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Genetic erosion over time of rice landrace agrobiodiversity

Brian V. Ford-Lloyd[1]*, Darshan Brar[2], Gurdev S. Khush[3], Michael T. Jackson[2] and Parminder S. Virk[2]

[1]*School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK,*
[2]*International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines and*
[3]*University of California, 39399 Blackhawk Place, Davis, CA 95616-7008, USA*

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Abstract

Changes in global biodiversity at the genetic level have proved difficult to determine for most organisms because of lack of standardized, repeated or historical data; this hampers the attempts to meet the convention on biological diversity (CBD) 2010 targets of reducing loss of genetic diversity, particularly of crop species. For rice, where germplasm and genetic data have been collected throughout South and Southeast Asia over many decades, contrary to popular opinion, we have been unable to detect a significant reduction of available genetic diversity in our study material. This absence of a decline may be viewed positively; over the 33-year timescale of our study, genetic diversity amongst landraces grown in traditional agricultural systems was still sufficiently abundant to be collected for *ex situ* conservation. However, if significant genetic erosion does take place in the future as a result of accelerating global warming and/or major changes in land use or agricultural practices, will it be catastrophic or gradual, and how will it be detected? We have shown a strong link between numbers of landraces collected (and therefore extant) and genetic diversity; hence, we have a clear indicator to detect loss of genetic diversity in the future. Our findings lend considerable support for *ex situ* conservation of germplasm; the more than substantial genetic resources already in genebanks are now safe. On the other hand, it is the germplasm growing in farmers' fields, continually adapting genetically to changing environmental conditions and evolving novel genetic forms, whose future has been much less certain but can now be effectively monitored using our criteria.

Keywords: CBD; conservation; genetic erosion; genetic resources; landraces; rice

Introduction

World governments, signatories to the targets set at the Johannesburg World Summit on Sustainable Development in 2002, left themselves and conservationists 8 years to undertake quite a significant task. This was to '… achieve by 2010 a significant reduction of the current rate of biodiversity loss at the global, regional and national level …' (UNEP, 2004; Balmford *et al.*, 2005). Included in the specified and so-called indicators for the 'components of biological diversity' is 'Trends in genetic diversity of domesticated plants and animals', where landraces will actually form the major component of domesticated plants. This proves to be highly problematic for a number of reasons. Among these is that, while the preservation of traditional crop varieties has progressed steadily over the last 40 or so years, guided by major international organizations such as Bioversity International (formerly IPGRI International Plant Genetic Resources Institute) and Food and Agriculture Organisation (FAO) that have attempted to estimate the numbers of samples in conservation (FAO, 1998), there have been few attempts to determine the extent to

* Corresponding author. E-mail: b.ford-lloyd@bham.ac.uk

which 'genetic diversity' *per se* has been captured and its loss reduced, in part because suitable data for analysis do not exist (Watson-Jones *et al.*, 2006; Keisa *et al.*, 2008). The numbers of samples under conservation in genebanks or landraces being maintained on-farm are often equated with the amount of genetic diversity, backed with little or no scientific evidence. Such proxy indicators of genetic erosion are rarely tested for their reliability because of the lack of suitable datasets. Identically named samples can sometimes be shown to be genetically different, while those given different names can be genetically the same (Appa Rao *et al.*, 2002). Possibly, the major problem is that, in order to effectively estimate the trends in genetic diversity, i.e. its loss, with a view to taking avoiding action, baseline data collected at a time point in the past are needed to compare with present-day values (Wilson *et al.*, 2004; Green *et al.*, 2005; Donald *et al.*, 2007). The problem is further exacerbated in that the agro-biodiversity component of biological diversity attracts little interest amongst those scientists and activists primarily involved with natural ecosystems. Indeed, even in a recent review of the CBD 2010 targets there was no mention of the genetic diversity of domesticated animals or plants (Balmford *et al.*, 2005), even though the 'proportion of products derived from sustainable use' (namely landraces) is quite specifically referred to in the CBD framework.

Since its foundation in 1960, the International Rice Research Institute (IRRI) has been working with national programme partners in Asia to preserve rice germplasm. Over 100,000 samples of rice, the majority being landraces, are now maintained for long-term storage in the International Rice Genebank. Rice breeding at IRRI has depended upon this diverse germplasm to supply its breeding programmes with sources of useful genes and, as part of the process, molecular genetic evaluation has been routinely applied to material collected since 1961. These data have not been specifically acquired for use in monitoring genetic erosion; the data have been generated to assist in breeding rice, and indeed the collection itself was developed firstly as a breeding resource, and secondly for the purposes of conservation. There are constraints on the analysis imposed by the structure of these data; nevertheless, we have undertaken analyses of what in reality is probably the largest dataset of its kind, to throw light on the question of genetic erosion in a major crop species. We have used allozyme data for 12 gene loci in up to 12,972 samples of landraces of rice collected from South and Southeast Asian countries over several decades.

Materials and methods

Data for 12 allozyme loci, *adh*1 (11), *amp*1 (2), *amp*2 (8), *amp*3 (6), *amp*4 (8), *est*1 (7), *est*2 (6), *est*5 (1), *est*9 (7), *pgi*1 (3), *pgi*2 (6) and *sdh*1 (12) (Glaszmann *et al.*, 1988; Khush *et al.*, 2003), were available for 12,972 accessions of mainly landraces from Bangladesh, Cambodia, India, Indonesia, Lao PDR, Philippines, Taiwan, Thailand and Vietnam (Supplementary Table S1, available online only at http://journals.cambridge.org). These allozyme loci covered eight of the rice chromosomes (Wu *et al.*, 1988; Cai *et al.*, 2004).

Individual landrace accessions grouped according to the date of collection over a 33-year period (1962–1995: Supplementary Table S1, available online only at http://journals.cambridge.org) were designated only for convenience as 'populations' and are referred to as such below. For each year of collection, there were between 5 and 1027 accessions. Information on actual year of collection was available only for 5641 accessions, all of which were used for analysis of 22 populations of variable sizes (as above); other analyses were based on sequential samples of 50 (Table 1) or 100 accessions (Table 2), taken for those years where there were actually 50 (or more; 17 populations) or 100 (or more; 15 populations) accessions available, respectively. Thirty populations of variable size (30–1130) organized according to the year of acquisition (rather than year of collection above) were also analysed where the assumption was that this would approximate the year of collection (Table 4).

Estimates per population were made of Nei's diversity (expected heterozygosity; Nei, 1978) and average number of alleles per locus (Lewis and Zaykin, 2001), and linear regression was performed of these variables against the fixed variables of the year of collection and population size (where population size varied).

Table 1. Diversity measured as richness (*A*) and evenness (*He*) in 'populations' of size $n = 50$ composed of accessions collected in different years

Population	*A*	He
1962	1.83	0.10
1967	2.25	0.32
1973	2.17	0.25
1974	2.33	0.28
1976	2.42	0.30
1977	2.17	0.24
1978	2.25	0.24
1979	2.42	0.33
1980	2.00	0.31
1982	1.75	0.21
1983	2.17	0.25
1984	2.08	0.21
1985	2.17	0.18
1986	2.00	0.30
1988	2.42	0.36
1989	1.83	0.16
1995	2.42	0.28

Table 2. Diversity measured as richness (*A*) and evenness (*He*) in 'populations' of size $n = 100$ composed of accessions collected in different years

Population	*A*	He
1962	2.00	0.15
1967	2.75	0.38
1973	2.33	0.23
1974	2.50	0.27
1977	2.25	0.24
1978	2.67	0.32
1980	2.08	0.29
1982	2.00	0.23
1983	2.25	0.24
1984	2.17	0.19
1985	2.00	0.15
1986	2.08	0.36
1988	2.67	0.37
1989	2.25	0.21
1995	2.50	0.23

In some cases, analysis of molecular variance was used to compare variances among populations (Supplementary Table S2, available online only at http://journals.cambridge.org).

Table 3. Diversity measured as richness (*A*) and evenness (*He*) in 'populations' of unequal sizes composed of accessions collected in different years

Population	*n*	*A*	He
1962	132	2.17	0.16
1967	126	2.83	0.37
1969	130	1.00	0.00
1972	11	1.83	0.34
1973	660	3.17	0.24
1974	121	2.58	0.27
1975	5	1.17	0.07
1976	50	2.42	0.30
1977	105	2.33	0.25
1978	56	2.25	0.24
1979	50	2.42	0.33
1980	327	2.33	0.30
1981	10	2.08	0.30
1982	518	2.33	0.22
1983	461	2.75	0.26
1984	486	2.42	0.20
1985	388	2.58	0.16
1986	483	2.25	0.35
1988	97	2.67	0.37
1989	360	3.00	0.20
1992	30	1.83	0.24
1995	1028	3.08	0.23

Results

The actual year of collection was known only for 5640 accessions. We therefore analysed 'populations' that were samples of landraces from individual years of collection where 'population' sizes (*n*) made up of randomly chosen accessions were set at either 50 or 100, and estimated the richness and evenness of genetic diversity by way of average number of alleles per locus (*A*) and expected heterozygosity (*He*), respectively (Tables 1 and 2). Regressions of both these estimates against the year of collection were found not to be significant. We also used this dataset, but taking individual years of collection to represent 22 'populations' of unequal sizes (*n* ranging from 5 to 1028) and again estimated *A* and *He* (Table 3), which were then regressed against the year of collection as well as *n*. Again, neither was significant against the year of collection ($P > 0.05$), but *A* was highly significant against *n* ($P = 0.006$).

Analysis of molecular variance was also used to compare variances among the so-called populations, allowing population differentiation over time to be assessed. When the variances of populations representing relatively short collecting time periods (e.g. 1 year) were compared, most, but not all, of the population pairwise comparisons were significant ($P < 0.05$) indicating population differentiation between samples collected in different years, but this applied equally to the pairs of populations that had been collected both close together in time (consecutively), and those that had been collected much further apart in time (Supplementary Table S2, available online only at http://journals.cambridge.org). This suggests that, while samples are clearly different genetically, other than in one case, this difference is not related to when the collections were made.

We also undertook analyses using the full 12,972 accession dataset, but because of the lack of availability of data for the actual year of collection, we used the year of acquisition by the International Rice Genebank as a proxy on the basis that this date must be the same as, or later than, the actual year of collection, but could not be earlier. As before, we used this dataset where individual years of collection represented 30 populations of unequal sizes (ranging from 30 to 1133) and estimated *A* and *He* (Table 4), which were then regressed against the year of collection and *n*, individually and in a multiple regression where year and *n* were the independent variables. Neither was significant against the year of collection ($P > 0.05$) in either analysis, but *A* was highly significant against *n* ($P = 0.000$) accounting for nearly half of the variance in *A* (adjusted R^2 of 0.437). Several other similar analyses were undertaken where 'populations' of equal sizes of accessions representing individual countries (Bangladesh and Cambodia) yielded non-significant results when the genetic diversity parameters were regressed against the year of collection.

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Table 4. Diversity measured as richness (*A*) and evenness (*He*) in 'populations' of unequal sizes composed of accessions that were entered into the genebank in different years

Population	*n*	*A*	He
1961	60	1.83	0.20
1962	511	3.25	0.31
1963	224	2.83	0.30
1966	53	2.58	0.32
1969	148	1.67	0.02
1970	328	3.00	0.31
1971	116	2.50	0.31
1972	342	2.42	0.33
1973	1133	3.50	0.33
1974	800	3.67	0.36
1975	333	3.17	0.32
1976	498	3.33	0.36
1977	574	3.42	0.29
1978	470	3.00	0.29
1979	807	3.08	0.27
1980	242	2.92	0.35
1981	371	2.67	0.27
1982	357	3.00	0.29
1983	981	2.92	0.27
1984	665	3.17	0.29
1985	551	2.75	0.23
1986	455	2.67	0.19
1987	607	2.50	0.34
1988	120	2.58	0.27
1989	179	2.83	0.35
1990	52	2.42	0.29
1991	525	2.92	0.32
1992	390	3.08	0.34
1993	30	1.83	0.24
1996	1028	3.08	0.23

Discussion

Human society, agriculture and earth's abundant plant resources have been co-evolving for more than 10,000 years (Esquinas-Alcazar, 2005). Complex interactions have resulted in the component of biodiversity known as agrobiodiversity manifested as locally adapted forms of crop plants or landraces (Camacho-Villa *et al.*, 2006). Selection by humans, either unconscious or deliberate, has resulted over the millennia in the varieties or landraces grown until relatively recently by many farmers throughout the world (Ford-Lloyd *et al.*, 2001). In the 1960s, however, it was recognized by certain scientists and FAO that these genetic resources were under the threat of extinction not least because of the sudden impact of the Green Revolution. Launched to provide a solution to the world's food problem, the Green Revolution exposed traditionally grown cereal landraces, particularly wheat and rice, to the threat of genetic erosion by way of their gradual replacement by a comparatively small number of modern high yielding varieties. To combat this threat, the scientific community launched a major campaign designed to conserve the threatened genetic diversity. From the 1960s to the present time, germplasm collecting has been undertaken on a large scale for the major world crops, with samples of germplasm being preserved *ex situ* in genebanks (Esquinas-Alcazar, 2005), while at the same time, on a much smaller scale, there have been some attempts to preserve landraces '*in situ*' on farms where traditional agriculture is still practiced (Stolton *et al.*, 2006). How effective has this conservation been, and to what extent does it address the CBD 2010 targets?

Analysis of this large, geographically and historically broad dataset has not revealed any consistent change or decline in the actual genetic diversity of traditional rice landraces in use by farmers over a 33-year time period, notwithstanding the release of the cultivars IR36 and IR64 for example, and their cultivation over millions of hectares, leading to zero diversity in those areas because of essentially monoculture. While being good news, this runs contrary to popular belief. Various scenarios can be presented to account for our results. Firstly, this clearly does not support an otherwise entirely plausible proposition that early on in the history of collecting rice, collectors only visited the locations where the greatest diversity was to be found, and subsequently visited areas that were less diverse. Another possibility is that collectors have been revisiting locations with high diversity rather than attempting to explore new areas. This would seem to be unlikely given the available collecting information and the geographic extent of collection, but if true, further indicates the absence of genetic erosion. Because the 'population' samples that we have used represent 'snapshots' of the rice being grown in farmers' fields, it may be possible to conclude from our results that genetic diversity in rice maintained *in situ* on-farm has in fact continued to survive throughout South and Southeast Asia for the 33-year time period covered by our study (although we have not been able to include in our study the many new landrace collections made since then by IRRI), although the actual area over which these are growing more recently compared to decades ago cannot be assessed.

There is another argument that could apply, and it is that there has been substantial overcollecting for *ex situ* conservation purposes over the study time period. It has been argued previously that even for predominantly inbreeding species such as rice, a relatively small sample of plants (as few as 172) randomly sampled would be sufficient to be sure of capturing within a species all alleles that occur at a frequency greater than 0.05 (Lawrence *et al.*, 1995). Ignoring the fact that many important genes for rice crop improvement have been discovered to occur at very low frequencies (Leung *et al.*, 2002), nevertheless, this is a frequency that is often assumed

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to represent a realistic figure to attain by way of germplasm collection for *ex situ* conservation purposes. The many thousands of accessions accumulated by IRRI that we have analysed will have achieved this several times over. Regression of population size against the year of collection shows a significant ($P = 0.034$) and positive association indicating that collecting activities have indeed increased over time; this could reflect the discovery of new sources of genetic diversity, but it could also reflect overcollecting.

What does this mean for meeting CBD 2010 targets? It is quite possible to argue that conservation has gone further than that which is needed, and that the germplasm resources of rice already stored in genebanks (particularly the International Rice Genebank at IRRI) ensure that future genetic erosion is effectively completely mitigated against, and not just reduced by the 70% required by the CBD. It also means that for the period over which these samples were taken, there has been no loss of genetic diversity in rice landraces detectable by way of this very large dataset, clearly a significant result for CBD purposes. This is consistent with the results from smaller studies on wheat (Donini *et al.*, 2000; Manifesto *et al.*, 2001; Khlestkina *et al.*, 2004) and on cultivated millet and sorghum (Bezancon *et al.*, 2008). It has often been argued that it is necessary to maintain genetic resources *in situ*, alongside conservation in genebanks, to allow for continued interaction of landrace genotypes with a changing environment, thus allowing for continued genetic adaptation on a local scale (Stolton *et al.*, 2006). This argument would seem to be important given the current concerns over climate change. Again, the genetic erosion over the timescale of our experiments has not been catastrophic; diversity has still been found to exist, but of course we do not know over what area of cultivation, nor across what agroecological conditions (given the lack of passport data).

This does not address the potential future loss of agrobiodiversity grown *in situ* in farmers' fields. The clear association between population size (the number of landrace accessions collected/acquired in any year) and genetic diversity richness validates for the first time the use of 'number of landraces' as an effective and simple proxy indicator that could readily be used to monitor the loss from farmers' fields of genetic diversity over time, provided that regular surveys were to be undertaken. Most landrace samples used in this study had unique names, aiding the process of surveying. Surveying however only provides information, and in itself does not prevent genetic or biodiversity loss, which can be achieved only by political action as and when it becomes necessary. In this study, we make no predictions about this possibility, but the precautionary principle would clearly dictate a need for the use of our simple proxy indicator for genetic erosion, and regular monitoring of landraces grown by farmers not just in Southeast Asia, but elsewhere in the world.

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