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EVALUATION OF THE POTENTIAL OF RFLPs FOR THE STUDY OF DISTINCTNESS, UNIFORMITY AND STABILITY IN SUNFLOWER

Document prepared by experts from France

Evaluation of the potential of RFLPs for the study of distinctness, uniformity and stability in sunflower

Y. X. ZHANG
GEVES, Le Magneraud, laboratoire de biochimie, BP52, 17700 SURGERES, France

Introduction

The GEVES (Groupe d'Etude et de Contrôle des Variétés et des Semences) is interested and implicated in the development of DNA profiling techniques for potential use in DUS testing of plant varieties, from several years. One of the research programs conducted by GEVES was the evaluation of the potentials of RFLP markers for distinctness and uniformity testing in sunflower.

Sunflower is a very important oil-seed production crop in France. Every year, there are more than one hundred of new hybrids applied for registration on the French list and new lines studied for plant breeder's rights. With this increasing number of new varieties and inbred lines, often selected from the similar genetic resources, distinction by the usual morphophysiological characters is reduced. Recently, RFLPs were reported to be promising molecular markers for the assessment of genetic variability among sunflower inbred lines (Berry et al., 1994; Gentzbittel et al., 1994; Zhang et al., 1995). Here we present our results on the interline variability (Distinctness) and intraline variability (Uniformity and Stability) obtained from RFLP analysis in sunflower.

Materials and methods

The study of interline variability was carried out on a set of 46 inbred lines of sunflower (public and protected lines), which represent the whole morphophysiological variability observed among the sunflower inbred lines maintained in the French reference collection in GEVES for DUS testing. Among these lines, several pairs are very close or not differentiated by morphological characters. For reason of simplicity and confidentiality, the lines were coded from number 1 to 46; lines 1 to 25 are maintainer (M) lines and lines 26 to 46 are restorer (R) lines. The leaves used for DNA isolation were collected from 5 plants per inbred line and bulked for each line.

To evaluate the variability within lines, 4 inbred lines were chosen for their importance in hybrid variety breeding : HA89, RHA266, CX and PAC2. For each line, 10 to 15 plants were analyzed by RFLPs. The leaves were harvested separately for each plant of the same line.

The methods used for DNA isolation and for RFLP analysis in this study were previously described (Gentzbittel et al., 1994). RFLP profiles observed in autoradiographs were scored visually. The presence or absence of a band in a gel lane was coded by 1 or 0 respectively.

Relationships among the sunflower inbred lines were studied by the estimation of Nei's similarity index *F* (Nei and Li, 1979) as well as Nei's distance *d,* expressed as the mean number of nucleotide substitutions per nucleotide site using the computer program developed by Gentzbittel and Nicolas (1990). A UPGMA dendrogram (Sneath and Sokal, 1973) was also constructed, based on the RFLP distances.

All the clones used as probes in our studies were prescreened and have detected polymorphism among sunflower inbred lines (Gentzbittel et al. 1994). The criteria of the choice of these clones were (1) genome coverage, (2) hybridization quality and (3) polymorphic content.

Results

1. Interline variability detected by RFLPs

Genetic variation among the 46 inbred lines of sunflower was assessed with 42 cDNA clones, combined with *Hin*dIII or *Eco*RI. The 42 probe-enzyme combinations produced a total of 203 fragments and 246 profiles across the 46 inbred lines of sunflower, corresponding an average of 4.8 fragments and 5.8 profiles per probe-enzyme combination. The number of RFLP profiles produced by each probe-enzyme combination ranged from 2 to 17 ; 62% of probe-enzyme combinations generated 2 to 5 profiles. The average gene diversity (H) (Nei, 1987) was 0.63 (S.E. = 0.83).

Nei and Li's similarity index *F* as well as the genetic distance *d*, calculated for the 1035 possible pairwise comparisons between the 46 sunflower inbred lines, are presented in Table 1. The *F* values ranged from 0.43 (for lines 4 and 31) to 0.98 (for lines 1 and 2), with an average of 0.63. Out of the 1035 possible pairwise combinations, 19 pairs of lines (1.84 %) had a *F* value more than 0.80; 7 pairs of lines (0.68 %) had a *F* value more than 0.90. The estimates of the distance *d* varied from 0.01 to 0.50 (Table 1).

Based on the RFLP distances estimated, an UPGMA dendrogram showing the relationships between the 46 inbred lines of sunflower was constructed (Fig. 1). Two main groups can be observed on the dendrogram: on the top a group of the R lines and on the bottom a group of M lines and there are several subgroups among each principal group. However, four R lines, 26, 38, 40 and 42 have been classed among the M lines. Likewise, two M lines, 5 and 13, have been classed among the R lines. The line 7 has been located out side the two main clusters. On the dendrogram, one can observe a triplet (lines 1, 2 and 18) and four pairs (lines 3 and 4, lines 29 and 34, lines 37 and 39, and lines 28 and 31) of lines which were very close and had a genetic distance less than 0.05 between them (Table 1). At morphophysiological level, lines 1, 2 and 18 can not be distinct; likewise for lines 29 and 34. Line 3 differentiated from line 4 only by one character - leaves denture. Lines 37 and 39 shared a common parent; likewise for the pair of lines 28 and 31. However, the two pairs of lines are declared distinct by morphophysiological characters.

2. Intraline variability of sunflower inbred lines revealed by RFLP

Evaluation of the intraline variation in four inbred lines of sunflower was performed with 30 probes, combined with *Hin*dIII or *Eco*RI. Ten plants for RHA266, 13 for CX, 14 for PAC2 and 15 for HA89 were studied. Evaluation of the variation within each line was based on the comparison of the fragment profiles revealed by each probe-enzyme combination. Table 2 shows the results from the comparison.

In line HA89, 2 of the 15 plants studied were different from the others by one and 9 probe-enzyme combinations respectively. For line CX, 3 of the 13 plants analyzed were differentiated with the other plants by 6, 1 and 4 probe-enzyme combinations respectively. Within line RHA266, 3 of the 10 plants were distinguished from the others by just one probe-enzyme combination. In line PAC2, no intraline variability has been detected by the 30 probe-enzyme combinations analyzed.

Discussion

The average number of RFLP variants detected by probe-enzyme combination across the 46 lines studies was about 6; this result confirmed the reports made by Berry et al. (1994) and by Gentzbittel et al.(1994) and Zhang et al. (1995) ; this means that the cultivated sunflower has a relatively high level of RFLP which is comparable with those reported in maize (Messmer et al., 1991, Smith et al; 1991, Livini al et. 1992). This level of polymorphism is three times higher than that revealed by isozymes in sunflower inbred lines (Quillet et al. 1992; Bourgoin-Grenèche, personal communication). The gene diversity calculated for the 46 inbred lines with this set of selected probes is on average 0.63, which is similar to that obtained on an other set of sunflower inbred lines (Zhang et al., 1995) but superior to the 0.49 reported by Berry et al. (1994).

The RFLP data obtained allowed a separation of M lines from R lines, with a few exceptions. This confirm the previous reports (Berry et al., 1994 ; Gentzbittel et al., 1994 ; Zhang et al., 1995) as well as the results obtained from isozymes (Bourgoin-Grenèche, personal communication), and is in concordance with the difference in morphology between these 2 types of lines in sunflower. Among the 46 lines analyzed, there are the 7 pairs of lines (1-2, 1-18, 2-18, 3-4, 29-34, 37-39 and 28-31) for them no difference (1-2, 1-18, 2-18, 3-4 and 29-34) or just some very small differentiation (37-39 and 28-31) can be observed from each other, based on morphological characters. From RFLP data, these pairs of lines showed the biggest similarity index values (> 0.90). Such a result proved the good precision of RFLPs on the estimation of genetic distance between inbred lines in sunflower.

Intraline variability has been detected by RFLP in three of the four lines analyzed; the degree of this variability varied according to line. This kind of variability has been already revealed by isozyme analysis and observed in the field on other sunflower inbred lines. There appear two explanations for some intraline variability on RFLP in cultivated sunflower. Firstly, the cultivated sunflower is an outbreeding species with forced selfpollination, secondly, RFLP loci have not been taken into consideration in the breeding programs until now. It should be indicated that these four inbreds examined in this study presented a good uniformity of morphological characters in the field. The heterogeneity may have a consequence in the study of distinctness based on DNA profiling because the more the degree of heterogeneity of a line is high, the more the distinctness of that line from the others is easy.

Conclusions

The results of the present study show that the RFLP data are potentially very useful in description and identification of sunflower inbred lines. RFLP has also been proved to be very powerful tools to determine the relations and to measure the genetic distance between lines. Compared with morphological characters, RFLPs have many advantages as descriptors of lines and varieties : unlimited number, independence of culture conditions, high level of polymorphism. This type of descriptor is especially useful to the cultivated sunflower which is extremely susceptible to the culture conditions ; the phenotype of a same inbred sunflower line may vary with the culture conditions and year. Thus, the whole set of reference varieties and lines has to be grown and observed each year and this is very expensive. The usual morphophysiological characters used for DUS testing in sunflower show more and more limits to describe correctly the numbers of inbred lines and varieties that increase year by year. In the future, the combination of both the classic morphophysiological characters and a genetic distance based on RFLP data in sunflower would improve the accuracy of description of inbred lines and varieties in the DUS testing and strengthen plant breeder's rights. Furthermore, the DNA markers like RFLPs might play an important role in the establishment of essential derivation because of its high number and good precision.

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Table 2. Intraline variability revealed by RFLPs in four sunflower inbred lines

Lines	Nb of plants analysed	Nb. of probe-enzyme combinations assayed	N° of the plant	Nb. of probe-enzyme combinations distinguishing this plant from the others
HA89 (CD)	15	29	p1	1
		29	p2	9
CX	13	30	p5	6
		30	p13	1
		30	p14	4
RHA266	10	30	p3	1
		30	p7	1
		30	p8	1
PAC2	14	30	0	0

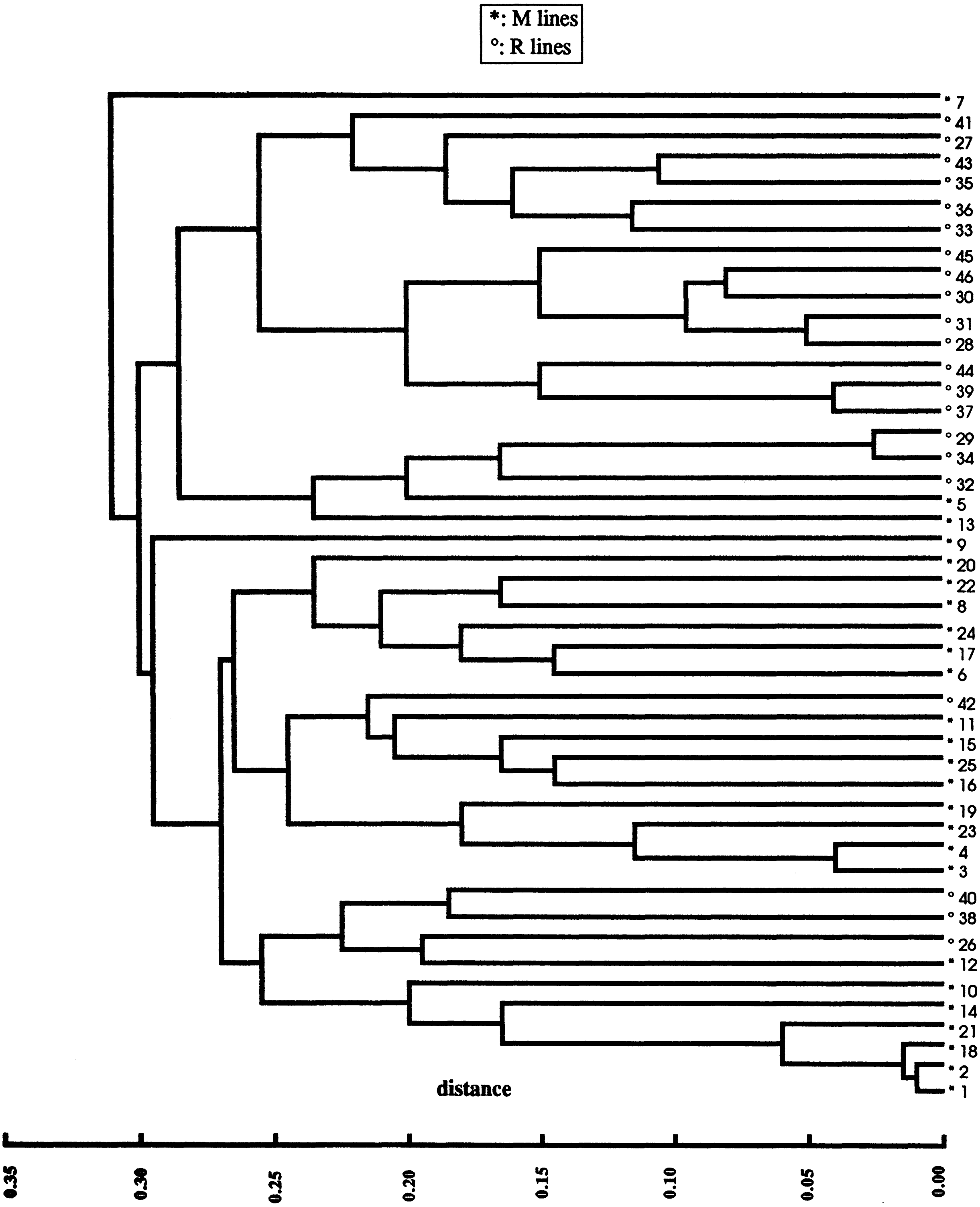

Fig. 1. UPGAM dendrogram generated from Nei's genetic distance estimates (Table 1) for the 46 sunflower inbred lines.

Table 1. Nei and Li's F index (below diagonal) and estimates of nucleotide substitutions per nucleotide site (above diagonal) for pairwise combinations among the 46 inbreds of sunflower

Lines	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
1		96	2224	2126	3064	2138	3106	2000	3078	1590	2186	2092	2395	1560	1995	1488	2005	127	2674	2360	1100	2058	1848	2529	2096	2259	2493	3985	3106	3071	4057	3797	3367	3176	2259	3367	3190	2058	3320	1892	3515	1850	2601	1885	3834	3298
2	0,98		2258	2159	2875	1989	3263	2129	2997	1714	2221	2028	2325	1678	2028	1613	2038	158	2496	2291	1213	2190	1701	2460	2129	2992	2325	3881	3022	3228	3952	3581	3160	2975	2099	3160	3100	1995	3227	1832	3298	1885	2635	1728	3729	3211
3	0,68	0,67		399	2735	2825	3479	2292	3314	2571	2186	2686	2722	2919	2715	2153	2919	2162	1967	2360	3492	3141	1246	3194	1906	2885	2919	4430	3106	3985	4658	3429	2359	2827	2359	2567	3453	3779	3190	2952	3141	1850	2292	3085	4288	3952
4	0,69	0,69	0,93		3064	3039	3610	2393	3194	2680	2505	2499	2836	2809	2606	1954	2596	2066	1781	2058	3367	2906	1009	3314	1724	3112	3031	4747	3479	4277	4986	3548	2462	3176	2259	2462	3589	3916	3453	3071	3022	1753	2000	3209	4613	4237
5	0,59	0,61	0,62	0,59		2144	2963	2801	2728	2885	2291	3262	2292	2487	2701	3126	3006	2760	2985	2563	3098	2997	2624	2941	2197	2974	2793	2708	2426	3160	2997	1822	2875	2005	2455	3449	2360	3470	2256	3160	3854	3645	3018	2394	2765	3382
6	0,69	0,71	0,61	0,59	0,69		2325	2110	1963	1807	2596	2293	2695	1686	2015	2559	1446	1907	1729	2261	2170	2357	1877	1625	2485	2746	1938	2695	2941	2801	3084	2004	2262	2587	2263	2857	3367	2455	3367	2695	2357	2997	2294	2392	3333	2587
7	0,59	0,57	0,55	0,54	0,60	0,67		1995	3092	3281	2622	2836	3336	2818	2836	2361	2601	2895	3263	3672	1772	2917	2861	3211	2190	3367	2818	3336	3372	3336	3281	3571	2906	3317	3126	2906	2590	2360	2952	2505	3281	3190	2715	2622	3170	3845
8	0,71	0,69	0,67	0,66	0,62	0,69	0,71		3262	2428	1954	2359	2257	2768	2062	1832	2358	1855	2529	2640	1940	1658	1913	2005	2162	2842	2985	3141	2394	2792	3333	3262	3436	2359	2629	3078	2219	2975	2658	2360	2126	2432	2885	2801	3100	3126
9	0,59	0,60	0,57	0,58	0,62	0,71	0,59	0,57		2567	3708	2929	2940	2801	3044	3141	2695	3098	2460	3367	2460	3750	2735	3538	3262	2358	2490	2940	3987	2606	2895	2857	2668	3919	2662	3112	3430	2895	3173	2827	3492	3538	3145	3071	3663	2708
10	0,76	0,74	0,64	0,63	0,61	0,73	0,57	0,66	0,64		3013	2122	2881	1848	2430	2616	2963	1635	2291	2952	2606	2836	2062	2784	2749	2601	3194	4513	3538	3773	4747	3131	3422	3479	2818	4237	3797	3192	4095	2432	3845	2855	3333	3013	4057	4021
11	0,68	0,68	0,68	0,65	0,67	0,64	0,63	0,71	0,53	0,59		2928	2253	2906	2150	1805	2792	2023	3245	2434	2432	2325	2291	2836	2053	2757	2058	2472	2289	2362	2658	3820	2881	2150	3106	2881	2325	2892	2213	2585	2658	2215	3157	2475	2639	2361
12	0,69	0,70	0,64	0,65	0,57	0,67	0,61	0,66	0,60	0,69	0,60		2467	1880	1961	2119	2162	2129	3092	2325	2640	2757	2292	2496	2062	1940	2668	3035	2611	3157	3106	30511	2749	2571	2426	2975	3551	2537	3415	2053	3106	2892	3009	2470	3245	2792
13	0,66	0,67	0,62	0,61	0,67	0,63	0,56	0,67	0,60	0,61	0,68	0,65		3126	2467	2545	2895	2428	3352	2881	3352	2432	2291	2291	1680	2749	2895	2810	2186	3430	2881	2842	2987	1954	2749	3352	2438	3502	2325	3300	3636	3283	3515	2940	2633	2801
14	0,76	0,74	0,60	0,62	0,65	0,74	0,61	0,62	0,62	0,72	0,61	0,72	0,58		2563	2126	2357	1604	2325	2963	1678	3078	2592	3018	2457	3051	3196	3884	3160	3367	3820	2516	3296	3112	3051	3415	3636	3314	3502	2784	3561	2871	2875	2792	4168	3228
15	0,71	0,70	0,62	0,64	0,63	0,70	0,61	0,70	0,59	0,66	0,69	0,71	0,65	0,64		1728	2359	1848	3211	2223	2325	2223	1906	2393	1602	2129	2066	2801	2722	3035	3352	3051	2749	2464	2129	2861	2903	2537	3026	2256	2757	1913	2258	2150	2989	3141
16	0,77	0,75	0,69	0,71	0,58	0,64	0,66	0,73	0,58	0,64	0,73	0,69	0,64	0,69	0,74		2640	1448	2836	1694	1797	1983	1415	3141	1468	2827	2749	3526	2928	3264	3876	3504	3192	2765	2190	2836	2877	2396	3003	2219	2845	2075	2646	2436	3644	3121
17	0,70	0,70	0,60	0,64	0,60	0,78	0,64	0,66	0,63	0,60	0,62	0,69	0,61	0,66	0,66	0,63		1952	2629	2529	2325	2226	2391	2011	2358	2941	2357	3492	3160	3245	3689	3026	2952	2997	2941	3179	3502	2635	3502	3009	2963	2757	2358	2571	4020	3595
18	0,98	0,97	0,69	0,70	0,62	0,72	0,61	0,72	0,59	0,75	0,70	0,69	0,66	0,75	0,72	0,78	0,71		2493	2193	996	1906	1625	2358	1855	2293	2325	3987	3245	3211	4057	3559	3031	2963	2197	3145	3085	2096	3209	1933	3280	1797	2629	1832	3840	3314
19	0,63	0,65	0,71	0,73	0,60	0,74	0,57	0,64	0,65	0,67	0,57	0,59	0,56	0,67	0,58	0,61	0,63	0,65		3176	3160	3176	1701	2885	2426	3504	2842	3743	3916	4165	3952	2293	2496	3457	2493	2929	3766	3176	3626	2987	2606	2505	2742	3000	4019	3457
20	0,66	0,67	0,66	0,70	0,64	0,67	0,53	0,63	0,56	0,60	0,66	0,67	0,61	0,60	0,68	0,74	0,64	0,68	0,58		2715	2188	1602	2258	2126	2818	2325	3372	2801	3502	3985	3245	2394	2537	1814	2606	3378	2952	3653	2765	3317	3357	2640	2774	3906	3352
21	0,82	0,81	0,55	0,56	0,59	0,68	0,73	0,71	0,65	0,64	0,66	0,63	0,56	0,74	0,67	0,73	0,67	0,84	0,58	0,62		2499	2668	2668	2635	2596	2132	3352	3515	2430	3422	3830	2929	3585	2701	2708	2737	2499	3227	1927	2606	2505	2742	2018	3729	2749
22	0,70	0,68	0,58	0,61	0,60	0,66	0,60	0,75	0,53	0,61	0,67	0,62	0,66	0,59	0,68	0,71	0,68	0,72	0,58	0,68	0,65		2567	1873	2749	2929	2851	2765	2153	2541	3071	3018	2940	2122	2601	2940	2399	2395	2512	2652	2188	3100	2749	2774	3208	2646
23	0,72	0,74	0,80	0,84	0,63	0,72	0,61	0,72	0,62	0,70	0,67	0,67	0,67	0,64	0,72	0,78	0,66	0,75	0,74	0,75	0,63	0,64		2325	1392	3098	2695	4092	3211	4092	4307	2963	2162	2708	1798	2563	2694	3126	2810	2715	2567	2153	2195	2395	3803	3654
24	0,64	0,65	0,58	0,57	0,60	0,75	0,58	0,70	0,54	0,62	0,61	0,65	0,67	0,59	0,66	0,58	0,70	0,66	0,61	0,67	0,63	0,72	0,67		3031	2985	2490	2606	2533	3057	2784	2651	2066	2393	2768	2563	2580	3009	2810	2827	1873	3672	2809	2952	3013	3044
25	0,69	0,69	0,72	0,74	0,68	0,65	0,68	0,69	0,57	0,62	0,70	0,70	0,74	0,65	0,75	0,77	0,66	0,72	0,66	0,69	0,63	0,62	0,78	0,59		2425	2559	3515	2940	3645	3333	2825	2742	2462	2228	2742	2892	2640	3013	3022	3585	2432	2460	2256	3490	3620
26	0,67	0,66	0,61	0,58	0,60	0,62	0,56	0,61	0,66	0,64	0,62	0,71	0,62	0,59	0,69	0,61	0,60	0,67	0,55	0,61	0,64	0,60	0,59	0,60	0,66		1952	2126	2258	2126	2193	2995	2005	2129	2391	3031	3336	2000	2964	2126	3280	2952	3179	2229	2470	2129
27	0,65	0,67	0,60	0,59	0,62	0,71	0,61	0,60	0,65	0,58	0,70	0,63	0,61	0,58	0,70	0,62	0,66	0,67	0,61	0,67	0,69	0,61	0,63	0,65	0,64	0,71		1515	2601	1691	1848	2325	1678	2359	1952	1855	2432	1848	2652	2674	2226	2122	1978	1866	2186	1528
28	0,51	0,52	0,47	0,45	0,63	0,63	0,56	0,58	0,60	0,46	0,65	0,59	0,61	0,51	0,62	0,55	0,55	0,51	0,53	0,56	0,56	0,62	0,50	0,64	0,55	0,69	0,77		1885	1125	487	1855	2223	1858	2325	2430	2325	1823	2215	2694	2119	3283	2464	2253	1101	1062
29	0,59	0,59	0,59	0,55	0,66	0,60	0,56	0,66	0,51	0,54	0,67	0,64	0,68	0,58	0,62	0,60	0,58	0,57	0,51	0,62	0,55	0,69	0,58	0,64	0,60	0,67	0,64	0,72		1694	1670	1798	1961	269	2159	2680	2144	2917	1833	2616	2917	3453	2715	2737	2215	1989
30	0,59	0,57	0,51	0,48	0,58	0,62	0,56	0,62	0,64	0,52	0,66	0,58	0,55	0,56	0,59	0,57	0,57	0,58	0,49	0,55	0,66	0,64	0,50	0,59	0,54	0,69	0,74	0,82	0,74		947	2526	2646	2053	2640	2987	2791	2541	2671	3049	2325	2903	2464	2585	1964	822
31	0,50	0,51	0,45	0,43	0,60	0,59	0,57	0,56	0,61	0,45	0,63	0,59	0,61	0,52	0,56	0,52	0,53	0,50	0,51	0,51	0,56	0,59	0,48	0,62	0,56	0,68	0,72	0,92	0,75	0,85		1830	2291	1738	2601	2606	2627	1892	2289	3000	2291	3490	2749	2545	1085	736
32	0,52	0,54	0,55	0,54	0,73	0,70	0,54	0,57	0,61	0,58	0,52	0,59	0,61	0,65	0,59	0,55	0,59	0,54	0,67	0,57	0,52	0,59	0,60	0,63	0,61	0,60	0,67	0,72	0,73	0,64	0,73		1838	1520	1760	2587	2715	2908	2715	3415	2695	3785	1783	2851	2156	2325
33	0,56	0,58	0,66	0,65	0,61	0,67	0,61	0,55	0,63	0,56	0,61	0,62	0,60	0,57	0,62	0,58	0,60	0,59	0,65	0,66	0,60	0,60	0,69	0,70	0,62	0,70	0,74	0,68	0,71	0,63	0,67	0,72		1658	1646	1133	2289	2499	1977	2430	2092	3209	1581	2652	2585	2428
34	0,58	0,60	0,61	0,58	0,70	0,64	0,57	0,66	0,51	0,55	0,69	0,64	0,71	0,58	0,65	0,62	0,60	0,60	0,55	0,64	0,54	0,69	0,63	0,66	0,65	0,69	0,66	0,72	0,95	0,70	0,74	0,77	0,75		2129	2428	2217	2871	1707	2467	2871	3264	2674	2580	2181	1961
35	0,67	0,69	0,66	0,67	0,65	0,67	0,58	0,63	0,63	0,61	0,59	0,66	0,62	0,59	0,69	0,68	0,60	0,68	0,65	0,73	0,63	0,64	0,73	0,62	0,68	0,66	0,71	0,67	0,69	0,63	0,64	0,73	0,75	0,69		1823	2505	2096	2845	2224	2292	2611	1028	2223	2255	2635
36	0,56	0,58	0,64	0,65	0,55	0,61	0,61	0,59	0,58	0,49	0,61	0,60	0,56	0,56	0,61	0,61	0,58	0,58	0,60	0,64	0,63	0,60	0,64	0,64	0,62	0,59	0,72	0,66	0,63	0,60	0,64	0,64	0,82	0,66	0,73		2976	2940	2976	2646	1995	2845	1411	3121	3320	2640
37	0,58	0,59	0,55	0,54	0,66	0,56	0,64	0,68	0,55	0,52	0,67	0,54	0,65	0,54	0,61	0,61	0,55	0,59	0,52	0,56	0,62	0,66	0,63	0,64	0,61	0,56	0,66	0,67	0,69	0,62	0,63	0,62	0,67	0,68	0,65	0,60		2745	380	2553	2745	2639	3137	1488	1700	2903
38	0,70	0,71	0,52	0,51	0,55	0,65	0,66	0,60	0,61	0,58	0,61	0,64	0,55	0,57	0,64	0,66	0,63	0,69	0,58	0,60	0,65	0,66	0,58	0,59	0,63	0,71	0,72	0,73	0,60	0,64	0,72	0,60	0,65	0,61	0,69	0,60	0,62		2745	1823	2502	2509	2749	1913	1733	2325
39	0,56	0,57	0,58	0,55	0,68	0,56	0,60	0,63	0,58	0,50	0,68	0,56	0,67	0,55	0,59	0,60	0,55	0,58	0,54	0,53	0,57	0,65	0,61	0,61	0,59	0,60	0,63	0,68	0,73	0,63	0,67	0,62	0,71	0,74	0,61	0,60	0,93	0,62		2438	2866	2519	3393	1685	1912	2549
40	0,72	0,73	0,60	0,59	0,58	0,63	0,65	0,66	0,61	0,66	0,64	0,70	0,57	0,62	0,68	0,68	0,59	0,71	0,60	0,62	0,71	0,63	0,62	0,61	0,59	0,69	0,63	0,63	0,64	0,59	0,60	0,56	0,66	0,65	0,68	0,63	0,64	0,73	0,65		2765	1905	2792	1941	3003	2687
41	0,55	0,57	0,58	0,59	0,52	0,66	0,57	0,69	0,55	0,52	0,63	0,59	0,54	0,54	0,62	0,61	0,60	0,57	0,64	0,57	0,64	0,68	0,64	0,72	0,54	0,57	0,68	0,69	0,60	0,67	0,67	0,63	0,69	0,61	0,67	0,71	0,62	0,65	0,61	0,62		2398	2224	2892	2829	2023
42	0,72	0,72	0,72	0,74	0,54	0,60	0,58	0,66	0,54	0,61	0,68	0,61	0,57	0,61	0,72	0,70	0,62	0,73	0,65	0,56	0,65	0,59	0,69	0,53	0,66	0,60	0,69	0,57	0,55	0,61	0,55	0,52	0,58	0,57	0,64	0,61	0,63	0,65	0,65	0,72	0,66		2765	2107	3245	2892
43	0,64	0,63	0,67	0,71	0,59	0,67	0,62	0,61	0,58	0,56	0,58	0,59	0,55	0,61	0,67	0,63	0,66	0,63	0,62	0,63	0,62	0,62	0,68	0,62	0,65	0,58	0,71	0,65	0,62	0,65	0,62	0,73	0,76	0,63	0,83	0,78	0,58	0,62	0,56	0,62	0,68	0,62		3035	2976	2784
44	0,72	0,74	0,59	0,58	0,66	0,66	0,63	0,62	0,59	0,59	0,65	0,65	0,60	0,62	0,69	0,65	0,64	0,73	0,60	0,62	0,70	0,62	0,66	0,60	0,68	0,68	0,72	0,68	0,62	0,64	0,64	0,61	0,63	0,64	0,68	0,58	0,77	0,72	0,74	0,71	0,61	0,69	0,59		2065	3049
45	0,52	0,53	0,48	0,46	0,62	0,56	0,58	0,59	0,53	0,50	0,63	0,57	0,63	0,49	0,60	0,54	0,50	0,52	0,50	0,51	0,53	0,58	0,52	0,59	0,55	0,65	0,68	0,82	0,68	0,71	0,83	0,69	0,64	0,68	0,68	0,56	0,74	0,74	0,72	0,60	0,61	0,57	0,60	0,70		1769
46	0,57	0,58	0,51	0,49	0,56	0,64	0,52	0,58	0,63	0,50	0,66	0,62	0,62	0,57	0,58	0,58	0,54	0,57	0,55	0,56	0,62	0,63	0,53	0,59	0,54	0,69	0,76	0,83	0,71	0,86	0,88	0,67	0,66	0,71	0,63	0,63	0,61	0,67	0,64	0,63	0,70	0,61	0,62	0,59	0,73	