic diversity than 10 genetically related varieties), no indicator has accounted for the additional role of within-variety allelic diversity. However, for a given genetic distance between heterogeneous varieties (*i.e.* landraces or population varieties), they have more genetic diversity than the same number of homogeneous varieties (*i.e.* pure line varieties) (Barcaccia et al., 2002; Dreisigacker et al., 2005; Zhang et al., 2006).

3. Development of a new composite indicator for crop genetic diversity in landscapes

As suggested by the CBD secretariat, “genetic diversity comprises the total genetic variation present in a population or species” and can be assessed when three different facets are combined: “(1) number of different entities (. . .); (2) evenness of the distribution of these entities, and (. . .) (3) the extent of the difference between the entities” (CBD, 2004, p. 8). To develop our comprehensive crop genetic diversity indicator, we were therefore concerned to account for (i) varietal richness, (ii) varietal evenness of spatial distribution and (iii) between-variety allelic diversity. We developed a composite indicator integrating these three levels to which we added the within-variety allelic diversity (Fig. 1).

The three main stages we followed to design an integrative indicator of genetic diversity were: (i) to account for the genetic diversity between-varieties, (ii) to weight allele frequencies according to the acreage share of the varieties carrying these alleles in a given geographical area and (iii) to include the variation that may exist within a variety.

3.1. Assessing genetic diversity between varieties

The theoretical population geneticist Nei (1973) quantified the gene diversity belonging to the same or to different populations (which he called “subdivided populations”), without regard to the

Table 1
What do indicators measure?

Components of genetic diversity that are accounted for					
Index/Indicator	Studies	Richness	Evenness of spatial distribution	Inter-varieties allelic diversity	Intra-varieties allelic diversity
Numbers of varieties	OECD (2001), Jarvis et al. (2008)	X			
SW (Shannon)	Martynov et al. (2006), Brennan and Bialowas (2001)	X	X (sensitive to rare varieties)		
Es (Simpson)		X	X (sensitive to lead varieties)		
J (Piélou)	Meul et al. (2005)	X (less sensitive to the number of varieties)	X (sensitive to the gap between the share of lead varieties and minor ones)		
H (Nei)	Roussel et al. (2004, 2005)	X		X	
H^*	This article	X	X	X	
$H_T{}^*$	This article	X	X	X	X

pattern of evolutionary forces such as mutation, selection and migration. Crop genetic resource scientists have since used this framework to quantify genetic diversity between varieties. The varieties grown in a given area at a given time can be considered as a subdivided population and each variety as a sub-population. To assess the genetic diversity between varieties, Nei's index takes into account the number of alleles and their frequencies in a set of varieties:

$$H = \frac{\sum_{j}\left(1-\sum_{i} p_{ij}^{2}\right)}{J}$$

H is the average Nei's diversity, p_{ij} is the frequency of allele i at locus j, J is number of loci.

Allelic frequencies were computed as: $p_{ij} = \sum_{k} G_{ijk}/K$, where K is the number of varieties, $G_{ijk} = 0.5$ if one allele of variety k at locus j is i, $G_{ijk} = 1$ if both alleles are i and $G_{ijk} = 0$ otherwise. Nei's index varies between 0, when one allele is fixed at each locus in the sample considered, and increases to a limit of 1, when the number of alleles at each locus increases and their frequencies are balanced.

3.2. *Integrating evenness of the spatial distribution of alleles*

In Nei's index, all varieties in the set are given the same weight without regard to their real spatial distribution in a given agricultural landscape. We included this variety *evenness* information (acreage share for all varieties in the geographical area considered) so as to get a picture of the evenness of the distribution of alleles in the landscape. Thus, these proportions were introduced to compute weighted allelic frequencies (p^*) and the diversity index becomes:

$$H* = \sum_{j} \frac{\left(1-\sum_{i} p{*}_{ij}^{2}\right)}{J}$$

with $p_{ij}^{*} = \sum_{k} \alpha_k \times G_{ijk}$ where α_k is the proportion of variety k in the geographical area considered. H^* provides information on the genetic diversity between varieties weighted by their share in the landscape. H^* index varies between 0 and 1. It is 0 when one allele is fixed at each locus in the area considered, and it increases towards

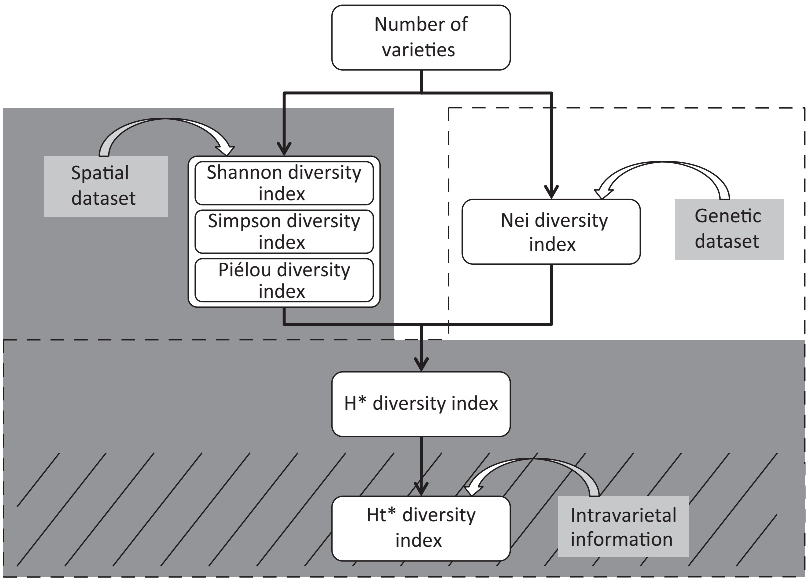

Fig. 1. Schematic of the different indexes and the information they include organized in four levels of complexicity.

1 when the number of alleles at each locus increases and when the proportion on the total acreage of each allele is balanced.

3.3. *Integrating within-variety diversity*

While steps 1 and 2 only assess between-variety genetic diversity, we incorporated the within-variety component in a third step by adapting Nei's expressions for the genetic diversity in the total subdivided population (Nei, 1973):

$$H_T = D_{ST} + H_S$$

where D_{ST} is the average genetic diversity between sub-populations (here: varieties) and H_S is the average genetic diversity within them. D_{ST} gives a measure of the absolute magnitude of genetic differentiation between sub-populations (varieties). However, in many cases, it is not possible to measure the genetic diversity within each variety nor the average within-variety diversity. An alternative route to get H_T is provided by the following Nei's equation (Nei, 1973):

$$G_{ST} = \frac{D_{ST}}{H_T}, \quad \text{thus} \quad H_T = \frac{D_{ST}}{G_{ST}}$$

where G_{ST} is the between-variety genetic differentiation relative to the total genetic diversity.

Considering the previously described H^* index as equivalent to a D_{ST} differentiation parameter allowed us to propose $H_T{}^*$ index as the final integrated indicator:

$$H_T* = \frac{H*}{G_{ST}}$$

Depending on the crop and on the region and varieties studied, G_{ST} will be estimated directly on the set of varieties or based on bibliography and other experiments (see below for an example).

4. Materials and methods for benchmarking several indicators

To test the new indicator and compare it to existing indexes for crop genetic diversity, we have analysed the bread wheat varieties that have been grown since 1878 in the *département* of Eure-et-Loir (*départements* are administrative divisions in France). We chose this dataset because Eure-et-Loir, with about 450.000 ha of cultivated land in the fertile South-western part of the Paris basin, ranks among the most productive wheat producing regions in 20th century Europe. Additionally, intensive historical research allowed us to gather reliable data on varieties acreage and share as far back as 1878.

4.1. *Data*

We gathered extensive sets of data on (a) allelic diversity of varieties, (b) the acreage share of each variety in the total wheat area in Eure-et-Loir for each year and (c) an estimation of the contribution of within-variety diversity (as compared to between-variety diversity) to the total genetic diversity.

4.1.1. *Historical data on varieties' acreage share*

Statistical services of the Wheat Board or/and of the Ministry of Agriculture started systematic surveys documenting (almost) yearly the acreage and share of wheat varieties in several of the French *départements* from the 1960s forwards. Besides this readily available data, we found additional data scattered in published or unpublished agricultural monographies, scientific journals, local scholarly societies' journals, and local archives. For the early 1950s, we could get quantified data from unpublished 1950 monographies conserved in the Wheat Board's archives and from the *Monographies départementales agricoles* launched in 1952 by the Ministry of Agriculture (Ministère de l'agriculture, 1959). The data for 1912 was derived from a study on the distribution of wheat varieties in France published by a professor at the National School of Agriculture of Grignon (Brétignière, 1912). Brétignière provides quantitative data on variety distribution, which could be complemented and corrected with several other qualitative sources of the same period (such as Garola, 1909). Thanks to these additional sources, we could reconstitute a fair picture of 1912. Yet, because we always took the most cautious decision as for the presence of a variety, the value of our $H_T{}^*$ indicator for 1912 should be considered as a minimum value rather than the best approximation. Finally, semi-quantitative data from Berthier (1878) and other contemporary sources allowed us to get a conservative estimate for 1878. As a whole, the acreage shares of wheat varieties in Eure-et-Loir have been documented for 46 different years between 1878 and 2006.

4.1.2. *Molecular data*

We used microsatellite markers (tandemly repeated DNA motifs of 1–6 bp in length) because they are highly polymorphic, neutral and distributed across the whole genome. Thus, they constitute a powerful and reliable tool to measure genetic diversity within and between varieties (Plaschke et al., 1995). Molecular data consisted of genotypes at 35 SSR markers chosen among the 42 polymorphic loci used to characterize diversity in Roussel et al. (2004, 2005). Microsatellites previously developed and mapped by Röder et al. (1998) (gwm markers) as well as one marker (cfd71) from INRA-Clermont-Ferrand (Guyomarc'h et al., 2003) which detects two loci, were selected to cover the whole bread wheat genome.

Genetic data were obtained by the genotyping of the 305 out of 343 bread wheats varieties reported as grown in Eure-et-Loir from 1878 to 2006 (see Suppl. Table 2). For DNA extraction, five seeds from self-pollinated ears of each of the accessions were sown in pots in glasshouse. When plantlets reached the 2-leaves stage, leaf samples were taken on the five plants and bulked. For each bulk, total DNA was extracted from 100 mg of fresh material following a protocol derived from the Dneasy 96 Plant Kit (Qiagen, Hilden, Germany). PCR protocols were adapted from Röder et al. (1998) and Guyomarc'h et al. (2003). Amplified fragments were separated on an ABI 3130xl semi-automatic sequencer (Applied Biosystems, Foster City, CA, USA) and analyzed using GeneMapper 3.7 (Applied Biosystems). Genotyping was performed by the Genotyping Platform at INRA Clermont-Ferrand. Data for 233 among these 305 varieties were obtained by Roussel et al. (2004, 2005), while data for the remaining 72 were obtained in 2009 for this study. Seeds of these 72 accessions were provided by several genebanks and by breeding companies (see Suppl. Table 2). Note that as only five individuals derived from self-pollination were used to genotype a variety, we could not assess within-variety diversity but rather the five individuals were considered as homozygous and genetically homogeneous and the rare multi-allelic profiles were replaced by missing data. Thus, G_{ijk} took only the two values 0 and 1 in this case.

4.2. *Estimation of within-variety diversity*

Because it is important to take within-variety into account whereas this information is not available for all varieties, values were estimated elsewhere from the bibliography. Although wheat is an autogamous species, the outcrossing rate is not zero (Enjalbert and David, 2000; Waines and Hegde, 2003). Due to pollen flow, seed admixture and mutation, a certain amount of variability can therefore be found within wheat varieties (Thomas et al., 2012). We identified three categories of varieties according to their level of homogeneity and the history of breeding: (1) landraces, *i.e.* the varieties obtained before 1884 (creation of the first hybrid "Dattel") or after 1884 but only based on mass selection, (2) old commercial lines, *i.e.* varieties developed after 1884 and before 1945 with pedigree selection, (3) modern pure lines, *i.e.* varieties obtained after 1945 and registered to the catalogue. Their respective share over time in the wheat area is given in Suppl. Table 3. Each category was then attributed a mean coefficient of within-variety diversity.

Landraces (L) – According to Harlan (1975), wheat landraces are varied and dynamic but always recognizable populations. In this study we considered landraces to be traditional local varieties and varieties derived by mass selection within these landraces. Ideally, we would assess within-landrace diversity by genotyping several individuals of each landrace, but in wheat, no such data were available on the genebank accessions we identified. In general, while diversity between accessions of wheat landraces has often been characterized (*e.g.* Raman et al., 2010), very few studies have assessed the diversity within wheat landraces. Yet, in the few cases where the genetic diversity within on farm cultivated landraces was analysed, a considerable level of H_S/H_T was found: 68% and 53% respectively within Turkish and Mexican landraces (Dreisigacker et al., 2005), 75.9% within Ethiopian populations (Mondini et al., 2010) and 52% within Italian emmer landraces (Barcaccia et al., 2002). Using all the identified studies assessing diversity within bread and tetraploid wheat landraces, we found G_{ST} (or F_{ST}) parameters ranging from 0.19 to 0.48 (0.30 in Omani durum wheat and 0.19 in bread wheat, Zhang et al., 2006; 0.33 between Turkish landraces and 0.47 between Mexican landraces, Dreisigacker et al., 2005; 0.48 in emmer landraces, Barcaccia et al., 2002). In a study of the genetic structure of Swedish barley landraces using 113-year-old seeds, the average G_{ST} estimated between all populations was 0.36 (Leino and Hagenblad, 2010) which is very much in line with the values estimated on current wheat landraces. An additional peculiarity of landraces is that they are usually grown by several farmers in a given location or in some cases on much broader areas and they are submitted to the different evolutionary forces including migration so that they can be understood as crop metapopulations (Alvarez et al., 2005). Thus, a realistic approach would take two levels of within-landrace diversity: between and within farms. For instance, in *Phaseolus vulgaris*, a self-pollinated species, Negri and Tiranti (2010) estimated F_{ST} between farms around 0.3 for the landrace “A Pisello”. In bread wheat, studying of the structure of genetic diversity of an old wheat variety maintained under on farm management conditions, “Rouge de Bordeaux”, Thomas et al. (2012) found a great variability within this variety, both at the within farm level (averaged $H_S = 0.16$) and between farms ($G_{ST} = 0.27$). Taking this diversity levels into account would further decrease G_{ST}. Yet, since it is very seldom documented in the literature, we used the published observed range for G_{ST} in cereal landraces was 0.19–0.48.

Old commercial lines (OL) – As they derive from 3 to 10 years of inbreeding, these varieties are expected to have a much higher level of homogeneity than landraces. Yet, as they have been cultivated and farm-saved on a large scale over many years (the turnover of varieties at the time was lower than nowadays and there was more than 97% farm-saved seed before World War II in France; Bonneuil and Thomas, 2009), they may maintain some heterogeneity compared to post 1945 registered varieties. We found no study testing the degree of difference between plants from the same variety name but cultivated at different times and places. The variability found within this category may depend on the level of fixation of the variety and more generally on the selection methods, the time/length and acreage cultivated as well as the seeds' origin (annual purchase of certified seed or farm-saved seed). Given this, we conservatively assumed a within-variety diversity (H_S) of old commercial lines as 1/10 of that of the landraces.

Modern pure lines (ML) – In France a national variety testing system emerged in the 1940s. In 1941, the *Comité Technique Permanent de la Sélection* was established overseeing the tests that all new varieties had to pass to be put to the market. These tests, which included an assessment of the distinction, uniformity and stability of the variety, were fully operational in 1945, resulting in the registration of only highly fixed pure line varieties (Bonneuil and Thomas, 2009, 2010). Furthermore, the formal seed market was promoted in the post-war decades and the use of farm-saved seeds decreased to about 50%. So – neglecting rare gene flow and mutation events, post 1945 varieties are considered in this study as genetically fixed and homogeneous, with all individual plants having the same genotype (no within-variety variability).

General expression for the indicator – Therefore, we set the G_{ST} coefficient to 0.4 for landraces (L), which is a rather conservative level (minimal risk of overestimating diversity) as compared to the 0.19–0.48 (average 0.35) documented in the literature discussed above. Additionally, we set G_{ST} coefficient to 0.94 ($= 1 - (1 - G_{ST(L)})/10 = 1 - 0.6/10$) for old commercial lines (OL) and 1 for modern lines (ML, corresponding to a null value for their within-variety diversity). These three coefficients were incorporated into the expression of $H_T{}^*$ – which may therefore exceed 1 since G_{ST} depends on the population considered – as follows:

$$H_T* = \frac{D_{ST}}{G_{ST_{(L)}} \times (\%L) + G_{ST_{(OL)}} \times (\%OL) + G_{ST_{(ML)}} \times (\%ML)}$$

With $\%L$, $\%OL$ and $\%ML$ being respectively the acreage share for landraces, old lines, and modern lines. Replacing further D_{ST} by its expression in terms of alleles' presence in the landscape makes:

$$H_T* = \frac{(1/J)\sum_j \left(1 - \sum_i \left(\sum_k \alpha_k \times G_{ijk}\right)^2\right)}{G_{ST_{(L)}} \times (\%L) + G_{ST_{(OL)}} \times (\%OL) + G_{ST_{(ML)}} \times (\%ML)}$$

4.3. Computing of indicators

A database was designed and built to stock all the historical and molecular data. It allows cross-referencing information on spatial distribution of bread wheat varieties for the documented years and molecular data for the 305 varieties. Functions allow the computation of the different indicators for each year. Some parameters such as G_{ST}, the required minimal area described with varieties, the minimal frequency of data to be completed at each locus, can be adjusted by the user before starting the computation. Initially, genetic data were obtained for 35 loci but an additional control on the minimal number of loci required for indicator computation was implemented in order to avoid estimation based on too few loci. When the number of loci fell below 10, it led to no estimation (missing data) of the indicators of genetic diversity.

The different indicators that have been computed were: (i) the number of varieties, (ii) the spatial indexes of Simpson (E_S) and Piélou (J), (iv) Nei's genetic index (H), the spatial genetic index (H^*) and the new integrative index ($H_T{}^*$).

We studied the effect of varying the parameters on the stability of the indicator $H_T{}^*$ in the case of the Eure-et-Loir *département* for 46 different years from 1878 to 2006.

We investigated (i) the impact of incompleteness of acreage share, (ii) the effect of varying the threshold for genetic data frequency (the maximum amount of missing data allowed at each locus) and (iii) the effect of varying the G_{ST} coefficients of the three categories of varieties (landraces, old commercial lines, modern pure lines) (see Suppl. Fig. 1 for the detailed results).

5. Results

In the sensitivity analysis, we found that missing data up to 30% only moderately affects the indicator values and trends except for the Piélou index, which should be used with particular care when missing information is suspected. In addition, since documentation tends to be less complete when going further in the past, the general bias might be an underestimation of crop diversity in the first half of the 20th century. So, in general the acreage data completeness threshold should be set to at least 70% depending on the objectives of each study. In the case of the Eure-et-Loir *département*, the documented acreage share of genetically characterized varieties was always higher than 80% of the total wheat area.

In a second step, we assessed the impact of varying the threshold for genetic data frequency between 0.75 and 1 on $H_T{}^*$. In general, this led to very similar values of the indicator. On this basis, we chose to set the frequency threshold to a rather stringent 0.95 level so as to maximize precision for allele frequency estimation at each locus. In the case of Eure-et-Loir, and with a frequency threshold of 0.95, the indicator was computed based on a minimal number of 22 loci (maximum = 35), which is rather satisfying.

Finally, we varied the G_{ST} coefficient from 0.3 to 1 for the landraces, from 0.5 to 1 for the old lines and from 0.94 to 1 for modern pure lines. The results (shown in Suppl. Fig. 1) indicate that setting the landrace G_{ST} to any value between 0.3 and 0.5 (the range found in the litterature), while leaving old lines G_{ST} at the same value, 0.94,

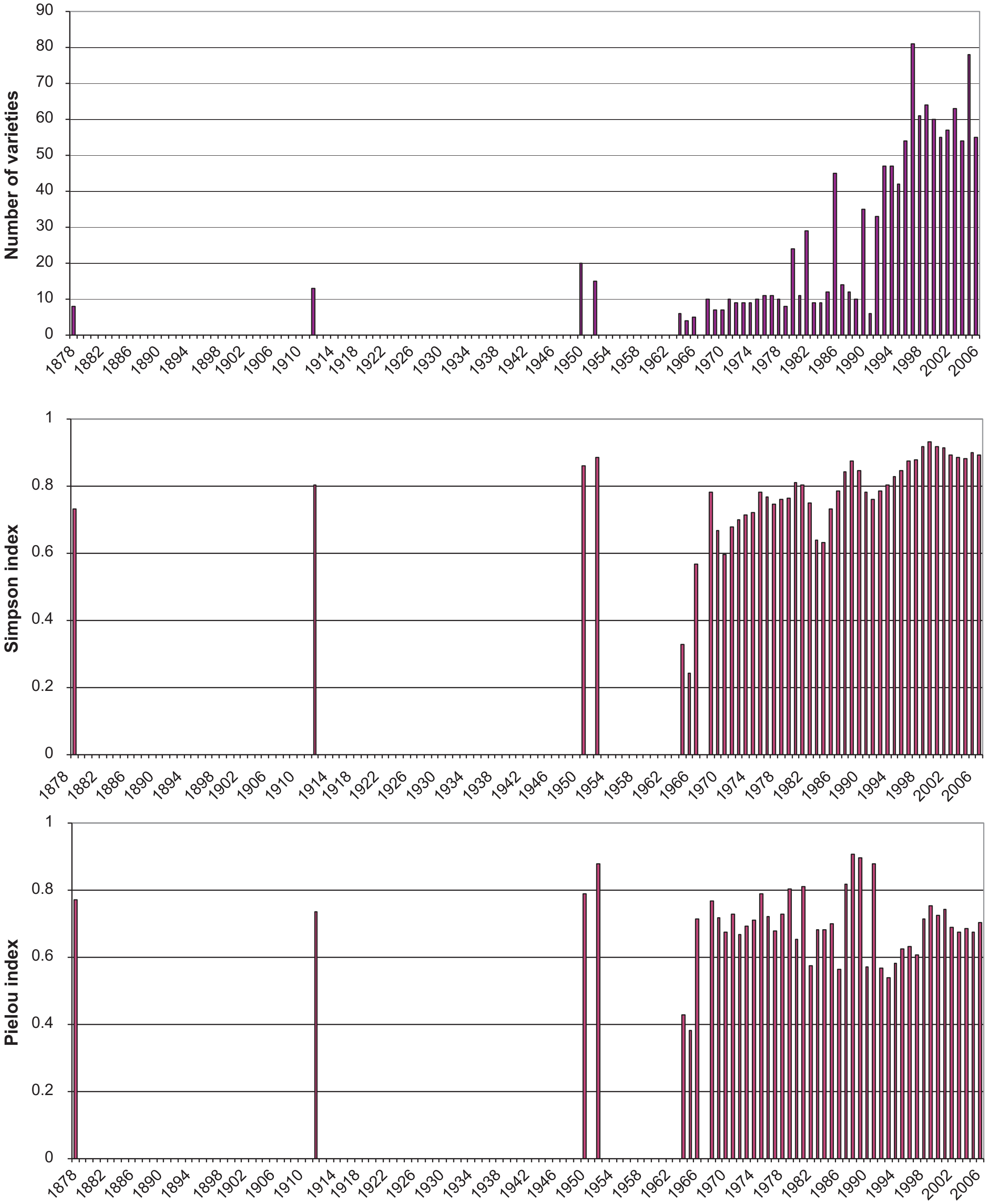

Fig. 2. Evolution of five index (the number of varieties, the Simpson index, the Pielou index, the Nei genetic diversity index, H and the spatial genetic diversity index, H^*) of wheat diversity from 1878 to 2006 in the *department* Eure-et-Loir.

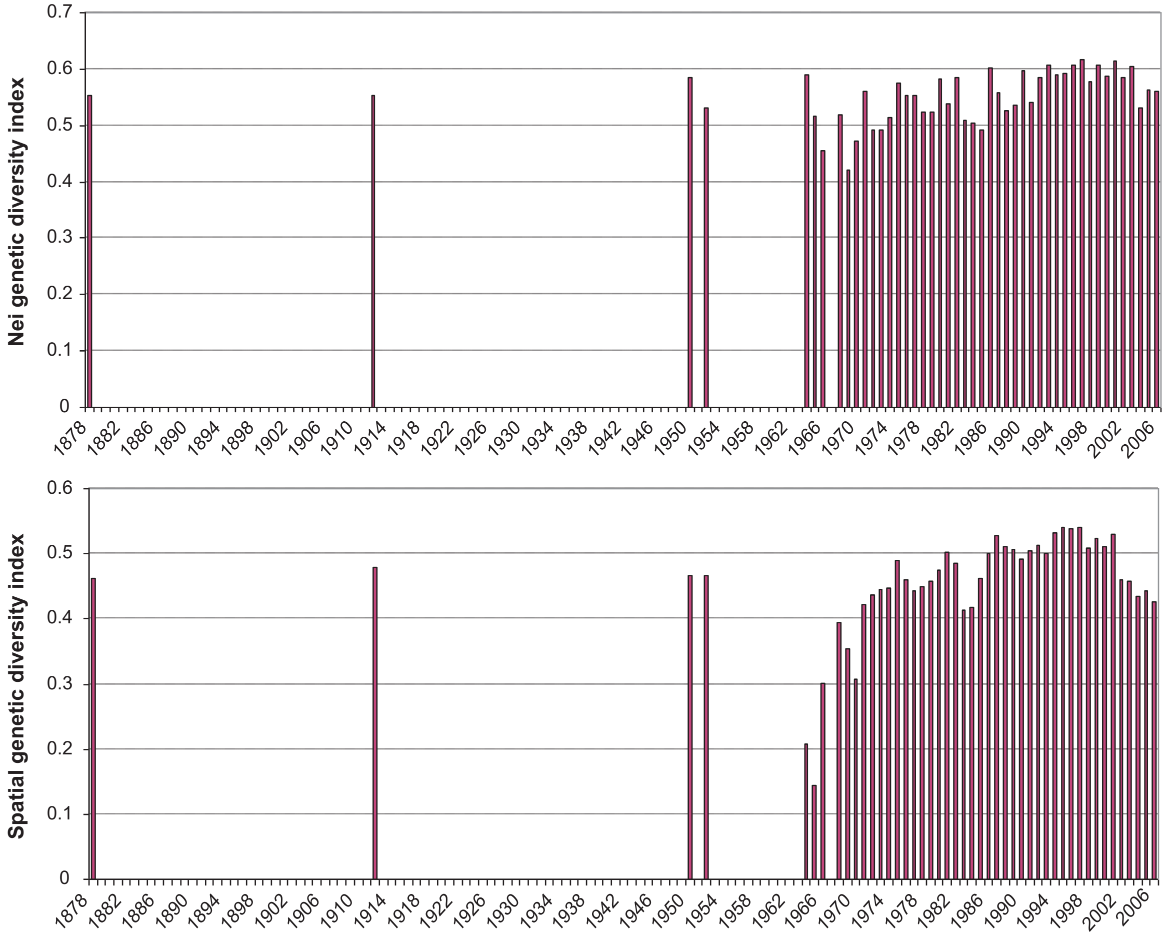

Fig. 2. (*Continued*).

does not alter the trends for H_T*. Yet, setting the G_{ST} coefficient of old lines below 0.8 significantly changed the trend of diversity evolution (high level of H_T* until the 50s and a fall afterwards). Thus, setting the old line G_{ST} coefficient to this high value (0.94) was very conservative and gave very little weight to genetic diversity within old lines.

For the *département* Eure-et-Loir, the acreage of wheat varieties has been obtained for 46 different years from 1878 to 2006. As shown in Fig. 2, the different indicators provide divergent pictures of wheat biodiversity temporal trends. While the number of varieties grown in the department and the spatial Simpson index drastically increased during the second half of the 20th century, Pielou's index, Nei's genetic diversity index and the spatial genetic diversity index, H*, stayed quite stable over time with some temporary drops (Fig. 2), and the new integrative H_T* index drastically decreased until the 1960s, then increased and finally decreased to the present time (Fig. 3). As expected, after 1964, there is almost no difference between H* and H_T* since almost exclusively modern varieties were grown during this period. Consequently, H_T* appeared most correlated with H* (0.69), while it was moderately correlated with the Nei, Simpson and Pielou indexes (resp. 0.35, 057, 0.38), and only slightly correlated with the number of varieties (0.19).

It is striking that the H_T* index displays almost opposite trends compared to the number of varieties index, suggesting that varietal richness *per se* contributes rather marginally to crop genetic diversity at landscape level. The integrative index, H_T*, dramatically decreased from 1.03 in 1878 to 0.49 in 1950 (Fig. 3), while none of the other indexes detected such an erosion (Fig. 2). This indicates that the extinction of landraces (Fig. 3) is the key factor for this decline. After 1950, H_T* fell again (below 0.2 in 1965). This trend is also detected by all the other indexes but Nei index, which is insensitive to spatial evenness. The lowest values observed in 1964–1965, can be thus attributed both to the limited varietal richness and to the dominance of a single modern variety in the landscape (80% of *Capelle Desprez* in 1964, Fig. 3). This may be also related to the Green Revolution period: after World War II, only a few old French varieties were used in breeding schemes to be crossed with a limited number of new lines introduced from Asian germplasm (Roussel et al., 2004). This produced a bottleneck effect which is detected by all the studied indexes. From the mid 1960s to the mid 1980s, H_T* increased up to its maximal value in the second half of the century (0.54 in 1995 which remains 14% below the 1912 value and 50% below the 1878 value). It is noteworthy that from 1965 to 1971, while the number of varieties is still very low, the Simpson, Piélou, H* and H_T* indices rose again (above 0.4 in 1971 for H_T*) as a result of a more balanced spatial distribution of these few varieties.

From the late 1980s to 2006, H_T* showed a downward trend down to a value of 0.42 in 2006 (Fig. 3). This trend is also partially confirmed by the Nei, Pielou and Simpson indexes, while the number of variety remained high (Fig. 2). This suggests that less dominant varieties (here, the five top varieties covered 74.1% of the wheat area in 1995 and 59.8% in 2006) may result in a lower genetic diversity if a larger set of the most frequent varieties are genetically more related.

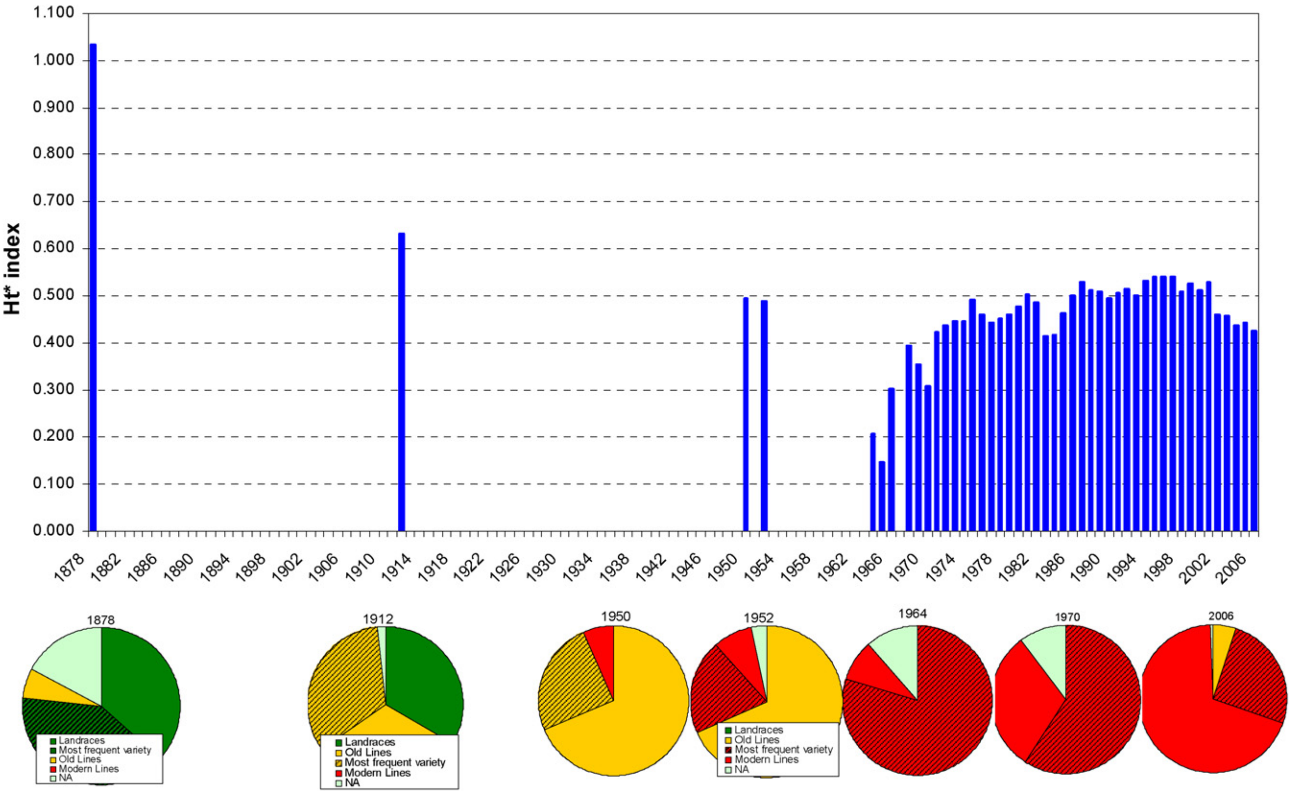

Fig. 3. Evolution of the new integrative indicator ($H_T{}^*$) of wheat diversity from 1878 to 2006 in the *department* Eure-et-Loir and shares of the three types of varieties (landraces, old lines and modern lines) and of the most frequent variety, for some years.

6. Discussion: the added value of the new indicator

In this study, we developed a new integrative indicator to assess crop biodiversity and we compared it to existing ones with a comprehensive historical dataset. The benchmarking study followed the behavior of a range of indicators to illustrate the multifaceted character of crop diversity. It appears that more varieties (the varietal richness factor) can mean less diversity when (i) their genetic structure is more similar (the effect of between-variety genetic diversity), or (ii) when more diverse landraces are replaced by many homogeneous lines (the effect of within-variety genetic diversity) or (iii) when one or a few varieties become dominant in the landscape (the spatial evenness effect). Furthermore, increased evenness in variety distribution (varietal evenness), for instance when top varieties represent a smaller proportion of the total cultivated area, can also mean less diversity when varieties are genetically more closely related.

This is why indicators which do not take into account this full range of factors (varietal richness, spatial evenness, between-variety genetic diversity and within-variety genetic diversity) may provide a misleading assessment of the state and trend of crop biodiversity. New integrative indicators such as $H_T{}^*$ are critically needed.

Integrative indicators such as $H_T{}^*$ can be used to help decision-makers to better monitor and manage crop diversity in agricultural landscapes. As several policy instruments emerge to prevent the erosion of crop biodiversity and its associated ecosystem services (such as biodiversity parks for maize and potato centers of origin in Central and South America, origin labelled product certification in many countries, the new EU directive on “conservation varieties” and Agro-Environmental Measure 214-G to save crop diversity in Europe, *etc.*), it is crucial to use relevant indicators to monitor and improve the efficiency of these policies on the ground. Compared to the commonly used crop diversity indicators (Nei’s index, the number of varieties), this integrative indicator has two main advantages. First, it takes into account the importance of the evenness component (and better than measurements of the acreage share of the top varieties as in OECD, 2001). Secondly, it does not neglect the within-variety diversity component, which allows the analysis of crop diversity trends over a longer time scale, and provides information to guide emerging policies to increase *in situ* crop diversity and its associated services (resilience to climate change and pest outbreaks), through the growing of heterogeneous varieties (populations or variety mixtures).

This indicator therefore represents a critical addition to the set of biodiversity indicators already developed by the various international initiatives to assess global biodiversity (GBO, CBD, OECD, SEBI, etc.). In a next step, it will be applied to all departments where wheat has been significantly grown in the last century in order to assess in more detail the trend in wheat biodiversity at the whole-country level. Moreover, $H_T{}^*$ can be used to assess crop biodiversity for various species at local, regional, national and global scale. While some countries already collect the relevant data (molecular data, spatial distribution data) on a regular basis for some crops, it might be necessary to optimize the monitoring and data collection for other crops and/or other countries. Although $H_T{}^*$ proposed here deals with within species diversity, a next step could be to design a global agricultural biodiversity indicator by associating the infraspecific indicators for all crop species and an inter-specific component. This indicator would provide the most comprehensive picture of crop diversity in agricultural landscape to date.

Ideally, a fuller account of agrobiodiversity would cover the complete diversity of agroecosystems, which also includes elements such as weeds, feral plants, hedges, fauna and soil biodiversity. The increasing knowledge of the positive effect of crop genetic diversity at the field scale on biodiversity as a whole (Chateil et al., submitted for publication), and on functional biodiversity assessed through ecological services (Hajjar et al., 2008), reinforces the interest of such a crop genetic diversity indicator.

The proposed new integrative indicators can also be used as output magnitude in metapopulation genetic simulations designed to compare various models of *ex situ* and *in situ* conservation and promote the most effective schemes.

7. Conclusion

In this study, we showed that integrative biodiversity indicators such as H_T^*, that take into account the full range of factors (varietal richness, spatial evenness, between-variety genetic diversity and within-variety genetic diversity) are critically needed for the assessment of the state and trend of crop biodiversity. Because it takes into account the importance of the evenness component and does not neglect the within-variety diversity component, it seems of particular interest to serve policy instruments that are currently introduced to prevent the erosion of crop biodiversity and its associated ecosystem services. In a first step, it needs to be applied at a larger geographical scale to document and compare wheat diversity state and trends and possibly detect (social, economical, political, agronomical, *etc.*) factors responsible for a loss or an increase in diversity. For a more comprehensive picture of agricultural biodiversity, it could be generalized to other crops and included in a global agrobiodiversity indicator where both crop and wild biodiversity would be taken into account.

The use of such indicators at local, regional, national and global level would foster more effective policies to enhance crop diversity as a key “ecological infrastructure”, helping maintain seeds and cropping systems' resilience to pests and climate change.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2012.04.002.

References

Alvarez, N., Garine, E., Khasah, C., Dounias, E., Hossaert-McKey, M., McKey, D., 2005. Farmers' practices, metapopulation dynamics, and conservation of agricultural biodiversity on-farm: a case study of sorghum among the Duupa in sub-sahelian Cameroon. Biol. Conserv. 121, 533–543.

Barcaccia, G., Molinari, L., Porfiri, O., Veronesi, F., 2002. Molecular characterization of emmer Italian landraces. Gen. Res. Crop Evol. 49, 415–426.

Berthier, H., 1878. Etude agricole sur le département de l'Eure-et-Loir. Colombon & Brulé, Paris.

Bonneuil, C., Thomas, F., 2009. Gènes, Pouvoirs et Profits. Quae-FPH, Paris.

Bonneuil, C., Thomas, F., 2010. Purifying landscapes: the Vichy regime and the genetic modernization of France. Hist. Stud. Nat. Sci. 40 (4), 532–568.

Brennan, J.P., Bialowas, A., 2001. Changes in characteristics of NSW wheat varieties 1965–1997. Economic Research Report No. 8, NSW Agriculture, Wagga Wagga.

Brétignière, L., 1912. Essai sur la répartition des variétés de blés cultivés en France. Renouard, Paris.

CBD, 2004. Indicators for assessing progress towards the 2010 target: trends in genetic diversity of domesticated animals, cultivated plants, and fish species of major socio-economic importance. http://www.cbd.int/doc/meetings/sbstta/sbstta-10/information/sbstta-10-inf-14-en.pdf.

Christiansen, M.J., Andersen, S.B., Ortiz, R., 2002. Diversity changes in an intensively bred wheat germplasm during the 20(th) century. Mol. Breed. 9, 1–11.

Chateil, C., Goldringer I., Tarallo, L., Kerbiriou, C., Le Viol, I., Ponge, J-F, Salmon, S., Gachet S.,Porcher E., Crop genetic diversity benefits farmland biodiversity in cultivated fields. Submitted for publication.

Donini, P., Law, J.R., Koebner, R.M.D., Reeves, J.C., Cooke, R.J., 2000. Temporal trends in the diversity of UK wheat. Theor. Appl. Genet. 100, 912–917.

Dreisigacker, S., Zhang, P., Warburton, M.L., Skovmand, B., Hoisington, D., Melchinger, A.E., 2005. Genetic diversity among CIMMYT wheat landraces accessions investigated with SSRs and implications for plant genetic resources management. Crop Sci. 45, 653–661.

EEA (European Environment Agency), 2007. Halting the loss of biodiversity by 2010: proposal for a first set of indicators to monitor progress in Europe. European Environment Agency. Technical report 11, Copenhagen. 186 pp.

Enjalbert, J., David, J.L., 2000. Inferring recent outcrossing rates using multilocus individual heterozygosity: application to evolving wheat populations. Genetics 156 (4), 1973–1982.

FAO, 2010. The Second Report on the State of the World's Plant Genetic Resources for Food and Agriculture. FAO, Rome.

Finckh, M.R., 2008. Integration of breeding and technology into diversification strategies for disease control in modern agriculture. Eur. J. Plant. Pathol. 121, 399–409.

Folke, C., Carpenter, S., Walker, B., Scheffer, M., Elmqvist, T., Gunderson, L., Holling, C., 2004. Regime shifts, resilience, and biodiversity in ecosystem management. Annu. Rev. Ecol. Evol. Syst. 35, 557–581.

Frison, E.A, Cherfas, J., Hodgkin, T., 2011. Agricultural biodiversity is essential for a sustainable improvement in food and nutrition security. Sustainability 3, 238–253.

Garola, C.V., 1894. Les céréales. Firmin-Didot, Paris (with revised editions in 1905, 1909, 1920 and 1925).

Goffaux, R., Goldringer, I., Bonneuil, C., Montalent, P., Bonnin, I., 2011. Quels indicateurs pour suivre la diversité des plantes cultivées? Le cas du blé tendre cultivé en France depuis un siècle. Rapport FRB, Série Expertise et synthèse, 2011, 44 p.

Guyomarc'h, H., Sourdille, P., Charmet, G., Edwards, K.J., Bernard, M., 2003. Characterisation of polymorphic microsatellite markers from Aegilops tauschii and transferability to the D-genome of bread wheat. Theor. Appl. Genet. 104, 1164–1172.

Hajjar, R., Jarvis, D.I., Gemmill-Herren, B., 2008. The utility of crop genetic diversity in maintaining ecosystem services. Agric. Ecosyst. Environ. 123, 261–270.

Hammer, K., Laghetti, G., 2005. Genetic erosion – examples from Italy. Genet. Res. Crops Evol. 52, 629–634.

Harlan, J.R., 1975. Our vanishing genetic resources. Science 188, 618–621.

Heal, G., Walker, B., Levin, S., Arrow, K., Dasgupta, P., Daily, G., Ehrlich, P., et al., 2004. Genetic diversity and interdependent crop choices in agriculture. Resour. Energy Econ. 26, 175–184.

Huang, X.Q., Wolf, M., Ganal, M.W., Orford, S., Koebner, R.M.D., Roder, M.S., 2007. Did modern plant breeding lead to genetic erosion in European winter wheat varieties? Crop Sci. 47, 343–349.

Jarvis, D.I., Brown, A.H.D., Cuong, P.H., et al., 2008. A global perspective of the richness and evenness of traditional crop-variety diversity maintained by farming communities. PNAS 105, 5326–5331.

Le Clerc, V., Cadot, V., Canadas, M., Lallemand, J., Guèrin, D., Boulineau, F., 2006. Indicators to assess temporal genetic diversity in the French Catalogue: no losses for maize and peas. Theor. Appl. Genet. 113 (7), 1197–1209.

Le Clerc, V., Bazante, F., Baril, C., Guiard, J., Zhang, D., 2005. Assessing temporal changes in genetic diversity of maize varieties using microsatellite markers. Theor. Appl. Genet. 110, 294–302.

Leino, M.W., Hagenblad, J., 2010. Nineteenth century seeds reveal the population genetics of landrace barley (*Hordeum vulgare*). Mol. Biol. Evol. 27, 964–973.

Mace, G.M., Baillie, J.E.M., 2007. The 2010 biodiversity indicators: challenges for science and policy. Conserv. Biol. 21 (6), 1406–1413.

Martynov, S.P., Dobrotvorskaya, T.V., Pukhalskiy, V.A., 2006. Dynamics of genetic diversity in winter common wheat *Triticum aestivum* L. cultivars released in Russia from 1929 to 2005. Russ. J. Genet. 42, 1137–1147.

Meul, M., Nevens, F., Reheul, D., 2005. Genetic diversity of agricultural crops in Flanders over the last five decades. Agron. Sustain. Dev. 25, 491–495.

Ministère de l'agriculture, 1959. Monographies agricoles départementales: 28 Eure-et-Loir. Documentation Française, Paris.

Mondini, L., Farina, A., Porceddu, E., et al., 2010. Analysis of durum wheat germplasm adapted to different climatic conditions. Ann. Appl. Biol. 156, 211–219.

Negri, V., Tiranti, B., 2010. Effectiveness of in situ and ex situ conservation of crop diversity. What a *Phaseolus vulgaris* L. landrace case study can tell us. Genetica 138, 985–998.

Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70, 3321–3323.

OECD, 2001. Environmental Indicators for Agriculture, vol. 3. OECD, Paris.

Ortiz, R., Lund, B., Andersen, S.B., 2003. Breeding gains and changes in morphotype of Nordic spring wheat (1901–1993) under contrasting environments. Genet. Res. Crops Evol. 50, 455–459.

Østergård, H., Finckh, M.R., Fontaine, L., Goldringer, I., Hoad, S.P., Kristensen, K., Lammerts Van Bueren, E.T., Mascher, F., Munk, L., Wolfe, M.S., 2009. Time for a shift in crop production: embracing complexity through diversity at all levels. J. Sci. Food Agric. 89, 1439–1445.

Pielou, E.C., 1966. Measurement of diversity in different types of biological collections. J. Theor. Biol. 13, 131–144.

Plaschke, J., Ganal, M.W., Roder, M.S., 1995. Detection of genetic diversity in closely-related bread wheat using microsatellite markers. Theor. Appl. Genet. 91, 1001–1007.

Raman, H., Stodart, B.J., Cavanagh, C., et al., 2010. Molecular diversity and genetic structure of modern and traditional landrace cultivars of wheat (*Triticum aestivum* L.). Crop Pasture Sci. 61, 222–229.

Reif, J.C., Zhang, P., Dreisigacker, S., Warburton, M.L., van Ginkel, M., Hoisington, D., Bohn, M., Melchinger, A.E., 2005. Wheat genetic diversity trends during domestication and breeding. Theor. Appl. Genet. 110, 859–864.

Röder, M.S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.H., Leroy, P., Ganal, M.W., 1998. A microsatellite map of wheat. Genetics 149, 2007–2023.

Roussel, V., Koening, J., Beckert, M., Balfourier, F., 2004. Molecular diversity in French bread wheat accessions related to temporal trends and breeding programmes. Theor. Appl. Genet. 108, 920–930.

Roussel, V., Leisova, L., Exbrayat, F., Stehno, Z., et Balfourier, F., 2005. SSR allelic diversity changes in 480 European bread wheat varieties released from 1840 to 2000. Theor. Appl. Genet. 111, 162–170.

Simpson, E.H., 1949. Measurement of diversity. Nature 163 (4148), 688.

Thomas, M., Demeulenaere, E., Dawson, J.C., Khan, A.R., Galic, N., Jouanne-Pin, S., Remoue, C., Bonneuil, C., Goldringer., I., 2012. On-farm dynamic management of genetic diversity: the impact of seed diffusions and seed saving practices on a population-variety of bread wheat. Evol. Appl., in press.

Waines, J.G., Hegde, S.G., 2003. Intraspecific gene flow in bread wheat as affected by reproductive biology and pollination ecology of wheat flowers. Crop Sci. 43, 451–463.

Wolfe, M.S., Baresel, J.P., Desclaux, D., Goldringer, I., Hoad, S., Kovacs, G., Löschenberger, F., Miedaner, T., Østergård, H., Lammerts Van Bueren, E.T., 2008. Developments in breeding cereals for organic agriculture in Europe. Euphytica 163, 323–346.

van de Wouw, M., van Hintum, T., Kik, C., van Treuren, R., Visser, B., 2010. Genetic diversity trends in twentieth century crop cultivars: a meta analysis. Theor. Appl. Genet. 120, 1241–1252.

Zhang, P., Dreisigacker, S., Buerkert, A., Alkhanjari, S., Melchinger, A.E., Warburton, M.L., 2006. Genetic diversity and relationships of wheat landraces from Oman investigated with SSR markers. Gen. Resour. Crop Evol. 53, 1351–1360.