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COMPARISON OF ANONYMOUS AND GENIC MICROSATELLITES FOR VARIETY
DISCRIMINATION IN WHEAT

prepared by experts from the United Kingdom

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**Comparison of Anonymous and Genic
Microsatellites (SSRs) for Variety
Discrimination in Winter Wheat.**

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Comparison of EST SSR's with Anonymous SSR's

Microsatellites from two sources were used to assess the variation that exists between 66 varieties of winter wheat.

- A set of 12 microsatellites generated from genomic libraries were used. This set is referred to as the *anonymous*. These are mapped.
- The 20 EST-derived or 'genic'-microsatellites were of high quality, amplifying clear products with few stutter bands. Their chromosomal locations are known and they are believed to have mostly 'housekeeping gene' functions (*personal communication*).

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Comparison of EST SSR's with Anonymous SSR's



The 66 winter wheat varieties comprise a UK and non-UK material. The UK set consists of 56 NIAB Recommended varieties (from many breeders and countries of origin from the past 60 years).


Ten non-UK varieties, with no *a priori* shared breeding histories, from

- Japan
- India
- China
- Greece
- New Zealand.

form the "world" or 'W' set.

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Comparison of EST SSR's with Anonymous SSR's




Eujayl *et al* (2001) used a set of EST derived SSRs to assess the genotypic variation among durum wheat cultivars and compared this data with that generated using anonymous-SSRs.

This revealed that the EST-SSRs were less polymorphic than the anonymous-SSRs, but were still informative tools for assessing genetic relationships.

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Comparison of EST SSR's with Anonymous SSR's



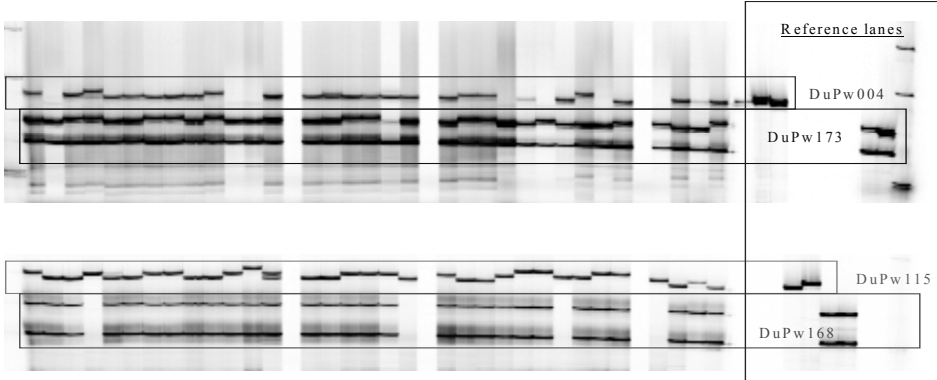
Details of the molecular techniques used in the SSR studies are available upon request from the authors.

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Comparison of EST SSR's with Anonymous SSR's



The profile obtained when 36 wheat varieties are amplified with 4 EST microsatellite primer pairs; DuP004, DuP173, DuPw115 and DuPw168



Reference lanes


DuPw004

DuPw173

DuPw115


DuPw168

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Comparison of EST SSR's with Anonymous SSR's


- The 20 microsatellites from expressed regions all produced scorable profiles when used to amplify the test DNAs. The number of alleles amplified by each microsatellite ranged from 1 to 7.
- A wide range of Polymorphic Information Content (PIC) values were observed in the set of EST microsatellites. A high PIC value indicates that a marker is more likely to distinguish between varieties.
- Three microsatellites showed no variation between this set of wheat varieties. The remaining 17 SSRs were polymorphic with an average PIC of 0.41.
- Two ESTs had PIC values of 0.759 and 0.747 respectively, indicating a high ability to distinguish these varieties of wheat.

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Comparison of EST SSR's with Anonymous SSR's

- The average PIC for the 17 polymorphic EST SSRs was 0.41 which is lower than the average PIC of 0.54 obtained for the set of 12 anonymous SSRs used to screen the same wheat material.
- Six of the seventeen polymorphic EST microsatellites amplified unique alleles that appeared only once in the test set of varieties. Rare alleles (i.e. those that appear with a frequency less than 0.05) are useful if they appear in a variety that will be studied in depth, e.g. for essential derivation (EDV) issues. In such cases, the presence of an allele that is particular to that variety allows easy detection of progeny.
- However, if a microsatellite amplifies the rare allele in only one variety and all other varieties are indistinguishable from each other, the microsatellite is of limited use for general discrimination. This will be reflected in the low PIC value.


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Comparison of EST SSR's with Anonymous SSR's

- Only the EST SSRs that were monomorphic for the whole set of varieties have been removed with little or no additional selection (in terms of PIC values etc.) applied.
- However, the winter wheat *anonymous* SSRs were heavily selected to be 'useful' (highly polymorphic) and so any direct comparison with EST SSRs will be biased in favour of the anonymous SSRs.
- In this set of varieties, the wheat EST SSRs have generally lower PIC values, lower levels of polymorphism and lower discrimination rate (-9%) compared to the *anonymous* SSRs. This result confirms Eujayl *et al* (2001)

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Comparison of EST SSR's with Anonymous SSR's

Individual SSR PIC Values

EST-SSR's	PIC Value	Anonymous SSR's	PIC Value
DuPw004	0.701	m3033	0.564
DuPw023	0.087	m3081	0.771
DuPw043	0.474	m3047	0.549
DuPw108	0.421	m3034	0.854
DuPw115	0.582	m3071	0.749
DuPw124	0.535	m3050	0.559
DuPw135	0.059	m3030	0.537
DuPw165	0.030	m3030	0.087
DuPw167	0.759	m3030	0.030
DuPw173	0.618	m3088	0.610
DuPw205	0.454	m3137	0.529
DuPw216	0.349	m3103	0.713
DuPw217	0.650	m3080	0.539
DuPw227	0.059	m3009	0.454
DuPw238	0.446		
DuPw254	0.088		
DuPw398	0.747		
Max	0.759	Max	0.854
Min	0.030	Min	0.030
Mean	0.415	Mean	0.539
Median	0.454	Median	0.554

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Comparison of EST SSR's with Anonymous SSR's



EST-SSR's	PIC Value	Anonymous-SSR's	PIC Value
Max	0.759	Max	0.854
Min	0.030	Min	0.030
Mean	0.415	Mean	0.539
Median	0.454	Median	0.554

Wheat PIC values from other published papers (excluding any monomorphic bands).


20 wheat lines; 49 primer pairs.
Average PIC 0.58; Range 0.18 - 0.84
Bryan et al (1997) TAG. 94; 557-563

55 wheat genotypes; 20 SSR's
Average PIC 0.76; Range 0.21 - 0.72
Prasad et al (2000) TAG. 100; 584-592

60 wheat cultivars; 42 SSR's (14 each genome)
Average PIC 0.66 (Genome a)
Average PIC 0.62 (Genome b)
Average PIC 0.61 (Genome d)
Stachel et al (2000) TAG. 100; 242-248

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Comparison of EST SSR's with Anonymous SSR's




The data were used to seek answers to two specific questions.

- Are there advantages from the use of EST SSRs in terms of establishing distinctness?
- Do the EST SSRs offer advantages in terms of establishment of robust 'minimum distance' estimators?

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Comparison of EST SSR's with Anonymous SSR's




Discrimination.

- Mirroring the distinctness test in DUS testing has been done for both UK+W (66 varieties) and UK only (56 varieties).
- Criteria to establish distinctness are based on the number of pattern differences exceeding the threshold for each variety compared to each other variety.
- The stringency of the criteria increases as the threshold requirement increases from 1 pattern difference to 5 differences.
- An approach based on 'genetic distance' is also being worked upon and will be presented at a further meeting.

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Comparison of EST SSR's with Anonymous SSR's




		Percentage Discrimination Rate Full 66 Variety Set			Difference (Anonymous-EST)
		UK(56)+W(10) (Anonymous+EST)	UK(56)+W(10) Anonymous	UK(56)+W(10) EST	
Number of SSR's		31	14	17	
Distinctness criteria in terms of the number alleles different	1	100.0	97.0	96.9	0.1
	2	100.0	90.9	78.1	12.8
	3	96.9	65.2	57.8	7.3
	4	87.5	39.4	29.7	9.7
	5	79.7	24.2	15.6	8.6

Individual use of anonymous or EST SSRs fails to give a 100% discrimination rate even at the 'weakest' distinctness criterion. There is not a complete 1:1 relationship between the set of non-distinct varieties using the two SSR sets (anonymous and EST).

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Comparison of EST SSR's with Anonymous SSR's



		Percentage Discrimination Rate UK Varieties Only			
		UK (56) (Anonymous+EST)	UK (56) Anonymous	UK (56) EST	Difference (Anonymous-EST)
Number of SSR's		31	14	17	
Distinctness criteria in terms of the number alleles different	1	100.0	96.4	96.3	0.1
	2	100.0	89.3	74.1	15.2
	3	96.3	58.9	51.9	7.1
	4	85.2	28.6	20.4	8.2
	5	75.9	14.3	3.7	10.6

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Comparison of EST SSR's with Anonymous SSR's




Minimum Distance.

The distance matrices are calculated using the City Block approach. Each row of these matrices relates to an individual varieties distance 'profile', as shown on the next slide. Such profiles can be compared pair-wise and correlations between the SSRs assessed.

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Comparison of EST SSR's with Anonymous SSR's



An Example of an Individual Variety Profile Distances


City Block														For Variety 66 Distance Minimum	For Variety 66 Distance Maximum		
	v Var1	v Var2	v Var3	v Var4	v Var5	v Var6	v Var7	v Var8	v Var9	v Var10	etc	v Var64	v Var65	v Var66			
Anonymous SSR's	Var 66	0.14	0.31	0.12	0.17	0.15	0.32	0.27	0.30	0.22	0.21	...	0.11	0.31	N/A	0.057	0.354
EST SSR's	Var 66	0.16	0.15	0.24	0.18	0.08	0.20	0.19	0.17	0.13	0.20	...	0.08	0.19	N/A	0.058	0.355

Distance estimate for Variety 66 v Variety 5 (by City Block method) for anonymous SSRs and EST SSRs

For Anonymous SSRs Distance = 0.15
For EST SSRs Distance = 0.08

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Comparison of EST SSR's with Anonymous SSR's



An Example of an Individual Variety Profile Distances

City Block														For Variety 66 Distance Minimum	For Variety 66 Distance Maximum		
	v Var1	v Var2	v Var3	v Var4	v Var5	v Var6	v Var7	v Var8	v Var9	v Var10	etc	v Var64	v Var65	v Var66			
Anonymous SSR's	Var 66	0.14	0.31	0.12	0.17	0.15	0.32	0.27	0.30	0.22	0.21	...	0.11	0.31	N/A	0.057	0.354
EST SSR's	Var 66	0.16	0.15	0.24	0.18	0.08	0.20	0.19	0.17	0.13	0.20	...	0.08	0.19	N/A	0.058	0.355

Minimum distance(MD) estimate for Variety 66 compared to all other varieties taken as rows out of the respective full pair-wise distance matrices.

For anonymous SSRs 0.057
For EST SSRs 0.058

For this example variety, chosen at random, the minimum distance estimates from the anonymous and EST SSRs are the same.

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SSR Minimum Distances for UK(56) + W(10) Varieties

Variety	ID	UK+W		UK+W
		Anonymous	EST	Correlations of SSR's [§]
		Min	Min	Variety Profile Pairwise
UKVar	2	0.06	0.01	0.56
UKVar	5	0.06	0.08	0.56
UKVar	3	0.06	0.01	0.55
UKVar	1	0.07	0.03	0.53
UKVar	8	0.06	0.01	0.52
UKVar	6	0.06	0.02	0.48
UKVar	19	0.05	0.00	0.46
UKVar	4	0.06	0.05	0.45
UKVar	27	0.03	0.03	0.44
UKVar	9	0.06	0.06	0.43
UKVar	13	0.05	0.03	0.42
UKVar	16	0.05	0.00	0.42
UKVar	10	0.06	0.05	0.38
UKVar	7	0.06	0.07	0.37
UKVar	17	0.03	0.03	0.36
UKVar	14	0.06	0.04	0.36
UKVar	26	0.07	0.02	0.35
UKVar	11	0.06	0.03	0.33
UKVar	18	0.02	0.03	0.31
UKVar	29	etc	etc	etc
UKVar	30	0.13	0.07	0.21
UKVar	31	0.07	0.04	0.14
UKVar	6	0.10	0.09	0.14
UKVar	34	0.04	0.08	0.12
UKVar	56	0.13	0.12	0.04
UKVar	35	0.05	0.13	-0.07
YU-18	W	0.06	0.06	0.43
Xian-8	W	0.11	0.08	0.37
Kopara	W	0.15	0.20	0.21
HD2188	W	0.15	0.10	0.11
Xios	W	0.12	0.10	0.04
Takahe	W	0.12	0.15	0.01
Mykonos	W	0.12	0.11	-0.01
Chinese_Spr	W	0.29	0.11	-0.17
Norin_6	W	0.19	0.17	-0.25
CS91	W	0.24	0.22	-0.29
Minimum		0.00	0.00	
Maximum		0.29	0.24	
Median		0.07	0.04	

§ Between profiles of anonymous and EST SSR's

Comparison of EST SSR's with Anonymous SSR's

Minimum distance estimates ranked by profile correlations between anonymous set and corresponding variety profile based on EST SSRs



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SSR Minimum Distances for UK(56) + W(10) Varieties


Variety	ID	UK+W		UK+W
		Anonymous	EST	Correlations of SSR's [§]
		Min	Min	Variety Profile Pairwise
UKVar	2	0.06	0.01	0.56
UKVar	5	0.06	0.08	0.56
UKVar	3	0.06	0.01	0.55
UKVar	1	0.07	0.03	0.53
UKVar	8	0.06	0.01	0.52
UKVar	6	0.06	0.02	0.48
UKVar	19	0.05	0.00	0.46
UKVar	4	0.06	0.05	0.45
UKVar	27	0.03	0.03	0.44
UKVar	9	0.06	0.06	0.43
UKVar	13	0.05	0.03	0.42
UKVar	16	0.05	0.00	0.42
UKVar	10	0.06	0.05	0.38
UKVar	7	0.06	0.07	0.37
UKVar	17	0.03	0.03	0.36
UKVar	14	0.06	0.04	0.36
UKVar	26	0.07	0.02	0.35
UKVar	11	0.06	0.03	0.33
UKVar	18	0.02	0.03	0.31
UKVar	29	etc	etc	etc
UKVar	30	0.13	0.07	0.21
UKVar	31	0.07	0.04	0.14
UKVar	6	0.10	0.09	0.14
UKVar	34	0.04	0.08	0.12
UKVar	56	0.13	0.12	0.04
UKVar	35	0.05	0.13	-0.07
YU-18	W	0.06	0.06	0.43
Xian-8	W	0.11	0.08	0.37
Kopara	W	0.15	0.20	0.21
HD2188	W	0.15	0.10	0.11
Xios	W	0.12	0.10	0.04
Takahe	W	0.12	0.15	0.01
Mykonos	W	0.12	0.11	-0.01
Chinese_Spr	W	0.29	0.11	-0.17
Norin_6	W	0.19	0.17	-0.25
CS91	W	0.24	0.22	-0.29
Minimum		0.00	0.00	
Maximum		0.29	0.24	
Median		0.07	0.04	

§ Between profiles of anonymous and EST SSR's

Comparison of EST SSR's with Anonymous SSR's

Modest profile correlations indicating a measure of agreement between distances generated from anonymous SSRs and the EST SSRs

Weak or negative correlations between corresponding SSRs




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SSR Minimum Distances for UK(56) + W(10) Varieties				
Variety	ID	UK+W		Correlations of SSR's ² Variety Profile Pairwise
		Anonymous	EST	
UKVar	2	0.06	0.01	0.56
UKVar	5	0.06	0.08	0.56
UKVar	3	0.06	0.01	0.55
UKVar	1	0.07	0.03	0.53
UKVar	8	0.06	0.01	0.52
UKVar	6	0.06	0.02	0.48
UKVar	19	0.05	0.00	0.46
UKVar	4	0.06	0.05	0.45
UKVar	27	0.03	0.03	0.44
UKVar	9	0.06	0.08	0.43
UKVar	13	0.05	0.03	0.42
UKVar	16	0.05	0.00	0.42
UKVar	10	0.06	0.05	0.38
UKVar	7	0.06	0.07	0.37
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UKVar	14	0.06	0.04	0.36
UKVar	26	0.07	0.02	0.35
UKVar	11	0.06	0.03	0.33
UKVar	18	0.02	0.03	0.31
UKVar	29	etc	etc	etc
UKVar	30	0.13	0.07	0.21
UKVar	31	0.07	0.04	0.14
UKVar	6	0.10	0.09	0.14
UKVar	34	0.04	0.08	0.12
UKVar	56	0.13	0.12	0.04
UKVar	35	0.05	0.13	-0.07
YU-18	W	0.06	0.06	0.43
Xian-8	W	0.11	0.08	0.37
Kopara	W	0.15	0.20	0.21
HD2189	W	0.15	0.10	0.11
Xios	W	0.12	0.10	0.04
Takahe	W	0.12	0.15	0.01
Myokonos	W	0.12	0.11	-0.01
Chinese_Spr	W	0.29	0.11	-0.17
Norin_6	W	0.19	0.17	-0.25
CS91	W	0.24	0.22	-0.29
Minimum		0.00	0.00	
Maximum		0.29	0.24	
Median		0.07	0.04	

Comparison of EST SSR's with Anonymous SSR's

Minimum of all minimum distances is 0.00 for both SSR sets. (Equivalent to a maximum similarity coefficient of 1.0)



§ Between profiles of anonymous and EST SSR's

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SSR Minimum Distances for UK(56) + W(10) Varieties				
Variety	ID	UK+W		Correlations of SSR's ² Variety Profile Pairwise
		Anonymous	EST	
UKVar	2	0.06	0.01	0.56
UKVar	5	0.06	0.08	0.56
UKVar	3	0.06	0.01	0.55
UKVar	1	0.07	0.03	0.53
UKVar	8	0.06	0.01	0.52
UKVar	6	0.06	0.02	0.48
UKVar	19	0.05	0.00	0.46
UKVar	4	0.06	0.05	0.45
UKVar	27	0.03	0.03	0.44
UKVar	9	0.06	0.08	0.43
UKVar	13	0.05	0.03	0.42
UKVar	16	0.05	0.00	0.42
UKVar	10	0.06	0.05	0.38
UKVar	7	0.06	0.07	0.37
UKVar	17	0.03	0.03	0.36
UKVar	14	0.06	0.04	0.36
UKVar	26	0.07	0.02	0.35
UKVar	11	0.06	0.03	0.33
UKVar	18	0.02	0.03	0.31
UKVar	29	etc	etc	etc
UKVar	30	0.13	0.07	0.21
UKVar	31	0.07	0.04	0.14
UKVar	6	0.10	0.09	0.14
UKVar	34	0.04	0.08	0.12
UKVar	56	0.13	0.12	0.04
UKVar	35	0.05	0.13	-0.07
YU-18	W	0.06	0.06	0.43
Xian-8	W	0.11	0.08	0.37
Kopara	W	0.15	0.20	0.21
HD2189	W	0.15	0.10	0.11
Xios	W	0.12	0.10	0.04
Takahe	W	0.12	0.15	0.01
Myokonos	W	0.12	0.11	-0.01
Chinese_Spr	W	0.29	0.11	-0.17
Norin_6	W	0.19	0.17	-0.25
CS91	W	0.24	0.22	-0.29
Minimum		0.00	0.00	0.56
Maximum		0.29	0.24	-0.29
Median		0.07	0.04	0.28

Comparison of EST SSR's with Anonymous SSR's

Example where minimum distances are 0.00 ...

For EST SSRs the pair M. Nimrod v Prof. Marchal. (if omitted gives minimum minimum distance of 0.009)


Not necessarily matched by distances calculated from the anonymous SSRs



§ Between profiles of anonymous and EST SSR's

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Comparison of EST SSR's with Anonymous SSR's




Conclusions

Within the 66 varieties evaluated in this study:-

- The EST-derived microsatellites were of high quality, amplifying clear products with few stutter bands.
- The PIC values of the EST-microsatellites were generally lower than those observed in anonymous microsatellites derived from genomic libraries. Comparison with recently published PICs in wheat show both anonymous and EST SSRs used in this study, to be within the observed ranges.
- EST SSRs are less polymorphic.

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Comparison of EST SSR's with Anonymous SSR's



Conclusions

- EST SSRs are probably less neutral, due to differential 'selection pressures', and thus may exhibit acceptable levels of uniformity (no data).
- On the basis of this study there is no advantage, in terms of establishing distinctness, from the use of EST derived SSRs ('expressed') over selected anonymous SSRs (not expressed).
- There is no change in the distribution of minimum distances observed nor in the minimum of these minimum distances.

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